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Review

The role of trace metals in photosynthetic electron transport in O_2-evolving organisms

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Abstract

Iron is the quantitatively most important trace metal involved in thylakoid reactions of all oxygenic organisms since linear (= non-cyclic) electron flow from H_2O to $NADP^+$ involves PS II (2–3 Fe), cytochrome b_6-f (5 Fe), PS I (12 Fe), and ferredoxin (2 Fe); (replaceable by metal-free flavodoxin in certain cyanobacteria and algae under iron deficiency). Cytochrome c_6 (1 Fe) is the only redox catalyst linking the cytochrome b_6-f complex to PS I in most algae; in many cyanobacteria and Chlorophyta cytochrome c_6 and the copper-containing plastocyanin are alternatives, with the availability of iron and copper regulating their relative expression, while higher plants only have plastocyanin. Iron, copper and zinc occur in enzymes that remove active oxygen species and that are in part bound to the thylakoid membrane. These enzymes are ascorbate peroxidase (Fe) and iron-(cyanobacteria, and most algae) and copper-zinc- (some algae; higher plants) superoxide dismutase. Iron-containing NAD(P)H-PQ oxidoreductase in thylakoids of cyanobacteria and many eukaryotes may be involved in cyclic electron transport around PS I and in chlororespiration. Manganese is second to iron in its quantitative role in the thylakoids, with four Mn (and 1 Ca) per PS II involved in O_2 evolution. The roles of the transition metals in redox catalysts can in broad terms be related to their redox chemistry and to their availability to organisms at the time when the pathways evolved. The quantitative roles of these trace metals varies genotypically (e.g. the greater need for iron in thylakoid reactions of cyanobacteria and rhodophytes than in other O_2-evolvers as a result of their lower PS II:PS I ratio) and phenotypically (e.g. as a result of variations in PS II:PS I ratio with the spectral quality of incident radiation).

Introduction

Trace metals play key roles in (non-cyclic) photosynthetic electron transport in the thylakoids of O_2-evolving organisms (Nicholls and Ferguson 1992; Kaneko et al. 1996; Merchant and Dreyfuss 1998). Mn is essential for O_2 evolution. Fe is involved in the PS II complex (cytochrome b_{559} and one additional Fe which is not in haem or part of a FeS complex), the cytochrome b_6-f complex (cytochrome b_{563}, cytochrome f, Rieske non-haem iron-sulfur complex containing 2 Fe), cytochrome c_6 (an alternate to Cu-containing plastocyanin), PS I complex (3 non-haem iron-sulfur complexes F_X, F_B and F_A, each containing 4 Fe) and ferredoxin (a protein with one non-haem iron-sulfur complex containing 2 Fe, an alternate to flavodoxin which contains no metals). Cu occurs in plastocyanin but, despite some claims, probably not in PS II. Ca^{2+}, at trace element levels, has a role in the thylakoid lumen in facilitating H_2O dehydrogenation and O_2 evolution (Hankamer et al. 1997).

In addition to these 'core' electron transport components involved in non-cyclic electron transport from H_2O to NADPH, there are also thylakoid compon-

ents which contain Fe, Cu and Zn that are involved in essential processes that are not part of the 'core' non-cyclic electron flow. These include the NAD(P)H dehydrogenase which may be involved in chlororespiration and cyclic electron flow around PS I; this complex contains non-haem Fe-sulfur centres. Additionally, the Mehler reaction involves the production of superoxide, which is converted to H_2O_2 using superoxide dismutase (containing Fe, Mn or Cu plus Zn when the whole range of organisms in which it occurs are considered); this H_2O_2 is ultimately converted to H_2O and O_2 using one of a number of enzymes. These generally involve Fe, such as the Fe-containing catalase (in a few 'primitive' cyanobacteria: Asada et al. 1993) or, with additional input of reductant from PS I, to H_2O using the Fe-containing ascorbate peroxidase (Asada 1992; Asada et al. 1993b). The thylakoid-bound and stromal ascorbate peroxidases are produced by alternative gene splicing in *Cucurbita moschata* (Mano et al. 1997). In some algae, glutathione peroxidase, containing the non-metal Se, occurs; it is considered here because it 'spares' Fe in the alternate peroxidase, i.e. ascorbate peroxidase. The higher plant glutathione peroxidase which seems to be a lipid hydroperoxidase, does *not* contain Se (Stadtman 1996; Eshdat et al. 1997; Mullineaux et al. 1998). These metal-containing catalysts, which are involved in the Mehler peroxidase reaction and may also be regarded as means of detoxifying active oxygen species, are a part of the thylakoid membrane, although there is a parallel set of enzymes in the stroma that catalyze the same reactions. It is also significant that Couture et al. (1994) have reported a nuclear-encoded, thylakoid-expressed haemoglobin (containing Fe) in *Chlamydomonas reinhardtii*.

Our objectives in this review are:

1. To analyse the functional role of the metals in relation to the polypeptides, porphyrins, etc. in which they are found;
2. To assemble and interpret the adaptation-acclimation-related variations among taxa in the use of alternate catalysts containing different, alternative, metals (Cu or Fe) or a metal (Fe) *versus* no metal;
3. To describe and interpret the adaptation-acclimation-related variations in the ratios of metal-containing catalysis among taxa;
4. To use data discussed in 2 and 3. to indicate differences in the metal costs of photosynthesis at a given rate (mol O_2 or mol C per second;
5. To relate the discussion in 1.–4. to evolution in terms of the chemical appropriateness of the metal to a given function and the evolutionary 'need' for that function at an earlier time when the availability of various metals was very different from the situation today, mainly as a result of a less oxidizing global environment earlier in the history of the biosphere.

We do not deal in detail with the involvement of trace metals in 'downstream' electron transport reactions associated with CO_2, NO_3^-, NO_2^-, N_2 and SO_4^{2-} assimilation which involve inputs, directly or indirectly, of photoproduced reductant and ATP.

For CO_2 assimilation, one trace metal requirement relates to the Zn in the carbonic anhydrase involved in CO_2 supply to Rubisco. Even when there is a functional CO_2 concentrating mechanism, there is usually some Rubisco oxygenase activity resulting in the production of phosphoglycolate and hence glycolate. Glycolate is metabolized in higher plants and some algae by Fe-containing catalase (Suzuki et al. 1991; Falkowski and Raven 1997; Iwamoto and Ikawa 1997). In other algae, glycolate is metabolized by glycolate dehydrogenase; here the reductant enters the respiratory chain which involves Fe and Cu (Raven 1988a, 1990; Suzuki et al. 1991; Falkowski and Raven 1997). Glyoxylate metabolism, insofar as this is known, involves a dehydrogenase step(s) which involves the respiratory chain, and hence Fe and Cu (Falkowski and Raven 1997). The alternative to glycolate metabolism is glycolate excretion (Falkowski and Raven 1997), which incurs no direct trace metal involvement (see H_2O_2 excretion: 2(c) below).

The trace metal requirements of the assimilation of NO_3^- and NO_2^- (cyanobacteria and eukaryotes) and NO_3^-, NO_2^- and N_2 (cyanobacteria only, at least among oxygenic organisms) have been dealt with in detail by Raven (1988a, 1990). The computed Fe and Mo requirement for growth at a given rate is significantly increased when NO_3^-, and especially N_2, rather than NH_4^+ is the N source (Raven 1988a, 1990).

The functional roles of the metals

Linear electron flow with $NADP^+$ as electron acceptor

Figure 1 shows the classic 'Z scheme' based on the concept of Hill and Bendall (1960), showing redox potential and emphasizing the places at which trace metals function. The quantities of trace metals indicated are for one protein (e.g. cytochrome c_6 or

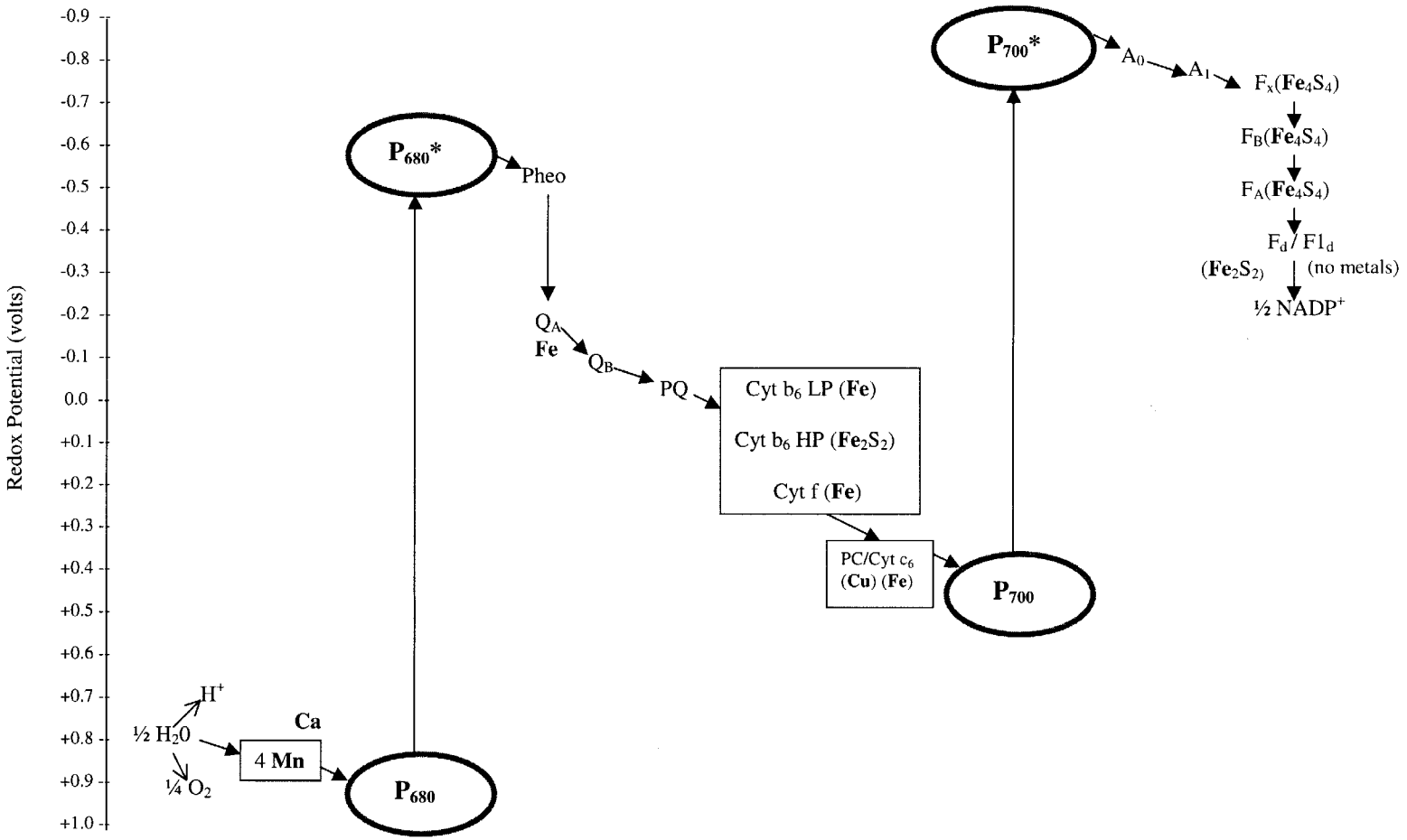

Figure 1. The 'Z scheme' of non-cyclic electron transport, showing the involvement of trace metals in various redox components in relation to their standard redox potential (modified from Falkowski and Raven 1997).

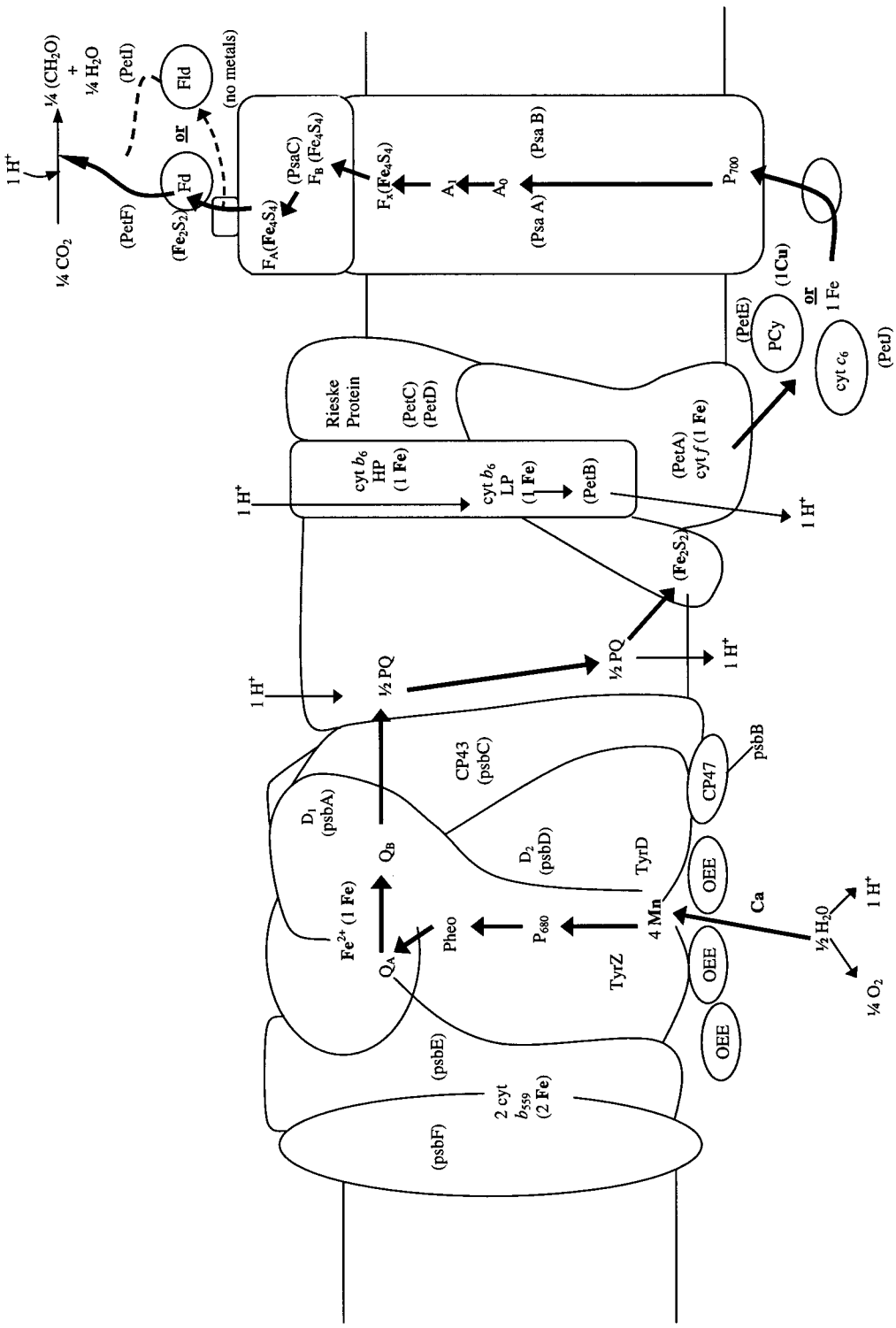

Figure 2. The spatial arrangement in the thylakoid membrane of the components of non-cyclic electron transport, showing the involvement of trace metals (modified from Falkowski and Raven, 1997, incorporating the likely arrangement of the b_6–f complex based on the crystal structure of the (larger) bovine b-c_1 complex from Iwata et al. 1998 and Smith 1998).

plastocyanin) or one complex (e.g. the cytochrome b_6-f complex).

Figure 2 indicates the spatial (as opposed to the energetic, Figure 1) disposition of the various trace metal-containing catalysts of linear electron transport. Also indicated are the stoichiometries of electron flow to vectorial H^+ fluxes; with a proton-motive Q cycle the H^+/e^- ratio is 3 which, with a H^+/ATP ratio of 4, produces 0.75 ATP per electron transferred from H_2O to $NADP^+$ (Falkowski and Raven 1997).

Table 1 gives molecular data on the proteins that bind trace metals in the linear electron transport chain, and indicates how the metals are bound. The data in Table 1 show that, with a 1:1:1:1:1 stoichiometry of PS II:cyt b_6-f:cyt c_6: PS I:ferredoxin, there are 23 Fe, 4 Mn and no Cu per 'linear electron transport chain', while with cytochrome c_6 replaced by plastocyanin and ferredoxin replaced by flavodoxin, the 'linear electron transport chain' with a 1:1:1:1:1 stoichiometry of PS II:cyt b_6-f:plastocyanin:PS I:flavodoxin has 20 Fe, 4 Mn and 1 Cu. The Fe content of these idealized linear electron transport chains assumes 2 cytochrome b_{559}, each with 1 Fe, per PS II; if, as is very possible there is only 1 cytochrome b_{559} per PS II, the number of Fe per linear electron transport chain here, and subsequently in this paper, should be reduced by 1. Table 2 lists the Fe content of the various complexes.

Cytochrome f, cytochrome c_6, the iron sulphur centres of the Rieske protein and the electron acceptor complex of PS 1, plastocyanin, ferredoxin and flavodoxin have a straightforward role in the linear electron transfer pathway.

The two haems of the cytochrome b_6 provide the transmembrane electron transfer path for the Q cycle mechanism in the cytochrome b_6-f complex.

Cytochrome b_{559} is an essential component of PS II, deletion of the genes for the polypeptide results in failure to assemble the complex. Under various experimental conditions, it may be either oxidised or reduced by the PS II reaction centre, it also has two redox potential forms. However its *in vivo* function is unknown. A role in protection against photoinhibition, possibly by providing a path for cyclic electron transfer around PS II, seems most likely (Whitmarsh et al. 1994; Prosil et al. 1996). There may also be a role for this cytochrome in cyclic electron flow round PS I (Miyake et al. 1995).

The function of the non-haem iron in PS II is also uncertain. Unlike the homologous Fe in the purple bacterial reaction centre it can undergo oxidation and reduction. It is structurally placed between the primary and secondary quinone electron acceptors but is not thought to act as an electron carrier between them as its redox potential, +400 mV, is too high for oxidation by the quinone (Diner and Petrouleas 1988), electron flow only occurring when it is in the Fe^{2+} form. Electron flow is also dependent on the presence of bicarbonate as a ligand to the Fe. Removal of the bicarbonate or replacement by formate inhibits electron transfer and may contribute to photoinhibition (Blubaugh and Govindjee 1988).

Mn is an essential component of the water oxidation complex. Four Mn atoms are organized in two interacting pairs in the form of μ-oxo bridged dimers (Yachandra et al. 1993, MacLachlan et al. 1994b; Riggs-Gelasco et al. 1996). Ca is also an essential component of the complex (Yocum 1991) possibly as an integral component of the Mn complex (MacLachlan et al. 1994a; Latimer et al. 1995). The Mn complex provides the redox system for the accumulation of the 4 oxidizing equivalents required for water oxidation, providing the gating mechanism between the single electron turnover of the reaction centre and the oxidation of water. Three of the five oxidation states of the water oxidizing complex are known to involve different Mn redox states (Messinger et al. 1997).

Not listed in Table 1 are two other c-type cytochromes which are associated with the photosynthetic apparatus of many cyanobacteria and algae, and which have not so far been shown to act in linear electron transport or, indeed, in any other essential redox reactions in the organisms in which it occurs (Kerfeld and Krogmann 1998).

One of these c-type cytochromes is cytochrome c_{549}LP, with a redox potential of -250 mV (Navarro et al. 1995). Cytochrome c_{549}LP has been shown to occur in cyanobacteria, red algae (Rhodophyta), diatoms (Heterokontophyta) and the ulvophycean *Bryopsis* (Chlorophyta) (Evans and Krogmann 1983). Although some reports indicate thylakoid membrane association of cytochrome c_{549}LP, with the molecule located toward the lumenal side of the membrane and essential to the structure of PS II in the organisms in which it occurs, in other cases (e.g. *Synechocystis* sp. strain 6803) this cytochrome appears to be entirely water-soluble (Navarro et al. 1995; Kerfeld and Krogmann 1998). If there is one molecule of cytochrome c_{549}LP per PS II reaction centre, then each 'linear electron transport chain' has one more Fe than in otherwise similar organisms which lack cytochrome c_{549}LP (e.g. higher plants). Cytochrome c_{549}LP can

Table 1. Thylakoid proteins involved in electron transport which bind trace metals

Gene	Polypeptide	Mass of mature polypeptide	Function	Metals bound	References
psbA	Hydrophobic D1 protein of PS II reaction centre	38 021	Catalysts of primary photochemistry of PS II	1 Fe per heterodimer as neither haem nor FeS; 4 Mn	
psbD	Hydrophobic D2 protein of PS II reaction centre	39 418			
psbE	Hydrophobic α-cyt b_{559}	4409	Photoprotection? (not a component of linear electron transport)	1 Fe per heterodimer as haem; 1 or 2 b_{559} (1 or 2 Fe) per PS II reaction centre	Hankamer et al. (1997); Hoganson and Babcock (1997); Krieger and Rutherford (1997); Rhee et al. (1998)
psbF	Hydrophobic β-cyt b_{559}	4195			
psbO	33 kDa extrinsic protein	26 539	Stabilizes Mn cluster bound to D1/D2; Cl^- binding	Ca^{2+} binding	
psbP	23 kDa extrinsic protein	20 210	Cl^- binding		
psbQ	16 kDa extrinsic protein	16 523	Cl^- binding		
psaA	Hydrophobic 83.2 kDa protein of PS I	83 200	Catalysts of primary photochemistry of Ps I	4 Fe per heterodimer as F_X [4 Fe – 4S]; 1 heterodimer per PS I, so 4 Fe per PS I	Evans et al. (1981) Golbeck (1992); Evans and Nugent (1993); Berry et al (1997); Scheller et al. (1997); Schubert et al. (1997)
psaB	Hydrophobic 82.5 kDa protein PS I	82 500			
psaC	Hydrophilic stromal proteim pf PS I	8900	Transfer of electrons from F_x to ferredoxin or flavodoxin *psaC*; 1 *psaC* per PS I, so 8 Fe per PS I	8 Fe per heterodimer as F_A [4 Fe – 4 S] and F_B [4 Fe – 4 S] per (1997); Schubert et al. (1997)	Evans and Nugent (1993); Scheller et al.

Table 1. Continued

Gene	Polypeptide	Mass of mature polypeptide	Function	Metals bound	References
PetF	Ferredoxin; hydrophilic stromal protein of PS I	11 000	Transfer of electrons from F_A to ferredoxin-NADP oxidoreductase and thence to $NADP^+$, to NO_2^- reductase to ferredoxin-dependent glutamate synthase, to ferredoxin-dependent thioredoxin reductase, and to monodehydro-ascorbate	2 Fe as *[2 Fe – 2 S]	Evans and Nugent (1993); Miyake and Asada (1994); Scheller et al. (1997)
PetA	Cytochrome *f* component of cytochrome b_6-*f*–FeS complex; hydrophobic	32 038	Redox reactions; H^+ pumping	1 Fe per monomer bound as haem; 1 cyt *f* per b_6-*f* FeS	
PetB	Cytochrome b_6 component of cytochrome b_6-*f* FeS complex; hydrophobic	24 166	Redox reactions, H^+ pumping	1 Fe per monomer bound as haem; 2 cyt b_6 per b_6-*f* comples;	Cramer et al. (1996 Hpe (1993)
PetC	Rieske non-haem Fe polypeptide; hydrophobic	19 116	Redox reactions	2 Fe per complex 2 Fe as [2 Fe – 2S];	
PetJ	Cytochrome c_6 (alternative to plastocyanin); (hydrophilic protein in lumen)	10 000	Electron transfer from b_6-*f*–FeS complex to Ps I	1 Fe per monomer bound as Fe	
PetE	Plastocyanin (alternative to cytochrome c_6); (hydrophilic protein in lumen)	10 000	Electron transfer *from b_6-*f*–FeS complex to PS I	1 Cu	Falkowski and Raven (1997)

Table 2. Fe content of proteins or protein complexes involved in photosynthetic electron transport. From references in Tables 1 and 4, and in the text

Protein or protein complex	Number of Fe atoms per or protein complex
PS II	(2-)3
PS I	12
Cytochrome b_6-f	5
Cytochrome c_6	1
Ferredoxin	2
Catalase	4
Fe superoxide dismutase	1
Ascorbate peroxidase	1
NAD(P)H/PQ oxidoreductase	8–18

function *in vitro* as a catalyst of cyclic electron flow, via *its* rapid reduction by at least some isoforms of ferredoxin and its oxidation by plastocyanin (or, presumably, cytochrome c_6) (Navarro et al. 1995; Kerfeld and Krogmann 1998). Another suggested role of cytochrome c_{549}LP is in anoxia tolerance via an interaction with (soluble, cytosolic or stromal) hydrogenase (Kerfeld and Krogmann 1998; Merchant and Dreyfuss 1998). Neither of these roles seems to be particularly compatible with location of cytochrome c_{549}LP toward the lumenal side of the thylakoid membrane.

