Coccolithophore Cell Biology: Chalking Up Progress

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Abstract

Coccolithophores occupy a special position within the marine phytoplankton because of their production of intricate calcite scales, or coccoliths. Coccolithophores are major contributors to global ocean calcification and long-term carbon fluxes. The intracellular production of coccoliths requires modifications to cellular ultrastructure and metabolism that are surveyed here. In addition to calcification, which appears to have evolved with a diverse range of functions, several other remarkable features that likely underpin the ecological and evolutionary success of coccolithophores have recently been uncovered. These include complex and varied life cycle strategies related to abiotic and biotic interactions as well as a range of novel metabolic pathways and nutritional strategies. Together with knowledge of coccolithophore genetic and physiological variability, these findings are beginning to shed new light on species diversity, distribution, and ecological adaptation. Further advances in genetics and functional characterization at the cellular level will likely to lead to a rapid increase in this understanding.

1. INTRODUCTION TO COCCOLITHOPHORES

The coccolithophores are an important group of marine phytoplankton characterized by their covering of external $CaCO_3$ plates called coccoliths. They emerged relatively recently in evolutionary timescales (~300 Ma) and have become major contributors to marine ecosystems and global biogeochemical cycles. The most abundant coccolithophore species in modern oceans is *Emiliania huxleyi*, which can form massive blooms in temperate and subpolar regions, producing up to 10^8 cells L^{-1}. Together with other ecologically significant species, the coccolithophores contribute up to half of the ~1.6 Pg y^{-1} of $CaCO_3$ produced in the pelagic zone (Balch et al. 2007). Coccolithophores influence surface-ocean biogeochemistry by fixing a significant amount of C through photosynthesis (the biological C pump) and by releasing CO_2 during coccolith formation (the carbonate counter-pump) (Rost & Riebesell 2004). The ballast of sinking coccolithophore calcite increases the burial flux of organic matter (Ziveri et al. 2007). Coccolithophores also contribute to global S cycling through their production of dimethylsulfoniopropionate (DMSP).

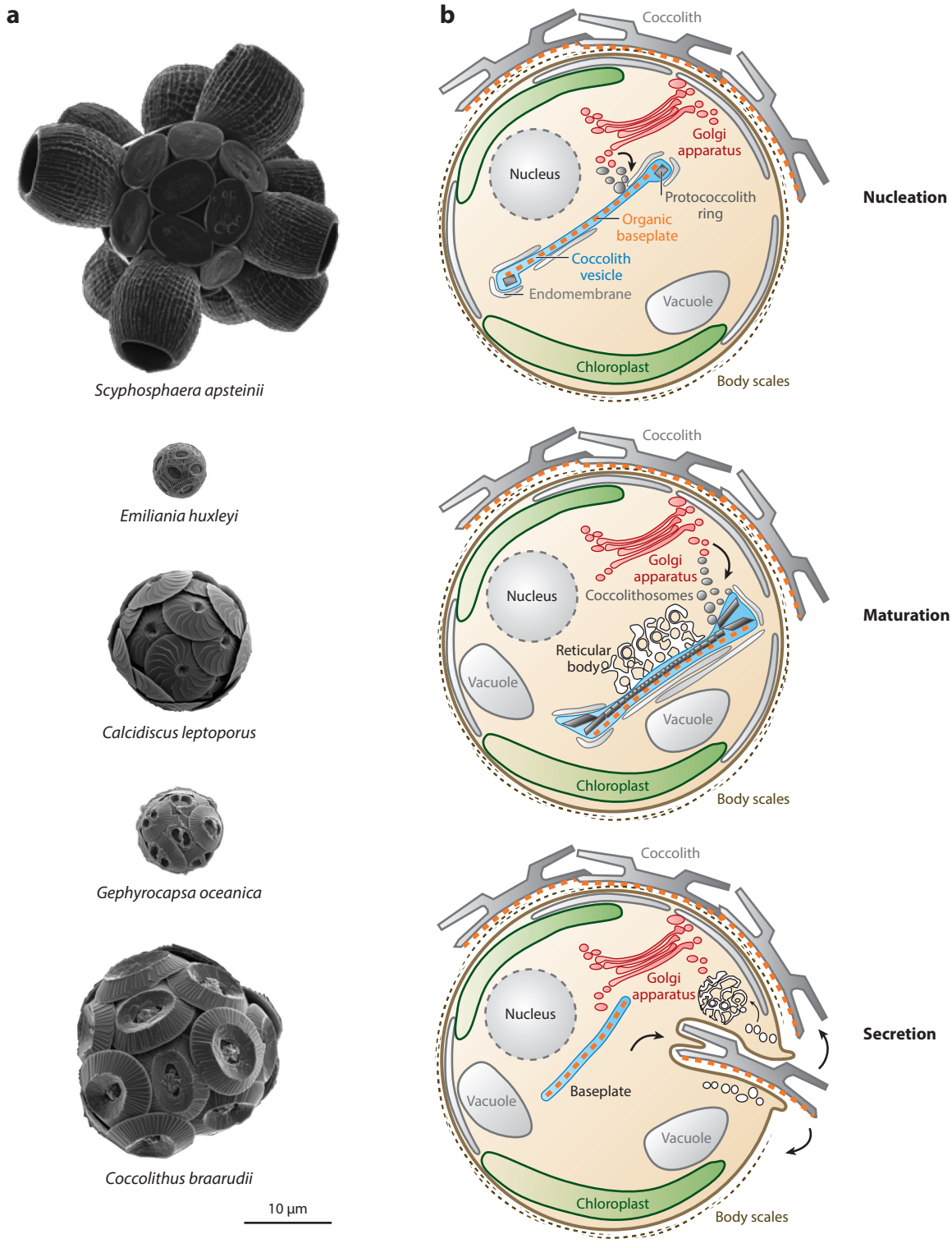
Coccolithophores exhibit remarkable metabolic features that underpin their ability to successfully compete with other species in the surface oceans. Most notably, unlike most calcifying organisms, they produce their calcite coccoliths in an intracellular compartment and subsequently secrete them onto the cell surface (Raven & Giordano 2009) (**Figure 1**). A better understanding of the ecophysiology of coccolithophores through studies of phenotypic and physiological plasticity, cell metabolism, microbial interactions, mechanisms of calcification, and genetic diversity will lead to an improved understanding of their biogeochemical impacts and responses to environmental change. Here, we highlight some of the most recent advances in these areas and offer suggestions of potential avenues for further research.

2. EVOLUTION OF COCCOLITHOPHORES

Coccolithophores belong to the Haptophyta lineage of eukaryotes, the position of which in the eukaryote tree of life has been much debated. Recent multigene phylogenies place the haptophytes as a sister group to the centrohelids in the Haptista, which shows some association to the Stramenopile-Alveolate-Rhizarian (SAR) supergroup but excludes cryptophytes (Burki et al. 2016). Haptophytes possess a plastid of red algal origin, although the mechanism through which this plastid was acquired is also the subject of considerable debate. A recent study suggested that plastids of red algal origin in many photosynthetic eukaryotes may have arisen from serial endosymbiotic events, with haptophytes acquiring their plastid from an ochrophyte (photosynthetic stramenopile) ancestor (Stiller et al. 2014). This hypothesis is in alignment with both ultrastructure and pigment associations between these two groups and presumably occurred early in the evolution of haptophytes, because chloroplast acquisition by the aplastidic ancestral cell is estimated to have taken place at ~1,100 Ma (De Vargas et al. 2007), although it is clear that much remains to be learned about haptophyte evolution.

Figure 1

Coccolithophores and intracellular calcification. (*a*) Scanning electron micrographs of several coccolithophores reproduced to the same scale. (*b*) Models illustrating the sequence of intracellular coccolith production. The process starts with the nucleation of peripheral calcite crystals onto an organic baseplate that is produced in a Golgi-derived coccolith vesicle (*top*). *Trans*-Golgi vesicle trafficking provides organic components. As the calcite coccolith matures (*middle*), the endomembranes associated with the coccolith vesicle become more complex, playing an important role in ion transport and coccolith morphology. Finally, coccoliths are released to the cell surface through exocytosis (*bottom*). Rapid recycling of the membrane components is likely required for new coccolith production.


a
Scyphosphaera apsteinii
Emiliania huxleyi
Calcidiscus leptoporus
Gephyrocapsa oceanica
Coccolithus braarudii
10 µm
b
Coccolith
Golgi apparatus
Nucleus
Protococcolith ring
Organic baseplate
Coccolith vesicle
Endomembrane
Vacuole
Chloroplast
Body scales
Nucleation
Coccolith
Golgi apparatus
Nucleus
Coccolithosomes
Reticular body
Vacuole
Vacuole
Chloroplast
Body scales
Maturation
Coccolith
Golgi apparatus
Nucleus
Baseplate
Vacuole
Vacuole
Chloroplast
Body scales
Secretion

Calcification (the precipitation of $CaCO_3$) occurs in diverse eukaryote lineages, suggesting that this trait has evolved on multiple independent occasions (Raven & Giordano 2009). It likely emerged in the coccolithophores close to the divergence of the Calcihaptophycidae and Prymnesiales (~310 Ma) (Liu et al. 2010), with the earliest fossil heterococcoliths and holococcoliths dated at 220 Ma and 185 Ma, respectively (De Vargas et al. 2007). Calcification in the haptophytes may have evolved independently on more than one occasion, as the phylogenetic position of *Braarudosphaera*, which produces atypical pentagonal nannoliths, remains uncertain (Hagino et al. 2016). The elevated Mg content of the pentaliths of *Braarudosphaera* suggests that they form extracellularly (Hagino et al. 2016), although the inability to grow this species in culture has hampered more detailed investigations into its physiology and evolutionary origins. Loss of calcification appears to have occurred at least once in the coccolithophores, as the Isochrysidales contain numerous noncalcifying lineages (e.g., *Isochrysis*). The calcifying members of the Isochrysidales (*Emiliania* and *Gephyrocapsa*) also lack holococcoliths in their haploid life cycle stages, suggesting that this trait either evolved after the divergence of the Isochrysidales or was lost in this lineage. Some members of the Coccolithales (*Pleurochrysis* and *Hymenomonas*) also lack holococcoliths, supporting independent loss.

