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Mar. Drugs **2015**, *13*, 5993-6018; doi:10.3390/md13095993

OPEN ACCESS

marine drugs

ISSN 1660-3397

www.mdpi.com/journal/marinedrugs

Review

Diatom-Specific Oligosaccharide and Polysaccharide Structures Help to Unravel Biosynthetic Capabilities in Diatoms

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Academic Editor: Véronique Martin-Jézéquel

Received: 29 June 2015 / Accepted: 11 September 2015 / Published: 18 September 2015

Abstract: Diatoms are marine organisms that represent one of the most important sources of biomass in the ocean, accounting for about 40% of marine primary production, and in the biosphere, contributing up to 20% of global CO_2 fixation. There has been a recent surge in developing the use of diatoms as a source of bioactive compounds in the food and cosmetic industries. In addition, the potential of diatoms such as *Phaeodactylum tricornutum* as cell factories for the production of biopharmaceuticals is currently under evaluation. These biotechnological applications require a comprehensive understanding of the sugar biosynthesis pathways that operate in diatoms. Here, we review diatom glycan and polysaccharide structures, thus revealing their sugar biosynthesis capabilities.

Keywords: microalgae; glycan; polysaccharide; diatom; exopolysaccharides; EPS; nucleotide sugars

1. Introduction

Among ocean phytoplankton, diatoms are highly diverse with an estimated 10^5 to 10^7 species [1]. Marine diatoms make up an important group: they contribute to approximately 40% of primary productivity in marine ecosystems and 20% of global carbon fixation [2–5]. Diatoms also participate in the ocean silica cycle [6–9], iron cycle [8,10–12] and nitrogen cycle [13–16]. Due to their high diversity and very specific metabolism, diatoms have been used as bio-indicators and filters for controlling and purifying contaminated water [17–20]. For example, some diatoms such as *Cylindrotheca fusiformis*, *Cyclotella cryptica*, *Phaeodactylum tricornutum*, *Skeletonema costatum,* and *Thalassiosira pseudonana* have been used to absorb high quantities of heavy metals [21–23]. Diatoms are also used in nanotechnology to produce living nano-scale structures because they can build a silica shell at room temperature from a very small amount of silica dissolved in water [24–27]. In parallel, diatoms have been explored as sources of bioactive metabolites. Such compounds have many uses in the food industry. For example, diatoms have long been used as feedstock in aquaculture [28] and more recently in human health and food supplements (Figure 1).

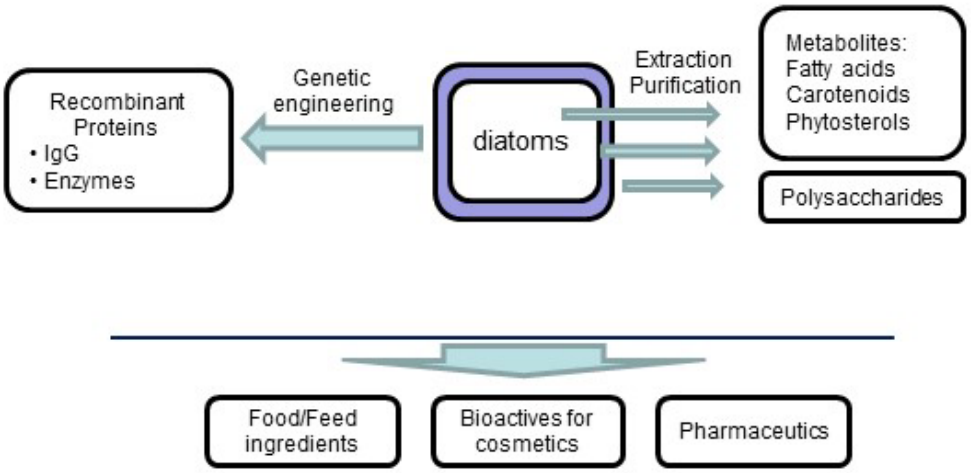

Figure 1. Applications of diatom active compounds in human health and food supplements.

Given their ability to produce carotenoids, phytosterols, vitamins, and antioxidants, diatoms have become valuable sources of food supplements for humans [29]. Moreover, they can synthesize large amounts of polyunsaturated fatty acids, which are bioactive substances proven to promote human health (e.g., decrease in frequency of cardiovascular diseases and cancers) and growth in animals [19,30,31]. Additionally, the major carotenoid of diatoms, the brown-colored fucoxanthin, is used as an antioxidant, anti-inflammatory, anti-diabetes and anti-cancer drug [32,33], as well as for its protective effect on liver, eyes, blood vessels, skin, and lungs [32,34]. Anti-inflammatory and immunostimulating activities of diatoms polysaccharides such as laminarin have also been reported as effective in various fish species [35–37]. Additionally, other polysaccharides such as chrysolaminarin from the diatom

Chaetoceros muelleri have been shown to be promising candidates as immuno-stimulatory food additives in aquaculture [38]. Chrysolaminarin isolated from the diatom *Synedra acus* shows anti-tumor activity by inhibiting the proliferation of human colon cancer cells and colony formation [39]. Recently, polysaccharides from algae (including diatoms) have attracted interest in the cosmetic industry: some sulfated polysaccharides have already been tested to prevent the accumulation and the activity of free radicals and reactive chemical species, therefore acting as protective systems against oxidative stress [40]. The use of diatoms is thus likely to expand in the future. Additionally, the diatom *P. tricornutum* has recently been evaluated as a potential solar-fueled expression system to produce bioplastics [41] and biopharmaceuticals. In the biopharmaceutical field, diatoms have successfully been used to produce functional monoclonal human IgG antibodies directed against the hepatitis B virus surface antigen [42,43]. Understanding post-translational modifications (including glycosylation processing) in diatoms is fundamental, because they determine the critical quality attributes that can influence folding, half-life, activity, and immunogenicity of biopharmaceuticals [44,45].

Glycoconjugates, such as glycans and polysaccharides, are assembled and modified within the endomembrane system [46]. Their synthesis involves three steps, the first being the formation of activated nucleotide sugars, such as NDP-sugars or NMP-sugars within the cytosol [47]. Then, the nucleotide sugars are actively transported to the endoplasmic reticulum (ER) and Golgi apparatus where they serve as donor substrates for glycosyltransferases (GT) that transfer a specific sugar from its activated nucleotide form to a specific acceptor leading to the extension of the glycoconjugates.

In this review, in regard to the capability of diatoms to synthesize glycoconjugates, we focus on the composition, structure and properties of diatom polysaccharides—whether they be intracellular, cell wall-bound or secreted in the culture medium—and on the structure and biosynthesis of *N*-glycans attached to proteins.

