REVIEW

# Starch metabolism in green algae

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Starch plays a central role in the life cycle as one of the principal sources of chemical energy. This polysaccharide accumulates in plastids in green algae and land plants, and both organisms have acquired various enzyme isoforms for each step of the metabolic pathway. Eukaryotic green microalgae present the critical photosynthetic functions as higher plants. However, due to the small size of their genome, gene redundancy is decreased, a feature that makes them an excellent model for investigating the properties of photosynthetic physiology. In the last decade, there has been an increasing demand for starch in many industrial processes, such as food, pharmaceutical, and bioethanol production. Thus, a better understanding of starch biosynthesis, in particular the structure–function relationship and regulatory properties of the enzymes involved in its production may provide a powerful tool for the planning of new strategies to increase plant biomass, as well as to improve the quality and quantity of this polymer.

Received: September 18, 2012
Revised: January 19, 2013
Accepted: January 21, 2013

**Keywords:**
Algae / Metabolism / Starch

## 1 Introduction

The algae are a greatly diverse group of photosynthetic organisms that are widely distributed on the planet and are critical for sustaining atmospheric and terrestrial conditions. They are present in many forms ranging from the small picoplankton living in the oceans to the macrophytic organisms which forms layers that resemble grass on the coasts [1–4]. The higher diversity among the algae is not only respect to size and shape, but also with respect to the formation of various chemical compounds through the different biosynthetic pathways [3].

Algae are economically important due to their biological role in ecosystems and as a source of commercially significant products such as food. Moreover, algae are also used as vitamin fount by the health food industry because of their high levels of vitamin A. In addition, these micro-organisms are used as feed additives for aquaculture, as coloring agents of food, and as fluorescent tags to localize, quantify or identify surface molecules for specific assays. Algae are also very important because they synthesize a number of different lipids and polysaccharides that serves as carbon storage compounds of high biological and commercial value. Some of the carbohydrates are anionic and bind and chelate several metals, thus helping to maintain a hydration surface around the cell [3, 5–8]. Finally, certain polysaccharides have anticoagulant properties [7], while others are used for making solid media or other products, such as ice creams, cosmetics, ceramics, cleaners, and toothpastes (http://www.nmnh.si.edu/botany/projects/algae/Alg-Prod.htm). Furthermore, very long chain PUFAs produced and stored in high levels by some marine microalgae could be beneficial for mammalian brain development [9, 10]. Besides its nutritional characteristics make them an interesting product that can be sold as health food products and can also be incorporated in infant food formulas for worldwide distribution [3].

Graham et al. (2000) have postulated that land plants evolved from green algae belonging to the Charophyceae [4] (Fig. 1). Charophytes or stoneworts are one of the largest

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**Abbreviations: ADPGlc,** ADP-glucose; **DBE,** debranching enzyme; **DPE,** disproportionating enzyme; **GBSS,** granule-bound SS; **GH,** glycoside hydrolase; **GT,** glycosyltransferase; **GWD,** glucan water dikinase; **SBE,** starch branching enzyme; **UDPGlc,** UDP-glucose

 

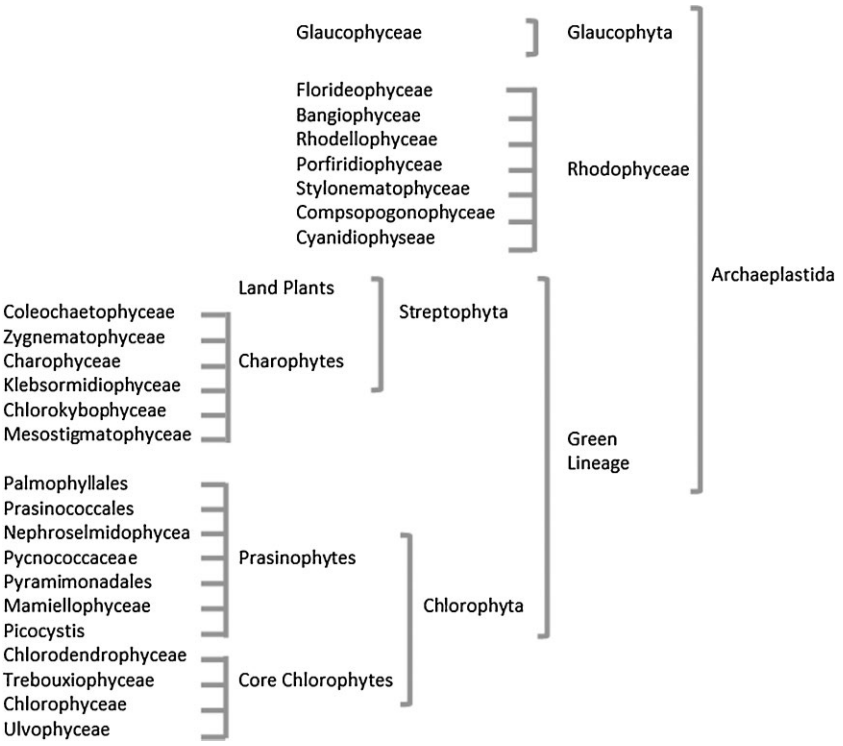


**Figure 1.** Schematic representation of the phylogeny of the Archeaplastida

and most structurally complex green algae. It has been reported the existence of six orders of green algae within this class: Charales, Zygnematales, Chlorokybales, Coleochaetales, Mesostigma, and Klebsormidiales [11, 12].

