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Diversity, Genomics, and Distribution of Phytoplankton-Cyanobacterium Single-Cell Symbiotic Associations

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Abstract

Cyanobacteria are common in symbiotic relationships with diverse multicellular organisms (animals, plants, fungi) in terrestrial environments and with single-celled heterotrophic, mixotrophic, and autotrophic protists in aquatic environments. In the sunlit zones of aquatic environments, diverse cyanobacterial symbioses exist with autotrophic taxa in phytoplankton, including dinoflagellates, diatoms, and haptophytes (prymnesiophytes). Phototrophic unicellular cyanobacteria related to *Synechococcus* and *Prochlorococcus* are associated with a number of groups. N_2-fixing cyanobacteria are symbiotic with diatoms and haptophytes. Extensive genome reduction is involved in the N_2-fixing endosymbionts, most dramatically in the unicellular cyanobacteria associated with haptophytes, which have lost most of the photosynthetic apparatus, the ability to fix C, and the tricarboxylic acid cycle. The mechanisms involved in N_2-fixing symbioses may involve more interactions beyond simple exchange of fixed C for N. N_2-fixing cyanobacterial symbioses are widespread in the oceans, even more widely distributed than the best-known free-living N_2-fixing cyanobacteria, suggesting they may be equally or more important in the global ocean biogeochemical cycle of N.

Despite their ubiquitous nature and significance in biogeochemical cycles, cyanobacterium-phytoplankton symbioses remain understudied and poorly understood.

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1. INTRODUCTION

Interactions between microorganisms and macroorganisms or other microorganisms dominate life on Earth and have been critical for the evolution of plants and animals. Consistent interactions with microbes are termed symbioses, but symbioses vary in the types of interactions and the effects on the organisms. Symbiotic interactions are characterized on the basis of benefits and costs for the partners involved and whether there are positive or negative effects of one on the other (commensalism, parasitism), or positive effects on both (mutualism). Microbial interactions are widespread and involve organisms among the three domains of life: Eukaryota, Archaea, and Bacteria.

Mutualism: a symbiotic interaction where both partners benefit

Heterotrophic: relating to organism that obtains C and energy from organic C by taking up dissolved organic matter or consuming plants, animals, or detritus

Autotrophic: relating to an organism that produces organic compounds from CO_2 and generally produces energy from light or from oxidizing inorganic compounds

Microbial interactions among two or more unicellular microbes (e.g., eukaryotic microalgae and prokaryotic bacteria) are particularly interesting, because they involve symbiotic interactions without the complexities of tissue and cellular development involved in symbiosis with multicellular organisms (139). In aquatic environments, unicellular microorganisms including cyanobacteria and eukaryotic microalgae (protists) dominate photosynthesis. All eukaryotic phytoplankton are the product of evolution of symbiotic interactions (32). Chloroplasts are the remnant of an early symbiosis with a cyanobacterium, or the subsequent engulfment of eukaryotic algae followed by retention as a chloroplast (4). There are numerous examples of symbioses involving eukaryotic plankton (heterotrophic or autotrophic) and other eukaryotes, bacteria (including cyanobacteria), or archaea. Extant interactions among unicellular microorganisms (protistan algae or protozoans) in the plankton range from interactions among those that are simply in close physical proximity [in what is sometimes called the phycosphere (2, 111)] to interactions with those that are attached (ectosymbionts) or intracellular (endosymbionts).

The associations or symbioses between cyanobacteria (unicellular or filamentous) and planktonic unicellular phototrophic eukaryotes (phytoplankton, or algae) are diverse and are widespread in the low-nutrient waters of the open ocean (24). Symbioses involving N_2-fixing cyanobacteria,

those that can fix atmospheric gaseous N_2 into biologically available ammonium, are particularly important in the biogeochemical cycling of N in the global ocean. In the last decade, an unusual symbiosis between a haptophyte and a unicellular cyanobacterium with a greatly reduced genome was discovered; it has a wide geographic distribution, being found to be active even in cold Arctic waters (55, 90, 112, 113, 123).

The biological mechanisms involved in these intriguing cyanobacterium–unicellular protistan alga symbioses are poorly known, largely because they remain uncultivated in stable cultures or enrichments and are present in relatively low, patchy numbers in ocean waters. Recent studies are beginning to elucidate the C and N exchanges involved, as well as possible other metabolic interactions.

The purpose of this review is to provide an overview of the diversity of currently known symbioses with cyanobacterium symbionts (cyanobionts) in the plankton, some of which have received little attention and are poorly known (**Figure 1**; **Supplemental Table 1**). We describe what is known about the genome and biological characteristics of interactions in the better-studied symbiotic systems of the N_2-fixing cyanobacterial symbioses with diatoms and haptophytes. The biogeochemical significance of N_2-fixing cyanobacterium-phytoplankton symbioses is discussed while knowledge gaps and areas of current research are highlighted. It is hoped that this review will guide renewed scientific efforts toward the unique and diverse cyanobacterium-phytoplankton symbioses in aquatic environments that are of interest and importance to understanding the evolution of organelles as well as the ecology of biogeochemical cycles.

Haptophyte: member of a group of single-celled eukaryotic microalgae, most of which are photosynthetic; named after an organelle called a haptonema

Cyanobiont: a cyanobacterial symbiont

Photosymbiosis: a partnership where photosynthetic microalgae live inside or on a heterotrophic host organism

Mixotrophic: relating to an organism that can switch between self-feeding (autotrophy) and consumption of organic substrates (heterotrophy)

2. CYANOBACTERIA IN SYMBIOSIS

Diverse interactions between cyanobacteria and protistan algae have been described largely from microscopic observations. Since many of these associations have only been described from observations of associations of cells in field samples, in many cases, the nature of the symbiotic interactions has not been demonstrated. Symbioses include those between cyanobacteria and heterotrophic (nonphototrophic) protists and those involving N_2-fixing cyanobacteria and phototrophic protists (**Figure 1**). Heterotrophic protists derive C and energy from other sources, for example, from grazing or phototrophic symbionts, while phototrophic (autotrophic) protists use light energy to fix CO_2 into organic matter. N_2-fixing cyanobacteria form partnerships with multicellular land plants and aquatic unicellular protists (108) with the obvious advantage to the eukaryote that they are a source of N. Non-N_2-fixing cyanobacteria form symbioses with nonphotosynthetic heterotrophic protists (photosymbiosis) with the obvious benefit of providing fixed C (organic C).

2.1. Cyanobacteria–Heterotrophic Protists

Protistan partners in cyanobacterial symbioses are diverse and span the eukaryotic tree of life. Several genera of nonphototrophic (heterotrophic) protists are associated with unicellular cyanobacteria, presumed to be a symbiotic interaction. Hosts include several genera of dinoflagellates (some dinoflagellates have lost photosynthetic capabilities and are heterotrophic; others are autotrophic or mixotrophic), radiolarians, tintinnids, silicoflagellates, and thecate amoebae (10, 16, 31, 36, 37, 50, 98, 115, 118) (**Figure 1*g*,*i*–*p***; **Supplemental Table 1**). These symbioses have been described largely by microscopy, and genetic diversity analyzed by molecular phylogeny primarily of the cyanobionts, with a few exceptions of identification of the host (7, 50).

