LAURIE-EVE RIOUX

CARACTÉRISATION STRUCTURALE ET ÉVALUATION DE L'ACTIVITÉ BIOLOGIQUE DE POLYSACCHARIDES EXTRAITS DE *SACCHARINA LONGICRURIS*

Thèse présentée
à la Faculté des études supérieures de l'Université Laval
dans le cadre du programme de doctorat en Sciences et technologie des aliments
pour l'obtention du grade de Philosophiae Doctor (Ph.D.)

DÉPARTEMENT DE SCIENCES DES ALIMENTS ET NUTRITION
FACULTÉ DES SCIENCES DE L'AGRICULTURE ET DE L'ALIMENTATION
UNIVERSITÉ LAVAL
QUÉBEC

2010

Résumé

Saccharina longicruris est une algue brune présente au Québec comportant des polysaccharides sulfatés (galactofucanes ou fucoïdane) et un β-glucane (laminarane) dont les structures n'ont jamais été caractérisées. Les fucoïdanes d'autres espèces algales ont démontré plusieurs activités biologiques telles qu'anticoagulante, anti-inflammatoire, antivirale et anti-tumorale. Quant au laminarane, des activités anti-tumorale au niveau du colon et anti-apoptose ont été observées. Les caractéristiques structurales des laminaranes et galactofucanes (fucoïdane dont le contenu en galactose est égal ou supérieur au fucose) extraits en mai (M05), août (A05), novembre 2005 (N05) et juin 2006 (J06) ont été étudiées. La caractérisation structurale du laminarane et du galactofucane a été ensuite approfondie. Les galactofucanes ont été dépolymérisés pour faciliter la détermination de la structure mais aussi pour étudier l'effet du poids moléculaire sur l'activité biologique. L'activité métabolique des fractions a été étudiée par l'utilisation de puces à ADN. Le profil d'expression génique de fibroblastes traités avec du laminarane et du galactofucane a montré des modulations du métabolisme du cycle cellulaire et de la matrice extracellulaire. Ces résultats ont été validés par des tests *in vitro* classiques pour confirmer les effets sur la croissance cellulaire, l'apoptose et la sécrétion de métalloprotéinase matricielle (MMP) et de collagène-I. Par exemple, le laminarane stimule la sécrétion de collagène-1, protéine importante pour la guérison des plaies alors que le galactofucane inhibe la prolifération cellulaire. Ces résultats ont permis d'identifier des caractéristiques structurales importantes pour la modulation de l'activité comme le poids moléculaire. Ils ont aussi permis de clarifier la relation entre la structure et la bioactivité du laminarane et du galactofucane.

Abstract

Saccharina longricruris is a brown seaweed present in Quebec containing galactofucan (fucoidan), a sulphated polysaccharides, and laminaran, a β-glucan. These polysaccharides were never analysed in term of structure and biological activities for this seaweed species. Bioactivities of fucoidans from other seaweed species were uncovered like: anticoagulant, anti-inflammatory, antiviral, antitumor and as contraceptive agent. For laminaran, anti-tumor activity in the gut and anti-apoptosis were established. Laminaran and galactofucan extracted from four harvest periods May (M05), August (A05), November 2005 (N05) and June 2006 (J06) were studied. Basic structural characterisation was realised to determine the relation between the harvest period and the structure of laminaran and galactofucan. Further structural analysis were realised to clarify the polysaccharides structure. Only galactofucan were depolymerised to improve the structural comprehension and to study the effect of low molecular weight polysaccharides on the biological activity. The effect of laminaran and galactofucan on the metabolic activity was studied using DNA microarray. The gene expression profile of fibroblasts treated with each polysaccharide fraction showed to influence the cell cycle progression and the extracellular matrix. Classical *in vitro* tests were performed to validate the results obtained with DNA microarray. Cell growth, apoptosis, matrix metalloproteinase (MMP) and collagen-I were analysed. Laminaran showed to stimulate the secretion of collagen-I, a protein involved during wound healing. Galactofucan showed an anti-proliferation activity. Structural differences were reported to influence the biological activities of these polysaccharides, for example the molecular weight. All the data were used in attempt to clarify the link between structure and the bioactivities of galactofucan and laminaran.

Avant-Propos

Tout d'abord, je voudrais remercier ma directrice, Sylvie Turgeon, pour son support, son écoute et ses encouragements. Merci pour toutes les petites rencontres de 5 minutes qui n'étaient pas prévues à ton horaire et qui me permettaient d'avancer encore plus vite. Merci Sylvie de m'avoir donné les outils pour accomplir mes élans de folies. Merci surtout d'avoir été pour moi un modèle et de m'avoir enseigné à être une bonne chercheure.

Un gros merci à mon co-directeur, Martin Beaulieu, pour son écoute et son soutien. Merci Martin, encore une fois, de m'avoir fait confiance et de m'avoir créé un projet sur mesure. Je me considère très chanceuse d'avoir eu un projet aussi stimulant du début à la fin. Merci à tes idées de génie qui arrivaient toujours juste au bon moment. Sans oublier ton risotto aux champignons sauvages, quel délice!

Je voudrais exprimer ma gratitude à Madame Véronique Moulin. Merci d'avoir contribué à approfondir mes connaissances au niveau des tests d'activités biologiques et d'avoir pris le temps de répondre à mes nombreuses questions.

Je voudrais aussi remercier les professionnels de l'INAF pour leurs grands supports durant mes expériences au laboratoire. Merci à Diane Gagnon, Alain Gaudreau, Pascal Dubé, Anne-Françoise Allain. Un gros merci au personnel du laboratoire pilote, Mélanie Martineau, Pascal Cliche, Gaétan Desnoyer, Jocelyne Giasson et Bernard Béliveau. Aussi, je voudrais remercier les stagiaires qui ont travaillé avec moi, Isabelle Parent et Laurence Cambron-Fortin. Les filles vos efforts et votre implication dans ce projet ont été importants pour sa réussite. Je tiens à remercier les membres de mon équipe de recherche particulièrement, Sandra Laneuville, pour tout son support scientifique, son écoute et ses encouragements. Un merci tout spécial pour Karen Vallverdu-Spitz, pour les pauses café et une belle fête de la St-Jean. Je voudrais faire un petit clin d'œil à mes collègues de labo, merci de m'avoir encouragé surtout les samedis et dimanches matins où il était toujours trop tôt pour faire du labo.

Je tiens à remercier les Biotechnologies Océanova inc. et le CRSNG-RDC pour leur support financier. Je remercie également le FQRNT pour m'avoir octroyé une bourse d'étude.

En terminant, je voudrais remercier ma famille et mes amis. À mes parents qui ont toujours été là pour me supporter et m'encourager. À mes amies : Caroline, Anne, Valérie L., Valérie A., Nadia, Carinne, Marie-Andrée et Audrey, merci de m'avoir donné l'occasion de m'évader en me parlant de tout sauf de science. Un million de fois merci à Steeve Larouche, mon conjoint et l'homme au plus beau sourire. Merci Steeve d'avoir été patient et compréhensif pour toutes ces fins de semaine passées au labo. Merci de m'avoir écoutée et encouragée quand ça n'allait pas. Merci d'avoir cru en moi.

Cette thèse a été rédigée sous forme d'article. Le premier chapitre intitulé « Revue de littérature » est une revue des caractéristiques structurales et des activités biologiques des polysaccharides extraits de l'algue *Saccharina longicruris*.

Le deuxième chapitre est intitulé « Effect of season on the composition of bioactive polysaccharides from the brown seaweed *Saccharina longicruris* » et cet article est publié dans la revue *Phytochemistry*. Ce chapitre porte sur l'effet de la saison sur les composantes structurales des polysaccharides extraits de l'algue. J'ai contribué à ce travail en réalisant en totalité les manipulations au laboratoire et la rédaction de l'article. Sylvie Turgeon et Martin Beaulieu ont apporté leur soutien scientifique dans les expérimentations au laboratoire et lors de la rédaction de l'article.

Le troisième chapitre est intitulé « Modulation of metabolic activities induced by a laminaran extract from brown seaweed *Saccharina longicruris* ». Ce chapitre sera publié sous la forme de deux articles, mais pour faciliter la compréhension de la thèse, il a été rédigé en un seul bloc. Ainsi, la section sur la structure du laminarane sera soumise dans la revue *Phytochemistry*. La section comprenant toute l'activité métabolique du laminarane sera publiée dans un autre journal. Ce chapitre met en évidence l'effet du laminarane sur l'expression des gènes liés à la prolifération cellulaire et à la matrice extracellulaire.

L'étude de puces à ADN a été réalisée par la compagnie Straticell (Gembloux, Belgique). La confirmation des résultats par des tests *in vitro* classique ont été réalisés par Véronique Moulin (LOEX). J'ai contribué à ce travail en réalisant l'extraction et la caractérisation structurale des fractions. De plus, j'ai analysé tout les résultats issus de l'étude de l'expression des gènes et des analyses *in vitro* classiques. J'ai aussi rédigé les articles. Sylvie Turgeon, Martin Beaulieu et Véronique Moulin ont apporté leur soutien scientifique dans les expérimentations au laboratoire et lors de la rédaction des articles.

Le quatrième chapitre est intitulé « Modulation of metabolic activities induced by a crude and depolymerised galactofucan extract from brown seaweed *Saccharina longicruris* ». Ce chapitre sera publié sous la forme de trois articles, mais pour faciliter la compréhension, il a été rédigé en un seul bloc. Ainsi, la section comprenant la structure de galactofucane brut et dépolymérisé sera soumise conjointement avec la structure du laminarane dans la revue *Phytochemistry*. Les résultats de l'activité métabolique du galactofucane natif et dépolymérisé seront publiés dans deux articles. Ce chapitre met en évidence l'effet du galactofucane brut et dépolymérisé sur l'expression des gènes liés à la prolifération cellulaire et à la matrice extracellulaire. L'étude de puces à ADN a été réalisée par la compagnie Straticell (Gembloux, Belgique). La confirmation des résultats par des tests *in vitro* classique ont été réalisés par Véronique Moulin (LOEX). J'ai contribué à ce travail en réalisant l'extraction et la caractérisation structurale des fractions. De plus, j'ai analysé tout les résultats issus de l'étude de l'expression des gènes et des analyses *in vitro* classiques. J'ai de plus effectué la rédaction des articles. Sylvie Turgeon, Martin Beaulieu et Véronique Moulin ont apporté leur soutien scientifique dans les expérimentations au laboratoire et lors de la rédaction des articles.

I am only as strong as the coffee I drink, the
hairspray I use and the friends I have.
To all of you that have touched my life.
Here's to you.
Anonym

Table des matières

Liste des tableaux

Liste des figures

Introduction

Les algues sont très versatiles de par leur utilisation dans plusieurs domaines d'intérêts tels que : la nutrition humaine et animale, la cosmétique et les fertilisants. La production totale annuelle d'algue a une valeur commerciale de 6 milliards de dollars américain dont 5 milliards sont destinés aux produits alimentaires pour consommation humaine. Au total, c'est 8 millions de tonnes d'algues fraîches qui sont destinés aux différents secteurs annuellement (FAO, 2003). La consommation des algues ne cesse d'augmenter au Japon, en Chine et en Corée alors, qu'elle reste modeste en Europe et en Amérique (Indegaard et al., 1991a). Traditionnellement, les algues étaient consommées à l'état frais ou blanchies dans des salades, des soupes ou en garnitures (Yuan, 2008). Plus récemment, la consommation des algues séchées a augmenté en popularité en occident avec l'apparition des sushis.

Les algues sont faibles en gras et contiennent beaucoup de minéraux et de vitamines. Des quantités importantes de protéines, d'acides gras polyinsaturés à longues chaînes et des fibres solubles et insolubles y sont présentes (Yuan, 2008). Parmi les fibres solubles, on retrouve des hydrocolloïdes tels que l'alginate, l'agar-agar, les carraghénanes, le fucoïdane et le laminarane qui sont présents en proportion importante dans les algues. Plusieurs de ces polysaccharides sont utilisés dans les aliments comme agent épaississant, gélifiant et émulsifiant. Récemment, certains ont aussi démontré de nombreuses activités biologiques notamment des activités anticoagulante, anti-inflammatoire, antivirale et au niveau de la stimulation du système immunitaire (Fitton et al., 2008; Nagaoka et al., 2000). Ces activités ont été rapportées pour différentes espèces d'algues et pour différents types de structure.

Plusieurs espèces d'algues sont présentes au Québec, particulièrement dans le fleuve Saint-Laurent. Parmi les espèces exploitables, l'algue brune *Saccharina longicruris*, croit exclusivement auprès des côtes de l'Atlantique Nord, expliquant le peu d'études portant sur cette dernière. Cette algue brune contient des polysaccharides particulièrement intéressants : les alginates, les fucoïdanes et les laminaranes qui ont démontré plusieurs activités biologiques chez d'autres espèces d'algues. Les fucoïdanes possèdent des activités

anticoagulante, anti-inflammatoire, antivirale, anti-tumorale et comme agent contraceptif (Berteau et al., 2003; Boisson-Vidal et al., 1995; Nagaoka et al., 2000). Plusieurs espèces d'algues ont été étudiées tels : *Fucus vesiculosus* (Nagaoka et al., 2000) et *Laminaria brasiliensis* (Pereira et al., 1999) qui ont montré des structures différentes. Le laminarane constitue la réserve énergétique des algues, l'équivalent du glycogène humain. Il est reconnu pour son activité anti-tumorale (Kuda *et al.*, 2005) et est impliqué dans la régulation de la mort cellulaire (Kim *et al.*, 2006). Dû à ses propriétés gélifiantes et épaississantes (McNeely et al., 1973), l'alginate est surtout intéressant comme ingrédient alimentaire puisqu'il est présent en grande quantité.

Le contenu en polysaccharides dans les algues est influencé par plusieurs facteurs environnementaux, biologiques et physiques. Par exemple, la période de récolte, l'espèce d'algue et le protocole d'extraction influencent les rendements en polysaccharide mais aussi la structure. Ceci aura des conséquences importantes sur l'activité biologique des polysaccharides. Le poids moléculaire, la nature des unités de base, le contenu en sulfates et leurs positions, le type de liaison glycosidique ainsi que de la géométrie de la molécule (Melo et al., 2002; Shanmugam et al., 2000) sont des caractéristiques structurales très importantes. Ces dernières influenceront positivement ou négativement l'activité selon le cas. Par exemple, un fucoïdane de faible poids moléculaire aura une activité anticoagulante supérieure comparativement au polysaccharide natif (Nishino et al., 1991).

L'objectif de cette thèse est de mieux comprendre le lien entre la structure et l'activité biologique de polysaccharides extraits de *Saccharina longicruris*. Ces travaux permettront d'évaluer le potentiel de ces extraits d'algues dans le domaine des aliments fonctionnels, des produits de santé naturels et des cosmétiques. Ces extraits représentent un potentiel de développement important pour le secteur des biomolécules d'origine marine au Canada.

Chapitre I :
Revue de littérature

1.1 Les algues

Les algues jouent un rôle important dans notre écosystème. Elles fixent le carbone en le transformant de la forme inorganique à organique. Elles sont aussi à la base de la chaîne alimentaire et sont consommées par plusieurs organismes. Les algues sont regroupées en trois grandes classes basées sur leur pigmentation : les algues brunes (*Phaeophyaceae*), rouges (*Rhodophyceae*) et vertes (*Chlorophyceae*). Les algues vertes sont peu exploitées mondialement et seules les espèces du genre *Monostroma*, *Caulerpa* et *Enteromorpha* sont cultivées commercialement. Pour les algues rouges, les espèces du genre *Porphyra*, *Gelidium*, *Gloiopeltis*, *Eucheuma* et *Gracilaria* sont les plus populaires. Les algues rouges sont bien connues pour leurs composés gélifiants tels : les carraghénanes et l'agar. Chez les polysaccharides extraits d'algues rouge, des activités anticoagulantes (Farias et al., 2000) et antivirales (Carlucci et al., 1997) ont été démontrées. Les algues brunes sont les plus exploitées à l'échelle mondiale. Plus de 1000 espèces d'algues brunes ont été dénombrées (Guiry et al., 2009). Les *Dictuotales*, les *Ectocarpales*, les *Fucales* et les *Laminariales* sont les ordres qui renferment le plus d'espèces (Figure 1.1). Plusieurs espèces ont une valeur commerciale importante tels les genres : *Laminaria*, *Undaria*, *Macrocystis*, *Sargassum* et *Fucus* (Ito et al., 1989). Toutes les algues brunes sont pluricellulaires, la plupart vivent en eau salée et sont abondantes sur les côtes tempérées en eau froide. Au Québec, différentes espèces sont présentes telles que : *Ascophyllum nodosum*, *Fucus vesiculosus* et *Saccharina longicruris*. Ces trois espèces sont recherchées pour leurs composés (polysaccharides et polyphénols) ayant des applications nutraceutiques et vertus antioxydantes (Plaza et al., 2008).

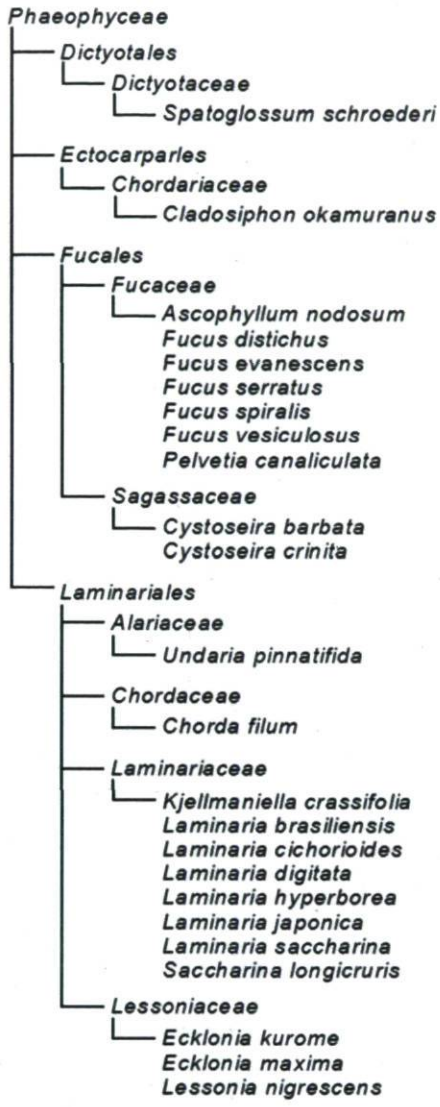

Figure 1.1 : Taxonomie des algues de la classe des *Phaeophyaceae* présentée dans cette thèse. Adapté de Guiry et collaborateurs (Guiry et al., 2009)

Parmi les espèces d'algues exploitables, *Saccharina longicruris* croit exclusivement auprès des côtes de l'Atlantique Nord, expliquant le peu d'études portant sur la caractérisation de ces polysaccharides. Les recherches réalisées se sont concentrées sur l'impact des vagues sur la croissance (Gerard et al., 1979), les rendements en polysaccharides en fonction de la période de récolte (Chapman et al., 1978; Souchet, 2004), sur l'impact des nutriments

présents dans l'eau au niveau de la croissance de l'algue (Anderson et al., 1981; Chapman et al., 1977; Gagné et al., 1982), sur l'étude des monosaccharides (blocs MM et GG) de l'alginate (Craigie et al., 1984) et sur la caractérisation rhéologique et des composantes structurales des polysaccharides extraits des algues brunes du Québec (Rioux et al., 2007a; Rioux et al., 2007b).

Les algues brunes se retrouvent sous la forme d'un thalle qui représente l'ensemble de l'appareil végétatif de l'algue (Figure 1.2). Le thalle se compose d'un crampon (semblable à une racine) qui le fixe à un stipe (semblable à une tige) qui supporte les frondes (semblable à des feuilles) (Campbell, 1995). D'autres espèces d'algues portent aussi des ballonnets qui permettent aux algues de flotter lorsqu'elles sont submergées.

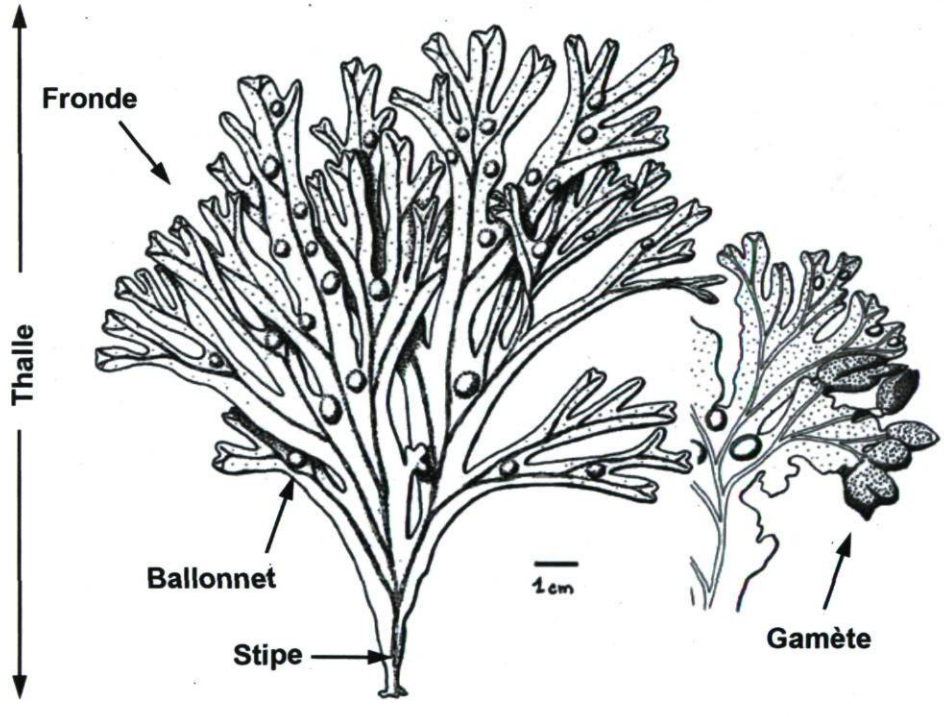

Figure 1.2 : Représentation d'un thalle de *Fucus vesiculosus*, un exemple d'algue brune. Adapté de Noailles (Noailles, 2003).

1.2 *Saccharina longicruris*

1.2.1 Physiologie

Saccharina longicruris anciennement nommé *Laminaria longicruris* (Lane et al., 2006) fait partie de l'ordre des *Laminariales* et de la famille des *Laminariaceaes* (Figure 1.3). De couleur jaune à brun pâle, elle se distingue par un stipe cylindrique creux au sommet. Cette algue possède un crampon ramifié et la fronde est simple et ondulée sur les côtés. Cette espèce peut atteindre de 2 à 7 mètres de long (Chapman, 1987). Sa population est élevée sur les côtes de la Nouvelle-Écosse et des thalles ont été retrouvés au Québec. Elle croit en grande concentration dans la Baie-des-Chaleurs, sur la côte sud de l'Île-d'Anticosti, aux Îles-de-la-Madeleine et allant de l'estuaire du Saint-Laurent jusqu'à l'Île-aux-Coudres (MAPAQ, 1993). Cette espèce est unique au Canada et très peu de recherches y ont été consacrées.

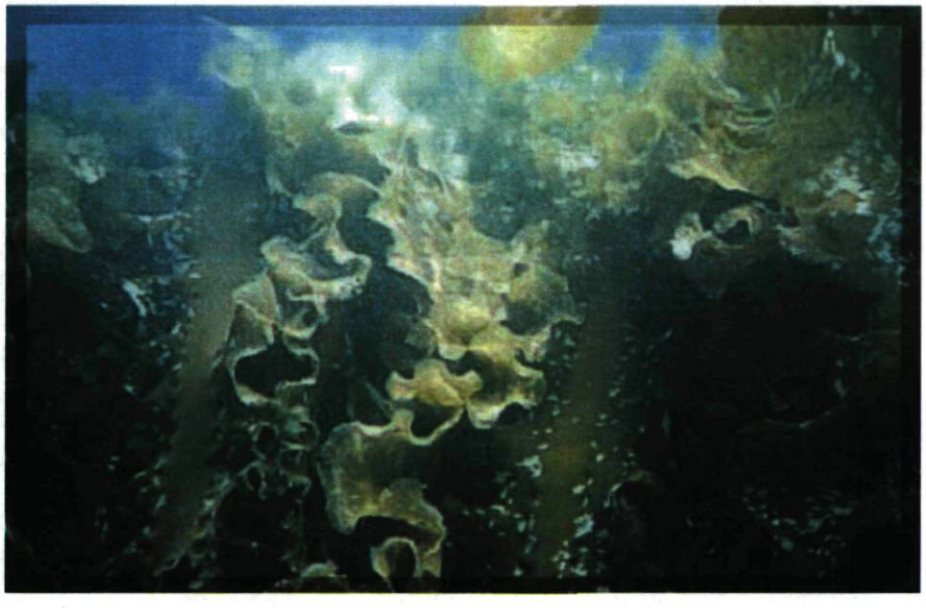

Figure 1.3 : Photo sous-marine de *Saccharina longicruris*.
(Poirier, 2002)

1.2.2 Composition

Cette espèce contient sur base sèche 27,7% de cendres, 12,4% de protéines, 2,1% de lipides et 57,8% de glucides (Rioux et al., 2007a). Plusieurs polysaccharides de structure tels que l'alginate et le fucoïdane et de réserve, comme le laminarane sont présents. Elle contient environ 0,2% de laminarane, 1,7% de fucoïdane, 20,0% d'alginate (Rioux et al., 2007a) et la fraction résiduelle serait composé majoritairement de cellulose et d'hémicellulose. Le contenu en laminarane des laminaires peut atteindre 30% des polysaccharides. Cependant, les rendements d'extractions sont influencés par plusieurs facteurs.

1.2.2.1 Facteurs influençant le contenu en polysaccharides

De manière générale, le contenu en polysaccharides, leur structure et leur l'activité biologique sont influencées par plusieurs facteurs. L'espèce de l'algue est très importante car certaines espèces contiennent moins de polysaccharides que d'autres. Par exemple, les *Fucaceas*, comme *A. nodosum*, contiennent moins de laminarane que les *Laminariaceaes* (Tableau 1.1).

Tableau 1.1 : Composition moyenne en polysaccharides des algues brunes

	Ascophyllum nodosum	*Laminaria digitata*
	g/100g d'algue sec	g/100g d'algue sec
Alginate	15 - 30	20 - 45
Fucoïdane	2 - 10	2 - 10
Laminarane	0 - 10	0 - 18

Adapté de Indergaard et collaborateur (Indegaard et al., 1991a).

D'autres facteurs influencent le contenu en polysaccharides tels : l'âge de la population, la période de récolte et les traitements post-récolte (séchage, entreposage, extraction, etc.) (Black et al., 1952; Haug et al., 1956; Zvyagintseva et al., 2003). Par exemple, la lyophilisation a un impact sur le poids moléculaire de l'alginate de sodium dû aux

différents cycles que subissent les échantillons (Wedlock et al., 1987). Plusieurs facteurs environnementaux peuvent modifier le contenu en polysaccharides tels : l'habitat, le degré d'exposition aux vagues, les courants marins, la lumière disponible pour la photosynthèse, la température de l'eau, la profondeur d'immersion des algues, les sels nutritifs et la salinité (Black, 1954; Black et al., 1949; Lobban et al., 1997; Percival et al., 1967). Par exemple, le maximum de photosynthèse se produirait entre six et dix mètres d'eau et non à la surface (Percival et al., 1967). Aussi, la disponibilité des sels nutritifs, principalement le nitrate, influence la productivité des algues (Edwards et al., 2006; Gordillo et al., 2006; Harlin et al., 1978) mais agit également sur la structure, la composition et la bioactivité des polysaccharides.

Pour le laminarane, plusieurs études ont montré l'impact des conditions environnementales sur les rendements en polysaccharide (Anderson et al., 1981; Chapman et al., 1978; Gagné et al., 1982). Au courant d'une année, la croissance de l'algue est dictée par l'intensité lumineuse. Par contre, lorsque l'algue se retrouve en période de restriction de nitrite et nitrate entre les mois de mai et août (selon le site de récolte), un stimulus ordonne la synthèse de laminarane dans les frondes. En conséquence, des quantités de l'ordre de 2 à 6% y sont trouvées selon la saison (Chapman et al., 1977; Gagné et al., 1982). En revanche, lorsque le taux de nitrite et nitrate est constant tout au long de l'année (sans limitation), la teneur en laminarane est généralement faible, moins de 1.8% (Anderson et al., 1981; Gagné et al., 1982).

Quant au fucoïdane, des études ont montré que sa concentration était influencée par la période de récolte (Black, 1954). Son maximum est généralement atteint à l'automne et au début de l'hiver pour *Laminaria* (Doner et al., 1973). De plus, le niveau d'exposition des algues à l'air (Percival et al., 1967) et l'âge des frondes (Zvyagintseva et al., 2003) semblent influencer la quantité de fucoïdane. Une étude a montré que les frondes plus âgées, 2 ans versus 0,8 an, contenaient plus de fucoïdane 6.5 versus 1.1% (Zvyagintseva et al., 2003).

Pour l'alginate, peu de fluctuations en fonction des saisons ont été rapportées. Néanmoins, lorsque le taux de laminarane est maximal, une plus faible quantité d'alginate serait présente dans les frondes (Percival et al., 1967).

1.3 Les polysaccharides

Les polysaccharides sont des polymères d'unités de monosaccharides et leurs nombre, et degré de polymérisation, varient selon la source. Plusieurs facteurs influencent la structure des polysaccharides. La nature des unités de base (monosaccharides), la liaison glycosidique, les groupements fonctionnels (carboxyle, sulfate, etc.) et le poids moléculaire influencent directement les propriétés des polysaccharides (Leung et al., 2006).

Les polysaccharides sont retrouvés dans les algues, les plantes ou synthétisés par des microorganismes qui produisent respectivement l'alginate, l'amidon et la gomme xanthane. Les β-glucanes sont une famille de polysaccharides retrouvées dans plusieurs sources différentes (plante, champignon, levure, etc.) qui sont largement étudiées (Tableau 1.2). Ces derniers possèdent tous la même caractéristique structurale soit d'être majoritairement composés de β-D-glucose mais le type de liaison glycosidique et leurs poids moléculaires diffèrent grandement. Ces polymères possèdent plusieurs activités intéressantes telles des activités anti-tumorale (Baba et al., 1986; Surenjav et al., 2006) et anti-infectieux (Williams, 1997).

Les polysaccharides sulfatés possèdent plusieurs activités biologiques. L'héparine et l'héparane sulfate sont des puissants anticoagulants (Zhang et al., 2005a) alors que le carraghénane a une activité antivirale (Yamada et al., 2000). Les polysaccharides sulfatés ont peu de similitudes d'un point de vue structural, la composition en monosaccharides, le type de liaison glycosidique et le poids moléculaire varient considérablement (Tableau 1.2). Seuls les groupements sulfate sont présents en plus ou moins grande proportion pour chaque polymère.

Tableau 1.2 : Caractéristiques structurales des β-glucanes et polysaccharides sulfatés.

Polysaccharides (PS)	Origine	Liaisons glycosidiques	Ramifications	Poids moléculaire (kDa)
β-glucanes				
β-glucane avoine et orge (Lazaridou et al., 2007)	Plante	(1,3)-β-D-glucose (1,4)-β-D-glucose	-	20-400
Glucane phosphate (Mueller et al., 2000)	Champignon	(1,3)-β-D-glucose	-	157
Grifolane (Bae et al., 2005; Iino et al., 1985)	Champignon	(1,3)-β-D-glucose	(1,6)-β-D-glucose	8-2000
Laminarane (Chizhov et al., 1998; Nelson et al., 1974)	Algue brune	(1,3)-β-D-glucose	(1,6)-β-D-glucose	5
Lentinane (Zhang et al., 2007)	Champignon	(1,3)-β-D-glucose	(1,6)-β-D-glucose	500
Scléroglucane (Mueller et al., 2000; Pretus et al., 1991)	Champignon	(1,3)-β-D-glucose	(1,6)-β-D-glucose	1020-1560
Zymosane (Chen et al., 2007a)	Champignon	(1,3)-β-D-glucose	(1,6)-β-D-glucose	-
PS sulfatés				
Carraghénane (Piculell, 1995)	Algue rouge	(1,3)-α-D-galactose (1,4)-β-D-galactose	-	300-600
Héparine (Zhang et al., 2005a)	Animale ou synthétique	(1,4)-α-D-glucosamine (1,4)-β-D-acide glucuronique (1,4)-α-L-acide iduronique	-	5-40
Héparane sulfate (Griffin et al., 1995; Zhang et al., 2005a)	Animale ou synthétique	(1,4)-α-D-glucosamine (1,4)-β-D-acide glucuronique (1,4)-α-L-acide iduronique	-	5-50
Fucoïdane (Boisson-Vidal et al., 1995; Rupérez et al., 2002)	Algue brune	(1,2)-α-L-fucose et/ou (1,3)-α-L-fucose et/ou (1,4)-α-L-fucose	(1,2)-α-L-fucose et/ou (1,4)-α-L-fucose	6-1600

Les polysaccharides extraits des algues marines sont très appréciés pour leurs propriétés gélifiantes et épaississantes et de plus en plus pour leurs activités biologiques multiples. À l'heure actuelle, des breuvages et des suppléments alimentaires (Beijing Gingko Group, 2007) contenant des extraits de polysaccharides d'algues ont été commercialisés. Leurs propriétés sont influencées par la composition et la structure des polysaccharides qui sont propres à chaque espèce d'algues permettant une grande diversité.

1.3.1 Le laminarane

Le laminarane est la réserve glucidique des algues brunes et se retrouve dans le ou les plastes de chaque cellule. De faible poids moléculaire (5000 Da environ), son degré de polymérisation est variable, généralement entre 20-25 unités selon la méthode de caractérisation utilisée (Chizhov et al., 1998; Nelson et al., 1974; Read et al., 1996). Un degré de polymérisation de 25 a été déterminé par EISM (*electrospray ionisation mass spectrometry*) (Read et al., 1996) alors qu'un degré de polymérisation de 33 a été déterminé par RMN (résonance magnétique nucléaire) (Kim et al., 2000), tous deux pour un extrait commercial de laminarane provenant de *Laminaria digitata*.

Deux formes de laminaranes sont présentes : soluble et insoluble. La première se caractérise par une solubilité totale dans l'eau froide alors que la deuxième est soluble dans l'eau chaude (Black et al., 1973). La solubilité du laminarane dépend aussi du nombre de ramifications présentes sur la chaîne. Plus le laminarane est ramifié, plus il est soluble en eau froide. Par contre, celui qui l'est faiblement, donc linéaire, peut devenir soluble à chaud (Lahaye et al., 1997a; Rupérez et al., 2002).

La structure et la composition du laminarane varient en fonction de l'espèce (Chizhov et al., 1998). Les premiers laminaranes caractérisés étaient uniquement composés de (1,3)-β-D-glucose (Barry, 1939). Plus tard, la présence de ramification intrachaîne (1,6)-β-D-glucosyle a aussi été démontrée (Peat et al., 1958). Des résidus D-mannitol (M-Chain) aux extrémités de certaines chaînes de laminarane ont été découverts alors que les autres extrémités sont composées de D-glucose (G-chain). Le ratio des chaînes M:G dépend de

l'espèce d'algue (Chizhov et al., 1998). Dans le cas de la source commerciale de *L. digitata*, un ratio M:G de 3 pour 1 a été observé par EISM (Read et al., 1996).

La conformation d'oligo-laminarane dans différents solvants tels l'eau, le diméthyle sulfoxide (DMSO), le diméthylformamide (DMF) à partir de modèle informatique a été présentée (Frecer et al., 2000). Les résultats ont montré que les oligo-laminaranes avaient une conformation différente dans le DMSO car, ce solvant permet d'accepter des protons provenant de la formation de liens hydrogène avec C4-OH et C6-OH. Dans l'eau et le DMF, le C4-OH est engagé comme donneur de proton de la formation de lien hydrogène avec le groupement *O*-glucopyranose précédent. Ces résultats montrent que, selon le solvant, la réactivité des laminaranes peut être différente.

1.3.2 Le fucoïdane

Les fucoïdanes sont des polysaccharides structuraux présents dans la paroi cellulaire des algues. On les retrouve aussi dans les concombres de mer et dans les œufs d'oursins (Nagaoka et al., 2000; Pereira et al., 1999). Ces fucoïdanes sont généralement linéaires et ils sont composés d'un tétrasaccharide répétitif dont les fucoses se distinguent par leur profil de sulfatation (Pereira et al., 1999). Au niveau des algues brunes, trois familles de polysaccharides sulfatés sont retrouvées: les fucoïdanes, les ascophyllanes et les sulfates de glycuronofucogalactanes (Lahaye et al., 1997a). Les trois fractions coexistent et les proportions dépendent de l'espèce et du tissu étudiés. La quantité de chacune des familles varie aussi en fonction de la période de récolte. Les ascophyllanes sont composés de chaînes d'acides mannuroniques avec des fucoses ramifiés (3-*O*-D-xylosyl-L-fucose-4-sulfate). Les glycuronofucogalactanes sont composés de chaînes linéaires de D-galactose avec des ramifications de L-fucose-3-sulfate et occasionnellement des acides uroniques. Le fucoïdane est un homofucane composé majoritairement de L-fucose, mais ce terme est employé en littérature avec beaucoup de confusion. Le terme fucanes est utilisé lorsque le polysaccharide est composé majoritairement de L-fucose avec moins de 10% d'autres monosaccharides (Berteau et al., 2003) alors que le terme fucoïdane correspond à un polymère hétérogène de fucoses avec une proportion plus ou moins grande d'autres

monosaccharides. Les galactofucanes sont des polysaccharides sulfatés eux aussi extraits des algues brunes contenant de grandes quantités de D-galactose (Hemmingson et al., 2006; Rocha et al., 2005). Ils sont régulièrement inclus dans la famille des fucoïdanes (Lee et al., 2004; Li et al., 2006; Ponce et al., 2003; Shevchenko et al., 2007).

Plusieurs études ont tenté de déterminer la structure exacte des fucoïdanes. Seuls quelques exemples de régularités dans la structure ont été découverts : les liaisons, les ramifications, la position des sulfates et les autres sucres semblent être variables (Ponce et al., 2003). Les fucoïdanes sont composés généralement de L-fucose, d'acide D-uronique, de D-galactose, de D-xylose, de sulfate et les proportions de chacun sont variables (Rupérez et al., 2002). De plus, la présence de D-glucose et de D-mannose a aussi été observée chez certaines espèces d'algues (Duarte et al., 2001).

Les *Fucaceaes*, dont fait partie *A. nodosum,* sont composés majoritairement de α-L–fucose lié en α-(1,3) et α-(1,4) (Chevolot et al., 1999; Chevolot et al., 2001; Daniel et al., 2001; Daniel et al., 1999; Daniel et al., 2007; Marais et al., 2001). Les chaînes latérales sont composées de simples et de multiples unités de fucosyle avec des ramifications en position 4. Daniel et collaborateurs (2001) ont montré la présence de groupements sulfate en position 2 et la possibilité d'en retrouver aussi en position 3 et 4 via l'utilisation d'enzyme spécifique. La présence de 2,3-*O*-sulfate et de 2,4-*O*-sulfate a aussi été identifiée. Récemment, la présence de L-fucose mono et di-sulfatés en position 2 a été identifiée (Daniel et al., 2007). Pour *F. vesiculosus*, deux modèles de structures de fucoïdanes ont été présentés (Figure 1.4). Sommairement, Percival et McDowell (1967) ont découvert que les fucoïdanes avaient deux structures possibles. La première consiste en des unités fucose liées en α-(1,2) avec la présence de groupement sulfate en position 4. La deuxième comprend les mêmes éléments que la première mais en plus avec la possibilité de lien α-(1,3) entre les fucoses. Patankar et collaborateurs (1993) ont plutôt affirmé que les unités fucose sont liées en α-(1,3) pour un mélange de fucoïdane commercial extrait de *F. vesiculosus*. Les fucoses terminaux ont aussi des ramifications liées en α-(1,2) ou α-(1,4). Dans le cas de *Fucus serratus* L., une étude récente a montré des différences structurales par rapport aux fucoïdanes d'*A. nodosum* et *F. vesiculosus* (Bilan et al., 2006). La chaîne

principale contient elle aussi des fucoses liés en α-(1,3) et α-(1,4) mais avec une prévalence plus élevée de groupement α-(1,4). La présence de ramification en position 2 et 4 a été observée ainsi que des groupements sulfate majoritairement en position 2 et parfois en position 4 (Bilan et al., 2006). Les groupements fucose des fucoïdanes natifs sont habituellement monosulfatés parfois disulfatés et certains sont non sulfatés. Enfin, les spectres RMN 1D des fucoïdanes extraits d'*A. nodosum* et de *F. vesiculosus* ont été comparés. Plusieurs similitudes structurales et biologiques ont été rapportées par contre, des différences importantes ont été démontrées lorsque comparées à des fucoïdanes de laminaires (Pereira et al., 1999).

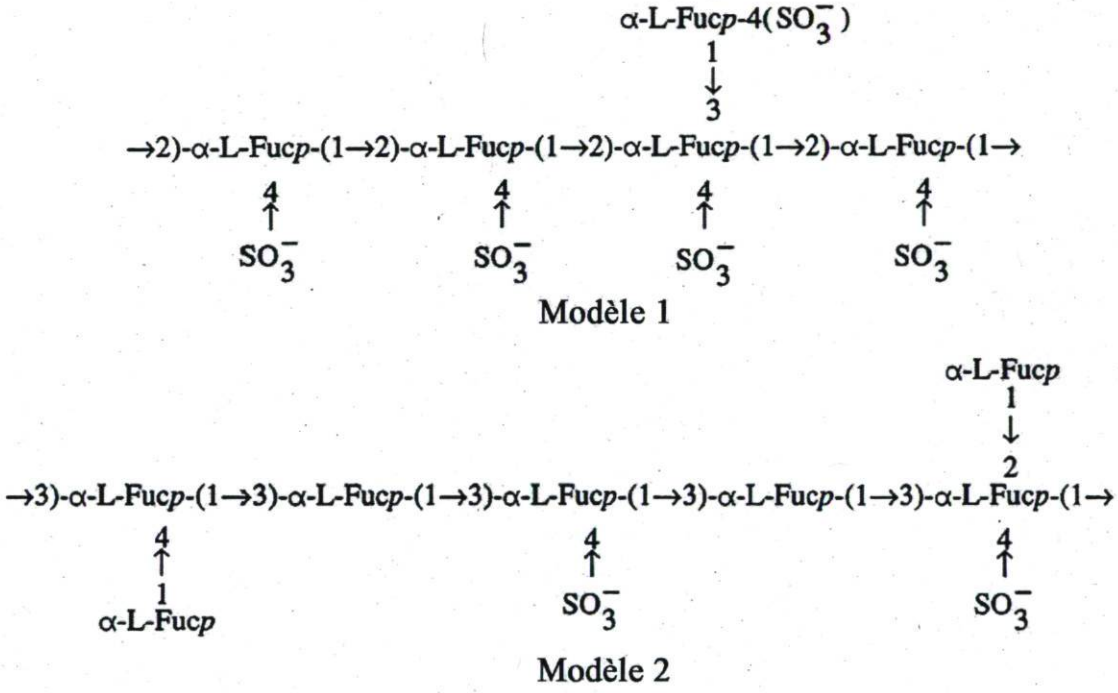

Figure 1.4 : Structure des fucoïdanes.
Modèle 1 proposé par Percival et McDowell (1967) et modèle 2 par Patankar et collaborateurs (1993). Adapté de Chevolot et collaborateurs (Chevolot et al., 1999).

Cependant, pour les *Laminariaceaes*, seule la structure partielle des fucoïdanes a été déterminée pour quelques espèces telles : *Ecklonia kurome*, *Chorda filum* et *Laminaria brasiliensis*. Ces espèces possèdent des fucoïdanes dont la structure principale est différente

de celle des *Fucaceaes* (Sakai et al., 2004). En effet, des liaisons α-(1,2) ont été identifiées chez *Laminaria brasiliensis* avec des ramifications contenant des fucoses sulfatés (Pereira et al., 1999). La présence de fucoses liés en α-(1,3) avec des ramifications en α-(1,2) a aussi été identifiée dans les fucoïdanes de *C. filum* (Chizhov et al., 1999).

Le poids moléculaire des fucoïdanes n'est pas encore établi dû aux variations structurales. Certains chercheurs affirment que le poids est d'environ 100 kDa (Patankar et al., 1993) alors que d'autres parlent plutôt de 6800 Da (Nishino et al., 1994). Selon Rupérez et collaborateurs (2002), le poids moyen serait 1600 kDa pour la majorité des fucoïdanes alors qu'une faible fraction serait de 43 kDa. Une fois extraits, les fucoïdanes sont très solubles dans l'eau tout comme à pH acide (Rupérez et al., 2002). Par contre, les fucoïdanes seraient difficilement hydrolysables dû à leurs structures complexes.

Une étude récente a montré qu'il était possible de faire directement la synthèse des fucoïdane (Ustyuzhanina et al., 2006). Les chercheurs ont synthétisé un octasaccharide linéaire sulfaté et un non sulfaté. Ils ont même réussi à recréer un tétrasaccharide ramifié en position 2. Ceci pourrait faciliter la compréhension de l'activité biologique de polymères de fucoses sulfatés.

1.3.2.1 Le galactofucane

Les galactofucanes sont des polysaccharides sulfatés contenants d'égales proportions de L-fucose et de D-galactose (Hemmingson et al., 2006; Rocha et al., 2005). Une étude a montré que l'unité de la chaîne principale contenait du β-D-galactose lié en β-(1,4) et partiellement sulfaté en position 3. De cette unité, 25% contiendrait un tétrasaccharide de α-L-fucose lié en α-(1,4) sulfaté en position 3 avec un ou deux α-L-fucose non-sulfaté et un β-xylose terminal lié en position 4 (Rocha et al., 2005). Une autre étude a montré un galactofucane avec des fucoses et des galactoses sur la chaîne principale (Hemmingson et al., 2006). Plus précisément, des fucoses liés en (1,3) avec des fucoses disulfatés en position 2 et 4 ont été déterminés. Aussi, des galactoses liés en (1,3) et des galactoses liés en (1,4) avec un sulfate en position 3 ont été montrés.

La caractérisation structurale de ces polysaccharides est complexe et mérite d'être encore approfondie. Il est clair que plusieurs similarités structurales ont été observées, notamment au niveau de la chaîne principale des fucoïdanes. Par contre, des variations au niveau du positionnement des ramifications et des groupements sulfates entraîneront sûrement des différences au niveau de l'activité des fucoïdanes.

1.3.3 L'alginate

L'alginate se retrouve dans la matrice intercellulaire de l'algue (Moe et al., 1995) ou plus spécifiquement dans la paroi cellulaire. Il donne de la flexibilité aux thalles, prévient la dessiccation et intervient dans les échanges d'ions. L'alginate est un sel dérivé de l'acide alginique. De structure linéaire, l'alginate se compose d'acide β-D-mannuronique (ou M) et d'acide α-L-guluronique (ou G) lié en β-(1,4) (ou α-1,4 dans le cas de bloc GG) (Figure 1.5). Les deux monomères forment des blocs de type M-M---M-M ou G-G---G-G appelés segments homogènes et ---M-G-M-G--- nommé segment mixte. Son poids moléculaire varie de 500 à 1000 kDa selon la source et la méthode d'extraction. Il est bien connu pour ses propriétés épaississantes, émulsifiantes, stabilisantes et gélifiantes (McNeely et al., 1973). Des activités biologiques lui sont conférées, mais sa capacité gélifiante est toutefois mieux reconnue.

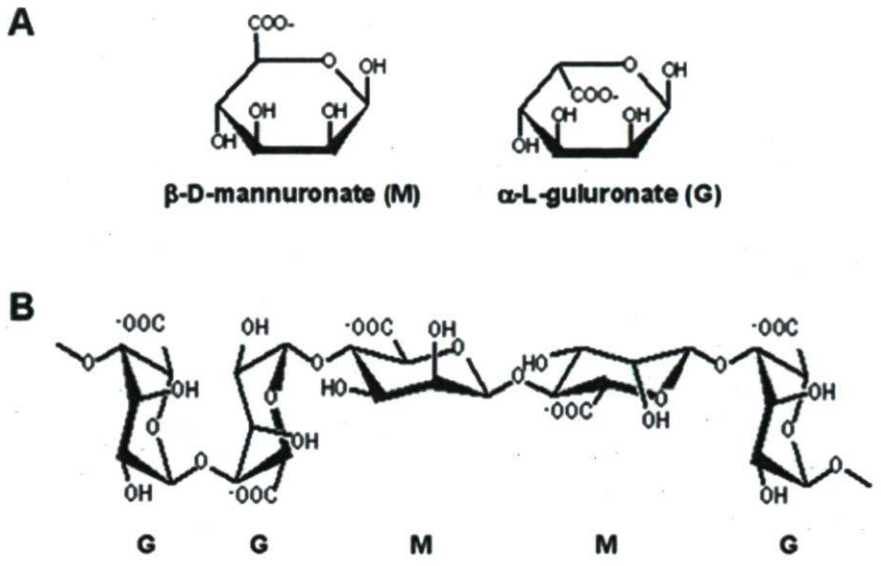

Figure 1.5 : Représentation des monomères (A) et des segments mixtes et homogènes (B). (Sriamornsak et al., 2007)

1.4 Étude des caractéristiques structurales

La caractérisation structurale des polysaccharides est une étape importante pour comprendre l'activité biologique. Plusieurs caractéristiques structurales telles que : le poids moléculaire, la charge, la conformation, la position des groupements sulfates ou la nature des liaisons glycosidiques sont particulièrement importantes. Voici le détail de méthodes qui permettent de mieux étudier les caractéristiques structurales des polysaccharides.

1.4.1 Nature des liaisons glycosidiques

La nature des liaisons glycosidiques peut être déterminée entre autre par la spectrométrie de masse couplée à la chromatographie en phase gaseuse (GC-MS). Cette technique nécessite de convertir les monosaccharides en dérivés volatils. Le principe de cette technique consiste à méthyler les fonctions hydroxyles libres et à hydrolyser le polysaccharide méthylé afin de libérer les fonctions hydroxyles engagées dans des liaisons glycosidiques

(Figure 1.6). L'analyse des acétates d'alditols partiellement méthylés (AAPM) formés permet de déterminer les sites de liaison par GC-MS après réduction (transformation en alditol) et acétylation.

Seuls les acides uroniques posent problème, car il peut se produire la formation de cétone et de produits β-éliminés qui empêcheront, par exemple, de connaître la nature de la liaison glycosidique de l'acide uronique (Björndal et al., 1970). La protection des groupements carboxyliques par l'introduction de deux atomes de deutérium doit être effectuée pour préserver ses groupements (York et al., 1985).

La chromatographie en phase gazeuse permet de distinguer les dérivés des résidus de même nature (hexoses, pentoses, déoxyhexoses...). Ces résidus ne doivent pas posséder les mêmes temps de rétention relatifs d'où l'importance d'utiliser des standards. La fragmentation de chaque type de AAPM est caractéristique de la position des groupements méthyle et acétyle ce qui permet de déduire pour le résidu correspondant la nature de la liaison glycosidique. Ainsi, le produit final de la préparation du AAPM à figure 1.6 correspond à un hexose substitué en position 3 (ou 1,3,5-tri-*O*-acétyl-1-deuterio-2,4,6-tri-*O*-méthyl hexitol). En présence de groupements fonctionnels (exemple : groupement sulfate), il est préférable d'analyser le polymère avant et après l'enlèvement du groupement pour identifier sa position sur le monosaccharide.

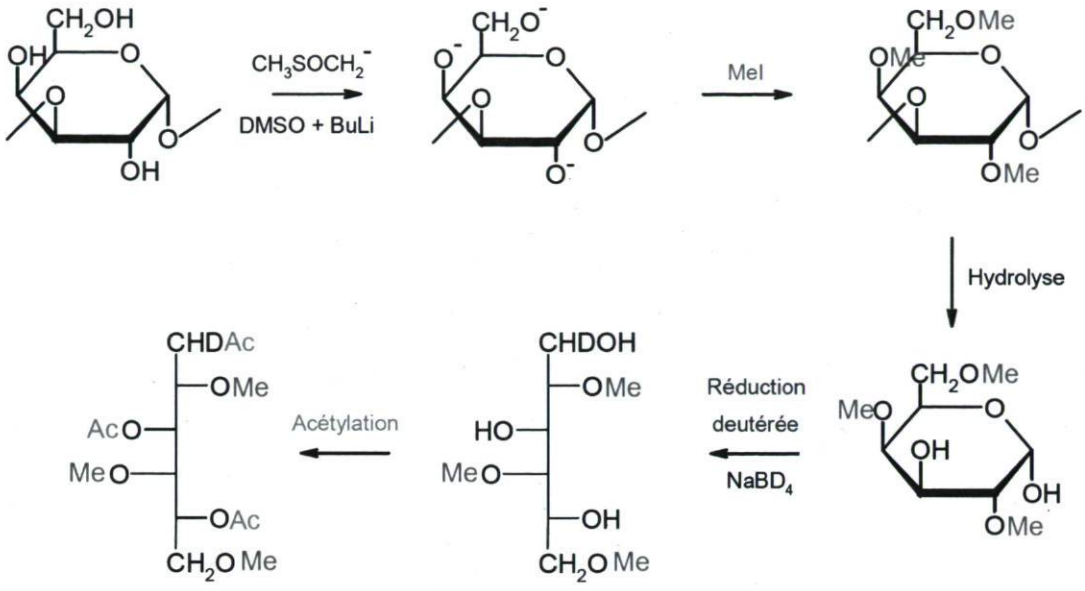

Figure 1.6 : Préparation des acétates d'alditols partiellement méthylés.
Adapté de Björndal et collaborateurs (Björndal et al., 1970).

1.4.1.1 L'hydrolyse des polysaccharides

Les polysaccharides natifs sont généralement moins actifs que les oligosaccharides (Roger et al., 2002). Il est plus difficile d'étudier la structure de gros polysaccharides que des oligosaccharides. Ainsi, l'hydrolyse de gros polysaccharides permet de clarifier les résultats obtenus en GC-MS avec le polysaccharide natif. Plusieurs méthodes permettant de réduire le poids moléculaire ont été étudiées afin de caractériser leur structure. L'hydrolyse peut être effectuée par voie chimique, enzymatique ou par dépolymérisation radicalaire. Il est généralement considéré que les deux dernières méthodes n'altèrent pas la structure globale du polysaccharide comparativement aux méthodes chimiques traditionnelles (Volpi et al., 1992). Le laminarane étant déjà un polysaccharide de faible poids moléculaire (5 kDa) nul besoin de l'hydrolyser. Par contre, les fucoïdanes ont des poids moléculaires nettement plus élevés et nécessitent une étape d'hydrolyse. De plus, l'utilisation de fucoïdane de faible poids moléculaire facilitera l'étude de sa structure puisqu'il sera plus facile de décortiquer les résultats en spectrométrie de masse, par exemple.

La dépolymérisation radicalaire permet de dégrader efficacement les polysaccharides afin de produire des oligo-fucoïdanes de petites tailles (environ 10kDa) (Nardella et al., 1996). Cette méthode consiste à libérer des radicaux libres HO• issus de la réaction entre un ion métallique (Cu^{2+}) et le peroxyde d'hydrogène. La réaction radicalaire du polysaccharide est suivie d'un réarrangement de la structure et du bris de la liaison glycosidique pour former des chaînes plus courtes (Volpi et al., 1992). Cette méthode s'est montrée efficace dans la production d'oligo-fucoïdanes possédant diverses activités biologiques et comme outils utile à la déduction de la structure (Colliec-Jouault et al., 2001; Nardella et al., 1996). De plus, cette méthode fût l'objet d'une étude portant sur la l'activité biologique et la caractérisation structurale de galactane sulfaté extrait d'algue rouge (Zúñiga et al., 2006).

L'hydrolyse enzymatique est une méthode immuable pour la production d'oligo-fucoïdanes et est un outil précieux pour la compréhension de la structure des polysaccharides. Récemment, une fucosidase a été isolée. Cette dernière dépolymérise le fucoïdane (2100 kDa), d'*Undaria pinnatifida*, en 7 oligo-fucoïdanes de structures différentes ayant de faibles poids moléculaires (305 à 3749 Da) (Woo-Jung et al., 2008). Daniel et collaborateurs (1999) ont eux aussi isolé des glycosidases mais à partir de mollusque. La L-fucosidase a montré une action sur les liaisons glycosidiques α alors que la D-galactosidase est active pour les β- et α-D-galactoses. Ces enzymes sont actives sur le fucoïdane extrait d'*A. nodosum* mais inefficace avec celui de *F. vesiculosus*. Les résultats se sont avérés négatifs, car l'enzyme est surtout active sur les liens α-(1,4) (Berteau et al., 2002) et ce fucoïdane n'en contient pas sur sa chaîne principale (Patankar et al., 1993; Percival et al., 1967). Parallèlement, une sulfoestérase capable d'enlever spécifiquement les groupements sulfates présents sur les L-fucoses a été identifiée (Daniel et al., 2001). Cette enzyme a montré des nouveautés structurales concernant la position des groupements sulfate (section 1.3.2). Une bactérie produisant une fucoïdane hydrolase a aussi été isolée à partir des effluents d'une usine de transformation d'alginate (Descamps et al., 2006). Ces effluents contiennent une grande quantité de fucoïdane de haut poids moléculaire. Le fucoïdane extrait de *Pelvetia canaliculata* a été dépolymérisé par cette enzyme ainsi que d'autres fucoïdanes extraits de *Fucus spiralis* et d'*A. nodosum*. Il est anticipé que l'enzyme est active envers les fucoïdanes ayant une structure composée de fucoses sulfatés alternés en

lien α-(1,3) et α-(1,4) (Descamps et al., 2006). Sakai et collaborateurs (2004) ont quant à eux extraits une fucosidase capable d'hydrolyser les fucoïdanes à partir de souches bactériennes marines. Cette enzyme est active envers les fucoïdanes extraits de *Kjellmaniella crassifolia* et partiellement active envers d'autres *Laminariales* telles : *Laminaria japonica*, *Lessonia nigrescens* et *Ecklonia maxima* (Sakai et al., 2004). Par contre, cette enzyme est inactive envers *A. nodosum* et *F. vesiculosus*. Ces résultats montrent que la structure des *Laminariales* est différente des *Fucales* et que les enzymes sont actives envers des structures bien précises de polysaccharides. On peut donc considérer les enzymes comme des outils de compréhension de la structure, car elles permettent de déduire le type de liaison glycosidique présent par exemple, un lien α-(1,4). Couplées à la technique de méthylation des polysaccharides et à la spectrométrie de masse, les enzymes spécifiques permettent de mieux étudier la structure des polysaccharides pour déterminer la nature des liaisons glycosidiques.

1.4.2 Séquence des unités de base de monosaccharides

Nous avons vu avec les méthodes précédentes qu'il était possible de déterminer le type de liaison glycosique mais qu'en est-il de la séquence des monosaccharides, c'est-à-dire l'ordre dans lequel les unités sont assemblées. La spectroscopie par RMN ou résonance magnétique nucléaire est l'outil d'analyse le plus utilisé en chimie, car elle permet d'obtenir des informations qualitatives et/ou quantitatives très précises sur l'échantillon analysé. Cette méthode utile mais très complexe permet de faire l'analyse structurale des polysaccharides. Elle permet entre autres de différencier les anomères alpha des anonères bêta. Elle permet aussi de déterminer les monosaccharides voisins. Cette méthode est utilisée en complémentarité avec la méthode d'hydrolyse des polysaccharides (section 1.4.1.1), car il est toujours plus facile d'étudier des oligosaccharides puisque cela permet de réduire le nombre de pics facilitant ainsi l'analyse. Cette méthode ne sera pas décrite en détails puisqu'elle n'a pas été utilisée dans cette thèse.

La RMN repose sur le magnétisme nucléaire. Cette technique permet de mesurer la capacité d'absorption que possèdent des molécules placées dans un champ magnétique. Certains

noyaux possèdent un spin nucléaire nul (^{14}C) alors que d'autres ont un spin nucléaire différent de zéro (^{1}H, ^{13}C). Les noyaux les plus souvent étudiés sont le proton (^{1}H), le carbone (^{13}C), le phosphore (^{31}P) et le fluor (^{19}F) qui présentent tous un spin nucléaire non nul. Ils agissent comme des aimants microscopiques et absorbent de l'énergie par un processus de résonance magnétique. Sous l'action d'un champ magnétique externe uniforme, le noyau peut prendre différentes orientations correspondant à des niveaux d'énergie différents (Hornak, 2002). L'un est de basse énergie, si le moment magnétique est parallèle et de même sens que le champ extérieur, alors que l'autre est d'énergie plus élevée, si le sens est contraire. La différence d'énergie ΔE entre ces deux états est proportionnelle au champ extérieur. La transition du niveau bas au niveau haut est possible due à l'absorption d'une radiation de fréquence ν telle que $\Delta E = h\nu$ (Hornak, 2002). Lorsque la transition a lieu, on dit qu'il y a résonance du noyau.

Il existe deux types de spectromètre RMN : un à balayage et un à transformée de Fourier. C'est ce dernier qui est le plus utilisé car il offre une résolution plus fine et une meilleure sensibilité. Les signaux émis par les échantillons suite à une courte impulsion permettent d'obtenir un spectre en fonction du temps. L'excitation causée par l'impulsion est détectée par une sonde. Le signal émis correspond à tous les pics d'absorption et un ordinateur doit ensuite transformer ce signal en fonction de la fréquence pour que chaque pic puisse être identifié avec l'aide d'une transformation de Fourier (Solomons et al., 2000). Ce processus est répété à chaque impulsion que subit l'échantillon permettant ainsi d'augmenter l'intensité du signal et d'éliminer le bruit de fond. Ainsi, la surface des pics est proportionnelle au nombre de noyaux qu'elle représente.

Les protons n'absorbent pas tous l'énergie à une même intensité de champ magnétique appliqué car la densité électronique et le mouvement des électrons du composé sont souvent différents. En présence d'un champ magnétique externe, on retrouve une circulation des électrons autour du proton qui génère un champ magnétique interne. Ce champ peut s'additionner au champ extérieur: c'est le phénomène de déblindage ou ce champ peut s'opposer au champ extérieur: c'est le phénomène de blindage (Solomons et al., 2000). Plus le blindage est intense, plus le proton doit être soumis à un champ extérieur fort

pour que se produise la résonance. Ceci se traduit par un déplacement des pics d'absorption vers la droite du spectre et l'inverse dans le cas du déblindage. L'intensité du blindage ou du déblindage dépend donc de l'environnement du proton donc, de la structure chimique du composé. Par exemple, la présence de groupement comme le chlore déplace la position des pics d'absorption des protons par rapport à la position qu'ils occuperaient s'ils étaient dépourvus de ces groupements. Ainsi, le mouvement des électrons dans les liaisons chimiques est appelé déplacement chimique et il est exprimé en ppm (parties par million). Pour établir le point zéro de l'échelle de déplacement chimique, un composé de référence, le tétraméthylsilane (TMS), est souvent ajouté à l'échantillon analysé (Teng, 2005). Le nombre de pics et la valeur du déplacement chimique permettent d'identifier le nombre et la nature des groupes de protons équivalents. La courbe d'intégration donne le nombre de protons de chaque type et la forme de chaque pic indique le nombre de protons voisins du proton étudié. En pratique, un proton ou un groupe de protons équivalents ayant n protons voisins donnera un signal constitué de (n+1) pics, appelé multiplet (singulet: 1 pic; doublet: 2 pics; triplet: 3 pics;....) (Teng, 2005). Il ne reste plus qu'à analyser le spectre pour identifier le composé.

La RMN apporte un certain nombre de renseignements relatifs à la structure d'une molécule comme sa stéréochimie. Par contre, beaucoup d'attention doit être portée à l'interprétation des spectres et plus le composé est complexe plus le spectre sera difficile à analyser.

1.4.3 Détermination du poids moléculaire

La connaissance du poids moléculaire d'un polymère est fondamentalement importante. Beaucoup de propriétés physiques, mécaniques et rhéologiques sont influencées par cette valeur notamment le rôle d'un polymère dans un aliment ou son impact dans un procédé ainsi que son activité biologique.

La détermination du poids moléculaire d'un polymère n'est pas sans complication. Dans le cas de polymère globulaire telles les protéines, plusieurs méthodes robustes peuvent être employées comme la diffusion de la lumière. Par contre, lorsque le polymère est linéaire ou

ramifié ou polydiperse comme dans le cas des polysaccharides, peu de méthodes sont alors disponibles. La polydispersité réfère à une distribution variable de poids moléculaire pour un même échantillon, c'est-à-dire qu'on retrouve plusieurs chaînes avec des degrés de polymérisation différents et donc des poids moléculaires différents. La polydispersité et la présence d'interactions polysaccharide-polysaccharide à concentration un peu plus élevée, accroît le degré de difficulté pour une estimation adéquate du poids moléculaire (Harding et al., 1991). Le poids moléculaire des polysaccharides est aussi influencé par le volume hydrodynamique, la nature de chaîne principale, la présence de ramification et de groupement fonctionnel. Ainsi, d'un point de vue structural les polysaccharides ne sont pas faciles à étudier et la détermination du poids moléculaire en décourage plus d'un. Par contre, leurs propriétés d'un point de vue médical, nutritionnel et fonctionnel sont dictées par les caractéristiques structurales, dont le poids moléculaire.

Plusieurs techniques sont disponibles. La viscosité intrinsèque est une technique simple et rapide par contre, lorsque les paramètres de l'équation de Mark-Houwink sont inconnus, cette méthode s'avère peu utile. La chromatographie haute performance d'exclusion moléculaire (HPSEC) est une méthode rapide, mais nécessite l'utilisation de standard dont les caractéristiques moléculaires sont similaires au polymère à l'étude. Dans le cas d'un nouveau polymère, les standards existants sont généralement peu appropriés et le poids moléculaire doit être rapporté en équivalent du standard (dextrane, pullulane, etc.). La diffusion statique (SLS) permet de déterminer le poids moléculaire de macromolécules de petite taille. Pour celles qui sont très grosses, cette méthode est peu appropriée, car certains principes de diffusion ne sont plus respectés. Finalement, la combinaison du HPSEC avec le MALLS (M*ulti angle laser light scattering*) offre beaucoup d'avantages, car c'est la seule méthode de détermination absolue du poids moléculaire. De plus, aucune calibration n'est requise ni l'utilisation de standard contrairement au HPSEC seul. Cette méthode permet de déterminer les différences de diffusion en fonction de l'angle. De plus, il est possible à partir de cette méthode de déterminer les constantes de l'équation de Mark-Houwink (Bertini et al., 2005). Lorsque le polymère est polydisperse ou lorsqu'il est de grande taille, son estimation est toutefois plus compliquée car le poids moléculaire est souvent surestimé (Podzimek, 2005).

1.4.3.1 HPSEC-MALLS

Cette technique combine le HPSEC et la diffusion de la lumière par MALLS. Cette méthode offre de nouvelles possibilités, car elle permet d'éviter les contraintes du HPSEC au niveau de l'utilisation de standard pour estimer le poids moléculaire. Le HPSEC-MALLS permet d'analyser la distribution d'échantillon polydisperse, d'obtenir de l'information sur les ramifications et la conformation moléculaire (Podzimek, 1994).

L'intensité de la lumière diffusée pour plusieurs angles (θ) et pour les solutions diluées est présentée par l'équation suivante :

$$(1.1)\ \frac{Kc}{R_\theta} = \left[1 + \frac{16\pi^2 R_G^2}{3\lambda^2}\sin^2\left(\frac{\theta}{2}\right)\right]\left(\frac{1}{M_W + 2A_2}\right)$$

où M_W correspond à la masse moléculaire moyenne en poids, R_G au rayon de giration, A_2 au deuxième coefficient du viriel, λ à la longueur d'onde dans le milieu, R_θ au ratio de Rayleigh, c à la concentration et K à une constante faisant intervenir le nombre d'Avogadro et la dn/dc (indice de réfraction en fonction de la concentration). Cette équation est valide seulement si aucune différence significative de changement de phase ou distorsion à incidence électrique n'est induite par les particules (Harding et al., 1991). Ce sont les critères de Rayleigh-Gans-Debye et ils sont généralement respectés pour des polysaccharides environ de $M_W < 20\text{x}10^6$ Da.

Ainsi, chaque angle du détecteur voit des grosseurs de particules différentes. Les petits angles voient les polysaccharides de haut poids moléculaire et vice versa. Pour estimer le poids moléculaire, il est nécessaire de connaître la concentration du polymère c_i à chaque tranche du pic et l'intensité de la lumière diffusée pour chaque détecteur (Reed, 1996). La valeur de c_i peut être déterminée en utilisant la constante absolue de calibration du détecteur d'indice de réfraction (CF) selon l'équation suivante :

$$(1.2)\ c_i = \frac{\Delta V_{RI,i} CF}{(dn/dc)}$$

où ΔV_{RI} correspond à la soustraction de la ligne de base de l'indice de réfraction pour chaque tranche.

Plusieurs modèles mathématiques peuvent être utilisés pour calculer le poids moléculaire. Les modèles de Zimm, Berry et Debye peuvent être utilisés à même le logiciel de l'appareil. Selon la grosseur des molécules, certains modèles sont plus appropriés que d'autre. Pour les molécules de faibles poids moléculaires (10 kDa) et de petite taille ($R_G << \lambda/20$), le modèle de Zimm ou Debye premier ordre est utilisé avec un seul angle (90°) de détection car les molécules de petite taille induisent une diffusion isotropique donc, P_θ est égal à 1 et l'équation 1.3 s'applique (Wyatt, 1993).

$$(1.3)\ \frac{Kc}{R_\theta} = \left(\frac{1}{P_\theta}\right)\left(\frac{1}{M_W} + 2A^2\right)$$

Ainsi, M_W peut être déterminé facilement si la concentration est assez faible pour s'assurer que $2A^2$ est très petit par rapport à $1/M_W$. Pour les molécules de poids moléculaires intermédiaires (100 à 1000 kDa), les modèles de Zimm et Berry en deuxième ordre sont les plus appropriés. Pour les hauts poids moléculaires (>1000 kDa), les modèles de Zimm et Berry en deuxième ordre sont les plus adéquats ou encore le modèle de Debye en quatrième ordre (Wyatt, 1993).

Dans la littérature, beaucoup de variations dans le choix des modèles peuvent être observées. Par exemple, pour le kappa et l'iota carraghénane de poids moléculaire allant de 89 à 738 kDa, le modèle de Zimm premier ordre a été utilisé (Viebke et al., 1995) ainsi que pour l'alginate (environ 200 kDa) (Turquois et al., 2000). Turquois et collaborateurs (2000) ont comparé les résultats obtenus avec le premier et le deuxième ordre. Ayant obtenu des résultats similaires, ils ont préconisé l'utilisation du premier ordre. Pour un exopolysaccharide isolé de champignon (M_W de 20 et 70 kDa), le modèle de Debye premier ordre a été utilisé (Lim et al., 2005). Dans une autre étude, les auteurs ont comparé chaque modèle (Zimm, Debye et Berry) avec le premier et le deuxième ordre pour de l'amidon (Chen et al., 2007c). Ils ont constaté que le modèle de Berry premier ordre était le plus

adéquat pour l'amylose (1000 kDa environ). Finalement, les modèles utilisés dans la littérature varient beaucoup, et ce même pour des polysaccharides de tailles semblables. Il est donc important de valider plusieurs modèles avant de faire son choix. De plus, pour beaucoup d'articles, le modèle n'est même pas spécifié.

A partir du modèle choisi, le logiciel de l'appareil calcule la masse moléculaire et le rayon de giration avec l'aide des équations suivantes (Jackson et al., 1989; Podzimek, 1994) :

la masse moléculaire moyenne en poids,

$$(1.4)\ M_W = \frac{\sum c_i M_i}{\sum c_i}$$

le rayon de giration moyen en poids,

$$(1.5)\ \left\langle R_G^2 \right\rangle_W = \frac{\sum c_i \left\langle R_g^2 \right\rangle_i}{\sum c_i}$$

où M_i correspond à une tranche d'un seul poids moléculaire et c_i la concentration d'une tranche. De plus, le calcul est effectué pour chacun des angles utilisés.

L'utilisation du HPSEC-MALLS offre beaucoup d'avantages, mais il y a aussi des limitations. La limite inférieure de détection est d'environ 10 kDa et pour les molécules de plus petite taille d'autres méthodes doivent être employées (exemple SLS). Pour les solutions de polymères de haut poids moléculaires, mais de faible concentration, l'estimation de la masse injectée n'est pas déterminée de façon précise influençant l'estimation de poids moléculaire (Wyatt, 1993). De plus, il faut aussi éviter les phénomènes d'absorption des molécules avec la matrice de la colonne et la séparation doit être causée uniquement par un mécanisme d'exclusion (Jackson et al., 1994). Les phénomènes d'étalement des pics sont aussi présents en HPSEC-MALLS dû à la présence de plusieurs détecteurs en série. En HPSEC, le poids est sous-estimé lors de l'étalement

d'un pic alors qu'avec le MALLS, le poids est surestimé. Parfois, un pic supplémentaire de volume d'élution inférieur est aperçu avec le détecteur à indice de réfraction, alors qu'il ne l'est pas en diffusion. Ceci peut être expliqué par la présence d'agrégats ou de molécules hautement ramifiées, mais à concentration très faible puisqu'ils sont détectés uniquement par indice de réfraction (Podzimek, 1994). Malgré ces problèmes, l'utilisation du HPSEC-MALLS est une méthode très efficace qui ne requiert pas de calibration contrairement au HPSEC standard.

1.5 Activités biologiques des polysaccharides extraits d'algues brunes

Les algues entières sont consommées en grande quantité en Asie et sont réputées avoir un impact bénéfique sur la santé. Des études épidémiologiques associent la consommation d'algues à une incidence réduite du cancer du sein chez des patientes Japonaises (Teas, 1983). Un effet anti-tumoral des algues brunes a été observé chez la souris (Noda et al., 1990). Une autre étude a aussi montré une réduction du nombre de cas d'artériosclérose et d'hypercholestérolémie chez les populations japonaises consommant beaucoup d'algues (Ito et al., 1989). Plusieurs études épidémiologiques comparant les Orientaux et les Occidentaux ont aussi montré une réduction des risques de développer des maladies cardiovasculaires lors d'une consommation régulière d'algues (Yuan, 2008). De manière générale, l'isolement des composés actifs permet une meilleure maîtrise de l'effet recherché.

