Tansley review

Biotic interactions as drivers of algal origin and evolution

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Summary

Biotic interactions underlie life's diversity and are the lynchpin to understand its complexity and resilience within an ecological niche. Algal biologists have embraced this paradigm, and studies building on the explosive growth in omics and cell biology methods have facilitated in-depth analysis of non-model organisms and communities from a variety of ecosystems. In turn, these advances have enabled a major revision of our understanding of the origin and evolution of photosynthesis in eukaryotes, bacterial-algal interactions, control of massive algal blooms in the ocean, and the maintenance and degradation of coral reefs. Here we review some of the most exciting developments in the field of algal biotic interactions and identify challenges for the next generation of scientists. We foresee the development of an algal knowledgebase that integrates ecosystem-wide omics data and the development of molecular tools/resources to perform functional analyses of individuals in isolation and in populations. These assets will allow us to move beyond mechanistic studies of a single species towards understanding the interactions amongst algae and other organisms both in the laboratory and in the field.

Key words: algae, algal blooms, endosymbiosis, organellogenesis, genomics, holobiont, symbiome, trophic interactions.

I. Introduction

Algae are key primary producers in aquatic environments and represent several emerging genetic model systems (Armbrust *et al.*, 2004; Hopes *et al.*, 2016; Nymark *et al.*, 2016). They also play an increasingly important role in human nutrition (FAO, 2014). Algal photosynthesis provides about one-half of the oxygen that we breathe, and their genomes reveal the story of a tangled past that traverses the tree of life through the processes of endosymbiosis and horizontal gene transfer (HGT) (Price *et al.*, 2012; Cenci *et al.*, 2017). Biotic interactions between algae and other eukaryotes (e.g., (Worden *et al.*, 2015) are extremely widespread in aquatic and terrestrial ecosystems. The degree to which nature has experimented with these relationships is wide-ranging, including interactions among organisms that maintain a few functional associations, to those that have evolved a highly integrated suite of functions. In addition to the intracellular interactions described below, algae also engage in extracellular/surface interactions in the phycosphere, which is the ecologically and physiologically integrated neighborhood inhabited by the alga (Bell & Mitchell, 1972). Epibiosis (surface colonization of one organism [the basibiont] by other attached organisms [epibionts]) will not be covered in great detail here, but occurs on all immersed surfaces in the aquatic environment, including those of micro and macroalgae, and is of paramount importance in the marine environment (Wahl *et al.*, 2012). Epibiotic interactions (e.g., alga-alga, alga-bacterium, alga-virus [see below]) play key roles in nutrient acquisition and recycling, metabolic flux, energy flow and developmental processes. In parallel with herbivory, epibiosis represents one of the most important interactions that can determine the fate of an alga and has been shown to shape entire marine communities (Korpinen *et al.*, 2007).

In this review, we focus on research that has contributed some of the most exciting insights concerning the ways in which biotic interactions shape algal evolution and physiology. This perspective recognizes that “symbiomes” or “holobionts” are important targets of study to elucidate the overall capacity of genomes to interact with the environment. Here symbiome refers to co-localized and co-evolving (i.e., under selection) taxa comprising a given consortium, whereas holobiont includes all physically associated taxa regardless of the nature of the biotic interaction (Boucias *et al.*, 2013; Bordenstein & Theis, 2015; Douglas & Werren, 2016; Tripp *et al.*, 2017). This revolution in understanding integrative ecosystem function has largely been driven by the occurrence of technological advances in fields such as genomics, proteomics, and cell biology. It is clear, however, that we are on the cusp of far greater advances, as the concept

of the symbiome informs our experimental approaches. Below, we discuss prominent examples of algal biotic interactions that have been selected to illustrate the importance of these interactions in a broad range of contexts ranging from deep evolutionary time to processes of key relevance in the current context of global climate change. The review will begin with a discussion of the origin of photosynthetic organelles based on endosymbiosis, and will then look at algal interactions in the coral symbioses and the threat that climate change imposes on this association. Lastly we will examine the role of bacteria in algal biology, and the arms race associated with alga-virus interactions.

II. Endosymbiosis

1. Complex biotic interactions explain plastid origin

1.1. Primary endosymbiosis in Archaeplastida

Algae originated as a consequence of primary plastid endosymbiosis, a process in which a mitochondrion-containing, single-celled eukaryote engulfed and retained a cyanobacterium that eventually became the photosynthetic organelle or plastid (Cavalier-Smith, 1982; Bhattacharya *et al.*, 2004). The product of this ca. 1.6 billion year old endosymbiotic event (Yoon *et al.*, 2004) eventually split into the three primary plastid lineages, the red algae, the glaucophyte algae, and the green algae plus plants (together, the supergroup Archaeplastida) (Adl *et al.*, 2012; Price *et al.*, 2012). Algae from these groups were themselves frequently engulfed by other protists, giving rise to a rainbow of serially derived plastids distributed throughout the tree of life (Palmer, 2003; Gould *et al.*, 2008) (Fig. 1a). The process of primary plastid capture has sometimes been depicted as a 'hungry' single-celled eukaryote engulfing a prokaryote followed by the subsequent evolution of a functional organelle. This portrayal begs the obvious question: if the process is so simple, then why has the event been so rare given that oceans and lakes are replete with phagotrophic protists that have been feeding on prokaryote prey for hundreds of millions of years? In fact, there are only two bona fide primary endosymbioses known that gave rise to widespread organelles over the long history of eukaryotes; the event from which all plastids originated, as explained above, and a prior event that led to the evolution of mitochondria. Other more taxonomically limited cases of organelle origin are associated with the photosynthetic amoeba lineage *Paulinella* (see below), the non-photosynthetic organelle of the trypanosomatids (Kostygov *et al.*, 2016; Morales *et al.*, 2016), and nitrogen fixing spheroid

