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REVIEW

THE ROLE AND EVOLUTION OF SUPEROXIDE DISMUTASES IN ALGAE[1]

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Superoxide dismutases (SOD) catalyze the disproportionation of the potentially destructive superoxide anion radical ($O_2^{\bullet -}$, a byproduct of aerobic metabolism) to molecular oxygen and hydrogen peroxide: $2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$. Based on metal cofactors, four known metalloforms of SOD enzymes have been identified: they contain either Fe, Mn, Cu and Zn, or Ni. Orthologs of all metalloforms are present in oxygenic photoautotrophs. The expression of SOD is highly regulated, with specific metalloforms playing an inducible protective role for specific cellular compartments. The various metalloforms of SOD are not distributed equally within either cyanobacteria or eukaryotic algae. Typically, cyanobacteria contain either an NiSOD alone or combinations of Mn and Ni or Fe and Mn metalloforms (CuZn is rare among the cyanobacteria). The bacillariophytes and rhodophytes retain an active MnSOD, whereas the chlorophytes, haptophytes, and embryophytes have either FeSOD or multiple combinations of Fe, Mn, and CuZnSODs. The NiSOD is a relatively novel SOD and has been generally excluded from evolutionary analyses. In both cyanobacteria and chlorophyte algae, the FeSOD metalloform appears to be associated with PSI, where its primary role is most likely to deactivate reactive oxygen produced by the Mehler reaction. The CuZnSOD also appears to be associated with the plastid but is phylogenetically more restricted in its distribution. In eukaryotic algae, SODs are all nuclear encoded and, based on nucleotide sequence, protein structures, and phylogenetic distributions, appear to have unique evolutionary histories arising from the lateral gene transfer of three distinct genes to the nucleus after the endosymbiotic acquisition of mitochondria and plastids. The varied phylogenetic histories and subcellular localizations suggest significantly different selection on these SOD metalloforms after the endosymbiont organelle-to-host gene transfer.

Key index words: **algae; antioxidant; evolution; Fenton chemistry; Haber-Weiss reaction; oxidative stress; phytoplankton; reactive oxygen species; SOD; superoxide dismutase**

Abbreviations: **E_o', standard reduction potential; GSB, green sulfur bacteria; NHE, normal hydrogen electrode; ROS, reactive oxygen species; SOD, superoxide dismutase**

The evolution of oxygenic photosynthesis in the early Proterozoic Eon exerted a powerful selective pressure on life (Dismukes et al. 2001, Knoll 2003, Falkowski et al. 2004a). Free atmospheric oxygen allowed metabolism to become "supercharged," whereby the energy extraction efficiency per mole of glucose increased over 400% relative to anaerobic fermentation. However, this metabolic benefit came at the price of potential damage to the metabolic machinery (Koppenol 1988). Reactive oxygen species (ROS), which under anaerobic conditions were present but almost certainly at very low concentrations, became relatively abundant. ROS can react with lipids, membranes, and proteins to cause irreversible damage to a cell. The most notable culprit within the ROS family is the superoxide anion radical ($O_2^{\bullet -}$), which is a metabolic byproduct of aerobic respiration and oxygenic photosynthesis (Falkowski and Raven 1997, Fridovich 1998). Both processes leak approximately 1%–4% of their electrons onto molecular oxygen to form superoxide (Halliwell 1982, Apel and Hirt 2004). Given the ubiquitous presence of ROS, it is not surprising that efficient defense mechanisms evolved to destroy ROS. In this article we review the biochemistry, physiological

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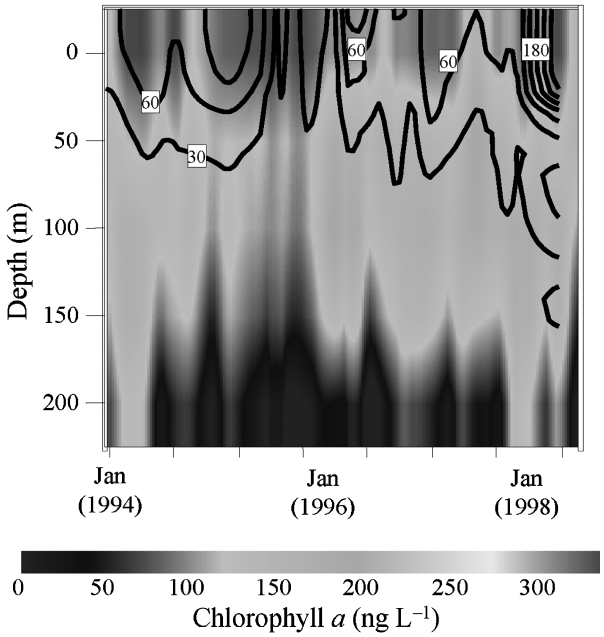


FIG. 1. Seasonal hydrogen peroxide and chl*a* at the Hawaii Ocean Times Series (HOT) station ALOHA. Data collected from January 1994 to December 1998 (Gasc et al. 2002). Color represents average chl in $ng \cdot L^{-1}$ and contour lines are of equal hydrogen peroxide concentrations in nM. Within the water column, algae are the most prominent source of ROS. Significant abiological ROS production would require the presence of free transition metals. At this location, the average trace metal concentrations are too low to produce (from Fenton reactions, see text) the level of ROS observed. Some work has suggested that in coastal and estuarine regions, colored dissolved organic matter may serve as both a source and a sink for ROS (Zepp et al. 1992, Blough and Zepp 1995, Andrews et al. 2000, Voelker et al. 2000). However, the low nutrient, low chl waters at the HOT station are unlikely to experience the same levels of colored dissolved organic matter and abiotically produced ROS.

function, and evolutionary history of the defense mechanisms in algae against ROS, focusing on the role of superoxide dismutase (SOD).

Reactive oxygen species accumulate both in the open ocean (Gasc et al. 2002) (Fig. 1) and in coastal zones (Zepp et al. 1992, Blough and Zepp 1995, Voelker et al. 2000). In the ocean, production of ROS appears to be largely biological. For example, in coastal areas dinoflagellates and raphidophycean algae are both sources of ROS. These organisms can produce ROS in the dark when exposed to a variety of stimulants, including lectins and iron-limited environments (Oda et al. 1992, 1997, Kim et al. 1999a). This response is temperature dependent, associated with the presence of an NADPH oxidase-like cell surface enzyme (Kim et al. 1999a,b, Twiner and Trick 2000), and may be used as a cell signaling mechanism or in quorum sensing (Joint et al. 2002, Apel and Hirt 2004). The extracellular target of ROS is not well known; however, there is experimental evidence that these molecules can reduce competition by killing or inhibiting the growth of bacteria as well as by reducing Fe found in iron complexes in bulk water. Thus, although ROS are potentially toxic, aquatic organisms may have capitalized on the chemistry associated with ROS production to increase their fitness. To do so, however, the organisms must have defense mechanisms that prevent the ROS from inflicting damage to themselves.

