Chapter 3

Phylogenetic Analysis of Sulfate Assimilation and Cysteine Biosynthesis in Phototrophic Organisms

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Summary

Sulfur is an essential nutrient for all organisms. The majority of sulfur in nature is found in inorganic form of sulfate, which has to be reduced and incorporated into bioorganic compounds. Assimilatory sulfate reduction occurs in various chemotrophic bacteria and fungi and in photosynthetic organisms, but is missing in animals and most prokaryotic and eukaryotic obligate parasites. Despite its central position in plant primary metabolism, the question of evolution of the pathway and origin of plant genes involved in sulfate assimilation has never been addressed. We have therefore made use of the vast amount of available sequence data to perform a phylogenetic analysis of sulfate assimilation genes from a range of lineages of photosynthetic organisms including photosynthetic bacteria, primary symbionts such as plants, green and red algae and various secondary and tertiary symbionts. The analysis revealed very complicated relations between the different lineages and different evolutionary histories of the individual genes of the pathway. Whereas, for example, plant sulfite reductase is clearly of a cyanobacterial origin, the other genes in the pathway, although being plastidial are, unusually, not of cyanobacterial origin. The clear separation between adenosine phosphosulfate- and phosphoadenosine phosphosulfate-reducing organisms seen in previous analyses has been lost with the inclusion of genes from diatom and cryptomonad secondary symbiont algae. In fact, a new variant of the key enzyme of sulfate assimilation, adenosine 5′-phosphosulfate reductase, lacking an iron sulfur cofactor, has been discovered. In addition, many interesting fusion proteins between various components of the pathway were uncovered in the newly sequenced algal genomes which open new exciting opportunities to improve the efficiency of the pathway or some of its reactions. In the chapter, protein phylogenies of seven enzymes of the pathway will be discussed in detail with relation to distribution of enzyme variants among prokaryotic and eukaryotic lineages, origin of plant genes, and the origin of genes in algae with secondary and tertiary plastids.

I. Introduction

A. Sulfate Assimilation

Sulfur occurs in nature in various oxidation states in inorganic, organic, and bioorganic compounds. Sulfur can readily change its oxidation state, therefore oxidized sulfur compounds serve as terminal electron acceptors in respiration of sulfate reducing bacteria and reduced sulfur compounds support chemotrophic or phototrophic growth of many bacteria and Achaea as electron donors. Sulfur is an essential nutrient for all organisms as it is part of cysteine and methionine, sulfur containing proteogenic amino acids with frequent catalytic and structural functions, coenzymes and prosthetic groups such as iron–sulfur clusters, thiamine, lipoic acid, coenzyme A, etc. In these metabolites sulfur is in a reduced form, however, the major form of sulfur available in nature is in the oxidized form of sulfate. Many microorganisms, algae, plants and fungi (but not animals) are able to take the sulfate up, reduce it and incorporate in amino acids in the pathway of

Abbreviations: APR – adenosine 5′-phosphosulfate reductase; APS – adenosine 5′-phosphosulfate; ATPS – ATP sulfurylase; CBS – cystathionine β-synthase; EST – expressed sequence tag; GSH – glutathione; LGT – lateral gene transfer; OAS – *O*-acetylserine; OASTL – *O*-acetylserine (thiol)lyase; PAPS – 3′-phosphoadenosine 5′-phosphosulfate; PPase – inorganic pyrophosphatase; SAT – serine acetyltransferase; SiR – sulfite reductase

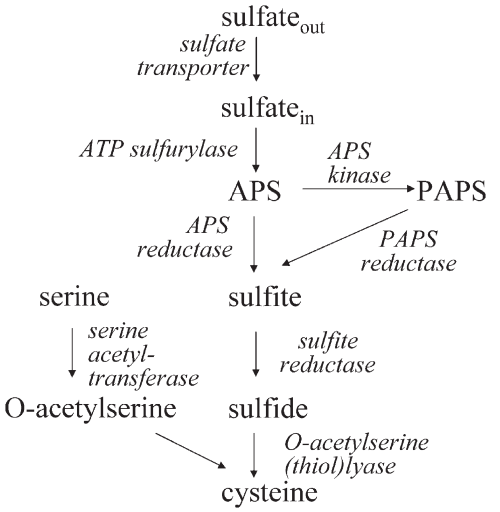

Fig. 1. Assimilatory sulfate reduction in plants.

