Chapter 1

Recent Advances in the Photosynthesis of Cyanobacteria and Eukaryotic Algae

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I. Algal Systematics

Since the publication of the 1st Edition, there have been very significant advances in a number of areas of algal classification and systematics. Within the Cyanobacteria several new Phyla have been added such as Malainabacteria (e.g. Thiel et al. 2018). In the eukaryotic algae there has been the addition in the Apicomplexa of *Chromera* (Chromerids) and *Vitrella* (Vitrellids), which have only Chl *a* (and seemingly have lost Chl *c*) (See Fig. 7, Chap. 10). In the other classes, much progress has been

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made by the use of "-omics" approaches to tidying up the various families (see Chaps. 2, 10 and 16). Fortunately this has not altered our conception of how the algae evolved or when, as outlined in the first edition. It is still an open question as to how the primary plastids evolved (see Chaps. 2 and 10).

The presence of new chlorophylls (Chl *d* and Chl *f*), and their biosynthesis is described, alongside the better-known chlorophylls, in Chap. 10.

II. Cyanobacteria

Cyanobacteria have received many, many studies in the last years, not least because of their potential for bioenergy production and value-added products (Larkum et al. 2012; Ortiz-Marquez et al. 2013; Savakis and Hellingwerf 2015; Heimann 2016). The cyanobacteria are a very ancient and diverse group. They go back to at least the Great Oxidation Event, at ~2.4 billion years ago (Shih and Matzke 2013). However, the symbiotic event that led to plastids occurred much later. Shih and Matzke 2013 estimate 0.9 billion years ago, using molecular clock techniques calibrated by gene duplications. However the establishment of red algae to ~1.2 BYA, and possibly by ~1.6 BYA must suggest a much earlier date (Butterfield 2000, 2015; Bengtson et al. 2017) The monophyletic hypothesis of plastid phylogeny (and perhaps even the shopping-bag model – see Chap. 10) would suggest that a single taxon or closely related set of taxa gave rise to the primary plastids. However, it is not certain that such a taxon or set of taxa would have survived to the present time and attempts to locate this have not been successful so far (Ochoa de Alda et al. 2014; Archibald 2015; Sánchez-Baracaldo et al. 2017).

III. Crystal Structures

The past decade has seen a quantum jump in the available crystal structures of photosynthetic molecules and in the definition of these structures. Previously, rather low resolution structures of Photosystem I (PSII) and II (PSI) were available In the past decade we have seen a 1.9 A resolution of PSII (Umena et al. 2011) with avoidance of X-ray damage (Suga et al. 2015) and several high resolution structures of PSI (Jordan et al. 2001; Nelson 2018) including the novel approach of Nathan Nelson to use virus derived gene structures.

The new crystal structures have enriched our understanding of the basics of photosynthesis in many ways. The elucidation of the oxygen evolving centre of Photosystem II (the Mn_4CaO_5 centre – see Suga et al. 2015) has revealed the elegant workings of the O_2-forming cycle and opened up the way for biotechnological approaches to synthetic structures for oxygen evolution. More than that the insights into how the special pair of PSII operates in relation to its electron donors (Y_z and Cyt b_{559}) and acceptors (Phaeophytin, non-haem iron and Q_A and Q_B) have also opened up the way to biomimetic approaches.

Likewise the structures of Photosystem I have also elucidated the specific structure of this system and shown that both arms of PsaA and PsaB are functional in transporting electrons to the electron acceptor of P700 (Jordan et al. 2001).

Interestingly, however, in certain cyanobacteria it has been discovered that two special Chlorophylls (absorbing in the near infra-red, NIR) not only act as light-harvesting pigments but are specifically required for the equivalent of P680 and P700. In the case of Chl *d* in *Acaryochloris marina* this is P694 and P710 (Itoh et al. 2007) and in Chl *f* organisms it is P698 and P718 (Nuernberg et al. 2018).

Structures of anoxygenic photosynthetic bacteria are also providing new insights into the possible evolution of photosynthetic reaction centres. In the case of PS bacteria with Reaction Centre II type centres (Rhodobacter and Chlorobiaceae) large differences exist between these and RCII of PSII. And in the case of Type I RCs the recent structural model of *Heliobacterium* has revealed a Ca^{2+} site with remarkable site homologies to the Mn_4CaO_5 centre site. This has suggested that this site was blocked in the *Heliobacterium* line specifically to prevent the formation of oxygen (Cardona et al. 2018). Furthermore, the effects of the presence of oxygen have been looked for and found in both PSI and PSII and have been used, in conjunction with other evidence, to argue for an early evolution of oxygen evolution from manganese centres in forebears of cyanobacteria, possibly before 3.5 billion years ago (BYA) (Cardona et al. 2018).