One of the major biochemical protective systems against ROS is provided by the SODs, found in all branches of the tree of life (Fig. 2). There are four known metalloforms of SOD, identified by their metal centers: Fe, Mn, CuZn, and Ni. Orthologs of all four forms have been found in photosynthetic organisms. The cellular SOD profile is variable between organisms and can change with ambient environmental conditions (Fee 1991, Amanatidou et al. 2001). Most research on SODs has focused on either medical or agricultural applications; however, given the polyphyletic origins of eukaryotic algae (Baldauf 2003), the SOD profiles of these organisms can be used to address the evolutionary history of these enzymes and vice versa. Indeed, given their phylogenetic trajectory and the variability of trace metals in aquatic ecosystems (Kremling and Streu 2001, Whitfield 2001, Anbar and Knoll 2002, Sanudo-Wilhelmy et al. 2002, Saito et al. 2003, Seyler and Boaventura 2003), algae serve as excellent models for understanding the environmental regulation of oxidative stress and the efficacy of SOD in preventing damage to cellular machinery.

SOURCE AND SINKS OF ROS IN ALGAE

Molecular oxygen has a triplet ground state, making it an excellent oxidizing agent (and hence, terminal electron acceptor) in aqueous solutions (Halliwell 1995). However, O_2 can be reduced to several intermediates besides H_2O. The overall four-electron reduction of molecular oxygen is thermodynamically a highly favored reaction (standard reduction potential $[E_o'] = +0.815$ V vs. normal hydrogen electrode [NHE] at pH 7.25) and occurs in mitochondria and aerobic heterotrophic prokaryotes (Table 1). All intermediate levels of reduced O_2 are invariably deficient in hydrogen atoms and thermodynamically more reactive than H_2O and hence comprise a suite of ROS. They include the superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroperoxy radical ($HO_2^{\bullet -}$), and the hydroxyl radical ($HO^{\bullet}$) (Gabig and Babior 1982).

Virtually all ROS found in aquatic environments are the result of biological production through redox reactions with O_2 (Boveris and Cadenas 1982, Han et al. 2001). $O_2^{\bullet -}$ is formed by the following reaction:

$$O_2 + e^- \rightarrow O_2^{\bullet -} \quad (1)$$

which has a standard reduction potential (E_o' vs. NHE, pH 7) of -0.33 V (Table 1). Potential sources of ROS include Mehler ("pseudo-cyclic") electron flow around PSI (Asada 1999) and in the mitochondria between