2. Monosaccharide Composition and Structures of Polysaccharides in Diatoms

Given the thousands of diatom species, with their large variety of forms, symmetry and cell wall shapes, the monosaccharide composition and structure of diatom glycoconjugates are likely to be highly specific. Diatoms are usually described as single cells with a protoplast embedded in a frustule—the name for the diatom cell wall—composed of two overlapping valves or thecae: the larger, upper epitheca and the smaller, lower hypotheca (Figure 2A). The frustule is composed of three successive layers: (1) the inner-most, organic layer, called the diatotepum, is in contact with the plasmalemma; (2) a mineral, silicified shell that contains organic matter; and finally (3) an external organic coat that is trapped in secreted mucilage, that we call here "cell wall-bound exopolysaccharides (EPSs)" (Figure 2B). Numerous studies have characterized the monosaccharide composition of cell wall polysaccharides, intracellular food storage polymers, and extracellular mucilage, but results must be carefully interpreted according to the extraction techniques used, which depend on the solubility of the respective components [48–53].

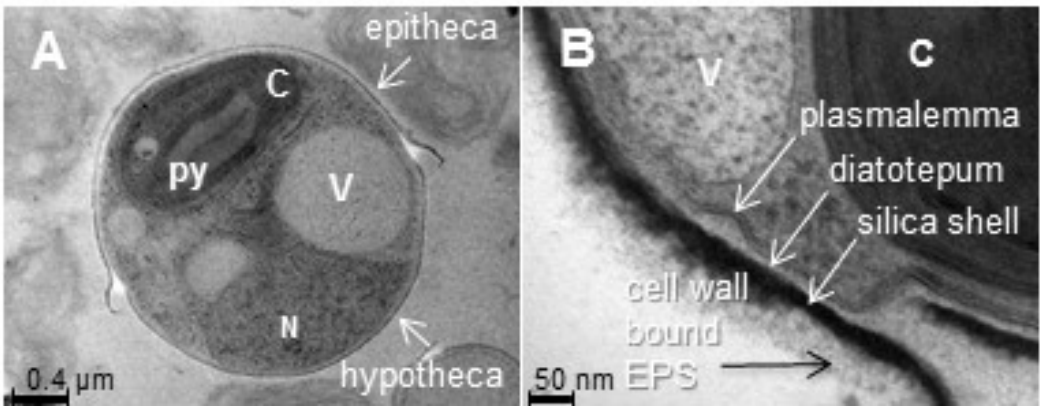

Figure 2. Ultrastructural organization of a diatom cell: transmission electron micrographs of *Phaeodactylum tricornutum* oval morphotype. The cells were embedded in LRW resin with 0.5% uranyl acetate in a methanol/Reynold's lead citrate solution. (**A**) general overview of a *P. tricornutum* oval cell. Scale bar = 0.4 µm; (**B**) zoom of the cell wall. Scale bar = 50 nm. N: nucleus; V: vacuole; C: chloroplast; py: pyrenoid; EPS: exopolysaccharides.

2.1. Insoluble Polysaccharides in Diatoms

2.1.1. Frustules, or Cell Wall Polysaccharides

In diatoms, cell wall silica is associated with organic matter, composed mainly of proteins [54–56], polyamines [57], and polysaccharides [58]. Three families of proteins have been isolated from *C. fusiformis* cell walls: frustulins, pleuralins, and silaffins (see reference [59] for a review). Long polyamine chains, together with silaffins, are likely involved in frustule biosynthesis. However, the location and role of each component involved in frustule biosynthesis are not well-understood. Experimental studies on the chemical composition of the organic matter in diatom cell walls have demonstrated that polysaccharide content dominates that of proteins and lipids [60], although X-ray photoelectron spectroscopy measurements of cell surface components in *T. pseudonana* appear to show that polysaccharides are not predominant [61]. To characterize and localize these polysaccharides, accurate sequential extraction is necessary for at least three reasons. First, depending on their composition and structure, polysaccharides have various water-solubility properties, which in turn vary with temperature and chemical treatments. Second, cell wall polysaccharides may be more or less tightly bound either together, to silica, or to other insoluble components of the frustule. Third, a secreted or an extracted substance is not necessarily water-soluble once outside the cell; thus an insoluble extracted polysaccharide is not necessarily a cell wall component.

Based on typical sequential extractions performed either on live or mechanically disrupted cells, we reviewed the monosaccharide composition reported in the three final fractions: (1) hot alkali soluble fraction; (2) hot alkali insoluble fraction; and (3) residual material that makes up the insoluble organic cell wall fraction (see Table 1). Different monosaccharide profiles have been observed in alkali soluble fractions: fucose dominates in *Thalassiosira gravida* and *Corethron hystrix*; rhamnose is mostly encountered in *Chaetoceros affinis* [62], galactose in *Thalassiosira weissflogii* [63] and mannose in *P. tricornutum*. Fractions with high levels of ribose were attributed to the cross-contamination of the extracts from intracellular content [50,62]. In the alkali insoluble fraction, the monosaccharide

composition profile is not that different from that found in insoluble organic cell walls in which mannose is preponderant. Insoluble organic cell walls, as summarized over 19 taxa in Table 1, and first shown by Coombs and Volcani [64], and reviewed by Hoagland *et al.* [48], contain fucose, galactose, glucose, mannose, xylose, glucuronic acid residues and, to a much lesser extent, rhamnose and arabinose. Although mannose is obviously the most abundant monosaccharide, fucose is dominant in *Nitzschia brevirostris*, whereas glucose predominates in *Melosira granulata* and *Cyclotella stelligera* [55], *Nitzschia curvilineata* and *Amphora salina* [53]. It is difficult to determine the abundance of other monosaccharide components due to their heterogeneous representation.

The best studied frustule polysaccharides have been extracted from *P. tricornutum*. Based on successive alkali extraction, deproteination, chromatographic separation, Percival and co-workers extracted a cell wall polysaccharide from *P. tricornutum* [65] that is mainly composed of mannose, glucuronic acid residues and sulfate groups. Mild acid hydrolysis of the polysaccharides combined with chemical analysis of the oligosaccharide fragments revealed moieties that should be present in the overall polysaccharide structure. Blocks of 3-linked mannose have been identified and are assumed to form the backbone of the polysaccharides (Figure 3A). Substitution at position 2 of the mannose of the main chain with di- and trisaccharides composed of mannose and glucuronic acid (Figure 3B), or with sulfate groups have also been described in *Pinnularia viridis* [52] as well as in *P. tricornutum* [66]. However, the detailed configuration of the linkage between residues, as well as the size and distribution of the ramification are still unknown. The cell wall monosaccharide composition of several diatom species includes high amounts of mannose and glucuronic acid and low, but more variable amounts of fucose and xylose. According to these results, the glucuronomannan described in *P. tricornutum* may be synthesized more generally by other diatoms [52,66,67]. Environmental conditions have been shown to influence the monosaccharide composition of cell wall polysaccharides, thus affecting their respective structures as illustrated in *P. tricornutum* [66]. Variations in culture conditions, such as phosphate limitation in the culture medium, an increase in salinity, switching culture from liquid to solid medium—all considered as stress conditions—may cause the observed enrichment in rhamnose, uronic acid, sulfate, and *O*-methylated sugars in the insoluble polymeric fraction. Such variation in monosaccharide composition is assumed to modify polysaccharide structures, enabling cells to adapt to environmental changes. Therefore, the effects of culture conditions on the monosaccharide composition of glycoconjugates must be considered when comparing experimental results.