Based on a phylogenetic analysis of the combined sequences of four genes, a small subunit rRNA gene (nuclear), *ATPB* and *rbcL* (chloroplastic), and *NAD5* (mitochondrial) of various green plants and charophycean green algae, Karol and col. (2001) reported that the Charales represent the closest green algae linked to land plants [13]. Additionally, taking into account their morphological characteristics alone, the Coleochaetales and the Charales were considered highly nearby affiliated with land plants [12, 14].

Starch biosynthesis is unique to the Archaeplastida supergroup, comprising Rhodophyceae (red algae), Chloroplastida (green algae and land plants), and a minor group called the Glaucophytes (Fig. 1). It was described that the synthesis of this polysaccharide evolved from the ancestral ability to make glycogen [15]. The Archaeplastida are generally considered to be monophyletic; i.e., all members are descended from a single ancestor in which a primary endosymbiotic event occurred entailing the uptake of a cyanobacterial cell (the symbiont) by a nonphotosynthetic eukaryotic cell (the host) [16]. Most cyanobacteria synthesize glycogen, as also occur in non-plant eukaryotes. However, the recent identification of new cyanobacterial species that make starch-like oligosaccharides with an intermediate type of chain length distribution between amylopectin and glycogen (designated as either semiamylopectin or cyanobacterial starch) suggests that the primary endosymbiont also had the ability to synthesize these kind of polymers [15, 17–20].

Differences in the starch biosynthetic pathways between the archaeplastidal lineages have arisen during subsequent evolution. Most notably, in green plants starch is synthesized in the plastid compartment, whereas in red algae and in glaucophytes its synthesis occurs in the cytosol. Interestingly, some rhodophyte species have reverted their metabolism to the synthesis of glycogen [15, 20].

There are four biochemical steps that are required for the synthesis of starch: substrate activation, chain elongation, chain branching, and chain debranching [15, 21] (Fig. 2). Phylogenetic analyses of the protein sequences of different starch metabolic enzymes have revealed a mixture of host- and symbiont-derived genes in each branch of the Archaeplastida [17, 22–24]. In green plants, the soluble (SS) and granule-bound SSs (GBSSs), which utilizes mainly ADP-glucose (ADPGlc) are derived from the symbiont; whereas SSs from red algae and glaucophytes utilizes mainly UDP-glucose (UDPGlc) being the soluble forms derived from the host, while the GBSS-like proteins are derived from the symbiont. The ancestry of other starch metabolic enzymes is also a mosaic; in all cases, starch branching enzymes (SBEs), phosphorylases, and β-amylases are derived from the ancestral host, whereas the disproportionating enzyme 1 (DPE1) protein and isoamylase (ISA) are proposed to come from the symbiont. The sequence of events that resulted in cytosolic starch biosynthesis in some Archaeplastida and plastidial starch biosynthesis in other organisms remains a subject of speculation [15, 17, 24].

 

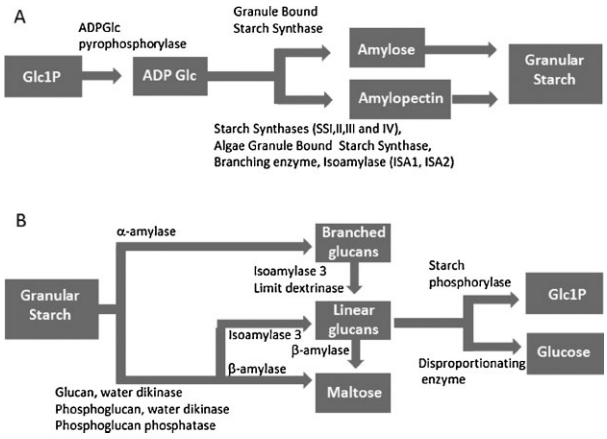


**Figure 2.** Starch synthesis (A) and degradation (B) pathways in chloroplasts. (A) The first step in starch biosynthesis is the production of ADPGlc via APGlc PPase. Then SSs catalyze the elongation of α-1,4-glucans by the transfer of the glucosyl moiety from the sugar nucleotide to the non-reducing end of the growing polyglucan chain. Soluble SSs forms are involved in amylopectin synthesis, whereas the GBSS forms participate in amylose synthesis, but also have an essential role in amylopectin production in *C. reinhardtii*. BE cleaves a linear glucose chain and transfers the cleaved portion to a glucose residue within an acceptor chain via an α-1,6 linkage to form a branch and ISA 1 and 2 facilitates granule crystallization by removing wrongly positioned branches. (B) PWD and GWD phosphorylate the surface of the starch granule, making it accessible for β-amylase action. Phosphate is concomitantly released by phosphoglucan phosphatase to allow complete degradation. Then, β-amylase hydrolyzes glucans producing maltose. Starch is also metabolized to branched glucans by α-amylase and to linear glucans by α-amylase, ISA3, and pullulanase. These linear glucans are further metabolized through β-amylase to maltose, through DPE to glucose or through starch phosphorylase to glucose-1-phosphate. Maltose and glucose are then transported from chloroplast to cytosol (Zeeman et al. 2010).

