PORPHYRA: COMPLEX LIFE HISTORIES IN A HARSH ENVIRONMENT: *P. UMBILICALIS, AN INTERTIDAL RED ALGA FOR GENOMIC ANALYSIS*

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1. Introduction

The red algal genus *Porphyra* ("nori," "laver") is species-rich and widely distributed (Brodie and Zuccarello, 2007). The gametophyte is characterized by a large, foliose blade or thallus, which in many locations alternates with a small, filamentous sporophyte (the shell-boring "conchocelis" phase). The reproductive phases among the *Porphyra* species can vary greatly, with essential details of reproductive life-histories summarized in Brodie and Irvine (2003) and Fig. 1. The life history of *Porphyra* was elucidated by Kathleen Drew (1949), who first demonstrated alternation between the blade and conchocelis phases in a British isolate of *Porphyra*, which she initially identified as *P. umbilicalis* but which was almost certainly *P. dioica* (Brodie and Irvine, 2003; Brodie et al., 2008). Drew's incisive observations allowed for greatly improved production of the economically important *P. yezoensis* (nori) in Japan, beginning in the 1950s (Ueda, 1958; as cited in Kafuku and Ikenoue, 1983).

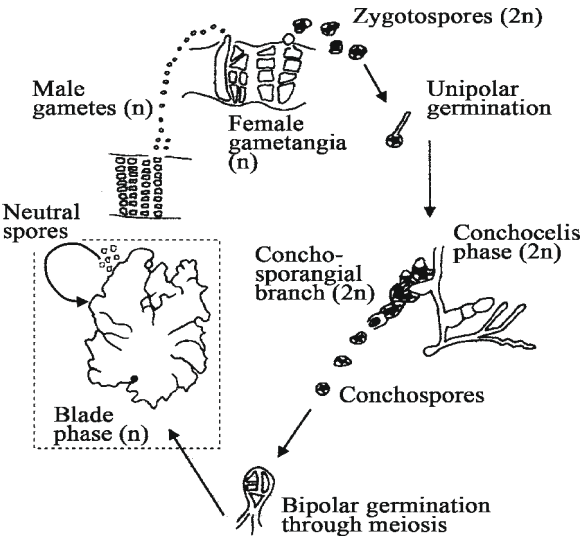

Figure 1. Heteromorphic, sexual life history of *Porphyra*. Both phases of the life history produce spores that regenerate each phase directly. The boxed stage is a focus of the *Porphyra* genomics' project (courtesy of M. Holmes and J. Brodie).

Table 1. Classes of Rhodophyta showing evolution of key characteristics in Bangiophyceae.

Class sensu Yoon et al. (2006)	Unicellular (U) or multicellular (M)	Asexual spores	Sexual reproduction	Complex life histories	Pit plugs
Cyanidiophyceae	U	–	–	–	–
Porphyridiophyceae	U	–	–	–	–
Rhodellophyceae	U	–	–	–	–
Compsopogonophyceae	M	Present	Present	–	–
Stylonematophyceae	M	Present	Present	–	–
Bangiophyceae	M	Present	Present	Present	Present
Florideophyceae	M	Present	Present	Present	Present

Today, the aquaculture crop of nori in Japan, China, and Korea is worth more than $1.4 billion/year (Yarish and Pereira, 2008). *Porphyra* is valued as human food because of its high level of protein (25–50%), vitamins (B_{12}, C), trace minerals, and dietary fiber (Noda, 1993; MacArtain et al., 2007). *Porphyra umbilicalis* is currently a target of pilot aquaculture in North America (Blouin et al., 2007).

Porphyra belongs to the order Bangiales (Bangiophyceae) (Table 1), which represents an ancient lineage with fossil records that provide evidence for sexual reproduction that dates to at least 1.2 BYA (Butterfield, 2000). Traditionally, *Porphyra* species have been largely delineated on the basis of blade characteristics (e.g., pigmentation, blade thickness, cell dimensions), but recent molecular analyses suggest that the genus is polyphyletic (Oliveira et al., 1995; Nelson et al., 2006). The filamentous (*Bangia*) and blade-like forms (*Porphyra*) of the Bangiophyceae exhibit convergent functional and morphological tendencies, which appear to have arisen numerous times during the evolution of these genera.