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2.1.1. Dinoflagellates. The physical associations and locations of cyanobionts of nonphototrophic protists have been best documented in heterotrophic dinoflagellates using transmission

electron microscopy (TEM), including some studies that also used immunolocalization of biomarkers for cyanobacteria. Based on the combined results of several studies (31, 33, 36, 59, 80, 120), the cell morphologies of the cyanobacterial symbionts (cyanobionts), including several ultrastructural characters, have recently been described (24) (**Supplemental Table 1**). Cyanobiont cells have been observed with no visible cell degradation, in the process of cellular division, that contain dense glycogen storage bodies and cyanophycin granules in some of them and with the

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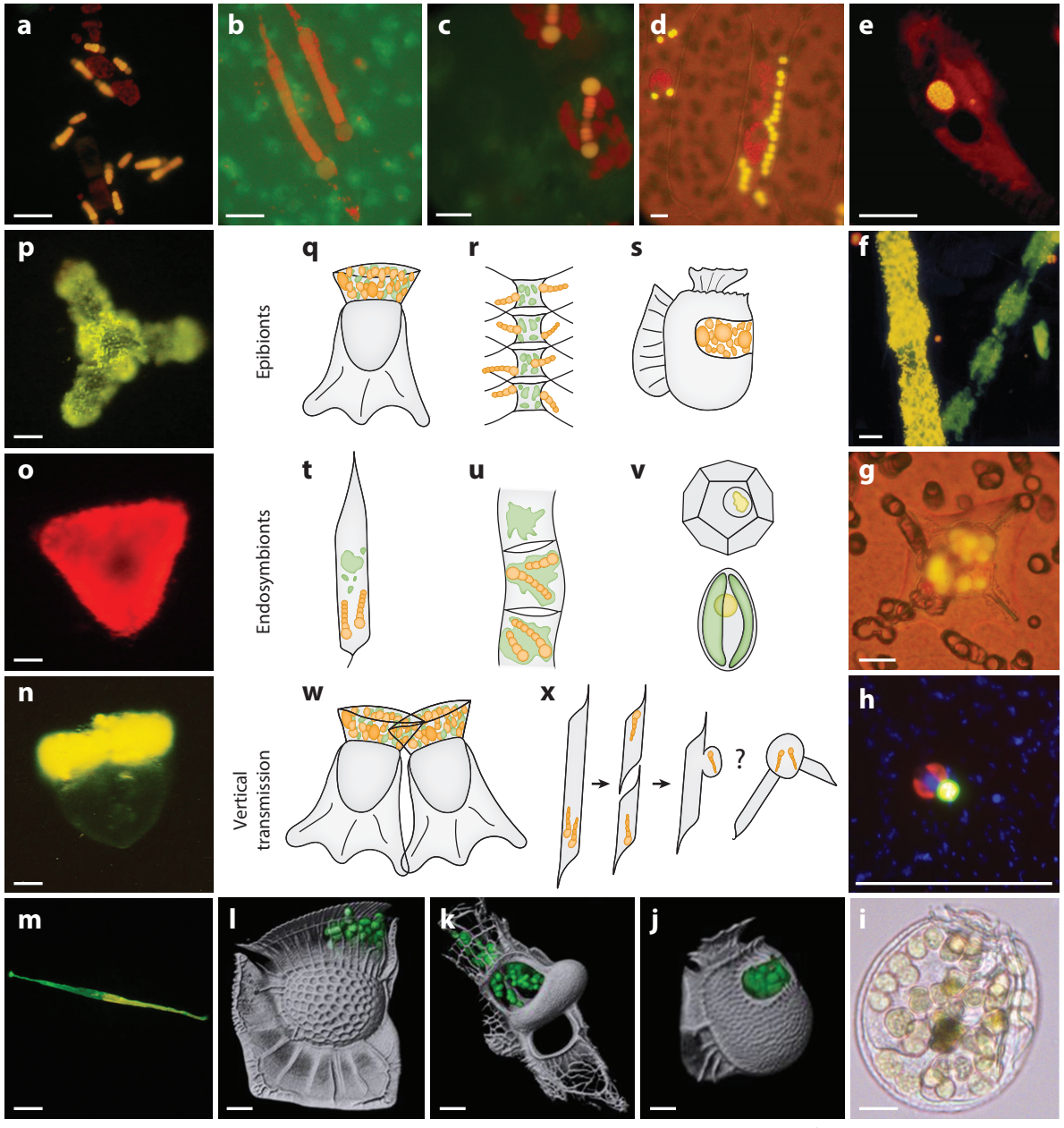

(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Micrographs of diversity of cyanobacterium-phytoplankton symbioses and conceptual diagrams of epibiotic, endosymbiotic, and vertical transmission in single-cell phytoplankton symbioses. Scale bars are approximately 10 μm, except in panel *f*, where the bar is 25 μm. Epi-fluorescent images using blue (*a–d,f,m,n,p*) and green (*o*) excitation filter sets to identify cyanobacteria by their phycobilin and chlorophyll pigments, which fluoresce yellow and red, respectively. Eukaryotic chloroplasts emit red under blue excitation. (*a–d*) Blue epi-fluorescent images of symbiotic diatoms from the open sea. Panel *b* adapted from Reference 39 with permission. (*e*) In situ hybridization to the 16S rRNA of the spheroid body inside a freshwater diatom. Reproduced with permission from Reference 107. (*f*) Tripartite symbiosis among diatom, protozoa, and cyanobacteria cells. Reproduced from Reference 10 with permission. (*g*) Symbiotic silicoflagellate. (*h*) CARD-FISH image of the UCYN-A1/haptophyte symbiosis. Reproduced from Reference 76 with permission. (*i*) Light micrograph of a symbiotic dinoflagellate with coccoid cyanobionts. Reproduced from Reference 45 with permission. (*j–l*) A series of confocal images of dinoflagellates with epibiotic symbionts. Images from Sébastien Colin, CNRS, Station Biologique de Roscoff, Tara Oceans Project. (*m*) Dinoflagellate with internal symbionts. (*n*) A symbiotic tintinnid. (*o,p*) Two symbiotic spongiose radiolarians. (*a–p*) Images by R.A. Foster unless otherwise noted. Center inset illustrations show epibionts, endosymbionts, and vertical transmission in cyanobacterium-phytoplankton symbioses [cyanobacterium symbionts are *yellow-orange* (but only contain chlorophyll) and protist chloroplasts are *green*]. The haptophyte may have flagella/haptonemas and spines. (*q–s*) Three examples of epibiont symbioses. Corresponding micrographs are panels *l*, *a*, and *j*, respectively. (*t–v*) Three examples of endobiont symbioses. Corresponding micrographs are *b*, *c*, and *h*, respectively. Illustration in panel *v* drawn based on photomicrograph in Reference 54. (*w,x*) Two examples of vertical transmission of cyanobacterial symbionts in dinoflagellate hosts (*w*) that asexually divide and pass on symbiont populations to daughter cells and diatom hosts (*x*) that reach a critical reduction in size after several generations of cell division; then the host diatom must pass through sexual reproduction (auxosporulation). Illustration of the proposed transfer of symbionts during lateral auxospore formation during the sexual phase adapted from References 127 and 128. Abbreviation: CARD-FISH, catalyzed reporter deposition fluorescence in situ hybridization.

presence of nitrogenase and phycoerythrin confirmed by antisera localization, suggesting that the cells are living, active symbionts, and not simply prey cells that have been grazed (33, 36, 61, 80) (**Supplemental Table 1**). In the girdle of symbiotic dinoflagellates, cyanobacterial cells are often observed mixed with coexisting bacteria cells, suggesting the possibility of tripartite symbiotic interactions (33, 36, 61). Less common and limited to one type of dinoflagellate host was the presence of an outer sheath surrounding the cyanobiont cells (36), which could be a strategy for avoiding symbiont cell degradation.