Table 1. Summary of the monosaccharide composition of diatom extracts: alkali soluble fraction, alkali insoluble fraction, and insoluble organic cell wall residues. Values are expressed in mol% of total monosaccharides detected in extracts. Horizontal sums of values lower than 100% indicate that some monosaccharides were not clearly identified in the corresponding study.

Monosaccharide		Ara	Fuc	Gal	Glc	Man	Rha	Rib	Xyl	3-O-MeFuc	2-MeGal	3/4-MeGal	GalA	GlcA	2-MeGlcA	ManA	2-MeRha	3-MeRha	2,3-diMeRha	3-MeXyl	4-MeXyl	Unknown	GlcNac
[a] *Chaeotoceros affinis*			11	18	2	6	52	—	7														
[a] *Chaeotoceros curvisetus*			32	31	1	6	16	10	4														
[a] *Chaeotoceros decipiens*			4	7	6	32	25	4	10														
[a] *Chaeotoceros debilis*			11	23	6	—	22	23	15														
[a] *Chaeotoceros socialis*			18	16	3	9	23	11	8														
[a] *Thalassiosira gravida*			43	12	4	7	—	27	7														
[a] *Corethron hystrix*	Alkali soluble		60	9	11	9	1	8	2														
[b] *Thalassiosira weissflogii*	fraction	1.94	6.98	36	19.5	17.9	4	3.91	7.31														
[c] *Phaeodactylum tricornutum*		0.3	2.3	1.7	2.4	55.6	11.7	—	5.4	nd	tr	—	1.9	6.7		12	nd	nd	—	nd	nd		
[d] *Phaeodactylum tricornutum* **O**		7	3	8	18	28	9	1	8			tr					tr			tr			
[d] *Phaeodactylum tricornutum* **F**			2	6	11	45	7		8			2		10									2
[e] *Stauroneis amphioxys*		—	1	9	7	50	2		2	2			nd	28	nd		nd	2	nd	—	—		
[d] *Phaeodactylum tricornutum* **O**	Alkali insoluble fraction		1	5	12	72	1	tr	1														
[d] *Phaeodactylum tricornutum* **F**		4	1	12	10	66	2	tr	4			1											
[f] *Nitzschia frustulum*		—	14	14	tr	32	5	—	tr													35	
[f] *Nitzschia angularis*		—	64	4	14	11	tr	—	tr													7	
[f] *Asterionella socialis*	Insoluble organic	—	tr	5	tr	22	tr	—	tr													78	
[f] *Cylindrotheca fusiformis*	cell walls	—	tr	10	tr	12	tr	—	tr													78	
[g] *Navicula pelliculosa*		—	9.2	9.9	25.9	48.5	3.8	—	3.1														
[g] *Melosira nummuloides*		0.3	25.6	3.7	0.9	56.8	1.8	—	10.9														

Table 1. *Cont.*

[g] ***Melosira granulata***	Insoluble organic cell walls	0.6	0.8	5.4	46.7	6.5	1.2	—	38.9										
[g] ***Cyclotella stelligera***		2	0.6	22.4	43.3	13.6	0	—	18										
[g] ***Cyclotella cryptica***		—	12.2	12.2	13	37.2	7.4	—	17.8										
[g] ***Nitzschia brevirostris Hust.***		1.7	42.5	9.5	14.6	20.2	2.4	4.7	4.3										
[h] ***Pinnularia viridis***		tr	1.5	7	13	54	9.5		11			—	—	—	2	1	1	—	—
[h] ***Craspedostauros australis***		tr	tr	2	5	69	2		4			2	2	12	—	—	—	2	—
[h] ***Thalassiosira pseudonana***		1	2	10	6	65	tr		5			—	10	—	—	tr	—	—	1
[h] ***Nitzschia navis-varingica***		2	1	3	7	64	2		1			tr	15	—	—	5	—	—	—
[i] ***Coscinodiscus radiatus***				tr	4.7	80.1	1.5		1.4			tr	12.4						
[i] ***Nitzschia curvilineata***				6.5	45.6	40.3	1.7		4.4			—	1.4						
[i] ***Amphora salina***				2.7	47.7	41.5	1.5		1.3			tr	5.5						
[i] ***Triceratium dubium***				tr	6.4	67.4	tr		6.3			5.6	1.5						12.8
[d] ***Phaeodactylum tricornutum* O**			3	3	11	61	12		2	4									
[d] ***Phaeodactylum tricornutum* F**		3	1	10	25	47	3	tr	6	2									

tr, trace (<0.8% mol); —, not detected; nd, not determined. Bright yellow: major compound, green: second-most major compound. [a] [62]; [b] [63]; [c] [66]; [d] [67]; [e] [68]; [f] [69]; [g] [55]; [h] [52]; [i] [53]. Morphotypes of *Phaeodactylum tricornutum*: F, fusiform; O, oval. Ara, arabinose; Fuc, fucose; Gal, galactose; GalA, galacturonic acid; Glc, glucose; GlcA, glucuronic acid; GlcNAc, *N*-acetylglucosamine; Man, mannose; ManA, mannuronic acid; Me, methyl group; Rha, rhamnose; Rib, ribose; Xyl, xylose.

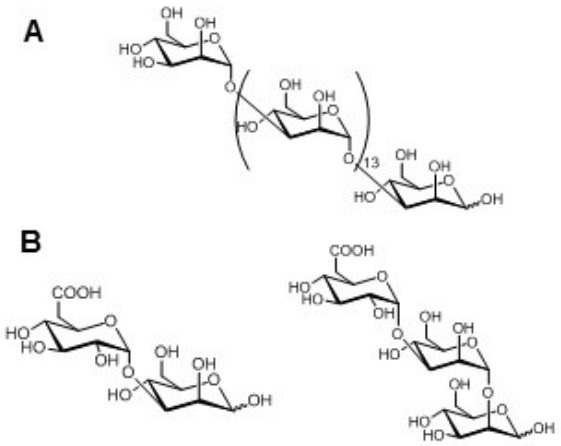

Figure 3. Drawings of hypothetical structures of oligosaccharides found in insoluble cell wall polysaccharides after mild acid hydrolysis of *Phaeodactylum tricornutum* cell wall extracts. (**A**) 1,3-linked mannopyranose chains; (**B**) oligosaccharide fragments. Although hypothetical alpha-linkages are shown here, there is no clear evidence for either alpha- or beta-linkages [65].