It is generally accepted that multicellularity arose independently in different major eukaryotic lineages (Baldauf, 2008). Detailed investigations indicate that many genetic building blocks critical for attaining multicellularity are shared by animal and plant model organisms. Some key innovations thought to have been important in the radiation of developmental patterns in red algae were already present in the common ancestor of *Porphyra*, while others had yet to develop. For example, pit plugs (= pit connections) are a strongly conserved feature of red algae, and are thought to have played important roles in the evolution of structural diversity, reproductive strategies, and ecological adaptation. Pit plugs are always present in the florideophyte red algae, where variations in their ultrastructure are highly informative in ordinal-level systematics (Pueschel, 1994). The relative importance of pit plugs to red algal metabolic circuits, core physiological functions, and evolutionary processes are largely unknown. Although pit plugs are invariably present in florideophytes, simpler and presumably ancestral forms of this structure are found in the conchocelis phase of members of the Bangiales (Bourne et al., 1970; Lee and Fultz, 1970; Ueki et al., 2008). Thus, comparative transcriptomics of gametophyte and sporophyte stages of *Porphyra* offer an unparalleled opportunity to identify genes encoding pit plug structural elements and to unravel genetic and metabolic networks involved in their biogenesis.

2. An Emphasis on the Genome of *Porphyra umbilicalis*

To date, the only red algal genomes for which there are complete or nearly complete sequences are the acidothermophiles *Cyanidioschyzon merolae* (Matsuzaki et al., 2004; Nozaki et al., 2007) and *Galdieria sulphuraria* (Barbier et al., 2005). These algae belong to the Cyanidiophyceae, which has most likely experienced extensive genome reduction and elevated gene divergence rates as a result of adaptation to the specialized, extreme habitats of the fumaroles and acidic waters in which they live. The genome of *C. merolae* (16.5 Mb) is highly compact with a low number of protein-encoding genes (~5,300), relatively few transposable elements (Nozaki et al., 2007), and the occurrence of introns in only 26 genes (Matsuzaki et al., 2004). The haploid genome of *P. purpurea* was determined by flow cytometry to be ~270 Mb (using nuclei isolated from protoplasts, Le Gall et al., 1993), a value that is many times larger than the genome sizes of *C. merolae* and *G. sulphuraria*. Therefore, it is reasonable to assume that the *Porphyra* genome contains many more protein-encoding genes than are present in these highly modified, unicellular taxa. The genomic complexity of *Porphyra* is expected to be more like that of free-living, mesophilic rhodophytes.

The chromosome number of *Porphyra* species tends to be low when compared with that of many other red algae. The haploid (n) chromosome number of *P. umbilicalis* is reported to range from 3 to 5 (rf. Table 4.1 in Cole, 1990), which may be correlated with the geographic distribution of different isolates, cryptic taxa, or misidentifications. Brodie and Irvine (2003) found n=4 in *P. umbilicalis* from British shores, and *P. purpurea* (the "type species") also has four chromosomes, whereas *P. yezoensis* generally has three (Wilkes et al., 1999). We chose to generate genomic DNA from a strain of *P. umbilicalis* that is abundant on the northeastern coast of the USA, because the blade reproduces asexually by neutral spores, making it possible to obtain large quantities of homogeneous genetic material. The imminent sequencing of the *P. umbilicalis* genome by the Joint Genome Institute and the subsequent analyses of its sequence will provide us with insights into (1) mechanisms associated with the transition from a unicellular life mode to one of multicellularity, including regulatory elements critical for that transition, (2) the development of the sexual phases of the life cycle, and (3) adaptive mechanisms that enable the organism to cope with physiological stresses, including desiccation, high light, and nutrient deprivation.

3. Asexual Reproduction

Four types of asexual spores are produced by the *Porphyra* blade (Nelson et al., 1999); they include archeospores, neutral spores, agamospores, and endospores. Understanding and exploiting asexual reproduction can be highly beneficial for commercial cultivation of *Porphyra*. For example, the production of asexual

spores results in an increased "set" of gametophytic thalli on the cultivation nets and allows for a longer cultivation period. Also, archeospores can be used for establishing diverse, valuable lines, including mutants that have a desirable texture or color. The different spore types are described below:

1. An archeospore (= monospore in older literature) is formed by differentiation of a vegetative blade cell into a single spore, which germinates into the blade/foliose phase. Some species of *Porphyra* form archeospores only on young thalli a few millimeters long, whereas other *Porphyra* species (e.g., *P. yezoensis*) continue producing archeospores on thalli several centimeters long. Recently, Kitade et al. (2008) reported the identification of candidate genes involved in asexual reproduction in *P. yezoensis*.
2. Neutral spores are formed by mitotic cleavage of blade cells; such spores germinate and develop into new blades. Gametophytic *P. umbilicalis* regenerates the blade directly through the production of neutral spores throughout the year on the coast of Maine. The viability of these spores declines in summer, and sexual reproduction has not been observed in this population (Blouin et al., 2007), in contrast to northeastern Atlantic *P. umbilicalis* where both sexual and asexual reproduction are observed (Brodie and Irvine, 2003).
3. Agamospores are formed by mitotic cleavage of blade cells, without fertilization, and under appropriate conditions may germinate into conchocelis filaments.
4. Endospores are formed by mitotic divisions of blade cells, and occur as an irregularly arranged and indefinite number of spores encased in a distinct envelope.
5. Two types of asexual spores (neutral conchospores and archeospores) that develop from the conchocelis stage are also known (Knight and Nelson, 1999; Nelson et al., 1999).

4. Genome Evolution

Genome evolution is a complex process, characterized by a series of sometimes overlapping evolutionary events. Lateral gene transfer (LGT, the transfer of genes between two different strains or species) is a ubiquitous phenomenon whereby exogenous genetic material is taken into an organism and is subsequently stably incorporated into its genome. The contribution of LGT to genome innovation and physiological diversity of life forms has been highlighted in recent years (Boucher et al., 2003). Using a phylogenetic approach for multigenomic analysis, previous studies have demonstrated that LGT is prominent among prokaryotes and some eukaryotes (Keeling and Palmer, 2008). Because many algae have lost their ancestral phagotrophic capacity, the extent of LGT between algae and other species or lineages may be limited, but LGT in algae has been noted in a number of studies, most of which involve intron sequences (e.g., between brown and red algae [Bhattacharya et al., 2001]; between fungi and red algae [Müller et al., 2005]). A prime example of LGT is provided by the location of genes that regulate plastid structure and function,

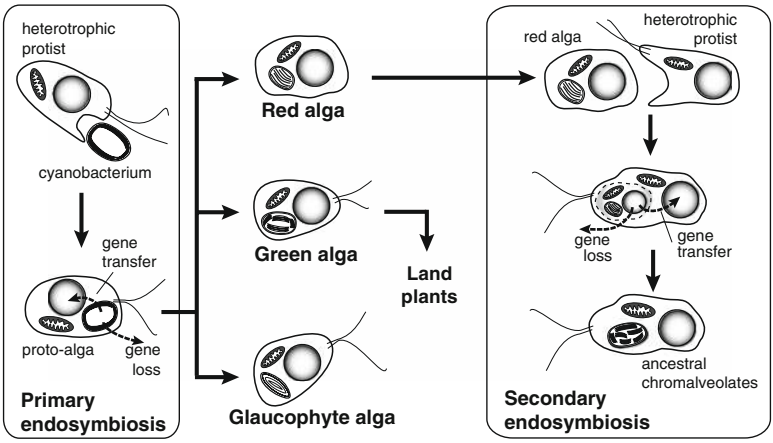

Figure 2. Endosymbiosis and gene transfer model leading from cyanobacteria to the red algae through primary endosymbiosis (*left*), and subsequently, through secondary endosymbiosis to other algal lineages including brown algae, cryptomonads, diatoms, dinoflagellates, haptophytes, and apicomplexans. Each transfer also resulted in gene losses (courtesy of Cheong Xin Chan).

which evolved from the ancestral genes of the engulfed primary endosymbiont, a cyanobacterium; these genes are now present in both the red algal chloroplast and nucleus (Fig. 2). Subsequent secondary and even tertiary endosymbiotic events produced additional algal lineages and led to further gene transfers in the new host organism (Reyes-Prieto et al., 2007).