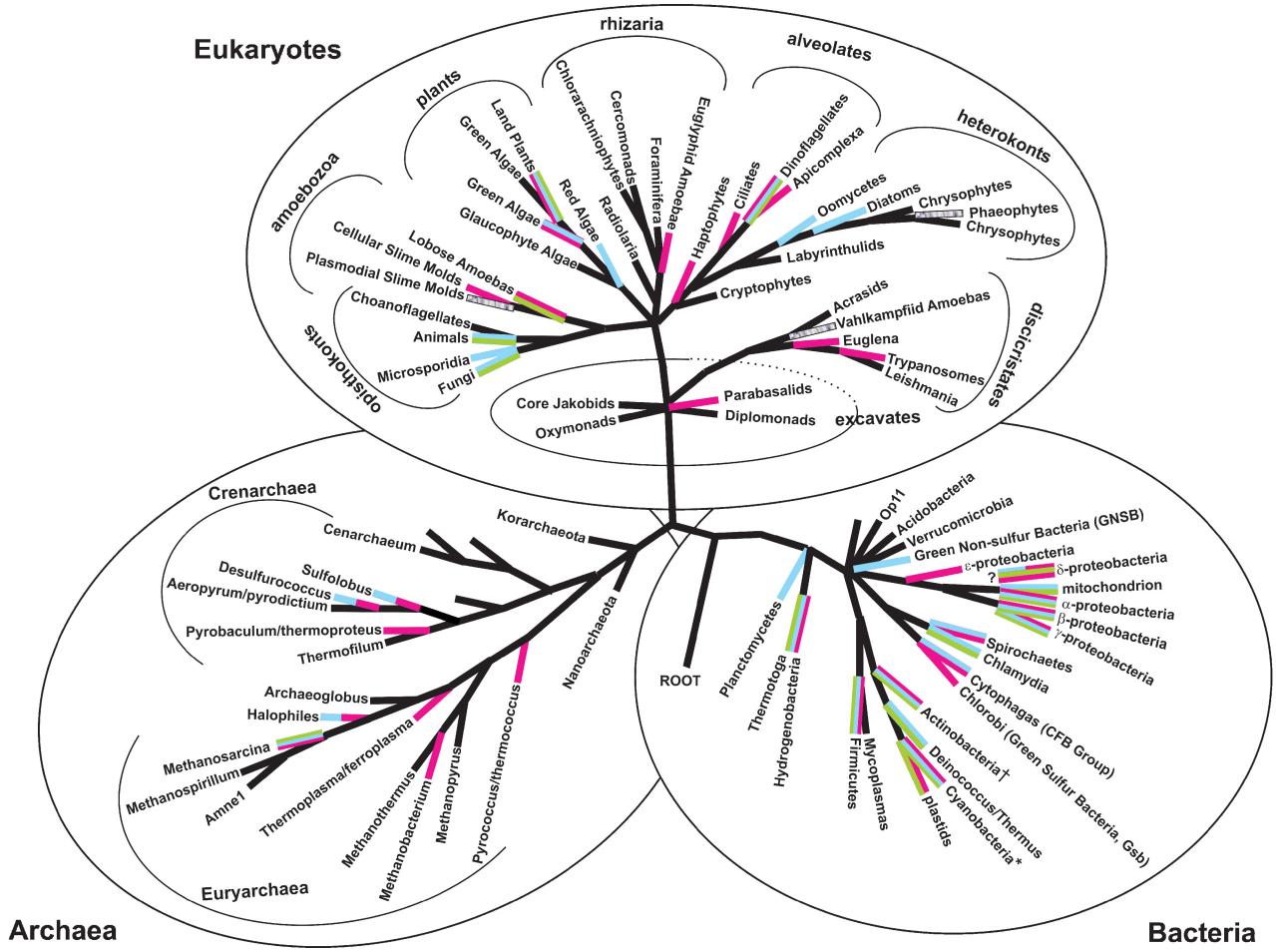


FIG. 2. Synthetic distribution based on known evidence from biochemical and genetic data of the various SODs over the tree of life (figure modified after Baldauf et al. 2004). All the potential routes for genetic inheritance are evident in SOD genes. The FeSOD is widely distributed between all major clades, whereas specifically MnSOD is more prevalent in Bacteria and Eukaryota. Fe and MnSOD have contrasting potential evolutionary histories. In eukaryotic photosynthetic autotrophs, the ancestral origin of FeSOD may be from the GSB ancestor of PSI in cyanobacteria, whereas MnSOD has a variety of possible origins, mainly the proteobacterial ancestor to PSII and mitochondria; both plastid metalloforms having been acquired through cyanobacteria. The CuZnSOD demonstrates multiple lateral gene transfers. Furthermore, organisms often possess multiple copies of CuZnSOD in their genomes, which are, in general, significantly phylogenetically distant. Branches are colored according to known SODs: magenta, FeSOD; cyan, MnSOD; cyan/magenta, Fe/MnSOD (cambialistic); green, CuZnSOD; and gray, unknown form of SOD. *The NiSOD genes are found in genomes of four cyanobacteria. The CuZnSOD is only found in *Gloeobacter* sp. †NiSOD and FeZnSOD are found in *Streptomyces* spp.

respiratory complexes II and III and cytochrome c oxidase (Dufour et al. 2000, Casteilla et al. 2001).

The most reactive ROS is the hydroxyl radical ($HO^{\bullet}$) which is generated by the Haber-Weiss reaction (Haber and Weiss 1934, Weiss 1935):

$$O_2^{\bullet -} + H_2O_2 \rightarrow OH^- + HO^{\bullet} + O_2 \qquad (2)$$

This reaction is further catalyzed by Fe released *in vivo* from Fe_4S_4 clusters by $O_2^{\bullet -}$:

$$[2Fe(II)2Fe(III)\text{-}4S] + O_2^{\bullet -} + 2H^+ \rightarrow [Fe(II)3Fe(III)\text{-}4S] + H_2O_2 \qquad (3)$$

where Fe(II) is released:

$$[Fe(II)3Fe(III)\text{-}4S] \rightarrow Fe(II) + [3Fe(III)\text{-}4S] \qquad (4)$$

This "free" Fe(II) either immediately reacts with H_2O_2 to produce hydroxyl radicals through the Fenton reaction (Fenton and Jackson 1899),

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + HO^{\bullet} + OH^- \qquad (5)$$

or is quickly oxidized,

$$Fe(II) + {}^{\bullet}OH \rightarrow Fe(III) + OH^- \qquad (6)$$

and is then available to react with the superoxide anion radical and start the reaction again (Bielski and Cabelli 1995). Because of the reactive nature of the hydroxyl radical, strong selective pressures favor cells that destroy the reactants (i.e. the superoxide anion radical and hydrogen peroxide) to benign products, ultimately H_2O (Liochev and Fridovich 1994, 1999).

TABLE 1. The four one-electron reactions for the reduction of O_2 to H_2O in aqueous solution and the corresponding reduction potentials.

	E_o' (V vs. NHE, pH 7)
$O_2 + e^- \rightarrow O_2^{\bullet -}$	−0.33
$O_2^{\bullet -} + e^- + 2H^+ \rightarrow H_2O_2$	+0.89
$H_2O_2 + e^- + H^+ \rightarrow H_2O + OH$	+0.38
$OH + e^- + H^+ \rightarrow H_2O$	+2.31

From Ho et al. (1995b).

The initial electron donation leading to the superoxide anion radical is unfavored and is the *main* factor limiting the reactivity of molecular oxygen (Table 1). Consequently, the oxidizing potential in O_2 cannot be accessed until after this first reduction, and thus molecular oxygen can coexist with reducing agents without reacting rapidly (Ho et al. 1995a,b). Although the reaction is not thermodynamically favored, electron equivalents produced *in vivo* often form $O_2^{\bullet -}$ if reducing agents, such as flavins and hydroquinones, are available. Once the initial electron donation produces superoxide anion radicals, the resulting molecule readily propagates free radical oxidation in a variety of biological molecules such as leukoflavins, tetrahydropterins, and catecholamines (Fridovich 1981) and inactivates iron–sulfur containing compounds (Fridovich 1997). Once produced, these ROS attack lipids, nucleic acids, and damage most cellular machinery (Voet and Voet 1990). Thus, despite the energetic advantage gained from using O_2 as a terminal electron acceptor, cells are required to maintain an efficient defense system against its byproducts.

THE SOD DEFENSE SYSTEM

Three major enzyme systems have evolved in oxygenic photoautotrophs to deactivate ROS: the SODs, catalases, and peroxidases (Asada 1999). Cells also maintain a suite of nonenzymatic antioxidants (including carotenoids and glutathione), but these are not addressed here because their chemistries generally overlap with the catalases and peroxidases (Halliwell 1999, Mallick and Mohn 2000).

The SODs are comprised of 150 to 220 amino acid residue subunits that form homodimeric or tetrameric protein complexes that coordinate specific metal cofactors. SOD catalyzes the dismutation of the superoxide anion radical into hydrogen peroxide and molecular oxygen according to (for scheme, see Table 2)

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (7)$$

which can be analyzed as two reduction half-reactions:

$$O_2 + e^- \rightarrow O_2^{\bullet -} \quad (8)$$

$$O_2^{\bullet -} + 2H^+ + e^- \rightarrow H_2O_2 \quad (9)$$

The redox mechanism toggles the active site metal between a reduced and oxidized form (i.e. either donating or accepting an electron). Thus, SOD is active

TABLE 2. The basic supuroxide dismutase ping-pong mechanism.