2.1.2. Chitinous Spines

Cyclotella and *Thalassiosira* species produce stiff and highly crystalline fibers of chitin (poly-*N*-acetyl-D-glucosamine), as demonstrated using chemical, crystallographic, and enzymatic methods [70–72]. Due to high crystallinity (chitin is probably the most crystalline polysaccharide material on earth); the crystal structure of chitin fibers has been resolved at the molecular level [73,74]. Diatoms secrete β-chitin (Figure 4), which has a crystalline structure similar to that described in worms [75–77], but different from that of arthropods, crustaceans, and fungi, which all synthesize α-chitin. α-Chitin shows anti-parallel chain packing, whereas β-chitin polymer chains show parallel packing, meaning that reducing ends all point out in the same direction. In diatoms, chitin fibers are excreted through specialized pores within the thecae called fultoportulae. Cross-sections examined under transmission electron microscopy show invaginations of the plasma membrane at the site of chitin polymerization [78–80]. Similar secretion systems have been reported for the giant tube worm *Riftia pachyptila* [81,82]. Crystallographic analyses of chitin fibers bound to thecae demonstrate that chitin polymerization occurs by elongation at the non-reducing end, consistent with the reducing chain end being the furthest from the biosynthesis site [83,84].

Genes encoding chitin synthase were discovered in the *T. pseudonana* genome. Homologous genes, but no chitin fibers, have been described in *Skeletonema costatum*, *Chaetoceros socialis*, *Lithodesmium undulatum* and *P. tricornutum*, suggesting a common origin of chitin synthase in diatoms, but also indicating potential occurrence of yet undescribed chitin [85]. Chitin occurs in the silica frustule of *T. pseudonana* [86] and is probably an underestimated component of diatom cell walls in general. Inhibition of chitin synthase or chitin crystallization mainly increases the sedimentation rate of diatoms. This effect suggests that chitin fibers are involved in the buoyancy of the dense siliceous diatom cells. The importance of chitin fibers in diatoms has also been highlighted: the chitin content accounts for an estimated 30% of the organic carbon pool in *Cyclotella* species [87].

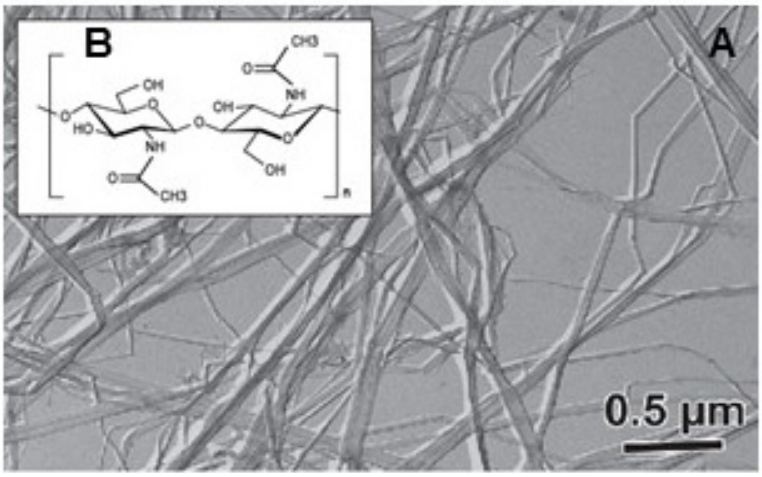

Figure 4. β-chitin fibers of *Thalassiosira* sp. (**A**) Transmission electron micrograph shadowed with tanta-lum/tungsten (Ta/W). (**B**): *N*-acetylglucosamine sequence of the chemical structure of chitin. The image was recorded and kindly provided by Dr. H. Chanzy, CERMAV-CNRS, France.

2.2. Soluble Polysaccharides in Diatoms

2.2.1. Food Storage Polysaccharides

The food storage polysaccharide in diatoms is a β(1,3) glucan, also called chrysolaminarin because it resembles the β(1,3) glucan found in chrysophyte algae [88]. This particular polysaccharide has been localized in the vacuole using aniline blue dye [89] and anti-β(1,3) glucan antibodies [51]. Vacuolar accumulation is enhanced during photosynthesis and is mobilized in the dark. β(1,3) glucan content can reach up to 20%–30% of dry matter during the exponential growth phase of the diatom [90] and up to 80% during the stationary phase.

Treating diatom cells with hot or boiling water is often sufficient to extract chrysolaminarin as the main polysaccharide component. Mild acid hydrolysis and freeze-drying also help cell wall disruption. Chrysolaminarin is insoluble in organic solvents and can be easily recovered by precipitation in alcohol or acetone [91–93]. The structure of diatom β(1,3) glucans was first described from a mixed bloom of freshwater diatom species that included *Nitzschia sigmoidea*, *Cymatopleura solea*, *Pinnularia* sp. and *Melosira varians* [91]. Since then, chrysolaminarin structures from several diatom species (*Skeletonema*, *Phaeodactylum*, *Chaetoceros*, *Thalassiosira*) have been studied using chemical analysis and spectroscopic methods such as NMR (Figure 5). The NMR spectra given in Figure 5 show high degrees of similarities between the β(1,3) glucan extracted from *Saccharina latissima* (Figure 5A) and from *P. tricornutum* (Figure 5B) , with the exception of fewer β(1,6) branching signals for chrysolamaninarin and a slightly higher reducing-end signal. The *P. tricornutum* β(1,3) glucan may have lower molecular weight or lack a mannitol residue at the reducing end.

