Algae are simple photosynthetic eukaryotes which—owing to their colonization of the oceans—are responsible for up to 50% of the planet’s atmospheric carbon fixation (22). They comprise a diverse group that can broadly be defined as unicellular (microalgae) or multicellular (macroalgae) photosynthetic organisms that lack roots, stems, leaves, conducting vessels, and complex sex organs (55). A single endosymbiotic event between a cyanobacterium-like organism and a nonphotosynthetic eukaryote is thought to have given rise to the three basal groups of algae: the chlorophyta (from which higher plants arose), the glaucophyta, and the rhodophyta (Fig. 1).

The chlorophyta and the rhodophyta, via secondary and tertiary endosymbiotic events with different nonphotosynthetic eukaryotes, gave rise to algal groups with complex plastids (Fig. 1). Some groups, such as the apicomplexans (e.g., Plasmodium falciparum) subsequently lost the ability to photosynthesize, although they still retain plastids. The number of symbioses that have occurred during the evolution of algae has been heavily debated, and the details are discussed elsewhere (46), but there are likely to have been several events. Consequently, it is not surprising that the physiology and metabolism of algae are extremely varied. For example, while the majority of green algae contain a highly structured cell wall comprising glycoproteins (19), euglenophyta simply contain a protein layer (known as the pellicle) beneath the cell membrane, and the cell walls of diatoms are made from silica (24). Dinophyta, euglenophyta, and heterokontophyta contain members that are phagotrophic on bacterial prey, but this characteristic is absent from the groups with simple plastids. Furthermore, although most algae are regarded as free-living organisms, many dinoflagellates are closely associated with corals, and members of several algal groups live with fungi as lichens (13), providing photosyntheate for their heterotrophic partner.

VITAMIN AUXOTROPHY IN ALGAE

The ability to photosynthesize has led to the perception of algae as autotrophic organisms requiring light and a mixture of inorganic nutrients only. However, studies by Lwoff and Dusin 1937 discovered that some members of the chlorophyta and cryptophyta require thiamine as a growth factor in culture (39). Over the following 40 years, many studies described algal species that required different combinations of three B vitamins: vitamin B₁₂ (cobalamin), vitamin B₆ (thiamine), and vitamin B₂ (biotin) (50). A compilation of all of the available data (see Table S1 in the supplemental material; summarized in Table 1) reveals the widespread nature of vitamin auxotrophy within the algal kingdom. Of 306 species surveyed, more than half required cobalamin, while 22% required thiamine and a smaller proportion (5%) required biotin. Remarkably, for all three vitamins, the algal species that have an obligate requirement for the different cofactors do not appear to fall into any one lineage, but rather auxotrophy is present in several unrelated phyla, indicating that it must have arisen independently several times through evolution. Even more surprisingly, this pattern is also mirrored within individual genera. For example, Hematococcus in the chlorophyta, Peridinium in the dinophyta, Hymenomonas in the haptophyta, and Nitzschia in the heterokontophyta all have species that require cobalamin and others that do not (see Table S1 in the supplemental material). Similarly, the dinoflagellate Gymnodinium brevis requires all three vitamins whereas G. spendent requires only cobalamin. For the auxotrophy to have evolved so frequently in algae, the simplest explanation is that it is due to the loss of a single gene. A plausible hypothesis would therefore be that species with a requirement for a particular vitamin have lost a gene involved in the biosynthesis of that cofactor. An intriguing parallel is seen with vitamin C auxotrophy in mammals, where loss of the terminal enzyme of the pathway has occurred in both primates and guinea pigs (10).