The components of the starch biosynthetic machinery that are found in all starch-synthesizing organisms are likely to have made an significant contribution at some stage in the evolution of glucan polymers that form starch granules. For example, GBSS-like proteins, the main enzymes that synthesize amylose in Chloroplastida, are present in all starch-synthesizing lineages examined thus far. Even though GBSS is not essential for amylopectin synthesis in higher plants, it is involved in amylopectin synthesis in *Chlamydomonas reinhardtii*, suggesting that their capacity to produce long glucan chains could be an important factor in the evolutionary transition to the synthesis of amylopectin-like rather than glycogen-like polymers [25]. It is worth mentioning that *C. reinhardtii* GBSSI is involved both, in amylose and amylopectin synthesis. Thus, it is possible to postulate that the subsequent acquisition of other SS isoforms in green plants made the original function of GBSS redundant, being the synthesis of amylopectin a secondary function.

It should be noted that there are two models for the synthesis of the amylopectin fraction: (i) the glucan-trimming model [26] based on experimental evidence in maize, *Arabidopsis* and *Chlamydomonas*, it was suggested that SS and SBE enzymes synthesize a soluble molecule called preamylopectin which is substrate for the debranching enzyme (DBE) and D-enzyme (a 4-α-glucanotransferase), that selectively remove some branches leading to the production of a insoluble amylopectin molecule; and (ii) the water-soluble polysaccharide (WSP)-clearing model, described for *Arabidopsis* [27], in which DBE would not act directly in the synthesis of amylopectin, but would recycle soluble products arising from the action of SS and SBE on maltooligosaccharides (MOSs).

Isoamylases, also present in all starch-synthesizing organisms, are other enzymes that have probably made an important contribution in starch metabolism evolution. Their original function was associated to glucan degradation (as is the case of some glycogen-synthesizing bacteria [28, 29]). However, their recruitment to glucan synthesis is likely to have been an important step toward the synthesis of glucan polymers that form starch granules. This step could be facilitated by gene duplication events that allowed the evolution of multiple isoforms with distinct substrate specificities (i.e., ISA1 and ISA2), whereas ISA3 is involved in starch degradation (Fig. 2). Further insight into the evolution of starch metabolism from ancestral glycogen metabolism will be facilitated by the recent inclusion of other model organisms from the different branches of the Archaeplastida [15, 21, 22, 30].

## 2 Genomes: Sequenced strains and genomic studies

Plant genomes are usually large and complex, having gene redundancy, duplications, and transposable elements among other features [31]. As a practical alternative, unicellular green algae are suitable for the study of numerous biological processes due to their simplest genomic, molecular, and physiological characteristics.

In the last years, several nuclear and organelle algae genomes have been sequenced. Some nuclear-sequenced genomes from green algae include those from *Ostreococcus tauri* [32], *Ostreococcus lucimarinus* [33], *C. reinhardtii* [34], *Micromonas pusilla* [35], *Bathycoccus prasinos* [36], *Chlorella variabilis* [37], *Coccomyxa subellipsoidea* [38], and *Volvox carteri* [39]. Genomes from *Dunaliella salina* [40], *Chlorella vulgaris* (http://www.jgi.doe.gov/sequencing/allinoneseqplans.php), *Nannochloris* (NCBI BioProject PRJNA84219), *Chlorella pyrenoidosa* (NCBI Bioproject PRJNA171991), *Trebouxia* sp. (NCBI BioProject PRJNA82781), and *Botryococcus braunii* (http://www.jgi.doe.gov/sequencing/allinoneseqplans.php) are in the process of being sequenced.

Besides, sequence material of the organelle genomes from *O. tauri* [41], *D. salina* [40], *C. reinhardtii* [42, 43], *Nephroselmis*

 

*olivacea* [44, 45], *Chaetosphaeridium globosum* [46], *C. vulgaris* [47], and *Mesostigma viride* [48, 49] are also available.

*C. reinhardtii* and *O. tauri* genomes are the best characterized, as documented in numerous publications [32–34]. Although *Chlamydomonas* has been a model organism since several decades ago [50], *Ostreococcus* has gained importance in the last years, since its first description in 1994 [51]. While *Ostreococcus* have a small and compact genome, with a low number of introns per gene, broad reduction of intergenic regions and small average transcript size [32, 33], *C. reinhardtii* presents a genome complexity comparable to Arabidopsis [34, 52].

