Sparing and Salvaging Metals in Chloroplasts

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1 INTRODUCTION

Of the essential inorganic minerals, the micronutrients (manganese, iron, copper, and zinc) have held special interest in plant physiology because of their chemistry, duality as both nutrient and toxin, availability, and biogeochemical history. The green tissues of plants readily exhibit visual symptoms of metal deficiency, and even before the role of metals in photosynthesis was understood, the importance of metals in plant nutrition was already evident. Chlorosis is a common symptom of poor metal nutrition, which manifests as a yellowing of green tissues owing to decreased abundance of chlorophyll. Because of this readily scored phenotype, the earliest work on trace metals focused on descriptive observations of metal nutrition.

It was not until the early to mid-1800s with the popularization of the mineral theory of plant nutrition, that a serious look at the requirement of metal salts for plant growth and health began (Figure 1). In the 1840s, Eusébe Gris reported that the application of iron salts to either the roots or directly to the leaves of some chlorotic plants resulted in reversing the symptom. As a result, iron deficiency became synonymous with chlorosis. Julius von Sachs is accredited with establishing the essentiality of iron in plant growth, and 40-years later Benjamin Moore reported that iron is indeed in the chloroplast and proposed that it plays a direct role in photosynthesis. In the next 30 years, the essentiality of manganese, copper, and zinc in plant growth and more specifically in photosynthesis was reported. While the necessity of metal salts in plant growth and in photosynthesis was apparent, the actual biological role of these ions remained elusive until the second half of the twentieth century, with the discovery of metal ions as cofactors for chloroplast-localized proteins.

We now know that linear electron flow requires the direct involvement of manganese, iron, and copper (Figure 2). A Mn_4CaO_5 cluster on the donor side of each photosystem II (PSII) monomer unit in the dimer catalyzes the extraction of electrons from water, initiating the sequence of electron flow. Within PSII, these electrons move to Q_A (a one-electron acceptor plastoquinone) and then to Q_B (a two-electron acceptor plastoquinone). A bicarbonate ion ligated to a non-heme iron is thought to facilitate this transfer. The cytochrome b_6f dimer contains both heme and iron–sulfur clusters. Electron transfer between cytochrome b_6f and the acceptor side of photosystem I (PSI) can involve either a small copper protein, plastocyanin (land plants, algae and cyanobacteria), or an equivalent heme protein (algae and cyanobacteria). At the terminal end of linear electron flow, PSI contains three 4Fe–4S clusters involved in electron transfer within the complex, and these ultimately reduce the 2Fe–2S protein ferredoxin, which provides the reducing power for several metabolic reactions including $NADP^+$ reduction.

In addition to the thylakoid membrane, many metal-dependent proteins localize to other chloroplast compartments (Table 1). They do not necessarily participate in photosynthetic electron transfer directly, but they serve as support staff

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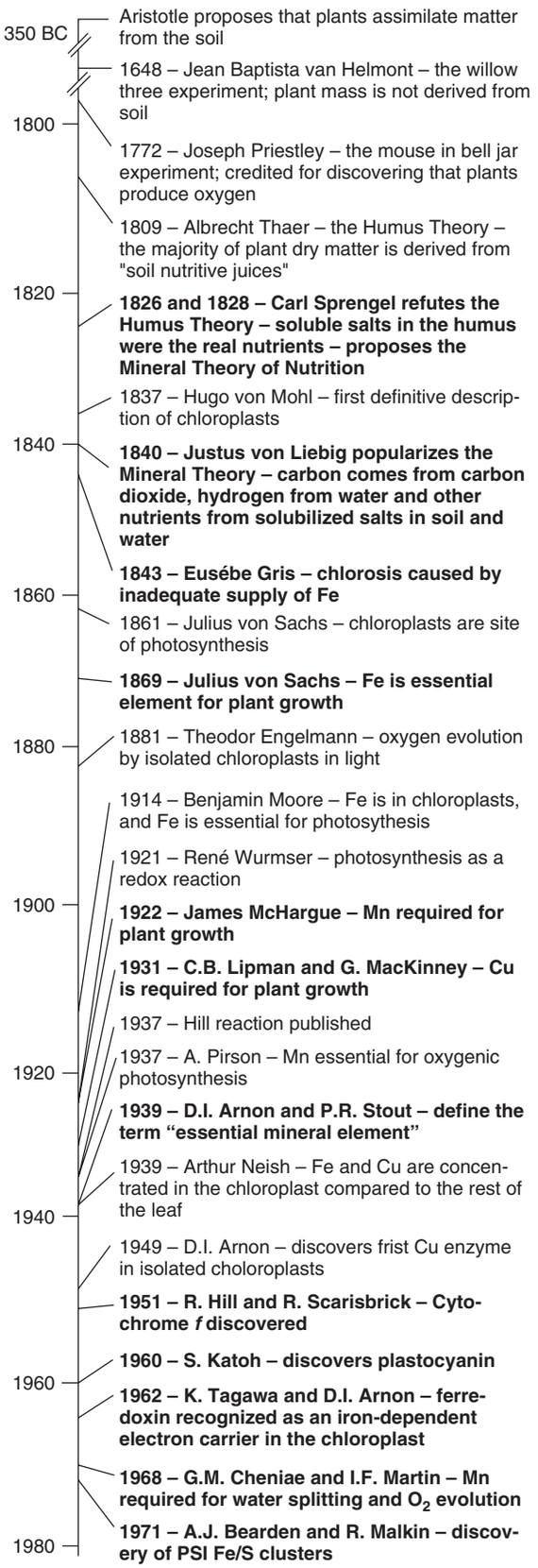

to ensure that the chloroplast is as productive as possible in situations of environmental light fluctuation or when detrimental by-products of the light reactions, such as reactive oxygen species, accumulate. The chloroplast is also the site of many metabolic pathways that are dependent on a transition metal at one or more steps. These include fatty acid biosynthesis, amino acid biosynthesis, nitrate and sulfate assimilation, and secondary metabolite synthesis. Metal ions also play central roles as cofactors in protein structure (such as zinc in ribosomes) and regulation.

2 METAL SPARING AND SALVAGING WITHIN THE CHLOROPLAST

Plants regulate metal assimilation and distribution to ensure a balance between supply and demand, but when demand exhausts the external provisions, mechanisms for conserving, redistributing, and prioritizing the metal cofactor are activated. These mechanisms include metal sparing, which is a regulated reduction in the abundance of metal-dependent proteins, and metal salvaging, which involves degradation of metal-bound proteins and recycling of the limiting cofactor. Metal-sparing mechanisms result in a decreased number of metal-dependent proteins through repression of gene expression or degradation of transcripts and apoprotein, while metal salvaging results in liberation and redistribution of the precious metal. Both metal sparing and salvaging ultimately lead to allocation of the limiting nutrient away from unnecessary proteins and toward indispensable proteins. An attempt is made to preserve core metal-dependent functions during the shortage, and when metal is resupplied to deficient cells, the cellular landscape is poised to prioritize metal cofactor delivery to those key proteins.