bodies in the rhopalodiacean diatoms (Nakayama *et al.*, 2014; Zehr *et al.*, 2016). The rarity of primary endosymbiosis has fascinated scientists for many years and is usually attributed to the extensive innovations required for organelle establishment. These include: a) events that lead to the protection of the nascent endosymbiont from host digestion; b) tailoring of processes critical for the exchange of metabolites between the endosymbiont and host cell (Facchinelli & Weber, 2011); c) the origin of an import system to move cytosolic proteins into the nascent organelle (Schleiff & Becker, 2011); d) foreign gene acquisition through HGT and the integration of the HGT-derived protein products into both host and newly developing organelle pathways (Cavalier-Smith, 2002; Karkar *et al.*, 2015); and e) movement of genes from the organelle to the host nucleus to escape Muller's ratchet, i.e., accumulation of mutations in non-recombining genomes (Felsenstein, 1974). Processes that would exacerbate the impact of Muller's ratchet and make relocation of genes from the organelle to the nuclear genome more imperative are the mutagenic effect of damaging reactive oxygen species (ROS) produced as a consequence of photosynthesis in the organelle (van Creveld *et al.*, 2015), and as yet unexplained processes associated with greater damage of DNA in organelles than in their aerobic bacterial ancestors (Raven, 2015). Explanations for why organelle genomes are retained include coordinated synthesis of complexes assembled in the organelle, and the regulation of transcriptional and post-transcriptional processes by the organelle redox state (van Creveld *et al.*, 2015).

The most critical innovation listed above, is the first, namely how a captured bacterial cell evades digestion by the host during the initial stages of plastid evolution. A potential answer to this question comes from recent work exploring the evolution of mitochondria. Current mitochondrial gene phylogenies indicate that this organelle originated from anciently diverged environmental Rickettsiales-like pathogens with relatively large gene inventories (Wang & Wu, 2015; Ball *et al.*, 2016c) whose descendants are now often found in association with protists (Martijn *et al.*, 2015). However, these taxa are distinct from the highly specialized animal parasites with streamlined genomes, such as the typhus agent *Rickettsia prowazekii* (Zomorodipour & Andersson, 1999), that were initially proposed as the alpha-proteobacterial candidates based on limited data that was collected over ten years ago (Emelyanov, 2003). The host of this mitochondrial endosymbiosis was likely to be a member of the recently discovered archaeal 'Asgard' superphylum (including the Lokiarchaeota and Heimdallarchaeota), which is the most closely related prokaryote to the eukaryote nuclear lineage (Spang *et al.*, 2015;

Zaremba-Niedzwiedzka *et al.*, 2017). Therefore, the increasingly widely accepted view is that an Asgard-like cell was infected by a relatively gene-rich Rickettsiales-like pathogen, thus laying the foundation for mitochondrial endosymbiosis and eukaryogenesis. By virtue of their existing ability to thrive in the intracellular environment, the ancestors of mitochondria were pre-adapted to switch from pathogenesis to endosymbiosis. These cells had evolved efficient solutions to deal with host innate immunity due to millions of years of coevolution with the Asgard lineage. These findings suggest that, to become a successful proto-endosymbiont, the invading cell needs to evade host defenses, which is more likely to be achieved by an intracellular pathogen adapted to the cytosolic lifestyle (Ball *et al.*, 2016b; Ball *et al.*, 2016c; Cenci *et al.*, 2017).

Application of this concept to the origin of plastids requires some modification because extant cyanobacteria are not intracellular pathogens and lack the inherent capacity to evade host defenses. We suggest two possible explanations for cyanobacterial survival. First, the Archaeplastida host of this endosymbiosis may have developed mutations that reduced the efficacy of its lytic/phagocytic functions. This provided the cyanobacterium sufficient residence time within a host food vacuole to evolve a character(s) beneficial to the host (e.g., secretion of fixed carbon or reduced nitrogen compounds), which allowed the establishment and spread of a founder population. This scenario is more likely to have occurred in oligotrophic waters, which lacked abundant prey. An alternative explanation is that the cyanobacterium was protected by a third 'player' that could withstand host defenses. This latter idea receives support from the finding that there are several dozen genes of chlamydial origin present in the nuclear genome of algae and plants (Huang & Gogarten, 2007; Becker *et al.*, 2008). Phylogenetic data suggest that these genes are from environmental strains with relatively large genomes, such as those that infect *Acanthamoeba*, and not the highly reduced human pathogens. In addition, many of the products of these nucleus-encoded genes are plastid targeted and perform specialized functions not associated with cyanobacteria (Huang & Gogarten, 2007; Moustafa *et al.*, 2008). These observations have led to the "ménage à trois" hypothesis (MATH) to explain the origin of plastids. In this scenario a Chlamydiales ancestor evolved from a pathogenic to symbiotic lifestyle, protecting the cyanobacterium in its inclusion vesicle (Ball *et al.*, 2013; Cenci *et al.*, 2017). Although the MATH remains controversial due largely to issues associated with 'deep time' gene phylogenies and the unresolved role of HGT in eukaryote evolution (Dagan *et al.*, 2013; Ball *et al.*, 2016a), its complexity reflects well-established biotic interactions. As

illustrated in Fig. 1b, it predicts that an elementary body (chlamydial infectious particle) escapes host defenses by remodeling the phagocytic membrane and by secreting chlamydial effector proteins that enable bacterial specific metabolites of photosynthesis such as ADP-glucose to enter the host cytosolic glycogen stores. Both glaucophytes and red algae store carbohydrates in their cytosol suggesting that the glycogen/starch pool may have provided an opportunity to buffer the unsynchronized demand and supply of carbon of the cyanobiont and its host. Several observations support this idea: a) enzymes involved in manipulating host carbohydrate metabolism are pathogen effectors secreted by the type-III secretion system (Gehre *et al.*, 2016); b) pathogenic Chlamydiae synthesize extracellular storage carbohydrates within parasitophorous vacuoles using analogous nucleotide-sugars and nucleotide-sugar transporters (Gehre *et al.*, 2016); c) nucleotide-sugar transporters of host origin are evolutionary ancestors of plastid carbon exporters in red and green algae, as well as in plastids of secondary or tertiary endosymbiotic origin (Moog *et al.*, 2015); and d) analysis of the tryptophan biosynthesis pathway in Archaeplastida shows that one-half (4/8) of the genes encoding proteins in this pathway are putatively of chlamydial origin, as are the *E. coli tyr/mtr* (tyrosine/tryptophan) transporter genes (Cenci *et al.*, 2016; Cenci *et al.*, 2017).