The other *c*-type cytochrome is cytochrome c_{552}(cyt *M*). This soluble cytochrome *c* has a redox potential similar to that of cytochrome c_6 and of plastocyanin, so that it could act as a third alternative to these two redox catalysis in certain cyanobacteria and algae (Kerfeld and Krogmann 1998; see 'Genetic and environmentally induced expression of alternate catalysts containing different metals or no metals' below). However, cyt *M* is present in much smaller amounts than cytochrome c_6 (or plastocyanin), which could constrain any obligatory role in linear (or cyclic) electron transport (Kerfeld and Krogmann 1998). Certainly the molecular genetic elimination of cytochrome *M* in a cyanobacterium has no observable phenotypic effect, albeit in cells that can express both cytochrome c_6 and plastocyanin (Kerfeld and Krogmann 1998; see also Manna and Vermaas 1997).

Table 3. Occurrence of plastocyanin and cytochrome c_6 (see Raven et al. 1989; Raven et al. 1990; Kerfeld and Krogmann 1998)

Taxon	Plastocyanin	Cytochrome c_6
Cyanobacteria (including Chloroxybacteria?)	In most	In all?
Rhodophyta	Absent	Present
Heterokontophyta		
Bacillariophyceae	Absent	Present
Chrysophyceae	(Absent)	Present
Fucophyceae (= Phaeophyceae)	Absent	Present
Synurophyceae	(Absent)	Present
Tribophyceae (= Xanthophyceae)	Absent	Present
Haptophyta	(Absent?)	Present
Cryptophyta	?	?
Dinophyta	?	?
Chlorophyta	In all?	In some
Embryophyta	Present	Absent
Euglenophyta	Absent	Present
Chlorachniophyta	?	?

Genetic and environmentally induced expression of alternate catalysts containing different metals or no metals

The major alternative catalysts in the linear electron transport pathway are the water-soluble proteins involved in the cytochrome b_6-f complex to P_{700} electron transfer (plastocyanin *or* cytochrome c_6) and the F_A to ferredoxin-$NADP^+$ reductase electron transfer (ferredoxin *or* flavodoxin). The former pair of redox carriers function on the lumenal side of the thylakoid membrane while the latter pair function on the stromal (eukaryotes) or cytosol (prokaryotes) side of the membrane.

Table 3 shows the taxonomic (phylogenetic) distribution of the occurrence of the members of the alternative pair plastocyanin/cytochrome c_6. Although it has previously been thought that some cyanobac-

teria lack the capacity to make plastocyanin, more recent evidence, genetic and other, indicates a very widespread capacity for plastocyanin synthesis in cyanobacteria *sensu lato*, i.e. including chloroxybacteria or prochlorophytes (Arudchandran et al. 1994; Clarke and Campbell 1996; Kaneko et al. 1996). Among the green algae (Chlorophyta), plastocyanin seems to be universal, with the capacity to produce cytochrome c_6 in many cases. Plastocyanin seems to be present in all higher plants (Embryophyta, i.e. bryophytes and tracheophytes) and there is no evidence for cytochrome c_6. The situation in the closest living relatives of the ancestors of the embryophytes, i.e. the class Charophyceae *sensu lato*, or Klebsomidiophyceae of the division Chlorophyta (van den Hoek et al. 1995), is unclear. All of the other groups of algae seem to lack plastocyanin and use only cytochrome c_6. This is true of the organisms that obtained their plastids by secondary endosymbiosis of a green eukaryote, i.e. the Euglenophyceae (the situation in the Chlorarachniophyceae is not clear), the red algae which, presumably, like the green algae, have chloroplasts derived from endosymbiosis of a cyanobacterium (but see Stiller and Hall 1997), as well as the Heterokontophyta and, probably, Haptophyta which obtained their plastids by secondary endosymbiosis of red algae. Use of plastocyanin or cytochrome c_6 in other significant non-green algal taxa, which also obtained their plastids by secondary endosymbiosis (the Cryptophyta and Dinophyta), is not clear. Present evidence shows that goes both cytochrome c_6 and plastocyanin in eukaryotes are invariably encoded by nuclear genes.

A number of provisos are needed in considering the distribution among algae of cytochrome c_6 and plastocyanin.

1. The older literature was confounded by confusion of cytochrome c_6 with cytochrome *f*, a point which was elegantly clarified by Wood (1977; see Falkowski and Raven 1997).
2. The use of visible wavelength spectrometry (a broad redox-dependent band centred at $\sim$ 580 nm) to characterize plastocyanin led to some 'false positives' in plastocyanin distribution, e.g. reports of its presence in red algae (see Raven et al. 1990; Falkowski and Raven 1997).
3. Nucleotide sequence data could be useful in showing whether the genetic information for only one, or for both, of the alternates is present in a given organism (e.g. Kaneko et al. 1996).
4. There is also the possibility that the gene for the cytochrome c_6 or plastocyanin expressed in the plastid of organisms (hosts) whose plastids arose from secondary endosymbiosis may not have arrived in the host with the nucleus of the eukaryote ingested in the secondary endosymbiosis that gave the host its plastid, with transfer of the genes encoding plastocyanin or cytochrome c_6 to the host nucleus.

The alternative is that the cytochrome c_6 (or plastocyanin) genes could have been present in the host nucleus from a prior round of plastid acquisition by primary (prokaryotic) or secondary (eukaryotic) endosymbiosis, a suggestion made by Cavalier-Smith (1982, 1992). Although this may not be the simplest explanation of the data for cytochrome c_6 (or plastocyanin), it has relevance for 'explaining' odd juxtapositions of various carotenoids in different algal taxa (Raven 1987a). Although there is evidence that a mutant cyanobacterium (*Synechocystis* sp. PCC 6803) lacking both plastocyanin (as a result of Cu deprivation) and cytochrome c_6 (by mutation) still have functionality in electron transfer from the cytochrome b_6-*f* complex to P_{700} culminating in photolithotrophic growth (Zhang et al. 1994), more recent work confirms that strain 6803 contains the genes for both cytochrome c_6 and plastocyanin (Kaneko et al. 1996) and that mutants of PCC 6803 with neither cytochrome c_6 nor plastocyanin are non-viable under conditions permitting phototrophic or chemoorganotrophic growth (Manna and Vermaas 1997). The Cu deficiency imposed on the *Synechocystis* sp. strain 6803 by Zhang et al. (1994) still permitted some expression of plastocyanin (and cytochrome oxidase) (Manna and Vermaas 1997). There is also the possibility that soluble cytochrome *M*, which is encoded in the genome of *Synechocystis* sp. strain 6803 (Kaneko et al. 1996) and which has a redox potential similar to that of cytochrome c_6 and of plastocyanin, could function in electron transfer from the cytochrome b_6-*f* complex to P_{700} (Kerfeld and Krogmann 1998). However, the level of expression of cytochrome *M*, at least in organisms that have normal expression of cytochrome c_6 and of plastocyanin, is much lower than that of these two redox proteins (Kerfeld and Krogmann 1998).

Plastocyanin and cytochrome c_6 have closely similar functional characteristics in the transfer of electrons between the cytochrome b_6-*f* complex and P_{700}. They also have similar molecular masses, so in terms of evolutionary optimality there is little reason to use one rather than the other. Much physiological evidence, now supported by molecular data on the control of transcription and translation, has accumulated on

the relative expression of plastocyanin and of cytochrome c_6 as a function of Cu and Fe availability, the Cu-containing plastocyanin being expressed more than the Fe-containing cytochrome c_6 when Cu availability is high but Fe availability is low, and *vice versa* for higher Fe availability than Cu availability (Falkowski and Raven 1997). Plastocyanin represents a larger fraction of the total known cellular component containing Cu than does cytochrome c_6 as a fraction of the total known cellular components containing Fe, especially in organisms lacking Cu–Zn superoxide dismutase (i.e. almost all algae, the Charophyceae *sensu lato* and peridinin-containing dinoflagellates being exceptions) the relative sparing effect on Cu use by expression of cytochrome c_6 rather than plastocyanin is greater than that on Fe use by the expression of plastocyanin rather than cytochrome c_6.

Less information is available on the occurrence of the other major alternative pair of catalysts, the iron-containing ferredoxin and the metal-free flavoprotein flavodoxin. It is believed (Raven et al. 1989) that the higher plants (bryophytes and tracheophytes) are unable to express flavodoxin and always rely on ferredoxin. Among the cyanobacteria and the algae, the alternative expression of ferredoxin and flavodoxin is common. However, by contrast with the cytochrome c_6-plastocyanin pair, there is only one reported case of the occurrence of flavodoxin to the exclusion of ferredoxin, i.e. the occurrence of flavodoxin but not ferredoxin in the marine red macroalga *Chondrus crispus* (see Raven et al. 1990).

In those algae with the potential to express either ferredoxin or flavodoxin, the 'normal' electron carrier between the sequence A_0 (a form of chlorophyll *a*, the initial electron acceptor of PS I), A_1 (phylloquinone) and the iron-sulfur complexes F_X, F_B and F_A, and the ferredoxin-NADP oxidoreductase, is ferredoxin. However, with Fe deficiency there is increasing expression of flavodoxin. Indeed, the expression of flavodoxin as detected immunologically is used as a bioassay for Fe availability in the ocean (La Roche et al. 1996). Unlike the alternative pair cytochrome c_6/plastocyanin, there is a significant difference between the catalytic effectiveness (on a molar basis) of ferredoxin and flavodoxin; ferredoxin has a higher specific reaction rate (mol electron transferred per mol protein per second) than does flavodoxin in their normal *in situ* place in non-cyclic electron transport. Although this may be a determinant of the timing of replacement of ferredoxin by flavodoxin during developing Fe deficiency in algae and cyanobacteria with the potential to express either (or both) catalysts, La Roche et al. (1996) found that flavodoxin expression in the phytoplankton of the N.E. Pacific began rather early in the development of Fe deficiency.

Linear electron flow with O_2 as electron acceptor: The Mehler peroxidase reaction

The overall reaction here is that of O_2 evolution by the water dehydrogenation reaction of the oxidizing side of PS II balanced by the reduction of O_2 to water by the reducing side of PS I. With 3 H^+ pumped per electron transferred (and per 2 photons absorbed), the H^+/ATP ratio of 4 gives 0.75 ATP produced per electron transferred and (with optimal excitation energy allocation to the two photosystems) 0.375 ATP produced per photon absorbed (Figure 3).

We now know (see 'Introduction', and Wiese et al. 1998) that the sequence of electron transport from the reducing side of PS I to O_2 involves superoxide and hydrogen peroxide as intermediates; this is termed the Mehler peroxidase reaction.

We have seen in the 'Linear electron flow with $NADP^+$ as electron acceptor' section above that linear electron transfer to $NADP^+$ with a 1:1:1:1:1 stoichiometry of trace metal-containing components has 20–23 Fe, 4 Mn and 0–1 Cu per electron transfer chain.

For the Mehler peroxidase reaction there is an additional need for trace metals as a result of the production of superoxide followed by its dismutation by a metal-containing superoxide dismutase (Fe, or Cu/Zn), and removal of the hydrogen peroxide produced by superoxide dismutase by the Fe-containing catalase or hydroperoxidase (Fe) in some cyanobacteria, glutathione peroxidase (Se) in some cyanobacteria and (eukaryotic) algae, and ascorbate peroxidase in most cyanobacteria and eukaryotic algae and all higher plants (Henry et al. 1976; Asada et al. 1980; Tel-Or et al. 1985; Shigeoka et al. 1987; Harrison et al. 1988; Price and Harrison 1988; de Jesus et al. 1989; Halliwell and Gutteridge 1989; Miyake et al. 1991; Shigeoka et al. 1991; Asada 1992; Asada et al. 1993b; Takeda et al. 1993; Kaneko et al. 1996; Stadtman 1996; Butow et al. 1997; Eshdat et al. 1997; Noctor and Foyer 1998; Obinger et al. 1998; Regelsberger et al. 1999) (Table 4). Not all of these enzymes are thylakoid-bound, but at least a fraction of cyanobacterial and plastidic superoxide dismutase and ascorbate peroxidase are thylakoid-associated (Asada et al. 1980; Mano et al. 1997).

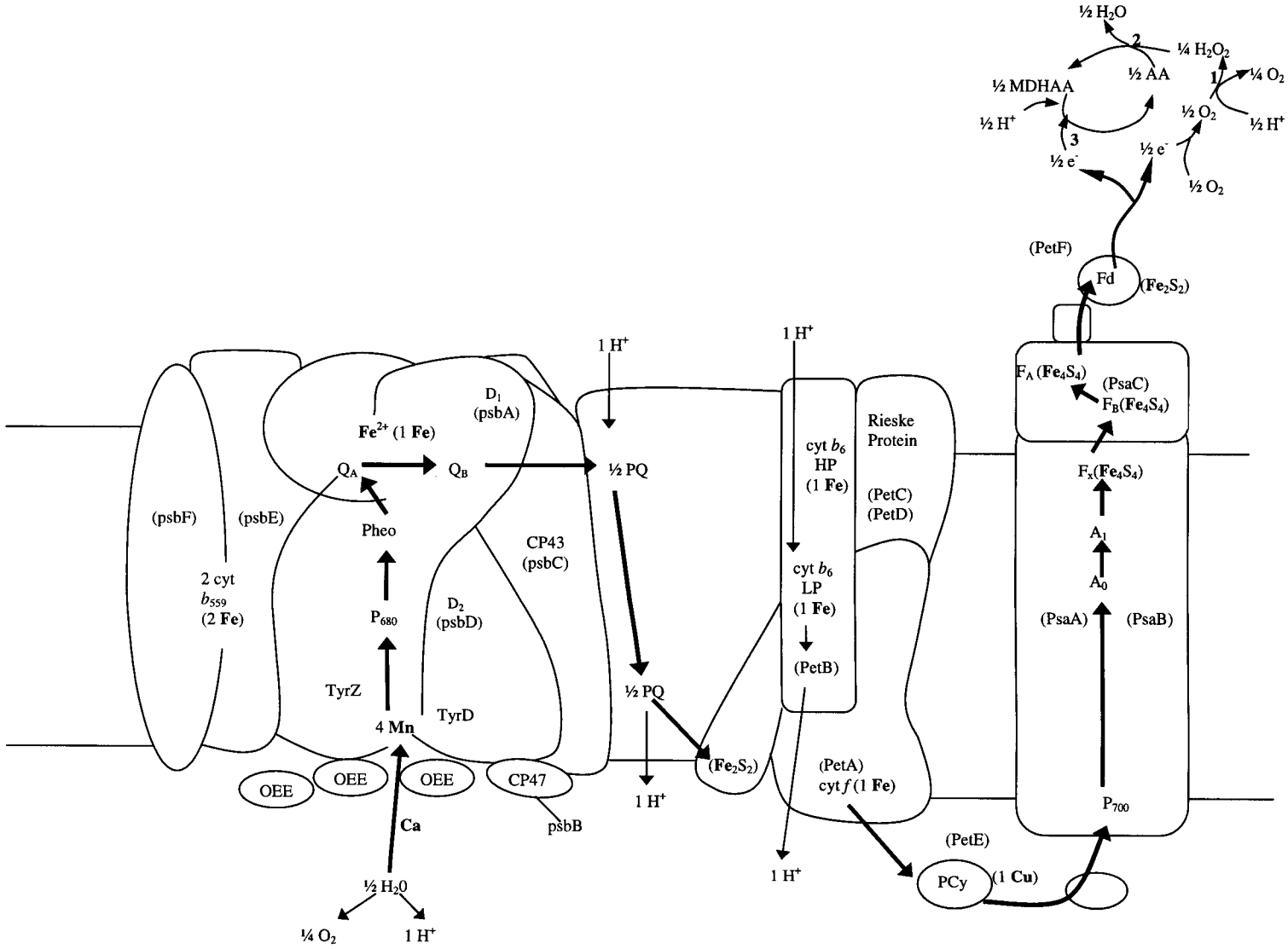

Figure 3. The Mehler peroxidase reactions at the reducing end of PS I in higher plants and charophycean algae. The reactions shown are for the thylakoid-associated enzymes superoxide dismutase (1) with 1 Cu and 1 Zn per molecule, ascorbate peroxidase (2) with 1 Fe per molecule and ferredoxin-monodehydroascorbate oxidoreductase (3) with no known trace metal requirement (Miyake and Asada 1992a,b; Miyake et al. 1993). A stromal pathway with the same trace metal electron, O_2 and H^+ stoichiometry involves NADPH, NADPH-oxidized glutathione oxidoreductase, and reduced glutathione-dehydroascorbate oxidoreductase.

Table 4. Turnover of metal-containing catalysts in different mechanisms of disposing of the $O_2^{\cdot -}$ produced in the Mehler reaction by PS I

Organisms	Superoxide dismutase turnovers per 4 electrons from PS I	H_2O_2-disposing enzyme turnovers per 4 electrons from PS I[b]
'Primitive' cyanobacteria	Two turnovers of Fe–SOD	Two turnovers of (Fe) catalase or hydroperoxidase
Many cyanobacteria; most eukaryotic algae (except Charophyceae and peridinin-containing Dinophyceae) (in absence of Se)	One turnover of Fe–SOD	One turnover of (Fe) ascorbate peroxidase[a]
Charophycean green algae; peridinin-containing Dinophyceae; higher plants	One turnover of Cu–Zn SOD	One turnover of (Fe) ascorbate peroxidase[a]
Chlamydomonas, many diatoms, grown with Se; some cyanobacteria	One turnover of Fe–SOD	One turnover of (Se) glutathione peroxidase[a]
(Aquatic plants?)	(Two turnovers of Fe–SOD (algae), Cu–Zn SOD (higher plants?)	No enzyme used; H_2O_2 efflux?)

[a]One turnover of ascorbate on glutathione peroxidase is based on the overall stoichiometry for the two electron reaction in which one ascorbate (or two GSH) is converted to one dehydroascorbate (or one GSSG). While this is appropriate for glutathione peroxidase, the ascorbate peroxidase reaction converts two ascorbate to two monodehydroascorbate. In the case of the stromal enzyme the two monodehydroascorbate molecules dismute to one ascorbate and one dehydroascorbate (which is reduced in a two electron reaction by GSH to regenerate ascorbate), while for the thylakoid-associated enzyme the two monodehydroascorbate molecules are reduced by ferredoxin to regenerate two ascorbate (see Miyake and Asada 1994).

[b]References: Henry et al. (1976); Asada et al. (1980); Price et al. (1987); Price and Harrison (1988); Harrison et al. (1988); de Jesus et al. (1989); Halliwell and Gutteridge (1989); Miyake et al. (1991); Shigeoka et al. (1991); Asada (1992); Miyake and Asada (1992a.b); Asada et al. (1993b); Miyake and Asada (1993); Takeda et al. (1993); Ogawa et al. (1995); Kaneko et al. (1996); Mano et al. (1997); Stadtman (1996); Butow et al. (1997); Eshdat et al. (1997); Regelsberger et al. (1999).

Attempts to compute the minimum requirement of Fe, Cu, Zn and Se downstream of ferredoxin in the various forms of the Mehler reaction relative to those of linear electron transport requires knowledge of the flux of electrons through the various reactions relative to those through the linear pathway, and of the specific reaction rate of the enzymes involved.

Dealing first with the stoichiometry issue, the simplest process is that found in 'primitive' cyanobacteria wherein (Fe) superoxide dismutase activity is followed by that of catalase. Here two turnovers of Fe superoxide dismutase and of Fe-containing catalase are needed for every four electrons which eventually end up in 2 H_2O (Table 4). This 'primitive' cyanobacterial state can be produced in higher plants; thus, expression of *Escherichia coli* catalase in chloroplasts of *Nicotiana tabacum* greatly reduces the expression of ascorbate peroxidase (Shikanai et al. 1998b). In other cyanobacteria and eukaryotic algae other than the Charophyceae in the Division Chlorophyta and the peridinin-containing dinoflagellates, the H_2O_2 produced by (Fe) superoxide dismutase is fur-

ther metabolized by Fe-containing ascorbate peroxidase. However, in this case, only half as much H_2O_2 is generated per electron flowing out of PS I as in the catalase case mentioned above. This is because only two of these electrons pass from the reducing end of PS I to H_2O via $O_2^{\cdot -}$ and H_2O_2: the other pair of electrons is routed via ferredoxin, NADPH and glutathione to reduce back to ascorbate the dehydroascorbate generated by ascorbate peroxidase in the case of soluble (stromal) ascorbate peroxidase, and *via* ferredoxin to reduce back to ascorbate the monodehydroascorbate generated by the thylakoid-bound ascorbate peroxidase (Table 4; Figure 3). For charophycean green algae, higher plants and peridinin-containing dinoflagellates the situation is as described for the ascorbate peroxidase-containing cyanobacteria and non-charophycean algae, except that the superoxide dismutase involved is the Cu–Zn type rather than the Fe form. A further stoichiometric variation is in those eukaryotic algae (*Chlamydomonas*; some diatoms) expressing glutathione peroxidase, where a pair of electrons is transferred via one turnover each of Fe superoxide dismutase and of Se-containing glutathione peroxidase (Table 4).

The final possibility was suggested by Collén (1994) and Collén and Pedersén (1996), who pointed out that the high membrane permeability of H_2O_2 makes H_2O_2 efflux an option for disposing of this intermediate in the Mehler peroxidase reaction in aquatic organisms with a large extracellular sink for H_2O_2 (see Patterson and Myers 1973) (Table 4). Collén (1994) and Collén and Pedersén (1996) found a significant efflux of H_2O_2 from the green marine (ulvophycean) macroalga *Ulva lactuca* relative to the (presumed) rate of H_2O_2 production in the Mehler reaction in the light, bearing in mind that the Ulvophyceae have glycolate dehydrogenase rather than the H_2O_2-generating glycolate oxidase which deals with the small glycolate production rate found in those ulvophyceans (e.g. *Ulva*) with a CO_2 concentrating mechanism (Suzuki et al. 1991; Raven 1997c).

A diffusive efflux of H_2O_2 in algae is rendered more plausible by the smaller inhibition of photosynthesis by (added) H_2O_2 in those algae tested than in higher land plants (Takeda et al. 1995; Korb and Raven, unpublished data). In *Chara*, a close freshwater relative of higher plants, photosynthetic CO_2 fixation appears to be more sensitive to H_2O_2 than in other algae (Korb and Raven, unpublished data). The high permeability of phospholipid bilayers to H_2O_2 (Takahashi and Asada 1983; Mathai and Sitavaman 1994), augmented by H_2O_2 permeation through aquaporins (Henzler and Steudle 1998) also renders a diffusive efflux of H_2O_2 in algae more plausible, although aquaporins may not occur in all plasmalemmas, or at all in plastid envelopes (Raven 1982, 1995). The 'basal' H_2O_2 permeability is equivalent to a water layer of 200 μm. This diffusion boundary layer thickness is similar to what may occur around many aquatic macrophytes in poorly stirred environments (Raven 1984).

