8 Reactive Oxygen Species (ROS)

Reactive oxygen species are comprised of singlet oxygen ($^1O_2$), hydroxyl radical (OH^2), hydrogen peroxide (H_2O_2) and a number of smaller constituents. All of these radicals have short half-lives and react with organic molecules to form oxidation products; singlet oxygen has a half-life of ~3.5 μs at normal temperatures. With lipids, lipid peroxides are formed which may be quite detrimental to normal membrane-based reactions. Thus the formation of ROS, which is unavoidable when electron transport processes are involved, is an activity, which is (a) curtailed as far as possible by special biochemical processes (see below) and (b) mitigated by repair processes. A prime example is in PSII where for reasons of proper functioning a carotenoid triplet valve cannot be put in place and D1 protein is actively degraded during photosynthesis and must be actively replaced.

A set of protective measures are set in place in eukaryotic alga systems to mitigate the formation of ROS. One of these is the alternate oxygenase (NOX). Another is the Mehler Ascorbate Peroxidase (MAP) pathway, whereby electrons are channeled out of the reducing side of PSI to oxygen, rather than feeding into NADP reduction. These reactions have only attracted attention relatively recently and their true importance is only now being realized by an increasing number of sophisticated investigations.

9 Evolution of Photosynthetic Proteins Involved in Photoprotection and Light Harvesting

Balancing efficient capture of light against the damaging effects of high light is a problem faced by all photosynthetic organisms and results in the phenomenon of photoinhibition. It is therefore clear why both oxygenic and anoxygenic photosynthetic organisms have evolved mechanism to deal with situations of low light and high light. As discussed at the beginning of this chapter, curiously there is no phylogenetic link between the light harvesting proteins of these two types of photosynthetic organisms. This is despite a clear phylogenetic link between the proteins of their Reaction Centres. For the proteins involved in light harvesting and photoprotection in oxygenic photosynthetic organisms there are some clear links. Furthermore, it is likely that in Cyanobacteria, lightharvesting proteins involving Chl evolved before phycobiliproteins; however, these isiA proteins bear no relation to the light-harvesting proteins of eukaryotic algae and land plants, on the one hand, nor to the anoxygenic photosynthetic bacteria, on the other. Nevertheless there is a clear link back to Cyanobacteria both for the light harvesting proteins of eukaryotic algae (and land plants) and the photoprotective proteins (Fig. 9). Some important recent advances have been made by the group of Adamska.

It has long been known that Cyanobacteria have a group of proteins, which broadly fit into the category of High Light- Induced Proteins (HLIP). These have a single membrane spanning helix, which binds Chl *a* and probably acts as a means of supplying Chl under high light stress. These proteins are homologous with small Chlorophyll-Binding (CB) proteins (OHP1, OHP2); also with a single membrane spanning helix, which occur in plastids of eukaryotic algae and land

plants (OHP1 occurs in green algae and land plants, OHP2 occurs in almost all algae and in land plants). Twohelix Stress Enhanced proteins (SEP) also occur in most eukaryotic algae and land plants and can be seen as gene duplications from HLIP/OHP. These two-helix proteins almost certainly gave rise by gene duplication to four-helix proteins, of which to PSBS and LHCSR are extant members, PSBS in green algae and LHCSR in eukaryotic algae and early land plants (liverworts and mosses). Early four helix proteins then likely evolved into the three-helix proteins, which include CAB/CAC proteins of many algae and land plants, the early light induced protein (ELIP) of green algae and land plants and a RedCAP (red lineage Chl a/b bindinglike) protein of red algae, cryptophytes, haptophytes and heterokontophytes, including diatoms . The evolutionary development of the RedCAP protein is not clear, and is probably lost in the events that led up to the formation of the primary plastids, over 1 Ga ago. During these events, glaucophytes did not inherit any of the new CAB/CAC proteins, red algae (but PSI only) and the red algal lineage inherited RedCAP proteins and green alga inherited CAB proteins (and associated, LHCSR, PSBS, ELIP and SEP proteins). During this time Chl *c* evolved and became the Chl that accompanied Chl *a* on the three-helix RedCAP (CAC) protein − not in red algae where only Chl *a* is bound, but in cryptophytes where Chl *c 2* is bound, and in haptophytes and heterokontophytes, where Chl *c 1* and *c 2* are bound (Fig. 9). As pointed out by Sturm *et al.* (2013), this would have required considerable genetic readjustment in the lineages with secondary plastids. However, why Chl *c* was used and not Chl *b* is unclear (Green 2011). In the green lineage, which includes Chlorophyceae, Euglenophyceae, Chlorarachniophyceae and land plants, CAB proteins with associated LHCSR, PSBS, ELIP and SEP proteins were inherited. However, during the evolution of land plants

LHCSR proteins were discarded in favor ofPSBS in the evolution vascular plants (see Fig. 9).