Tryptophan starvation may have been a mechanism used by the host of the primary plastid to combat chlamydial infection (Bonner *et al.*, 2014). Tryptophan biosynthesis is by far the most costly amino acid for cells to synthesize. In comparison to the eukaryotic host and the cyanobiont, sensitivity of the chlamydial symbiont to tryptophan starvation would have been exacerbated by the energy requirements for its synthesis (Bonner *et al.*, 2014). This biotic interaction would therefore have selected for movement of the chlamydial *trp* operon to the cyanobacterial endosymbiont genome to ensure high levels of gene expression. Cenci *et al*. (2016) posit that the chlamydial *trp* operon transfer occurred via conjugation during co-localization of chlamydial and cyanobacterial cells in inclusion vesicles. At a later time, some *trp* genes were moved to the Archaeplastida nuclear genome by endosymbiotic gene transfer (EGT) (Martin & Herrmann, 1998) from the cyanobacterial plastid forerunner. The MATH is reinforced not only by functional considerations, but also gene numbers. Chlamydiae HGTs are not scattered randomly among the organisms of the tree of life, but rather, an outsize contribution (ca. 30-50 genes, depending on the lineage being studied) is found when compared to other non-cyanobacterial prokaryotic gene acquisitions in Archaeplastida nuclear genomes (Huang &

Gogarten, 2007; Deschamps, 2014). In addition, analysis of the plastid proteome shows that despite having >50-fold more proteobacterial than chlamydial sequences in current genome databases (e.g., National Center for Biotechnology Information), Proteobacteria and Chlamydiae genes represent the largest contribution to plastid functions (46 and 24 genes, respectively, in *Arabidopsis thaliana)*, with only 13 from alpha-Proteobacteria (Qiu *et al.*, 2013). The MATH provides a testable model that can be used to study the steps that led to plastid origin. Beyond its specific predictions (Ball *et al.*, 2013; Cenci *et al.*, 2017), this theory highlights the complexity of biotic interactions that underlie endosymbiosis. In the future, the aim should be to develop systems in the laboratory to study the processes underlying endosymbiosis so that we can move beyond trees and diagrams, to allow experimental elucidation of mechanisms underlying organellogenesis.

1.2. Origin of the *Paulinella* chromatophore

The concept of multiple microbes contributing to plastid evolution in the Archaeplastida, may also explain the maintenance and evolution of the plastid (termed the chromatophore) in *Paulinella chromatophora* (Marin *et al.*, 2005; Nowack *et al.*, 2008; Yoon *et al.*, 2009). In this case, there is currently no evidence for chlamydial-facilitated organelle origin. However, over 200 bacterium-derived HGTs have been found in the nuclear genome of this species that complement gene losses from the chromatophore genome. Specifically, many missing components of critical endosymbiont pathways, such as for amino acid and peptidoglycan biosynthesis and DNA replication, have been compensated for by the acquisition of a variety of prokaryotic donor genes via HGT (Nowack *et al.*, 2016). Access to these foreign genes was likely facilitated by phagotrophic uptake of bacteria by the host amoeba, followed by HGT of DNA to the amoeba nuclear genome. Once activated, nucleus-encoded gene products were relocated to the chromatophore, possibly by trafficking through the secretory system, where they could replace components of the pathways encoded on the chromatophore genome (Nowack & Grossman, 2012). It should be stressed that a response to Muller's ratchet acting on the chromatophore genome (leading to genome reduction) in *P. chromatophora* is certainly expected, but surprisingly, it is not primarily EGT and rerouting of host proteins in this relatively 'young' endosymbiosis (i.e., 90-140 million years old; Delaye *et al.*, 2016) that facilitates this process, but rather, repurposing of environmental DNA as a result of biotic interactions.

2. Complex biotic interactions explain the symbiosis between algae and corals

2.1. Maintenance of the symbiosis

Corals are the structural and trophic foundation of coral reefs, which support about 30% of all described marine species (Wilkinson, 2004). Critically, reef-building corals are a symbiosis between the coral animal *per se* and photosynthetic dinoflagellates in the genus *Symbiodinium* (Figs. 2a, 2b3). *Symbiodinium* are also key algal symbionts in a wide range of coral reef animals, including sea anemone, sponges, jellyfish, and clams. The coral-*Symbiodinium* association is one of relaxed specificity: individual corals can harbor alternative and multiple symbiont types simultaneously, and a *Symbiodinium* type may associate with a range of coral hosts (Silverstein *et al.*, 2012). Upon acquisition of *Symbiodinium* by host gastrodermal cells (within which the algal cells reside), the *Symbiodinium* are physically separated from the cytoplasm by a host-derived vacuole known as the symbiosome (Roth *et al.*, 1988). Exposure to competent *Symbiodinium* cells triggers an initial stress response in the coral *Acropora digitifera*, resulting in transient suppression of protein synthesis and mitochondrial metabolism (Mohamed *et al.*, 2016). This finding supports the hypothesis that the symbiosome is a phagosome that has undergone early arrest (Shinzato *et al.*, 2011; Mohamed *et al.*, 2016).

