BNL-222840-2022-JAAM

The Mosaic Landscape of Algal Metal Transport and Usage

C. E. Blaby-Haas

To be published in "Microbial Metabolism of Metals and Metalloids"

March 2022

Biology Department

Brookhaven National Laboratory

U.S. Department of Energy

USDOE Office of Science (SC), Biological and Environmental Research (BER) (SC-23)

DISCLAIMER

Chapter 3: "The mosaic landscape of algal metal transport and usage"

Crysten E. Blaby-Haas
Biology Department, Brookhaven National Laboratory, Upton, NY 11973 and Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY 11794
cblaby@bnl.gov

Abstract

Like all other eukaryotes, metal ions are essential nutrients for algae, which must be assimilated from the environment. At the same time, in excess, metal ions are inherently toxic. Reflecting the complex evolutionary relationships among the alga groups, algal genomes encode a variety of widely conserved and unique transport proteins to handle the balance between nutrient and toxin. Each genomic repertoire enables algal species to thrive in environments with unique biogeochemical characteristics compared to non-algal species commonly used as reference organisms for metal homeostasis. As a result, the study of algal metal homeostasis broadens our understanding of how phenotypically and taxonomically divergent eukaryotes have evolved to perform photosynthesis in disparate environments. These niches are as varied as marine versus freshwater, aquatic versus deserts, polar versus tropical, and free-living versus endosymbiotic. While access to genome sequences and predicted gene models is increasing, enabling identification of characterized proteins through computational genomics analyses, the next stage is developing transformation approaches and RNA-guided endonuclease systems for understanding the molecular and biological function of metal-regulated genes unique to algae.

Key Words: transition metals; copper, zinc, iron, manganese, molybdenum, photosynthesis, algae, metalloproteins

Section Headings:

1. Introduction
2. What are algae and where can they be found?
3. Metal usage
4. Metal transport
    - 4.1. Endocytosis-mediated iron transport
    - 4.2 Metal permeases
        - 4.2.1 An overview of assimilation
        - 4.2.2 An overview of export
        - 4.2.4 Permease substrates
        - 4.2.4 Metal permease families in algae
    - 4.3 Metallochaperones
5. Conclusions

1. Introduction

In the early 1900s, to sum his observations of animal behavior, Victor Shelfold wrote "…the habitat is the mold into which the organism fits" (Shelford 1913). This statement holds true across scales from learned behavior down to the selective pressures imparted by environments on genomes. Indeed, the habitat is the mold into which the genome fits. Any organisms that fail to fit that mold are outcompeted and fade from existence in the niche. With the development of whole-genome sequencing technologies, those inherited adaptations that passed the filter of natural selection can be elucidated and compared across lineages and environments. The rise of RNA-guided endonucleases as genetic tools is opening the door to experimental validation of those genome-based insights, providing a means to erase individual adaptative strategies and quantify the impact on condition-specific fitness.

For metal ions, two filters exist: competition for metal ions and avoidance of their toxicity. Metals provide proteins with chemistry or folding properties that are not easily achieved with just the amino acid sidechains of polypeptides. As a result, metals are essential nutrients. At the same time, metal ions are inherently toxic to biology. Transition metals can accept and donate electrons, a function that biology has capitalized on, but if allowed to react with oxygen or reactive oxygen species, these metals can catalyze oxidative damage to membranes and proteins. Zinc, although not redox active, can bind indiscriminately to proteins either inactivating them by replacing the cognate catalytic metal or by generating protein aggregates. Therefore, metal ions have left their mark on genomes as protein cofactors, and multi-layered strategies for handling deficiency and toxicity exist.

The guiding principle underlying metal homeostasis is to ensure a supply of metal ions to metal-dependent proteins while avoiding potential toxicity of excess metal ions. Subsequently, both global and local metal bioavailabilities across time have shaped gene repertoires reflected in the resulting lifestyles and metabolism of different algal species. The ability of individual algae to thrive in environments with unique geochemistry, compared to non-algal species commonly used as reference organisms for metal homeostasis, combined with access to their gene and protein sequences, provides an opportunity to broaden our understanding of metal transport and usage across the eukaryotic tree of life. The availability of 'omics technologies, such as transcriptomics and proteomics, enables genome-wide surveys of gene-specific responses to metal deficiency and excess. These datasets often reveal both conserved responses across algal and non-algal lineages and organism-specific or lineage-specific adaptations. Those genes found to be highly differentially expressed then often become targets for follow-on experimental characterization, largely through reverse genetics and molecular biology techniques. As discussed in this chapter, these

studies have revealed commonalities in metal homeostasis with non-algal lineages, including land plants, fungi and animals, and novelty that has contributed to the success of algae in complex and competitive habitats.

2. What are algae and where can they be found?

An alga is any eukaryotic organism that can perform photosynthesis and is not a land plant. Because of this pre-molecular (i.e. pre-genome sequencing) definition, the algal group is unified based on phenotype, not molecular phylogenetics. Algae are distributed throughout the eukaryotic tree of life; they do not have a single common ancestor in the traditional sense. Instead, the proverbial saying 'You are what you eat' could never be truer. The major lineages of algae are related through independent instances of endosymbiosis during which a eukaryotic host engulfs and retains either a cyanobacterium, producing a primary plastid, or an established alga, producing a secondary (or tertiary or more complex) plastid. The distinguishing feature of primary plastids compared to the more complex plastids is the number of membranes surrounding the plastid. Primary plastids have two membranes, while complex plastids, with a few exceptions (Wetherbee et al. 2019), have three to four membranes. Many of the stolen genes from the endosymbiont are ultimately transferred to and incorporated into the host's nuclear genome; a process often referred to as endosymbiotic gene transfer. The algal group is, therefore, polyphyletic, because the most recent common ancestor of all eukaryotic algae was not an alga, and many algae are more closely related to non-photosynthetic protists than they are to other algae.

There are also grey areas in the use of the terms alga and algae. With the advent of DNA sequencing and molecular phylogenetics, the term "non-photosynthetic algae" can now be found in the literature referring to organisms that are phylogenetically embedded in algal lineages but have colorless plastids, because they have lost the ability to perform photosynthesis. These types of algae provide insightful comparator species for phylogenetically profiling gene sets important for photosynthesis (Hadariová et al. 2017; Gawryluk et al. 2019). There are also several organisms that are sometimes referred to as algae but do not have a self-replicating plastid. These acquired phototrophs either have a symbiotic relationship with an alga, such as *Paramecium bursaria* with endosymbiotic *Chlorella* spp. (Karakashian 1975), or are able to engulf and steal plastids from algae (kleptoplasty), such as *Mesodinium rubra* (previously named as *Myrionecta rubra*), which acquires plastids from cryptophyte algal prey (Johnson and Stoecker 2005). In these cases, feeding on algae is needed to replenish their supply of plastids.

Because of these complex evolutionary histories and deep evolutionary roots, the gene repertoires of algae are diverse. The green, red and glaucophyte algae, like their sister lineage the land plants, share genes from

their shared last common ancestor, including both genes from the last common ancestor of Archaeplastida and genes from the cyanobacterium-like endosymbiont that became the shared primary chloroplast. This lineage likely dates back to an algal ancestor that existed at least 1.5 billion years ago (Blaby-Haas and Merchant 2019). Algae that do not have a primary plastid, such as diatoms or Euglena, share genes from an ancient protist-like ancestor and genes from a red-alga-like or green-alga-like endosymbiont, respectively, that became their chloroplast. The spread of plastids across the eukaryotic tree of life through endosymbiosis, retention, and endosymbiotic gene transfer has occurred multiple independent times, with some cases of complex serial endosymbiosis (Stiller et al. 2014).

The lifestyles, environments, and morphology of individual algal subgroups are as diverse as their evolutionary relationships. As an example, the Chlorophyte lineage (also known as the green algae, which share a relatively recent common ancestor with the land plants) contains both the smallest and largest known free-living single-celled eukaryotes, *Ostreococcus tauri* (Courties et al. 1994) and *Caulerpa taxifolia* (Ranjan et al. 2015), respectively. Multicellular green algal species can range from 20 μM (the colonial alga *Tetrabaena socialis* (Arakaki et al. 2013)) to three feet in size (the seaweed *Ulva lactuca* (Steffensen 1976)). The largest alga, *Macrocystis pyrifera* (North 1971), a brown alga in the SAR supergroup, can reach 200 feet in length. Algae can be found in temperate and tropical soil, fresh water, and the oceans, as well as extreme environments. The halophilic green alga *Dunaliella salina* inhabits the Northern arm of the Great Salt Lake, Utah, where the NaCl concentration is oversaturated (Brock 1975). Red algae in the order *Cyanidiales* thrive at pH 0.5-3 and high temperature (50-55°C) (Castenholz and McDermott 2010). Psychrophilic green algae, such as *Chlamydomonas raudensis* (UWO 241), inhabit permanently ice-covered lakes in Antarctica (Pocock et al. 2004), while snow algae often cover glaciers like pink Kool-Aid (because of a red carotenoid pigment) (Bidigare et al. 1993) and diatoms inhabit brine channels in sea ice (Mock and Junge 2007). Because of these and other drastically different algal species, unique adaptations involving metal homeostasis are expected but remain under investigated.