Until now, however, it has not been possible to test this hypothesis because nothing was known of the biosynthetic routes for vitamins in the algal kingdom and only very little was known about the roles of the cofactors in algal metabolism. With the advent of genome sequencing, our ability to address questions like this has been revolutionized. Currently, the sequences of four algal genomes are available. The first to become available was that of P. falciparum (25), a unicellular nonphotosynthetic apicomplexan, which lives as a parasite in insects and humans and is the causative agent of the devastating tropical disease malaria. The release of the P. falciparum genome sequence was quickly followed by that of Chlamydomonas reinhardtii (www.jgi.doe.gov), a unicellular green alga, which is commonly isolated from soils in North America. Two further genome sequences were released in 2004; Thalassiosira pseudonana is an ecologically important centric diatom found
in many of the world’s oceans (2), and *Cyanidioschyzon merolae* is a unicellular thermophilic red alga isolated from sulfate-rich hot springs (pH 1.5, 45°C) (42). In this article, we use the genome sequence data available for these four species to investigate the question of vitamin metabolism in algae, thus providing the first clues as to why and how some algae have a requirement for these cofactors. Each vitamin will be discussed in turn before we focus on possible routes for their acquisition by algae, which, given the extremely low free concentrations of these nutrients in the natural environment, are likely to be complex.

**BIOTIN**

Biotin (vitamin B₇) was discovered in 1901 as a growth-promoting factor for yeast (69) and was finally isolated and purified in 1941 (64). Biotin is a cofactor for several essential carboxylase enzymes (62), including acetyl coenzyme A (CoA) carboxylase, which is involved in fatty acid synthesis, and so is universally required. The molecule consists of an imidazole ring fused to a sulfur-containing tetrahydrothiophene ring with a fatty acid side chain (Fig. 2). In eubacteria, the first precursor for biotin synthesis is pimeloyl-CoA but the source of this differs among different species. Thereafter, the concerted action of four enzymes, BioF, BioA, BioD, and BioB, converts pimeloyl-CoA to biotin (20) (Fig. 2). In the budding yeast *Saccharomyces cerevisiae*, homologues of bioA, bioD, and bioB, but not bioF, are present, so the source of 7-keto-8-aminopelargonic acid remains unknown. The higher plant *Arabidopsis thaliana* contains genes for BioF, BioA (also called BIO1), and BioB (also called BIO2) (3, 49), but the genome does not appear to contain a gene with sequence similarity to known bioD genes. Since *A. thaliana* can synthesize biotin de novo, the absence of a bioD gene from the genome suggests that in higher plants the conversion of 7,8-diaminopelargonic acid to

![FIG. 1. Summary of algal evolution. The three basal groups chlorophyta, rhodophyta, and glaucocystophyta are shown with green, red, and blue plastids, respectively. Those groups derived from the chlorophyta and rhodophyta by secondary endosymbioses are shown with the appropriate colored plastids. Tertiary endosymbiotic events are not shown in this diagram. The boxed phyla contain at least one organism with a sequenced genome.](image1)

![FIG. 2. The biotin biosynthetic pathway as elucidated in eubacteria.](image2)

| Phylum               | No. of species surveyed | No. of species requiring: |
|----------------------|-------------------------|---------------------------|
| Chlorophyta          | 148                     | 44                        |
| Rhodophyta           | 13                      | 12                        |
| Cryptophyta          | 6                       | 5                         |
| Dinophyta            | 27                      | 24                        |
| Euglenophyta         | 15                      | 13                        |
| Haptophyta           | 17                      | 10                        |
| Heterokontophyta     | 80                      | 47                        |
| Total                | 306                     | 155                       |

*Only those species that have had their cobalamin, thiamine, and biotin requirements assessed have been included in this survey, and for this reason those data do not include any glaucocystophytes, chloroarachnophytes, or apicomplexans. A requirement for biotin is found only in species that contain complex plastids, i.e., those that have arisen as the result of secondary and tertiary endosymbiosis with a eukaryotic alga. Furthermore, every species that requires biotin also requires cobalamin, thiamine, or both.*
and C. merolae, and P. falciparum\(^a\)