Novel insights into algal starch metabolism have been developed from the analysis of the genomes of the green algae mentioned above. Ral's work in 2004 was the first comprehensive analysis of *O. tauri* starch genomics, granule morphology, and partitioning mechanisms [53]. In this work the presence and expression of storage polysaccharide metabolism genes by reverse transcription (RT)-PCR was verified and proved that, in spite of *O. tauri* small-genome size, this picoalga exhibits the same degree of complexity as that of vascular plants regards to the starch metabolism pathways [53]. Their results showed that most Prasinophyceae starch metabolism enzymes have been conserved throughout evolution; however, *O. tauri*, unlike Arabidopsis and other plants, do not seem to have any protein related to glycogenin, a self-glucosylating glycosyltransferase (GT) that acts as a primer for the synthesis of glycogen in yeasts and mammals [54].

More recently, a comparative bioinformatic study of six algal genomes (two *Chlorophyceae*: *C. reinhardtii* and *V. carterii*, and four Prasinophytae: *O. tauri* and *O. lucimarinus* and two *M. pusilla* strains) suggested that the complex metabolic pathway of glucan storage is conserved in photosynthetic organisms [55]. These algae harbor all the starch biosynthetic pathway steps, characteristic of higher plants (Fig. 2), with at least one ADPGlc pyrophosphorylase (ADPGlc PPase), a GBSSI, SSSs I-IV (SSI-SSIV), SBEI and SBEII, ISA1 and ISA2, with the exception of *O. tauri* for which no SSIV gene sequence was found [53, 56]. It was reported that SSIV regulates starch granule number in Arabidopsis and it would also participate in starch granule priming [57]. In addition, all the characterized algae contain at least one gene encoding each enzyme involved in starch degradation, such as ISA3, pullulanase, D-enzyme, α-amylase, glucan water dikinase (GWD), phosphoglucan water dikinase (PWD), and starch excess 4 (SEX-4) phosphatase, an enzyme required for the removal of phosphate groups from starch in Arabidopsis [58, 59]. It is important to note that the D-enzyme was also associated to amylopectin synthesis in *C. reinhardtii* [26, 60, 61].

Interestingly, each analyzed algae contains more SSIII-like genes than *Arabidopsis*. Besides, *Micromonas* strains contain two copies of SSI and SSII genes whereas *Chlamydomonas* and *Volvox* only have one duplicated SSI-like sequence. Until now, the functional significance of these additional sequences is unknown [58].

Regards *Chlamydomonas*, given the broad ESTs generated for this algae in several nutritional conditions [62–64], Deschamps et al. (2008) verified the presence of ESTs corresponding to starch metabolism genes, and also described the transit peptides for chloroplast localization in many related enzymes [55, 58].

Several works have been reported about the regulation of algae starch metabolism. Monnier et al. (2010) conducted a genome-wide analysis of gene expression in *O. tauri* cells and described the fundamental contribution of transcriptional regulation during the light-dark cycle [65]. Furthermore, this work suggested the occurrence of a circadian regulation of starch content in *Ostreoccocus*, as it was previously reported in *Chlamydomonas* based on the analysis of ADPGlc PPase activity and the expression of GBSSI and SSIII transcripts [25]. Accordingly, Ral et al. (2006) demonstrated a strong functional relationship between GBSSI and SSIII in *Chlamydomonas*, two enzymes that play an essential role in the synthesis of long glucan chains within amylopectin as described above [25].

In Arabidopsis, although the transcription of starch metabolism genes is regulated by circadian clock, protein levels appear to remain relatively constant throughout the circadian cycle [66]. Thus, the starch content in plant tissues is not thought to be under circadian clock control. Accordingly, these results suggest that regulatory mechanisms for starch metabolism in green algae are dissimilar from those in plants, being the transcriptional regulation more important in these unicellular photosynthetic organisms [25].

Sorokina et al. (2011) have proposed a modeling approach integrating data from microarray analysis with a stoichiometric reconstruction of starch metabolism in *O. tauri* for the purpose of predicting the dynamics of the starch content in the light/dark cycle [67]. In addition, after performing an in silico experiment of gene deletion they have described the contribution of each starch metabolism enzyme for the glucan storage profile. In particular, the deletion of GWD, α-amylase, and starch phosphorylase (Fig. 2) decreased the starch degradation rate, while the deletion of phosphoglucomutase promotes its degradation. On the other hand, the deletion of the maltose transporter increases the starch synthesis rate, whereas the deletion of fructose-1,6-bisphosphatase and fructose bisphosphate aldolase genes had an opposite effect [67]. Moreover, Sorokina et al. have also identified the ADPGlc PPase, GBSSI, α-amylases, GWD, and the maltose transporter as potential targets of transcriptional regulation, confirming the presence of different regulatory mechanisms of starch metabolism in *O. tauri* respect to land plants [67].