3. Metal usage

Because of their role in enzyme catalysis and protein folding, metal ions are essential nutrients, but which metals and at what concentrations are needed by an individual algal species can vary. Based on studies of reference algal species with sequenced nuclear genomes (Table 1), algae have a relatively large requirement for iron as the most abundant and irreplaceable inorganic cofactor for linear electron transport during photosynthesis and respiration. In addition to iron, algae require several metals from the d-block of the periodic table, specifically zinc, copper, manganese, and molybdenum. Some algae, such as the prasinophytes, use nickel for urease (Collier et al. 2009), while cobalt, in the form of cobalamin (vitamin

142 B_{12}), is needed for one of two classes of methionine synthase (Helliwell et al. 2011). Since no alga is known
143 to be able to synthesize B_{12}, this vitamin must be acquired from bacteria (Croft et al. 2005).
144
145 Table 1. Common reference algal species for metal homeostasis with sequenced nuclear genomes.

Species	Lineage	Environment	Type of plastid	Genome
Chlamydomonas reinhardtii	Plant	Freshwater/soil	primary	(Merchant et al. 2007; Blaby et al. 2014)
Ostreococcus tauri	Plant	Marine	primary	(Derelle et al. 2006)
Phaeodactylum tricornutum	Diatom	Marine	complex	(Bowler et al. 2008; Rastogi et al. 2018)
Thalassiosira pseudonana	Diatom	Marine	complex	(Armbrust et al. 2004)

146
147 The types of reactions and classes of proteins that bind and utilize specific metal ions is determined by the
148 inherent chemistry of each metal ion. In nature, the zinc ion exhibits a single oxidation state (+2) and is
149 often used as a cofactor to stabilize protein structure or in hydrolytic reactions where it serves as an
150 electrophile. Depending on the specific ligand environment, copper, iron, and manganese ions, which can
151 exist in different oxidation states, are also employed for hydrolytic reactions but, unlike zinc, can function
152 in electron transfer reactions. Molybdenum has only been found in eukaryotes as the constituent of a
153 tricyclic pyranopterin compound named the molybdenum cofactor (Moco) and functions in electron transfer
154 within a small number of proteins. Typically, zinc, copper and manganese are not associated with prosthetic
155 groups. Iron, on the other hand, is versatile, being directly associated with amino acids in some enzymes,
156 as in Fe-dependent superoxide dismutase, and incorporated into organic or inorganic cofactors like heme
157 and Fe-S clusters, as found in the photosynthetic complexes. These iron-bound cofactors associate with
158 proteins as prosthetic groups and provide an additional means for tuning the redox potential of the iron
159 atom to catalyze electron transfer.
160
161 Because individual proteins have evolved to harness the chemistry of specific metal ions, substitution of
162 metal ions for one another is not a common occurrence. Nevertheless, such adaptive mechanisms have been
163 described for algae and examples of nutritive substitution phenomena involving replacement of one metal
164 ion for another are available. In some cases, the underlying genetics is understood. Carbonic anhydrase in

eukaryotes is characterized as a zinc-dependent enzyme, but during growth under low zinc conditions with cadmium supplementation, the carbonic anhydrase CDCA1 from the marine diatom *Conticribra weissflogii* (previously *Thalassiosira weissflogii*) is active with cadmium instead of zinc (Lane et al. 2005). The cambialistic (capable of functioning with multiple metal atoms) nature of this enzyme is thought to represent a zinc-sparing mechanism that provides a selective advantage for diatoms growing in zinc-poor ocean waters. In contrast to such promiscuous proteins, interchangeable proteins that require different metal cofactors have also been characterized in algae. The copper-regulated switch between copper-dependent plastocyanin and Fe-dependent cytochrome c_6 is a well-characterized strategy for recycling and sparing copper (Kropat et al. 2015). In contrast to these examples, a cobalt/zinc nutritive substitution has been widely described for different types of algae (Sunda and Huntsman 1995; Intwala et al. 2008; Saito and Goepfert 2008), but the underlying genetics and mechanism for this phenotype are not known. Based on the previous examples, zinc and cobalt may be interchangeable cofactors because of the existence of cambialistic proteins, or some algae may have interchangeable sets of zinc-dependent and cobalt-dependent protein isoforms. Discovering the proteins that are responsible for this type of cofactor switch promises to reveal novel proteins and homeostatic regulation unique to algae.

4. Metal transport

Like all other eukaryotes, algal cells are surrounded by a plasma membrane with intracellular membranes delineating sub-compartments and organelles. Metal-dependent proteins are found throughout the cell and are typically translocated into organelles as unfolded polypeptides. As such, transporters are needed for passing metals across multiple membranes, either to correct deficiency or toxicity. However, our understanding of metal transporters in the plasma membrane is far more advanced than our understanding of metal transport across the membranes of organelles. Two main types of transport have been described in algae, endocytosis-mediated and permease-mediated.

4.1 Endocytosis-mediated iron transport

Endocytosis is a process by which the plasma membrane surrounds a target by invagination to form a vesicle in the cytosol. The cargo is then sorted, often associated with endosomal acidification (Elkin et al. 2016). Although endocytosis may be responsible for the uptake of other metal ions or metal-chelate molecules, only iron uptake by endocytosis has been described for algae. Four separate families of algal proteins have been implicated in endocytosis-mediated iron assimilation: transferrin, phytotransferrin, ISIP1 (iron starvation-induced protein 1), and ferrichrome-binding protein (FBP).

An animal-like transferrin was first discovered in the unicellular green alga *D. salina* as a salt-induced, 150 kDa, plasma membrane bound protein and was initially referred to as p150 (Sadka et al. 1991). Cloning and sequencing of the corresponding cDNA revealed sequence similarity to animal transferrin (Fisher et al. 1996). While animal transferrins are composed of two homologous domains, the *D. salina* transferrin has three homologous domains and is often referred to as TTf for triplicated transferrin (Fisher et al. 1998). Like animal transferrins, expression is induced by iron deficiency, and TTf binds and facilitates assimilation of the oxidized ion of iron, Fe^{3+} (Fisher et al. 1998). Unlike animals, TTf forms a complex with a multi-copper ferroxidase (D-Fox), p130b and a second transferrin-like protein, DTf (*D. salina* transferrin) (Paz et al. 2007a). D-Fox is likely responsible for oxidation of Fe^{2+} prior to binding to TTf, as has been suggested for ceruloplasmin (Eid et al. 2014). The function of DTf is unknown since the protein does not bind iron (Schwarz et al. 2003). The function of p130b is also unknown, but it is structurally similar to ISIP1 from the diatom *Phaeodactylum tricornutum*, which is also induced by iron deficiency (Allen et al. 2008). Shared structural features with the low-density lipoprotein receptor LDLR, a cell-surface receptor in humans, led to the original hypothesis that p130b and ISIP1 are iron-deficiency-responsive receptors (Lommer et al. 2012). Subsequent characterization in *P. tricornutum* led to the conclusion that ISIP1 is involved in the endocytosis-mediated assimilation of siderophores by FBP1, a protein with sequence similarity to the periplasmic component of the bacterial Fe^{3+}-hydroxamate transport system (Sutak et al. 2012; Kazamia et al. 2018). Therefore, by analogy, p130b may function in the endocytosis of TTf in *D. salina*. However, while multiple algal and land plant genomes encode transferrin-like proteins, only diatoms and *D. salina* have a protein similar to p130b or ISIP1 (Figure 1).

Not only was *D. salina* the first alga for which an animal-like transferrin was found, but this family is also uniquely expanded. At least 10 separate genes encoding proteins with similarity to the transferrin PFam domain PF00405 are present in the available genome assembly (Polle et al. 2020). When other algae and land plants have transferrin, there typically are only one or two. A similarly sized expansion (7 animal-like tansferrins) is also found in the genome of the macroalga *Caulerpa lentillifera,* an edible seaweed commonly referred to as sea grapes (Arimoto et al. 2019). For *D. salina*, the unusual number of transferrins may relate to its unique tolerance of hypersaline environments where iron bioavailability is relatively low. *C. lentillifera*, however, is not an extremophile and is often found throughout the coastal Asia-Pacific region. What is unique about *C. lentillifera* and other members of the *Caulerpa* genus is that they appear to be complex plant-like seaweeds with structures analogous to leaves, stems and roots but are actually composed of a single cell with multiple nuclei (Coneva and Chitwood 2015). The absence of compartmentalization afforded by being multi-cellular must come with its own challenges, such as having to rely on diffusion and cytoplasmic streaming for distribution of metal ions across relatively large

distances. By assimilating iron with transferrin and endocytosis, these siphonous macroalgae may be able to selectively shuttle iron throughout the expansive cytoplasm with vesicle-mediated trafficking, releasing the iron where and when needed.