| Gene         | Model (length [amino acids]) |
|--------------|-------------------------------|
| C. reinhardtii | T. pseudonana | C. merolae | P. falciparum |
| bioF         | e\_gwW.18.89.1           | 2,889.1 (370) | CML225C (433) | chr12.glimmerm\_1307 (630) |
| bioA         | e\_gwH.100.1.1 (675)     | 128.16.1 (417) | CMG023C (802) |
| bioB         | Chlre2\_kg.scaffold\_300026 (PS) | 10.577.1 (324) | CML210C (379) |
| thiS         | e\_gwW.11.33.1 (270)     | 54,113.1 (385) | CMV092C (67) |
| thiE         | e\_gwH.31.56.1 (396)     | 20.15.1 (432) | CMM289c (539) |
| iscS         | estExt\_fgenesh2\_pg.C\_710047 (744) | 6.114.1 (703) | CMT234C (453) |
| thG          | estExt\_fgenesh2\_pg.C\_360009 (637) | 169.11.1 (254) | chr7.phat\_291 (1,250) |
| thiH/O       | MTC\_estExt\_fgenesh2\_pg.C\_120281 (567) | 7.273.1 (353) | chr13.genefinder.27r (584) |
| thiC         | estExt\_fgenesh2\_pg.C\_630009 (637) | 12.129.1 (636) | CMG017C (673) |
| thiD         | mtc\_168251 (711)        | 54.158.1 (267) | CMO125C (297) |
| thiE         | mtc\_168251 (7110)       | 54.123.1 (1,930) | chr5.glimmerm\_537 (310) |
| TPK          | gwW.1.690.1 (358)        | 30.73.1 (217) | MAL6PI.285 (545) |
| thiM         | estExt\_gwp\_1H.C\_30409 (242) | 65.17.1 (1,248) | PFL1920c (302) |
| metE         | Chlre2\_kg.scaffold\_S2000007 (842) | 65.17.1 (1,248) | CMJ234C (767) |
| metH         | estExt\_GenewiseW\_1.C\_30026 (1,357) | 80.8.1 (579) |
| cblE         | e\_gwH.14.9.1 (1,381)   | 2.1075.1 (301) | E\_histolytica, like P. falciparum, is an obligate parasite that can presumably obtain biotin from its host, while D. discoideum is a slime mold that preys on soil microorganisms. The genomes of these two amoebae contain a gene with sequence similarity to bioF, while D. discoideum also contains a gene with sequence similarity to bioA. Given our current knowledge of biotin biosynthesis in eukaryotes, it is not possible to conclude how biotin auxotrophy arose initially in these lineages. Nevertheless, the simplest explanation is that it was caused by the loss of a single biosynthetic gene, although this might not be the same gene in every case.

**THIAMINE**

Like biotin, thiamine also plays a pivotal role in intermediary carbon metabolism. The active form of the vitamin is thiamine pyrophosphate (TPP), which is essential for all organisms. The cofactor associates with a number of enzymes involved in primary carbohydrate and branched-chain amino acid metabolism, including pyruvate dehydrogenase, transketolase, \(\alpha\)-ketoadic decarboxylase, and \(\alpha\)-ketoadic oxidase (57). Recent work on the biosynthesis of thiamine has mainly concentrated on three prokaryotic organisms, *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, and *Bacillus subtilis* (5). Thiamine consists of a thiazole and a pyrimidine moiety, which are produced in separate branches of the biosynthetic pathway before being coupled together to produce thiamine phosphate. This is then further phosphorylated to produce the active cofactor TPP (Fig. 3). Many of the genes encoding thiamine biosyn-
thetic enzymes from bacteria have been cloned, and in several cases the structures of the enzymes have been solved (60). We have a less complete understanding of the pathway in eukaryotes, and what knowledge there is comes mainly from the yeast S. cerevisiae. The overall pathway is similar to that in bacteria, with thiamine monophosphate formed from thiazole and pyrimidine moieties, but the enzymes involved appear to be different. None of the bacterial genes have homologues in the yeast genome. In contrast, one enzyme of the thiazole branch, thi4, and one pyrimidine biosynthetic gene, thi5, have been cloned from yeast, but neither shows any sequence similarity to the bacterial enzymes. Furthermore, thiL is absent and the terminal enzyme of the pathway is thiamine pyrophosphokinase (TPK), which pyrophosphorylates thiamine to form thiamine pyrophosphate (Fig. 3).