Whether there were strong coevolutionary relationships between the emergence of calcification in haptophytes and the physicochemical properties of the oceans remains unclear (Raven & Giordano 2009). A recent study revisited the hypothesis that intracellular calcification evolved as a strategy to avoid the cytotoxicity of Ca^{2+} under the higher levels of Ca^{2+} in which coccolithophores arose. A calcifying strain of *E. huxleyi* showed resilience to increased levels of Ca^{2+} representative of the Cretaceous, whereas several noncalcifying phytoplankton and a noncalcifying strain of *E. huxleyi* were unable to tolerate these higher Ca^{2+} levels (Müller et al. 2015). This most likely demonstrates the efficiency of the Ca^{2+} transport and sequestration system in calcifying coccolithophores that can overcome the additional burden of Ca^{2+} influx imposed under these conditions. Whether the higher environmental Ca^{2+} levels in which coccolithophores evolved acted to select for the evolution of an entire intracellular calcification system remains highly speculative. As Raven & Crawfurd (2012) pointed out, cellular mechanisms that maintain low free cytosolic Ca^{2+} evolved early in eukaryote evolution, well before the emergence of intracellular calcification in coccolithophores.

3. CELL BIOLOGY, LIFE CYCLE, AND ECOLOGICAL NICHES

3.1. Life Cycle Transitions

Coccolithophores exhibit both calcified haploid and diploid life cycle phases that can reproduce asexually (Frada et al. 2009, Houdan et al. 2004, Nöel et al. 2004, Young et al. 2005). Diploid cells produce structurally complex calcite crystal heterococcoliths and dominate natural populations. In many species, the periodic haploid phase produces holococcoliths made up of simple calcite rhombohedra (Geisen et al. 2002, Young et al. 1999). Transitional coccospheres comprising holo- and heterococcoliths have been described for most major taxonomic groups, primarily from field specimens (Cros et al. 2000, Geisen et al. 2002, Young et al. 2005), suggesting that coccolithophores readily undergo life phase transitions in natural populations. In some species within the Pleurochrysidaceae and Hymenomonadacae, the heterococcolith-bearing phase alternates with a noncalcifying haploid phase (Fresnel 1994, Nöel et al. 2004).

Oviedo et al. (2015) proposed that nutrient-poor pelagic waters favor motile haploid holococcolithophore assemblages, whereas diploid cells are better adapted for warmer, nutrient-rich coastal waters. Nutrient-driven diploid-haploid niche partitioning may also underlie the depth

distributions of coccolithophores, as observed in the northwest Mediterranean, with nutrient-depleted upper oligotrophic waters favoring haploid holococcolith-bearing cells and deeper, nutrient-rich waters favoring diploid heterococcolith-bearing cells (Cros & Estrada 2013, Oviedo et al. 2015). Accordingly, the diploid *Coccolithus braarudii* and *Calcidiscus leptoporus* sustain higher growth rates than haploid motile cells under high inorganic nutrient levels (Houdan et al. 2006). Moreover, Nöel et al. (2004) were able to induce transitions from haploid cells to heterococcolith diploid cells of the oceanic *Calyptrosphaera sphaeroidea* by increasing trace metals and vitamins in the culture medium. They also found that a decreased temperature causes diploid-to-haploid transitions in this species. Switching to a haploid and potentially mixotrophic mode of nutrition presumably enables these cells to sustain growth rates that would otherwise not be possible under inorganic nutrient limitation (see Section 3.2). Consistent with this, addition of organic C stimulates the growth of haploid cells of *C. braarudii* and *C. leptoporus*, and these cells are known to actively phagocytose bacteria (Houdan et al. 2006). Increased turbulence inhibits the growth of haploid motile *C. braarudii* and induces phase transitions to the diploid nonmotile phase (Houdan et al. 2006). Finally, both laboratory and field experiments have suggested that viral infection promotes a shift from susceptive diploid to resistant (noncalcifying) haploid *E. huxleyi* (Frada et al. 2008, 2012), implying that alternating life history phases could be a crucial response to the presence of pathogens that ensures the long-term persistence of the resident population (**Figure 2**).

The alternating haploid-diploid life cycle of coccolithophores, combined with the quite different physiological capabilities of these two cell types, likely represents a successful niche partitioning

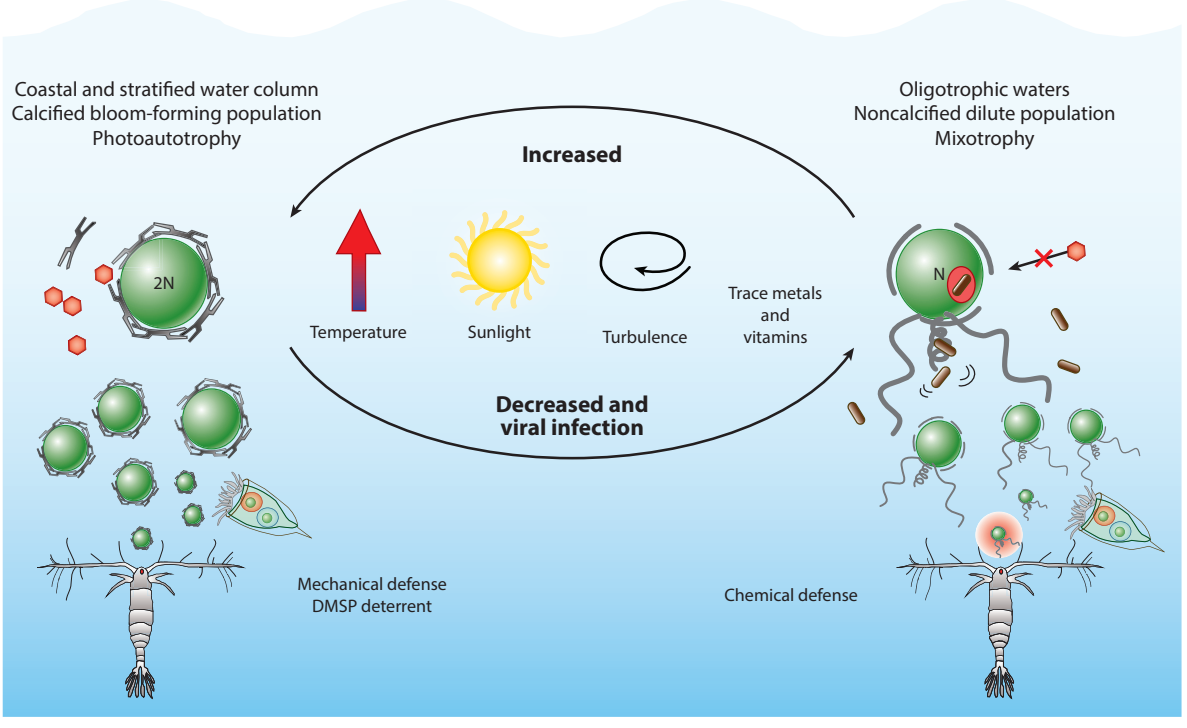


Figure 2

The major abiotic and biotic factors that appear to influence life cycle phase transitions in coccolithophores. Abbreviation: DMSP, dimethylsulfoniopropionate.

strategy under varying abiotic and biotic pressures. The haploid phase is also the precursor to sexual reproduction through syngamy, which potentially contributes to microadaptation in response to the prevailing conditions. The high genomic variability among diploid calcifying *E. huxleyi* strains (Read et al. 2013) points to a strong adaptation signal among extant isolates. Interestingly, a recent study showed that the haploid gene content of diploid strains varies considerably among biogeographical isolates. Using sequencing and competitive genome hybridization, von Dassow et al. (2015) demonstrated that the haploid genome content is diminished in some diploid strains. For example, 23% of a set of *E. huxleyi* genes associated with cilia or flagella function (including genes encoding three of the critical dynein heavy-chain proteins) are missing from the genome sequence of the CCMP1516 strain, suggesting that it has lost the ability to form functional flagella (von Dassow et al. 2015). Whether this loss occurred during the ~20 years this strain remained in culture under stable high-nutrient conditions is unclear. However, a targeted polymerase chain reaction of 83 diploid strains isolated from different oceanographic regions showed that 37 strains associated with warmer oligotrophic waters lacked two dynein heavy-chain genes essential for flagella motility, which suggests that the ability to undergo phase transitions to motile haploid cells was not advantageous for *E. huxleyi* under these generally warmer, stable, low-nutrient conditions (von Dassow et al. 2015). This finding is seemingly at odds with the studies of holococcolith-bearing species described above, which demonstrated that oligotrophic conditions favor the transition to the haploid stage. A more detailed understanding of the ecological drivers of life cycle transitions in coccolithophores is needed. It will be interesting to see whether strains that have lost the ability to produce motile haploid cells are susceptible to the *E. huxleyi* virus (EhV; see below).