Where

$$SOD + O_2^{\bullet -} \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} SOD - O_2^{\bullet -} \xrightarrow{k_2} \text{products}$$

k_1 = second-order association rate
k_{-1} = first-order enzyme-substrate dissociation constant
k_2 = first-order catalytic rate constant

$$k_f = k_2/K_m = k_1k_2/(k_{-1} + k_2)$$

where:
k_f = second-order rate constant
K_m = Michaelis-Menten constant

2^{nd} order catalytic rate (k_{cat}/K_m) $\approx 10^9\ M^{-1} \cdot sec^{-1}$
Note: The catalytic process is diffusion limited; that is, $k_f = k_1$ because $k_2 \gg k_{-1}$

From Falconi et al. (2002).

whether the metal center is oxidized or reduced. For example, if Fe(III) is present at the active site, then the enzyme acts as an oxidant and O_2 is produced. If Fe(II) is present, the enzyme acts as a reductant and produces H_2O_2. This basic redox bifunctionality has been verified for all metalloforms of SOD regardless of the metal cofactor. The perpetual cycling of the redox state of the active site metal cofactor explains why SOD catalysis proceeds at a near diffusion-limited rate of approximately $10^9\ M^{-1} \cdot s^{-1}$, which is four orders of magnitude faster than the spontaneous dismutation of the superoxide anion radical. Thus, SOD removes superoxide and thereby precludes the development of Haber-Weiss and Fenton chemistries and therefore the production of even more radicals. All SODs provide this efficient defensive capability despite major differences in their structures.

Iron and manganese SODs. Based on amino acid alignments, Fe and MnSOD are approximately 50% similar (Fridovich 1998, Fink and Scandalios 2002) and appear to have evolved from a gene duplication event from a common ancestor. These two SOD types are typically homodimers or tetramers that contain one metal atom per 200 to 220 amino acid residue subunit with molecular masses between 14 to 30 kDa (Steinman 1982a). Certain Archaea express a dual metal or cambialistic SOD; that is, they may have either Fe or Mn at the active site in the same protein. Although this may not be surprising based on structural similarity to both the obligate FeSOD and MnSOD, it is the exception rather than the rule (Edward et al. 1998). It is not known whether there are cambialistic SODs in algae.

Although there is a high degree of similarity between Fe and MnSOD, two specific amino acid residues differentiate them: residue 77 (glutamine in FeSOD and glycine in MnSOD) and 146 (alanine in FeSOD and either glutamine or histidine in MnSOD) (Weatherburn 2001). Based on structural studies, these amino acid residues are critical to the redox

activity of the metal cofactor; thus, although it is possible to substitute Fe for Mn in the active site of MnSOD (or vice versa), the resulting complex exhibits little or no catalytic activity. The lack of activity is likely due to standard electrical potential differences (E_o' vs. NHE, pH 7) (Vance and Miller 1998, Renault et al. 2000). These differences underlie the considerable distance between the redox potentials of the two metal centers. For example, the active site Fe redox potential, E_o', is much lower for Fe when Fe is in the active site of the MnSOD protein than in FeSOD (Vance and Miller 1998, Renault et al. 2000). The standard reduction potential, E_o', is a thermodynamic parameter that indicates the energetically favored direction for a reaction. If E_o' is lowered, Fe(III) is stabilized when substituted in MnSOD and hence the ability of the metal to accept electrons is thermodynamically impeded (Batinic-Haberle 2002, Batinic-Haberle et al. 2004). The substituted metal center (stabilized as an oxidized species) cannot then oxidize $O_2^{\bullet -}$ to O_2 (one of the two half-reactions of $O_2^{\bullet -}$ disproportionation; see previous section). Thus, mutations in the binding site in SODs not only are critical for metal selectivity but modulate or "tune" the E_o' to facilitate enzymatic activity.

An important difference between the Fe and MnSODs is their intracellular location (Fig. 3). The FeSOD is typically localized in the chloroplasts and the cytoplasm (Kliebenstein et al. 1998, Fink and Scandalios 2002). In contrast, MnSOD is almost always found in mitochondria (Kitayama et al. 1999, Wu et al. 1999, Okamoto et al. 2001a,b). This localization appears to have changed during the evolutionary radiations as some cyanobacteria, which have both Fe and MnSOD, have MnSOD in both periplasmic *and* thylakoid membranes (Herbert et al. 1992, Chen et al. 2001, Li et al. 2002). Importantly, in cyanobacteria, the factor that determines the localization is an N terminal, hydrophobic, transmembrane helix tail on the MnSOD (Atzenhofer et al. 2002, Regelsberger et al. 2002). Thus,