Animal-like transferrins have only been identified in plants and green algae in the chlorophyte and streptophyte lineages (Bai et al. 2016; Blaby-Haas and Merchant 2017), while most algae contain proteins similar to phytotransferrin (pTF) from *P. tricornutum* (Figure 1). The use of the "phyto" prefix may be confusing since this family is not found in land plants. Instead, phytotransferrin homologs are specific to algae and can be found as soluble proteins, such as the FEA (Fe-assimilating) proteins in the green alga *Chalmydomonas reinhardtii* (Allen et al. 2007), or membrane-bound proteins, such as Ot-FEA1 in the green alga *O. tauri* (Lelandais et al. 2016; Scheiber et al. 2019). The first member of this alga-specific family to be discovered was *HCR1* from *Alvikia littoralis* (previously *Chlorococcum littorale*), a marine green alga from the chlorophyte lineage. The gene was isolated as a high-CO_2 and iron-deficiency induced transcript (Sasaki et al. 1998). Subsequently, a homolog from the freshwater/soil green alga *C. reinhardtii*, originally named *H43*, was identified as a cadmium- and iron-deficiency induced transcript (Rubinelli et al. 2002). After the identification of a second iron-deficiency-induced *H43*-like gene in *C. reinhardtii*, these proteins were renamed FEA (Merchant et al. 2006; Allen et al. 2007). In *C. reinhardtii,* the FEA proteins are secreted, and in a cell-wall-less strain, FEA1/FEA2 are lost to the supernatant correlating with increased sensitivity to iron deficiency (Allen et al. 2007). The mechanism responsible for FEA-mediated iron assimilation is uncharacterized, but recombinant expression of *FEA1* in *Arabidopsis thaliana* and cassava leads to increased root iron content (Narayanan et al. 2011; Ihemere et al. 2012).

Phytotransferrin was originally named ISIP2 and was discovered in an iron-responsive transcriptomics study in *P. tricornutum* (Allen et al. 2008). Follow-on characterization of this protein resulted in increasing evidence for a function analogous to transferrin leading to the name phytotransferrin (Morrissey et al. 2015; McQuaid et al. 2018). Recent analysis of protein-protein interactions and microscopy suggests that after endocytosis phytotransferrin may be directly sorted to the chloroplast with vesicle trafficking together with ISIP1 and FBP1 (Turnšek et al. 2021). Whether such vesicle-mediated transport of iron and potentially other metal ions to the chloroplast exists outside diatoms is currently unknown. *D. salina*, which has a primary plastid like other chlorophytes, appears to internalize iron into acidic compartments, which are thought to act as an intermediary storage station, as in animals (Paz et al. 2007b). Therefore, the functional analogy between transferrins and phytotransferrins in algae may end after internalization. In contrast to land plants and algae with primary plastids, the chloroplast of diatoms is surrounded by four membranes, where the outer envelope (called the chloroplast endoplasmic reticulum (CER)) is continuous with the nuclear

envelope and is proposed to have originated from the host endomembrane. Vesicle-mediated iron transport within the endomembrane system may have been an adaption necessary for supplying nutrients to the engulfed alga ancestor of the plastid, and this strategy has been maintained.

4.2 Metal permeases

Metal permeases are membrane-spanning proteins that provide routes for metal ions to cross biological membranes. Some permeases form a selective channel for metal ions to travel through the membrane while leveraging concentration gradients to drive transport. Other permeases leverage ATP hydrolysis or protonmotive force. Based largely on characterization of non-algal homologs, metal permeases can be divided into two main functional groups based on their direction of transport relative to the cytosol. Transporter families typically fall into one group or the other, but exceptions to such broad classifications often occur in the literature. These exceptions may be due to functional divergence within a transporter family or, in some cases, may be a consequence of relying solely on phenotypes of gene knockouts without additional information, such as transporter localization or orientation in the membrane, with which to contextualize reverse-genetics data. Therefore, the following generalizations are a useful jumping-off point for functional annotation, but experimentation is critical for establishing functional capabilities, since diversification is central to the evolution of adaptive mechanisms.

4.2.1 An overview of assimilation

Members of group A typically transport metal ions into the cytoplasm, either across the plasma membrane during assimilation or across intracellular membranes for release of intracellular stores. Expression of the corresponding genes often, but not always, increases during metal deficiency. Group A permeases include the NRAMP, ZIP, FTR, CTR, MOT1, and MOT2 families (Figure 2). The NRAMP family was named after the first member to be studied, Natural-Resistance-Associated Macrophage Protein 1, from mouse (Vidal et al. 1993). NRAMPs are most often found to function in iron and manganese uptake as proton-metal symporters. The ZIP (Zrt-, Irt-like Proteins) family is named after the first members to be characterized, the zinc transporters Zrt1p (Zhao and Eide 1996a) and Zrt2p (Zhao and Eide 1996b) from the yeast *Saccharomyces cerevisiae* and IRT1, the major iron-uptake protein in *A. thaliana* roots (Eide et al. 1996). Those ZIP transporters characterized to date transport divalent metal ions, and most play biological roles in either zinc or iron transport. The FTR (Fe transporter) family was first identified as part of a high-affinity iron transport complex containing Fet3p, a multi-copper oxidase (MCO), in *S. cerevisiae* (Stearman et al. 1996). Unlike NRAMP and ZIP, which can transport ferrous iron, FTR transports ferric iron. CTR (Cu transporter) was also first discovered in *S. cerevisiae* (Dancis et al. 1994). Unlike the three transporters

described so far, CTRs transport monovalent cations. MOT1 and MOT2 (Molybdenum transporters) are independent families of oxianion molybdate (MoO_4^{-2}) transporters (Tejada-Jiménez et al. 2007, 2011).

4.2.2 An overview of export

Members of group B typically transport metal ions out of the cytoplasm. Within this group are distributive transporters, which provide metal for organelle-localized metalloproteins that are either synthesized in organelles or imported as unfolded polypeptides. When present in the endomembrane system, group B transporters pump metal into membrane-delineated compartments for storage or efflux out of the cell. These families are also often found in the vacuole's boundary membrane. Since not all algae have vacuoles as described for yeast and plants, these transporters may be found in other lysosome-like organelles that function to detoxify and store metal ions (Blaby-Haas and Merchant 2014). Expression of the corresponding genes often, but not always, increases during metal excess. Exceptions are the distributive transporters PAA1 (P-type ATPase of Arabidopsis 1) and PAA2 (P-type ATPase of Arabidopsis 2) from the Cu-ATPase family, which supply copper for the biogenesis of plastocyanin in the chloroplast. The genes encoding these transporters are down-regulated in the shoots of *A. thaliana* plants when exposed to excess copper (del Pozo et al. 2010). Members of group B include the CDF, P_{1B}-type ATPases, GDT1, ferroportin, and Ccc1/VIT1 families (Figure 2). The first member of the CDF (Cation Diffusion Facilitator) family was identified in *S. cerevisiae* as a gene responsible for zinc tolerance, *ZRC1* (zinc resistance conferring) (Kamizono et al. 1989). Comparison of this protein with a second protein from yeast responsible for cobalt tolerance, Cot1p (Conklin et al. 1992), and a bacterial protein involved in tolerance to multiple metals, CzcD (Nies 1992), led to the hypothesis that these make up a family of metal transporters responsible for metal resistance (Nies and Silver 1995). P_{1B}-type ATPases couple ATP hydrolysis to monovalent (copper) or divalent metal transport (zinc, cadmium, or iron) depending on the specific subfamily and sequence determinants (Purohit et al. 2018). Members of the GDT1 family (formerly named UPF0016) were first described as Ca^{2+} effluxers (Demaegd et al. 2013; Wang et al. 2016), but were later found to also function as Mn^{2+} transporters in different organisms and different cellular compartments (Fisher et al. 2016; Schneider et al. 2016; Potelle et al. 2016). Ferroportins (Fpn) are most often described as iron exporters, but a plant homolog, IREG2 is thought to detoxify Ni^{2+} (Schaaf et al. 2006). Ccc1p, the founding member from *S. cerevisiae*, and VIT1, the founding member from *A. thaliana*, both mediate transport of Fe^{2+} into the vacuole (Li et al. 2001; Kim et al. 2006).