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Partial 16S rRNA gene sequences obtained from DNA amplified from individual symbiotic partner cells (planktonic dinoflagellates) confirmed the majority of cyanobiont sequences to be phototrophic, non-N_2-fixing cyanobacteria genera (37). The 16S rRNA gene sequences were similar (99–100% identical) to those of *Synechococcus* (and *Prochlorococcus*), and only a small subset of sequences associated with one dinoflagellate (*Histioneis*) were similar to other species of cyanobacteria, including known N_2-fixing cyanobacteria (37). Sequences obtained from one symbiotic individual cell included sequences from other bacteria (noncyanobacteria) that confirmed results of TEM studies showing mixed populations (31, 33, 36, 61). More recently, Farnelid et al. (33) showed that two genera of symbiotic dinoflagellates had associated alpha-, beta- and gammaproteobacteria based on *nifH* (which encodes the nitrogenase enzyme for N_2 fixation) gene sequences. A small subset of *nifH* sequences were similar to those from anaerobic bacteria, and in general the putative N_2-fixing symbionts were not specific for a single host type, and mixed assemblages were identified in individual hosts (33). The endosymbionts of the intertidal pond-dwelling dinoflagellate *Sinophysis canaliculata* (**Figure 1*i***) were similar to the other symbiotic dinoflagellates in the plankton, based on ultrastructural characters and autofluorescence patterns; however, the 16S rRNA gene sequences suggested that the symbionts were N_2-fixing unicellular cyanobacteria (31, 45). Thus, there appear to be complex assemblages involving diazotrophic cyanobacteria and N_2-fixing heterotrophic bacteria in some heterotrophic dinoflagellates.

N_2 fixation: The reduction of N_2 gas to ammonia

2.1.2. Tintinnid. Epi-fluorescent observations have been recorded for a tropical tintinnid, *Codonella* sp., with a high density of fluorescing cyanobacterial cells within the oral groove of

its lorica (16) (**Figure 1*n***). Micrographs by TEM also showed the presence of two bacterial cell morphotypes (coccoid and rod-like) co-occurring with numerous cyanobacteria in the process of cell division (36) (**Supplemental Table 1**). Some tintinnids are heterotrophic, but others are mixotrophic. The cyanobionts of *Codonella* were similar in morphology and 16S rRNA sequence to *Synechococcus* (36, 37). Other tintinnid genera are known to graze and retain the chloroplast of their algal prey, a process termed kleptoplastidy (116).

Endosymbiosis: a partnership between one or more organisms where one partner (symbiont) is enclosed within the other partner's (host's) cytoplasm

Chromatophore: pigment-containing intracellular body/organelle (plastid) in the thecate amoeba *Paulinella*

Rubisco: abbreviation for ribulose-1,5-bisphosphate carboxylase/oxygenase; responsible for the first major step in C fixation

Heterocyst: a specialized cell in cyanobacteria where N_2 fixation occurs but photosynthetic O_2 is not produced

2.1.3. Radiolarians. The endosymbionts of the radiolarian *Dictyocoryne truncatum* were initially identified as bacterial and brown algal symbionts (3). More recently, Foster et al. (36, 37) identified some of the endosymbionts as non-N_2-fixing cyanobacteria (*Synechococcus* and *Prochlorococcus*) based on autofluorescence (**Figure 1*o,p***), cell morphology (size and thylakoid distribution), protein content (localization of phycoerythrin), and 16S rRNA sequence. Radiolarian cells are characteristically composed of a central capsule that separates the cell into inner (endoplasm) and outer (ectoplasm) portions. The bacterial cells were reported to co-occur with cyanobacteria in the central capsule of both radiolarian genera (36). Hence, mixed populations of cyanobacteria and bacteria are common in many symbioses (dinoflagellates, tintinnids, radiolarians), yet how these mixed populations interact with one another and their respective hosts is unknown.

2.1.4. Amoebae. The endosymbiosis between freshwater amoeba *Paulinella chromatophora* and its chromatophores is a relatively recently evolved primary photosymbiosis (85, 100, 101). There have now been marine species described (71, 97). *P. chromatophora* has been extensively studied and recently reviewed (103), and therefore it will only briefly be summarized here. The two photosynthetic chromatophores of *P. chromatophora* are similar in pigmentation and ultrastructure to *Synechococcus* cells (70). However, the chromatophores are much longer (25–30 μm) in cell length than *Synechococcus* cells (0.8–1.2 μm), and they are phylogenetically related to alphacyanobacteria (the lineage of cyanobacteria containing form 1A Rubisco), which distinguishes them from the betacyanobacteria (the lineage of cyanobacteria containing form 1B Rubisco) plastid progenitor (85).

2.2. N_2-Fixing Cyanobacteria and Phototrophic Protists

The best-studied cyanobacterium-phototroph symbioses involve unicellular and filamentous heterocyst-forming strains of N_2-fixing cyanobacteria (**Supplemental Table 1**). Heterocysts are specialized cells for N_2 fixation (135). Thus far no symbioses between filamentous non-heterocyst-forming cyanobacteria and eukaryotic algae have been described, although it is likely that they exist. Diatoms with associated N_2-fixing filamentous heterocyst-forming cyanobacteria were among the first marine planktonic symbioses described (67, 79, 104). However, relatively little is known about the functional relationships of the symbiosis since enrichments or cultures are not stable and can only be maintained temporarily (127, 128).