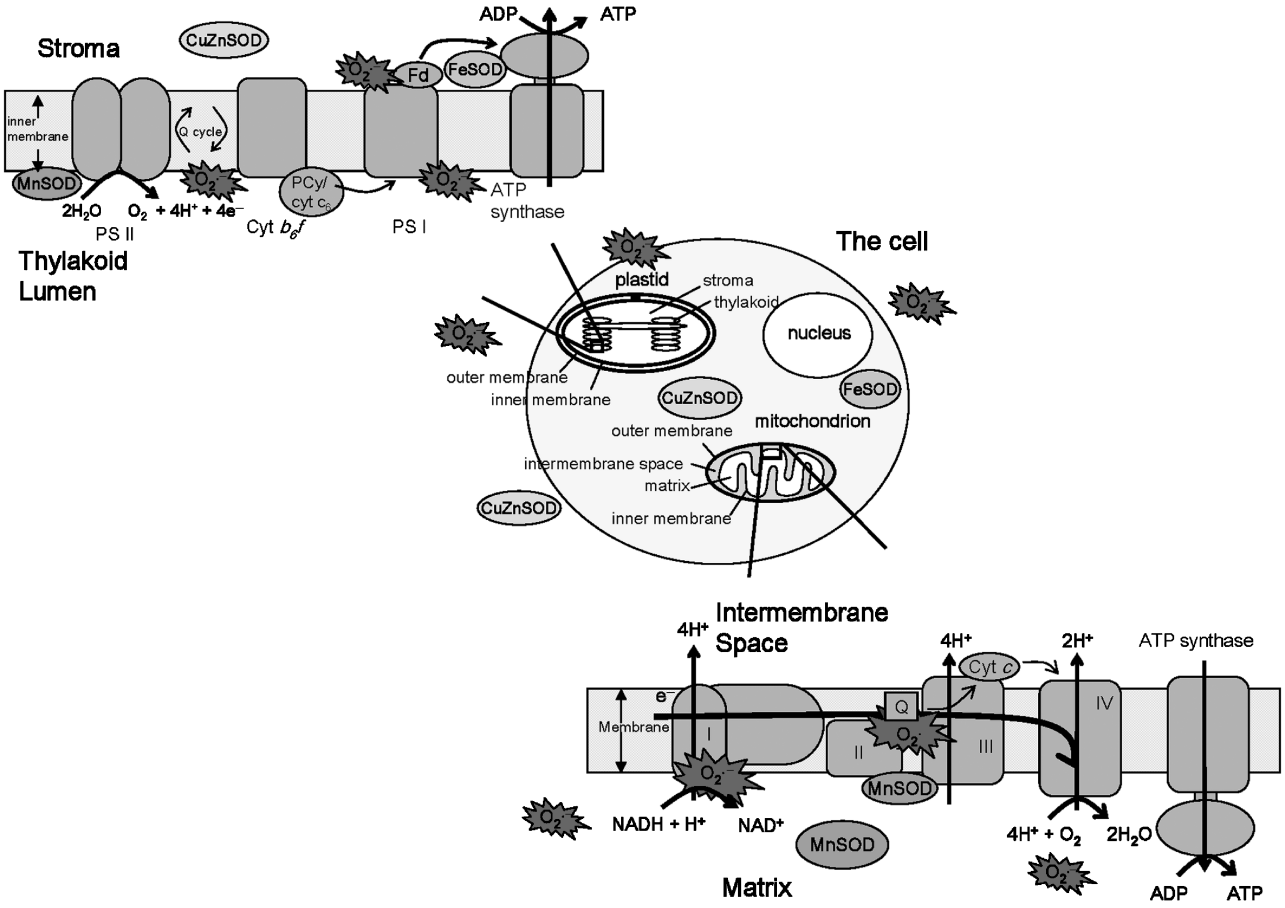


FIG. 3. Subcellular localization of SODs. This conceptual scheme summarizes all known locations of SODs within eukaryotic cells. An important attribute of SODs is that they are poised and ready at the metabolic site of superoxide production. Soluble forms of SOD found in the cytoplasm are typically CuZnSOD in embryophytes. Occasionally, soluble FeSOD can be found, but both cytoplasmic and extracellular SODs are characteristic of multicellular organisms that have cell signaling pathways through which ROS are exchanged. The chloroplast can be associated with Fe, Mn, and/or CuZnSOD. In diatoms, MnSOD can be found in the chloroplast, most likely associated with the lumen side of PSII (unpublished data). The CuZnSOD and FeSOD may fill the same role on the stromal side of PSI, with the former dominantly found in embryophytes and charophytes and the latter in the cyanobacteria, chlorophytes, and dinoflagellates. Much research is still needed to completely understand the SODs within other algal plastids. The mitochondria have MnSOD almost exclusively. No other SOD has been found associated with these organelles in photosynthetic autotrophs.

the overall tertiary structure of Fe and MnSODs remains similar.

Copper-zinc SODs. CuZnSOD has different primary and tertiary structures than Fe and MnSOD and almost certainly evolved independently. The CuZn enzymes have between 150 to 160 amino acid residues per subunit and are homodimeric; each monomer has a molecular weight between 31 to 33 kDa (Steinman 1982a, Fridovich 1998). For each subunit there is one Cu and one Zn atom, potentially allowing for two active sites per enzyme. This enzyme is stable with reports of the second-order rate constant (k_f) maintaining stability in 8 M urea for several hours at room temperature (Steinman 1982a). It can also withstand multiple freeze–thaw cycles and prolonged refrigeration once purified. This stability may arise from the high glycine content (13%–17%), which contributes to extensive β-pleated sheet conformation (Chen et al. 2001). The CuZnSOD is typically localized in the chloroplasts of higher plants and/or free in the cytosol (Wu et al. 1999) (Fig. 3). However, in metazoans, $<1\%$ of total cellular CuZnSOD (if present) may be accounted for in the mitochondrial intermembrane space (Okado-Matsumoto and Fridovich 2001, Inarrea 2002); it is typically found soluble both within cell cytoplasm and also in extracellular spaces. The discovery of CuZnSOD in prokaryotes (Steinman 1982b, Bannister and Parker 1985, Steinman 1985, Benov and Fridovich 1994, Benov and Fridovich 1996) ended the speculation that this metalloform of the enzyme evolved after the divergence of the three domains of life. In prokaryotes, CuZnSOD has been found in the periplasm of α, β, and γ proteobacteria. There are very few data known regarding CuZnSOD in eukaryotic algae (Okamoto et al. 1998).

Nickel SODs. The NiSOD has a completely different structure from either the Fe or MnSODs or the CuZnSODs; it was first discovered, cloned, and characterized in the bacterial genus, *Streptomyces* (Youn et al. 1996, Barondeau et al. 2004, Wuerges et al. 2004). Additionally, a survey of available genomes suggests this form of SOD may be active in cyanobacteria as well (Fig. 4) (Palenik et al. 2003). Given that the most abundant cyanobacteria on Earth are *Prochlorococcus* sp. and *Synechococcus* sp., SOD may reveal a major global importance for nickel (Partensky et al. 1999, Palenik et al. 2003).

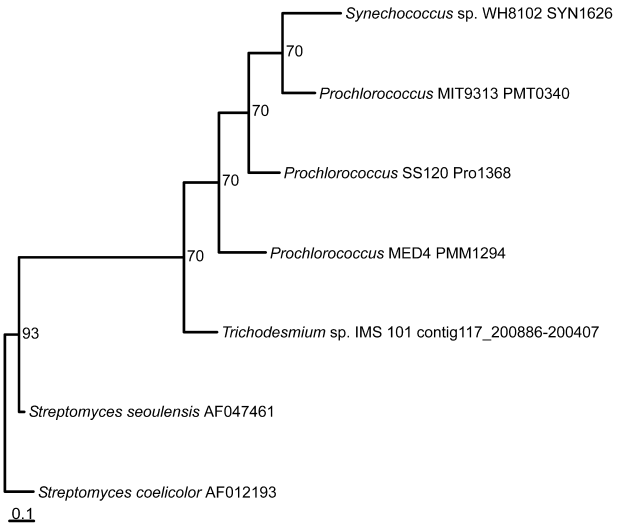


FIG. 4. Phylogenetic tree of NiSOD genes. The NiSODs have been biochemically characterized in the two *Streptomyces* sp. shown. The cyanobacterial sequences represented here are derived from genomic data, but no biochemical or physiological data are yet available for the activity of NiSODs in cyanobacteria. Interestingly, the three *Prochlorococcus* sp. strains shown possess only the gene for NiSOD; they do not contain genetic information for any other known form of SOD. The evolutionary significance of NiSODs is not yet clear, but the increase in genomic data will help resolve this issue. This may prove to be a major sink for Ni in environments where these are the dominant organisms. This unrooted tree was generated using the Genetic Database Environment (GDE) sequence alignment editor (Smith et al. 1994) based on a DNA maximum likelihood approach using fastDNAml (Felsenstein 1981, Olsen et al. 1994); the branching pattern is supported by bootstrap analysis (100 replicates). The tree has been modified for clarity. Accession numbers are given for the *Streptomyces* sp. and open reading frame identification numbers are given for all the cyanobacteria. Scale bar represents the number of substitutions per nucleotide site.