In addition to the genomic information, *Chlamydomonas* and *O. tauri* are excellent model organisms because of the existence of several genetic and molecular tools and appli-

 

cations as well as the possibility to achieve stable mutants using different approaches [68].

The development of selectable markers [50, 69–73] allowed the transformation of the plastid and nuclear genomes of Chlamydomonas [69, 74–76]. Gene function can be evaluated using classical chemical or physical mutagenesis [77], antisense or RNAi suppression of gene activity [78, 79] insertional mutagenesis and gene disruption by homologous recombination, although the last one is still inefficient in nucleus. In the chloroplast genome it is possible to insert a DNA fragment at an exact position, whereas in the nuclear genome, DNA integrates randomly, making impossible to inactivate any particular gene. In addition, many reporter genes are available to elucidate gene expression regulation [80, 81], as well as several reporter molecules to enable trace gene and gene products within particular compartments in the cell [82].

Regarding *O. tauri*, it has been recently developed at the François-Yves Bouget laboratory many tools for gene functional analysis including gene overexpression, antisense knockdown, and stably transformed reporter cell lines to analyze transcriptional and translational activity under different growth conditions [83, 84].

On the other hand, five genomes from red algae (*C. merolae*, *P. umbilicalis*, *C. crispus*, *G. andersonii*, and *G. sulphuraria*) and one for Glaucophytes (*C. paradoxa*) have recently been sequenced including unicellular and multicellular species [85, 86]. Their starch metabolic pathways are well conserved all over the lineage. Surprisingly, Rhodophyceae need fewer than 12 genes to accumulate complex starch granules very similar to Chloroplastida starch [87]. Initially it was reported that floridean starch from red alga lacks amylose, but some Rhodophyta lineages accumulate this polysaccharide [18, 19].

## 3 Green algae

### 3.1 *Chlamydomonas*

*Chlamydomonas* genus is polyphyletic, since it is distributed in at least five distinct lineages and represents more than 600 species being *Chlamydomonas reinhardtii* the most characterized member [88–90].

Traditionally the genus *Chlamydomonas* comprises all biflagellate green algae, approximately 10 μm long, in which two flagella of the same length emerge closely spaced, coated by a multilayered cell wall and having a unique chloroplast with pyrenoid(s), a protein complex composed mainly of an aggregation of RuBisCO, surrounded by starch, called pyrenoidal starch [91].

*Chlamydomonas* has been widely used as a model system for the study of photosynthesis, chloroplast biogenesis, flagellar function, cell–cell recognition, cell cycle control, and circadian rhythm because of its well-defined genetics, and the development of efficient methods for nuclear and chloroplast transformation [92, 93]. In addition, due to its high growth rate, the microalgae can be easily cultured, obtaining high yields by utilizing the sunlight as energy source [94, 95]. Particularly, *C. reinhardtii* is well-known as a photoautotrophic microorganism, having a great ability to fix $CO_2$ and accumulating large quantities of starch. Therefore, the study and characterization of *Chlamydomonas* becomes an excellent opportunity to understand the mechanisms involved in starch biosynthesis [96, 97].

Different molecular analyses of starch biosynthetic genes were performed in *C. reinhardtii* mutants defective in starch biosynthesis [98, 99]. Some of these mutants include strains defective for *STA7* (encoding a DBE), resulting in the synthesis of a glycogen-like polysaccharide instead of starch [100]. Izumo et al. (2011) reported the effects of the GBSSI-defective mutation (*STA2*) on the production of pyrenoidal starch in *C. reinhardtii*. It was suggested that, in *Chlamydomonas*, GBSSI is required for the formation of a stable normally thick pyrenoidal starch sheath without impacting either on the $CO_2$-concentrating mechanism (CCM) or cell growth. Besides, it has been demonstrated the requirement of GBSSI to obtain high levels of crystallinity of the pyrenoidal starch granule due to the GBSSI induced starch granule fusion as also reported in maize [101].

Other experimental evidences provided by Van den Koornhuyse et al. (1996) showed that *C. reinhardtii*, mutants defective either for phosphoglucomutase or ADPGlc PPase-large subunit, accumulates polysaccharides similar to transient starch. Transient starch is defined as the polysaccharides found in plant storage organs prior to storage starch and amylose synthesis [102].

Furthermore, three distinct starch phosphorylase activities were detected in *C. reinhardtii*, two plastidial enzymes (PhoA and PhoB) and a single extraplastidial form (PhoC), all of them displaying higher affinity for glycogen as in vascular plants. Starch phosphorylases are involved in the phosphorolytic degradation of starch, catalyzing the reversible transfer of glucosyl units from glucose-1-phosphate to the non-reducing end of the α-1,4-D-glucan chains with the release of phosphate [103]. The two *C. reinhardtii* plastidial phosphorylases would function as homodimers containing two PhoA (91-kDa) subunits and two PhoB (110-kDa) subunits. PhoA and PhoB differ in their inhibition sensitivity by ADPGlc and their affinity for MOSs. Molecular analysis established that the *C. reindhartii* gene *STA4* encodes for PhoB, and it was reported that *STA4* deficient strains display a significant decrease in the amount of starch during storage. This finding correlates with the accumulation of abnormally shaped granules containing a higher proportion of amylose and a modified amylopectin structure [104].