Well-characterized examples of metal-sparing and metal-salvaging mechanisms at the sub-cellular level exist because of the availability of single-cell reference organisms such as cyanobacteria and algae. Here, we will focus on sub-cellular acclimation to metal deficiency specifically in context of the chloroplast, although these strategies may operate to economize and re-distribute metal at every level in the plant.

Figure 1 Historically significant events in understanding the role of trace metals in plant nutrition. The origin of scholarly discourse on plant nutrition is commonly traced to Aristotle and his students who formalized the role of soil in plant survival. Aristotle's notion that plants absorb their matter from soil was refuted 2000 years later by Jean Baptista van Helmont's five-year willow tree experiments, although he erroneously concluded that plant matter was absorbed from water. In the next 300 years, our current understanding of plant physiology was shaped with the discovery of photosynthesis and the role of inorganic minerals as nutrients and then, more specifically, as protein cofactors. Seminal discoveries in plant metal nutrition are in bold

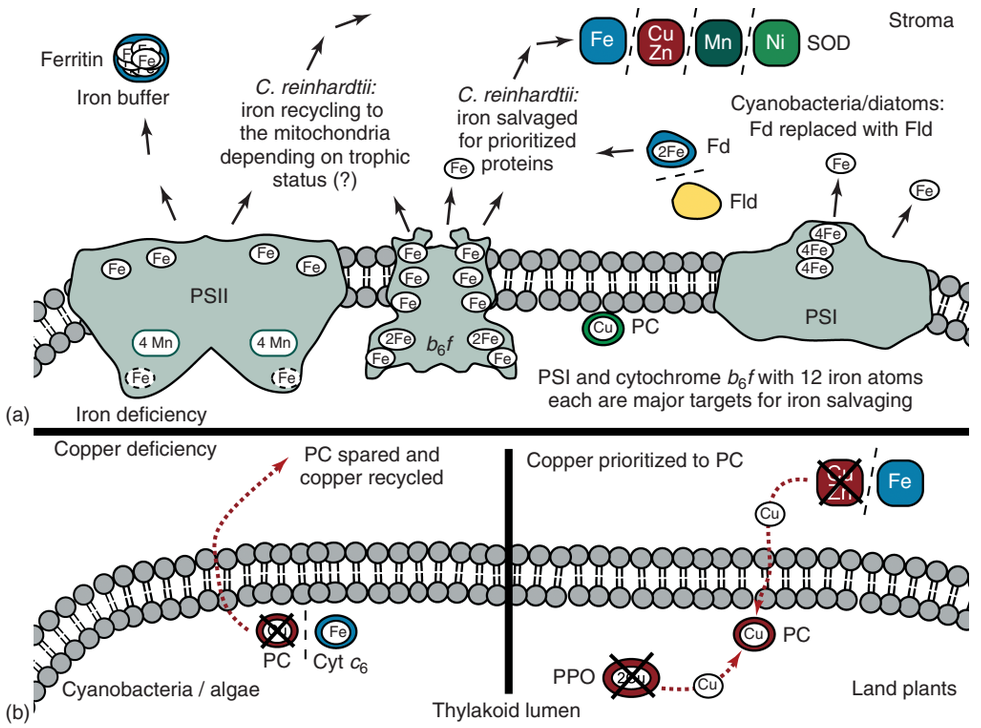

Figure 2 Iron and copper sparing and salvaging within the chloroplast. Assuming 1:1:1:1:1 stoichiometry of functional complexes (dimers for PSII and Cytb_6f and either monomer or trimer for PSI), linear electron flow from PSII to ferredoxin is estimated to require 56 iron ions (using plastocyanin; 57 with cytochrome c_6) in cyanobacteria and 30 iron ions (using plastocyanin; 31 using cytochrome c_6) in land plants and algae. Iron found in Cytc_{550}, which is only present in the PSII complex of cyanobacteria and some red and brown algae, is surrounded by a dashed circle. (a) The iron-salvaging pathways as described for *C. reinhardtii*, cyanobacteria, and diatoms. In *C. reinhardtii*, iron is salvaged from the degradation of the ETC complexes and buffered by ferritin. A hierarchy of iron reallocation is established and involves maintenance of FeSOD in the chloroplast and respiration in the mitochondria during photoheterotrophic growth. Replacement of SOD isoforms (either transiently or permanently) and a switch between ferredoxin and flavodoxin are also popular metal-sparing strategies in photosynthetic microbes, particularly phytoplankton. (b) The two major copper-sparing and -salvaging pathways are shown. During copper deficiency in land plants, plastocyanin maturation is prioritized through downregulation of dispensable copper-bound proteins (dotted red line). In some cyanobacteria and algae, plastocyanin is dispensable and copper may be recycled to other compartments (dotted red line)

2.1 The Membranes of the Chloroplast—Barriers to Metal Mobilization

As pointed out, the importance of metals as essential plant nutrients was reported in the literature over a century and a half ago. However, we are just now beginning to understand how the plant ensures proper acquisition and distribution of these nutrients to the correct proteins in the correct compartments.

To maintain metal homeostasis, photosynthetic eukaryotes have a unique challenge to overcome. The chloroplast is composed of three membranes: the outer- and inner-envelope membranes and the thylakoid membrane, and these membranes delineate three compartments: the intermembrane (interenvelope) space, the stroma, and the thylakoid lumen. The chloroplast machinery translocates most (if not all) metal-dependent proteins unfolded and cofactor free. Within the chloroplast, the imported polypeptide is processed to its mature form and sorted to its final destination, which may be the inter-membrane space, envelope membrane, stroma, thylakoid membrane, or lumen. For metalloproteins, insertion of the cofactor is usually one of the very last steps in protein maturation [except for lumen proteins that are translocated by the twin-arginine translocase (TAT) pathway described below] and occurs once the protein has reached its final destination. This has been experimentally demonstrated for cytochrome c_6 heme attachment, Mn_4CaO_5 cluster assembly in PSII, and copper insertion in plastocyanin.[1]

Therefore, the cofactor must be present in the final compartment. The outer envelope of archaeplastidic chloroplasts is analogous to the outer membrane of bacteria, and metal-chelates may freely diffuse across this porous membrane into the intermembrane space. The inner membrane is analogous to the plasma membrane of bacteria, but how metal crosses this membrane is largely unknown. A P_{1B}-type ATPase PAA1 is proposed to pump copper into the stroma, as is a second ATPase HMA1, which is also implicated in