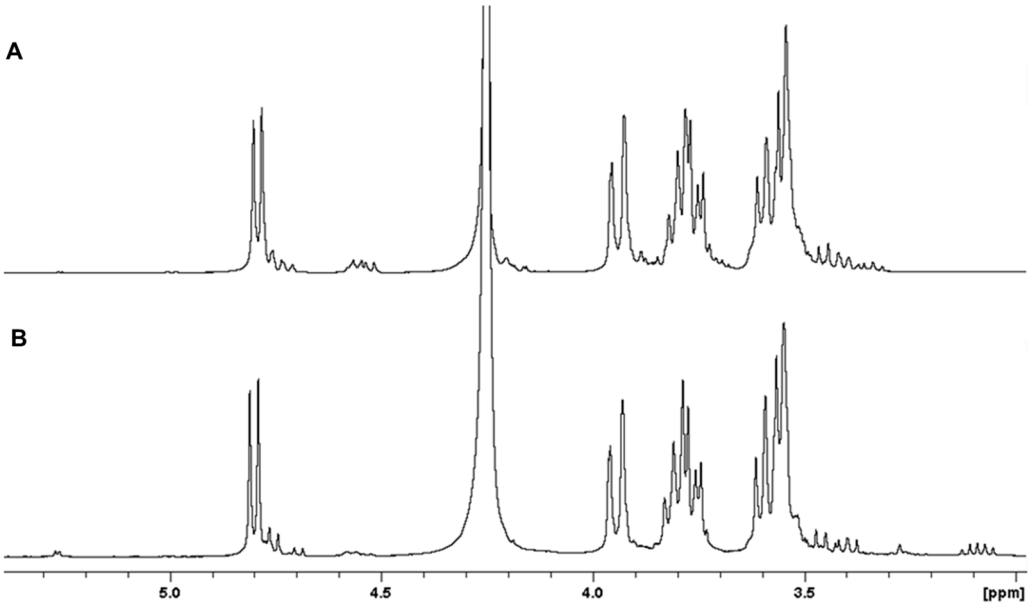

Figure 5. Structural analysis of β(1,3) glucan, a food storage polysaccharide. ^{1}H NMR spectra of (**A**) laminarin from *Saccharina latissima* and (**B**) β(1,3) glucan (chrysolaminarin) extracted from *Phaeodactylum tricornutum* (400 MHz, 353 K). Chrysolaminarin contains fewer β(1,6) branching signals (4.5–4.6 ppm) than laminarin. The slightly higher reducing end signal at 5.26 ppm (α-anomer) in the chrysolaminarin spectrum can be attributed to a lower molecular weight or the absence of a mannitol residue at the reducing end.

Based on the numerous studies, a general picture of diatom chrysolaminarin structure has emerged. It is usually composed of a β(1,3) glucan backbone chain ramified with β(1,6) glucose and sometimes with β(1,2) glucose. The length of the backbone chain and the degree of ramification vary with the diatom species (Table 2). During the growth phase of *T. weissflogii* and *C. muelleri*, the structure of chrysolaminarin does not change noticeably, suggesting that culture conditions do not influence the chrysolaminarin structure [94].

Table 2. Overview of the structural features of diatom chrysolaminarins. For comparison, *Laminaria digitata* laminarin has a degree of polymerization (DP) of 20–30 residues and a degree of branching (DB) of 0.05, [95]. Yield extraction of chrysolaminarin is expressed in % of diatom dry weight.

Species	Mw/DP	Branching	Yield% (*w*/*w*)	Reference
Phaeodactylum tricornutum	nd	Some β(1,6) branching	14%	[92]
Skeletonema costatum	6–13 kDa	Some β(1,6) and β(1,2) branching	32%	[96]
Stauroneis amphioxys	4 kDa, DP ~24	Some β(1,6) and β(1,2) branching	nd	[97]
Achnanthes longipes	nd	Small degree of β(1,6) and β(1,2) branching	nd	[98]
Pinnularia viridis	>10 kDa	Small degree of β(1,6) branching	nd	[99]

Table 2. *Cont.*

Aulacoseira baicalensis	3–5 kDa	nd	0.9%	[93]
Stephanodiscus meyerii	40 kDa	β(1,6)/β(1,3) DB 0.053 [a]	0.5%	
Stephanodiscus meyerii	2–6 kDa	β(1,6)/β(1,3) DB 0.25 [a]	0.4%	
Aulacoseira baicalensis	nd	β(1,6)/β(1,3) DB 0.11 [a] Mannitol detected	0.6%	
Chaetoceros muelleri	DP 22–24	β(1,6)/β(1,3) DB 0.006–0.009	nd	[94]
Thalassiosira weissflogii	DP 5–13	No branching	nd	
Chaetoceros debilis	4.9 kDa, DP 30	β(1,6) 37% of total residue	10%	[100]

nd: not determined; [a] calculated based on published data.

Based on the biological activity of β(1,3) glucans (see Section 1), laminarin is currently marketed for its ability to stimulate macrophages leading to immuno-stimulatory, anti-tumor and wound-healing activities [101].

2.2.2. Exopolysaccharides

Diatoms synthesize extracellular mucilage, which mainly consists of complex heteroglycans. Although EPS usually refers to exopolysaccharides, in its broad sense it includes all extracellular polymeric substances, which have high carbohydrate contents [99,102,103], and can even be used to mean any macromolecule secreted from the plasmalemma (see review by Hoagland *et al.*, reference [48]). EPSs have been described in many forms, such as stalks, tubes, apical pads, adhering films, fibrils, and cell coatings, which imply that EPS components have a wide variety of morphologies, ranging from highly crystalline rigid fibrils to highly hydrated mucilaginous capsules, and including polymers that are tightly bound to or integrated in the cell wall. In this section, we focus only on soluble EPSs.

Excretion of EPSs by diatoms provides a food source for heterotrophic organisms and affects the erodibility of biofilms [102–104]. EPS production rates and their monosaccharide compositions differ according to the growth phase and the physiological status of the cells [68,103–105]. EPS secretion depends on environmental conditions such as nutrient availability, daily fluctuations, irradiance, and even metal toxicity [106–110]. Studies have shown that nitrogen (*N*) and phosphate (*P*) limitations affect the production rate of EPSs as well as their monosaccharide compositions. For example, *N* or *P* limitation have been shown to stimulate EPS production in various diatoms [66,108,110]. Under *P*-limited conditions in *C. fusiformis*, monosaccharide composition shows an increase in galactose and a decrease in glucose, whereas the composition of the remaining monosaccharides is almost unaffected [108]. EPS production also increases under *P*-depleted conditions in other diatom species [109] with reduced glucose content in *Cylindrotheca closterium*. Likewise, fucose and rhamnose appear to be involved in adhesion, either by enrichment in some biofilm EPS structures with those residues, or by modification of the linkage types of those residues [67,111]. Mass spectrometry on *T. pseudonana* EPSs has shown that the degree of polymerization and the distribution of EPSs vary in response to nutrient depletion and different nutrient sources [110].

As in cell wall polysaccharides, the monosaccharide composition of EPSs can vary drastically depending on the extraction method [112]. However, diatom EPSs have two general compositional features: (1) they consist of heteropolysaccharides that can be sulfated and (2) they contain rhamnose, fucose, galactose, glucose, mannose, xylose and/or uronic acids as well as some arabinose in lower

proportions (Table 3). Furthermore, diatom EPSs have high proportions of methylpentoses compared with intracellular soluble and cell wall polysaccharides. To date, no complete fine structure has been resolved for diatom EPSs and only a few studies report data on linkages. However, the available data show a large diversity of linkages found in diatom EPSs, with many glycosyl residues being typical of branched structures (Table 3).

Table 3. Structural characteristics of some exopolysaccharides (EPSs) in diatoms: monosaccharide composition, sulfate substitution, linkages. The data are non-exhaustive and only include soluble EPSs recovered from culture media.