Thiamine was the first vitamin found to be an algal growth factor (39). Early studies on the specificity of this requirement showed that in some cases thiamine auxotrophy could be relieved by addition of the thiazole moiety to the growth medium, in others cases the pyrimidine moiety was sufficient, while in the final group of auxotrophs the full thiamine molecule was essential for growth (50). These studies show that in algae the thiamine biosynthetic pathway follows the same general pattern as in other organisms, with two separate branches to make each of the moieties, which are then combined together to make thiamine (Fig. 3). Furthermore, the presence of some parts of the pathway in thiamine auxotrophs suggests that they require the vitamin because they have lost one or more of the essential genes involved in its biosynthesis.

C. reinhardtii, C. merolae, and T. pseudonana do not require thiamine or any of the intermediates in its biosynthesis for growth, demonstrating that they can synthesize the vitamin de novo. BLAST searches with the thiamine biosynthetic genes from E. coli, S. enterica serovar Typhimurium, and B. subtilis against the genome of the red alga C. merolae demonstrates that it has all of the genes necessary to synthesize thiamine monophosphate via the bacterial route (Fig. 3 and Table 2). However, it does not contain a gene with similarity to bacterial thiamine monophosphate kinase (ThiL) and instead has a homologue of the yeast TPK. The current versions of the C. reinhardtii and T. pseudonana genomes suggest they contain the genes for most of the enzymes in the pathway, but they do not contain genes with sequence similarity to the short bacterial thiS gene, and C. reinhardtii also lacks a gene with sequence similarity to thiG, which is involved in the synthesis of thiazole phosphate. Many of the enzymes in the thiazole branch are similar to those involved in molybdopterin biosynthesis, and so one must be careful when assigning a role to these proteins purely on the basis of sequence similarity. Unlike T. pseudonana and C. merolae, C. reinhardtii contains a gene with sequence similarity to thiM, which in eubacteria is involved in scavenging the thiazole moiety from the environment (60). However, in C. reinhardtii this gene appears to be essential for thiamine biosynthesis, since mutations in thiM lead to thiamine auxotrophy (21). This suggests that synthesis of the thiazole moiety in C. reinhardtii follows a route different from that in eubacteria. Another difference in C. reinhardtii is that the ThiD and ThiE proteins are predicted to be part of the same large polypeptide (mtc_168251), with the central region corresponding to thiD and the 3’ end containing thiE. The N terminus of
the protein has no bacterial homologue, and the significance of the fusion protein thus remains unknown.

A further complication when extrapolating biochemical pathways from genome sequences is that the thiazole branch of the pathway initially involves the formation of deoxy-xylulose-5-phosphate (DXP) from glyceraldehyde-3-phosphate and pyruvate. In many organisms, DXP is also the precursor to isoprenoids. There are two known routes to isoprenoids, the DXP pathway and the mevalonate (MEV) pathway (37). Animals use the MEV pathway, while eu-bacteria use either the DXP or the MEV pathway (53). Higher plants have the ability to synthesize isoprenoids via both routes; the MEV pathway is in the cytosol and endoplasmic reticulum (as it is in animals), whereas the DXP pathway is confined to the plastids. The chlorophytes, such as *Scenedesmus obliquus*, *C. reinhardtii*, and *Chlorella fusca* (14), and *P. falciparum* (53) use the DXP pathway exclusively, whereas the euglenophyte *Euglena gracilis* uses only the MEV pathway (14) and has an obligate requirement for thiamine, suggesting that DXP is not used in the biosynthesis of either thiamine or isoprenoids in this organism. The rhodophyte *Cyanidium caldarium* and the heterokontophyte *Ochromonas danica* use both the DXP and MEV pathways (14), but while *C. caldarium* does not require thiamine, *O. danica* has an obligate requirement for the vitamin. This demonstrates that it is not simply the ability to synthesize DXP that determines whether or not an alga has a requirement for thiamine.

The thiamine requirement of *P. falciparum* has not been categorically established, although previous reports have suggested that it possesses the enzymes that catalyze the final steps in the pathway (6). The *P. falciparum* genome has a complement of thiamine biosynthesis genes similar to that of *C. reinhardtii*, with the exception of *thiC*, suggesting that it cannot synthesize the pyrimidine moiety from 5-aminomimidazole ribonucleotide. It also lacks either a *thl* or a TPK gene but does have a gene for ThiM. Given the parasitic lifestyle of *P. falciparum*, it is quite possible that it is able to acquire either thiamine or its constituent parts from its host.