Archaeplastida

A eukaryotic supergroup of organisms with primary plastids. Includes Chlorophyceae (including streptophytes) Rhodophyceae and Glaucophyceae.

Chromalveolates

A group of algae proposed by Cavalier Smith, which are in the lineage that inherited genes from red algae and have secondary (and tertiary) plastids. Depending
on how it is defi ned it includes Cyptophyceae, Haptophyceae, Heterokontophyceae (including diatoms), Chromerids and apicomplexans. The group is largely consonant with the group Chromista used in this book (see AlgaeBase: http://www.algaebase.org/).

CAB/CAC proteins

As used here, this term refers specifi cally to the CAB/CAC superfamily that contain three transmembrane α-helices binding Chl *a* and Chl *b* or Chl *c* .Coded for by Lhc genes.

LHC superfamily

Proteins that contain a characteristic transmembrane domain called the LHC motif, but do not necessarily bind Chls.

LHCSR

Stress-induced LHC protein involved in fl exible NPQ in algae, liverworts and mosses.

NPQ

Non-photochemical quenching of chlorophyll fluorescence. Used as a proxy for photoprotective thermal ndissipation of excess light energy in photosynthesis.

OCP

Orange carotenoid protein involved in fl exible NPQ in many phycobilisome-containing cyanobacteria.

PBS

Phycobilisome, the major soluble light-harvesting antenna in many cyanobacteria, red algae and glaucophytes. It is composed of water-soluble phycobiliproteins
and is peripherally attached to thylakoids, where it transfers absorbed light energy to the reaction centers of PSII and PSI.

PSBS

A four-helix protein in the LHC superfamily that is involved in fl exible NPQ in green algae and land plants. This protein does not appear to bind pigments.

Xanthophyll cycle

An interconversion of xanthophylls that involves one or two de-epoxidation reactions occurring in high light (with a reverse epoxidation reaction in limiting light). Three types of xanthophyll cycles are known: violaxanthin cycle, diadinoxanthin cycle, and lutein epoxide cycle.

Nitrogen Fixation in Cyanobacteria

Oil, natural gas and coal are not only our primary energy sources but also serve as the feedstock of several derived synthetic materials such as diesel, plastics and pharmaceuticals. Rapid consumption of these fuels has not only led to their near exhaustion but has also been attributed as one of the primary drivers of global warming. Recent research findings have shown that microorganisms may hold a solution to the twin crises of global warming and rapidly declining rate of fossil fuels. Photosynthetic microorganisms have the potential to mitigate carbon dioxide at the source and produce high-end biofuels, including zero carbon fuels such as hydrogen and high-carbon fuels such as biodiesel. Cyanobacteria in particular have gained a lot of attention in recent years because of their potential applications in the sector of bioenergy (Mata et al. 2010). Two reasons have made them especially powerful: they have minimal nutrient requirements and have the potential to be genetically modified. This is in sharp contrast to bacteria that require carbohydrates as feedstock, which significantly adds to the budget of an entire project. On the other hand, cyanobacteria are known to harvest light more efficiently than terrestrial plants. In fact, cyanobacteria are responsible for 20–30 % of Earth's photosynthetic productivity and are known to convert solar energy into biomass-stored chemical energy at the rate of ~450 TW (Pisciotta et al. 2010). Additionally, bulk cultivation of cyanobacteria for commercial application is much more feasible as compared to plants as they have a faster

growth rate and can be grown on non-arable, non-productive land. Cultivation of cyanobacteria for biotechnological applications thus poses no threat to food crops, in contrast to

the cultivation of plants for the same end. Furthermore, cyanobacteria are also capable of utilizing a wide variety of water sources including wastewater (Tamagnini et al. 2007) to produce both biofuels and valuable co-products (Parmar et al. 2011). In view of this, research groups are now considering growing marine cyanobacteria in bags on the surface of oceans to generate biomass for extraction of cyanobacterial fuel and value-added co-products.