Coral reefs thrive in nutrient-poor waters. In return for shelter (e.g., from ultraviolet radiation, predation), *Symbiodinium* photosynthesis may provide >90% of the fixed carbon requirement (Muscatine & Porter, 1977) of the hosts. A critical limitation of photosynthesis is access to dissolved inorganic carbon. Since they have no direct access to ambient seawater, *Symbiodinium* cells depend on the host for delivery of inorganic carbon (C_i; CO_2 or HCO_3^-) (see Fig. 2c). When net photosynthesis takes place some C_i is generated via respiration, but in corals the predominant C_i supply to photosynthesis is its accumulation within host tissue from external sources (Shinzato *et al.*, 2011). The concentration of C_i in the host tissue can be ~70-fold that of seawater, which represents a steeper gradient than is observed for most organisms that use a carbon concentrating mechanism (CCM) (Shinzato *et al.*, 2011). C_i accumulation by the host could also be related to the existence of an acidified space between the algal cell and the symbiosome membrane, and the presence of an uncharacterized HCO_3^- transporter in the symbiosome membrane and carbonic anhydrase activity in the acidified space. The host also

appears to have an active role in regulating photosynthesis in the symbionts (Barott *et al.*, 2015; Bhattacharya *et al.*, 2016).

The algae of the holobionts also accumulate C_i (Walker *et al.*, 1980; Barott *et al.*, 2015). This is likely related to the fact that dinoflagellates such as *Symbiodinium* have Form II ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO), which shows low CO_2:O_2 selectivity and, probably, a low affinity for CO_2 (Leggat *et al.*, 2000). Other symbioses have different roles for the animal in C_i supply to *Symbiodinium*; e.g., in tridacnid giant clams, where the symbionts are extracellular, the haemolymph is the immediate source of C_i. The C_i concentration in the haemolymph in the light is lower than that in seawater (Muscatine & Porter, 1977), thus there is no evidence for accumulation of C_i to higher concentrations than in seawater during influx through the gill epithelium. In this case, the accumulation of C_i by the photobiont presumably plays an even more vital role in algal primary production.

The symbiotic relationship between *Symbiodinium* and its coral hosts determines not only the rate of coral-reef growth (calcium carbonate deposition), but also how corals respond to environmental stress (Voolstra *et al.*, 2015). A modest episodic period of increased temperature of the ocean surface (e.g., a few days at 1-2°C above the mean summer minimum) can set off a cascade of photoinhibition, the decoupling of carbon flow between the symbiont and host (breakdown of symbiosis), oxidative damage, and physical loss of symbiont cells (Wooldridge, 2013). This process, known as coral bleaching, leaves the coral host at risk for starvation, disease, and death unless the symbiosis is soon re-established (Hoegh-Guldberg, 1999). In this way, algae are essential for survival and maintenance of coral reef ecosystems. The impact of current environmental change on the health of the symbiotic association is particularly alarming, especially in recent years. For example, >90% of the 911 reefs surveyed in 2015-2016 at the Great Barrier Reef (the world's largest continuous reef system) showed signs of severe bleaching (Albright *et al.*, 2016).

2.2. Omics perspective on the coral symbiosis

The cellular and molecular processes of symbiosis that are actively being explored include recognition, capture of the symbiont in the symbiosome, proliferation of symbionts in host tissue, loss of symbionts from the host tissue, and metabolic exchange and nutrient trafficking between *Symbiodinium* and the host across multiple membranes. There is much to be learned about these

topics from a broader genomic and molecular evolutionary perspective. *Symbiodinium* is classified into nine clades based on phylogenetic markers, although they represent a highly divergent group of dinoflagellate species (LaJeunesse *et al.*, 2005; Wham & LaJeunesse, 2016) and may include >100 species capable of forming symbiotic associations with corals. Hurdles in studying genomic and molecular aspects of the coral system include difficulties associated with the establishment of axenic cultures of the various *Symbiodinium* types, their slow growth, and the size and complexity of their genomes. The dinoflagellate nuclear genome can be massive, up to 250 Gbp in size (LaJeunesse *et al.*, 2005; Lin, 2011), and exhibits unusual features including non-canonical nucleotides, atypical intron-exon splice signals (Lin, 2011), and RNAs that are trans-spliced (Zhang *et al.*, 2007). RNA editing of transcripts has been described in mitochondrial and plastid genomes (Lin, 2011; Jackson & Waller, 2013; Mungpakdee *et al.*, 2014), whilst the plastid genome is comprised of distinct DNA minicircles, each containing a gene, a few genes, or in some cases, no genes (Zhang *et al.*, 1999; Howe *et al.*, 2008). The nuclear genomic features are set against a backdrop of gene or genome-fragment duplications, and abundant non-coding repetitive elements (McEwan *et al.*, 2008; Shoguchi *et al.*, 2013). Three genome sequences of *Symbiodinium* from distinct clades were recently published (Shoguchi *et al.*, 2013; Lin *et al.*, 2015; Aranda *et al.*, 2016), and their estimated genome sizes of 1.1-1.5 Gbp are smaller than the earlier estimates of 3-5 Gbp (LaJeunesse *et al.*, 2005). In addition, these genomes share little sequence similarity; i.e., <1% of total sequenced reads from *S. kawagutii* mapped onto the genome assembly of *S. minutum*, and vice versa (Lin *et al.*, 2015). These results indicate a high level of genome divergence among distinct *Symbiodinium* clades.

Further complexity in corals comes from a three-way functional complementarity between the coral host, the dinoflagellate, and the associated microbiome of bacteria and viruses (Ziegler *et al.*, 2017). For example, the incomplete cysteine biosynthesis pathway in the coral *Acropora digitifera* (Shinzato *et al.*, 2011; Shinzato *et al.*, 2014) is compensated for by *Symbiodinium* (Shoguchi *et al.*, 2013; Lin *et al.*, 2015), whereas bacteria likely play a key role in regulating the availability of nitrogen to the coral host and algae and in resistance to thermal stress (Radecker *et al.*, 2015; Ziegler *et al.*, 2017). Nevertheless, given the diversity of *Symbiodinium* species, a 'one-reference-genome-fits-all' assumption will not be possible for studying the coral-dinoflagellate symbiosis and interactions, and additional genome data from the different species types/clades will be necessary. An effective approach would be to integrate

multi-omics data from the coral and the associated *Symbiodinium* and microbiome; i.e. the holobiont (Bordenstein & Theis, 2015), to tease apart the individual contributions of each component in sustaining a healthy holobiont. Availability of additional data from free-living dinoflagellates will help address key questions including the evolutionary events and functional innovations that lead to the transition from a free-living to a symbiotic lifestyle. At the same time, tractable lab model systems are being developed (Shapiro *et al.*, 2016) that will enable the study of cellular mechanisms that underlie the response to elevated temperature and pathogens. Findings from such studies will inform strategies for conservation of and risk mitigation for reef ecosystems.