Les études démontrant des activités biologiques pour les extraits de laminaranes, fucoïdanes, galactofucanes et alginates seront présentées dans les sections suivantes.

1.5.1 Le laminarane

Le laminarane possède diverses activités biologiques notamment au niveau de la stimulation du système immunitaire et un effet cytotoxique sur les cellules des tumeurs (Nagaoka et al., 2000). De par sa structure, le laminarane n'est pas hydrolysé lors de son

passage dans la première partie du tractus gastro-intestinale ce qui permet de le considérer comme une fibre alimentaire (Devillé et al., 2004). De plus, le laminarane n'est pas retrouvé dans les fèces des rats, suggérant qu'il est utilisé par la microflore intestinale. Le laminarane natif est soumis à la dépolymérisation via la laminaranase. Les oligomères libérés sont métabolisés par la microflore grâce à leurs β-glucosidases constitutives (Michel et al., 1999). Par un test *in vitro*, le laminarane natif est fermenté deux heures après la mise en contact de la microflore alors que les oligo-laminaranes le sont immédiatement. Il a été suggéré que le délai observé pour le polymère natif est dû à une adaptation des bactéries conduisant à l'activation d'enzymes spécifiques (Michel et al., 1999; Michel et al., 1996). Les oligo-laminaranes sont considérés comme des substrats d'intérêt dû à leurs propriétés immunostimulantes. Ces derniers pourraient exercer une action spécifique sur les cellules immunitaires dans les plaques de Peyer lors de leur passage dans l'intestin grêle (Michel et al., 1999). Aussi, la fermentation du laminarane dans le colon de rat peut avoir plusieurs effets physiologiques avantageux notamment au niveau du transit intestinal, de l'intégrité de l'épithélium intestinal et au niveau de la composition des fèces et de la microflore (Devillé et al., 2004). Une étude *in vivo* avec des porcelets a montré une réduction significative d'entérobactéries, de bifidobactéries et de lactobacilles dans le caecum et le colon (Reilly et al., 2008). Les porcelets ont été nourris avec une diète enrichie en extrait d'algue contenant 170 ppm de laminarane et 136 ppm de fucoïdane. Une autre étude mais cette fois chez le rat, a montré que le laminarane ne stimulait pas la croissance de souches probiotiques telles les bifidobactéries et les lactobacilles (Devillé et al., 2007) alors que deux autres études, une *in vitro* et l'autre *in vivo*, avaient démontré le contraire (Kuda et al., 1998; Lahaye et al., 1997b). Michel et collaborateurs (1999) ont attribuée la croissance de souches probiotiques à la présence de ramification en β-(1,6). Or, Devillé et collaborateurs (2007) utilisaient eux aussi un laminarane avec des ramifications en β-(1,6) mais sans la stimulation de souches probiotiques. La croissance de souches probiotiques pourrait donc être associée à une conformation plus spécifique telle que dans le cas du lentinane, β-glucane de champignon, où la conformation en simple ou triple hélice influence l'activité (Surenjav et al., 2006).

Plus encore, la fermentation du laminarane induit une forte production d'acides gras de courtes chaînes, de propionate et de butyrate lors de test *in vivo* (Devillé et al., 2007; Kuda et al., 2005). La production de butyrate est très intéressante, car elle pourrait avoir un effet anti-tumoral chez les patients atteints de cancer du colon (Kuda et al., 2005). Également, une diète enrichie en laminarane protège les rats à l'étude contre les hépatotoxicités engendrées par les lipopolysaccharides d'*Escherichia coli* en modulant la réponse immunitaire (Neyrinck et al., 2007). Des laminaranes sulfatés de faibles poids moléculaires ont aussi montré une activité anti-tumorale chez la souris (Jolles et al., 1963) alors que des laminaranes sulfatés chimiquement ont montré une activité anticoagulante (Ito et al., 1989). Récemment, une étude a démontré une protection contre l'apoptose, c'est-à-dire la mort cellulaire, en utilisant du laminarane natif et des oligo-laminaranes (Kim et al., 2006). Les résultats ont montré que 50% des cellules contrôles sont mortes après trois jours alors que celles traitées avec des oligo-laminaranes et du laminaranes natifs survivaient. Par ailleurs, 85% des cellules traitées ont survécu jusqu'à 2 semaines. Pour comprendre comment les laminaranes protégeaient les thymocytes de souris (cellules lymphoïdes) de la mort cellulaire, une deuxième expérimentation a été réalisée avec les oligo-laminaranes en utilisant des puces à ADN (section 1.6). Lors de cette deuxième partie, plusieurs gènes réduisant l'apoptose ont été exprimés alors qu'une protéine responsable de la mort cellulaire a été réprimée de façon importante (Kim et al., 2006).

D'autres β-glucanes de structure semblable au laminarane sont extraits des champignons (Basidiomycotina et Ascomycotina) tels : le lentinane et le grifolane (Zhang et al., 2007). Ils possèdent eux aussi des activités biologiques telle une activité anti-tumorale (Iino et al., 1985; Sakai et al., 1968; Surenjav et al., 2006). Une étude *in vivo* chez la souris a comparé l'effet anti-tumoral du lentinane à celui du laminarane. Les résultats ont montré que le laminarane était dépourvu d'activité anti-tumorale alors que le lentinane inhibait la croissance des cellules cancéreuses (Baba et al., 1986). Au niveau de la structure, la présence majoritaire de lien β-(1,6) dans les β-glucanes semble inhiber l'activité anti-tumorale et ceci a été attribué a une grande flexibilité des chaînes (Zhang et al., 2007). De plus, la conformation en triple hélice semble être nécessaire. L'activité biologique ne

semble donc pas être uniquement influencée par la structure de la chaîne de base mais par d'autres facteurs tels : le poids moléculaire ou la présence de ramification.

D'autres activités ont aussi été observées chez les β-glucanes telles : anti-inflammatoire, immunomodulatrice et antivirale (Zhang et al., 2007). De plus, les β-glucanes stimuleraient les fonctions des macrophages et des neutrophiles (Williams, 1997) en favorisant la réparation des tissus (Wei et al., 2002). Ces actions se font grâce à trois récepteurs de β-glucanes : dectine-1, récepteur scavenger (scavenger receptor ou SR) et récepteur du complément 3 (complement receptor ou CR3) (Brown et al., 2005; Leung et al., 2006). Plusieurs paramètres structuraux tels que : la charge, la solubilité, le poids moléculaire, le degré de ramification et la conformation sont reconnus pour influencer la capacité des β-glucanes à se lier à ces récepteurs (Leung et al., 2006). Des études ont montré que le laminarane est capable de se lier à un récepteur spécifique sur les monocytes, possiblement dectine-1 (Gantner et al., 2003; Kikkert et al., 2007). Finalement, la capacité du laminarane à se fixer à un récepteur de β-glucane de la lignée cellulaire U937 dépourvue de CR3 a été étudiée. Les résultats ont montré que le laminarane se lie préférentiellement à un des deux sites du récepteur permettant l'activation de certaines voies métaboliques (Mueller et al., 2000) telles que l'activation du NFκβ (nuclear factor kappa β), une protéine impliquée dans la réponse immunitaire (Battle et al., 1998). Des résultats similaires ont été observés avec les fibroblastes (Kougias et al., 2001).

1.5.2 Le fucoïdane

L'activité biologique des polysaccharides sulfatés est connue depuis longtemps (Chargaff et al., 1936). Les fucoïdanes ne présentent aucune toxicité, n'induisent pas d'inflammation et pourraientt servir d'agents anticoagulant, anti-inflammatoire, antiviral, d'anti-tumoral et contraceptif (Boisson-Vidal et al., 1995; Nagaoka et al., 2000). Leur activité dépend du poids moléculaire, de la nature des unités de base, du contenu en groupements sulfate et de leurs positions, du type de liaison ainsi que de la géométrie de la molécule (Melo et al., 2002; Shanmugam et al., 2000). Fait intéressant, la FDA (*Food and Drug Administration*) aux États-Unis a inscrit les fucoïdanes à titre de nouveau supplément alimentaire (US Food

and Drug Administration, 2000). Par contre, il est spécifié que même si le produit semble sécuritaire, il manque de l'information pour homologuer l'ingrédient. Ceci ouvre donc la voie à la mise en marché de suppléments alimentaires d'origine marine.

1.5.2.1 Activité anticoagulante

L'activité anticoagulante des fucoïdanes est l'une des activités les plus importantes de ce polysaccharide. Plusieurs études ont été réalisées et des résultats ont été obtenus à partir de tests *in vitro* (Church et al., 1989; Grauffel et al., 1989) et *in vivo* (Boisson-Vidal et al., 2000; Mauray et al., 1995; Trento et al., 2001).

Afin de mieux comprendre le rôle du fucoïdane, les principales étapes de la coagulation sont expliquées. Lors d'une lésion d'un vaisseau sanguin cinq d'étapes se succèdent afin d'éviter les hémorragies et les risques de thrombose (Marieb, 1999). La **première étape** est le spasme vasculaire qui provoque la vasoconstriction du vaisseau induit par les cellules endothéliales et les plaquettes. La **deuxième étape** est la formation du clou plaquettaire qui consiste à former un bouchon temporaire sur la brèche. La coagulation constitue la **troisième étape** et peut emprunter la voie intrinsèque ou la voie extrinsèque toutes deux déclenchées par des lésions aux tissus (Figure 1.7). Dans la voie intrinsèque, tous les facteurs sont présents dans le sang par contre, lorsque le sang est exposé un facteur tissulaire (libéré par les cellules abîmées) la voie extrinsèque s'amorce à la place de la voie intrinsèque. Chacune des voies est suivie d'une cascade de réactions vers l'activation d'un facteur commun, le facteur X. Suite à son activation, le facteur X_a se complexe pour former l'activateur de prothrombine. Dans le cas d'étude *in vitro*, la voie intrinsèque est empruntée et le raisonnement diagnostique d'interprétation des tests de coagulation (temps de céphaline activé, etc.) pourra se faire à partir de ce schéma (Schved, 2005). Par contre, avec les modèles *in vivo*, c'est principalement la voie extrinsèque qui est utilisée, car il y infiltration de sang dans les tissus (Colman et al., 2006; Zhang et al., 2005a). Cette voie comporte moins d'étape et est plus rapide que la voie intrinsèque. Ensuite, l'activateur de prothrombine catalyse la transformation de la prothrombine, une protéine plasmatique, en une enzyme active appelée thrombine. La thrombine enlève ensuite certains peptides du

fibrinogène et le transforme en fibrine. Ces derniers se lient aux plaquettes et s'entremêlent pour former la charpente d'un caillot pour colmater le vaisseau jusqu'à sa guérison définitive.

Ensuite survient la **quatrième étape**, la rétraction et réfection du caillot. La rétraction correspond à la stabilisation du caillot provoquée par les plaquettes qui provoquent une traction sur les filaments de fibrine et une expulsion du sérum. Le processus de cicatrisation vient alors de débuter. Par la suite, la reconstruction de la paroi vasculaire débute.

Finalement, lors de la fibrinolyse (**cinquième étape**) le caillot est éliminé dès que la cicatrisation est terminée. La fibrinolyse résulte de l'action d'une enzyme, la plasmine, qui est capable de dégrader la fibrine. Cette enzyme est présente en grande quantité dans le caillot mais reste inactive jusqu'au moment où les cellules endothéliales sécrètent l'activateur tissulaire du plasminogène. Ainsi, la plasmine s'attaque au caillot afin de le dissoudre pour éviter par exemple, des embolies.

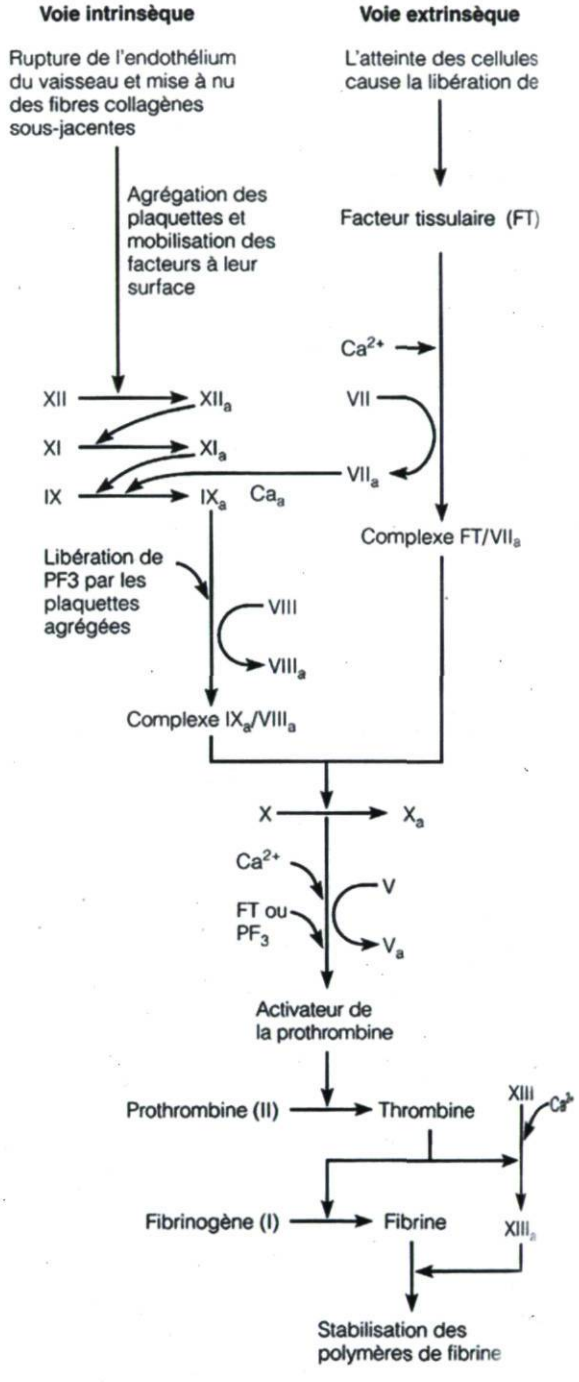

Figure 1.7 : Diagramme montrant les phases de formation du clou plaquettaire et celles des voies intrinsèques et extrinsèques de la coagulation.
(Marieb, 1999)

Lors d'une transfusion sanguine ou d'une opération, la coagulation du sang n'est pas un phénomène souhaitable. Des médicaments comme l'héparine et la warfarine sont administrés aux patients pour limiter ce phénomène. Les fucoïdanes ont été proposés comme alternative à l'héparine, car ils ont moins de risque de contenir des virus ou des prions puisque celle-ci provient fréquemment du porc ou du boeuf. Des études ont montré que les fucoïdanes sont des anticoagulants puissants semblables à l'héparine, mais ayant un mécanisme d'action différent. Une étude a dévoilé l'effet des fucoïdanes sur les premières étapes de la coagulation : l'activation plaquettaire (Dürig et al., 1997). Les résultats ont montré que l'effet était influencé par la dose, le poids moléculaire et le nombre de groupement sulfaté des fucoïdanes. Curieusement, les fractions les plus actives étaient celles les moins sulfatées et de hauts poids moléculaires. Ce phénomène serait influencé par le degré de ramification du polysaccharide. Plus le fucoïdane est ramifié, plus la coagulation des plaquettes serait importante (Mourão et al., 1999). Une étude *in vivo* réalisée chez le babouin a montré après injection de fucoïdane, une forte agrégation plaquettaire lors d'une xénotransplantation (Alwayn et al., 2000), transfert de cellules, tissus ou organe entre des individus de deux espèces différentes. Cependant, l'activation plaquettaire n'est pas un processus souhaitable lorsque le système ne subit pas de dommage et pourrait engendrer la formation de caillot chez des personnes saines provoquant ainsi des thromboses ou des embolies (Marieb, 1999). D'autres études *in vivo* ont démontré le potentiel anticoagulant des fucoïdanes en agissant sur la thrombose. Du fucoïdane a été injecté par intraveineuse à des lapins dix minutes avant induction de la thrombose. Les résultats ont montré que les fucoïdanes inhibent l'action du facteur X_a et l'action anti-thrombose persiste plus longtemps qu'avec l'héparine (Mauray et al., 1995).

D'autres travaux ont montré que les fucoïdanes extraits de *F. vesiculosus* étaient plus efficaces que l'héparine au niveau du retard de l'action de la thrombine sur le fibrinogène (Ito et al., 1989). Aussi, les fucoïdanes extraits de *F. vesiculosus* et *Pelvetia canaliculata* ont affiché une activité anticoagulante par des tests *in vitro* en agissant comme activateur de l'antithrombine III et de l'héparine cofacteur II, deux inhibiteurs de thrombine (Colliec et al., 1991; Sinniger et al., 1993). Des effets similaires ont aussi été rapportés par un test *in vitro* avec des fucoïdanes extraits de *Laminaria cichorioides* (Yoon et al., 2007). Aussi,

ceux extraits de *L. brasiliensis* inhibent *in vitro* l'action de la thrombine et du facteur X_a, des enzymes pro-coagulantes (Figure 1.7) (Pereira et al., 1999). Ces fucoïdanes ont aussi montré une activité anticoagulante supérieure aux fucoïdanes extraits d'*A. nodosum* et *F. vesiculosus,* et ce, avec un contenu en sulfates inférieur aux deux autres (Pereira et al., 1999). Ceci laisse supposer que le contenu en sulfates n'est pas le seul élément influençant l'activité biologique, mais que la densité de charges et la structure (liaisons glycosidiques, monosaccharides) sont aussi des facteurs importants. D'un point de vue structural, il a été démontré que la présence de groupements sulfate en position 4 était nécessaire pour inhiber la thrombine via l'action de l'héparine cofacteur II (Pereira et al., 2002). D'autres ont établi qu'il y avait une relation entre l'activité anticoagulante et le poids moléculaire des fucoïdanes extraits d'*Eklonia kurome* (Nishino et al., 1991). Plus les fractions sont de faibles poids moléculaires (13 kDa) plus l'activité est importante. Par contre, le poids moléculaire optimum serait propre à chaque espèce expliquant pourquoi une étude a observée une activité anticoagulante supérieure avec du fucoïdane de haut poids moléculaire (Dürig et al., 1997). D'autres ont remarqué qu'en réduisant le nombre de groupements sulfate et le poids moléculaire, les activités anticoagulante et anti-thrombose diminuaient (Boisson-Vidal et al., 2000). Finalement, des fucoïdanes extraits d'invertébrés marins ont montré par modélisation que la conformation est une caractéristique essentielle pour l'obtention d'une activité anticoagulante (Becker et al., 2007). Par conséquent, il est important de choisir les fucoïdanes possédant les bonnes caractéristiques structurales pour obtenir l'activité souhaitée.

1.5.2.2 Activité anti-inflammatoire

La réaction inflammatoire est un mode de réponse de l'organisme à une agression de type infectieuse, immunologique, tumorale, etc. Cette réaction est souvent bénéfique mais il arrive parfois qu'elle endommage les cellules et les tissus. On appelle système du complément, un groupe de protéines sériques impliqué dans la phagocythose et la lyse des bactéries. Ce système peut être activé selon la voie classique ou alternative (Figure 1.8).

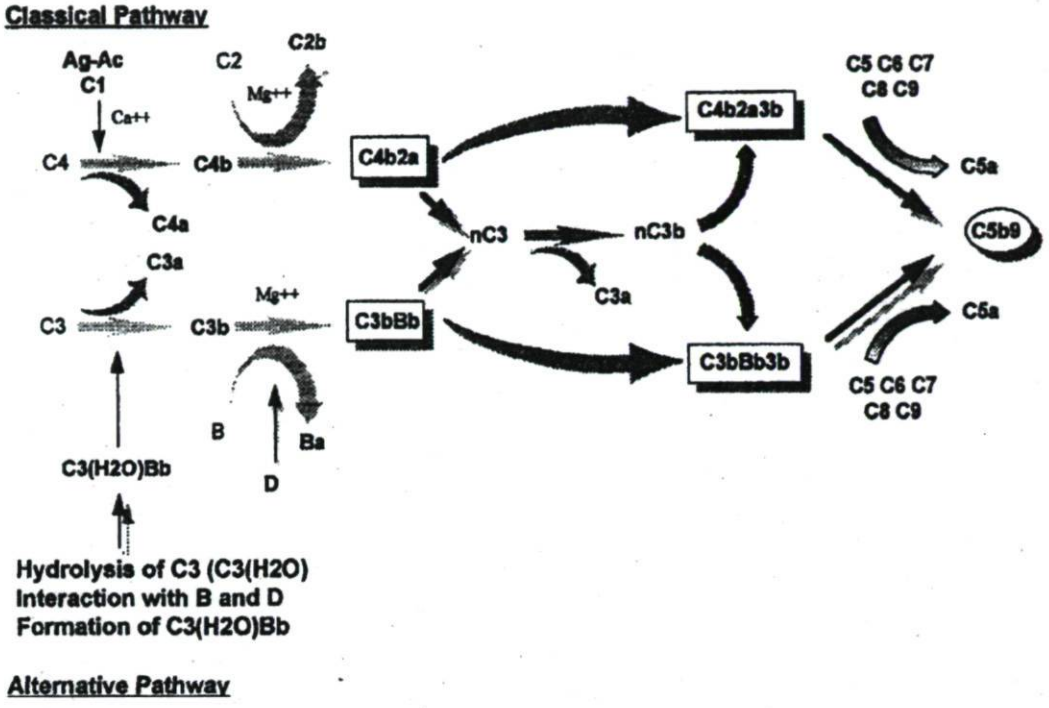

Figure 1.8 : Schéma du système du complément.
(Boisson-Vidal et al., 1995)

Les fucoïdanes de faibles poids moléculaires se sont montrés efficaces contre l'inhibition du système du complément *in vitro*. Ces fucoïdanes sont 40 fois plus actifs que l'héparine pour l'inhibition de la voie classique et égaux à l'héparine pour l'inactivation de la voie alternative (Boisson-Vidal et al., 1995). Les fucoïdanes de faibles poids moléculaires inhibent l'action de la convertase C3 en limitant l'activation de C1 ou par la segmentation de C4 par C1. Les fucoïdanes ont aussi la capacité d'inhiber la voie alternative en empêchant la liaison entre le facteur B et C3 (Blondin et al., 1994). D'ailleurs, un polysaccharide de faible poids moléculaire et contenant des monosaccharides tels le galactose et l'acide glucuronique est essentiel pour obtenir une activité. L'utilisation de l'héparine *in vivo* n'est pas adéquate pour inhiber le système du complément car il s'agit d'un anticoagulant à la dose d'inhibition (Boisson-Vidal et al., 1995). Cependant, les fucoïdanes de faibles poids moléculaires induisent une activité anticoagulante plus faible avec une dose molaire équivalente à l'héparine.

L'interaction entre les fucoïdanes et les sélectines a aussi été démontrée (Foxall et al., 1992; Omata et al., 1997). Les sélectines sont des molécules d'adhésion retrouvées à la surface des leucocytes (L-sélectines), des plaquettes (P-sélectines) ou des cellules endothéliales (E- et P-sélectines) (Ley et al., 2004). Lors d'une réaction inflammatoire, les leucocytes se déplacent vers le site infecté à travers le flux sanguin. Des interactions entre les sélectines et leurs ligands respectifs (oligosaccharides sulfatés) permettent l'adhérence des leucocytes sur l'endothélium pour effectuer un phénomène de roulement (Figure 1.9a) (Ley et al., 2004). Au site d'inflammation, les cellules endothéliales expriment des chémokines. Ces dernières activent l'expression d'intégrines spécifiques qui se retrouvent à la surface des leucocytes et permettront la liaison avec des molécules d'adhésion cellulaire (ICAM-1, VCAM-1 et PECAM-1) (Gaudry et al., 1998). Cette liaison a pour rôle d'arrêter le roulement des leucocytes (Figure 1.9b) et favoriser leur traversée de l'endothélium. Une fois traversés, les leucocytes vont détruire le tissu endommagé et éliminer les agents pathogènes. Cependant, lors de certaines réactions inflammatoires chroniques ou aiguës, les leucocytes peuvent avoir des effets nocifs (Gaudry et al., 1998). L'utilisation de molécules permettant d'éviter la liaison entre les leucocytes et les sélectines, empêcherait le roulement des leucocytes et par conséquent, leur traversée de l'endothélium. L'utilisation du fucoïdane est tout à fait appropriée, car ce polysaccharide possède des groupements sulfate tout comme les ligands des sélectines. Ainsi, les fucoïdanes pourraient agir en bloquant l'interaction entre les sélectines et leurs ligands.

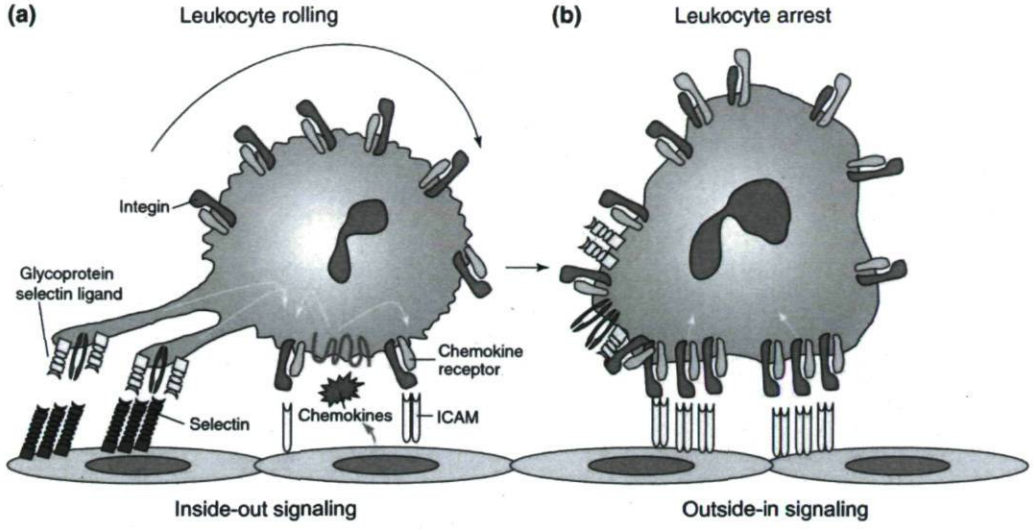

Figure 1.9 : (a) Roulement du leucocyte par la liaison entre la sélectine et son ligand. (b) Arrêt du leucocyte par la liaison entre l'intégrine et molécule d'adhésion cellulaire (ICAM).
(Takada et al., 2007)

Des études *in vivo* ont montré que les fucoïdanes empêchent la migration de deux types de leucocytes (neutrophiles et éosinophiles) vers les sites d'inflammation (Shimaoka et al., 1996; Teixeira et al., 1997). D'autres effets ont été rapportés, par exemple : la perfusion des fucoïdanes s'avère utile pour réduire l'infiltration de neutrophiles (leucocytes) dans les blessures myocardiques après une reperfusion ou une ischémie chez le rat (Omata et al., 1997). Une étude *in vivo* a été réalisée pour tester la capacité anti-inflammatoire des fucoïdanes commerciaux lors d'une méningite bactérienne (Granert et al., 1999). Lors d'une méningite, la réponse inflammatoire est causée par des cytokines telles : TNF-α (*tumor necrosis factor*) et IL1 (*interleukin-1*). Ces cytokines provoquent l'accumulation de leucocytes dans le liquide cérébrospinale ce qui peut causer des dommages neurologiques. Des études ont montré qu'une réduction des leucocytes dans l'espace sous-arachnoïdien peut réduire la mortalité (Tuomanen et al., 1989). L'utilisation *in vivo* des fucoïdanes permet de réduire la concentration de leucocytes en réduisant la concentration de TNF-α et de IL1 (Granert et al., 1999). Pour finir, une vaste étude portant sur neuf fucoïdanes d'algues brunes a montré des résultats intéressants sur l'inhibition des P-sélectines par des

tests *in vitro* et *in vivo*. Les fucoïdanes de *L. saccharina*, *L. digitata*, *F. evanescens*, *F. serratus*, *F. distichus*, *F. spiralis* et *A. nodosum* ont significativement inhibé les P-sélectines (Cumashi et al., 2007). Par contre, *Cladosiphon okamuranus* et *F. vesiculosus* n'ont pas permis de les inhiber. Ces résultats sont étonnants puisque les fucoïdanes d'*A. nodosum* et *F. vesiculosus* sont connus pour avoir une structure similaire (voir section 1.3.2). Encore une fois, la structure des fucoïdanes serait responsable des différences d'activités entre les diverses espèces d'algues.

1.5.2.3 Activité antivirale

Les fucoïdanes possèdent des activités antivirales *in vitro* en inhibant l'infection des cellules hôtes par *Herpes simplex* (Adhikari et al., 2006), cytomégalovirus et VIH (Baba et al., 1988b; Hoshino et al., 1998). Le mécanisme d'action des fucoïdanes contre ces virus se situe au niveau de l'inhibition de l'attachement du virus sur les cellules hôtes (Baba et al., 1988b; Hoshino et al., 1998). Dans le cas du VIH, les fucoïdanes empêcheraient la liaison entre la glycoprotéine 120 et le récepteur CD4 des cellules hôtes (Figure 1.10) empêchant la formation d'un syncytium (cellule de grosse taille) (Baba et al., 1990; McClure et al., 1991). Ainsi, les fucoïdanes joueraient une rôle protecteur en limitant la réplication du virus et aussi en empêchant la formation de syncytium (Baba et al., 1990; Béress et al., 1993).

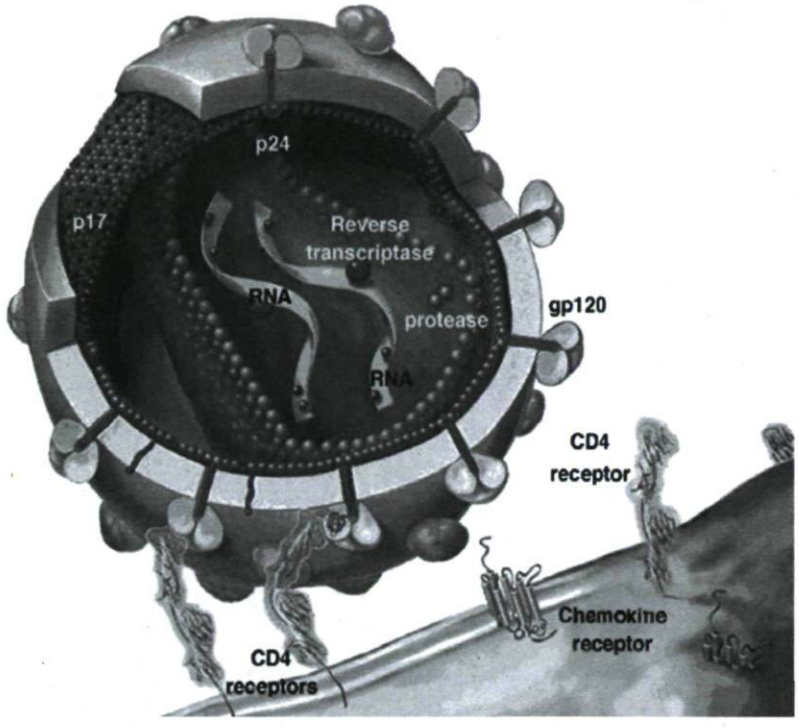

Figure 1.10 : Mécanisme d'attachement du VIH sur les cellules hôtes.
(Cobain, 2003)

Pour *Herpes simplex* type 2, une étude *in vitro* a montré que les fucoïdanes doivent être présents lors de la phase d'absorption du virus afin d'éviter l'appariement du virus avec les cellules hôtes (Feldman et al., 1999). D'autres ont montré par des tests *in vitro*, que même 2 heures après l'infection, les fucoïdanes inhibaient la réplication d'*Herpes simplex* type 1 (Hoshino et al., 1998). Il est possible que le polysaccharide s'attache à certaines membranes moléculaires de la cellule hôte ce qui empêcherait la pénétration du virus.

La relation entre la structure et l'activité antivirale de fucoïdanes extraits de l'algue brune *Stoechospermum marginatum* a été réalisée (Adhikari et al., 2006). Les résultats ont montré qu'une fraction à haute teneur en fucose, faiblement sulfatée (13%) et de poids moléculaire d'environ 40 kDa, a un potentiel antiviral contre *Herpes simplex*. La teneur en sulfate est importante car, les fractions désulfatées ont montré une diminution du potentiel antiviral de 14 à 64 fois (Adhikari et al., 2006). Aussi, l'activité antivirale des fucoïdanes pourrait être

induite par la conformation en L des fucoses (Venkateswaran et al., 1989). Pour le caractère anti-HIV, les fucoïdanes doivent avoir un poids moléculaire plus grand que 5000 Da et idéalement être di-sulfatés pour inhiber la formation de syncytium (Baba et al., 1990). D'autres ont aussi montré qu'un poids moléculaire compris entre 10 et 20 kDa favorisait une activité anti-HIV (Béress et al., 1993).

1.5.2.4 Activité anti-proliférative

La prolifération d'une cellule implique la réplication de l'ADN et sa répartition en deux cellules filles. Ces étapes sont appelées cycle cellulaire. Les phases de réplication de l'ADN (phase S) et de la mitose (phase M) sont séparées par deux phases : G1 et G2 (Figure 1.11). Pendant la phase G1, la cellule analyse si son environnement et sa taille sont propices à la prolifération avant d'enclencher la réplication de l'ADN (phase S). Lors de la phase G2, la cellule vérifie que l'ADN est bien répliqué avant de poursuivre avec la mitose, division de la cellule mère en deux cellules filles. Chacune des phases se succèdent grâce à l'apparition simultanée de cyclines et de protéines kinase cdk (*cyclin dependant kinase*). On retrouve aussi des inhibiteurs de cdk (p21, p27, p57) qui lorsque sécrétés durant la phase G1, empêchent la progression du cycle cellulaire.

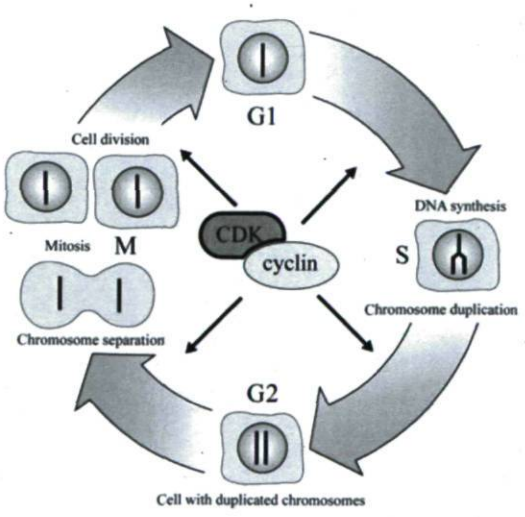

Figure 1.11 : Représentation des phases du cycle cellulaire.
(Hartwell et al., 2001)

Plusieurs études ont démontré que le fucoïdane et l'héparine inhibaient la proliferation de plusieurs lignées cellulaires (Ellouali et al., 1993; Haroun-Bouhedja et al., 2000; Kanabar et al., 2005; Logeart et al., 1997b; Patel et al., 2002). Des cellules du muscle lisse vasculaire traitées à l'héparine ont montré une augmentation de l'expression du gène p27 (un inhibiteur de cdk) et de la sécretion de la protéine (Fasciano et al., 2005). Ceci a empêché l'activation du cdk-2 et conséquemment a inhibé la progression du cycle cellulaire. D'autres ont proposé que l'inhibition du cycle cellulaire soit causée par l'inactivation de ERK (Patel et al., 2002), une protéine requise pour la transcription de la cycline D (phase G1) (Robinson et al., 1997). Par ailleurs, d'autres travaux ont aussi montré que le fucoïdane inhibait la prolifération de cellules cancereuses lui conférant ainsi une activité anti-tumorale (Aisa et al., 2005; Teruya et al., 2007).

1.5.2.4.1 Activité anti-tumorale

L'apparition d'une tumeur est reliée à un disfonctionnement au niveau du mécanisme de croissance cellulaire (Boisson-Vidal et al., 1995). L'apparition de métastase implique le transit de cellules cancéreuses à travers les vaisseaux sanguins permettant le déplacement des cellules cancéreuses vers d'autres tissus. Une fois dans le tissu, les cellules cancéreuses prolifèrent. Ce processus fait intervenir des enzymes produites par les cellules cancéreuses qui solubilisent la matrice. L'invasion des tissus sains est réalisée via des protéines d'adhésion (laminine, fibronectine) qui se lient aux récepteurs des cellules cancéreuses (Soeda et al., 1994). De plus, les cellules cancéreuses produisent leur propre facteur de croissance (FGFs et etc.) qui permettent leur adhésion aux cellules endothéliales et stimulent leur croissance (Boisson-Vidal et al., 1995). De nouveaux vaisseaux sanguins sont créés pour alimenter la tumeur, ce processus s'appelle l'angiogénèse.

L'activité anti-tumorale des fucoïdanes peut se faire grâce à l'inhibition des enzymes, de l'adhésion des cellules, par un effet cytotoxique ou par un effet anti-angiogénique. (Boisson-Vidal et al., 1995). Les fucoïdanes d'*A. nodosum* de faibles poids moléculaires (de 15 à 140 kDa) inhibent la prolifération des cellules cancéreuses de différentes lignées cellulaires (Ellouali et al., 1993). De plus, les fractions de poids moléculaires inférieurs à 15 kDa avaient une activité beaucoup plus faible. Une autre étude a montré que la densité

de charges de différents polysaccharides (fucoïdane, carraghénane, dextrane sulfaté, etc.) est importante pour leur bioactivité. Toutefois, la position des groupements sulfate serait aussi essentielle (Belford et al., 1993). D'ailleurs, les fucoïdanes de faibles poids moléculaires ne semblent pas entraîner une action anti-prolifération aussi importante que ceux de hauts poids moléculaires (Logeart et al., 1997a).

La formation de tumeur pourrait être inhibée en empêchant la liaison entre les récepteurs des cellules cancéreuses et des protéines d'adhésion de la matrice. Ainsi, les fucoïdanes natifs ont la capacité d'inhiber l'attachement des cellules cancéreuses à la laminine (protéine d'adhésion) pour une concentration allant de 2.5 à 25 µg/ml (Soeda et al., 1994). Pareillement, les fucoïdanes de faibles poids moléculaires pourraient inhiber l'adhésion cellule-cellule des cellules endothéliales de l'aorte de bovin (Glabe et al., 1983).

D'autres études ont porté sur l'activité anti-métastatique des fucoïdanes. Les métastases sont des foyers secondaires cancéreux disséminés par voie sanguine ou lymphatique. En théorie, en inhibant l'adhésion des cellules, la formation de métastases devrait être restreinte. Une étude a été réalisée sur la relation entre le nombre de métastases et le traitement avec divers polysaccharides sulfatés. Les fucoïdanes ont permis d'inhiber la formation de métastase chez les cellules d'adénocarcinomes mammaire de rat (Coombe et al., 1987) et des adénocarcinomes de poumon implantés chez la souris (Alekseyenko et al., 2007). Par ailleurs, certains pensent que le mécanisme d'action anti-métastatique serait peut-être relié à leurs activités anti-inflammatoires (Parish et al., 1987).

Une étude a été réalisée sur la capacité des fucoïdanes natifs et des fucoïdanes enrichis en sulfates à inhiber l'angiogénèse des tumeurs chez la souris. Les fucoïdanes enrichis ont montré une activité anti-angiogénique plus importante que chez les fucoïdanes natifs (Koyanagi et al., 2003). D'autres études ont été réalisées sur le caractère pro-angiogénique des fucoïdanes. Ces derniers auraient la capacité de stimuler la formation de nouveaux vaisseaux sanguins permettant d'alimenter les tissus suite à une ischémie (arrêt ou diminution en apport sanguin) (Lake et al., 2006; Luyt et al., 2003; Matou et al., 2002). Ceci est contradictoire avec les résultats des activités anti-tumorales et anti-angiogéniques

de ces molécules car, une étape importante du développement de la tumeur est sa vascularisation. Ceci serait attribuable à des différences dans la structure des fucoïdanes. En effet, les trois études ont utilisé un fucoïdane de faible poids moléculaire allant de 5000 à 15000 Da alors que celle montrant une activité anti-angiogénique utilisait un fucoïdane natif. D'autres ont aussi montré que les fucoïdanes de poids moléculaire inférieur à 8 kDa n'avaient aucun effet sur la prolifération de cellulaire (Logeart et al., 1997a). Donc, le poids moléculaire semble être une caractéristique structurale importante pour la prolifération des cellules et donc, l'activité anti-tumorale.

1.5.2.4.2 L'apoptose

L'apoptose (mort cellulaire) a un rôle important dans le développement et l'homéostasie des organismes multicellulaires. Ce mécanisme permet d'éliminer une cellule sénescente ou défaillante (cause : cancer, mutation, virus, bactérie etc.) en occasionnant peu ou pas d'inflammation comparativement au processus de nécrose. L'apoptose est activée par la voie extrinsèque via le récepteur de mort (*death receptor*) ou par la voie intrinsèque via la mitochondrie (Figure 1.12) (Chipuk et al., 2005). Les deux voies conduisent à l'activation de caspases. Ces dernières induiront de la protéolyse permettant de détruire la structure de la cellule pour former des corps apoptotiques (*apoptotic body*) qui seront éliminés par phagocytose.

Dans certain cas, l'héparine et le fucoïdane peuvent induire l'apoptose. Une étude *in vitro* a montré que l'héparine stimule l'apoptose de lymphoblastes par l'activation de caspases (Erduran et al., 2007). Un fucoïdane riche en sulfate engendre l'activation des caspases-3 et -7, des intermédiaires dans la réaction d'apoptose, chez une lignée cellulaire leucémique U937 (Teruya et al., 2007). D'autres ont proposé que le mécanisme par lequel l'héparine induit l'apoptose de cellules cancéreuses serait relié à l'inhibition d'AKt (Ueda et al., 2009). AKt est impliqué dans la prolifération cellulaire et l'apoptose (Franke et al., 2003). AKt phosphoryle plusieurs protéines pro-apoptiques ce qui conduit à leur inactivation et conséquemment stimule l'apoptose. Une autre étude *in vitro* a montré une réduction de la prolifération suivie d'une augmentation de l'apoptose de cellules cancéreuses traitées avec du fucoïdane (Aisa et al., 2005). Les auteurs ont observé une activation de la caspase-3 et

ont montré que le fucoïdane agirait sur la voie intrinsèque de l'apoptose. Ils ont aussi montré une inactivation d'ERK, qui comme nous l'avons vu précédemment empêche la progression du cycle cellulaire.

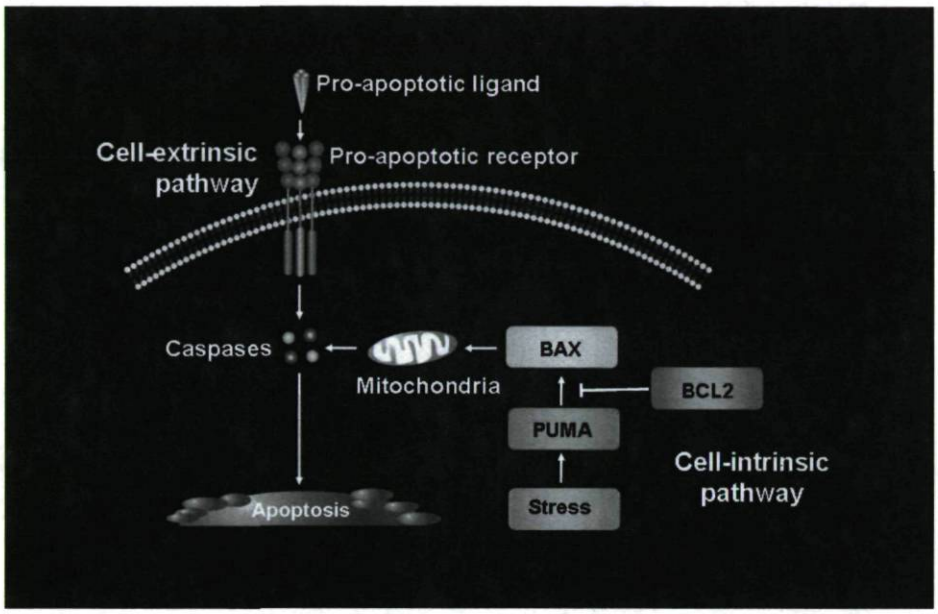

Figure 1.12 : Les deux principales voies d'activation de l'apoptose.
(Ashkenazi, 2002)

1.5.2.5 Activité contraceptive

Les fucoïdanes ont aussi montré une action potentielle contraceptive chez l'humain (Mahony et al., 1993; Mahony et al., 1991). Lors de la fécondation, le spermatozoïde doit franchir plusieurs étapes avant d'atteindre la zone pellucide puis le noyau de l'ovule (Figure 1.13). À la surface de la zone pellucide, trois types de glycoprotéines ZP1, ZP2 et ZP3 sont retrouvées chez la souris (Wassarman, 1988). L'attachement entre l'acrosome et la zone pellucide est effectué via la glycoprotéine ZP3. Chez l'humain, entre 2 et 4 glycoprotéines sont présentes dans la zone du pellucide mais le mécanisme d'action de l'attachement de l'acrosome est encore inconnu (Mahony et al., 1991; Wassarman, 2008).

Certaines études suggèrent que les ZP3 et ZP4 seraient impliqués (Chakravarty et al., 2008).

Plusieurs polysaccharides peuvent moduler l'adhésion oviducte-spermatozoïde, l'interaction spermatozoïde-ovocyte et au niveau de l'implantation de l'embryon (Tanghe et al., 2004). Actuellement, aucune recherche n'a étudié le barrage de l'implantation de l'embryon par l'utilisation de polysaccharides sulfatés. Par contre, une étude a démontré que des protéines d'adhésion (laminine, fibronectine) et des récepteurs (protéines) sont retrouvés à la surface de l'embryon pour assurer l'implantation dans l'utérus (Farach et al., 1987). Ces protéines peuvent se lier à l'héparine. Or, les fucoïdanes sont capable de contrer l'action des protéines d'adhésion (voir section 1.5.2.4.1) mais peuvent-ils empêcher l'implantation d'un embryon? D'autres études devront être réalisées pour le découvrir.

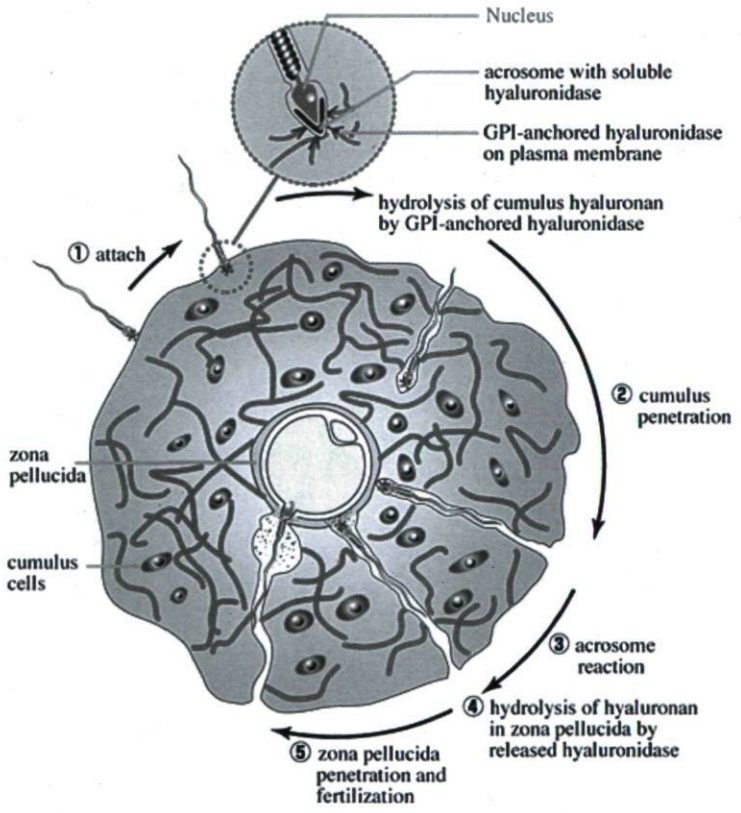

Figure 1.13 : Étapes de la fécondation de l'ovule par le spermatozoïde. (Glycoforum, 1998)

Une étude a montré que les spermatozoïdes s'attachent aux cellules épithéliales de l'oviducte (trompes de Fallope chez l'humain) jusqu'à ce qu'un signal, provenant de l'ovule, les détache (Talevi et al., 2001). L'adhésion à la paroi de l'oviducte est une étape cruciale pour la fécondation. Les polysaccharides sulfatés empêchent la liaison entre l'oviducte et les spermatozoïdes du bovin (Talevi et al., 2001). Par ailleurs, le prétraitement du sperme avec des fucoïdanes empêche aussi l'adhésion des spermatozoïdes au niveau de l'oviducte bovin. Cependant chez l'humain, aucune étude n'a été réalisée sur ce sujet.

Par ailleurs, les fucoïdanes préviendraient la liaison entre les glycoprotéines de la zone pellucide de l'ovule et le spermatozoïde chez l'humain (Oehninger et al., 1990; Oehninger et al., 1992). Plus précisément, une analyse *in vitro* a montré que les fucoïdanes empêchaient la liaison entre l'acrosome et les glycoprotéines (Mahony et al., 1991) (étape 3 Figure 1.14). Le marquage par un agent fluorescent des fucoïdanes a permis d'identifier qi'ils étaient attachés à la surface des spermatozoïdes au niveau de la membrane de l'acrosome (Mahony et al., 1993) limitant ainsi l'adhésion aux glycoprotéines. D'autres études, chez le hamster et la souris, ont montré que les fucoïdanes inhibaient significativement la fusion entre le spermatozoïde et l'ovocyte (Boldt et al., 1989; Dravland et al., 1988)

D'un point de vue structural, les fucoïdanes de faible poids moléculaire 16kDa (Boisson-Vidal et al., 1995) et de source commerciale (Mahony et al., 1993) ont démontré respectivement une activité contraceptive *in vivo* chez la souris et *in vitro* chez l'humain. Les résultats étaient comparables entre les deux études. La présence de groupements sulfates semble cependant être essentielle pour cette activité (Talevi et al., 2001).

Les fucoïdanes ont aussi été retrouvés à la surface des œufs d'oursins (Berteau et al., 2003). Ces fucoïdanes jouent un rôle critique dans la fécondation des œufs au niveau de l'activation de l'acrosome (Mourão, 2007). Cependant, seuls les fucoïdanes d'une espèce active spécifiquement l'acrosome de la même espèce. Il est tout à fait étonnant de voir que, d'un côté les fucoïdanes puissent agir comme agent contraceptif alors que chez les oursins, ils agissent comme des médiateurs de la fécondation.

En terminant, l'activité contraceptive et antivirale des fucoïdanes rend ce polysaccharide très attrayant pour la formulation de nouveau contraceptif. Cependant, trop d'informations sont manquantes au niveau de la structure et du mécanisme d'action des fucoïdanes pour pouvoir le commercialiser.

1.5.2.6 Autres activités

Les fucoïdanes ont montré d'autres activités très intéressantes. Par exemple, le fucoïdane de faible poids moléculaire (< 16kDa) protègerait le réseau de fibre élastique de la peau contre la protéolyse enzymatique en inhibant l'action des MMP2 (gélatinase A) et MMP3 (stromelysine 1) (Senni et al., 2006). Ces résultats suggèrent que ce fucoïdane pourrait servir au traitement de certaines pathologies inflammatoires où il y a une dégradation incontrôlée de la matrice extracellulaire (emphysème, parodontite ou ulcère de la jambe) (Senni et al., 2006). Le fucoïdane a aussi monté un potentiel à prévenir et traiter le photo-vieillissement de la peau en inhibant la production de MMP1 (collagénase) lors d'un traitement UVB de fibroblastes humains normaux cultivés *in vitro* (Moon et al., 2008).

Une activité antioxydante a aussi été rapportée pour le fucoïdane. Les résultats ont montré que les oligo-fucoïdanes pouvaient lier les radicaux hydroxyles ($HO^{\cdot}$), inhiber l'hémolyse des érythrocytes (globule rouge) et chélater le Fe^{2+}. Les oligo-fucoïdanes (5 kDa) ne peuvent cependant pas lier le radical superoxyde ($O_2^{\cdot -}$) (Wang et al., 2007), espèce chimique très toxique. Par contre, l'utilisation de fucoïdane natif (170 kDa) inhibe les radicaux superoxydes (de Souza et al., 2007; Veena et al., 2007). Encore une fois, la structure est très importante pour la modulation de l'activité.

Finalement, le fucoïdane extrait de *Cladosiphon okamuranus* a montré un effet sur l'attachement de la bactérie *Helicobacter pylori in vitro* et *in vivo* à la mucine (glycoprotéine) et la muqueuse gastrique (Shibata et al., 2003). Ces résultats montrent le potentiel du fucoïdane pour prévenir les désordres gastriques comme les ulcères récurrents et réduire les risques associés au développement du cancer de l'estomac.

1.5.3 Le galactofucane

Plusieurs activités ont été spécifiquement attribuées aux galactofucanes quoique très semblables à celles des fucoïdanes. Des études ont montré une activité antivirale contre le virus d'*Herpes simplex* type 1 et 2 avec l'aide de galactofucane (Hemmingson et al., 2006; Thompson et al., 2004). Une activité anti-thrombotique deux fois plus importante a aussi été démontrée pour les galactofucanes comparativement à l'héparine lors d'une étude *in vivo* (Rocha et al., 2005). De plus, l'utilisation de fucoïdanes et de galactofucanes de poids moléculaire allant 10 à 80 kDa a montré des effets similaires sur l'activité anticoagulante (Nishino et al., 1991). Les résultats ont également montré que les fractions de poids moléculaire inférieur à 30 kDa avaient une meilleure activité.

1.5.4 L'alginate

De par ses propriétés physiques, l'alginate est très utile pour produire des pansements qui faciliteront la guérison des patients. L'alginate du pansement est solubilisé par les fluides du corps grâce à l'échange entre les ions sodiques des fluides et des ions calciques du gel (FAO, 2003).

Des études ont montré le caractère fermentescible de l'alginate de sodium par des bactéries du colon (Michel et al., 1996). La formation de dérivé d'alginate produit par la flore intestinale lors de la fermentation des oligo-alginates pourrait avoir des effets positifs sur la muqueuse digestive (Michel et al., 1999). De plus, l'alginate enrichi en groupement mannuronique peut stimuler la production d'une cytokine (TNF, *tumor necrosis factor*) par les monocytes humains. L'activité serait reliée à la présence de liaison β-(1,4) di-équatoriale et au degré de polymérisation (Espevik et al., 1993). La production du TNF est importante car cette cytokine joue un rôle important dans la réponse immunitaire et inflammatoire (Otterlei et al., 1991). De plus, l'action des alginates enrichis en groupements mannuroniques est équivalente à l'action des lipopolysaccharides qui sont connus pour stimuler la production de cytokines (TNF et interleukine 6) (Otterlei et al., 1993).

L'alginate a aussi montré un activité anti-tumorale (Fujihara et al., 1984), laquelle serait encore une fois reliée à la présence de groupement mannuronique (Fujihara et al., 1992). Une activité antivirale a aussi été démontrée chez plusieurs espèces de poisson (Cheng et al., 2008). De plus, il peut réduire l'absorption intestinale du glucose, des triglycérides et du cholestérol (Paxman et al., 2008) et avoir un effet sur la glycémie postprandiale (Torsdottir et al., 1991).

L'alginate peut aussi servir de matrice d'encapsulation pour véhiculer des molécules actives ou sensibles telles que des vaccins (Li et al., 2008b), des vitamines (Chen et al., 2007b) et des bactéries probiotiques (Ding et al., 2009). L'acide alginique et l'alginate de sodium permettent aussi de protéger l'estomac et l'œsophage contre l'acidité découlant du reflux gastrique (Dettmar et al., 2007). L'alginate est un excellent chélateur de strontium et de radium et permet de bloquer l'empoisonnement par des métaux lourds (Indegaard et al., 1991b).

1.6 Nouvelles approches de détection de l'activité biologique

1.6.1 Introduction

La recherche d'activité biologique induite par différents extraits (polysaccharides, bêta-carotène, etc.) est très populaire aujourd'hui. Par contre, le criblage de ces activités est complexe et nécessite plusieurs étapes. Les méthodes traditionnelles tels les modèles *in vivo* (Devillé et al., 2004; Granert et al., 1999; Noda et al., 1990; Omata et al., 1997) et *in vitro* (Baba et al., 1988a; Colliec et al., 1994; Dürig et al., 1997; Grauffel et al., 1989; Roger et al., 2002) sont souvent utilisés. Les modèles *in vivo* sont particulièrement fiables pour identifier de tels effets, mais présentent des contraintes économiques et éthiques. Ces contraintes peuvent être partiellement évitées par l'utilisation de modèles *in vitro*, leur utilisation étant d'une grande utilité pour étudier des effets spécifiques déjà anticipés. Par contre, ces modèles présentent une approche qui rend coûteuse l'analyse systématique d'un grand nombre d'effets et son aspect réductionniste exclut la possibilité d'identifier des effets

insoupçonnés absents de la batterie de tests. De plus une question subsiste : les modèles *in vitro* reflètent-ils la réalité? Par exemple, lors de la coagulation du sang, seule la voie intrinsèque est analysée dans des modèles *in vitro* (Marieb, 1999) alors qu'en réalité, la voie extrinsèque est présente dès que le sang s'est infiltré dans le tissu. Or, la voie extrinsèque comporte beaucoup moins d'étapes et est donc plus rapide. Ceci laisse présager des différences importantes dans les résultats obtenus.

D'autres méthodes telles que les puces à ADN peuvent être également employées. Avec l'avancement de la technologie et le séquençage du génome humain, les puces à ADN s'avèrent être une méthode de choix pour étudier le degré d'expression génique d'ARN extrait de cellules. Les puces à ADN se présentent sous la forme d'un support solide contenant quelques centaines à plusieurs milliers de fragments de gènes (séquence nucléique), et qui, dans un mélange de molécules, s'apparient avec des séquences nucléiques complémentaires, permettent de détecter la présence des mêmes gènes dans des cellules soumises à l'analyse. Le caractère unique de cette méthode repose sur l'appariement spécifique des nucléotides complémentaires par hybridation. Les puces d'ADN permettent d'exposer des cellules humaines en culture à différents traitements et d'analyser ensuite leur profil d'expression génique. Cette méthode est idéale pour réaliser des comparaisons systématiques, car l'expression de plusieurs gènes peut être mesurée simultanément. Cet outil extrêmement puissant permet l'identification de nouvelles voies métaboliques et de gènes d'intérêt. Les domaines d'applications des puces à ADN sont extrêmement divers. Elles sont utilisées pour comprendre le fonctionnement normal d'une cellule ou d'un tissu, ou son fonctionnement altéré par un environnement particulier, une mutation ou par une pathologie (cancer, diabète, maladie cardiovasculaire, ou encore infection virale ou bactérienne). Des puces sont fabriquées pour une très grande diversité de génomes de bactéries, de parasites, de champignons, de plantes et de vertébrés.

Plusieurs d'études ont été réalisées au niveau de la dermatologie (Bernard et al., 2002; Curto et al., 2002; Gazel et al., 2003; Iyer et al., 1999) et du cancer de la prostate (Dulinska et al., 2005; Handayani et al., 2006) avec différentes molécules. Par contre, aucune n'a été réalisée avec des cellules humaines traitées avec des polysaccharides d'algues. Une autre

recherche s'est intéressée à l'utilisation du chlorure de gadolinium et d'un polysaccharide extrait de la plante *Angelica sinensis* afin d'étudier les gènes associés aux maladies du foie (Ding et al., 2003). L'étude du profil d'expression génique induit par des oligochitosanes a aussi été réalisée sur des cellules cancéreuses provenant des os. L'extrait saccharidique s'est montré efficace pour interagir au niveau de la multiplication et de la différenciation cellulaire (Ohara et al., 2004). Récemment, une étude a montré comment les laminaranes protégeaient les thymocytes de la mort cellulaire grâce à l'utilisation de la technique des puces à ADN (Kim et al., 2006).

Il existe un intérêt très fort de la communauté scientifique pour cet outil, mais aussi une certaine méconnaissance de son potentiel et de ses limites. Plusieurs technologies sont actuellement disponibles telles les puces à oligonucléotides, à dépôt ou à jet d'encre. Les puces à ADN offrent plusieurs avantages comparativement aux méthodes traditionnelles. Par contre, beaucoup d'effort et d'attention doivent être apportés à la planification et au design de l'expérience, à l'analyse de l'expression des gènes et à l'interprétation des résultats (Zakharkin et al., 2005). Pour un bref rappel des notions de génomiques, veuillez consulter l'annexe 1.

1.6.2 Les puces à ADN

Durant les dernières années, beaucoup d'efforts ont été mis sur le diagnostic et le traitement de plusieurs maladies. Les mécanismes physiologiques des cellules saines ont aussi été étudiés lors de cette période. Toutes ces découvertes résultent en plusieurs années de recherche sur les facteurs génétiques et environnementaux qui régulent le fonctionnement des cellules (Seta et al., 2002). Historiquement, ces études portaient sur un seul gène et une protéine à la fois. En revanche, les fonctions biologiques complexes et l'apparition de certaines maladies, tel le cancer, impliquent l'action de plusieurs milliers de gènes simultanément. La découverte de la séquence du génome humain a permis le développement de plusieurs méthodes utiles en génie génétique telle l'amplification de l'ADN par PCR et la théorie de Venter. Cette théorie propose d'étudier directement

l'ARNm plutôt que de séquencer tout le génome pour identifier les gènes (Baldi et al., 2002). L'ARNm étant la réplique quasi parfaite de la séquence codante de l'ADN, son analyse peut-être utilisée pour mesurer l'expression génique. Le niveau d'expression de chaque gène est influencé par des facteurs environnementaux (la température, le stress, la lumière et d'autres signaux qui induisent des changements dans le niveau d'hormone ou d'autres substances indicatrices) et génétiques (la séquence d'ADN du gène, les promoteurs, etc.). Pour toutes ces raisons, l'étude de l'ARN fournit de l'information non seulement au niveau du potentiel génétique d'un organisme, mais aussi sur les changements dynamiques dans l'expression des gènes. Les puces à ADN peuvent être utilisées pour quantifier cette information. Elles permettent de visualiser rapidement des différences d'expression entre les gènes du génome complet d'un organisme. Sur une seule puce, l'ensemble du génome humain peut être analysé en une seule étape.

Des puces à ADN commerciales existent sur le marché (Schena et al., 1999). Les platesformes disponibles peuvent contenir de 10 000 à 47 000 gènes. Par contre, jusqu'à maintenant seulement 10 000 à 12 000 gènes humains ont une fonction connue. L'utilisation de puce à ADN repose entièrement sur le procédé d'hybridation où un simple brin fluorescent est hybridé sur le support solide de la puce.

Il existe principalement deux grands types de puces celles à dépôt (*spotting*) ou à jet d'encre et les puces à oligonucléotides avec la synthèse *in situ* des sondes oligonucléotidiques sur une surface solide. Ces deux types de puces sont très différents au niveau des manipulations, de la planification de l'expérience, des méthodes graphiques de contrôle de la qualité des données (diagnostic), de la normalisation et de la transformation des données (Baldi et al., 2002). Les modes de fonctionnement seront décrits brièvement avec une attention plus particulière aux puces à dépôt qui ont été utilisées dans ce projet.

1.6.2.1 Puces à oligonucléotides

Les puces à oligonucléotides (Figure 1.14) sont créées à partir de courts brins d'ADN ou sondes. Ces derniers sont photo-chimiquement ajoutés sur une puce de verre, un nucléotide

à la fois par un procédé de photolithographie (Fodor et al., 1993). Cette technique, issue de la microélectronique s'apparente à celle de la gravure. Elle repose sur la protection ou l'exposition à la lumière, par un jeu de pochoirs, de zones définies de la puce afin de rendre réactifs les groupements chimiques photosensibles désirés. Les sondes contiennent environ 25 bases d'un gène qui peut en contenir 1000. Les 25 bases sont comparées au reste du génome pour s'assurer qu'ils ne correspondent à aucun autre gène. Vingt-deux sondes appartenant à différentes sections du gène et en plusieurs copies sont réparties sur la puce. Onze correspondent exactement à la séquence du gène (*perfect match*), les 11 autres présentent un mésappariement (*mismatch*) sur la position centrale où le nucléotide a été remplacé par son nucléotide complémentaire (Warrinton et al., 2000). Dans l'analyse des puces Affymetrix™, la quantification de l'ARN ne sera pas basée sur le signal d'hybridation d'une seule sonde, mais sur la combinaison des données de ces 22 hybridations. Le traitement informatique des mesures est donc une composante essentielle pour optimiser la qualité de la mesure. Des puces Affymetrix™ sont disponibles pour des mammifères (homme, souris, rat, chien, porc, poule, bovin), des végétaux (maïs, tomate, riz, soya, citron), des bactéries (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* et *Pseudomonas aeruginosas*) et plusieurs autres organismes (http://www.affymetrix.com/products/arrays/index.affx).

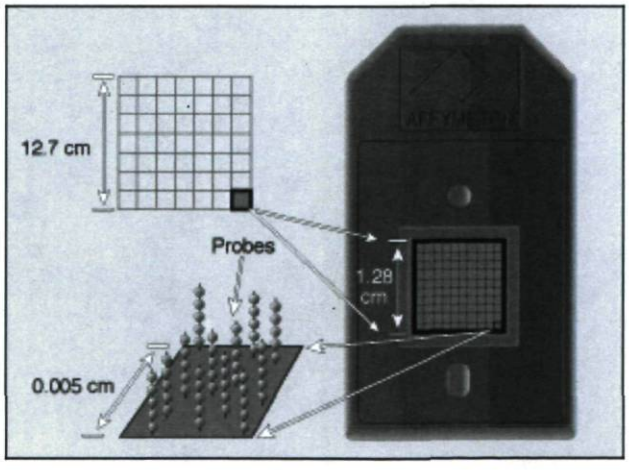

Figure 1.14 : Représentation d'une puce Affymetrix™.
(Rinehart, 2004)

Les puces sont scellées dans un emballage plastique afin de les protéger de l'environnement. De plus, elles sont vendues avec un volume fixe de réactif pour assurer la validité des résultats et elles sont facilement manipulables (Schena et al., 2000). Les puces Affymetrix™ offrent l'avantage d'analyser plusieurs milliers de gènes simultanément, facilitant les recherches de nature exploratoire. La technique d'Affymetrix™ ne se contente pas d'accélérer le diagnostic. Elle apporte deux avantages supplémentaires au regard des autres techniques moléculaires: son pouvoir résolutif et le traitement en parallèle de plusieurs questions diagnostiques. Au niveau des désavantages, leur coût est beaucoup plus élevé que les puces à ADN avec dépôt ou à jet d'encre. Ces puces sont peu compatibles avec les systèmes de lecture à l'exclusion des systèmes Hewlett Packard (Schena et al., 2000). Un système de lecture spécialisé est utilisé avec ces puces. En présence d'un échantillon et d'un contrôle, deux puces doivent être utilisées alors qu'une serait nécessaire pour les puces à dépôt avec deux colorants (Knudsen, 2002).

1.6.2.2 Puces à ADN à dépôt

Les puces avec dépôt (spotting) ont été très utilisées jusqu'à présent (Figure 1.15). La fabrication de ces puces consiste en un dépôt direct d'ADN ou sondes à raison de 1000 sondes/cm². Cette tâche est effectuée par un robot qui dépose les sondes sur une lame de verre de bonne qualité traitée chimiquement avec de la poly-L-lysine pour lier les sondes de façon covalente par rayon UV (Southern, 2001). Les sondes sont généralement formées d'ADN double brin de 200 à 2000 paires de bases amplifiées par PCR ou récemment des oligonucléotides de 22 à 70 bases, donc de l'ADN simple brin. Les sondes sont utilisées pour fixer spécifiquement des fragments d'ARN total ou messager (cibles) et de l'ADN complémentaire (ANDc).

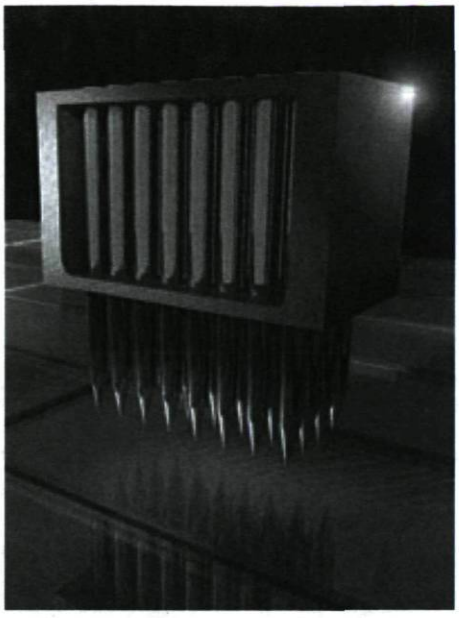

Figure 1.15 : Appareillage pour le dépôt des molécules d'ADN.
(Brown, 2000)

Les puces à dépôt offrent l'avantage d'être compatible avec plusieurs platesformes de détection à fluorescence, des systèmes de fabrication robotisée et avec plusieurs microscopes existants. De plus, l'utilisation de faible volume d'hybridation est envisageable. Il est aussi possible de mettre seulement les gènes d'intérêt sur la puce ce qui n'est pas le cas avec les puces à oligonucléotides. De plus, l'utilisation de deux colorants sur une puce permet d'analyser l'échantillon et le contrôle sur la même puce (Knudsen, 2002). En règle générale, l'utilisation des puces à dépôt représente un coût total plus faible comparativement aux puces Affymetrix™. Ces puces donnent d'excellents résultats dans la mesure où un nombre suffisant de contrôles est présent sur la puce et qu'un traitement statistique est effectué. Un des désavantages se situe au niveau de la lame de microscope qui est susceptible aux contaminations (poussières, etc.) puisqu'elle est exposée à l'air. Ce problème peut-être contourné par l'utilisation d'une chambre dépourvue de poussière pour la fabrication de la puce. Finalement, la création de la lame nécessite plusieurs étapes critiques ce qui peut occasionner quelques variations (par exemple : l'humidité) nécessitant un contrôle de la qualité.

1.6.2.3 Puces à ADN à jet d'encre

Les puces à jet d'encre utilisent une technologie appelée piézoélectrique pour déposer quelques picolitres de réactifs à un endroit précis sur la lame (Schena et al., 1999). Brièvement, un courant électrique est appliqué sur un cristal qui contracte une membrane. En se relâchant, la membrane libère une seule goutte provenant du réservoir par l'orifice, d'un volume d'un dixième de picolitres (Blanchard, 1998). Le réservoir se remplit de nouveau par capillarité. Une seule goutte est libérée à chaque impulsion de tension appliquée au cristal. Ce système peut déposer les sondes, ADNc, etc., par un jet faible sur la lame à une vitesse de 1000 gouttes par seconde. Grâce au contrôle informatique, il est possible de déplacer l'orifice du système à différents endroits sur la lame pour y déposer précisément les différents réactifs (Blanchard, 1998).

En plus des avantages énumérés pour les puces à dépôt, cette méthode permet de produire des puces identiques très rapidement. Par contre, la création de puce à haute densité est un peu plus complexe. Deux gouttelettes trop rapprochées auront tendance à s'étendre et à se mélanger. Un espacement d'au moins 600 microns est nécessaire avec des gouttelettes de 40 picolitres (Blanchard, 1998). Sinon, d'autres méthodes doivent être utilisées pour empêcher la dispersion des gouttelettes.

Chaque type de puce possède ses avantages et ses inconvénients. Elles requièrent de l'appareillage différent et les coûts sont aussi variables en fonction du système choisi. Voici un tableau qui permet de résumer les principales caractéristiques de chaque type de puce (Tableau 1.3). Il est à noter que d'autres compagnies offrent les services de fabrication de puces sur mesures (exemple : Agilent et Nimblegen). Ces derniers offrent à leurs clients d'isoler certains gènes d'intérêts sur une puce.

Tableau 1.3 : Comparaison des différentes technologies de fabrication des puces à ADN

Critères	**Photolithographie** (Oligonucléotides)	**Piézoélectrique** (Jet d'encre)	**Dépôt** (Dépôt)
Synthèse combinatoire	Oui	Oui	Non
Jet d'encre	Non	Oui	Non
Impression à la surface	Non	Non	Oui
Pochoir ou masque requis	Oui	Non	Non
Retracer l'échantillon	Non	Non	Oui
Densité (cm^{-2})	244 000	10 000	6500
Longueur minimum	25 nucléotides	Aucune	Aucune
Matériels hybridés sur la puce	Oligos	Oligos et ADNc	Oligos et ADNc
Coût	Élevé	Modéré	Faible
Applications	Expression génique et détection des mutations	Expression génique et détection des mutations	Expression génique et détection des mutations
Compagnies effectuant la commercialisation des puces	Affymetrix™	Incyte, Packard Intruments, Protogene, Rosetta	Cartesian, Genetix, Genometrix, Gene Machines, Genetic Microsystems, Hyseq, Molecular Dynamics Norgren Systems, Synteni, Telechem, Agilent

Adapté de Schena et collaborateur (Schena et al., 1999)

1.6.2.4 Limites d'utilisation des puces à ADN

Les puces possèdent d'innombrables avantages que ce soit au niveau de la rapidité d'exécution et de la possibilité d'étudier plusieurs gènes simultanément. En revanche, cette technique est relativement nouvelle et manque de balises solides au niveau du langage et des contrôles utilisés (Gazel et al., 2003). De plus, leur acceptation est parfois lente car la démonstration de la reproductibilité des résultats est absente dans plusieurs articles. L'utilisation d'un seul expérimentateur permet souvent de limiter la variation entre les répétitions (Warrinton et al., 2000). Rarement, les variations expérimentales et biologiques sont mesurées et caractérisées. Ceci a comme conséquence que plusieurs études ont rapporté des résultats sans définition statistique du niveau de signification (Baldi et al., 2002). Aussi, les puces génèrent des milliers de résultats qu'il faut analyser, interpréter et archiver. La présence de faux positifs fait partie des risques encourus avec cette méthode par contre, des méthodes efficaces d'analyse peuvent limiter ce problème.