3.2. Mixotrophy

Bacterivory in oligotrophic ecosystems is dominated by picoeukaryote algae (Hartmann et al. 2012), with small, flagellated, noncalcifying haptophyte taxa contributing up to 30% of bacterivory in oligotrophic coastal waters (Unrein et al. 2014). These haptophytes acquire and incorporate C and N from labeled *Prochlorococcus* and *Synechococcus*, implying that they can be significant grazers of picocyanobacteria, redirecting C within marine food webs (Ward & Follows 2016). Given the mixotrophic origins of the haptophytes (De Vargas et al. 2007), it is not surprising that coccolithophores possess the genes associated with the maintenance of a phagosomal pathway. Although phagocytotic behavior has generally been attributed to the haploid motile (and haptonemal-bearing) life phase of coccolithophores, transcripts of genes related to phagocytosis in *E. huxleyi* are more abundant in diploid, nonmotile, calcifying cells (Rokitta et al. 2011). If phagocytosis occurs in these diploid cells, it does so in the absence of a haptonemal appendage and while the cell is covered with a layer of coccoliths.

Much remains to be learned about the nutritional capability of coccolithophores to determine whether they can be significant grazers of bacteria and to assess their ability to occupy alternate ecological niches in order to overcome inorganic nutrient limitation. This is of particular interest considering climate change scenarios in which increased sea surface temperature and stratification may favor mixotrophic modes of nutrition (Mitra et al. 2014, Wilken et al. 2013).

4. BIOTIC INTERACTIONS

4.1. Bacteria

Although there are several well-described mutualistic interactions between eukaryote phytoplankton and bacteria (see Cooper & Smith 2015 and references therein), functional interactions between bacteria and coccolithophores remain largely uncharacterized. A survey of bacteria

associated with cultured *E. huxleyi* and *C. braarudii* highlighted a species-rich community of alpha- and gammaproteobacteria, including several taxa that are also associated with other phytoplankton, such as *Marinobacter* and *Marivita* (Green et al. 2015). Of interest are bacteria that may be more specific to the unique coccolithophore phycosphere. These include hydrocarbon-degrading bacteria and a Bacteroidetes diversity dominated by Sphingobacteria as opposed to the Flavobacteria that are more typical of diatoms and dinoflagellates. The presence of species of Acidobacteria known to be associated with organisms that secrete carbonate biominerals led to the proposal that these acid-secreting bacteria could degrade coccolith calcite and access coccolith-associated polysaccharides (CAPs) as a source of organic C (Green et al. 2015).

How coccolithophore-bacteria associations influence nutrient exchange and C flow is unknown. However, comparing axenic and nonaxenic cultures, Van Oostende et al. (2013) showed that the presence of bacteria in cultures of P-limited *E. huxleyi* results in an altered composition of dissolved polysaccharides and a greater production of extracellular particulate organic matter. Thus, bacterial activity can modify the pattern of organic matter produced and released by coccolithophores, thereby influencing export production. Moreover, intracellular pools of lipids and alkenones are likely dependent on bacterial assemblages associated with coccolithophores (Segev et al. 2016), which warrants further investigation, given the importance of the alkenones as temperature paleoproxies.

Evidence of mutualistic interactions between bacteria and coccolithophores is limited. Seyedsayamdost et al. (2011) described a mutualism between the bloom-associated roseobacter *Phaeobacter gallaeciensis* and *E. huxleyi* in which the bacterium produces antibiotics and auxin, which are presumed to support a growth-enhancing relationship in which the bacterium derives C and S from DMSP produced by the algae. However, *P. gallaeciensis* opportunistically switches from mutualist to pathogen as *E. huxleyi* approaches stationary or senescing stages. The cue for this so-called Jekyll-and-Hyde transition by *P. gallaeciensis* is *p*-coumaric acid, a lignin-like compound released by aging *E. huxleyi* cells. In response to *p*-coumaric acid, *P. gallaeciensis* produces a suite of secondary metabolites, including potent algicides called roseobacticides (Seyedsayamdost et al. 2011). This complex metabolic interaction has an interesting twist, as isotope labeling demonstrates that the bacteria incorporate the *p*-coumaric acid into the biosynthetic pathway of the roseobacticides, resulting in a virulent hybrid molecule derived from both host and pathogen (Seyedsayamdost et al. 2014). Moreover, the DMSP derived from the algae during the mutualistic phase is important in providing a source of S for toxin production (Seyedsayamdost et al. 2014) (**Figure 3**).

The potential for detrimental interactions with bacteria is also demonstrated by the high sensitivity of *E. huxleyi* to the algicidal marine gammaproteobacterium *Pseudoalteromonas piscicida* (Harvey et al. 2016). In this case, a soluble quorum-sensing alkyl-quinolone was purified and found to mediate mortality in *E. huxleyi* at nanomolar concentrations, whereas the green alga *Dunaliella tertiolecta* and the diatom *Phaeodactylum tricornutum* were insensitive to this compound. Nevertheless, all three species were susceptible to exudates of *P. piscicida*, suggesting the production of a cocktail of compounds that confers broad algicidal activity (Harvey et al. 2016).

These recent developments demonstrate that coccolithophores have a complex and underexplored repertoire of symbiotic, mutualistic, and antagonistic interactions with bacteria. Characterizing these interactions is important to determine the relative contributions of bacteria and viruses to coccolithophore population dynamics and associated biogeochemical cycles (**Figure 3**).

4.2. Viruses

The complete genome sequence and transcription profile of EhV-86, a large DNA virus that infects *E. huxleyi* (Wilson et al. 2005), yielded important insights into the host–virus dynamic.

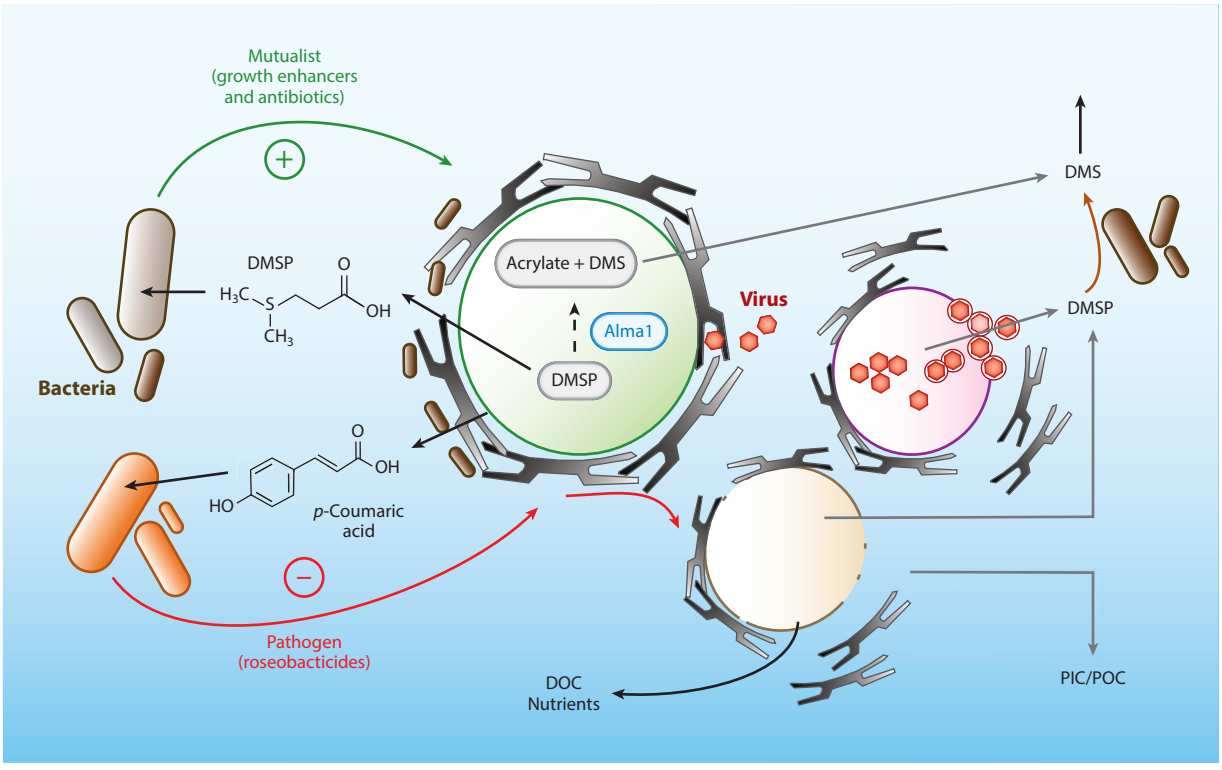


Figure 3

Bacterial and viral interactions with *Emiliania huxleyi* and their intersection with DMSP metabolism. Aside from its osmoprotective properties, DMSP serves other functional roles, including hindering grazing activity, contributing to antioxidant capacity, and sustaining bacterial mutualisms. The recently discovered DMSP lyase Alma1 in *E. huxleyi* indicates that endogenous control of DMSP pools can be dynamically regulated in coccolithophores. Release of DMSP upon cell death leads to rapid turnover and increased DMS production through bacteria-mediated cleavage. Abbreviations: DOC, dissolved organic carbon; DMS, dimethyl sulfide; DMSP, dimethylsulfoniopropionate; PIC, particulate inorganic carbon; POC, particulate organic carbon.

Both mesocosm (Pagarete et al. 2011) and mesoscale studies have demonstrated the ability of the virus to regulate bloom dynamics in natural populations, especially under relatively stable physical conditions (Lehahn et al. 2014). Research by several groups over the last decade has focused on the viral infection mechanism (**Figure 4**).