4.2.3 Permease substrates

Unlike endocytosis-mediated transport that appears to be specific to iron, metal permeases are often able to transport multiple different metal ions. For instance, although characterized members of the NRAMP family

have specificity against Ca^{2+} and Mg^{2+}, they can transport several d-block divalent metal ions with a preference for Fe^{2+} and Mn^{2+} over Zn^{2+} (Illing et al. 2012; Bozzi et al. 2016). Other metal permeases that transport divalent metal ions have an analogous lack of selectivity when comparing first row transition metals and zinc. Ferroportin is best known as a H^{+}/Fe^{2+} antiporter (Pan et al. 2020), but can also transport Co^{2+}, Zn^{2+} and Ni^{2+} (Schaaf et al. 2006; Mitchell et al. 2014). Since these permeases often have a broad spectrum of substrates, the biologically relevant substrate is typically predicted based on the conditions under which the permease is expressed. For instance, members of the ZIP family are predicted to be responsible for zinc transport when the gene is expressed during zinc deficiency, or iron when expressed during iron deficiency. However, even though structural and mechanistic models are now available for the ZIPs, sequence determinants for substrate preference or whether a preference exists are unknown (Hu 2020). Since the assimilation of non-limiting metal ions during zinc- or iron-deficiencies would lead to mis-metallation of apo-proteins, such knowledge gaps point to the possibility that additional factors, such as periplasmic metal-binding proteins, exist in the cell to mediate substrate specificity.

Indeed, such proteins are co-expressed with ZIPs during zinc deficiency in algae. In *C. reinhardtii*, a protein structurally similar to AztD, a WD40-like periplasmic zinc chaperone characterized in bacteria (Handali et al. 2015; Neupane et al. 2019), is induced during zinc limitation (Malasarn et al. 2013). Based on the recent reference genome assembly (Goodstein et al. 2012; Blaby et al. 2014), the gene encoding this protein is head-to-head with *ZRT1* that encodes one of the main zinc transporters from the ZIP family in *C. reinhardtii*. This proximity suggests that the two genes could share a bi-directional promoter, and evolution has selected for tight co-regulation of this putative zinc chaperone and the zinc transporter. A protein similar to TroA, a zinc-binding component of a bacterial ATP-binding cassette transport system (Lee et al. 1999), is expressed during zinc limitation in *Emiliania huxleyi*, a unicellular alga from the haptophyte lineage, which is commonly found in zinc-depleted open-ocean waters (Shire and Kustka 2021). Whether these algal proteins function in an analogous way to their bacterial counterparts is unknown. Like other eukaryotes, algae do not contain multi-subunit ABC transport complexes, such as ZnuABC from bacteria. Therefore, if these proteins do function as secreted zinc chaperones in algae, they may interact with and lend substrate specificity to the ZIPs.

Selectivity of metal ion transport has been achieved to a certain extent by the CTR family and Cu-ATPases that transport Cu^{1+} and the MCO/FTR complex that transports Fe^{3+} (Figure 2). These transporters can electrochemically separate the monovalent cuprous ion and trivalent ferric ion, respectively, from the other potentially competing divalent metal ions, such as zinc that only exists in nature as a divalent ion. However, the CTR family can also transport Ag^{1+}, and, as a result, under conditions that these transporters are

expressed, such as copper-deficiency, cells are sensitive to Ag^{1+} toxicity (Howe and Merchant 1992). Presumably, from the point-of-view of natural selection, the cost associated with increased Ag^{1+} toxicity is offset by the benefit afforded by excluding divalent metal ions. A similar bargain likely exists for the MCO/FTR transporter, since analogous ferric transporters from bacteria can also transport Ga^{3+} and Al^{3+} (Anderson et al. 2004).

4.2.4 Metal permease families in algae

With some notable exceptions, much of our understanding of algal metal permeases is derived from bioinformatic analyses. Membrane-spanning transporters are often difficult to work with *in vitro* and, for those algae that are genetically tractable, genetic redundancy due to overlapping gene functions often obfuscates phenotypes of gene-specific mutants. Robust bioinformatic analyses rely on the availability of high-quality algal genome sequences and gene models (Blaby-Haas and Merchant 2019; Hanschen and Starkenburg 2020). With these resources in hand, sequence similarity and phylogenetics of metal permease families can be used to leverage experimentally defined knowledge from reference organisms, such as *S. cerevisiae* and *A. thaliana*, for understanding metal transport in algae. One consequence is that much of our understanding of algal metal transport comes from experimental results with similar proteins in non-algal species. Therefore, a key step in leveraging protein family knowledge derived from other organisms is contextualizing that information with experimentation in algae and shedding light on functional divergence and tailoring that has occurred during evolution. Depending on the genetic tractability and culturability of a target algal species, such experimentation could be as simple as assaying condition-specific gene expression or as complex as reverse genetic analyses. Algal genomes also encode metal transport proteins that have yet to be found in non-algal genomes. These proteins are often found through metal-responsive transcriptome and proteome analyses. The algal-specific FEA (Rubinelli et al. 2002; Allen et al. 2007) and phytotransferrin families (McQuaid et al. 2018) discussed above, highlight the need for experimentation in algae beyond omics-based technologies.

Genes encoding ancient metal transport families shared with other eukaryotes or with bacteria are found in algal genomes. As a result, close homologs may be found in either the plant, fungal, animal, protist or bacterial lineages (Hanikenne et al. 2005; Blaby-Haas and Merchant 2012). Even within the same transporter family, algal genomes often encode some members that are closely related to fungal sequences and other members that are more closely related to plant sequences, reflecting the complex evolution that has resulted in modern-day algal genomes. An example is the CTR family in the green alga *C. reinhardtii*. Two homologs, CTR1 and CTR2, are similar to available fungal sequences, while the homolog COPT1 is similar to land plant sequences (Page et al. 2009). *C. reinhardtii* also has members of transport families that

are more similar to bacterial sequences, such as the divalent metal ion permeases, NRAMP1 and NRAMP2, which are members of the MntH-like subfamily (Blaby-Haas and Merchant 2012). Because of such evolutionary relationships at the protein family level, an understanding of protein phylogeny can help determine which functional knowledge, for instance derived from fungal, bacterial, or plant homologs, may be more relevant to understanding the function of a particular algal protein.

4.3 Metallochaperones

In addition to membrane-bound transporters, soluble proteins are responsible for transporting metal ions. These proteins, referred to as metal chaperones or metallochaperones, ensure that the correct metals are delivered to the right proteins. Like other eukaryotes, algal genomes encode Atx1-like copper chaperones (Pufahl et al. 1997; Merchant et al. 2020) and, for those algae that have a Cu-Zn superoxide dismutase, a CCS-like copper chaperone (VC et al. 1997; Foflonker and Blaby-Haas 2020). Green algae also share a plant-specific copper chaperone that delivers copper to the chloroplast (Blaby-Haas et al. 2014). Unlike other eukaryotes, algae have an unusually large number of nucleotide-dependent metallochaperones (NMCs) that are often expressed only during zinc limitation.

The molecular function of the NMC family (also referred to as CobW or COG0523 (Haas et al. 2009)) is not yet fully resolved. Most homologs contain conserved GTPase and metal-binding motifs. This family has shared ancestry with HypB and UreG, two GTPases involved in nickel insertion in Ni-Fe hydrogenase and Ni-urease, respectively (Haas et al. 2009). This shared ancestry plus the conservation of GTPase and metal-binding suggests that these putative chaperones also function in metalloprotein biogenesis. Most eukaryotic genomes only contain one homolog, with some gene expansion in human (5 homologs) and plants (3 to 4 homologs). Algal genomes, however, can encode upwards of 27 NMCs as found for *E. huxleyi*. Three of these NMCs are significantly increased in abundance during zinc deficiency (Shire and Kustka 2021). The *C. reinhardtii* genome encodes 12 NMCs. Of these, *ZCP1* and *ZCP2* respond specifically to zinc, and these 2 proteins are the two most abundant soluble proteins during zinc limitation (Haas et al. 2009; Hsieh et al. 2013; Malasarn et al. 2013). Although *E. huxleyi* and *C. reinhardtii* are both algae and share some genes, because of endosymbiotic gene transfer from a red alga in an ancestor of *E. huxleyi*, their last common ancestor likely dates to the first eukaryotes, suggesting that the large number of NMCs in these algae is due to convergent evolution. Clearly, the NMCs are providing important metal-related functions for algae from different environments and with different evolutionary backgrounds.