The much larger size and, presumably, greater number of genes in the genome of *P. umbilicalis* compared to *C. merolae* will provide additional information to test the idea of Plantae monophyly. This includes a better knowledge of:

1. The diversity of genes encoding enzymes of common metabolic pathways that function in plastids of *Porphyra* and other Plantae members (e.g., amino acid, lipid, and cofactor biosynthesis)
2. LGT between *Porphyra* and other algal lineages (e.g., Calvin-Benson Cycle enzymes in plastids; Reyes-Prieto and Bhattacharya, 2007)
3. Putative gene fusions that may have occurred in the chromosome of the ancestral host prior to the radiation of the Plantae (Gross et al., 2008)

Other phylogenetically relevant genes are those that encode products that function in plastids (Fig. 2) and that are common to plants and algae of the green, red, and glaucophyte lineages, but absent in cyanobacteria. These genes originated *de novo* from the chromosome of the host or were recruited from pre-existing genes to express a new function in the organelle. Examples that can be examined in the *P. umbilicalis* genome include the light-harvesting protein family, solute transporters on the inner plastid membrane, and the TOC/TIC plastid protein translocons (Kalanon and McFadden, 2008).

Table 2. Genome size (Mb) and percentage of the genome corresponding to transposable elements (TE).

Organism	Assembly size (Mb)	TE (~genome %)
Ostreococcus tauri	12.6	10
Cyanidioschyzon merolae	16.5	0.7
Thalassiosira pseudonana	34.5	2
Arabidopsis thaliana	140.1	15
Oriza sativa	430	14
Homo sapiens	3,000	44

Transposable elements (TEs) are believed to play a fundamental role in genome evolution, adaptation to diverse environmental conditions and speciation (Kazazian, 2004), and to be a major source of variation in genome size among eukaryotes. TEs are considered mobile elements, able to move to other locations within the genome. At this juncture, there is limited evidence of a linear relationship between the total number of transposable elements in the genome of an organism and genome size (Kidwell, 2002); however, it is still likely that the ~270 Mb genome of *P. umbilicalis* will have more TEs than the relatively few that are found in *C. merolae* (Table 2.). The number and features of TEs in the *P. umbilicalis* genome will augment our limited understanding of the role of these elements in red algal evolution.

It will be important to determine the genome content and possible regulatory roles of small RNAs (~20–40 nt RNAs) in *P. umbilicalis*. Small RNAs can regulate gene expression through a variety of mechanisms, including RNA decay, DNA methylation, or translation efficiency (Wu and Belasco, 2008; Vazquez, 2006). Small RNAs (for example microRNAs [miRNA], and small-interfering RNAs [siRNA]) are incorporated into protein complexes (e.g., Argonaute/Piwi proteins, with PIWI and PAZ domains) that ultimately control their regulatory function (Takeda et al., 2008). When a BLAST search was conducted (L. Rymarquis, unpublished) against the ~30,000 sequences from various *Porphyra* species present in the NCBI database, it was observed that several ESTs of *P. yezoensis* could potentially encode proteins containing the PIWI and PAZ domains characteristic of Argonaute/Piwi family proteins. Analyses of such sequences, as well as small RNA sequences, will ultimately determine what types of small RNAs, if any, are produced by *P. umbilicalis* and may provide clues to their mode of action.

5. Associations with Other Organisms

Various types of associations between red algae and other organisms have been noted, but are still little studied. Some associations are disadvantageous, such as the pathogenic responses elicited when cultivated *Porphyra* is infected by the oomycete *Pythium porphyrae*. This fungus, which is the causative agent of red rot

disease in *Porphyra*, can infect and encyst on many different red algae, as shown by infection experiments of Uppalapati and Fujita (2000). Zoospores attach to *Porphyra* and *Bangia*, with a successful invasion of the algal tissue being dependent on sulfated galactans (porphyran, commercial agar, agarose, and carrageenans).

Vitamin B_{12} is needed as a growth factor by several algal species, serving as a cofactor for methionine synthase (METH). Croft et al. (2005) reported that the vitamin could be supplied by symbiotic bacteria. On the other hand, Droop (2007) has proposed that in the natural environment, there is sufficient cobalamin in seawater for algal growth. Whether or not *Porphyra* requires exogenous B_{12} vitamins for growth remains to be determined, but Takenaka et al. (2003) found that axenically grown *P. yezoensis* contained nearly the same concentration of B_{12} as wild plants. Some red algae do not require B_{12}; for example, *Porphyridium purpureum* (*P. cruentum*) has been grown axenically in a chemically defined artificial seawater medium for decades (E. Gantt, personal observation). Genomic analysis of *Cyanidioschyzon merolae* (Matsuzaki et al., 2004) revealed that it encodes an alternative, B_{12}-independent form of methionine synthase, METE. The *Porphyra* sequencing project will provide the first genomic information about the role of B_{12} in a multicellular red alga, and may have implications in farming of seaweed for food, and/or for the production of vitamins.