The current use of photosynthesis to produce biofuels follows a biphasic, indirect process. In the first step, biomass is generated using the energy provided by the sun, which in a second step is converted into biofuels. The presence of cellulose in the biomass is a major bottleneck in the fermentation process and requires energy-intensive pretreatment steps using heat or chemical reagents. Further downstream processing of biofuels such as ethanol requires yet another energy-intensive step of distillation after fermentation (Tanksale et al. 2010). Thus, in such cases yields are limited by the amount of biomass that can be fermented. However, the problem can be circumvented if cyanobacterial hydrogen is considered as the final goal, as its production does not rely on biomass digestion but instead employs a direct approach. Therefore, considering the different array of biofuels, biohydrogen production using photosynthetic organisms appears promising.

The journey of hydrogen production from cyanobacteria to be used as a fuel has come a long way since Benemann introduced the concept in the 1970s (Benemann and Weare

1974). Today the availability of techniques such as large scale sequencing, global transcriptomics and in depth proteomic analysis promises to take this research to greater heights. In fact, with the advent of synthetic biology the possibility to create novel pathways has already rekindled new hopes (Heidorn et al. 2011). In this chapter, the fundamentals and recent advances made in the field of cyanobacterial hydrogen production will be analyzed in detail.

Enzymes Related to Hydrogen Production in Cyanobacteria

There are two types of enzymes that are directly related to cyanobacterial hydrogen production: hydrogenases and nitrogenase. Structurally and functionally these enzymes differ. The primary role of the enzymes within the biological system is unique. While nitrogenase is primarily involved in nitrogen fixation, the role of uptake hydrogenase is in conservation of energy by catalyzing the oxidation (uptake) of the hydrogen released by nitrogenase during nitrogen fixation. In addition, bidirectional Hox-hydrogenase acts as an electron valve within the cell to release excess reducing equivalents as molecular hydrogen. The Hox-hydrogenase may also be involved in uptake activity, essential to preserve the redox balance of the cell. Another major difference between the catalyzed reactions is in terms of energy requirements. Nitrogenase catalyzes hydrogen production in an energy-intensive reaction requiring 16 ATP molecules per molecule of hydrogen evolved in addition to reductants, while hydrogenase does not have any energy requirements (Watt et al. 1975). Moreover, it has been estimated that the light required for hydrogen evolution by nitrogenase is approximately 9 ± 1 quanta/H_2. In principle, hydrogenase requires less than 1 quantum/H_2 and should therefore exhibit three times the efficiency of any nitrogenase-mediated system (Benemann 1994).

Cyanobacterial Nitrogenase

Nitrogen is needed by the organism for synthesis of amino acids and nucleotides. Nitrogenases are complex enzymes and catalyze the reaction shown in Eq. (2):

$$8\,H^+ + 8e^- + N_2 + 16MgATP \rightarrow$$
$$2NH_3 + 16MgADP + 16P_i + H_2 \qquad (2)$$
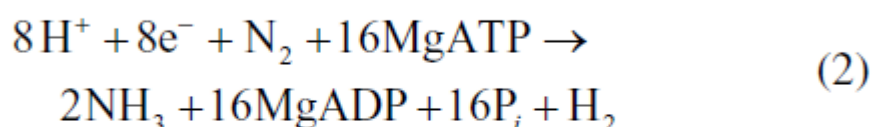
Nitrogenase appears to be a highly conserved enzyme complex. It is found in a diverse group of prokaryotes from the Bacteria and Archaea, but is not encoded in any eukaryotic genome (Berman-Frank et al. 2003). The phylogenetic ancestry of nitrogenase reveals a single common ancestor to all the nitrogenases sequenced so far, and also indicates its origin at a time prior to Earth's oxygenation (Broda and Pesheck 1983). Since the energy-intensive reaction requires 16 mol ATP for every mole of nitrogen fixed, the enzyme is activated only when the organism does not have an easy access to any other inorganic nitrogen sources present in the surrounding media.