An important early observation in the EhV-86 genome was the presence of a suite of genes that are derived from host–virus horizontal gene transfer (Monier et al. 2009) and code for the biosynthesis of sphingolipids, which are expressed during the lytic infection cycle (Wilson et al. 2005). Studies have since shown that the virus reprograms host lipid metabolism, stimulating the production of highly saturated triacylglycerols (Malitsky et al. 2016), suppressing the host glycosphingolipid pathway, and promoting the production and incorporation of viral glycosphingolipids (vGSLs) (Rosenwasser et al. 2014). At least in the early stages of infection, the virions bud from the host and retain a lipid envelope that is derived from the host (Mackinder et al. 2009) but is highly enriched in saturated triacylglycerols (Malitsky et al. 2016) and contains vGSLs (Fulton et al. 2014, Vardi et al. 2009).

The infection mechanism of EhV appears to involve recognition of components in lipid raft microdomains of the host membrane. Uninfected cells have a diverse lipid raft proteome, the

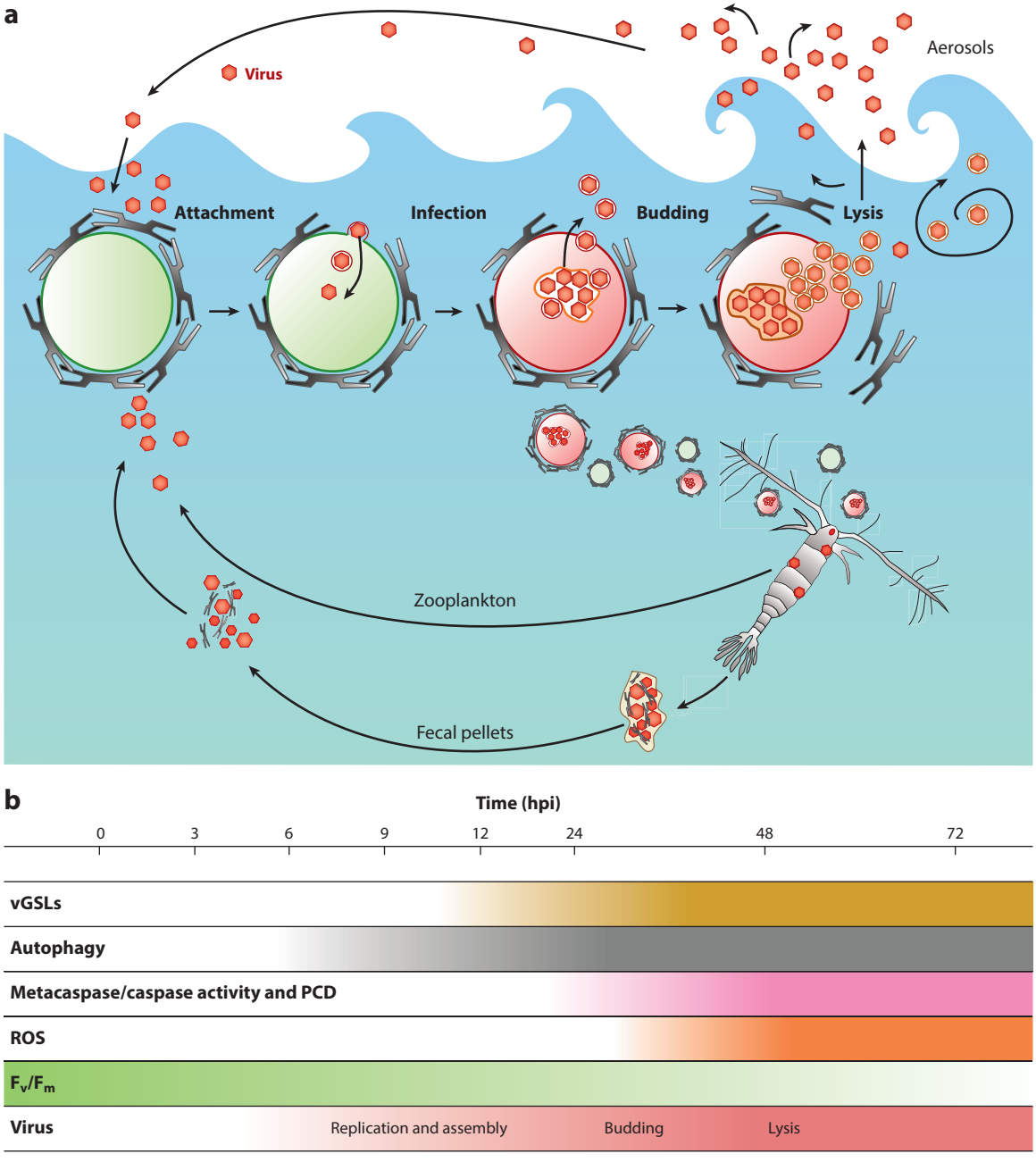


Figure 4

Major cellular events during EhV infection of diploid *Emiliania huxleyi*. (*a*) The main stages of infection of *E. huxleyi* by viral particles in the ocean and the potential viral transmission mechanisms that have been identified. (*b*) A timeline and the main cellular events that have been examined in laboratory cultures of *E. huxleyi*. Abbreviations: EhV, *Emiliania huxleyi* virus; F_v/F_m, in vivo maximum quantum yield of photosystem II photochemistry, decline of which is an indicator of metabolic stress; hpi, hours postinfection; PCD, programmed cell death; ROS, reactive oxygen species; vGSL, viral glycosphingolipid.

composition of which is rapidly altered upon infection by EhV (Rose et al. 2014). Of particular interest is a Toll-like interleukin receptor protein present in host lipid rafts that may interact with a virus-associated C-type lectin, possibly mediating attachment and viral translocation across the membrane. Whether lipid rafts play a role in both viral entry and egress has not been fully resolved. Susceptibility to infection appears to also be strongly correlated with the presence of sialic-acid glycosphingolipids (sGSLs) in diploid cells (Hunter et al. 2015). The absence of sGSLs from the lipidome of resistant haploid cultures suggests a mechanism that could explain the basis for the diploid-to-haploid escape strategy during virus-induced bloom termination (Frada et al. 2008, 2012). The events that trigger a switch to haploid and resistant cells upon viral infection are yet to be established.

A further innovation of EhV is the proviral utilization of the host autophagy pathway. Schatz et al. (2014) recently demonstrated that the lytic phase of EhV infection corresponds with increased expression of autophagy-related genes (ATG genes) along with ultrastructural changes (multimembrane vesicles), increased lysosomal activity, and a protein marker (Atg8) for autophagy associated with the membranes surrounding viral particles. Viral entry and DNA replication are unaffected by inhibitors of autophagy, whereas assembly and exit from the cell are suppressed, suggesting that the co-option of the autophagy pathway is a late-stage cellular interaction that promotes intracellular encapsulation of the virions in modified host membranes and propagation by burst release (Schatz et al. 2014) (**Figure 4**). Viral particles are coated with several layers of host membrane enriched with triacylglycerols and vGSLs and facilitate the envelope fusion of viral particles with uninfected host membranes (Mackinder et al. 2009).

Terminal events following infection and in response to increasing vGSLs include increased reactive oxygen species production (Evans et al. 2006), rapid degradation of cellular components, reduction of photosynthetic efficiency, and induction of host caspase and metacaspase activity that is necessary to promote viral production (**Figure 4**). This suggests that host–virus coevolution resulted in strong selection for viruses that co-opt the phytoplankton programmed-cell-death pathway in their infection strategy (Bidle et al. 2007, Vardi et al. 2009). These cellular events have since been confirmed in natural populations of *E. huxleyi* (Vardi et al. 2012).

How phytoplankton viruses propagate in natural ecosystems is not well understood, although the coccolithophore model has yielded important discoveries. Multiple viral transmission mechanisms likely play a critical role in the ecology of *E. huxleyi*. Zooplankton (Frada & Vardi 2015) and aerosolization (Sharoni et al. 2015) have the potential to increase the dispersal of competent EhV particles through the water column and over large scales, respectively, which could facilitate rapid infection and termination of coccolithophore blooms. The local diffusion and encounter rates of viral particles determine infection of the host cell at the microscale. Zooplankton-mediated dispersal may be an important determinant of infection at the mesoscale owing to their nonrandom diffusivity in a patchy prey landscape (**Figure 4**). The half-life of aerosolized viral particles exposed to sunlight or UV is only 20 min. Nevertheless, the 1,000-times-greater diffusivity of aerosols compared with particles in the water column suggest that aerosols could be a highly effective transmission mechanism over larger oceanographic realms.

Advances in understanding the host–virus infection dynamic have enabled deeper ecological questions to be addressed. For example, vGSLs have successfully been used as in situ biomarkers for viral infection in natural populations (Vardi et al. 2012), allowing interrogation at the population level to reveal the degree of genetic and metabolic variability among natural host–virus populations. Such biological and ecological insights will enable viral impacts on nutrient fluxes, microbial food webs, and C export from the surface ocean to be more clearly defined and to be incorporated effectively into ecosystem models (Weitz et al. 2015).

5. COCCOLITHOPHORE METABOLISM AND PHYSIOLOGICAL VERSATILITY

Coccolithophores exhibit unique metabolic traits that contribute to their physiological versatility. Genomic, proteomic, metabolomic, and biochemical approaches have been restricted largely to *E. huxleyi* and may not reflect the full metabolic diversity of other coccolithophore lineages.

5.1. Carbon Metabolism

In most photosynthetic organisms, the major C storage compounds are α- and β-glucans (e.g., starch in land plants and green algae). Although *E. huxleyi* produces a water-soluble β-glucan, quantitative analyses of C fluxes during photosynthesis suggest that β-glucan is only a minor sink (<1%) for fixed C (Tsuji et al. 2015). Instead, C is predominantly stored in low-molecular-weight compounds (such as mannitol), acidic polysaccharides, alkenones, and other lipids (Obata et al. 2013, Tsuji et al. 2015). The absence of significant storage glucans and the primary accumulation of C into mannitol and alkenones are distinct features of C metabolism in *E. huxleyi* (Obata et al. 2013, Tsuji et al. 2015).