Table 1 Metal-dependent pathways in the chloroplast

	Gene (protein)	Cofactor	[Transcript] metal replete	[Transcript] metal deplete	[Protein] metal replete	[Protein] metal deplete
The light reactions						
PSII	*psbE* (Cytb_{559})	heme				
Lp, Ga dimer: 4Fe, 8Mn	*psbF*					
Cb, Ra, Dt dimer: 6Fe, 8Mn	*psbA* (D1) *psbD* (D2)	Fe–S				↓ (–Fe)
	psbA (D1) *psbC* (CP43)	Mn_4CaO_5				
Cyanobacterial/diatom-specific	*psbV* (Cytc_{550})	Heme				
Cytb_6f	*petB* (Cytb_6)	Heme (×3)				
Dimer: 12Fe	*petA* (Cyt*f*)	Heme				↓ (–Fe)
	PETC (Rieske iron–sulfur protein)	[2Fe–2S]				
Plastocyanin (1Cu) Lp, Ga, Dt, Cb	*PETE1* (Ath)	Cu	182	129 (–Cu)		
	PETE2 (Ath)	Cu	1681	1529 (–Cu)		
	PCY1 (Cre)	Cu	7100	6200 (–Cu)	1350	nd (–Cu)
Cyt c6 (1Fe) Ga, Ra, Dt, Cb	*CYC6* (Cre)	Heme	0.8	2677 (–Cu)	nd	285 (–Cu)
PSI	*psaC*	[4Fe–4S] (×2)				
Lp, Ga monomer: 12Fe	*psaA*	[4Fe–4S]				↓ (–Fe)
Cbtrimer: 36Fe	*psaB*					
Fd (2Fe)	Characterized and chloroplast-localized in *A. thaliana*					
	FD1 (Ath)	[2Fe–2S]	81			
	FD2 (Ath)	[2Fe–2S]	1230			
	FDC1 (Ath)	[2Fe–2S]	59			
	FDC2 (Ath)	[2Fe–2S]	35			
	Characterized and chloroplast-localized in *C. reinhardtii*					
	PETF (Cre)	[2Fe–2S]	8278	5065 (–Fe)	87 ± 35	nd (–Fe)
	FDX2 (Cre)	[2Fe–2S]	8	3.1 (–Fe)		
	FDX3 (Cre)	[2Fe–2S]	21	144 (–Fe)	nd	45 ± 7 (–Fe)
	FDX5 (Cre)	[2Fe–2S]	17	28 (–Fe)		
	FDX6 (Cre)	[2Fe–2S]	41	362 (–Fe)		↓ (–Fe)
Carbon metabolism						
Associates with glyceraldehyde-3-phosphate dehydrogenase and phosphoribulokinase	*CP12-1* (Ath)	Cu	523	483 (–Cu)		
	CP12-2 (Ath)	Cu	180	181 (–Cu)		
	CP12-3 (Ath)	Cu	6	6 (–Cu)		
Carbonic anhydrase	*CAH1* (Cre)	Zn	12			
	CAH8 (Cre)	Zn	101			
Alcohol dehydrogenase	*ADH1* (Cre)	Fe	205			
Alternative electron transfer						
Type I NAD(P)H dehydrogenase	*NDF4* (Ath)	[2Fe–2S]	34			
Missing from Cre	*ndhI*	[4Fe–4S] (x2)				
	ndhK	[4Fe–4S]				
Plastid terminal oxidase (PTOX)	*IM1* (Ath)	di-iron	48			
	PTO1 (Cre)	di-iron	87	42 (–Fe)		
	PTO2 (Cre)	di-iron	27	11 (–Fe)		
Succinate dehydrogenase	*SDH2* (Cre)	[2Fe–2S] [4Fe–4S] [3Fe–4S]	324	190 (–Fe)		
	SDH3 (Cre)	Heme	324	147 (–Fe)		
	SDH4 (Cre)		314	150 (–Fe)		
Carotenoid biosynthesis						
Carotenoid hydroxylase	*CYP97A3* (Cre)	Heme	14	43 (–Fe)		
	CYP97B3 (Cre)	Heme	24	21 (–Fe)		
	CYP97C1 (Cre)	Heme	19	63 (–Fe)		
4-Hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	*HDS* (Cre)	[4Fe–4S]	49	110 (–Fe)	125 ± 37	23 ± 7 (–Fe)
4-Hydroxy-3-methylbut-2-enyl diphosphate reductase	*HDR* (Cre)	[4Fe–4S]	95	102 (–Fe)	61 ± 7	nd (–Fe)
Carotenoid metabolism						
β-Carotene isomerase	*D27* (Cre)	Fe	27	18 (–Fe)		

Table 1 (*Continued.*)

	Gene (protein)	Cofactor	[Transcript] metal replete	[Transcript] metal deplete	[Protein] metal replete	[Protein] metal replete
Carotenoid cleavage dioxygenases						
Strigolactone biosynthesis	*CCD7* (Cre)	Fe	48	43 (–Fe)		
	CCD8 (Cre)	Fe	16	19 (–Fe)		
Abscisic acid biosynthesis	*NCED2* (Ath)	Fe	0.3			
	NCED3 (Ath)	Fe	6			
	NCED6 (Ath)	Fe				
	NCED5 (Ath)	Fe	1.5			
	NCED9 (Ath)	Fe	0.1			
Tetrapyrrole biosynthesis						
Aerobic Mg-Proto IX monomethyl ester cyclase	*CTH1* (Cre)	di-iron	394	81 (–Cu)		
	CRD1 (Cre)	di-iron	147	1857 (–Cu)		
Chlorophyllide *a* oxygenase	*CAO* (Cre)	[2Fe–2S] Fe	88	222 (–Cu)		
Tetrapyrrole metabolism						
Pheophorbide *a* oxygenase	*PAO* (Ath)	[2Fe–2S] Fe	134			
	PAO1 (Cre)	[2Fe–2S] Fe	8	3 (–Fe)		
	PAO2 (Cre)	[2Fe–2S] Fe	1	4 (–Fe)		
	PAO3 (Cre)	[2Fe–2S] Fe	4	5 (–Fe)		
Regulation						
Ferredoxin-thioredoxin reductase	*FTR* (Cre)	[4Fe–4S]	109	75 (–Fe)	20 ± 4	8 ± 3 (–Fe)
Nitrogen assimilation						
Fd-glutamate synthase	*GLU1* (Cre)	[3Fe–4S]	39	84 (–Fe)	108 ± 8	43 ± 7 (–Fe)
NADH-glutamate synthase	*GLT1* (Cre)	[3Fe–4S]	81	28 (–Fe)	46 ± 10	13 ± 2 (–Fe)
Ferredoxin-dependent nitrite reductase	*NIR1* (Ath)	[4Fe–4S] heme	200			
Sulfate assimilation						
Ferredoxin-sulfite reductase	*SIR* (Cre)	[4Fe–4S] heme	121	20 (–Fe)		
Detoxification						
Ascorbate peroxidase	*SAPX* (Ath)	Heme	76			
	TAPX (Ath)	Heme	86			
	APX1 (Cre)	Heme	143	74 (–Fe)		
Superoxide dismutase	*CSD2* (Ath)	Cu/Zn	571	12 (–Cu)		
	FSD1 (Ath)	Fe	14	1566 (–Cu)		
	FSD3 (Ath)	Fe	41	46 (–Cu)		
	FSD1 (Cre)	Fe	224	241 (–Fe)	340 ± 42	320 ± 87 (–Fe)
	MSD3 (Cre)	Mn	1	546 (–Fe)	nd	73 ± 21 (–Fe)
Alkenal/one oxidoreductase	*AOR* (Ath)	Zn	154			
Truncated hemoglobins	*THB1* (Cre)	Heme	2	0.8 (–Fe)		
Defense						
Polyphenol oxidase	*PPO* (Poplar)	Cu (x2)				
Fatty acid metabolism						
Lipoxygenase	*LOX 2*(Ath)	Non-heme iron	1460			
	LOX3 (Ath)	Non-heme iron	45			
	LOX4 (Ath)	Non-heme iron	23			
Stearoyl-ACP desaturase	*FAB2* (Cre)	di-iron	183	465 (–Fe)	47 ± 19	23 ± 9 (–Fe)
Allene oxide synthase	*AOS* (Ath)	Heme	321			
Carboxytransferase beta subunit of the acetyl-CoA carboxylase	*accD*	Zn				
Protein import						
Translocon	*TIC55* (Ath)	[2Fe–2S] Fe	85			
Amino acid biosynthesis						
Dihydroxy acid dehydratase	*DHAD* (Cre)	[2Fe–2S]	132	52 (–Fe)		
Isopropylmalate dehydratase, large subunit	*LEUC1* (Cre)	[4Fe–4S]	270	301 (–Fe)	159 ± 18	54 ± 1 (–Fe)
Glycine betaine biosynthesis						
Choline monooxygenase	*CMO*(spinach)	[2Fe–2S]				