6. Cytoskeletons, Cell Division, and Flagellar Genes

Forty-four different cell-cycle genes and ten different cytoskeletal genes have been identified from the existing *P. yezoensis* EST data set (S. Lin and Y.Y. Zhuang, unpublished analysis). The cell-cycle-related genes include core regulatory genes such as cyclin kinases (*cdc2* and other CDKs), CDK regulators, cyclins (e.g., cyclin E, which regulates the transition from G_1 to S phase; cyclin B, which regulates the G_2 to M transition), and the anaphase promotion complex proteins, plus a proliferating cell nuclear antigen (PCNA). Phylogenetic analysis reveals a high divergence of these genes across different algal groups and other organisms. According to this analysis, *Porphyra* does not appear to be particularly ancient with respect to the core components of the cell cycle, although information on genetic regulatory networks that control cell cycle progression and cytoskeletal function in algae is still very limited, particularly for *Porphyra*.

Red algae have no flagella or basal bodies (centrioles), structures involved in motility, mitosis, and sensory functions in many organisms. These structures are considered to be features of the ancestral protist that gave rise to the red algal lineage, but have been lost during red algal evolution, as also occurred in some green algae and during the evolution of land plants (Merchant et al., 2007). Based on this hypothesis, red algal genomes may contain remnant genes or pseudogenes that encode sequences that resemble flagellar and basal body proteins. Tubulin and kinesin are known from *Cyanidioschyzon*, but dynein is absent, consistent with the absence of a flagellum.

Many red algal spores (e.g., archeospores, tetraspores, carpospores; see Pickett-Heaps et al., 2001) exhibit an amoeboid type of cell motility, which has also been observed in neutral spores of *P. umbilicalis* (Brodie and Irvine, 2003; N. Blouin, personal observation). Analysis of this motility in archeospores of *Porphyra pulchella* (Ackland et al., 2007) demonstrated that pseudopodial activity was dependent on an actin/myosin cytoskeleton, with the actin filaments arrayed in short and long peripheral bundles.

7. Photosynthesis, and Light Absorbing Molecules

The only chlorophyll present in *P. umbilicalis*, as in other red algae, is chlorophyll *a*. Earlier reports claiming the presence of chlorophyll *d* in certain red algae are now thought to reflect the adherence of chlorophyll *d* producing cyanobacterial epiphytes to the algal surface. Enzymes required for the biosynthesis of chlorophyll *a* as ones use in green plants (Masuda, 2008) are known. Genes encoding many of these essential biosynthetic enzymes also occur in *Cyanidioschyzon* and in the cyanobacterium *Synechocystis* PCC 6803. While very likely, it remains to be determined whether *P. umbilicalis* employs a similar biosynthetic pathway to chlorophyll *a*.

It is very likely that the plastid genome of *P. umbilicalis* is very similar to that of its relative *P. purpurea,* which has a chloroplast genome of about 191 kb (Reith and Munholland, 1995), larger than that of *Cyanidioschyzon* (ca. 150 kb) (Ohta et al., 2003). Current estimates predict that the plastid genome of *Porphyra* encodes over 200 proteins one third of which are not present in the *Arabidopsis* chloroplast genome (Reith and Munholland, 1995). Despite the large size of the red algal chloroplast genome and its cyanobacterial origin, several notable genes expected to reside on the plastid genome have not been found; these include *ndh* (energy conversion protein), *infA* (translation factor), and *clpP* (ATP-dependent protease). The genes encoding reaction center proteins of photosystem (PS) I and II, and most of the genes encoding phycobilisome polypeptides (major light harvesting complex of PS II, see later) are encoded on the chloroplast genome.

In *P. umbilicalis*, the major PS II antenna, the phycobilisomes, are generally ellipsoidal in shape (Algarra et al., 1990). The predominant pigment within phycobilisomes is rhodophycean phycoerythrin (R-PE) with absorbance maxima (A_{max}) at ca. 498, 542, and 565 nm. R-phycocyanin (A_{max} of 553 and 615 nm) and allophycocyanin (A_{max} of 650 nm) are important but occur in relatively lower amounts, which is rather typical for PE-containing rhodophytes (Gantt et al., 2003). Functionally significant for extended light absorption is the development of chlorophyll-carotenoid binding proteins (LHC I) associated with PS I (Wolfe et al., 1994). Because small proteins with domains having homology to LHC I polypeptides are present in cyanobacteria (Dolganov et al., 1995), it is assumed that following the primary endosymbiosis, fusion of genes encoding these cyanobacterial proteins (as well as the movement of the genes to the nuclear genome of the host) was critical for the evolution of the LHC gene family. Phycobilisomes in *Porphyridium purpureum (cruentum)*