Nitrogenases are classified depending upon the presence of metals present in the active site. The active site contains iron as one of the metal cofactors, the other being either molybdenum, vanadium or an additional iron ion (Zehr et al. 2003; Burgess and Lowe 1996; Eady 1996). Amongst all types of nitrogenase, molybdenum nitrogenase occurs most

commonly in nature (Zehr et al. 2003), and is therefore described in some detail here. Nitrogenase is a complex enzyme and comprises two protein units. The smaller protein is called dinitrogen reductase (Fe protein or protein II), while the larger unit is called dinitrogenase (MoFe protein, or protein I) and contains the active site. The dinitrogenase is a heterotetramer ($\alpha_2\beta_2$) of about 220–240 kDa; the α and β subunits are encoded by *nifD* and *nifK*, respectively. The smaller dinitrogenase reductase is a homodimer (γ_2) of about 60–70 kDa and is encoded by *nifH* (Howard and Rees 1996). The smaller unit comprises redox active Fe_4S_4 clusters similar to those present in smaller molecular weight electron carriers. It is thus responsible for mediating the electrons from the external donor (ferredoxin or flavodoxin) to the active site (Burgess and Lowe 1996). The larger subunit comprises two types of clusters, a P cluster, and an M cluster. The P cluster is a Fe_8S_7 center and is thought to function as a relay that accepts electrons from the redox active Fe_4S_4 center of the smaller subunit and further transfers them to the M cluster. The M cluster is the active site and comprises an inorganic Fe_7MoS_9C component (the FeMo cofactor) and an organic component, homocitrate (Howard and Rees 1996; Spatzal et al. 2011). The FeMo cofactor is the postulated site of substrate binding and reduction, while the specific role of homocitrate has not yet been elucidated (Bothe et al. 2010). It is to be noted that both the Fe_4S_4 center of the small subunit and the Fe_8S_7 P center are highly oxygen labile. In fact, the Fe_4S_4 center is much more oxygen-sensitive and gets

irreversibly damaged on exposure to oxygen (Postgate 1998). The problem of oxygen sensitivity is enhanced in photosynthetic cyanobacteria owing to the oxygen-evolving

PSII. To circumvent this issue, cyanobacteria have evolved different mechanisms to separate nitrogenase activity and oxygen evolution. This is ensured either by the localization of the nitrogenase in the microaerobic environment of the heterocyst, or by temporal separation of the photosynthetic oxygen evolution and nitrogen reduction activities. Both mechanisms are further discussed below. Nitrogenase is known to be inhibited by carbon monoxide (Christiansen et al. 2000). However, the chemistry is only partially understood.

Besides nitrogen reduction, nitrogenase can catalyze other reactions that involve a reactant with a triple bond. These include nitriles (RCN), isonitriles (RNC), hydrogen cyanide (HCN), nitrous oxide (N_2O), and acetylene (C_2H_2), as well as the double bond compound azide (N_3^-). Of particular interest for nitrogen research is the reduction of acetylene to ethylene. Ten to twelve percent acetylene in the headspace is routinely used to estimate the nitrogenase activity of a culture using gas chromatography (Carpenter 1983; Capone 1993).

Nitrogenase in Non-heterocystous Cyanobacteria

Some unicellular (such as *Synechococcus* and *Gloeothece*) and certain filamentous cyanobacteria (such as *Trichodesmium*) are known to possess nitrogenase. They have all developed unique strategies to safeguard their nitrogenase from oxygen. In certain organisms like *Synechococcus*, nitrogen fixation is temporally separated in the diurnal cycle. Oxygenic photosynthesis is known to take place in the daytime (under light), while nitrogenase is known to function in dark when PSII is inactive and the low amounts of oxygen present in the environment are consumed by high-rate respiration (Bothe et al. 2010). This occurs mostly when the organism forms dense mats or biofilms. However, this is not the case with all unicellular cyanobacteria. In *Cyanothece*, nitrogenase is known to be under the circadian control (Min and Sherman 2010). It can thus reduce nitrogen in air even under continuous light. It does so by temporarily inactivating PSII and increasing the rate of respiration (Reddy et al. 1993). The filamentous cyanobacterium *Trichodesmium* shows division of labor, where some cells carry out nitrogen fixation while others carry out oxygenic photosynthesis. Ohki and Taniuchi (2009) showed that 77 % of all vegetative cells in *Trichodesmium* stained positive for nitrogenase, indicating the absence of any heterocyst-like structure for nitrogenase protection.