(*continued overleaf*)

Table 1 (*Continued.*)

	Gene (protein)	Cofactor	[Transcript] metal replete	[Transcript] metal deplete	[Protein] metal replete	[Protein] metal replete
Proteases						
FstH	*FTSH1* (Ath)	Zn	136			
	FTSH2 (Ath)	Zn	692			
	FTSH5 (Ath)	Zn	345			
	FTSH7 (Ath)	Zn	16			
	FTSH8 (Ath)	Zn	130			
	FTSH9 (Ath)	Zn	62			
Peptidase M50 family protein	*ARASP* (Ath)	Zn	19			
	AT1G05140 (Ath)	Zn	47			
Stromal processing peptidase	*SPP* (Ath)	Zn	152			
Algal anaerobiosis						
Hydrogenase	*HYDA1* (Cre)	[4Fe–4S] 2Fe	157	171 (–Fe)		
	HYDA2 (Cre)	[4Fe–4S] 2Fe	33	22 (–Fe)		
Zinc-finger proteins						
Involved in chloroplast and palisade cell development	*VAR3* (Ath)	Zn	22			
Ribosomal subunits	*rpl33* (Ath)	Zn				
	S26 (Ath)	Zn	65			
Cyclic electron flow	*PGRL1A* (Ath)	Zn	321			
	PGRL1B (Ath)	Zn	62			
Chloroplast splicing factor	*APO1* (Ath)	Zn	34			

The chloroplast is a major site for the localization of metal-dependent proteins and enzymes. In addition to the role of manganese, iron, and copper in photosynthetic electron transfer, the chloroplast houses several metal-dependent metabolic pathways. This table is not all-inclusive, but an attempt to document some of the well-studied pathways is made. Chloroplast localization was curated from either the literature or the Plant Proteome DataBase (PPDB) (http://ppdb.tc.cornell.edu/). To estimate abundance, RNA-Seq data from Bernal *et al*.[21] for *A. thaliana* (Ath), or from Castruita *et al*.,[8] and Urzica *et al*.[9] for *C. reinhardtii* (Cre) were used. Because of how these datasets are generated, transcript abundance for genes encoded in the chloroplast genome is not available. As the protein and transcript abundance of metal-dependent proteins does not necessarily correlate, especially when metal sparing or salvaging is active, protein abundance is also listed if available.[8,9] If proteome data are not available, immunoblot evidence was used (denoted by arrow indicating direction of abundance compared to metal-replete condition). Abbreviations: Lp, land plants; Ga, green algae; Cb, cyanobacteria; Ra, red algae; Dt, diatoms.

zinc export and calcium transport. PIC1/TIC21 may transport iron into the stroma, but this protein is also thought to be a component of the protein translocation machinery. Metal-dependent proteins also reside in the lumen. Heme is translocated into the thylakoid via the cytochrome *c* synthesis (CCS) system, which couples heme transport to cytochrome maturation, and a third P_{1B}-type ATPase PAA2 pumps copper for plastocyanin maturation.

Cofactor loading in a compartment other than the one in which the protein functions has also been demonstrated.[2] One of the three translocation machineries for protein transport into the thylakoid, the TAT pathway, accepts folded proteins. In cyanobacteria, this route is proposed to transport metalloproteins that require cofactor loading in the cytoplasm before they reach the periplasmic space. Metalloproteins (such as the Rieske iron–sulfur protein and the copper-dependent polyphenol oxidase) are also substrates for the TAT pathway in chloroplasts, suggesting cofactor loading in the stroma before transport to the lumen.

While transport into the chloroplast is at least known to occur, the bidirectional flux of metals within the chloroplast or between non-vacuole organelles in the plant cell is unexplored. When metal-dependent proteins fall victim to metal-salvaging processes, proteins on the waiting list may be in a separate compartment. Therefore, the spatial demands on the intracellular metal supply change during metal sparing and salvaging, and this requires intracellular movement of metals. In the green alga, *Chlamydomonas reinhardtii*, prioritization of chloroplast-independent energy metabolism during metal deficiency even suggests redistribution of the salvaged metal between organelles.[3,4]

As we will see, recent advances in transcriptomics and proteomics are providing a novel insight into the scope and ubiquity of metal-sparing and -salvaging mechanisms. The next step will be to discover the extent to which metals are redistributed throughout the chloroplast, and the cell and the molecular details governing metal redistribution.

3 BACK-UPS

Metal sparing and salvaging may be accompanied by replacement of the targeted metalloprotein with a back-up that

uses a different metal cofactor (one that is available) or no cofactor at all. Specific examples will be given in the following sections. By expressing a back-up, the cell can reduce metal expenditure without sacrificing its function.

The existence of metalloprotein back-ups underscores the redundancy of metal-catalyzed chemistry—two metals can catalyze the same reaction. However, except for documented cases of cambialism, metalloproteins are generally specific for one metal. This means that a separate protein must be produced to catalyze the reaction with the alternative non-limiting cofactor. Even when a protein is genuinely cambialistic, the various metal ions may not provide the same level of activity. There are very few examples—perhaps only two—in the literature of cambialism as a proposed mechanism to overcome metal-limitation (vide infra).

Carbonic anhydrases are responsible for the reversible hydration of CO_2 to bicarbonate and a proton. Classically, these enzymes use a zinc-bound hydroxide to catalyze this reaction, but during growth under low zinc with cadmium supplementation, the carbonic anhydrase CDCA1 from the marine diatom *Thalassiosira weissflogii* contains cadmium instead of zinc and is still active. This unique class of carbonic anhydrase is a structural mimic of the unrelated β-carbonic anhydrase dimer and can readily exchange cadmium for zinc to yield an even more active enzyme.[5] The cambialistic nature of this enzyme, which is important in carbon sequestering, is thought to represent a zinc-sparing mechanism and provide a selective advantage for diatoms growing in zinc-poor ocean waters.