Other unique aspects of coccolithophore metabolism are evident from the presence of novel enzymes or their unusual localization. For example, pyruvate carboxylase is commonly found in the cytoplasm or mitochondria of eukaryotes, where it plays an important role in replenishing tricarboxylic acid (TCA) cycle intermediates. However, in *E. huxleyi*, pyruvate carboxylase is plastid localized, leading to the proposal that it plays a novel role by acting to regulate C flux to amino acid skeletons within the plastid (Tsuji et al. 2015). Transcriptomic studies suggest that *E. huxleyi*, like diatoms, possesses an ornithine-urea cycle that plays a similar role in N redistribution during N limitation (McKew et al. 2015, Rokitta et al. 2014). N limitation also led to elevated expression of a mitochondrial malate-quinone oxidoreductase in *E. huxleyi* (Rokitta et al. 2014). This enzyme enables direct transfer of electrons from malate to quinone, representing an alternative input to the mitochondrial electron transport chain that is not dependent on the activity of the TCA cycle. Malate-quinone oxidoreductase is present in dinoflagellates and some other alveolates but is notably absent from diatoms (Danne et al. 2013, Rokitta et al. 2014). Interestingly, metabolite profiling studies of *E. huxleyi* using gas chromatography–mass spectrometry could not detect malate, whereas malate accumulates significantly in the cells of land plants (up to 350 mM) (Obata et al. 2013). These findings suggest that malate plays a very different role in coccolithophore metabolism and that coccolithophores are much less reliant on the activity of the TCA cycle than land plants are.

5.2. Osmoprotectants

Coccolithophores accumulate a range of metabolites that can act as osmoprotectants, including polyols (mannitol), quaternary ammonium compounds (glycine betaine and homarine), and the tertiary sulfonium compound DMSP (Gebser & Pohnert 2013). DMSP is a major metabolite in many haptophytes, although it is absent from some haptophyte lineages, such as *Pavlova*, which accumulates cyclitols (e.g., D-1,4/2,5-cyclohexanetetrol) instead (Kobayashi et al. 2007). DMSP production by coccolithophores and other marine phytoplankton plays an important role in the global S cycle, as DMSP is the precursor of the climate-active gas dimethyl sulfide (DMS). Both DMSP and DMS act as powerful infochemicals that can influence a wide variety of biotic interactions at both the microscale (e.g., chemotaxis of bacteria and alteration of zooplankton trophic behavior) and macroscale (e.g., as chemoattractants for birds, turtles, and fish) (Fredrickson & Strom 2009, Garren et al. 2014, Savoca & Nevitt 2014, Seymour et al. 2010, Steinke et al. 2006,

Wolfe et al. 1997). Coccolithophores accumulate high amounts of DMSP (up to 400 mM in *E. huxleyi*), the concentration of which is influenced by light intensity, salinity, growth phase, and diel cycle and also differs significantly among strains (Darroch et al. 2015, Franklin et al. 2010, Keller et al. 1999, Steinke et al. 1998). Gebser & Pohnert (2013) demonstrated that the major zwitterionic metabolites in *E. huxleyi* are DMSP, glycine betaine, and homarine and that the ratio of these osmolytes is remarkably constant over a range of salinities (ratios of approximately 100:6:10). These results suggest that all three of these osmolytes are regulated by similar mechanisms in response to changes in salinity. N limitation results in much lower cellular concentrations of the quaternary ammonium compounds (glycine betaine and homarine), although little or no compensatory increase in the cellular concentration of DMSP was observed under these conditions (Keller et al. 1999).

Bacteria and algae both contain enzymes (DMSP lyases) that can cleave DMSP to generate DMS, which contributes a major flux of S to the atmosphere. Significant progress in the past decade has led to the identification of bacterial DMSP lyases that are thought to play a major role in DMS production in the oceans (Moran et al. 2012). However, the recent identification of an *E. huxleyi* gene product, Alma1, as a specific and highly active algal DMSP lyase indicates that coccolithophores can directly cleave the DMSP they produce to generate DMS and acrylate (Alcolombri et al. 2015). The *E. huxleyi* enzyme shares no sequence similarity with the DMSP lyases found in bacteria, although related enzymes are present in marine phytoplankton that accumulate DMSP, including other haptophytes (e.g., *Phaeocystis antarctica* and *Prymnesium parvum*) and dinoflagellates (e.g., *Symbiodinium* spp.) (Alcolombri et al. 2015). Levels of *Alma1* gene expression and protein abundance in *E. huxleyi* correlate closely with DMSP lyase activity. The discovery of Alma1 fills an important missing link in the marine S cycle and will aid estimations of the relative contributions of phytoplankton and bacteria to global DMS production. The Alma1 protein has two conserved cysteines essential for its enzymatic activity, which is sensitive to oxidants. This suggests that changes in cellular redox status could modulate DMSP lyase activity, which may be linked to the proposed antioxidant role for DMSP (Darroch et al. 2015, Sunda et al. 2002), as rates of DMSP cleavage would decrease in response to oxidative stress.

6. RECENT INSIGHTS INTO FUNCTIONAL ROLES OF CALCIFICATION

The most striking metabolic specialization in coccolithophores is calcification itself. However, the functional roles of this calcification remain uncertain (Raven & Crawfurd 2012, Taylor & Brownlee 2016, Young 1994), and several hypotheses have been proposed relating to nutrient uptake, photosynthesis, and protection from abiotic and biotic stressors. Several recent studies have provided intriguing new insights. A potential role for calcification in the utilization of HCO_3^- as a source of CO_2 for photosynthesis has been widely discussed (Berry et al. 2002, Raven & Crawfurd 2012). Although photosynthesis and calcification interact metabolically (see Section 6.3), an obligatory dependence of photosynthesis on calcification, at least in *E. huxleyi*, is not well supported by recent studies (Herfort et al. 2004, Leonardos et al. 2009, Trimborn et al. 2007). Indeed, under conditions with low dissolved inorganic carbon, photosynthesis may compete with calcification for HCO_3^- (Bach et al. 2013).

6.1. Defense Against Grazers and Pathogens

Although the coccosphere may be expected to have a protective role, the evidence remains equivocal. For example, the presence of a coccosphere does not prevent ingestion of *E. huxleyi* by either

copepods or microzooplankton predators (Harris 1994), although highly modified articulated coccoliths of members of the Syracosphaeraceae could act as a more direct mechanical deterrence (Young et al. 2009). Recent evidence has shown that haploid noncalcifying *E. huxleyi* cells possess inducible grazing defense properties, whereas calcifying cells do not, indicating complex relationships between prey and grazer activity (Kolb & Strom 2013). Harvey et al. (2015) recently showed that grazers feeding on calcified *E. huxleyi* strains have significantly lower growth rates than those feeding on noncalcified strains, the proposed mechanism being reduced digestion efficiency in the food vacuole or phagosome when feeding on calcified cells compared with noncalcified cells. Thus, calcification is integrated into a range of traits (including production of DMSP and other undefined metabolites) that can influence the degree of top-down control at the population level. In the case of pathogens, susceptibility and infection rates appear to be determined by a variety of metabolic interactions unrelated to calcification (see Section 6.3). Indeed, diploid calcifying cells of *E. huxleyi* were shown to be susceptible to viral infection, whereas haploid cells were not, leading to the Cheshire cat diploid-to-haploid escape strategy hypothesis (Frada et al. 2008).

6.2. Modulation of the Diffusion Boundary Layer

A comparison of isogenic calcifying and noncalcifying isolates of *E. huxleyi* showed that the noncalcifying strain exhibits higher growth rates than the calcifying strain under stable, nutrient-replete conditions (Bartal et al. 2015), consistent with the considerable energetic cost of calcification. However, under moderately turbulent growth conditions, the ability to produce coccoliths conferred mechanical resilience and sustained levels of nitrate acquisition, possibly via stabilization of the diffusion boundary layer at the cell surface. Mitchell et al. (2013) proposed a similar role for diatom frustules based on the diffusional bias caused by their fine-scale architecture, which could enhance uptake in patchy nutrient environments. These observations emphasize the need to better understand the microenvironment between the coccosphere and cell membrane and how the coccoliths and associated structures can influence this microenvironment.

6.3. Modulating the Light Field and Energy Balance

It has been speculated that coccoliths alter the light field experienced by the cell in either a photoprotective or photoenhancing role (Nanninga & Tyrrell 1996, Quinn et al. 2005). Recent work on isolated *E. huxleyi* coccoliths suspended in solution and aligned in a magnetic field showed that both enhancement and inhibition of incident light scattering is possible, although the effect on light intensity was less than 5% (Mizukawa et al. 2015). Similar conclusions can be drawn from experiments comparing photosynthetic parameters in diploid calcifying *E. huxleyi* cells with those in haploid noncalcifying *E. huxleyi* cells (Houdan et al. 2005). The light saturation kinetics are similar in both cell types, although photoinhibition was observed only in the haploid noncalcifying strain.

The remarkable resistance to photoinhibition by calcified strains of *E. huxleyi* led to the hypothesis that calcification may provide an alternative energy sink in response to high light levels. Inhibition of calcification in low-Ca^{2+} seawater led to downregulation of photosynthetic pigments and C fixation (Xu & Gao 2012), and these cells were also more susceptible to UV radiation (Xu et al. 2011). Moreover, sudden increases in light intensity from subsaturating growth irradiance enhanced calcification within minutes in diploid *E. huxleyi*, suggesting a mechanism for rapid dissipation of excess energy in addition to changes in light-harvesting pigment content (Ramos et al. 2012). The relatively stable proteome of *E. huxleyi* during photoacclimation from subsaturating to suprasaturating light levels (McKew et al. 2013a,b) implies that, at a steady state, the calcification

machinery operates substantially below its maximum potential and can respond rapidly to altered environmental conditions.