IV. Light Harvesting

Cyanobacteria have continued to provide interesting new mechanisms and pigments. The phycobilisome, which is present in most cyanobacteria, has been shown to be under control in supplying energy to the reaction centres of thylakoids though the Orange Carotenoid Pigment. This is a mechanism of Non-photochemical Quenchin (NPQ) or Down Regulation and is described in Chap. 10 and 12. In terms of pigments the biggest breakthrough has been the discovery of Chl *f* (Chen et al. 2010) in cyanobacteria from stromatolites of the Hamelin Pool, Shark Bay and its subsequent finding in a range of other cyanobacteria (Ho et al. 2016); Nuernberg et al. 2018). Chl *f* may be the last chlorophyll to be discovered (Schliep et al. 2013); however, there has continued to be progress with all the other chlorophylls (Chap. 10) and in the case of near infra red (NIR) driven systems there has been surprising progress. In the first edition of this book in 2004 Chl *d* was a new chlorophyll (see Chap. 3 of that book) and the only other established absorber of NIR was the chlorophyll *a* of *Ostreobium* (Chap. 11). In the meanwhile Chl *d* organisms have been found extensively and many eukaryotic algae have been found with Chl *a* able to absorb in the NIR (see Chap. 11 and Wolf et al. 2018).

In Eukaryotic algae there have been continuous advances in terms of mechanisms and a general survey of these is given in Chap.10 with more detailed descriptions given in Chaps. 3, 11, 14, 15, 16 and 17. Perhaps the most exciting and controversial aspect of light harvesting is the role of coherent energy transfer in pigment beds and this is dealt with in detail in Chap. 15. There is now good evidence that coherent energy transfer occurs in certain members of the Cryptophyceae (Chap. 15) and also evidence that it plays a role in desert algae (Bar-Eyal et al. 2015, 2017).

In eukaryotic algae with primary plastids (i.e with two envelope membranes; viz. Chlorophyta, Rhodophyta and Glaucophyta) all have received intense investigation in term of their light harvesting. In the Chlorophyceae (a class in the Chlorophyta), *Chlamydomonas reinhardtii* has continued to be the favourite organism because it can be easily genetically transformed (Chap. 4). This has led to a much better understanding of how the supply of light energy is balanced between the two photosystems and how the reaction centres are protected from damagingly high levels of solar radiation, by non-photochemical quenching and other mechanisms (Chaps. 3, 10, 11, 12, 16 and 17). Like the vascular plant *Arabidopsis thaliana*, *C. reinhardtii* will continue to be a well-used organism into the future.

However, other algae are emerging for their use in microalgal biotechnology. Perhaps foremost here is *Nannochloropsis gaditana*, a member of the Eustigmatophyceae (with secondary plastids), which is transformable, possesses only Chl *a,* and which can be made to produce large amounts of lipids (Radakovits et al. 2012). However there is a wide choice of microalgae, and even macroalgae, now available (Larkum et al. 2012; Borowitzka et al. 2016).

In particular, the red algae (Rhodophyta) are now receiving close attention, despite their complex phycobilisome structure (Ago et al. 2016; Zhang et al. 2017) (see Chap. 11).

In secondary plastids the structure of a four- (or three-) membrane envelope and complex thylakoid structure has not deterred significant advances in our understanding of the structure and light-harvesting mechanisms, especially in the diatoms (Wang et al. 2019) (see Chaps. 12 and 16).

V. Photoinhibition

Photoinhibition continues to be one of the most challenging topics in algal research. Clearly algae have evolved mechanisms to protect against photoinhibition of one photosystem or the other. And this involves down regulation of high energy supply to the photosytems by Non-photochemical quenching and other mechanisms (see Chap. 10). In this volume there is a welcome chapter devoted to this topic alone (Chap. 13).

Both Photosystem I (Tikkanen et al. 2014) and Photosystem II (Andersson and Aro 2001) are involved in photoinhibition. Generally PS I is more resistant to photoinhibition, although it has recently been shown that Flavodiiron is safety valve for PSI under fluctuating light and when the system is overstressed inhibition can occur and lead to serious inhibition of photosynthesis (Jokel et al. 2018). On the other hand it seems that photoinhibition of PSII is an unavoidable consequence of water splitting (i.e. the large E_m of $P_{680}/P_{680}+$) and the inability of placing carotenoids close to P680 (Chap. 10); the net result of this is the unavoidable production of singlet oxygen (see Chaps. 10 and 17) which constantly impairs D1 of PSII (Andersson and Aro 2001).