The efflux of *all* of the H_2O_2 produced in the Mehler reaction would obviate the need for H_2O_2-metabolizing enzymes such as catalase, ascorbate peroxidase or glutathione peroxidase, provided that the H_2O_2 levels were reduced to values similar to those achieved by these enzymes. Such an absence of H_2O_2-metabolizing enzymes would mean two turnovers of Fe superoxide dismutase per four electrons from PS I used in producing 2 H_2O_2. However, all O_2-evolvers have activities of at least one of the H_2O_2-metabolizing enzymes, so the efflux possibility suggested by Collén (1994) apparently does not obviate the need for enzymic H_2O_2 disposal. Furthermore, the rates of H_2O_2 efflux in *Enteromorpha intestinalis* and *Codium fragile* in the light (and the dark) are much lower (Korb and Raven, unpublished) than the rate measured in *Ulva lactuca* (a very close relative of *Enteromorpha*) by Collén (1994) and Collén and Pedersén (1996). The conclusion about diffusive H_2O_2 efflux is that it is not a universal and major means of disposing of H_2O_2 produced in the Mehler reaction in aquatic organisms, and hence of possibly economizing on metal use.

The very low membrane permeability of $O_2^{\cdot -}$, and its very high reactivity (Takahashi and Asada 1983) mean that there is no possibility of replacing superoxide dismutase by the efflux of $O_2^{\cdot -}$ in aquatic organisms.

Quantification of minimal metal requirements for the Mehler (peroxidase) reaction requires knowledge of the specific reaction rate (on a catalytic metal basis) of the enzymes involved at the *in vivo* substrate concentrations. Specific reaction rate is not a simple function of the (sole) substrate concentration for two of the enzymes considered here, i.e. superoxide dismutase and catalase, since these dismutation reactions involve two $O_2^{\cdot -}$ (and 2 H^+) and two H_2O_2 respectively.

Assuming a maximum specific reaction rate of 400 s^{-1} for our idealised linear redox chain with a 1:1:1:1:1 stoichiometry at light saturation (i.e. 400 electrons transferred per redox chain per second: see

Raven 1990), then we can use published specific reaction rates of the enzymes to compute how much of the enzyme is needed to consume the active oxygen species derived from the reaction of ferredoxin, etc., with O_2. Superoxide dismutase has a specific reaction rate of 10^6 s^{-1} (mol $O_2^{\cdot -}$ mol^{-1} enzyme s^{-1}, i.e. mol electron mol^{-1} enzyme s^{-1}), so that only $400/10^6$ or 4.10^{-4} mol SOD would be needed per redox chain *if* the steady-state $O_2^{\cdot -}$ level were sufficient to saturate the enzyme ($\sim$ 1 mol m^{-3}): Schomburg and Stephan (1994). However, a higher ratio of superoxide dismutase to redox chain would be needed if the steady-state $O_2^{\cdot -}$ concentration at the active centre of superoxide dismutase is lower, and especially if diffusion of $O_2^{\cdot -}$ from each of 5000 redox chains to a superoxide dismutase molecule leads to high concentrations of $O_2^{\cdot -}$ at the site of $O_2^{\cdot -}$ generation, which might cause more damage than would otherwise be the case; with 1 Fe, or 1 Cu + 1 Zn per superoxide dismutase molecule, there must be at least 4×10^{-4} mol Fe, or 4×10^{-4} mol Cu + Zn per mol redox chain. These computed SOD requirements apply when catalase is the agent removing H_2O_2; if peroxidase is used, only half as much superoxide dismutase, and hence Fe or Cu + Zn is needed per linear redox chain (Tables 4 and 5). The volume-based quantities of Cu–Zn superoxide dismutase in chloroplasts (Asada and Takahashi 1987), together with the data in Table 4.1 of Lawlor (1993), yields values of up to 0.5 mol superoxide dismutase per mol electron transport chain, i.e. 10^3 times the computed minimum value. Recent data show even higher values of 1.0 mol superoxide dismutase per mol PS I (or per mol electron transport chain): Ogawa et al. (1995). This apparent excess capacity (see Table 5) is related to maintaining steady-state $O_2^{\cdot -}$ concentrations at far below the concentration needed to saturate superoxide dismutase (Asada and Takahashi 1987). However, transgenic experiments have shown that SOD activity in the chloroplast may restrict the rate of the Mehler (peroxidase) reaction under some conditions (Arisi et al. 1998).

One of the enzymes disposing of H_2O_2, i.e. catalase, has a very high specific reaction rate at substrate (H_2O_2) saturation, i.e. 16 000 s^{-1} (Schomburg et al. (1994). In view of the complex kinetics of catalase, it is difficult to assess the rate at the 'normal' *in vivo* H_2O_2 concentration of perhaps 0.1 mol m^{-3} H_2O_2 but would be at most one-tenth of the cited H_2O_2-saturated rate or 1600 s^{-1}. In terms of the redox reactions of H_2O_2 and of linear electron flow, this corresponds to an electron flux of 3200 mol electron mol^{-1} catalase s^{-1}, so that the minimum catalase content needed is 400/3200 or 0.125 mol catalase per mol non-cyclic redox chain; with 4 Fe per molecule this means 0.5 mol Fe per mol redox chain (Table 5).

Ascorbate peroxidase has a H_2O_2 (and ascorbate) saturated specific reaction rate of 323 mol H_2O_2 mol^{-1} enzyme s^{-1}, or 646 mol electron mol^{-1} enzyme s^{-1} (Schomburg et al. 1994). Because two electrons pass through the linear redox chain per electron passing through H_2O_2 into ascorbate peroxidase, the minimum ascorbate peroxidase needed is 400/1252 or 0.32 mol enzyme (0.32 mol Fe) per mol linear redox chain (Table 5). More enzyme is needed if the H_2O_2 level is less than the 0.15 mol m^{-3} H_2O_2 needed to saturate the enzyme (Table 5). Data in Asada and Takahashi (1987) on the quantity of ascorbate peroxidase per unit chloroplast volume and data in Table 4.1 of Lawlor (1993) give values of 0.02–0.1 mol ascorbate peroxidase per mol linear redox chain, i.e. rather less than the value computed above. However, more recent data (Miyake et al. 1993) show that there is about 1 mol ascorbate peroxidase (half thylakoid-bound) per mol linear redox chain, i.e. in excess of what is required, and consistent with a lower steady-state concentration of H_2O_2 in chloroplasts than is needed to saturate the enzyme.

For glutathione peroxidase the reported specific reaction rate is 3100 mol H_2O_2 mol^{-1} enzyme s^{-1} at substrate (H_2O_2) saturation (Schomburg et al. 1994). Because the affinity for at least organic hydroperoxidases can be high (K_m 0.006 mol m^{-3}) this specific rate may also apply to the reaction with *in vivo* concentrations of H_2O_2 in chloroplasts. An *in vivo* rate of 3100 mol H_2O_2 mol^{-1} enzyme s^{-1} means a rate of 6200 mol electron mol^{-1} enzyme s^{-1}. As the stoichiometry of electron flow through glutathione peroxidase to that through the non-cyclic redox chain in the Mehler peroxidase reaction is, as for ascorbate peroxidase, 1:2, the minimum required ratio of ascorbate peroxidase to linear redox chains is 400/12,400 or 0.032 mol enzyme per mol redox chain. With 2 Se per molecule this corresponds to 0.064 mol Se per mol redox chain (Table 5).

These computations of the minimum requirement for trace metals in the Mehler catalase and Mehler peroxidase reactions are summarised in Table 5. To put these values on a whole thylakoid basis it is necessary to know what fraction of the electron flow through the linear redox chain can flow through the $O_2^{\cdot -}$-consuming and H_2O_2-consuming reactions of the Mehler catalase and Mehler peroxidase reactions.

Table 5. Computed (in text) ratio of the *minimum* content of enzyme (and their trace metals) dealing with active O species per idealized non-cyclic redox chain for the 'Mehler catalase' reaction and for the 'Mehler peroxidase' reaction using ascorbate or glutathione peroxidase

Reaction	Enzyme	Computed minimum (and observed) content per non-cyclic redox chain	Conputed minimum (and observed) trace metal content per non-cyclic redox chain
'Mehler catalase' in 'ancestral' Catalase	Fe superoxide dismutase	4.10^{-4}	4.10^{-4} (Fe)
	Catalase or hydroperoxidase	0.125	0.5 (Fe)
'Mehler ascorbate peroxidase' in most cyanobacteria and algae other than Charophyceae and peridinin-containing Dinophyceae	Fe superoxide dismutase	2.10^{-4}	2.10^{-4} (Fe)
	Ascorbate peroxidase	0.32	0.32 (Fe)
'Mehler glutathione peroxidase' in some algae	Fe superoxide dismutase	2.10^{-4}	2.10^{-4} (Fe)
	glutathione peroxidase	0.032	0.064 (Se)
'Mehler ascorbate peroxidase' in Charophyceae; peridinin-containing Dinophyceae and higher plants	Cu, Zn superoxide dismutase	2.10^{-4} (1.0)	2.10^{-4} (Cu) (1.0 (Cu)) 2.10^{-4} (Zn) (1.0 (Zn))
	Ascorbate peroxidase	0.32 (0.5)	0.32 (Fe)

Where data are available (Miyake et al. 1993; Ogawa et al. 1995), the observed contents are given in brackets beside the computed values.

In some cyanobacteria and chlorophyte microalgae, the O_2 uptake and evolution rate in the Mehler reaction in the induction phase upon illumination after a dark period is equal to the CO_2 fixation and O_2 evolution rates in subsequent steady-state photosynthesis, i.e. the electron flow through the reactions involving active oxygen species can equal the maximum capacity of linear electron flow to CO_2 (Radmer and Kok 1976; Radmer, Kok and Ollinger, 1978; Radmer and Ollinger 1980; 1981; Kana 1990, 1992, 1993). Data from higher plants do not generally show such a high potential for the Mehler peroxidase reaction relative to linear electron flow to other acceptors (Badger 1985). Thus, in at least some algae, and cyanobacteria, the ratios cited in Table 5 can be taken as referring to the *whole* linear electron transport catalytic capacity, while in other organisms it could refer to a smaller fraction (Osmond and Grace 1995; Biehler and Fock 1996; Cheeseman et al. 1997) of the overall catalytic capacity of the linear redox chain.

With 3 Fe per PS II, 5 Fe per cyt b_6-f, 12 Fe per PS II, 2 Fe per ferredoxin and 1 Fe per ascorbate peroxidase, and a 1:1:1:1:1 ratio of these 5 catalysts, the Fe requirement of the Mehler peroxidase reaction is 23 Fe per electron transport chain. This ratio relates to a higher plant, where there is the additional requirement of 2 Cu (1 Cu for plastocyanin, 1 Cu for Cu–Zn superoxide dismutase, both present at 1 molecule per electron transport chain). In algae with cytochrome c_6 rather than plastocyanin, and Fe–SOD rather than Cu–Zn SOD, the Fe requirement is 25 Fe and 0 Cu per electron transport chain. The Fe requirement is reduced by 2 if flavodoxin replaces ferredoxin.

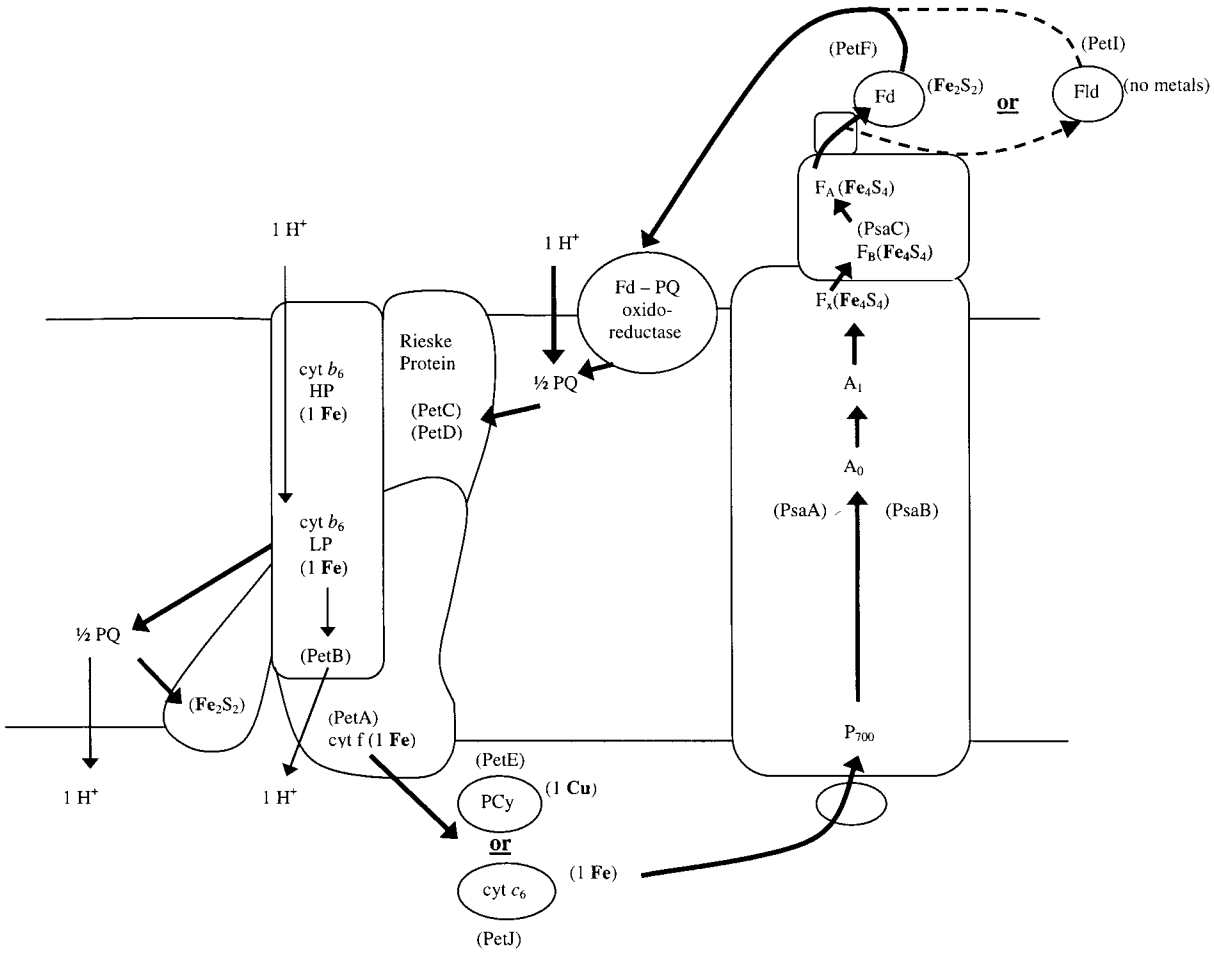

Figure 4. The spatial arrangement in the thylakoid membrane of the components of cyclic electron transport with involvement of ferredoxin-PQ oxidoreductase, showing involvement of trace metals.

Cyclic electron transport, chlororespiration and the role of NAD(P)H dehydrogenase

Cyclic electron transport around PS I (Figures 4 and 5), with associated H^+ pumping and ADP phosphorylation, can be demonstrated *in vivo*, in chloroplasts *in vitro* and (with supplementation of redox catalysts) in isolated thylakoids (Raven 1976a,b; Herbert et al. 1990; Heber and Walker 1992; Bendall and Manusso 1995). The extent to which cyclic electron transport and photophosphorylation occur in parallel with the non-cyclic or Mehler peroxidase variants of linear electron transport in the same thylakoid membrane is unclear (Bendall and Manusso 1995). Also unclear is the maximum capacity for cyclic electron transport under optimal redox conditions and at light saturation, although the available data suggest a value of 10% or less of the capacity for linear-chain electron transport except in specialized cells such as the N_2-fixing heterocysts of certain filamentous cyanobacteria and the bundle sheath cells of NADPme-C_4 flowering plants (Raven 1976a,b; Cha and Mauzerall 1992; Fork and Herbert 1993; Asada et al. 1993a; Bendall and Manusso 1995; Malkin and Canaani 1994).

Whatever the pathway(s) of cyclic electron transport around PS I *in vivo* there seems to be a role for A_0, A_1, F_X, F_B, F_A and ferredoxin or flavodoxin at the reducing end of the pathway, and for PQ, the cytochrome b_6-f complex and cytochrome c_6 or plastocyanin at the oxidizing end of the pathway (Bendall and Manusso 1995; Miyake et al. 1995; Scheller 1996). Even this incomplete pathway, with no link between ferredoxin or flavodoxin and PQ, has a very substantial requirement for trace metals. Thus, with a 1:1:1:1: stoichiometry of

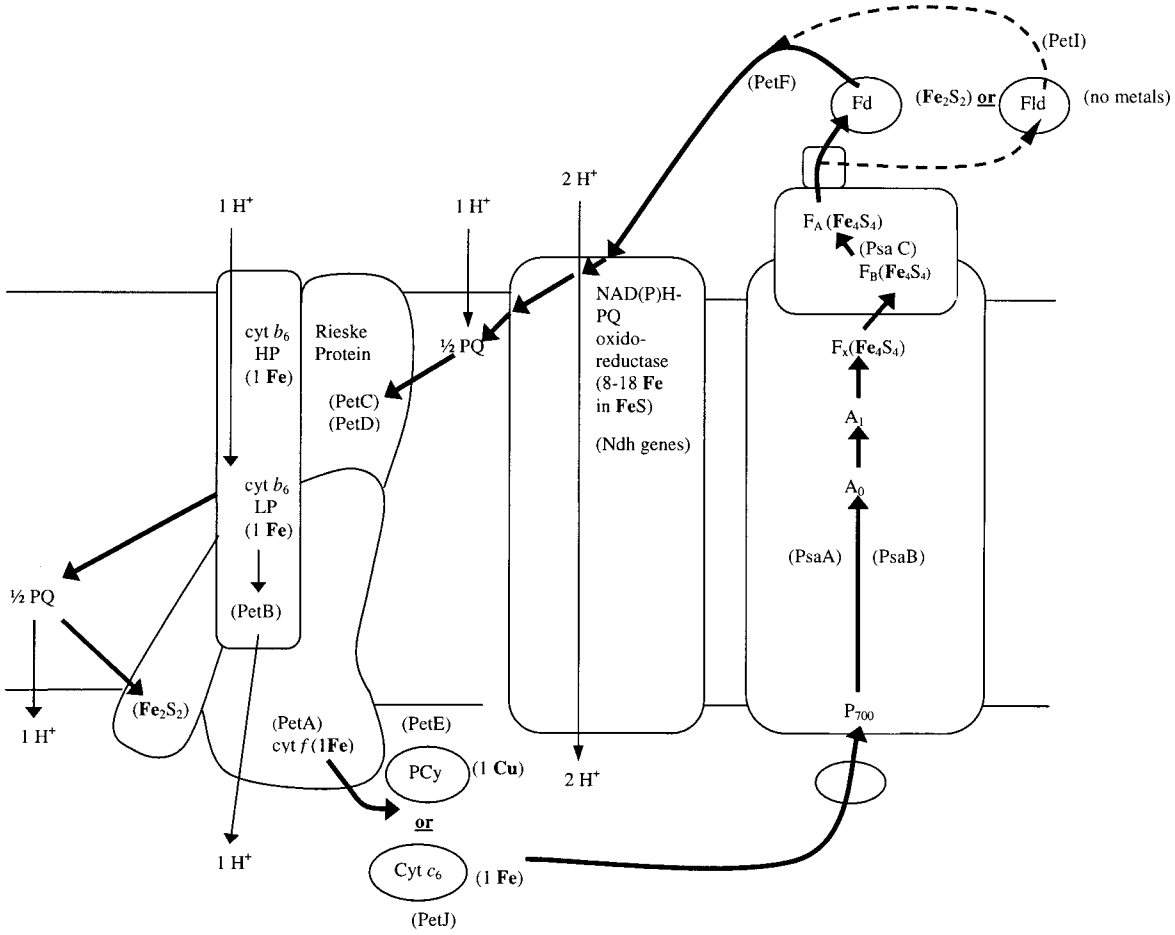

Figure 5. The spatial arrangement in the thylakoid membrane of the components of cyclic electron transport with involvement of NAD(P)H-PQ oxidoreductase, showing involvement of trace metals.

cytochrome b_6-f, cytochrome c_6, PS I and ferredoxin there is a requirement for 20 Fe, no Mn and no Cu for each cyclic electron transport chain, while with a 1:1:1:1:1 stoichiometry of cytochrome b_6-f, plastocyanin, PS I and flavodoxin there are 17 Fe, no Mn and 1 Cu in each cyclic electron transport chain. These trace metal requirements apply to those pathways of cyclic electron transport that do not contain Fe for the ferredoxin (or flavodoxin) to PQ segment of cyclic electron transport, e.g. a 'direct' ferredoxin-PQ oxidoreductase (Bendall and Manusso 1995) or some sequence involving the *psaE* gene product (Yu et al. 1993; Zhao et al. 1993).

A significantly greater Fe requirement would follow from involvement of NAD(P)Hdh (NAD(P)H: PQ oxidoreductase) in cyclic electron flow (Mi et al. 1992a.b; Bendall and Manusso 1995; Scheller 1996; Endo et al. 1997a,b, 1998; Teicher and Scheller 1998). Depending on the assumed Fe content of the NAD(P)H-PQ oxidoreductase (8–18 Fe per complex: Siedow 1995; Brandt 1997), a cyclic electron pathway could have from 25 Fe (1:1:1:1:1 stoichiometry of NAD(P)H-PQ oxidoreductase (8 Fe):cytochrome b_6-f complex: plastocyanin:PS I:flavodoxin) to 38 Fe (1:1:1:1:1 stoichiometry of NAD(P)H-PQ oxidoreductase (18 Fe), cytochrome b_6-f complex:cytochrome c_6: PS I:ferredoxin). Before discussing these data in the context of the iron requirement, and photon cost, of cyclic photophosphorylation, we shall briefly consider a second possible function of NAD(P)-PQ oxidoreductase, i.e. chlororespiration.

Chlororespiration (Bennoun 1982; Godde 1982; Bruce et al. 1983; Bennoun 1983; Caron et al. 1986; Klöck et al. 1989; Klöck et al. 1989; Scherer 1990; Wilhelm and Duval 1990; Avelange and Rebeille 1991; Peltier and Schmidt 1991; Walker 1992; Groom et al. 1993; Ting and Owens, 1993; Bennoun 1994; Schmetterer 1994; Buchel and Garab 1995; Endo and Asada 1996; Casper-Lindley and Björkman 1997; Buchel et al. 1998; Feild et al. 1998) involves the oxidation of carbohydrate within chloroplasts in darkness or in low light, ultimately using O_2 as the terminal electron acceptor, and appears to function in generating ATP in the dark and in low light.

The pathway of chlororespiration is unclear. Studies on mutants of *Chlamydomonas reinhardtii* (Bennoun 1993) show that chlororespiration does not obligatorily involve the Rieske Fe–S protein or the cytochrome *f* components of the cytochrome b_6-*f* complex, plastocyanin, or the CF_0–CF_1 ATP synthetase (see also Feild et al. 1998, for work on higher plants). Belkhodja et al. (1998) found more expression of chlororespiratory (dark PQ reduction) in Fe-deficient leaves of higher plants than of controls, a result which would not be predicted from the involvement of the Fe-rich NAD(P)H-PQ oxidoredustase or cytochrome b_6-*f* complex. Evidence on the nature of the terminal oxidases, and hence of their trace metal content, does not yield a clear picture (Bennoun 1982; Buchel and Garab 1995; Goyal and Tolbert 1996; Wiekkowski and Bojko 1997; Feild et al. 1998). Indeed, Rich et al. (1998; see also Bennoun 1994), having taken great care to minimize contamination from other organelles, conclude that higher plant chloroplasts lack NAD(P)H-PQ oxidoreductase and plastoquinol oxidase, and that they lack cytochrome *c* or alternative oxidase.