ENVIRONMENTAL REGULATION OF SODS IN ALGAE

Visible light stress. Although the biology of visible (400–700 nm) light stress has been an area of active research for vascular plant systems over the last two decades, the impact of high irradiance on the ecology of algae has been under-emphasized (Cullen and Lewis 1995). Very few studies have been conducted on algal cultures grown at irradiance values greater than 1000 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (for reference, the maximum solar irradiance on Earth at local noon is 2200 μmol photons $\cdot m^{-2} \cdot s^{-1}$), which is unfortunate because most of the ocean photosynthetic carbon fixation is often light saturated (Falkowski and Raven 1997). These high light levels can suppress photosynthetic rates due to the photochemical production of ROS *in vivo*, which can damage the photosynthetic apparatus (Critchley 1994, Nickelsen and Rochaix 1994, Telfer and Barber 1994).

Irradiance experiments showed that different metalloforms of SOD have selective inducible protective functions related to their subcellular distribution. As described above, in cyanobacteria MnSOD is typically embedded in membranes, whereas FeSOD is soluble. Studies using a variety of mutants, inhibitors, and light conditions have demonstrated that FeSOD is associated with the photoprotection of PSI (Herbert et al. 1992, Thomas et al. 1998). Field data also illustrated the selective protective functions for the SODs, as FeSOD was largely associated with nitrogen-fixing heterocysts that only contain PSI (Canini et al. 1998). Specifically, cells exposed to higher irradiances showed a dramatic increase in SOD activity and content. Thus, different metalloforms of SOD protect different cellu-

lar proteins and can provide an *in vivo* tool to study cellular responses to oxidative stress (Lesser and Stochaj 1990).

Ultraviolet radiation stress. Within the photosynthetic machinery, UV-B (280–320 nm) radiation inhibits PSII (Iwanzik et al. 1983, Kulandaivelu and Noorundeen 1983, Renger et al. 1989, Schofield et al. 1995) by degrading the D1/32 kDa protein complex (Greenberg et al. 1989, Richter et al. 1990, Melis et al. 1992, Jansen et al. 1993). Because the quinones, which are integral prosthetic components of PSII, absorb UV-B light (Greenberg et al. 1989, Melis et al. 1992, Jansen et al. 1993), it has been hypothesized that damage occurs beyond the photosynthetic reaction molecule, probably at the primary (Q_A) and secondary (Q_B) quinone electron acceptors in the reaction centers (Prasil et al. 1996).

In both green algae and diatoms, SODs exhibit a dose-dependent regulation in response to UV-B radiation (Malanga and Puntarulo 1995, Malanga et al. 1997, Rijstenbil 2002). Interestingly, the ROS produced with UV-A (320–400 nm) do not stimulate a significant increase in SOD. Therefore, the differences in the protective response in nonenzymatic antioxidants and SOD probably reflect the specific and different target sites of the UV-A and UV-B damage (Rijstenbil 2002, 2003).

Nutrient stress. Oxidative stress occurs under nutrient limitation as cellular metabolic rates are disrupted and the cellular scaffolding is degraded. For example, photosynthetic machinery, which represents a significant fraction of the total cellular protein, is translationally impaired and cellular components are catabolized to maintain photosynthetic activity (Falkowski et al. 1989; Falkowski and Raven 1997). Because certain key constituents of PSII reaction centers are destroyed, instead of driving photosynthesis, absorbed light is dissipated via alternative pathways and frequently leads to the production of ROS. Additionally, under acute nutrient limitation, the respiratory degradation of cellular proteins and membranes can lead to the production of ROS species. In cyanobacteria, nitrogen limitation resulted in an increase in FeSOD expression exclusively associated with PSI in heterocysts but no change in MnSOD (Liu et al. 2000, 2002).

Metal toxicity. Excess free metal ions can initiate Fenton reactions (Pinto et al. 2003). Many cyanobacteria have been shown to produce extracellular metal chelators that serve both as nutritive (to modulate the uptake of micronutrient trace metals) and antioxidant buffers (to prevent Fenton chemistry) (Ahner and Morel 1995, Martinez et al. 2000). Although intracellular chelation is a major preventive mechanism in many organisms, this phenomenon is poorly understood in algae. The most comprehensive group of metal toxicity studies examined various SOD responses under both acute and chronic metal conditions (Okamoto et al. 1996, 2001a,b, Okamoto and Colepicolo 1998). In dinoflagellates, lethal metal levels, most notably Cu, elicited a 53% increase in total SOD activity within hours of exposure and was mirrored by increased lipid peroxidation. The specific response, however, varied depending on metal (Okamoto et al. 2001a,b). Metal stress also initiated an increase in mRNA transcript levels of *sodB* (which encodes FeSOD); however, the translation of these transcripts appeared to be regulated by a circadian rhythm, so that conclusive evidence for the direct regulation of FeSOD by ROS has yet to be demonstrated. Responses to high metal concentrations have also been found in diatoms and green algae (Rijstenbil et al. 1994, Canini et al. 1998).