Species	Monosaccharides	Sulfate [a] (wt%)	Linkages	Reference
Amphora sp. F1	GlcA(1.6)/Gal(1.1)/Fuc(1) [b]	9.7	nd [c]	[113]
Amphora sp. F2	GlcA(2.8)/Fuc(1)/Gal(0.8)	18.2	nd	
Amphora holsatica	UA/Rha/Fuc/Glc/Xyl/Ara [d]	nd	nd	[114]
Amphora rostrata	Fuc(41)/Gal(32)/UA(23)/ Man(9)/Rha(8)	10	nd	[115]
Asterionella socialis [e]	Rha(70)/Man(7)/2 Unk(23)	nd	nd	[69]
Chaetoceros affinis [e]	Fuc(39)/Rha(35)/Gal(26)	8.7	t-Fuc [f], 2,3-Fuc, 3,4-Fuc, 3-Fuc/2-Rha, t-Rha, 3-Rha, 3,4-Rha/3-Gal, t-Gal, 4-Gal [f]	[116,117]
Chaetoceros curvisetus [e]	Fuc(35)/Gal(10)/Rha(3)	7	2-Fuc [f], t-Fuc [f], 2,3-Fuc, 3-Fuc, 2,3-Fuc [f], 3,4-Fuc, 3,5-Fuc [f]/ 3-Gal, 2,3-Gal, t-Gal/2-Rha, t-Rha	[118]
Chaetoceros debilis [b]	Fuc(30)/Gal(29)/Rha(17)/ Man(10)/Xyl(9)/Glc(5)	nd	nd	[62]
Chaetoceros decipiens [e]	Rha(34)/Fuc(32)/Gal(17)/ Man(7)/Xyl(5)/Glc(5)	nd	nd	
Coscinodiscus nobilis [b]	Fuc(34)/Man(19)/Glc(16)/ Rha(15)/GlcA(9)/Xyl(6)	16.7	3-Fuc/6-Man/3-Glc/2-Rha/ t-Xyl with Fuc and Rha branched or sulfated	[119]
Cylindrotheca closterium	Xyl(46)/Glc(23)/Rha(15)/ Gal(12)/Man(4)/UA(5)	0	nd	[107]
Cylindrotheca fusiformis	Gal(38)/Glc(26)/Xyl(13)/Rha(13)/UA(7)/Man(5)	31	nd	[108]
Cyclotella nana [e]	Rha(33)/Gal(14)/Glc(11)/ Man(10)/Rib(8)/Xyl(7)/ 2 Unk(17)/GlcA(?)	nd	nd	[69]
Melosira nummuloides	UA/Rha/Fuc/Glc/Xyl/Ara/Gal [d]	nd	nd	[114]
Navicula directa	UA/Rha/Fuc/Glc/Ara/Gal/Xyl [d]	nd	nd	
Navicula incerta [e]	Rha(33)/Fuc(20)/Man(10)/Xyl (9)/Gal(8)/GlcA(?)/3 Unk(20)	nd	nd	[69]
Navicula salinarum	Glc(41)/Xyl(20)/Gal(19)/ Man(14)/Rha(5)/UA(21)	6.3	nd	[107]

Table 3. *Cont.*

Navicula subinflata	Glc(94)/UA(9)	9.6	nd	[120]
Nitzschia angularis [e]	Rha(20)/Gal(17)/Fuc(16)/ Ara(8)/Man(7)/Xyl(7)/GlcA(?)/ 2 Unk(25)/	nd	nd	[69]
Nitzschia frustulum [e]	Man(34)/Rha(24)/Gal(8)/ GlcA(?)/2 Unk(34)	9	nd	
Pinnularia viridis	Rha(29)/Gal(23.5)/Xyl(17)/ Glc(7.5)/Man(6.5)/Fuc(6)	nd	3-Rha, 3,4-Rha, 2,3-Rha, 2-Rha, t-Rha/ 3-Gal, 3,6-Gal/ t-Xyl, 2,4-Xyl, 4-Xyl/4-Glc/ 4-Man, t-Man/t-Fuc, 2-Fuc	[99]
Thalassiosira sp. F1	Man(51)/Rha(19)/Fuc(8)/Xyl(6)	nd	t-Man, 4,6-Man, 4-Man/3-Rha, 2-Rha/3-Fuc, t-Fuc/t-Xyl, 2-Xyl	[121]
Thalassiosira sp. F2	Man(57)/Xyl(19)/GlcA(6)/ GalA(5)	nd	4-Man, t-Man, 2-Man/4-Xyl, t-Xyl/t-GlcA/t-GalA	

[a] Sulfate percentages represent % weight of isolated polymers; [b] Only the sugars with contents of >5% are reported. Ara, arabinose; Fuc, fucose; Gal, galactose; Glc, glucose; GlcA, glucuronic acid; Man, mannose; Rha, rhamnose; Rib, ribose; UA, uronic acid; Unk, unknown; Xyl, xylose. Numbers in brackets following abbreviations give relative proportions of monosaccharide residues expressed as mol%, wt%, molar ratio, *etc.*, as reported. The sugars are ordered from high to low percentages for each species; [c] nd, not determined; [d] The ratio varies with hydrolysis conditions; [e] Data also available in the review [48]; [f] Glycosyl linkages expressed as the position(s) of substitution in addition to C-1 (t-Fuc, terminal fucosyl; 3-Rha, 3-rhamnosyl); Subscript "f" following sugar abbreviation indicates furanose form.

3. Structures and Biosynthesis of Protein *N*-Glycans in Diatoms

N-glycosylation is a major co- and post-translational modification of proteins in eukaryotes occurring in both the ER and the Golgi apparatus (Figure 6, [122]). In this process, a lipid-linked oligosaccharide composed of three glucose (Glc), nine mannose (Man) and two GlcNAc residues ($Glc_3Man_9GlcNAc_2$) is first assembled by the stepwise addition of monosaccharides on a dolicholpyrophosphate on the cytosolic side of and then in the lumen of the ER [123]. This oligosaccharide precursor is then transferred by the oligosaccharyl transferase (OST) complex onto the asparagine residues of consensus Asn-X-Ser/Thr sequences of a protein [123]. In 3.5% of studied cases, other sequences such as Asn-X-Cys, Asn-X-Val are glycosylated in endogenous or recombinant proteins produced in mammals or plant cells [124–126]. The glycoprotein is deglucosylated by α-glucosidases I and II and then reglucosylated by an uridine diphosphate (UDP)-glucose glycoprotein glucosyl transferase (UGGT) to ensure proper folding of the nascent protein through its interaction with ER-resident chaperones, such as calnexin and calreticulin [127]. These ER events are conserved in eukaryotes because they are crucial for efficient protein folding [127]. Bioinformatic analyses demonstrate that most of the genes encoding enzymes involved in the biosynthesis of the dolicholpyrophosphate-linked oligosaccharide, named asparagine-linked glycosylation (ALG) [128], are predicted in the genomes of diatoms (*P. tricornutum* [129], *T. pseudonana* [130], *Fragilariopsis cylindrus* [131] and *Aureococcus anophagereffens* [132]) (Figure 6), [133–135]. The only exception is ALG 10, an α(1,2)-glucosyl transferase responsible for the transfer of the terminal Glc residue of the triglucosyl extension of the *N*-glycan precursor, for which no

homology has been found (Figure 6) [133,135]. In addition to ALG genes, genes encoding subunits of the oligosaccharyl transferase have also been identified in diatom genomes, especially in *P. tricornutum* (Figure 6), in which α-glucosidase II (but not α-glucosidase I) as well as ER-resident UGGT and chaperones such as calreticulin are also predicted (Figure 6) [133,135]. These proteins are key elements of the quality control of proteins occurring in the ER. Large oligomannosides, with sizes of up to $Man_9GlcNAc_2$, have been found in *P. tricornutum* glycoproteins [133], suggesting that the synthesis of the oligosaccharide precursor and the quality control of secreted proteins may occur in a similar manner as that observed in other eukaryotes. However, in the ER, α-glucosidase I appears to remove the terminal α(1,2)-glucosyl transferase that is transferred by ALG10. Absence of ALG 10 and α-glucosidase I genes in *P. tricornutum* suggests that the *N*-glycan precursor is not fully glucosylated in diatoms into $Glc_3Man_9GlcNAc_2$ (Figure 6) [133,135].