Regardless of the environmental drivers that may have led to the evolution of intracellular calcification, the selective advantage of calcite production in modern coccolithophores is likely to be multifarious and remains enigmatic. Understanding these functional roles of calcification is important but will continue to be a challenge, given the interdependency of the cellular and metabolic processes involved.

7. OPENING THE BLACK BOX OF VITAL EFFECTS IN COCCOLITHOPHORES

Well-preserved coccolithophore calcite and alkenones in ocean sediments are used to reconstruct physicochemical properties of the surface oceans. The carbonate structures produced by coccolithophores and foraminifera have been utilized to develop a range of geochemical proxies, because elements and isotopes in the mineral theoretically reflect their abundances in seawater and allow for paleoreconstructions of environmental conditions in the surface oceans. Marine biogenic $CaCO_3$ proxies include Mg/Ca, Sr/Ca, $^{18}O/^{16}O$ (paleothermometry), and $^{13}C/^{12}C$ (dissolved inorganic carbon and ocean productivity), and these proxies are increasingly relevant tools for understanding past climate events and informing ecological scenarios that may arise from the predicted future Anthropocene climate (Levin et al. 2015).

The physiological processes that mediate biogenic $CaCO_3$ precipitation play a critical role in stable isotope incorporation that can dramatically deviate from thermodynamic predictions. These so-called vital effects are due to the biologically controlled transport of ions and organic compounds into the compartment that promotes a saturated state favoring nucleation and calcite precipitation. Foraminifera $CaCO_3$ proxies such as the Mg/Ca paleothermometer are well advanced and robust (Hermoso 2014, Levin et al. 2015), although considerable variation in Mg incorporation appears to be driven by cellular metabolic processes (Spero et al. 2015), and isotopic fractionation of $\delta^{13}C$ and $\delta^{18}O$ is also significantly influenced by photosymbionts (Takagi et al. 2015). Hermoso (2014) has extensively covered coccolithophore proxies. The multiple ion transporters and endomembrane compartments involved in the transcellular pathway of inorganic substrates for intracellular coccolith production have a significant but largely uncharacterized influence on ion and isotope fractionation. Species differences in calcite precipitation, associated organic material, and coccolith digenesis are also poorly understood. Further implementation of robust coccolith proxies will require an improved mechanistic understanding of calcification.

8. CALCIFICATION MECHANISM

The mechanism of coccolithophore calcification has been studied extensively in the decades since the pioneering work of Paasche (1968), and advances in the intracellular model of calcification have been covered in several comprehensive reviews (Brownlee & Taylor 2004; Brownlee et al. 2015; Paasche 2001; Westbroek et al. 1989; Young et al. 1999, 2005). Despite this work, the mechanistic details of coccolith production are surprisingly incomplete.

8.1. Ultrastructure and Role of Intracellular Membranes

Coccoliths are produced in an intracellular Golgi-derived vacuole [generally referred to as the coccolith vesicle (CV)] that has a complex relationship with the endomembrane system. The basic sequence of events has been well described at the ultrastructural level in several species (see

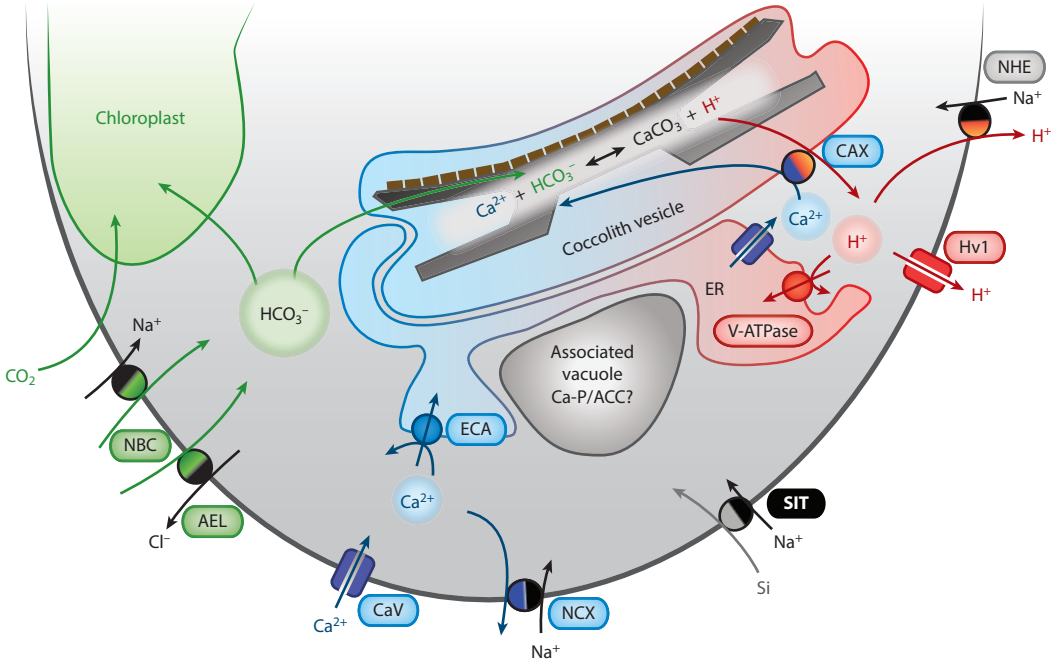


Figure 5

Model of ion transport during calcification in coccolithophores. Of particular importance is the efficient movement of Ca^{2+} and H^+ without compromising the cytoplasmic homeostasis of these ions. The endomembrane system that surrounds the coccolith vesicle likely plays a critical role. The role of Si in coccolith production has yet to be determined, but it may be important in stabilizing an intermediate form of $CaCO_3$. Abbreviations: ACC, amorphous $CaCO_3$; AEL, anion-exchange-like transporter belonging to the solute carrier 4 family; CaV, voltage-activated Ca^{2+} channel; CAX, Ca^{2+}/H^+ exchanger; ECA, ER-type Ca^{2+} ATPase; ER, endoplasmic reticulum; NBC, Na^+/HCO_3^- cotransporter; NCX, Na^+-dependent Ca^{2+} exchanger; NHE, Na^+/H^+ exchanger; SIT, Si transporter; V-ATPase, vacuolar-type H^+-ATPase.

Figure 1*b*), but the mechanism by which coccoliths are produced and secreted is not fully understood. The endomembrane system most likely plays an important role in supplying the inorganic and organic substrates for calcification through targeted *trans*-Golgi trafficking and via direct association with the CV (**Figure 5**). For example, a system of anastomosing tubules known as the reticular body is closely associated with the coccolith compartment and is important in the delivery of organic and inorganic substrates for calcification and in determining the fine-scale morphology of the mineral structure (Drescher et al. 2012, Taylor et al. 2007).

The scale of secretion of the coccoliths themselves—which are transferred to the cell surface via a single exocytotic event—suggests that potentially novel features of exo- and endocytosis are required to coordinate the process and efficiently retrieve the membrane (see **Figure 1*b***). Lee et al. (2015) recently found evidence of a modified membrane trafficking system (MTS) in the haptophyte complement of post-Golgi adaptor protein (AP) complexes. Loss of AP3 (which targets the multivesicular body and lysosome) and AP5 (which targets the *trans*-Golgi network and multivesicular body) and multiple expansions of AP4 (which mediates *trans*-Golgi network trafficking to the plasma membrane and endosomes) are common among calcifying and noncalcifying haptophytes (Lee et al. 2015), suggesting that a specialized MTS may have been necessary for the genesis of haptophyte body scales prior to the evolution of calcified scales. A further unique AP4 expansion within calcifying haptophytes indicates additional specialization of the MTS specific

for biomineralization. Moreover, diploid-specific expression of several syntaxin and soluble NSF attachment protein receptor (SNARE) homologs that function in vesicle engagement and fusion with target membranes (Mackinder et al. 2011) implies that additional specificity in the MTS could be achieved through differential transcription and translation of MTS genes in calcifying cells. A closer analysis of expression patterns of MTS-related genes and the localization of their proteins during calcification and viral infection could provide important information on the coordination of membrane dynamics during these processes.

Regulation of the cytoskeleton must play an important role in directing calcification because it actively interacts with the MTS, may influence the shape of the CV, and probably controls vesicle and cell movements associated with coccolith secretion. However, there is little detailed information on the cytoskeletal dynamics of coccolithophores. Unsurprisingly, inhibitors of actin and microtubules result in gross distortions of coccoliths (Langer et al. 2010), but the specific roles that these structures play in calcification have not been resolved.

8.2. Role of Organic Components in Calcification

Biomineral deposition is invariably associated with organic material that plays a critical role in regulating the process. In the case of coccolithophores, the precise regulatory mechanisms of organic baseplate scales and coccolith-associated organic material are largely unresolved, although recent functional studies have provided important new information.

8.2.1. Coccolith-associated polysaccharides. Although proteins are generally the predominant organic components of $CaCO_3$ biomineral structures (e.g., those of corals and molluscs), polysaccharides are the major organic components associated with calcite production in coccolithophores. The CAPs make up 2% of the coccolith mass in *Pleurochrysis carterae* (Okumura et al. 2012), and up to 28% of ^{14}C incorporation is dedicated to extracellular polysaccharides in *E. huxleyi*, reinforcing the importance of CAPs in the calcification process (Kayano & Shiraiwa 2009).