Requirement for cyclic photophosphorylation

The requirement for cyclic photophosphorylation is apparently variable among different photosynthetic cells; on a unit chromophore basis the requirement is probably highest in heterocysts of cyanobacteria and bundle sheath cells of C_4 NADPme plants (see Bendall and Manusso 1995). In other photosynthetic cells the *in vivo* capacity for cyclic photophosphorylation is apparently lower, i.e. only about one-tenth that of linear photophosphorylation (Raven 1976a). Although this restricted capacity may relate to the capacity of ATP-consuming reactions *in vivo* under conditions that isolate cyclic photophosphorylation as the ATP source, it is worth considering possible Fe-economies of limiting the capacity of the ferredoxin/NAD(P)H to PQ (i.e. cyclic-specific and chlororespiration-specific) part of the pathway by restricting the quantity of the catalysts of this redox pathway. The *in vivo* specific reaction rate of mitochondrial NADH-UQ oxidoreductase is high relative to that of subsequent redox complexes in the mitochondrial electron transport chain (Siedow 1995; Whitehouse and Moore 1995) and bearing in mind the low demand for the products of NAD(P)H-PQ oxidoreductase in cyclic redox reactions, considerable Fe economies could occur if the NAD(P)H-PQ oxidoreductase content was very low. However, there must be a lower limit on the number of NAD(P)H-PQ oxidoreductase complexes per unit light-harvesting pigment if the high photon yield of cyclic photophosphorylation *in vivo* (Raven 1976a) is to be explained. This is because there must be limits on excitation energy migration from the totality of pigments associated with PS I reaction centres to the few PS I reaction centres associated (albeit via the membrane-dissociable ferredoxin or flavodoxin) with the restricted number of NAD(P)H-PQ oxidoreductase complexes, which then interact (via the mobile PQH_2/PQ pool) with the cytochrome b_6-*f* complex and thence (via membrane-dissociable cytochrome c_6 or plastocyanin) to the oxidizing end of the same few PS I reaction centres. Of course, similar quantitative arguments (complexes per unit chlorophyll) apply to any non-Fe-requiring means of electron transfer from ferredoxin or flavodoxin to PQ, but without the implications for Fe cost.

Now that we have considered the two putative client reactions for a functional NAD(P)H-PQ oxidoreductase, we can consider the evidence that this Fe-costly redox complex is involved in these reactions, and the consequences of its involvement relative to other, relatively uncharacterized, redox reactions.

The occurrence of NAD(P)H-PQ oxidoreductase genes

The genes encoding this complex are found in the genome of cyanobacteria (Kaneko et al. 1996) and (with some exceptions) in the plastid genome of 'green plants', i.e. the higher plants or embryophytes, and the green algae *sensu lato* including not only the Chlorophyta but also the euglenoids and chlorarachniophytes, which obtained plastids by (second-

ary) endosymbiosis of unicellular members of the green algae *sensu stricto* (Bhattacharya and Medlin 1998). Organisms within the green plants that lack the plastid-located NAD(P)H-PQ (*ndh*) genes include some flowering plant parasites, some *Pinus* spp., two *Chlamydomonas* spp. and *Euglena gracilis* (de Pamphilis and Palmer 1990; Haberhauser and Zetschke 1994; Wakagusi et al. 1994).

However, there are no known occurrences of *ndh* genes in the plastid genome of red algae, nor of those algae (e.g. diatoms, brown algae, cryptophytes, haptophytes) which obtained their plastids by (secondary) endosymbiosis of unicellular members of the red algae (Reith 1995). Thus, any plastid-expressed NAD(P)H-PQ oxidoreductases in these organisms lacking *ndh* genes in their plastid genome must have these genes for this redox complex in their nuclei; although this is not impossible, there is apparently no evidence that it occurs (Reith 1995; Martin et al. 1998).

In summary, plastid-located *ndh* genes are found in many but not all of the O_2-evolving eukaryotes tested. Although a nuclear location of these genes in organisms lacking the plastidial genes is possible, there is no apparent evidence that this is the case, despite the occurrence of cyclic electron transport and photophosphorylation (Raven 1976a,b, 1984; Cha and Mauzerall 1992; Fork and Herbert 1993; Malkin and Canaani 1994; Bendall and Manusso 1995) and chlororespiration (Caron et al. 1986; Büchel and Garab 1995) in organisms both with and without known NAD(P)H-PQ oxidoreductase genes.

The expression of NAD(P)H-PQ oxidoreductase genes

The NAD(P)H-PQ oxidoreductase genes are expressed in cyanobacteria where they function in normal dark respiration as well, apparently, as cyclic electron flow and photophosphorylation (see below, and Scherer 1990; Mi et al. 1992a, b, 1995; Schmetterer 1994; Tanaki et al. 1997). In eukaryotes it is clear that steps must be taken to prevent interference from the mitochondrially expressed NADH-UQ oxidoreductase in the immunological or functional assay of plastidial NAD(P)H-PQ oxidoreductase (Rich et al. 1998; Teicher and Scheller 1998). Thus, while there is evidence for expression of NAD(P)H-PQ oxidoreductase in thylakoids of green algae *sensu stricto* (Godde 1982) and of higher plants (Berger et al. 1993; Guedeney et al. 1996; Kubicki et al. 1996; Endo et al. 1997a,b; Wiekkowski and Bojko 1997; Burrows et al. 1998; Sazanov et al. 1998a,b; Shikanai et al. 1998a; Teicher and Scheller 1998), it is important to bear in mind the need for rigorous exclusion of mitochondrial contamination.

Quantitation of expression has been less frequently attempted. There are semi-quantitative indications that, as expected if there is a predominant role of cyclic electron flow in bundle sheath cells of NADPme-C_4 plants and NAD(P)H-PQ oxidoreductase is involved in this cyclic electron flow, there is greater expression of NAD(P)H-PQ oxidoreductase in bundle sheath chloroplasts than in mesophyll chloroplasts of the NADPme-C_4 plant *Sorghum bicolor* (Kubicki et al. 1996). More quantitative estimates include those of Sazanov et al. (1998a) who found one NAD(P)H-PQ oxidoreductase complex per 100 (linear) 'electron transport chain' in the C_3 *Pisum sativum* chloroplasts, and Burrows et al. (1998) who found one NAD(P)H-PQ oxidoreductase per 70 PS II in the C_3 *Nicotiana tabaccum*. As there are 22 Fe per linear electron transport chain with 3 Fe in PS II, 5 Fe in the cytochrome b_6-f complex, 12 Fe in PS I and 2 Fe in ferredoxin, and 8–18 Fe per NAD(P)H-PQ oxidoreductase (see above), there is 86–275 times as much Fe in the components of linear electron transport as in NAD(P)H-PQ oxidoreductase.

The low level of expression of NAD(P)H-PQ oxidoreductase relative to that of the components of the linear electron transport pathway in eukaryotes imposes constraints on the capacity of electron flow through the NAD(P)H-PQ oxidoreductase relative to that of linear electron transport. One feature that may help to offset the low level of expression of NAD(P)H-PQ oxidoreductase is that it might, by analogy with the respiratory NAD(P)H-UQ oxidoreductase have a high specific reaction rate relative to other photosynthetic redox agents; NAD(P)H-UQ oxidoreductase has a higher specific reaction rate than do subsequent respiratory redox catalysts (Whitehouse and Moore 1995). This permits the same steady state electron flux during NADH oxidation by O_2 to occur through the NAD(P)H-UQ oxidoreductase as occurs through the more abundant cytochrome b-c_1 complex and the even more abundant cytochrome oxidase (Siedow 1995; Whitehouse and Moore 1995).

However, the inequality of abundance is much greater in the case of the plastid NAD(P)H-PQ oxidoreductase. Even if the maximum electron transport rate through the NAD(P)H-PQ oxidoreductase could approach a value that is 70 (*Nicotiana*) – 100 (*Pisum*) times that through linear electron trans-

port, there might be constraints on the rate at which electrons could be delivered from the NAD(P)H-PQ oxidoreductase to the cytochrome b_6-f complex *via* PQ/PQH_2, despite the known lateral transport properties of PQ/PQH_2 in the thylakoid membrane, from PS II in appressed to cytochrome b_6-f and PS I in non-appressed regions of eukaryotic green plant thylakoids. In fact NAD(P)H-PQ oxidoreductase occurs with PS I and cytochrome b_6-f in non-appressed thylakoids of *Nicotiana*, thus diminishing the extent of transport of PQ/PQH_2 that would be needed. Nevertheless, the low level of expression and the likelihood that the NAD(P)H-PQ oxidoreductase can only catalyze a specific rate of electron flow which is a few-fold greater than the specific flux through the linear electron transport chain means that the level of expression of NAD(P)H-PQ oxidoreductase would only permit a light-saturated electron flux through the NAD(P)H-PQ oxidoreductase at 5–10% of the linear electron flux. Although the flux-limiting steps in chlororespiration are not known, it is of interest that the chlororespiratory rate in *Helianthus annus* is only 1/300 that of linear electron transport at light saturation (Feild et al. 1998).

Spatial constraints may apply to the light-limited rate of electron transfer through NAD(P)H-PQ oxidoreductase if it is functional in cyclic electron transport. Here the argument involves the number of PS I complexes with which each of the rare NAD(P)H-PQ oxidoreductase complexes can inteact. If this number is less than the overall ratio of PS I to NAD(P)H-PQ oxidoreductase, then a maximal photon yield of cyclic electron transport demands that excitation energy is transferred efficiently from PS I units that are not in redox contact with NAD(P)H-PQ oxidoreductase to those that are in such contact. Here, the argument does not concern the maximum specific reaction rate of NAD(P)H-PQ oxidoreductase; these quantum yield considerations are all within the ceiling imposed by this maximum specific reaction rate. We shall return to this photon yield question again under 'Fe requirements for cyclic, and Mahler reaction electron transport and H^+ pumping: Fe and photon costs' section below.

Evidence for functioning of NAD(P)H-PQ oxidoreductase in cyclic electron flow and chlororespiration

A powerful tool in assessing the role of NAD(P)H-PQ oxidoreductase in cyclic electron flow and chlororespiration, and in metabolism more generally, is to inactivate one or more of the *ndh* genes that encode parts of the NAD(P)H-PQ oxidoreductase complex. Generally, deletions or inactivations have no phenotypic effect under optimal growth conditions.

The absence of a phenotypic effect of NAD(P)H-PQ oxidoreductase deletion under optimal growth conditions has been reported for cyanobacteria (Schluchler et al. 1993; Yu et al. 1993; Zhao et al. 1993; Howitt et al. 1996; Klugehammer et al. 1997; Sültemeyer et al. 1997; Ohkawa et al. 1998) and for C_3 flowering plants (Rochaix 1997; Burrows et al. 1998; Sazanov et al. 1998a,b; Shikanai et al. 1998a; Nixon and Malinga 1999; Roldán 1999). These results indicate that if any NAD(P)H-PQ oxidoreductase-requiring process, such as cyclic electron flow and photophosphorylation, and chlororespiration, are needed for growth under optimal conditions then there must be a bypass (or bypasses) of the step involving NAD(P)H-PQ oxidoreductase, which permits the function to be fulfilled to an extent that does not interfere with normal growth and development.

However, phenotypic effects of preventing NAD(P)H expression have been found under sub-optimal growth conditions in cyanobacteria. *Synechocystis* sp. strain PCC 7002 had a reduced rate of photolithotrophic growth at low (but not high) photon flux density, and was unable to grow photoorganotrophically on glycerol when PS II activity was inhibited by DCMU (Schluchler et al. 1993). These two effects can be attributed to decreases in cyclic photophosphorylation and/or chlororespiration, and cyclic photophosphorylation, respectively. These effects were mimicked when the *psaE* gene product (a component of PS I with no direct redox capabilities) was absent, either on its own or in combination with a lack of *ndhF* (NAD(P)H-PQ oxidoreductase (Yu et al. 1993; Zhao et al. 1993).

The other set of sub-optimal growth conditions in which the absence of NAD(P)H-PQ oxidoreductase manifests itself is in response to low inorganic C supply, i.e. when CO_2 concentrating mechanisms (CCMs) are maximally expressed (Klugehammer et al. 1997; Sültemeyer et al. 1997; Ogawa et al. 1985; Ogawa 1991; Ohkawa et al. 1998). Here, it seems to be the

HCO_3^--transporting rather than the CO_2-transporting variant of CCM seems to be affected by the absence of NAD(P)H-PQ oxidoreductase (Sültemeyer et al. 1997). Although these findings seem to be consistent with the operation of cyclic electron flow via NAD(P)H-PQ oxidoreductase in this light-dependent HCO_3^- transport, evidence from P_{700} redox kinetics indicates that NAD(P)H-PQ oxidoreductase is *not* essential for cyclic electron flow in *Synechocystis* sp. strain 7002 (Sültemeyer et al. 1997). Similar results were found for mutants lacking *psaE* as for those lacking *ndhF* (i.e. lacking NAD(P)H-PQ oxidoreductase) (Sültemeyer et al. 1997), a somewhat different conclusion to that arrived at for the same strain by Schluchler et al. (1993), Yu et al. (1993) and Zhao et al. (1993).

No such direct effect on phenotype has been investigated for higher plants lacking NAD(P)H-PQ oxidoreductases under sub-optimal conditions. However, Sazanov et al. (1998b) showed a reduced rate of PQ photoreduction in heat-stressed *Nicotiana tabacum* lacking NAD(P)H-PQ oxidoreductase relative to wild type.

The absence of plastid-encoded NAD(P)H-PQ oxidoreductase is the norm for a number of green eukaryotic algae and higher plants and for all other eukaryotic O_2-evolvers, despite the evidence for cyclic electron transport and for chlororespiration in these organisms (see 'Cyclic electron transport, chlororespiration and the role of NAD(P)H dehydrogenase' and 'The occurrence of NAD(P)H-PQ oxidoreductase gene' sections above).

Other evidence relating to the function of NAD(P)H-PQ oxidoreductase in cyclic electron transport and in chlororespiration concerns the sensitivity of these processes to inhibitors that are allegedly specific to NAD(P)H-PQ (or UQ) oxidoreductases such as rotenone and amytal, and the phenotypic oxidoreductase nucleotide specificity of the complex (Bendall and Manusso 1995; Miyake et al. 1995; Scheller 1996; Darrouzet et al. 1998). The inhibitor evidence is somewhat ambivalent. As to nucleotide specificity, many reports indicate complete, or substantial, selectivity for NADH rather than NADPH. This permits a role in chlororespiratory use of glycolytic NADH, but not of NADPH from the oxidative pentose phosphate pathway or from PS I unless a transhydrogenase is present; the occurrence of such an enzyme in plastids and cyanobacteria is equivocal at best (Schluchler et al. 1993; Bendall and Manusso 1995). However, NAD(P)H-PQ oxidoreductase from *Synechocystis* PCC 6803 was NADPH-specific and had ferredoxin-$NADP^+$ reductase activity (Matsuo et al. 1998). Similar results were found for Teicher and Scheller (1998) for the *Hordeum vulgare* and by Funk et al. (1999) for the *Zea mays* thylakoid enzymes.

The conclusion is that the NAD(P)H-PQ oxidoreductase may well be a component of a cyclic electron transport pathway, but not necessarily the only pathway of cyclic electron transport in a given organism. Of the alternative pathways from ferredoxin to PQ a commonly suggested, but poorly characterized, possibility is a ferredoxin-PQ oxidoreductase (Bendall and Manusso 1995); this possible metal (Fe?) involvement is unclear. It is also not known if all variants of chlororespiration involve NAD(P)H-PQ oxidoreductase.

Fe requirements for cyclic, and Mehler reaction electron transport and H^+ pumping: Fe and photon costs

We have seen in 'Cyclic electron transport, chlororespiration and the role of NAD(P)H dehydrogenase' section above that the Fe content per 'idealized' cyclic electron transport chain containing NAD(P)H-PQ oxidoreductase is 27-37 Fe for the case of plastocyanin rather than cytochrome c_6, and ferredoxin rather than flavodoxin, depending on the assumptions about the Fe content of NAD(P)H-PQ oxidoreductase, while in the absence of NAD(P)H-PQ oxidoreductase the value is 19 Fe. The Fe cost of ATP synthesis by this pathway depends on the H^+/e^- ratio and the H^+/ATP ratio that are assumed. The H^+/e^- ratio of cyclic electron transport is generally thought to be 2. This accords with the 'Q-cycle' operation of the cytochrome b_6-f complex, but has no role for H^+ pumping by the NAD(P)H-PQ oxidoreductase which, by analogy with the mitochondrial NADH-UQ oxidoreductase, would have a H^+/e^- ratio of 2 (Siedow 1995; Whitehouse and Moore 1995; Brandt 1997). An H^+/e^- ratio of 4 (2 from NAD(P)H-PQ oxidoreductase, 2 from cytochrome b_6-f complex) is consistent with early data summarized by Heath (1972) and more recent work of Heber et al. (1995a). With an H^+/ATP ratio of 4 (Falkowski and Raven 1997), an H^+/e^- ratio of 4 would give, with 1 electron cycled per photon contributing excitation energy to PS I, one ATP per photon absorbed by pigments contributing to light harvesting by PS I reaction centres effective in cyclic electron flow (see 'The expression of NAD(P)H-PQ oxidoreductase genes' section) above. *In vivo* estimates from the 1970s of the photon yield of cyclic photophosphorylation also gave val-

ues of 1 ATP per photon absorbed by PS I (Raven 1976a,b), although at that time the interpretation was in terms of an H^+/e^- ratio of 2 and an H^+/ATP ratio of 2. The absence of the NAD(P)H-PQ oxidoreductase H^+ pump (or its equivalent) would, on current views of the H^+/ATP, give only 0.5 ATP per photon absorbed by pigments related to PS I reaction centres effective in cyclic electron flow. These computed photon yields of ATP production by cyclic photophosphorylation may be compared with the values for the Mehler peroxidase reaction where 2 photon are absorbed and used by PS II and PS I to move 1 electron from H_2O to O_2 and pump 3 H^+ and hence produce 0.75 ATP. The net result is that 0.75 ATP is made with 2 photons or 3/8 (0.375) ATP per photon absorbed. This value is lower than the *in vivo* estimates of 1 ATP per photon from the 1970s for cyclic photophosphorylation based on total photons absorbed by PS I using irradiation at 700 nm (see above). However, it is closer to values predicted from current perceptions of the H^+/ATP ratio (4) and an H^+/e^- ratio of 2 with (potentially) all PS I units participating and an H^+/e^- ratio of 4. Some PS I units are presumably not involved in cyclic electron transport involving NAD(P)H-PQ oxidoreductase due to lack of connection to a (scarce) NAD(P)H-PQ oxidoreductase (see 'The expression of NAD(P)H-PQ oxidoreductase genes' section above). The predicted photon yield of cyclic photophosphorylation would become even more comparable to that of ATP supply from the Mehler peroxidase reaction (0.375 ATP per photon absorbed; see below) if 400–700 nm photons absorbed by pigments that cannot transfer excitation energy to PS I reaction centres, are taken into account (see Raven 1976a,b).

The Fe cost of ATP synthesis by the cyclic photophosphorylation, with or without involvement of NAD(P)H-PQ oxidoreductase can be approached as follows. If we assume a maximum *in vivo* specific reaction rate of PS I of 400 mol electron per mol PS I per second in linear electron flow (based on the compilation in Raven 1990), and a capacity for cyclic electron flow of 10% of the linear rate, i.e. 40 mol electron per mol PS I per second, the maximum rate of electron transport with NAD(P)H-PQ oxidoreductase operative is then 1.08–1.48 mol electron per mol Fe in the cyclic redox pathway per second for the range of Fe contents cited in 'The occurrence of NAD(P)H-PQ oxidoreductose genes' section above. This calculation is for a pathway using plastocyanin rather than cytochrome c_6 and ferredoxin rather than flavodoxin. This means that, with an ATP/e^- ratio of 1, then 1.08–1.48 mol ATP produced per mol Fe in the cyclic redox pathway per second (Table 6). For a cyclic pathway lacking NAD(P)H-PQ oxidoreductase, the Fe content for the plastocyanin/ferredoxin alternative is 19 Fe per cyclic redox chain, and the ATP/e^- ratio is 0.5. The rate of cyclic electron transport per Fe is then 2.1 mol electron per mol Fe per second and the rate of ATP synthesis is 1.05 mol ATP per mol Fe per second (Table 6).

The computations of Fe costs show that, even with the highest assumed Fe content of the NAD(P)H-PQ oxidoreductase, the rate of ATP synthesis is, for a given electron flow rate, very similar on a mol Fe basis for the NAD(P)H-PQ oxidoreductase-containing redox sequence as for those that lack that complex and catalyse electron flow from ferredoxin (or flavodoxin) to PQ with a redox system that lacks Fe (or any other metal) and that does not pump H^+. In the context of limiting factors other than Fe in the natural environment the NAD(P)H-PQ oxidoreductase-containing cyclic redox pathway can have a photon yield of ATP production which is twice that of a cyclic redox pathway lacking NAD(P)H-PQ oxidoreductase (see Raven 1988a, 1990 and Sunda and Huntsman 1997 for consideration of Fe and light colimitation).

We now compare the mol ATP produced per mol Fe in the redox chain per second for pseudocyclic photophosphorylation (Mehler peroxidase reaction) with cyclic photophosphorylation with and without NAD(P)H-PQ oxidoreductase, as the source of ATP without a net reduction of oxidant. The Mehler peroxidase pathway ('Linear electron flow with O_2 as electron acceptor: the Mahler peroxidase reaction' section above) with plastocyanin rather than cytochrome c_6 and ferredoxin rather than flavodoxin has a minimum Fe, Cu and Zn content of 23 Fe, 1 Cu and 1 Zn (including the Fe, Cu and Zn needed for enzymes of $O_2^{\cdot -}$ and H_2O_2 metabolism) (Table 5) for a linear chain with all complexes at equal concentration. With $3H^+/e^-$ in the Mehler peroxidase reaction and 4 H^+/ATP, linear electron flow of 40 mol electron per mol complex per second (the same as that assumed for cyclic electron flow) means that 1.36 ATP synthesized per mol Fe in the redox chain per second (Table 6). This is within the range of rates computed for cyclic electron flow using NAD(P)H-PQ oxidoreductase with the highest and lowest likely Fe contents of the NAD(P)H-PQ oxidoreductase complex, and higher than the rate for a cyclic redox sequence lacking NAD(P)H-PQ oxidoreductase (Table 6). The photon cost of ATP synthesis in the Mehler peroxidase reaction, assuming equal photon allocation to PS I and PS II, is 0.375 ATP

Table 6. Computed (in text) Fe costs of ATP synthesis in cyclic and O_2-linked linear (Mehler peroxidase) electron transport pathway with electron flow at 40 mol electron per mol complex per second

Reaction sequence	Mol ATP per mol Fe in redox sequence per second
Cyclic electron flow with NAD(P)H-PQ oxidoreductase, assuming 18 Fe per NAD(P)H-PQ oxidoreductase complex	1.08
Cyclic electron flow with NAD(P)H-PQ oxidoreductase, assuming 8 Fe per with NAD (P)H-PQ oxidoreductase complex	1.4
Cyclic electron flow without NAD(P)H-PQ oxidoreductase, i.e. assuming a non Fe-containing, non H^+-pumping 'ferredoxin-PQ oxidoreductase'	1.05
O_2-linked linear (Mehler peroxidase) electron flow, including the observed Fe content of the ascorbate peroxidase involved in this reaction sequence	1.30

All pathways assumed to have equal quantities of all redox complexes, and to use plastocyanin rather than cytochrome c_6 and ferredoxin rather than flavodoxin. This 'plastocyanin' variant of the pathways is most commonly associated with the non Fe-containing Cu–Zn superoxide dismutase in the Mehler peroxidase reaction.

per photon absorbed (2 photons absorbed per electron transported; 3 H^+ per electron transported; 4 H^+ per ATP: Falkowski and Raven 1997).

Three further considerations must be borne in mind when comparing the Fe requirements for ATP synthesis by cyclic electron flow and by the Mehler peroxidase reaction. One is that components of the Mehler peroxidase reaction have the additional role of disposing of active oxygen species other than those generated by PS I (Noctor and Foyer 1998). A second consideration is that the cyclic electron transport may be more effective in acidifying the thylakoid lumen in high light, thereby increasing non-photochemical quenching of excitation energy, than is the case for the Mehler peroxidase electron transport (Heber and Walker 1992; cf. Osmond and Grace 1995). A further role for coupled cyclic electron around PS I is in the regulation of PS II (Heber et al. 1995b). The final consideration is that the NAD(P)H:PQ oxidoreductase may be essential for chlororespiration as well as cyclic electron transport.