assimilatory sulfate reduction (Fig. 1). In this pathway the entry of sulfate into cells is facilitated by sulfate transporters. The chemically stable sulfate has to be activated before reduction by adenylation with ATP to adenosine 5′-phosphosulfate (APS) in a reaction catalyzed by ATP sulfurylase (ATPS). APS can be directly reduced to sulfite by APS reductase (APR) or phosphorylated by APS kinase to form 3′-phosphoadenosine 5′-phosphosulfate (PAPS). PAPS is reduced to sulfite or serves as a source of activated sulfur for various sulfonation reactions modifying proteins, saccharides or secondary compounds. PAPS reductase uses thioredoxin as an electron donor, whereas APS reductase can be either thioredoxin-dependent or react with glutathione (see section III.D). APS dependent pathway is more frequent, being found in plants, algae, and most bacteria, while the PAPS reductase seems to be restricted to fungi, some enteric γ-proteobacteria and many (but not all) cyanobacteria. Sulfite produced by APR or PAPS reductase is then reduced to sulfide by sulfite reductase (SiR). The reaction requires the transfer of six electrons provided by NADPH in chemotrophic organisms or by ferredoxin in phototrophs, thus linking sulfate assimilation with photosynthesis. Sulfide is then incorporated into the amino acid skeleton of *O*-acetylserine or *O*-acetylhomoserine (in fungi) to form cysteine or homocysteine respectively in a reaction catalyzed by *O*-acetyl(homo)serine-(thiol)lyase (OASTL). The activated precursor is provided from serine and acetyl-coenzyme A by serine acetyltransferase (SAT). SAT and OASTL form a multienzyme complex often called cysteine synthase. The pathway of plants was subjected to many comprehensive reviews (Leustek et al., 2000; Hawkesford and Wray, 2000; Saito, 2004; Kopriva and Rennenberg, 2004; Rausch and Wachter, 2005; Kopriva, 2006) and is described in great detail elsewhere in this book.

It should be noted that sulfate is reduced in many bacteria and Archaea in a dissimilatory manner as an electron acceptor for respiration. The dissimilatory sulfate reduction often utilizes the same enzyme activities as sulfate assimilation, i.e., ATP sulfurylase, APS reductase, and sulfite reductase, but the structure of these enzymes is very different (Hansen, 1994; Stahl et al., 2002) and will therefore not be discussed here in detail. The biochemistry and molecular biology of dissimilatory sulfur metabolism will be described in section IV.

B. Photosynthetic Organisms

This chapter focuses on the evolution of sulfate assimilation in plants and other photosynthetic organisms. The rationale for this limitation is firstly the clear physiological link of sulfate assimilation to photosynthesis and, secondly, the vast amount of new sequence data available for photosynthetic organisms, including various algae that have not yet been thoroughly exploited. Photosynthesis uses energy from light to produce chemical energy and is therefore essential for life on Earth. Photosynthesis occurs in plants, algae, and several groups of photosynthetic bacteria. Whereas in plants and algae photosynthesis is confined to chloroplasts, in photosynthetic bacteria it occurs directly in the cytoplasm. The photosynthesizing bacteria can be separated in two major groups. Cyanobacteria possess chlorophyll and are capable of oxygen generation whereas other photoautotrophic bacteria absorb light with the help of bacteriochlorophyll and exist in an anoxygenic environment.

The ability for phototrophic life in bacteria is not limited to a phylogenetically distinct group. Bacteriochlorophyll dependent photosynthesis is found in five classes of bacteria: (1) green sulfur bacteria (*Chlorobium, Chlorobaculum*, and *Pelodictyon*), (2) *Chloroflexus* division called also green non-sulfur bacteria (*Chloroflexus, Roseiflexus*), (3) *Heliobacteriaceae*, within the low GC Gram positive bacteria, (4) purple sulfur bacteria of γ-proteobacteria (*Chromatium, Thiocapsa, Ectothiorhodospira*) and (5) purple non-sulfur bacteria spread between α- (e.g., *Rhodobacter, Rhodopseudomonas, Roseovarius, Rhodospirillum*) and β-proteobacteria (*Rho-*

doferax, Rubrivivax) (Imhoff, 1992). These bacteria use hydrogen, sulfide, thiosulfate, or ferrous iron as electron donors to support their anoxic, phototrophic growth. A special physiological group is capable of aerobic photosynthesis, it includes species such as *Roseobacter, Roseococcus*, or *Porphyrobacter*, which use light energy only as a supplement and are not capable of photoautotrophic growth (Yurkov and Beatty, 1998). More details on taxonomy of anoxygenic photosynthetic bacteria are available in Chapter 14.