5 Conclusions

Largely through ‘omics-based experiments, significant progress has been made in identifying conserved and unique metal-transport strategies in algae. Transporters encoded in these genomes can be identified by similarity based techniques, aided by high-quality genome assemblies and gene model predictions. Likewise, technologies, such as RNAseq and iTRAQ-based proteomics, are available to quantify gene expression at the genome-wide scale in response to metal ion availability. However, the intracellular location of most metal transporters and substrate preferences are unknown. Development of reverse-genetic techniques enabled by RNA-guided endonucleases, such as CRISPR-Cas9, and the characterization of mutants defective in genes identified in ‘omics experiments are expected to yield considerable new discoveries. Insights into novel functional capabilities and adaptions that have evolved are critical for understanding the role of taxonomically diverse algae in distinct ecosystems. There are notable interactions between metals, such as the need for increased copper transport to metallate FOX1 during iron deficiency, which can complicate the interpretation of loss-of-function phenotypes for ascribing molecular function. Therefore, inter-disciplinary studies are needed to understand the role of predicted algal proteins at both the molecular and biological levels. The number of genes of unknown or uncertain function that are predicted in algae genomes points to yet-to-be discovered strategies (Blaby-Haas and Merchant 2019). As algae are increasingly the focus of biotechnological innovation in bioenergy and bioproduction, understanding these processes will also provide novel resources for engineering algal metabolism.

6 Acknowledgments

CB is supported by the US Department of Energy, Office of Science, Office of Biological, and Environmental Research, as part of the Quantitative Plant Science Initiative SFA at Brookhaven National Laboratory and under Award Number DE-SC0018301.

460 References

461 Allen AE, LaRoche J, Maheswari U, et al (2008) Whole-cell response of the pennate diatom
462 *Phaeodactylum tricornutum* to iron starvation. Proc Natl Acad Sci U S A 105:10438–10443.
463 https://doi.org/10.1073/pnas.0711370105

464 Allen MD, Del Campo JA, Kropat J, Merchant SS (2007) FEA1, FEA2, and FRE1, encoding two
465 homologous secreted proteins and a candidate ferrireductase, are expressed coordinately with FOX1
466 and FTR1 in iron-deficient *Chlamydomonas reinhardtii*. Eukaryot Cell 6:1841–1852.
467 https://doi.org/10.1128/EC.00205-07

468 Anderson DS, Adhikari P, Nowalk AJ, et al (2004) The hFbpABC transporter from *Haemophilus*
469 *influenzae* functions as a binding-protein-dependent ABC transporter with high specificity and
470 affinity for ferric iron. J Bacteriol 186:6220–6229. https://doi.org/10.1128/JB.186.18.6220-
471 6229.2004

472 Arakaki Y, Kawai-Toyooka H, Hamamura Y, et al (2013) The simplest integrated multicellular organism
473 unveiled. PLoS One 8:e81641

474 Arimoto A, Nishitsuji K, Higa Y, et al (2019) A siphonous macroalgal genome suggests convergent
475 functions of homeobox genes in algae and land plants. DNA Res 26:183–192.
476 https://doi.org/10.1093/dnares/dsz002

477 Armbrust EV, Berges JA, Bowler C, et al (2004) The genome of the diatom *Thalassiosira Pseudonana*:
478 Ecology, evolution, and metabolism. Science (80-) 306:79–86.
479 https://doi.org/10.1126/science.1101156

480 Bai L, Qiao M, Zheng R, et al (2016) Phylogenomic analysis of transferrin family from animals and
481 plants. Comp Biochem Physiol Part D Genomics Proteomics 17:1–8

482 Bidigare RR, Ondrusek ME, Kennicutt MC, et al (1993) Evidence a photoprotective for secondary
483 carotenoids of snow algae. J Phycol 29:427–434. https://doi.org/10.1111/j.1529-
484 8817.1993.tb00143.x

485 Blaby-Haas CE, Merchant SS (2017) Regulating cellular trace metal economy in algae. Curr Opin Plant
486 Biol 39:88–96. https://doi.org/10.1016/j.pbi.2017.06.005

487 Blaby-Haas CE, Merchant SS (2012) The ins and outs of algal metal transport. Biochim Biophys Acta -
488 Mol Cell Res 1823:1531–1552. https://doi.org/10.1016/j.bbamcr.2012.04.010

489 Blaby-Haas CE, Merchant SS (2019) Comparative and Functional Algal Genomics. Annu. Rev. Plant
490 Biol. 70:605–638

491 Blaby-Haas CE, Padilla-Benavides T, Stübe R, et al (2014) Evolution of a plant-specific copper
492 chaperone family for chloroplast copper homeostasis. Proc Natl Acad Sci U S A 111:E5480–E5487.
493 https://doi.org/10.1073/pnas.1421545111

494 Blaby-Haas CECE, Merchant SSS (2014) Lysosome-related organelles as mediators of metal
495 homeostasis. J Biol Chem 289:28129–28136. https://doi.org/10.1074/jbc.R114.592618
496 Blaby IK, Blaby-Haas CE, Tourasse N, et al (2014) The Chlamydomonas genome project: A decade on.
497 Trends Plant Sci 19:. https://doi.org/10.1016/j.tplants.2014.05.008
498 Bowler C, Allen AE, Badger JH, et al (2008) The Phaeodactylum genome reveals the evolutionary history
499 of diatom genomes. Nat 2008 4567219 456:239–244. https://doi.org/10.1038/nature07410
500 Bozzi AT, Bane LB, Weihofen WA, et al (2016) Conserved methionine dictates substrate preference in
501 Nramp-family divalent metal transporters. Proc Natl Acad Sci U S A 113:10310–10315.
502 https://doi.org/10.1073/pnas.1607734113
503 Brock TD (1975) Salinity and the ecology of Dunaliella from Great Salt Lake. Microbiology 89:285–292
504 Castenholz RW, McDermott TR (2010) The Cyanidiales: ecology, biodiversity, and biogeography. In:
505 Red algae in the genomic age. Springer, pp 357–371
506 Collier JL, Baker KM, Bell SL (2009) Diversity of urea-degrading microorganisms in open-ocean and
507 estuarine planktonic communities. Environ Microbiol 11:3118–3131
508 Coneva V, Chitwood DH (2015) Plant architecture without multicellularity: quandaries over patterning
509 and the soma-germline divide in siphonous algae. Front Plant Sci 0:287.
510 https://doi.org/10.3389/FPLS.2015.00287
511 Conklin DS, McMaster JA, Culbertson MR, Kung C (1992) COT1, a gene involved in cobalt
512 accumulation in *Saccharomyces cerevisiae*. Mol Cell Biol 12:3678–3688.
513 https://doi.org/10.1128/MCB.12.9.3678-3688.1992
514 Courties C, Vaquer A, Troussellier M, et al (1994) Smallest eukaryotic organism. Nature 370:255
515 Croft MT, Lawrence AD, Raux-Deery E, et al (2005) Algae acquire vitamin B 12 through a symbiotic
516 relationship with bacteria. Nature 438:90–93
517 Dancis A, Yuan DS, Haile D, et al (1994) Molecular characterization of a copper transport protein in S.
518 cerevisiae: An unexpected role for copper in iron transport. Cell 76:393–402.
519 https://doi.org/10.1016/0092-8674(94)90345-X
520 del Pozo T, Cambiazo V, González M (2010) Gene expression profiling analysis of copper homeostasis in
521 *Arabidopsis thaliana*. Biochem Biophys Res Commun 393:248–252.
522 https://doi.org/10.1016/j.bbrc.2010.01.111
523 Demaegd D, Foulquier F, Colinet AS, et al (2013) Newly characterized Golgi-localized family of proteins
524 is involved in calcium and pH homeostasis in yeast and human cells. Proc Natl Acad Sci U S A
525 110:6859–6864. https://doi.org/10.1073/pnas.1219871110
526 Derelle E, Ferraz C, Rombauts S, et al (2006) Genome analysis of the smallest free-living eukaryote
527 Ostreococcus tauri unveils many unique features. Proc Natl Acad Sci 103:11647–11652.