Plusieurs limitations sont observées en général avec les puces. Tout d'abord, les puces permettent d'étudier un transcriptome à la fois, c'est-à-dire plusieurs gènes exprimés ou réprimés à un temps précis. Donc, l'activité enzymatique et le niveau de traduction sont pour le moment ignorés lors de l'analyse. Aussi, l'effet de l'épissage alternatif, processus englobant l'excision des introns et la réunion des exons, sur le niveau d'expression n'est pas non plus mesuré (Knudsen, 2002). Rappelons, que l'ARNm est une molécule instable qui est sensible aux dégradations enzymatiques, car la quantité d'ARN représente l'équilibre entre la synthèse et la dégradation. Un ARNm plus sensible, sera plus difficilement détectable d'un point de vue statistique. En terminant, plusieurs étapes de validations sont requises pour valider l'expression des gènes (PCR en temps réel) et vérifier la sécretion de la protéine (western blot, Elisa, etc.).

1.6.3 Identification d'activités par puce à ADN à dépôt

Cette section a pour but de montrer les étapes importantes pour identifier des activités biologiques par l'utilisation de puces à ADN. Elle permet aussi de présenter la

méthodologie qui a été employée au cours de cette thèse pour étudier l'activité biologique du laminarane, du galactofucane natif et dépolymérisé.

1.6.3.1 Planification de l'expérience

La planification de l'expérience est sans nul doute l'étape la plus importante lors de l'utilisation de puce à ADN. Dû à leur coût important, il serait dommage de ne pouvoir effectuer les comparaisons voulues ou avoir insuffisamment de puissance pour identifier les facteurs d'intérêts. Ainsi, une expérience bien planifiée permet d'estimer la variabilité des données et permet de contrôler certains effets expérimentaux qui amélioreront la qualité des données. Idéalement, des réplications techniques (même ARN) et biologiques (répliquer les puces avec de l'ARN indépendant) doivent être réalisées pour estimer respectivement la variance expérimentale et la variance biologique (Labbe, 2006; O'Connell, 2002; Parmigiani et al., 2003; Zakharkin et al., 2005). Répliquer le nombre de puces est très important car il arrive parfois que certains effets expérimentaux soient plus importants que l'effet d'intérêt. En règle générale, il faut prévoir à l'avance les effets d'intérêt et planifier l'expérience en conséquence.

1.6.3.2 Les modèles cellulaires

Le choix du type de cellule influence la réponse biologique et détermine le type d'application visée. Les fibroblastes et les kératinocytes sont les modèles de peau les plus courants. Les kératinocytes sont retrouvés dans l'épiderme et forment des couches qui aident à contenir des fluides et offrent une barrière contre l'environnement (Curto et al., 2002). Les kératinocytes sont généralement employés en dermatologie (Bernard et al., 2002) et pour des applications cosmétiques. Les fibroblastes situés sous le derme sont dérivés du mésenchyme et synthétisent des composants extracellulaires essentiels assurant le support de la structure tissulaire (Curto et al., 2002). De ce fait, les cellules fibroblastes offrent l'avantage d'être plus représentatives car elles se retrouvent partout dans les tissus conjonctifs qui tapissent le corps humain (Marieb, 1999). De plus, ces cellules de peaux ont été utilisées pour l'étude du vieillissement de la peau (Curto et al., 2002; Sudel et al., 2005). Outre le choix des cellules, le protocole de culture des cellules est aussi un aspect important

à maîtriser pour obtenir de l'homogénéité dans les résultats (Iyer et al., 1999). Les cultures cellulaires préalablement sélectionnées sont exposées aux extraits pour ensuite récupérer l'ARN total des cellules ou seulement l'ARNm (Dale et al., 2002).

Dans le cadre de cette étude, des fibroblastes normaux humains ont été utilisés. Pour chaque culture avec traitement, un contrôle a été réalisé.

1.6.3.3 Préparation du matériel génétique

L'ARN récupéré des cellules est transformé en ADN complémentaire (ADNc) qui est en fait la copie complémentaire de l'ARNm effectué par RT-PCR (Figure 1.16). Ici, le choix des amorces est très important pour produire de l'ADNc issus d'ARNm uniquement. Pour ce faire, la queue poly-A de l'ARNm peut être utilisée pour former un complexe avec un oligo(dT) synthétique, un court polymère de désoxythymidine (ou ADN synthétique contenant des nucléotides T) (Dale et al., 2002). Pour l'utilisation des puces à dépôt, l'ADNc doit être ensuite amplifié par PCR pour avoir des quantités suffisantes de matériel. Cette méthode offre l'avantage de pouvoir détecter de faible quantité d'ARNm.

Lors de la transcription inverse, des marqueurs sont ajoutés à l'ADNc à partir des amorces pour détecter l'activité. Un (biotine) ou deux (Cy3 et Cy5) marqueurs peuvent être utilisés. Dans le cadre du projet, l'ADNc est marqué par de la biotine.

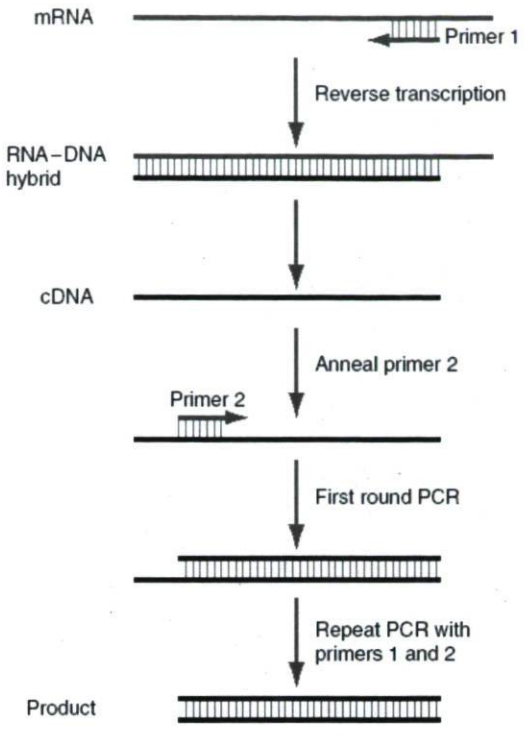

Figure 1.16 : Schéma de la réaction de transcription inverse par PCR. (Dale et al., 2002)

1.6.3.4 Hybridation et lavage sur la puce

Pour ce projet, la puce « *DualChip® human aging* » de la compagnie Eppendorf sera utilisée. Cette puce dédiée contient 240 gènes dont 13 gènes domestiques *(housekeeping genes)* (Figure 1.17). Les gènes domestiques correspondent à des gènes qui sont toujours transcrit peu importe les conditions expérimentales par la cellule et permettent de normaliser les résultats (section 1.6.3.5). Six standards internes provenant des gènes de *Lycopersicon esculentum* ont aussi été ajoutés. Ces derniers sont associés aux mécanismes spécifiques des plantes et leurs séquences ne sont nullement reliées aux gènes des mammifères. De plus, des contrôles positifs et négatifs sont inclus pour la normalisation et la détection des gènes (Eppendorf International, 2008b).

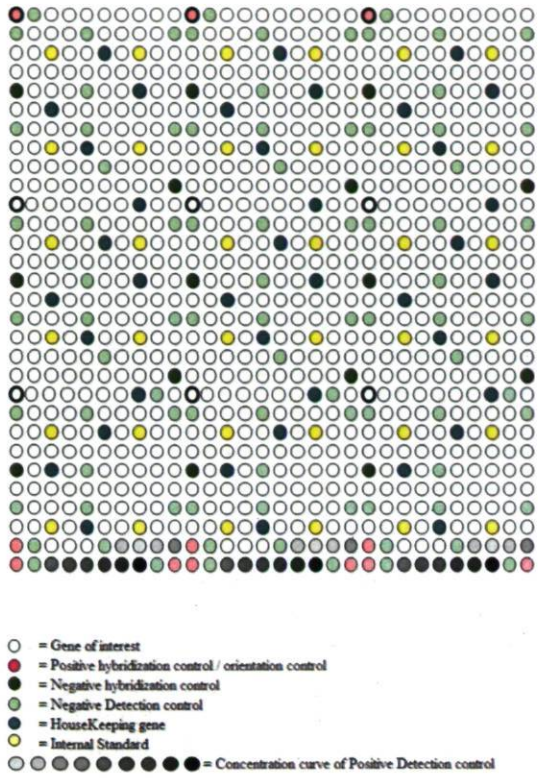

Figure 1.17 : Design de la puce *DualChip® human aging*.
(Eppendorf International, 2008a)

Les chaînes d'ADNc fragmentées et marquées sont hybridées de 14 à 16 heures sur la puce via un appareil spécialisé qui permet d'agiter les puces à vitesse et température constantes. L'hybridation consiste en l'appariement entre deux brins complémentaires ADN-ADN, ADN-ARN ou entre un support solide (puce) et de l'ARN pour former une double hélice stable. Ainsi sur les puces à ADN, seules les chaînes d'ADNc ayant trouvé leurs homologues seront détectées dû au marquage par la biotine. Lorsque les échantillons d'ADNc sont mis en contact avec la puce, un temps d'attente est nécessaire afin que les chaînes d'ADNc retrouvent les sondes d'ADN spécifiques parmi les centaines de gènes présents sur la puce. Puis, un mélange de streptavidine (ou anti-biotine) couplé à des nanoparticules d'or est ajouté à la puce (Figure 1.18). La streptavidine va se fixer directement sur la biotine. Finalement, la puce est incubée avec un colorant contenant du

sel d'argent ($AgNO_3$) qui se fixe sur les particules d'or permettant la détection des chaînes d'ADNc hybridées (Alexandre et al., 2001).

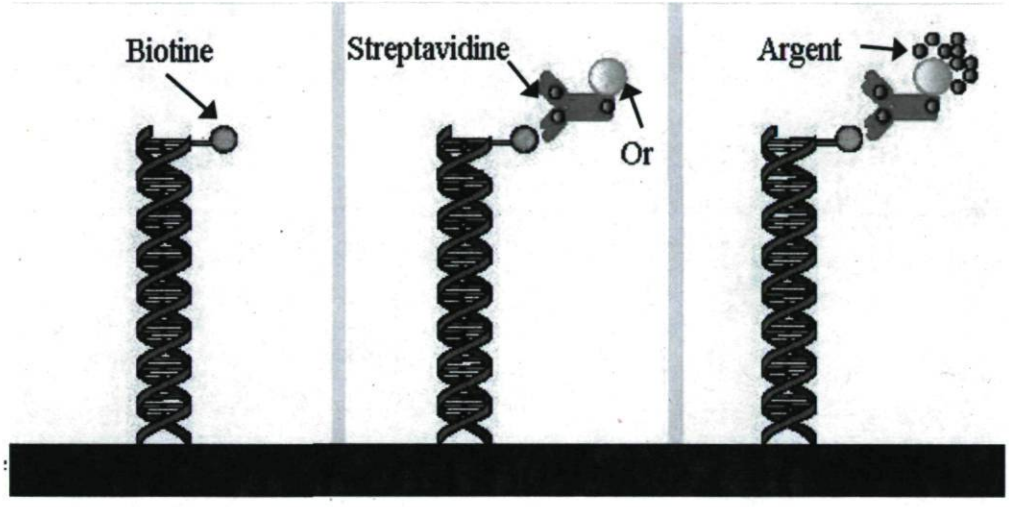

Figure 1.18 : Coloration de l'ADNc hybridé sur la puce.
Adapté d'Alexandre et collaborateurs
(Alexandre et al., 2001).

1.6.3.5 Cueillette des données et quantification

Par la suite, les puces sont lues par une caméra DCC (dispositif à couplage de charge). Plusieurs compagnies offrent différents modèles de scanneur. Dans le cadre du projet, le scanneur Silverquant (Eppendorf, Allemagne) a été utilisé (Figure 1.19). Ce système est en mesure de délimiter chaque « spot » de colorant argent et d'évaluer l'intensité de gris en fonction de la quantité d'ADNc hybridé. Les puces sont numérisées très rapidement à raison de 2 puces de 4 cm^2 à une résolution de 12 µm en 40 secondes (Margaine et al., 2007). L'intensité du signal de chaque « spot » a été calculée en soustrayant le bruit de fond moyen. Un signal de 2,5 fois plus grand que le bruit de fond a été accepté comme étant un signal significatif d'une hybridation (de Longueville et al., 2002).

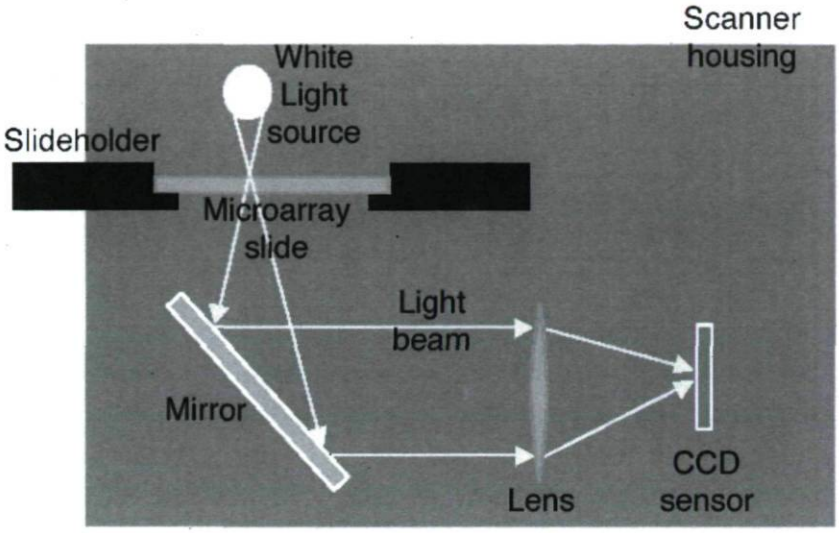

Figure 1.19 : Vue schématique du système optique.
Adapté de Margaine et collaborateurs
(Margaine et al., 2007).

Lorsque les données brutes sont générées, leur analyse est réalisée pour identifier le degré d'expression des gènes. Il arrive parfois qu'on retrouve des biais dans les données, par exemple : un problème au niveau du scanneur, une différence dans la concentration d'ADNc sur les puces, une hybridation inégale sur une même puce ou une hybridation inégale entre puces. Pour pallier à ces problèmes, une étape de linéarité et deux étapes de normalisation sont réalisées.

La linéarité des résultats est réalisée avec les six standards de concentrations différentes qui permettent de calculer une équation pour réaliser la linéarité de tous les autres gènes (Eppendorf International, 2008b). Un ratio entre le contrôle et le traitement est ensuite calculé pour chaque gène. Ainsi, le logiciel classe les gènes en trois catégories de ratio : *quantitative*, *qualitative* et *meaningless* (Figure 1.20). Par la suite, les ratios de chaque gène sont normalisés.

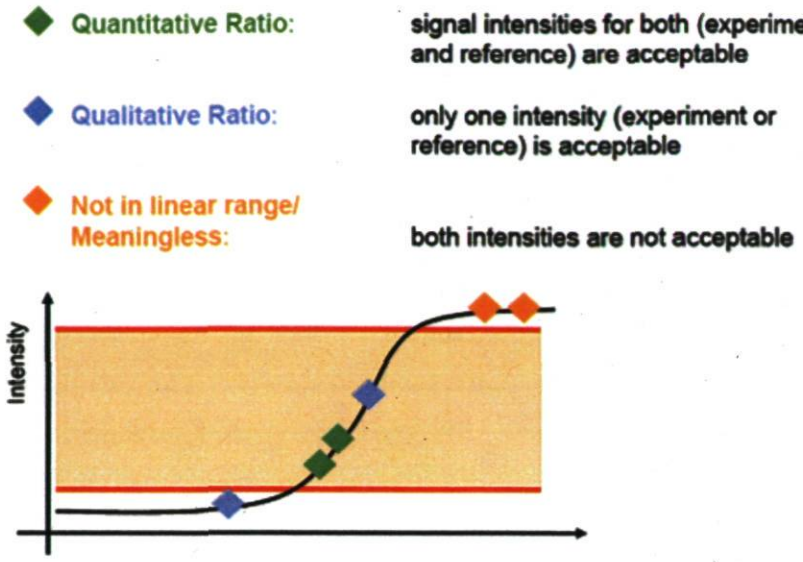

Figure 1.20 : Intervalle de linéarité des gènes.
(Bebermeier et al., 2008)

La première étape de normalisation est réalisée avec l'aide des standards internes de faibles et de hautes concentrations provenant des gènes de *Lycopersicon esculentum*. Deux standards internes sont retrouvés par zone permettant de déterminer un facteur de normalisation par zone. Ce facteur est calculé à partir des ratios entre les contrôles et les traitements de la même zone. Ensuite, le facteur de normalisation est utilisé pour normaliser les gènes de sa zone. Idéalement, un ratio entre 0,75 et 1,25 doit être obtenu sinon, cela indique que la transcription inverse n'était pas adéquate chez les contrôles et les traitements et que la quantification des gènes n'est pas conforme.

Ensuite, une deuxième étape de normalisation est nécessaire pour s'assurer de l'intégrité et de la pureté de l'ARNm (de Longueville et al., 2002). Treize gènes domestiques (*Housekeeping genes*) sont utilisés pour réaliser cette étape. Ces derniers doivent avoir un signal 2 fois plus grand que le bruit de fond et un signal plus grand que les contrôles négatifs. Les gènes domestiques ne correspondant pas à ces critères sont éliminés du calcul pour déterminer le facteur de normalisation. Ce dernier est calculé à partir des moyennes de

ratios de gènes domestiques retenus. Ainsi, le ratio entre les contrôles et les traitements de chaque gène est également corrigé avec ce nouveau facteur.

La compagnie Eppendorf distribue un logiciel (*Silverquant analysis software*) effectuant toutes les opérations fondamentales de l'analyse des puces telles : correction du bruit de fond, quantification et normalisation pour une comparaison entre puce, détermination de la présence d'un gène transcrit et si celui-ci est exprimé différemment (Margaine et al., 2007). Idéalement, un logiciel gratuit, bien documenté et publié facilite l'analyse des résultats tel : BioConductor (Duboit et al., 2003; Zakharkin et al., 2005). Par contre, ces logiciels sont difficilement utilisables par les non-statisticiens.

Dans le cadre du projet, nous avons choisi une puce qui contenait beaucoup de standard. Cette dernière offre l'avantage d'assurer un contrôle sur la validité des résultats.

1.6.3.6 Analyse statistique

Le logiciel *Silverquant analysis software* permet l'analyse statistique lors d'une expérience comparant un contrôle et un traitement. Le but est de déterminer si le niveau d'expression de plusieurs gènes entre un contrôle et un traitement est significativement différent. Ainsi, un coefficient de variation et un intervalle de confiance sont déterminés (Chen et al., 1997).

Le coefficient de variation est déterminé à partir des ratios de gènes domestiques sélectionnés qui sont supposés être stables entre le contrôle et le traitement. Le niveau de signification des ratios des gènes d'intérêts est déterminé comme suit : un ratio en dehors de l'intervalle de confiance de 95% est significativement différent alors qu'un ratio en dehors d'un intervalle de confiance de 99% est significativement très différent (de Longueville et al., 2002; de Magalhães et al., 2004). Par traitement informatique, il est possible de cibler rapidement les gènes ou les groupes de gènes qui provoquent ou atténuent certaines activités. Lors de l'analyse, ces gènes sont regroupés par catégorie fonctionnelle afin d'identifier les gènes responsables d'activités biologiques intéressantes.

1.7 Hypothèse et objectifs

La littérature a montré que les polysaccharides extraits des algues possèdent plusieurs activités biologiques et que ces dernières pouvaient être influencées par l'espèce d'algue. Pour étudier les polysaccharides de *Saccharina longicruris*, l'utilisation des puces à ADN s'avère être une méthode de choix puiqu'elle permet de découvrir des activités insoupçonnées contrairement aux méthodes traditionnelles (*in vitro* et *in vivo*). Chaque espèce d'algue contient des polysaccharides possédant des structures disctinctes et peu est connu de ceux issus de *Saccharina longicruris*. De plus, les rendements d'extraction des polysaccharides et leurs structures sont influencés par plusieurs facteurs dont la saison. Les unités de base (monosaccharides), le poids moléculaire ainsi que le degré de sulfatation varient aussi selon la saison.

L'hypothèse de recherche est donc la suivante :

De par leurs structures uniques, le laminarane et le fucoïdane (galactofucane) extraits de *Saccharina longicruris* devraient posséder des activités biologiques d'intérêt. Selon la littérature, l'activité biologique serait influencée par les variations structurales d'où l'importance de mieux comprendre le rôle entre la structure et l'activité biologique des polysaccharides.

Les objectifs visés sont :

1. Extraction et détermination de la composition de base du laminarane natif, du galactofucane natif et du galactofucane fractionné pour chaque période de récolte de l'algue.
2. Production d'oligo-galactofucane par dépolymérisation et analyse structurale des polysaccharides natifs et dépolymérisés.
3. Vérification de l'activité biologique des polysaccharides natifs et des oligosaccharides selon la période de récolte la plus intéressante.
4. Détermination de la relation entre la structure et l'activité biologique des polysaccharides.

Chapitre II :
Effect of season on the composition of bioactive polysaccharides from the brown seaweed *Saccharina longicruris*

2.1 Résumé

Les caractéristiques structurales du laminarane et du galactofucane extraits de l'algue brune Saccharina longicruris ont été étudiées pour quatre périodes de récolte (M05, A05, N05 et J06). Le laminarane brut a été purifié et le galactofucane brut a été fractionné par chromatographie d'échange ionique avec des paliers de NaCl (0,5; 1 et 2 M) pour isoler trois fractions de galactofucanes. Les résultats montrent des variations au niveau de la composition en monosaccharides pour les différentes périodes de récoltes. Le laminarane purifié contient entre 45,1% et 69,1% de D-glucose alors que le niveau de D-mannitol est similaire d'une fraction à l'autre (moins de 1,7%). Pour le galactofucane brut, les pourcentages de L-fucose et de D-galactose ont varié selon la période de récolte. Pour M05, la quantité de L-fucose était plus élevée que le D-galactose (21,5 vs 111%) alors que l'inverse a été observé pour les autres périodes : A05 (18,5 vs 36,6%), N05 (20,9 vs 36,8%) et J06 (12,8 vs 19,6%). Les galactofucanes fractionnés, 0,5 et 2 M, ont le même profil de monosaccharides que les galactofucanes bruts. Par contre, la fraction 1 M contient plus de L-fucose que de D-galactose et ce pour toutes les périodes de récolte. Le galactofucane brut de M05, A05 et N05 contient de 19,9 à 21,5% de groupements sulfate, alors que J06 en contient seulement 14.3%. Le galactofucane fractionné 2 M contient une plus grande proportion de groupements sulfate (27,1-36,9%) que les autres fractions, comme la fraction 1 M par exemple, qui contient entre 9,2 et 15,9% de groupements sulfate. La prochaine étape sera d'étudier l'activité biologique des laminaranes et des galactofucanes pour tenter de relier la structure à l'activité.

2.2 Abstract

The structural features of laminarans and galactofucans extracted from the brown seaweed *Saccharina longicruris* were determined for four harvest periods (M05, A05, N05 and J06). Crude laminarans were purified and crude galactofucans were fractionated using DEAE Sepharose anion exchange chromatography with increasing levels of NaCl (0.5, 1 and 2 M). The results showed differences in terms of their monosaccharide compositions. Purified laminaran contained a high proportion of D-glucose, between 45.1% and 69.1%, with a higher amount in M05 and A05, while the amount of D-mannitol remained constant (less than 1.7%). Crude galactofucans from M05, A05, and N05 contained 19.9% to 21.5% of sulphates, where J06 had only 14.3%. The 2 M fractionated galactofucans contained a higher proportion of sulphate groups, from 27.1% to 36.9%, for each harvest period, while the 1 M fraction contained 9.2% to 15.9% of sulphates. An important variation in the amount of L-fucose and D-galactose was observed for crude and fractionated galactofucans. In M05, a higher content of L-fucose was observed for crude galactofucans compared to that observed for D-galactose (21.5% vs. 11.1%), whereas the opposite was found for A05 (18.5% vs. 36.6%), N05 (20.9% vs. 36.8%), and J06 (12.8% vs. 19.6%). Also, the 0.5 and 2 M fractions were similar to the crude galactofucans. A05, N05, and J06 contained lower amounts of L-fucose than D-galactose, while the M05 fractions showed the opposite behaviour. However, the 1 M fraction showed a higher amount of L-fucose than D-galactose for each harvest period. The next step will be to study the biological activity of the fractions and to attempt to relate this activity to the structure of the galactofucan and laminaran fractions.

2.3 Introduction

Seaweeds have always been of great interest in Asian culture as marine food sources (Fleurence, 1999). For example, Japanese people consume over 1.6 kg of dry seaweed per year *per capita* (Fleurence, 1999). Conversely, in Europe and North America seaweeds are mostly used for either animal nutrition or for their hydrocolloids such as alginate (Indegaard et al., 1991a). Seaweeds contain few lipids and are a good source of proteins, vitamins, and minerals (Ito et al., 1989). Also, seaweeds contain a large array of nutraceutical components, including antioxidant and bioactive polysaccharides such as fucoidan and laminaran (Plaza et al., 2008).

Laminaran is a small polysaccharide of 5 kDa with a degree of polymerization (DP) ranging from 20 and 25 (Chizhov et al., 1998; Nelson et al., 1974). Initially, laminaran was reported as a polymer of D-glucose linked via β-(1,3) bonds (Barry, 1939), but later intra-chain branching linked via β (1,6) bonds was also observed (Peat et al., 1958). Also, some chains were terminated by D-mannitol residues (Nelson et al., 1974). Laminaran has several biological activities: most studies that have been done on the effect of laminaran in the gastrointestinal tract (Devillé et al., 2004; Devillé et al., 2007; Michel et al., 1999; Michel et al., 1996), or on either its antitumor (Sakai et al., 1968) or its anti-coagulant activity of a derivative sulphated laminaran (Ito et al., 1989).

Fucoidans are α-L-fucose polysaccharides containing sulphate groups and minor monosaccharides, such as D-galactose, D-xylose, D-glucose, D-mannose, and D-uronic acid. Their structures are complex, the polymers are heterogeneous and no defined regularity has been observed (Kusaykin et al., 2008). Galactofucans are also isolated from brown seaweeds and are often included in the fucoidan family. Galactofucans are sulphated polysaccharides containing an equivalent proportion of L-fucose and D-galactose (Hemmingson et al., 2006; Rocha et al., 2005). One study showed a backbone dominated by both 3-linked α-fucose and β-galactose (Hemmingson et al., 2006), while another showed that the main chain was a 4-linked β-galactose with branches of oligosaccharides composed of 3-sulfated, 4-linked α-fucose (Rocha et al., 2005). Moreover, the biological

activities of galactofucans have been reported for brown seaweeds such as *Undaria pinnatifida*, *Spatoglossum schroederi*, and *Ecklonia kurome*. Anti-coagulant, anti-thrombosis, or antiviral activities are known in galactofucans (Hemmingson et al., 2006; Nishino et al., 1991; Rocha et al., 2005).

The structure and the biological activities of laminaran and galactofucan are thought to be influenced by environmental factors, such as water temperature, nutritive salt, salinity, waves, sea current, and depth of immersion (Black, 1954; Black et al., 1949; Lobban et al., 1997; Percival et al., 1967). The extraction protocol and the harvest period are other important factors (Black et al., 1952; Haug et al., 1956). The availability of nutritive salt, mostly nitrate, influences seaweed productivity (Edwards et al., 2006; Gordillo et al., 2006; Harlin et al., 1978), but also impacts the structure, composition, and consequently the biological activity of polysaccharides. For laminaran, some researchers have shown the impact of environmental factors on laminaran storage (Anderson et al., 1981; Chapman et al., 1978; Gagné et al., 1982) but few on the composition (Zvyagintseva et al., 2003). For fucoidan, some studies have shown an impact of the harvest period (Black, 1954), the exposure of the seaweed to air (Percival et al., 1967), and the fronds age (Zvyagintseva et al., 2003) on the level of fucose.

Brown seaweed, *Saccharina longicruris*, is a species found in North America and more precisely, in eastern Canada. A member of the *Laminariaceae*, this species has been used in a small number of studies. No research has yet been conducted on the seasonal variation of the fucoidan (galactofucan) and laminaran content and composition in this species. The main objective of this research, therefore, was to characterize the polysaccharide composition of this species according to the harvest period.

2.4 Experimental

2.4.1 Algal materials

Laminaran and galactofucan were extracted from *Saccharina longicruris* harvested by diving in May (M05), August (A05) and November 2005 (N05) and June 2006 (J06). The harvest site was located between Percé (48°31'59" North – 64°12'59" West) and L'Anse-à-Beaufils (48°28'29" North – 64°18'30" West) (Québec, Canada) at an average distance of 100 m from the shore. A total of 10 kg of seaweeds were collected for each harvest period. The stipes were removed and only the fronds were kept for the study. The fronds were milled in a Desintegrator (Rietz Manufacturing Co., Santa Rosa, USA), fitted with perforated plates of 4.65 mm, vacuum sealed, and kept at -30 °C until use.

2.4.2 Chemical composition

The crude seaweed analyses (proteins, ash and lipids) were carried out (AOAC, 1990). The sulphur in the galactofucans was quantified by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) using the model Optima 4300DV from Perkin-Elmer (Boston, USA) equipped with Winlab32 software. The sulphate content was deduced from the amount of sulphur determined by ICP using the following equation: % sulphate group = 3.22 x S (Roger et al., 2004).

2.4.3 Extraction procedures

Laminarans and galactofucans were extracted from the milled seaweeds (Nishino et al., 1989). Seaweeds (150 g) were mixed with HPLC grade H_2O and 1% (w/v) of $CaCl_2$. Each mixture was stirred at 85 °C for 4h, and then centrifuged (16 887 g, 20 min). Each supernatant was isolated by vacuum filtration on Whatman #4 filters. Each filtrate was mixed with 1 volume of 2% NaCl and 2 volumes of EtOH (95:5, v/v), and then stirred for 1h at room temperature and stored at -20 °C for 48h. Each mixture was centrifuged (16 887g, 10 min) to isolate the pellets (crude galactofucans) and the supernatants (crude laminarans which contained a mixture of laminarans, galactofucans and polyphenols).

EtOH was evaporated from each supernatant, and the resulting supernatant fractions were dialyzed with 1 kDa membranes. The pellets were hydrated and dialyzed using a 15 kDa cut-off membrane. Galactofucan and laminaran fractions were freeze-dried and kept at 4 °C until use. The residual fraction of extraction, containing mostly a mixture of alginate, cellulose, hemicellulose and protein, was freeze-dried and weighed. Extractions were made in triplicates for each harvest period.

Fractions will be presented as galactofucan M05, A05, N05 or J06 which refers to their respective harvest period.

2.4.4 Crude laminaran purification

The crude laminaran fractions were treated with 5% NaCl and 8 volumes of ethanol 95% to remove undesirable contaminants (polyphenols). Then, purified laminaran was isolated with Centricon Ultracel® YM-30 using 1 M NaCl following the Millipore (Billerica, MA, USA) protocol to remove small traces of galactofucans. Each fraction was dialyzed with a 1 kDa membrane and freeze-dried.

Fractions will be presented as laminaran M05, A05, N05 or J06 which refers to their respective harvest period.

2.4.5 Galactofucan fractionation

Crude galactofucans were fractionated by a DEAE Sepharose CL-6B anion exchanger (Sigma, USA) equilibrated with 0.5 M NaCl solution. Each sample (200 mg) was applied to the column (2.5 x 35 cm) and eluted stepwise by NaCl solutions of increasing concentrations of 0.5, 1 and 2 M at a flow rate of 1.4 min/mL. The elution of the polysaccharides was followed by the phenol-sulphuric acid method (Dubois et al., 1956) which uses glucose as the standard. Absorbance measurements were obtained in triplicate with a microplate reader (Molecular Devices, Ottawa, Canada). This procedure was

performed two times for each extraction and the corresponding fractions were combined, dialyzed and freeze-dried.

Fractions will be presented as fractionated galactofucan M05, A05, N05 or J06 which refers to their respective harvest period.

2.4.6 Monosaccharide analysis

Neutral sugars were identified as trimethylsilyl derivatives after acidic methanolysis of the polymer and subsequent GC analysis (Kamerling et al., 1975; Montreuil et al., 1986). Methanolysis was performed in 2 M MeOH-HCl at 100 °C for 4 h for galactofucan and 2 h for laminaran, and the methyl glycosides were converted to the corresponding per-*O*-trimethylsilylated derivatives. The derivatives were separated and quantified by GC on a HP 5890A system equipped with a FID detector and a CP-Sil-5CB fused silica column (60 m x 0.25 mm, Chrompack, Varian) in a split/splitless mode using helium at a flow of 1.5 mL/min as the gas carrier. The oven temperature was programmed at 50 °C for 1 min, 20 °C/min to 120 °C, and then 2 °C/min to 240 °C. The injector and detector temperatures were maintained respectively at 290 °C and 300 °C. The monosaccharides were identified according to their retention times and quantified using an internal standard method involving *myo*-inositol. All analyses were made in triplicates.

2.4.7 HPSEC-molecular weight determination of laminarans

The polysaccharide weight average, Mw, was determined by HPSEC (High Performance Size Exclusion Chromatography. The HPSEC system consisted of a Beckman 126 HPLC pump (Beckman Coulter Canada, Inc., Mississauga, ON, Canada), an injection loop of 100 µL, an analogue Beckman interface module Gold 406 (Beckman Coulter Canada, Inc., Mississauga, ON, Canada) and a Hitachi L-7490 refractometer (sensitivity ½) (Hitachi High-Technologies Canada, Inc., Rexdale, ON, Canada). The molecular weight values were analyzed with Gold software version V810 using the molecular weight function. A

molecular weight calibration curve was constructed with five polyethylene glycol (PEG) standards from 985 to 12 400 Da (Phenomenex, inc., Torrance, CA, USA).

Two columns were used in series: TSK-guard column PWXL (6 mm X 40 mm) and a TSK-G3000 PWXL (7.5 mm X 300 mm) (Tosoh Bioscience, Montgomeryville, USA). The molecular weight separation range for the polysaccharides was established from 1 to 60 kDa by the supplier. The mobile phase consisted of a filtrated (0.22 μm) 0.1 M NaCl solution obtained with HPLC grade H_2O. The flow rate was 0.8 mL/min and analyses were performed at room temperature. The samples were dissolved in 0.1 M NaCl solution to reach a concentration of 1 mg/mL, and filtered (0.45 μm) to eliminate dust particles.

2.4.8 HPSEC-MALLS-molecular weight determination of galactofucans

The polysaccharide weight averages, Mw, were determined by HPSEC-MALLS (High Performance Size Exclusion Chromatography-Multiangle Laser Light Scattering). The HPSEC system consisted of a LKB 2150 HPLC pump (GE Healthcare Bio-Sciences Corp., Milford, USA), a Hewlett Packard series 1100 auto-injector with an injection loop of 100 μL and a Wyatt 903 refractometer (Wyatt Technology, Santa Barbara, USA). The MALLS apparatus consisted of a Wyatt Dawn-DSP laser photometer (Wyatt Technology, Santa Barbara, USA) equipped with a K5 flow cell and a He-Ne laser operating at $\lambda = 632.8$ nm.

Three columns were used in series: TSK-guard column PWXL (6 mm X 40 mm), TSK-G6000PW (7.5 mm X 300 mm) and a TSK-G4000 PWXL (7.5 mm X 300 mm) (Tosoh Bioscience, Montgomeryville, USA). The mobile phase consisted of a filtered (0.22 μm) 0.1 M NaCl solution obtained with HPLC grade H_2O. The flow rate was 0.5 mL/min and analyses were performed at room temperature. The samples were dissolved in 0.1 M NaCl solution and filtered (0.45 μm) to eliminate dust particles. The MALLS instrument was placed directly after the HPSEC columns and before the refractive index detector (DRI).

Prior to measurements, a Dawn apparatus was calibrated using HPLC grade toluene and normalized using a 47 300 Da pullulan standard from a P-82 kit (Shodex, Japan) in 0.1 M

NaCl. The performance of the HPSEC-MALLS system was checked with monodisperse pullulan of various molecular weights. A dn/dc value of 0.129 for galactofucans was used (Rioux et al., 2007a).

Data were collected from the DRI and MALLS and evaluated with the ASTRA software 4.70.07. Since galactofucans are polydisperse polysaccharides, only average weights were compared. Results were estimated using second-order Zimm.

2.4.9 Statistical analyses

The composition and structural data were analyzed using SAS 9.1.3 software (SAS Institute Inc., Cary, USA). The effect of the harvest period was analyzed as a 4 (harvest period) x 3 (extraction) factorial experiment by analysis of variance (ANOVA). A comparison between the harvests periods was analyzed with the measure of the least significance difference (LSD) at a significant level of 5%. Means with the same letter are not significantly different (at 0.05). The GC measurements were analyzed with a factorial experiment. The effect of the harvest period was analyzed as a 4 (harvest period) x 3 (extraction) by ANOVA for each monosaccharide. A comparison between harvest periods was analyzed for each monosaccharide with the measure of the least significance difference (LSD) at a significant level of 5%. Means with the same letter are not significantly different (at 0.05).

2.5 Results and discussion

2.5.1 Brown seaweed (*S. longicruris*) composition

The brown seaweed composition (protein, lipid, and ash) was determined for each harvest period: May 2005 (M05), August 2005 (A05), November 2005 (N05) and June 2006 (J06) (Table 2.1). Seasonal trends were observed in the concentration of proteins, lipids and ashes. Significantly higher amounts of protein (12.2%), lipid (0.8%) and ash (26.3%) were found in M05, whereas the amounts of each component decreased, respectively, by 5.0%, 0.3% and 22.6% by the end of the summer (A05). In N05, the levels of protein and ash

began to increase once more to 9.3% and 30.5% respectively, while the level of lipids remained constant (0.5%). Moreover, the seaweed composition was significantly different in M05 and J06.

The yields of extraction of crude galactofucans, crude laminarans, and residual fractions are presented in Figure 2.1. The proportion of crude laminaran in M05 and A05 (around 5.3%) was significantly higher than the amount found in N05 and J06, in which less than 2.5% was recovered. For galactofucans, higher amounts were recovered in M05 (4.5%), while lower amounts were obtained for A05, N05, and J06 (1.6 to 2.4%). The residual fraction content increased throughout the year, while the amount of laminaran and galactofucan decreased. This fraction mostly contained alginate, cellulose and hemicellulose, but also lipids, proteins and minerals. A significantly higher amount of a residual fraction was found in J06 (95%). Lower levels were found for M05, A05 and N05, from 53% to 70%. For each component, significant differences were observed between M05 and J06. These variations could be related to many factors such as the growth cycle and environmental factors.

Statistics on environmental factors such as water temperature, salinity and presence of nutritive salts were analyzed from January 2004 to December 2006 to search for changes that might explain the variation between M05 and J06, for polysaccharide, protein, lipid and ash levels. Two buoys were analyzed near the harvest zone, the Gaspé Current (49°24'00" N - 66°19'00" W) and the Shédiac (47°47'00" N - 64°02'00" W) sites. Undoubtedly, no differences were observed in the water temperature and salinity among each year (data not shown) (Fisheries and Oceans Canada, 2008). The amount of nitrite and nitrate at Shédiac site was similar between 2004 and 2006 (Figure 2.2). A low level of nutritive salt was present in June (0.5mmol/m^3), whereas in November, the level increased (4mmol/m^3). For the Gaspé Current site, the level of nitrate and nitrite was similar to the Shédiac site but with slight differences (Figure 2.2) (Fisheries and Oceans Canada, 2008). Higher amounts in nitrate and nitrite were present in June 2006 (11 mmol/m^3) as compared to 2005 (5 mmol/m^3). The impact of higher amounts of nitrate and nitrite on the production and structure of galactofucans was never established. However, it has an important influence on the levels of laminaran (Chapman et al., 1977). The decline in nutritive salts in

the water gives a stimulus to the seaweed to produce laminaran and to reduce its overall growth. In Chapman and Craigie's (1977) studies, a low level of nitrate was found from March to October (at 6 m depth) which is comparable to what was found in the present study. However, the maximum level of laminaran was found in September (Chapman et al., 1978), while in our study the maximum was reached in August. Also, their study showed an important reduction of laminaran in November which was also observed in this study. However, another study has shown that when no restriction in nitrate occurs, the amount of laminaran was relatively low throughout the year (less than 1.8%) (Anderson et al., 1981).

The fluctuation of the levels of proteins, lipids, ash and carbohydrates from May to November may be attributed to the seaweed growth cycle. The *S. longicruris* growth cycle starts during the winter when sporulation occurs (Chapman, 1987). At this period, protein and lipid are synthesized, the level of ash increases, and the amount of laminaran decreases (Black et al., 1949). Then, the elongation period begins, and the plant grows fast (2-3 cm/day) (Chapman, 1987). With the decline of nutritive salt in the water (August), the growth is reduced and laminaran accumulates in the fronds (Chapman et al., 1977; 1978). When the maximum laminaran level is attained in the fronds, the proportions of alginate, cellulose, and protein are minimal (Percival et al., 1967) as well as the level of ash (Haug et al., 1956). Then, a rise occurs in the protein content at the same time as the nitrite and nitrate level increases. This indicates another growth period, but much less intense than in spring (Black et al., 1949). An important drop in the laminaran level was also observed, which might be caused by further growth. The growth slowly decreased with photoperiod reduction (November, December) (Anderson et al., 1981). Winter growth then takes place under reduced light, while seaweeds use laminaran as a source of carbon to follow their development (Chapman et al., 1977). The fucoidan level in *Laminariaceae* species, was shown to be minimum in March/April and it accumulated in the fronds as photosynthesis continued (Black, 1954) which was the opposite in this findings. Moreover, the L-fucose content decreased with the depth of immersion with *Laminariaceae* species (Black, 1954). Differences between M05 and J06 could also be attributed to frond age. Previous studies have shown that young fronds accumulate less laminaran and fucan than older ones (Zvyagintseva et al., 2003). In our study, M05 contained older fronds than J06, and

consequently had higher amounts of laminarans and galactofucans. The fronds observation showed important differences in term of appearance between M05 and J06 (data not shown). In M05, many parts of the fronds were missing and they were fragile and viscous. On the other hand, J06 fronds were entire and firm. Clear evidence of frond age on the amount of laminaran and galactofucan could be expected. Young fronds (0.8 year old) of *L. cichorioides* contained 1.1% and 0.2% of fucan and laminaran respectively, while older fronds (2 years old) contained 6.5% and 6.5% of each polysaccharide (Zvyagintseva et al., 2003). This was also noted in another study, where an higher amount of fucoidan was found when fronds were decaying and sporangia were maturing as compared with young fronds (Honya et al., 1999). In this study, 4.5% and 5.3% of galactofucan and laminaran were found in M05, while only 1.6% of galactofucan and 2.2% of laminaran were found in J06.

Finally, significant variations in the amounts of protein, lipid, ash and carbohydrate (laminaran and galactofucan) were observed between M05 and J06. These differences could be attributed to the level of nutritive salts, the growth cycle and the frond age. Other factors, which could not be verified, such as sea current, waves, thickness of ice cover, and intertidal environment could also have an influence on the seaweed constituents. Each factor could impact either individually or simultaneously the growth, and thus of the accumulation of polysaccharides, proteins, lipids and ash.

2.5.2 Purified laminaran analysis

Laminaran characterisation (Table 2.2) showed significant differences among harvest period. A higher amount of D-glucose was found in M05 (69.1%) and A05 (66.1%), while 45.1% and 56.0% for N05 for J06, respectively. On the other hand, the level of D-mannitol remained constant for each harvest period, ranging between 0.9% and 1.7%. Differences were observed in the D-glucose content between M05 and J06. Some experiments have been conducted on the monosaccharide composition of laminaran (Zvyagintseva et al., 2003). Results showed no variation in the proportion of D-glucose during the year, while

the frond age could be responsible. Also, their research has not tested the effect of fronds age on the monosaccharide content of laminaran.

The measured molecular weights were comparable for all tested fractions. N05 showed a slightly higher molecular weight at 3.06 kDa, while the other varied from 2.56 to 2.97 kDa, expressed in equivalents of PEG standards. However, M05 and J06 seemed to have lower molecular weights, which might be explained by the use of laminaran as a carbon source during winter growth (Chapman et al., 1977). The molecular weight results were lower than the values reported in the literature (Chizhov et al., 1998; Nelson et al., 1974). Chizhov and collaborators (1998) determined the molecular weight of laminaran MALDI-TOF MS for *Laminaria hyperborea* and *Laminaria cichorioides*. The molecular weights were around 4.26 kDa. Thus, no comparison was possible because the reported data used a different method.

2.5.3 Crude galactofucan analysis

The characterization of crude galactofucans is given in Table 2.3. From May to November, the sulphate contents varied from 19.9% to 21.5%, while in J06 the amount decreased significantly (14.3%). This could be attributed to the higher level of nitrate in May 2006 as compared to May 2005. The changes in nitrate content could also have influenced the seaweed growth cycle and thus the galactofucan composition. High molecular weight galactofucans were isolated for each harvest period and no significant variation was observed. The molecular weights varied from 2051 to 2378 kDa. Since these fractions were crude (no purification), having a high molecular weight was expected. Other research has also shown high molecular weight fucans. Crude fucans from *Pelvetia canaliculata* with a molecular weight of 1700 kDa were isolated (Kloareg et al., 1986). The molecular weights were determined by light scattering and the conditions were similar to our study.

The monosaccharide compositions of crude galactofucans (Table 2.4) were significantly different among the harvest periods. Results showed few variation in D-xylose, D-mannose, D-glucose, D-galacturonic acid, and D-glucuronic acid content through out the

year. However, the L-fucose and D-galactose contents showed significant variations. In M05, the amount of L-fucose was higher than the amount of D-galactose (21.5% vs. 11.1%), while the opposite was found for the other harvest periods; A05 (18.5% vs. 36.6%), N05 (20.9% vs. 36.8%), and J06 (12.8% vs. 19.6%). Moreover, J06 contained significantly less L-fucose than the other harvest period. Still, the influence of the growth cycle could have contributed to influence the monosaccharide composition as well as the fronds' age. Research has shown that old fronds contained a higher amount of L-fucose than young fronds from 100% to 87% (in molar percentage) for the hot water extract (Zvyagintseva et al., 2003). This could explain differences in the monosaccharide composition between M05 and J06.

2.5.4 Fractionated galactofucans

Crude galactofucans were fractionated in order to investigate how the sulphate groups and the monosaccharides were distributed with an increasing NaCl concentration of 0.5, 1 and 2 M. No molecular weight determination was performed due to low fractionation yields. Fraction 0.5 M (Table 2.5) showed significant sulphate group variations among the harvest periods. M05 and J06 showed lower amounts of sulphates (8.8% and 8.4%) than A05 and N05 (18.2% and 20.2%). Moreover, no significant variation was observed in the 1 M fraction, as the sulphate levels varied from 9.2% to 15.9%. In the 2 M fraction, higher contents of sulphates were found. In J06, a significantly lower amount of sulphates (27.1%) was found, while the other harvest period contained 30.5-36.0% of sulphates. Obviously, high NaCl concentrations will lead to higher sulphate content. However, 1 M fraction showed lower amount of sulphate groups for A05 and N05 than for 0.5 M fraction. This could be related to polysaccharide structure. The crude galactofucan fractions contained a mixture of different structures with various amounts of negatively charged molecules (sulphate and carboxylic groups) according to the harvest period. These molecules had an important impact on the affinity of the polysaccharide fractions with the column resins at 0.5 M NaCl. Thus, higher molecular weight fraction can overlap and protect some negatively charged molecules from interacting with the resin. In this case, the fraction would be eluted with 0.5 M instead of 1 M NaCl, even if it contained higher level of

sulphate groups. This was observed in other research (Li et al., 2006), where an higher molecular weight polysaccharide fraction with higher sulphate group content was eluted first.

The monosaccharide composition of each fraction showed important variation according to the harvest period especially between M05 and J06 (Figure 2.3). Focusing on the proportion of L-fucose versus D-galactose in which the main variation took place, we find that the 0.5 M fraction showed a higher content in D-galactose than L-fucose for each harvest period: M05 15.3% vs. 10.6%, A05 34.1% vs. 13.4%, N05 43.7% vs. 14.6% and J06 18.8% vs. 8.2%. The 1 M fraction is, however, different, as a higher amount of L-fucose was found for each harvest period: between 12.1% and 18.6%. In the 2 M fraction, the monosaccharide composition was comparable to the crude galactofucan pattern. In M05, a higher amount of L-fucose was found in comparison to D-galactose (24.8% vs. 14.9%), while A05 contained 14.9% vs. 50.0%, N05 22.0% vs. 53.2% and J06 17.5% vs. 56.8% respectively. Concerning the other monosaccharides, D-xylose, D-mannose, D-glucose, D-galacturonic acid, and D-glucuronic acid, a lower amount and few differences were observed during the year (data not shown) for each fraction.

A high amount of D-galactose was found in crude and fractionated galactofucans (0.5 and 2 M) for A05, N05 and J06. Other research had found a high proportion of other monosaccharides than L-fucose in fucoidan. For example, in brown seaweed *Laminaria gurjanovae*, fractionated fucoidans were found by anion exchange chromatography on DEAE-cellulose with 2 M NaCl with 50% L-fucose, 41% D-galactose and 2% of D-mannose (Shevchenko et al., 2007). In *Spatoglossum schroederi*, a molar ratio of 1:2:2 of fucose, galactose and sulphate was found for fractionated galactofucan obtained by ion exchange chromatography with a potent antithrombotic activity (Rocha et al., 2005).

2.6 Conclusion

After analysis, the laminaran composition was alike other research, where an higher amount was isolated in A05 due to a low level of nitrate. Moreover, the monosaccharide

composition and the molecular weights were quite similar for each harvest period. Furthermore, both crude and fractionated galactofucans showed structural differences according to the harvest period. A significant high proportion of D-galactose was found in A05, N05, and J06, which is interesting from the perspective of a biological activity study. Significant variation was found between M05 and J06 in terms of the seaweed composition and polysaccharide structural characteristics. These differences could be attributed to the nutritive salt present in the water, the growth cycle, and/or the fronds age. Further research with GC-MS analysis will help us to determine if L-fucose and/or D-galactose are mono and/or disulphated. The next step will be to link structure to the biological activity of laminaran and galactofucan with DNA microarrays.

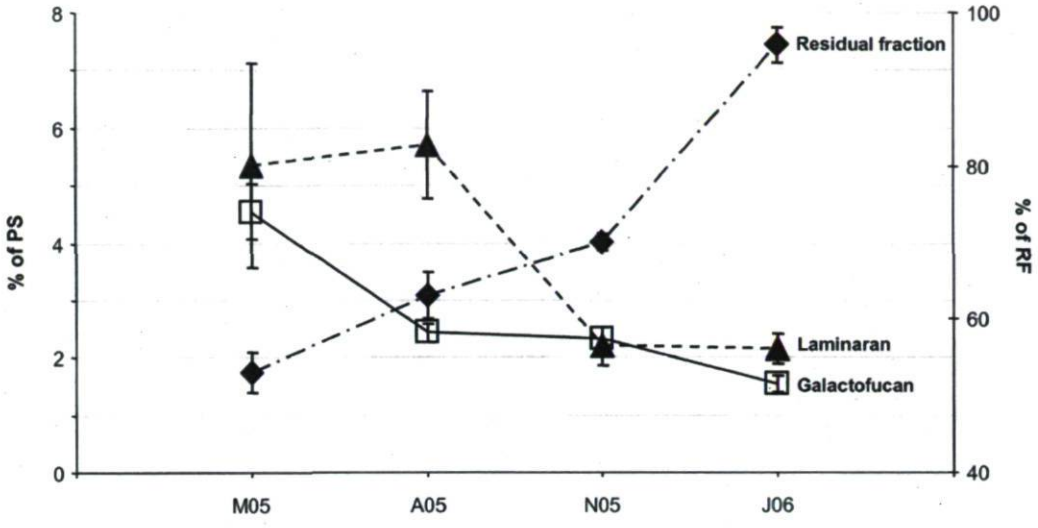

Figure 2.1 : Yields of extraction of crude polysaccharides (PS) and residual fraction (RF) for each harvest period.
Results presented on basis of dried seaweed.

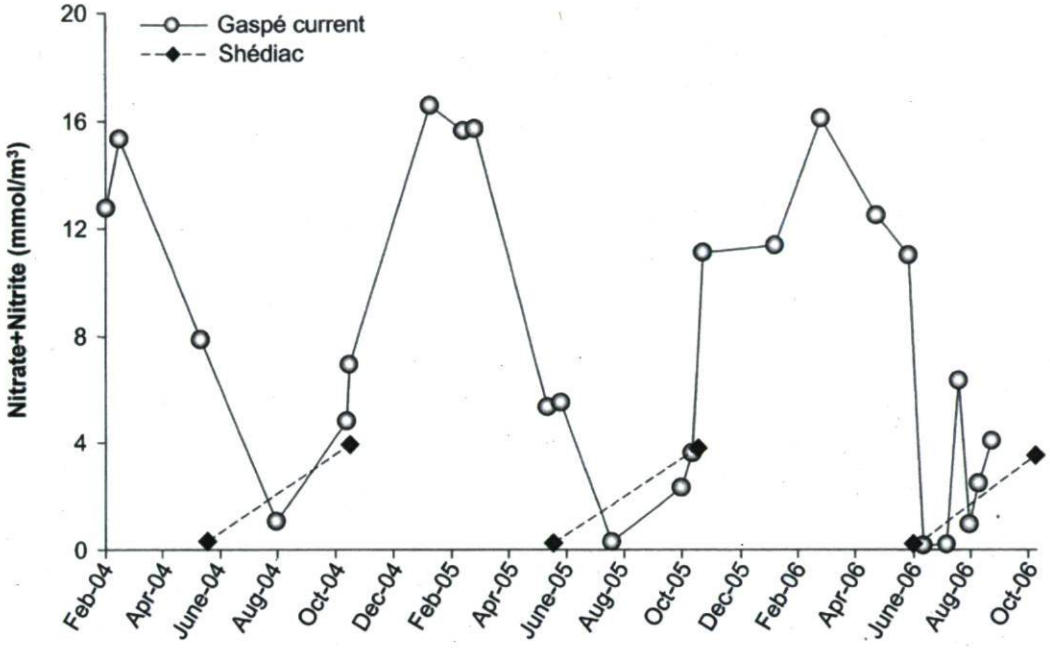

Figure 2.2 : Total amount of nitrate and nitrite monitored in the water at the Gaspé Current and Shédiac sites between January 2004 and December 2006.
Data provided by the department of Fisheries and Ocean Canada (2008).

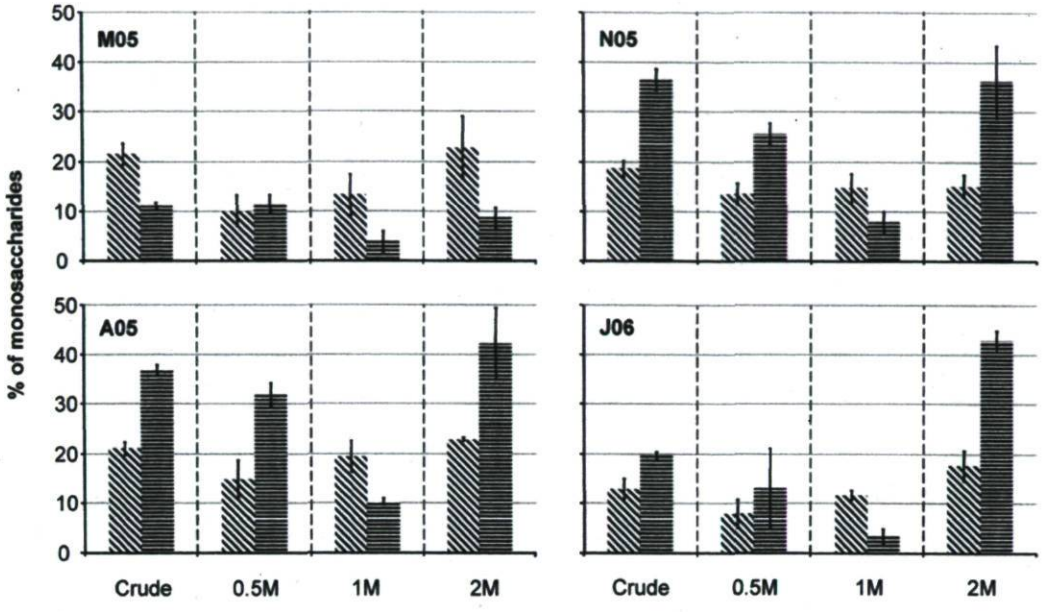

Figure 2.3 : L-fucose and D-galactose contents of crude and fractionated galactofucans with 0.5, 1 and 2M concentration of NaCl for each harvest period:

L-fucose

D-galactose

Table 2.1 : Composition of crude seaweed *Saccharina longicruris* (% of dry weight).

Harvest period	Proteins (%)	Lipids (%)	Ashes (%)
M05	12.2 ± 0.6 **d**	0.8 ± 0.1 **b**	26.3 ± 0.5 **b**
A05	5.0 ± 0.2 **a**	0.3 ± 0.1 **a**	22.6 ± 0.1 **a**
N05	9.3 ± 0.3 **b**	0.5 ± 0.1 **a**	30.5 ± 0.3 **c**
J06	10.9 ± 0.3 **c**	0.4 ± 0.1 **a**	31.2 ± 0.4 **d**

Columns with the same letter are not significantly different at $p < 0.05$.

Table 2.2 : Content (%) of monosaccharides and molecular weights of purified laminarans from *Saccharina longicruris*.

Harvest period	D-Glucose (%)	D-Mannitol (%)	Molecular weight kDa
M05	69.1 ± 7.7 **c**	1.1 ± 0.2 **a**	2.88 ± 0.24 **a**
A05	66.1 ± 4.7 **bc**	1.7 ± 0.0 **b**	2.97 ± 0.13 **a**
N05	45.1 ± 9.3 **a**	0.9 ± 0.1 **a**	3.06 ± 0.43 **a**
J06	56.0 ± 1.5 **ab**	1.1 ± 0.1 **a**	2.56 ± 0.16 **a**

Columns with the same letter are not significantly different at $p < 0.05$.

Table 2.3 : Global composition of crude galactofucans from *Saccharina longicruris*.

Harvest period	Sulphates (%)	Molecular weight (kDa)
M05	19.9 ± 0.7 **b**	2379 ± 254 **a**
A05	20.3 ± 1.2 **b**	2147 ± 529 **a**
N05	21.5 ± 1.1 **b**	2188 ± 644 **a**
J06	14.3 ± 0.9 **a**	2051 ± 177 **a**

Columns with the same letter are not significantly different at $p < 0.05$.

Table 2.4 : Content (%) of monosaccharides of crude galactofucans from *Saccharina longicruris*.

Harvest period	L-fucose (%)	D-galactose (%)	D-xylose (%)	D-mannose (%)	D-glucose (%)	D-galacturonic acid (%)	D-glucuronic acid (%)
M05	21.5 ± 2.1 **b**	11.1 ± 0.6 **a**	3.5 ± 0.3 **b**	5.5 ± 0.2 **b**	1.4 ± 0.1 **b**	0.6 ± 0.2 **b**	4.2 ± 0.3 **b**
A05	18.5 ± 1.7 **b**	36.6 ± 2.1 **c**	2.5 ± 0.2 **a**	3.5 ± 0.2 **a**	1.9 ± 0.2 **c**	0.0 ± 0.0 **a**	3.5 ± 0.2 **ab**
N05	20.9 ± 1.3 **b**	36.8 ± 1.0 **c**	2.3 ± 0.3 **a**	3.1 ± 0.1 **a**	0.7 ± 0.4 **a**	0.0 ± 0.0 **a**	2.9 ± 0.2 **a**
J06	12.8 ± 2.0 **a**	19.6 ± 0.8 **b**	3.5 ± 0.7 **b**	5.6 ± 0.9 **b**	0.8 ± 0.3 **a**	0.0 ± 0.0 **a**	5.0 ± 0.7 **c**

Columns with the same letter are not significantly different at $p < 0.05$.

Table 2.5 : Content (%) of sulphate groups of fractionated galactofucans isolated with 0.5, 1 and 2 M concentration of NaCl.

Harvest period	0.5M		1M		2M	
M05	8.8 ± 0.8	**a**	15.9 ± 6.7	**a**	36.0 ± 2.0	**b**
A05	18.2 ± 1.5	**b**	11.6 ± 0.4	**a**	30.5 ± 1.0	**b**
N05	20.2 ± 1.7	**b**	14.1 ± 0.9	**a**	31.2 ± 2.2	**b**
J06	8.4 ± 3.9	**a**	9.2 ± 0.7	**a**	27.1 ± 2.1	**a**

Columns with the same letter are not significantly different at $p < 0.05$.

Chapitre III :
Modulation of metabolic activities induced by a laminaran extract from brown seaweed *Saccharina longicruris*

3.1 Résumé

Le laminarane est un β-glucane extrait des algues brunes connu pour son activité anti-tumorale dans le colon et est impliqué dans la régulation de la mort cellulaire (apoptose). Le laminarane a été extrait des frondes de *Saccharina longicruris* récoltées à plusieurs périodes de récolte (M05, A05, N05 et J06). La composition en monosaccharides, la position des liaisons glycosidiques et le poids moléculaire ont été déterminés. L'activité biologique a été étudiée à l'aide de puces à ADN et les résultats ont été confirmés par des tests *in vitro* classiques (croissance cellulaire, apoptose, sécrétion de MMP et collagène). Les laminaranes contiennent entre 50,6 et 68,6% de D-glucose et environ 1,3% de D-mannitol. La présence de lien β-(1,3) au niveau de la chaîne principale a été confirmée alors qu'il y aurait présence de ramification en position 6 et en position 2. M05 semble moins ramifié que les autres fractions ce qui pourrait influencer sa conformation en solution. Le poids moléculaire est comparable pour chacune des périodes de récolte, variant entre 2900-3300Da. L'étude de l'effet du laminarane sur le profil d'expression génique de fibroblastes a mis en évidence des modulations du métabolisme du cycle cellulaire et au niveau de la matrice extracellulaire. Ainsi, le laminarane de M05 ne montre aucun effet sur la croissance des cellules alors que A05, N05 et J06 montre une réduction de la croissance cellulaire tant au niveau des gènes qu'au niveau des tests *in vitro* classique. Cette différence est d'autant plus importante que M05 stimule l'expression des gènes des télomérases qui ont pour fonction d'empêcher le raccourcissement des télomères, phénomène lié au vieillissement des cellules. De plus, les fibroblastes traités avec M05 et N05 ont montré une augmentation de la sécrétion de collagène, une protéine utile lors du processus de guérison d'une plaie. Ces travaux montrent que M05 semble différent des autres laminaranes au niveau de son activité. Cette différence serait associé à la structure du laminarane qui pour cet extrait, montre un taux de ramification inférieur aux autres ce qui pourrait influencer sa conformation en solution et donc son activité.

3.2 Abstract

Laminaran is a β-glucan extracted from brown seaweed that showed an anti-apoptotic and an anti-tumoral activity in the gut. Laminaran was extracted from brown seaweed *Saccharina longicruris* at four harvest periods (M05, A05, N05, and J06). Molecular weights, monosaccharides composition, and glycosidic linkage were determined. DNA microarrays were used to determine the gene expression profile of fibroblasts treated with laminaran. Classical *in vitro* tests (cell growth, apoptosis, MMP, and collagen secretion) were realised to validate the DNA microarray results. Laminaran fraction showed a molecular weight ranging from 2900-3300Da. Laminaran contained between 50.6 and 68.6% of D-glucose and an average of 1.3% of D-mannitol. Presence of β-(1,3) linkage between D-glucose in the main chain was detected. Branching in position 6 and in position 2 was noticed. M05 contained less branching than the other laminaran fractions which might influence its conformation in solution. The gene expression profile showed an influence on the expression of genes involve in the cell cycle and with the extracellular matrix. Results showed no negative or positive effect on the cell cycle regulation for fibroblasts treated with M05, while the gene expression of fibroblasts treated with A05, N05 and J06 showed a reduction of cell proliferation. This was validated with the cell growth experiment. Also, M05 has up-regulated the expression of telomerase genes (TERC and TERT), which are known to prevent telomere shortening and ageing. Furthermore, M05 and N05 showed a stimulation of the secretion of collagen-I, a protein beneficial during wound healing. The results showed that M05 has a different activity than the other laminaran fraction. This could be partially explained by the lower level of branching that could influence M05 conformation in solution and would thus, influence its activity.

3.3 Introduction

β-glucan from various sources (plants, mushrooms, moulds, yeast, barley, etc.) were largely studied. They share the same structural characteristics, where they are all mainly composed of β-D-glucose. β-glucan from oat and barley contains D-glucose linked in β-(1,3) and β-(1,4) with a molecular weight between 20-400 kDa (Lazaridou et al., 2007). Fungal β-glucans like: lentinan, grifolan, scleroglucan, and zymosan are composed of D-glucose linked in β-(1,3) and β-(1,6) (Chen et al., 2007a; Mueller et al., 2000), while glucan phosphate is composed of D-glucose only linked in β-(1,3) (Mueller et al., 2000). Broad molecular weight distributions were found for these fungal β-glucan. Grifolan was reported to have a wide molecular weight distribution ranging from 8-2000 kDa (Bae et al., 2005; Iino et al., 1985), while scleroglucan molecular weight varies between 1020-1560 kDa (Mueller et al., 2000; Pretus et al., 1991). Lentinan and glucan phosphate showed respectively a molecular weight of 500 kDa (Zhang et al., 2007) and 157 kDa (Mueller et al., 2000). Laminaran is the glycosidic reserve of brown seaweed. It is a small glucan of 5 kDa with a degree of polymerisation between 20 and 25 (Chizhov et al., 1998; Nelson et al., 1974). Laminaran is present in two forms: soluble and insoluble. The first form is characterized by a complete solubility in cold water, while the other is only soluble in hot water (Black et al., 1973; Percival et al., 1967). Solubility is also influenced by the presence of branching. The higher the branching content the higher is the solubility in cold water. Two types of laminaran have been described, one type with chains that are terminated by D-mannitol residues (M-series) and the other type with chains terminated by D-glucose residues (G-series) (Nelson et al., 1974). Laminaran is composed of D-glucose linked in β-(1,3) (Barry, 1939) with intrachain branching linked in β-(1,6) (Peat et al., 1958). Ratios of the two types of laminaran as well as their structure vary according to the seaweed species and environmental factors such as nutritive salts and the fronds age (Chizhov et al., 1998; Rioux et al., 2009). These factors are believed to influence the biological activity of laminaran.

Several biological activities were attributed to the β-glucan. Lentinan was found to have an antitumor activity (Baba et al., 1986; Surenjav et al., 2006), while glucan phosphate from

Saccharomyces cerevisiae is a potent anti-infective agent (Williams et al., 1996). Moreover, β-glucan is known to have an important impact on the macrophage and complement pathway activation (Leung et al., 2006). Three β-glucan receptors were identified such as: scavenger receptor (SR), complement receptor 3 (CR3) and dectin-1 (Brown et al., 2005; Leung et al., 2006). Several structural parameters such as: charge, solubility, molecular weight, degree of branching, and conformation are known to influence the binding ability of β-glucan on these receptors (Leung et al., 2006). Two β-glucan receptors have been found on human dermal fibroblast (HDF), which activates proinflammatory intracellular signalling pathway and up-regulates cytokine genes expression (Kougias et al., 2001). Authors showed that laminaran was able to only bind one of the two sites, while glucan phosphate was able to interact with both sites. This was also seen with human promonocytic cell line (U937) (Mueller et al., 2000). Results showed that laminarans interact preferentially with one of the two sites present on U937, which can stimulate intracellular signalling pathways. Another study showed that laminaran was able to bind a specific receptor on monocytes, possibly dectin-1 which contains two isoforms (Gantner et al., 2003; Kikkert et al., 2007). The authors believed that laminaran blocks B isoform. These researches indicate that laminaran might have various impacts on different cell line.

Laminaran was further investigated in terms of a fermentative effect in the gut (Devillé et al., 2004; Devillé et al., 2007; Michel et al., 1999; Michel et al., 1996). These researches have determined that laminaran was used by the microbial population in the gut (Devillé et al., 2007; Michel et al., 1999). Moreover, an important production of butyrate was observed which could have an important role in the protection against colon cancer (Devillé et al., 2007). Another *in vivo* study realised with piglets showed a significant reduction of enterobacteria, bifidobacteria and lactobacilli population in the caecum and in the colon with a diet supplemented with seaweed extracts containing 170 ppm of laminaran and 136 ppm of fucoidan (Reilly et al., 2008). Besides, the dietary supplementation of laminaran protects rats against hepatotoxicity induced by lipopolysaccharide (LPS) by modulating immune response (Neyrinck et al., 2007). Other activities such as anti-tumor (Jolles et al., 1963) and anti-apoptosis (Kim et al., 2006) were found with native laminaran and oligo-

laminaran, while sulphated laminaran derivatives were found to have anticoagulant activity (Ito et al., 1989). Since, laminaran has a totally different structure to the other β-glucans, other biological activities are anticipated.

Aim of this work is to test various samples of laminaran from *Saccharina longicruris* using DNA microarray to search for novel biological activities by the analysis of the genes expression profile. Several biological activities were validated with classical *in vitro* test. Moreover, a relationship between the structure and biological properties of laminaran is proposed.

3.4 Experimental

3.4.1 Algal materials

Laminaran was extracted from *Saccharina longicruris* harvested in May (M05), August (A05), November 2005 (N05) and June 2006 (J06) at Gaspé (Québec, Canada). The seaweeds were milled in a Desintegrator (Rietz Manufacturing Co., Santa Rosa, USA) fitted with perforated plates of 4.65 mm, vacuum sealed and kept at -30°C until use.

3.4.2 Extraction procedures

Crude laminaran was extracted from milled seaweeds (Nishino et al., 1989). Seaweeds (300 g) were mixed with HPLC grade water and 1% (w/v) of $CaCl_2$. Each mixture was stirred at 85°C during 4h and then, centrifuged (16 887 g, 20 min). The supernatant was isolated by vacuum filtration on Whatman #4 filters. Each filtrate was mixed with 1 volume of 2% NaCl and 2 volume of EtOH (95:5, v/v) and then, stirred during 1h at room temperature and stored at -20°C during 48h. Each mixture was centrifuged (16 887 g, 10 min) to isolate the pellet (crude galactofucan) and the supernatant (crude laminaran containing a mix of laminaran, galactofucan and polyphenol) separately. The ethanol was evaporated from the supernatant and then, all fractions were dialysed with 1 kDa membranes during 48h and then freeze-dried. At least three extractions per harvest period were realised and pooled

together to generate enough starting material for the bioactivity study and structural characterisation.

Crude laminaran fractions were resuspended in water at a concentration of 0.1%. Laminaran was purified by ultrafiltration with a 50 kDa Prep/Scale-TFF membrane (Millipore, Canada) to remove galactofucan from the mixture. The filtrates were freeze-dried. The filtrates were resuspended in water and added on a low pressure column with Amberlite resin (Sigma, Canada) to remove traces of polyphenols. Purified laminaran fraction was eluted with 5 column volume of water and polyphenol was isolated with 5 column volume of ethanol (95:5, v/v).

Fractions will be presented as laminaran M05, A05, N05 or J06 which refers to their respective harvest period.

3.4.3 Structural characterisation of the laminaran extracts

3.4.3.1 Monosaccharides analysis

Neutral sugars, after acidic methanolysis of the polymer and subsequent GC analysis were identified as trimethylsilyl derivatives. Methanolysis was performed in 2 M MeOH-HCl (Sigma, Canada) at 100°C for 2h and the methyl glycosides were converted to the corresponding per-*O*-trimethylsilylated derivatives (Kamerling et al., 1975; Montreuil et al., 1986). The derivatives were separated and quantified by GC on a HP 5890A system equipped with a FID detector and a CP-Sil-5CB fused silica column (60 m x 0.25 mm, Chrompack, Varian) in a split/splitless mode using helium at a flow of 1.5 mL/min as the gas carrier. The oven temperature was programmed at 50°C for 1 min, 20°C/min to 120°C, and then 2°C/min to 240°C. Injector and detector temperature were maintained respectively at 290°C and 300°C. The monosaccharides were identified according to their retention times and quantified using an internal standard method involving *myo*-inositol. All analyses were made in triplicates.

3.4.3.2 Methylation analysis

Methylation analysis was performed using modifications of three methods (Hakomori, 1964; Hellerqvist, 1990; Waeghe et al., 1983). First, laminaran (2 mg) was treated with Dowex cation exchange resin (Sigma, Canada) and freeze-dried. Then, laminaran hydroxyl groups were methylated with methyl iodide in dimethyl sulfoxide (Sigma, Canada) and with lithium 1-butanide (Sigma, Canada) as anion. Methylated laminaran samples were purified with Sep-Pak C18 (Waters, Canada) eluted with acetonitrile and dried under nitrogen flow. The purified methylated laminaran was hydrolysed with 2 M trifluoroacetic acid for 2h at 100°C and reduced with NaBD4. Then, derivatives were acetylated with acetic anhydride (Sigma, Canada) and pyridine (Sigma, Canada). Samples were diluted with methylene chloride (Sigma, Canada) prior to analysis. Samples were analysed by gas chromatography-mass spectrometry (GC/MS) carried on Agilent Technologies (Mississauga, ON, Canada) model 6890N Network GC system. A CP-Sil-5CB fused silica column (60 m x 0.25 mm, Chrompack, Varian) using helium as carrier was used at a flow rate of 1.2 mL/min. The oven temperature was programmed at 50°C for 1 min, 20°C/min to 120°C, and then 2°C/min to 240°C. Injector and detector temperature were maintained respectively at 290°C and 300°C. Ionization was carried out in electron impact (EI, 70 eV). The monosaccharides were identified according to their retention times and mass fragments.