Cyanobacteria are a morphologically diverse group of bacteria. Because of their ability to photosynthesize they have been described in the past as 'blue-green algae' (Stafleu et al., 1972) and their classification is still based more on phenotypical characters than molecular data. Cyanobacteria are divided into five major orders (*Chroococcales, Nostocales, Oscillatoriales, Pleurocapsales*, and *Stigonematales*) (Waterbury, 2001). Cyanobacteria are the accepted ancestors of all plastids (Nelissen et al., 1995; Cavalier-Smith, 2002). The primary endosymbiosis which gave rise to primary plastids is thought to have been a single event after which much of the cyanobacterial genome was transferred to the nucleus and the glaucophytes diverged, followed by red and green algae, the latter of which also gave rise to plants (McFadden, 2001; Martin et al., 2002; Rodriguez-Ezpeleta et al., 2005). This theory is strongly corroborated by phylogenetic analyses that show strong support for cyanobacteria and plants and algae as sister taxa in phylogenies based on the majority of plastid proteins (Martin et al., 2002; Chu et al., 2004). The great diversity of today's algal world is, however, due to further endosymbiosis events in which red or green algae were engulfed by eukaryotic hosts and gave rise to secondary and tertiary plastids (Archibald and Keeling, 2002; Bhattacharya et al., 2004; Patron et al., 2006). These complex plastids are surrounded by three or more membranes (Schwartzbach et al., 1998) and require new mechanisms of protein transport (Sulli et al., 1999; Waller et al., 2000; van Dooren et al., 2001; Patron et al., 2005). Euglenoids and chlorarachniophytes have secondary plastids of a green algal origin, though neither plastid nor host cell are directly related. Heterokonts, haptophytes, cryptophytes, apicomplexans, and dinoflagellates (collectively, chromalveolates) all possess secondary endosymbionts derived from red algae and although the bulk of molecular evidence supports a single red-algal endosymbiotic event (Fast et al., 2001; Archibald and Keeling, 2002; Yoon et al., 2002a; Cavalier-Smith, 2003; Harper and Keeling, 2003; Bhattacharya et al., 2004; Patron et al., 2004; Waller et al., 2006), the relatedness of the plastid is still a matter of contention (Bodyl, 2004; Sánchez Puerta et al., 2005). To add to the complexity, in some dinoflagellates, such as *Kryptoperidinium* or *Karlodinium*, the secondary plastid was replaced by a plastid from another secondary alga, resulting in tertiary plastid (Chesnick et al., 1997; Yoon et al., 2002b; Patron et al., 2006). A consequence of these complex evolutionary histories is that the genomes of such secondary and tertiary symbionts are a mosaic drawn from many sources, which makes analyses of evolutionary origins of single genes a very difficult task.

C. Genomics of Photosynthetic Organisms

The technological progress in high throughput DNA sequencing created vast amount of data, most of which is publicly available for analysis. This is especially true for bacterial genomes, where since the completion of the first genome of *Haemophillus influenzae* (Fleischmann et al., 1995) 599 bacterial genomes have been fully sequenced (NCBI Entrez Genome Project, November 2007). To date there are complete sequences for 29 cyanobacterial genomes, as well as many photosynthetic bacteria: 4 green sulfur bacteria (*Chlorobium tepidum, C. chlorochromatii, C. phaeobacteroides, Pelodictyon luteolum*), a green non-sulfur bacterium *Chloroflexus aurantiacus*, 12 species and strains of purple non-sulfur bacteria (such as *Rhodobacter sphaeroides, Rhodopseudomonas palustris, Rhodospirillum rubrum, Rhodoferax ferrireducens*, or *Rubrivivax gelatinosus*), and 6 aerobic phototrophic bacteria (e.g., *Erythrobacter litoralis, Roseobacter* sp., and *Roseovarius nubinhibens*). Many plant and algal genomes have also been completely sequenced, e.g., *Arabidopsis thaliana* (The Arabidopsis Genome Initiative, 2000), *Oryza sativa* (Goff et al., 2002; Yu et al., 2002), *Populus trichocarpa* (http://genome.jgi-psf.org/Poptr1/Poptr1.home.html), *Physcomitrella patens* (http://moss.nibb.ac.jp/), *Selaginella moellendorffii* (http://www.jgi.doe.gov/ sequencing/why/CSP2005/selaginella.html), *Chlamydomonas reinhardtii* (http://genome.jgi-psf.org/Chlre3/Chlre3.home.html; Grossman, 2005), *Cyanidioschyzon merolae* (http://merolae.biol.s.u-tokyo.ac.jp/; Matsuzaki et al., 2004), and *Thalassiosira pseudonana* (Armbrust et al., 2004). In