2.2.1. Diatoms. Several strains of the heterocyst-forming cyanobacterium *Richelia intracellularis* and one strain of *Calothrix rhizosoleniae* are symbiotic with several genera of diatoms (40, 43, 63) (**Figure 1*a–c***). There is some confusion in the nomenclature based on microscopy identifications for the various heterocyst-forming strains (38, 49, 63, 67, 98) (**Supplemental Table 1**). Only *C. rhizosoleniae* has been isolated into long-term culture (strain SC01); however, after isolation, the host died, and SC01 continued to grow asymbiotically (38). The location of the symbionts varies from externally attached to partially or fully integrated into the host (13, 79, 98, 127, 128) (**Figure 1*r,t,u***). In all, N_2 fixation has been measured and shown to transfer N to the host diatom using ^{15}N tracer experiments and nanoSIMS (nanoscale secondary ion mass spectroscopy)

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(39). Based on *nifH* gene sequence phylogeny, the heterocyst-forming cyanobacteria symbionts can be divided into partner-specific clades, het-1 (host: *Rhizosolenia clevei*), het-2A (host: *Hemiaulus hauckii*), het-2B (host: *H. membranaceus*), and het-3 (host: *Chaetoceros compressus*), with 91.1% sequence identity (43). The strains are often quantified by *nifH* qPCR, and the various symbioses estimated by the *nifH* copy abundance of the symbionts (19, 41). The host diatoms were recently identified by 18S rRNA and *rbcL* (encodes large subunit of Rubisco) genetic sequences, and sequences clustered according to the genus they belong to (*Rhizosolenia*, *Hemiaulus*, or *Chaetoceros*) (13). Thus, there are distinct partnerships between diatom species and N_2-fixing cyanobacterial strains, although the degree and driver of specificity are not yet well known.

Diatoms have also been observed associated with unicellular N_2-fixing cyanobacterial symbionts (**Supplemental Table 1**). A unicellular cyanobacterium similar in morphology, pigmentation, and nucleotide (16S rRNA and *nifH* gene) sequence to the free-living unicellular diazotroph *Crocosphaera watsonii* (13, 90, 134) has been reported associated with the chain-forming centric diatom *Climacodium frauenfeldianum* (17) (**Figure 1*d***). Thus, it appears that these coccoid symbionts are likely *C. watsonii*. At least six strains of *C. watsonii* have been isolated, and six genomes have draft sequences that show unusual genome sequence conservation; however, none of these strains were symbiotic (5). It would be interesting to know whether the symbiotic *C. watsonii* also has genome sequence conservation or has modified genome content reflecting the symbiotic lifestyle. The 18S rRNA gene and *rbcL* sequences for *C. frauenfeldianum* were recently reported, and concatenated gene phylogenies placed the *C. frauenfeldianum* sister to symbiotic *Hemiaulus* spp. (13). In the case of these symbioses, N_2 fixation by the cyanobacterium and N transfer to the partner diatom have also been shown at the cellular level, with the use of ^{15}N tracer experiments and nanoSIMS (39).

There are a few reports of observations of heterocyst-forming cyanobacteria and unicellular cyanobacteria living with other diatom genera (15, 52, 99, 120, 129). To our knowledge, there are no further studies or reports on these symbioses (**Supplemental Table 1**).

A unique three-part cosmopolitan symbiosis also exists between a diatom, an aplastidic colonial protozoan, and a unicellular cyanobacterium (*Synechococcus*) (10, 44, 48, 57) (**Figure 1*f*, Supplemental Table 1**). This symbiosis has been exclusively studied by microscopy, including several electron microcopy studies that have described the diatom frustules as devoid of chloroplasts but housing epiphytic protozoans embedded with numerous cyanobacteria. It is speculated that the protozoan provides motility and exudates to support the diatom growth, while the cyanobacterium supports the protozoan by supplying reduced N (10, 57, 120).

A few freshwater diatoms belonging to the family Rhopalodiaceae form N_2-fixing symbioses; however, their symbionts are referred to as inclusions or spheroid bodies (29) (**Figure 1*e***). The two best-studied diatoms are *Rhopalodia gibba* (93) and *Epithemia turgida* (77). The spheroid bodies are nonphotosynthetic, N_2-fixing, and considered obligate symbionts (29, 35). Spheroid bodies of *R. gibba* possess internal membranes similar to those of cyanobacteria but lack chlorophyll autofluorescence and are surrounded by a double membrane (1, 29, 107). Between 1 and 10 spheroid bodies are present in each host, and the number is dependent on N concentration in the medium, increasing in number with decreasing N concentration (28).

2.2.2. Haptophytes. A marine N_2-fixing planktonic cyanobacterial symbiosis was relatively recently identified (122, 124, 136) (**Figure 1*b***). The cyanobacterium, discovered initially from a *nifH* gene and transcript sequences amplified from seawater, was ultimately shown to be a symbiont. This symbiont was initially presumed to be a free-living unicellular cyanobacterium based on its phylogenetic relationship to unicellular cyanobacteria diazotrophs (137). For years, the lifestyle of UCYN-A, formerly known as Group A, remained an enigma since it could not be visualized by fluorescence microscopy as related cyanobacteria can, and its daytime maximum

qPCR: quantitative polymerase chain reaction; a DNA amplification method for detecting genes and organisms

Diazotroph: a N_2-fixing microorganism

UCYN-A: unicellular cyanobacteria N_2-fixing group A; an uncultivated group of cyanobacteria that is symbiotic with a marine haptophyte (prymnesiophyte)

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nifH gene expression was opposite of that expected for unicellular diazotrophs (138). Ultimately sequencing of the genome led to the discovery of the symbiosis (124, 136), since the unusually small genome (1.44 Mbp) lacked key metabolic pathways for cyanobacteria and implied a symbiotic life history (124, 136). UCYN-A, or "*Candidatus* Atelocyanobacterium thalassa," is symbiotic with a haptophyte partner (prymnesiophyte) and is now known to associate with at least three distinctly different species or strains in the *Braarudosphaera bigelowii* group (20, 21, 26, 34, 122, 123). Six sublineages based on *nifH* are defined for UCYN-A (UCYN-A1 through UCYN-A6) (125): Three are currently known to be symbiotic (UCYN-A1, UCYN-A2, UCYN-A3) with *B. bigelowii* (UCYN-A2) or a *B. bigelowii* relative (UCYN-A1, and presumably UCYN-A3) (21, 34). The three known haptophyte partners differ in cell size estimated from microscopy and flow cytometric sorting by side scatter: 1–3 μm (UCYN-A1 host), 3–5 μm (UCYN-A3 host), and 7–10 μm (UCYN-A2 host) (21, 76, 122, 123) (**Supplemental Table 1**).

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3. FUNCTIONAL AND GENOMIC CHARACTERISTICS OF CYANOBACTERIUM-PHYTOPLANKTON SYMBIOSIS

Symbioses between two (or more) unicellular microorganisms have a number of requirements and characteristics. These characteristics include the evolution of the genome(s), the functions that benefit partners, behavioral and morphological modifications that have evolved as a result of the symbiosis, the physical location of the two unicellular partners in relation to each other, and the means by which the symbiosis is maintained by vertical transmission or horizontal acquisition from the environment (**Figure 1*w*,*x***). It is important to note that since few of the symbioses have been brought into isolation, our understanding of the symbiotic interactions of these interesting symbioses is limited. Most information has come from field observations, expeditions, and experimentation.