Superoxide dismutase (SOD) from several bacterial species is a second example of cambialistic metal sparing. Divergent evolution has led to the iron- and manganese-dependent SODs, while convergent evolution has led to the unrelated classes of CuZnSODs and NiSODs. As we will see, these classes are commonly used as back-ups for each other in metal-sparing strategies, but only a unique subclass of the iron- and manganese-dependent SODs is cambialistic. The cambialistic SODs are found mainly in prokaryotes and may resemble an ancestor that existed before the evolution of the two distinct iron and manganese types.[6] While the distinct SODs are selective for one metal or the other, the cambialistic SODs are active with either cofactor. The concentration of manganese and iron in the culture medium determines which cofactor the cambialistic SOD will use.

Back-up proteins generally arise by convergent evolution and do not share sequence similarity. A notable exception is the manganese- and iron-dependent SOD family mentioned earlier. In most cases, the existence of unrelated functionally equivalent protein families with different metal cofactors is known for decades before their role as a back-up enzyme during metal-deficiency acclimation is known. These strategies are invariably uncovered through gene expression analysis, as the back-up is expressed only under metal deficiency. Because they may not have recognizable domains, several back-ups could remain unnoticed in the list of metal-deficiency-induced genes of unknown function.

An example is the *folE2* gene commonly found in bacterial zinc regulons.[7] GTP cyclohydrolase I (encoded by *folE* in *Escherichia coli*) catalyzes the conversion of GTP to 7,8-dihydroneopterin triphosphate, a substrate for several biosynthetic pathways including de novo folate synthesis in bacteria and plants. Absence of the corresponding gene in several bacterial genomes led to the discovery of a non-homologous gene encoding a functionally equivalent enzyme named *folE2*. Until the discovery of this function, *folE2*, referred to at that time as *yciA*, was a gene of unknown function regulated by zinc nutrition. As these genomes contain the gene encoding the canonical enzyme, which is zinc-dependent, the designation of YciA/FolE2 as a zinc-independent back-up for FolE has been proposed.

4 REFERENCE ORGANISMS FOR SUB-CELLULAR METAL SPARING AND SALVAGING

Although cyanobacteria lack the complexity of photosynthetic eukaryotes, they are free-living relatives of the original chloroplast. Several cyanobacterial genomes are sequenced, and the fortuitous arrangement of co-regulated genes into operons can aid in functional genomics studies of genes conserved between the lineages.

With the seminal work on plastocyanin and cytochrome c_6 substitution, *C. reinhardtii* has become a premier reference for probing analogous metal-responsive events. In addition to the genome, the transcriptomes of *C. reinhardtii* under copper- and iron-deficiency situations have been sequenced.[8,9]

The analyzes of individual phytoplankton and phytoplankton communities have provided a novel insight into distinctive strategies for acclimating to metal deficiency and novel adaptations to metal-poor aquatic environments. Compared to life on land, aquatic environments have unique metal nutrient concentrations and speciations. Both spatial and temporal changes in metal concentration influence the biochemistry of marine microorganisms, and in some cases, phytoplankton species have adapted to chronic metal deficiency by remodeling the genome to reflect the repertoire corresponding to genes induced in terrestrial algae during metal deficiency. Many oceanic algal species have evolved the ability to grow at much lower external and intracellular concentrations of iron, zinc, and manganese and, when possible, functionally equivalent proteins have permanently replaced those that are dependent on these metals.

5 COPPER

Copper and iron are the only trace metals concentrated in the chloroplast compared to the rest of the leaf.[10,11] This

Table 2 Transcripts involved in *A. thaliana* copper economy

Gene	Description	Cu^-/Cu^+	miRNA	Location
AT2G28190	CSD2 (copper/zinc superoxide dismutase)	0.02	mir398	Stroma
AT2G29130	LAC2 (laccase; copper ion binding)	0.02	mir397	Apoplastic
AT2G02850	ARPN (plantacyanin; copper ion binding)	0.06	mir408	Apoplastic
AT1G08830	CSD1 (copper/zinc superoxide dismutase)	0.06	mir398	Cytoplasm
AT1G12520	CCS1 (copper chaperone for superoxide dismutase)	0.07	miR398	Stroma/cytoplasm
AT2G30210	LAC3 (laccase; copper ion binding)	0.2	mir408	Apoplastic
AT1G72230	Plastocyanin-like domain-containing protein	0.2	mir408	Apoplastic
AT2G38080	IRX12/LAC4 (laccase; copper ion binding)	0.4	mir397	Apoplastic
AT3G15640	Cytochrome *c* oxidase family protein (Cox5b-1)	0.4	mir398	Mitochondrion
AT5G60020	LAC17 (laccase; copper ion binding)	0.6	mir397	Apoplastic
AT2G44790	UCC2 (uclacyanin; copper ion binding)	0.5	mir408	Apoplastic

The fold difference in transcript abundance from shoots of plants grown in the absence (Cu^-) and presence (Cu^+) of copper supplementation is given according to Bernal *et al*.[21]

observation can be attributed to the prevalence of these metals in the electron transport chain (ETC), but while iron is central to multiple steps in linear electron flow, only one step and only one protein, plastocyanin, is dependent on copper. In land plants and most algae, plastocyanin is responsible for the oxidation of the b_6f complex and reduction of PSI during linear electron flow (Figure 2). Exceptions include some red and brown algae that are solely reliant on cytochrome c_6 (iron-dependent) for this step. In most chloroplasts, a bottleneck in photosynthesis can form during copper deficiency, where electron flow through plastocyanin becomes the limiting step. Therefore, copper-sparing mechanisms in chloroplasts described so far center on this small soluble protein in the thylakoid lumen. The best-characterized responses are a plastocyanin/cytochrome c_6 switch in algae and microRNAs (miRNAs)-imposed copper economy in land plants (Figure 2 and Table 2).

5.1 Cytochrome c_6 Replaces Plastocyanin During Copper Deficiency

In the late 1970s, it became apparent that a soluble heme protein substitutes for plastocyanin in response to copper nutrition in some algae and cyanobacteria. This heme protein, originally referred to as cytochrome *c*-552 or *c*-553 based on α-absorption maximum and eventually renamed cytochrome c_6 based on function, serves as a back-up protein and facilitates an effective copper-sparing mechanism: electron transfer can occur without interruption, absence of plastocyanin minimizes the chloroplast demand for copper, and available copper may be redirected to other proteins. Plastocyanin is estimated to be one of the most abundant proteins in the lumen, and this switch spares a significant amount of copper. The reciprocal accumulation of cytochrome c_6 and plastocyanin is widespread among algae and cyanobacteria, but was presumably lost from land plants and lost or never gained by some red, green, and brown algae, which contain either plastocyanin or cytochrome c_6.[12]

While copper regulates transcription of the gene encoding cytochrome c_6 in both cyanobacteria and algae, the regulation of plastocyanin abundance occurs by various mechanisms.[13] However, each case represents a copper-sparing mechanism, because each results in a lower copper quota in the cell (for cyanobacteria) or the cell and the chloroplast (for algae). In the cyanobacteria *Anabaena* sp. PCC 7937, *Anabaena* sp. PCC 7120, *Synechocystis* sp. PCC 6803, and *Prochlorothrix hollandica*, plastocyanin mRNA abundance decreases in response to decreasing copper concentrations. In green algae, three distinct mechanisms have been found. Plastocyanin abundance in *Scenedesmus obliquus* is regulated at the level of mRNA accumulation, whereas in *Pediastrum boryanum*, mRNA accumulates in both copper-replete and -deficient cells, but the transcript from copper-deficient cells is truncated, resulting in loss of the initiation codon, and hence it is not translated. In *C. reinhardtii*, mRNA abundance is independent of the medium copper concentration, and an unidentified protease, which is active specifically during copper deficiency, targets plastocyanin for degradation. This degradation is not an example of metal salvaging, because the protease (or proteases) targets the mature but cofactor-free form.[14] Presumably, the cost of maintaining plastocyanin expression, translation, and localization to the thylakoid in *C. reinhardtii* is offset by the advantage of re-establishing plastocyanin function that much faster after copper resupply.