VI. Dinoflagellates and Coral Bleaching

Dinoflagellate single-celled algae are a curious example of algae with secondary plastids; in this instance with 3 envelope membranes and an enslaved nuclear fragment with affinities to red algae (Douglas et al. 2001). Few studies have focused on these interesting organisms since the publication of the first edition. The exception here is the great current interest in coral bleaching. Corals are a symbiosis between dinoflagellates (in the Symbiodiniaceae, generally called zooxanthellae) and the coral animal (in the Cnidaria). For unresolved reasons in many coral reefs around the world Symbiodiniaceae leave the coral host at seawater temperatures of 30–32 °C. In Chap. 17, it is suggested that reactive oxygen species (ROS – hydrogen peroxide and singlet oxygen) trigger the bleaching process. The reason why ROS should be produced is probably related to the fact that dinoflagellates have a Type II Rubisco, which is impaired at temperatures above 30 °C. ROS are implicated in many second messenger systems in higher plants, with much information recently obtained from *Arabidiopsis thaliana* for hydrogen peroxide signaling (Noctor et al. 2018). The hypothesis, which was proposed a long time ago and is still current, is that over-reduction of PSI as a result of inhibition of the Calvin-Benson Cycle generates hydrogen peroxide, which triggers the release of the zooxanthellae (Chap. 17).

VII. Carbon Uptake and Metabolism (See Chap. 7 & 8)

Carbon Metabolism

Photosynthetic carbon reduction cycle (PCRC) is the core of autotrophic carbon assimilation in cyanobacteria and algae. There is limited evidence of C_4 or C_3-C_4 metabolism as a precursor to C_3 biochemistry in algae, and no evidence of C_4 or C_3-C_4

metabolism in cyanobacteria (Raven et al. 2017). Regulation of the PCRC differs in (e.g.) diatoms from the canonical green algal and embryophyte mechanism (Jensen et al. 2017). The reactions of the PCRC that have the greatest control strength have been determined for a cyanobacterium, and are similar to those in embryophytes (Janasch et al. 2018. Although the phosphoglycolate synthesis by Rubisco is limited by CCMs in most algae, there is usually a significant flux through phosphoglycolate; not all of the resulting glycolate is excreted, and the pathway of glycolate metabolism is not clear in diatoms (it is not the photorespiratory carbon oxidation cycle nor the tartronic semialdehyde pathway) (Raven et al. 2017). Diatoms are further discussed in Chap. 16).

Inorganic Carbon Acquisition

Most algae, and all cyanobacteria, have CCMs (Raven et al. 2017). The structural, mechanism and control of the CCM in cyanobacteria is well understood with diffusive carbon dioxide entry, and active bicarbonate transporters, at the plasmalemma, energized carbon dioxide conversion to bicarbonate in the cytosol, and bicarbonate conversion to carbon dioxide in carboxysomes that contain all of cell's Rubisco in a liquid-like condensate (Wang et al. 2019). Among eukaryotic algae, the best understood organism is *Chlamydomonas reinhardtii*, where the Rubisco, contained in the pyrenoid, is in a liquid-like matrix (Freeman Rosenzweig et al. 2017), similar to that in carboxysomes. However, how the pyrenoid functions are performed in algae, including most macroalgae with CCMs, lacking pyrenoids needs further investigations.

VIII. Water-Water Cycles (See Chap. 8)

Recent advances in understanding how a fraction of the oxygen produced in photosystem II is reconverted to (ultimately) water downstream of photosystem II have focused on PTOX and flavodi-iron proteins, accepting electrons from, respectively, photosystem II (e.g. Houille-Vernes et al. 2011) and photosystem I (e.g. Shimakawa et al. 2015). These water-water cycles, and Asada-Halliwell-Foyer water-water cycle involving both photosystems and ascorbate peroxidase, have a number of potential roles, including providing additional ATP as an alternative to cyclic electron and transport and hence phosphorylation, or oxidative phosphophorylation, when the ATP:NADPH supplied by linear electron flow is lower than that required in metabolism. This function would conflict with a role of water-water cycles in dissipation of excess excitation energy unless there is facultative uncoupling, for which there is no evidence. Energy dissipation by photorespiration is subject to an analogous constraint in terms of the carboxylation:oxygenation ratio of Rubisco that is regulated solely by the CO_2:O_2 ratio at the active site, but energy dissipation can be increases by replacing the photorespiratory carbon oxidation cycle or tartronic semialdehyde pathway with conversion of glycolate, via glyoxalate, oxalate and formate, to CO_2 via a pathway not coupled to ATP synthesis, or, less likely, glycolate excretion (Eisenhut et al. 2008).

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