3.1. Genome Adaptations for a Symbiotic Life

Important and interesting characteristics of symbioses are how genomes evolve during adaptation to the symbiotic lifestyle. Gene loss, reductions of genome size, and decreases in GC content are common character changes in symbiont genomes, especially for endosymbionts (92). In phytoplankton-cyanobacterium symbiosis, gene loss and genome reduction are most pronounced in the N_2-fixing cyanobacterial symbionts: UCYN-A, the spheroid bodies of the freshwater *Rhopalodia* diatoms, and one of the *R. intracellularis* strains of marine diatoms. How the host has compensated for symbiont gene loss and function is not yet known since no genomes are publicly available for any of the phytoplankton hosts of cyanobacterial symbionts.

Decreased GC percentage compared to free-living counterparts is observed in three examples. The GC content of the two UCYN-A strains sequenced is 31–32% in UCYN-A1 and UCYN-A2 and 33.4% in the freshwater diatom spheroid bodies in comparison to 40% in the evolutionarily closely related but free-living *Cyanothece* ATCC 51442 (132). Similarly, the GC content of the diatom symbiont genomes range from 34% to 39% in *R. intracellularis* strains and *C. rhizosoleniae* strain SC01 (59, 60, 95, 124), in comparison to the 46% GC of the free-living, heterocyst-forming *Anabaena variabilis* ATCC 29413 genome (121).

Genome sizes of the symbiotic strains are greatly reduced compared to free-living counterparts. The best model for genome reduction in endosymbiosis or transition to organelle is the *P. chromatophora* symbiosis. The chromatophore genome is 1.02 Mbp. Most importantly, the nuclear genome of the eukaryotic partner *P. chromatophora* has been sequenced (9.6 Gbp), and genes have been lost from the symbiotic chromatophore and some of the genes transferred to the

eukaryotic genome through both endosymbiotic gene transfer (EGT) and horizontal gene transfer (HGT) (102). Interestingly, the HGT genes are derived from diverse bacterial lineages and outnumber the EGT genes, some of which are necessary for chromatophore function and replication (102).

There are draft genome sequences for the filamentous heterocyst-forming *R. intracellularis* strains that are associated with *H. hauckii* (3.2 Mbp), *H. membranaceus* (2.2 Mbp), and *R. clevei* (5.4 Mbp) (59, 60). There is also a draft genome sequence for the *C. rhizosoleniae* SC01 strain, which is the largest (5.97 Mbp) of the group. Recently, Parks et al. (105) assembled and reported presumed draft *Richelia* genome sequences (7.9 Mbp, 8.01 Mbp, 8.05 Mbp, and 2.23 Mbp) from publicly available metagenomic data [metagenome assembled genome (MAG)]. The genome sizes (>7 Mbp) for three of the MAGs are more similar to free-living heterocyst-forming cyanobacteria (e.g., *A. variabilis* ATCC 29413: 7.1 Mbp); however, the GC contents of the MAGs are lower (37%) and more similar to that of *C. rhizosoleniae* SC01 and the *R. intracellularis* strain that associates with *R. clevei* (39%) (60, 121). Although the phylogenetic identity of the MAGs has not been confirmed, the MAGs may be useful for completing genome sequences.

The smallest *Richelia* genome is 3.2 Mbp (60) and is highly reduced compared to the 7.1 Mbp genome of another heterocyst-forming cyanobacterium, *A. variabilis* ATCC 29413 (121). The genomes of unicellular symbionts of freshwater diatoms are even smaller. The partial genome of the *R. gibba* spheroid body is estimated to be 2.6 Mbp, and the complete genome of the spheroid body of *E. turgida* is 2.79 Mbp (73, 95). The haptophyte symbiont UCYN-A genome is only 1.44 Mbp, so all three unicellular cyanobiont genomes are substantially smaller than the 5.4 Mbp genome of the free-living *Cyanothece* ATCC 51442 (132). Thus, the genome reductions in the cyanobacteria of cyanobacterium-phytoplankton symbioses are substantial, being up to 75% in UCYN-A and 50% in the spheroid bodies of freshwater diatoms and ranging 23–54% in the heterocyst-forming symbionts of marine diatoms.

The reduction in genome size is due to the loss of genes. The lost genetic information differs dramatically even in these symbioses where the cyanobacteria function the same way in providing fixed N to the host/partner. The *E. turgida* spheroid body genome has some similarity in size and gene loss to the UCYN-A symbiont genomes of marine haptophytes (8, 95, 124). Genomes of the UCYN-A1 and -A2 symbiont strains are similar in size (1.44 Mbp and 1.48 Mbp, respectively), sharing 96.6% (1,159 of 1,200) of protein-coding genes with high synteny but only 86% amino acid sequence identity (8), and lack the same basic metabolic pathways. Interestingly there is a loss of photosynthetic capability in both the haptophyte/UCYN-A symbiosis and the spheroid bodies; however, this occurred by different mechanisms. The *Epithemia* spheroid bodies have lost both photosystem I and II (PSI and PSII), whereas UCYN-A has lost only PSII (95, 125, 136). The loss of PSII is important, as this is the O_2-producing photosystem, and hence UCYN-A circumvents inactivation of the O_2-sensitive nitrogenase enzyme for N_2 fixation. Additionally, both have lost the genes for C fixation (Rubisco) and no longer can fix C. In the haptophyte–UCYN-A symbiosis, the host haptophyte is photosynthetically active (123). Although in both symbiotic systems photosynthesis is nonfunctional, the *nif* operon remains, along with uptake hydrogenases (*hupL*, *hupS*), which is important for recovery of energy from H_2 evolved by nitrogenase (8, 95, 124).

In contrast, the diatom endosymbiont *R. intracellularis* genome lacks some N assimilatory pathways including ammonium transporters and glutamine:2-oxoglutarate aminotransferase (GOGAT), but not the N_2 fixation genes (60). GOGAT functions in the glutamine synthase (GS)-GOGAT, the universal pathway common to all cyanobacteria, to produce glutamate from glutamine produced by GS and a C skeleton (2-oxoglutarate). Hilton and coworkers (60) proposed an alternative pathway for assimilating N_2-derived ammonium by glutamate dehydrogenase (GDH) and C skeletons provided by the host, a situation reminiscent of that of some terrestrial

symbioses (114). However, the substitution of GDH for GOGAT is unlikely since GDH is generally a catabolic enzyme, and therefore another alternative may exist. The mechanisms underlying the metabolite exchanges (and transport) in all the symbioses are largely unstudied and are an open area for future research.

Oligotrophic: relating to an organism that can live in environments characterized by low nutrient conditions

3.2. Partner Functions

The benefits and dependencies of the cyanobacterium-phytoplankton partnerships have only recently been demonstrated in a few of the more accessible, easily sampled, examples. The advantages for the partners of these symbioses are not always clear, except for those involving N_2-fixing cyanobacteria.

3.2.1. Protection. The host partner involved in an endosymbiotic or epibiotic relationship may provide protection, for example from grazing, when the host is larger or more recalcitrant to predation because of spines, shells, or motility (46). Examples also exist in ciliate-bacterium symbioses where the bacterium provides protection to its respective host partners by production of refractile bodies (R-bodies) (46). Defensive roles played by host and/or symbiont are not known in cyanobacterial symbioses, although diatoms may provide a protective shell for the heterocyst-forming filamentous cyanobacteria (e.g., *Richelia*) and spheroid bodies.