Genetic and environmentally driven variations in the ratio of trace metal-containing catalysts in the thylakoid membrane

Background

It is now clear that the major trace metal-containing complexes in the thylakoid membrane are not generally present in equal quantities in a given genotype grown under a given set of conditions, that these ratios are susceptible to environmental (growth condition) influences, and that there are substantial genetic (large-scale taxonomic or phylogenetic) variations in the range of ratios of these components.

Genetically determined variations in trace metal-containing catalysts

Most data are available for variations in the ratio of PS II to PS I as a function of genetic and environmental factors, with rather fewer data on the ratio of cytochrome b_6-f complex, and even fewer data on the ratios plastocyanin/cytochrome c_6 and on ferredoxin/flavodoxin to the photosystems. The

best established genetic variation in content of metal-containing catalysts is for the ratio of PS II to PS I. The highest ratio of PS II to PS I is found in cyanobacteria *sensu stricto* (i.e. excluding chloroxybacteria) and Rhodophyta (Cunningham et al. 1989; Malkin et al. 1990; Rögner et al. 1990; Murakami et al. 1997; Falkowski et al. 1998 cf. Greene and Gerard 1990; Kübler and Davison 1993). The ratio in these organisms is almost always less than 1, is commonly 1:3–1:5, is sometimes as low as 1:9, or is even 1:25 for the non-heterocystous N_2–fixing marine cyanobacterium *Trichodesmium* spp. Higher ratios of PS II to PS I, usually more than 1.0 and as high as 4.0, are found for the chloroxybacteria, Chlorophyta and their Embryophyta (higher plant) derivatives and the Euglenophyta as well as for the Heterokontophyta (diatoms, brown algae), Dinophyta and Haptophyta; (Falkowski et al. 1981; Dubinsky et al. 1986; Falkowski et al. 1986; Friedmann and Alberte 1986; Chow and Anderson 1987; Smith and Melis 1987; Sukenik et al. 1987; Guenther et al. 1988; Raven 1988a; Falkowski et al. 1989; Lee and Whitmarsh 1989; Wilhelm et al. 1989; Burger-Wiersma and Matthijs 1990; Chow et al.1990; Raven 1990; Bullerjahn and Post 1993; Trissl and Wilhelm 1993; Iglesias-Prieto and Trenck 1994; Partensky et al. 1997; cf. Caron et al. 1988). No data were found for the Cryptophyta or Chlorarachniophyta. These variations in the ratio of two major trace metal-containing complexes mean that, on a total chromophore basis, the Fe content in the two photosystems is greatest for cyanobacteria and Rhodophyta with a high ratio of the (high Fe) PS I to the (low-Fe) PS II, while the Fe content of the two photosystems is smallest for Heterokontophyta and such Chlorophyta as *Mantoniella* (Wilhelm et al. 1989) with a high ratio of PS II to PS I. By contrast, the Mn (in PS II only) content per unit total chromophore *may* be lower in cyanobacteria and Rhodophyta which have a lower PS II:PS I ratio than in organisms with higher PS II:PS I ratios.

The variations in PS II:PS I given above apparently represent genetically determined differences among major taxa (Divisions). Within these higher taxa there are genetically determined differences relating to the habitat in which the organisms normally grow. Thus, while the typical range of PS II/PS I ratios in sun-adapted terrestrial higher plants (1.7–1.8) overlaps with that of shade-adapted terrestrial higher plants (1.19–1.88) (Chow et al. 1990a), the lowest value for the shade-adapted species is lower than that for the sun-adapted species. This mimics the acclimatory effect seen when sun-adapted plants are grown at low PFDs, e.g. PS II/PS I in *Pisum sativum* is 1.79 when grown at high PFD and is 1.27 when grown at low PFD (Chow et al. 1990a). We shall see later ('Environmental (acclimatory) variations in trace metal-containing catalysts' section below) that this acclimatory effect of low growth PFD in decreasing PS II/PS I is not universal, so that in some eukaryotic algae growth PFD does not alter PS II/PS I, while in others PS II/PS I increases as the growth of PFD is decreased.

Environmental (acclimatory) variations in trace metal-containing catalysts

The PS II:PS I ratio, and the PS II:total chromophore and PS I:total chromophore ratios, vary with the photon flux density for growth and the nitrogen and iron supply for growth. Fe deficiency lowers the thylakoid protein content, and the activation state (but not the quantity) of Rubisco (Evans 1996). Berges et al. (1996) showed that N deficiency increased the PS II:PS I ratio in the marine green alga *Dunaliella tertiolecta* from 1.0 in N-replete cells to 2.4 in N-starved cells, and in the marine diatom *Thalassiosira weissflogii*, from 0.45 in N-replete to 1.4 in N-starved cells.

More relevant to *in vivo* functioning in terms of the effectiveness with which organisms use the potentially limiting resources Fe and Mn, are the rates of CO_2 fixation per unit Fe or Mn at light and CO_2 saturation. The PS II:PS I ratio differences, and more importantly the PS I and PS II content per unit chromophore, combined with data on photosynthetic rate per unit chromophore, indicate that the cyanobacteria and red algae should have the most Fe-costly CO_2 fixation (mol Fe needed to yield a fixation rate of one mol CO_2 per second), while diatoms, brown algae and the green alga *Mantoniella* should be the least Fe-costly (Raven 1988a, 1990; Wilhelm et al. 1989). This analysis ignores the Fe used in the cytochrome b_6-f complex, cytochrome c_6 (if present), ferredoxin (if present), thylakoid NAD(P)H-PQ oxidoreductase (Endo et al. 1997a,b; Mi et al. 1997; Matsuo et al. 1997), thylakoid Fe-superoxide dismutase (Asada et al. 1980; Asada 1992; Asada et al. 1993b) and thylakoid ascorbate peroxidase (Asada 1992; Asada et al. 1993b). The Fe content on a unit light-harvesting chromophore basis in all of these thylakoid-associated components, except the cytochrome b_6-f complex, cytochrome c_6 and ferredoxin, is poorly understood. Although the

Fe content per molecule of Fe superoxide dismutase and per molecule of ascorbate peroxidase is known, the number of thylakoid-associated molecules of these enzymes on a total chromophore basis is rather poorly characterized (Asada et al. 1980; Hewitt 1983; Asada 1992, 1993b). Price et al. (1998) have elegantly shown the lower limit of expression of the cytochrome b_6-f complex compatible with the wild-type phenotype in *Nicotiana*.

Variations in the light supply for growth can cause acclimation of the PS I and PS II content of photosynthetic cells, and hence in the computed Fe cost of photosynthesis in terms of the high Fe PS I. In some cases, e.g. the marine green microalga *Dunaliella tertiolecta*, there is no significant effect of PFD for growth on the PS II:PS I ratio, although there is an almost 3-fold increase in the reaction centre content per cell in cells grown at low PFDs and a rather larger proportional increase in chlorophylls *a* and *b* per cell (Falkowski et al. 1981). A very different acclimation is apparent in the marine diatom *Skeletonema costatum*, where the PS II:PS I is more than twice as high for cells grown at the lowest PFD tested as in those grown at the highest PFD. There is an approximately 1.5-fold increase in PS II and in chlorophylls *a* and *c* per cell, and an approximately 1.5-fold decrease in PS I per cell (Falkowski et al. 1981). Neither alga shows a large change in light-saturated photosynthetic rate on per cell basis as a function of PFD for growth (Falkowski et al. 1981), so that the light-saturated photosynthesis rate per PS I decreased from 136 (high light grown) to 45 (low light grown) electrons per PS I per second in *Dunaliella tertiolecta*, and increased from 231 (high light grown) to 285 (low light grown) electrons per PS I per second in *Skeletonema costatum*.

Photosynthesis at low PFDs for these two algae grown under similar conditions has been measured by Falkowski and Owens (1980). At low PFDs for measurement the photosynthetic rate on a chlorophyll basis for *Dunaliella tertiolecta* is independent of the PFD for growth, so that the photosynthetic rate per PS I measured at 20 μmol photon m^{-2} s^{-1} is about 5 electrons per PS I per second regardless of the PFD for growth (Falkowski and Owens 1980; Falkowski et al. 1981). *Skeletonema costatum* cells grown at low PFDs have a lower chlorophyll-based O_2 evolution rate at low PFDs for measurement. Photosynthetic rates measured at a PFD of 20 μmol photon m^{-2} s^{-1} are 135 electrons per PS I per second for high light cells, and 100–111 electrons per PS I per second for cells grown at low PFDs (Falkowski and Owens 1980; Falkowski et al. 1981).

These computations show that, although low-light-acclimated *Skeletonema* cells have a lower photosynthetic rate per PS I than cells acclimated to high light, the rates are much higher than in *Dunaliella* cells where there is little or no acclimation of light-limited rates of photosynthesis per PS I as a PFD for growth. The computed electron transfer rates per PS I can be directly translated to electron transfer rates per atom of Fe in PS I by division of 12.

The increase in PS II:PS I with acclimation to lower PFDs in *Skeletonema costatum* cannot be generalised to all diatoms. Although the PS II:PS I ratio is higher in *Phaeodactylum tricornutum* (Friedmann and Alberte 1986) and *Cylindrotheca fusiformis* (Smith and Melis 1988) acclimated to lower PFDs, *Thalassiosira weisflogii* has an essentially constant PS II:PS I regardless of the PFD for growth (Dubinsky et al. 1986). However, as in *Skeletonema costatum*, the PS I (and PS II) per unit chlorophyll decrease with lower PFDs for growth, as is also the case for the haptophyte *Isochrysis galbana* and the dinoflagellate *Prorocentrum micans* (Dubinsky et al. 1986).

For another heterokont, the giant kelp *Macrocystis pyrifera* (Smith and Melis 1987), photosynthetic laminae (blades) growing at the surface had a PS II:PS I ratio of 1.79, while those growing at 20 m depth had a PS II:PS I ratio of 2.22, with an increased ratio of chlorophyll to PS II and to PS I in the deeper-growing blades. This acclimation in terms of PS II:PS I for blades growing at lower PFDs, i.e. at greater depths, is in the same direction as in the heterokont (diatom) *Skeletonema costatum* discussed above. There are no comparable photosynthetic data to permit assessment of specific reaction rate of the photosystems, and hence Fe cost, at the growth PFDs. The change in PS II:PS I with depth in *Macrocystis* is unlikely to be a response to a changed ratio of radiation exciting PS II to that exciting PS I with depth because the radiation exciting PS I relative to PS II probably decreases with depth (Kirk 1994). Thus, the depth response of *Macrocystis*, when interpreted in spectral terms, is the opposite of the acclimation of photosystem ratio as a response to light quality in cyanobacteria (Fujita et al. 1987; Murakami et al. 1997) and higher plants (Chow et al. 1990a,b). The light quality effect in cyanobacteria and higher plants involves a higher PS II:PS I ratio when growth occurs with a predominance of radiation exciting Photosystem I, and *vice versa*.

Further variations in the effect of photon flux density for growth, independent of far-red enrichment in shade light for terrestrial plants in nature, are a *decrease* in PS II:PS I with a lower photon flux density for growth in the flowering plant *Pisum sativum* (Chow and Anderson 1987, 1990b; Lee and Whitmarsh 1989; Chow et al. 1990b), and the absence of any effect of photon flux density for growth on the PS II: PS I ratio in the unicellular red alga *Porphyridium cruentum* (Cunningham et al. 1989).

The variability in acclimatory effects of photon flux density on PS II:PS I ratio is also seen in the effects of photon flux density for growth on the PS II:cytochrome b_6-f complex ratio (see Table 3 of Raven 1990; see also Wilhelm and Wild 1984a,b, for effects of photon flux density for growth of *Chlorella* on the PS I:cytochrome b_6-f complex ratio, and Chow and Hope 1998 for the PS II:cytochrome b_6-f complex:PS I in *Nicotiana tabacum*). The *direction* of change of PS II:cytochrome b_6-f complex as a result of varied photon flux density for growth is not always in the same as that for the change in PS II:PS I ratio.

We have emphasized the role of the light climate on phenotypic (acclimatory) variations in the ratio of Fe-containing components of thylakoids. Another obviously important determinant of the production of Fe-containing catalysts of thylakoids is the supply of Fe (Evans 1996; Imsande 1998; Behrenfeld and Kolber 1999).

Greene et al. (1991) examined the effect of Fe sufficiency and Fe limitation on the content of PS I, PS II and the cytochrome b_6-f complex of the diatom *Phaeodactylum tricornutum*. Fe deficiency reduced the content of PS I and PS II reaction centres, but not that of the cytochrome b_6-f complex, on a chlorophyll *a* basis. As Fe deficiency very substantially reduced the chlorophyll *a* content per cell, the content of all three complexes (PS I, PS II, cytochrome b_6-f) is greatly reduced on a cell basis. The reduction in the content per cell of the three complexes is greatest for PS I and least for the cytochrome b_6-f complex with a change in the PS II:cytochrome b_6-f complex:PS I ratio from 2.9:7.9:1 in Fe-replete cells to 4.2:19:1 in Fe-deficient cells. The greater decrease in PS I than of the other complexes accords with minimizing the Fe content of the thylakoids. Greene et al. (1991) using the Fe/complex values quoted by Raven (1990), calculated that about 40% of the cellular Fe in Fe-deficient *Phaeodactylum tricornutum* can be accounted for by PS I, PS II and the cytochrome b_6-f complex. Hewitt (1983) cites 18.5% as the fraction of leaf Fe in higher plants that can be accounted for in known photosynthetic and non-photosynthetic components.

Greene et al. (1991) found that the content of the Fe-containing PS I and PS II complexes on a chlorophyll *a* basis is decreased in Fe-deficient cells. This suggests that, predominantly, the reduced chlorophyll *a* content per cell in Fe-deficient organisms relates to the decreased content of Fe-containing pigment–protein complexes, while the pigment–protein complexes which do *not* contain Fe are associated in a stoichiometric way. It is known that some steps in the chlorophyll synthesis pathway are Fe-dependent. Thus, Figure 9.1 of Marschner (1995) identifies an Fe requirement in the conversion of coproporphyrinogen to protoporphyrinogen, and in the conversion of Mg protoporphyrin to protochlorophyllide. We note that the Fe requirement in converting succinyl CoA plus glycine to δ-aminolevulinic acid shown in Figure 9.1 of Marschner (1995) is not relevant to chlorophyll synthesis since this invariably involves the glutamate pathway of δ-aminolevulinic acid synthesis: Falkowski and Raven (1997). However, the evidence of Greene et al. (1991) suggests that the Fe requirement for chlorophyll synthesis is less significant than a decreased content of Fe-containing photosynthetic apoproteins in causing the lower chlorophyll content of Fe-deficient cells.

Restricted Fe supply also interacts with other growth rate limiting resource inputs such as a low incident photon flux density. Kudo and Harrison (1997) measured the critical Fe value, i.e. the Fe content of cells that was just sufficient to achieve the maximum possible specific growth rate under a given set of growth conditions, as a function of the light supply in the cyanobacterium *Synechococcus*, and found a higher Fe requirement for low light-grown than high light-grown cells. This agrees with suggestions of Raven (1998a, 1990) that low light adapted (or acclimated) cells, which usually have more pigment per cell and often have fewer reaction centre complexes per unit chlorophyll (but sometimes more per cell) than high light adapted or acclimated cells, have lower achieved rates of electron transport through Fe-containing redox catalysts under their low-light growth conditions than do high light adapted (or acclimated) cells under their high light growth conditions. Therefore cells growing at low light need more Fe per unit C fixed per second than do cells growing in high light (Raven 1990).

One possibility for offsetting this greater Fe cost of C fixation during photosynthesis at low photon

flux densities is a reduction in cell size, and thus in the package effect. The package effect (Kirk 1994) involves a lower specific absorption coefficient of pigment, and hence a lower efficiency of light absorption per unit pigment, within suspended particles relative to that in a monodisperse solution at the same concentration of pigment (mol m^{-3}). This effect increases, for a given concentration of pigment per unit volume of particle, with increasing size of the particle (subthylakoid particles; whole thylakoids; chloroplasts; cells; multicellular organs). The package effect means that the rate of photon absorption per pigment molecule is, for a given incident photon flux density, greater for structures with a smaller package effect. For a given ratio of pigment to thylakoid Fe (or Mn), the greater efficiency of photon absorption and hence the increased rate of excitation energy transfer to reaction centres and of electron flow through Fe- (and Mn-) containing catalysts, should be greater in cells with a smaller package effect (Raven 1986, 1987b, 1997c, 1998a, 1999). Thus, a smaller package effect can not only lead to an increased rate of photosynthesis per unit pigment at low photon flux densities, but can also increase the rate of photosynthesis per unit Fe (or Mn) (Raven 1998a, 1999). The work of Sunda and Huntsman (1997) and Van Leeuwe et al. (1997) are examples to which this package effect argument can be applied in relation to photosynthetic rates of marine phytoplankton cells when Fe and light are limiting growth rates.

Work in which the ratio of the major iron-containing complexes in thylakoids has been altered phenotypically in response to treatments other than Fe supply, i.e growth under putatively Fe-sufficient growth conditions, includes photon flux density (400–700 nm) for growth (e.g. Falkowski et al. 1981; Dubinsky et al. 1986; Wilhelm et al. 1989; Burger-Wiersma and Matthijs 1990; Greene and Gerard 1990), the spectral composition of the radiation supplied for growth (e.g. Fujita et al. 1987; Chow et al. 1990b; Murakami et al. 1997), the inorganic C supply (Manodori and Melis 1987; Murakami et al. 1997; cf. Li and Canvin 1998) and the external Na^+ concentration (Murakami et al. 1997). Where variations in incident photon flux density of a given spectral composition influence the ratio of PS I to PS II, it is invariably to decrease the ratio at lower photon flux densities, although there is sometimes no phenotypic influence of the rate of photon supply for growth on the PS I:PS II ratio. For those spectral effects that relate predominantly to the fraction of incident light absorbed by antenna pigments serving particular light reactions, it is found that light predominantly exciting PS I leads to an increased PS II:PS I ratio and *vice versa*. Restricted inorganic C supply and high Na^+ decreased the PS II:PS I ratio in *Synechocystis* (Manodori and Melis 1987; Murakami et al. 1997).

These effects may be related to the influence of the various environmental factors on the redox state of PQ and/or the cytochrome b_6-*f* complex, a high PQH_2/PQ ratio decreasing expression of PS II and *vice versa* (see Hihara et al 1998; Allen 1993; Pfannschmidt et al. 1999). For changes in photon flux density, a tendency for reactions downstream of both photoreactions, CO_2 fixation and the Mehler reaction, to have a greater capacity to accept electrons than PS I and PS II have to supply them after a decrease in incident photon flux could lead to a lower PQH_2/PQ ratio and a smaller extent of reduction of the b_6-*f* complex. This could cause differential gene expression such that the photoinhibition-sensitive PS II is expressed more at lower than at higher photon flux densities relative to the expression of PS I, with the *possibility* of an increase in the overall photon yield of photosynthesis. However, this sort of mechanism does not *guarantee* an increase in the overall photon yield and poses problems at high photon flux densities, where photoinhibition *sensu lato* can lower the PQH_2/PQ ratio and *decrease* the effective PS II:PS I ratio.

For spectral variations in light supply, the logical chain is less tortuous. Light that preferentially excites PS I causes a decrease in the PQH_2/PQ ratio and increases expression of PS II, balancing the excitation inputs to PS I and to PS II. The reverse occurs with light that preferentially excites PS II.

In the case of restricted inorganic C supply, there is expression (or increased expression) of inorganic carbon concentrating mechanisms dependent on cyclic electron transport, if not cyclic photophosphorylation (Klugehammer et al. 1997; Sültemeyer et al. 1997; Ohkawa et al. 1998; cf. Ogawa et al. 1985; Ogawa 1991). High Na^+ concentrations increase the rate of the directly or indirectly ATP-powered active Na^+ efflux (Murakami et al. 1997). In both cases, the argument an increased rate of ATP consumption would, if ATP is only available from non-cyclic photophosphorylation, lead to an increased NADPH/$NADP^+$ ratio and hence to an increased PQH_2/PQ ratio. This increase in PQH_2/PQ not only leads (in most organisms) to a state transition increasing the ratio of excitation energy reaching PS I relative to PS II but also, if the increased PQH_2/PQ is long sustained (more

than minutes), to changed degrees of gene expression (Allen 1993). These longer-term effects yield an increased expression of PS I, with the likelihood of increased ATP supply by cyclic photophosphorylation. However, it may be difficult with such redox control of the capacity for cyclic photophosphorylation, to avoid over-reduction of the redox carriers of the cyclic pathway. This is due to reductant inputs from the reducing end of both PS II and PS I, unless cyclic photophosphorylation involves distinct PS I units having no redox contact with PS II. This would have important implications for flexibility of operation of thylakoid reactions.

Overall, the treatments that produce a high PQH_2/PQ would favor an increase in the high Fe-demanding PS I relative to the low Fe-demanding PS II; the influence on the cytochrome b_6-f complex, with its intermediate Fe requirement, is variable, sometimes tracking PS II and sometimes, more closely following PS I expression (Wilhelm et al. 1989; Murakami et al. 1997). It would be teleologically appropriate if the greater relative Fe demand engendered by a high PQH_2/PQ ratio resulted in enhanced Fe acquisition by cell surface Fe(III) reductases. This would require that Fe(III) supply to the cell surface would not be limiting.

These predictions of Fe costs of photosynthetically supported growth are at least qualitatively validated for Fe contents and growth rates of microalgae as a function of differences in combined N source (NH_4^+ or NO_3^-) (Raven 1988a; Rueter, 1993; Maldonado and Price 1996; Kudo and Harrison 1997; cf. Henley and Yin 1998). However, the Fe costs of growth as predicted by Raven (1988a) are greatly (10-fold) in excess of the values measured for an *oceanic* diatom (*Thalassiosira oceanica*) adapted to the low-Fe open ocean habitat, although they are closer to the values measured for a *coastal* diatom (*Thalassiosira weisflogii*) from a higher-Fe habitat (Sunda et al. 1991; Sunda and Huntsman 1995a). This difference between coastal and open ocean phytoplankton organisms is also seen for coccolithophorids and dinoflagellates, oceanic coccolithophorids being the most Fe-efficient of the organisms tested by Brand (1991). The least Fe-efficient organisms tested by Brand (1991) were coastal and other marine cyanobacteria (see Wilhelm et al. 1996; Muggli and Harrison 1997). The high Fe cost of growth of cyanobacteria (low PS II:PS I) relative to chlorophyll *b*-containing prasinophytes and chlorophyll *c*-containing raphidophytes, dinophytes, coccolithophorids (high PS II:PS I) agrees with predictions of Raven (1988a, 1990). However, the very low Fe cost of growth in some oceanic eukaryotic phytoplankton organisms is a considerable challenge to our belief that we understand the Fe content of particular catalysts, their *in vivo* specific reaction rate, and the stoichiometry of their reaction products with C assimilation and growth (Raven 1988a, 1990).

Mn presents a less complex case than than Fe, and the achieved Mn cost of growth (Sunda and Huntsman 1986, 1998) is in reasonable agreement with prediction (Raven 1990; Sunda and Huntsman 1998). The prediction here is based on the use of Mn predominantly in PS II, with some involved in Mn superoxide dismutase in mitochondria of eukaryotes and in thylakoids of cyanobacteria (Okada et al. 1979).

For Cu and Zn, the other trace metals involved in thylakoid-associated and other electron transport reactions neither estimates of the metal cost of growth nor measurements of the actual metal cost of growth are available.

Cu has a major photosynthetic (thylakoid) role in plastocyanin. Plastocyanin is not encoded in the genome of all organisms, nor, if encoded, is it always expressed (Falkowski and Raven 1997); the ratio of Cu/Fe availability is a major determinant of expression (Falkowski and Raven 1997). The suggestion that there is a necessary occurrence of Cu in PS II (Droppa et al. 1984; Droppa and Horváth 1990), seems to result from nuclear contamination of PS II preparations (Avellano et al. 1994). A well substantiated role for Cu in the thylakoids of peridinin-containing dinoflagellates, Charophyceae *sensu lato* and Embryophyta is in thylakoid-associated Cu–Zn superoxide dismutase (Henry et al. 1976; Asada et al. 1980; de Jesus et al. 1989; Butow et al. 1997; cf. Asada et al. 1977). Roles of Cu elsewhere in photosynthetic organisms include stroma and cytosol Cu–Zn superoxide dismutase (in peridinin-containing dinoflagellates, Charophyceae *sensu lato* and Embryophyta) cytochrome oxidase, and certain other oxidases.