The two single-celled amoebae *E. histolytica* and *D. discoideum* require thiamine for growth, and none of the genes specific for thiamine biosynthesis are found in their genomes. Thus, although the currently available genome sequences do not allow us to determine the initial process leading to thiamine auxotrophy, it appears that once this has arisen, there is no selection pressure for the retention of any of the biosynthetic genes and these are lost from the genome.

**COBALAMIN**

Cobalamin is a cobalt-containing tetapyrrole related to chlorophyll and heme (Fig. 4A). Minot and Murphy first identified this cofactor in the 1920s, when they described how they were able to cure the symptoms of pernicious anemia with liver extracts (44). The active factor was isolated (61) and crystallized (51) in 1948; it was given the name vitamin B_{12} or, as it contained cobalt, cobalamin. Cobalamin acts as a cofactor for enzymes that catalyze either rearrangement-reduction reactions or methyl transfer reactions. In bacteria there are more than 20 cobalamin-dependent enzymes (40), including those important for methanogenesis, but in eukaryotes there are many fewer. In animals, there are two, methionine synthase and methylmalonyl-CoA mutase, which is involved in the utilization of odd-chain fatty acids (40). Higher plants have no cobalamin-dependent enzymes and so neither utilize nor synthesize cobalamin.

Cobalamin biosynthesis has been well characterized in bacteria. There are essentially two alternative routes, comprising up to 20 enzymatic steps from the tetapyrrole primogenitor uroporphyrinogen III (66). The first to be characterized was the so-called late-insertion pathway (4, 63), which has an absolute requirement for molecular oxygen (58) and in which the cobalt ion is inserted into the tetapyrrole macrocycle after ring contraction. The second route is called the early-insertion pathway (54), where the cobalt ion is chelated before ring contraction and which can operate under anaerobic conditions. All archaea and many eu-bacteria are able to synthesize cobalamin de novo, but several eu-bacteria lack the biosynthetic pathway. An example of the latter is *E. coli*, which utilizes cobalamin from the environment if it is available but is able to alter its metabolism in the absence of the cofactor.

More than half of all microalgae surveyed (Table 1; see Table S1 in the supplemental material) (11) have an obligate requirement for exogenous vitamin B_{12}, leading to the remarkable conclusion that auxotrophy is the norm rather than the exception in the algal kingdom, despite the fact that these organisms are photosynthetic. Of the algal species that did not require an exogenous supply for growth, some were found to take up cobalamin if it was available (11; see below). However, when grown in its absence, the cells did not contain measurable amounts of cobalamin. This demonstrates that, rather than being able to synthesize it, these vitamin B_{12}-independent algae had no need for the cofactor in their metabolism, a situation similar to that found in *E. coli*.

Inspection of the available algal genome sequences confirmed these observations. *T. pseudonana* has an obligate requirement for vitamin B_{12}, but *C. reinhardtii* and *C. merolae* do...
not require the vitamin. BLAST searches of the *C. reinhardtii*, *C. merolae*, and *P. falciparum* genomes did not identify any genes with sequence similarity to known cobalamin biosynthetic genes, and while a gene with sequence similarity to *cbiP*, encoding adenosyl-cobyrinic acid-a,c-diamide synthase, is present in the genome of *T. pseudonana* (new V2.0 genewise 7.511.1), this organism does not possess any other genes required for cobalamin biosynthesis (11). Thus, algae do not have the ability to synthesize cobalamin de novo, indicating that cobalamin auxotrophy is likely to have arisen because of an obligate requirement for the cofactor in algal metabolism rather than from the inability to synthesize it. It is interesting that this is different from the situation observed for thiamine and biotin auxotrophy, which appears to have arisen because of the loss of one or more genes involved in the biosynthesis of the cofactors.

Soon after the isolation of vitamin B$_{12}$ as the mammalian anti-pernicious anemia factor (44), *E. gracilis* was shown to require the vitamin for growth (52). Early studies showed that the requirement of many auxotrophic algae for vitamin B$_{12}$ was reduced, but not completely removed, if methionine was added to the culture medium (29). This observation can now be explained by the fact that cobalamin is a cofactor for methionine synthase. More-recent studies (30) have shown that *E. gracilis* contains a vitamin B$_{12}$-dependent methionine synthase (also called MetH), consistent with the idea that cobalamin plays a role in algal methionine biosynthesis.