III. Biotic interactions within the phycosphere

3.1 Alga-bacterium biotic interactions

Interactions between algae and bacteria are likely to be universal in the environment. Many notable examples are species specific, such as the green seaweed *Ulva mutabilis,* which relies on different bacterial strains for successful morphogenesis (Spoerner *et al.*, 2012). In the laboratory, rather than forming the typical blade- or tube-like morphology, axenic gametes of *U. mutabilis* develop into callus-like aggregates of undifferentiated cells with abnormal cell walls. These findings suggest the existence of chemical signaling between bacteria and the alga, and potentially, complementarity of metabolic pathways. Similar interactions have also been found between the bacterium *Sulfitobacter pseudonitzschiae* and the diatom *Pseudo-nitzschia multiseries* (Amin *et al.*, 2015), and bacteria have been shown to facilitate acclimation of the brown seaweed *Ectocarpus siliculosis* to a freshwater environment (Dittami *et al.*, 2016). Another striking example of this phenomenon is the ‘Jekyll-and-Hyde’ (named by the authors) relationship between the roseobacter *Phaeobacter gallaeciensis*, a biofilm forming symbiont of the bloom-forming haptophyte alga *Emiliania huxleyi* (Seyedsayamdost *et al.*, 2011). Under normal growth conditions *P. gallaeciensis* secretes antibiotics and growth phytohormones (e.g. the auxin indole-3-acetic acid) that appear to benefit the alga. However, as the algal population ages, the bacteria shift their small molecule biosynthesis pathways to the production of algaecides, and act as an *E. huxleyi* pathogen (Seyedsayamdost *et al.*, 2011; Segev *et al.*, 2016). A different type of biotic interaction involves capture and ‘farming’ of the cryptophyte alga

Teleaulax amphioxeia by its host ciliate, *Mesodinium rubrum*, to extract nutrients from the captured, intact alga (Qiu *et al.*, 2016).

More general interactions are seen with bacteria that play a key role in providing micronutrients to algae. Examples are essential organic compounds such as thiamine (vitamin B_1) and cobalamin (vitamin B_{12}). These compounds are required as enzyme cofactors, but many phytoplankton species are unable to synthesize them. Only prokaryotes (and only then, a subset of both Eubacteria and Archaea) can synthesize cobalamin *de novo* (Warren *et al.*, 2002), and levels free in the aquatic environment are generally too low to support algal growth (Sañudo-Wilhelmy *et al.*, 2012). Direct provision of the vitamin from bacteria to algae has been demonstrated in the laboratory (Croft *et al.*, 2005; Wagner-Döbler *et al.*, 2010; Kazamia & Smith, 2014; Durham *et al.*, 2015) and evidence that similar exchanges occur in the natural environment comes from correlations observed between the presence of B_{12}-producing bacteria and algal blooms (Gobler *et al.*, 2007; Bertrand *et al.*, 2015). There is still specificity in this interaction, however, demonstrated by the fact that, whilst cyanobacteria are B_{12} producers, they make a variant known as pseudocobalamin which is considerably less bioavailable to eukaryotic algae than cobalamin, the variant produced by many heterotrophic bacteria (Helliwell *et al.*, 2016). Thus provision of photosynthate from the algae may provide the signal to attract and retain cobalamin-producers within the phycosphere. As well as B_{12}, recent studies have demonstrated that bacteria can also provide either thiamine (vitamin B_1) or its precursors to phytoplankton (McRose *et al.*, 2014; Paerl *et al.*, 2015), and because these organic micronutrients are often limiting in the ocean (Sañudo-Wilhelmy *et al.*, 2012), thiamine-producing bacteria could potentially regulate phytoplankton blooms.

The specificity and extent of such algal-bacterial interactions in the natural environment remain to be determined however. One exciting development that will enable better understanding of the diverse and multifaceted ways in which algal cells interact with their biotic and abiotic environments is the explosion of metagenomics and metatranscriptomics information that is being produced by projects such as the TARA Oceans Expedition (Bork *et al.*, 2015). Current analyses of the 'interactome' in the photic zone have revealed novel partnerships and unexpected factors controlling community structure (Lima-Mendez *et al.*, 2015). Together with mechanistic examinations of algal physiology and biochemistry in laboratory conditions (e.g. (Durham *et al.*, 2015), these omics-enabled analyses will fundamentally change our views of

how algae sense and survive in the current world, and how resilient they may be to fluctuating conditions wrought by climate change.

3.2. Host-virus arms race during algal blooms

3.2.1. Viral control of algal blooms

Many algal species exhibit the phenomenon of 'blooms', for example 'red tides', where there is a massive increase in cell numbers over a short period, frequently as a result of changing environmental conditions, such as agricultural run-off or ocean upwelling. In some cases, these can pose threats to human health (so-called harmful algal blooms; HABs) due to the toxins that are produced by the algae and/or associated bacteria (Petitpas *et al.*, 2014). Such blooms are ephemeral events of exceptionally high primary productivity that regulate the flux of nutrients and metabolites across aquatic food webs. These large-scale events also contribute to global net primary production, one-half of which is provided by oceanic phytoplankton (Behrenfeld *et al.*, 2006). Several key biotic interactions can control the extent and fate of phytoplankton blooms in the ocean, among them top-down regulation by grazers, interactions with algicidal bacteria, and viral infection (Bidle, 2015). Viruses play a key role in this process because they infect many marine algal species, such as the major 'brown tide' alga *Aureococcus anophagefferens* (Moniruzzaman *et al.*, 2016), resulting in cessation of phytoplankton blooms. Viruses are the most abundant biological entities in the marine environment and are considered to be major ecological, evolutionary and biogeochemical drivers of marine microbial life (Suttle, 2007). Moreover, they enhance the diversity and composition of the microbial communities by facilitating HGT among their hosts.