ALGAE SODS IN AN EVOLUTIONARY CONTEXT

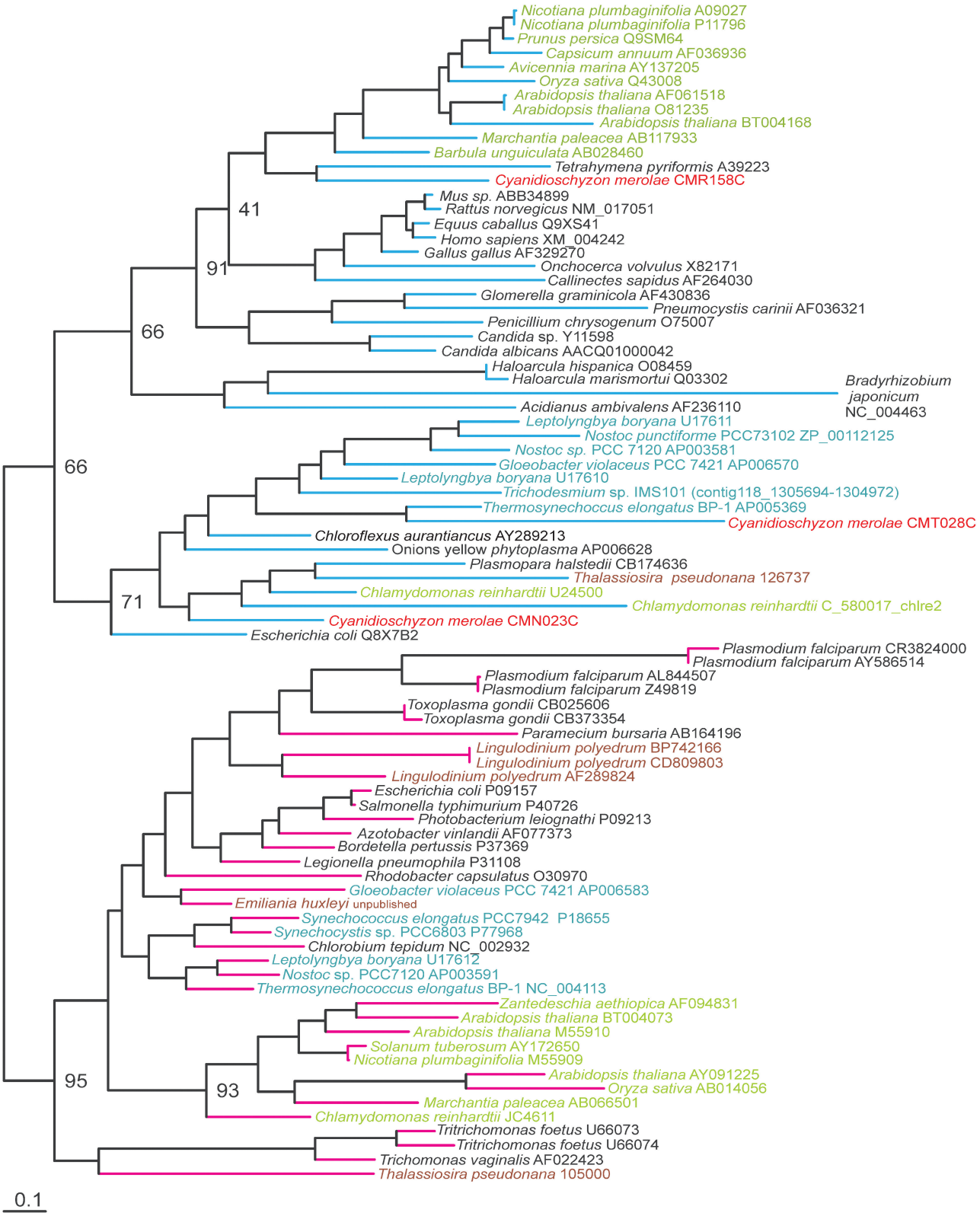
There are three possible sources of the separate SOD genes in eukaryotic algae: 1) an archezoon (i.e. the protoeukaryotic host that existed before the acquisition of organelles), 2) lateral gene transfer from the genome of the donors of organelles to the host cell, and 3) lateral gene transfer independent of organelle acquisition (Martin and Russell 2003).

All mitochondria likely originated from a common eubacterial ancestor belonging to the α-proteobacteria, which was acquired by an archeozoon through a single endosymbiotic event (Gray et al. 1999, Martin et al. 2001). In contrast, the origin of plastids is more diverse (Delwiche 1999, Palmer 2003). Primary plastids are derived from the endosymbiotic acquisition of cyanobacteria and found in chlorophytes, prasinophytes, rhodophytes, and embryophytes, whereas secondary plastids were acquired from primary plastid algae through a few endosymbiotic events involving several heterotrophic eukaryotic cells. Secondary plastid algal phyla, for which SOD genetic data are available, include a diatom (Bacillariophyceae, e.g. *Thalassiosira pseudonana*), a haptophyte (e.g. *Emiliania huxleyi*), and dinoflagellates (Dinophyceae, e.g. *Lingulodinium polyedrum*) (Okamoto and Colepicolo 1998, Okamoto et al. 2001a, 2001b, Armbrust et al. 2004, unpublished data). As part of endosymbiotic processes, gene transfers occurred from the endosymbiont genome(s) to the host nucleus including genes encoding for SOD.

Figure 2 shows all known SODs superimposed over the tree of life. The FeSODs dominate the Archaea, including a few cambialistic enzymes that change depending on environmental conditions. The MnSOD gene, *sodA*, is more widely distributed in the bacteria and eukaryota. Most organisms that possess MnSOD also have FeSOD, CuZnSOD, or all three. Important exceptions to this are the green nonsulfur bacteria, rhodophytes, and diatoms. The CuZnSODs are widely distributed over the entire tree. We first consider the dispersal of the Fe and MnSOD genes based on available genetic and biochemical data.

Phylogenetic trees suggest that Fe and MnSOD are derived from a common ancestor via a gene duplication event (Fig. 5). The FeSOD cluster contains the basal eukaryotic group, parabasalids, that do not ap-

pear to have mitochondria. This cluster then divides into a strongly supported group containing green plants exclusively and a diverse group containing cyanobacteria, the green sulfur bacterium (GSB) *Chlorobium tepidum*, proteobacteria, and eukaryotes (*E. huxleyi*, alveolates). Interestingly, dinoflagellate and other al-



veolate SODs appear, in this context, to have been derived from proteobacteria. A similar situation is found in most dinoflagellates that contain the type 2 RUBISCO, apparently acquired from α-proteobacteria by lateral gene transfer (Delwiche and Palmer 1996). This differs from other plastid targeted proteins, such as glyceraldehyde-3-phosphate dehydrogenase, where phylogenies suggest all secondary red plastid targeted genes have a common origin (Takishita et al. 2004).