The CAPs are acidic in nature, comprising a backbone of D-mannose residues and a series of side chains that include D-ribose, L-arabinose, D-xylose, L-rhamnose, and D-galacturonic acid residues and ester-bound sulfate groups (Fichtinger-Schepman et al. 1981, Kayano & Shiraiwa 2009). The steric arrangement of the carboxyl groups of the acidic residues confers the ability to bind both free Ca^{2+} and the Ca^{2+} of calcite crystal surfaces (Henriksen et al. 2004). The type of CAP produced appears to be species specific (Borman et al. 1982, Hirokawa et al. 2005, Marsh et al. 1992, Ozaki et al. 2007), and even within a single species, CAPs may play distinct roles during coccolith production. For example, in *P. carterae*, acidic polysaccharides (PS1 and PS2) are associated predominantly with excreted coccoliths and are thought to play a role in Ca^{2+} transport and crystal growth, whereas a mannose-, xylose-, and sulfate-rich polysaccharide (PS3) is proposed to play a role in governing morphology during later stages of coccolith development (Marsh et al. 2002). The biosynthetic pathway for CAP production is unknown, although it is assumed to occur via the endomembrane and Golgi system, with delivery to the developing coccolith compartment via *trans*-Golgi vesicle transport (Marsh 1994).

How CAPs regulate coccolith morphology is also poorly understood, although they may in part determine the coccolith crystal shape by inhibiting calcite growth at acute steps of calcite crystals (Henriksen et al. 2004, Kayano et al. 2011). Inorganic precipitation experiments have demonstrated that the pH and ionic composition of the medium strongly affect the interaction of the CAPs with the mineral surface. Selective binding of CAPs to the acute step edges of rhombic calcite crystals is promoted between pH 3.4 and 7.7 and in the presence of K^+, Na^+, Sr^{2+}, and Ca^{2+}

ions. CAP attachment to the acute step edges of calcite drives crystal morphology away from the rhombic form, allowing extension along the *c* axis (Kayano et al. 2011). Basic pH and the presence of Mg^{2+} prevent site-specific absorption of CAP (Henriksen & Stipp 2009), thereby favoring the rhombic calcite morphology. In *E. huxleyi* cultures, elevated Mg^{2+} in the growth medium results in aberrant coccoliths (Herfort et al. 2004), whereas elevated Sr^{2+} does not significantly alter coccolith morphology (Langer et al. 2006). Although it is not possible to extrapolate the concentration of these ions in the medium to the site of calcification, these results are consistent with inorganic experiments showing that CAP interactions with calcite are strongly influenced by Mg^{2+} ions (Henriksen & Stipp 2009, Henriksen et al. 2004).

A future challenge is to understand the ontogenetic and temporal chemistry of the CV. Regulation of calcite morphology could conceivably be achieved through temporal modulation of CAP interactions with the calcite surface by fluctuations in CV pH, as well as Ca^{2+}, carbonate species, and other metal cations, such as Mg^{2+}. Given the close association of the CV and endomembranes and the developing calcite crystal (**Figure 5**), it would be interesting to consider the glycolipid and glycoprotein complement of these membranes in order to assess whether membrane-associated oligosaccharide residues could play a direct role in altering crystal growth that leads to fine-scale morphological features, such as pores (Drescher et al. 2012).

In addition to the role of CAPs in the mineralization process itself, their integration into the coccolith structure has important biogeochemical implications. Hassenkam et al. (2011) argued that the notable lack of thermodynamically favored Ostwald ripening of calcite crystals in coccolith-dominated chalk deposits is due to the large amount of organic material associated with them, a striking observation that illustrates the geological influence of CAPs. Indeed, intracrystalline CAPs can be recovered from fossil coccoliths from 70 Ma and retain their ability to interact with calcite surfaces in inorganic experimental systems (Sand et al. 2014). CAPs are also critical in resisting coccolith dissolution, significantly influencing digenesis and the burial flux of inorganic C (Hassenkam et al. 2011). Moreover, the stable and recalcitrant intracrystalline organic C in ancient coccolith deposits suggests that a significant fraction is unavailable for remineralization. Given that up to ~15% of cellular organic C may be allocated to CAPs, this fraction is important to consider when assessing export of inorganic and organic C. CAPs and their precursors could also play important roles in the coagulation of cells and coccoliths, affecting the ballasting of calcite (Chow et al. 2015).

8.2.2. Coccolith-associated proteins. Although the role of matrix proteins in coccolith production appears to be limited and is likely confined to the baseplate scale, a gene encoding a glutamic-acid-, proline-, and aspartic-acid-rich protein (GPA) with Ca^{2+}-binding motifs is associated with coccolith morphology in *E. huxleyi* and *Gephyrocapsa oceanica* (Corstjens et al. 1998). The GPA protein was isolated from CAP fractions, suggesting a role in coccolith growth and morphology. Experiments using the quantitative polymerase chain reaction technique showed strong regulation of the GPA-encoding gene, with upregulation in noncalcifying haploid cells and in calcifying diploid cells in which calcification was suppressed by a low-Ca^{2+} treatment (Mackinder et al. 2011). Although this result is counterintuitive, there are several possible explanations for it, including an inhibitory role for GPA at high concentrations. Without a clearer understanding of how calcification is regulated by organic components in general, it is difficult to draw firm conclusions. The GPA-encoding gene has not been detected in the transcriptomes of any other coccolithophore species to date, suggesting that pelagic, bloom-forming species in the family Noëlaerhabdaceae may have unique organic regulatory components that underlie mechanistically distinct calcification processes among coccolithophores (see Section 8.5).

8.3. Ion Transport

The calcification process (**Figure 5**) presents a remarkable case of transport physiology, requiring some of the highest sustained transcellular fluxes of Ca^{2+}, HCO_3^-, and H^+ of any known eukaryote cell (Brownlee & Taylor 2004, Brownlee et al. 2015). Comparative transcriptomics has identified transport genes likely to be specifically associated with calcification (Mackinder et al. 2011, von Dassow et al. 2009). Of particular relevance are a Ca^{2+}/H^+ exchanger (CAX3), a vacuolar-type H^+-ATPase (V-ATPase), and a Na^+-dependent K^+/Ca^{2+} exchanger (NCKX). Inorganic C fluxes are likely mediated by one or more HCO_3^- transporters in the solute carrier 4 (SLC4) family. Additional constitutive transporters, such as Ca^{2+} channels and Ca^{2+}-ATPases (the calcium ATPase SERCA-like proteins), likely facilitate transcellular transport of Ca^{2+} (**Figure 5**).

The coccolithophore cell faces the challenge of maintaining a large transcellular flux of Ca^{2+} from seawater to the coccolith-forming compartment without disturbing the low cytosolic Ca^{2+} concentration. Likewise, a mechanism of removal of H^+ generated by calcification that avoids catastrophic acidosis of the cytosol is required. The endomembrane pathway and its arrangement with the CV offer solutions to this problem that also meet some of the necessary charge-balancing requirements (Raven & Crawfurd 2012) (**Figure 5**). Moreover, the presence of a closely associated endomembrane system may also explain the paradox presented by biochemical purification of a V-ATPase from CV-enriched membranes in *Pleurochrysis* (Corstjens et al. 2001). The orientation of a V-ATPase is the reverse of that required to remove H^+ from the CV, but its association with the CV may be due to copurification of closely associated endomembranes in which a V-ATPase could act to sequester H^+ released from the CV (**Figure 5**).

Based on gene expression studies, Mackinder et al. (2010, 2011) proposed a model for Ca^{2+} accumulation in which Ca^{2+} is concentrated in a CV precursor compartment prior to delivery to the calcification site. Consistent with this model is the recent demonstration of a vacuolar-like compartment in calcifying *E. huxleyi* cells that concentrates a disordered Ca^{2+} phase and makes close contact with the CV (Sviben et al. 2016). Some of the features of the precursor vesicles associated with coccolith production are reminiscent of Ca^{2+}- and P-rich acidocalcisomes that have been identified in a variety of microorganisms, including apicomplexan parasites (Rohloff et al. 2011). Whether such a compartment plays a direct role in coccolithophore calcification remains to be determined.

8.4. The Problem of Protons

The formation of $CaCO_3$ from Ca^{2+} and HCO_3^- external substrates (Bach et al. 2013) necessitates the production of H^+, most likely at the site of $CaCO_3$ precipitation, that needs to be removed from the CV and ultimately the cytosol to prevent acidosis (Brownlee et al. 2015). Evidence from both gene expression studies (Mackinder et al. 2011) and flux modeling (Holtz et al. 2013) is consistent with a role for Ca^{2+}/H^+ antiporters (with a H^+:Ca^{2+} stoichiometry of at least 2:1 in the CV membrane) and V-ATPases in the recycling of H^+ into the endomembrane system and the accumulation of Ca^{2+} in a precursor calcification compartment (Brownlee et al. 2015, Taylor et al. 2011). The coccolithophore plasma membrane's high permeability for H^+ (Suffrian et al. 2011), which results from the activity of voltage-dependent H^+ channels (Taylor et al. 2011) that activate upon cytosolic acidification and/or depolarization of membrane potential, provides an effective high-capacity H^+ efflux pathway that can alleviate transient imbalances in H^+ production between calcification and H^+ consumption through metabolism and buffering. This role for H^+ channels in cellular pH homeostasis represents a unique and highly novel aspect of coccolithophore biology.