3.2.2. Phototrophy. In many of the planktonic symbioses, the hosts are heterotrophic and/or have lost their photosynthetic capacity (e.g., dinoflagellates), while the cyanobacteria remain or are presumed photosynthetically active. In these cases, the role of the phototroph is clear in providing organic C, but to date, transfer of fixed C from the cyanobacteria to the partner/host has not been demonstrated. Taylor (120) hypothesized that the dinoflagellate-cyanobiont relationship could be a temporary condition, with the cyanobacterium ultimately being consumed. This possible scenario is reminiscent of the farming strategy in ciliate-alga symbioses where algal density is controlled by grazing by the ciliate host (65). Based on observations of remnant eukaryotic cells in the food vacuoles of ectosymbiont-bearing dinoflagellates, Tarangkoon et al. (119) suggested that the hosts utilize a multiple-resource strategy (photosymbiosis and phagotrophy) to cope with living in the low-nutrient oligotrophic habitat.

In N_2-fixing diatom symbioses where the host is also photosynthetic, it is still not resolved whether both partners are active in phototrophy. In terrestrial symbioses involving cyanobacteria, the cyanobionts are largely nonphotosynthetic and live heterotrophically from the reduced C (e.g., sugars) supplied by their hosts (108). However, in these terrestrial systems, the cyanobionts reside in the dark, which is quite different from the upper well-lit surface waters of oceans and lakes. Harke and others (56) recently showed that gene expression was coordinated in wild populations of the *Rhizosolenia-Richelia* symbiosis in order for the host to supply C in exchange for N. For example, nitrogenase genes in the *Richelia* symbionts were significantly coexpressed with transporters of photosynthesis-derived carbohydrates in the host diatom *Rhizosolenia*. Moreover, the upregulation of a transport receptor [e.g., tripartite ATP-independent periplasmic (TRAP)] in the cyanobacterium *Richelia* was hypothesized to lead to import of host-diatom-derived sugar substrates (56). A scenario for C transport/import from the host is likely, especially in more internal symbionts, but TRAP transporters are known to be specific for carboxylates and not sugars (106).

In the UCYN-A–haptophyte symbiosis, UCYN-A has lost its C fixation ability, and therefore the reduced C from the photosynthetic haptophyte is presumably exchanged for fixed N from UCYN-A (123). UCYN-A has lost the O_2-evolving PSII, and therefore the nitrogenase enzyme

is less inhibited (nitrogenase is sensitive to inactivation by O_2); however, it still needs electrons to fuel N_2 fixation. UCYN-A has retained PSI, which is expressed (94), but it is still unclear whether PSI generates ATP through cyclic photophosphorylation or there is an electron source that also generates reductant. Recent work has attempted to resolve how UCYN-A and its partner coordinate C and N metabolism by examining patterns of gene expression in UCYN-A. Using daily gene transcription profiles of wild UCYN-A1 and UCYN-A2 populations, Muñoz-Marín et al. (94) proposed a model (see figure 7 in Reference 94) of electron flow in UCYN-A to fuel N_2 fixation during the day. Interestingly, UCYN-A has shifted its daily gene expression pattern relative to other unicellular cyanobacteria, presumably to facilitate N_2 fixation in the light period to coordinate with energy provided by host photosynthesis during the day (94). The model assumes that UCYN-A uses host-derived carbohydrates to fuel daytime N_2 fixation, and interestingly it is the same scheme proposed for heterocysts in filamentous heterocyst-forming cyanobacteria (84).

Nutricline: A region in the water column (or sediment) where nutrient concentrations decline rapidly with depth

3.2.3. Vitamins. In the plankton, vitamin availability can limit growth as much as trace elements and nutrients. For example, many eukaryotic algae are not able to synthesize vitamin B_{12} (cobalamin), and in the plankton a primary source of cobalamin is bacterially derived (22). Evidence is accumulating for the role of vitamins in a few of these cyanobacterial symbioses, and it includes genome mining and in situ transcription. For example, despite highly reduced genomes, UCYN-A symbionts have retained the entire synthesis pathway for the vitamin B_{12} variant pseudocobalamin; hence, it must play an important role (94). Pseudocobalamin is unusable by most eukaryotes (87), and interestingly, in the field study of *Rhizosolenia-Richelia* symbiosis, transcripts for altering pseudocobalamin to a usable form by the host diatom were detected, along with transcripts for cobalamin biosynthesis and biotin synthase by the symbiont (56). Hence it is possible that the cyanobacterial symbionts are relieving their respective hosts of vitamin stress, in addition to providing N.

3.3. Behavioral and Morphological Aspects

Behavioral modifications (e.g., migration patterns) are less pronounced in planktonic symbiosis compared to other symbiotic systems (e.g., invertebrate symbioses); however, since some protists are capable of modest motility (flagella, diatom ballasting for buoyancy and sinking), the hosts may play a role in maintaining position in the sunlit upper waters or transit vertically to deeper waters, where higher concentrations of nutrients are available (nutricline) (91, 129, 130). In culture, ascending (floating) symbiotic diatoms (*H. hauckii*) were shown to produce transparent exopolymer particles as a strategy for neutral and positive buoyancy despite forming dense chains (68). Similar to asymbiotic diatoms, symbiotic diatoms form colonies, especially when present at high densities (blooms). Colony formation is an evolved trait and believed to be a strategic adaptation for survival in the plankton (64).

Morphological modifications are generally less prevalent in unicellular symbiosis, such as the specialized cavities found in many multicellular plants. One noteworthy exception is within the dinophysoids. There is a gradual invagination of the large posterior cup-shaped girdle in *Ornithocercus* that houses the epibionts to form a deepened pocket at the base of the girdle in *Histioneis*, which is further reduced and enclosed into a small greenhouse in *Citharistes* (120) **(Figure 1*j–l*)**. The opening to the chamber in *Citharistes* is slightly larger than the cell diameter of the symbionts and perhaps imposes a size-based selection for certain symbiotic partners (37) and makes it difficult for the symbionts to escape.

In the heterotroph-cyanobacterium symbiosis, the cyanobacterial partners either are internal (endosymbionts) without any fixed position/specialized chamber (e.g., central capsule of

radiolarians) or are attached externally (ectobionts) (e.g., girdle of dinoflagellates, tintinnids) (**Figure 1*i–q,s***). In both cases, the cyanobionts often co-occur with other bacteria. Mixed populations are common in ectobionts, perhaps as a result of continual renewal of the symbiont population from the environment (horizontal transmission) or maybe as a strategy for increasing symbiont performance. For example, in terrestrial mutualisms, mixed populations are also common, and in some they act to stabilize the mutualism by creating competition among the populations that results in increased overall fitness (69).