Higher plants do not require vitamin B$_{12}$ for methionine biosynthesis because they contain vitamin B$_{12}$-independent methionine synthase (MetE) and not MetH. By contrast, animals contain MetH and not MetE and thus require cobalamin. The recent genome-sequencing projects have demonstrated that both MetH and MetE can be found in different algae. While *T. pseudonana* contains MetH only and *C. merolae* contains MetE only, *C. reinhardtii* contains both enzymes (Table 2). In the presence of vitamin B$_{12}$, *C. reinhardtii* uses MetH but in the absence of the vitamin it uses MetE (11). This phenomenon is analogous to the situation in eubacteria such as *E. coli*, which also switch between MetE and MetH, depending upon the availability of exogenous cobalamin (68). MetH has a much higher turnover rate than MetE, and so it is a preferred route for methionine synthesis when cobalamin is present (27). Interestingly, the two obligate parasites *P. falciparum* and *E. histolytica* do not appear to contain either methionine synthase, suggesting that they may acquire methionine from their hosts. In contrast, the genome of *D. discoideum*, like that of *C. reinhardtii*, contains both metE and metH.

The fact that addition of methionine does not completely remove vitamin B$_{12}$ auxotrophy in algae prompted some investigators to look for other vitamin B$_{12}$-dependent enzymes. A vitamin B$_{12}$-dependent ribonucleotide reductase has been partially purified from *E. gracilis* (28), suggesting that this organism may require cobalamin for DNA biosynthesis. This is consistent with the observation that DNA biosynthesis appears to be inhibited during vitamin B$_{12}$ deprivation. However, the vitamin B$_{12}$-dependent type II ribonucleotide reductase, which is generally thought to be present in prokaryotes only, is one of three isoforms of ribonucleotide reductase (33). Other studies have shown that ribonucleotide reductase activity increases in *E. gracilis* during vitamin B$_{12}$ deficiency (8), suggesting that, as with many bacteria, there is more than one isoform of ribonucleotide reductase in this organism.

An alternative explanation for the reduction in DNA biosynthesis during vitamin B$_{12}$ deprivation is that it is a result of a perturbation of folate metabolism which results from reduced methionine synthase activity; this enzyme uses folate as a cofactor. Such a metabolic abnormality, which is termed “folate trapping,” is characteristic of vitamin B$_{12}$ deficiency in humans (59). Vitamin B$_{12}$ auxotrophy in the green alga *Lobomonas rostrata* can only be rescued when both folate and methionine are added to the culture medium together (11), demonstrating that folate trapping also occurs in algae and providing an explaining as to why earlier studies (29) could only partially rescue vitamin B$_{12}$ auxotrophy in algae with the addition of methionine alone.

Crude cell extracts of *E. gracilis* have been reported to contain methylmalonyl-CoA mutase activity (67), leading the authors to suggest that this organism contains a third vitamin B$_{12}$-dependent enzyme. This enzyme catalyzes the reversible conversion of succinyl-CoA to methylmalonyl-CoA. In mammals, methylmalonyl-CoA mutase is essential for the degradation of odd-chain fatty acids (40), but in other organisms, the enzyme has a role in anaerobic metabolism during propionate fermentation, as well as in the biosynthesis of branched-chain fatty acids. *E. gracilis* is able to grow on propionate (67), providing further evidence that an active methylmalonyl-CoA mutase may be present in the cell. A methylmalonyl-CoA mutase gene is present in the genome of *T. pseudonana*, and there is also an expressed sequence tag with sequence similarity to this gene from the diatom *Phaeodactylum tricornutum* (PTMM04237). Furthermore, the enzyme has recently been purified from the vitamin B$_{12}$-dependent haptophyte *Pleurochrysis carterae* (45). Interestingly, all of the algae that have been found to contain methylmalonyl-CoA mutase have complex plastids.