8.5. Silicon and New Paradigms for Calcification

Calcification in the coccolithophores evolved at a time (~300 Ma) when the dissolved Si concentrations of the surface ocean were much greater than they are today. The subsequent expansion of the diatoms at the beginning of the Cenozoic (from 66 Ma) led to a dramatic decline in the dissolved Si concentrations in the surface ocean (Siever 1992), which in turn resulted in a decrease in the extent of silica produced by other silicified organisms, such as the heavily silicified sponges and radiolarians (Lazarus et al. 2009, Maldonado et al. 1999). The ability of diatoms to draw down dissolved Si is due to high-affinity Na^+-coupled Si transporters (SITs) in their plasma membranes that facilitate uptake of silicic acid against a concentration gradient, leading to its eventual depletion from the surrounding seawater. Until recently, SITs had been identified only in stramenopiles (diatoms and chrysophytes) and siliceous choanoflagellates (Marron et al. 2013). However, Durak et al. (2016) recently described a SIT homolog in *Prymnesium neolepis*, an unusual silicifying haptophyte. Remarkably, they also found a SIT in the coccolithophore *Scyphosphaera apsteinii* as well as closely related SIT-like (SITL) proteins in three coccolithophore species (*S. apsteinii*, *C. braarudii*, and *C. leptoporus*). They further showed that each of these species is highly sensitive to Ge, an analog of Si that acts as a competitive inhibitor of Si uptake. The growth of these coccolithophores in low-Si seawater amended with 5-μM Ge resulted in highly aberrant coccoliths, and this inhibitory effect was reversed by the addition of 100-μM Si. Prolonged growth at a very low Si concentration (<0.1 μM) also resulted in the production of aberrant coccoliths, indicating that Si is required for calcification. In stark contrast, no inhibitory effects of Ge (up to 20 μM) were observed in *E. huxleyi* and *G. oceanica*, which are bloom-forming species that do not possess SITs or SITLs.

The role of Si in coccolithophore calcification remains to be determined. Small amounts of Si are detectable in the coccoliths of *S. apsteinii*, suggesting that Si may play a direct role in coccolith formation (Drescher et al. 2012). Recent advances have shown that Si can act to stabilize amorphous $CaCO_3$ (ACC) (Ihli et al. 2014, Kellermeier et al. 2010). It is therefore possible that Si stabilizes the otherwise labile ACC phase in the development of coccoliths, which can then undergo a transition to crystalline calcite in combination with the coccolith-associated organic components. There is no conclusive evidence for the involvement of ACC in coccolithophore calcification, although small Ca^{2+}-rich membrane-bound granules known as coccolithosomes appear to be an integral part of calcification, at least in the early stages of coccolith production in *P. carterae* and *Hymenomonas carterae*. A transition from amorphous ACC present in the CV to calcite at the onset of calcification is difficult to reconcile with the fact that the very first $CaCO_3$ that appears to precipitate onto the baseplate scale is in the form of a highly ordered ring of rhomboid calcite crystallites, the protococcolith ring (Young et al. 1999); however, this ontogenetic model of coccolith growth is derived from the non-Si-requiring *E. huxleyi*, and it is premature to rule out a contribution of ACC to calcification in all groups. High-resolution analytical measurements of coccolithophore cells and their calcite coccoliths throughout development, comparing Si-requiring and non-Si-requiring species, are now needed to determine the mechanism of Si regulation of calcification, whether ACC or some other intermediate phase is involved in the process, and the degree to which Si is incorporated into the calcite.

The long-held concept that the ecological niche of coccolithophores is partially defined by their lack of a requirement for Si is derived largely from studies of *E. huxleyi* (Tyrrell & Merico 2004). The presence of SIT/SITL transporters and Ge sensitivity in a broader range of coccolithophores indicates considerable physiological diversity in their Si requirements. Although the Si quota of coccolithophores is likely to be small, the ability of certain species (e.g., *E. huxleyi* and *G. oceanica*) to entirely avoid a requirement for Si may confer a competitive advantage in

specific environments, such as Si-depleted waters following a diatom bloom. A wider phylogenetic analysis should reveal whether a requirement for Si is an ancestral trait in coccolithophores and identify whether the dramatic depletion of dissolved Si from surface waters during the Cenozoic provided selective pressure to uncouple calcification from Si in some coccolithophore lineages, such as the Noëlaerhabdaceae. The identification of this major mechanistic difference among ecologically important coccolithophore species again highlights the need to study a multitude of species in laboratory-based studies in order to address how the differing Si requirements influence competitive interactions of coccolithophores with their ecosystem.

9. COCCOLITHOPHORE DISTRIBUTION, DIVERSITY, AND ADAPTATION

Much of our understanding of coccolithophore physiology relates to *E. huxleyi*, although it is becoming clear that other coccolithophore lineages may exhibit considerably different physiological attributes (Durak et al. 2016, Rickaby et al. 2010). Although *E. huxleyi* is the most abundant coccolithophore species in modern oceans, many of the other, larger coccolithophores, such as *C. pelagicus* and *C. leptoporus*, contribute significantly to global calcite production (Daniels et al. 2014). Coccolithophore species exhibit distinct vertical and latitudinal zonation (Boeckel & Baumann 2008, Okada & Honjo 1973, Winter et al. 1994), with species diversity greatest in the stable, low-nutrient environments found at low latitudes. In more variable regimes with higher nutrients found at higher latitudes, coccolithophore species diversity is lower, and assemblages are often dominated by *Emiliania* (Brun et al. 2015). Vertical zonation is pronounced in the communities at higher latitudes. For example, in the equatorial Atlantic, the characteristic coccolithophores of the oligotrophic surface waters are *Umbellosphaera irregularis* and *Umbellosphaera tenuis*, whereas the typical coccolithophores of the lower photic zone are *Florisphaera profunda* and *Gladiolithus flabellatus* (Kinkel et al. 2000). *E. huxleyi* is distinct from many other species in that it is common in all photic zones. The pronounced vertical zonation of coccolithophore species may be driven by factors such as light, temperature, and nutrients, which are all likely to contribute to diversity in coccolithophore physiology. Many coccolithophore species, particularly those from the lower photic zone, have not yet been isolated in laboratory culture, and so it is likely that the true breadth of coccolithophore physiology is yet to be discovered.

Intraspecific genetic diversity in coccolithophores also contributes to their physiological and morphological diversity. Strains of *E. huxleyi* can be assigned to a series of different morphotypes based on the morphology of their coccoliths (Young & Westbroek 1991). Strain-specific differences in pigments and the composition of lipid biomarkers such as alkenones and alkenes have also been observed, although these could not be assigned to different morphotypes (Conte et al. 1995). The sequencing of the *E. huxleyi* genome revealed pronounced genetic variability among strains, even in those that have been isolated from similar geographical locations (Read et al. 2013). *E. huxleyi* strains possess a core genome that is common to all strains, as well as an additional complement of genes that differ markedly among strains. Read et al. (2013) proposed that this pan-genome enabled physiological plasticity and contributed to the ecological success of *E. huxleyi* in diverse marine environments. Recently, detailed phylogenetic studies have provided insight into potential mechanisms underlying the genetic diversity exhibited by *Emiliania*. For example, *Emiliania* shows evidence for introgressive hybridization with older *Gephyrocapsa* clades, a process that would result in extensive genetic mixing (Bendif et al. 2015).

The predicted changes in ocean carbonate chemistry have led to considerable interest in the ability of coccolithophores to adapt to changes in their environment. A full discussion of the implications of environmental change for coccolithophore biology is beyond the scope of this

article (for excellent reviews, see Meyer & Riebesell 2015, Raven & Crawfurd 2012, Ridgwell et al. 2009, Rost et al. 2008), but it is important to note the capacity for adaptation when considering genetic and physiological diversity among strains. Recent evidence suggests that the physiological properties of *E. huxleyi* strains isolated from differing geographical locations relate to the carbonate chemistry of the seawater from which they were isolated (Rickaby et al. 2016). This could reflect the ability of *E. huxleyi* to adapt to its environment or could represent the selection of strains that exhibit a competitive advantage from a standing genetic stock. Laboratory experimental evolution approaches have suggested that both processes are likely to contribute to adaptive evolution within *E. huxleyi* populations over relevant timescales (Lohbeck et al. 2012).

10. CONCLUDING REMARKS

Remarkable new discoveries of cell physiology, microbial interactions, metabolism, and biomineralization continue to emerge. These discoveries have important implications for understanding ecosystem linkages and the role coccolithophores play in marine biogeochemical cycles. Advances have been achieved largely through a combination of genomics, transcriptomics, proteomics, and metabolomics together with targeted functional characterization of specific genes. The rapidly increasing genomic and transcriptomic resources in coccolithophores and other haptophytes provide an unprecedented opportunity to understand the molecular basis of physiological versatility and diversity. The lack of stable transformation and reverse genetic systems is a bottleneck that now limits progress in understanding specific processes such as calcification. A multidisciplinary approach that combines functional characterization of genes with high-resolution ultrastructure and analytical chemistry promises to yield answers to some of the most pressing questions in coccolithophore calcification.

Much of our understanding of coccolithophore biology comes from studies of *E. huxleyi*, but there is clearly considerable physiological diversity among ecologically important coccolithophore species as well as genetic diversity within species. It is important to understand how these differences influence the distribution and ecological role of coccolithophores, and gaining that understanding will require the use of comparative physiology, ecology, and genomics to study a broader range of coccolithophore species representing the four major families.

A better understanding of the unique physiology of coccolithophores will help provide inputs compatible with trait-based ecosystem models, which have great potential for describing the biogeography of phytoplankton and their responses to environmental variables (Follows & Dutkiewicz 2011). The future is promising, with the community increasingly adopting an interdisciplinary approach from bench to field in order to understand how the unique physiological versatility and metabolic repertoire of coccolithophores define their ecology and responses to climate change.

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