Zn has a role in thylakoids in Cu–Zn superoxide dismutase in the Dinophyta (or at least the 'normal' peridinin-containing representatives), Charophyceae *sensu lato* and Embryophyta (Henry et al. 1976; Asada et al. 1980; de Jesus et al. 1989; Butow et al. 1997; cf. Asada et al. 1977) and a non-electron transport role for any thylakoid-associated carbonic anhydrase (Karlson et al. 1996, 1998; Raven, 1997a,d; Stemler 1997), regardless of which of the three gene families of carbonic anhydrase might be involved because they all involve Zn (Hewett-Emmett and Tashian 1996). Non-thylakoid roles and locations for Zn in

photosynthetic organisms include Cu–Zn superoxide dismutase in stroma and cytosol (Charophyceae and Embryophyta) and thylakoid, stroma, cytosol and extracellular carbonic anhydrase required for inorganic carbon assimilation using diffusive CO_2 entry and when active inorganic carbon transport occurs, as well as in 'Zn fingers' and some phosphatases and other enzymes (Vallee and Galdes 1984; Williams and Fraústo da Silva 1996; Raven 1997d; Karlson et al. 1998). It is of interest that *Chlorella pyrenoidosa* requires a much higher Zn concentration in the growth medium for photolithotrophy (0.77 mmol m^{-3}) than for chemoorganotrophy (77 nmol m^{-3}). This presumably reflects a very large Zn requirement for carbonic anhydrase in this alga (Eyster 1964) which lacks Cu–Zn superoxide dismutase. Although HCO_3^- has a protective role on the oxidising lumen side of PS II (Klimov et al. 1997; Hulsebosch et al. 1998), it is not known if this involves the intrathylakoid carbonic anhydrase (Karlson et al. 1998). Eyster (1964) also showed the expected (Raven 1988a) greater need for Fe (18 mmol m^{-3}) and Mn (0.1 mmol m^{-3}) in photolithotrophic than in chemoorganotrophic growth (Fe 1 μmol m^{-3} and Mn 1 μmol m^{-3}, respectively). However, Tortel et al. (1996) showed a *higher* Fe:C ratio in marine chemoorganotrophic bacteria than in eukaryotic phytoplankton. This is contrary to the predictions of Raven (1988a) *if* the achieved C assimilation rates in the work of Tortel et al. (1996) are equal for the chemoorganotrophs and the photolithotrophs. The maximum specific growth rates of the chemoorganotrophic bacteria in Figure 1 of Tortel et al. (1996) are about five times those for eukaryotic phytoplankton at the same temperature (20 °C; Raven 1986). This means that the C assimilation rate per unit (catalytic) Fe in the chemoorganotrophic cells is higher than in the eukaryotic photolithotrophs, as predicted by Raven (1988a). Further work is needed with specific attention to the achieved *in situ* growth rate of chemoorganotrophic bacteria (Pakulski et al. 1996) and phytoplankton in the same, Fe-limited, conditions (Pakulski et al. 1996). The situation is further complicated by the high Fe costs of growth found for phagotrophic marine protozoa (Chase and Price 1997).

Evolution of trace metal requirements in thylakoid reactions

In addition to the chemical 'fitness' of a given trace metal to fulfill a given role in thylakoid redox reactions, a consideration of the evolution of such roles requires that we consider the requirement, as perceived by an inadequately informed human observer from, so frequently, several billion years distance for that role at a given time as well as the availability of trace elements at that time. It is also necessary to include any known or likely needs for a given trace metal in any other roles in the organism.

The main change in the environment of the earth that has influenced the availability of Fe, Mn, Cu and Zn over the last 4 billion years has been the increased O_2 level, and increased redox potential (biological scale, with H_2 electrode negative, O_2 electrode positive). Because photodissociation of H_2O vapor can only produce O_2 at $\leq 10^{-8}$ of the present atmospheric level, the accumulation of O_2 in the original $CO_2/N_2/H_2O$ vapor atmosphere depends on the occurrence of O_2-producing photosynthesis. O_2 accumulation also depends on the oxidation of all of the primeval O_2-consuming inorganic oxidants (mainly Fe^{2+}, S^{2-}) and on the sequestration of the organic C counterpart of the O_2 as products of photosynthesis so that it cannot recombine with the O_2 by biotic (respiratory) or abiotic means.

There is evidence for Rubisco-based photosynthetic CO_2 assimilation, the core of CO_2 fixation process in all extant O_2-evolvers, as long as 3.85 billion years ago (Schidlowski 1988; Mojzis et al. 1996; Rosing 1999; cf. Eiler 1997), and there is fossil evidence for cyanobacteria, all of which today have the capacity for O_2 evolution, from 3.45 billion years ago. However, O_2 build-up *globally* to more than about 10^{-8} of the present atmospheric level did not occur until about 2 billion years ago, approximately coincidental with the origin of eukaryotes. If photosynthetic O_2 evolution indeed began as early as 3.45 billion years ago, then the absence of a global O_2 build-up until more than a billion years later could be related to the oxidation of Fe^{2+} to Fe^{3+} and S^{2-} to SO_4^{2-}.

The significance of this change from global anoxia before 2 billion years ago to (irregularly) increasing O_2 levels after 2 billion years ago for metal availability is as follows. Fe^{2+} and Mn^{2+} were relatively abundant in the anoxic ocean before 2 billion years ago. Cu was unavailable because it was present as cuprous ion (Cu^+), which was precipitated as the very insoluble Cu_2S, and Zn (Zn^{2+}) was very probably less available than it is in today's oxygenated ocean due to the high dissolved S^{2-} level (Williams and Frausto da Silva 1996; Raven 1997b,d, 1998d). This global scenario permits the 'luxurious' use of Fe and Mn in

the photosynthetic redox catalysts PS I and PS II, cytochrome b_6-f complex, cytochrome c_6, ferredoxin and NAD(P)H dehydrogenase. Perhaps this is not true in Fe- or MnSOD, in catalase or in ascorbate or glutathione peroxidases, because their substrates $O_2^{-\cdot}$ and H_2O_2 would not have been readily available in early anoxic oceans (although FeSOD is found in some extant obligately anaerobic bacteria: Asada et al. 1980). However, this scenario would not favor the use of Cu in plastocyanin or of Cu and Zn in Cu–Zn superoxide dismutase, which in any case would not have a substrate in the absence of significant free O_2. Oceanic dissolved Fe (Fe^{2+}) could also have had a role in providing a UV screen for early biota near the ocean surface before significant atmospheric O_2 built up, permitting the production of an atmosphere O_3 screen for UV and paralleling the occurrence of very low UV-absorbing Fe^{3+} in the ocean (Cleaves and Miller 1998; cf. Mulkidjianian and Junge 1997).

This view has an early recruitment of Fe and Mn into the redox reactions of O_2-evolving photosynthesis. Many of the Fe-containing components could have been derived from earlier Fe-containing catalysts in chemolithotrophs, phototrophs which could not evolve O_2, or chemoorganotrophs using an electron acceptor for respiratory electron transport. Such an electron acceptor could be abiotically generated NO_2^-, derived from $2\ CO_2 + N_2 \rightarrow 2\ CO_2 + 2\ NO^{\cdot}$, or $N_2 + 2\ H_2O \rightarrow 2\ H_2 + 2\ NO^{\cdot}$, energized by lightning or by meteorite impacts (Mancinelli and McKay 1988; Summers and Chang 1993; Yung and McElroy 1997; Raven and Yin 1998). Fe could have had earlier important involvement in the origin of life via continued production of the UV-labile NH_3 needed for amino-acid and nucleotide base synthesis from NO_2^- derived from the $NO^{\cdot}$ generated by lightning or bolide impacts (Summers and Chang 1993; cf. Falkowski 1997). However, the need for NH_3 synthesis would be negated by an origin and early evolution of life using magmatic NH_3 at hydrothermal vents well out of the range of UV-B penetration (Russell and Hall 1997). Further possible involvement of Fe in the chemolithotrophic evolution of life has been proposed by Wächterhäuser and by Russell (summarized by Maden 1995; see Russell and Hall 1997), using FeS, and leading to FeS protein catalysts consistent with very early occurrence of chemolithotrophic cells (Pace 1997). A delayed recruitment of Cu and Zn into redox catalysts could have been a result of one or more of:

1. Unavailability of Cu and Zn in the early anoxic ocean;
2. The occurrence of Fe and Mn-containing catalysts that would perform the same function (Fe or Mn superoxide dismutases; cytochrome c_6 instead of plastocyanin); and
3. The absence of substrates for the catalysts (little or no O_2^- for superoxide dismutase or H_2O_2 for catalase or ascorbate peroxidase when O_2 was $\leq 10^{-8}$ of the present level).

A number of considerations suggest that this is an oversimplification of the sequence of metal use, or at least under-estimates the time for which Cu has functioned in redox reactions. One line of evidence comes not from photosynthesis *per se* but from respiration. Schäfer et al. (1996) point out that the Cu-containing cytochrome oxidase seems to have a time of origin, based on molecular phylogenetic considerations, which considerably predates the global occurrence of O_2 at above 10^{-8} of the present atmospheric level, i.e. it originated well before 2 billion years ago. This presents two O_2-related problems: one is the global absence of O_2, the 'normal' substrate for electron donation by cytochrome oxidase; the other is the global absence of available Cu for synthesis of cytochrome oxidase in the global absence of O_2. A second problem with a later origin of Cu-dependent catalysts, this time directly related to O_2-evolving photosynthesis, is the phylogenetic distribution of plastocyanin. The widespread occurrence of plastocyanin in cyanobacteria indicates an origin early in the evolution of cyanobacteria (e.g. Kaneko et al. 1996). Of course, the distribution patterns of the cytochrome oxidase and plastocyanin could be explained by lateral gene transfer. This may help to explain the distribution of plastocyanin among extant cyanobacteria, but it is very unlikely to be the cause of the (apparently) monophyletic origin and subsequent phylogenetic distribution of cytochrome oxidase.

This 'Cu-anomaly' can be rationalized by the evolution of Cu-requiring catalysts in oxygenated microenvironments in a globally anoxic environment. There seems to be no such problem with the timing of the evolution of another photosynthesis-related, Zn-containing, redox enzyme, i.e. the Cu, Zn form of superoxide dismutase because it did not appear until after the origin of eukaryotes 2 billion years ago. Sporadic presence of the enzyme in prokaryotes could be explained by lateral gene transfer. This also fits with the use of Zn in zinc fingers in eukaryotes. However, there are arguments that Zn could have been involved earlier than 2 billion years ago in carbonic anhydrase, which is not a photosynthetic *redox* enzyme, although

Co could have substituted for it here (Morel et al. 1994; Sunda and Huntsman 1995b; Raven 1997d). The relatively low early availability of Zn relative to its present availability, and especially to the ready availability of Mg throughout the history of life, does not favor the early use of Zn in chlorophylls in place of mg. Although Zn occurs in bacteriochlorophyll in the acidophilic photosynthetic bacterium *Acidiphilium rubrum* (Wakao et al. 1996) Zn-chlorophyll has not been reported from O_2-evolvers.

Although not strictly a metal, Se is a trace element that is present as selenocysteine in the (H_2O_2-removing) Fe-containing glutathione peroxidase (Stadtman 1996), a significant peroxidase in some algae (*Chlamydomonas*; many diatoms) and perhaps some cyanobacteria (see 'Linear electron flow with O_2 as electron acceptor: the Mehler peroxidose reaction' section above). It is of interest that glutathione peroxidase of higher plants, where it is a minor component relative to ascorbate peroxidase, has cysteine rather than selenocysteine and has very low maximum specific activity relative to the Se-containing mammalian enzymes (Eshdat et al. 1997). The use of selenocysteine rather than cysteine may to be a primitive feature related to anoxic or low O_2 habitats as -SeH is much more readily auto-oxidized by O_2 than is -SH (Lee and Berry 1996). However, the occurrence of Se-containing glutathione peroxidase in O_2-evolving organisms means that it can function in cellular environments with O_2 concentrations in excess of those in an air-equilibrated solution (see Raven et al. 1994).

The high Fe (and Mn) requirements of thylakoid redox reactions, and hence the high Fe requirement for photolithotrophic growth, were probably a significant contribution to the natural selection pressures related to the evolution of the range of mechanisms for acquiring Fe from low-solubility Fe(III) complexes found in cyanobacteria, algae and higher plants in aerobic environments. These mechanisms involve surface acidification and reduction of Fe(III) to Fe(II), which is then taken up as Fe^{2+} by specific transporters in the plasmalemma (in eukaryotic primary producers other than grasses), or production of siderophores that chelate Fe(III), with uptake of the Fe(III) siderophore complex by specific transporters in the plasmalemma (cyanobacteria, grasses) (Raven 1988b; Hutchins 1995; Marschner 1995; Walls et al. 1995). The terrestrial higher plants thus have both the cyanobacterial (siderophores in grasses) and eukaryotic algal (Fe(III) reduction in a wide range of vascular plants) mechanisms. The Fe requirement for thylakoid redox reaction in higher plants is, of course, relatively immutable, subject to modification only by changes in the ratio of the complexes for the two Photosystems and other redox complexes in the thylakoid membrane. There is no substitution of ferredoxin by flavodoxin (slightly reducing the Fe requirement) or substituting plastocyanin by cytochrome c_6 (thereby slightly increasing the Fe requirement).

An enigmatic 'Fe-sparing' effect of V has been reported by Meisch and Beilig (1975) and Wilhelm and Wild (1984a), but not in higher plants (Hewitt 1983). This differential effect was attributed to an effect of V on a non-enzymatic transamination of δ-aminolevulinic acid in algae but not higher plants (Meisch et al. 1978; Hewitt 1983). However, it is now clear that the (enzymic) glutamate pathway of δ-aminolevulinic synthesis is ubiquitous for chlorophyll synthesis in O_2-evolvers (Falkowski and Raven 1997). There is no evidence that Fe is replaced by V in any Fe-containing catalyst of electron transport in photosynthesis, or that V was involved in the evolution of the Fe-containing catalysts of photosynthetic processes.

Conclusions

The involvement of trace metals in thylakoid reactions can in large part be explained by their redox chemistry and the availability during the evolution of the particular reaction sequence.

Although the quantitative role of trace metals in thylakoids is well understood for the major trace metal-containing complexes of linear electron transport from H_2O to $NADP^+$ and thence to CO_2, there are many fewer data on minor components. These minor components include the NAD(P)H-PQ oxidoreductase, which may be involved in chlororespiration and in cyclic electron flow and photophosphorylation, and the superoxide dismutases and peroxidases associated with the Mehler peroxidase reaction.

Major variations in Fe costs of linear electron transport occur via phylogenetically determined and acclimatory changes in the content of major Fe-containing complexes (PS II, cytochrome b_6-f, PS I). However, the acclimatory changes are not overtly driven by Fe availability but rather by changes in light quantity and quality. By contrast, variations in Fe availability alter the ferredoxin/flavodoxin ratio in some algae and cyanobacteria, while changes

in Cu and Fe availability alter the cytochrome c_6/plastocyanin ratio in some green algae and cyanobacteria. These switches have a relatively minor impact on overall Fe costs of linear electron transport, but have a very large impact on the Cu costs of linear electron transport.

Note added in proof

Hippler et al. (1998) give a detailed review of the use of *Chlamydomonas* genetics in studying biogenetic pathways, including H_2 metabolism. Hoefnagel et al. (1998) consider the interdependence between chloroplasts and mitochondria in the light and the dark, including a critical consideration of chlororespiration. Finally, Stewart and Brudwig (1998) review the stoichiometry and functioning of cytochrome b_{559} of Photosystem II.

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References

Allen JF (1993) Redox control of gene expression and the function of chloroplast genomes – an hypothesis. Photosynth Res 36: 950-102

Arisi A-CM, Cornic G, Jouanin L and Foyer CH (1998) Overexpression of iron superoxide dismutase in transformed poplar modifies the regulation of photosynthesis at low CO_2 partial pressures or following exposure to the preoxidant herbicide methyl viologen. Plant Physiol 117: 565–574

Arudchandran A, Seeburg D, Burkhart W and Bullerjahn GS (1994) Nucleotide sequence of the *pet E* gene encoding plastocyanin from the photosynthetic prokaryote, *Prochlorothrix hollandica*. Biochim Biophys Acta 1188: 447–449

Asada K (1992) Ascorbate peroxidase – a hydrogen peroxide scavenging enzyme in plants. Physiol Plant 85: 235–241

Asada K and Takahashi M (1987) Production and scavenging of active oxygen in photosynthesis. In: Kyle DJ, Osmond CB and Arntzen CJ (eds) Photoinhibition, pp 227–287. Elsevier, Amsterdam

Asada K, Kanematsu S and Uchida K (1977) Superoxide dismutases in photosynthetic organisms: Absence of CuZn enzyme in eukaryotic algae. Arch Biochem Biophys 179: 243–256

Asada K, Kanematsu S, Okada S and Hayakana T (1980) Phylogenetic distribution of three types of superoxide dismutase in organisms and in cell organelles. In: Bannister JV and Hill HAO (eds) Chemical and Biochemical Aspects of Superoxide and Superoxide Dismutase, pp 136–153. Elsevier, New York

Asada K, Heber U and Schreiber U (1993a) Electron flow to the intersystem chain from stromal components and cyclic electron flow in maize chloroplasts, as detected in intact leaves by monitoring redox change of P_{700} and chlorophyll fluorescence. Plant Cell Physiol 34: 39–50

Asada K, Miyake C, Sano S and Amako K (1993b) Scavenging of hydrogen peroxide in photosynthetic organisms – from catalase to ascorbate peroxidase. In: Kellinder KG, Rasmusssen SK, Penel C and Greppin H (eds) Plant Peroxidases: Biochemistry and Physiology, pp 243–250. University of Geneva

Avelange M-H and Rebeille F (1991) Mass spectrometric demonstration of O_2 gas exchange during a dark-to-light transition in higher plant cells. Evidence for two individual O_2-uptake components. Planta 183: 158–165

Avellano JB, Schröder WP, Sandmann G, Chueca A and Barón M (1994) Removal of nuclear contaminants and non-specifically Photosystem II-bound copper from Photosystem II preparations. Physiol Plant 91: 369–374

Badger MR (1985) Photosynthetic oxygen exchange. Annu Rev Plant Physiol 36: 27–53

Behrenfeld MJ and Kolber ZS (1999) Widespread iron limitation of phytoplankton in the South Pacific Ocean. Science 283: 840–843

Belkhodja R, Morales F, Quilez R, Lopez Millan AF, Abadia A and Abadia J (1998) Iron deficiency causes changes in chlorophyll fluorescence due to the reduction in the dark of the Photosystem II acceptor. Photosynth Res 56: 265–276

Bendall DS and Manusso RS (1995) Cyclic photophosphorylation and electron transport. Biochim Biophys Acta 1229: 23–38

Bennoun P (1982) Evidence for a respiratory chain in the chloroplast. Proc Natl Acad Sci 79: 4252–4256

Bennoun P (1993) Effects of mutations and of ionophores on chlororespiration in *Chlamydomonas reinhardtii*. FEBS Lett 156: 363–365

Bennoun P (1994) Chlororespiration revisited: mitochondrial-plastid interactions in *Chlamydomonas*. Biochim Biophys Acta 1186: 59–66

Berger S, Ellersiek U, Westhoff P and Steinmüller K (1993) Studies on the expression of NDH-H, a subunit of the NAD(P)H-plastoquinone-oxidoreductase of higher plant chloroplasts. Planta 190: 25–31

Berges JA, Charlebois DO, Mauzerall DG and Falkowski PG (1996) Differential effect of nitrogen limitation on the photosynthetic efficiencies of Photosystems I and II in microalgae. Plant Physiol 110: 689–696

Berry MC, Bratt PJ and Evans MCW (1997) Relaxation properties of the Photosystem I electron transfer components: Indications of the relative position of the electron transfer cofactors in Photosystem I. Biochim Biophys Acta 1319: 163–176

Bhattacharya D and Medlin L (1998) Algal phylogeny and the origin of land plants. Plant Physiol 116: 9–15

Biehle K and Fock H (1996) Evidence for the contribution of the Mehler-peroxidase reaction in dissipating excess electrons in drought-stressed wheat. Plant Physiol 112: 265–272

Blubaugh DJ and Govindjee (1988) The molecular mechanisms of the bicarbonate effect at the plastoquinone reductase site of photosynthesis. Photosynth Res 19: 85–128

Brand LC (1991) Minimum iron requirement of marine phytoplankton and the implications for the biogeochemical control of new production. Limnol Oceanogr 36: 1756–1771

Brandt U (1997) Proton translocation by membrane-bound NADH: Ubiquinone oxidoreductase (complex I) through redox-gated ligand conduction. Biochim Biophys Acta 1318: 79–91

Bruce D, Vidaver W, Colbow K and Popovic R (1983) Electron transport-dependent chlorophyll *a*-fluorescence quenching by O_2 in various algae and higher plants. Plant Physiol 73: 886–888

Büchel C and Garab G (1995) Evidence for the operation of a cyanide-sensitive oxidase in chlororespiration in the thylakoids of the chlorophyll *c*-containing alga *Pleurochloris meiringensis*. (Xanthophyceae). Planta 197: 69–75

Büchel C, Zsíros O and Garab G (1998) Alternative cyanide-sensitive oxidase interacting with photosynthesis in *Synechocystis* PCC6803. Ancestor of the terminal oxidase of chlororespiration? Photosynthetica 35: 223–231

Bullerjahn GS and Post AF (1993) The prochlorophytes: Are they more than just chlorophyll *a/b* containing cyanobacteria? CRC Crit Revs Micro 19: 43–59

Burger-Wiersma T and Matthijis HCP (1990) The Biology of the Prochlorales. In: Codd GA (ed) Autotrophic Microbiology and One-Carbon Metabolism, pp 1–24. Kluwer Academic Publishers, Dordrecht, The Netherlands

Burrows PA, Sazanov LA, Svab Z, Maliga P and Nixon PJ (1998) Identification of a functional respiratory complex in chloroplasts through analysis of tobacco mutants containing disrupted plastid *ndh* genes. EMBO J 17: 868–876

Butow BJ, Wynne D and Tel-Or E (1997) Superoxide dismutase activity in *Peridinium gatunense* in Lake Kinneret: Effect of light regime and carbon dioxide concentration. J Phycol 33: 787–793

Caron L, Berkaloff C, Duval, J-C and Jupin H (1986) Chlorophyll fluorescence transients from the diatom *Phaeodactylum tricornutum*: Relative rates of cyclic phosphorylation and chlororespiration. Photosynth Res 10: 131–139

Caron L, Mortain-Bertrand A and Jupin H (1988) Effect of photoperiod on photosynthetic characteristics of two marine diatoms. J Exp Mar Biol Ecol 123: 211–226

Casper-Lindley C and Björkman O (1997) Nigericin insensitive post-illumination reduction in fluorescence yield in *Dunaliella tertiolecta* (Chlorophyta). Photosynth Res 50: 209–222

Cavalier-Smith T (1982) The origin of plastids. Biol J Linn Soc 17: 289–306

Cavalier-Smith T (1992) The number of symbiotic origins of organelles. Biosystems 28: 91–106

Cha Y and Mauzerall DC (1992) Energy storage as linear and cyclic electron flows in photosynthesis. Plant Physiol 100: 1869–1877

Chase Z and Price NM (1997) Metabolic consequences of iron deficiency in heterotrophic marine protozoa. Limnol Oceanogr 42: 1673–1684

Cheeseman, JM, Herendeen LB, Cheeseman AT and Clough BF (1997) Photosynthesis and photoprotection in mangroves under field conditions. Plant Cell Environm 20: 579–588

Chow WS and Anderson JM (1987) Photosynthetic responses of *Pisum sativum* to an increase in irradiance during growth. II Thylakoid membrane components. Aust J Plant Physiol 14: 9–19

Chow WS, Anderson JM and Melis A (1990a) The Photosystem stoichiometry in thylakoids of some Australian shade-adapted species. Aust J Plant Physiol 17: 665–674