addition, a large number of EST projects have been initiated for several diverse algae such as *Bigelowiella natans, Cyanophora paradoxa, Glaucocystis nostochinearum, Heterocapsa triquetra, Karlodinium micrum*, and *Pavlova lutheri*, within the Protist EST Program (http://amoebidia.bcm.umontreal.ca/public/pepdb/welcome.php). Clearly, this wealth of sequence information enables now to study the evolution of metabolic pathways and the plasticity of photosynthetic organisms.

II. Occurrence of Sulfate Assimilation in Different Taxa

Although sulfate assimilation is essential for autotrophic growth and synthesis of cysteine and methionine, the pathway seems to be readily dispensable when the lifestyle of organism allows. It is absent in all metazoans, which satisfy their need for reduced sulfur by ingestion of sulfur containing amino acids cysteine or methionine. Interestingly, whereas plants can synthesize methionine from cysteine and not vice versa, animals possess the enzymes for the reverse transsulfuration pathway to synthesize cysteine from methionine and not the methionine biosynthetic genes (Leustek et al., 2000; Stipanuk, 2004). Therefore, methionine is an essential amino acid for animal nutrition. Another group of organisms lacking sulfate assimilation pathway are numerous bacteria and protists adapted to parasitism. In bacterial species that have undergone a significant genome reduction, the sulfate assimilation operon is almost invariably lost (Sakharkar et al., 2004; Nozaki et al., 2005). This is enabled by the adaptation of the nutrition of these parasites for metabolites provided by the host. Eukaryotic parasitic protists such as certain apicomplexans and the ciliates, interestingly the sister taxa to dinoflagellates, as well as parasitic fungi such as microsporidia, presumably scavenge reduced sulfur compounds from their host (Fulton and Grant, 1956; Payne and Loomis, 2006). A third group of organisms usually lacking sulfate assimilation are Archaea and bacteria using dissimilatory sulfide (or thiosulfate) oxidation or sulfate reduction for respiration and energy conversion. The habitats of such organisms always contain sulfide (Perez-Jimenez and Kerkhof, 2005), therefore, there is no need for sulfate assimilation to sustain cysteine biosynthesis. In total, from the 551 completely sequenced bacterial genomes 300 revealed a capacity for sulfate assimilation judged by the presence of the *cysH* gene encoding APS/PAPS reductase and the same was true for 5 out of 48 sequenced Archaea.

On the other hand, sulfate assimilation is present in plants, algae, and fungi. In plants and primary algae (green, red, and glaucophytes) the reductive part of the pathway, i.e., APR and SiR, is confined to plastids, whereas ATPS and APS kinase are present in plastids and in the cytosol and cysteine synthesis takes place in the plastids, cytosol, and mitochondria (Leustek et al., 2000; Rotte and Leustek, 2000; Koprivova et al., 2001; Wirtz et al., 2004). There is not enough information on localization of the pathway in secondary and tertiary symbionts, but in many cases, e.g., the stramenopile (diatom) *Thalassiosira pseudonana*, the genes seem to encode proteins with pre-sequences that suggest a chloroplast location. The only remarkable exception is *Euglena gracilis*, which possesses a sulfate-reducing pathway in the mitochondria (Brunold and Schiff, 1976; Saidha et al., 1988). Yeasts and other fungi seem to reduce sulfate in the cytosol.

III. Phylogenetic Analysis of Sulfate Assimilation Genes

The recent increase in availability of sequence information from various algae and plants enables a wide sampling of taxa for thorough phylogenetic analysis of photosynthetic organisms. The questions of the origin of plant genes and metabolic diversity of plants and algae can be now addressed with much better resolution. In addition, the possibility of using the genomic information for phylogenomics allows for the reconstruction of the evolutionary history of organisms (Delsuc et al., 2005), which is especially important for the complex algal lineages. Since sulfate assimilation is essential for plants and algae and is directly linked with photosynthesis we have undertaken a detailed phylogenetic analysis of the genes involved in the pathway.