### 3.2 *Ostreococcus*

*Ostreococcus tauri* is a unicellular green alga, discovered in 1994 in the Thau Lagoon in France using flow cytometry

 

[51, 105]. Each cell has a very simple structural organization with a diameter minor than 1 μm, lacking cell wall and flagella, and containing one large nucleus, a single chloroplast and mitochondria, one Golgi apparatus and a reduced cytoplasmic compartment [106]. It is the smallest free-living eukaryote identified to date and has the smallest eukaryotic genome [51, 105]. More recently, three-dimensional images of the *O. tauri* cell ultrastructure in a near-native state were obtained using the new technology electron cryotomography [107].

Based on its chlorophyll pigments, carotenoids [51] and its 18S rDNA sequence, it was reported that *O. tauri* belongs to the Prasinophycee class [32], an early branch in the lineage of green plants.

Other members of the *Ostreococcus* genus have been found in different marine ecosystems. Strain diversity was analyzed by sequencing their rDNA internal transcribed spacer regions, using pulsed-field gel electrophoresis and by the characterization of its pigment composition [108]. As a result, four different ecotypes have been defined regard its light intensity adaptation, reinforcing results obtained by Guillou et al. [109], by clustering small subunit rDNA sequences of *Ostreococcus*. Clade A comprised strains isolated from surface down to 65 m depth. Clade B included strains from the zone of 90–120 m depth; and sequences of strains OTH95 (Thau Lagoon) and RCC 501 (from Mediterranean Sea, 0–5 m depth) constituted clades C and D, respectively, both adapted to high light intensity [108]. These strains present different adaptation to environmental conditions faced at surface and the bottom of the oceanic photic zone. Deep strains show high sensitivity to photoinhibition at high light intensities, whereas surface strains do not grow at lowest light intensities.

It was described that *O. tauri* accumulates only one starch granule inside its chloroplast by using a pathway of comparable complexity as occur in higher plants or *Chlamydomonas*, using ADPGlc as the glycosyl donor substrate [53, 110, 111].

*In vitro* assays showed that *O. tauri* presents ADPGlc PPase and GBSSI activities [53]. The former enzyme was activated by 3-phosphoglycerate and inhibited by orthophosphate, as previously reported for land plants and cyanobacteria [112–115]. However, *O. tauri* ADPGlc PPase is not redox-regulated and present a modified functionality, with its large subunit leading catalysis [116]. Accordingly to previous publications [18, 54], Sorokina et al. (2011) postulated the occurrence of a strong connection between genetic regulation and metabolic function in *O. tauri*, essentially as a result of the relative weakness of the redox regulation of starch metabolism because of the absence of the redox-target sequences of the known redox regulated enzymes in plants, such as GWD, ADPGlc PPase and α-amylase [63]. This results suggests either, that redox regulation appeared later in evolution or that the algae have developed a different mechanism for the redox control of ADPGlc PPase and the other mentioned enzymes [55, 58].

As mentioned above, *Ostreococcus* lacks genes related to yeast or mammal glycogenin. *O. tauri* starch granule partitioning mechanism could explain the absence of these proteins, making unnecessary the existence of a primer to start *de novo* starch synthesis. It was reported that during plastid division, the starch granule is elongated and is divided in two new granules that are segregated into each recently formed chloroplast [53]. The requirements for initiating the crystalline growth of the granule are contained in the existing structure of the polysaccharide and the plastid division machinery. Besides, it has been proposed that the localized synthesis and degradation would regulate starch granule partitioning in *O. tauri*. The presence of a pullulanase associated to the starch granule may reflect a function of this enzyme in the partitioning process [53].

This hypothesis is further reinforced by the fact than *Ostreococcus* never degrades its starch completely, even after a prolonged incubation in the dark. A similar fact occurs in *Chlamydomonas*, where starch is not fully degraded under different tested conditions, and also seems to lack glycogenins [53].

Another interesting data is the fact that *Ostreococcus* genome lacks SSIV gene. As mentioned, SSIV controls the number of starch granules in Arabidopsis and is supposed to participate in polysaccharide biosynthesis priming or in starch granule priming. Mutants of Arabidopsis lacking SSIV display a single large granule for each chloroplast instead of the many smaller starch granules present in wild-type plants [55, 57]. The absence of SSIV in *O. tauri* would be also a direct consequence that this alga does not require starch granule priming or does not need to maintain a certain number of starch granules. In contrast, the same genus member *O. lucimarinus* contains one SSIV-like sequence and present several starch grains in its chloroplast [55, 56]. It remains to be determined whether this alga contains a genuine SSIV or if SSIV would have a different role in this case.