3.4.3.3 Electrospray ionisation mass spectrometry

Sample preparation and data analyses were performed using modification of Read method (Read et al., 1996). Samples were prepared as describe above (section 3.4.3.2). Briefly, samples (2 mg) were pre-treated with Dowex cation exchange resin (Sigma, Canada) and methylated. Then, samples were purified with Sep-Pak C18 (Waters, Canada) eluted with acetonitrile. Samples were prepared by adding 50% of the acetonitrile mix and 50% solution containing 0.01% sodium acetate in 50:50 v/v water:MeOH.

Analyses were performed on a Leco High-Throughput Unique Time of flight (TOF) mass spectrometer (MS) (Mississauga, ON, Canada). The instrument was equipped with a

syringe direct injector (Harvard) at a flow rate of 50 µL/min. The flow was interfaced with an high flow atmospheric pressure ionization electrospray (ES) chamber. Conditions for the analysis in the positive ion mode included an electrospray voltage of 2 kV, a nebulizer pressure of 250 kPa and a desolvation temperature of 210°C. Data were collected on ChromaTOF (Leco) over a mass range of m/z 50-6000 at 2.08 spectra per second. The instrument was calibrated using an ES tuning mix (mass ranging from 118.08 to 2721.89 m/z) (Agilent technologies). Since the highest calibration peak within the mix was 2721.89, the calibration was slightly adjusted to use G23 as an internal calibration reference. Then, the other masses were determined in relation with this peak. In order to improve the sensibility, around 1250 scans were acquired and averaged.

3.4.3.4 HPSEC-molecular weight determination of laminaran

The polysaccharide weight average, Mw, was determined by HPSEC (High Performance Size Exclusion Chromatography (Rioux et al., 2009). Briefly, TSK-guard column PWXL (6 mm X 40 mm) and a TSK-G3000 PWXL (7.5 mm X 300 mm) (Tosoh Bioscience, Montgomeryville, USA) were used in series. The mobile phase consisted of a filtrated (0.22 µm) 0.1 M NaCl solution obtained with HPLC grade H_2O. The flow rate was 0.8 mL/min and analyses were performed at room temperature. The samples were dissolved in 0.1 M NaCl solution to reach a concentration of 1 mg/mL, and filtered (0.45 µm) to eliminate dust particles. A molecular weight calibration curve was constructed with five polyethylene glycol (PEG) standards from 985 to 12 400 Da (Phenomenex, inc., Torrance, CA, USA). Laminaran molecular weight was reported as equivalent of PEG.

3.4.4 Gene expression study

3.4.4.1 Cytotoxicity assessment of laminaran extract

Human dermal fibroblasts (HDF) from newborn were classically grown in DMEM (Dulbecco's Modified Eagle's Medium) (Invitrogen, UK) with 10% of fetal calf serum (Invitrogen, UK) and 100 µg/mL of penicillin/streptomycin. Cells were maintained in a

humidified incubator at 37°C with 5% of CO_2 atmosphere. Cells were kept in culture under exponential growth conditions and were harvested with trypsin/EDTA solution when cells reached a population doubling of 33. HDFs were plated in 24 well plates at 30 000 cells/well.

Cell viability was tested by applying laminaran at concentration of 0.004 to 2.5 mg/mL in cultured cells for 24h to determine the optimal concentration for the gene expression study. A solution of 2.5 mg/mL of MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide) (Sigma-Aldrich, Germany) was applied and incubated for 2h at 37°C in a humidified incubator with 5% CO_2. Medium was removed and lysis solution was added. After 2h at 37°C, 200 µL of lysis solution was transferred in 96 well plates and read at 570 nm in a microplate reader. Cytotoxicity was expressed in percent relative to untreated cells control as a decrease of mitochondrial dehydrogenase measured by formazan production from MTT. Cell viability was realised in triplicates for each harvest period. High cell survival was observed at 2.5 mg/mL which was the concentration used for the gene expression experiment.

3.4.4.2 Cell culture

The study was carried out with HDF cultured in the same conditions listed above. For this experiments 500 000 cells were seeded in 75 cm^2 flasks. At 80% confluence, cells were treated with laminaran at a concentration of 2.5 mg/mL for 24h. Three cell cultures were realised for each laminaran extract.

3.4.4.3 Extraction of total RNA

Total RNA extraction was performed with RNAgents Total RNA Isolation System (Promega). The culture medium was decanted from each flask and the cells were washed 2 times with ice-cold sterile PBS. Cells were lysed with 300 µL of chilled denaturing solution and scrapped. The lysed cells were transferred in an RNAse-free tube and homogenised. For the RNA extraction, 30 µL of 2 M sodium acetate was added in the lysate and mixed thoroughly by inverting the tube 4-5 times. 300 µL of phenol:chloroform:isoamyl alcohol

were added to the tube, mixed carefully by inversion 3-5 times and then shaked vigorously for 10 sec. The tubes were chilled on ice during 15 min. This mixture was centrifuged (10 000g, 20 min) at 4°C. The top aqueous phase that contained the RNA, was removed carefully and transferred to a new RNAse-free tube. RNA precipitation was realised by adding an equal volume of isopropanol to the aqueous phase and incubated for 30 minutes at -20°C. The RNA pellet was isolated by centrifugation (10 000 g, 20 min) at 4°C. The pellets were washed by adding 1 mL of ice-cold 75% ethanol and centrifuged (10 000g, 20 min) at 4°C. When the pellets were dried, they were resuspended in an appropriate volume of nuclease-free water and stored at -80°C.

3.4.4.4 RNA integrity and concentration verification

The integrity and quality of RNA template were checked. The concentration was determined by using an aliquot of the isolated RNA diluted in TE (10 mM Tris-HCl (pH 8), 1 mM EDTA). Its concentration was determined by Nanodrop (Isogen ND-1000) against TE as blank solution by spectrometric measurement. The integrity of total RNA was assessed by visualization of intact ribosomal RNA bands with Agilent Bioanalyzer 2100 analysis. Total RNA from higher eukaryotes should have a ribosomal band size of 1.9 kb for the 18S-RNA and 4.7 kb for 28S-RNA.

3.4.4.5 Reverse Transcription and incorporation of a biotin-dNTP mixture during cDNA synthesis

For the cDNA synthesis with biotin labeling, a reaction mix (1) was prepared with 10µg of total RNA, Internal Standard mix-Silverquant, and Oligo$(dT)_{12\text{-}18}$ Primer (0.5µg/mL) for a final volume of 10µL. The reaction mix was incubated at 70°C for 10 min and chilled on ice for 5 min. A second reaction mix (2) was prepared with 5X First-Strand buffer, DTT (0.1 M), Biotin-dNTP and RNaseOut (40 U/µL). Nine µL of reaction mix 2 was added to reaction mix 1 (10 µL) on ice and incubated at room temperature for 5 min. SuperScript™ II Reverse Transcriptase (200 U/µL) was added to the reaction two times and incubated at 42°C for 90 min and then incubated at 70°C for 15 min. RNase H (2U/µL) was added to the

reaction and incubated at 37°C for 20 min. The reaction was ended by heating to 95°C for 3 min. The samples were placed on ice and then hybridization was performed.

3.4.4.6 Hybridization on DualChip® Human Aging (Eppendorf, 0038 950 003)

The DualChip® Human Aging contains 227 genes involved in stress and ageing related pathways (extracellular matrix, keratinocyte differentiation, DNA damage, oxidative stress, cell signalling, cell proliferation, DNA repair, apoptosis, and inflammation) and 13 housekeeping genes for data normalization (complete gene list on the chip is available in Annex 2). There were control probes for six different internal standards used to determine the quality for both the labelling reaction, the hybridization, and to normalize the data. Several controls were present on the chip to check the hybridization step, the orientation of the array, and unspecific background. The method was based on a system with two arrays (a control and a test) per glass slide with three identical subarrays per array. Positive and negative controls were spotted on each subarray in order to control the reliability of the experimental data.

The hybridization mix was prepared in the following order: Biotin-labeled cDNA, Biotin-HybControl, RNase-free water, Silverquant hybridization additive, HybriBuffer A and HybriBuffer B for a final volume of 100 µL. The hybridization mix was heated for 5 min at 60°C. 100 µL of hybridization mix was dispensed slowly into the injection port of the hybridization frame to avoid inserting air bubbles. The surface around the injection port of the hybridization frame was carefully dried. The aluminum sealing pad was applied to the top of the hybridization frame to seal the injection port. Immediately after the hybridization frames sealing, the DualChip® was place placed in the Thermoblock for slides. The thermoblock lid was closed and the DualChip® slides were incubated overnight (12–16h) at 60°C in the thermomixer with a mixing frequency of 1400 rpm.

3.4.4.7 Silverquant detection

After the hybridization, the slides were removed from the thermoblock and incubated for 2 min at room temperature. Both hybridization frames were removed and the slide was

immediately immersed in the wash buffer. The slides were washed in three one-minute sessions. The wash buffer was removed and, the slides were incubated in pre-blocking buffer for 10 min at room temperature. The pre-blocking buffer was removed and the slides were incubated in the diluted gold-conjugated Anti-biotin antibody for 45 min at room temperature under gentle agitation (300 rpm). After incubation with the gold-conjugated Anti-biotin antibody, the box was emptied and the slides were washed in four one-minute sessions using wash Buffer. The slides were incubated with rinsing buffer for one minute. Correct amount of Silverquant solution and Silverquant solution A were added and slides were incubated for 5 min at room temperature under agitation (300 rpm). At the end of these 5 min, the staining solution (pink-grey solution) was removed; the slides were washed twice during 1 min using distilled water at room temperature. The backside of the slides and the barcode sticker were wiped using a paper towel, then immediately the slides were centrifuged (600 rpm, 5 min) to dry.

3.4.4.8 Array scanning

After staining, the array was scanned using Eppendorf Silverquant scanner and following Silverquant software recommendations. The Silverquant Analysis software is then used for spot finding and quantification.

3.4.4.9 Data quantification

Array images obtained were normalised and quantified using Silverquant Analysis software, following several steps:

The intensity of every DNA spot was determined by using the local background subtractions. A signal at least 2.5 fold higher then the local background was accepted. An intensity ratio between the sample and the test were determined by averaging the intensity of the triplicate DNA spot on the chip.

For each array, the experiment was compared to control, corresponding to RNA from untreated cells. After spot detection, normalisation with internal standards was performed. Internal standards were composed of external plant RNA added in the mix of the reverse-

transcription reaction, allowing the evaluation of its efficacy. Ratios of internal standards were used to evaluate efficacy. The ratios should be included between 0.75 and 1.25. Outside these ranges, it can be concluded that the reverse-transcription reaction was not equally efficient in the control and the experiment, and that gene quantification would not be correct or artefactual.

A second step of normalisation with 13 housekeeping genes was realised because internal standard does not include purity and quality of the RNA. The variance of the normalised housekeeping genes was used to provide an estimate of the expected variance. This will lead to a predicted confidence interval for testing the significance of the ratio obtained. Ratios were significantly different when they were outside the 95% confidence interval (Anova) (de Longueville et al., 2002; de Magalhães et al., 2004). Only significant genes were presented (P-values < 0.05 or 0.01). Also, an average value was calculated from the normalised ratios of the three replicates.

3.4.5 Classical *in vitro* tests

3.4.5.1 Cell types and cell growth determination

Human dermal fibroblasts were isolated from human skin biopsies as described in (Moulin et al., 2002). All procedures involving patients were reviewed and approved by the Research Ethical Committee of the CHA (Centre hospitalier affilié universitaire de Québec). Cells were cultured in DMEM and 10% FBS (fetal bovine serum) (Moulin et al., 2004) and were used at passage 6. Cells were seeded in 12-wells plates (BDBiosciences, Mississauga, ON, Canada) at a density of 15 000 cells per well. After 24h, one well by plate was trypsined and cells counted using Coulter® counter. Other wells were then washed with medium without FBS and treated with laminaran in DMEM with 0.5% FBS (Moulin et al., 1997). Culture media were changed every two days and cells were trypsined and counted after 6 days of treatment using a Coulter® counter.

3.4.5.2 Detection of apoptosis using propidium iodide

Cells were plated in 6-wells plates at a density of 50 000 cells per wells and cultured during 5 days. Cells were then washed with medium without FBS and then treated with laminaran in DMEM with 0.5% FBS. After 24h treatment, floating and trypsined cells were pooled and fixed in cold 70% ethanol. Then, the fixed cells were re-suspended in PBS containing RNase and propidium iodide as previously described (Larochelle et al., 2004). Fluorescence was measured with a flow cytometer. Cells that contained less than 2n DNA content (sub G0) in the cycle analysis profile were considered to be apoptotic (apoptotic bodies).

3.4.5.3 Quantitative determination of collagen-I secretion

Cell culture supernatants collected 48h after the last medium change were centrifuged to remove any particulate material, and stored at -70°C until assessed. At the used supernatant dilutions, the culture media and tested samples were not found to interfere with any of the assays. Quantitative determination of collagen-I secretion was indirectly detected using a Procollagen I C-peptide (PIP) *in vitro* enzyme immunoassay (EIA) kit (Takara Bio Inc., Wisconsin, USA). The amount of the free propeptides stochiometrically reflects the amount of secreted collagen-I molecules (Corriveau et al., 2009).

3.4.5.4 Total MMP activity

Total MMP activity was determined in the same supernatant than for collagen assay using the SensoLyteTM 520 Generic MMP assay kit (Anaspec, San Jose, CA). This kit can detect simultaneously the activity of MMP1, 2, 7, 8, 9, 12, 13 and 14. Results reflect the overall MMP and metalloprotease inhibitor balance. The culture media and tested samples used were not found to interfere at the dilutions used.

3.4.5.5 Statistical analysis

Presented results were the average ± standard deviation of the results. Statistical differences were computed by the Student's t-test. The results were considered significant when P-values were < 0.05 (Scherrer, 1984).

3.5 Results and discussion

3.5.1 Structural characterisation of laminaran fractions

The effect of the harvest season on the structural composition was previously established for each laminaran extracts (Rioux et al., 2009). Results showed structural differences between M05 and J06 which could be linked to the amount of nutritive salt present in the water, the growth cycle of the seaweed, and/or the frond age.

D-glucose content varies from 50 to 69% for each harvest period, whereas the level of D-mannitol remained constant for each harvest period, ranging between 1.2 and 1.6% (Table 3.1). The measured molecular weights by HPSEC were comparable for all tested fractions ranging from 2894 to 3317 Da expressed in equivalents of polyethylene glycol (PEG) standards (Table 3.1). However, molecular weight results obtained by electrospray ionization mass spectrometry (ESIMS) were higher. Results varying from 3900 to 5800 m/z were obtained, while another research has found a molecular weight varying from 4300 to 6500 m/z (Read et al., 1996) for a commercial laminaran obtained from Sigma Chemical. In many cases, m/z value is numerically equal to the molecular (ionic) mass in Dalton when the ions encountered in mass spectrometry have just one charge ($z=1$). The results obtained by HPSEC are just an estimate of the true laminaran molecular weight because the system was calibrated with PEG, whereas the molecular weight obtained by ESIMS was determined by the true mass of each monosaccharide units. The use of PEG as HPSEC standards thus resulted in an underestimation of the molecular weight.

The degree of polymerization of laminaran extracts was determined using ESIMS. Results showed a slight difference of degree of polymerisation (d.p.) for M- and G-chains (chains

terminated by a mannitol or a glucose residue). A degree of polymerisation of 17-25 (M-series) and 18-26 (G-series) were found for M05 and A05 (Table 3.1). However, lower degrees of polymerisation were found for N05 (d.p. $G_{17-24}M$; G_{18-25}) and J06 (d.p. $G_{16-24}M$; G_{17-25}). Spectra have shown different ratios of M:G-series according to the harvest period. A ratio of 1:1 was found for M05, N05 and J06, while a ratio of about 1:1.2 was found for A05 (Figure 3.1). This can be explained by the seaweed growth cycle. At this period, lower amount of laminaran is found in the seaweed and consequently lower amount of mannitol was isolated compared to other harvest period (Anderson et al., 1981). Different results were found for commercial laminaran from *Laminaria digitata*. ESIMS showed that the G-series contains 22-28 glucosyl residue and M-series contains 20-30 glucosyl residues linked to a mannitol residue (Read et al., 1996). They also found a ratio of M:G series of 3:1. Others have demonstrated that laminaran from *Cystoseira barbata* and *Cystoseira crinita* have no M-chain with a degree of polymerisation up to 31 with an average around 23 glucose units (Chizhov et al., 1998). This gives important insight that laminaran structure varies according to the seaweed species.

Linkage analysis realised by GC-MS showed the presence of a 3-linked glucopyranose backbone ranging from 76.8 to 87.4% (Table 3.2) and 1-mannitol was detected at a concentration lower than 0.5% (data not shown). The presence of 2- and 6-substituted glucopyranose residue were determined for M05, A05 and N05 in a proportion of 2.1 to 7.3%, while only 2-substituted glucopyranose residues were found for J06 in a proportion of 13.8%. Branching at position *O*-2 has never been detected for laminaran except for commercial laminaran from Sigma which contained low amount of substitution at *O*-2 (Adams et al., 2008). Other β-glucan are substituted at position *O*-2 and they exhibit antitumor activity (Zhang et al., 2007). Finally, the amount of branched residues is equivalent or lower than the terminal one, indicating that there is no more than one branching per chain except for J06 which might contain two branching points per chain. M05 seems to be less branched than the other fraction, which might impact its behaviour in solution.

Structural characterisation did not show clear distinct structural features between harvest periods. Linkage analysis tends to show that M05 is less branched than the other fractions which could affect the laminaran solubility and its conformation in solution. The higher the branching content the higher is the solubility in cold water. Laminaran from M05 was more hardily dissolved than the other fractions in the culture media (DMEM) in which fibroblast cells were grown. DMEM contained salts, amino acids, vitamins and glucose, which might have modified laminaran conformation in solution. Other research showed that laminaran conformation is influenced by the solvent (Frecer et al., 2000). Besides, several researches showed a critical impact of the polymer structure and its conformation on its binding capacity to a receptor (Leung et al., 2006; Mueller et al., 2000). Mueller and co-workers showed that β-glucan affinity to a receptor is based primarily on conformation in solution and to lesser extent to the branching frequency, molecular weight and charge of the polymer (Mueller et al., 2000). Conformational study of oligo-laminaran showed that de C6-OH could form a proton donor hydrogen bond to the C4-OH group oxygen in water (Frecer et al., 2000). M05 was found to have less *O*-6 branching than A05 and N05 which could reveal a different conformation. Further analyses would be necessary to verify this hypothesis.

Our data showed a slight structural difference between each fraction which indicates possible variation in the gene expression profile. These results are important to control the biological activity of laminaran fractions throughout the year.

3.5.2 Metabolic activities induced by laminaran extracts

DNA microarray analysis was used to determine the gene expression profile modifications after fibroblast treatment with 4 laminaran fractions: May (M05), August (A05), November 2005 (N05), and June 2006 (J06). Only significant gene expression modifications (P-values < 0.05 or 0.01) when compared to their respective control on the same microarray chip, are presented. Results showed significant variation in the gene expression profile among each laminaran extracts (Table 3.3). Genes involved in the cell cycle regulation, chromosomal processing, extracellular matrix, immune, and inflammations were significantly up- or

down-regulated compared to the untreated cells. Genes from the same functional activity were regrouped and analysed together for all extracts. Classical *in vitro* tests were performed to validate the results obtained with DNA microarray. Cell growth, apoptosis, matrix metalloproteinase (MMP) and collagen-I secretion were analysed.

3.5.2.1 Effect on the cell cycle

3.5.2.1.1 Gene expression on fibroblasts treated with laminaran

To regulate the cell cycle, several proteins are expressed or repressed at certain time point. For example, G1 is controlled by cyclin/Cdk complexes. Cell passage through G1 is controlled by ordered expression of cyclin D and E, which associates respectively with Cdk4/6 and Cdk2/3. There are also some Cdk inhibitors (p21, p27 and p57) which will interfere with the formation of cyclin/Cdk complexes. The over expression of these proteins will force the cell to exit the cycle until the concentration of these proteins is reduced. Variation in the expression of several genes involved in the cell cycle regulation was shown after laminaran treatments (Figure 3.2). M05 treatments showed opposite results than A05, N05 and J06 treatments. Most genes expressions were down-regulated in presence of A05, N05 and J06 with an average ratio of -1.9 to -6.0. A05 and N05 treatments showed to have an important proportion of down-regulated genes, while J06 treatment showed few. The expression of most cyclins (CCNB1, CCNE1, CCNF, CCNH), cyclin-dependant kinase (Cdk) inhibitors (p57 and p27), proliferating cell nuclear antigen (PCNA), retinoblastoma 1 (RB1), c-myc binding protein (cmyc), thrombospondin 1 (TSP1), kinesin family member 2C (KIF2C) and 23 (KIF23), polymerase alpha 2 (POLA2), ataxia telangiectasia mutated (ATM), E2F transcription factor 1 (E2F1), antigen identified by monoclonal antibody (Ki-67), and topoisomerase II alpha (TOP2A) genes were down-regulated after laminaran treatments (A05, N05 and/or J06). The down-regulation of the gene expression of several cyclins inhibits the G1 and G2 phase progression especially, CCNB1, which is essential for G2 progression. The cmyc gene expression is involved in many biological functions like apoptosis, extracellular matrix, and cell cycle. The down-regulation of cmyc gene expression was observed after A05, N05, and J06 treatment; and is thought to reduce cell proliferation. This was confirmed after N05 treatment with the inhibition of Ki-67 gene

expression, a proliferation marker (Takagi et al., 2001). Also, RB1 and E2F1 proteins form a complex destabilised by Cdk-4 to liberate E2F1, which are required for the cell to enter S phase. The down-regulation of RB1 and E2F1 gene expressions indicates that the cell might not replicate its DNA (Adams et al., 1996). Also, KIF23 and -2C proteins are required for the progression through M phase (Kim et al., 1997a) and both genes expressions were down-regulated in presence of N05. The ATM gene is involved in the genome stability, cellular responses to DNA damage and cell cycle control and its expression was also down-regulated (Savitsky et al., 1997). PCNA protein is mostly expressed during a normal DNA replication or during DNA damage (Sengupta et al., 2005). Moreover, the expression of POLA2 protein is essential because it contributes to extend of the lagging strand during DNA replication (Collins et al., 1993), while TOP2A protein is involved during chromosome segregation and condensation that occur during M-phase (Watt et al., 1994). PCNA, POLA2 and TOP2A genes expression were all significantly down-regulated indicating, once again, that the cell cycle is arrested. This can be validated with the chromosomal processing genes (telomeric repeat binding factor 1 (TRF1), histone 1, centromere protein A and F) gene expressions that were also down-regulated (Table 3.3). These genes are essential during M-phase indicating that the cell won't undergo mitosis. Finally, the Cdk inhibitors genes, p57 and p27, were also down-regulated in presence of some laminaran extracts indicating that the cell cycle arrest is not caused by DNA damage. Thus, all the data seems to indicate that laminaran extracts from A05, N05, and J06 reduce cell proliferation.

After M05 treatment, only CCNF and tumor protein p53 (p53) gene expressions were significantly up-regulated with an average ratio of 2.1 and 4.6 respectively. This indicates that there is little difference between cells treated with laminaran M05 and control which will have no impact (positive or negative) on cell proliferation. CCNF protein is involved during the interphase (M-phase) (Bai et al., 1994) and the gene up-regulation indicates that the cells were able to undergo mitosis. The over expression of p53 is often associated with DNA damage. The up-regulation of p53 stimulates p21 gene expression, which interacts with Cdk resulting in the cell cycle arrest before entering S phase (Harper et al., 1995). The expression of p21 gene was not differentially up-regulated in this experiment indicating

that the cell cycle was not arrested. Also, it is unlikely that cell death occurs due to the over expression of p53, because BCLX gene, an apoptosis inhibitor, expression was up-regulated. In a case of DNA damage, the ATM gene is expressed in respond to double-strand DNA breaks which ATM protein will later phosphorylates p53 (Sengupta et al., 2005) and has for consequence of inducing apoptosis, DNA repair and cell cycle arrest (Bain et al., 2007). ATM gene expression was up-regulated without being significant in M05 (data not shown) and is thought to play an important role with M05 in telomere activity.

3.5.2.1.1.1 Effect on telomeres

Telomeres are specialized DNA-protein complex located at ends of linear chromosomes and consist of the same base sequence (TTAGGG) repeated several times. Telomeres allow chromosomal stability, protect chromosome ends against cellular exonucleases and non-homologous end-joining (NHEJ), and distinguish chromosome ends from DNA breaks (De Boeck et al., 2009). During chromosome duplication, the leading strand replicates in the 5′ to 3′ direction smoothly to the end of its template. However, lagging strand synthesis is unable to copy the parental strand completely, inevitably leaving the daughter strand shorter than the parental strand. All following replication results in a gradual loss of DNA at the chromosome end. The very end of the telomere is formed with a single-stranded 3′ containing the TTAGGG which is called the G-overhang. This overhang can loop back and invade homologous double-stranded telomeric tracts to form a large lasso-like structure called t-loop which would cap the end of the telomere (Verdun et al., 2007) via TRF1 protein. The formation of this structure has been proposed to mimic a DNA double-stranded break which involves the same proteins like ATM and would take place during the G2 phase (Verdun et al., 2005). Thus, the expression of ATM gene during telomere maintenance suggests that p53 was not phosphorylated and will not arrest the cell cycle progression (Verdun et al., 2005), but can accelerate telomere shortening if ATM is down-regulated (Metcalfe et al., 1996). When telomeres become critically too short, the cell cycle arrests and cells enter apoptosis (Hao et al., 2004), which might be a consequence to ageing (Aubert et al., 2008). Certain types of cells can bypass telomere shortening by producing telomerase that would add TTAGGG sequence on the leading strand (De Boeck et al.,

2009). Telomerase-reverse transcriptase (TERT) and telomerase RNA component (TERC) proteins are known to extend life span of the cells and prevent senescence (Bodnar et al., 1998; Lin et al., 2008). Both TERT and TERC genes expressions were up-regulated in this study when M05 was used with an average ratio of 4.4 and 2.6 respectively (Table 3.3). This up-regulation was unpredictable because these genes are not usually expressed in normal fibroblasts explaining why human fetal fibroblasts typically senesce after 60-80 population doubling (Campisi, 1996). Only, certain stem cells or germline populations which have long or indefinite life-spans express telomerase (Forsyth et al., 2002). Stimulation of telomerase expression could be useful in age-related diseases like dyskeratosis congenital, ataxia telangiectasia, and many others (Aubert et al., 2008). Our results suggest that HDF treated with laminaran from M05 could extend telomere length to ultimately prevent ageing. To confirm this hypothesis, further analyses are required.

3.5.2.1.2 Classical in vitro test on fibroblasts treated with laminaran

The effect of laminaran on the cell growth was validated. Cells were cultured during 6 days in presence of laminaran extracts, M05 and N05 at three concentrations (1.25, 2.5 and 5 mg/mL) (Figure 3.3). Results showed that M05 treatment did not modify cell number at a concentration of 1.25 and 2.5 mg/mL compare to the control (SVF 0.5%). However, there was a significant reduction of the cell number when a concentration of 5 mg/mL was used. This was also observed in presence of N05 at every concentration. The number of apoptotic cells were determined and showed to be significant for fibroblasts (~ 90%) treated with laminaran M05 compared to the control at each concentration (Figure 3.4) but the sample has interfered with the method (discussed next). Fibroblasts exposed with laminaran N05 showed few apoptotic bodies (< 5%).

The gene expression profile is complementary with the results obtained with the classical *in vitro* test. As seen earlier, cells treated with M05 seems to be able to proliferate normally as compared to the control. Cell growth related-gene expression was not modified for the concentration 2.5 mg/ml tested. It has been shown that human mammary epithelial cells

expressing telomerase proliferate normally compared to the control (Smith et al., 2003). Following N05 treatment, the reduction of proliferation was linked to the decrease of most of the cyclins and cell cycle mediator gene expression as previously detected. No apoptosis was observed in fibroblast treated with laminaran N05 (Figure 3.4) as also seen in the gene expression study. Several apoptosis genes like caspase7 (CAS7), clusterin (APOJ) and BCL2-associated X (Bax) genes expressions were down-regulated (Table 3.3). Notice that BCL2-like 1 (BCLX), an anti-apoptotic gene, was also down-regulated for N05 treatment indicating that this gene is not involved in the protection against apoptosis. Although, M05 showed a significant increase of the sub G0 cells number, it has to be noted that the laminaran samples probably have interfered with the results because M05 extract seem to have different affinity for the solvent compare to N05 extract as explained in the section 3.5.1. M05 has lower branching than N05 (Table 3.2), which might explain why M05 extract was more hardily dissolve in the culture media and interfered with the analysis. Visual observation of the cell treated with M05 showed no cell death and the number of cells afters 6 days was equivalent to the control. This confirms that M05 treatment does not stimulate cell death and could prevent apoptosis due to the up-regulation of the expression of BCLX, an anti-apoptotic gene (Table 3.3). Other research has shown that laminaran and oligo-laminaran from *Laminaria japonica* were found to have an anti-apoptotic activity on mouse thymocytes (Kim et al., 2006). This was determined by *in vitro* analysis and DNA microarray using a mouse chip. Other low molecular α-glucan has shown a reduction of cell proliferation via the induction of apoptosis against colon cancer cells and the glucans were less effective on human normal fibroblasts (Lavi et al., 2006), while others showed no apoptosis activity on promonocytic cell line treated with a β-glucan (Battle et al., 1998).

3.5.2.2 Effect on the extracellular matrix

3.5.2.2.1 Gene expression on fibroblasts treated with laminaran

Laminaran treatment modified the level of expression of extracellular matrix (ECM) gene by fibroblasts. Notice that there was no significant difference of ECM genes expression when A05 and J06 were used in comparison with the control. Nevertheless, several ECM genes expressions were significantly up-regulated when M05 was added to the culture

media. Matrix metalloproteinase 14 (MMP14), dermatopontin (DPT) and fibromodulin (FMOD) genes expressions were significantly up-regulated with an average ratio from 1.9 to 2.4 (Figure 3.5). FMOD protein has the ability to interact with collagen-I and –II and to control the fibril formation of fibrillar collagen. FMOD protein was found to be down-regulated in senescent cells. FMOD gene knock-out mice show a decreased tendon stiffness with irregular collagen fibrils that often show a reduced diameter (Svensson et al., 1999). DPT protein can inhibit cell adhesion; interacts with transforming growth factor β (TGF-β) resulting in the loss or the enhancement of its biological activity according to the system, and accelerates collagen fibrillogenesis (Okamoto et al., 2006). It is not clear if DPT protein interacts with collagen but it might regulate the diameter and the architecture of collagen fibrils. Collagen gene expressions were not significantly altered in this study compared to the control. MMP14 is a receptor and an activator of MMP2. A deficiency in MMP14 protein causes mouse arthritis development and connective tissue disease (Holmbeck et al., 1999). Importantly, tumor necrosis factor-α (TNF-α), a key mediator in immune and inflammatory response, can stimulate the expression of MMP14. In our assay, TNF-α and MMP2 genes were not significantly differently expressed compared to the control when M05 was used.

After N05 treatment, there was a significant down-regulation of MMP1, MMP14 and collagen-VI gene expression with an average ratio ranging from -2.5 to -8.0 (Figure 3.5). In usual, MMP are not express in normal healthy tissues (Parks et al., 1998), but are found increased in senescent or cancer cells or during wound healing (Fisher et al., 2009; Giambernardi et al., 1998; Gill et al., 2008). MMP are not only involves in ECM but can alter cell growth, apoptosis, cell-cell communication, cell migration and tumor (McCawley et al., 2001). MMP1 cleaves peptide bonds in native collagen resulting in the formation of fragmented collagen (Fisher et al., 2009), but not collagen-VI (Welgus et al., 1981). MMP14 cleaves collagen-I, -II and -III (Ohuchi et al., 1997). Collagen-VI gene expression is up-regulated during wound healing of human skin (Oono et al., 1993) and collagen-VI protein promote proliferation of serum starved fibroblasts (Ruhl et al., 1999), but this gene was down-regulated in our study.

3.5.2.2.2 Classical in vitro test on fibroblasts treated with laminaran

The specific activity of MMP secreted after laminaran treatment was confirmed with an *in vitro* test (Figure 3.6) assaying the whole catalytic properties of culture supernatant of treated cells. The results reflect a balance between MMP and TIMP, their inhibitor, quantities. Results showed a significant and a concentration dependant increase of the specific activity of MMP when M05 was used. When N05 was added, the MMP activity was not significantly different of the control whatever the concentration of N05 used. Fibroblast treated with laminaran increased the collagen-I secretion compared to the control (Figure 3.7). After M05 treatment, there was a dose dependant increase of collagen-I. However, fibroblasts treated with N05 showed a maximum of collagen-I secretion at 2.5 mg/ml. The amount of collagen type-I was found double with N05 treatment compared to M05 treatment at 2.5 mg/mL (42 ± 4 vs. 21 ± 4 ng/1000 cells).

There was a good correlation between the results obtained with the MMP specific activity at concentration 2.5 mg/mL and the gene expression study with M05 extracts. Following M05 treatment, fibroblasts showed an increase of MMP14 gene expression (Figure 3.5) and a significant increase of MMP secretion (Figure 3.6). N05 treatment has decreased MMP1 and MMP14 gene expression, while the MMP secretion remained similar to the control. Collagen-I was not significantly altered compare to the control in the gene expression study with M05 and N05 treatments. However, collagen-I secretion was increased compared to the control with both treatments. The discrepancy between both experiments can be explained by the protocols. The amount of collagen-I secretion was determined from cultured fibroblast treated with laminaran during 6 days, while the cell culture for the DNA microarray experiment was realised after 24h of treatment. Results also showed that fibroblasts treated with M05 secreted less collagen-I compared to N05. The lower level of collagen-I found with M05 treatment could be linked to the higher secretion of MMP. MMP14 cleaves collagen-I, -II and -III (Ohuchi et al., 1997). Also, both DPT and FMOD showed to interact or affect collagen-I (Okamoto et al., 2006; Viola et al., 2007).

In aged skin, higher amount of MMP1 are found compare to young skin (Fisher et al., 2009). MMP1 cleaves peptide bonds in native collagen resulting in the formation of

fragmented collagen which negatively affect the functional and structural integrity of skin. Our data showed that MMP1 gene expression was significantly down-regulated in presence of N05. Besides, a grifolan from mushroom *Grifola frondosa* induced a reduction of MMP1 secretion after UVA treatment of HDF (Bae et al., 2005). Ultraviolet irradiations are known to increase MMP-1 secretion which induces photodamage and skin ageing. This gives us a hint that laminaran might inhibit MMP1 secretion which will have for advantage of limiting collagen-I degradation.

β-glucan combined with collagen in a meshed reinforced wound dressing was shown to be a valuable treatment of partial thickness burns (Delatte et al., 2001). In addition, others have shown that glucan phosphate can stimulate the production of collagen-1 and -3 by HDF (Wei et al., 2002) probably through the recognition of glucan phosphate with two β-glucan receptor found on HDF (Kougias et al., 2001). Authors showed that laminaran was able to bind to only one of the two receptors as shown in an another cell line (Mueller et al., 2000).

The results obtained in this study combined with the information provided in the litterature indicate that laminaran could be valuable to improve wound healing. One crutial step in this process is the migration and the proliferation of fibroblast leading to an increase of the collagen-I and -III secretion (Mutsaers et al., 1997). However in aged skin, this process takes more time leading to a reduction of fibroblasts proliferation and collagen secretion (Ashcroft et al., 2002). Thus, laminaran could ultimately spead up wound healing in aged and normal skin by increasing fibroblasts proliferation and collagen secretion at the site of injury. Further analysis will be required to verify this hypothesis.

3.5.2.3 Effect on the immune and inflammatory pathway

As seen earlier, laminaran can interact with one of the two glucan receptors present on HDF. Moreover, glucan phosphate interacts with two β-glucan receptors on fibroblasts stimulating the gene expression of pro-inflammatory cytokine like interleukin 6 (IL6) and nuclear factor-kappa β (NFκβ) nuclear binding activity (Kougias et al., 2001). Others

showed that β-glucan derived molecules were able to stimulate IL6 gene expression by myofibroblasts (van Tol et al., 1999). The activation of NFκβ results in the production of several immune and inflammatory proteins such as: IL1β, IL6, IL8, ICAM-1 (Barnes et al., 1997). It is a reasonable hypothesis that laminaran could also stimulate immune and inflammatory genes since it can interact with one of the two β-glucan receptors. Our gene expression study showed that some immune and inflammatory genes expressions were down-regulated for N05, while no change was observed when A05 was used (Table 3.3). Moreover, gene expression of several cytokine was up-regulated in presence of M05 (IL8, IL11, GMCSF) and J06 (IL1β, IL6, GMCSF). The expression of TERT gene in fibroblast was also shown to up-regulate several cytokines such as: IL1α and β, IL6, IL8 and GMCSF (Kanzaki et al., 2003). The expression of immune and inflammatory genes is required to protect against infection. However, further analysis will be necessary to confirm if laminaran can modulate inflammation or an immune response.

3.5.2.4 Relation between telomere extension and the ECM

Most studies focusing on telomere showed that an increase of the expression of telomerase can prevent cell ageing. It has been demonstrated that the telomerase up-regulation in *in vitro* aged fibroblasts can restore dermal integrity (Funk et al., 2000). Using DNA microarray in young, senescent and telomerase expressing fibroblasts, authors have demonstrated that telomerase expression increase in aged cells can restore the molecular phenotype of young cells. The collagen-I and -III expression were found to be similar in young and telomerase expressing fibroblasts. However, the expression of MMP (3 and 10) and immune/inflammatory genes was reduced in telomerase expressing fibroblasts compared to senescent fibroblasts, but not completely inhibited. Our data showed higher level of collagen and MMP secretion in fibroblast treated with M05 compare to the control. But, MMP3 and MMP10 could not have been secreted because the activation time was too short to allow their secretion and the genes were not differentially expressed compared to the control. Funk and collaborator (2000) showed similarities with our research concerning collagen secretion and immune/inflammatory gene. Authors showed that telomerase expression could bring back the molecular phenotype of aged cells to a younger state and

the expression of MMP, collagen and immune/inflammatory gene remain elevated. We believed that laminaran M05 could possibly extent telomere length and prevent ageing. The others fractions (A05, N05, and J06) does not seem to have this ability.

3.5.3 Relation between structural characteristics of laminaran extracts and their metabolic activities

All the data presented here tend to show that laminaran M05 is different from the other extracts (A05, N05 and J06) according to the gene expression study. This was also validated by classical *in vitro* test, where cell growth, apoptosis, MMP specific activities, and the level of collagen-I secretion of fibroblasts treated with laminaran gave different results. Structural differences observed for the laminaran extracts could explain the variation observed in the metabolic activities.

Structural characterisation did not show up clear distinct structural feature. Linkage analysis tends to show that M05 could have less branching at position *O*-6 than A05 and N05, which would result in a different conformation in solution. Branched laminaran has a better solubitlity than linear one (Lahaye et al., 1997a). Laminaran and other β-glucan (glucan phosphate, scleroglucan) showed distinct structural features. Glucan phosphate is a nonbranched polymer composed of D-glucose linked in β-(1,3) with a molecular weight of 157 kDa, while scleroglucan is a much larger polymer (1020 kDa), highly branched with D-glucose linked in β-(1,3) with branching at position *O*-6 (Mueller et al., 2000). On the other hand, the laminaran used in this study is a small molecular weight (4-6 kDa) glucan with D-glucose linked in β-(1,3) with branching at position *O*-2 and *O*-6. Scleroglucan has the most rigid conformation in solution followed by glucan phosphate and laminaran (Mueller et al., 2000). Scleroglucan forms triple helix in water, while laminaran and glucan phosphate form single helix (Mueller et al., 2000). However, other showed that laminaran and glucan phosphate were able to form triple helix but with low sodium hydroxide concentration (Williams et al., 1991). Laminaran was showed to formed triple helix from freeze-dried DMSO solution (Saitô et al., 1986). The conformation of laminaran is strongly influenced by the solvent in which the polymer is dissolved (Frecer et al., 2000).

The polymer structure and its conformation have been proven to influence its binding capacity to a β-glucan receptor (Leung et al., 2006; Mueller et al., 2000) and consequently affect its biological activity. Dectin-1 is mostly expressed on monocyte/macrophage and neutrophil cells (Brown et al., 2005). Dectin-1 induces innate immune responses to fungal pathogens (Adams et al., 2008) and mediates various cellular functions like the production of cytokines (Brown, 2006). The binding affinity of several β-glucan to dectin-1 is dependant of the polymer chain length and side chain branching (Adams et al., 2008). Synthetic glucans showed with molecular modeling that six glucose units were essential for one helical turn. Thus, at least six glucose units linked in β-(1,3), one glucose side chain branch, and one full helical turn are required for the interaction with dectin-1. Also, high molecular weight β-glucan has a superior affinity with dectin-1. Many dectin-1 molecules could bound large glucan through the repeating units, increasing the affinity in an additive way (Adams et al., 2008). Glucan phosphate showed the highest affinity with dectin-1 followed by scleroglucan and laminaran. Scleroglucan (1020 kDa) lowest affinity compared to glucan phosphate (157 kDa), could be explained by the presence of glucose linked in β-(1,4) in scleroglucan limiting the interaction with many dectin-1 (Adams et al., 2008).

Scleroglucan and laminaran interacts selectively with one site on human promonocytic cell line (U937), while glucan phosphate interacts with both sites. Glucan phosphate showed lower binding affinity than scleroglucan and laminaran probably caused by the presence of phosphate groups and the absence of side chain branching (Mueller et al., 2000). On fibroblast, glucan phosphate was capable to bind to both β-glucan receptor, while laminaran was able to bind to only one receptor (Kougias et al., 2001). Also, the binding affinity was equivalent for both polymers based on molar concentration.

Up to now, it is not possible to relate a specific structure to a specific activity. Each β-glucan receptor showed good binding affinity for different polymer structure. It is clear that each receptor seems to have better affinity with some specific structure that they will most likely meet in the body. The recognition and interaction of β-glucan polymer with its

receptor is important, for example, for host defence against fungal infection (Adams et al., 2008).

3.6 Conclusion

Laminaran extract from brown seaweed *Saccharina longicruris* influenced fibroblast behaviour according to the harvest period. Laminaran can selectively to influence the fibroblast growth, the synthesis of MMP and collagen, and the expression of telomerase genes according to the harvest period. These results lead us to think that some laminaran could prevent fibroblast ageing and be valuable during wound healing by increasing the secretion of collagen-I. These activities are probably linked to the polymer conformation in solution and by the presence of side chain branching. Further analysis will be required to verify this hypothesis.

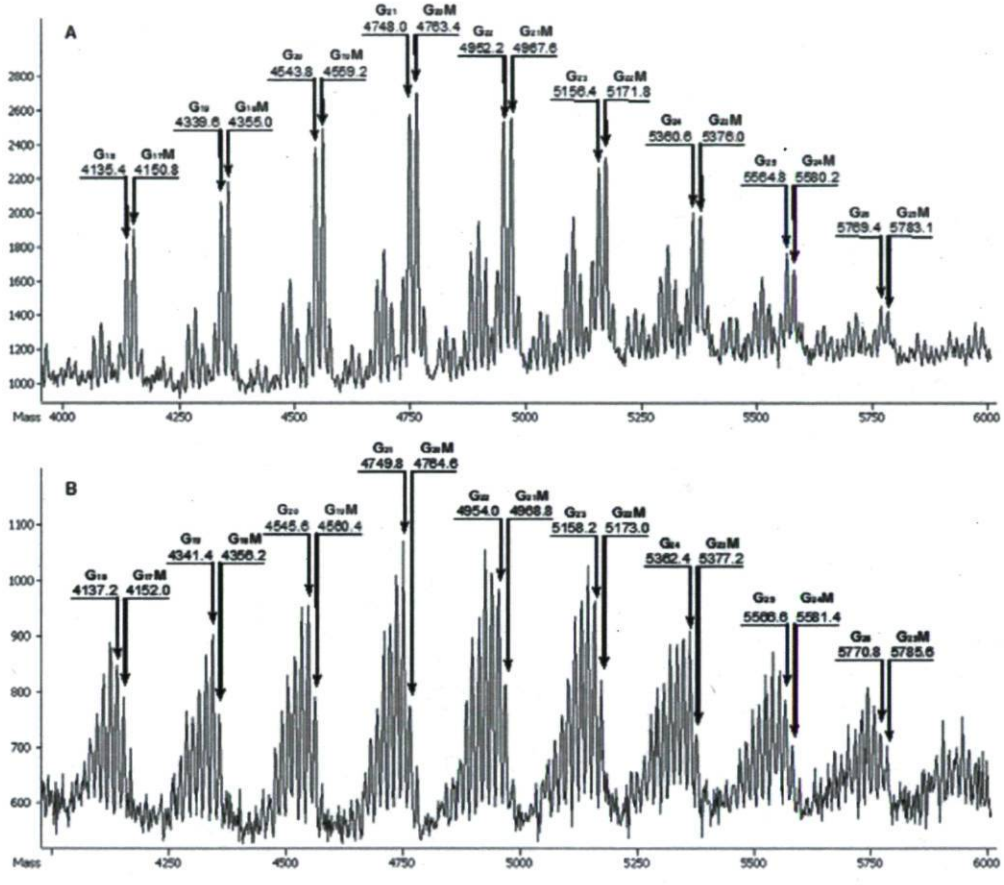

Figure 3.1 : Electrospray ionisation mass spectra of laminaran from (A) M05 and (B) A05.

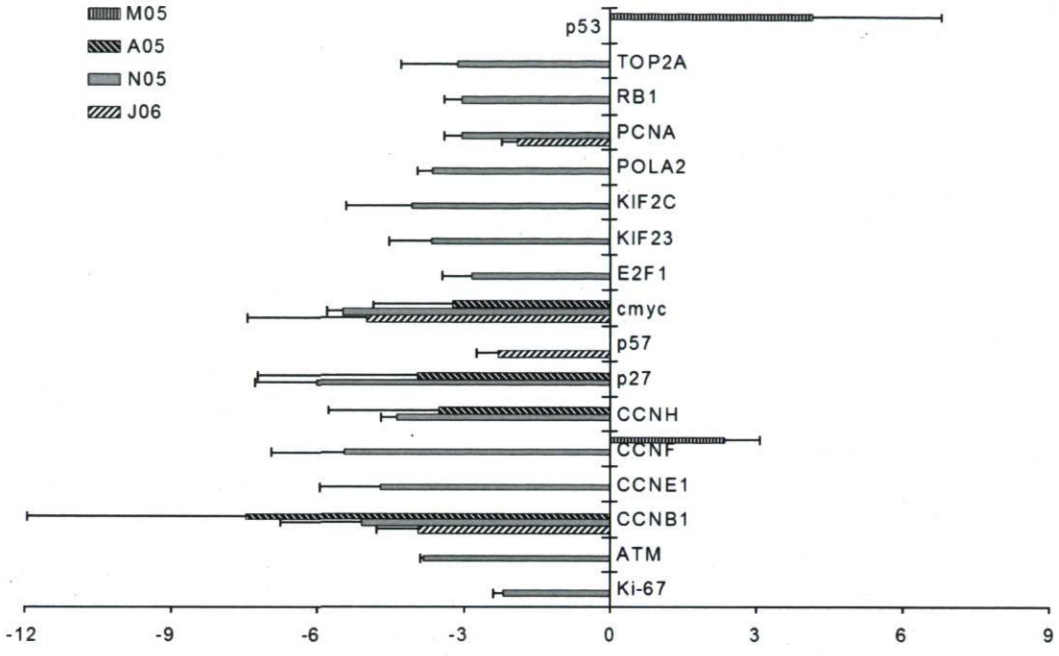

Figure 3.2 : Effect of laminaran on the cell cycle regulation.
Data are the average ratio ± SD carried out in three different experiments. Only significant up- and down-regulated genes were presented.

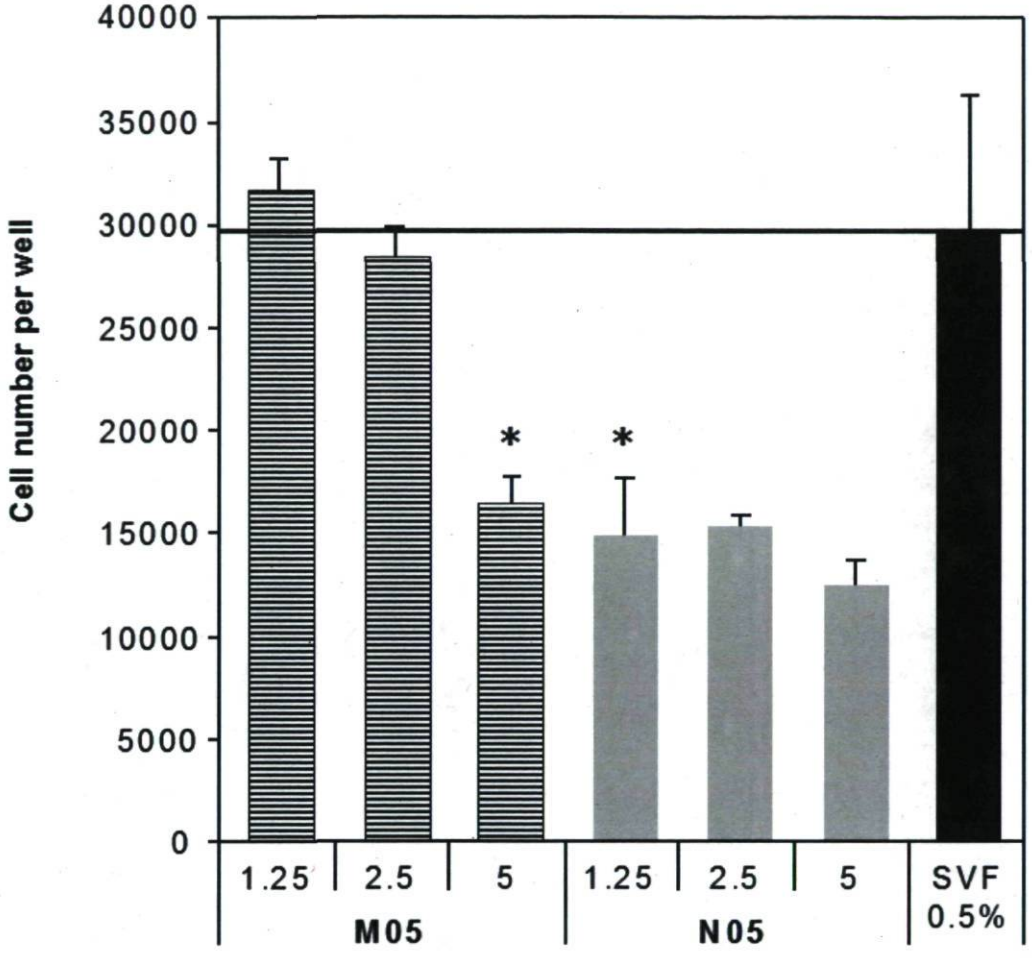

Figure 3.3 : Cell growth of human dermal fibroblasts treated with laminaran.
The number of cell was evaluated after 6 days in culture with laminaran extracts at different concentrations (1.25, 2.5 and 5mg/mL). Data are the means ± SD carried out in triplicate. Significant differences between cells treated with SVF 0.5% (control) and laminaran were calculated using Student test (*P<0.05).

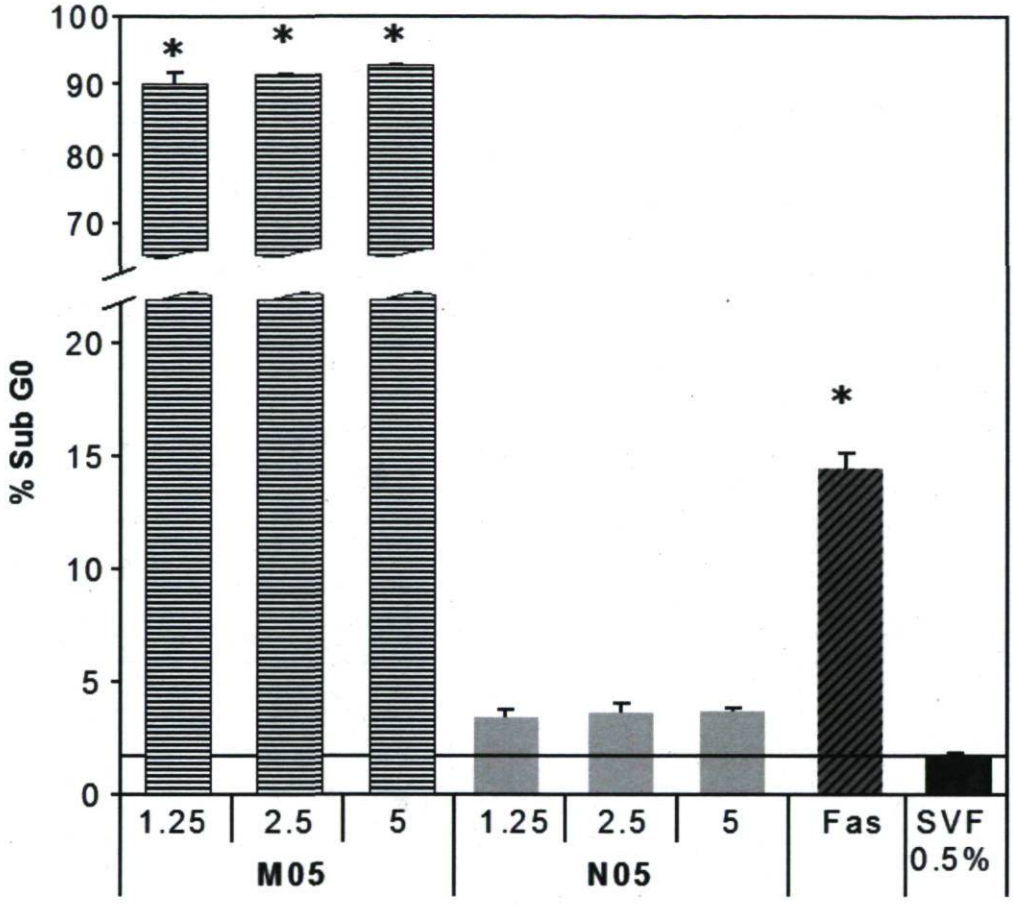

Figure 3.4 : The apoptotic cells in human dermal fibroblasts treated with laminaran. Percentage of cells stained with propidium iodide in sub G0 (apoptosis) phase after 24 h cell treatment with laminaran (1.25, 2.5 and 5 mg/mL) was presented. Fas was used as a positive control to induce apoptosis. Data are the means ± SD carried out in triplicate. Absence of error bars signifies an error which was too small to be illustrated. Significant differences between cells treated with SVF 0.5% (control) and laminaran were calculated using Student test (*P<0.05).

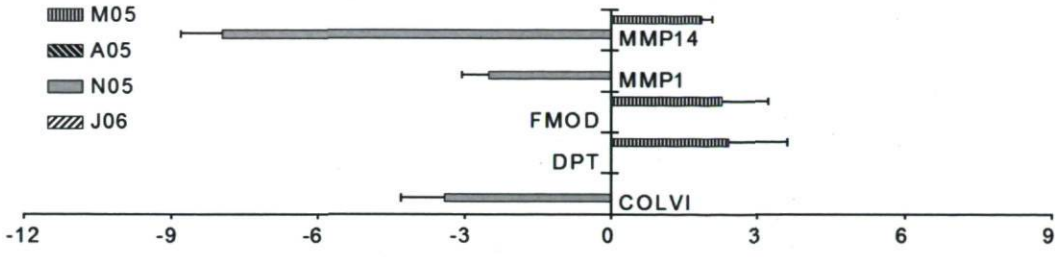

Figure 3.5 : Effect of laminaran on the matrix extracellular genes.
Data are the average ratio ± SD carried out in three different experiments. Only significant up- and down-regulated genes were presented.

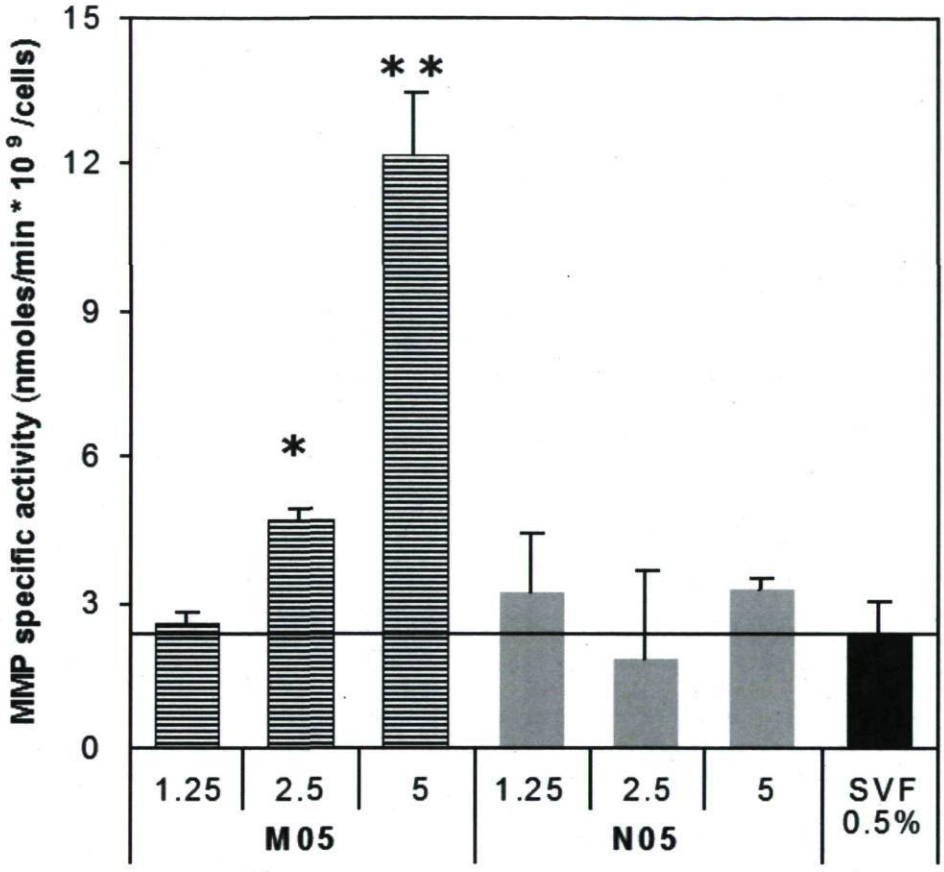

Figure 3.6 : MMP specific activity in human dermal fibroblasts treated with laminaran. Results reflect the overall MMP quantification from the supernatants of cell in culture with laminaran extracts at different concentrations (1.25, 2.5 and 5mg/mL). Data are the means ± SD carried out in triplicate. Significant differences between cells treated with SVF 0.5% (control) and laminaran were calculated using Student test (*P<0.05 and **P<0.01).

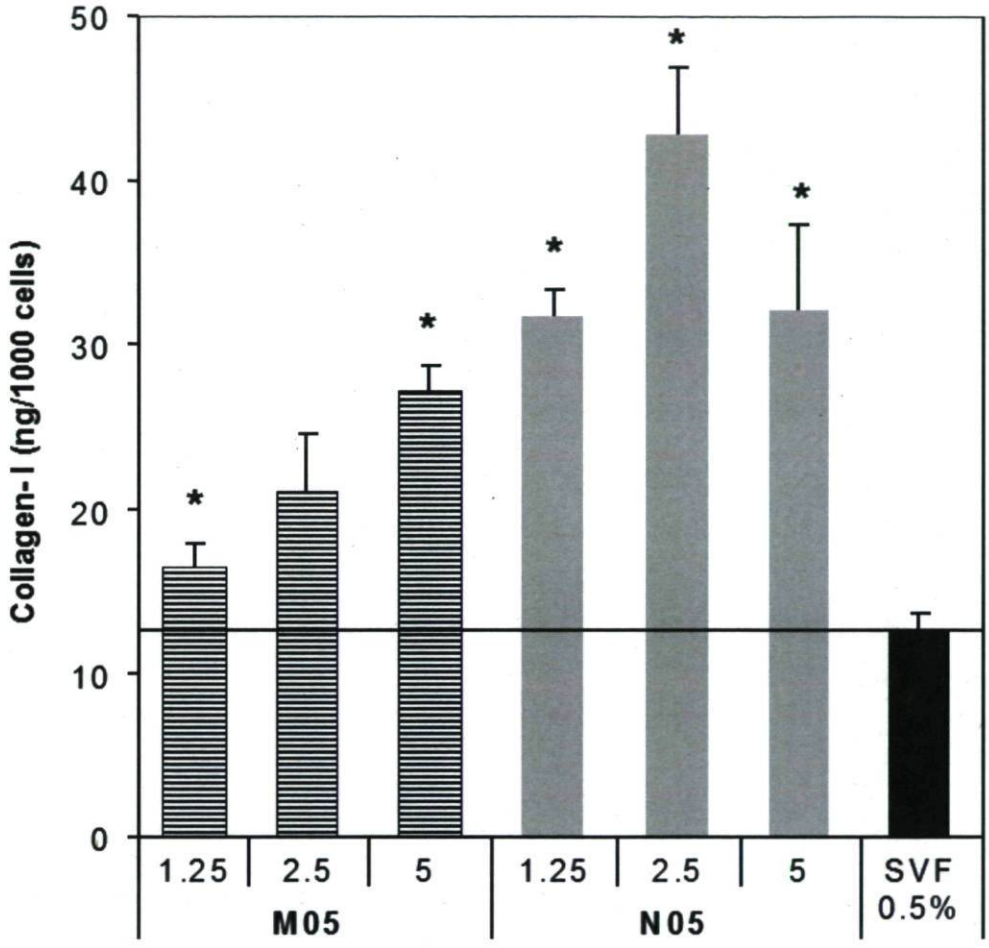

Figure 3.7 : Collagen-I secretion in human dermal fibroblasts treated with laminaran. Results reflect the collagen-I secretion from the supernatants of cell in culture with laminaran extracts at different concentrations (1.25, 2.5 and 5mg/mL). Data are the means ± SD carried out in triplicate. Significant differences between cells treated with SVF 0.5% (control) and laminaran were calculated using Student test (*P<0.05).

Table 3.1 : Composition in monosaccharide and molecular weight of laminaran from *Saccharina longicruris* for each harvest period.

Harvest period	D-glucose (%)	D-mannitol (%)	Mw* (Da)	Degree of polymerisation M-series	Degree of polymerisation G-series	Observed masse average (m/z)
M05	68.2 ± 4.3	1.2 ± 0.1	2894	17-25	18-26	4100-5800
A05	64.0 ± 4.5	1.3 ± 0.0	3125	17-25	18-26	4100-5800
N05	68.6 ± 3.5	1.6 ± 0.1	3317	17-24	18-25	4100-5600
J06	50.6 ± 9.8	1.2 ± 0.1	2995	16-24	17-25	3900-5600

* Molecular weights in equivalent of polyethylene glycol (PEG).

Table 3.2 : Analysis of methylated reduced laminaran alditol acetates from *Saccharina longicruris* from four harvest period.

Glycosyl residue	Position of *O*-methyl group	Deduced position of substitution	Detector response (%)			
			M05	A05	N05	J06
Glucosyl	2,3,4,6	Terminal	5.8	8.0	6.7	7.2
	2,4,6	3	87.4	76.8	84.2	79.0
	4,6	2,3	4.7	7.3	4.8	13.8
	2,4	3,6	2.1	4.1	4.3	nd

nd: not detected

Table 3.3 : Gene expression* from human dermal fibroblasts treated with laminaran.

General function	Gene names	Symbol	Swissprot accession number	Average ratio			
				M05	A05	N05	J06
Apoptosis	BCL2-associated X protein	BAX	Q8WXU1			-8.9 ± 2.2	
	BCL2-like 1	BCLX	Q07817	1.7 ± 0.7		-11.0 ± 6.0	
	Caspase 7	CAS7	P55210			-3.4 ± 0.9	
	Clusterin (apolipoprotein J)	APOJ	P10909			-2.3 ± 0.2	-2.1 ± 0.4
Cell cycle	Antigen identified by monoclonal antibody Ki-67	Ki-67	P46013			-2.2 ± 0.2	
	Ataxia telangiectasia mutated	ATM	Q13315			-3.8 ± 0.0	
	Cyclin B1	CCNB1	P14635		-7.5 ± 4.5	-5.1 ± 1.7	-3.9 ± 0.9
	Cyclin E1	CCNE1	P24864			-4.7 ± 1.2	
	Cyclin F	CCNF	P41002	2.3 ± 0.7		-5.4 ± 1.5	
	Cyclin H	CCNH	P51946		-3.5 ± 2.2	-4.4 ± 0.3	
	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	p27	P46527		-4.0 ± 3.3	-6.0 ± 1.3	
	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)	p57	P49918				-2.3 ± 0.4
	C-myc binding protein	cmyc	Q99417		-3.2 ± 1.6	-5.5 ± 0.3	-5.0 ± 2.5
	E2F transcription factor 1	E2F1	Q01094			-2.8 ± 0.6	
	Kinesin family member 23	KIF23	Q02241			-3.6 ± 0.9	
	Kinesin family member 2C	KIF2C	Q99661			-4.1 ± 1.3	
	Polymerase alpha 2	POLA2	Q14181			-3.6 ± 0.3	
	Proliferating cell nuclear antigen	PCNA	P12004			-3.1 ± 0.4	-1.9 ± 0.3
	Retinoblastoma 1	RB1	P06400			-3.0 ± 0.4	
	Topoisomerase (DNA) II alpha	TOP2A	P11388			-3.1 ± 1.2	
	Tumor protein p53	p53	P04637	4.1 ± 2.6			
Chromosomal processing	Centromere protein A	CENPA	P49450		-5.1 ± 3.1	-6.8 ± 1.6	-3.5 ± 1.1
	Centromere protein F	CENPF	P49454			-3.4 ± 0.7	
	Histone 1, H3i	H3FF	P16106			-2.6 ± 1.0	-2.5 ± 1.4
	Telomeric repeat binding factor 1	TRF1	P54274			-4.1 ± 0.7	
Extracellular matrix	Collagen, type VI, alpha 2	COLVI	P12110			-3.4 ± 0.9	
	Dermatopontin	DPT	Q07507	2.4 ± 1.2			
	Fibromodulin	FMOD	Q06828	2.3 ± 1.0			
	Matrix metalloproteinase 1 (interstitial collagenase)	MMP1	P03956			-2.5 ± 0.5	
	Matrix metalloproteinase 14 (membrane-inserted)	MMP14	P50281	1.9 ± 0.2		-8.0 ± 0.8	
Immune and inflammatory	Colony stimulating factor 2 (granulocyte-macrophage)	GMCSF	P04141	2.1 ± 0.4			5.3 ± 1.4
	Intercellular adhesion molecule 1	ICAM-1	P05362	2.1 ± 0.5		-2.6 ± 1.1	2.9 ± 1.5
	Interleukin 1, beta	IL1β	P01584				11.9 ± 5.5
	Interleukin 6 (interferon, beta 2)	IL6	P05231				15.9 ± 4.4
	Interleukin 8	IL8	P10145	10.5 ± 12.8			
	Interleukin 11	IL11	P20809	2.0 ± 0.1		-3.0 ± 0.7	
	Interleukin 11 receptor, alpha	IL11R	Q16542			-2.3 ± 0.2	
	Secreted phosphoprotein 1 (osteopontin)	OPN	P10451	8.6 ± 9.7			
Telomere maintenance	Telomerase RNA component	TERC	/	2.6 ± 0.5		-5.7 ± 3.5	
	Telomerase-reverse transcriptase	TERT	O14746	4.4 ± 3.4			

*Only significant differences between treated and untreated cells genes expression were noted.

Chapitre IV :
Modulation of metabolic activities induced by crude and depolymerised galactofucan extracted from brown seaweed *Saccharina longicruris*

4.1 Résumé

Le galactofucane (fucoïdane) est un polysaccharide sulfaté extrait des algues brunes qui a montré des activités anticoagulante, anti-thrombose, anti-inflammatoire, antivirale et anti-tumorale. Le galactofucane a été extrait de l'algue brune *Saccharina longicruris* en mai (M05), août (A05), novembre 2005 (N05) et juin 2006 (J06). Les galactofucanes bruts de M05 et N05 ont été dépolymérisés (RDP) durant 2 et 4 heures pour obtenir au total 4 fractions (M05 RDP2H, M05 RDP4H, N05 RDP2H et N05 RDP4H). La composition en monosaccharides, la position des liaisons glycosidiques et le poids moléculaire ont été déterminés pour chaque fraction. L'activité biologique a été étudiée à l'aide de puces à ADN et les résultats ont été confirmés par l'utilisation de tests *in vitro* classiques (croissance cellulaire, apoptose, sécrétion de MMP et de collagène). Les galactofucanes bruts montrent un poids moléculaire variant entre 638-1529 kDa alors que celui des fractions RDP est inférieur à 30 kDa. Les galactofucanes ont une structure complexe suite à la dépolymérisation. Les fractions RDP contiennent plus de groupements sulfates (30-39%) comparativement aux fractions brutes (13-20%). Des unités de fucopyranoses liées en (1,3) avec des groupements sulfate en position 4 et des unités de galactopyranoses liées en (1,6) avec des sulfates en position 3 ont été identifiés pour les galactofucanes bruts et RDP. Les fractions M05 et J06 ont montré une plus faible proportion de galactopyranoses liés en (1,6) possédant des sulfates en position 3 comparativement aux fractions A05 et N05. L'effet du galactofucane brut et RDP sur le profil d'expression génique de fibroblastes a mis en évidence des modulations du métabolisme du cycle cellulaire et au niveau de la matrice extracellulaire. Les galactofucanes bruts et RDP influencent la croissance des fibroblastes, l'apoptose, la sécrétion de MMP et de collagène-I en fonction de la période de récolte. Les fractions brutes ont une activité antiproliférative et peuvent entrainer une dégradation de la matrice extracellulaire due à une sécrétion accrue de MMP. Le galactofucane M05 stimule l'apoptose mais le mécanisme est encore inconnu. Les fractions RDP augmentent la sécrétion de collagène-I et maintiennent la croissance des fibroblastes. Ainsi, les galactofucanes bruts et dépolymérisés ont des activités métaboliques opposées. Ces différences sont attribuées au poids moléculaire, au taux et à la position des groupements sulfates qui varient d'une fraction à l'autre.

4.2 Abstract

Galactofucan (fucoidan) is a sulphated polysaccharide extracted from brown seaweed that have been shown to have anticoagulant, antitumoral, anti-thrombosis, anti-inflammatory, and antiviral activities. Galactofucan was extracted from brown seaweed *Saccharina longicruris* at four harvest periods (M05, A05, N05, and J06). Crude galactofucan M05 and N05 were depolymerised (RDP) during 2 or 4 hours to obtained 4 RDP fractions (M05 RDP2H, M05 RDP4H, N05 RDP2H, and N05 RDP4H). Molecular weights, monosaccharide composition, and glycosidic linkage by GC-MS were determined. DNA microarrays were used to determine the gene expression profile of fibroblasts treated with crude and RDP galactofucan. Classical *in vitro* tests (cell growth, apoptosis, MMP, and collagen secretion) were realised to validate the DNA microarray results. Crude galactofucan fractions showed a molecular weight ranging from 638-1529 kDa, while the RDP fractions have a molecular weight lower then 30 kDa. The structure of the galactofucan fractions remained complex after the depolymerisation. RDP fractions showed to be more sulphated (30-39%) than the crude fractions (13-20%). Crude and RDP fractions contained 3-linked fucopyranose 4-sulphate and 6-linked galactopyranose 3-sulphate. Galactofucans isolated from M05 and J06 contained less 6-linked galactopyranose 3-sulphate than A05 and N05 fractions. The gene expression profile of fibroblasts treated with galactofucan showed an influence on the expression of genes involved in the cell cycle and with the extracellular matrix components. Crude and RDP galactofucans influenced fibroblast growth, apoptosis, synthesis of MMP and collagen according to the harvest period. Crude galactofucan showed an anti-proliferative activity and an increase of the extracellular matrix (ECM) degradation caused by the increase of MMP secretion. Only, M05 stimulated apoptosis, but the mechanism is still unknown. RDP fraction increased collagen-I secretion and maintained fibroblasts proliferation. Thus, crude and RDP galactofucan exhibited different metabolic activities. This could be attributed to the molecular weight, the amount and the position of the sulphate groups that vary for each fraction.

4.3 Introduction

Sulphated polysaccharides are usually found in animal tissues and seaweeds and possess several biological activities (Zhang et al., 2004). Heparin is sulphated polysaccharide composed of a disaccharide repeating unit containing uronic acids (L-iduronic acid or D-glucuronic acid) (1,4) linked to D-glucosamine (Mulloy et al., 2000; Zhang et al., 2005a). Different patterns of *N*- and *O*-sulphation and *N*-acetylation were found. Unfractionated and low molecular weight (LMW) heparins are both used as anticoagulant drug. Besides this activity, heparin has a wide range of physiological effects such as effect on the secretion of extracellular matrix proteins (Ferrao et al., 1993; Gogly et al., 1999; Tyagi et al., 1997), influence on the expression of mitogen-activated gene (Fasciano et al., 2005) and activation of apoptosis (Erduran et al., 2007; Ueda et al., 2009).

Fucoidan, a sulphated polysaccharide extracted from brown seaweed, is believed to follow a similar mechanism of action to heparin (Patel et al., 2002) concerning the affinity on the cell surface (Logeart et al., 1997a; Logeart et al., 1997b). Some authors shown that fucoidan affects the secretion of extracellular matrix proteins (Moon et al., 2008), influences cell proliferation (Haroun-Bouhedja et al., 2000; Koyanagi et al., 2003) and can induce apoptosis (Aisa et al., 2005). Anticoagulant, antitumoral, anti-thrombosis, anti-inflammatory, and antiviral activities are well-known for fucoidan (Berteau et al., 2003; Boisson-Vidal et al., 1995).