A major conclusion in sequencing the draft genomes of the *Richelia/Calothrix* symbionts of marine diatoms was that the genome size and content were related to symbiont cellular location (60). Smaller genomes were associated with endosymbionts. The symbiont cellular location is a continuum in the marine diatom symbiosis, from external (*C. rhizosoleniae*) to pseudo internal (*R. intracellularis* resides between the diatom plasma membrane and diatom frustule in *R. clevei*) to completely internal (inside the plasma membrane; *R. intracellularis* in *H. hauckii*) (13, 62, 127, 128) (**Figure 1*a-c,r,t,u***). Recently, using confocal laser scanning microscopy (CLSM), Caputo et al. (13) showed that the *R. intracellularis* endosymbionts have partially penetrated the diatom cytoplasm and reside centrally, partially surrounded by the diatom chloroplast and in close proximity to the diatom nucleus. The CLSM results were only partially confirmed by TEM. In freshwater *Rhopalodia* diatoms, the cyanobacterial bodies are similarly centrally located, typically in pairs next to the host nucleus and separated by a cytoplasmic membrane of the diatom (29, 73).

The reported location of the cyanobiont in the haptophyte/UCYN-A symbiosis varies in different studies, which may be due to the different strains, different habitats, or different life cycle stages (**Figure 1*b,v***). Hagino et al. (53) demonstrated one to two spheroid bodies associated with *B. bigelowii* cells by TEM that had a three-layered envelope reminiscent of gram-negative bacteria. The spheroid (inclusion) bodies of freshwater diatoms and the putative N_2-fixing symbionts of a few dinoflagellates are also separated from the host by a membrane (1, 29, 31, 35, 41, 45, 70, 107) (**Supplemental Table 1**). Enclosure in a membrane could be important to avoid host digestion, a scenario common to the endosymbiotic algae of ciliates (65). A membrane separation from the host cytoplasm is also conducive to protecting the O_2-sensitive nitrogenase enzyme from the O_2 evolved by the host haptophyte chloroplasts. Similar observations of one to two UCYN-A cells per host have been documented in numerous catalyzed reporter deposition fluorescence in situ hybridization studies (11, 75, 76, 86, 123) (**Figure 1*b***); however, it has been presumed these may be loosely attached. A recent study described a symbiosome-like compartment either free in the plankton or carried by *B. bigelowii* containing variable but higher numbers (three to ten cells) of UCYN-A2-lineage cells (20). Symbiosome is an adopted term from legume-rhizobium symbioses and describes the compartment in the plant cell occupied by the diazotrophic bacteria (30). Unfortunately, the cellular location of the UCYN-A symbionts has been difficult to determine due to the lack of cultures and the need to use destructive procedures in order to visualize by microscopy.

Supplemental Material >

3.4. Partner Maintenance and Acquisition

Unicellular symbioses require that the symbionts be vertically transmitted, or be horizontally acquired from a free-living population (**Figure 1*w,x***). Observations of vertical transmission are common in dinoflagellate-cyanobacterium symbioses (figure 19.1L in Reference 24) (**Figure 1*w***). To maintain vertical transmission in unicellular symbioses the partners must grow at the same rate; otherwise, one partner outgrows the other and the symbiosis is lost. The latter has been shown in only one of the diatom symbioses (*R. clevei–R. intracellularis*), where asynchronous growth led to host cells outgrowing their symbionts when the host cells divided asexually (127). However, Harke and coworkers (56) recently reported a rather synchronous cell division in *Rhizosolenia-Richelia*

populations based on the high coexpression of putative cell division marker genes (*ftsH*) in both the host and symbiont. Moreover, transcription of silicic acid transporters peaked in expression in the host *Rhizosolenia* and was prior to the *ftsH* signal, arguing in favor of partner-synchronized cell division (56). However, one must consider that a synchronous division pattern could be eventually disrupted, e.g., if sexual reproduction (auxospore formation) is required to restore the host cell diameter, as in other diatoms. In fact, it was proposed that reinfection of *Richelia* to asymbiotic *Rhizosolenia* likely occurred during auxosporulation (127) (**Figure 1*x***). A thorough description (in German) of the vertical transmission of the spheroid bodies during auxospore formation in wild freshwater diatom (*E. turgida* and *R. gibba*) populations has been provided by Geiter (47). Combining such observations with more modern molecular approaches, e.g., transcriptomics, would undoubtedly provide insights into how partners synchronize (or do not) growth.

In other symbioses, the symbionts are acquired from the surrounding environment (horizontal transmission). In planktonic symbioses this is particularly important and risky since if there is a horizontally transferred phase of the life cycle, encounter rates of partners may limit reinfection given the dilute nature of the environment (139). A plausible solution is to develop partnerships with highly abundant and widely distributed partners that co-occur in the free-living state (25). Acquisition strategies are largely presumed based on observations of one or more of the partners partially living in an asymbiotic state; with the exception of *Synechococcus*- and *Prochlorococcus*-like symbionts, free-living phases for symbionts are not well characterized. Elaborate mechanisms for cell signaling, recognition, and infection are known for many terrestrial plant and invertebrate-bacterium symbioses (23, 88) but are unknown for unicellular symbioses such as in the plankton.

4. ECOLOGICAL DISTRIBUTIONS AND IMPLICATIONS FOR BIOGEOCHEMISTRY

Knowledge of the distributions of cyanobacterium-phytoplankton symbioses is constrained by oceanography: the oceans are large and under-sampled with respect to time and space. Although diverse, most of the cyanobacterium-phytoplankton symbioses are in low abundances (from undetectable to a few cells per milliliter) relative to free-living microbial components of the plankton (16). This low abundance makes them difficult to study and difficult to detect in the large global sequencing surveys (14), but also suggests that there are likely to be many more symbioses yet to be described.

Phytoplankton-cyanobacterium symbioses are important in ocean biogeochemistry. In general, marine phytoplankton symbioses with cyanobacteria are commonly found in low-nutrient, warm, tropical, and subtropical waters (16, 24, 119). In fact, some have suggested that they are indicator organisms for oligotrophy [e.g., dinoflagellate photosymbiosis (51), UCYN-A (109)]. The significance of non-N_2-fixing symbioses is very difficult to quantify, partially because both partners can obtain energy from light, and it is not known in detail what the mechanisms and effects on growth and productivity are. In contrast, N_2-fixing phytoplankton-cyanobacterium symbioses have clear biological, ecological, and ecosystem significance, since they provide N for eukaryotic phytoplankton partner species. Although reports of many of the symbioses described in this review are from relatively few anecdotal observations, there have been substantial efforts to quantify the distribution and significance of N_2-fixing symbioses, because of the quantitative significance of N_2 fixation as a N source in the N-limited waters of the open ocean.