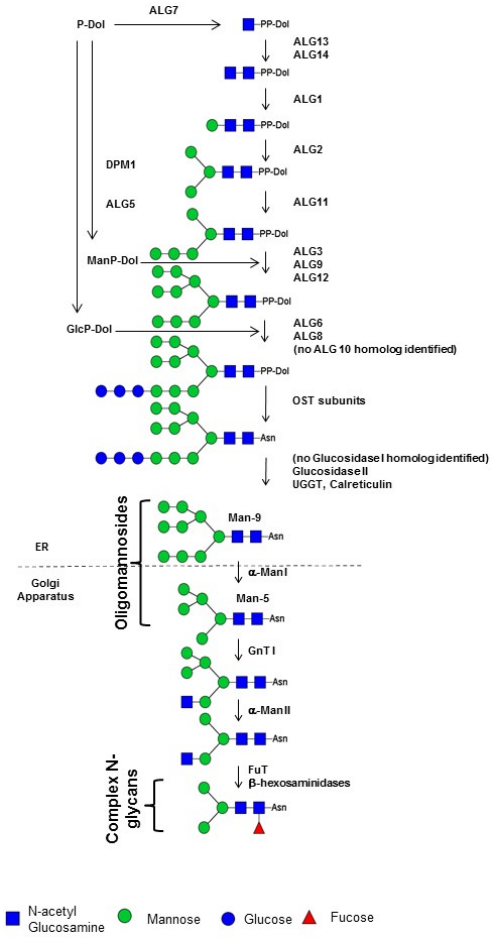

Figure 6. Proposed *N*-glycosylation pathway in *Phaeodactylum tricornutum*. Sequences predicted in the *P. tricornutum* genome are shown in bold. *ALG10* and glucosidase I genes have not been identified so far. The *N*-glycan structures presented in this figure are as given in Varki *et al.* [121]. ER: endoplasmic reticulum; DPM1: dolichol-phosphate mannosyl transferase; ALG: asparagine-linked glycosylation; PP-Dol: pyrophosphate dolichol; P-Dol: dolichol phosphate; OST: oligosaccharyl transferase; Asn: asparagine; UGGT: UDP-glucose glycoprotein glucosyl transferase; GnT: N-acetylglucosaminyl transferase; α-Man: α-Mannosidase; FuT: fucosyl transferase; Man-5 to Man-9: oligomannoside bearing 5 to 9 mannose residues.

In contrast to ER events, evolutionary adaptation of *N*-glycan processing in the Golgi apparatus has given rise to a variety of organism-specific complex structures [136]. First, α-mannosidases (α-Man I) degrade the oligosaccharide precursor into oligomannosides ranging from $Man_9GlcNAc_2$ to $Man_5GlcNAc_2$ (Man-9 to Man-5) (Figure 6). *N*-acetylglucosaminyl transferase I (GnT I) then transfers a first *N*-acetylglucosaminyl (GlcNAc) residue onto Man-5 and initiates the synthesis of a large variety of structurally different complex-type *N*-glycans. This processing continues with the removal of two mannosyl residues and then decoration of the *N*-glycans by the action of a specific repertoire of glycosyl transferases such as α-fucosyl transferases (FuT). Therefore, mature proteins leaving the secretory pathway carry organism-specific complex *N*-glycans allowing the protein to acquire a set of glycan-mediated biological functions [137,138]. Searches in diatom genomes for candidate genes encoding Golgi glycosidases and glycosyl transferases involved in *N*-glycan processing have led to the identification of α-Man I, GnT I and a FuT (putative α(1,3)-FuT) candidates [133,134], (Figure 6). The GnT I gene predicted in the *P. tricornutum* genome has been demonstrated to encode an active functional enzyme able to restore the maturation of *N*-linked glycans into complex-type *N*-glycans in the CHO Lec1 mutant which is affected in its endogenous GnT I [133]. Moreover, structural analysis of glycans *N*-linked to proteins secreted by the diatom *P. tricornutum* indicate that these oligosaccharides are processed through a GnT I-dependent pathway into partially fucosylated $Man_3GlcNAc_2$ (Figure 6) [133]. This truncated and fucosylated *N*-linked glycan likely results from the trimming of two mannose residues from Man-5 by an α-Man II and then transfer of an α(1,3)-fucose residue [133]. Later, the terminal GlcNAc introduced by the Golgi *P. tricornutum* GnT I are probably eliminated by β-hexosaminidases, as previously described in land plants and insects [139,140]. Two putative β-hexosaminidases have already been identified in *P. tricornutum* [133].

In addition, diatoms may have glycoproteins bearing *O*-glycans. For example, Swift and Wheeler showed that some proteins associated with the silica shell, called frustule-associated components (FACs), can be easily extracted from cell walls with an EDTA-based treatment [56]. These fractions can contain glycoproteins. In *C. fusiformis*, EDTA-soluble proteins [141], called frustulins, bear a glycan moiety composed of rhamnose (25%), galactose (20%), xylose (20%), glucose (2%), and mannose (1%). Although first described as proteoglycans [142], glycoproteins have also been isolated [99] in *Craspedosauros australis* extracted with urea and exhibit a xylose-rich composition (41%), with lower amounts of galactose, rhamnose, and mannose (14%, 12%, 10%, respectively). More work needs to be done to fully characterize the glycan structures of such glycoproteins and determine their biosynthetic pathway.