The MnSOD cluster divides into two moderately supported sister groups (Fig. 5). The first branches into two sister clusters, one containing Euryarcheota and the α-proteobacterium *Bradyrhizobium* and the other including eukaryotic groups (Metazoa, fungi, higher plants, green and red algae, and ciliates). Accordingly, and in agreement with the mitochondrial localization of MnSOD, this cluster is consistent with a mitochondrial inheritance of MnSOD. The second main MnSOD cluster divides into a group mostly represented by cyanobacteria and a diverse group including bacteria and eukaryotes. This latter group contains green and red algae and two stramenopile sequences, including *T. pseudonana*.

One interpretation of this phylogenetic analysis is that Mn and FeSODs are very ancient molecules that were selected before the oxidation of Earth, approximately 2.3 billion years ago (Bekker et al. 2004), when these two metals were relatively abundant in the ocean. The retention of these two proteins in eukaryotes reflects the history of the endosymbiotic appropriation of mitochondria and the two photosystems in oxygenic plastids. The MnSOD appears to have been inherited by eukaryotic algae through proteobacteria, whose ancestors were the progenitors of both mitochondria and PSII (Michel and Deisenhofer 1988, Martin and Russell 2003), which eukaryotic algae inherited by organelle acquisition. In contrast, the biochemical association of FeSOD with PSI in extant plastids and phylogenetic distribution of the protein suggest that it was acquired through GSB, the closest extant relative to the progenitors of PSI in cyanobacteria (Baymann et al. 2001). Functional *sodB* may have been lost during the evolution, leading to its absence in the bacillariophytes and rhodophytes (Matsuzaki et al. 2004). Genomic analysis suggests that diatoms have retained two pseudogenes for FeSOD (Armbrust et al. 2004). But evidence for the sole use of the MnSOD metalloform (apparently in a variety of posttranslationally modified forms) has been shown biochemically in the diatom *Thalassiosira pseudonana* and *T. oceanica* (Peers and Price 2004, unpublished data). Further biochemical and molecular genetic data are needed to confirm this phenomenon.

The wide distribution of CuZnSOD suggests multiple lateral gene transfers between evolutionarily diverse organisms. For example, there are typically multiple copies of the genes for CuZnSOD in higher plants (Jesus et al. 1989, Grace 1990, Fink and Scandalios 2002). There are generally two types of CuZnSODs in organisms that contain this enzyme: in higher plants they can be cytoplasmic or chloroplastic forms, whereas metazoa have cytoplasmic and extracellular forms. These forms are all phylogenetically related to the bacterial forms (Fink and Scandalios 2002). Moreover, the gene encoding CuZnSOD (*sodC*) is found in a myriad of dsDNA virus genomes, further suggesting that it is readily transferred between prokaryotic and eukaryotic hosts. This could also account for the apparently recent acquisition of *sodC* in the euryarchaeon *Methanosarcina acetivorans* (Fig. 2) (Galagan et al. 2002). A few studies have linked presence of CuZnSOD or FeSOD as proof of the endosymbiotic origins of plastids and mitochondria (Jesus et al. 1989, Grace 1990); however, given the frequent occurrence of lateral gene transfer of *sodC*, such an interpretation may not be valid.

CONCLUSIONS AND FUTURE DIRECTIONS

In summary, examples of the four known metalloforms of SOD, which are distinguished by their metal cofactor, Fe, Mn, CuZn, and Ni, have been identified in cyanobacteria and eukaryotic algae. However, very little is known regarding the location, regulation, and the cause of this metalloform diversity. From an evolutionary perspective, phylogenetic relationships among the various SODs provide key insight into the history of organelles. Specifically, eukaryotic algae show a spectrum of SODs whose nuclear-encoded genes are derived from endosymbiotic events. Altogether, Mn and FeSOD address several issues regarding the evolution of photoautotrophic eukaryotes related to the inheritance of proteins directly from organelles and specifically plastid history. A group of MnSODs seems to have remained closely related among diverse algal phyla. In contrast, FeSOD may reflect dramatic evolutionary changes related to plastid endosymbiosis. It highlights the early divergence between the green and red plastid lineages (Grzebyk et al. 2003). It also suggests different

FIG. 5. Unrooted phylogenetic tree for Fe and Mn SOD proteins. The phylogenetic clusters reflect the evolutionary history of these nuclear encoded genes. The MnSOD branches follow a mitochondrial origin, whereas the FeSOD patterns are likely due to origination from the plastid symbiont. The FeSOD is likely derived from the ancestral progenitors of PSI in cyanobacteria, the GSB. Interestingly, MnSOD may have originated from the proteobacterial source of PSII, which is also associated with the origin of mitochondria (see text). The SOD amino acid sequences were aligned using CLUSTALX (Thompson et al. 1994, 1997) and the Genetic Data Environment (GDE) (Smith et al. 1994) multiple sequence editor. A maximum likelihood tree was constructed using PHYML (Guindon and Gascuel 2003) applying an empirical model of evolution (Whelan and Goldman 2001), and the branching pattern is supported by bootstrap analysis (100 replicates). The scale represents the expected number of substitutions per amino acid position. The tree has been modified for clarity. The FeSOD representatives are indicated with magenta branches, whereas MnSOD representatives are in cyan. Species text color key: green, chlorophytes and higher plants; red, rhodophytes; aqua, cyanobacteria; brown, chromophyte algae. Accession numbers of the sequences are next to each taxon.

evolutionary history of acquisition of plastids between the diatoms, haptophytes, and dinoflagellates, which may challenge the hypothesis of a single endosymbiotic acquisition of secondary plastids (Cavalier-Smith 1999, Palmer 2003, Falkowski et al. 2004a, Grzebyk et al. 2004).

For algae, SODs are a window into past events and the importance of the genetic transfers that occurred. Efforts should focus on using the SODs' homologous origins and multiple metal employments to further understand the success of different algal forms in contrasting environments. The regulation of specific metalloforms of SOD by light, nutrients, and other environmental pressures may also help to determine the necessity through evolutionary time of particular trace metal requirements for organelles and the whole cell. We need a better understanding of SOD as representative of the first in line of the enzymatic antioxidant arsenal. The importance of lateral gene transfers in algae should be further explored as a tool to understand the extant biodiversity. Algal SODs may provide key information to help understand this stochastic evolutionary process. Finally, we may then be able to relate these data back to the evolutionary history of algae and begin to understand why these organisms are so drastically diverse and different between the ocean and land.

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