This conclusion would suggest that there is an advantage of using plastocyanin over cytochrome c_6. We do not know what the advantage is, but we have noted that there are many other metabolic changes in the plastid coincident with the use of cytochrome c_6. One of these changes is in the level of unsaturation of thylakoid membrane galactolipids, which would influence membrane fluidity—presumably of relevance for a diffusible electron carrier.[8] Another major change is the upregulation of enzymes in tetrapyrrole biosynthesis.[8] Specifically, expression of the gene *CPX1* encoding coproporhyrinogen (coprogen) III oxidase, which functions prior

to the branch point between heme and chlorophyll biosynthesis, increases in a copper-deficiency-dependent manner. One thought is that this response may supply the extra heme required for cytochrome c_6 maturation. Nevertheless, the extra heme is probably only marginal over the amount required for other photosynthetic complexes, and *CPX1* mRNA, protein, and activity increase about 5- to 10-fold. Furthermore, the expression of three genes encoding enzymes in the chlorophyll biosynthetic pathway is also increased during copper deficiency in *C. reinhardtii*: *CRD1* encodes a component of the aerobic Mg-Proto IX monomethyl ester cyclase, *CGL78*/*YCF54* encodes an ortholog to a protein that forms a complex with and is required for function of the Mg-Proto IX monomethyl ester cyclase,[15] and *CAO* encodes chlorophyllide *a* oxygenase, which is involved in chlorophyll *b* biosynthesis. We note that all three enzymes are oxygen-dependent. *CRD1*, *CGL78*, and *CPX1* are direct targets of the Cu-responsive transcription factor CRR1, suggesting a specific adaptation in *C. reinhardtii* of the tetrapyrrole pathway in response to copper deficiency—probably to increase flux through chlorophyll biosynthesis. However, the specific consequence of this adjustment remains unknown.

5.1.1 Copper Recycling

Instead of copper salvaging, copper recycling may exist in cyanobacteria and algae. Not only can photosynthesis still function without plastocyanin because of the presence of cytochrome c_6, but plastocyanin ultimately serves as a copper storage protein. The copper-bound plastocyanin presumably succumbs to normal protein turnover, and reestablishment of the cellular copper equilibrium would result in redirection of copper away from the thylakoid. Whether altering the copper distribution in the cell would occur in a passive manner or involve regulation of transporters and/or metal-binding factors such as metal chaperones is presently not known. Targets for prioritized copper allocation in *C. reinhardtii* include cytochrome oxidase, which acquires copper in the mitochondrion inter-membrane space, and ferroxidase, which acquires copper in the trans-Golgi en route to the plasma membrane. As ferroxidase is required for high-affinity iron uptake and, therefore, dispensable in an iron-replete situation, the prioritized target may be cytochrome oxidase. Indeed, ferroxidase protein abundance decreases during growth in iron-replete, copper-limited medium.[8] Under copper-replete conditions, plastocyanin is estimated to be at least 10-fold more abundant than cytochrome oxidase,[14] but reduction of the copper quota may be a larger advantage than copper recycling as copper-starved (successive culturing in copper-limited medium[16]) *C. reinhardtii* cultures do not display visible signs of copper deficiency.

5.2 miRNAs and the Cu-Economy Model in Land Plants

The lifestyles of the fast-growing, motile, single-celled photosynthetic microbes and of slower-growing, stationary, multicellular land plants have resulted in quite different evolutionary strategies to overcome fluctuations in copper availability. The most dramatic difference is that a plastocyanin back-up is not encoded in the genomes of land plants (as of August 2012), and cyanobacteria and algae successfully cope with copper deficiency, whereas land plants do not. Copper-deficient plants show symptoms of stunted growth, chlorosis, photosynthetic and morphological defects, and experience desiccation.[17] Copper-sparing mechanisms do exist in land plants, but the inability to sacrifice plastocyanin without consequences on photosynthetic efficiency appears to have a large impact on survival. The presumed goal of copper sparing and salvaging in land plants is to prioritize copper for plastocyanin, but the plant can spare and salvage only a finite amount of copper to maintain an adequate level of plastocyanin. However, these mechanisms suffice in the face of fluctuating copper availability and mild copper deficiency.

As characterized in *Arabidopsis thaliana* and *Populus trichocarpa*, post-transcriptional regulation of non-essential copper-dependent proteins and synthesis of back-up enzymes are the primary responses to copper-deficiency in land plants.[18] In 2003, using DNA microarrays, Wintz *et al*.[19] noted that sub-optimal copper nutrition leads to a reduction in the abundance of the CuZnSOD-encoding transcripts (*CSD1* and *CSD2*) and those for the associated copper chaperone (*CCS*). Downregulation of these genes was later found to result from targeted transcript degradation due to a microRNA. miRNAs are short (ca. 21 nucleotides) non-coding RNAs that usually negatively regulate protein abundance by targeting transcripts for degradation or inhibiting translation.[20] While the transcription factor CRR1 regulates copper-dependent expression of cytochrome c_6 in *C. reinhardtii*, a CRR1 ortholog in *A. thaliana*, SPL7, regulates the expression of the copper miRNAs. Both regulators share a GTAC recognition site, which constitutes the core of a Cu-response element (CuRE). The element is located upstream of the transcription start site.[21]

Copper limitation in *A. thaliana* induces the expression of four Cu-miRNA families. These families are central to the Cu-miRNA-mediated model for Cu-economy proposed by Burkhead *et al*.[18] (Table 2). The transcripts for copper proteins that are dispensable such as several phytocyanins (targets of miR408) and laccases (targets of miR397, miR408, and miR857), and copper proteins that can be replaced with copper-independent back-ups such as CuZnSOD (target of miR398) are degraded. The outcome is a reduced demand on the copper pool, estimated to be by about 70%,[21] and putatively a higher success of plastocyanin metallation. An alternative or mutual result is that transcript degradation eliminates the competition, and during copper re-supply, plastocyanin is positioned to preferentially acquire copper. Reflective of selectively reduced protein synthesis during copper deficiency,

photosynthetic efficiency recovers sooner than do the activities of CuZnSOD and polyphenol oxidase in *P. trichocarpa*.[22]

Of the known targets of Cu-miRNAs in *A. thaliana*, only CSD2 and its chaperone CCS1 are in the chloroplast. SOD is an important detoxification enzyme responsible for the dismutation of superoxide to hydrogen peroxide. In green leaves, nearly 90% of SOD activity is in the chloroplast,[23] and because the reducing side of PSI produces superoxide, CSD2 localizes to the stroma. Therefore, CSD2 may directly compete for copper heading toward plastocyanin in the thylakoid lumen. Land plants also encode iron-dependent superoxide dismutases (FeSODs), and copper deficiency leads to an increase in the abundance of the FeSOD-encoding transcript, *FSD1*, concurrently with the degradation of *CSD2* transcript.[21] The presumed role of *FSD1* expression is compensation for the lack of CuZnSOD activity.