N_2 fixation in the oceans has historically been believed to be important in the central, open-ocean, low-nutrient waters of the major ocean basins (the gyres of the North and South Atlantic and Pacific Oceans and the Indian Ocean) and the Baltic Sea (12, 78, 81). Historically it was believed that most N_2 fixation was due to the free-living non-heterocyst-forming filamentous

cyanobacterium *Trichodesmium* in the oligotrophic oceans, and free-living heterocyst-forming genera (*Nodularia* and *Aphanizomenon*) in estuarine systems including the Baltic Sea (12, 27, 110). More recently, the significance of the previously known diatom symbioses and the haptophyte symbioses has been discovered. The diatom-symbiotic populations have a cosmopolitan distribution but appear rather patchy in regions where they form blooms: in regions influenced by riverine plumes (e.g., Amazon, Congo, Mekong, Niger, Orinoco) or, in contrast, in the open ocean gyres (e.g., North Pacific) (9, 18, 41, 42, 66, 82, 83, 117, 126, 130). There are a few reports of symbiotic diatoms in coastal temperate environments, for example, near California and Hawaii (58, 72, 133). In general, heterocyst-forming cyanobacteria dominate brackish or benthic habitats; thus, the planktonic symbionts of diatoms are remarkably widespread and are some of the most ubiquitous marine heterocyst-forming cyanobacteria. The UCYN-A/haptophyte symbioses have been found to have one of the most expansive geographic ranges of the planktonic symbioses (or free-living marine N_2-fixing cyanobacteria), being reported in tropical, in temperate, and, more recently, in coastal habitats and at higher latitudes, including the Danish Strait and the Bering, Chukchi, and Beaufort Seas (6, 13, 55, 86, 89, 90, 96, 112, 113, 125).

Previous studies have shown that during blooms, the *R. clevei–* and *H. hauckii–R. intracellularis* symbioses were extremely important new sources of N, *R. clevei–R. intracellularis* providing up to 33 mg N/(m^2·d) to the North Pacific gyre (83) and *H. hauckii–R. intracellularis* providing 45 mg N/(m^2·d) to the tropical North Atlantic (18). These values for total N_2 fixation exceed the estimates of N flux from below the euphotic zone; some have extrapolated that 30–60% of the excess productivity can be accounted for by symbiotic N_2-fixing diatoms, supporting 35–48% of the phytoplankton-based N demand (126, 133). For comparison, areal rates of fixation for wild populations of *Trichodesmium* spp., often considered the most significant N_2-fixing microorganisms in the ocean, are in the range 0.1–12.57 mg N/(m^2·d) (12). The symbiotic diatoms also sink rapidly, unlike *Trichodesmium* spp., and hence contribute substantially to C sequestration to the deep sea and global C and N cycling (66, 117).

More recently, the activity of individual microbial populations has been estimated using stable isotope (^{15}N) labeled substrates (N_2) coupled to measurements of uptake rates into single cells by nanoSIMS. The method has been applied to several field populations of symbiotic N_2-fixing diatoms, and UCYN-A/haptophyte symbioses. There are fewer measures for the symbiotic diatoms and rates vary by an order of magnitude [1.15–50.5 fmol N/(cell·h)], while rates of UCYN-A populations vary by several orders of magnitude (14, 39, 74, 75, 76, 86). An interesting application of the single-cell rates is to estimate what fraction of the host growth the symbiotic partner supports. For symbiotic N_2-fixing diatoms growing at background densities, Foster et al. (39) assumed a growth rate reported earlier (127, 128) and estimated that nearly all (97.3%) the host N-based growth was supported by their heterocyst-forming partners.

Recent reports for UCYN-A were similarly high in warm tropical waters [average of 12 fmol N/(cell·d) for UCYN-A1 and 220 fmol N/(cell·d) for UCYN-A2] and unexpectedly were within the same magnitude in cooler Arctic waters [average of 7.6 fmol N/(cell·d) for UCYN-A1 and 13.0 fmol N/(cell·d) for UCYN-A2] (55, 86). N_2 fixation has not been routinely measured or considered in polar planktonic waters and has been ignored in biogeochemical models until recently (131). The single-cell rates of fixation by UCYN-A are also comparable to rates estimated for wild populations of *Trichodesmium* spp.; in fact, Martínez-Pérez et al. (86) estimated 5–10 times higher growth rates by the UCYN-A symbioses compared to coexisting *Trichodesmium* spp. in the North Atlantic Ocean. The single-cell rates are important for determining the relative contribution of different diazotrophs since the contribution of individual groups can be estimated, which is difficult in bulk water measurements (39, 46, 86). These measurements have underscored the potential significant role of UCYN-A/haptophyte symbioses in the N cycle (55, 86).

5. CONCLUSIONS

Recent studies have highlighted the biological, ecological, and biogeochemical significance of phytoplankton-cyanobacterium symbioses. The symbioses occur with a diverse array of phototrophic, heterotrophic, and mixotrophic protists in the ocean plankton, including dinoflagellates, tintinnids, radiolarians, silicoflagellates, diatoms, and haptophytes. They have an array of physiological, morphological, and genomic adaptations of evolutionary and biological interest. The N_2-fixing symbioses vary tremendously in activity, host/partner type, size, and, presumably, sinking speeds. The relatively recent discovery of the UCYN-A/haptophyte symbioses has underscored the importance of cyanobacterium-phytoplankton symbioses in ocean biogeochemical cycles, particularly since they are found in the widest distribution of planktonic N_2-fixing symbioses documented so far, even in the Arctic Ocean. The diverse characteristics and importance in biogeochemical cycles described in this review will hopefully inspire students and researchers to make yet more discoveries in cyanobacterium-phytoplankton symbioses in aquatic ecosystems.

SUMMARY POINTS

1. Planktonic partnerships are diverse and are modern counterparts of the ancient evolutionary event that led to evolution of chloroplasts.
2. Cyanobacteria are symbionts with a wide variety of heterotrophic protists in the plankton including dinoflagellates, radiolarians, silicoflagellates, and tintinnids.
3. N_2-fixing cyanobacteria are symbiotic with a variety of phytoplankton species, most commonly with diatoms and most recently discovered with a widely distributed haptophyte.
4. Several continuums can be observed in the planktonic symbiosis with cyanobacteria, including how symbionts are physically associated, whether they are vertically transferred from one generation to the next, genome size and content, and extent of genome reduction.
5. The interactions involved between partners are poorly known but may involve other metabolic interactions beyond simple exchange of fixed C for fixed N, such as controlling vitamin availability.
6. Cyanobacterium-phytoplankton symbioses are widely distributed in subtropical and tropical waters, and there is recent evidence of both presence and activity in temperate and polar habitats.
7. Current understanding of planktonic symbiosis has become largely based on culture-independent methods that have limited and replaced cultivation efforts. It does, however, provide necessary information for cultivation strategy, hypothesis generation and testing, and a means for identifying new symbioses.

FUTURE ISSUES

1. Why is there an abundance of mixed populations in many of the photosymbioses? What is the function of the co-occurring bacteria?
2. How do planktonic symbioses propagate? How are life cycles of partners coordinated?

3. How are partners acquired, selected, and transmitted? What drives their selectivity? Are there free-living forms?
4. What metabolites other than the obvious (N, C, vitamins) are exchanged between partners?
5. What tools and/or information would help to identify and cultivate new symbioses?
6. What is the quantitative global significance of phytoplankton-cyanobacterium symbioses?

NOTE ADDED IN PROOF

Single-cell genome sequencing recently confirmed that the cyanobacterial symbionts of *Ornithocercus magnificus* (**Figure 1*l***) are related to *Prochlorococcus* and *Synechococcus* (95a).

DISCLOSURE STATEMENT

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