Chow WS, Goodchild DJ, Miller CI and Anderson JM (1990b) The influence of high levels of brief or prolonged supplementary far-red illumination during growth on the photosynthetic characteristics, composition and morphology of *Pisum sativum* chloroplasts. Plant Cell Environ 13: 135–145

Clarke AK and Campbell D (1996) Inactivation of the*Pet E* gene for plastocyanin lowers photosynthetic capacity and exacerbates chilling induced photoinhibition – in the cyanobacterium *Synechococcus*. Plant Physiol 112: 1551–1561

Cleaves HJ and Miller SL (1998) Oceanic protection of prebiotic organic compounds from UV radiation. Proc Natl Acad Sci 95: 7260–7263

Collén J (1994) Production of hydrogen peroxide and volatile hydrocarbons by macroalgae. Acta Universitas Upsaliensis, Uppsala

Collén J and Pedersén M (1996) Production, scavenging and toxicity of hydrogen peroxide in the green seaweed *Ulva rigida*. Eur J Phycol 31: 265–271

Couture M, Chamberland H, St-Pierre B and Guertin M (1994) Nuclear gene encoding chloroplast hemoglobins in the unicellular green algae *Chlamydomonas eugametos*. Mol Gen Genet 243: 185–187

Cramer WA, Soriano GM, Ponomarev M, Huang D, Zhang H, Martinez SE and Smith JL (1996) Some new structural aspects and old controversies concerning the cytochrome b_6-*f* complex of oxygenic photosynthesis. Annu Rev Plant Physiol and Plant Mol Biol 47: 477–508

Cunningham FX Jr, Dennenberg RS, Mustardy L, Jursinic PA and Gantt E. (1989) Stoichiometry of Photosystem I, Photosystem II and phycobilisomes in the red alga *Porphydridium cruentum* as a function of growth irradiance. Plant Physiol 91: 1179–1187

Darrouzet E, Issartel J-P, Lunardi J and Dupuis A (1998) The 49-kDa subunit of NADH-ubiquinone oxidoreductase (Complex I) is involved in the binding of piericidin and retenone, two quinone-related inhibitors. FEBS Lett 431: 34–38

de Jesus MD, Tabatabai F and Chapman DJ (1989) Taxonomic distribution of copper-zinc superoxide dismutase in green algae and its phylogenetic importance. J Phycol 24: 767–772

de Pamphilis CW and Palmer JD (1990) Loss of photosynthetic and chlororespiratory genes from the plastid genome of a parasitic flowering plant. Nature 348: 337–339

Diner BA and Petrouleas V (1988) Q_{400}, the non-haem iron of the Photosystem II iron-quinone complex. Biochim Biophys Acta Acta 985: 107–125

Droppa M and Horváth G (1990) The role of copper in photosynthesis. CRC Crit Rev Plant Sci 9: 111–123

Droppa M, Terry N and Horváth G (1984) Effects of Cu deficiency on photosynthetic electron transport. Proc Natl Acad Sci USA 81: 2369–2273

Dubinsky Z, Falkowski PG and Wyman K (1986) Light harvesting and utilization by phytoplankton. Plant Cell Physiol 27: 1335–1349

Eiler JN, Mojzsis SJ and Arrhenius G (1997) Carbon isotope evidence for early life. Nature 386: 665

Endo T and Asada K (1996) Dark induction of the non-photochemical quenching of chlorophyll fluorescence by acetate in *Chlamydomonas reinhardtii*. Plant Cell Physiol 37: 551–555

Endo T, Mi H, Shikanai T and Asada K (1997a) Donation of electrons to plastoquinone by NAD(P)H dehydrogenase and by ferredoxin-quinone reductase in spinach chloroplasts. Plant Cell Physiol 38: 1272–1277

Endo T, Shikanai T, Hashimoto T, Yamada Y, Yokota A and Asada K (1997b) Cyclic electron transport mediated by NAD(P)H dehydrogenase in tobacco chloroplasts. Plant Cell Physiol 38: S527

Endo T, Shikanai T, Sato F and Asada K (1998) NAD(P)H dehydrogenase-dependent, antimycin A-sensitive electron donation to plastoquinone in tobacco chloroplasts. Plant Cell Physiol 39: 1226–1231

Eshdat Y, Holland D, Faltin Z and Ben-Hayyim G (1997) Plant glutathione reductases. Physiol Plant 100: 234–240

Evans EH, Rush JD, Johnson CE, Evans MCW and Dickson DPE (1981) The nature of centre X of Photosystem I reaction centres

from the cyanobacterium *Chlorogloea fritschii* determined by Mossbauer spectrometry. Eur J Biochem 118: 81–84

Evans JR (1996) Developmental constraints on photosynthesis: Effects of light and nutrition. In: Baker NR (eds) Photosynthesis and the Environment, pp 281–304. Kluwer Academic Publishers, Dordrecht, The Netherlands

Evans MCW and Nugent JHA (1993) Structure and function of the reaction centre cofactors of oxygenic organisms. In: Norris JR and Diesenhoffer J (eds) The Photosynthetic Reaction Center, Vol I, pp 391–415. Academic Press, New York

Evans PK and Krogmann DW (1983) Three *c*-type cytochromes from the red alga *Porphyridium cruentum*. Arch Biochem Biophys 227: 494–510

Eyster C (1964) Micronutrient requirements for green plants, especially algae. In: Jackson DF (ed) Algae and Man, pp 86–119. Plenum Press, New York

Falkowski PG (1997) Evolution of the nitrogen cycle and its influence on biological sequestration of CO_2 in the ocean. Nature 387: 272–275

Falkowski PG and Owens TG (1980) Light-shade adaptation. Two strategies of marine phytoplankton. Plant Physiol 66: 592–595

Falkowski PG and Raven JA (1997) Aquatic Photosynthesis. Blackwell Science, Malden, MA

Falkowski PG, Owens TG, Ley AC and Mauzerall DC (1981) Effects of growth irradiance on the ratio of reaction centres in two species of marine phytoplankton. Plant Physiol 68: 969–973

Falkowski PG, Dubinsky Z and Wyman K (1985) Growth-irradiance relationships in phytoplankton. Limnol Oceanogr 30: 311–321

Falkowski PG, Sukenik A and Herzig R (1989) Nitrogen limitation of *Isochrysis galbana* (Haptophyceae). II. Relative abundance of chloroplast proteins. J Phycol 25: 471–478

Falkowski PG, Barber RT and Smetack V (1998) Biogeochemical controls and feedbacks on ocean primary productivity. Science 281: 200–206

Feild TS, Nedbal L and Ort DR (1998) Nonphotochemical reduction of the plastiquinone pool in sunflower leaves originates from chlororespiration. Plant Physiol 116: 1209–1218

Fork DC and Herbert SK (1993) Electron transport and photophosphorylation by Photosystem I *in vivo* in plants and cyanobacteria. Photosynth Res 36: 149–168

Friedmann AL and Alberte RS (1986) Biogenesis and light regulation of the major light harvesting chlorophyll-protein complexes of diatoms. Plant Physiol 80: 43–51

Fujita Y, Murakami A and Ohki K (1987) Regulation of photosystem composition in the cyanobacterial photosynthetic system: The regulation occurs in response to the redox state of the electron pool located between the two Photosystems. Plant Cell Physiol 28: 283–292

Funk E, Schäfer E and Steinmüller K (1999) Characterization of the complex 1-homologous NAD(P)H-plastoquinone-oxidoreductase (NDH-complex) of maize chloroplasts. J Plant Physiol 154: 16–23

Godde D (1982) Evidence for a membrane bound NADH-plastiquinone-oxidoreductase in *Chlamydomonas reinhardtii*. Arch Microbiol 131: 197–202

Golbeck JH (1992) Structure and function of Photosystem I. Annu Rev Plant Physiol and Plant Mol Biol 43: 293–324

Goyal A and Tolbert NE (1996) Association of glycolate oxidation with photosynthetic electron transport in plant and algal chloroplasts. Proc Natl Acad Sci USA 93: 3319–3324

Greene RM and Gerard VA (1990) Effects of high-frequency light fluctuation on growth and photoacclimation of the red alga *Chondrus crispus*. Mar Biol 105: 357–364

Greene RM, Geider RJ and Falkowski PG (1991) Effect of iron limitation on photosynthesis in a marine diatom. Limnol Oceanogr 36: 1772–1782

Groom Q, Kramer DM, Crofts AR and Ort DR (1993) The nonphotochemical reduction of plastoquinone in leaves. Photosynth Res 36: 205–215

Guedeney G, Corneille S, Cuine S and Peltier G (1996) Evidence for association of *ndh*B, *ndh*J gene products and ferredoxin-NADP-reductase as components of a chloroplastic NAD(P)H dehydrogenase complex. FEBS Lett 378: 277–280

Guenther JE, Nemsen JA and Melis A. (1988) Photosystem stoichiometry and chlorophyll antenna size in *Dunaliella salina* (green algae). Biochim Biophys Acta 934: 108–117

Haberhauser G and Zetschke K (1994) Functional loss of all *ndh* genes in an otherwise relatively unaltered plastid genome of the holoparasitic flowering plant *Cuscuta reflexa*. Plant Mol Biol 24: 217–222

Halliwell B and Gutteridge JMC (1989) Free Radicals in Biology and Medicine, second edition. Oxford University Press, Oxford

Hankamer B, Barber J and Boekema EJ (1997) Structure and membrane organization of Photosystem II in green plants. Annu Rev Plant Physiol Plant Mol Biol 48: 641–671

Harrison PJ, Yu PW, Thompson PA, Price NM and Phillips DJ (1988) Survey of selenium requirements in marine phytoplankton. Mar Ecol Prog Ser 47: 89–96

Heath RL (1972) Light requirements for H^+ transport by isolated chloroplast as measured by the bromocresol purple indicator. Biochim Biophys Acta 256: 645–655

Heber U and Walker DA (1992) Concerning a dual function of coupled cyclic electron transport in leaves. Plant Physiol 100: 1621–1626

Heber U, Bukhov NG, Neimanis S and Kobayashi Y (1995a) Maximum $H^+/h\upsilon_{PSI}$ stoichiometry of proton transport during cyclic electron transport in intact chloroplasts is at least two, but probably higher than two. Plant Cell Physiol 36: 1639–1647

Heber U, Gerst U, Krieger A, Niemanis S and Kibayashi Y (1995b) Coupled cyclic electron transport in intact chloroplasts and leaves of C_3 plants. Does it exist? If so, what is its function? Photosynth Res 46: 269–275

Henley WJ and Yin Y (1998) Growth and photosynthesis of marine *Synechococcus* (Cyanophyceae) under iron stress. J Phycol 34: 94–103

Henry LEA, Halliwell B and Hall DO (1976) The superoxide dismutase activity of various organisms measured by a new and rapid assay technique. FEBS Lett 66: 303–306

Henzler T and Steudle E (1998) Water and solute permeability across water channels. J Exp Bot 49: 105

Herbert SK, Fork DC and Malkin S (1990) Photoaccoustic measurements of *in vivo* energy storage by cyclic electron flow in algae and higher plants. Plant Physiol 94: 926–934

Hewett-Emmett D and Tashian RE (1996) Functional diversity, conservation and convergence in the evolution of the α-, β- and δ-carbonic anhydrase gene families. Mol Phylogenetics Evol 5: 50–77

Hewitt EJ (1983) A perspective on mineral nutrition: Essential and functional minerals in plants. In: Robb PA and Pierpoint WB (eds) Metals and Micronutrients: Uptake and Utilization by Plants, pp 277–323. Academic Press, London

Hihara Y, Sonoike K and Ikeuchi M (1998) A novel gene, *pmgA*, specifically regulates Photosystem stoichiometry in the cyanobacterium *Synechocystis* sp. PCC 6803 in response to high light. Plant Physiol 117: 1205–1216

Hill R and Bendall F (1960) Function of two cytochrome components in chloroplasts: A working hypothesis. Nature 186: 136–137

Hippler H, Redding K and Rochaix J-D (1998) *Chlamydomonas* genetics, a tool for the study of bioenergetic pathways. Biochim Biophys Acta 1367: 1–62

Hoefnagel MHN, Atkin OK and Wiskich JT (1998) Interdependence between chloroplasts and mitochondria in the light and the dark. Biochim Biophys Acta 1366: 235–255

Hoganson CW and Babcock GT (1997) A metalloradical mechanism for the generation of oxygen from water in photosynthesis. Science 277: 1953–1956

Hope AB (1993) The chloroplast cytochrome *bf* complex: A critical focus on function. Biochim Biophys Acta 1143: 1–22

Howitt CA, Whelan J, Price GD and Day DA (1996) Cloning, analysis and inactivation of the *ndhK* gene encoding a subunit of NADH quinone oxidoreductase from *Anabaena* PCC 7120. Eur J Biochem 240: 173–180

Hulsebosch RJ, Allaklendiev SI, Klimov VV, Picord R and Hoff AJ (1998) Effect of bicarbonate on the S_2 multiline EPR signal of the oxygen-evolving complex in Photosystem II membrane fragments. FEBS Lett 424: 146–148

Hutchins DA (1995) Iron and the marine phytoplankton community. Prog Phycol Res11: 1–49

Iglesias-Prieto R and Trench RK (1994) Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density. Mar Biol 113: 163–175

Imsande J (1998) Iron, sulfur, and chlorophyll deficiencies: A need for an integrative approach in plant physiology. Physiol Plant 103: 139–144

Iwamoto K and Ikawa T (1997) Glycolate metabolism and subcellular distribution of glycolate oxidase in *Spatoglossum pacificum* (Phaeophyceae, Chromophyta). Phycol Res 45: 77–83

Iwata S, Lee JW, Okada K, Lee JK, Iwata M, Rasmussen B, Link TA, Ramaswarmy S and Jap BK (1998) Complete structure of the 11-subunit bovine mitochondrial cytochrome bc_1 complex. Science 281: 64–71

Kana TM (1990) Light-dependent oxygen cycling measured by an oxygen-18 isotope dilution method. Mar Ecol Prog Ser 64: 293–300.

Kana TM (1992) Relationship between photosynthetic oxygen cycling and carbon assimilation in *Synechococcus* WH 7803 (Cyanophyta). J Phycol 28: 304–308

Kana TM (1993) Rapid oxygen cycling in *Trichodesmium thiebautii*. Limnol Oceanogr 38: 18–24

Kaneko T, Sato S, Kotani H, Tanaka A, Asamizu E, Nakamura Y, Miyajima N, Hirosawa M, Sugiura M, Sasamoto S, Kimura T, Hosouchi T, Matsuno A, Muraki A, Nakasaki N, Nanio K, Okumura S, Shimpo S, Takeuchi C, Wada T, Watanabe A, Yarrada M, Yasuda M and Tabata S (1996) Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. DNA Res 3: 109–136

Karlson J, Clarke A, Chen Z, Mason CB, Moroney JV and Samuelsson G (1996) Chloroplastic carbonic anhydrase of *Chlamydomonas reinhardtii*: Molecular cloning, sequencing and Western blot analysis of high C_i-requiring mutants. In: Melkonian M (ed) Abstracts of the First European Phycological Congress Cologne, August 1996. Abstract 248, p 36

Karlson J, Clarke A, Chen Z, Hugghins SY, Park YI Husic HD, Moroney JV and Samuelsson G (1998) A novel α-type carbonic anhydrase associated with the thylakoid membrane in *Chlamydomonas reinhardtii* is required for growth at ambient CO_2. EMBO J 17: 1208–1216

Kerfeld CA and Krogmann DW (1998) Photosynthetic cytochromes *c* in cyanobacteria, algae and plants. Annu Rev Plant Physiol and Plant Mol Biol 49: 397–425

Kirk JTO (1994) Light and Photosynthesis in Aquatic Ecosystems, second edition. Cambridge University Press, Cambridge

Klimov VV, Baranov SV and Allaklendiev SI (1997) Bicarbonate protects the donor side of Photosystem II against photoinhibition and thermoinactivation. FEBS Lett 418: 243–246

Klöck G, Sültemeyer DF, Fock HP and Kreuzberg K (1989) Gas exchange in intact isolated chloroplasts from *Chlamydomonas reinhardtii* during starch degradation in the dark. Physiol Plant 75: 109–113

Klugehammer B, Sültemeyer D, Badger MR and Price GD (1997) Analysis of the CO_2 concentrating mechanism in the marine cyanobacterium, *Synechococcus* PCC7002: The role of NdhD3, forming part of a NADH dehydrogenase complex. Abst 13th Annual Meeting Australian Society for Phycology and Aquatic Botany, Hobart, 22–24 January, 1997, pp 80–81

Krieger A and Rutherford AW (1997) Comparison of chloride-depleted and calcium-depleted Photosystem II: The midpoint potential of Q_A and susceptibility to photodamage. Biochim Biophys Acta 1319: 91–98

Kubicki A, Fuuk E, Westhoff P and Steinmüller K (1996) Differential expression of plastome-encoded *ndh* genes in mesophyll and bundle-sheath chloroplasts of the C_4 plant *Sorghum bicolor* indicates that the complex-I-homologous NAD(P)H-plastoquinone oxidoreductase is involved in cyclic electron transport. Planta 199: 276–281

Kübler JE and Davison IR (1993) Thermal acclimation of light-use characteristics of *Chondrus crispus* (Rhodophyta). Eur J Phycol 30: 189–195

Kudo I and Harrison PJ (1997) Effect of iron nutrition on the marine cyanobacterium *Synechococcus* grown on different N sources and irradiances. J Phycol 33: 232–240

La Roche J, Boya PW, McKay RML and Geider RJ (1996) Flavodoxin as an *in situ* marker for iron stress in phytoplankton. Nature 382: 802–805

Latimer MJ, Derose VJ, Mukerji I, Yachandra VK, Sauer K and Klein MP (1995) Evidence for the proximity of calcium to the manganese cluster of Photosystem II: Determination by x-ray absorption spectroscopy. Biochem 34: 10898–10909

Lawlor DW (1993) Photosynthesis: Molecular, Physiological and Environmental Processes, second edition. Longman Scientific and Technical, Harlow

Lee SC and Berry MJ (1996) Knowing when not to stop: Selenocysteine incorporation in eukaryotes. Trends Biochem Sci 21: 203–205

Lee WJ and Whitmarsh J (1989) Photosynthetic apparatus of pea thylakoid membranes. Response to growth light intensity. Plant Physiol 89: 932–940

Li Q and Canvin DT (1998) Energy source for HCO_3^- and CO_2 transport in air-grown cells of *Synechococcus* UTEX 625. Plant Physiol 116: 1125–1132

MacLachlan DJ, Nugent JHA and Evans MCW (1994a) A XANES study of the manganese complex of inhibited PS II membrane indicates manganese redox changes between the modified S_1, S_2 and S_3 states. Biochim Biophys Acta 1185: 103–111

MacLachlan DJ, Nugent JHA, Bratt PJ and Evans MCW (1994b) The effects of calcium depletion on the O_2-evolving complex in spinach PS II. Biochim Biophys Acta 1186: 186–200

Maden BEH (1995) No soup for starters? Autotrophy and the origin of metabolism. Trends Ecol 10: 111–116

Maldonado MT and Price NM (1996) Influence of N substrate on Fe requirements of marine centric diatoms. Mar Ecol Prog Ser 141: 161–172

Malkin S and Canaani O (1994) The use and characteristics of the photoaccoustic method in the study of photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 45: 493–526:

Malkin S, Herbert SK and Fork DC (1990) Light distribution, transfer and utilization in the marine red alga *Porphyra perforata* from photoaccoustic energy-storage measurements. Biochim Biophys Acta 1016: 177–189

Mancinelli RL and McKay CP (1988) The evolution of nitrogen cycling. Origin Life Evolution Biosphere 18: 311–325

Manna P and Vermaas W (1997) Lumenal proteins involved in respiratory electron transport in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Mol Biol 35: 407–416

Mano S, Yamaguchi K, Hayashi M and Nishimura M (1997) Stromal and thylakoid-bound ascorbate peroxidases are produced by alternative splicing in pumpkin. FEBS Lett 413: 21–26

Manodori A and Melis A (1987) Cause and effect relationship between environmental conditions and photosystem stoichiometry in *Synechococcus* 6301. In: Biggins J (ed) Progress in Photosynthesis Research, Volume II, pp 249–252. Nijhoff, Dordrecht, The Netherlands

Marschner H (1995) Mineral Nutrition of Higher Plants, second edition. Academic Press, London

Martin W, Stoebe B, Goremykin V, Hansmann S, Hasegawa M and Kowallik KV (1998) Gene transfer to the nucleus and the evolution of chloroplasts. Nature 393: 162–165

Mathai JC and Sitavaman V (1994) Stretch sensitivity of transmembrane mobility of hydrogen peroxide through voids in the bilayer. Role of cardiolipin. J Biol Chem 269: 17784–17793

Matsuo M, Endo T and Asada K (1997) Purification of NAD(P)H dehydrogenase complex (NDH) in *Synechocystis* PCC6803. Plant Cell Physiol 38: 528

Matsuo M, Endo T and Asada K (1998) Properties of the respiratory NAD(P)H dehydrogenase isolated from the cyanobacterium *Synechocystis* PCC 6803. Plant Cell Physiol 39: 263–267

Meisch H-U and Bielig H-J (1975) Effect of vanadium on growth, chlorophyll formation and iron metabolism in unicellular green algae. Arch Microbiol 105: 77–82

Meisch H-U, Hoffmann H and Reinle W (1978) Vanadium catalysis in the nonenzymatic transamination of δ-aminolevulinic acid. Z Naturforsch 33C: 623–628

Merchant S and Dreyfuss BW (1998) Post-translational assembly of photosynthetic metalloproteins. Annu Rev Plant Physiol Plant Mol Biol 49: 25–51

Messinger J, Nugent JHA and Evans MCW (1997) Detection of an epr multiline signal for the S_0 state in Photosystem II. Biochem 36: 11055–11060

Mi H, Endo T, Schreiber U, Ogawa T and Asada K (1992a) Electron donation from cyclic and respiratory flows to the photosynthetic chain is mediated by pyridine nucleotide dehydrogenase in the cyanobacterium *Synechocystis* PCC6803. Plant Cell Physiol 33: 1233–1237

Mi H, Endo T, Schreiber U and Asada K (1992b) Donation of electrons from cytosolic components to the intersystem chain in the cyanobacterium *Synechococcus* sp. PCC7002 as determined by the reduction of $P_{700}{}^{+}$. Plant Cell Physiol 33: 1099–1105

Mi H, Endo T, Ogawa T and Asada K (1995) Thylakoid membrane-bound, NADPH-specific pyridine nucleotide dehydrogenase complex mediates cyclic electron transport in the cyanobacterium *Synechocystis* sp. PC 6803. Plant Cell Physiol 36: 661–668

Mi H, Endo T, Matsuo M, Ogawa T and Asada K (1997) Inhibition of NAD(P)H dehydrogenase (NDH) by HQNO in the cyanobacterium *Synechocystis* PCC6803. Plant Cell Physiol 38: 528

Miyake C and Asada K (1992a) Thylakoid-bound ascorbate peroxidase in spinach chloroplasts and photoreduction of its primary oxidation product monodehydroascorbate radicals in thylakoids. Plant Cell Physiol 33: 541–553

Miyake C and Asada K (1992b) Thylakoid-bound ascorbate peroxidase scavenges hydrogen peroxide photoproduced – photoreduction of monodehydroascorbate radical. In: Murata N (ed) Research in Photosynthesis, II, pp 563–566. Kluwer Academic Publishers, Dordrecht, The Netherlands

Miyake C, Michihata F and Asada K (1991) Scavenging of hydrogen peroxide in prokaryotic and eukaryotic algae: acquisition of ascorbate peroxidase during the evolution of cyanobacteria. Plant Cell Physiol 32: 33043

Miyake C, Cao W-H and Asada K (1993) Purification and molecular properties of the thylakoid-bound ascorbate peroxidase in spinach chloroplasts. Plant Cell Physiol 34: 881–889