The majority of transcripts targeted by Cu-miRNAs encode proteins that are or are predicted to be secreted (Table 2). Although these proteins are apoplastic, metallation would occur in the secretory system before export,[24] and the reduced synthesis of these proteins may lead to rerouting of copper to the chloroplast. The presence of miR397, miR398, and miR408 gene families is conserved in *P. trichocarpa*, which contains an additional miRNA family, miR1444. miR1444 is upregulated during copper deficiency and regulates the abundance of transcripts encoding polyphenol oxidase.[22] Polyphenol oxidases are binuclear copper proteins in the thylakoid lumen, which are absent in both *A. thaliana* and *C. reinhardtii*. The presence of this miRNA family reveals tailoring of Cu-economy to gene repertoire.

6 IRON

Studies in the 1940s established that chloroplasts contain roughly 80% of the cellular iron found in green leaves.[25,26] The majority of this iron is present in the proteins of the photosynthetic ETC (Figure 2). The simplest calculation of iron content per ETC (23–24 iron) assumes a molar ratio of 1:1 for each iron-containing complex, and that each complex is a monomer. Therefore, this value is an underestimate because both PSII and cytochrome b_6f are dimers, PSI is a trimer in cyanobacteria but a monomer in eukaryotes, and the stoichiometries of these complexes are generally not 1:1. For instance, the PSI:PSII ratio in cyanobacteria can vary from 4:1 to 1:1 depending on the nutritional supply of iron, and a constitutive ratio of 1:10 was found for a diatom adapted to chronically low-iron abundance. Regardless of the absolute iron requirement of the ETC, the chloroplastic demand is relatively high, and the bioavailability of iron in aerobic soils is low. Therefore, acquired iron deficiency compared to deficiency in other transition metals is common in agriculture. Iron deficiency also contributes to reduced primary production in the oceans. Then, it is of no surprise that iron was one of the first inorganic nutrients studied in plant fitness.

Considering the historical significance of iron in plant nutrition, sparing and salvaging in the context of the chloroplast is underexplored in land plants. This gap in knowledge may be because of the difficulty in controlling iron deficiency, which is necessary to avoid noise from the individual context of different cell types in a tissue. Therefore, iron sparing and salvaging in the context of photosynthesis is best understood in cyanobacteria and algae, where homogeneous populations grown under strictly defined conditions of iron nutrition are easily attained. Well-characterized examples include remodeling of the photosynthetic machinery and back-ups.

6.1 Iron Sparing and Salvaging in *C. reinhardtii*: Remodeling of the Thylakoid Membrane Protein Content

Physiology and expression of key genes involved in acclimating to iron status define four stages of iron nutrition in *C. reinhardtii*.[27] Cells will accumulate two- to five-fold more iron in the iron-excess stage (200 μM medium iron content) compared to the iron-replete stage (20 μM medium iron content), and, as a consequence, are sensitive to excess excitation energy. In the iron-replete situation, genes encoding iron uptake pathways are expressed at basal levels, and luxury iron consumption corresponds to one-sixth of the available medium iron content (ca. 3 μM iron).

As the cells become iron deficient (3–1 μM medium iron content), classic iron-deficiency chlorosis is not evident, but a programmed response that includes induction of iron uptake and remodeling of the photosynthetic architecture is initiated.[28] Various iron-containing proteins in the chloroplast are degraded, including PSI, the cytochrome b_6f complex, and ferredoxin, and the light-harvesting complex associated with the remaining PSI is remodeled. ETC remodeling is not unique to *C. reinhardtii*. Similar iron-deficiency responses such as decreasing the ratio of PSI to PSII are observed in other algae and cyanobacteria.[29]

In *C. reinhardtii*, not all iron-dependent proteins in the chloroplast are reduced in abundance. Maintenance of FeSOD suggests salvaging of iron within the chloroplast, while maintenance of respiration during growth on acetate suggests salvaging of iron between the chloroplast and mitochondrion.[3,27] Iron released from the degradation of ETC complexes is buffered by ferritin, an iron-storage protein whose expression is increased during iron deficiency in *C. reinhardtii*. In contrast, ferritin expression in other eukaryotes, including land plants, is increased during iron excess.[30] Ferritin, by binding the released iron, serves as a buffer during the salvage process.

The fourth stage, iron limitation (≤0.5 μM medium iron content), is marked by chlorosis and diminished growth rate. Loss of PSI and cytochrome complexes is evident

with a reduction in abundance to <1% in acetate-grown *C. reinhardtii* cells compared to iron-replete cultures. In the absence of acetate, however, these complexes are maintained, suggesting that the complexes are sacrificed in the previous situation for iron salvaging.

A recent analysis of transcript abundance using the RNA-Seq method during the three iron nutrition stages has further illuminated iron-sparing and -salvaging strategies of *C. reinhardtii*. Generally, transcripts encoding proteins with Fe–S clusters were reduced in abundance, while transcripts encoding some heme-bound proteins were increased in abundance (Table 1). This trend may represent routing of iron away from a subset of proteins and ensure maintenance of another subset. The reduction of Fe–S proteins in the chloroplast may also be an attempt to control photooxidative stress. Fe–S clusters are particularly labile in the presence of superoxide, which is produced by the photoreduction of dioxygen by PSI, especially when the function of the Fe–S clusters in PSI is compromised by iron deficiency. Superoxide destroys solvent-exposed Fe–S clusters (including potentially those from PSI), releasing Fe^{3+}, which can then react with hydrogen peroxide, creating the highly cytotoxic hydroxyl radical, which cannot be enzymatically destroyed.

6.2 Back-Ups

6.2.1 Ferredoxin/Flavodoxin—Iron Salvaging

Ferredoxin and flavodoxin are iron-dependent and iron-independent electron transfer proteins, respectively. Positioned at the terminal end of linear electron flow, they serve as switchboards providing reducing power for several pathways involved in metabolism and regulation. Flavodoxin, known at the time of its discovery as phytoflavin, was first isolated from the cyanobacterium *Synechococcus elongatus* and found to the substitute for ferredoxin in the photoreduction of $NADP^+$ by isolated chloroplasts.[31] The connection to iron sparing was made by Knight *et al*.[32], who isolated flavodoxin from the bacterium *Clostridium pasteurianum* grown in low-iron medium. They proposed that flavodoxin has a similar role during iron deficiency as ferredoxin has during iron sufficiency, and thus the name ''flavor-'', as it contains a flavin and ''-doxin'' because of its equivalence with ferredoxin. Schönheit *et al*.[33] found that ferredoxin was actively degraded when the *C. pasteurianum* culture consumed the bulk of the iron from the medium. The cells continued to grow and a second Fe–S protein, pyruvate synthase, was maintained. They proposed an iron-salvaging mechanism during which iron is recycled from ferredoxin.