are primarily, if not exclusively, associated with PS II, and only secondarily transfer excitation energy to PS I. Enhancing the light absorbance capacity for both photosystems represents a significant evolutionary advantage. The association of LHCs directly with the PS I reaction centers was established in *Porphyridium* (Wolfe et al., 1994), but can be expected to occur in other red algae based on comparisons of LHC protein sequences and the occurrence and amount of pigments that function in light absorption (Gantt et al., 2003).

Red algae are relatively diverse in the composition of their carotenoids, pigments that may both enhance light absorbance capacity and protect against photo-oxidation. The red alga *C. merolae* synthesizes only a few β-ring carotenoids (i.e., β-carotene and zeaxanthin; Cunningham et al., 2006). Other red algae produce carotenoids with ε-rings as well as β-rings (i.e., α-carotene [β, ε-carotene] and lutein), and still others accumulate epoxycarotenoids such as antheraxanthin and violaxanthin (Marquardt and Hanelt, 2004; Schubert et al., 2006). Lutein and ß-carotene are the predominant carotenoids of *P. umbilicalis* (Fig. 3). and of other species of *Porphyra* (Shimma and Taguchi, 1966; Schubert et al., 2006), with lesser amounts of α-carotene and zeaxanthin also typically observed. Epoxycarotenoids such as antheraxanthin and violaxanthin were not detected in a recent analysis (Fig. 3), and have not been identified in other *Porphyra* species (Schubert et al., 2006). Interestingly, the lutein in dried *P. yezoensis* was reported to be present largely as a cis-geometrical isomer (Shimma and Taguchi, 1966), which is potentially an artifact associated with drying of the samples.

Of particular interest regarding the carotenoid pathway of *P. umbilicalis* is the identity of the enzyme catalyzing the formation of ε-rings. The ε-ring cyclase of plants and green algae is thought to have originated by duplication of a gene encoding a ß-ring cyclase, and plant ε-cyclase enzymes retain some ability to

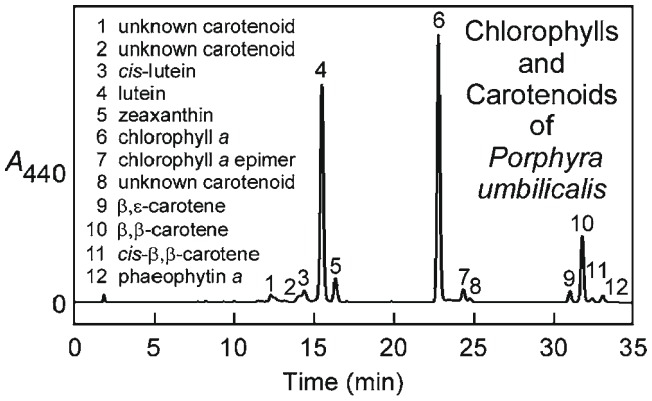

Figure 3. HPLC elution profile of lipid-soluble pigments extracted from a *P. umbilicalis* thallus from the coast of Maine. Extraction and analysis was as in Cunningham et al. (2006), except the mobile phase gradient was 50% B (ethyl acetate) in A (acetonitrile/methano/triethylamine, 90/10/0.1) (courtesy of F.X. Cunningham).

produce β-rings as well as ε-rings (Cunningham et al., 2006). Also of interest is the origin of the enzyme or enzymes responsible for 3-hydroxylation of carotenoid β- and ε-rings, thereby enabling the synthesis of zeaxanthin and lutein. *Cyanidioschyzon merolae* has a plastid gene encoding a polypeptide similar in sequence to cyanobacterial CrtR-type ß-carotene 3-hydroxylases, but the chloroplast genomes of *P. yezoensis* and *P. purpurea* do not have such a gene, and a gene encoding a polypeptide similar to CrtR or to plant or green algal CrtZ-type ß-carotene 3-hydroxylases is not apparent in the available *Porphyra* EST sequences.