Miyake C, Schreiber U and Asada K (1995) Ferredoxin-dependent and Antimycin A-sensitive reduction of cytochrome *b*-559 by far-red light in maize thylakoids; participation of a monodial-reducible cytochrome *b*-559 in cyclic electron flow. Plant Cell Physiol 36: 743–748

Mojzis SJ, Arrhenius G, McKeegan KD, Harrison TM, Nutman AP and Friend CRL (1996) Evidence for life on earth before 3800 million years ago. Nature 384: 55–59

Morel FMM, Reinfelder JR, Roberts SB, Chamberlain CP, Lee JG and Yee D (1994) Zinc and carbon co-limitation of marine phytoplankton. Nature 369: 740–742

Muggli DL and Harrison PJ (1997) Effects of iron on two oceanic phytoplanktons grown in natural NE subarctic Pacific seawater with no artificial chelaters present. J Exp Mar Biol Ecol 212: 225–237

Mulkidjianian Y and Junge W (1997) On the origin of photosynthesis as inferred from sequence analysis – a primordial UV-protection as common ancestor of reaction centers and antenna proteins. Photosynth Res 31: 27–42

Mullineaux PM, Karpinski S, Jiménez A, Cleary SP, Robinson C and Creisser GP (1998) Identification of cDNAs encoding plastid-targeted glutathione peroxidase. Plant J 13: 375–379

Murakami A, Kim S-J and Fujita Y (1997) Changes in photosystem stoichiometry in response to environmental conditions for cell growth observed with the cyanophyte *Synechocystis* PCC6714. Plant Cell Physiol 38: 392–397

Navarro JA, Hervas M, De Le Gedra B and De La Rosa MA (1995) Purification and physicochemical properties of the low-potential cytochrome c_{549} from the cyanobacterium *Synechocystis* sp. PCC6803. Arch Biochem Biophys 318: 46–52

Nicholls DG and Ferguson SJ (1992) Bioenergetics 2. Academic Press, London

Nixon PJ and Maliga P (1999) Chlororespiration: Only half a story. Trends Plant Sci 4: 51

Noctor G and Foyer CH (1998) Ascorbate and glutathione: Keeping active oxygen under control. Annu Rev Plant Physiol and Plant Mol Biol 49: 249–279

Obinger C, Regelsberger G, Pircher A, Strasser G and Peschek GA (1998) Scavenging of superoxide and hydrogen peroxide in blue-green algae (cyanobacteria). Physiol Plant 104: 693–698

Ogawa T (1991) A gene homologous to the subunit-2 gene of NADH dehydrogenase is essential to inorganic carbon transport of *Synechocystis* PCC 6803. Proc Natl Acad Sci 88: 4275–4279

Ogawa T, Omata T, Miyano A and Inowe Y (1985) Photosynthetic reactions involved in the CO_2-concentrating mechanism in the cyanobacterium, *Anacystis nidulans*. In: Lucas WJ and Berry JA (eds) Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms, pp 287–304. American Society of Plant Physiologists, Rockville, MD

Ogawa K, Kanematsu S, Takabe K and Asada K (1995) Attachment of CuZn-superoxide dismutase to thylakoid membranes at the site of superoxide generation (PS I) in spinach chloroplasts: detection by immuno-gold labelling after rapid freezing and substitution method. Plant Cell Physiol 36: 565–573

Ohkawa H, Murakami A, Sonoda M and Ogawa T (1998) Analysis of five *ndhD* mutants of *Synechocystis* PCC6803. Plant Cell Physiol 39: s19

Okada S, Kanematsu S and Asada K (1979) Intracellular distribution of manganese and ferric superoxide dismutases in blue-green algae. FEBS Lett 103: 106–110

Osmond CB and Grace SC (1995) Perspectives on photoinhibition and photorespiration in the field: quintessential inefficiencies of the light and dark reactions of photosynthesis? J Exp Bot 46: 1351–1362

Pace NR (1997) A molecular view of microbial diversity and the biosphere. Science 276: 734–740

Pakulski JD, Coffin RB, Kelley CA, Holde, SL, Downer R, Aas P, Lyons Mm and Jeffrey WH (1996) Iron stimulation of Antarctic bacteria. Nature 383: 133–134

Partensky F, La Roche J, Wyman K and Falkowski PG (1997) The divinyl-chlorophyll *a/b*-protein complexes of two strains of the oxyphototrophic marine prokaryote *Prochlorococcus* – characterization and response to changes in growth irradiance. Photosynth Res 51: 209–222

Patterson COP and Myers T (1973) Photosynthetic production of hydrogen peroxide by *Anacystis nidulans*. Plant Physiol 51: 104–109

Peltier G and Schmidt GW (1991) Chlororespiration: An adaptation to nitrogen deficiency in *Chlamydomonas reinhardtii*. Proc Natl Acad Sci USA 88: 4791–4795

Pfannschmidt T, Nilsson A and Allen JF (1999) Photosynthetic control of chloroplast gene expression. Nature 397: 625–628

Prosil, O, Kolber Z, Berry JA and Falkowski PG (1996) Cyclic electron flow around PS II *in vivo*. Photosynth Res 48: 395–410

Price NM and Harrison PJ (1988) Specific selenium-containing macromolecules in the marine diatom *Thalassiosira pseudonana*. Plant Physiol 86: 192–199

Price NM, Thompson A and Harrison PJ (1987) Selenium: An essential element for growth of the coastal marine diatom *Thalassiosira pseudonana* (Bacillariophyceae). J Phycol 23: 1–9

Price NM, von Caemmerer S, Evans JR, Siebke K, Andersen JM and Badger MR (1998) Photosynthesis is strongly reduced by antisense suppression of chloroplastic cytochrome *bf* complex in transgenic tobacco. Aust J Plant Physiol 25: 445–452

Radmer RK and Kok B (1976) Photoreduction of O_2 primes and replaces CO_2 assimilation. Plant Physiol 58: 336–340

Radmer RK and Ollinger O (1980) Light-driven uptake of oxygen, carbon dioxide and bicarbonate by the green alga *Scenedesmus*. Plant Physiol 65: 723–729

Radmer RK and Ollinger O (1981) The variability of light-driven oxygen uptake in algae. Plant Physiol 67: 119.

Radmer RK, Kok B and Ollinger O (1978) Kinetics and apparent K_m of O_2 cycle under conditions of limiting carbon dioxide fixation. Plant Physiol 61: 915–917

Raven JA (1976a) Division of labour between chloroplasts and cytoplasm. In: Barber J (ed) The Intact Chloroplast, pp 403–443. Elsevier, Amsterdam

Raven JA (1976b) Transport in algal cells. In: Lüttge U and Pitman Mg (eds) Transport in Cells and Tissues: Encyclopedia of Plant Physiol, new series, Vol IIA, pp 129–188. Springer-Verlag, Berlin

Raven JA (1982) The energetics of freshwater algae; energy requirements for biosynthesis and volume regulation. New Phytol 92: 10–20

Raven JA (1984) Energetics and Transport in Aquatic Plants. A.R. Liss, New York

Raven JA (1986) Physiological consequences of extremely small size for autotrophic organisms in the sea. In: Platt TR and Li WKW (eds) Photosynthetic Picoplankton, pp 1–70. Canadian Bulletin of Fisheries and Aquatic Sciences 214

Raven JA (1987a) Biochemistry, biophysics and physiology of chlorophyll *b*-containing algae: Implications for taxonomy and phylogeny. Progr Phycol Res 5: 1–122

Raven JA (1987b) The role of vacuoles. New Phytol 106: 357–422

Raven JA (1988a) The iron and molybdenum use efficiencies of plant growth with different energy, carbon and nitrogen sources. New Phytol 109: 279–285

Raven JA (1988b) Algae. In: Baker DA and Hall JL (eds) Solute Transport in Plant Cells and Tissues, pp 166–219. Longman Scientific and Technical, Harlow

Raven JA (1990) Predictions of Mn and Fe use efficiencies of phototrophic growth as a function of light availability for growth and C assimilation pathway. New Phytol 116: 1–18

Raven JA (1995) Costs and benefits of low intracellular osmolarity in cells of freshwater algae. Funct Ecol 9: 701–707.

Raven JA (1997a) CO_2 concentrating mechanisms: A direct role for thylakoid lumen acidification? Plant Cell Environ 20: 147–154

Raven JA (1997b) The role of marine biota in the evolution of terrestrial biota: Gases and genes. Biogeochemistry 39: 139–164

Raven JA (1997c) The vacuole: A cost-benefit analysis. Adv Bot Res 25: 59–86

Raven JA (1997d) Inorganic carbon acquisition by marine autotrophs. Adv Bot Res 27: 85–209

Raven JA (1998a) Small is beautiful. Funct Ecol 12: 503–513

Raven JA (1998b) Extrapolating feedback processes from the present to the past. Phil Trans R Soc London 353: 19–28

Raven JA (1999) Picophytoplankton. Prog Phycol Res, in press

Raven JA and Yin Z-H (1998) The past, present and future of nitrogenous compounds in the atmosphere and their interactions with plants. New Phytol 139: 205–219

Raven JA, Johnston AM and bin Surif M (1989) The photosynthetic apparatus as a phyletic character. In: Green JC, Leadbeater BSC and Diver WL (eds) The Chromophyte Algae: Problems and Perspectives, pp 63–84. The Systematics Association Special Volume No 33. Clarendon Press, Oxford

Raven JA, Johnston AM and MacFarlane JJ (1990) Carbon metabolism. In: Sheath RG and Cole KM (eds) The Biology of the Red Algae, pp 171–202. Cambridge University Press, Cambridge

Raven JA, Johnston AM, Kübler JE, and Parsons R (1994) The influence of natural and experimental high O_2 concentration on O_2-evolving photolithotrophs. Biol Revs 69: 61–94

Regelsberger G, Obinger C, Zoder R, Altman F and Peschek G (1999) Purification and characterization of a hydroperoxidase from the cyanobacterium *Synechocystis* PCC 6803: Identification of its gene by peptide mass mapping using matrix assisted laser desorption ionization time-of-flight mass spectrometry. FEMS Microbiol Lett 170: 1–12

Reith M (1995) Molecular biology of rhodophyte and chromophyte plastids. Annu Rev Plant Physiol and Plant Mol Biol 46: 549–575

Rhee K-H, Morris EP, Barber J and Kühlbrandt W (1998) Three-dimensional structure of the plant Photosystem II reactive centre at 8Å resolution. Nature 396: 283–286

Rich PR, Hoefnagel MHN and Wiskich JT (1998) Possible chlororespiratory reactions of thylakoid membranes. In: Moeller IM, Gardeström P, Glimelius K and Glaser E (eds) From Gene to Function, pp 17–23. Backhuys Publishers, Leiden

Riggs-Gelasco P, Mei R, Yocum CF and Penner-Hahn JE (1996) Reduced derivatives of the Mn cluster in the oxygen evolving complex Photosystem II, an EXAFS study. J Am Chem Soc 118: 2387–2399

Rochaix J-D (1997) Chloroplast reverse genetics: New insights into the function of plastid genes. Trends Plant Sci 2: 419–425

Rögner M, Nixon PJ and Diner BA (1990) Purification and characterization of Photosystem I and Photosystem II core complexes from wild-type and phycocyanin-deficient strains of the cyanobacterium *Synechocystis* PCC6803. J Biol Chem 265: 6169–6196

Roldán M (1999) Can chlororespiration in plants help to explain the controversial phenotype of *ndh* mutants? Trends Plant Sci 4: 50

Rosing MT (1999) ^{13}C-depleted carbon microparticles in >3700-Ma sea-floor sedimentary rocks from West Greenland. Science 283: 674–676

Rueter JG (1993) Limitation of primary productivity in the oceans by light, nitrogen and iron. In: Yamamoto HY and Smith CM (eds) Photosynthetic Responses to the Environment, pp. 126–135. American Society for Plant Physiol, Rockville, MD

Russell MJ and Hall AJ (1997) The emergence of life from iron monosulphide bubbles at a submarine hydrothermal redox and pH front. J Geol Soc London 154: 377–402

Sazanov LA Burrows PA and Nixon PJ (1998a) The plastid *ndh* genes code for a NADH-specific dehydrogenase: Isolation of a complex 1 analogue from pea thylakoid membranes. Proc Natl Acad Sci USA 95: 1319–1324

Sazanov LA, Burrows PA and Nixon PJ (1998b) The chloroplast *ndh* complex mediates the dark reduction of the plastoquinone pool in response to heat stress in tobacco leaves. FEBS Lett 429: 115–118

Schäfer G, Purschke W and Schmidt CL (1996) On the origin of respiration: Electron transport proteins from Archaea to man. FEMS Microbiol Revs 18: 173–188

Scheller HV (1996) *In vitro* cyclic electron transport in barley follows two independent pathways. Plant Physiol 110: 187–194

Scheller HV, Naver H and Moller BL (1997) Molecular aspects of Photosystem I. Physiol Plant 100: 842–851

Scherer S (1990) Do photosynthetic and respiratory electron transport chains share redox proteins? Trends Biochem Sci 15: 458–462

Schidlowski M (1988) A 3,800-million-year isotopic record of life from carbon in sedimentary rocks. Nature 333: 313–318

Schluchler WM, Zhao J and Bryant DA (1993) Isolation and characterization of the *ndhF* gene of *Synechococcus* sp. PCC7002 and initial characterization of an interposon mutant. J Bacteriol 175: 3343–3352

Schmetterer G (1994) Cyanobacterial respiration. In: Bryant DA (ed) The Molecular Biology of Cyanobacteria, pp 409–435. Kluwer Academic Publishers, Dordrecht, The Netherlands

Schomburg D and Stephan D (eds) (1994) Enzyme Handbook, Volume 8. Springer Verlag, Berlin

Schomburg D, Salzmann M and Stephan D (eds) (1994) Enzyme Handbook, Volume 7. Springer Verlag, Berlin

Schubert WD, Klukas O, Krauss N, Saenger W, Fromme P and Witt HT (1997) Photosystem I of *Synechococcus elongatus* at 4 Angstrom resolution: A comprehensive structure analysis. J Mol Biol 272: 741–769

Shigeoka S, Takeda T and Hanaoka T (1991) Characterization and immunological properties of selenium-containing glutathione peroxidase induced by selenite in *Chlamydomonas reinhardtii*. Biochem J 275: 623–627

Shikanai T, Endo T, Hashimoto T, Yamada Y, Asada K and Yokota A (1998a) Directed disruption of the tobacco *ndhB* gene impairs cyclic electron flow around PS I. Proc Natl Acad Sci USA 95: 9705–9709

Shikanai T, Takeda T, Yamauchi H, Sano S, Tomizawa K-I, Yokota A and Shigeoka S (1998b) Inhibition of ascorbate peroxidase under oxidative stress in tobacco having bacterial catalase in chloroplasts. FEBS Lett 428: 47–51

Siedow JN (1995) Bioenergetics: The mitochondrial electron transfer chain. In: Levings III CS and Vasil IK (eds) The Molecular Biology of Plant Mitochondria, pp 281–312. Kluwer Academic Publishers, Dordrecht, The Netherlands

Smith BM and Melis A (1987) Photosystem stoichiometry and excitation distribution in chloroplasts from surface and minus 20 m blades of *Macrocystis pyrifera*, the giant kelp. Plant Physiol 84: 1325–1330

Smith JL (1998) Secret life of cytochrome bc_1. Science 281: 58–59

Smith PM and Melis A (1988) Photosynthetic responses of diatoms to growth irradiance. Plant Physiol 86: 132a

Stadtman TC (1996) Selenocysteine. Annu Rev Biochem 65: 83–100

Stemler AJ (1997) The case for chloroplast thylakoid carbonic anhydrase. Physiol Plant 99: 348–353

Stewart DH and Brudwig GW (1998) Cytochrome b_{559} of photosystem II. Biochim Biophys Acta 1367: 63–87

Stiller JW and Hall BD (1997) The origin of red algae: Implications for plastid evolution. Proc Natl Acad Sci USA 94: 4520–4525

Sukenik A, Bennett J and Falkowski P (1987) Light-saturated photosynthesis-limitation by electron transport or carbon fixation. Biochim Biophys Acta 891: 205–215

Sültemeyer D, Price GD, Bryant DA and Badger MR (1997) *PsaE* and *ndhF* mediated electron transport affects bicarbonate transport rather than carbon dioxide uptake in the cyanobacterium *Synechococcus* sp. PCC 7002. Planta 201: 36–42

Summers DP and Chang S (1993) Prebiotic ammonia from reduction of nitrite by iron (II) on the early earth. Nature 365: 630–633

Sunda WG and Huntsman SA (1986) Relationships among growth rate, cellular manganese concentrations, and manganese transport kinetics in estuarine and oceanic species of the diatom *Thalassiosira*. J Phycol 22: 259–270

Sunda WG and Huntsman SA (1995a) Iron uptake and growth limitation in oceanic and coastal phytoplankton. Mar Chem 50: 189–206

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

Sunda WG and Huntsman SA (1997) Interrelated influence of iron, light and cell size on marine picophytoplankton growth. Nature 390: 389–392

Sunda WG and Huntsman SA (1998) Interactive effects of external manganese, the toxic metals copper and zinc, and light in controlling cellular manganese and growth in a coastal diatom. Limnol Oceanogr 43: 1467–1475

Sunda WG, Swift DG and Hutchison SA (1991) Low iron requirement for growth in oceanic phytoplankton. Nature 351: 55–57

Suzuki K, Iwamoto K, Yokoyama S, Ikawa T (1991) Glycolate-oxidizing enzymes in algae. J Phycol 27: 492–498

Takahashi M-A and Asada K (1983) Superoxide anion permeability of phospholipid membranes and chloroplast thylakoids. Arch Biochem Biophys 226: 558–566

Takeda T, Nakano Y and Shigeoka S (1993) Effects of selenite, CO_2 and illumination on the induction of selenite-dependent glutathione peroxidase in *Chlamydomonas reinhardtii*. Plant Sci 94: 81–88

Takeda T, Yokota Y and Shigeoka S (1995) Resistance of photosynthesis to hydrogen peroxide in algae. Plant Cell Physiol 36: 1089–1095

Tanaki Y, Katada S, Ishikawa H, Ogairana T and Takabe T (1997) Electron flow from NAD(P)H dehydrogenase to Photosystem I is required for adaptation to salt shock in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Cell Physiol 38: 1311–1318

Teicher HB and Scheller HV (1998) The NAD(P)H dehydrogenase in barley thylakoids is photoactivable and uses NADPH as well as NADH. Plant Physiol 117: 525–532

Tel-Or E, Nuflejt M and Packer L (1985) The role of glutathione and ascorbate in hydroperoxide removal in cyanobacteria. Biochem Biophys Res Commun 132: 533–539

Ting CS and Owens TG (1993) Photochemical and nonphotochemical fluorescence quenching processes in the diatom *Phaeodactylum tricornutum*. Plant Physiol 101: 1323–1330

Tortel PD, Maldonado MT and Price NM (1996) The role of heterotrophic bacteria in iron-limited ocean ecosystems. Nature 383: 330–332

Trissl HW and Wilhelm C (1993) Why do thylakoids from higher plants form membrane stacks? Trends Biochem Sci 18: 415–419

Vallee BL and Galdes A (1984) The metallobiochemistry of zinc enzymes. Advs Enzymol 56: 283–430

van den Hoek C, Mann DG and Jahns HM (1995) Algae: An Introduction to Phycology. Cambridge University Press, Cambridge

Van Leeuwe MA, Stefels J and de Baar HJW (1997) The light harvesting capacity of Antarctic phytoplankton remains under iron limitation. Phycologia 34 (supplement) 116

Wakao N, Yokoi N, Isoyama N, Hiraishi A, Shimada, K, Kobayashi M, Kise H, Iwaka M, Itoh S, Takaichi S and Sakurai Y (1996) Discovery of natural photosynthesis using Zn-containing bacteriochlorophyll in an aerobic bacterium *Acidiphilium rubrum*. Plant Cell Physiol 37: 889–893

Wakasugi T, Tsudzuki T, Shibata M and Hira A (1994) Loss of all *ndh* genes as determined by sequencing the entire chloroplast genome of black pine (*Pinus thungergii*). Proc Natl Acad Sci USA 91: 9794–9798

Walker JE (1992) The NADH: ubiquinone oxidoreductase (complex I) of respiratory chains. Quarterly Rev Biophys 25: 253–324

Walls ML, Price NM and Bruland KW (1995) Iron chemistry in seawater and its relationship to phytoplankton: A workshop report. Mar Chem 48: 157–182

Whitehouse DG and Moore AL (1995) Regulation of oxidative phosphorylation in plant mitochondria. In: Levings III CS and Vasil IK (eds) The Molecular Biology of Plant Mitochondria, pp 313–344. Kluwer Academic Publishers, Dordrecht, The Netherlands

Whitmarsh J, Samson G and Poulson M (1994) Photoprotection in Photosystem II – the role of cytochrome b_{559}. In: Baker NR and Bowyer JR (eds) Photoinhibition of Photosynthesis. From Molecular Mechanisms to the Field, pp 75–93. Bios Scientific Publishers, Oxford

Wiekkowski S and Bojko M (1997) The NADPH-dependent electron flow in chloroplasts of the higher plants. Photosynthetica 34: 481–496

Wiese C, Shi L-H and Heber V (1998) Oxygen reduction in the Mehler reaction is insufficient to protect Photosystems I and II of leaves against photoactivation. Physiol Plant 102: 437–446

Wilhelm C and Wild A (1984a) The variability of the photosynthetic unit in *Chlorella*. I. The effect of vanadium on photosynthesis, productivity P-700 and cytochrome *f* in undiluted and homocontinous cultures of *Chlorella*. J Plant Physiol 115: 115–124

Wilhelm C and Wild a (1984b) The variability of the photosynthetic unit in *Chlorella*. II. The effect of light intensity and cell development on photosynthesis, P-700 and cytochrome *f* in homocontinous and synchronous cultures of *Chlorella*. J Plant Physiol 115: 125–135

Wilhelm C and Duval J-C (1990) Fluorescence induction kinetics as a tool to detect a chlororespiratory activity in the prasinophycean alga, *Mantoniella squamata*. Biochim Biophys Acta 1016: 197–202

Wilhelm C, Krämer P and Lenartz-Weiler I (1989) The energy distribution between the photosystems and light-induced changes in the stoichiometry of system I and II reaction centers in the chlorophyll *b*-containing alga *Mantoniella squamata* (Prasinophyceae). Photosynth Res 20: 221–233

Wilhelm SW, Maxwell DP and Trick CG (1996) Growth, iron requirements, and siderophore production in iron-limited *Synechococcus* PCC7002. Limnol Oceanogr 41: 89–97

Williams RJP and Fraústo da Silva JJR (1996) The Natural Selection of the Chemical Elements. Clarendon Press, Oxford.

Wood PM (1997) The roles of *c*-type cytochromes in algal photosynthesis: extraction from algae of a cytochrome similar to higher plant cytochrome *f*. Eur J Biochem 72: 605–613

Yachandra VK, Derose VJ, Latimer MJ, Mukerji I, Sauver K and Klein MP (1993) Where plants make oxygen – a structural model for the photosynthetic oxygen-evolving manganese cluster. Science 260: 675–679

Yocum CF (1991) Calcium activation of photosynthetic water oxidation. Biochim Biophys Acta 1059: 1–15

Yu I, Zhao J, Huhlenhoff V, Bryant DA and Golbeck JH (1993) PsaE is required for *in vivo* cyclic flow around Photosystem I in the cyanobacterium *Synechococcus* sp. PCC7002. Plant Physiol 103: 171–180

Yung YL and McElvoy MB (1979) Fixation of nitrogen in the prebiotic atmosphere. Science 203: 1002–1004

Zhang L, Pakrasi HB and Whitmarsh J (1994) Photoautotrophic growth of the cyanobacterium *Synechocystis* sp. PCC6803 in the absence of cytochrome c_{553} and plastocyanin. J Biol Chem 269: 5036–5042

Zhao J, Snyder WB, Mühlenhoff U, Rhiel E, Warren PV, Golbeck JH and Bryant DA (1993) Cloning and characterization of the *psaE* gene of the cyanobacterium *Synechococcus* sp. PCC7002: Characterization of a *psaE* mutant and overproduction of the protein in *Escherichia coli*. Mol Microbiol 9: 183–194