https://doi.org/10.1073/PNAS.0604795103
Eid C, Hémadi M, Ha-Duong NT, El Hage Chahine JM (2014) Iron uptake and transfer from ceruloplasmin to transferrin. Biochim Biophys Acta - Gen Subj 1840:1771–1781. https://doi.org/10.1016/J.BBAGEN.2014.01.011
Eide D, Broderius M, Fett J, Guerinot M Lou (1996) A novel iron-regulated metal transporter from plants identified by functional expression in yeast. Proc Natl Acad Sci U S A 93:5624–5628. https://doi.org/10.1073/pnas.93.11.5624
Elkin SR, Lakoduk AM, Schmid SL (2016) Endocytic pathways and endosomal trafficking: a primer. Wiener Medizinische Wochenschrift 2016 1667 166:196–204. https://doi.org/10.1007/S10354-016-0432-7
Fisher CR, Wyckoff EE, Peng ED, Payne SM (2016) Identification and characterization of a putative manganese export protein in *Vibrio cholerae*. J Bacteriol 198:2810–2817. https://doi.org/10.1128/JB.00215-16
Fisher M, Gokhman I, Pick U, Zamir A (1996) A structurally novel transferrin-like protein accumulates in the plasma membrane of the unicellular green alga *Dunaliella salina grown* in high salinities. J Biol Chem 272:1565–1570
Fisher M, Zamir A, Pick U (1998) Iron Uptake by the Halotolerant Alga DunaliellaIs Mediated by a Plasma Membrane Transferrin. J Biol Chem 273:17553–17558
Foflonker F, Blaby-Haas CE (2020) Co-locality to co-functionality: Eukaryotic gene neighborhoods as a resource for function discovery. Mol Biol Evol. https://doi.org/10.1093/molbev/msaa221
Gawryluk RMR, Tikhonenkov D V., Hehenberger E, et al (2019) Non-photosynthetic predators are sister to red algae. Nat 2019 5727768 572:240–243. https://doi.org/10.1038/s41586-019-1398-6
Goodstein DM, Shu S, Howson R, et al (2012) Phytozome: A comparative platform for green plant genomics. Nucleic Acids Res 40:. https://doi.org/10.1093/nar/gkr944
Haas CE, Rodionov DA, Kropat J, et al (2009) A subset of the diverse COG0523 family of putative metal chaperones is linked to zinc homeostasis in all kingdoms of life. BMC Genomics 10:1–21. https://doi.org/10.1186/1471-2164-10-470
Hadariová L, Vesteg M, Hampl V, Krajčovič J (2017) Reductive evolution of chloroplasts in non-photosynthetic plants, algae and protists. Curr Genet 2017 642 64:365–387. https://doi.org/10.1007/S00294-017-0761-0
Handali M, Roychowdhury H, Neupane DP, Yukl ET (2015) AztD, a Periplasmic Zinc Metallochaperone to an ATP-binding Cassette (ABC) Transporter System in *Paracoccus denitrificans*. J Biol Chem 290:29984–29992. https://doi.org/10.1074/JBC.M115.684506
Hanikenne M, Krämer U, Demoulin V, Baurain D (2005) A comparative inventory of metal transporters

562 in the green alga *Chlamydomonas reinhardtii* and the red alga *Cyanidioschizon merolae*. Plant
563 Physiol 137:428–446. https://doi.org/10.1104/pp.104.054189
564 Hanschen ER, Starkenburg SR (2020) The state of algal genome quality and diversity. Algal Res
565 50:101968. https://doi.org/10.1016/J.ALGAL.2020.101968
566 Helliwell KE, Wheeler GL, Leptos KC, et al (2011) Insights into the evolution of vitamin B12
567 auxotrophy from sequenced algal genomes. Mol Biol Evol 28:2921–2933
568 Howe G, Merchant S (1992) Heavy metal-activated synthesis of peptides in *Chlamydomonas reinhardtii*.
569 Plant Physiol 98:127–136. https://doi.org/10.1104/pp.98.1.127
570 Hsieh SI, Castruita M, Malasarn D, et al (2013) The proteome of copper, iron, zinc, and manganese
571 micronutrient deficiency in *Chlamydomonas reinhardtii*. Mol Cell Proteomics 12:65–86.
572 https://doi.org/10.1074/mcp.M112.021840
573 Hu J (2020) Toward unzipping the ZIP metal transporters: structure, evolution, and implications on drug
574 discovery against cancer. FEBS J. https://doi.org/10.1111/FEBS.15658
575 Ihemere UE, Narayanan NN, Sayre RT (2012) Iron biofortification and homeostasis in transgenic cassava
576 roots expressing the algal iron assimilatory gene, *FEA1*. Front Plant Sci 3:171.
577 https://doi.org/10.3389/fpls.2012.00171
578 Illing AC, Shawki A, Cunningham CL, Mackenzie B (2012) Substrate profile and metal-ion selectivity of
579 human divalent metal-ion transporter-1. J Biol Chem 287:30485–30496.
580 https://doi.org/10.1074/jbc.M112.364208
581 Intwala A, Patey TD, Polet DM, Twiss MR (2008) Nutritive substitution of zinc by cadmium and cobalt
582 in phytoplankton isolated from the Lower Great Lakes. J Great Lakes Res 34:1–11
583 Johnson MD, Stoecker DK (2005) Role of feeding in growth and photophysiology of *Myrionecta rubra*.
584 Aquat Microb Ecol 39:303–312
585 Kamizono A, Nishizawa M, Teranishi Y, et al (1989) Identification of a gene conferring resistance to zinc
586 and cadmium ions in the yeast *Saccharomyces cerevisiae*. Mol Gen Genet MGG 1989 2191
587 219:161–167. https://doi.org/10.1007/BF00261172
588 Karakashian MW (1975) Symbiosis in *Paramecium bursaria*. Symp Soc Exp Biol 145–173
589 Kazamia E, Sutak R, Paz-Yepes J, et al (2018) Endocytosis-mediated siderophore uptake as a strategy for
590 Fe acquisition in diatoms. Sci Adv 4:eaar4536. https://doi.org/10.1126/SCIADV.AAR4536
591 Kim SA, Punshon T, Lanzirotti A, et al (2006) Localization of iron in Arabidopsis seed requires the
592 vacuolar membrane transporter VIT1. Science (80-) 314:1295–1298.
593 https://doi.org/10.1126/science.1132563
594 Kropat J, Gallaher SD, Urzica EI, et al (2015) Copper economy in Chlamydomonas: Prioritized allocation
595 and eallocation of copper to respiration vs. Photosynthesis. Proc Natl Acad Sci U S A 112:2644–

2651. https://doi.org/10.1073/pnas.1422492112
Lane TW, Saito MA, George GN, et al (2005) A cadmium enzyme from a marine diatom. Nature 435:42
Lee Y-H, Deka RK, Norgard M V., et al (1999) *Treponema pallidum* TroA is a periplasmic zinc-binding protein with a helical backbone. Nat Struct Biol 1999 67 6:628–633. https://doi.org/10.1038/10677
Lelandais G, Scheiber I, Paz-Yepes J, et al (2016) *Ostreococcus tauri* is a new model green alga for studying iron metabolism in eukaryotic phytoplankton. BMC Genomics 17:1–23
Li L, Chen OS, Ward DMV, Kaplan J (2001) CCC1 Is a Transporter That Mediates Vacuolar Iron Storage in Yeast. J Biol Chem 276:29515–29519. https://doi.org/10.1074/jbc.M103944200
Lommer M, Specht M, Roy A-S, et al (2012) Genome and low-iron response of an oceanic diatom adapted to chronic iron limitation. Genome Biol 2012 137 13:1–21. https://doi.org/10.1186/GB-2012-13-7-R66
Malasarn D, Kropat J, Hsieh SI, et al (2013) Zinc deficiency impacts CO2 Assimilation and disrupts copper homeostasis in *Chlamydomonas reinhardtii*. J Biol Chem 288:10672–10683. https://doi.org/10.1074/jbc.M113.455105
McQuaid JB, Kustka AB, Oborník M, et al (2018) Carbonate-sensitive phytotransferrin controls high-affinity iron uptake in diatoms. Nature 555:543–537. https://doi.org/10.1038/nature25982
Merchant SS, Allen MD, Kropat J, et al (2006) Between a rock and a hard place: Trace element nutrition in Chlamydomonas. Biochim Biophys Acta - Mol Cell Res 1763:578–594. https://doi.org/10.1016/j.bbamcr.2006.04.007
Merchant SS, Prochnik SE, Vallon O, et al (2007) The Chlamydomonas genome reveals the evolution of key animal and plant functions. Science (80-) 318:245–250
Merchant SS, Schmollinger S, Strenkert D, et al (2020) From economy to luxury: Copper homeostasis in Chlamydomonas and other algae. Biochim Biophys Acta - Mol Cell Res 1867:118822. https://doi.org/10.1016/j.bbamcr.2020.118822
Mitchell CJ, Shawki A, Ganz T, et al (2014) Functional properties of human ferroportin, a cellular iron exporter reactive also with cobalt and zinc. Am J Physiol - Cell Physiol 306:C450–C459. https://doi.org/10.1152/ajpcell.00348.2013
Mock T, Junge K (2007) Psychrophilic Diatoms. Springer, Dordrecht, pp 343–364
Morrissey J, Sutak R, Paz-Yepes J, et al (2015) A Novel Protein, Ubiquitous in Marine Phytoplankton, Concentrates Iron at the Cell Surface and Facilitates Uptake. Curr Biol 25:364–371. https://doi.org/10.1016/J.CUB.2014.12.004
Narayanan NN, Ihemere U, Chiu W, et al (2011) The iron assimilatory protein, FEA1, from *Chlamydomonas reinhardtii* facilitates iron-specific metal uptake in yeast and plants. Front Plant Sci 2:67. https://doi.org/10.3389/fpls.2011.00067