Fucoidans are L-fucose polysaccharides containing sulphate groups and minor monosaccharides such as: D-galactose, D-xylose, D-glucose, D-mannose and D-uronic acid. No define regularity has never been observed in the fine structure (Kusaykin et al., 2008). Fucoidan from the *Fucaceae* family are well documented. Structural features of fucoidan from *Ascophyllum nodosum* showed L-fucose linked in α-(1,3) and α-(1,4) with lateral chain composed of single and multiple fucosyl units with branching in position 4 (Chevolot et al., 1999; Chevolot et al., 2001; Daniel et al., 2001; Daniel et al., 1999; Marais et al., 2001). The presence of sulphate groups at position *O*-2 and possibly at position *O*-3 and *O*-4 was discovered (Daniel et al., 2001). On the other hand, galactofucans are also

isolated from brown seaweeds and are often included in the fucoidan family. Galactofucans are sulphated polysaccharides containing an equivalent proportion of L-fucose and D-galactose (Hemmingson et al., 2006; Rocha et al., 2005). In one study, a backbone dominated by both 3-linked α-fucose and β-galactose was found (Hemmingson et al., 2006), while in another the main chain was formed with a 4-linked β-galactose with branches of oligosaccharides composed of 3-sulfated, 4-linked α-fucose (Rocha et al., 2005). Furthermore, anticoagulant, anti-thrombosis, and antiviral activities have been reported for galactofucans from brown seaweeds *Undaria pinnatifida*, *Spatoglossum schroederi*, and *Ecklonia kurome* (Hemmingson et al., 2006; Nishino et al., 1991; Rocha et al., 2005).

All the activities reported for heparin, fucoidan and galactofucan are closely related to the structure of the polysaccharide. The composition in monosaccharide, amount and position of sulphate groups, molecular weight and conformation are recognised to influence its bioactivity. For example, LMW heparins are known to improve the anticoagulant activity, to have longer plasma half-life and a better bioavailability at low doses, and a more predictable dose response (Hirsh et al., 2001). Others showed that the anti-proliferative activity of fucoidan and heparin is related to its molecular weight (Kanabar et al., 2005; Logeart et al., 1997a). Fractions with a molecular weight superior to 15 kDa inhibited cell proliferation, while smaller fractions maintained cell proliferation (Logeart et al., 1997a). Thus, it is predictable that crude and LMW fucoidan could have different activity.

Due to their different structures, galactofucans are believed to induce distinctive metabolic activities compare to fucoidan and heparin. The aim of this work is to test various samples of galactofucan isolated from *Saccharina longicruris* on fibroblasts using DNA microarray to search for novel activities by the mean of the gene expression profile studies. Several biological activities were validated with classical *in vitro* tests. Moreover, a relationship between the structure and biological properties of galactofucan is proposed.

4.4 Experimental

4.4.1 Algal materials

Galactofucan was extracted from *Saccharina longicruris* harvested in May (M05), August (A05), November 2005 (N05) and June 2006 (J06) at Gaspé (Québec, Canada). The seaweeds were milled in a disintegrator (Rietz Manufacturing Co., Santa Rosa, USA) fitted with perforated plates of 4.65mm, vacuum sealed and kept at -30°C until use.

4.4.2 Extraction procedures

Galactofucans were extracted from milled seaweeds (Nishino et al., 1989). Seaweeds (300 g) were mixed with HPLC grade H_2O and 1% (w/v) of $CaCl_2$. Each mixture was stirred at 85°C for 4h, and then centrifuged (16 887 g, 20 min). The supernatant was isolated by vacuum filtration on Whatman #4 filters. Each filtrate was mixed with 1 volume of 2% NaCl and 2 volumes of EtOH (95:5, v/v), and then stirred for 1h at room temperature and stored at -20 °C for 48h. Each mixture was centrifuged (16 887 g, 10 min) to isolate the pellets containing crude galactofucans. The pellets were hydrated and dialyzed using a 15 kDa cut-off membrane during 48h. Crude galactofucan fractions were then freeze-dried. At least three extractions per harvest period were realised and pooled together to generate enough starting material for the bioactivity study and structural characterisation.

Fractions will be presented as crude galactofucan M05, A05, N05 or J06 which refers to their respective harvest period.

4.4.3 Radical depolymerisation (RDP)

RDP of crude galactofucan was realised (Nardella et al., 1996). Galactofucan (3 g) was solubilised overnight in 200 mL of HPLC grade H_2O. The galactofucan solution was mixed with 600 mg of copper acetate monohydrate in a temperature controlled reactor (60°C). The pH was maintained at 7.5 by automatic addition of 1 M NaOH. Hydrogen peroxide (H_2O_2,

12.5% (v/v)) solution was added with a peristaltic pump at a flow rate of 43.2 mL/h. After, 2h (RDP 2H) or 4h (RDP 4H), the mixture was vacuum filtrated with Whatman GF/C filters. The reaction was stopped by adding 2 g of $NaBH_4$ previously dissolved in 30mL of HPLC grade H_2O. The solution was stirred during 2h, neutralised with 10 N of acetic acid and filtrated two times on Whatman GF/C filters, one time with Whatman #4 filters and one time Whatman #1 filters. The filtrate was concentrated with a rotative evaporator and purified by Chelex 100 chelating resin (Bio-Rad) to remove copper from the medium. The solution was dialysed using a 1 kDa cut-off membrane during 48h and freeze-dried.

Fractions will be presented as RDP galactofucan M05 (M05 RDP2H or M05 RDP4H) and N05 (N05 RDP2H or N05 RDP4H) which refers to their respective harvest period.

4.4.4 Structural characterisation of the galactofucans extracts

4.4.4.1 Desulphation (DS)

50 mg of crude or RPD galactofucan fractions were purified with a Dowex cation exchange resin (Sigma, Canada). Pyridium salt was formed by adding 500 µL of pyridine (Sigma) and freeze-dried. 4 mL of anhydrous DMSO and 1 mL of MeOH were added. The mixture was heated at 100°C during 4h under stirring. The solution was neutralised, dialysed using 1 kDa cut-off membrane (4°C, 48h) and freeze-dried. Residual sulphate groups' content was verified with IR and ICP-OES and less than 1% of sulphate groups were found in each fraction.

Sulphur content of the galactofucans was quantified by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) using the model Optima 4300DV from Perkin-Elmer (Boston, USA) equipped with Winlab32 software. The sulphate content was deduced from the amount of sulphur determined by ICP using the following equation: % sulphate group = 3.22 x S (Roger et al., 2004).

4.4.4.2 Monosaccharides analysis

Neutral sugars, after acidic methanolysis of the galactofucan and subsequent GC analysis were identified as trimethylsilyl derivatives as described in chapter 2.

4.4.4.3 Methylation analysis

Methylation analysis of crude and RDP galactofucan was performed using modification of three methods (Hakomori, 1964; Hellerqvist, 1990; Waeghe et al., 1983) as described in chapter 3.

4.4.4.4 HPSEC-molecular weight determination of depolymerised galatofucans

The polysaccharide weight average, Mw, was determined by HPSEC (High Performance Size Exclusion Chromatography (Rioux et al., 2009). Two columns were used in series: TSK-guard column PWXL (6 mm X 40 mm) and a TSK-G4000 PWXL (7.5 mm X 300 mm) (Tosoh Bioscience, Montgomeryville, USA). The molecular weight separation range for the polysaccharides was established from 2 to 30 kDa polyethylene glycol (PEG) equivalent by the supplier. A molecular weight calibration curve was constructed with five PEG standards from 1400 to 20 100 Da (Phenomenex, inc., Torrance, CA, USA) to estimate the polysaccharides' Mw.

4.4.4.5 HPSEC-MALLS-molecular weight determination of galactofucans

The polysaccharide molecular weight averages, Mw, were determined by HPSEC-MALLS (High Performance Size Exclusion Chromatography-Multiangle Laser Light Scattering) as detailed in chapter 2.

4.4.4.6 Infrared spectroscopy

Infrared spectroscopy analyses were realised on crude and depolymerised galactofucan as well as the desulphated fractions. A Nicolet 506 apparatus was used and spectra between

750 and 3000 cm^{-1} were recorded. The samples were analysed as KBr pellets (1 mg of galactofucan mix with 100 mg of KBr).

4.4.5 Gene expression study

4.4.5.1 Cytotoxicity assessment of crude and RDP galactofucan extracts

Human dermal fibroblasts (HDF) from newborn were classically grown as presented in chapter 3.

Cell viability was realised has described in chapter 3, but with slight modifications. The test was realised by applying crude galactofucan at concentrations of 0.125 to 2 mg/mL and RDP galactofucan at concentrations of 0.5 to 2 mg/mL in cultured cells for 24h to determine the optimal concentration for the gene expression study. High cell survival was observed for crude and RDP galactofucan at 2 and 1 mg/mL respectively, which was the concentration used for the gene expression experiment.

4.4.5.2 Cell culture

The study was carried out with HDF cultivated in the same conditions listed above. For this experiments 500 000 cells were seeded in 75 cm^2 flasks. At 80% confluence, cells were treated with crude galactofucan at a concentration of 2 mg/mL and RDP galactofucan at a concentration of 1 mg/mL during 24h. Three cell cultures were realised for each crude and RDP galactofucan extract.

4.4.5.3 Extraction of total RNA

The extraction was performed with the RNeasy Mini Kit (Qiagen). The culture medium was decanted from each flask and cells were washed 2 times with sterile PBS. Cells were detached with trypsin and a medium containing 10% of foetal bovine serum was added. Cells were transferred in a tube and centrifuged (300 g, 5 min). The pellet was rinsed 2 times with PBS and cells were disrupted by adding 350µL of Buffer RLT. Lysed cells were

transferred in an RNAse-free tube and homogenised. For the RNA extraction, 1 volume of 70% ethanol was added in the lysate and mixed thoroughly by pipetting. 700 µL of the sample were transferred to an RNeasy spin column and centrifuged (8000 g, 15 sec). 700 µL of Buffer RW1 were added to the column and centrifuged (8000 g, 15 sec). 500 µL of Buffer RPE were added to the column and centrifuged (8000 g, 15 sec). 500 µL of Buffer RPE was added to the column and centrifuged (8000 g, 2 min). For the RNA elution, 30 µL of RNAse-free water were added to the column and the RNA was stored at -80°C.

4.4.5.4 RNA integrity and concentration verification

The integrity and quality of RNA templates were checked as described in chapter 3.

4.4.5.5 Reverse Transcription and incorporation of a biotin-dNTP mixture during cDNA synthesis

The cDNA were synthesised and biotin labelled as detailed in chapter 3.

4.4.5.6 Hybridization on DualChip® Human Aging (Eppendorf, 0038 950 003)

The DualChip® Human Aging contains 227 genes involved in stress and ageing related pathways (extracellular matrix, keratinocyte differentiation, DNA damage, oxidative stress, cell signalling, cell proliferation, DNA repair, apoptosis, and inflammation) and 13 housekeeping genes for data normalization (the gene list is available in Annex 2). The complete presentation of the chip and the hybridization method are detailed in chapter 3.

4.4.5.7 Silverquant detection

The method is detailed in chapter 3.

4.4.5.8 Array scanning

The method is detailed in chapter 3.

4.4.5.9 Data quantification

The method is described in chapter 3.

4.4.6 Classical in vitro tests

Classical *in vitro* tests were performed to validate the results obtained with DNA microarray. Cell growth, apoptosis, matrix metalloproteinase (MMP) and collagen-I secretion were analysed. All the methods used are exhaustively described in detail in chapter 3.

4.5 Results and discussion

4.5.1 Structure of crude and depolymerised galactofucans

The monosaccharide compositions of crude and depolymerised (RDP) galactofucans showed differences among the fractions. Results showed few variations in D-xylose, D-mannose, D-glucose, D-galacturonic acid, and D-glucuronic acid contents for the crude galactofucan according to the harvest period (Table 4.1), while the L-fucose and D-galactose contents showed important variations. In M05, the amount of L-fucose was equivalent to the amount of D-galactose (12.7% vs. 16.7%), while a lower proportion of L-fucose compared to D-galactose was found for the other crude fractions; A05 (12.9% vs. 36.8%), N05 (14.4% vs. 33.1%), and J06 (9.4% vs. 25.0%). These results suggest that M05 has a different structure than the other fractions which could lead to different biological activities.

Crude galactofucans were treated by radical depolymerisation (RDP) to reduce their molecular weight to gain a better knowledge of its structure and to verify the effect of molecular weight on the biological activity. As A05, N05 and J06 had similar composition, only crude M05 and N05 were depolymerised during 2 or 4 hours. The longer is the depolymerisation, the smaller is the polysaccharide. Thus, a depolymerisation time of 2h

should produce galactofucan with a higher molecular weight distribution than a 4h treatment.

The monosaccharide composition of the RDP fractions was different than their respective crude fractions (Table 4.1). M05 RDP2H and 4H showed lower amounts of D-xylose, D-mannose, D-glucose, and D-glucuronic acid compared to the crude M05 fraction. Moreover, the proportion of L-fucose was increased for M05 RDP2H and 4H (25.5% and 19.9 % respectively), while the amount of D-galactose remained similar (14.2% and 9.6%). For N05 RDP2H and 4H, the proportion of L-fucose and D-galactose remained similar to the crude one. 6.8% and 11.6% of L-fucose were found respectively for N05 RDP2H and 4H, while 31.1% and 23.8% of D-galactose was found. Also, N05 RDP2H and 4H showed a reduction of D-xylose, D-mannose, D-glucose, and D-glucuronic acid compared to the crude N05 fraction.

Crude fractions M05, A05 and N05 showed a proportion of sulphate groups varying between 17.6-20.0%, while J06 contained 13.1% (Table 4.1). RDP2H fractions contained less sulphate groups than the RDP4H fractions. M05 RDP2H and N05 RDP2H contained respectively 35.6% and 30.3%, while M05 RDP4H and N05 RDP4H contained 38.7% and 32.4% of sulphate groups. The molecular weight of crude and RDP fraction was determined by MALLS and/or HPSEC. Results showed that M05 has an higher molecular weight (1529 kDa) compared to A05 (851.0 kDa), N05 (638.0 kDa) and J06 (765.0 kDa) that were similar. RDP fractions showed an important reduction of the molecular weight which was anticipated. But, there was only a slight difference between fraction RDP2H and 4H. M05 RDP2H and 4H showed a molecular weight of 23.3 kDa and 4.8 kDa respectively. N05 RDP2H showed a molecular weight of 30.2 kDa, while N05 RDP4H has a molecular weight between 6.1-10.4 kDa according to the analytical method used. Others have isolated a galactan of 8.5 kDa after 3h of depolymerisation (Zúñiga et al., 2006), while a 7.7-8.3 kDa fucoidan was isolated after 5h of depolymerisation (Nardella et al., 1996).

Authors showed with a depolymerised fucoidan of 8.3 kDa, a small increase of L-fucose (31.3% vs. 36.4%) and sulphate groups (26.1% vs. 29.7%) content compared to the crude

fraction (Nardella et al., 1996). Another research with a galactan (8.5 kDa) made the same observation; depolymerisation only causes a small increase in the level of galactose (43.4% vs. 49.8%) and sulphate groups (22.2% vs. 24.7%) compared to the crude fraction (Matsuhiro et al., 2005; Zúñiga et al., 2006). Both fucoidan (Nardella et al., 1996) and galactan (Zúñiga et al., 2006) showed a reduction in the level of uronic acid content in the depolymerised material compared to the crude fraction. The same tendency was observed in our study for M05 and N05 RDP. Moreover, the M05 RDP fractions showed an increase of the level of L-fucose compared to the crude M05 fraction, but the level of D-galactose remained the same. The level of L-fucose and D-galactose remained the same for the N05 RDP extracts. However, both M05 and N05 RDP fractions showed an important increase in the amount of sulphate groups.

The infrared spectra of crude and RDP galactofucan confirmed significant amount of sulphate groups (Figure 4.1). The bands at 1230 and 1260 cm^{-1} were assigned to asymmetrical stretching of S=O (Lijour et al., 1994) (Figure 4.1A, C and D). This was supported by the disappearance of these bands after solvolytic desulphation of crude extract (Figure 4.1B). The sulphate band at 850 cm^{-1} was reported to be related to a C-4 axial position on fucose (Patankar et al., 1993) or galactose (Melo et al., 2002). This was observed for all crude and depolymerised fractions (Figure 4.1A, C and D). A shoulder was observed at 820 cm^{-1} in all crude galactofucans (Figure 4.1A), while depolymerised fraction showed bands at 820 and 835 cm^{-1} (Figure 4.1C and D). For galactan, bands at 820 and 830 cm^{-1} consisted respectively of a 2-sulfated galactose and a 6-sulfated galactose (Melo et al., 2002; Mollet et al., 1998). For fucoidan, a shoulder at 820 cm^{-1} or so suggested sulphate groups in equatorial position at C-2 and/or C-3 (Marais et al., 2001; Patankar et al., 1993). Sulphate groups' assignation is determined by methylation analysis. Finally, the large band between 1030-1168 cm^{-1} or so was assigned to an hemiacetal stretching (Patankar et al., 1993). The peak at 1425 cm^{-1} or so was coherent with the presence of uronic acid (Rupérez et al., 2002) and the peaks heights were different for each crude fraction. Results showed that J06 should have a higher proportion of uronic acids than the other fraction. This was confirmed by the monosaccharide composition. J06 has a total of

6.2% of uronic acids, while M05, A05 and N05 contained respectively 3.5%, 2.3% and 4.2%.

Methylation analysis was performed on each fraction (Table 4.2). Results showed a complex and heterogeneous structure. The presence of L-fucose, D-galactose, D-xylose, and D-glucose as terminal units indicates the presence of a highly branched polysaccharide. Only traces (< 1%) of fuco-and galactofuranose residues were found, thus the pyranosyl terminology was used all over this paper. At this point, it is not clear whether the results were issued from a glycosyl linkage or a sulphate ester substitution. Thus, the results will be presented as substitution patterns.

For the crude fraction, results showed the presence of 3-substituted fucopyranose (11.8-13.5%) with a smaller proportion of 4-substituted fucopyranose (3.5-7.8%). There was also a large proportion (> 5.9%) of 2,3- and 3,4-disubstituted fucopyranose for most fractions except for N05 (~3%). Also, 2,3,4-trisubstituted fucopyranose were found, ranging from 6.9-12.2%. The presence of 3- and 6-substituted galactopyranose residues were found in higher proportion, 5.7-12.0% for A05 and N05, while M05 and J06 contained less than 3.9%. Similar observation was realised with 4,6-disubstituted galactopyranose which was higher in A05 and N05 (~8%) compared to M05 and J06 (~3%). All crude fractions contained a large proportion (> 5.1%) of 3,6-disubstituted galactopyranose residue. M05 and J05 contained larger amounts of 2,3,6-trisubstituted mannopyranose and terminal xylose. Each crude fraction also contained equivalent proportion of 4-substituted xylopyranose and terminal glucose. As seen in the GC results, the total of fucosyl units were equivalent for each crude fraction (~50%). However, A05 and N05 contained much more galactosyl units (~50%) than M05 and J06 which contained less than 30%. This was not completely in agreement with the GC results because J06 had a higher proportion of D-galactose than M05 which was not reflected in the GC-MS results. Thus, M05 and J06 were not really different in terms of structure as we have anticipated.

The RDP showed similar substitution patterns to their respective crude fraction. However, no glucose substitution was observed and lower levels of terminal mannosyl and xylosyl

units were found in RDP fraction. As seen in the crude fractions, RDP M05 and N05 were different. M05 RDP fraction showed low levels of 3- and 6-substituted galactopyranose residue (<3.3%) compared to N05 RDP (6.4-9.3%). This is in agreement with the GC analysis, where smaller amounts of D-galactose were found in M05 RDP compared to N05 RDP.

After solvolytic desulphation (DS), there were few remaining sulphate groups (< 1%, data not shown) which was confirmed by FTIR (Figure 4.1). For the crude and RDP fractions, the content of 4- and 6-substituted galactopyranose increased with the concomitant decrease of respectively 3,4- and 3,6-disubstituted galactopyranose contents. Also, results showed the disappearance of 3,4,6-trisubstituted galactopyranose with the simultaneous increase of 4,6-disubstituted galactopyranose. These data suggests the presence of sulphate groups at position *O*-3. Moreover, only little amount of sulphate groups at position *O*-2 can be found on galactose units caused by the decrease of 2,3-disubstituted galactopyranose. Simultaneously, 2,3- and 3,4-disubstituted fucopyranose as well as 2,3,4-trisubstituted fucopyranose decreased considerably although, there was no increase of 3- and/or 4-substituted fucopyranose after desulphation. Besides, the total amount of fucosyl residues decreases drastically for each fraction after desulphation with the concomitant increase of an unknown glycosyl substituent. This could be caused by the desulphation step which might initiate the rearrangement of fucose units after sulphate groups' removal. FTIR results showed an important proportion of axial sulphate groups at position *O*-4. Since D-galactose was mostly *O*-3 sulphated, only L-fucose could be sulphated at position *O*-4. This was in agreement with the disappearance of 2,4- and 3,4-disubstituted fucopyranose. It was not excluded that L-fucose units might have been di-sulphated due to the disappearance of 2,3,4-trisubstituted fucopyranosyl units but it could not have been verified in this experiment. The proportion of 3,4-disubstituted fucopyranosyl was much larger (11.4-20.9%) in M05 and M05 RDP when compared to N05 and N05 RDP. This suggests that sulphate groups might have been mostly substituted on fucosyl units for M05 and M05 RDP. This could be explained by the amounts of sulphate groups in the RDP fraction. M05 RDP 2H and 4H contained more sulphate groups (~5%) than N05 RDP 2H and 4H (Table 4.1).

In summary, all fractions contained 3-linked fucopyranose 4-sulphate. This amount was increased in the RDP fraction because they contained higher amounts of sulphate groups. Also, all fractions contained 6-linked galactopyranose 3-sulphate and 4,6-linked galactopyranose, which in M05, M05 RDP and J06 was 3-sulphated. A similar galactofucan from *Undaria pinnatifida* was isolated in another study but with a smaller molecular weight (290 kDa vs. 1529 kDa for M05 and 638 kDa for N05). A galactofucan backbone dominated by both a 3-linked fucopyranose 2,4-disulphate and 3-linked galatopyranose was found (Hemmingson et al., 2006). A small proportion of 6-linked galactopyranose 3-sulphate was also observed. Another highly purified galactofucan from *U. pinnatifida* with a much smaller molecular weight (9 kDa) was studied (Lee et al., 2004). The structure analysis showed a backbone dominated with 3-linked fucopyranose with 3-, 4- and 6-linked galactopyranose. Sulphate groups were at position *O*-2 on the fucose units, while they were at position *O*-3 or *O*-6 on the galactose residues (Lee et al., 2004). Both research showed an antiviral activity against *Herpes simplex* type 1 and 2 and cytomegalovirus, but the higher molecular weight galactofucan (290 kDa) needed a lower dose of polysaccharide to induce an antiviral activity (IC_{50}) (Hemmingson et al., 2006; Lee et al., 2004). Experimental designs were different in both studies, but they tend to show that purified polysaccharides does not always provide an amplified activity because the high molecular weight fractions showed a higher content in sulphate groups (0.94 sulphate ester groups per sugar residue vs. 0.72) than the lower molecular weight fractions. Others showed that the antiviral activity was reduced of 14-62 times when the fucoidan was desulphated (Adhikari et al., 2006).

The low molecular weight galactofucan (9 kDa) showed similarities with our RDP fraction specially N05 because this fraction also contained 3-and 4- linked galactopyranose and a higher proportion of 6-linked galactopyranose (Lee et al., 2004). The major difference was on the sulphate groups' position, no sulphate groups at position *O*-2 on the fucose units and at position *O*-6 of galactose residues groups were found in our study. The position of sulphate groups was found to be important for anticoagulant activity where sulphate groups need to be at position *O*-4 (Pereira et al., 2002). In the case of an antiviral activity, the amount seems to be more important than the position of sulphate groups (Hemmingson et

al., 2006; Lee et al., 2004). It is clear that according to the seaweed species, the structure changes. It was interesting to see that 9 kDa and 290 kDa galactofucans from *U. pinnatifida* and the galactofucan from *S. longicruris* share structural similarities even if, the latter was much more complex and heterogeneous. Interestingly enough, both seaweeds are part of the same order: the *Laminariales*.

4.5.2 Metabolic activities induced by crude and depolymerised galactofucans extracts

DNA microarray analysis was used to determine the gene expression profile modifications after fibroblast treatment with 4 crude galactofucan fractions: May (M05), August (A05), November (N05) 2005, and June 2006 (J06). Four depolymerised (RDP) galactofucan fractions from M05 and N05 were also studied (M05 RDP 2H, M05 RDP4H, N05 RDP2H and N05 RDP4H) (Table 4.1). Only significant gene expression modifications (P-values < 0.05 or 0.01) when compared to their respective control on the same microarray chip were presented. Results obtained with each crude (Table 4.3) and RDP (Table 4.4) galactofucan extracts were separately presented. Genes involved in the cell cycle regulation, chromosomal processing, extracellular matrix, immune, and inflammation were significantly up- or down-regulated compared to the untreated cells. Genes from the same functional activity were grouped and analysed together for all extracts. Classical *in vitro* tests were performed to validate the results obtained with DNA microarray. Cell growth, apoptosis, matrix metalloproteinase (MMP) and collagen-I secretion were analysed.

4.5.2.1 Anti-proliferative activity of crude galactofucan extracts

4.5.2.1.1 Gene expression on fibroblasts treated with crude galactofucan

Several genes involved in the cell cycle regulation were significantly up- or down regulated after all galactofucan fraction treatments like: cyclin-dependent kinase inhibitor 1B (p27), inhibitor of DNA binding 1 (ID1) and 2 (ID2) (Figure 4.2). The secretion of p27 protein inhibits the phosphorylation of retinoblastoma 1 (RB1) by the complex of cyclin E/cyclin-

dependant kinase (cdk)-2 during the G1 phase. This outcome limits the cell cycle progression by restricting the transition into S phase. The expression of p27 gene was up-regulated (from 3.2 to 8.3 fold increased) after treatment with all crude extracts. Moreover, ID1 and ID2 proteins are necessary to enter S phase (Hara et al., 1994) and the expression of these genes was down-regulated after treatment with each galactofucan extract with a ratio ranging from -1.9 to -7.7.

Other genes involved in the cell cycle regulation were significantly modified after treatment with one or many fractions. The expression of cyclin B1 (CCNB1), E2F transcription factor 1 (E2F1), early growth response 1 (EGR1), kinesin family member 23 (KIF23), and proto-oncogene protein c-fos (FOS) gene was all down-regulated, while cyclin F (CCNF), E2F transcription factor 5 (E2F5), E3 ubiquitin-protein ligase Mdm2 (MDM2), and ataxia telangiectasia mutated (ATM) gene expression was up-regulated compared to the control (Figure 4.2). FOS protein is required for G0 to G1 transition (Kovary et al., 1992) and this gene was down regulated after J06 treatment. E2F5 protein is required for G1 arrest (Gaubatz et al., 2000) and the gene was up-regulated after N05 treatment. Moreover, E2F1 protein stimulates the cell to enter in the S phase (Adams et al., 1996) and the expression of this gene was down-regulated after A05 treatment. Also, CCNB1, EGR1 and KIF23 proteins are required to enter or during mitosis (Kim et al., 1997a; Pines et al., 1989; Suggs et al., 1990). The expression of these genes was down-regulated during one or many galactofucan extract treatments, indicating that the cell cycle might be arrested after these treatments. In the case of DNA damage, the ATM gene is expressed in respond to double-strand DNA breaks. ATM protein will then phosphorylate p53 (Sengupta et al., 2005) and limit its interaction with MDM2 (Proctor et al., 2008). This event will increase p53 protein levels causing growth arrest, apoptosis and DNA repair (Sengupta et al., 2005). ATM gene expression was up-regulated after A05 and N05 treatment, but p53 gene expression was not significantly modified compared to the control in those fractions. MDM2 protein promotes the rapid degradation of p53 protein (Proctor et al., 2008). MDM2 gene expression was only up-regulated when M05 was used and ATM gene was not significantly expressed compared to the control when this fraction was added. Conversely, CCNF is involved

during the interphase (M-phase) (Bai et al., 1994) and its expression was curiously up-regulated after M05 treatment.

Expression of other genes not specifically involved in the cell cycle progression but negatively influencing the cell cycle was up-regulated. Among these, small proline-rich protein 1B (SPRR1B) expression was up-regulated after M05 treatment (Table 4.3). This protein is induce after IL1β and IL6 treatment of human corneal epithelial cells (Li et al., 2008a). SPRR1B over expression enhances entry of cells into the G0 phase of the cell cycle (Tesfaigzi et al., 2003). Expression of the chromosomal processing gene, centromere protein F, was down-regulated in J06 treated cells (Table 4.3). This protein is essential during the G2 to M phase transition and during mitosis (Ashar et al., 2000).

Each gene significantly up- and down-regulated contributes to arrest the cell cycle, indicating that crude galactofucan extracts could have an anti-proliferative activity. Heparin also possess an anti-proliferative activity caused by the up-regulation of the expression of p27 gene and its protein in vascular smooth muscle cells (Fasciano et al., 2005). In these cells, the up-regulation of p27 protein inhibits the activation of cdk-2 and prevented the cells to enter S phase. The up-regulation of expression of p27 gene after crude galactofucan treatment might inhibit the cell cycle in a similar manner then the one observed with heparin. Further analyses are required to verify this hypothesis.

4.5.2.1.2 Classical in vitro test on fibroblasts treated with galactofucan

Cell growth modification after M05 and N05 treatments were studied to validate previous results obtained with DNA microarray technique. Results have shown a significant reduction of cell number after 6 days in presence of M05 compared to the control for each concentration tested (Figure 4.3). In presence of N05 extracts, cells number has decreased rapidly, after 48h, for the 2 and 4 mg/mL concentration (data not shown). Only the 1mg/mL concentration has shown little amounts of remaining cells. This could indicate that galactofucan N05 might kill the cells faster than M05 by either an apoptosis or necrosis mechanism. Thus, the amount of apoptotic bodies was determined. For fibroblast treated with M05, significant amounts of apoptotic bodies were found at 2 and 4 mg/mL (22% and

46% respectively) compared to the control after 24h (Figure 4.4). This could suggest that the cell growth reduction observed with galactofucan M05 occurs through an apoptosis mechanism. For fibroblast treated with N05 extracts, only 3 and 5% of apoptotic bodies were observed respectively at the 1 and 2 mg/mL concentration, while at 4mg/mL, there was a significant increase of apoptotic bodies (~25%) after 24h (Figure 4.4). This indicates that the reduction of cell growth observed at 4 mg/mL was induced by apoptosis mechanism, while at 1 and 2 mg/mL this could have occurred through a necrosis mechanism. Remember that when working with DNA microarray, the ARN is extracted from the cell at a certain time point. Also, each method used has different level of sensitivity, where apoptosis rate could be significantly increased with one method but not with another one. Based on these differences in methods, it could be normal to see variations between the propidium iodide test and the gene expression study.

The results concerning the apoptosis rate obtained with the classical *in vitro* tests are unexpected because of the results of the DNA microarray technique. The gene expression study did not show any sign of apoptosis induction after M05 and N05 treatment. For example, Bax gene was not differentially expressed compared to the control (Table 4.3). Apoptosis is activated by the extrinsic pathway through the death receptor or the intrinsic pathway through the mitochondria (Chipuk et al., 2005). Bax is a pro-apoptotic gene expressed during the intrinsic pathway. Also, Bax allow the release of the mitochondrial *cytochrome c*, which will result in apoptosome formation via the apoptotic-protease-activating factor-1 (APAF1) (Green et al., 1998). This apoptosome recruits and activates procaspase-9, which, in turn, activates effector caspases. In this study, apoptosis might not be activated via Bax because this gene is known to be down-regulated by IL6 (Lotem et al., 1995), and IL6 was up-regulated in cells treated with each extracts. Clusterin (CLU) inhibits apoptosis and is believed to interfere with Bax activation in the mitochondria (Zhang et al., 2005b). CLU gene was significantly up-regulated in each galactofucan extracts (Table 4.3). Also, insulin-like growth factor binding protein 3 (IGFBP3) is an important stimulator of apoptosis (Rajah et al., 1997), but this gene was down-regulated with each galactofucan ranging from -2.5 to -3.3 (Table 4.3) in our study.

On the other hand, N05 treatment seems to induce cell death by a necrosis mechanism. This mechanism is followed by an inflammatory reaction because necrotinised cells are not recognised by phagocytes. Heat shock protein 70, a marker of necrosis, plays an important role in activating specific signal for the immune system (Proskuryakov et al., 2003). This gene expression was not differentially altered compared to the control in our study. Other proteins (calreticuline, oligonucleosomes) and carbohydrates may play an important role (Melcher et al., 1999).

Heparin and heparan sulphate can inhibit fibroblast cell proliferation. In certain cases, the inhibitory action is not caused by cell death because, after removing the media containing the polysaccharide, cells are able to proliferate normally (Ferrao et al., 1993). The mechanism of action by which heparin inhibits cell proliferation without activating the apoptosis mechanism, could be linked to the intracellular interaction of heparin with transcription factors such as transforming growth factor-β (TGF-β). However in this study, TGF-β expression was not significantly modified compared to the control. Also, heparin could compete with Apaf-1 (apoptosis protease activating factor-1) for binding to *cytochrome c* to limit caspase 9 activation (Young, 2008), which would have for consequence of inhibiting apoptosis. Conversely, heparin induces apoptosis by stimulating caspase 8 and 3 (Erduran et al., 2007). Unfractionated heparin also showed to generate apoptosis through the suppression of AKt (Ueda et al., 2009). AKt is involved in cell growth, proliferation and apoptosis (Franke et al., 2003). AKt phosphorylates several pro-apoptotic proteins, such as Bad, leading to their inactivation. Phosphorylated Bad dislocates Bcl-xL, which can play its anti-apoptotic role (Datta et al., 1997). Inactivation of AKt triggers apoptosis induction by causing the dephosphorylation of Bad, which promotes its binding with Bcl-xL (Ueda et al., 2009). This activates the mitochondrial apoptotic cascade resulting in the *cytochrome c* release and caspase 9 activation. A sulphated polysaccharide from green algae *Capsosiphon fulvescens* induces apoptosis through the inactivation of AKt (Kwon et al., 2007).

Commercial fucoidan (Sigma) has a mitogenic activity with Balb/c 3T3 fibroblasts (Belford et al., 1993). Others showed an anti-proliferative activity with high molecular weight

fucoidan (> 100 kDa) (Ellouali et al., 1993; Haroun-Bouhedja et al., 2000; Kanabar et al., 2005; Koyanagi et al., 2003; Logeart et al., 1997a; Patel et al., 2002). This difference of activity could be attributed by the use of diverse cell lines that might respond differently when treated with fucoidan. Fucoidan from Sigma inhibited cell proliferation and induced apoptosis with human lymphoma cell line (Aisa et al., 2005). Results showed that caspase 3 is activated and the mitochondrial electric potential is decreased demonstrating that fucoidan induces apoptosis (Aisa et al., 2005). An oversulphated fucoidan (32.8%) also exhibited an anti-proliferative activity and stimulated apoptosis through the activation of caspase 3 and 7 in a leukemia cell line (Teruya et al., 2007). Aisa and coworkers (2005) suggest that apoptosis is influenced by the down regulation of ERK signalling pathway. ERK signalling pathway is involved in cell proliferation, because it is required for cyclin D transcription (Robinson et al., 1997). Also, the anti-proliferative activity of heparin (Daum et al., 1997; Hedin et al., 1998; Ottlinger et al., 1993; Pukac et al., 1997) and fucoidan (Patel et al., 2002) is linked to the inactivation of ERK in vascular smooth muscle. Others showed that ERK must be down-regulated and simultaneously, JNK-p38 MAPK, must be up-regulated to induce apoptosis (Xia et al., 1995) because p38 inhibits cyclin D transcription (Robinson et al., 1997). Besides, the activation of p38 inactivates ERK in fibroblast and promote apoptosis (Li et al., 2003). Thus, the cellular decision of death or survival is determined by the balance between ERK, JNK and p38 signalling pathways. Fucoidan from Sigma increase the activity of JNK and p38 in murine macrophage cells (Hsu et al., 2001). The gene p38 is not activated in Aisa and coworkers (2005) study but they did not test JNK. However, our gene expression study has shown the up-regulation of MAP2K1 (or MEK1), an activator of ERK. Further studies will be necessary to confirm by which mechanism apoptosis was induced with the M05 galactofucan extract.

Several studies, including the present work, showed that sulphated polysaccharides have an anti-proliferative activity and for a number of fractions, this happen through an apoptosis or necrosis. The structure of the polysaccharides as well as its conformation can modulate the activity (Garg et al., 2000; Garg et al., 2003; Kanabar et al., 2005; Koyanagi et al., 2003). This could explain why M05 treatment has induced apoptosis, while N05 treatment has induced necrosis after 24h. The relation between the structural characteristics of galactofucan and their activities will be discussed in section 4.5.3.

4.5.2.2 Effect on the extracellular matrix of crude galactofucan extracts

4.5.2.2.1 Gene expression on fibroblasts treated with crude galactofucan

Galactofucan extracts modified the level of expression of extracellular matrix (ECM) gene. ECM constituents are important in physiological situations because they influence cell growth differentiation, cell adhesion, mobility and spreading (D'ortho et al., 1997). The expression of collagen-III (COL3A1), connective tissue growth factor (CTGF), elastin (ELN), fibronectin 1 (FN1), thrombospondin (THBS1), and transgelin (TAGLN) gene was down-regulated, while cathepsin H (CTSH) and S (CTSS), collagen-XV (COL15A1), fibromodulin (FMOD), and matrix metalloproteinase (MMP1, 3, 7, 10, 11, 14, 15) gene expression was up-regulated compared to the control (Figure 4.5).

Usually, MMP genes are not expressed in normal healthy tissues (Parks et al., 1998) but are expressed in senescent cells, cancer cells or during wound healing (Fisher et al., 2009; Giambernardi et al., 1998; Gill et al., 2008). MMP are not only involved in ECM degradation, but can alter cell growth, apoptosis, cell-cell communication, cell migration, and tumor (McCawley et al., 2001). MMP1 gene was up-regulated in presence of each galactofucan extracts ranging from 1.9 to 3.9. All other MMP (3, 7, 10, 11, 14, and 15) genes were up-regulated in presence of one or many extracts ranging from 1.7 to 3.0 (Figure 4.5). MMP3 gene expression was up-regulated when cells were treated with M05 and that can be linked to the fact that the nuclear presence of active MMP3 protein could results in apoptosis (Si-Tayeb et al., 2006). MMP7 gene expression was only up-regulated during M05 treatment and its protein is also involved in apoptosis by cleaving cell surface molecules such as Fas-ligand (FasL) (Ii et al., 2006). Soluble FasL is a stimulator of death receptor Fas, which activates apoptosis extrinsic pathway. Also, MMP7 is found in noninjured tissues and is required for wound healing by mediating wound-induced epithelial migration (Page-McCaw et al., 2007). But, MMP7 protein is also capable of degrading elastin (ELN) (Murphy et al., 1991), which is contradictory with the previous statement, indicating that the experiment conditions must be different. ELN is a major component of the elastic fiber found in mammalian tissues and it is the molecular component that gives extensibility. ELN gene was significantly down-regulated in all

extracts ranging from -2.8 to -4.4. ELN protein can be degraded by other substrates such as MMP3 and 14. MMP14 and 15 are MMP2 activator (D'ortho et al., 1997), but MMP2 was not differentially expressed compared to the control in our study. A deficiency in MMP14 causes mouse to develop arthritis and connective tissue disease (Holmbeck et al., 1999). Importantly, tumor necrosis factor-α (TNF-α), a key mediator in immune and inflammatory response, can stimulate the expression of MMP14 gene. TNF-α gene was not differentially expressed compared to the control in the current study.

FN1 and TAGLN genes expressions were down-regulated after M05 and J06 treatment with a ratio ranging from -1.7 to -2.1. FN1 is an adhesion molecule and an important matrix component to promote survival and growth of various cells including fibroblasts (Guo et al., 1997). MMP3, 7, 14 and 15 degrade FN1 protein into fragments that are being used by fibroblasts to migrate into wound (D'ortho et al., 1997). TAGLN is an actin binding protein mostly found in smooth muscle cells and this gene expression has been showed to be down-regulated in fibroblasts cells (Lawson et al., 1997).

CTGF protein can induce ECM synthesis by stimulation of collagen-I and FN1 synthesis (Frazier et al., 1996). CTGF gene expression as FN1 and COL3A1 ones was down-regulated after J06 treatment. THBS1 protein can stimulate (Krishnaswami et al., 2002) or inhibit cell growth throughout the up-regulation of p21 (Yamauchi et al., 2007). THBS1 expression was found to be down-regulated by J06 treatment but p21 was not differentially express compare to the control. CTSS gene expression was up-regulated in presence of M05. This protein attenuates the basic fibroblast growth factor (bFGF)-mediated binding of FGF receptor (Liuzzo et al., 1999) which is required for mitogenesis (Ornitz et al., 1992). CTSS also plays a role in the degradation of extracellular proteins (Liuzzo et al., 1999). CTSS production is stimulated by IL1β, which gene expression was also up-regulated by M05 treatment.

CTSH (3.4), COL15A1 (1.9), CST6 (2.8), and FMOD (2.7) gene expression was up-regulated by only N05 treatment (Figure 4.5). CTSH protein degrades many extracellular matrix components, such as proteoglycans, laminin, and collagens II, IX, and XI

(Dickinson, 2002). FMOD protein has the ability to interact with collagen-I and -II and to control the fibril formation of fibrillar collagen (Svensson et al., 1999). FMOD gene expression was found to be down-regulated in senescent cells (Bevilacqua et al., 2005) and its expression was up-regulated in the current study. Collagen-XV protein is found in most basement membrane zones of human tissues and contains highly interrupted collagenous domain for attachment to chondroitin sulphate and heparin sulphate (Myers et al., 2007). Collagen-XV protein might be involved in the survival and stabilization of muscle fibers and endothelial cells on the subjacent basement membrane (Eklund et al., 2001).

4.5.2.2.2 Classical in vitro test on fibroblasts treated with crude galactofucan

The effect of galactofucan on the ECM was only validated with M05 and N05 galactofucan extracts. After 6 days with crude galactofucan extracts, no collagen-I secretion has been detected (data not shown). This was coherent with the gene expression study, where no variation of collagen-I gene expression was detected compared to the control. The whole catalytic property of culture supernatant of treated cells was assayed for the determination of MMP specific activity. The results reflect a balance between MMP and TIMP, their inhibitor, quantities. After M05 treatment, the specific activity of MMP was significantly increased compared to the activity assayed on the control supernatants (Figure 4.6). However, the activity was decreased when M05 concentration was enhanced. These results correlate with the gene expression study, where several MMP gene expressions were up-regulated (MMP1, 3, 6, 10, 15) during M05 treatment. When fibroblasts were treated with N05, at 1 mg/mL, the MMP activity was similar than the control MMP activity, while no activity was detected for the other concentrations (Figure 4.6). However, the gene expression study has shown an up-regulation of MMP1, 11 and 15 in presence of N05 (Figure 4.2). This discrepancy, in both experiments, can be explained by the protocols. The secretion of MMP were determined with cultured fibroblast treated with galactofucan during 6 days, while the cell culture for the DNA microarray experiment was realised after 24h of treatment.

Fucoidan (Sigma) inhibits MMP1 secretion on fibroblasts treated by UVB (Moon et al., 2008). Ultraviolet irradiations are known to increase MMP1 production, which induces

photodamage and skin ageing. MMP1 is up-regulated via UVB-induced ERK and JNK signalling pathway, but not p38 (Kim et al., 2005). Conversely, others showed that p38 block ERK pathway (at MEK level) and inhibit MMP1 gene expression (Westermarck et al., 2001). Moon and coworkers (2008) showed that fucoidan, in non-UVB-induced cells, increase the expression of phosphorylated (p-) ERK and JNK, while UVB-induced cells treated with fucoidan showed a reduction of p-ERK with a reduction of MMP1.

Heparin inhibits the secretion of MMP3 and MMP9 by arterial smooth muscle cells after their stimulation by phorbol ester (Kenagy et al., 1994). Similarly, heparin decreases MMP-1 secretion in dermal fibroblasts (Gogly et al., 1999) and reduces MMP1 and 3 production by gingival fibroblasts (Gogly et al., 1998). Also, heparin decreases MMP1 and 3 secretion, when gingival fibroblast are treated with IL1β, an inducer of these MMP (Gogly et al., 1998). IL1β was significantly up-regulated after M05 treatment (Table 4.3). Thus, IL1β could have contributed to up-regulate MMP1 and/or 3 genes with M05 treatment. Others showed that LMW heparin stimulates MMP1 and MMP2 secretion by heart fibroblasts (Tyagi et al., 1997).

Thus, the difference between our results and the literature could be linked to the polymers structure and concentration, cell lines and passage. For example, fucoidan from Sigma is a polydisperse polymer blend. Two researches with the same fucoidan showed in one case an inhibition of proliferation (Aisa et al., 2005) and the other, a stimulation of proliferation (Belford et al., 1993). Thus, the polymer structure could differ from batch to batch explaining such variation in the activity.

As seen in the gene expression study, no collagen-I was secreted when M05 or N05 were used (data not shown). Reduction of collagen-I and –III secretion combined with an anti-proliferation activity is observed for gingival fibroblast treated with heparin (Senni et al., 1998). The total collagen synthesis and proliferation are also reduced compared to the control for smooth muscle cells treated with heparin and low molecular weight fucoidan (Logeart et al., 1996). Both previous studies used overconfluent cells to minimise the anti-proliferative activity of heparin and/or fucoidan. Conversely, fibroblast treated with

unfractionated heparin and fucoidan from *Fucus spiralis* showed an increase in collagen-I synthesis compared to the control when proliferation is inhibited in subconfluent fibroblast cultures (Ferrao et al., 1993). However, they showed a reduction of collagen-I synthesis compared to the control in confluent fibroblast cultures treated with heparin and fucoidan. Others showed a reduction of collagen-I in keloid fibroblasts treated with heparin (Tan et al., 1991). The dissimilarity in the expression of collagen could reside in cell passage and confluence, duration of the experiment, concentration of heparin, structure of the polysaccharide and quantification method that were different in each experiment.

Several researches realised with heparin showed its anti-proliferative activity (Cavari et al., 1993; Ferrao et al., 1993; Logeart et al., 1996; San Antonio et al., 1992). This activity is believed to be linked to the capacity of heparin to bound with several component of the ECM (San Antonio et al., 1992). Thrombospondin, fibronectin and collagen have heparin binding sites. Heparin can bind to collagen-I which will reduce fibroblasts adhesion to collagen-I and inhibit cell growth (San Antonio et al., 1992). However, galactofucan reduced collagen-I secretion compared to the untreated cells and thus, fibroblasts proliferation is reduced by another mechanism.

4.5.2.3 Other metabolic activities of crude galactofucan

Several genes involved in fibrinolysis, dissolution of clots, were up-regulated (Table 4.3). Plasminogen activator tissue (t-PA) was only significantly up-regulated after A05 and N05 treatment, whereas plasminogen activator urokinase (u-PA) was significantly up-regulated after all treatments. Heparin can stimulate t-PA activity (Edelberg et al., 1990) and u-PA (Conrad, 1998) increasing the conversion of plasminogen to plasmin. Plasminogen activator inhibitor 2 (PAI2), an inhibitor of u-PA, was only up-regulated after M05 and J06 treatment. Heparin reduced plasminogen activator inhibitor 1 (PAI1) (Conrad, 1998), this can explain why this gene was weakly expressed by galactofucan treatment compared to the control. Also, PAI1 is the only protein capable of controlling the level of both t-PA and u-PA. Thus, A05 and N05 treatment might be helpful in the dissolution of clots to prevent thrombosis because both PAI1 and PAI2 are not differentially expressed compared to the

control. Others showed that fucoidan is able to stimulate t-PA to induce plasma clot lysis and shortened the lysis time (Soeda et al., 1992). The higher is the amount of sulphate groups the higher is the activity. Fucoidan is also able to stimulate u-PA mRNA expression in macrophage (Falcone et al., 1991). Besides, t-PA and u-PA could be stimulated by pro-inflammatory cytokines (Levi et al., 2005). PAI2 is also expressed in fibroblasts (Kruithof et al., 1995) after stimulation with inflammatory cytokines (Fish et al., 2006). IL1β and TNF-α both showed to stimulate t-PA and u-PA activation (van der Poll et al., 1991). In our study, however, IL1β gene expression was significantly up-regulated only by M05 treatment, while TNF-α gene was not differentially expressed compared to the control. Thus, other genes might be responsible for the expression of t-PA and u-PA.

Other genes involved in the oxidative metabolism, defence system, immune and inflammatory reaction were up- and/or down-regulated with several extracts (Table 4.3). For example, during an inflammatory response, leukocytes migrate to the infected sites through the blood stream. Leukocyte surface is enclosed with adhesion molecules, p-selectin. When p-selectin links to ICAM-1, a selectin receptors found at the surface of endothelial cells, the leukocyte rolling is stopped and allowed the leukocytes to migrate through the endothelium at the site of inflammation (Ley et al., 2004). ICAM-1 is found at the surface of fibroblast and its expression increases during inflammation by inflammatory cytokines (Rothlein et al., 1988). High level of ICAM-1 is found on endothelial cells when treated with dermatan sulphate, but not chondroitin sulphate nor heparin sulphate (Penc et al., 1999). Fucoidan showed to interfere with leukocyte rolling by binding to p-selectin present at the surface of the endothelium (Heinzelmann et al., 1998) inhibiting the migration of leukocytes at inflammation (Berteau et al., 2003; Omata et al., 1997) to reduce chronic inflammation.

Finally, more analysis will be required to confirm if galactofucan fraction can have a fibrinolytic activity and by which mechanism it is activated. Also, it will be interesting to validate if the immune and inflammatory genes have an impact on the anti-proliferation activity of crude galactofucan.

4.5.2.4 Effect of depolymerised galactofucan on the cell cycle

4.5.2.4.1 Gene expression on fibroblasts treated with RDP galactofucan

Several genes involved in the cell cycle regulation were expressed significantly up- or down-regulated after depolymerised (RDP) galactofucan treatments (Figure 4.7). Antigen identified by monoclonal antibody Ki-67 (Ki-67), cyclins (CCNB1, CCND1, CCND3, CCNE1, CCNF, and CCNG1), cyclin-dependent kinase inhibitors (p16, p21, p27, and p57), early growth response 1 (EGR1), E2F transcription factor 1 (E2F1), inhibitor of DNA binding 2 (ID2), kinesin family member 2C (KIF2C) and 23 (KIF23), topoisomerase (DNA) II alpha (TOP2A), Transcription factor AP-2 alpha (TFAP2A), beta (TFAP2B) and gamma (TAFP2C), and tumor protein p53 (p53) were significantly up- and/or down-regulated in cells after treatment with one or many RDP fractions.

To regulate the cell cycle, several proteins are expressed or repressed at certain time point. For example, G1 is controlled by cyclin/Cdk complexes. Cell passage through G1 is controlled by ordered expression of cyclin D and E, which associates respectively with Cdk4/6 and Cdk2/3. There are also some Cdk inhibitors (p21, p27 and p57) which interfere with the formation of cyclin/Cdk complexes. The over expression of these proteins forces the cell to exit the cycle until their concentrations are reduced.

Different levels of gene expression linked to the cell cycle regulation were detected after 24h of treatment with each RDP galactofucan extracts (Figure 4.7). First, cyclins (CCNB1, CCND1, CCND3, and CCNE1) gene expressions were significantly down-regulated compared to the control in presence of one or many extracts. Cdk inhibitors (p16, p21, p27 and p57) were up-regulated and inhibit the phosphorylation of retinoblastoma 1 (RB1) by the complex of cyclin E/cyclin-dependant kinase-2 during the G1 phase. This will result in the reduction of the cell cycle progression. The expression of p27 gene was up-regulated (from 3.9 to 10.8 fold increased) after most extracts treatments except for M05 RDP2H treatment (Figure 4.7). The expression of p21 gene was up-regulated in presence of M05 RDP2H, while both p16 and p57 gene expression were up-regulated after M05 RDP4H treatment. Moreover, the up-regulation of p53 stimulates p21 gene, which protein interacts

with Cdk resulting in the cell cycle arrest before entering S phase (Harper et al., 1995). The over expression of p53 is often associated with DNA damage and this gene expression was up-regulated in presence of M05 RDP4H and N05 RDP2H. M05 RDP4H treatment has stimulated the up-regulation of CCNG1 gene expression, a transcriptional target of p53 gene (Okamoto et al., 1994) and involved in G2/M phase arrest in response to DNA damage (Kimura et al., 2001). Also, RB1 and E2F1 protein form a complex destabilised by Cdk-4 to liberate E2F1, which is required for the cell to enter S phase. The down-regulation E2F1 gene expression indicates that the cell might not replicate its DNA (Adams et al., 1996). Thus, the expressions of cyclin, Cdk inhibitors, p53, E2F1 genes all contribute to arrest the cell cycle during G1 preventing its transition through S phase.

EGR1 and KIF2C proteins are required to achieve mitosis (Kim et al., 1997a; Suggs et al., 1990). TOP2A protein is involved during chromosome segregation and condensation occurring during M-phase (Watt et al., 1994). These genes expression, EGR1, KIF2C, and TOP2A, were all down-regulated in presence of one or many galactofucan extracts, indicating that the cell cycle might be arrested. Ki-67 gene, a proliferation marker (Takagi et al., 2001), expression was down-regulated only for M05 RDP2H treatment. TFAP2C gene expression was down-regulated after M05 RDP4H treatment and is required for cell proliferation (Eckert et al., 2005). In addition, chromosomal processing genes expression (centromere protein A and F) were also down-regulated only after the treatment with RDP2H fractions (Table 4.4). These genes are essential during M-phase indicating that the cell will not undergo mitosis. Also, small proline-rich protein 1B (SPRR1B) expression is induced by IL1β and IL6 treatment in human corneal epithelial cells (Li et al., 2008a). SPRR1B over expression enhances entry of cells into the G0 phase of the cell cycle (Tesfaigzi et al., 2003). This gene expression was up-regulated in presence of M05 RDP4H (Table 4.4) and could have influenced the amount of cells in the G0 phase.

Conversely, ID2 protein is necessary to enter S phase (Hara et al., 1994) and this gene expression was up-regulated after M05 RDP2H treatment. Transcription factor AP-2 alpha (TFAP2A) and beta (TFAP2B) gene expressions were up-regulated after the treatment with M05 RDP4H. TFAP2A gene expression was also up-regulated in presence of M05 RDP2H.

The up-regulation of the expression of TFAP2 genes are required for cell proliferation (Eckert et al., 2005). Moreover, CCNF and KIF23 protein are involved during the M-phase (Bai et al., 1994; Kim et al., 1997a) and these genes expressions showed to be up-regulated after M05 RDP4H treatment. Thus, the up-regulation of the expression of these genes could allow completion of the cell cycle.

Others showed that Dalteparin, a LMW heparin, reduce cell proliferation and induce apoptosis through the secretion of p21 and p27 proteins (Chen et al., 2008). Heparan sulphate negatively regulates the cell cycle by inhibiting retinoblastoma, cyclin A and CDK1 protein expression (Manton et al., 2006). However, heparan sulphate disaccharide increases the secretion of cyclin D and activates MAPK with an increase of colon cancer cell proliferation (Fishman et al., 2002). These results could be linked to the molecular weight of the polymer (see section 4.5.3). LMW heparin and fucoidan have no impact on cell proliferation when the molecular weight is smaller than 6 and 8 kDa respectively (Kanabar et al., 2005; Logeart et al., 1997a).

Each RDP fraction has induced a different profile of gene expression. In overall, M05 RDP fraction profiles seem different to the N05 RDP fraction profiles especially, M05 RDP4H and N05RDP4H. This variation was not surprising because the crude galactofucan and laminaran fractions (chapter 3) also showed important differences attributed to the harvest period and fronds age (chapter 2). Also, the gene expressions analyses have shown that according to the depolymerisation time (2h or 4h) the extracts could possess different activity which could be linked to the structure of the polysaccharide. Finally, all the data has indicated that RDP galactofucan extracts reduce cell proliferation, but M05 RDP fractions seem to behave differently. Several genes expressions were only up-regulated after M05 RDP treatments (TFAP2A-B, CCNF, KIF23) and might allow the cells to continue their proliferation. Other analysis would be required to confirm this hypothesis.

4.5.2.4.2 Classical in vitro test on fibroblasts treated with RDP galactofucan

The effect of RDP galactofucans on cell growth was only validated using M05 RDP4H and N05 RDP4H (Figure 4.8). The number of cells treated with both RDP galactofucans was

similar than the control, independently the concentration. These results have indicated that both RDP fractions have no effect on cell proliferation. Apoptosis have been assayed and treatment with RDP fraction did not modified the number of sub G0 cells compared to the control (data not shown).

The gene expression study has shown opposite results especially when N05 RDP4H was used. Many genes that are implicated in cell proliferation were down-regulated or up-regulated and let think that cells can decrease their growth when the treatment was present. However, several genes (TFAP2A-B, CCNF, KIF23) expressions were only up-regulated with M05 RDP4H treatment. These genes have the ability to allow the progression of the cell cycle. Several genes involved in the apoptosis mechanism were up-regulated and in overall, they tend to show that RDP galactofucan does not stimulate the apoptosis mechanism. After M05 RDP4H treatment, BAX (pro-apoptosis) and BCLX (anti-apoptosis) genes expressions were both down-regulated, while caspase 8 was up-regulated (Table 4.4). Clusterin (CLU) gene expression was significantly up-regulated in presence of each RDP fractions (Table 4.4). CLU inhibits apoptosis and is believed to interfere with Bax activation in the mitochondria (Zhang et al., 2005b).

RDP galactofucan treatment, showed no effect on the proliferation of fibroblasts. This might be explained by the expression of mitogen-activated protein kinase (MAPK) which control a series of event such as: growth, apoptosis, inflammation (Pearson et al., 2001). MAP2K1 (or MEK1) and MAP2K2 (MEK2) were significantly up-regulated in M05 and/or N05 RDP4H treated cells (Table 4.4). MAP2K1 and MAP2K2 are both activator of ERK which is required for cyclin D transcription (Robinson et al., 1997). After M05 RDP4H treatment, both CCND1 and CCND3 gene expressions were down-regulated. Moreover, MAPK8 (JNK1) protein may induce apoptosis or enhance cell proliferation in certain circumstances when accompanied, for example, by activation of ERK (Barr et al., 2001). MAPK8 gene expression was significantly down-regulated after M05 RDP4H treatment and significantly up-regulated in presence of N05 RDP2H.

In literature, two commercial low molecular weight (LMW) heparins, Dalteparin (Mw ≈ 5 kDa) and Nadroparin (Mw ≈ 4.5 kDa) showed opposite results. In the first research, LMW heparin increased fibroblast proliferation and collagen production, suggesting an acceleration in wound healing (Esen et al., 2009), while in the other, inflammation and cell proliferation are reduced and apoptosis stimulated (Civelek et al., 2007). LMW fucans (20-40 kDa) showed a reduction of proliferation with various tumor cell lines (Alekseyenko et al., 2007; Ellouali et al., 1993). The different cell lines responded differently to LMW fucans in term of the concentration used to induce the inhibition (Ellouali et al., 1993). Rabbit and rat smooth muscle cells proliferation are reduced with LMW fucoidan (8 and 19 kDa) (Deux et al., 2002; Logeart et al., 1997b). Others showed that LMW fucoidan (4 and 16 kDa) increases the proliferation of endothelial cells (Giraux et al., 1998; Zemani et al., 2005), while other detected no proliferation with another LMW fucoidan (5.1 kDa) (Lake et al., 2006).

Variation between the experiments could be attributed to the protocol used, where healing and normal fibroblasts modulate cell proliferation differently (Agarwal et al., 2006). During wound healing, fibroblasts increase their proliferation (Agarwal et al., 2006). This explained why opposite results were obtained between the experiments. Also, different cell lines with different structures of LMW fucoidan influence positively or negatively the proliferation.

4.5.2.5 Effect of depolymerised galactofucan on the extracellular matrix

4.5.2.5.1 Gene expression on fibroblasts treated with RDP galactofucan

Treatment with RDP galactofucan extracts modified the level of expression of some extracellular matrix (ECM) related-gene (Figure 4.9). Collagen-I (COL1A1) gene expression was down-regulated, while cathepsin D (CTSD) and H (CTSH), collagen-III (COL3A1) and -XV (COL15A1), connective tissue growth factor (CTGC), elastin (ELN), fibromodulin (FMOD), matrix metalloproteinase (MMP2, 10, 11, 15), tissue inhibitor metalloproteinase 2 (TIMP2), thrombospondin (THBS1), and transgelin (TAGLN) genes expression was up-regulated (Figure 4.9).

In usual, MMP are not expressed in normal healthy tissues (Parks et al., 1998) and are found in senescent, cancer cells or during wound healing (Fisher et al., 2009; Giambernardi et al., 1998; Gill et al., 2008). MMP are not only involved in ECM, but can alter cell growth, apoptosis, cell-cell communication, cell migration, and tumor (McCawley et al., 2001). MMP (2, 10, 11, and 15) genes expression was up-regulated after one or many extracts treatment (from 1.9 to 13.6 fold increased). MMP10 gene expression was up-regulated in presence of M05 RDP4H. However, it is not transcriptionally active in fibroblasts indicating that the protein won't be secreted (Nagase, 1998). MMP11 gene is expressed in fibroblasts (Giambernardi et al., 1998) but has very weak activity toward ECM molecules (Murphy et al., 1993). MMP15 is a MMP2 activator (D'ortho et al., 1997). MMP2 gene expression was up-regulated in presence of M05 RDP2H. MMP2 degrades several types of collagen, elastin and fibronectin (Giambernardi et al., 1998; Nagase et al., 2006). ProMMP2, generated after the translation, has the ability to form a tight complex with TIMP2 and is required for MMP2 activation (Nagase et al., 2006). Moreover, TIMP2 stimulates fibroblast proliferation (Corcoran et al., 1995). MMP2, TIMP2 and MMP15 were up-regulated by M05 RDP2H treatment, while TIMP2 and MMP15 were up-regulated with N05 RDP4H treatment (Table 4.4).

Our study has shown that collagen-I gene expression was down-regulated with M05 RDP4H treatment. Conversely, collagen-III gene expression was up-regulated in presence of M05 RDP2H and N05 RDP4H. Collagen-XV is found in most basement membrane zones of human tissues and contains highly interrupted collagenous domain for attachment to chondroitin sulfate and heparin sulphate (Myers et al., 2007). Collagen-XV might be involved in the survival and stabilisation of muscle fibers and endothelial cells on the subjacent basement membrane (Eklund et al., 2001). ELN is the elastic protein found in mammalian tissue and it is the molecular component that gives extensibility. ELN was up-regulated with M05 RDP2H.

THBS1 protein can stimulate (Krishnaswami et al., 2002) or inhibit cell growth throughout the up-regulation of p21 (Yamauchi et al., 2007). THBS1 gene expression was up-regulated

with by each RDP galactofucan treatment and p21 gene expression was up-regulated only with M05 RDP2H treatment (Table 4.4). Moreover, transgelin (TAGLN) is an actin binding protein mostly found is smooth muscle cells and showed to be down-regulated in fibroblasts (Lawson et al., 1997). TAGLN gene expression was significantly up-regulated in presence of M05 RDP4H (Table 4.4). CTGF protein can induce ECM synthesis by stimulation collagen-I and fibronectin (FN1) synthesis (Frazier et al., 1996). CTGF gene expression was up-regulated with M05 RDP4H treatment. FMOD protein has the ability to interact with collagen-I and -II and to control the fibril formation of fibrillar collagen (Svensson et al., 1999). FMOD gene expression was found to be down-regulated in senescent cells (Bevilacqua et al., 2005) and was up-regulated in our study. CTSD gene expression was up-regulated for M05 RDP2H treatment and CTSH was up-regulated for M05 and N05 RDP4H treatments. Cathepsin D (CTSD) uses ECM as a substrate (Liaudet-Coopman et al., 2006). Cathepsin H (CTSH) can degrade many extracellular matrix components, such as proteoglycans, laminin, and collagens II, IX, and XI (Dickinson, 2002).

Once again, each RDP galactofucan treatment induces a different profile of gene expression for ECM. In overall, M05 RDP fractions seem different to N05 RDP fractions especially, M05 RDP4H and N05RDP4H. These differences could be attributed to the diverse structure of the polymer fraction.

4.5.2.5.2 Classical in vitro test on fibroblasts treated with RDP galactofucan

The effect of galactofucan on the ECM was only validated with M05 RDP4H and N05 RDP4H galactofucan extracts. The whole catalytic property of culture supernatant of treated cells was assayed for the determination of MMP specific activity. The results reflect a balance between MMP and TIMP quantities, TIMP being MMP inhibitor. Results showed little MMP specific activity in supernatants which was not significantly different compared to the control (data not shown). Fibroblasts treated with M05 and N05 RDP4H have a higher collagen-I secretion compared to the control (Figure 4.10). The amount of secreted collagen-I seems to be concentration dependant when cells were treated with N05 RDP4H.

Classical *in vitro* test showed opposite results to the gene expression profile. Following the gene expression experiment, the amount of collagen-I secretion could be down-regulated after M05 RDP4H treatment, while the secretion was in fact increased at a concentration of 1mg/mL that is the concentration used in the gene expression study. Equivalent amount of MMP secretion compared to the control was found with the classical *in vitro* test, while MMP2, 10, 11, 15 and TIMP2 gene expression was up-regulated. TIMP2 gene expression was up-regulated with N05 RDP4H treatment and is a inhibitor of all MMP and MT-MMP (Lambert et al., 2004). Since the MMP test reflects a balance between MMP and TIMP, it could be hypothesised that the MMP secretion is not visible due to the inhibition of MMP by TIMP2. This discrepancy, in both experiments, can also be explained by the protocols. The secretion of collagen-I and MMP were determined with cultured fibroblast treated with RDP galactofucan during 6 days, while the cell culture for the DNA microarray experiment was realised after 24h of treatment.

LMW heparin (1-9kDa) decreases MMP1, increases MMP2 and TIMP2 and have no impact on TIMP1 secretion by dermal fibroblasts (Gogly et al., 1999). Others showed that LMW heparin increases MMP1 and MMP2 secretion by heart fibroblasts (Tyagi et al., 1997). Gingival fibroblasts treated with LMW heparin (1-9kDa), reduced the secretion levels of MMP1 and 3 (Gogly et al., 1998). Also, LMW heparin diminishes MMP1 and 3 levels, when gingival fibroblasts are treated with IL1β, an inducer of these MMP (Gogly et al., 1998). LMW fucoidan (16 kDa) inhibits TIMP1 and MMP2 secretion, while no basal expression MMP3 is detected (Senni et al., 2006). However, LMW fucoidan is able to reduce MMP3 secretion after a pre-treatment with IL1β. Authors showed an increase of complex formation with and without IL1β. LMW fucoidan increase the complex formation between MMP2 or MMP3 with TIMP1 (Senni et al., 2006). In our research, MMP1, MMP3 and TIMP1 were not differentially expressed compared to the control in the gene expression study and no modulation of the MMP activity was found with the classical *in vitro* test. These results indicate that RDP galactofucan could limit MMP activity by the formation of complexes with TIMP. Thus, LMW fucoidans and possibly galactofucan could be useful to control matrix destruction in diseases such as emphysema, periodontitis, and chronic leg ulcer (Senni et al., 2006).

LMW heparin (1.5-3.0 kDa) stimulates collagen-I, -III and -V synthesis by HDF (El Nabout et al., 1989). Administration of LMW heparin (Nadroparin) after rats underwent full-thickness surgical incision of the Achilles tendon followed by primary repair, showed an increase of collagen synthesis in fibroblast, fibrillar collagen in the ECM and new vascular tissue formation (Esen et al., 2009). Authors suggest that heparin accelerates tendon healing.

Vascular smooth muscle cells treated with LMW fucoidan showed an anti-proliferative activity and reduced collagen synthesis (Logeart et al., 1996). Others showed that mice treated with intra muscular injection of LMW heparin (2-3 kDa) also reduce collagen-III synthesis (Asselot et al., 1989). The mice are not injured explaining why there is no increase of collagen because healing and normal cells (fibroblasts) modulate proliferation and collagen synthesis differently (Agarwal et al., 2006). During wound healing, fibroblasts increase their proliferation to produce large quantities of collagen-I and -III (Agarwal et al., 2006; Mutsaers et al., 1997). Thus, RDP galactofucan extract could be valuable during wound healing because it has increased fibroblast proliferation and collagen secretion.

4.5.2.6 Other metabolic activities of depolymerised galactofucan

Other genes involved in the oxidative metabolism, defence system, immune and inflammatory reaction were up- and/or down-regulated with several extracts (Table 4.4). As seen with crude galactofucan, RDP fraction showed the up-regulation of ICAM-1. Fucoidan is believed to interfere with leukocyte rolling by binding to p-selectin present at the surface of the endothelium (Heinzelmann et al., 1998) inhibiting the migration of leukocytes (Berteau et al., 2003; Omata et al., 1997) to reduce chronic inflammation.

In opposition to the crude galactofucan fractions, RDP fraction does not seem to exhibit a fibrinolytic activity. Only, PAI2 gene was strongly down-regulated in M05 RDP2H and 4H extracts. Others showed that the fibrinolytic activity is reduced with LMW fucoidan (Soeda et al., 1993). The fibrinolytic activity observed only for crude galactofucan was shown to be influenced by the amount of sulphate groups and molecular weight. For crude fucoidan

(100-150 kDa), the activity increases with the increase of sulphate groups (Soeda et al., 1992). The fibrinolytic activity of oversulphated fucoïdane decrease with the decrease of its molecular weight (Soeda et al., 1993). This could explain why no fibrinolytic activity was observed with RDP galactofucan fraction.

4.5.3 Relation between the structural characteristics of crude and RDP galactofucan extracts and their activities

All the data presented here tend to show that crude and RDP galactofucans induce different metabolic activities on cells according to the gene expression study. This was also validated by classical *in vitro* tests, where cell growth, apoptosis and MMP and collagen-I secretion by fibroblasts gave different results. Several structural differences were observed between each crude fraction especially between M05 and N05. Similar observations were made with RDP galactofucans from M05 and N05. All crude and depolymerised fractions contained 3-linked fucopyranose 4-sulphate and 6-linked galactopyranose 3-sulphate. However, M05 contained less 6-linked galactopyranose 3-sulphate than N05. Structural differences observed for the crude and galactofucan extracts could explain the variation observed in the metabolic activities.

Most studies were conducted to verify the effect of molecular weight or the degree and position of sulphate groups of sulphated polysaccharides on their biological activities. Several heparin fractions were tested to determine the effect of molecular weight on endothelial cell proliferation stimulated by fibroblast growth factor (FGF-2), a cell proliferation stimulator (Khorana et al., 2003). Unfractionated (12-15 kDa), LWM (3 and 6 kDa), octasaccharide (2.4 kDa), pentasaccharide (1.7 kDa) and tetrasaccharide (1.2 kDa) of heparin were used with FGF-2. Unfractionated heparin, 6 and 3 kDa fractions reduced FGF-2 dependent proliferation, while the oligosaccharides had no effect (Khorana et al., 2003). An anti-proliferative activity is also observed with airway smooth muscle cells treated with two unfractionated (17-19 and 30 kDa) and LMW (6, 5 and 3 kDa) heparins (Kanabar et al., 2005). A similar study was conducted with crude (556 kDa) and LMW (97, 26, 16, 8 and 5.5 kDa) fractions of fucoidan (Logeart et al., 1997a). LMW fraction 8 and

5.5 kDa have no effect on cell proliferation, while the others fractions (556, 97, 26, and 16 kDa), inhibit vascular smooth muscle cell proliferation. The authors suggest that a degree of polymerisation of 30 units was required to have a significant reduction of proliferation (Logeart et al., 1997a). Comparable results were obtained with fibroblast (Ellouali et al., 1994) and tumor cells (Ellouali et al., 1993) treated with fucoidans of various molecular weights.

Heparan sulphate is structurally related to heparin but contains less sulphate groups (Zhang et al., 2005a). Oversulphated heparin and heparan sulphate added to pulmonary artery smooth muscle cells showed few differences between each other concerning the anti-proliferative activity (Garg et al., 2003). These polysaccharides have different content in *N*- and *O*-sulphate groups, *N*-acetyl groups and the proportion of uronic acids (Garg et al., 2003). The authors showed that the ratio between iduronic acid and glucuronic acid is not essential for the anti-proliferative activity, while *N*-sulphated and 6-*O*-sulphated groups were important (Garg et al., 2000). Heparin binding affinity to granulocyte colony stimulating factor (GCSF), a proliferation enhancer protein (Marino et al., 2003), was verified in terms of sulphate groups present on the polysaccharide chain. Results showed that 2,3-*O*-sulphate groups were more important than *N*-sulphate groups for heparin binding capacity (Liang et al., 2008). If heparin anti-proliferative activity is mediated through an heparin binding properties, this tells us that 2,3-*O* sulphate groups could alter this activity. Others showed that 20% of sulphate groups were required for fucoidan to have an anti-proliferative activity (Haroun-Bouhedja et al., 2000). The fraction containing 20% of sulphate groups has a molecular weight of 15 kDa, while the fraction containing 18.4% of sulphate groups and a molecular weight of 9.5 kDa showed no impact on cell proliferation. Others showed that LMW fucoidan of 8 kDa has a weak antiproliferative activity (Logeart et al., 1997a) and we demonstrated that RDP galactofucan (<10 kDa) have no effect on proliferation. Thus, the absence of proliferation with the 18.4% sulphate groups could be attributed to its molecular weight. Still, an oversulphated fucoidan (32.8% of SO_3^-) reduces cell proliferation, while the crude fraction (13.5% of SO_3^-) had no impact (Teruya et al., 2007).