The genomes of some algae, particularly marine diatoms, contain genes for both ferredoxin and flavodoxin.[34] The presence of both genes does not necessarily equate to the ability of that organism to functionally substitute one for the other in response to iron. While reciprocal expression is found in the oceanic diatom *T. weissflogii*, flavodoxin is not induced during iron deficiency in the coastal relative *Thalassiosira pseudonana*. Flavodoxin induction by iron deficit is widespread in bacteria and proposed as a marker of iron deficiency in the oceans. Less well explored, mainly because of the dearth of robust genetic tools, is the ability of flavodoxin to functionally substitute for ferredoxin in algae.

Flavodoxin is missing from the genomes of sequenced land plants and most algae. Nevertheless, transgenic tobacco lines expressing a cyanobacterial flavodoxin in chloroplasts gained a fitness advantage when grown on iron-deficient medium.[35] This and other studies of recombinant flavodoxin have incited curiosity as to why flavodoxin was lost during the transition to land. The simplest answer is that during the evolution of modern plants, flavodoxin did not provide a selective benefit, and as in the case of so many genes inherited from the original cyanobacterial symbiont, the flavodoxin gene was simply lost.

Even in the absence of flavodoxin, iron sparing involving ferredoxin does occur in *C. reinhardtii*. Of the ferredoxins in the chloroplast, ferredoxin-2 is predicted to reduce nitrate reductase and, therefore, may be unnecessary during growth in the presence of ammonium and can be spared if iron nutrition is suboptimal.

6.2.2 Superoxide Dismutase

With four distinct isoforms having four different metal cofactor requirements, when multiple SODs make their way into the same genome, the stage is set for a metal-sparing strategy. As we saw with copper economy, the CuZnSOD in the chloroplast of land plants is downregulated reciprocally with the induction of FeSOD. In bacteria, where CuZnSOD is missing, the reciprocal presence of FeSOD in iron-replete medium and MnSOD in iron-deficient medium is widespread.

Some algae also lack CuZnSOD and rely solely on FeSOD (or MnSOD in the case of *T. pseudonana*) activity in the chloroplast. Therefore, the induction of an MnSOD during iron deficiency in *C. reinhardtii* pointed to an iron-sparing mechanism in the chloroplast.[36] However, recent work has shown that while the MnSOD is produced and localizes to the chloroplast, FeSOD is actually maintained while other iron-dependent proteins are lost.[27]

6.2.3 Fructose-Bisphosphate Aldolase

The expression of a metal-free isozyme of fructose-bisphosphate aldolase has also been proposed to serve as a back-up for the metal-dependent isozyme in the chloroplast during iron limitation.[37] While the expression of the metal-free form is responsive to iron nutrition in numerous systems, dependence of the metal-dependent form on iron has not been shown. Therefore, whether expression of fructose-bisphosphate aldolase is an example of iron sparing or involved in metabolic remodeling is yet to be determined.

6.3 Chronic Iron Limitation

Iron fertilization experiments in the 1990s established that photosynthesis in the open ocean is chronically limited by the abundance and bioavailability of iron. The phytoplankton species in these communities (cyanobacteria, diatoms, and algae) have adapted to the iron deficit with permanent iron-sparing: reduction in the abundance of iron-requiring proteins and reduction in the total number of genes encoding iron-requiring proteins. Putative *sodN* genes are present in the pico-prymnesiophyte metagenome, the four pico-prasinophyte genomes, and *Phaeodactylum tricornutum*, and while they may also contain genes for CuZnSOD or MnSOD, these genomes do not contain genes for FeSOD.[38] Like land plants, some diatoms that inhabit the open ocean use plastocyanin instead of cytochrome c_6, while their coastal cousins constituently use cytochrome c_6. Other differences include the ratio of iron-rich ETC complexes. The oceanic diatom *Thalassiosira oceanica* has up to fivefold lower PSI and up to sevenfold lower cytochrome b_6f complex concentrations than the coastal diatom *T. weissflogii*.[39]

In nitrogen fixation, the nitrogenase enzyme system catalyzes the reduction of dinitrogen to ammonia. This system consists of two proteins: a 4Fe:4S enzyme and a molybdenum–iron enzyme with an 8Fe:7S cluster and a Mo:7Fe:9S cofactor. In the cyanobacterium, *Crocosphaera watsonii*, nitrogen fixation occurs at night and transcripts involved in photosynthesis decline, while transcripts for the nitrogenase system increase in the evening as photosynthesis winds down. The most obvious explanation for temporal separation of these two processes is the incompatibility between molecular oxygen and the nitrogenase complex. Nevertheless, recent quantification of protein abundance during the metabolic switch has led to the hypothesis that cyanobacteria recycle iron between the nitrogenase proteins and photosynthesis.[40] This process, referred to as ''hot-bunking,'' is estimated to reduce the cellular iron quota by around 40% and appears to be an adaptation to the low bioavailability of iron in the open ocean, as regulation by iron status is not indicated.

7 ZINC

Although zinc is an abundant metal cofactor, few examples of zinc sparing and salvaging in plants are available, and none is presently known to operate in the chloroplast. One hurdle to the study of sub-cellular zinc homeostasis is the lack of techniques applicable to the study of this spectroscopically silent ion. In addition, the ease with which the zinc cofactor loads into the apoprotein in vitro generally precludes the need to add exogenous zinc to enzyme assays. Therefore, several enzymes may be erroneously labeled as not requiring a cofactor. In bacteria, zinc sparing and salvaging are well-known strategies for acclimation to zinc limitation, and this is mainly because of the identification of zinc-responsive regulons using bioinformatic approaches.[13] Examples of these include reciprocal expression of zinc-dependent vs zinc-independent isoforms of ribosomal subunits, metabolic enzymes, and regulatory proteins. Perhaps, the role of zinc in the chloroplast has been overlooked by the plant world because of its inability to perform redox chemistry (in fact, zinc is not a transition metal), but interest in zinc and acclimation to zinc deficiency is gaining prominence.[41] Diatoms have provided us with several examples of strategies to overcome zinc limitation: the use of a cadmium-activated carbonic anhydrase discussed earlier and a cobalt-substituted carbonic anhydrase. Whether these scenarios occur in the chloroplast is yet to be determined. The abundance of the carbonic anhydrases in algae for operation of the carbon concentrating mechanisms suggests that they may be sources for zinc salvage. Yet, their function is essential in low CO_2 environments, and back-up versions of these enzymes may well occur in the algae of the plant lineage as well.

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9 ABBREVIATIONS AND ACRONYMS

CCS = cytochrome synthesis; CuRE = Cu-response element; ETC = electron transport chain; FeSODs = iron-dependent superoxide dismutases; miRNAs = microRNAs; miRNAs = microRNAs; PPDB = Plant Proteome DataBase; PSII = photosystem II; PSI = photosystem I; SOD = Superoxide dismutase; TAT = twin-arginine translocase.

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