### 3.3 *Micromonas*

The Prasinophyte *M. pusilla* was the first picoplanktonic species described by Butcher et al. in 1960 [117]. *M. pusilla* is a diminute (1–2 μm) green alga with a pear-shaped naked cell body, one chloroplast with pirenoidal starch, a single posterior flagellum and a characteristic swimming behavior [118, 119]. According to the literature, *M. pusilla* is the most ubiquitous and cosmopolitan species of all picoeukaryotes described at the present [120]. *M. pusilla* becomes predominant in the picoeukaryotic community along all the year in many coastal systems such as the English Channel [121]. Recent studies based on phylogenetic analysis of several genes from these species collected worldwide revealed the existence of three [109] to five [122] phylogenetically discrete clades, suggesting that this taxon is a complex of cryptic

 

**Table 1.** Storage glucan characteristics from representative photosynthetic organisms

| | | Embryophyta | Chlorophyta | Chlorophyta | Chlorophyta | Rhodophyta | Glaucophyta |
|---|---|---|---|---|---|---|---|
| | | *A. thaliana* | *O. tauri* | *C. reinhardtii* | *M. pusilla* | *C. merolae* | *C. paradoxa* |
| Storage glucan | Name | Starch | Starch | Starch | Starch | Floridean starch | Floridean starch |
| | Glycosid bonds | α-1,4 | α-1,4 | α-1,4 | α-1,4 | α-1,4 | α-1,4 |
| | Branches | α-1,6 | α-1,6 | α-1,6 | α-1,6 | α-1,6 | α-1,6 |
| | Structure | Granules | Unique granule | Granules | Granule | Granules | Granules |
| | Cell location | Plastidial | Plastidial | Plastidial | Plastidial | Cytosolic | Cytosolic |
| | Molecular composition | Amylose/ amylopectin | Amylose/ amylopectin | Amylose/ amylopectin | Amylose/ amylopectin | Semi-amylopectin | Amylose/ amylopectin |
| Metabolic pathway | Glucose donor | ADPGlc | ADPGlc | ADPGlc | ADPGlc | UDPGlc | UDPGlc |
| | Complexity | High | High | High | High | Low | Low |
| Enzyme sets | ADPGlc PPase | 6 | 2 | 3 | 3 | – | |
| | SSS (ADPG) | 5 | 5 | 7 | 8 | – | |
| | SSS (UDPG) | – | – | – | – | 1 | 1 |
| | GBSS | 1 | 1 | 2 | 1 | 1 | 1 |
| | SBE | 3 | 2 | 3 | 3 | 1 | 1 |
| | Isoamylase | 3 | 3 | 3 | 3 | 2 | 1 |
| | Direct DBE | 3 | | 3 | 3 | | |
| | Pullulanase | 1 | 1 | 1 | 1 | | |
| | Phosphorylases | 2 | 2 | 2 | 3 | 1 | 1 |
| | Glucanotransferase | 1 | 1 | 1 | 1 | – | |
| | Transglucosidase | 1 | 1 | 1 | 1 | 1 | |
| | β-amylases | 9 | 2 | 3 | 2 | 1 | |
| | GWD | 3 | 4 | 4 | 3 | 1 | |
| | References | [135] | [53] | [111] | [55, 58] | [87, 136] | [17, 30] |

species which started to diverge during the late Cretaceous period [119, 122, 123].

However, to date no clear morphological, ecophysiological, or biogeographical differentiation between strains or clades of this species had been reported, except for one lineage described as purely Arctic [124].

In the work of Deschamps et al. (2008), it has been described at least 32 genes involved in starch metabolism on *M. pusilla*: 3 ADPGlc PPases, 8 SSs, 1 GBSS, 3 SBE, 3 DBE, 1 pullulanase, 3 phosphorylases, 1 D-enzyme, 1 DPE2, 2 β-amylases, 3 isoamylases, and 3 GWD (Table 1). However, at the present there is no information about mutants in starch metabolism genes from Micromonas [55, 58].

## 4 Red algae and Glaucophytes

Red algae (Rhodophyceae) are one of the oldest groups of marine organisms with nearly 6000 species. The color of these algae is due to the phycoerythrins pigments which absorbs blue light and reflect red light [16, 125]. Rhodophyceae are photosynthetic eukaryotes which accumulate starch granules outside the plastids named floridean starch. These granules contain all the major features found in Chloroplastida starch. In spite of the initial report that floridean starch lacked amylose [18, 19], it was demonstrated that some red alga lineages such as the Porphyridiales also accumulate this glucan fraction [126, 127].

The extra-plastidic starch synthesis is performed by an UDPGlc-selective α-glucan synthase, unlike what happens in plants, where the synthesis occurs within plastids, but similar to the cytosolic synthesis of glycogen that occurs in other eukaryotes. Viola et al. (2001), suggested that given the arising consensus of the monophyletic origin of plastids, the capacity for starch synthesis might have selectively evolved from an α-glucan synthesizing machinery of the host ancestor and its endosymbiont in red algae and green algae, respectively [128].