4. Nucleotide Sugar Biosynthesis in Diatoms

Monosaccharides represent the building blocks of glycans and polysaccharides (see Section 1). They are usually synthesized and converted into nucleotide sugars through a cytosolic interconversion metabolism (KEGG map 00520; [143]. In turn, nucleotide sugars—which are universal sugar donors—are involved in the formation of polysaccharides, glycoproteins, proteoglycans, and glycolipids. This metabolism is highly conserved in prokaryotes and eukaryotes and involves a set of phosphorylases, epimerases and reductases, as well as fructose-6-phosphate amino transferases enabling the synthesis of aminosugars. Nucleotide sugars may also result from the salvage pathway that involves the hydrolysis

of glycans to free sugars, their phosphorylation and finally their nucleotidylation. Searches in diatom genomes (*P. tricornutum* [129], *T. pseudonana* [130], *Fragilariopsis cylindrus* [131], and *Aureococcus anophagefferens* [132] for genes encoding cytosolic enzymes of both the interconversion and salvage pathways have led to the identification of putative candidates for the synthesis of UDP-sugars such as UDP-galactose (UDP-Gal), UDP-galacturonic acid (UDP-GalA), UDP-glucuronic acid (UDP-GlcA), UDP-xylose (UDP-Xyl) and UDP-rhamnose (UDP-Rha) that are directly derived from UDP-Glc (Figure 7). Moreover, gene predictions also include enzymes required for the synthesis of the guanine diphosphate (GDP)-sugars originating from Man-6P (Figure 7). Aminosugar such as GlcNAc biosynthesis likely occurs by amination of the C2 on fructose-6P (Fru-6P) as reported in other organisms. Other gene predictions based on diatom genomes include genes encoding several sugar phosphorylases of the salvage pathway (Figure 7). However, neither UDP-galacturonate decarboxylases, nor UDP-arabinose 4-epimerases required for L-arabinose biosynthesis are predicted in these genomes. Other than arabinose, predicted nucleotide sugar metabolism is generally in agreement with the sugar compositions of polysaccharides and glycans isolated from diatoms.

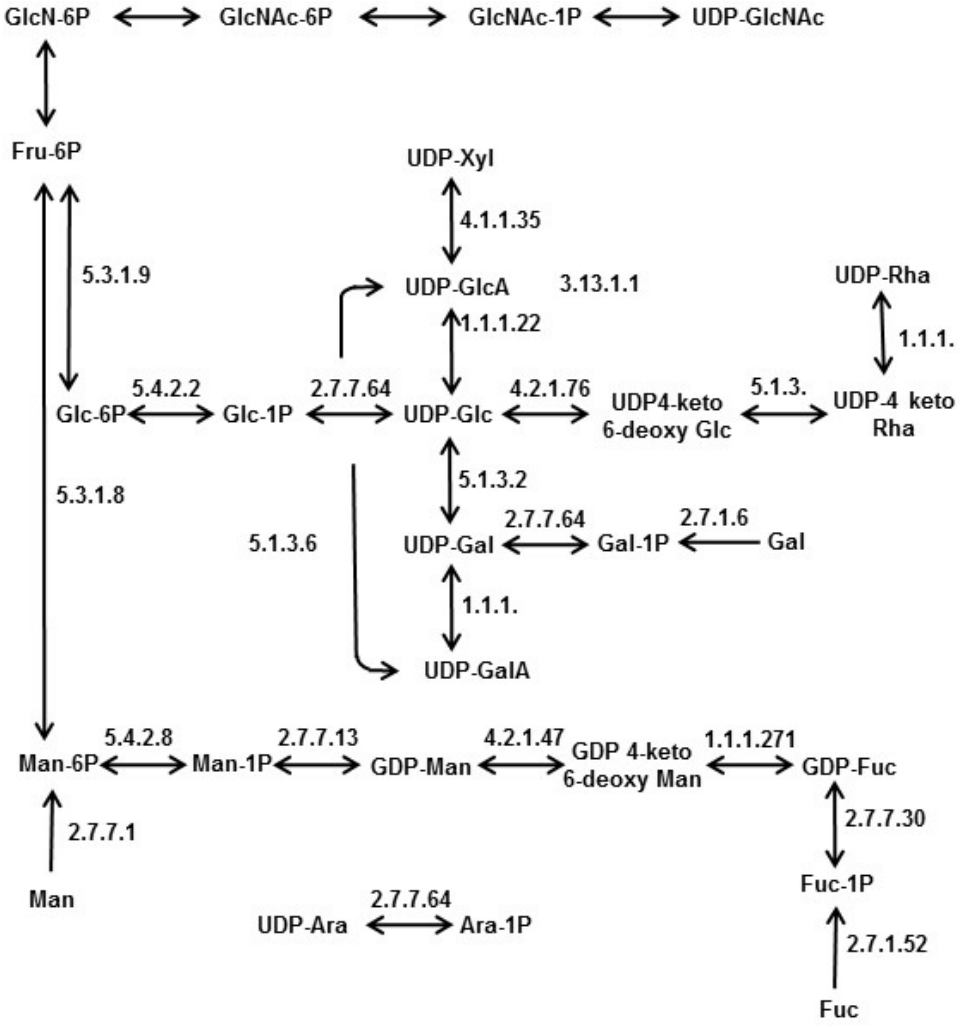

Figure 7. Predicted nucleotide sugar metabolism in diatoms based on bioinformatics analyses of the genomes from *Phaeodactylum tricornutum* [129], *Thalassiosira pseudonana* [130], *Fragilariopsis cylindrus* [131] and *Aureococcus anophagefferens* [132].

5. Conclusions and Perspectives

The abundant literature cited in this review demonstrates that monosaccharide composition is highly variable in diatom EPSs. The variation in physiological conditions greatly influences their composition, suggesting that diatoms modulate their polysaccharide biosynthesis machinery to adapt to environmental conditions. EPSs have been shown to be heteropolysaccharides. Branched and sulfated glucuronomannans are thought to be ubiquitous and thus representative of diatom cell walls. β-chitin fibers have also been found in some diatom species. Chrysolaminarin is a common β(1,6) ramified β(1,3) polyglucan for food storage in diatoms. Although numerous efforts have been made to determine the structures of diatom polysaccharides, there is currently a lack of information regarding their biosynthesis pathways, as well as their cell localization and organization.

With regard to protein *N*-glycosylation, gene prediction analysis suggests that diatoms are equipped with most of the eukaryotic genes encoding ER-resident essential players, such as sugar transferases and chaperones. Some candidate genes involved in subsequent Golgi events of *N*-glycosylation have also been found in *P. tricornutum*, justifying further investigations and characterization of diatom *N*-glycosylation pathways. When looking at potential nucleotide sugar synthesis in diatom genomes, key enzyme genes were predicted for almost all monosaccharides, with the exception of those for arabinose synthesis, although this sugar was detected in some cell wall polysaccharides. In the coming years, the increasing demand for marine polysaccharides and for recombinant therapeutic glycoproteins produced in microalgae will lead the scientific community to carry out more research to better understand diatom polysaccharide and glycan structures, as well as their respective biosynthesis pathways and cell localizations.

Acknowledgments

Part of the work described in this review was funded by the University of Rouen, GRR-VASI, GRR-IRIB as well as the *Institut Universitaire de France*, (B.G.; C.B.; P.L. and M.B.). T.L.C. and W.H. received funding from the European Community Seventh Framework Programme (FP7) under grant agreement No. 286354.

The authors would like to thank Azzedine Driouich for critical reading and valuable advice on this manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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