Recent reports have highlighted a novel inventory of auxiliary metabolic genes found in the genomes of marine viruses that were previously thought to be restricted to the genomes of their hosts (Enav *et al.*, 2014; Rosenwasser *et al.*, 2016) with functions including photosynthesis, the pentose phosphate pathway, phosphate regulation, sulfur metabolism, polysaccharide synthesis, sphingolipid metabolism, and DNA/RNA processing. These genes can expand metabolic capabilities within the infected phototrophs and affect the flux of metabolites and infochemicals to the phycosphere. Viruses infecting terrestrial plants are typically small RNA viruses that encode a few genes and therefore their life cycle is tightly integrated with and

dependent on the cellular processes of their host plants (Roossinck, 1997). In contrast, viruses that infect eukaryotic algae can have a high burst size (i.e., number of viruses released from each infected cell), and have genomes of 160 to 560 kbp that encode up to 600 proteins (Wilson *et al.*, 2009). Thus, these viruses require substantial resources such as fatty acids, amino acids, nucleotides, and energy to facilitate replication and assembly. Nevertheless, there is still no fundamental understanding of how such large viruses rewire the metabolism of their photosynthetic host to support their unique life cycle.

Although the ecological importance of host-virus interactions is well recognized, the ability to assess their functional/ecological impact is limited to current approaches that focus mainly on quantification of viral abundance, gene content and diversity (Brum & Sullivan, 2015). Developing laboratory-based model systems for ecologically relevant algal-virus interactions, coupled with a molecular toolbox and genomic and post-genomics resources have deepened our mechanistic understanding of these interactions and their ecological impact (Fig. 3) (Read *et al.*, 2013).

3.2.2. *Emiliania huxleyi*-EhV- an important host-pathogen model system

The cosmopolitan coccolithophore *E. huxleyi* is a unicellular alga that forms massive oceanic blooms covering thousands of square kilometers (Tyrrell & Merico, 2004). The intricate calcite exoskeleton of *E. huxleyi* accounts for approximately one third of total marine $CaCO_3$ production (Monteiro *et al.*, 2016). *E. huxleyi* is also a major producer of dimethyl sulfide (DMS), a bioactive gas with a significant climate-regulating role that enhances cloud formation (Alcolombri *et al.*, 2015). Therefore, biotic interactions that regulate the fate of these blooms play a profound role in determining atmospheric conditions and nutrient cycling in the ocean. Annual *E. huxleyi* spring blooms are frequently terminated by infection with a specific large dsDNA virus (EhV) (Schroeder *et al.*, 2002) that belongs to the Coccolithoviruses group within the monophyletic Phycodnaviridae, a family of nucleocytoplasmic large DNA viruses. This model host-virus interaction spans more than 10 orders of spatial magnitude, from the individual cell (~10^{-6} m) to mesoscale oceanic eddies (~10^{5} m) (Lehahn *et al.*, 2014). The system is physiologically well characterized and has the great advantage of a wealth of genomic information from the alga (Read *et al.*, 2013) and from specific viral strains with different degrees of susceptibility to viral infection. Genome analysis of EhV revealed a cluster of putative

sphingolipid biosynthetic genes (Wilson *et al.*, 2005). Production of glycosphingolipids is strongly induced during viral infection. These lipids are major constituents of EhV membranes and can induce host programmed cell death (PCD) during lytic infection in cultures and during natural blooms (Vardi *et al.*, 2012). Indeed, during lytic infection, EhV triggers hallmark PCD responses, including production of ROS (Vardi *et al.*, 2012; Sheyn *et al.*, 2016), induction of caspase activity, metacaspase expression and compromised membrane integrity (Bidle *et al.*, 2007). Viral infection also induced remodeling of the host antioxidant gene network and redox metabolism through co-induction of glutathione and H_2O_2 synthesis, both essential for successful viral replication (Sheyn *et al.*, 2016). Viral infection "engineers" sphingolipid metabolism of the host by causing down-regulation of host sphingolipid biosynthesis genes while the viral genes are highly up-regulated (Rosenwasser *et al.*, 2014), resulting in altered substrate specificity of serine palmitoyl-CoA transferase activity (Ziv *et al.*, 2016). The viral enzymes have different substrate specificities from those of the host and regulate the production of virus-specific glycosphingolipids composed of unusual hydroxylated C17 sphingoid-bases (t17:0) (Ziv *et al.*, 2016). These virus-specific sphingolipids are essential for assembly and infectivity by the virion. Combined transcriptomic and metabolomic analyses over the course of an *E. huxleyi* viral infection revealed major, rapid transcriptome remodeling that elicited elevated de novo fatty acid synthesis to support viral assembly and a high demand for viral internal lipid membranes (Rosenwasser *et al.*, 2014). Remodeling of lipid metabolism was mediated by accumulation of distinct lipid droplets containing highly saturated triacylglycerols (TAGs) (Malitsky *et al.*, 2016). Stored TAGs may serve as energy and lipid reservoirs that are catabolized for viral assembly during later stages of infection.

These approaches, which involved rigorous quantification of the rewired metabolism during algal-virus interactions have provided fundamental insights into the strategies employed during their biochemical "arms race". Identification of specific metabolites synthesized during these interactions may yield biomarkers for sensitive detection of active viral infection in the marine environment (Vardi *et al.*, 2009).

IV. Future prospects

As described in this review, there has been significant progress in studies of the algal symbiome that stress the primacy of biotic factors in algal growth and productivity in the environment.