630 Neupane DP, Fullam SH, Chacón KN, Yukl ET (2019) Crystal structures of AztD provide mechanistic
631 insights into direct zinc transfer between proteins. Commun Biol 2019 21 2:1–12.
632 https://doi.org/10.1038/s42003-019-0542-z
633 Nies DH (1992) CzcR and CzcD, gene products affecting regulation of resistance to cobalt, zinc, and
634 cadmium (czc system) in *Alcaligenes eutrophus*. J Bacteriol 174:8102–8110.
635 https://doi.org/10.1128/JB.174.24.8102-8110.1992
636 Nies DH, Silver S (1995) Ion efflux systems involved in bacterial metal resistances. J Ind Microbiol 1995
637 142 14:186–199. https://doi.org/10.1007/BF01569902
638 North WJ (1971) Growth of individual fronds of the mature giant kelp, Macrocystis. Nov hedwigia
639 Beihefte
640 Page MD, Kropat J, Hamel PP, Merchant SS (2009) Two Chlamydomonas CTR copper transporters with
641 a novel cys-met motif are localized to the plasma membrane and function in copper assimilation.
642 Plant Cell 21:928–943. https://doi.org/10.1105/tpc.108.064907
643 Pan Y, Ren Z, Gao S, et al (2020) Structural basis of ion transport and inhibition in ferroportin. Nat
644 Commun 11:1–11. https://doi.org/10.1038/s41467-020-19458-6
645 Paz Y, Katz A, Pick U (2007a) A Multicopper Ferroxidase Involved in Iron Binding to Transferrins in
646 *Dunaliella salina* Plasma Membranes. J Biol Chem 282:8658–8666.
647 https://doi.org/10.1074/jbc.M609756200
648 Paz Y, Shimoni E, Weiss M, Pick U (2007b) Effects of Iron Deficiency on Iron Binding and
649 Internalization into Acidic Vacuoles in *Dunaliella salina*. Plant Physiol 144:1407–1415.
650 https://doi.org/10.1104/PP.107.100644
651 Pocock T, Lachance M-A, Pröschold T, et al (2004) Identification of a psychrophilic green alga from
652 Lake Bonney Antarctica: *Chlamydomonas raudensis* Ettl. (UWO 241) *Chlorophyceae*. J Phycol
653 40:1138–1148
654 Polle JEW, Calhoun S, McKie-Krisberg Z, et al (2020) Genomic adaptations of the green alga *Dunaliella*
655 *salina* to life under high salinity. Algal Res 50:101990.
656 https://doi.org/10.1016/J.ALGAL.2020.101990
657 Potelle S, Morelle W, Dulary E, et al (2016) Glycosylation abnormalities in Gdt1p/TMEM165 deficient
658 cells result from a defect in Golgi manganese homeostasis. Hum Mol Genet 25:1489–1500.
659 https://doi.org/10.1093/hmg/ddw026
660 Pufahl RA, Singer CP, Peariso KL, et al (1997) Metal ion chaperone function of the soluble Cu(I)
661 receptor Atx1. Science (80-) 278:853–856. https://doi.org/10.1126/science.278.5339.853
662 Purohit R, Ross MO, Batelu S, et al (2018) Cu+-specific CopB transporter: Revising P1B-type ATPase
663 classification. Proc Natl Acad Sci 115:2108–2113. https://doi.org/10.1073/PNAS.1721783115

Ranjan A, Townsley BT, Ichihashi Y, et al (2015) An intracellular transcriptomic atlas of the giant coenocyte *Caulerpa taxifolia*. PLoS Genet 11:e1004900

Rastogi A, Maheswari U, Dorrell RG, et al (2018) Integrative analysis of large scale transcriptome data draws a comprehensive landscape of *Phaeodactylum tricornutum* genome and evolutionary origin of diatoms. Sci Reports 2018 81 8:1–14. https://doi.org/10.1038/s41598-018-23106-x

Rubinelli P, Siripornadulsil S, Gao-Rubinelli F, Sayre RT (2002) Cadmium- and iron-stress-inducible gene expression in the green alga *Chlamydomonas reinhardtii*: Evidence for H43 protein function in iron assimilation. Planta 215:1–13. https://doi.org/10.1007/s00425-001-0711-3

Sadka A, Himmelhoch S, Zamir A (1991) A 150 kilodalton cell surface protein is induced by salt in the halotolerant green alga *Dunaliella salina* . Plant Physiol 95:822–831

Saito MA, Goepfert TJ (2008) Zinc-cobalt colimitation of *Phaeocystis antarctica*. Limnol Oceanogr 53:266–275

Sasaki T, Kurano N, Miyachi S (1998) Cloning and characterization of high-CO_2-specific cDNAs from a marine microalga, *Chlorococcum littorale*, and effect of CO_2 concentration and iron deficiency on the gene expression. Plant Cell Physiol 39:131–138. https://doi.org/10.1093/oxfordjournals.pcp.a029349

Schaaf G, Honsbein A, Meda AR, et al (2006) AtIREG2 encodes a tonoplast transport protein involved in iron-dependent nickel detoxification in *Arabidopsis thaliana* roots. J Biol Chem 281:25532–25540. https://doi.org/10.1074/jbc.M601062200

Scheiber IF, Pilátová J, Malych R, et al (2019) Copper and iron metabolism in *Ostreococcus tauri* – the role of phytotransferrin, plastocyanin and a chloroplast copper-transporting ATPase. Metallomics 11:1657–1666. https://doi.org/10.1039/C9MT00078J

Schneider A, Steinberger I, Herdean A, et al (2016) The evolutionarily conserved protein PHOTOSYNTHESIS AFFECTED MUTANT71 is required for efficient manganese uptake at the thylakoid membrane in Arabidopsis. Plant Cell 28:892–910. https://doi.org/10.1105/tpc.15.00812

Schwarz M, Sal-Man N, Zamir A, Pick U (2003) A transferrin-like protein that does not bind iron is induced by iron deficiency in the alga *Dunaliella salina*. Biochim Biophys Acta - Proteins Proteomics 1649:190–200. https://doi.org/10.1016/S1570-9639(03)00185-7

Shelford VE (1913) Animal Communities in Temperate America: As Illustrated in the Chicago Region: a Study in Animal Ecology, 2nd edn. University Press, Chicago, IL

Shire DM, Kustka AB (2021) Proteomic responses of the coccolithophore *Emiliania huxleyi* to zinc limitation and trace metal substitution. Environ Microbiol. https://doi.org/10.1111/1462-2920.15644

Stearman R, Yuan DS, Yamaguchi-Iwai Y, et al (1996) A Permease-Oxidase Complex Involved in High-Affinity Iron Uptake in Yeast. Science (80-) 271:1552–1557.

https://doi.org/10.1126/SCIENCE.271.5255.1552

Steffensen DA (1976) Morphological variation of Ulva in the Avon-Heathcote Estuary, Christchurch. New Zeal J Mar Freshw Res 10:329–341

Stiller JW, Schreiber J, Yue J, et al (2014) The evolution of photosynthesis in chromist algae through serial endosymbioses. Nat Commun 2014 51 5:1–7. https://doi.org/10.1038/ncomms6764

Sunda WG, Huntsman SA (1995) Cobalt and zinc interreplacement in marine phytoplankton: Biological and geochemical implications. Limnol Oceanogr 40:1404–1417

Sutak R, Botebol H, Blaiseau P-L, et al (2012) A Comparative Study of Iron Uptake Mechanisms in Marine Microalgae: Iron Binding at the Cell Surface Is a Critical Step. Plant Physiol 160:2271–2284. https://doi.org/10.1104/PP.112.204156

Tejada-Jiménez M, Galván A, Fernández E (2011) Algae and humans share a molybdate transporter. Proc Natl Acad Sci U S A 108:6420–6425. https://doi.org/10.1073/pnas.1100700108

Tejada-Jiménez M, Llamas Á, Sanz-Luque E, et al (2007) A high-affinity molybdate transporter in eukaryotes. Proc Natl Acad Sci U S A 104:20126–20130. https://doi.org/10.1073/pnas.0704646104

Turnšek J, Brunson JK, Martinez Viedma MDP, et al (2021) Proximity proteomics in a marine diatom reveals a putative cell surface-to-chloroplast iron trafficking pathway. Elife 10:1–113. https://doi.org/10.7554/ELIFE.52770

VC C, LW K, J S, et al (1997) The copper chaperone for superoxide dismutase. J Biol Chem 272:23469–23472. https://doi.org/10.1074/JBC.272.38.23469

Vidal SM, Malo D, Vogan K, et al (1993) Natural resistance to infection with intracellular parasites: Isolation of a candidate for Bcg. Cell 73:469–486. https://doi.org/10.1016/0092-8674(93)90135-D

Wang C, Xu W, Jin H, et al (2016) A Putative Chloroplast-Localized Ca^{2+}/H^{+} Antiporter CCHA1 Is Involved in Calcium and pH Homeostasis and Required for PSII Function in Arabidopsis. Mol Plant 9:1183–1196. https://doi.org/10.1016/j.molp.2016.05.015