Oversulphated heparin and heparan sulphate anti-proliferative activity is not influenced by the ratio between iduronic acid and glucuronic acid (Garg et al., 2003). Others showed similar results with fucoidan, where the amount of fucose does not seem to influence the activity (Ellouali et al., 1993). Thus, the monosaccharide composition does not seem to be important for the proliferation of cells but is essential for the anticoagulant activity of heparin. Heparin contains an anti-thrombin binding domain composed of a pentasaccharide with specific monosaccharides, *N*- and *O*-sulphate groups composition (Thunberg et al., 1982). Several researches have tried to determine a specific sequence responsible for the anticoagulant activity of fucoidan, but failed to found one. Fucoidan is a heterogeneous polymer and no defined regularity has been observed (Kusaykin et al., 2008). It was ruled out that the presence of *O*-2 and -4 sulphate groups influences the activity (Pereira et al., 2002). The conformation of the polysaccharides might influence as well the activity. The spacing between sulphate groups will force the polysaccharide to adopt a certain conformation which will affect its binding capacity to thrombin (Becker et al., 2007; Pereira et al., 2002).

We observed that crude galactofucan M05 showed an apoptotic activity, while none was observed with N05. M05 galactofucan fraction has a higher molecular weight than N05 and contained equivalent amounts of L-fucose and D-galactose. Also, M05 fraction sulphate groups are mostly located on the fucopyranose units, whereas they were mostly located on the galactopyranose units for N05. Thus, these differences could have an important impact on the activity. We also observed that crude galactofucan (638-1529 kDa) have an anti-proliferative activity, while RDP4H galactofucan (< 10 kDa) showed had no effect on proliferation. The effect RDP2H galactofucans (23.3-30.2 kDa) on fibroblasts proliferation was not tested. According to Logeart and collaborators (1997), this fraction might inhibit cell proliferation. The amount of sulphate groups also seems to be important. Crude fucoidan containing 13.5% showed no impact on cell proliferation (Teruya et al., 2007). Thus, crude galactofucan J06 might not inhibit cell growth because it contains 13.1% of sulphate groups. This could be linked to the structure of the polysaccharide. Thus, the molecular weight, the amount and the position of sulphate groups are important for the anti-proliferative activity of galactofucan. Further analysis will be required to verify how these

structural characteristics affect the apoptosis as well as the secretion of MMP and collagen-I.

4.6 Conclusion

The structure of crude and RDP galactofucan extracts from brown seaweed *Saccharina longicruris* was investigated and showed interesting difference according to the harvest period. All fractions contained 3-linked fucopyranose 4-sulphate and 6-linked galactopyranose 3-sulphate. M05 and J06 contained less 6-linked galactopyranose 3-sulphate than A05 and N05. The structure of crude fractions remained complex after the depolymerisation. RDP fraction showed to be more sulphated than the crude fraction, with a much smaller molecular weight (< 30 kDa). Crude and RDP galactofucan exhibit different metabolic activities probably linked to the polymer molecular weight, the amount and the position of the sulphate groups. The monosaccharide composition and the conformation might also influence its activities.

Galactofucan influenced fibroblast gene expression according to the harvest period. Crude and RDP galactofucans seems to influence fibroblasts growth, the apoptosis, the synthesis of MMP and collagen according to the harvest period. Crude galactofucan showed an anti-proliferative activity with a possible increase of the ECM degradation by fibroblasts caused by the secretion of MMP. Only, M05 has stimulated cell death, but the mechanism is still unknown. ERK/JNK/p38 signalling pathways, AKt or MMP could be responsible for the apoptosis activation. This fraction could be interesting in cancer treatment, but further analysis will be required. RDP fraction could be valuable during wound healing by increasing collagen-I secretion and did not modified cell proliferation. Further analysis will be required to verify this hypothesis.

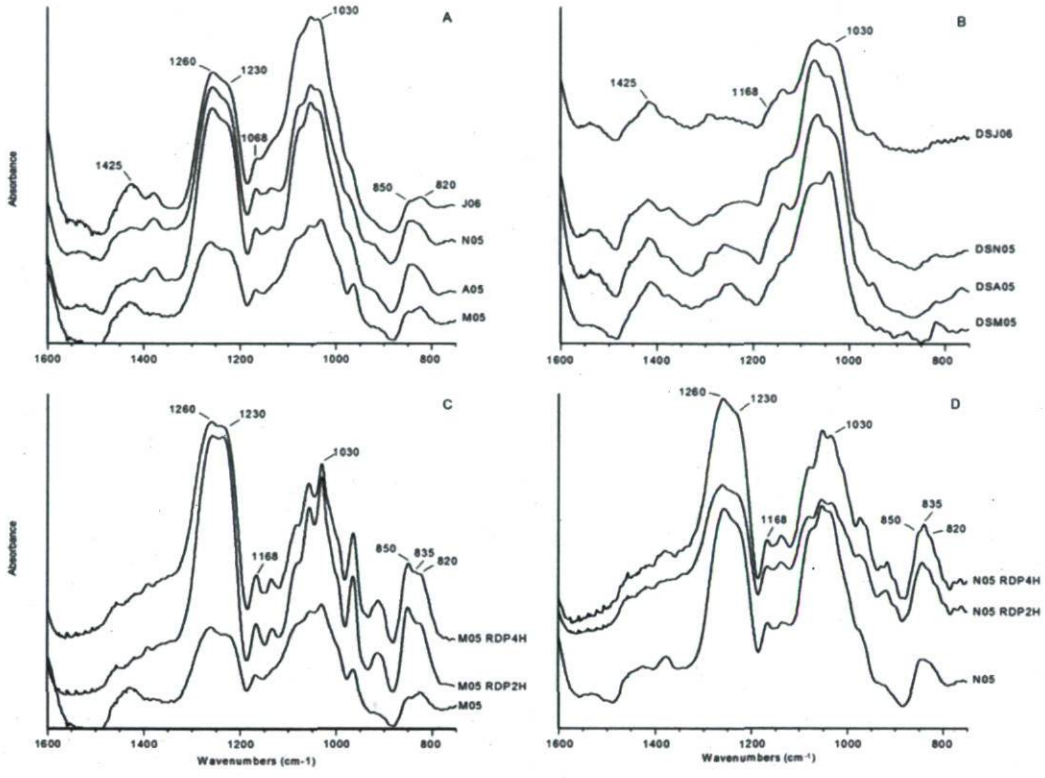

Figure 4.1 : IR spectra in KBr pellet of crude (A), desulphated (B) and depolymerised galactofucan M05 (C) and N05 (D).

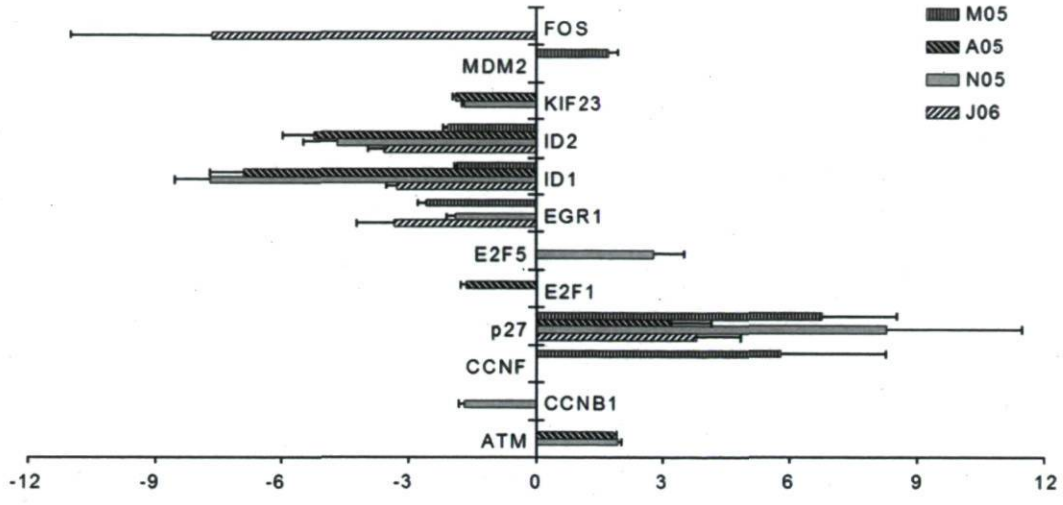

Figure 4.2 : Effect of crude galactofucan on the cell cycle regulation.
Data are the average ratio ± SD carried out in three different experiments. Only significant up- and down-regulated genes are presented.

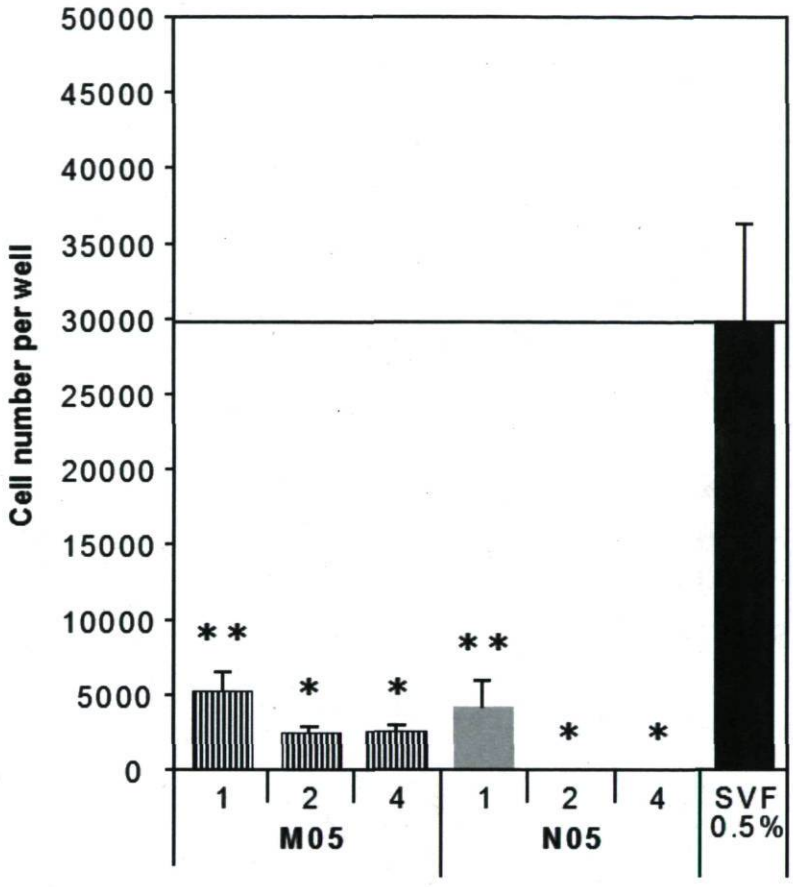

Figure 4.3 : Cell growth of normal dermal fibroblasts treated with crude galactofucan. The number of cell was evaluated after 6 days in culture with galactofucan at different concentrations (1, 2 and 4 mg/mL). Data are the means ± SD carried out in triplicate. Significant differences between cells treated with SVF 0.5% (control) and galactofucan were calculated using Student test (*$P < 0.05$ and **$P < 0.01$).

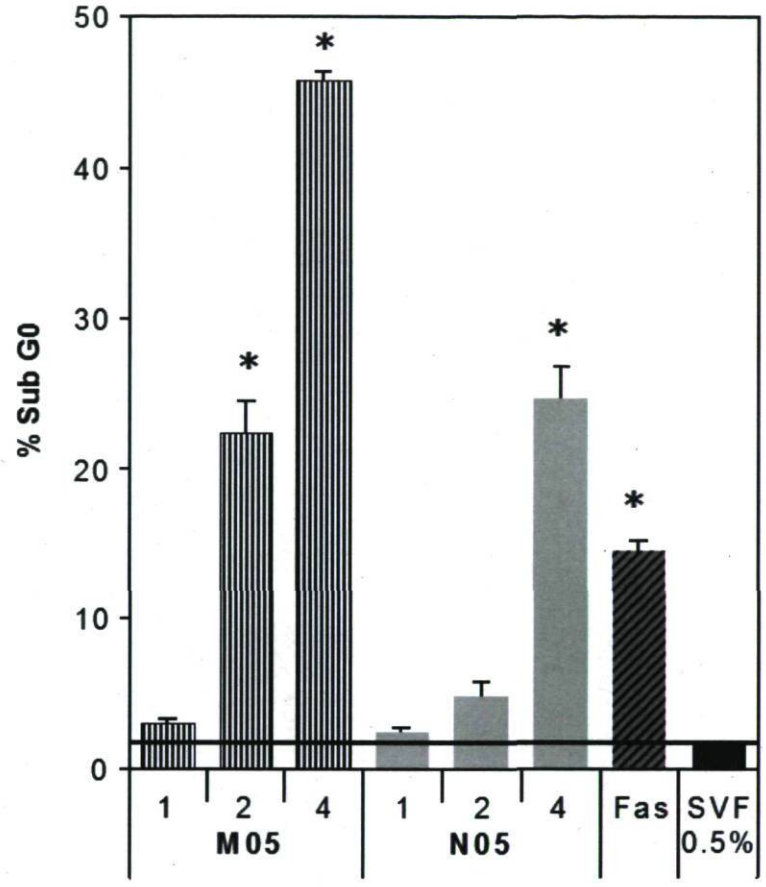

Figure 4.4 : The apoptotic cells in normal dermal fibroblasts treated with crude galactofucan.
Percentage of cells stained with propidium iodide in sub G0 (apoptosis) phase after 24 h cell treatment with galactofucan (1, 2 and 4 mg/mL) was presented. Fas was used as a positive control to induce apoptosis. Data are the means ± SD carried out in triplicate. Absence of error bars signifies an error which was too small to be illustrated. Significant differences between cells treated with SVF 0.5% (control) and galactofucan were calculated using Student test (*$P < 0.05$).

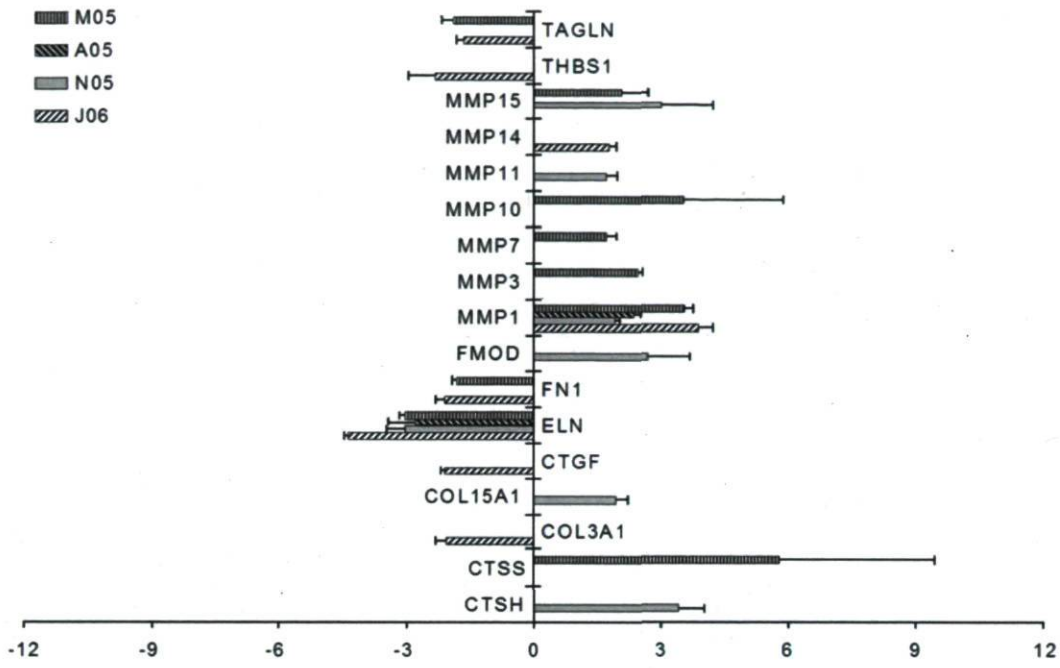

Figure 4.5 : Effect of crude galactofucan on the matrix extracellular genes.
Data are the average ratio ± SD carried out in three different experiments. Only significant up- and down-regulated genes are presented.

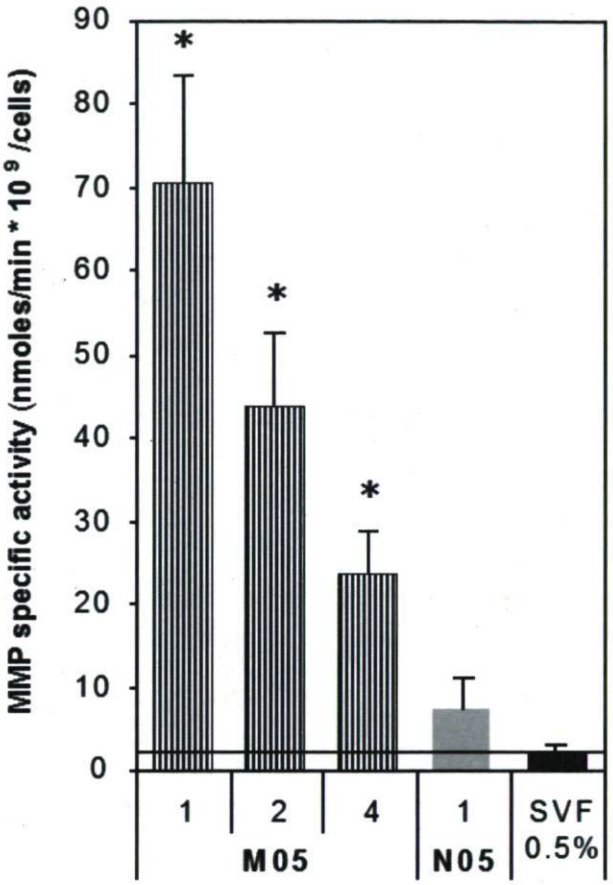

Figure 4.6 : MMP specific activity in normal dermal fibroblasts treated with crude galactofucan.
Results reflect the overall MMP quantification from the supernatants of cell in culture with galactofucan extracts at different concentrations (1, 2 and 4 mg/mL). Data are the means ± SD carried out in triplicate. Significant differences between cells treated with SVF 0.5% (control) and galactofucan were calculated using Student test (*P < 0.05).

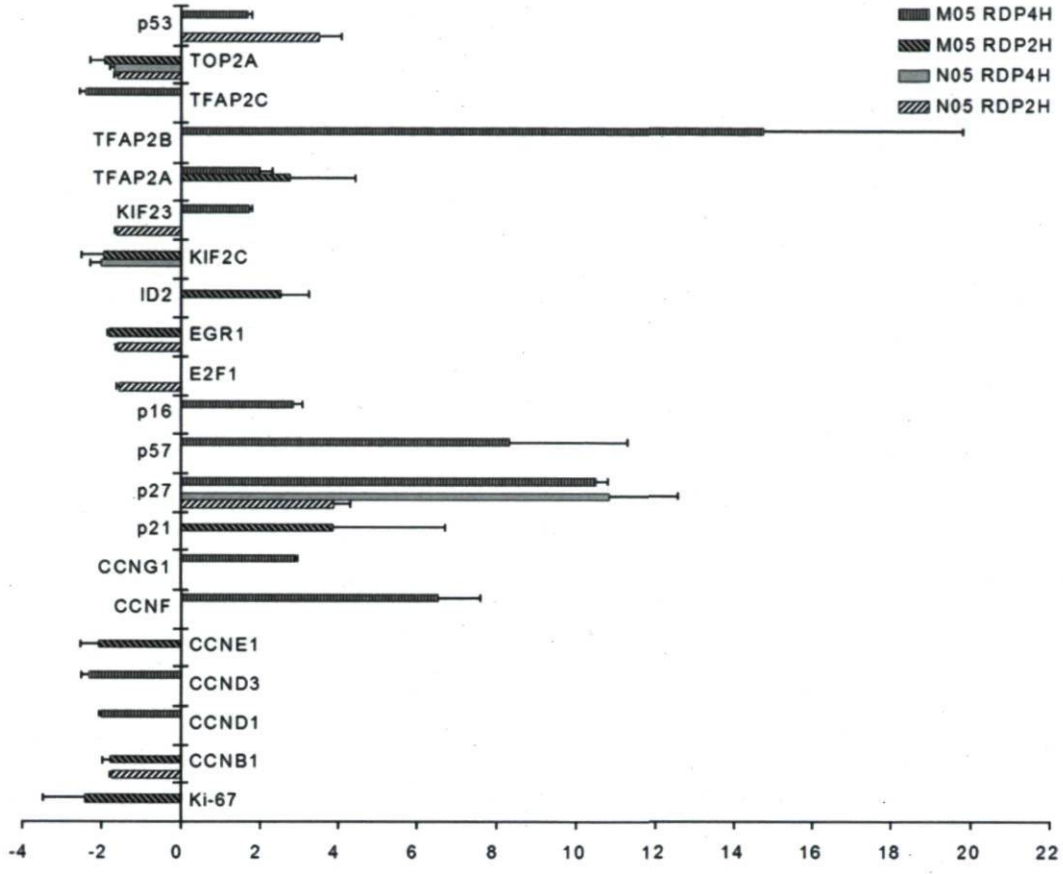

Figure 4.7 : Effect of depolymerised galactofucan on the cell cycle regulation.
Data are the average ratio ± SD carried out in three different experiments. Only significant up- and down-regulated genes are presented.

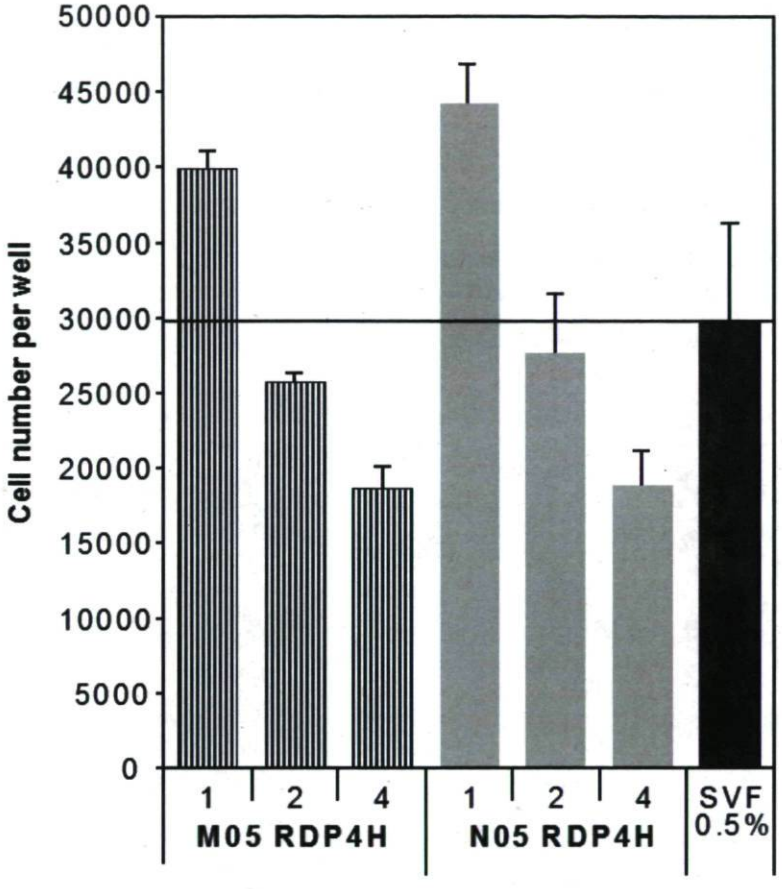

Figure 4.8 : Cell growth of normal dermal fibroblasts treated with depolymerised (RDP) galactofucan.
The number of cell was evaluated after 6 days in culture with RDP galactofucan at different concentrations (1, 2 and 4 mg/mL). Data are the means ± SD carried out in triplicate. Significant differences between cells treated with SVF 0.5% (control) and RDP galactofucan were calculated using Student test (*$P < 0.05$).

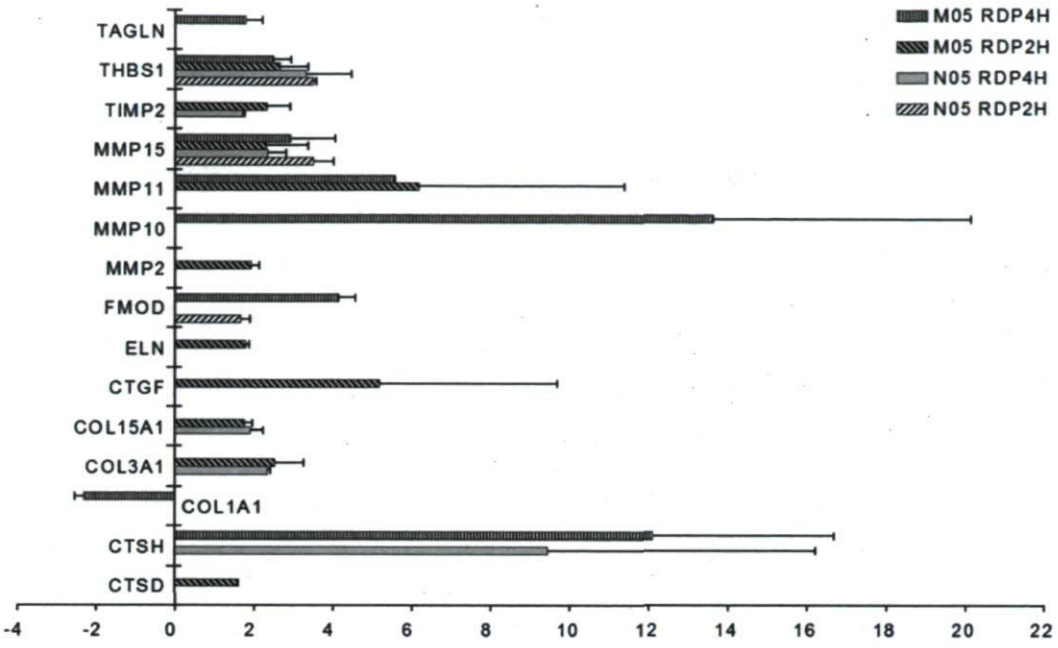

Figure 4.9 : Effect of depolymerised galactofucan on the matrix extracellular genes. Data are the average ratio ± SD carried out in three different experiments. Only significant up- and down-regulated genes are presented.

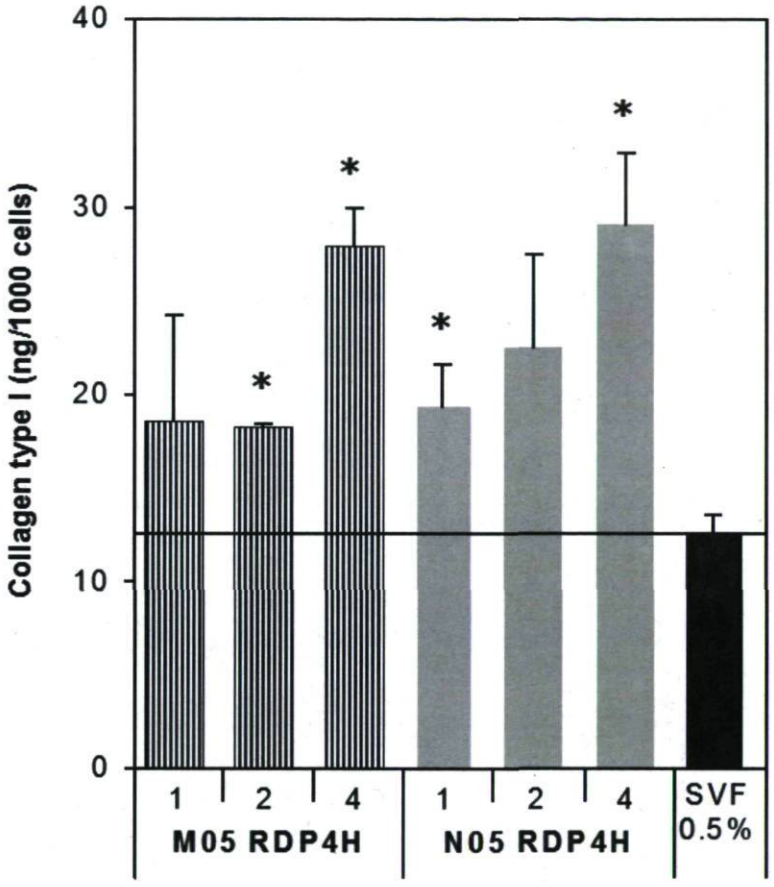

Figure 4.10 : Collagen type I secretion in normal dermal fibroblasts treated with depolymerised (RDP) galactofucan.
Results reflect the collagen type I secretion from the supernatants of cell in culture with RDP galactofucan extracts at different concentrations (1, 2 and 4 mg/mL). Data are the means ± SD carried out in triplicate. Significant differences between cells treated with SVF 0.5% (control) and RDP galactofucan were calculated using Student test (*$P < 0.05$).

Table 4.1 : Composition in monosaccharide, sulphate groups, and molecular weight of crude and depolymerised (RDP) galactofucan.

Fraction	L-fucose	D-galactose	D-xylose	D-mannose	D-glucose	D-galacturonic ac.	D-glucuronic ac.	Sulphates (%)	Mw (kDa)	
									MALLS	HPSEC*
M05	12.7 ± 2.6	16.7 ± 0.7	2.4 ± 0.3	4.2 ± 0.1	1.0 ± 0.1	nf	3.5 ± 0.2	17.6 ± 0.2	1 529.0	nd
A05	12.9 ± 1.4	36.8 ± 2.3	1.6 ± 0.4	2.9 ± 0.2	1.2 ± 0.3	nf	2.3 ± 0.7	20.0 ± 0.3	851.0	nd
N05	14.4 ± 1.9	33.1 ± 6.2	2.1 ± 0.2	2.7 ± 0.8	1.4 ± 0.8	1.0 ± 2.0	3.2 ± 0.9	19.1 ± 0.4	638.0	nd
J06	9.4 ± 1.1	25.0 ± 3.4	3.1 ± 0.4	5.6 ± 1.2	1.0 ± 0.3	0.8 ± 1.4	5.4 ± 0.7	13.1 ± 0.6	765.0	nd
M05 RDP2H	24.5 ± 7.6	14.2 ± 3.7	1.5 ± 0.2	2.1 ± 0.2	nf	0.3 ± 0.5	0.9 ± 0.9	35.6 ± 1.0	23.3	nd
M05 RDP4H	19.9 ± 6.4	9.6 ± 5.1	0.8 ± 0.6	0.8 ± 0.6	0.1 ± 0.1	0.4 ± 0.8	0.1 ± 0.2	38.7 ± 0.7	4.8	4.8
N05 RDP2H	6.8 ± 3.5	31.1 ± 9.2	nf	1.0 ± 0.4	0.1 ± 0.2	0.9 ± 1.0	0.3 ± 0.3	30.3 ± 1.1	30.2	nd
N05 RDP4H	11.6 ± 4.7	23.8 ± 7.1	nf	0.3 ± 0.3	nf	1.7 ± 0.6	nf	32.4 ± 0.9	10.4	6.1

* Molecular weights in equivalent of polyethylene glycol (PEG).
nf: not found
nd: not dertermined

Table 4.2 : Analysis of methylated reduced galactofucan alditol acetates.

Glycosyl residue	Position of *O*-methyl group	Deduced position of substitution	Detector response (%)															
			M05	DS M05	M05 RDP2H	DS M05 RDP2H	M05 RDP4H	DS M05 RDP4H	A05	DS A05	N05	DS N05	N05 RDP2H	DS N05 RDP2H	N05 RDP4H	DS N05 RDP4H	J06	DS J06
Fucosyl	2,3,4	*p* Terminal	4.9	6.8	9.1	2.6	3.5	4.6	7.5	3.9	11.4	5.9	3.9	2.1	15.8	5.8	8.1	6.1
	2,3	4	7.8	2.4	7.6	2.2	9.0	5.0	3.5	1.1	4.3	0.8	2.7	1.6	6.2	3.0	3.6	1.5
	2,4	3	13.5	2.9	21.7	2.8	15.4	8.1	12.8	10.1	11.9	10.6	8.4	8.3	15.1	12.6	11.8	6.8
	3,4	2				1.1		5.1					0.2		3.2			
	2	3,4	11.4		16.0		20.9	4.2	6.7	1.3	3.1	1.8	7.9	0.8	3.0	2.1	8.0	0.8
	3	2,4	1.6			1.4	3.4				0.5				1.6	0.7	4.0	
	4	2,3	5.9		9.6		8.5		6.5		3.5		8.5		3.1	1.3	6.1	
		2,3,4	12.2		7.9		13.6		9.1		6.9		13.6		2.4		9.9	
Total			*57.5*	*12.2*	*71.7*	*10.1*	*74.4*	*27.0*	*46.1*	*16.4*	*41.6*	*19.1*	*45.3*	*12.8*	*50.4*	*25.6*	*51.6*	*15.2*
Galactosyl	2,3,4,6	*p* Terminal	2.0	7.9	3.3	5.0	1.5	6.7	5.8	12.8	9.0	11.1	3.8	12.5	9.5	13.7		9.9
	2,4,6	3	3.9	5.4	2.4	4.4	3.3	5.0	5.7	6.6	6.4	7.5	5.7	10.0	6.4	8.0	2.7	5.8
	2,3,6	4	2.0	3.8	1.0	4.0	2.0	6.2	3.7	6.9	4.2	5.9	3.0	9.9	2.6	8.6		4.5
	2,3,4	6	3.1	13.0	3.0	4.3	2.0	5.4	8.4	16.3	12.0	16.8	6.4	13.9	9.3	13.2	2.2	14.8
	4,6	2,3			0.5								0.8		0.8	0.5	2.1	
	3,6	2,4	1.7	2.9	0.6	1.6			1.4	2.1	1.1	2.2	1.0	0.8	0.7	0.9	2.8	2.5
	2,6	3,4	1.8		1.0		2.6		2.0	0.9	1.0	0.8	3.7	1.3	0.9	1.5	3.9	
	2,4	3,6	5.1		3.3		5.7		6.8	1.2	5.5	1.4	9.0	3.6	6.5	3.8	5.4	1.2
	2,3	4,6	3.6	8.9	3.2	2.9	2.0	5.0	7.9	6.1	8.5	8.0	7.3	5.0	5.6	5.9	2.7	6.6
	2	3,4,6	3.7		1.6		3.9		2.5		1.3		5.1		2.0	0.7	5.4	
	3 or 4	2,4,6 or 2,3,6			1.1		1.2		1.6		1.1		3.9		2.2	0.6		
		2,3,4,6			0.4	3.4			0.5				1.8		1.2	0.4		
Total			*26.7*	*42.0*	*21.5*	*25.6*	*24.1*	*28.2*	*46.3*	*52.9*	*50.1*	*53.6*	*51.5*	*56.9*	*47.7*	*57.9*	*27.2*	*45.3*
Other glycosyl				30.7		46.6		33.6		20.0		19.9		24.3		10.3		23.5
*Misc.**			15.7	15.2	6.7	17.8	1.5	11.2	7.6	10.7	8.3	7.4	3.2	6.0	1.9	6.2	21.3	16.0

*Miscellaneous residues, primarily glucosyl, mannosyl and xylosyl.

Table 4.3 : Gene expression* from human dermal fibroblasts treated with crude galactofucan.

Function	Gene names	Symbol	Swissprot accession number	Average ratio			
				M05	A05	N05	J06
Apoptosis	Clusterin (apolipoprotein J)	CLU	P10909	2.6 ± 0.9	3.7 ± 0.4	4.7 ± 0.6	1.9 ± 0.2
Cell cycle	Ataxia telangiectasia mutated	ATM	Q13315		1.8 ± 0.1	1.9 ± 0.1	
	Cyclin B1	CCNB1	P14635			-1.7 ± 0.1	
	Cyclin F	CCNF	P41002	5.8 ± 2.5			
	Cyclin-dependent kinase inhibitor 1B	p27	P46527	6.8 ± 1.8	3.2 ± 1.0	8.3 ± 3.2	3.8 ± 1.1
	E2F transcription factor 1	E2F1	Q01094		-1.7 ± 0.1		
	E2F transcription factor 5, p130-binding	E2F5	Q15329			2.8 ± 0.7	
	Early growth response 1	EGR1	P18146	-2.6 ± 0.2		-1.9 ± 0.2	-3.3 ± 0.9
	Inhibitor of DNA binding 1	ID1	P41134	-1.9 ± 0.0	-6.9 ± 0.8	-7.7 ± 0.8	-3.3 ± 0.2
	Inhibitor of DNA binding 2	ID2	Q02363	-2.1 ± 0.1	-5.2 ± 0.7	-4.7 ± 0.8	-3.6 ± 0.4
	Kinesin family member 23	KIF23	Q02241		-1.9 ± 0.1	-1.7 ± 0.0	
	E3 ubiquitin-protein ligase Mdm2	MDM2	Q00987	1.7 ± 0.3			
	Proto-oncogene protein c-fos	FOS	P01100				-7.7 ± 3.3
Chromosomal processing	Centromere protein F	CENPF	P49454				-1.9 ± 0.1
Defense system	Prostaglandin G/H synthase 1	PTGS1	P23219	1.8 ± 0.1	2.7 ± 0.3	3.1 ± 0.3	1.8 ± 0.4
	Prostaglandin G/H synthase 2	PTGS2	P35354	2.7 ± 0.1			
Extracellular matrix	Cathepsin H	CTSH	P09668			3.4 ± 0.8	
	Cathepsin S	CTSS	P25774	5.8 ± 3.7			
	Collagen, type III, alpha 1	COL3A1	P02461				-2.1 ± 0.2
	Collagen, type XV, alpha 1	COL15A1	P39059			1.9 ± 0.3	
	Connective tissue growth factor	CTGF	P29279				-2.1 ± 0.1
	Elastin	ELN	P15502	-3.0 ± 0.1	-2.8 ± 0.6	-3.0 ± 0.4	-4.4 ± 0.1
	Fibronectin 1	FN1	P02751	-1.8 ± 0.1			-2.1 ± 0.2
	Fibromodulin	FMOD	Q06828			2.7 ± 1.0	
	Matrix metalloproteinase 1 (interstitial collagenase)	MMP1	P03956	3.5 ± 0.2	2.4 ± 0.2	1.9 ± 0.1	3.9 ± 0.4
	Matrix metalloproteinase 3 (stromelysin 1)	MMP3	P08254	2.4 ± 0.1			
	Matrix metalloproteinase 7 (matrilysin)	MMP7	P09237	1.7 ± 0.3			
	Matrix metalloproteinase 10 (stromelysin 2)	MMP10	P09238	3.5 ± 2.4			
	Matrix metalloproteinase11 (stromelysin 3)	MMP11	P24347			1.7 ± 0.3	
	Matrix metalloproteinase 14 (membrane-inserted)	MMP14	P50281				1.8 ± 0.2
	Matrix metalloproteinase 15 (membrane-inserted)	MMP15	P51511	2.1 ± 0.6		3.0 ± 1.2	
	Thrombospondin 1	THBS1	P07996				-2.3 ± 0.6
	Transgelin	TAGLN	Q01995	-1.9 ± 0.3			-1.7 ± 0.2
Immune and inflammatory	Colony stimulating factor 2 (granulocyte-macrophage)	GMCSF	P04141	11.2 ± 4.5			9.1 ± 1.3
	Intercellular adhesion molecule 1 (CD54)	ICAM-1	P05362	10.3 ± 3.9	1.8 ± 0.1	5.5 ± 2.7	4.3 ± 2.4
	Interleukin 1, beta	IL1β	P01584	15.6 ± 5.8			
	Interleukin 6 (interferon, beta 2)	IL6	P05231	36.2 ± 5.6	4.2 ± 0.7	7.2 ± 3.2	13.2 ± 3.9
	Interleukin 8	IL8	P10145	120.1 ± 4.7	4.6 ± 1.7	9.1 ± 2.7	51.9 ± 3.3
	Interleukin 15	IL15	P40933	3.0 ± 2.1		3.3 ± 1.6	
	Interleukin 11 receptor, alpha	IL11R	Q16542			2.1 ± 0.2	
	Secreted phosphoprotein 1 (osteopontin)	SPP1	P10451	1.7 ± 0.2			
Oxidative metabolism	Glutathione peroxidase 1	GPX1	P07203	-1.9 ± 0.4			
	Heme oxygenase (decycling) 1	HMOX1	P09601	1.8 ± 0.2	1.9 ± 0.2	2.0 ± 0.3	2.0 ± 0.3
	Superoxide dismutase 2, mitochondrial	SOD2	P04179	4.4 ± 0.1		2.1 ± 0.0	2.1 ± 0.2
Telomere maintenance	Telomerase RNA component	TERC				5.8 ± 0.4	2.9 ± 1.4
Others	Mitogen-activated protein kinase kinase 1	MAP2K1	Q02750	2.1 ± 0.7			
	Insulin-like growth factor binding protein 3	IGF3	P17936	-2.5 ± 0.0	-2.8 ± 0.6	-3.2 ± 0.0	-3.3 ± 0.2
	Plasminogen activator inhibitor 2	PAI2	P05120	4.8 ± 0.3			3.7 ± 0.5
	Plasminogen activator,Urokinase	u-PA	P00749	3.8 ± 0.3	3.8 ± 0.2	3.2 ± 0.4	4.1 ± 0.2
	Tissue plasminogen activator	t-PA	P00750		1.8 ± 0.2	1.6 ± 0.1	
	Small proline-rich protein 1B (cornifin)	SPRR1B	P22528	1.7 ± 0.2			

* Only significant differences between treated and untreated cells genes expressions were noted.

Table 4.4 : Gene expression* from human dermal fibroblasts treated with depolymerised galactofucan.

Function	Gene names	Symbol	Swissprot accession number	Average ratio			
				M05 RDP4H	M05 RDP2H	N05 RDP4H	N05 RDP2H
Apoptosis	BCL2-associated X protein	BAX	Q8WXU1	-1.9 ± 0.0			
	BCL2-like 1	BCLX	Q07817	-3.9 ± 0.1			
	Caspase 8	CASP8	Q14790	3.9 ± 1.2			
	Clusterin (apolipoprotein J)	CLU	P10909	2.3 ± 0.1	3.0 ± 0.5	3.2 ± 0.7	3.8 ± 0.2
Cell cycle	Antigen identified by monoclonal antibody Ki-67	KI-67	P46013		-2.4 ± 1.0		
	Cyclin B1	CCNB1	P14635		-1.8 ± 0.2		-1.7 ± 0.1
	Cyclin D1	CCND1	P24385	-2.0 ± 0.0			
	Cyclin D3	CCND3	P30281	-2.3 ± 0.2			
	Cyclin E1	CCNE1	P24864		-2.1 ± 0.5		
	Cyclin F	CCNF	P41002	6.5 ± 1.1			
	Cyclin G1	CCNG1	P51959	2.9 ± 0.1			
	Cyclin-dependent kinase inhibitor 1A	p21	P38936		3.8 ± 2.9		
	Cyclin-dependent kinase inhibitor 1B	p27	P46527	10.5 ± 0.3		10.8 ± 1.7	3.9 ± 0.4
	Cyclin-dependent kinase inhibitor 1C	p57	P49918	8.3 ± 3.0			
	Cyclin-dependent kinase inhibitor 2A	p16	P42771	2.8 ± 0.3			
	E2F transcription factor 1	E2F1	Q01094				-1.6 ± 0.0
	Early growth response 1	EGR1	P18146		-1.8 ± 0.0		-1.6 ± 0.1
	Inhibitor of DNA binding 2	ID2	Q02363		2.5 ± 0.7		
	Kinesin family member 2C	KIF2C	Q99661		-1.9 ± 0.5	-2.0 ± 0.3	
	Kinesin family member 23	KIF23	Q02241	1.7 ± 0.1			-1.6 ± 0.0
	Transcription factor AP-2 alpha	TFAP2A	P05549	2.0 ± 0.3	2.8 ± 1.7		
	Transcription factor AP-2 beta	TFAP2B	Q92481	14.7 ± 5.1			
	Transcription factor AP-2 gamma	TFAP2C	Q92754	-2.4 ± 0.2			
	Topoisomerase (DNA) II alpha	TOP2A	P11388		-1.9 ± 0.3	-1.7 ± 0.1	-1.6 ± 0.1
	Tumor protein p53	p53	P04637	1.7 ± 0.1			3.5 ± 0.6
Chromosomal processing	Centromere protein A	CENPA	P49450		-2.0 ± 0.0		-1.6 ± 0.1
	Centromere protein F	CENPF	P49454		-8.6 ± 7.2		-5.7 ± 2.0
Extracellular matrix	Cathepsin D	CTSD	P07339		1.6 ± 0.0		
	Cathepsin H	CTSH	P09668	12.1 ± 4.6		9.5 ± 6.7	
	Collagen, type I, alpha 1	COL1A1	P02452	-2.3 ± 0.2			
	Collagen, type III, alpha 1	COL3A1	P02461		2.5 ± 0.8	2.4 ± 0.1	
	Collagen, type XV, alpha 1	COL15A1	P39059		1.8 ± 0.2	1.9 ± 0.3	
	Connective tissue growth factor	CTGF	P29279		5.2 ± 4.5		
	Elastin	ELN	P15502		1.8 ± 0.1		
	Fibromodulin	FMOD	Q06828	4.1 ± 0.4			1.7 ± 0.2
	Matrix metalloproteinase 2 (collagenase)	MMP2	P08253		1.9 ± 0.2		
	Matrix metalloproteinase 10 (stromelysin 2)	MMP10	P09238	13.6 ± 6.5			
	Matrix metalloproteinase 11 (stromelysin 3)	MMP11	P24347	5.5 ± 0.0	6.2 ± 5.2		
	Matrix metalloproteinase 15 (membrane-inserted)	MMP15	P51511	2.9 ± 1.1	2.3 ± 1.1	2.3 ± 0.5	3.5 ± 0.5
	Tissue inhibitor metalloproteinase 2	TIMP2	P16035		2.3 ± 0.6	1.7 ± 0.0	
	Thrombospondin 1	THBS1	P07996	2.5 ± 0.4	2.6 ± 0.7	3.3 ± 1.2	3.5 ± 0.1
	Transgelin	TAGLN	Q01995	1.8 ± 0.4			
Immune and inflammatory	Chemokine (C-C motif) ligand 5	CCL5	P13501	1.9 ± 0.2			
	Colony stimulating factor 2 (granulocyte-macrophage)	GMCSF	P04141	4.4 ± 3.4			
	Intercellular adhesion molecule 1 (CD54)	ICAM-1	P05362	5.7 ± 0.5	3.3 ± 1.1	5.0 ± 2.4	4.1 ± 0.9
	Interleukin 6 (interferon, beta 2)	IL6	P05231	1.9 ± 0.1			3.9 ± 3.4
	Interleukin 15	IL15	P40933	7.2 ± 0.7		3.4 ± 1.1	
	Interleukin 11 receptor, alpha	IL11R	Q16542	3.5 ± 0.6		2.4 ± 0.8	2.8 ± 0.1
	Secreted phosphoprotein 1 (osteopontin)	SPP1	P10451	2.7 ± 0.1			
Oxidative metabolism	Glutathione peroxidase 1	GPX1	P07203	-5.9 ± 0.2			
	Heme oxygenase (decycling) 1	HMOX1	P09601	1.7 ± 0.1	2.6 ± 0.1	2.3 ± 0.9	1.8 ± 0.1
Telomere maintenance	Telomerase RNA component	TERC	/	34.8 ± 4.9			
Others	Mitogen-activated protein kinase kinase 1	MAP2K1	Q02750	97.5 ± 7.7		68.8 ± 58.1	
	Mitogen activated protein kinase kinase 2	MAP2K2	P36507	1.8 ± 0.3			
	Mitogen-activated protein kinase 8	MAPK8	P45983	-4.7 ± 2.5			1.7 ± 0.1
	Plasminogen activator inhibitor 2	PAI2	P05120	-9.3 ± 1.9	-6.0 ± 3.8		
	Small proline-rich protein 1B (cornifin)	SPRR1B	P22528	49.1 ± 18.4			

* Only significant differences between treated and untreated cells genes expressions were noted.

Conclusion

Cette étude présente pour la première fois les caractéristiques structurales et les activités biologiques des laminaranes et des galactofucanes extraits de l'algue brune *Saccharina longicruris* et ce, en fonction de la période de récolte. L'hypothèse du travail a été validée, car il a été démontré que le laminarane et le galactofucane de *Saccharina longicruris* possèdent plusieurs activités biologiques intéressantes. Ces dernières sont influencées par la structure du polysaccharide, d'où l'importance de mieux comprendre la relation entre la structure et l'activité biologique des polysaccharides.

Dans un premier temps, le laminarane et le galactofucane bruts ont été extraits et la composition de base a été déterminée pour chaque période de récolte. De plus, chaque galactofucane brut a été fractionné par chromatographie d'échange ionique avec trois paliers de concentration de NaCl (0,5; 1 et 2 M). Cette étape a permis d'établir que les rendements d'extraction du laminarane étaient supérieurs en août, dus aux faibles taux de nitrites et nitrates présents dans l'eau. Peu de fluctuations au niveau de la composition en monosaccharides et du poids moléculaire ont été observées pour chaque période de récolte. L'isolement des galactofucanes bruts a donné des rendements plus importants en M05, probablement dû au fait que les frondes étaient plus matures en M05 au cours de cette période. Les galactofucanes bruts et fractionnés ont présenté des variations structurales importantes en fonction de la période de récolte. Les galactofucanes récoltés en A05, N05 et J06 ont montré un taux plus élevé de D-galactose contrairement à celui récolté en M05, qui contient une proportion plus importante de L-fucose. Les galactofucanes isolés dans la fraction 0,5 et 2 M NaCl conservent le même profil que les galactofucanes natifs alors que la fraction 1 M NaCl possède du L-fucose en plus grande proportion. Les galactofucanes bruts et fractionnés isolés de M05, A05 et N05 ont un taux de sulfatation supérieur à celui de J06 excepté pour la fraction 0,5M NaCl où les galactofucanes isolés de M05 et de J06 ont un taux équivalent. Le poids moléculaire des galactofucanes natifs est similaire pour chaque période de récolte. Par contre, pour les galactofucanes fractionnés, le poids moléculaire n'a pu être analysé dû à de faibles rendements de fractionnement. Contrairement à ce qui avait été anticipé, les galactofucanes isolés de M05 et de J06

semblent montrer une structure différente. Ces variations ont été attribuées aux sels nutritifs présents dans l'eau, au cycle de croissance de l'algue et à l'âge des frondes.

Dans un deuxième volet, une analyse structurale plus approfondie des polysaccharides a été réalisée. Le galactofucane ayant un poids moléculaire élevé, il a été dépolymérisé pour faciliter l'analyse de sa structure mais aussi pour étudier l'activité biologique de galactofucane de faible poids moléculaire. Seuls les extraits provenant de l'isolement de M05 et de N05 ont été dépolymérisés car leurs compositions en monosaccharides étaient très différentes. De plus, l'isolat de N05 contient un taux de galactofucane légèrement plus élevé que celui de J06. Pour les galactofucanes natifs et dépolymérisés, les unités de L-fucose sont liées en (1,3) avec des groupements sulfate en position 4. Les unités D-galactose sont liées en (1,6) avec des groupements sulfate en position 3. Le poids moléculaire des fractions brutes est similaire (638-851 kDa) pour les galactofucanes isolés de A05, N05 et J06, mais inférieur à celui isolé de M05 (1529 kDa). Les fractions dépolymérisées 2H ont un poids moléculaire entre 23 et 30 kDa alors que les fractions 4H ont un poids inférieur à 10 kDa. Lors de l'étude du laminarane, la présence de lien β-(1,3) au niveau de la chaîne principale a été confirmée ainsi que la présence de ramifications en position 6 et en position 2. Par contre, le laminarane isolé de J06 ne montre aucune ramification en position 6. De plus, les laminaranes provenant de M05 semblent moins ramifiés que les autres fractions ce qui pourrait influencer son interaction avec les solvants et sa conformation en solution. Le poids moléculaire et le degré de polymérisation est comparable pour chacune des périodes de récolte.

Dans un troisième temps, nous avons étudié l'activité biologique des polysaccharides bruts et dépolymérisés choisis selon les périodes de récolte les plus intéressantes. L'utilisation des puces à ADN a permis de réaliser des comparaisons systématiques, car l'expression de plusieurs gènes après le traitement de 24h sur des fibroblastes a pu être mesurée simultanément. Cet outil extrêmement puissant a permis l'identification de gènes d'intérêt notamment au niveau du cycle cellulaire mais aussi, au niveau de la matrice extracellulaire. Des tests *in vitro* classiques ont été réalisés pour valider les effets observés avec les puces à ADN. Les effets sur la croissance cellulaire, l'apoptose, la sécrétion de MMP et de

collagène-I ont été analysés. Nous avons montré que le laminarane influence le comportement des fibroblastes en fonction de la période de récolte. Il module la croissance, la synthèse des MMP et de collagène ainsi, que l'expression des gènes des télomérases. Ces résultats laissent penser que le laminarane pourrait prévenir le vieillissement des cellules et être utile dans le processus de guérison d'une plaie en stimulant l'augmentation de la sécrétion du collagène-I. Nous avons aussi montré que les galactofucanes bruts et dépolymérisés influencent le comportement des fibroblastes en fonction de la période de récolte. Ils modulent la croissance, l'apoptose, la synthèse des MMP et de collagène. Les fractions brutes ont une activité antiproliférative ainsi qu'une activité de dégradation de la matrice extracellulaire causée par l'augmentation de la sécrétion des MMP. Le galactofucane extrait de M05 stimule l'apoptose par le biais d'un mécanisme qui n'est pas encore connu alors que l'extrait provenant de la période N05 indurait la mort cellulaire par nécrose. Les fractions dépolymérisées augmentent la sécrétion de collagène-I et ne modifient pas la croissance des fibroblastes. Ainsi, les galactofucanes bruts et dépolymérisés ont des activités métaboliques opposées. Les fractions brutes laissent penser qu'elles pourraient avoir un effet anti-tumoral alors que les fractions dépolymérisées pourraient être utiles dans le processus de guérison d'une plaie.

Dans un quatrième temps, la relation entre la structure et l'activité biologique des polysaccharides a été analysée. Au niveau du laminarane, peu de différences marquantes ont été observées entre les extraits à l'exception de l'extrait provenant de M05 qui semble moins ramifié que les autres fractions, affectant sa conformation en solution. La conformation en solution (formation d'hélice), le degré de polymérisation et la présence de chaînes latérales semblent être les caractéristiques structurales les plus importantes pour favoriser la liaison entre le laminarane et les récepteurs de β-glucane (Leung et al., 2006). La différence d'activité biologique observée chez le laminarane isolé de M05 pourrait être liée à sa capacité à se lier au récepteur de β-glucane via sa conformation en solution. Les galactofucanes ont présenté des différences importantes au niveau de la composition en monosaccharides, du taux de sulfatation et du poids moléculaire. Nous avons montré que le poids moléculaire influence l'activité métabolique des galactofucanes, en effet les fractions brutes de hauts poids moléculaires réduisent la prolifération cellulaire alors que les

fractions dépolymérisées (<10 kDa) maintiennent la prolifération des fibroblastes. Le taux de sulfatation influence également la prolifération cellulaire mais nous n'avons pas été en mesure d'établir comment ces groupements influencent l'activité. Néanmoins, il semblerait qu'un taux d'environ 20% de groupements sulfate serait requis pour inhiber la prolifération cellulaire (Haroun-Bouhedja et al., 2000). De plus, la position des groupements sulfates aurait un impact important car, cela influencerait aussi la conformation du polysaccharide et donc sa capacité à se lier à certains récepteurs. C'est cette liaison qui permettrait d'activer certaines fonctions métaboliques des galactofucanes. À partir des résultats obtenus, nous avons une meilleure compréhension de la relation entre la structure et l'activité biologique des polysaccharides. Ceci permettra de probablement générer de nouveaux cosméceutiques et nutraceutiques à base de polysaccharides d'algues ayant pour fonction de prévenir le vieillissement ou de favoriser le processus de guérison des plaies.

En vue de compléter ces travaux, il serait pertinent de valider si les laminaranes extraits de *Saccharina longicruris* sont effectivement capables de se lier aux récepteurs de β-glucane présents sur les fibroblastes. Ceci permettrait de mieux comprendre le mécanisme d'action pour la prévention du vieillissement et la guérison des plaies. Afin de valider l'activité télomérase du laminarane isolé de M05 sur la prévention du vieillissement des cellules, il serait essentiel de valider l'expression du gène (RT-PCR) et la sécrétion de la protéine (essai TRAP) (Kim et al., 1997b). Par la méthode Flow-Fish (Rufer et al., 1998), il serait aussi possible de vérifier si les fibroblastes traités avec du laminarane ont des télomères plus long que les cellules contrôles. Ceci nous permettrait de confirmer le caractère anti-âge du laminarane. Il serait aussi justifié d'étudier les effets du laminarane sur d'autres lignées cellulaires et de valider le caractère réversible de l'expression des télomérases en présence et en absence de laminarane. L'amélioration du processus de guérison des plaies engendrée par le laminarane pourrait être vérifiée dans un modèle tridimensionnel de plaie cutanée. Le cas échéant, plusieurs applications potentielles pourraient en découler, telle l'utilisation de laminarane dans une crème anti-âge ou crème améliorant le processus de cicatrisation pour des patients atteints d'acnée.

Au niveau des galactofucanes, il serait aussi pertinent de vérifier s'ils ont la capacité de se lier à certains récepteurs présents sur les fibroblastes et vérifier comment la structure influence cette liaison. Il est reconnu que l'héparine a la capacité d'interagir avec plusieurs protéines dont la thrombine pour empêcher la coagulation du sang et la thrombose. Il serait fort intéressant de valider si les galactofucanes peuvent influencer la coagulation du sang mais surtout s'ils peuvent interagir avec d'autres protéines pour moduler d'autres activités. Il serait aussi intéressant de valider par quel mécanisme l'apoptose est stimulée par le galactofucane brut extrait de M05. Aussi, il serait important de vérifier si l'activité antiproliférative observée peut se traduire en activité antitumorale en traitant des lignées cellulaires cancéreuses avec des galactofucanes bruts. Pour les galactofucanes dépolymérisés 2H, la relation structure-fonction indique que les fucoidanes et galactofucanes de poids moléculaire inférieur à 10 kDa n'ont aucun effet sur la prolifération cellulaire. Il serait pertinent de valider si les galactofucanes de poids moléculaire variant entre 23 et 30 kDa inhibent la prolifération cellulaire. Il serait également pertinent de comprendre comment les groupements sulfate influencent l'activité car, nous savons que dans certains cas, l'enrichissement en groupements sulfate amplifie l'activité. Or, nous avons observé que les fractions dépolymérisées contenant un haut taux de sulfates (~30%) n'inhibent pas la prolifération. Il serait donc pertinent de trouver le poids moléculaire et le taux de sulfatation optimal qui permettent de moduler la prolifération cellulaire. De plus, il serait pertinent de valider comment la structure des polysaccharides affecte la synthèse de MMP et de collagène car ceci pourrait engendrer une activité favorisant le processus de guérison des plaies pour les galactofucanes dépolymérisés. L'utilisation d'un modèle tridimensionnel de plaie cutanée pourrait aussi être intéressante pour valider cette activité.

Il aurait été intéressant d'analyser la structure des laminaranes et des galactofucanes par RMN, ceci nous aurait peut-être permis de voir plus de différences de structure entre les périodes de récolte et nous aurait permis préciser la relation entre la structure et la bioactivité des polysaccharides.

En terminant, l'utilisation des puces à ADN est un outil puissant contrairement aux tests *in vitro* et *in vivo,* qui rendent coûteuse l'analyse systématique d'un grand nombre d'effets et leur aspect réductionniste exclut la possibilité d'identifier des effets insoupçonnés absents de la batterie de tests. Les puces à ADN permettent l'identification de gènes d'intérêts, par contre, des étapes de validation (PCR en temps réel) doivent être réalisées pour confirmer l'expression des gènes. Aussi, l'utilisation de tests Elisa ou de Western blot sont requis afin de confirmer la sécrétion des protéines et de valider l'activité biologique.

Bibliographie

Adams, E. L., Rice, P. J., Graves, B., Ensley, H. E., Yu, H., Brown, G. D., Gordon, S., Monteiro, M. A., Papp-Szabo, E., Lowman, D. W., Power, T. D., Wempe, M. F., Williams, D. L. (2008). Differential high-affinity interaction of dectin-1 with natural or synthetic glucans is dependent upon primary structure and is influenced by polymer chain length and side-chain branching. *Journal of Pharmacology and Experimental Therapeutics*, 325(1): 115-123.

Adams, P. D., Kaelin, W. G. (1996). The cellular effects of E2F overexpression in *Transcriptional Control of Cell Growth: The E2F Gene Family*, P. J. Farnham(Ed.). Berlin, Springer: 79-93.

Adhikari, U., Mateii, C. G., Chattopadhyay, K., Pujol, C. A., Damonte, E. B., Ray, B. (2006). Structure and antiviral activity of sulfated fucans from *Stoechospermum marginatum*. *Phytochemistry*, 67(22): 2474-2482.

Agarwal, C., Britton, Z. T., Alaseirlis, D. A., Li, Y. X., Wang, J. H. C. (2006). Healing and normal fibroblasts exhibit differential proliferation, collagen production, alpha-SMA expression, and contraction. *Annals of Biomedical Engineering*, 34(4): 653-659.

Aisa, Y., Miyakawa, Y., Nakazato, T., Shibata, H., Saito, K., Ikeda, Y., Kizaki, M. (2005). Fucoidan induces apoptosis of human HS-Sultan cells accompanied by activation of caspase-3 and down-regulation of ERK pathways. *American Journal of Hematology*, 78(1): 7-14.

Alekseyenko, T. V., Zhanayeva, S. Y., Venediktova, A. A., Zvyagintseva, T. N., Kuznetsova, T. A., Besednova, N. N., Korolenko, T. A. (2007). Antitumor and antimetastatic activity of fucoidan, a sulfated polysaccharide isolated from the Okhotsk sea *Fucus evanescens* brown alga. *Bulletin of Experimental Biology and Medicine*, 143(6): 730-732.

Alexandre, I., Hamels, S., Dufour, S., Collet, J., Zammatteo, N., De Longueville, F., Gala, J. L., Remacle, J. (2001). Colorimetric silver detection of DNA microarrays. *Analytical Biochemistry*, 295(1): 1.

Alwayn, I. P. J., Appel, J. Z., Goepfert, C., Buhler, L., Cooper, D. K. C., Robson, S. C. (2000). Inhibition of platelet aggregation in baboons: therapeutic implications for xenotransplantation. *Xenotransplantation*, 7(4): 247-257.

Anderson, M. R., Cardinal, A., Larochelle, J. (1981). An alternate growth-pattern for *Laminaria longicruris*. *Journal of Phycology*, 17(4): 405-411.

AOAC (1990). Officials methods of analysis. Washington, DC, Association of Official Analytical Chemists.

Ashar, H. R., James, L., Gray, K., Carr, D., Black, S., Armstrong, L., Bishop, W. R., Kirschmeier, P. (2000). Farnesyl transferase inhibitors block the farnesylation of CENP-E and CENP-F and alter the association of CENP-E with the microtubules. *Journal of Biological Chemistry*, 275(39): 30451-30457.

Ashcroft, G. S., Mills, S. J., Ashworth, J. J. (2002). Ageing and wound healing. *Biogerontology*, 3(6): 337-345.

Ashkenazi, A. (2002). Targeting death and decoy receptors of the tumour-necrosis factor superfamily. *Nature Reviews. Cancer*, 2(6): 420-430.

Asselot, C., Labatrobert, J., Kern, P. (1989). Heparin fragments regulate collagen phenotype and fibronectin synthesis in the skin of genetically diabetic mice. *Biochemical Pharmacology*, 38(6): 895-899.

Aubert, G., Lansdorp, P. M. (2008). Telomeres and aging. *Physiological Reviews*, 88(2): 557-579.

Baba, H., Kunimoto, T., Nitta, K., Sato, K., Hashimoto, S., Kohno, M., Kita, Y., Ogawa, H. (1986). Rapid tumor-regression and induction of tumor-regressing activity in serum by various immune-modulating agents. *International Journal of Immunopharmacology*, 8(6): 569-572.

Baba, M., Nakajima, M., Schols, D., Pauwels, R., Balzarini, J., Declercq, E. (1988a). Pentosan polysulfate, a sulfated oligosaccharide, is a potent and selective anti-HIV agent *in vitro*. *Antiviral Research*, 9(6): 335-343.

Baba, M., Schols, D., Pauwels, R., Nakashima, H., Declercq, E. (1990). Sulfated polysaccharides as potent inhibitors of HIV-induced syncytium formation - A new strategy towards aids chemotherapy. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology*, 3(5): 493-499.

Baba, M., Snoeck, R., Pauwels, R., Declercq, E. (1988b). Sulfated polysaccharides are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, and human immunodeficiency virus. *Antimicrobial Agents and Chemotherapy*, 32(11): 1742-1745.

Bae, J. T., Sim, G. S., Lee, D. H., Lee, B. C., Pyo, H. B., Choe, T. B., Yun, J. W. (2005). Production of exopolysaccharide from mycelial culture of *Grifola frondosa* and its inhibitory effect on matrix metalloproteinase-1 expression in UV-irradiated human dermal fibroblasts. *FEMS Microbiology Letters*, 251(2): 347-354.

Bai, C., Richman, R., Elledge, S. J. (1994). Human Cyclin-F. *EMBO Journal*, 13(24): 6087-6098.

Bain, P., Shaw, G., Patel, B. (2007). Induction of p53-regulated gene expression in human cell lines exposed to the cyanobacterial toxin cylindrospermopsin. *Journal of Toxicology and Environmental Health*, 70(19): 1687-1693.

Baldi, P., Hatfield, G. W. (2002). *DNA microarrays and gene expression: from experiments to data analysis and modeling*. Cambridge, Cambridge University Press, 213 p.

Barnes, P. J., Larin, M. (1997). Nuclear factor-κB - A pivotal transcription factor in chronic inflammatory diseases. *New England Journal of Medicine*, 336(15): 1066-1071.

Barr, R. K., Bogoyevitch, M. A. (2001). The c-Jun N-terminal protein kinase family of mitogen-activated protein kinases (JNK MAPKs). *International Journal of Biochemistry and Cell Biology*, 33(11): 1047-1063.

Barry, V. (1939). Constitution of laminarin - Isolation of 2,4,6-trimethylglucopyranose. *Scientific Proceedings of the Royal Dublin Society*, 22: 59-67.

Battle, J., Ha, T. Z., Li, C. F., Della Beffa, V., Rice, P., Kalbfleisch, J., Browder, W., Williams, D. (1998). Ligand binding to the (1 -> 3)-beta-D-glucan receptor stimulates NF kappa B activation, but not apoptosis in U937 cells. *Biochemical and Biophysical Research Communications*, 249(2): 499-504.

Bebermeier, J.-H., Stiliaras, A., Herzer, P. (2008). "DualChip® expression-profiling: Data processing with Silverquant-labeling." Retrieved December 1st, 2008, from http://www.stagbio.fr/int/index.php?sitemap=4.1&pb=6c5019d984feae98&action=support&contentid=1&catalognode=34980&productpage=7.

Becker, C. F., Guimaraes, J. A., Mourão, P. A. S., Verli, H. (2007). Conformation of sulfated galactan and sulfated fucan in aqueous solutions: Implications to their anticoagulant activities. *Journal of Molecular Graphics and Modelling*, 26(1): 391-399.

Beijing Gingko Group. (2007). "FucoMax™ Fucoidan." Retrieved December 1st, 2008, from http://www.fucomax.com.

Belford, D. A., Hendry, I. A., Parish, C. R. (1993). Investigation of the ability of several naturally-occurring and synthetic polyanions to bind to and potentiate the biological activity of acidic fibroblast growth factor. *Journal of Cellular Physiology*, 157(1): 184-189.

Béress, A., Wassermann, O., Bruhn, T., Béress, L., Kraiselburd, E. N., Gonzalez, L. V., Demotta, G. E., Chavez, P. I. (1993). A new procedure for the isolation of anti-HIV compounds (polysaccharides and polyphenols) from the marine alga *Fucus vesiculosus*. *Journal of Natural Products*, 56(4): 478-488.

Bernard, F. X., Pedretti, N., Rosdy, M., Deguercy, A. (2002). Comparison of gene expression profiles in human keratinocyte mono-layer cultures, reconstituted

epidermis and normal human skin; transcriptional effects of retinoid treatments in reconstituted human epidermis. *Experimental Dermatology*, 11(1): 59-74.

Berteau, O., McCort, I., Goasdoue, N., Tissot, B., Daniel, R. (2002). Characterization of a new alpha-L-fucosidase isolated from the marine mollusk *Pecten maximus* that catalyzes the hydrolysis of alpha-L-fucose form algal fucoidan (*Ascophyllum nodosum*). *Glycobiology*, 12(4): 273-282.

Berteau, O., Mulloy, B. (2003). Sulfated fucans, fresh perspectives: structures, functions, and biological properties of sulfated fucans and an overview of enzymes active toward this class of polysaccharide. *Glycobiology*, 13(6): 29R-40R.

Bertini, S., Bisio, A., Torri, G., Bensi, D., Terbojevich, M. (2005). Molecular weight determination of heparin and dermatan sulfate by size exclusion chromatography with a triple detector array. *Biomacromolecules*, 6(1): 168-173.

Bevilacqua, M. A., Iovine, B., Zambrano, N., D'Ambrosio, C., Scaloni, A., Russo, T., Cimino, F. (2005). Fibromodulin gene transcription is induced by ultraviolet irradiation, and its regulation is impaired in senescent human fibroblasts. *Journal of Biological Chemistry*, 280(36): 31809-31817.

Bilan, M. I., Grachev, A. A., Shashkov, A. S., Nifantiev, N. E., Usov, A. I. (2006). Structure of a fucoidan from the brown seaweed *Fucus serratus L. Carbohydrate Research*, 341(2): 238-245.

Björndal, H., Hellerqv.Cg, Lindberg, B., Svensson, S. (1970). Gas-liquid chromatography and mass spectrometry in methylation analysis of polysaccharides. *Angewandte Chemie International Edition*, 9(8): 610-619.

Black, W. A. P. (1954). The seasonal variation in the combined L-fucose content of the common British laminariaceae and fucaceae. *Journal of the Science of Food and Agriculture*, 5(9): 445-448.

Black, W. A. P., Dewar, E. T. (1949). Correlation of some of the physical and chemical properties of the sea with the chemical constitution of the algae. *Journal of the Marine Biological Association of the United Kingdom*, 28(3): 673-699.

Black, W. A. P., Dewar, E. T. (1973). Laminaran in *Industrial Gums*, R. L. Whistler(Ed.). New York, Academic Press: 137-145.

Black, W. A. P., Dewar, E. T., Woodward, F. N. (1952). Manufacture of algal chemicals .4. Laboratory-scale isolation of fucoidin from brown marine algae. *Journal of the Science of Food and Agriculture*, 3(3): 122-129.

Blanchard, A. (1998). Synthetic DNA array in *Genetic Engineering*, J. K. Setlow(Ed.). New York, Plenum Press. 20: 111-124.

Blondin, C., Fischer, E., Boissonvidal, C., Kazatchkine, M. D., Jozefonvicz, J. (1994). Inhibition of complement activation by natural sulfated polysaccharides (fucans) from brown seaweed. *Molecular Immunology*, 31(4): 247-253.

Bodnar, A. G., Ouellette, M., Frolkis, M., Holt, S. E., Chiu, C. P., Morin, G. B., Harley, C. B., Shay, J. W., Lichtsteiner, S., Wright, W. E. (1998). Extension of life-span by introduction of telomerase into normal human cells. *Science (New York, N.Y.)*, 279(5349): 349-352.

Boisson-Vidal, C., Chaubet, F., Chevolot, L., Sinquin, C., Theveniaux, J., Millet, J., Sternberg, C., Mulloy, B., Fischer, A. M. (2000). Relationship between antithrombotic activities of fucans and their structure. *Drug Development Research*, 51(4): 216-224.

Boisson-Vidal, C., Haroun, F., Eliouali, M., Blondin, C., Fisher, A. M., de Agostini, A., Jozefonvicz, J. (1995). Biological activities of polysaccharides from marine algae. *Drugs of the Future*, 20: 1237-1249.

Boldt, J., Howe, A. M., Parkerson, J. B., Gunter, L. E., Kuehn, E. (1989). Carbohydrate involvement in sperm-egg fusion in mice. *Biology of Reproduction*, 40(4): 887-896.

Brown, G. D. (2006). Dectin-1: a signalling non-TLR pattern-recognition receptor. *Nature Reviews Immunology*, 6(1): 33-43.

Brown, G. D., Gordon, S. (2005). Immune recognition of fungal beta-glucans. *Cellular Microbiology*, 7(4): 471-479.

Brown, P. O. (2000). "The mguide version 2.0." Retrieved December 1st, 2008, from http://cmgm.stanford.edu/pbrown/mguide/index.html.

Calladine, C. R., Drew, H. R., Luisi, B. F., Travers, A. A. (2004). *Understanding DNA: The Molecule & How It Works*. San Diego, Elsevier Ltd, 334 p.

Campbell, N. A. (1995). *Biologie*. Saint-Laurent, Édition du Renouveau Pédagogique Inc, 1190 p.

Campisi, J. (1996). Replicative senescence: An old lives' tale? *Cell*, 84(4): 497-500.

Carlucci, M. J., Scolaro, L. A., Errea, M. I., Matulewicz, M. C., Damonte, E. B. (1997). Antiviral activity of natural sulphated galactans on herpes virus multiplication in cell culture. *Planta Medica*, 63(5): 429-432.

Cavari, S., Ruggiero, M., Vannucchi, S. (1993). Antiproliferative effects of heparin on normal and transformed NIH/3T3 fibroblasts. *Cell Biology International*, 17(8): 781-786.

Chakravarty, S., Bansal, P., Sutovsky, P., Gupta, S. K. (2008). Role of proteasomal activity in the induction of acrosomal exocytosis in human spermatozoa. *Reproductive Biomedicine Online*, 16(3): 391-400.

Chapman, A. R. O. (1987). The wild harvest and culture of *Laminaria longicruris* in Eastern Canada in *Case studies of seven commercial seaweed resources*, M. S. Doty, J. F. Caddy and B. Santelices(Ed.). Rome, Food and Agriculture Organization of the United Nations. 281.