Exposure to high light, especially when coupled with desiccation or nutrient limitation, can cause severe damage to the photosynthetic apparatus as well as to other cellular constituents and processes. This damage results largely from the accumulation of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) (Halliwell and Gutteridge, 1999). The seasonal production of some antioxidants by *P. umbilicalis* was studied by Sampath-Wiley et al. (2008). These workers observed an accumulation of the highest levels of antioxidants during the summer, with levels of glutathione reductase, catalase, and carotenoids being higher during times of emersion relative to immersion. Diffusion could also play a role in eliminating H_2O_2 from *Porphyra* because of its thin blade (Collén et al., 1995). Analysis of the genome of *P. umbilicalis* will help researchers decipher potential mechanisms by which this alga, and potentially other desiccation-tolerant, high-light-resistant algae can cope with extremely high excitation pressure.

Mycosporine-like amino acids (MAAs) provide protection against UV radiation that can damage the cell's DNA and photosynthetic apparatus (Shick and Dunlap, 2002; Sinha et al., 2007). While *Porphyra* spp. can synthesize a number of different MAAs (shinorine and porphyra-334; Takano et al., 1979; Sinha et al., 2007), the details of their synthesis and their precise intracellular functions remain to be determined. More work is required to understand how the rich arrays of light-absorbing molecules in the red algae are co-regulated.

8. Carbohydrates: Storage and Cell Wall Composition

Unlike other photosynthetic eukaryotes that synthesize and store carbohydrate reserves in the plastid, red algae synthesize and store starch in the cytoplasm (Viola et al., 2001). This "floridean" starch consists of a backbone of α-1,4 linked glucan with α-1,6 branches, and it is structurally more similar to amylopectin than to glycogen. Floridoside (2-O-α-D-galactopyranosylglycerol) is the main source of soluble storage carbon in red algae, with a carbon transport function much like that of sucrose (Viola et al., 2001). Like sucrose, floridoside is synthesized via a phosphorylated intermediate in the cytosol. The genes for enzymes in floridoside metabolism have been identified in *C. merolae* and *G. sulphuraria* (Barbier et al., 2005), but are yet to be characterized in *Porphyra.* In *Gracilaria tenuistipitata*, the nucleotide sugar UDP Glucose (UDPGlc) appears to be the preferred substrate

for synthesis of floridean starch. The synthesis and accumulation of floridean starch present something of an evolutionary puzzle: features of its synthesis (from UDPGlc in the cytoplasm) are similar to features of glycogen synthesis in fungi and animals, whereas the structure of floridean starch is more akin to the carbon reserves accumulated in the plastids of chlorophytes.

The inner cell walls of the blade phase of the *Porphyra* thallus are reticulate and multilayered with xylan microfibrils in an amorphous matrix; these provide structural support for the thallus. The outer wall matrix is less structured and consists largely of porphyran (up to 30% dry wt.), a linear polymer of galactans that are sulfated to different degrees (Mukai et al., 1981). On the outer surface of the cell wall, a morphologically distinct cuticle of neutral polysaccharides is found; the cuticle is also protein-rich, unlike the waxy cuticle of vascular plants. The polysaccharide chains associated with the cell wall are synthesized in the Golgi complex from sugar nucleotide units (Cole et al., 1985).

An interesting evolutionary question is the occurrence of crystalline cellulose in the filamentous conchocelis phase of *P. leucosticta* (Gretz et al., 1986), and its absence in the gametophytic blade. In red algae in general, and in *Porphyra* in particular, different polymers fulfill the same function in the cell wall in different cell types or at different phases of the life cycle. Understanding the biosynthesis of the *Porphyra* cell wall over its life cycle and the specific functional associations of the individual wall components may provide important insights into the dynamics of cell wall function, structure, and biosynthesis, especially as they relate to the daily hydration extremes experienced in the intertidal zone. Genomic studies will also contribute to our understanding of the evolution of cellulose synthesizing enzymes.

9. Circadian and Circannual Rhythms

Porphyra species occupy distinct areas of the intertidal and subtidal zones, which suggests that different species may have different tolerances for water loss. *P. umbilicalis* is common in the high and mid-intertidal zones, and as is true of many intertidal *Porphyra* species, it dries to a fraction of its wet weight during daytime low tides. The molecular mechanisms for desiccation tolerance are currently unknown, but some may be regulated by an internal clock. Anticipating predictable environmental changes, on both diurnal and seasonal time scales, would prepare *Porphyra* for rhythmic changes in the environment. Indeed, growth and cell division occur principally in the dark phase of a 16:8 L:D cycle, and *P. umbilicalis* cells enter prophase near the end of the light period (Lüning et al., 1997). These rhythms persisted in constant light, and the free-running rhythm found for growth under constant green or red light was observed to be intensity-dependent, whereas growth under constant blue light was arhythmic (Lüning et al., 1997; Lüning, 2001). Lüning (2001) suggested that the circadian oscillator was especially sensitive to blue light but uses all visible wavelengths in synchronization of the rhythm. The existing data supported cryptochrome, phytochrome, and/or