Wetherbee R, Jackson CJ, Repetti SI, et al (2019) The golden paradox – a new heterokont lineage with chloroplasts surrounded by two membranes. J Phycol 55:257–278. https://doi.org/10.1111/JPY.12822

Zhao H, Eide D (1996a) The yeast ZRT1 gene encodes the zinc transporter protein of a high-affinity uptake system induced by zinc limitation. Proc Natl Acad Sci U S A 93:2454–2458. https://doi.org/10.1073/pnas.93.6.2454

Zhao H, Eide D (1996b) The ZRT2 gene encodes the low affinity zinc transporter in *Saccharomyces cerevisiae*. J Biol Chem 271:23203–23210. https://doi.org/10.1074/jbc.271.38.23203

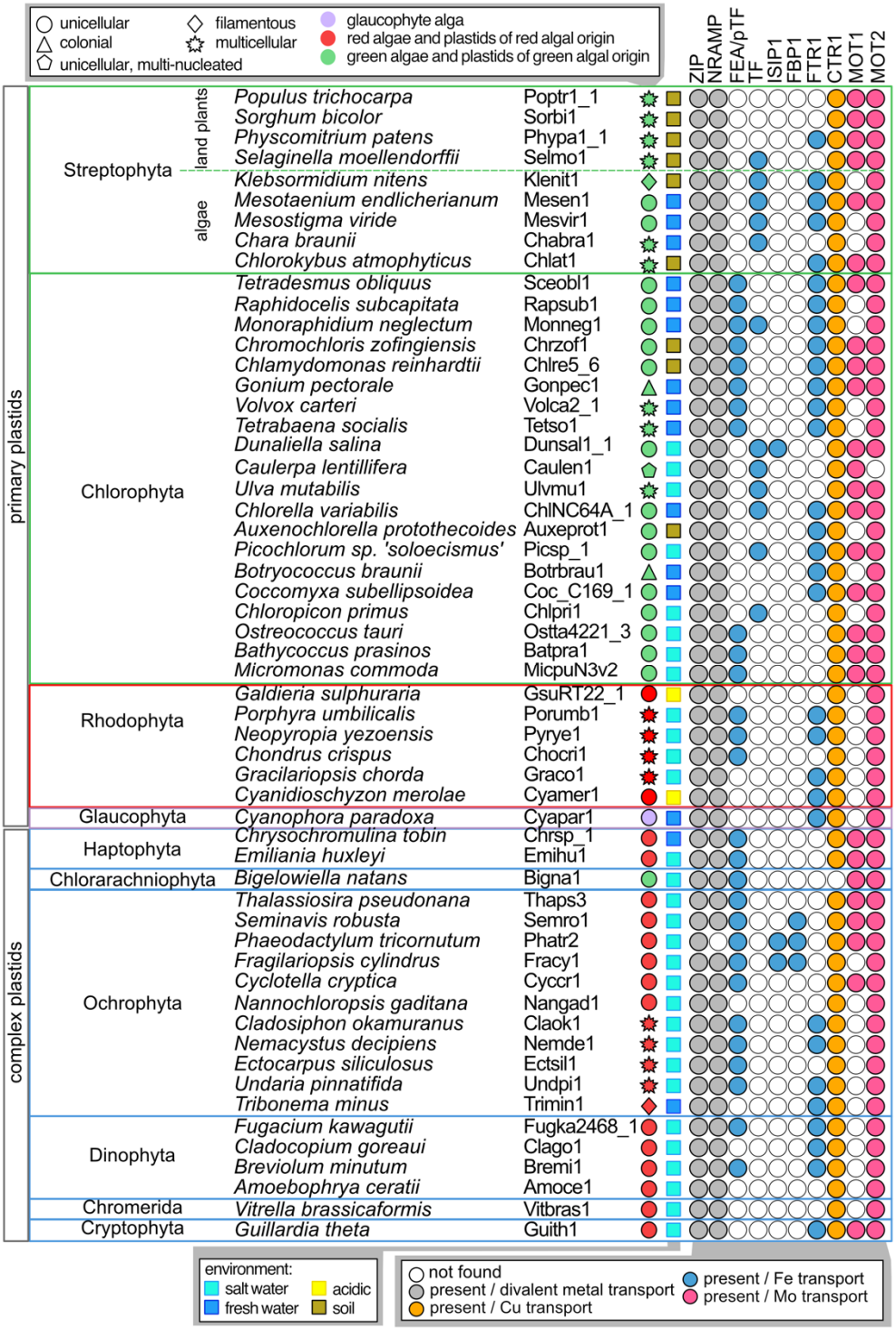

Figure 1 Presence of genes predicted to encode homologs of known metal transport proteins. Proteins were identified using either presence of a corresponding PFam domain or sequence similarity. The taxonomic class, genome identifiers from the Phycocosm database, physiological characteristics and environment are indicated.

734

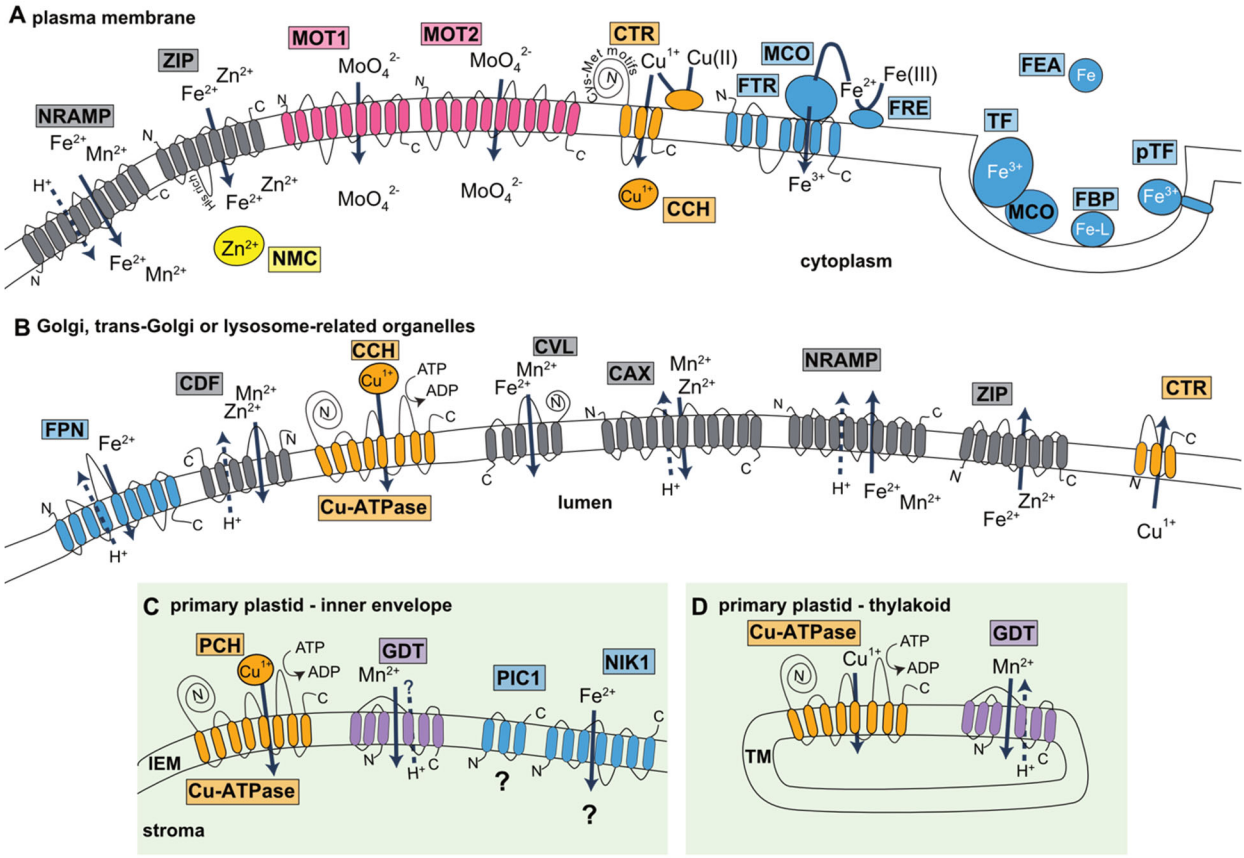

Figure 2 Metal transport proteins found in algae. A, transport proteins often found to localize to the plasma membrane. B, transporters often found to localize to intracellular membranes of the endomembrane system. C and D, transporters identified in the land plant *Arabidopsis* that are conserved in the green algae. Abbreviations not found in the text: CCH, Cu chaperone; PCH, plastid Cu chaperone; FRE, ferric reductase; CVL, Ccc1/VIT1-like; PIC1, permease in chloroplasts 1; NIK1, nickel transporter family; CAX, cation/proton exchanger.