These analyses have provided significant mechanistic insight into emerging systems across the algal tree of life. Nevertheless, there still remain many gaps in our knowledge and approaches. For example most studies at the functional level have focused on "pairs" such as bacteria/microalgae or viruses/microalgae, whereas these are likely to be much more complex in the natural environment. Similarly, whilst studies of microbial communities during annually reoccurring phytoplankton blooms provide clues about microalgae/bacteria interactions at the community level and in relation to changing environmental conditions, including those driven by global change (e.g. (Needham & Fuhrman, 2016), few address specific interactions. This is important because short-term fluctuations of environmental parameters (e.g., diurnal fluctuations) may be buffered by biotic interactions and are therefore invisible to the investigator, which would lead to the conclusion that they are not important, even though they might have an impact over a longer time scale. Furthermore, most studies do not look beyond correlations based on co-occurrence networks, which, while providing useful preliminary data on who interacts with whom, do not provide insights into the biological processes that orchestrate these interactions.

To tackle these challenges, future studies should include detailed biochemical analyses of metabolites both in environmental samples *in situ* and under controlled laboratory conditions, using either natural or synthetic communities. The combined analyses of natural and synthetic communities and the use of microbial mutants that impact specific pathways will help determine activities associated with ecosystem function. Genome editing applied to model microalgae and bacteria in combination with biochemical analyses of processes that govern their interactions will provide a step change in understanding how significant these interactions are in relation to abiotic drivers of biological diversity such as temperature, nutrients, seasonality and solar irradiance. By studying communities across global-scale environmental gradients such as coastal/open sea, surface/deep ocean, or polar/tropics, it should be possible to identify commonalities between taxonomically distinct, yet functionally equivalent communities.

Finally, metagenomic data is of vital importance to this field, but need to be combined with functional studies. We are now presented with an overwhelming amount of genomics and meta data and the time has come to start ferreting out the biological 'meaning' of this information using algal model systems, genetic tools, and functional genomics to understand gene function and cellular mechanism and connect these insights with in-depth studies of

physiology, metabolism, and life cycle phenotypes. Adding the new dimension of single cell analysis is another emerging area that will likely fundamentally chmage how we interpret algal diversity, behavior, and acclimation strategies. With these integrative approaches, we may even be able to provide key insights into how global change not only impacts the diversity of specific taxa but the complex interacting communities of species in the ocean that underpin marine ecosystem services responsible for the health and well-being of human societies.

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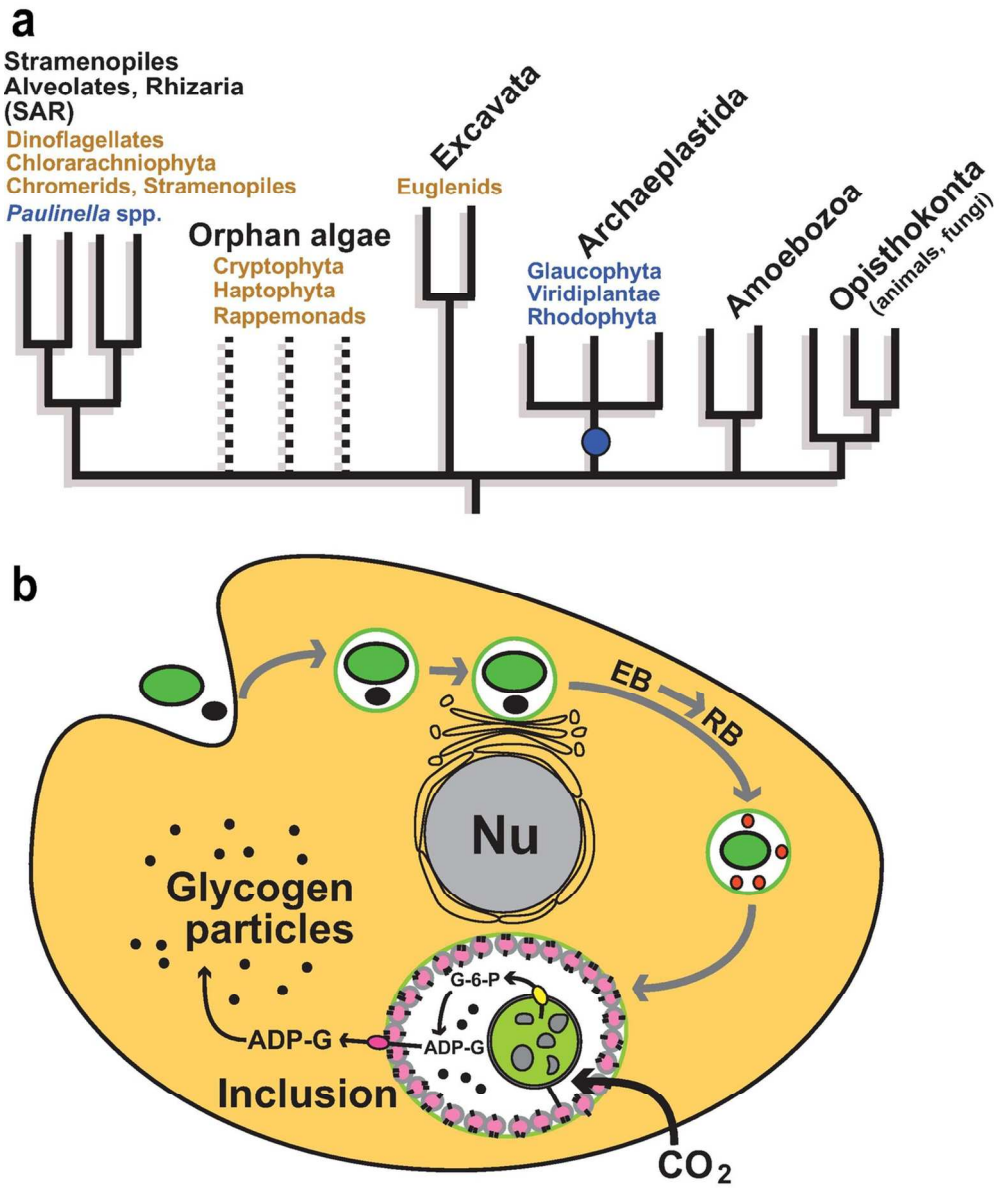
Figure legends