Some uncultured unicellular marine cyanobacteria that lack oxygen evolution and CO_2 fixation capacity have been recently identified. Whole genome sequencing of such organisms has revealed the presence of nitrogenase in their DNA (Zehr et al. 2008).

a honeycomb-like structure (Pastor and Hess 2012). The specialized honeycomb membrane structure and the "necklike" interconnections with the vegetative cells effectively reduce the gas exchange area (Merino-Puerto et al. 2011). Moreover, the respiratory enzymes are embedded in the membrane located at the cell junctions. This architecture effectively reduces the amount of oxygen diffusing into the heterocyst cells (Kumar et al. 2010). To study the regulatory machinery of the cell in response to heterocyst differentiation, several mutants have been created over the years. More recently, RNA sequencing and proteomic studies have further confirmed these findings (Flaherty et al. 2011; Ow et al. 2008).

It is still unknown which vegetative cells in a non-nitrogen fixing filament will differentiate into heterocysts (Fan et al. 2005). However, it is known that the differentiation is under the regulation of several factors. In the absence of easily accessible nitrogen sources in the medium, transcriptional factor NtcA, a DNA-binding dimer, is triggered (Herrero et al. 2004). Another associated protein, DevH, is expressed and has been characterized in *Anabaena* PCC 7120 (Hebbar and Curtis 2000). NtcA belongs to the CRP (cyclic AMP receptor protein) family of proteins, and is the main 2-oxoglutarate sensor for the initiation of heterocyst differentiation. 2-oxoglutarate provides the carbon skeleton for the

incorporation of inorganic nitrogen (as ammonium), and serves as a signal molecule of the relative organic carbon/nitrogen (C/N) content in the cells (Kumar et al. 2010). NtcA is crucial for the early steps of heterocyst differentiation. Other molecules and ions, including calcium and cyclic-di-GMP, have been identified as signals for the C/N imbalance (Zhao et al. 2005; Shi et al. 2006; Kumar et al. 2010). In *Anabaena* PCC 7120, the calcium-binding luminescent protein aequorin was used to detect the enhanced intracellular calcium levels following nitrogen deprivation (Torrecilla et al. 2004). Zhao et al. (2005) showed that the increased calcium ion concentrations are related to decreased CcbP, a calcium-sequestering protein. More than 500 proteins are differentially expressed in heterocysts during cellular transformation from vegetative cells, showing that this complex process is under the control of many genes (Kumar et al. 2010). The deletion of the *all2874* gene, which encodes a diguanylate cyclase, caused a significant reduction in hetero-

cyst frequency and reduced vegetative cell size (Neunuebel and Golden 2008). This indicated cyclic GMP as a signal for heterocyst differentiation.

Another factor that has been identified in heterocyst differentiation is the protein HetR, a serine protease that is known to bind to the promoters of several heterocyst-related genes, such as *hetR*, *hetP*, *hetA*, *hetZ*, and *patS*. It is however not known how the DNA-binding activity of HetR relates to its regulatory effects (Zhou et al. 1998; Zhou and Wolk 2003; Zhao et al. 2005). Knockout strains of *hetR* gene were found

to have lost their ability to differentiate into heterocysts, while overexpression of HetR increased the frequency of occurrence of heterocysts (Buikema and Haselkorn 1991, 2001). In the absence of any NtcA-binding site on its promoter, HetR is found to be autoregulated (Buikema and Haselkorn 2001). However, NtcA and HetR are also found to regulate each other in an interdependent manner such that in the deletion mutants of *ntcA*, *hetR* is not induced, while in the deletion mutant of *hetR*, *ntcA* is only transiently expressed (Frias et al. 1994; Muro-Pastor et al. 2002).

HetR is localized in the heterocyst with the help of HetF. This was identified in *hetF* deletion mutants (Wong and Meeks 2001), which failed to develop differentiated heterocysts. On the other hand, in the absence of nitrogen, overexpression of HetF produced a multiple contiguous heterocyst phenotype (Wong and Meeks 2001). It is also suggested that HetF acts as a protease (Risser and Callahan 2008) and thus plays a role in regulating the accumulation of HetR within the heterocysts (Wong and Meeks 2001).