In mammalian cells, methylmalonyl-CoA mutase is located in the mitochondrion. The proteins CblA and CblB are thought to be responsible for the intracellular transport of cobalamin into the mitochondria of mammalian cells (15, 16). Proteins with sequence similarity to both CblA and CblB can be found in the genome of *T. pseudonana* (Table 2), suggesting that the methylmalonyl-CoA mutase in this alga is likely to be located in the mitochondrion and that the machinery for the intracellular transport of cobalamin is conserved between animals and algae. Not surprisingly for organisms that do not contain methylmalonyl-CoA mutase, *C. reinhardtii*, *C. merolae*, and *P. falciparum* do not possess genes with sequence similarity to *cblA* and *cblB* (Table 2) but both *C. reinhardtii* and *T. pseudonana* contain a gene with sequence similarity to *cbiE*, which encodes methionine synthase reductase, required in organisms containing MetH.

Why is it that so many algae have an absolute requirement for vitamin B$_{12}$? The exact role of methylmalonyl-CoA mutase in algae is not known, but the fact that *E. gracilis* can use propionate as a carbon source suggests that it allows these organisms to grow heterotrophically when vitamin B$_{12}$ is available. The isolation of an expressed sequence tag encoding this enzyme from *P. tricornutum*, a vitamin B$_{12}$-independent alga, indicates that the presence of this enzyme in an algal cell does not in itself result in cobalamin auxotrophy. Instead, vitamin B$_{12}$ auxotrophy appears to be determined by the enzymes in-
volved in methionine biosynthesis. The red alga C. merolae does not require vitamin B_{12} and, like higher plants, contains metE only. In contrast T. pseudonana, which does require vitamin B_{12}, contains metH only, whereas C. reinhardtii possesses both enzymes. It seems likely that, as with many eubacteria, early eukaryotes contained both metE and metH and later lost one of the genes. In animals and some algae such as T. pseudonana, loss of metE in an environment that must have contained a readily available source of the vitamin resulted in the creation of a vitamin B_{12}-auxotrophic organism.

**ACQUISITION OF VITAMINS**

The requirement for biotin, thiamine, and cobalamin by so many disparate algae indicates that the vitamins are available in the environment and that mechanisms exist for their uptake into algal cells. These three vitamins are all water soluble and comparatively stable, suggesting that they can be rescued by salvage. Indeed, thiamine-scavenging pathways are known in animals, fungi, and eubacteria (60). These vitamins are cofactors for a limited number of enzymes and are thus required in small quantities, reducing the pressure on biosynthetic flux and making salvage a viable option.

However, the uptake of these compounds is not as simple as it may at first seem because their concentration in the natural environment is extremely low. Indeed, the minute amount of these organic micromolecules has made them difficult to measure (50). The concentration of vitamin B_{12} in seawater is thought to vary between 0 and 3 ng/liter (9), and while higher levels have been reported in some freshwaters (12, 34), these levels are generally too low to support algal growth. Several studies have shown that different vitamin B_{12}-dependent algae require at least 10 ng/liter cobalamin in order to grow (50). Similarly, the concentrations of both thiamine and biotin in the natural environment are below that normally required in culture, with thiamine levels typically varying between 8 and 15 ng/liter at different points in the Pacific Ocean and biotin varying between 1 and 4 ng/liter in the same regions (9). In the case of thiamine, the stability of the cofactor at the alkaline pH of seawater has been shown to be dependent on the temperature of the water, declining sharply between 10°C and 30°C (26). This makes acquisition of the free cofactor from solution an unlikely route for many marine organisms.

The observation that only trace amounts of these vitamins were present in natural waters led several investigators to examine whether these compounds influence the productivity, and succession, of different species. Menzel and Spaeth (43) reported that moderate diatom blooms occurred in the Sargasso Sea when cobalamin concentrations were at their highest, and several other studies have shown a link between algal productivity and vitamin concentrations (56, 65). Such observations led to suggestions that algae were significant contributors to the pool of vitamins found in these waters (43). While this may be true for thiamine and biotin, it cannot be the case for cobalamin since the biosynthetic pathway is not present in any eukaryotic organism (11).