On the other hand, Glaucophytes are a small group of microscopic algae found in freshwater environments. There are only about 13 species of glaucophytes, and although not particularly common in nature they are important because they occupy a pivotal position in the evolution of photosynthesis in eukaryotes. They also represent an intermediate in the transition from endosymbiont to plastids due to the presence of the prokaryotic peptidoglycan layer between their two membranes [129].

Price et al. (2012) performed an exhaustive analysis of the genome and transcriptome data from *Cyanophora paradoxa* and they have provided evidence for a single origin of the primary plastid in the eukaryote supergroup Plantae [86]. Indeed, several putative carbohydrate metabolism enzymes in *C. paradoxa* were identified using the Carbohydrate-Active

 

enZymes (CAZy) database [130] (see also The Cyanophora paradoxa Genome project, http://dblab.rutgers.edu/cyanophora/home.php). It was reported that the genome of this alga encodes 84 glycoside hydrolases (GHs) and 128 GTs, which is more than those present in *O. lucimarinus* (30 GHs and 69 GTs), but less than in *A. thaliana* (400 GHs and 468 GTs). It was also described that many of the above *C. paradoxa* proteins are involved in starch metabolism. Particularly, the major protein is phylogenetically related to the GT5 UDP-Glc specific enzyme of heterotrophic eukaryotes, suggesting that UDPGlc is the main nucleotide-sugar donor for starch synthesis in this alga [17, 30, 58].

Furthermore, another gene in the glaucophyte genome was detected whose product is related to the SSIII–SSIV from plants. This gene is phylogenetically related to glucan synthase in Chlamydiae, Cyanobacteria, and some Proteobacteria, possibly playing a role in linking the metabolism of the host and the endosymbiont. Because SSIII and SSIV enzymes uses preferentially ADPGlc in bacteria and plants [30, 131–134], it is possible to postulate that *C. paradoxa* or, alternatively, the common ancestor of Viridiplantae and Glaucophytes may have used both, ADPGlc or UDPGlc for starch synthesis [86].

Table 1 resumes the main storage polysaccharide characteristics from the members of Green Linage *Arabidopsis*, *O. tauri*, and *C. reinhardii*, the red alga *C. merolae*, and the Glaucophyte *C. paradoxa* [13, 53, 86, 111, 135, 136].

## 5 Biofuels: Biotechnological applications and uses of algae starch

The importance of a variety of renewable biofuels has been renovated due to the volatility of petroleum fuel costs and consequences resulting from the greenhouse emissions [137]. The interest in photosynthetic algae (microalgae and macroalgae) as a possible biofuels resource has considerably increased in the last years. Some algae species have higher biomass production rates compared to terrestrial plants [138]. In addition, many eukaryotic microalgae are able to store important amounts of energy rich compounds, such as starch and triacylglycerol (TAG) that can be utilized for the production of different biofuels, including biodiesel and ethanol [139].

Carbohydrates can be metabolized into a multiplicity of biofuels, such as ethanol, butanol, hydrogen, lipids, and/or methane. Polyglucans are accumulated in microalgae in a variety of ways. As we mentioned above the phyla Chlorophyta and Rhodophyta store α-1,4 and branched α-1,6 glucans [99].

While the use of algae with enriched starch content is conventional for the production of bioethanol, another attractive exploitation of starch from algae might be the production of hydrogen, which may be realized soon [140–142]. It has been described that sulfur limitation could be one of the ways to promote hydrogen production [143, 144]. In this way, recently it was shown that some *Chlorella* strains can produce and accumulate a significant volume of hydrogen gas under anaerobic conditions and sulfur deprivation as it was also reported for *C. reinhardtii* [145]. Another example might be *Chlorococcum*, that was also proposed for bioethanol production via dark fermentation of starch [146, 147]. Furthermore, this alga was also evaluated as a source of lipid for biodiesel production [148–150].

Unicellular microalgae are at the vanguard of research efforts directed at developing model systems and their corresponding technologies for the production of hydrogen and other fuels [138, 151, 152]. Compared with terrestrial plants, microalgae are much more efficient in converting sunlight into chemical energy, and need less water for cultivation [138]. Many species of algae that grow in salt water, are also able to grow on various conditions, and do not accumulate recalcitrant lignocellulosic biomass [138]. Actually, genetic and biotechnological manipulation techniques have been developed for some species, and are increasingly being applied to optimize biofuel production in several algal systems [152].

*This work was supported by grants from Biotechnology Program from Universidad Nacional de General San Martin (UNSAM) (PROG07F/2-2007), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP 00237) and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, PICT 2010–0543 and 0069, and PICT 2011 – 0982). J. B. is a postdoctoral fellow from CONICET. M. V. B., M. M., and D. G. C. are research members from CONICET.*

*The authors have declared no conflict of interest.*

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