The proteins PatS and HetN are also found to regulate heterocyst differentiation; their overexpression downregulates heterocyst frequency (Wu et al. 2004). PatS is a small polypeptide whose C-terminal pentapeptide RGSGR (PatS-5) inhibits heterocyst differentiation when added exogenously or when expressed from a heterocyst-specific promoter (Yoon and Golden 1975). HetN contains an identical RGSGR motif that has been shown to be a functional moiety (Higa et al. 2012).

The products of *hetN* and *patA* genes are known to regulate heterocyst differentiation by positive regulation of *hetR* (Liang et al. 1992). It is possible that PatA influences heterocyst development by attenuating the negative effects of the main inhibitory signals of heterocyst pattern formation, PatS and HetN (Orozco et al. 2006). Mutants of *patA* were found to develop heterocysts at the end of the filaments (Liang et al. 1992).

The *hetC* gene encodes a member of the family of ATP-binding cassette type exporters. It is required for an early step in the differentiation of heterocysts as observed by heterologous expression of a PhetC-GFP reporter construct, which showed an increase in expression in proheterocysts and heterocysts (Khudyakov and Wolk 1997; Muro-Pastor et al. 1999). Two other novel genes, *hetL* and *asr1734*, have been shown to be involved in regulating heterocyst development, but their exact roles and biochemical functions remain unclear. Also, overexpression of another protein, HetP, promotes differentiation even in *hetR* mutants. Its role remains unclear (Higa and Callahan 2010).

After the early heterocyst precursors have been developed, the cell undergoes further morphological and metabolic changes. These include the deposition of a thickened cell wall as described in the early part of this section, in addition to the expression of cytochrome *c* oxidase, nitrogenase and hydrogenases. In *Anabaena* PCC 7120, the final steps of differentiation encode three rearrangement processes in *nifD* of nitrogenase, *hupL* of uptake hydrogenase and *fdxN* of ferredoxin (Golden et al. 1985, 1988, Golden 1997). Golden and Weist (1988) studied these processes in detail and found that a 11 kb DNA element is excised by XisA in *nifD*, a 55 kb element is excised from *fdxN* by XisF (Golden et al. 1988; Carrasco et al. 1994) and finally a 10.5 kb element is excised from *hupL* by XisC (Carrasco et al. 2005). It is only after these programmed rearrangements that the heterocyst is fully functional and is able to fix nitrogen. This rearrangement is not found in the *hupSL* of *N. punctiforme* PCC 73102 (Oxelfelt et al. 1995). The physiological consequence of this difference has not yet been ascertained.

Nutrients and Their Acquisition: Phosphorus Phoysilogy in Microalgae

Phosphorus is fundamental to life, serving an integral role in aspects of cellular metabolism ranging from energy storage, to cellular structure, to the very genetic material that encodes all life on the planet. Weathering of phosphorus rich rocks is the major source of new phosphorus into aquatic environments. This phosphorus is utilized and transformed by cyanobacteria and eukaryotic algae driving complex metabolic and biogeochemical dynamics. For reviews on the biogeochemical dynamics of phosphorus. Dissolved organic phosphorus and its cycling in marine systems is comprehensively reviewed in Karl 2014 there are recent summaries of marine cellular phosphorus dynamics, stress responses, and interactions with the marine phosphorus cycle. This chapter focuses on phosphorus physiology in microalgae including cyanobacteria and eukaryotic groups. Many of the examples come from studies with marine species, so care should be applied when extrapolating to freshwater taxa, although many of the responses and underlying themes are consistent. This chapter also does not focus on phosphorus in macroalgae. There are many reviews focused on phosphorus physiology or metabolism in eukaryotic algae, and cyanobacteria which should be referred to for additional details on all of the topics highlighted in the following sections. Knowledge about cellular phosphorus dynamics in microalgae has been rapidly advancing with new methods and more sensitive approaches. This chapter builds upon the rich literature highlighted above with a primary focus on findings leveraged from technical developments in cell sorting, molecular 'omic tools, and advances in 31 P NMR, and mass spectrometry. The chapter focuses on how these advances have expanded understanding in the following sections; (2) Phosphorus in the

cell, (3) Inorganic phosphorus utilization, (4) Organic phosphorus utilization, (5) Phosphorus stress responses, (6) Methodological advances, and (7) Emerging themes and ongoing challenges.