opsins as potential photoreceptors for the rhythm (Lüning, 2001). *Porphyra* also undergoes many developmental transitions (e.g., gametophytic to sporophytic transitions in the life cycle) that are regulated by photoperiod (e.g., Tseng, 1981). Rapid progress in identification of the photoreceptors involved in these processes should be possible when the draft genome of *P. umbilicalis* becomes available.

10. Nutrient Acquisition

The *Porphyra* blade is one or two cells thick (a species-dependent character), which provides the organism with an extremely high surface area to volume ratio for rapid nutrient uptake (Kraemer et al., 2004). Interestingly, Kim et al. (2007) found that *P. umbilicalis* was able to take up nitrate from the surrounding medium more rapidly than several species with thinner thalli. Nitrogen uptake experiments on *P. yezoensis* and *P. purpurea* found that NH_4^+ was preferred over other nitrogen sources, such as NO_3^- (Kraemer et al., 2004). This information is particularly relevant for mixed aquaculture designs. An understanding of mechanisms involved in nitrogen acquisition in *Porphyra* is clearly important from an economic perspective, because *Porphyra umbilicalis,* and several other species, have been used in demonstration-scale, integrated multitrophic aquaculture systems (IMTAs) in the northeastern US. *Porphyra* functions as an extractive component of aquaculture systems by removing ammonium and other finfish wastes that serve as required nutrients for the alga (Carmona et al., 2006; Blouin et al., 2007).

Many of the components involved in sulfate uptake and assimilation in *P. yezoensis* were identified from nucleotide sequences present in the EST database (www.kazusa.or.jp/en/plant/porphyra/EST/) (Asamizu et al., 2003). In *P. purpurea* and *P. yezoensis*, cDNAs were isolated that are likely to encode known sulfate transporters and enzymes involved in reductive assimilation of sulfate (adenosine 5′-phosphosulfate kinase, sulfate adenyltransferase, cysteine synthase, sulfite reductase) (Minocha et al., 2008). Furthermore, analyses of cDNA libraries offer the potential for identification of enzymes that specifically catalyze the sulfonation of carrageenans or agars, which will greatly aid in our overall understanding of the flux of sulfur metabolites in *Porphyra,* the mechanisms involved in sulfonation, and functions of sulfonated polysaccharides.

11. Future Approaches and Challenges

There are many uncertainties about the ways in which *Porphyra* cells adjust their physiology/metabolism to accommodate stresses such as desiccation or to undergo cellular differentiation and development. The imminent sequencing of the *P. umbilicalis* genome by the Joint Genome Institutes, the availability of a diversity of *Porphyra* species with different tolerances to desiccation, and the finding that tolerant species can rapidly rehydrate and activate cellular metabolism make

Porphyra the genus of choice for developing a model system to define the critical mechanisms that enable organisms to cope with extreme water loss. At this juncture, there are also many molecular and genomic tools that would provide new insights into the desiccation process and its consequences. These tools include EST sequences, which have already been generated to some extent for *P. yezoensis* and *P. haitanensis* (Nikaido et al., 2000; Lee et al., 2000; Fan et al., 2007); the construction of BAC and plasmid libraries; further generation of mutants (Miura, 1990; Niwa et al., 1993; Mitman and van der Merr, 1994; Zhang et al., 2005) and especially selected mutant libraries; the development of a transformation system (Fukuda et al., 2008); and the application of high throughput technologies for generating in-depth transcriptome information (e.g., by 454 sequencing of the transcriptome when there is no complete genome sequence and Illumina sequencing when there is a fully sequenced genome), which can be used for identifying differentially expressed genes (Pearson et al., 2001). For these reasons, and many others, the *Porphyra* genome offers unique opportunities to investigate what was probably one of the first eukaryotic forays into structural complexity. It is quite likely to provide exciting and novel insights into the evolution of adaptation processes, the control of complex developmental programs, and the establishment of multicellularity in eukaryotes.

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