Chapman, A. R. O., Craigie, J. S. (1977). Seasonal growth in *Laminaria longicruris* - Relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Marine Biology*, 40(3): 197-205.

Chapman, A. R. O., Craigie, J. S. (1978). Seasonal growth in *Laminaria longicruris* - Relations with reserve carbohydrate storage and production. *Marine Biology*, 46(3): 209-213.

Chargaff, E., Bancroft, F. W., Brown, M. S. (1936). Studies on the chemistry of blood coagulation II on the inhibition of blood clotting by substances of high molecular weight. *Journal of Biological Chemistry*, 115: 155-156.

Chen, J. Z., Seviour, R. (2007a). Medicinal importance of fungal beta-(1 -> 3), (1 -> 6)-glucans. *Mycological Research*, 111: 635-652.

Chen, L. Y., Subirade, M. (2007b). Effect of preparation conditions on the nutrient release properties of alginate-whey protein granular minicrospheres. *European Journal of Pharmaceutics and Biopharmaceutics*, 65(3): 354-362.

Chen, M. H., Bergman, C. J. (2007c). Method for determining the amylose content, molecular weights, and weight- and molar-based distributions of degree of polymerization of amylose and fine-structure of amylopectin. *Carbohydrate Polymers*, 69(3): 562-578.

Chen, X. J., Xiao, W., Qu, X., Zhou, S. Y. (2008). The effect of dalteparin, a kind of low molecular weight heparin, on lung adenocarcinoma A549 cell line *in vitro*. *Cancer Investigation*, 26(7): 718-724.

Chen, Y., Dougherty, E. R., Bittner, M. L. (1997). Ratio-based decisions and the quantitative analysis of cDNA microarray images. *Journal of Biomedical Optics*, 2(4): 364-374.

Cheng, A. C., Chen, Y. Y., Chen, J. C. (2008). Dietary administration of sodium alginate and kappa-carrageenan enhances the innate immune response of brown-marbled grouper *Epinephelus fuscoguttatus* and its resistance against *Vibrio alginolyticus*. *Veterinary Immunology and Immunopathology*, 121(3-4): 206-215.

Chevolot, L., Foucault, A., Chaubet, F., Kervarec, N., Sinquin, C., Fisher, A. M., Boisson-Vidal, C. (1999). Further data on the structure of brown seaweed fucans: relationships with anticoagulant activity. *Carbohydrate Research*, 319: 154-165.

Chevolot, L., Mulloy, B., Ratiskol, J., Foucault, A., Colliec-Jouault, S. (2001). A disaccharide repeat unit is the major structure in fucoidans from two species of brown algae. *Carbohydrate Research*, 330(4): 529-535.

Chipuk, J. E., Green, D. R. (2005). Do inducers of apoptosis trigger caspase-independent cell death? *Nature Reviews Molecular Cell Biology*, 6(3): 268-275.

Chizhov, A. O., Dell, A., Morris, H. R., Haslam, S. M., McDowell, R. A., Shashkov, A. S., Nifant'ev, N. E., Khatuntseva, E. A., Usov, A. I. (1999). A study of fucoidan from the brown seaweed *Chorda filum*. *Carbohydrate Research*, 320(1-2): 108-119.

Chizhov, A. O., Dell, A., Morris, H. R., Reason, A. J., Haslam, S. M., McDowell, R. A., Chizhov, O. S., Usov, A. I. (1998). Structural analysis of laminarans by MALDI and FAB mass spectrometry. *Carbohydrate Research*, 310(3): 203-210.

Church, F. C., Meade, J. B., Treanor, R. E., Whinna, H. C. (1989). Antithrombin activity of fucoidan - The interaction of fucoidan with heparin cofactor-II, antithrombin-III, and thrombin. *Journal of Biological Chemistry*, 264(6): 3618-3623.

Civelek, A., Ak, K., Kurtkaya, O., Tekeli, A., Isbir, S., Nargileci, E., Arsan, S., Sav, A. (2007). Effect of a low molecular weight heparin molecule, dalteparin, on cellular apoptosis and inflammatory process in an incisional wound-healing model. *Surgery Today*, 37(5): 406-411.

Cobain, E. (2003). "CD4 and the HIV virus." Retrieved December 1st, 2008, from http://www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2003/Cobain/geneprotein.html.

Colliec-Jouault, S., Chevolot, L., Helley, D., Ratiskol, J., Bros, A., Sinquin, C., Roger, O., Fischer, A.-M. (2001). Characterization, chemical modifications and *in vitro* anticoagulant properties of an exopolysaccharide produced by *Alteromonas infernus*. *Biochimica et Biophysica Acta*, 1528(2-3): 141-151.

Colliec, S., Boisson-vidal, C., Jozefonvicz, J. (1994). A low molecular weight fucoidan fraction from the brown seaweed *Pelvetia canaliculata*. *Phytochemistry*, 35(3): 697-700.

Colliec, S., Fischer, A. M., Taponbretaudiere, J., Boisson, C., Durand, P., Jozefonvicz, J. (1991). Anticoagulant properties of a fucoidan fraction. *Thrombosis Research*, 64(2): 143-154.

Collins, K. L., Russo, A. A. R., Tseng, B. Y., Kelly, T. J. (1993). The role of the 70-Kda subunit of human DNA polymerase-alpha in DNA-replication. *EMBO Journal*, 12(12): 4555-4566.

Colman, R. W., Clowes, A. W., George, J. N., Goldhaber, S. Z., Marder, V. J. (2006). Overview of hemostasis in *Hemostasis and thrombosis : basic principles and clinical practice*, R. W. Colman, V. J. Marder, A. W. Clowes, J. N. George and S. Z. Goldhaber(Ed.). Philadelphia, Lippincott Williams and Wilkins: 3-16.

Conrad, H. E. (1998). *Heparin-binding proteins*. San Diego, Academic Press, 527 p.

Coombe, D. R., Parish, C. R., Ramshaw, I. A., Snowden, J. M. (1987). Analysis of the inhibition of tumor-metastasis by sulfated polysaccharides. *International Journal of Cancer*, 39(1): 82-88.

Corcoran, M. L., Stetler-Stevenson, W. G. (1995). Tissue inhibitor of metalloproteinase-2 stimulates fibroblast proliferation via a cAMP-dependent mechanism. *Journal of Biological Chemistry*, 270(22): 13453-13459.

Corriveau, M.-P., Boufaied, I., Lessard, J., Chabaud, S., Senécal, J.-L., Grodzicky, T., Chartier, S., Raymond, Y., Moulin, V. J. (2009). The fibrotic phenotype of systemic sclerosis fibroblasts varies with disease duration and severity of skin involvement: reconstitution of skin fibrosis development using a tissue engineering approach. *The Journal of Pathology*, 217: 534-542.

Craigie, J. S., Morris, E. R., Rees, D. A., Thom, D. (1984). Alginate block structure in phaeophyceae from Nova Scotia - Variation with species, environment and tissue-type. *Carbohydrate Polymers*, 4(4): 237-252.

Cumashi, A., Ushakova, N. A., Preobrazhenskaya, M. E., D'Incecco, A., Piccoli, A., Totani, L., Tinari, N., Morozevich, G. E., Berman, A. E., Bilan, M. I., Usov, A. I., Ustyuzhanina, N. E., Grachev, A. A., Sanderson, C. J., Kelly, M., Rabinovich, G. A., Iacobelli, S., Nifantiev, N. E. (2007). A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. *Glycobiology*, 17(5): 541-552.

Curto, E. V., Lambert, G. W., Davis, R. L., Wilborn, T. W., Dooley, T. P. (2002). Biomarkers of human skin cells identified using DermArray DNA arrays and new bioinformatics methods. *Biochemical and Biophysical Research Communications*, 291(4): 1052-1064.

D'ortho, M.-P., Will, H., Atkinson, S., Butler, G., Messent, A., Gavrilovic, J., Smith, B., Timpl, R., Zardi, L., Murphy, G. (1997). Membrane-type matrix metalloproteinases 1 and 2 exhibit broad-spectrum proteolytic capacities comparable to many matrix metalloproteinases. *European Journal of Biochemistry*, 250(3): 751-757.

Dale, J. W., von Schantz, M. (2002). *From genes to genomes*. West Sussex, John Wiley & sons, 360 p.

Daniel, R., Berteau, O., Chevolot, L., Varenne, A., Gareil, P., Goasdoue, N. (2001). Regioselective desulfation of sulfated L-fucopyranoside by a new sulfoesterase from the marine mollusk *Pecten maximus* - Application to the structural study of algal fucoidan (*Ascophyllum nodosum*). *European Journal of Biochemistry*, 268(21): 5617-5626.

Daniel, R., Berteau, O., Jozefonvicz, J., Goasdoue, N. (1999). Degradation of algal (*Ascophyllum nodosum*) fucoidan by an enzymatic activity contained in digestive glands of the marine mollusc *Pecten maximus*. *Carbohydrate Research*, 322(3-4): 291-297.

Daniel, R., Chevolot, L., Carrascal, M., Tissot, B., Mourão, P. A. S., Abian, J. (2007). Electrospray ionization mass spectrometry of oligosaccharides derived from fucoidan of *Ascophyllum nodosum*. *Carbohydrate Research*, 342(6): 826-834.

Datta, S. R., Dudek, H., Tao, X., Masters, S., Fu, H. A., Gotoh, Y., Greenberg, M. E. (1997). Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell*, 91(2): 231-241.

Daum, G., Hedin, U., Wang, Y. X., Wang, T., Clowes, A. W. (1997). Diverse effects of heparin on mitogen-activated protein kinase-dependent signal transduction in vascular smooth muscle cells. *Circulation Research*, 81(1): 17-23.

De Boeck, G., Forsyth, R. G., Praet, M., Hogendoorn, P. C. (2009). Telomere-associated proteins: cross-talk between telomere maintenance and telomere-lengthening mechanisms. *The Journal of Pathology*, 217(3): 327-344.

de Longueville, F., Surry, D., Meneses-Lorente, G., Bertholet, V., Talbot, V., Evrard, S., Chandelier, N., Pike, A., Worboys, P., Rasson, J.-P., Le Bourdelles, B., Remacle, J. (2002). Gene expression profiling of drug metabolism and toxicology markers using a low-density DNA microarray. *Biochemical Pharmacology*, 64(1): 137-149.

de Magalhães, J. P., Chainiaux, F., de Longueville, F., Mainfroid, V., Migeot, V., Marcq, L., Remacle, J., Salmon, M., Toussaint, O. (2004). Gene expression and regulation in H_2O_2-induced premature senescence of human foreskin fibroblasts expressing or not telomerase. *Experimental Gerontology*, 39(9): 1379-1389.

de Souza, M. C. R., Marques, C. T., Dore, C. M. G., da Silva, F. R. F., Rocha, H. A. O., Leite, E. L. (2007). Antioxidant activities of sulfated polysaccharides from brown and red seaweeds. *Journal of Applied Phycology*, 19(2): 153-160.

Delatte, S. J., Evans, J., Hebra, A., Adamson, W., Othersen, H. B., Tagge, E. P. (2001). Effectiveness of beta-glucan collagen for treatment of partial-thickness burns in children. *Journal of Pediatric Surgery*, 36(1): 113-118.

Descamps, V., Colin, S., Lahaye, M., Jam, M., Richard, C., Potin, P., Barbeyron, T., Yvin, J. C., Kloareg, B. (2006). Isolation and culture of a marine bacterium degrading the sulfated fucans from marine brown algae. *Marine Biotechnology*, 8(1): 27-39.

Dettmar, P. W., Hampson, F. C., Taubel, J., Lorch, U., Johnstone, L. M., Sykes, J., Berry, P. J. (2007). The suppression of gastro-oesophageal reflux by alginates. *International Journal of Clinical Practice*, 61(10): 1654-1662.

Deux, J. F., Meddahi-Pelle, A., Le Blanche, A. F., Feldman, L. J., Colliec-Jouault, S., Bree, F., Boudghene, F., Michel, J. B., Letourneur, D. (2002). Low molecular weight fucoidan prevents neointimal hyperplasia in rabbit iliac artery in-stent restenosis model. *Arteriosclerosis Thrombosis and Vascular Biology*, 22(10): 1604-1609.

Devillé, C., Damas, J., Forget, P., Dandrifosse, G., Peulen, O. (2004). Laminarin in the dietary fibre concept. *Journal of the Science of Food and Agriculture*, 84(9): 1030-1038.

Devillé, C., Gharbi, M., Dandrifosse, G., Peulen, O. (2007). Study on the effects of laminarin, a polysaccharide from seaweed, on gut characteristics. *Journal of the Science of Food and Agriculture*, 87(9): 1717-1725.

Dickinson, D. P. (2002). Cysteine peptidases of mammals: Their biological roles and potential effects in the oral cavity and other tissues in health and disease. *Critical Reviews in Oral Biology & Medicine*, 13(3): 238-275.

Ding, H., Shi, G. G., Yu, X., Yu, J. P., Huang, J. A. (2003). Modulation of $GdCl_3$ and Angelica Sinensis polysaccharides on differentially expressed genes in liver of hepatic immunological injury mice by cDNA microarray. *World Journal of Gastroenterology*, 9(5): 1072-1076.

Ding, W. K., Shah, N. P. (2009). An improved method of microencapsulation of probiotic bacteria for their stability in acidic and bile conditions during storage. *Journal of Food Science*, 74(2): M53-M61.

Doner, L. W., Whistler, R. L. (1973). Fucoidan in *Industrial Gums*, R. L. Whistler(Ed.). New York, Academic Press: 115-121.

Dravland, J. E., Mortimer, D. (1988). Role for fucose-sulfate-rich carbohydrates in the penetration of zona-pellucida-free hamster eggs by hamster spermatozoa. *Gamete Research*, 21(4): 353-358.

Duarte, M. E. R., Cardoso, M. A., Noseda, M. D., Cerezo, A. S. (2001). Structural studies on fucoidans from the brown seaweed *Sargassum stenophyllum*. *Carbohydrate Research*, 333(4): 281-293.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3): 350-356.

Duboit, S., Yang, J. Y. H. (2003). Bioconductor R packages foe exploratory analysis and normalization of cDNA microarray data in *The analysis of gene expression data: an overview of methods and software*, G. Parmigiani, E. S. Garrett, R. A. Irizarry and S. L. Zegger(Ed.). New York, Springer: 73-101.

Dulinska, J., Gil, D., Zagajewski, J., Hartwich, J., Bodzioch, M., Dembinska-Kiec, A., Langmann, T., Schmitz, G., Laidler, P. (2005). Different effect of beta-carotene on proliferation of prostate cancer cells. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 1740(2): 189-201.

Dürig, J., Bruhn, T., Zurborn, K. H., Gutensohn, K., Bruhn, H. D., Beress, L. (1997). Anticoagulant fucoidan fractions from *Fucus vesiculosus* induce platelet activation *in vitro*. *Thrombosis Research*, 85(6): 479-491.

Eckert, D., Buhl, S., Weber, S., Jager, R., Schorle, H. (2005). The AP-2 family of transcription factors. *Genome Biology*, 6(13): 246.

Edelberg, J. M., Pizzo, S. V. (1990). Kinetic analysis of the effects of heparin and lipoproteins on tissue plasminogen activator mediated plasminogen activation. *Biochemistry*, 29(25): 5906-5911.

Edwards, K. F., Pfister, C. A., Van Alstyne, K. L. (2006). Nitrogen content in the brown alga *Fucus gardneri* and its relation to light, herbivory and wave exposure. *Journal of Experimental Marine Biology and Ecology*, 336(1): 99-109.

Eklund, L., Piuhola, J., Komulainen, J., Sormunen, R., Ongvarrasopone, C., Fassler, R., Muona, A., Ilves, M., Ruskoaho, H., Takala, T. E. S., Pihlajaniemi, T. (2001). Lack of type XV collagen causes a skeletal myopathy and cardiovascular defects in mice. *Proceedings of the National Academy of Sciences of the United States of America*, 98(3): 1194-1199.

El Nabout, R., Martin, M., Remy, J., Robert, L., Lafuma, C. (1989). Heparin fragments modulate the collagen phenotype of fibroblasts from radiation-induced subcutaneous fibrosis. *Experimental and Molecular Pathology*, 51(2): 111-122.

Ellouali, M., Boisson-Vidal, C., Jozefonvicz, J. (1994). Antiproliferative effect and interaction of fucans with cells. *Colloids and Surfaces B: Biointerfaces*, 2(1-3): 305.

Ellouali, M., Boissonvidal, C., Durand, P., Jozefonvicz, J. (1993). Antitumor activity of low molecular weight fucans extracted from brown seaweed *Ascophyllum nodosum*. *Anticancer Research*, 13: 2011-2019.

Eppendorf International. (2008a). "DualChip® human aging v. 1.1 (gene list)." Retrieved December 1st, 2008, from http://www.eppendorf.com/int/index.php?sitemap=1&pb=06c3ba3d42e36570&action=document&contentid=1&docnode=32440&docnode=32441.

Eppendorf International. (2008b). "Silverquant® analysis software: User Manual Version 2.1." Retrieved December 1st, 2008, from http://www.stagbio.fr/int/index.php?sitemap=4.1&pb=10ec38c59031923c&action=support&contentid=1&catalognode=34980&productpage=8.

Erduran, E., Deger, O., Albayrak, D., Tekelioglu, Y., Ozdemir, T. (2007). *In vitro* investigation of the apoptotic effect of heparin on lymphoblasts by using flow cytometric DNA analysis and fluorometric caspase-3 and -8 activities. *DNA and Cell Biology*, 26(11): 803-808.

Esen, E. C., E., Ozogul, C., Tasci, A. G., Sipahioglu, S., Gemalmaz, H. C., Ozturk, A. M., Dogramaci, Y. (2009). The effect of low-molecular-weight heparin on rat tendon healing. *Acta Orthopaedica et Traumatologica Turcica*, 43(1): 54-61.

Espevik, T., Otterlei, M., Skjakbraek, G., Ryan, L., Wright, S. D., Sundan, A. (1993). The involvement of CD14 in stimulation of cytokine production by uronic acid polymers. *European Journal of Immunology*, 23(1): 255-261.

Falcone, D. J., McCaffrey, T. A., Vergilio, J. A. (1991). Stimulation of macrophage urokinase expression by polyanions is protein kinase C-dependent and requires protein and RNA synthesis. *Journal of Biological Chemistry*, 266(33): 22726-22732.

FAO (2003). *A guide to the seaweed industry*. Rome, FAO, 105 p.

Farach, M. C., Tang, J. P., Decker, G. L., Carson, D. D. (1987). Heparin/heparan sulfate is involved in attachment and spreading of mouse embryos *in vitro*. *Developmental Biology*, 123(2): 401.

Farias, W. R. L., Valente, A. P., Pereira, M. S., Mourão, P. A. S. (2000). Structure and anticoagulant activity of sulfated galactans - Isolation of a unique sulfated galactan from the red algae *Botryocladia occidentalis* and comparison of its anticoagulant action with that of sulfated galactans from invertebrates. *Journal of Biological Chemistry*, 275(38): 29299-29307.

Fasciano, S., Patel, R. C., Handy, I., Patel, C. V. (2005). Regulation of vascular smooth muscle proliferation by heparin - Inhibition of cyclin-dependent kinase 2 activity by p27(kip1). *Journal of Biological Chemistry*, 280(16): 15682-15689.

Feldman, S. C., Reynaldi, S., Stortz, C. A., Cerezo, A. S., Damonte, E. B. (1999). Antiviral properties of fucoidan fractions from *Leathesia difformis*. *Phytomedicine*, 6(5): 335-340.

Ferrao, A. V., Mason, R. M. (1993). The effect of heparin on cell proliferation and type-I collagen synthesis by adult human dermal fibroblasts. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1180(3): 225-230.

Fish, R. J., Kruithof, E. K. O. (2006). Evidence for serpinB2-independent protection from TNF-alpha-induced apoptosis. *Experimental Cell Research*, 312(3): 350-361.

Fisher, G. J., Quan, T., Purohit, T., Shao, Y., Cho, M. K., He, T., Varani, J., Kang, S., Voorhees, J. J. (2009). Collagen fragmentation promotes oxidative stress and elevates matrix metalloproteinase-1 in fibroblasts in aged human skin. *American Journal of Pathology*, 174(1): 101-114.

Fisheries and Oceans Canada. (2008). "Oceanographic Data Management System." Maurice Lamontagne Institute Mont-Joli (Québec) Canada. from http://www.osl.gc.ca/sgdo (searched on September 9, 2008)

Fishman, S., Brill, S., Papa, M., Halpern, Z., Zvibel, I. (2002). Heparin-derived disaccharides modulate proliferation and erb-B2-mediated signal transduction in colon cancer cell lines. *International Journal of Cancer*, 99(2): 179-184.

Fitton, J. H., Irhimeh, M. R., Teas, J. (2008). Marine algae and polysaccharides with therapeutic applications in *Marine nutraceuticals and functional foods*, C. Barrow and F. Shahidi(Ed.). Boca Raton, CRC Press: 345-366.

Fleurence, J. (1999). Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends in Food Science & Technology*, 10(1): 25-28.

Fodor, S. P. A., Rava, R. P., Huang, X. H. C., Pease, A. C., Holmes, C. P., Adams, C. L. (1993). Multiplexed biochemical assays with biological chips. *Nature*, 364(6437): 555-556.

Forsyth, N. R., Wright, W. E., Shay, J. W. (2002). Telomerase and differentiation in multicellular organisms: Turn it off, turn it on, and turn it off again. *Differentiation*, 69(4-5): 188-197.

Foxall, C., Watson, S. R., Dowbenko, D., Fennie, C., Lasky, L. A., Kiso, M., Hasegawa, A., Asa, D., Brandley, B. K. (1992). The 3 members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl lewis oligosaccharide. *Journal of Cell Biology*, 117(4): 895-902.

Franke, T. F., Hornik, C. P., Segev, L., Shostak, G. A., Sugimoto, C. (2003). PI3K/Akt and apoptosis: size matters. *Oncogene*, 22(56): 8983-8998.

Frazier, K., Williams, S., Kothapalli, D., Klapper, H., Grotendorst, G. R. (1996). Stimulation of fibroblast cell growth, matrix production, and granulation tissue formation by connective tissue growth factor. *Journal of Investigative Dermatology*, 107(3): 404-411.

Frecer, V., Rizzo, R., Miertus, S. (2000). Molecular dynamics study on the conformational stability of laminaran oligomers in various solvents. *Biomacromolecules*, 1(1): 91-99.

Fujihara, M., Iizima, N., Yamamoto, I., Nagumo, T. (1984). Purification and chemical and physical characterization of an antitumour polysaccharide from the brown seaweed *Sargassum fulvellum*. *Carbohydrate Research*, 125(1): 97-106.

Fujihara, M., Nagumo, T. (1992). The effect of the content of D-mannuronic acid and L-guluronic acid blocks in alginates on antitumor activity. *Carbohydrate Research*, 224: 343-347.

Funk, W. D., Wang, C. K., Shelton, D. N., Harley, C. B., Pagon, G. D., Hoeffler, W. K. (2000). Telomerase expression restores dermal integrity to *in vitro*-aged fibroblasts in a reconstituted skin model. *Experimental Cell Research*, 258(2): 270-278.

Gagné, J. A., Mann, K. H., Chapman, A. R. O. (1982). Seasonal patterns of growth and storage in *Laminaria longicruris* in relation to differing patterns of availability of nitrogen in the water. *Marine Biology*, 69(1): 91-101.

Gantner, B. N., Simmons, R. M., Canavera, S. J., Akira, S., Underhill, D. M. (2003). Collaborative induction of inflammatory responses by dectin-1 and toll-like receptor 2. *Journal of Experimental Medicine*, 197(9): 1107-1117.

Garg, H. G., Thompson, B. T., Hales, C. A. (2000). Structural determinants of antiproliferative activity of heparin on pulmonary artery smooth muscle cells. *American Journal of Physiology Lung Cellular and Molecular Physiology*, 279(5): L779-L789.

Garg, H. G., Yu, L. Y., Hales, C. A., Toida, T., Islam, T., Linhardt, R. J. (2003). Sulfation patterns in heparin and heparan sulfate: effects on the proliferation of bovine pulmonary artery smooth muscle cells. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 1639(3): 225-231.

Gaubatz, S., Lindeman, G. J., Ishida, S., Jakoi, L., Nevins, J. R., Livingston, D. M., Rempel, R. E. (2000). E2F4 and E2F5 Play an Essential Role in Pocket Protein-Mediated G1 Control. *Molecular Cell*, 6(3): 729-735.

Gaudry, M., Hakim, J. (1998). Chimiotactisme des polynucléaires neutrophiles humains in *L'inflammation*, F. Russo-Marie, A. Peltier and B. S. Polla(Ed.). Paris, John Libbey Eurotext: 501-511.

Gazel, A., Ramphal, P., Rosdy, M., De Wever, B., Tornier, C., Hosein, N., Lee, B., Tomic-Canic, M., Blumenberg, M. (2003). Transcriptional profiling of epidermal keratinocytes: Comparison of genes expressed in skin, cultured keratinocytes, and reconstituted epidermis, using large DNA microarrays. *Journal of Investigative Dermatology*, 121(6): 1459-1468.

Gerard, V. A., Mann, K. H. (1979). Growth and production of *Laminaria longicruris* (phaeophyta) populations exposed to different intensities of water movement. *Journal of Phycology*, 15(1): 33-41.

Giambernardi, T. A., Grant, G. M., Taylor, G. P., Hay, R. J., Maher, V. M., McCormick, J. J., Klebe, R. J. (1998). Overview of matrix metalloproteinase expression in cultured human cells. *Matrix Biology*, 16(8): 483-496.

Gill, S. E., Parks, W. C. (2008). Metalloproteinases and their inhibitors: Regulators of wound healing. *International Journal of Biochemistry and Cell Biology*, 40(6-7): 1334-1347.

Giraux, J. L., Matou, S., Bros, A., Tapon-Bretaudière, J., Letourneur, D., Fischer, A. M. (1998). Modulation of human endothelial cell proliferation and migration by fucoidan and heparin. *European Journal of Cell Biology*, 77(4): 352.

Glabe, C. G., Yednock, T., Rosen, S. D. (1983). Reversible disruption of cultured endothelial monolayers by sulfated fucans. *Journal of Cell Science*, 61: 475-490.

Glycoforum. (1998). "Role of hyaluronan during ovulation and fertilization." Retrieved December 1st, 2008, from http://www.glycoforum.gr.jp/science/hyaluronan/HA03/HA03E.html.

Gogly, B., Dridi, M., Hornebeck, W., Bonnefoix, M., Godeau, G., Pellat, B. (1999). Effect of heparin on the production of matrix metalloproteinases and tissue inhibitors of metalloproteinases by human dermal fibroblasts. *Cell Biology International*, 23(3): 203-209.

Gogly, B., Hornebeck, W., Groult, N., Godeau, G., Pellat, B. (1998). Influence of heparin(s) on the interleukin-1-beta-induced expression of collagenase, stromelysin-1, and tissue inhibitor of metalloproteinase-1 in human gingival fibroblasts. *Biochemical Pharmacology*, 56(11): 1447-1454.

Gordillo, F. J. L., Aguilera, J., Jimenez, C. (2006). The response of nutrient assimilation and biochemical composition of Arctic seaweeds to a nutrient input in summer. *Journal of Experimental Botany*, 57(11): 2661-2671.

Granert, C., Raud, J., Waage, A., Lindquist, L. (1999). Effects of polysaccharide fucoidin on cerebrospinal fluid interleukin-1 and tumor necrosis factor alpha in pneumococcal meningitis in the rabbit. *Infection and Immunity*, 67(5): 2071-2074.

Grauffel, V., Kloareg, B., Mabeau, S., Durand, P., Jozefonvicz, J. (1989). New natural polysaccharides with potent antithrombic activity - Fucans from brown algae. *Biomaterials*, 10(6): 363-368.

Green, D. R., Reed, J. C. (1998). Mitochondria and apoptosis. *Science*, 281(5381): 1309-1312.

Griffin, C. C., Linhardt, R. J., Vangorp, C. L., Toida, T., Hileman, R. E., Schubert, R. L., Brown, S. E. (1995). Isolation and characterization of heparan sulfate from crude porcine intestinal mucosal peptidoglycan heparin. *Carbohydrate Research*, 276(1): 183-197.

Guiry, M. D., Guiry, G. M. (2009). "*AlgaeBase*. World-wide electronic publication." National University of Ireland, Galway. from http://www.algaebase.org (searched on August 12, 2009)

Guo, N. H., Krutzsch, H. C., Inman, J. K., Roberts, D. D. (1997). Thrombospondin 1 and type I repeat peptides of thrombospondin 1 specifically induce apoptosis of endothelial cells. *Cancer Research*, 57(9): 1735-1742.

Hakomori, S. I. (1964). Rapid permethylation of glycolipid, and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. *Journal of Biochemistry*, 55(2): 205-208.

Handayani, R., Rice, L., Cui, Y. H., Medrano, T. A., Samedi, V. G., Baker, H. V., Szabo, N. J., Shiverick, K. T. (2006). Soy isoflavones alter expression of genes associated with cancer progression, including interleukin-8, in androgen-independent PC-3 human prostate cancer cells. *Journal of Nutrition*, 136(1): 75-82.

Hao, L. Y., Strong, M. A., Greider, C. W. (2004). Phosphorylation of H2AX at short telomeres in T cells and fibroblasts. *Journal of Biological Chemistry*, 279(43): 45148-45154.

Hara, E., Yamaguchi, T., Nojima, H., Ide, T., Campisi, J., Okayama, H., Oda, K. (1994). Id-related genes encoding helix-loop-helix proteins are required for G_1 progression and are repressed in senescent human fibroblasts. *Journal of Biological Chemistry*, 269(3): 2139-2145.

Harding, S. E., Varum, K. M., Stokke, B. T., Smidsrod, O. (1991). Molecular weight determination of polysaccharides in *Advances in Carbohydrate Analysis*, C. A. White(Ed.). Greenwich, JAI Press Ltd: 63-144.

Harlin, M. M., Craigie, J. S. (1978). Nitrate uptake by *Laminaria longicruris* (Phaeophyceae). *Journal of Phycology*, 14(4): 464-467.

Haroun-Bouhedja, F., Ellouali, M., Sinquin, C., Boisson-Vidal, C. (2000). Relationship between sulfate groups and biological activities of fucans. *Thrombosis Research*, 100(5): 453-459.

Harper, J. W., Elledge, S. J., Keyomarsi, K., Dynlacht, B., Tsai, L. H., Zhang, P. M., Dobrowolski, S., Bai, C., Connellcrowley, L., Swindell, E., Fox, M. P., Wei, N. (1995). Inhibition of cyclin-dependent kinases by p21. *Molecular Biology of the Cell*, 6(4): 387-400.

Hartwell, L. H., Hunt, R. T., Nurse, P. M. (2001). "The Nobel prize in physiology or medicine 2001." Retrieved February 5th, 2009, from http://nobelprize.org/nobel_prizes/medicine/laureates/2001/press.html.

Haug, A., Jensen, A. (1956). Seasonal variation in chemical compositions of *Laminaria digitata* from different parts of the Norwegian coast. International Seaweed Symposium, Trondheim 2, 10-15.

Hedin, U., Daum, G., Clowes, A. W. (1998). Heparin inhibits thrombin-induced mitogen-activated protein kinase signaling in arterial smooth muscle cells. *Journal of Vascular Surgery*, 27(3): 512-520.

Heinzelmann, M., Polk, H. C., Miller, F. N. (1998). Modulation of lipopolysaccharide-induced monocyte activation by heparin-binding protein and fucoidan. *Infection and Immunity*, 66(12): 5842-5847.

Hellerqvist, C. G. (1990). Linkage analysis using lindberg method in *Methods in Enzymology*, J. A. McCloskey(Ed.), Academic Press: 554-573.

Hemmingson, J. A., Falshaw, R., Furneaux, R. H., Thompson, K. (2006). Structure and antiviral activity of the galactofucan sulfates extracted from *Undaria pinnatifida* (Phaeophyta). *Journal of Applied Phycology*, 18(2): 185-193.

Hirsh, J., Warkentin, T. E., Shaughnessy, S. G., Anand, S. S., Halperin, J. L., Raschke, R., Granger, C., Ohman, E. M., Dalen, J. E. (2001). Heparin and low-molecular-weight heparin - Mechanisms of action, pharmacokinetics, dosing, monitoring, efficacy, and safety. *Chest*, 119(1): 64S-94S.

Holmbeck, K., Bianco, P., Caterina, J., Yamada, S., Kromer, M., Kuznetsov, S. A., Mankani, M., Gehron Robey, P., Poole, A. R., Pidoux, I., Ward, J. M., Birkedal-Hansen, H. (1999). MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. *Cell*, 99(1): 81-92.

Honya, M., Mori, H., Anzai, M., Araki, Y., Nisizawa, K. (1999). Monthly changes in the content of fucans, their constituent sugars and sulphate in cultured *Laminaria japonica*. *Hydrobiologia*, 399: 411-416.

Hornak, J. P. (2002). The basics of NMR. Rochester, Center for Imaging Science, Rochester Institute of Technology.

Hoshino, T., Hayashi, T., Hayashi, K., Hamada, J., Lee, J. B., Sankawa, U. (1998). An antivirally active sulfated polysaccharide from *Sargassum horneri* (Turner) C. Agardh. *Biological & Pharmaceutical Bulletin*, 21(7): 730-734.

Hsu, H. Y., Chiu, S. L., Wen, M. H., Chen, K. Y., Hua, K. F. (2001). Ligands of macrophage scavenger receptor induce cytokine expression via differential

modulation of protein kinase signaling pathways. *Journal of Biological Chemistry*, 276(31): 28719-28730.

Ii, M., Yamamoto, H., Adachi, Y., Maruyama, Y., Shinomura, Y. (2006). Role of matrix metalloproteinase-7 (matrilysin) in human cancer invasion, apoptosis, growth, and angiogenesis. *Experimental Biology and Medicine*, 231(1): 20-27.

Iino, K., Ohno, N., Suzuki, I., Sato, K., Oikawa, S., Yadomae, T. (1985). Structure-function relationship of antitumor beta-1,3-glucan obtained from matted mycelium of cultured *Grifola frondosa*. *Chemical & Pharmaceutical Bulletin*, 33(11): 4950-4956.

Indegaard, M., Minsaas, J. (1991a). Animal and human nutrition in *Seaweed Resources in Europe. Uses and Potential*, M. D. Guiry and G. Blunden(Ed.). Chichester, John Wiley & Sons: 21-64

Indegaard, M., Østgaard, K. (1991b). Polysaccharides for food and pharmaceutical uses in *Seaweed Resources in Europe. Uses and Potential*, M. D. Guiry and G. Blunden(Ed.). Chichester, John Wiley & Sons: 169-183.

Ito, K., Hori, K. (1989). Seaweed: Chemical composition and potential food uses. *Food Reviews International*, 5(1): 101-144.

Iyer, V. R., Eisen, M. B., Ross, D. T., Schuler, G., Moore, T., Lee, J. C. F., Trent, J. M., Staudt, L. M., Hudson, J., Boguski, M. S., Lashkari, D., Shalon, D., Botstein, D., Brown, P. O. (1999). The transcriptional program in the response of human fibroblasts to serum. *Science*, 283(5398): 83-87.

Jackson, C., Barth, H. G. (1994). Size exclusion chromatography with multiple detectors: inherent problems in data analysis. *Trends in Polymer Science*, 2(6): 203.

Jackson, C., Nilsson, L. M., Wyatt, P. J. (1989). Characterization of biopolymers using a multi-angle light scattering detector with size exclusion chromatography. *Journal of Applied Polymer Science: Applied Polymer Symposium* 43: 99-114.

Jolles, B., Remington, M., Andrews, P. S. (1963). Effects of sulphated degraded laminarin on experimental tumour growth. *British Journal of Cancer*, 17(1): 109-115.

Kamerling, J. P., Gerwig, G. J., Vliegenthart, J. F. G., Clamp, J. R. (1975). Characterization by gas-liquid chromatography mass spectrometry and proton-magnetic-resonance spectroscopy of pertrimethylsilyl methyl glycosides obtained in methanolysis of glycoproteins and glycopeptides. *Biochemical Journal*, 151(3): 491-495.

Kanabar, V., Hirst, S. J., O'Connor, B. J., Page, C. P. (2005). Some structural determinants of the antiproliferative effect of heparin-like molecules on human airway smooth muscle. *British Journal of Pharmacology*, 146(3): 370-377.

Kanzaki, Y., Onoue, F., Sakurai, H., Ide, T. (2003). Telomerase upregulates expression levels of interleukin (IL)-1 alpha, IL-1 beta, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor in normal human fibroblasts. *Biochemical and Biophysical Research Communications*, 305(1): 150-154.

Kenagy, R. D., Nikkari, S. T., Welgus, H. G., Clowes, A. W. (1994). Heparin inhibits the induction of three matrix metalloproteinases (stromelysin, 92-kD gelatinase, and collagenase) in primate arterial smooth muscle cells. *Journal of Clinical Investigation*, 93(5): 1987-1993.

Khorana, A. A., Sahni, A., Altland, O. D., Francis, C. W. (2003). Heparin inhibition of endothelial cell proliferation and organization is dependent on molecular weight. *Arteriosclerosis Thrombosis and Vascular Biology*, 23(11): 2110-2115.

Kikkert, R., Bulder, I., de Groot, E. R., Aarden, L. A., Finkelman, M. A. (2007). Potentiation of Toll-like receptor-induced cytokine production by (1 -> 3)-beta-D-glucans: implications for the monocyte activation test. *Journal of Endotoxin Research*, 13(3): 140-149.

Kim, H. H., Shin, C. M., Park, C. H., Kim, K. H., Cho, K. H., Eun, H. C., Chung, J. H. (2005). Eicosapentaenoic acid inhibits UV-induced MMP-1 expression in human dermal fibroblasts. *Journal of Lipid Research*, 46(8): 1712-1720.

Kim, I. G., Jun, D. Y., Sohn, U., Kim, Y. H. (1997a). Cloning and expression of human mitotic centromere-associated kinesin gene. *Biochimica et Biophysica Acta*, 1359(3): 181-186.

Kim, K.-H., Kim, Y.-W., Kim, H. B., Lee, B. J., Lee, D. S. (2006). Anti-apoptotic activity of laminarin polysaccharides and their enzymatically hydrolyzed oligosaccharides from *Laminaria japonica*. *Biotechnology Letters*, 28(6): 439-446.

Kim, N. W., Wu, F. (1997b). Advances in quantification and characterization of telomerase activity by the telomeric repeat amplification protocol (TRAP). *Nucleic Acids Research*, 25(13): 2595-2597.

Kim, Y. T., Kim, E. H., Cheong, C., Williams, D. L., Kim, C. W., Lim, S. T. (2000). Structural characterization of beta-D-(1 -> 3,1 -> 6)-linked glucans using NMR spectroscopy. *Carbohydrate Research*, 328(3): 331-341.

Kimura, S. H., Ikawa, M., Ito, A., Okabe, M., Nojima, H. (2001). Cyclin G1 is involved in G2/M arrest in response to DNA damage and in growth control after damage recovery. *Oncogene*, 20(25): 3290-3300.

Kloareg, B., Demarty, M., Mabeau, S. (1986). Polyanionic characteristics of purified sulfated homofucans from brown algae. *International Journal of Biological Macromolecules*, 8(6): 380-386.

Knudsen, S. (2002). *A Biologist's Guide to Analysis of DNA Microarray Data*. New York, John Wiley & Sons, Inc., 125 p.

Kougias, P., Wei, D., Rice, P. J., Ensley, H. E., Kalbfleisch, J., Williams, D. L., Browder, I. W. (2001). Normal human fibroblasts express pattern recognition receptors for fungal (1-->3)-beta-D-glucans. *Infection and Immunity*, 69(6): 3933-3938.

Kovary, K., Bravo, R. (1992). Existence of different Fos/Jun complexes during the G0-to-G1 transition and during exponential growth in mouse fibroblasts: differential role of Fos proteins. *Molecular and Cellular Biology*, 12(11): 5015-5023.

Koyanagi, S., Tanigawa, N., Nakagawa, H., Soeda, S., Shimeno, H. (2003). Oversulfation of fucoidan enhances its anti-angiogenic and antitumor activities. *Biochemical Pharmacology*, 65(2): 173.

Krishnaswami, S., Ly, Q. P., Rothman, V. L., Tuszynski, G. P. (2002). Thrombospondin-1 promotes proliferative healing through stabilization of PDGF. *Journal of Surgical Research*, 107(1): 124-130.

Kruithof, E. K. O., Baker, M. S., Bunn, C. L. (1995). Biological and clinical aspects of plasminogen activator inhibitor type 2. *Blood*, 86(11): 4007-4024.

Kuda, T., Goto, H., Yokoyama, M., Fujii, T. (1998). Fermentable dietary fiber in dried products of frown algae and their effects on cecal microflora and levels of plasma lipid in rats. *Fisheries Science*, 64(4): 582-588.

Kuda, T., Yano, T., Matsuda, N., Nishizawa, M. (2005). Inhibitory effects of laminaran and low molecular alginate against the putrefactive compounds produced by intestinal microflora *in vitro* and in rats. *Food Chemistry*, 91(4): 745-749.

Kusaykin, M., Bakunina, I., Sova, V., Ermakova, S., Kuznetsova, T., Besednova, N., Zaporozhets, T., Zvyagintseva, T. (2008). Structure, biological activity, and enzymatic transformation of fucoidans from the brown seaweeds. *Biotechnology Journal*, 3(7): 904-915.

Kwon, M. J., Nam, T. J. (2007). A polysaccharide of the marine alga *Capsosiphon fulvescens* induces apoptosis in AGS gastric cancer cells via an IGF-IR-mediated PI3K/Akt pathway. *Cell Biology International*, 31(8): 768-775.

Labbe, A. (2006). Atelier sur l'analyse statistique de données de puces à ADN, Université Laval, 17-18 mai 2006

Lahaye, M., Kaeffer, B. (1997a). Seaweed dietary fibres: structure, physico-chemical and biological properties relevant to intestinal physiology. *Sciences des Aliments*, 17(6): 563-584.

Lahaye, M., Michel, C., Benard, C., Kouman, S., Kaeffer, B., Cherbut, C. (1997b). Seaweeds: a possible source of non-digestible oligosaccharides in *Non-digestible oligosaccharides: healthy food for the colon?* Wageningen, Proceedings of the International Symposium: 47-56.

Lake, A. C., Vassy, R., Di Benedetto, M., Lavigne, D., Le Visage, C., Perret, G. Y., Letourneur, D. (2006). Low molecular weight fucoidan increases VEGF(165)-induced endothelial cell migration by enhancing VEGF(165) binding to VEGFR-2 and NRP1. *Journal of Biological Chemistry*, 281(49): 37844-37852.

Lambert, E., Dasse, E., Haye, B., Petitfrere, E. (2004). TIMPs as multifacial proteins. *Critical Reviews in Oncology Hematology*, 49(3): 187-198.

Lane, C. E., Mayes, C., Druehl, L. D., Saunders, G. W. (2006). A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) supports substantial taxonomic re-organization. *Journal of Phycology*, 42(2): 493-512.

Larochelle, S., Langlois, C., Thibault, I., Lopez-Valle, C. A., Roy, M., Moulin, V. (2004). Sensitivity of myofibroblasts to H_2O_2-mediated apoptosis and their antioxidant cell network. *Journal of Cellular Physiology*, 200(2): 263-271.

Lavi, I., Friesem, D., Geresh, S., Hadar, Y., Schwartz, B. (2006). An aqueous polysaccharide extract from the edible mushroom *Pleurotus ostreatus* induces anti-proliferative and pro-apoptotic effects on HT-29 colon cancer cells. *Cancer Letters*, 244(1): 61-70.

Lawson, D., Harrison, M., Shapland, C. (1997). Fibroblast transgelin and smooth muscle SM22 alpha are the same protein, the expression of which is down-regulated in many cell lines. *Cell Motility and the Cytoskeleton*, 38(3): 250-257.

Lazaridou, A., Biliaderis, C. G. (2007). Molecular aspects of cereal β-glucan functionality: Physical properties, technological applications and physiological effects. *Journal of Cereal Science*, 46(2): 101-118.

Lee, J. B., Hayashi, K., Hashimoto, M., Nakano, T., Hayashi, T. (2004). Novel antiviral fucoidan from sporophyll of *Undaria pinnatifida* (Mekabu). *Chemical & Pharmaceutical Bulletin*, 52(9): 1091-1094.

Leung, M. Y. K., Liu, C., Koon, J. C. M., Fung, K. P. (2006). Polysaccharide biological response modifiers. *Immunology Letters*, 105(2): 101-114.

Levi, M., van der Poll, T. (2005). Two way interactions between inflammation and coagulation. *Trends in Cardiovascular Medicine*, 15(7): 254-259.

Ley, K., Kansas, G. S. (2004). Selectins in T-cell recruitment to non-lymphoid tissues and sites of inflammation. *Nature Reviews Immunology*, 4(5): 325-336.

Li, B., Wei, X. J., Sun, J. L., Xu, S. Y. (2006). Structural investigation of a fucoidan containing a fucose-free core from the brown seaweed, *Hizikia fusiforme*. *Carbohydrate Research*, 341(9): 1135-1146.

Li, S., Nikulina, K., DeVoss, J., Wu, A. J., Strauss, E. C., Anderson, M. S., McNamara, N. A. (2008a). Small proline-rich protein 1B (SPRR1B) is a biomarker for squamous metaplasia in dry eye disease. *Investigative Ophthalmology and Visual Science*, 49(1): 34-41.

Li, S. P., Junttila, M. R., Han, J. H., Kahari, V. M., Westermarck, J. (2003). p38 mitogen-activated protein kinase pathway suppresses cell survival by inducing dephosphorylation of mitogen-activated protein/extracellular signal-regulated kinase kinase. *Cancer Research*, 63(13): 3473-3477.

Li, X. Y., Kong, X. Y., Shi, S. A., Zheng, X. L., Guo, G., Wei, Y. Q., Qian, Z. Y. (2008b). Preparation of alginate coated chitosan microparticles for vaccine delivery. *Bmc Biotechnology*, 8.

Liang, A. Y., Liu, X. J., Du, Y. G., Wang, K. Y., Lin, B. C. (2008). Further characterization of the binding of heparin to granulocyte colony-stimulating factor: Importance of sulfate groups. *Electrophoresis*, 29(6): 1286-1290.

Liaudet-Coopman, E., Beaujouin, M., Derocq, D., Garcia, M., Glondu-Lassis, M., Laurent-Matha, V., Prébois, C., Rochefort, H., Vignon, F. (2006). Cathepsin D: newly discovered functions of a long-standing aspartic protease in cancer and apoptosis. *Cancer Letters*, 237(2): 167-179.

Lijour, Y., Gentric, E., Deslandes, E., Guezennec, J. (1994). Estimation of the sulfate content of hydrothermal vent bacterial polysaccharides by Fourier-transform infrared-spectroscopy. *Analytical Biochemistry*, 220(2): 244-248.

Lim, J. M., Joo, J. H., Kim, H. O., Kim, H. M., Kim, S. W., Hwang, H. J., Yun, J. W. (2005). Structural analysis and molecular characterization of exopolysaccharides produced by submerged mycelial culture of *Collybia maculata* TG-1. *Carbohydrate Polymers*, 61(3): 296-303.

Lin, J., Jin, R., Zhang, B., Chen, H., Bai, Y. X., Yang, P. X., Han, S. W., Xie, Y. H., Huang, P. T., Huang, C., Huang, J. J. (2008). Nucleolar localization of TERT is unrelated to telomerase function in human cells. *Journal of Cell Science*, 121(13): 2169-2176.

Liuzzo, J. P., Petanceska, S. S., Moscatelli, D., Devi, L. A. (1999). Inflammatory mediators regulate cathepsin S in macrophages and microglia: A role in attenuating heparan sulfate interactions. *Molecular Medicine*, 5(5): 320-333.

Lobban, C. S., Harrison, P. J. (1997). *Seaweed ecology and physiology*. Cambrige, Cambridge University Press, 359 p.

Logeart, D., Letourneur, D., Jozefonvicz, J., Kern, P. (1996). Collagen synthesis by vascular smooth muscle cells in the presence of antiproliferative polysaccharides. *Journal of Biomedical Materials Research*, 30(4): 501-508.

Logeart, D., Prigent-Richard, S., Boisson-Vidal, C., Chaubet, F., Durand, P., Jozefonvicz, J., Letourneur, D. (1997a). Fucans, sulfated polysaccharides extracted from brown seaweeds, inhibit vascular smooth muscle cell proliferation. II. Degradation and molecular weight effect. *European Journal of Cell Biology*, 74(4): 385.

Logeart, D., Prigent-Richard, S., Jozefonvicz, J., Letourneur, D. (1997b). Fucans, sulfated polysaccharides extracted from brown seaweeds, inhibit vascular smooth muscle cell proliferation. I. Comparison with heparin for antiproliferative activity, binding and internalization. *European Journal of Cell Biology*, 74(4): 376.

Lotem, J., Sachs, L. (1995). Regulation of bcl-2, bcl-X_L and bax in the control of apoptosis by hematopoietic cytokines and dexamethasone. *Cell Growth and Differentiation*, 6(6): 647-653.

Luyt, C. E., Meddahi-Pelle, A., Ho-Tin-Noe, B., Colliec-Jouault, S., Guezennec, J., Louedec, L., Prats, H. E., Jacob, M. P., Osborne-Pellegrin, M., Letourneur, D., Michel, J. B. (2003). Low-molecular-weight fucoidan promotes therapeutic revascularization in a rat model of critical hindlimb ischemia. *Journal of Pharmacology and Experimental Therapeutics*, 305(1): 24-30.

Mahony, M. C., Clark, G. F., Oehninger, S., Acosta, A. A., Hodgen, G. D. (1993). Fucoidin binding-activity and its localization on human spermatozoa. *Contraception*, 48(3): 277-289.

Mahony, M. C., Oehninger, S., Clark, G. F., Acosta, A. A., Hodgen, G. D. (1991). Fucoidin inhibits the zona pellucida-induced acrosome reaction in human spermatozoa. *Contraception*, 44(6): 657-665.

Manton, K. J., Sadasivam, M., Cool, S. M., Nurcombe, V. (2006). Bone-specific heparan sulfates induce osteoblast growth arrest and downregulation of retinoblastoma protein. *Journal of Cellular Physiology*, 209(1): 219-229.

MAPAQ (1993). Séminaire sur la valorisation des algues du Québec, 1er septembre 1992

Marais, M. F., Joseleau, J. P. (2001). A fucoidan fraction from *Ascophyllum nodosum*. *Carbohydrate Research*, 336(2): 155-159.

Margaine, S., Hamels, S., Collet, J., Zammatteo, N., Mueller-Ibeler, J., von Goetz, F., Warnon, S., Van Huffel, C., Alexandre, I. (2007). "Application No 148: Silverquant® microarray detection and scanning system." Retrieved December 1st, 2008, from http://www.eppendorf-biochip.com/int/index.php?l=251&sitemap=4.2&pb=f77b8e299874dc7b&action=support&contentid=1&catalognode=66022&productpage=7.

Marieb, E. N. (1999). *Anatomie et physiologie humaines*. Saint-Laurent, Édition du Renouveau Pédagogique Inc., 1194 p.

Marino, V. J., Sterin-Prync, A. E., Roguin, L. P. (2003). Change in the accessibility of an epitope of the human granulocyte-colony stimulating factor after binding to receptors. *Cytokine*, 22(1-2): 5-11.

Matou, S., Helley, D., Chabut, D., Bros, A., Fischer, A. M. (2002). Effect of fucoidan on fibroblast growth factor-2-induced angiogenesis *in vitro*. *Thrombosis Research*, 106(4-5): 213-221.

Matsuhiro, B., Conte, A. F., Damonte, E. B., Kolender, A. A., Matulewicz, M. C., Mejías, E. G., Pujol, C. A., Zúñiga, E. A. (2005). Structural analysis and antiviral activity of a sulfated galactan from the red seaweed *Schizymenia binderi* (Gigartinales, Rhodophyta). *Carbohydrate Research*, 340(15): 2392-2402.

Mauray, S., Sternberg, C., Theveniaux, J., Millet, J., Sinquin, C., Taponbretaudiere, J., Fischer, A. M. (1995). Venous antithrombotic and anticoagulant activities of a fucoidan fraction. *Thrombosis and Haemostasis*, 74(5): 1280-1285.

McCawley, L. J., Matrisian, L. M. (2001). Matrix metalloproteinases: they're not just for matrix anymore! *Current Opinion in Cell Biology*, 13(5): 534-540.

McClure, M. O., Whitby, D., Patience, C., Gooderham, N. J., Bradshaw, A., Cheingsong-Popov, R., Weber, J. N., Davies, D. S., Cook, G. M. W., Keyness, R. J., Weiss, R. A. (1991). Dextrin sulphate and fucoidan are potent inhibitors of HIV infection *in vitro*. *Antiviral Chemistry and Chemiotherapy*, 2(3): 149-156.

McNeely, W. H., Pettitt, D. J. (1973). Algin in *Industrial Gums*, R. L. Whistler(Ed.). New York, Academic Press: 49-81.

Melcher, A., Gough, M., Todryk, S., Vile, R. (1999). Apoptosis or necrosis for tumor immunotherapy: what's in a name? *Journal of Molecular Medicine*, 77(12): 824-833.

Melo, M. R. S., Feitosa, J. P. A., Freitas, A. L. P., de Paula, R. C. M. (2002). Isolation and characterization of soluble sulfated polysaccharide from the red seaweed *Gracilaria cornea*. *Carbohydrate Polymers*, 49(4): 491-498.

Metcalfe, J. A., Parkhill, J., Campbell, L., Stacey, M., Biggs, P., Byrd, P. J., Taylor, A. M. (1996). Accelerated telomere shortening in ataxia telangiectasia. *Nature Genetics*, 13(3): 350-353.

Michel, C., Benard, C., Lahaye, M., Formaglio, D., Kaeffer, B., Quemener, B., Berot, S., Yvin, J. C., Blottiere, H. M., Cherbut, C. (1999). Algal oligosaccharides as functional foods: *In vitro* study of their cellular and fermentative effects. *Sciences des Aliments*, 19(3-4): 311-332.

Michel, C., Lahaye, M., Bonnet, C., Mabeau, S., Barry, J. L. (1996). *In vitro* fermentation by human faecal bacteria of total and purified dietary fibres from brown seaweeds. *British Journal of Nutrition*, 75(2): 263-280.

Moe, S. T., Draget, K. I., Skjåk-Braek, G., Smidsrød, O. (1995). Alginates in *Food Polysaccharides and Their Applications*, A. M. Stephen(Ed.). New York, Marcel Dekker: 245-286.

Mollet, J. C., Rahaoui, A., Lemoine, Y. (1998). Yield, chemical composition and gel strength of agarocolloids of *Gracilaria gracilis*, *Gracilariopsis longissima* and the newly reported *Gracilaria cf. vermiculophylla* from Roscoff (Brittany, France). *Journal of Applied Phycology*, 10(1): 59-66.

Montreuil, J., Bouquelet, S., Debray, H., Fournet, B., Spik, G., Strecker, G. (1986). Glycoproteins in *Carbohydrate analysis: a practical approach*, M. F. Chaplin and J. F. Kennedy(Ed.). Washington DC, IRL Press: 143-204.

Moon, H. J., Lee, S. R., Shim, S. N., Jeong, S. H., Stonik, V. A., Rasskazov, V. A., Zvyagintseva, T., Lee, Y. H. (2008). Fucoidan inhibits UVB-induced MMP-1 expression in human skin fibroblasts. *Biological & Pharmaceutical Bulletin*, 31(2): 284-289.

Moulin, V., Auger, F. A., OConnorMcCourt, M., Germain, L. (1997). Fetal and postnatal sera differentially modulate human dermal fibroblast phenotypic and functional features *in vitro*. *Journal of Cellular Physiology*, 171(1): 1-10.

Moulin, V., Larochelle, S., Langlois, C., Thibault, I., Lopez-Valle, C. A., Roy, M. (2004). Normal skin wound and hypertrophic scar myofibroblasts have differential responses to apoptotic inductors. *Journal of Cellular Physiology*, 198(3): 350-358.

Moulin, V., Plamondon, M. (2002). Differential expression of collagen integrin receptor on fetal vs. adult skin fibroblasts: implication in wound contraction during healing. *British Journal of Dermatology*, 147(5): 886-892.

Mourão, P. A. S. (2007). A carbohydrate based mechanism of species recognition in sea urchin fertilization. *Brazilian Journal of Medical and Biological Research*, 40(1): 5-17.

Mourão, P. A. S., Pereira, M. S. (1999). Searching for alternatives to heparin - Sulfated fucans from marine invertebrates. *Trends in Cardiovascular Medicine*, 9(8): 225-232.

Mueller, A., Raptis, J., Rice, P. J., Kalbfleisch, J. H., Stout, R. D., Ensley, H. E., Browder, W., Williams, D. L. (2000). The influence of glucan polymer structure and solution conformation on binding to (1-->3)-beta-D-glucan receptors in a human monocyte-like cell line. *Glycobiology*, 10(4): 339.

Mulloy, B., Mourão, P. A. S., Gray, E. (2000). Structure/function studies of anticoagulant sulphated polysaccharides using NMR. *Journal of Biotechnology*, 77(1): 123-135.

Murphy, G., Cockett, M. I., Ward, R. V., Docherty, A. J. P. (1991). Matrix metalloproteinase degradation of elastin, type IV collagen and proteoglycan. A quantitative comparison of the activities of 95 kDa and 72 kDa gelatinases, stromelysins-1 and -2 and punctuated metalloproteinase (PUMP). *Biochemical Journal*, 277: 277-279.

Murphy, G., Segain, J. P., Oshea, M., Cockett, M., Ioannou, C., Lefebvre, O., Chambon, P., Basset, P. (1993). The 28-kDa N-terminal domain of mouse stromelysin-3 has the general properties of a weak metalloproteinase. *Journal of Biological Chemistry*, 268(21): 15435-15441.

Mutsaers, S. E., Bishop, J. E., McGrouther, G., Laurent, G. J. (1997). Mechanisms of tissue repair: from wound healing to fibrosis. *The International Journal of Biochemistry & Cell Biology*, 29(1): 5-17.

Myers, J. C., Amenta, P. S., Dion, A. S., Sciancalepore, J. P., Nagaswami, C., Weisel, J. W., Yurchenco, P. D. (2007). The molecular structure of human tissue type XV presents a unique conformation among the collagens. *Biochemical Journal*, 404: 535-544.

Nagaoka, M., Shibata, H., Kimura-Takagi, I., Hashimoto, S., Aiyama, R., Ueyama, S., Yokokura, T. (2000). Anti-ulcer effects and biological activities of polysaccharides from marine algae. *BioFactors*, 12: 267-274.

Nagase, H. (1998). Stromelysins 1 and 2 in *Matrix metalloproteinases*, W. C. Parks and R. P. Mecham(Ed.). San Diego, Academic Press: 43-84.

Nagase, H., Visse, R., Murphy, G. (2006). Structure and function of matrix metalloproteinases and TIMPs. *Cardiovascular Research*, 69(3): 562-573.

Nardella, A., Chaubet, F., Boisson-Vidal, C., Blondin, C., Durand, P., Jozefonvicz, J. (1996). Anticoagulant low molecular weight fucans produced by radical process and ion exchange chromatography of high molecular weight fucans extracted from the brown seaweed *Ascophyllum nodosum*. *Carbohydrate Research*, 289: 201-208.

Nelson, T. E., Lewis, B. A. (1974). Separation and characterization of soluble and insoluble components of insoluble laminaran. *Carbohydrate Research*, 33(1): 63-74.

Neyrinck, A. M., Mouson, A., Delzenne, N. M. (2007). Dietary supplementation with laminarin, a fermentable marine beta (1-3) glucan, protects against hepatotoxicity induced by LPS in rat by modulating immune response in the hepatic tissue. *International Immunopharmacology*, 7(12): 1497-1506.

Nishino, T., Aizu, Y., Nagumo, T. (1991). The relationship between the molecular-weight and the anticoagulant activity of 2 types of fucan sulfates from the brown seaweed *Ecklonia kurome*. *Agricultural and Biological Chemistry*, 55(3): 791-796.

Nishino, T., Takabe, Y., Nagumo, T. (1994). Isolation and partial characterization of a novel beta-D-galactan sulfate from the brown seaweed *Laminaria angustata var. longissima*. *Carbohydrate Polymers*, 23(3): 165-173.

Nishino, T., Yokoyama, G., Dobashi, K., Fujihara, M., Nagumo, T. (1989). Isolation, purification, and characterization of fucose-containing sulfated polysaccharides from the brown seaweed *Ecklonia kurome* and their blood-anticoagulant activities. *Carbohydrate Research*, 186(1): 119-129.

Noailles, M.-C. (2003). "*Fucus vesiculosus* : Individu entier et détail d'un rameau fertile qui porte des réceptacles. ." Retrieved December 1st, 2008, from http://www.sb-roscoff.fr/INVENTAIRES/InvAlgues/index.algues.php?action=illustration&nom=F_vesic_dessin_modif.jpg&id_algue=104.

Noda, H., Amano, H., Arashima, K., Nisizawa, K. (1990). Antitumor-activity of marine-algae. *Hydrobiologia*, 204: 577-584.

O'Connell, J. (2002). RT-PCR in Biomedecine in *RT-PCR Protocols*, J. O'Connell(Ed.). Totowa, Humana Press. 193: 3-18.

Oehninger, S., Acosta, A., Hodgen, G. D. (1990). Antagonistic and agonistic properties of saccharide moieties in the hemizona assay. *Fertility and Sterility*, 53(1): 143-149.

Oehninger, S., Clark, G. F., Fulgham, D., Blackmore, P. F., Mahony, M. C., Acosta, A. A., Hodgen, G. D. (1992). Effect of fucoidin on human sperm zona-pellucida interactions. *Journal of Andrology*, 13(6): 519-525.

Ohara, N., Hayashi, Y., Yamada, S., Kim, S. K., Matsunaga, T., Yanagiguchi, K., Ikeda, T. (2004). Early gene expression analyzed by cDNA microarray and RT-PCR in osteoblasts cultured with water-soluble and low molecular chitooligosaccharide. *Biomaterials*, 25(10): 1749-1754.

Ohuchi, E., Imai, K., Fujii, Y., Sato, H., Seiki, M., Okada, Y. (1997). Membrane type 1 matrix metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules. *Journal of Biological Chemistry*, 272(4): 2446-2451.

Okamoto, K., Beach, D. (1994). Cyclin G is a transcriptional target of the p53 tumor suppressor protein. *EMBO Journal*, 13(20): 4816-4822.

Okamoto, O., Fujiwara, S. (2006). Dermatopontin, a novel player in the biology of the extracellular matrix. *Connective Tissue Research*, 47(4): 177-189.

Omata, M., Matsui, N., Inomata, N., Ohno, T. (1997). Protective effects of polysaccharide fucoidin on myocardial ischemia-reperfusion injury in rats. *Journal of Cardiovascular Pharmacology*, 50(6): 717-724.

Oono, T., Specks, U., Eckes, B., Majewski, S., Hunzelmann, N., Timpl, R., Krieg, T. (1993). Expression of type VI collagen mRNA during wound healing. *Journal of Investigative Dermatology*, 100(3): 329-334.

Ornitz, D. M., Yayon, A., Flanagan, J. G., Svahn, C. M., Levi, E., Leder, P. (1992). Heparin is required for cell-free binding of basic fibroblast growth factor to a soluble receptor and for mitogenesis in whole cells. *Molecular and Cellular Biology*, 12(1): 240-247.

Otterlei, M., Ostgaard, K., Skjakbraek, G., Smidsrod, O., Soonshiong, P., Espevik, T. (1991). Induction of cytokine production from human monocytes stimulated with alginate. *Journal of Immunotherapy*, 10(4): 286-291.

Otterlei, M., Sundan, A., Skjakbraek, G., Ryan, L., Smidsrod, O., Espevik, T. (1993). Similar mechanisms of action of defined polysaccharides and lipopolysaccharides - Characterization of binding and tumor necrosis factor alpha induction. *Infection and Immunity*, 61(5): 1917-1925.

Ottlinger, M. E., Pukac, L. A., Karnovsky, M. J. (1993). Heparin inhibits mitogen-activated protein kinase activation in intact rat vascular smooth muscle cells. *Journal of Biological Chemistry*, 268(26): 19173-19176.

Page-McCaw, A., Ewald, A. J., Werb, Z. (2007). Matrix metalloproteinases and the regulation of tissue remodelling. *Nature Reviews Molecular Cell Biology*, 8(3): 221-233.

Parish, C. R., Coombe, D. R., Jakobsen, K. B., Bennett, F. A., Underwood, P. A. (1987). Evidence that sulfated polysaccharides inhibit tumor metastasis by blocking tumor-cell-derived heparanases. *International Journal of Cancer*, 40(4): 511-518.

Parks, W. C., Sudbeck, B. D., Doyle, G. R., Saariahlo-Kere, U. (1998). Matrix metalloproteinases in tissue repair in *Matrix metalloproteinases*, W. C. Parks and R. P. Mecham(Ed.). San Diego, Academic Press: 263-298.

Parmigiani, G., Garrett, E. S., Irizarry, R. A., Zegger, S. L. (2003). The analysis of gene expression data: an overview of methods and software in *The analysis of gene expression data: an overview of methods and software*, G. Parmigiani, E. S. Garrett, R. A. Irizarry and S. L. Zegger(Ed.). New York, Springer: 1-45.

Patankar, M. S., Oehninger, S., Barnett, T., Williams, R. L., Clark, G. F. (1993). A revised structure for fucoidan may explain some of its biological-activities. *Journal of Biological Chemistry*, 268(29): 21770-21776.

Patel, M. K., Mulloy, B., Gallagher, K. L., O'Brien, L., Hughes, A. D. (2002). The antimitogenic action of the sulphated polysaccharide fucoidan differs from heparin in human vascular smooth muscle cells. *Thrombosis and Haemostasis*, 87(1): 149-154.

Paxman, J. R., Richardson, J. C., Dettmar, P. W., Corfe, B. A. (2008). Alginate reduces the increased uptake of cholesterol and glucose in overweight male subjects: a pilot study. *Nutrition Research*, 28(8): 501-505.

Pearson, G., Robinson, F., Gibson, T. B., Xu, B. E., Karandikar, M., Berman, K., Cobb, M. H. (2001). Mitogen-activated protein (MAP) kinase pathways: Regulation and physiological functions. *Endocrine Reviews*, 22(2): 153-183.

Peat, S., Whelan, W. J., Lawley, H. G. (1958). The structure of laminarin. 1. The main polymeric linkage. *Journal of the Chemical Society*: 724-728.

Penc, S. F., Pomahac, B., Eriksson, E., Detmar, M., Gallo, R. L. (1999). Dermatan sulfate activates nuclear factor-kappa b and induces endothelial and circulating intercellular adhesion molecule-1. *Journal of Clinical Investigation*, 103(9): 1329-1335.

Percival, E., McDowell, R. H. (1967). *Chemistry and enzymology of marine algal polysaccharides*. London, Academic Press, 219 p.

Pereira, M. S., Melo, F. R., Mourão, P. A. S. (2002). Is there a correlation between structure and anticoagulant action of sulfated galactans and sulfated fucans? *Glycobiology*, 12(10): 573-580.

Pereira, M. S., Mulloy, B., Mourão, P. A. S. (1999). Structure and anticoagulant activity of sulfated fucans - Comparison between the regular, repetitive, and linear fucans from echinoderms with the more heterogeneous and branched polymers from brown algae. *Journal of Biological Chemistry*, 274(12): 7656-7667.

Piculell, L. (1995). Gelling Carrageenans in *Food Polysaccharides and Their Applications*, A. M. Stephen(Ed.). New York, Marcel Dekker: 205-244.

Pines, J., Hunter, T. (1989). Isolation of a human cyclin cDNA: Evidence for cyclin mRNA and protein regulation in the cell cycle and for interaction with $p34^{cdc2}$. *Cell*, 58(5): 833-846.

Plaza, M., Cifuentes, A., Ibanez, E. (2008). In the search of new functional food ingredients from algae. *Trends in Food Science & Technology*, 19(1): 31-39.

Podzimek, S. (1994). The use of GPC coupled with a multiangle laser-light scattering photometer for the characterization of polymers on the determination of molecular-weight, size, and branching. *Journal of Applied Polymer Science*, 54(1): 91-103.

Podzimek, S. (2005). A review of the applications size-exclusion chromatography-multiangle light scattering to the characterization of synthetic and natural polymers in *Multiple Detection in Size-Exclusion Chromatography*, A. M. Striegel(Ed.). Washington DC, American Chemical Society: 94-112.

Poirier, C. (2002). "*Laminaria longicruris*." Retrieved December 1st, 2008, from http://www.plongeetech.com/.

Ponce, N. M. A., Pujol, C. A., Damonte, E. B., Flores, M. L., Stortz, C. A. (2003). Fucoidans from the brown seaweed *Adenocystis utricularis*: extraction methods, antiviral activity and structural studies. *Carbohydrate Research*, 338(2): 153-165.

Pretus, H. A., Ensley, H. E., McNamee, R. B., Jones, E. L., Browder, I. W., Williams, D. L. (1991). Isolation, physicochemical characterization and preclinical efficacy evaluation of soluble scleroglucan. *Journal of Pharmacology and Experimental Therapeutics*, 257(1): 500-510.

Proctor, C. J., Gray, D. A. (2008). Explaining oscillations and variability in the p53-Mdm2 system. *BMC Systems Biology*, 2: 75-75.

Proskuryakov, S. Y., Konoplyannikov, A. G., Gabai, V. L. (2003). Necrosis: a specific form of programmed cell death? *Experimental Cell Research*, 283(1): 1-16.

Pukac, L. A., Carter, J. E., Ottlinger, M. E., Karnovsky, M. J. (1997). Mechanisms of inhibition by heparin of PDGF stimulated MAP kinase activation in vascular smooth muscle cells. *Journal of Cellular Physiology*, 172(1): 69-78.

Rajah, R., Valentinis, B., Cohen, P. (1997). Insulin like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor-beta 1 on programmed cell death through a p53- and IGF-independent mechanism. *Journal of Biological Chemistry*, 272(18): 12181-12188.

Read, S. M., Currie, G., Bacic, A. (1996). Analysis of the structural heterogeneity of laminarin by electrospray-ionisation-mass spectrometry. *Carbohydrate Research*, 281(2): 187-201.

Reed, W. F. (1996). Coupled multiangle light-scattering and viscosimetric detectors for size exclusion chromatography with application to polyelectrolyte characterization. in *Stategies in Size Exclusion Chromatography*, M. Potschka and P. L. Dubin(Ed.). Washington DC, American Chemical Society: 7-34.

Reilly, P., O'Doherty, J. V., Pierce, K. M., Callan, J. J., O'Sullivan, J. T., Sweeney, T. (2008). The effects of seaweed extract inclusion on gut morphology, selected intestinal microbiota, nutrient digestibility, volatile fatty acid concentrations and the immune status of the weaned pig. *Animal*, 2(10): 1465-1473.

Rinehart, C. A. (2004). "Advanced molecular genetics-biology 566." Retrieved December 1st, 2008, from http://bioweb.wku.edu/courses/biol566/Images/stemAffyChip.jpg.

Rioux, L.-E., Turgeon, S. L., Beaulieu, M. (2007a). Characterization of polysaccharides extracted from brown seaweeds. *Carbohydrate Polymers*, 69(3): 530-537.

Rioux, L.-E., Turgeon, S. L., Beaulieu, M. (2007b). Rheological characterisation of polysaccharides extracted from brown seaweeds. *Journal of the Science of Food and Agriculture*, 87(9): 1630-1638.

Rioux, L.-E., Turgeon, S. L., Beaulieu, M. (2009). Effect of season on the composition of bioactive polysaccharides from the brown seaweed *Saccharina longicruris*. *Phytochemistry*, 70(8): 1069-1075.

Robinson, M. J., Cobb, M. H. (1997). Mitogen-activated protein kinase pathways. *Current Opinion in Cell Biology*, 9(2): 180-186.

Rocha, H. A. O., Moraes, F. A., Trindade, E. S., Franco, C. R. C., Torquato, R. J. S., Veiga, S. S., Valente, A. P., Mourao, P. A. S., Leite, E. L., Nader, H. B., Dietrich, C. P. (2005). Structural and hemostatic activities of a sulfated galactofucan from the brown alga *Spatoglossum schroederi* - An ideal antithrombotic agent? *Journal of Biological Chemistry*, 280(50): 41278-41288.

Roger, O., Colliec-Jouault, S., Ratiskol, J., Sinquin, C., Guezennec, J., Fischer, A. M., Chevolot, L. (2002). Polysaccharide labelling: impact on structural and biological properties. *Carbohydrate Polymers*, 50(3): 273-278.

Roger, O., Kervarec, N., Ratiskol, J., Colliec-Jouault, S., Chevolot, L. (2004). Structural studies of the main exopolysaccharide produced by the deep-sea bacterium *Alteromonas infernus*. *Carbohydrate Research*, 339(14): 2371-2380.

Rothlein, R., Czajkowski, M., Oneill, M. M., Marlin, S. D., Mainolfi, E., Merluzzi, V. J. (1988). Induction of intercellular adhesion molecule 1 on primary and continuous cell lines by pro-inflammatory cytokines. Regulation by pharmacologic agents and neutralizing antibodies. *Journal of Immunology*, 141(5): 1665-1669.

Rufer, N., Dragowska, W., Thornbury, G., Roosnek, E., Lansdorp, P. M. (1998). Telomere length dynamics in human lymphocyte subpopulations measured by flow cytometry. *Nat Biotech*, 16(8): 743-747.

Ruhl, M., Sahin, E., Johannsen, M., Somasundaram, R., Manski, D., Riecken, E. O., Schuppan, D. (1999). Soluble collagen VI drives serum-starved fibroblasts through S phase and prevents apoptosis via down-regulation of bax. *Journal of Biological Chemistry*, 274(48): 34361-34368.

Rupérez, P., Ahrazem, O., Leal, J. A. (2002). Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*. *Journal of Agricultural and Food Chemistry*, 50(4): 840-845.