Sulphur and Algae: Metabolism, Ecology and Evolution

Sulphur is one of the main components of algal cells, with a cell quota typically very similar to that of phosphorus. The importance of S is not simply quantitative; it is also associated with its presence in numerous pivotal structural and functional compounds such as the amino acids cysteine and methionine, non-proteic thiols (glutathione), sulpholipids, vitamins and cofactors, cell wall constituents. Sulphur is also a constituent of dimethylsulphoniopropionate (DMSP), which in some algae can represent a very large portion of cell S and is involved in algal responses to a variety of abiotic and biotic stresses, in addition to being indicted of an important role in climate control. Algae acquire S as sulphate (SO_4^{2-}), the most abundant form of inorganic S in nature, in which S appears with its highest oxidation number (+VI). Sulphur is however assimilated in the organic matter as sulphide (S^{2-}), where S appears with its lowest oxidation number (−II) (Fig. 1). A non trivial amount of reducing power is thus required for S assimilation. In vascular plants, this reducing power can be generated both from the photosynthetic and the respiratory electron transfer chain; in algae, the dependence of S assimilation from photosynthesis seems to be tighter. In both algae and plants, S assimilation mostly takes place in the chloroplast; the only known exception is *Euglena gracilis*,[1] which reduces sulphate into the mitochondrion.

Micronutrients

In the 40 years since the chapter 'Inorganic nutrients' was written by O'Kelley in the book '*Algal Physiology and Biochemistry*' (Stewart 1974), our understanding of the types, amounts, and roles of micronutrients in microalgae has expanded enormously, as has our ability to measure and decipher their activities, fate and behavior in cells and the surrounding environment. This chapter aims to provide a state-of-the art account of micronutrients in microalgae. Unlike the original chapter by O'Kelley, which included the macronutrient elements Sulfur, Potassium, Calcium and Magnesium, the reader is referred to other chapters in this book for an update on those elements. Given the extensive literature towards our understanding of some micronutrients, the reader is also referred to chapters "Iron" (Fe), "Selenium in Algae" (Se), and "Silicification in the Microalgae" (Si) which are dedicated exclusively to each of these micronutrient elements.

Although each micronutrient is considered on an element- by-element basis briefly below, following O'Kelley, it is recognized that each functions in the presence of others and is affected by them, such that these interactions, as we know them, will also be examined. The reader is referred to excellent reviews and/or treatises published over the decades since O'Kelley's chapter, particularly the book by Fraustoda Silva and Williams (2001) and papers by Raven (1988, 1990, Raven *et al.* (1999). More recent papers have begun to reveal the nature of yet-to-be-discovered metalloproteins involved in biochemistry and physiology and these understudied and unknown roles and activities of micronutrients will be the subject of future research efforts. Unlike other groups of organisms, microalgae have polyphyletic origins. They are not only morphologically but also physiologically and biochemically

heterogeneous, making generalizations about their micronutrient requirements challenging. As O'Kelley pointed out in 1974, whereas higher plants are thought to have essentially the same elemental requirements, there appear to be differences in elemental requirements between algal species. This includes the obvious fundamental requirements for Si in diatoms and some chrysophytes (see chapter "Silicification in the Microalgae", Finkel 2016) and Ca in coccolithophores, but also the lesser known and understood differences in micronutrient requirements between prokaryotes and eukaryotes as well as between eukaryotes. In addition, micronutrient requirements are known to differ between oceanic, coastal (neritic) and freshwater microalgae. The majority of examples in this Chapter will be microalgae that have a coastal and oceanic origin; this is in no way intended to discount the importance of freshwater systems. Anthropogenic inputs of micronutrients to the environment exceed inputs from natural sources by 10- to 100- fold, particularly to lakes, rivers, and the coastal ocean. There has been a concurrent steady increase in their concentrations in the biota, altering ecological stoichiometries, food webs and trophic movement of these elements. We also raise concerns of a new emergent pollutant (engineered nanoparticles), which will likely also be the focus of future studies of micronutrient effects on microalgae.