The fact that only prokaryotes have the ability to synthesize cobalamin implies that all of the vitamin B_{12} found in algae, and indeed animals, must originally have been produced by bacteria. Fogg and Kurata noted that many algae grew more rapidly in the presence of bacteria and thus concluded that the latter produce usable B vitamins for the algae (23, 36). More-recent work has provided firm evidence for this, since the cobalamin-dependent red alga Porphyridium purpureum can be sustained in defined culture medium lacking exogenous vitamin B_{12} by the marine bacterium Halomonas sp. In return, because there is no carbon source in the medium, the bacteria appear to be able to use the products of algal photosynthesis to grow (11). Halomonas sp. and others, such as Saccharophagus degradans, have been shown to degrade complex algal carbohydrates (18, 31). These symbiotic interactions between bacteria and algae appear to be widespread since a number of diverse algae are able to acquire vitamin B_{12} from bacteria (11). The lack of cobalamin in the environment, combined with the fact that more than half of all algae require the vitamin (Table 1), suggests that many algae form these symbiotic interactions in order to obtain the cofactor. Although there is no evidence that algae acquire thiamine directly from bacteria, such an interaction would explain why the level of free vitamin in natural waters does not limit algal growth. In support of this theory, Menzel and Spaeth, following their studies in the Sargasso Sea, found no evidence to suggest that vitamins limited algal productivity (43).

A number of dinoflagellate, cuglenoid, and heterokont algae are phagotrophic on bacterial prey, as is the amoeba D. discoideum. Furthermore, some dinoflagellates are known to contain intracellular bacteria (48). In terms of organic micronutrient acquisition, this provides an obvious route by which these organisms are able to take up their vitamins. All of the biotin-requiring algae fall into these groups, so the major route to biotin acquisition may be phagotrophy, and in organisms that do not have the ability to ingest bacteria, there may be strong evolutionary pressure to retain a functional biotin biosynthetic pathway. One other noteworthy point is that these phagotrophic groups include species that contain cobalamin-dependent methylmalonyl-CoA mutase (Fig. 1), suggesting perhaps that, like humans, they use this enzyme for the degradation of odd-chain fatty acids from their prey.

Algal-bacterial interactions are not limited to delivery of vitamins. Halomonas sp. has also been shown to improve the growth of the green alga Daniella balwardii under iron-deficient conditions, suggesting that the latter may be able to utilize bacterial siderophores (7, 35). Zoospores of the macroalga Ulva pertusa have been shown to recognize the quorum-sensing N-acetyl-l-homoserine lactone molecules released by bacterial biofilms, thereby facilitating the adherence of the zoospores to the surface (32). Even more remarkably, the morphology of the related alga Monostroma oxypermium is dependent on a growth factor, thallusin, synthesized by marine bacteria; in the absence of the bacteria, the algal thallus does not form and instead the alga grows as a loose association of single cells (41).

**CONCLUSIONS**

Vitamins are defined as organic micromolecules that must be obtained in the human diet. The observation that three of these vitamins are also essential for many photosynthetic algae, which are generally assumed to be completely autotrophic, is surprising. We have used the emerging genome sequences to
start to understand how this has arisen. For biotin and thiamine, the requirement for an exogenous supply is likely due to the loss of one or more key biosynthetic enzymes (Table 2). In contrast, cobalamin biosynthesis is absent from algae altogether and auxotrophy has arisen because of the loss of a cobalamin-independent methionine synthase. There is now clear recognition that prokaryotes occur if there is a reliable external supply in their environment.

In dispensing with the need to produce them, but this can only occur if there is a reliable external supply in their environment. At least for cobalamin, this comes from a symbiotic relationship with bacteria. There is now clear recognition that prokaryotic and eukaryotic organisms associate with each other (47) in order to exchange metabolites (7, 35) or to exploit unique cofactors are complicated to synthesize and required in trace amounts only, it is possible that there is a selective advantage in dispensing with the need to produce them, but this can only occur if there is a reliable external supply in their environment. It now seems likely that eukaryotic algae rely on other organisms for a source of essential vitamins, at least in some cases via a beneficial symbiosis. In the coming decades, both the enzymology and the regulation of these metabolic processes are likely to be explored in molecular detail.

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