Saitô, H., Tabeta, R., Sasaki, T., Yoshioka, Y. (1986). A high-resolution solid-state ^{13}C NMR study of (1→3)-β-D-glucans from various sources. Conformational characterization as viewed from the conformation-dependent ^{13}C chemical shifts and its consequence to gelation property. *Bulletin of the Chemical Society of Japan*, 59(7): 2093-2101.

Sakai, S., Takada, S., Kamasuka, T., Momoki, Y., Sugayama, J. I. (1968). Antitumor action of some glucans - Especially on its correlation to their chemical structure. *Gann*, 59(6): 507-512.

Sakai, T., Kawai, T., Kato, I. (2004). Isolation and characterization of a fucoidan-degrading marine bacterial strain and its fucoidanase. *Marine Biotechnology*, 6(4): 335-346.

San Antonio, J. D., Lander, A. D., Wright, T. C., Karnovsky, M. J. (1992). Heparin inhibits the attachment and growth of Balb/c-3T3 fibroblasts on collagen substrata. *Journal of Cellular Physiology*, 150(1): 8-16.

Savitsky, K., Platzer, M., Uziel, T., Gilad, S., Sartiel, A., Rosenthal, A., ElroyStein, O., Shiloh, Y., Rotman, G. (1997). Ataxia-telangiectasia: Structural diversity of untranslated sequences suggests complex post-transcriptional regulation of ATM gene expression. *Nucleic Acids Research*, 25(9): 1678-1684.

Schena, M., Davis, R. W. (1999). Genes, genomes and chips in *DNA microarrays: a pratical approach*, M. Shena(Ed.). Oxford, Oxford University Press: 1-16.

Schena, M., Davis, R. W. (2000). Technology Standards for Microarray Research in *Microarray Biochip Technology*, M. Schena(Ed.). Natick, Eaton Publishing: 1-18.

Scherrer, B. (1984). *Biostatistique*. Montreal, Paris, Morin, G., 850 p.

Schved, J. F. (2005). Hémostase : physiologie et exploration en pratique courante in *Coagulation, thrombose et médecine physique*, P. Codine, N. Kotzki and J. Pélissier(Ed.). Paris, Masson: 1-9.

Secko, D. (2003). "A monks flourishing garden : The basics of molecular biology explained." Retrieved December 1st, 2008, from http://www.scq.ubc.ca/a-monks-flourishing-garden-the-basics-of-molecular-biology-explained/.

Sengupta, S., Harris, C. C. (2005). p53: Traffic cop at the crossroads of DNA repair and recombination. *Nature Reviews Molecular Cell Biology*, 6(1): 44-55.

Senni, K., Borchiellini, C., Duchesnay, A., Pellat, B., Letourneur, D., Kern, P. (1998). Antiproliferative polysaccharides modulate distribution and phenotypic expression

of collagens by gingival fibroblasts. *Journal of Biomedical Materials Research*, 40(1): 164-169.

Senni, K., Gueniche, F., Foucault-Bertaud, A., Igondjo-Tchen, S., Fioretti, F., Colliec-Jouault, S., Durand, P., Guezennec, J., Godeau, G., Letourneur, D. (2006). Fucoidan a sulfated polysaccharide from brown algae is a potent modulator of connective tissue proteolysis. *Archives of Biochemistry and Biophysics*, 445(1): 56-64.

Seta, K., Kim, H. W., Yuan, Y., Lu, G., Spicer, Z., Kim, R., Ferguson, T., Pathrose, P., Millhorn, D. E. (2002). Gene expression microarrays: A platform for discovery in biological systems. *Microfabricated Sensors*, 815: 3-21.

Shanmugam, M., Mody, K. H. (2000). Heparinoid-active sulphated polysaccharides from marine algae as potential blood anticoagulant agents. *Current Science*, 79(12): 1672-1683.

Shevchenko, N. M., Anastyuk, S. D., Gerasimenko, N. I., Dmitrenok, P. S., Isakov, V. V., Zvyagintseva, T. N. (2007). Polysaccharide and lipid composition of the brown seaweed *Laminaria gurjanovae*. *Russian Journal of Bioorganic Chemistry*, 33(1): 88-98.

Shibata, H., Iimuro, M., Uchiya, N., Kawamori, T., Nagaoka, M., Ueyama, S., Hashimoto, S., Yokokura, T., Sugimura, T., Wakabayashi, K. (2003). Preventive effects of *Cladosiphon* fucoidan against *Helicobacter pylori* infection in Mongolian gerbils. *Helicobacter*, 8(1): 59-65.

Shimaoka, M., Ikeda, M., Iida, T., Taenaka, N., Yoshiya, I., Honda, T. (1996). Fucoidin, a potent inhibitor of leukocyte rolling, prevents neutrophil influx into phorbol-ester-induced inflammatory sites in rabbits lungs. *American Journal of Respiratory and Critical Care Medicine*, 153(1): 307-311.

Si-Tayeb, K., Monvoisin, A., Mazzocco, C., Lepreux, S., Decossas, M., Cubel, G., Taras, D., Blanc, J. F., Robinson, D. R., Rosenbaum, J. (2006). Matrix metalloproteinase 3 is present in the cell nucleus and is involved in apoptosis. *American Journal of Pathology*, 169(4): 1390-1401.

Sinniger, V., Taponbretaudiere, J., Millien, C., Muller, D., Jozefonvicz, J., Fischer, A. M. (1993). Affinity-chromatography of sulfated polysaccharides separately fractionated on antithrombin-III and heparin Cofactor-II immobilized on concanavalin-a-sepharose. *Journal of Chromatography-Biomedical Applications*, 615(2): 215-223.

Smith, L. L., Coller, H. A., Roberts, J. M. (2003). Telomerase modulates expression of growth-controlling genes and enhances cell proliferation. *Nature Cell Biology*, 5(5): 474-479.

Soeda, S., Ishida, S., Honda, O., Shimeno, H., Nagamatsu, A. (1994). Aminated fucoidan promotes the invasion of 3 LL cells through reconstituted basement membrane - Its possible mechanism of action. *Cancer Letters*, 85(1): 133-138.

Soeda, S., Ohmagari, Y., Shimeno, H., Nagamatsu, A. (1993). Preparation of oversulfated fucoidan fragments and evaluation of their antithrombotic activities. *Thrombosis Research*, 72(3): 247-256.

Soeda, S., Sakaguchi, S., Shimeno, H., Nagamatsu, A. (1992). Fibrinolytic and anticoagulant activities of highly sulfated fucoidan. *Biochemical Pharmacology*, 43(8): 1853-1858.

Solomons, T. W. G., Fryhle, C. B. (2000). *Chimie Organique*. Mont-Royal, Modulo, 1151 p.

Souchet, N. (2004). *Étude de l'influence de la période de récolte de l'algue brune Laminaria longicruris sur sa composition en polysaccharides bioactifs*. Mémoire de maîtrise, Université Laval, Québec, 80 p.

Southern, E. M. (2001). DNA Microarrays: History and Overview in *DNA arrays: methods and protocols*, J. B. Rampal(Ed.). Totowa, Human Press: 1-16.

Sriamornsak, P., Sungthongjeen, S. (2007). Modification of theophylline release with alginate gel formed in hard capsules. *Aaps Pharmscitech*, 8(3).

Sudel, K. M., Venzke, K., Mielke, H., Breitenbach, U., Mundt, C., Jaspers, S., Koop, U., Sauermann, K., Knussmann-Hartig, E., Moll, I., Gercken, G., Young, A. R., Stab, F., Wenck, H., Gallinat, S. (2005). Novel aspects of intrinsic and extrinsic aging of human skin: Beneficial effects of soy extract. *Photochemistry and Photobiology*, 81(3): 581-587.

Suggs, S. V., Katzowitz, J. L., Tsaimorris, C., Sukhatme, V. P. (1990). cDNA sequence of the human cellular early growth response gene Egr-1. *Nucleic Acids Research*, 18(14): 4283-4283.

Surenjav, U., Zhang, L., Xu, X. J., Zhang, X. F., Zeng, F. B. (2006). Effects of molecular structure on antitumor activities of (1 -> 3)-beta-D-glucans from different *Lentinus Edodes*. *Carbohydrate Polymers*, 63(1): 97-104.

Svensson, L., Aszodi, A., Reinholt, F. P., Fassler, R., Heinegard, D., Oldberg, A. (1999). Fibromodulin-null mice have abnormal collagen fibrils, tissue organization, and altered lumican deposition in tendon. *Journal of Biological Chemistry*, 274(14): 9636-9647.

Takada, Y., Ye, X., Simon, S. (2007). The integrins. *Genome Biology*, 8(5): 215.

Takagi, M., Sueishi, M., Saiwaki, T., Kametaka, A., Yoneda, Y. (2001). A novel nucleolar protein, NIFK, interacts with the forkhead associated domain of Ki-67 antigen in mitosis. *Journal of Biological Chemistry*, 276(27): 25386-25391.

Talevi, R., Gualtieri, R. (2001). Sulfated glycoconjugates are powerful modulators of bovine sperm adhesion and release from the oviductal epithelium *in vitro*. *Biology of Reproduction*, 64(2): 491-498.

Tan, E. M. L., Peltonen, J. (1991). Endothelial cell growth factor and heparin regulate collagen gene expression in keloid fibroblasts. *Biochemical Journal*, 278: 863-869.

Tanghe, S., Van Soom, A., Duchateau, L., Nauwynck, H., De Kruif, A. (2004). Carbohydrates and glycoproteins involved in bovine fertilization *in vitro*. *Molecular Reproduction and Development*, 68(4): 492-499.

Teas, J. (1983). The dietary intake of *Laminaria*, a brown seaweed, and breast cancer prevention,. *Nutrition and Cancer*, 4(3): 217-222.

Teixeira, M. M., Hellewell, P. G. (1997). The effect of the selectin binding polysaccharide fucoidin on eosinophil recruitment *in vivo*. *British Journal of Pharmacology*, 120(6): 1059-1066.

Teng, Q. (2005). *Structural Biology: Practical NMR Applications*. New York, Springer Science, 295 p.

Teruya, T., Konishi, T., Uechi, S., Tamaki, H., Tako, M. (2007). Anti-proliferative activity of oversulfated fucoidan from commercially cultured *Cladosiphon okamuranus* TOKIDA in U937 cells. *International Journal of Biological Macromolecules*, 41(3): 221-226.

Tesfaigzi, Y., Wright, P. S., Belinsky, S. A. (2003). SPRR1B overexpression enhances entry of cells into the G_0 phase of the cell cycle. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 285(4): L889-L898.

Thompson, K. D., Dragar, C. (2004). Antiviral activity of *Undaria pinnatifida* against herpes simplex virus. *Phytotherapy Research*, 18(7): 551-555.

Thunberg, L., Bäckström, G., Lindahl, U. (1982). Further characterization of the antithrombin-binding sequence in heparin. *Carbohydrate Research*, 100(1): 393-410.

Torsdottir, I., Alpsten, M., Holm, G., Sandberg, A. S., Tolli, J. (1991). A small dose of soluble alginate-fiber affects postprandial glycemia and gastric emptying in humans with diabetes. *Journal of Nutrition*, 121(6): 795-799.

Trento, F., Cattaneo, F., Pescador, R., Porta, R., Ferro, L. (2001). Antithrombin activity of an algal polysaccharide. *Thrombosis Research*, 102(5): 457-465.

Tuomanen, E. I., Saukkonen, K., Sande, S., Cioffe, C., Wright, S. D. (1989). Reduction of inflammation, tissue-damage, and mortality in bacterial meningitis in rabbits treated with monoclonal antibodies against adhesion-promoting receptors of leukocytes. *Journal of Experimental Medicine*, 170(3): 959-968.

Turquois, T., Gloria, H. (2000). Determination of the absolute molecular weight averages and molecular weight distributions of alginates used as ice cream stabilizers by using multiangle laser light scattering measurements. *Journal of Agricultural and Food Chemistry*, 48(11): 5455-5458.

Tyagi, S. C., Kumar, S., Katwa, L. (1997). Differential regulation of extracellular matrix metalloproteinase and tissue inhibitor by heparin and cholesterol in fibroblast cells. *Journal of Molecular and Cellular Cardiology*, 29(1): 391-404.

Ueda, K., Inoue, S., Zhang, Y., Kutsuna, T., Inoue, S., Noto, K., Arai, N., Noguchi, M. (2009). Heparin induces apoptosis through suppression of AKt in oral squamous cell carcinoma cells. *Anticancer Research*, 29(4): 1079-1088.

US Food and Drug Administration. (2000). "New dietary ingredients in dietary supplements." Retrieved 27 août, 2009, from http://www.fda.gov/Food/DietarySupplements/ucm109764.htm.

Ustyuzhanina, N., Krylov, V., Grachev, A., Gerbst, A., Nifantiev, N. (2006). Synthesis, NMR and conformational studies of fucoidan fragments. Part 8. Convergent synthesis of branched and linear oligosaccharides. *Synthesis*, (23): 4017-4031.

van der Poll, T., Levi, M., Buller, H., van Deventer, S. J., de Boer, J. P., Hack, C. E., ten Cate, J. W. (1991). Fibrinolytic response to tumor necrosis factor in healthy subjects. *Journal of Experimental Medicine*, 174(3): 729-732.

van Tol, E. A. F., Holt, L., Li, F. L., Kong, F. M., Rippe, R., Yamauchi, M., Pucilowska, J., Lund, P. K., Sartor, R. B. (1999). Bacterial cell wall polymers promote intestinal fibrosis by direct stimulation of myofibroblasts. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 277(1): G245-G255.

Veena, C. K., Josephine, A., Preetha, S. P., Varalakshmi, P. (2007). Beneficial role of sulfated polysaccharides from edible seaweed *Fucus vesiculosus* in experimental hyperoxaluria. *Food Chemistry*, 100(4): 1552-1559.

Venkateswaran, P. S., Millman, I., Blumberg, B. S. (1989). Interaction of fucoidan from *Pelvetia fastigiata* with surface-antigens of hepatitis-B and Woodchuck hepatitis viruses. *Planta Medica*, (3): 265-270.

Verdun, R. E., Crabbe, L., Haggblom, C., Karlseder, J. (2005). Functional human telomeres are recognized as DNA damage in G2 of the cell cycle. *Molecular Cell*, 20(4): 551-561.

Verdun, R. E., Karlseder, J. (2007). Replication and protection of telomeres. *Nature*, 447(7147): 924-931.

Viebke, C., Borgstrom, J., Piculell, L. (1995). Characterization of kappa-carrageenan and iota-carrageenan coils and helices by MALLS/GPC. *Carbohydrate Polymers*, 27(2): 145-154.

Viola, M., Bartolini, B., Sonaggere, M., Giudici, C., Tenni, R., Tira, M. E. (2007). Fibromodulin interactions with type I and II collagens. *Connective Tissue Research*, 48(3): 141-148.

Volpi, N., Mascellani, G., Bianchini, P. (1992). Low molecular weight heparins (5-kDa) and oligoheparins (2-kDa) produced by gel permeation enrichment or radical process: Comparison of structures and physicochemical and biological properties. *Analytical Biochemistry*, 200(1): 100-107.

Waeghe, T. J., Darvill, A. G., McNeil, M., Albersheim, P. (1983). Determination, by methylation analysis, of the glycosyl-linkage compositions of microgram quantities of complex carbohydrates. *Carbohydrate Research*, 123(2): 281-304.

Wang, P., Jiang, X. L., Jiang, Y. H., Hu, X. K., Mou, H. J., Li, M., Guan, H. H. (2007). In vitro antioxidative activities of three marine oligosaccharides. *Natural Product Research*, 21(7): 646-654.

Warrinton, J. A., Dee, S., Trulson, M. (2000). Large-scale genomic analysis using Affymetrix GeneChip® probe arrays in *Microarray Biochip Technology*, M. Schena(Ed.). Natick, Eaton Publishing: 119-148.

Wassarman, P. M. (1988). Zona pellucida glycoproteins. *Annual Review of Biochemistry*, 57: 415-442.

Wassarman, P. M. (2008). Zona pellucida glycoproteins. *Journal of Biological Chemistry*, 283(36): 24285-24289.

Watt, P. M., Hickson, I. D. (1994). Structure and function of type II DNA topoisomerases. *Biochemical Journal*, 303: 681-695.

Wedlock, D. J., Fasihuddin, B. A., Phillips, G. O. (1987). Factors influencing the molecular weight of sodium alginate preparations. *Food Hydrocolloids*, 1(3): 207-213.

Wei, D., Zhang, L., Williams, D. L., Browder, I. W. (2002). Glucan stimulates human dermal fibroblast collagen biosynthesis through a nuclear factor-1 dependent mechanism. *Wound Repair and Regeneration*, 10(3): 161.

Welgus, H. G., Jeffrey, J. J., Eisen, A. Z. (1981). The collagen substrate specificity of human skin fibroblast collagenase. *Journal of Biological Chemistry*, 256(18): 9511-9515.

Westermarck, J., Li, S. P., Kallunki, T., Han, J. H., Kahari, V. M. (2001). p38 mitogen-activated protein kinase-dependent activation of protein phosphatases 1 and 2A inhibits MEK1 and MEK2 activity and collagenase 1 (MMP-1) gene expression. *Molecular and Cellular Biology*, 21(7): 2373-2383.

Williams, D. L. (1997). Overview of (1->3)-beta-D-glucan immunobiology. *Mediators of Inflammation*, 6(4): 247.

Williams, D. L., McNamee, R. B., Jones, E. L., Pretus, H. A., Ensley, H. E., Browder, I. W., Diluzio, N. R. (1991). A method for the solubilization of a (1→3)-β-D-glucan isolated from *Saccharomyces cerevisiae*. *Carbohydrate Research*, 219: 203-213.

Williams, D. L., Mueller, A., Browder, W. (1996). Glucan-based macrophage stimulators - A review of their anti-infective potential. *Clinical Immunotherapeutics*, 5(5): 392-399.

Woo-Jung, K., Kim, S. M., Lee, Y. H., Kim, H. G., Kim, H. K., Moon, S. H., Suh, H. H., Jang, K. H., Park, Y. I. (2008). Isolation and characterization of marine bacterial strain degrading fucoidan from Korean *Undaria pinnatifida* sporophylls. *Journal of Microbiology and Biotechnology*, 18(4): 616-623.

Wyatt, P. J. (1993). Light-scattering and the absolute characterization of macromolecules. *Analytica Chimica Acta*, 272(1): 1-40.

Xia, Z., Dickens, M., Raingeaud, J. l., Davis, R. J., Greenberg, M. E. (1995). Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science*, 270(5240): 1326-1331.

Yamada, T., Ogamo, A., Saito, T., Uchiyama, H., Nakagawa, Y. (2000). Preparation of O-acylated low-molecular-weight carrageenans with potent anti-HIV activity and low anticoagulant effect. *Carbohydrate Polymers*, 41(2): 115-120.

Yamauchi, M., Imajoh-Ohmi, S., Shibuya, M. (2007). Novel antiangiogenic pathway of thrombospondin-1 mediated by suppression of the cell cycle. *Cancer Science*, 98(9): 1491-1497.

Yoon, S. J., Pyun, Y. R., Hwang, J. K., Mourão, P. A. S. (2007). A sulfated fucan from the brown alga *Laminaria cichorioides* has mainly heparin cofactor II-dependent anticoagulant activity. *Carbohydrate Research*, 342(15): 2326-2330.

York, W. S., Darvill, A. G., McNeil, M., Stevenson, T. T., Albersheim, P. (1985). Isolation and characterization of plant cell walls and cell wall components. *Methods in Enzymology*, 118: 3-40.

Young, E. (2008). The anti-inflammatory effects of heparin and related compounds. *Thrombosis Research*, 122(6): 743-752.

Yuan, Y. V. (2008). Marine algal constituents in *Marine nutraceuticals and functional foods*. Boca Raton, CRC Press: 259-296.

Zakharkin, S. O., Kim, K., Mehta, T., Chen, L., Barnes, S., Scheirer, K. E., Parrish, R. S., Allison, D. B., Page, G. P. (2005). Sources of variation in Affymetrix microarray experiments. *BMC Bioinformatics*, 6.

Zemani, F., Benisvy, D., Galy-Fauroux, I., Lokajczyk, A., Colliec-Jouault, S., Uzan, G., Fischer, A. M., Boisson-Vidal, C. (2005). Low-molecular-weight fucoidan enhances the proangiogenic phenotype of endothelial progenitor cells. *Biochemical Pharmacology*, 70(8): 1167-1175.

Zhang, F., Yoder, P. G., Linhardt, R. J. (2005a). Synthetic and natural polysaccharides with anticoagulant properties in *Polysaccharides: Structural Diversity and Functional Versatility*, S. Dimitriu(Ed.). New York, Marcel Dekker: 773-794.

Zhang, H. L., Kim, J. K., Edwards, C. A., Xu, Z. H., Taichman, R., Wang, C. Y. (2005b). Clusterin inhibits apoptosis by interacting with activated Bax. *Nature Cell Biology*, 7(9): 909-915.

Zhang, M., Cui, S. W., Cheung, P. C. K., Wang, Q. (2007). Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity. *Trends in Food Science & Technology*, 18(1): 4-19.

Zhang, W., Chuang, Y.-J., Swanson, R., Li, J., Seo, K., Leung, L., Lau, L. F., Olson, S. T. (2004). Antiangiogenic antithrombin down-regulates the expression of the proangiogenic heparan sulfate proteoglycan, perlecan, in endothelial cells. *Blood*, 103(4): 1185.

Zúñiga, E. A., Matsuhiro, B., Mejías, E. (2006). Preparation of a low-molecular weight fraction by free radical depolymerization of the sulfated galactan from *Schizymenia binderi* (Gigartinales, Rhodophyta) and its anticoagulant activity. *Carbohydrate Polymers*, 66(2): 208-215.

Zvyagintseva, T. N., Shevchenko, N. M., Chizhov, A. O., Krupnova, T. N., Sundukova, E. V., Isakov, V. V. (2003). Water-soluble polysaccharides of some far-eastern brown seaweeds. Distribution, structure, and their dependence on the developmental conditions. *Journal of Experimental Marine Biology and Ecology*, 294(1): 1-13.

Annexe 1 :
Rappel sur les gènes et le génome

Les gènes et le génome

Le génome est contenu dans le noyau des cellules eucaryotes et chaque noyau possède une copie complète du génome. Ce dernier est réparti en segments appelés chromosome. Chez l'humain, 46 chromosomes dont 22 paires homologues et deux chromosomes sexuels (X et Y) sont retrouvés (Calladine et al., 2004). L'ensemble comprenant 3,2 milliards de paires de bases. Chaque chromosome contient différents gènes (50 000 à 100 000 chez les humains) ayant des fonctions biologiques bien précises (Schena et al., 2000). Les gènes représentent une séquence ordonnée de nucléotides qui occupent une position précise sur un chromosome déterminé et qui constituent une information génétique dont la transmission est héréditaire. Les chromosomes possèdent la capacité de se répliquer et de subir des mutations. Les gènes représentent les unités physiques et fonctionnelles élémentaires de l'hérédité. Certains d'entre eux correspondent à une portion d'une chaîne d'ADN qui dicte la nature des protéines à synthétiser.

Les acides nucléiques

Il existe deux types d'acides nucléiques : l'ARN (acide ribonucléique) et l'ADN (acide désoxyribonucléique). Ces molécules permettent aux organismes de se reproduire. Ainsi, l'ADN fournit les directives pour sa propre réplication assurant la continuité de la vie alors que l'ARN sert plutôt d'intermédiaire dans la circulation de l'information génétique de l'ADN aux protéines. Les bases composant l'ADN et l'ARN sont réparties en deux catégories : les purines : l'adénine (A) et la guanine (G), et les pyrimidines qui comprennent la cytosine (C) et la thymine (T) ou l'uracile (U) pour l'ARN seulement. La structure chimique des bases est telle que A se joint spécifiquement à T/U et G s'associe avec C (et pas avec aucune autre base). Ainsi, un groupement purine est toujours associé à un groupement pyrimidine avec l'aide de liaisons hydrogène selon le modèle de Watson-Crick (Calladine et al., 2004). Les règles d'appariement des nucléotides sont à la base de la méthode d'hybridation expérimentale qui utilise, les segments d'ARN messager (ARNm) isolés de cellules pour retranscrire le segment ADN à l'origine de l'ARNm servant à

identifier le gène initial. D'autres méthodes, comme la réaction en chaîne de la polymérase (PCR) utilise aussi les règles d'appariement pour répliquer l'ADN rapidement.

La transcription et la traduction : Du gène à la protéine

Les gènes détiennent toutes les informations permettant la synthèse de protéines spécifiques, mais ils ne les construisent pas directement. C'est l'ARN qui est responsable d'assurer le lien entre l'information génétique et la synthèse des protéines. La transcription est la synthèse de l'ARN sous la direction de l'ADN, donc des gènes.

Pour effectuer la transcription, l'ADN double brin doit être déroulé partiellement via l'ARN polymérase, une enzyme (Figure A1.1). Cette dernière écarte les deux brins et lie les nucléotides de l'ARN les uns aux autres au fur et à mesure que leurs bases s'apparient le long du brin codant pour former l'ARN prémessager. Ensuite, l'ARN prémessager subit une maturation dans le noyau. Des enzymes excisent les introns, fragments non codants d'un gène situé entre deux exons, et regroupent les exons, fragments codants d'un gène, afin de former un ARNm avec une séquence codante continue. Par la suite, l'ARNm se dirige vers le cytoplasme jusqu'au ribosome. L'assemblage d'une protéine est effectué via le ribosome avec l'aide de l'ARN de transfert (ARNt) et cette étape est appelée traduction. L'ARNt a pour fonction de transférer vers le ribosome les unités provenant de la réserve d'acides aminés du cytoplasme. Pour chaque triplet de nucléotides (codon) présents sur l'ARNm, l'ARNt identifie un acide aminé précis en respectant les règles d'appariement. Par exemple, TTT correspond à la phénylalanine alors qu'AAA correspond à la lysine. Il peut y avoir plusieurs codons (1 à 6) pour un acide aminé. Ainsi, l'ARNm est décodé par l'ARNt pour former la protéine en question. Les protéines peuvent être composées de 20 acides aminés différents et l'ordre de ces acides aminés est variable d'une protéine à l'autre.

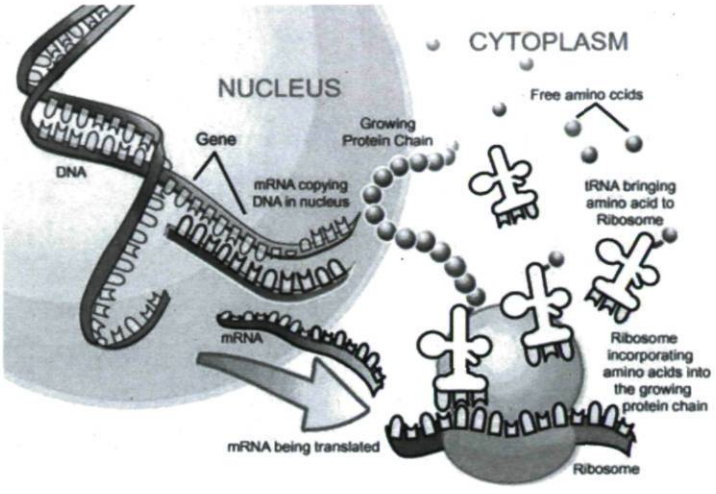

Figure A1.1 : Représentation globale de la transcription et de la traduction de l'ARNm pour la synthèse de protéines spécifiques.
(Secko, 2003)

Annexe 2 :
Gènes présents sur la puce à ADN
DualChip® human Aging 1.1

eppendorf

DualChip® human aging version 1.1

Gene name	Common Gene Symbol	Official Gene Symbol	Accession Number	Swissprot	General Function	Specific Function
ADAM metallopeptidase domain 1	ADAM1	ADAM1	Y09232.1	/	/	/
Actin, beta	Actl	ACTB	NM_001101.2	P02570	Cell Communication / Focal adhesion / Adherens junction / Tight junction / Leukocyte transendothelial migration / Regulation of actin cytoskeleton	Housekeeping gene
Aldolase A, fructose-bisphosphate	Aldo	ALDOA	NM_000034.1	P04075	Glycolysis / Gluconeogenesis / Pentose phosphate pathway / Fructose and mannose metabolism / Carbon fixation	Housekeeping gene
Alpha-tubulin ubiquitous	Tubu	K-ALPHA-1	NM_006082.1	P05209	Gap junction	Housekeeping gene
Amphiregulin (schwannoma-derived growth factor)	AREG	AREG	NM_001657.1	P15514	Cell proliferation	Growth factor activity
Amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65)	FE65	APBB1	L77864.1	O00213	Alzheimer's disease	Cytosolic anchoring site that can prevent the gene product's nuclear translocation
Annexin A1	ANX1	ANXA1	NM_000700.1	P04083	Calcium ion binding	Potential anti-inflammatory activity
Antigen identified by monoclonal antibody Ki-67	Ki-67	MKI67	NM_002417.2	P46013	Cell cycle	Required for maintening cell proliferation
Apolipoprotein B	APOB	APOB	NM_000384.1	P04114	Lipid transporter activity	Recognition signal for the cellular binding
Apolipoprotein E	APOE	APOE	M12529.1	P02649	Neurodegenerative Disorders / Alzheimer's disease	Essential for the normal catabolism of triglyceride-rich lipoprotein constituents
Ataxia telangiectasia mutated	ATM	ATM	U26455.1	Q13315	Cell cycle / Apoptosis / Toll-like receptor signaling pathway	Important cell cycle checkpoint kinase
B-cell CLL/lymphoma 2	BCL2	BCL2	NM_000633.1	P10415	Neurodegenerative Disorders / Apoptosis / Focal adhesion / Amyotrophic lateral sclerosis (ALS) / Prion disease	Blocks the apoptotic death of some cells such as lymphocytes
BCL2-associated X protein	BAX	BAX	NM_004324.1	Q8WXU1	Neurodegenerative Disorders / Apoptosis / Amyotrophic lateral sclerosis (ALS)	Involved in a wide variety of cellular activities
BCL2-like 1	BCLX	BCL2L1	NM_001191.1	Q07817	Neurodegenerative Disorders / Apoptosis / Jak-STAT signaling pathway / Amyotrophic lateral sclerosis (ALS)	Involved in a wide variety of cellular activities
Bone morphogenetic protein 2	BMP2	BMP2	NM_001200.1	P12643	Cytokine-cytokine receptor interaction / Hedgehog signaling pathway / TGF-beta signaling pathway	Induces bone and cartilage formation
Breast cancer 2, early onset	BRCA2	BRCA2	NM_000059.1	P51587	DNA repair	Functions as a tumor suppressor gene
Calnexin	CANX	CANX	NM_001746.1	P27824	Antigen processing and presentation	Plays a central role in the quality control of protein folding ; Chaperone
Caspase 7	CAS7	CASP7	NM_001227.2	P55210	Neurodegenerative Disorders / MAPK signaling pathway / Apoptosis / Alzheimer's disease / Dentatorubropallidoluysian atrophy (DRPLA)	Plays a central role in the execution-phase of cell apoptosis
Caspase 8	CAS8	CASP8	X98172.1	Q14790	Neurodegenerative Disorders / MAPK signaling pathway / Apoptosis / Toll-like receptor signaling pathway / Huntington's disease / Dentatorubropallidoluysian atrophy (DRPLA)	Involved in the programmed cell death induced by Fas and various apoptotic stimuli
Cathepsin D (lysosomal aspartyl peptidase)	CTSD	CTSD	M11233.1	P07339	Protein metabolism	Involved in the pathogenesis of several diseases
Cathepsin H	CTSH	CTSH	NM_004390.1	P09668	Proteolysis	Important in the overall degradation of lysosomal proteins
Cathepsin S	CTSS	CTSS	M90696.1	P25774	Antigen processing and presentation	May participate in the degradation of antigenic proteins
Cathepsin Z	CTSZ	CTSZ	AF136273.1	Q9UBR2	Proteolysis	Lysosomal cysteine proteinase and member of the peptidase C1 family
Cell division cycle 42 (GTP binding protein, 25kDa)	CDC42	CDC42	NM_001791.2	P21181	MAPK signaling pathway / Axon guidance / Focal adhesion / Adherens junction / Tight junction / T cell receptor signaling pathway / Leukocyte transendothelial migration / Regulation of actin cytoskeleton	Controls diverse cellular functions including cell morphology, migration, endocytosis and cell cycle progression
Centromere protein A	CENPA	CENPA	U14518.1	P49450	Nucleosome assembly	Specify the mitotic behavior of chromosomes
Centromere protein F	CENPF	CENPF	U30872.1	P49454	Chromosomal processing	Control of mitosis
Chemokine (C-C motif) ligand 5	RANTES	CCL5	NM_002985.1	P13501	Cytokine-cytokine receptor interaction / Toll-like receptor signaling pathway / Epithelial cell signaling in Helicobacter pylori infection	Involved in immunoregulatory and inflammatory processes; C-C chemokine activity
Clusterin (apolipoprotein J)	APOJ	CLU	J02908.1	P10909	Apoptosis	Complement activation ; plasma glycoprotein
C-myc binding protein	cmyc	MYCBP	NM_012333.2	Q99417	Transcription factor activity	Stimulates the activation of E box-dependent transcription by MYC
Collagen, type I, alpha 1	COL1A1	COL1A1	NM_000088.2	P02452	Cell Communication / Focal adhesion / ECM-receptor interaction	Major component of type I collagen
Collagen, type III, alpha 1	COL3A1	COL3A1	NM_000090.2	P02461	Cell Communication / Focal adhesion / ECM-receptor interaction	Encodes a fibrillar collagen
Collagen, type VI, alpha 2	COLVI	COL6A2	NM_058175.1	P12110	Cell Communication / Focal adhesion / ECM-receptor interaction	Importance of this collagen in organizing matrix components
Collagen, type XV, alpha 1	COL15A1	COL15A1	NM_001855.1	P39059	Cell differentiation	May function to adhere basement membranes to underlying connective tissue stroma
Colony stimulating factor 2 (granulocyte-macrophage)	GMCSF	CSF2	M11220.1	P04141	Cytokine-cytokine receptor interaction / Jak-STAT signaling pathway / Hematopoietic cell lineage / Natural killer cell mediated cytotoxicity / T cell receptor signaling pathway / Fc epsilon RI signaling pathway	Cytokine that controls the production, differentiation, and function of granulocytes and macrophages
Connective tissue growth factor	CTGF	CTGF	U14750.1	P29279	Growth factors and cytokines	Epidermal development
Creatine kinase, brain	CKB	CKB	M16364.1	P12277	Urea cycle and metabolism of amino groups / Arginine and proline metabolism	Energy homeostasis
Cyclin A1	cyA	CCNA1	NM_003914.2	P78396	Cell cycle	Functions in cell cycle
Cyclin B1	CyB1	CCNB1	NM_031966.1	P14635	Cell cycle	Involved in mitosis

Gene name	Common Gene Symbol	Official Gene Symbol	Accession Number	Swissprot	General Function	Specific Function
Cyclin D1	CND1	CCND1	NM_053056.1	P24385	Cell cycle / Wnt signaling pathway / Focal adhesion / Jak-STAT signaling pathway	Regulatory subunit of CDK4 or CDK6
Cyclin D2	CND2	CCND2	NM_001759.2	P30279	Cell cycle / Wnt signaling pathway / Focal adhesion / Jak-STAT signaling pathway	Regulatory subunit of CDK4 or CDK6
Cyclin D3	CND3	CCND3	NM_001760.2	P30281	Cell cycle / Wnt signaling pathway / Focal adhesion / Jak-STAT signaling pathway	Regulatory subunit of CDK4 or CDK6
Cyclin E1	CyE1	CCNE1	NM_001238.1	P24864	Cell cycle	Regulators of CDK kinases; essential for the control of the cell cycle at the G1/S transition
Cyclin F	CCNF	CCNF	NM_001761.1	P41002	Cell cycle	Member of the F-box proteins
Cyclin G1	CCNG	CCNG1	U53328.1	P51959	Cell cycle	Member of the cyclin family
Cyclin H	CCNH	CCNH	NM_001239.2	P51946	Cell cycle	Phosphorylates and activates cyclin-dependant protein kinases in the regulation of cell cycle progression
Cyclin-dependent kinase 2	CDK2	CDK2	NM_001798.2	P24941	Cell cycle	Essential for cell cycle G1/S phase transition
Cyclin-dependent kinase 4	CDK4	CDK4	U79269.1	P11802	Cell cycle / Tight junction / T cell receptor signaling pathway	Important for cell cycle G1 phase progression
Cyclin-dependent kinase 5, regulatory subunit 1 (p35)	p35	CDK5R1	NM_003885.1	Q15078	Cyclin dependent protein kinase activity	Development of the central nervous system
Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	p21	CDKN1A	U03106.1	P38936	Cell cycle	Regulator of cell cycle progression at G1
Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	p27	CDKN1B	NM_004064.2	P46527	Cell cycle	Controls the cell cycle progression at G1
Cyclin-dependent kinase inhibitor 1C (p57, Kip2)	p57	CDKN1C	NM_000076.1	P49918	Cell cycle	Inhibits G1-specific CDK-cyclin complexes,negative regulator of cell proliferation.
Cyclin-dependent kinase inhibitor 2A (p16, inhibits CDK4)	p16	CDKN2A	L27211.1	P42771	Cell cycle	Regulatory roles of CDK4 and p53 in cell cycle G1 progression
Cystatin E/M	CST6	CST6	U62800.1	Q15828	Cysteine protease inhibitor activity	Cysteine protease inhibitor activity
Cytokeratin 2	CYT2A	KRT2B	M99063.1	P35908	Cytoskeleton organization and biogenesis	Structural molecule activity
Dermatopontin	DPT	DPT	XM_001897.8	Q07507	Protein binding	Functions in cell-matrix interactions and matrix assembly
Desmoglein 1	DSG1	DSG1	AF097935.1	Q02413	Cell Communication	Calcium-binding transmembrane glycoprotein
Dihydrofolate reductase	DHFR	DHFR	NM_000791.2	P00374	One carbon pool by folate / Folate biosynthesis	Dihydrofolate reductase
DNA-damage-inducible transcript 3	G153	DDIT3	S40706.1	P35638	MAPK signaling pathway	DNA damage response; Transcription co-repressor
DnaJ (Hsp40) homolog, subfamily B, member 1	HSP40	DNAJB1	D49547.1	P25685	Protein folding	Response to unfolded protein
E2F transcription factor 1	E2F1	E2F1	NM_005225.1	Q01094	Cell cycle	Plays a crucial role in the control of cell cycle and action of tumor suppressor
E2F transcription factor 5, p130-binding	E2F5	E2F5	U31556.1	Q15329	Cell cycle / TGF-beta signaling pathway	Plays a crucial role in the control of cell cycle and action of tumor suppressor
E3 ubiquitin-protein ligase MDM2	MDM2	MDM2	NM_002392.2	Q00987	Cell cycle	Transcription factor ; acts as a major regulator of the tumor suppressor p53
Early growth response 1	EGR1	EGR1	NM_001964.1	P18146	Transcription factor activity	Functions as a transcriptional regulator
Early growth response 2	EGR2	EGR2	NM_000399.2	P11161	Transcription factor activity	Transcription factor
Early growth response 3	EGR3	EGR3	NM_004430.1	Q06889	Transcription factor activity	Participates in the transcriptional regulation of genes in controling biological rhythm
Elastin	ELN	ELN	NM_000501.1	P15502	Cell proliferation	Extracellular matrix structural constituent
Electron-transfer-flavoprotein, beta polypeptide	ETFB	ETFB	NM_001985.1	P38117	Electron transporter activity	Mitochondrial fatti acid catabolism
Enhancer of polycomb homolog 2 (Drosophila)	EPC1	EPC2	AF286904.1	Q96RR7	/	/
Eukaryotic translation elongation factor 1 alpha 1	EF1A	EEF1A1	AY043301.1	P04720	Protein biosynthesis	Responsibles for the enzymatic delivery of aminoacyl tRNAs to the ribosome
Eukaryotic translation initiation factor 3, subunit 6 48kDa	INT6	EIF3S6	NM_001568.1	P60228	Protein biosynthesis	Binds to the 40S ribosome and promotes the binding of methionyl-tRNAi and mRNA
Eukaryotic translation initiation factor 4A, isoform 1	EIF-4A	EIF4A1	NM_001416.1	P04765	Protein biosynthesis	ATP-dependent single stranded DNA-binding protein
Ewing sarcoma breakpoint region 1	EWSR1	EWSR1	NM_005243.1	Q01844	Transcription	/
Feline sarcoma oncogene	FES	FES	X52192.1	P07332	Axon guidance	Activity is required for maintenance of cellular transformation
Fibromodulin	FMOD	FMOD	NM_002023.2	Q06828	Transforming growth factor beta receptor complex assembly	May participate in the assembly of the extracellular matrix
Fibronectin 1	fibro	FN1	X02761.1	P02751	Cell Communication / Focal adhesion / ECM-receptor interaction / Regulation of actin cytoskeleton	Involved in cell adhesion and migration processes
Filaggrin	FLG	FLG	M60502.1	P20930	Cell differentiation	Implicated in a number of keratinizing disorders
Fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	VEGFR1	FLT1	NM_002019.2	P17948	Cytokine-cytokine receptor interaction / Focal adhesion	Important for the control of cell proliferation and differentiation
Galactosidase, beta 1	GLB1	GLB1	M34423.1	P16278	Galactose metabolism / N-Glycan degradation / Glycosaminoglycan degradation / Glycerolipid metabolism / Glycosphingolipid metabolism	Catalyses cleavage of galactose
Glucose-6-phosphate dehydrogenase	G6PD	G6PD	NM_000402.1	P11413	Pentose phosphate pathway / Glutathione metabolism	Main function is to produce NADPH
Glucosidase, alpha; acid (Pompe disease, glycogen storage disease type II)	GAA	GAA	NM_000152.2	P10253	Galactose metabolism / Starch and sucrose metabolism	Degradation of glycogen to glucose in lysosomes
Glutathione peroxidase 1	GPX	GPX1	M21304.1	P07203	Glutathione metabolism / Arachidonic acid metabolism / Amyotrophic lateral sclerosis (ALS)	Detoxification of hydrogen peroxide and is one of the most important antioxidant enzymes in humans
Glutathione S-transferase pi	GSTP1	GSTP1	NM_000852.2	P09211	Glutathione metabolism	Functions in xenobiotic metabolism and play a role in susceptibility to cancer, and other diseases

Gene name	Common Gene Symbol	Official Gene Symbol	Accession Number	Swissprot	General Function	Specific Function
Glutathione S-transferase theta 1	GSTT1	GSTT1	NM_000853.1	P30711	Glutathione metabolism	Catalyzes the conjugation of reduced glutathione to a variety of electrophilic and hydrophobic compounds
Glyceraldehyde-3-phosphate-dehydrogenase	GAPD	GAPDH	NM_002046.2	P04406	Glycolysis / Gluconeogenesis / Neurodegenerative Disorders /	Housekeeping gene
Growth factor receptor-bound protein 2	GRB2	GRB2	NM_002086.1	P29354	Neurodegenerative Disorders / MAPK signaling pathway / Dorso-ventral axis formation / Focal adhesion / Gap junction / Jak-STAT signaling pathway / Natural killer cell mediated cytotoxicity / T cell receptor signaling pathway / Insulin signaling pathway / Huntington's disease	Contains one SH2 domain and two SH3 domains
Heat shock 27kDa protein 1	HSP27	HSPB1	AB020027.1	P04792	MAPK signaling pathway	Responses to unfolded protein
Heat shock 70kDa protein 4	HSP70	HSPA4	AB023420.1	P34932	Protein folding	Responses to unfolded protein
Heat shock 70kDa protein 6 (HSP70B')	HSP70B'	HSPA6	NM_002155.1	P17066	Protein folding	Chaperones
Heat shock protein 90kDa alpha (cytosolic), class A member 1	HSP90-a	HSP90AA1	X15183.1	P07900	Antigen processing and presentation	Molecular chaperon, ATPase activity
Heme oxygenase (decycling) 1	hox2	HMOX1	NM_002133.1	P09601	Porphyrin and chlorophyll metabolism	Cleaves heme to form biliverdin
Heparin-binding EGF-like growth factor	HBEGF	HBEGF	M60278.1	Q14487	Epithelial cell signaling in Helicobacter pylori infection	Receptor activity
Hepatic leukemia factor	HLF	HLF	M95585.1	Q16534	Circadian rhythm	Accumulates according to a robust circadian rhythm
Hexokinase 1	HK1	HK1	M75126.1	P19367	Glycolysis / Gluconeogenesis / Fructose and mannose metabolism / Galactose metabolism / Starch and sucrose metabolism / Streptomycin biosynthesis / Aminosugars metabolism	Housekeeping gene
Histone 1, H2bk	H2B/5	HIST1H2BK	NM_080593.1	Q60814	Chromosomal processing	Responsible for the nucleosome structure
Histone 1, H3i	H3FF	HIST1H3I	NM_003533.2	P16106	Chromosomal processing	Responsible for the nucleosome structure within chromosomal fiber in eukaryotes
Histone 1, H4i	H4FM	HIST1H4I	NM_003495.2	/	Chromosomal processing	Responsible for the nucleosome structure within chromosomal fiber in eukaryotes
HLA-B associated transcript 1	BAT1	BAT1	Z37166.1	Q13838	Protein metabolism	Involved in a number of cellular functions including initiation of translation, RNA splicing, and ribosome assembly
HtrA serine peptidase 1	PRSS11	HTRA1	NM_002775.1	Q92743	Proteolysis	Member of the trypsin family of serine proteases
Hypoxanthine phosphoribosyltransferase 1	HPRT	HPRT1	NM_000194.1	P00492	Purine metabolism	Housekeeping gene
Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	ID1	ID1	X77956.1	P41134	TGF-beta signaling pathway	Can inhibit the DNA binding and transcriptional activation ability of basic HLH proteins
Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	ID2	ID2	M97796.1	Q02363	TGF-beta signaling pathway	Transcriptional regulators
Insulin-like growth factor 1 receptor	IGF1R	IGF1R	NM_000875.2	P08069	Focal adhesion / Adherens junction	Plays a critical role in transformation events
Insulin-like growth factor 1	IGF1	IGF1	X57025.1	P01343	Focal adhesion	Plays important roles in mammalian growth and development
Insulin-like growth factor binding protein 2	IGF2	IGFBP2	M35410.1	P18065	Growth factor and cytokines	Insulin-like growth factor binding
Insulin-like growth factor binding protein 3	IGF3	IGFBP3	X64875.1	P17936	Growth factor and cytokines	Insulin-like growth factor binding
Insulin-like growth factor binding protein 5	IGF5	IGFBP5	M65062.1	P24593	Growth factor and cytokines	Insulin-like growth factor binding
Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	ICAM-1	ICAM1	J03132.1	P05362	Cell adhesion molecules (CAMs) / Natural killer cell mediated cytotoxicity / Leukocyte transendothelial migration	Typically expressed on endothelial cells and cells of the immune system
Interferon, gamma	IFNG	IFNG	X13274.1	P01579	Cytokine-cytokine receptor interaction / TGF-beta signaling pathway / Jak-STAT signaling pathway / Natural killer cell mediated cytotoxicity / T cell receptor signaling pathway / Type I diabetes mellitus	Immunoregulatory functions
Interleukin 1, alpha	IL1	IL1A	NM_000575.1	P01583	MAPK signaling pathway / Cytokine-cytokine receptor interaction / Apoptosis / Hematopoietic cell lineage / Type I diabetes mellitus	Involved in various immune responses, inflammatory processes, and hematopoiesis
Interleukin 1, beta	IL1β	IL1β	M15330.1	P01584	MAPK signaling pathway / Cytokine-cytokine receptor interaction / Apoptosis / Toll-like receptor signaling pathway / Hematopoietic cell lineage / Type I diabetes mellitus	Important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis
Interleukin 10	IL10	IL10	NM_000572.1	P22301	Cytokine-cytokine receptor interaction / Jak-STAT signaling pathway / T cell receptor signaling pathway	Pleiotropic effects in immunoregulation and inflammation ; involved in the regulation of the JAK-STAT signaling pathway
Interleukin 11	IL11	IL11	NM_000641.1	P20809	Cytokine-cytokine receptor interaction / Jak-STAT signaling pathway / Hematopoietic cell lineage	Stimulates the T-cell-dependent development of immunoglobulin-producing B cells
Interleukin 11 receptor, alpha	IL11R	IL11RA	NM_004512.1	Q16542	Cytokine-cytokine receptor interaction / Jak-STAT signaling pathway / Hematopoietic cell lineage	Receptor for interleukin-11
Interleukin 12A	IL12	IL12A	M65291.1	P29459	Cytokine-cytokine receptor interaction / Toll-like receptor signaling pathway / Jak-STAT signaling pathway / Type I diabetes mellitus	Acts on T and natural killer cells
Interleukin 15	IL15	IL15	NM_000585.1	P40933	Cytokine-cytokine receptor interaction / Jak-STAT signaling pathway	Induces the activation of JAK kinases
Interleukin 2	IL2	IL2	U25676.1	P01585	Cytokine-cytokine receptor interaction / Jak-STAT signaling pathway / T cell receptor signaling pathway / Type I diabetes mellitus	Important for the proliferation of T and B lymphocytes
Interleukin 3 (colony-stimulating factor, multiple)	IL3	IL3	M20137.1	P08700	Cytokine-cytokine receptor interaction / Apoptosis / Jak-STAT signaling pathway / Hematopoietic cell lineage / Fc epsilon RI signaling pathway	Potent growth promoting cytokine
Interleukin 4	IL4	IL4	NM_000589.1	P05112	Cytokine-cytokine receptor interaction / Jak-STAT signaling pathway / Hematopoietic cell lineage / T cell receptor signaling pathway	T Cell proliferation; Cytokine
Interleukin 6 (interferon, beta 2)	IL6	IL6	NM_000600.1	P05231	Cytokine-cytokine receptor interaction / Toll-like receptor signaling pathway / Jak-STAT signaling pathway / Hematopoietic cell lineage	Interleukin-6 receptor binding
Interleukin 8	IL8	IL8	NM_000584.1	P10145	Cytokine-cytokine receptor interaction / Toll-like receptor signaling pathway / Leukocyte transendothelial migration	One of the major mediators of the inflammatory response

Gene name	Common Gene Symbol	Official Gene Symbol	Accession Number	Swissprot	General Function	Specific Function
Involucrin	IVL	IVL	M13903.1	P07476	Cell differentiation	Component of the keratinocyte crosslinked envelope
Jun D proto-oncogene	JUND	JUND	NM_005354.2	P17535	MAPK signaling pathway	Member of the JUN family ; it has been proposed to protect cells from p53-dependent senescence and apoptosis
Keratin 1 (epidermolytic hyperkeratosis)	KRT1	KRT1	NM_006121.2	P04264	Cell Communication	Member of the keratin gene family
Keratin 10	KRT10	KRT10	NM_000421.1	P13645	Cell Communication	Belongs to the superfamily of intermediate filament (IF) proteins
Keratin 14	KRT14	KRT14	NM_000526.2	P02533	Cell Communication	Structural molecule activity
Keratin 16	KRT16	KRT16	AF061812.1	P08779	Cell Communication	Member of the keratin gene family
Keratin 17	KRT17	KRT17	X62571.1	Q04695	Cell Communication	Marker of basal cell differentiation in complex epithelia
Keratin 19	KRT19	KRT19	NM_002276.1	P08727	Cell Communication	Responsible for the structural integrity of epithelial cells
Keratin 6A	KRT6A	KRT6A	NM_005554.2	P02538	Cell Communication	Member of the keratin gene family
Kinesin family member 23	KNSL5	KIF23	NM_004856.4	Q02241	Chromosomal processing	Microtubule motor, Kinesin
Kinesin family member 2C	KNSL6	KIF2C	NM_006845.2	Q99661	Cell cycle	Microtubule motor, Kinesin,implicated in intracellular transport events
Lymphotoxin alpha (TNF superfamily, member 1)	TNFB	LTA	NM_000595.2	P01374	Cytokine-cytokine receptor interaction / Antigen processing and presentation / Type I diabetes mellitus	Plays a role in apoptosis; mediates a large variety of inflammatory, immunostimulatory, and antiviral responses
Malate dehydrogenase 1, NAD (soluble)	MDH	MDH1	NM_005917.1	P40925	Citrate cycle (TCA cycle) / Pyruvate metabolism / Glyoxylate and dicarboxylate metabolism / Carbon fixation / Reductive carboxylate cycle (CO2 fixation)	Housekeeping gene
Matrix metalloproteinase 1 (interstitial collagenase)	MMP1	MMP1	NM_002421.2	P03956	Extracellular matrix	Interstitial collagenase, cleaves collagens (type I, II, III, VII and X) ; involved in the breakdown of extracellular matrix in normal physiological processes
Matrix metalloproteinase 10 (stromelysin 2)	MMP10	MMP10	NM_002425.1	P09238	Collagen catabolism	Involved in the breakdown of extracellular matrix in normal physiological processes
Matrix metalloproteinase 11 (stromelysin 3)	MMP11	MMP11	NM_005940.1	P24347	Extracellular matrix	Involved in physiologic and pathologic processes associated with extracellular matrix remodeling
Matrix metalloproteinase 12 (macrophage elastase)	MMP12	MMP12	NM_002426.1	P39900	Extracellular matrix	Degrades elastin,important in tissue remodeling and repair during development and inflammation
Matrix metalloproteinase 13 (collagenase 3)	MMP13	MMP13	NM_002427.1	P45452	Extracellular matrix	Involved in articular cartilage turnover and cartilage pathophysiology
Matrix metalloproteinase 14 (membrane-inserted)	MMP14	MMP14	NM_004995.2	P50281	Extracellular matrix	This activity may be involved in tumor invasion
Matrix metalloproteinase 15 (membrane-inserted)	MMP15	MMP15	NM_002428.1	P51511	Extracellular matrix	Activates progelatinase A
Matrix metalloproteinase 2 (gelatinase A)	MMP2	MMP2	NM_004530.1	P08253	Extracellular matrix	Encodes an enzyme which degrades type IV collagen, the major structural component of basement membranes
Matrix metalloproteinase 3 (stromelysin 1)	MMP3	MMP3	NM_002422.2	P08254	Extracellular matrix	Encodes an enzyme which degrades fibronectin, laminin, collagens III, IV, IX, and X, and cartilage proteoglycans
Matrix metalloproteinase 7 (matrilysin)	MMP7	MMP7	NM_002423.2	P09237	Extracellular matrix	Degrades proteoglycans, fibronectin, elastin and casein
MCM2 minichromosome maintenance deficient 2, mitotin (S. cerevisiae)	MCM2	MCM2	D21063.1	P49736	Cell cycle	Involved in the initiation of eukaryotic genome replication
Metallothionein 2A	MT2A	MT2A	V00594.1	P02795	Copper ion homeostasis	Metal ion binding
Methionine sulfoxide reductase A	MSRA	MSRA	AF183420.1	Q9UJ68	Oxidoreductase activity	Repair of oxidative damage to proteins to restore biological activity
Mevalonate kinase (mevalonic aciduria)	MVK	MVK	M88468.1	Q03426	Biosynthesis of steroids	Key intermediate in isoprenoid and sterol synthesis
Mitogen activated protein kinase 9	JNK2	MAPK9	U09759.1	P45984	MAPK signaling pathway / Wnt signaling pathway / Focal adhesion / Toll-like receptor signaling pathway / Insulin signaling pathway / Adipocytokine signaling pathway / Type II diabetes mellitus	This kinase blocks the ubiquitination of tumor suppressor p53
Mitogen activated protein kinase kinase 2	MEK2	MAP2K2	NM_030662.2	P36507	MAPK signaling pathway / Gap junction / Natural killer cell mediated cytotoxicity / Long-term potentiation / Long-term depression / Regulation of actin cytoskeleton / Insulin signaling pathway	Known to play a critical role in mitogen growth factor signal transduction
Mitogen-activated protein kinase 8	JNK1	MAPK8	L26318.1	P45983	MAPK signaling pathway / Wnt signaling pathway / Toll-like receptor signaling pathway / Insulin signaling pathway / Adipocytokine signaling pathway / Type II diabetes mellitus	Required for TNF-alpha induced apoptosis
Mitogen-activated protein kinase kinase 1	erk 1	MAP2K1	NM_002755.2	Q02750	MAPK signaling pathway / Dorso-ventral axis formation / Focal adhesion / Gap junction / Natural killer cell mediated cytotoxicity / Long-term potentiation / Regulation of actin cytoskeleton / Insulin signaling pathway	Involved in many cellular processes such as proliferation, differentiation, transcription regulation and development
Mitogen-activated protein kinase kinase 4	JNKK1	MAP2K4	NM_003010.1	P45985	MAPK signaling pathway / Toll-like receptor signaling pathway / Fc epsilon RI signaling pathway / Epithelial cell signaling in Helicobacter pylori infection	The roles of this kinase in mediating survival signal in T cell development, as well as in the organogenesis of liver.
MYC associated factor X	MAX	MAX	NM_002382.2	P28912	MAPK signaling pathway	Transcription factor; forms heterodimers with MYC, MAD and regulates gene expression
NCK adaptor protein1	NCK1	NCK1	NM_006153.2	P16333	Axon guidance / T cell receptor signaling pathway	An adaptor protein involved in transducing signals from receptor tyrosine kinases
Nerve growth factor receptor (TNFR superfamily, member 16)	NGFR	NGFR	M14764.1	P08138	Neurodegenerative Disorders / Cytokine-cytokine receptor interaction	Apoptosis
Neuregulin 1	NRG1	NRG1	M94165.1	Q02297	Neurogenesis	Plays a role in motor and sensory neuron development
Neurofibromin 1	NF1	NF1	NM_000267.1	P21359	MAPK signaling pathway	Negative regulator of the ras signal transduction pathway
Nuclear receptor subfamily1, group D, member 1	EAR1	NR1D1	NM_021724.1	P20393	Circadian rhythm	Possible receptor for triiodothyronine
Ornithine decarboxylase 1	ODC	ODC1	NM_002539.1	P11926	Urea cycle and metabolism of amino groups	Polyamine biosynthesis
P21/Cdc42/Rac1-activated kinase 1 (STE20 homolog, yeast)	PAK	PAK1	NM_002576.2	Q13153	MAPK signaling pathway / Axon guidance / Focal adhesion / Natural killer cell mediated cytotoxicity / T cell receptor signaling pathway / Regulation of actin cytoskeleton	Targets for the small GTP binding proteins Cdc42 and Rac; regulates cell motility and morphology

Gene name	Common Gene Symbol	Official Gene Symbol	Accession Number	Swissprot	General Function	Specific Function
PDZK1 interacting protein 1	MAP17	PDZK1IP1	U21049.1	Q13113	Oncogenesis	May play an important role in tumor biology
Peptidylprolyl isomerase E (cyclophilin E)	cyc	PPIE	AF042385.1	Q9UNP9	Protein folding	Housekeeping gene
Peroxiredoxin 6	AOP2	PRDX6	NM_004905.1	P30041	Phenylalanine metabolism / 2,4-Dichlorobenzoate degradation / Butanoate metabolism / Methane metabolism / Stilbene, coumarine and lignin biosynthesis / Alkaloid biosynthesis II	Involved in redox regulation of the cell ; may play a role in the regulation of phospholipid
Pitrilysin metallopeptidase 1	MP1	PITRM1	AF061243.1	Q95204	Metallopeptidase activity	Enzyme activator activity
Plasminogen activator tissue	TPA	PLAT	NM_000930.2	P00750	Complement and coagulation cascades	Serine protease that converts inactive plasminogen to plasmin
Plasminogen activator, Urokinase-receptor	uPAR	PLAUR	NM_002659.1	Q03405	Complement and coagulation cascades	Role in localizing and promoting plasmin formation
Plasminogen activator,Urokinase	PLAU	PLAU	NM_002658.1	P00749	Complement and coagulation cascades	Serine protease involved in degradation of the extracellular matrix and possibly tumor cell migration and proliferation
Polo-like kinase 1 (Drosophila)	PLK	PLK1	U01038.1	P53350	Cell cycle	May be required for cell division and may have a role during G1 or S phase
Poly (ADP-ribose) polymerase family, member 1	ADPR	PARP1	J03473.1	P09874	DNA repair / synthesis	Involved in the regulation of various important cellular processes such as differentiation, proliferation, and tumor transformation
Polymerase (DNA directed), alpha 2 (70kD subunit)	POLA2	POLA2	NM_002689.2	Q14181	Purine metabolism / Pyrimidine metabolism / DNA polymerase	May play an essential role at the early stage of chromosomal DNA replication
Proliferating cell nuclear antigen	PCNA	PCNA	NM_002592.1	P12004	Cell cycle	Involved in the RAD6-dependent DNA repair pathway
Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	COX1	PTGS1	NM_000962.2	P23219	Arachidonic acid metabolism	Cyclooxygenase
Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	COX2	PTGS2	NM_000963.1	P35354	Arachidonic acid metabolism	Key enzyme in prostaglandin biosynthesis
Proteasome (prosome, macropain) 26S subunit, non-ATPase, 1	PSMD1	PSMD1	NM_002807.1	Q99460	Proteasome	Cleaves peptides in an ATP/Ubiquitin-dependent process in a non-lysosomal pathway
Proteasome (prosome, macropain) 26S subunit, non-ATPase, 11	PSMD11	PSMD11	AB003102.1	Q00231	Protasome	An essential function of a modified proteasome, the immunoproteasome, is the processing of class I MHC peptides
Proteasome (prosome, macropain) 26S subunit, non-ATPase, 12	PSMD12	PSMD12	NM_002816.1	Q00232	Proteasome	Cleaves peptides in an ATP/Ubiquitin-dependent process in a non-lysosomal pathway
Proteasome (prosome, macropain) 26S subunit, non-ATPase, 6	PSMC6	PSMC6	NM_002806.1	Q92524	Proteasome	Cleaves peptides in an ATP/Ubiquitin-dependent process in a non-lysosomal pathway
Proteasome (prosome, macropain) subunit, alpha type, 2	PSMA2	PSMA2	NM_002787.1	P25787	Proteasome	Processing of class I MHC peptides
Proteasome (prosome, macropain) subunit, alpha type, 3	PSMA3	PSMA3	NM_002788.1	P25788	Proteasome	Processing of class I MHC peptides
Pyruvate kinase muscle	PKM2	PKM2	M26252.1	P14618	Glycolysis / Gluconeogenesis / Purine metabolism / Pyruvate metabolism / Carbon fixation / Insulin signaling pathway / Type II diabetes mellitus	Catalyzes the production of phosphoenolpyruvate from pyruvate and ATP
RAR-related orphan receptor A	RORA1	RORA	U04897.1	P35398	Signal transduction	Involved in organogenesis and differentiation
Related RAS viral (r-ras) oncogene homolog	RRAS	RRAS	NM_006270.2	P10301	MAPK signaling pathway / Dorso-ventral axis formation / Axon guidance / VEGF signaling pathway / Focal adhesion / Tight junction / Gap junction / Natural killer cell mediated cytotoxicity / T cell receptor signaling pathway / B cell receptor signaling pathway / Fc epsilon RI signaling pathway / Long-term potentiation / Long-term depression / Regulation of actin cytoskeleton / Insulin signaling pathway	
Ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease)	RET	RET	NM_000323.2	P07949	Protein-tyrosine kinase activity	Transduces signals for cell growth and differentiation
Retinoblastoma 1 (including osteosarcoma)	RB1	RB1	NM_000321.1	P06400	Cell cycle	Is an embryonic malignant neoplasm of retinal origin
Ribonucleotide-reductase M1 polypeptide	RRM1	RRM1	NM_001033.1	P23921	Purine metabolism / Pyrimidine metabolism	Ribonucleotide reductase essential for the production of deoxyribonucleotides before DNA synthesis in S phase
Ribosomal protein L13a	23kd	RPL13A	X56932.1	P40429	Ribosome	Housekeeping gene
Ribosomal protein L3	RPL3	RPL3	NM_000967.2	P39023	Ribosome	Catalyzes protein synthesis
Ribosomal protein S10	RPS10	RPS10	NM_001014.2	P46783	Ribosome	Catalyzes protein synthesis
Ribosomal protein S9	S9	RPS9	NM_001013.2	P46781	Ribosome	Housekeeping gene
S100 calcium binding protein A10	S100A10	S100A10	M81457.1	P08206	Calcium ion binding	involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation
S100 calcium binding protein A11 (calgizzarin)	S100A11	S100A11	D38583.1	P31949	Signal transduction	involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation
S100 calcium binding protein A7 (psoriasin 1)	PSOR1	S100A7	M86757.1	P31151	Epidermis development	involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation
S100 calcium binding protein A8 (calgranulin A)	S100A8	S100A8	NM_002964.2	P05109	Inflammatory response	Functions in the inhibition of casein kinase and as a cytokine ; involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation
Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	OPN	SPP1	NM_000582.1	P10451	Cell Communication / Focal adhesion / ECM-receptor interaction	Probably important to cell-matrix interaction
Secreted protein, acidic, cysteine-rich (osteonectin)	Oste	SPARC	NM_003118.1	P09486	Basement membrane	Inhibits cell-cycle progression, and influences the synthesis of extracellular matrix
Serine dehydratase	SDH	SDS	NM_006843.1	P20132	Glycine, serine and threonine metabolism / Cysteine metabolism	Housekeeping gene
Serpin peptidase inhibitor, clade B (ovalbumin), member 2	PAI2	SERPINB2	J02685.1	P05120	Plasminogen activator activity	Inhibits urokinase-type plasminogen activator
Serpin peptidase inhibitor, clade E, member 1	PAI1	SERPINE1	M14083.1	P05121	Complement and coagulation cascades	Acts as "bait" for tissue plasminogen activator, urokinase, and protein C
SHC (Src homology 2 domain containing) transforming protein 1	SHC	SHC1	U73377.1	P29353	Focal adhesion / Natural killer cell mediated cytotoxicity / Insulin signaling pathway	Couples activated growth factor receptors to signaling pathway

Gene name	Common Gene Symbol	Official Gene Symbol	Accession Number	Swissprot	General Function	Specific Function
Signal transducer and activator of transcription 5A	STAT5A	STAT5A	L41142.1	P42229	Jak-STAT signaling pathway	Member of the STAT protein family
SMAD, mothers against DPP homolog 1 (Drosophila)	SMAD1	SMAD1	U59423.1	Q15797	TGF-beta signaling pathway	Mediates the signals of the bone morphogenetic proteins (BMPs), which are involved in a range of biological activities including cell growth, apoptosis, morphogenesis, development and immune responses
Small proline-rich protein 1B (cornifin)	SPRR1B	SPRR1B	NM_003125.1	P22528	Keratinization	Keratinocyte protein
Sorcin	SRI	SRI	NM_003130.1	P30626	Signal transduction	calcium channel regulator activity
STE20-like kinase (yeast)	SLK	SLK	NM_014720.1	Q9H2G2	DNA repair / synthesis	protein serine/threonine kinase activity
Stomatin (EPB72)-like 2	SLP2	STOML2	AF282596	O60376	Receptor binding	/
Superoxide dismutase 2, mitochondrial	SoD	SOD2	NM_000636.1	P04179	Oxidative metabolism	Member of the iron/manganese superoxide dismutase family
Synuclein, gamma (breast cancer-specific protein 1)	SNCG	SNCG	NM_003087.1	O76070	Signal transduction	Plays a role in neurofilament network integrity
Telomerase RNA component	TERC	TERC	U86046.1	/	Telomere maintenance	ribonucleoprotein polymerase
Telomerase reverse transcriptase	TERT	TERT	AF018167.1	O14746	Telomere maintenance	Plays a role in cellular senescence
Telomeric repeat binding factor (NIMA-interacting) 1	TRF1	TERF1	U40705.1	P54274	Chromosomal processing	Functions as an inhibitor of telomerase
Thrombospondin 1	TSP1	THBS1	NM_003246.1	P07996	Cell Communication / TGF-beta signaling pathway / Focal adhesion / ECM-receptor interaction	Adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions
Thromboxane-A2-receptor	TBXA2R	TBXA2R	D38081.1	P21731	Calcium signaling pathway / Neuroactive ligand-receptor interaction	Stimulator of platelet aggregation
Thymidine kinase 1, soluble	TK1	TK1	NM_003258.1	P04183	Pyrimidine metabolism	Generates thymidylate for DNA synthesis
Thymidylate synthetase	TYMS	TYMS	NM_001071.1	P04818	Pyrimidine metabolism / One carbon pool by folate	Cofactor to maintain the dTMP (thymidine-5-prime monophosphate) pool critical for DNA replication and repair
TIMP metallopeptidase inhibitor 1	TIMP1	TIMP1	NM_003254.1	P01033	Extracellular matrix	Natural inhibitors of the matrix metalloproteinases, a group of peptidases involved in degradation of the extracellular matrix
TIMP metallopeptidase inhibitor 2	TIMP2	TIMP2	NM_003255.2	P16035	Extracellular matrix	Natural inhibitors of the matrix metalloproteinases, a group of peptidases involved in degradation of the extracellular matrix
Topoisomerase (DNA) II alpha 170kDa	TOP2	TOP2A	NM_001067.2	P11388	DNA repair / synthesis	Controls and alters the topologic states of DNA during transcription ; involved in processes such as chromosome condensation, chromatid separation
Transcription factor AP-2 alpha	TFAP2A	TFAP2A	M36711.1	P05549	Transcription factor activity	Interacts with inducible viral and cellular enhancer elements to regulate transcription of selected genes
Transcription factor AP-2 beta	TFAP2B	TFAP2B	X95594.1	Q92481	Transcription factor activity	Interacts with inducible viral and cellular enhancer elements to regulate transcription of selected genes
Transcription factor AP-2 gamma	TFAP2C	TFAP2C	NM_003222.2	Q92754	Transcription factor activity	Interacts with inducible viral and cellular enhancer elements to regulate transcription of selected genes
Transferrin receptor (p90, CD71)	TFR	TFRC	NM_003234.1	P02786	Hematopoietic cell lineage	Housekeeping gene
Transforming growth factor, alpha	TGFA	TGFA	NM_003236.1	P01135	Cell proliferation	biologically active polypeptides
Transforming growth factor, beta 1 (Camurati-Engelmann disease)	TGFB1	TGFB1	NM_000660.1	P01137	MAPK signaling pathway / Cytokine-cytokine receptor interaction / Cell cycle / TGF-beta signaling pathway	Controls proliferation, differentiation, and other functions in many cell types
Transforming growth factor, beta receptor II (70/80kDa)	TGFBR II	TGFBR2	D50683.1	P37173	MAPK signaling pathway / Cytokine-cytokine receptor interaction / TGF-beta signaling pathway / Adherens junction	Regulates the transcription of a subset of genes related to cell proliferation
Transgelin	SM22	TAGLN	M95787.1	Q01995	Cell structure	Involved in calcium interactions and contractile properties of the cell
Transglutaminase 1 (K polypeptide epidermal type 1, protein-glutamine-gamma-glutamyltransferase)	TGM1	TGM1	NM_000359.1	P22735	Keratinization	Catalyzes the cross-linking of proteins and the conjugation of polyamines to proteins
Transmembrane 4 L six family member 1	L6	TM4SF1	M90657.1	P30408	/	play a role in the regulation of cell development, activation, growth and motility
Tumor necrosis factor (TNF superfamily, member 2)	TNFa	TNF	NM_000594.1	P01375	MAPK signaling pathway / Cytokine-cytokine receptor interaction / Apoptosis / TGF-beta signaling pathway / Toll-like receptor signaling pathway / Hematopoietic cell lineage / Natural killer cell mediated cytotoxicity / T cell receptor signaling pathway / Adipocytokine signaling pathway / Type II diabetes mellitus / Type I diabetes mellitus	Functions through its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR
Tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	OPG	TNFRSF11B	NM_002546.2	O00300	Cytokine-cytokine receptor interaction	Functions as a negative regulator of bone resorption
Tumor necrosis factor receptor superfamily, member 1A	TNFRSF1A	TNFRSF1A	NM_001065.2	P19438	MAPK signaling pathway / Cytokine-cytokine receptor interaction / Apoptosis / Adipocytokine signaling pathway	This receptor can activate NF-kappaB, mediate apoptosis, and function as a regulator of inflammation
Tumor necrosis factor receptor superfamily, member 1B	TNFRSF1B	TNFRSF1B	NM_001066.1	P20333	Cytokine-cytokine receptor interaction / Adipocytokine signaling pathway	Mediates the recruitment of two anti-apoptotic proteins, c-IAP1 and c-IAP2
Tumor protein p53 (Li-Fraumeni syndrome)	p53	TP53	AF307851.1	P04637	MAPK signaling pathway / Cell cycle / Apoptosis / Wnt signaling pathway / Amyotrophic lateral sclerosis (ALS) / Huntington's disease	Plays an essential role in the regulation of cell cycle
Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	PLA2	YWHAZ	M86400.1	P29312	Cell cycle	Housekeeping gene
Tyrosine hydroxylase	TH	TH	NM_000360.1	P07101	Tyrosine metabolism / Parkinson's disease	involved in the conversion of tyrosine to dopamine
Ubiquitin-conjugating enzyme E2C	UBE2C	UBE2C	NM_007019.1	O00762	Ubiquitin mediated proteolysis	Required for the destruction of mitotic cyclins and for cell cycle progression
V-fos FBJ murine osteosarcoma viral oncogene homolog	c-fos	FOS	NM_005252.2	P01100	MAPK signaling pathway / Toll-like receptor signaling pathway / B cell receptor signaling pathway	Regulators of cell proliferation, differentiation, and transformation
V-myb myeloblastosis viral oncogene homolog (avian)-like 2	MYBL2	MYBL2	X13293.1	P10244	Transcription factor activity	Nuclear protein involved in cell cycle progression
V-raf-1 murine leukemia viral oncogene homolog 1	RAF1	RAF1	X03484.1	P04049	MAPK signaling pathway / Dorso-ventral axis formation / Focal adhesion / Gap junction / Natural killer cell mediated cytotoxicity / Long-term potentiation / Long-term depression / Regulation of actin cytoskeleton / Insulin signaling pathway	MAP kinase kinase kinase (MAP3K) which functions downstream of the Ras family