Fig. 1 Evolutionary history of algae. a) Schematic tree of eukaryotes showing the polyphyletic origins of algae. Plastids derived through primary cyanobacterial endosymbiosis are shown in blue text and when derived through secondary or higher order endosymbioses are shown in brown text. The major clades are often referred to as supergroups, except the orphan algae that do not yet have a stable position in molecular phylogenies. b) The ménage à trois hypothesis (MATH) for primary plastid origin in the Archaeplastida ancestor. The MATH proposes that environmental Chlamydiales played a direct role in plastid endosymbiosis vis-à-vis a tripartite relationship between the host, captured cyanobacterium, and a chlamydial symbiont (Ball *et al.*, 2013; Ball *et al.*, 2016a). Under this view, a chlamydial infectious particle (EB: elementary body, black circle) enters a host cell together with a cyanobacterium (turquoise circle). The EB remodels the phagocytic membrane into a chlamydia-controlled inclusion, thereby escaping host defenses. The EB differentiates into reticulate bodies (RBs; pink circles) that attach to the inclusion and secrete chlamydial effector proteins corresponding to glycogen metabolism enzymes into both the inclusion and the host cytosol. The cyanobacterium recruits chlamydial transporters through conjugation with Chlamydiae that allow the export of glucose-6-phosphate (G6P) through the UhpC transporter (yellow circle in the cyanobacterial cell envelopes). The G6P feeds glycogen synthesis within the inclusion through the ADP-G dependent chlamydial pathway of glycogen metabolism. Excess ADP-G in the inclusion exits through a host derived NST (nucleotide sugar transporter, red circle) and is incorporated into the host glycogen pool. This hypothetical sequence of steps is believed to have led to the establishment of the long-term symbiosis.

Fig. 2 Dinoflagellate symbionts in corals. a) *Acropora millepora* and b) *A. tenuis* showing tentacles associated with individual coral polyps and tissue color (with individual cells visible in *A. tenuis*) associated with a high abundance of *Symbiodinium* within the coral gastrodermis tissue layers (photo credits: Jean-Baptiste Raina). c) Metabolic exchange and nutrient trafficking between the coral animal and its *Symbiodinium* symbionts and extracellular microbes.

Fig. 3 Genome-enabled technologies can define distinct metabolic state of life cycle states during host-pathogen/symbiont interactions. The metabolic state of diverse life cycle states during host-

pathogen/symbiont interactions can be defined by combined high-throughput approaches including transcriptomics, proteomics, and metabolomics. Dimension reduction analyses such as principal component analysis (PCA) can be used to define the specific metabolic states of the algal host, its pathogen or free-living symbiont, and the unique metabolism of infected algae. Such characterization provides metabolic fingerprints that can serve as novel biomarkers to assess host-pathogen interactions in the marine environment, and to unravel the strategies employed during biochemical "arms race" of host-pathogen co-evolution. Figure modified from Rosenwasser *et al*. (2016).



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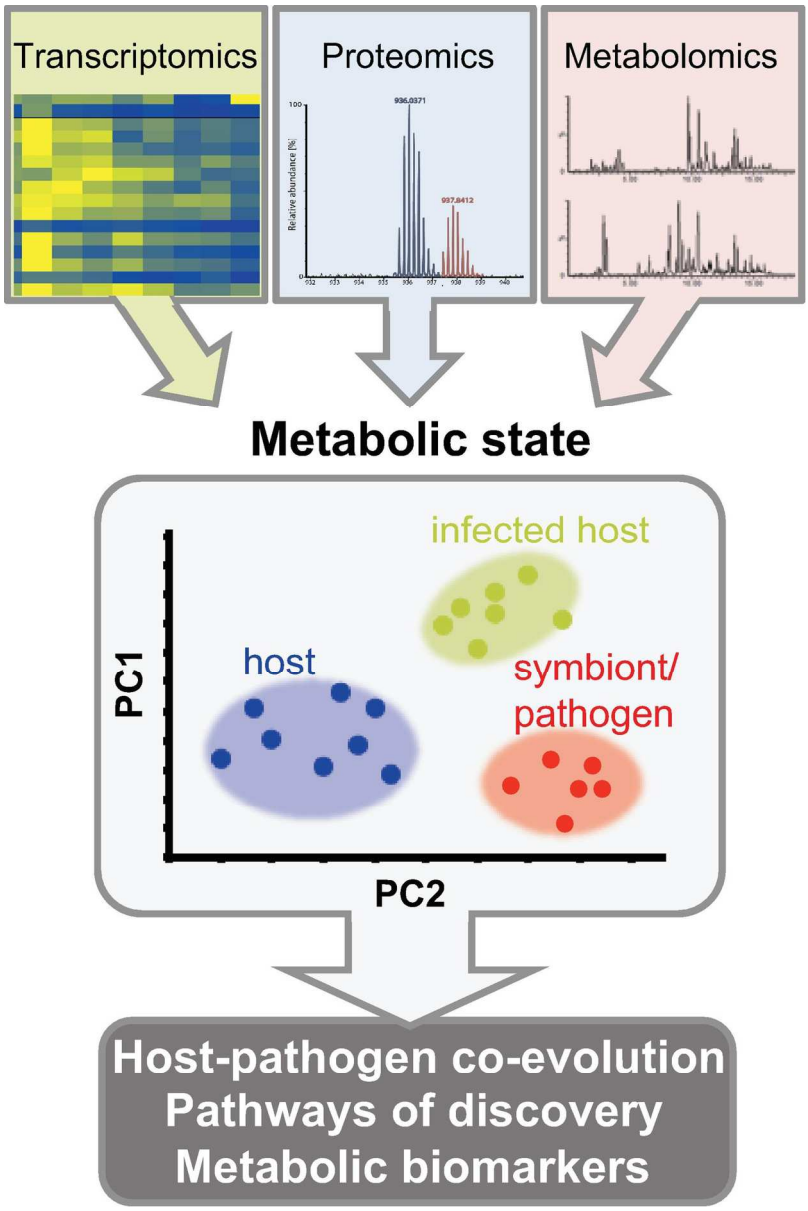

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