A. Sulfate Transporters

1. Types of Sulfate Transporters

Before sulfate can be reduced it has to be taken up into the cells through the plasma membrane and, in organisms that reduce sulfate in the

plastid, further transport across the membranes of cellular organelles is also required. In addition, in plants a long distance sulfate transport from roots to the shoots requires additional transport steps between cells of different tissues. The transport of sulfate is facilitated by transporters via a proton coupled co-transport (Hawkesford et al., 1993), or anion exchange and sodium co-transport in animals (Markovich, 2001). Because of the various transport steps, a large number of specific transporters with different affinities for sulfate exist in plants (reviewed in Buchner et al., 2004, see also Chapter 2). On the other hand, most microorganisms possess only a simple sulfate uptake system (Sirko et al., 1990; Laudenbach and Grossman, 1991; Kertesz, 2001). The ATP dependent uptake is accomplished by a permease complex composed from three cytoplasmic membrane components CysA, CysT, and CysW and a sulfate binding protein SbpA in the periplasmic space (SulT family transporter). In addition to this ABC type of transporter, some bacteria possess a sulfate transporter from the major facilitator superfamily (MFS; Kertesz, 2001). Yeast and filamentous fungi possess two isoforms of high affinity sulfate transporters (Cherest et al., 1997; Van de Kamp et al., 2000). These are single membrane proteins of 80–95 kDa with 10 or 11 predicted transmembrane domains. *P. chrysogenum* with a mutation in the SutB transporter are unable to grow on sulfate as sole sulfur source, whereas *S. cerevisiae* with both transporters disrupted are able to grow at very high sulfate concentrations (30 mM) indicating a presence of a low affinity sulfate uptake system, at least in yeast. Animals also contain multiple sulfate uptake systems: three sodium dependent transporters NaSi-1, SUT-1, and NaDC-1 and at least five anion exchangers (Markovich, 2001).

In higher plants sulfate transporters are encoded by a large family of 14 genes in Arabidopsis and 15 genes in rice. The genes can be divided into five groups according to sequence similarity (see Chapter 2). The individual genes have distinct affinities for sulfate and tissue-specific expression. Surprisingly in the completely sequenced genomes of the lower plants *P. patens* and *S. moellendorffii* only genes from groups 1 and 4, i.e., high affinity and vacuolar transporters, respectively, were identified. The green alga *Chlamydomonas* contains three 'plant-type' sulfate transporter genes and another nuclear gene encoding a SulP transporter responsible for sulfate transport into the chloroplast. SulP is closely related to the ABC transport system from cyanobacteria and similar genes are found in chloroplast genomes of several other green algae and the lower plants *Mesostigma viride, Marchantia polymorpha*, and *Anthoceros formosae*. The transporter responsible for sulfate uptake into plastids of higher plants still remains to be identified. In the complete genomes of the red alga *C. merolae* and the stramenopile, *T. pseudonana*, which has a secondary plastid of red-algal origin, only genes encoding a bacterial type MFS sulfate transporter are found. The prokaryotic MSF transporters are found in several other algae with plastid of red-algal origin, such as the haptophytes *Pavlova lutheri* and *Isochrysis galbana* and the dinoflagellate *Karlodinium micrum*, additionally a 'plant-type' transporter was found in *P. lutheri*.

2. Phylogenetic Analysis

An analysis of the full-length sequences reveals most of the streptophytes form a well-supported clade and, although *Arabidopsis* and *O. sativa* isoforms form five moderate- to well-supported sub-clades indicating that these duplicated in the ancestors of tracheophytes, the multiple isoforms from the lower plants *P. patens* and S. *moellendorffii* seem to have duplicated independently within those lineages (Fig. 2). The transporters of the green-lineage group together with strong support but are not related to cyanobacteria, as is often the case for transporters. In contrast, the red-lineage plastid transporters from *C. merolae* and *T. pseudonana* fall within a strongly supported clade of bacteria including cyanobacteria. The transporters from *P. patens* and *S. moellendorffii*, which cluster with group 1 transporters when only plant sequences are analyzed are positioned basal to the node separating low affinity (group 2) and high affinity (group 1) transporters of higher plants. Thus, their function as high or low affinity transporters has to be verified experimentally.

B. ATP Sulfurylase

1. Biochemical Properties

ATP sulfurylase (EC 2.7.7.4) catalyzes activation of sulfate by adenylation to APS. The formation of

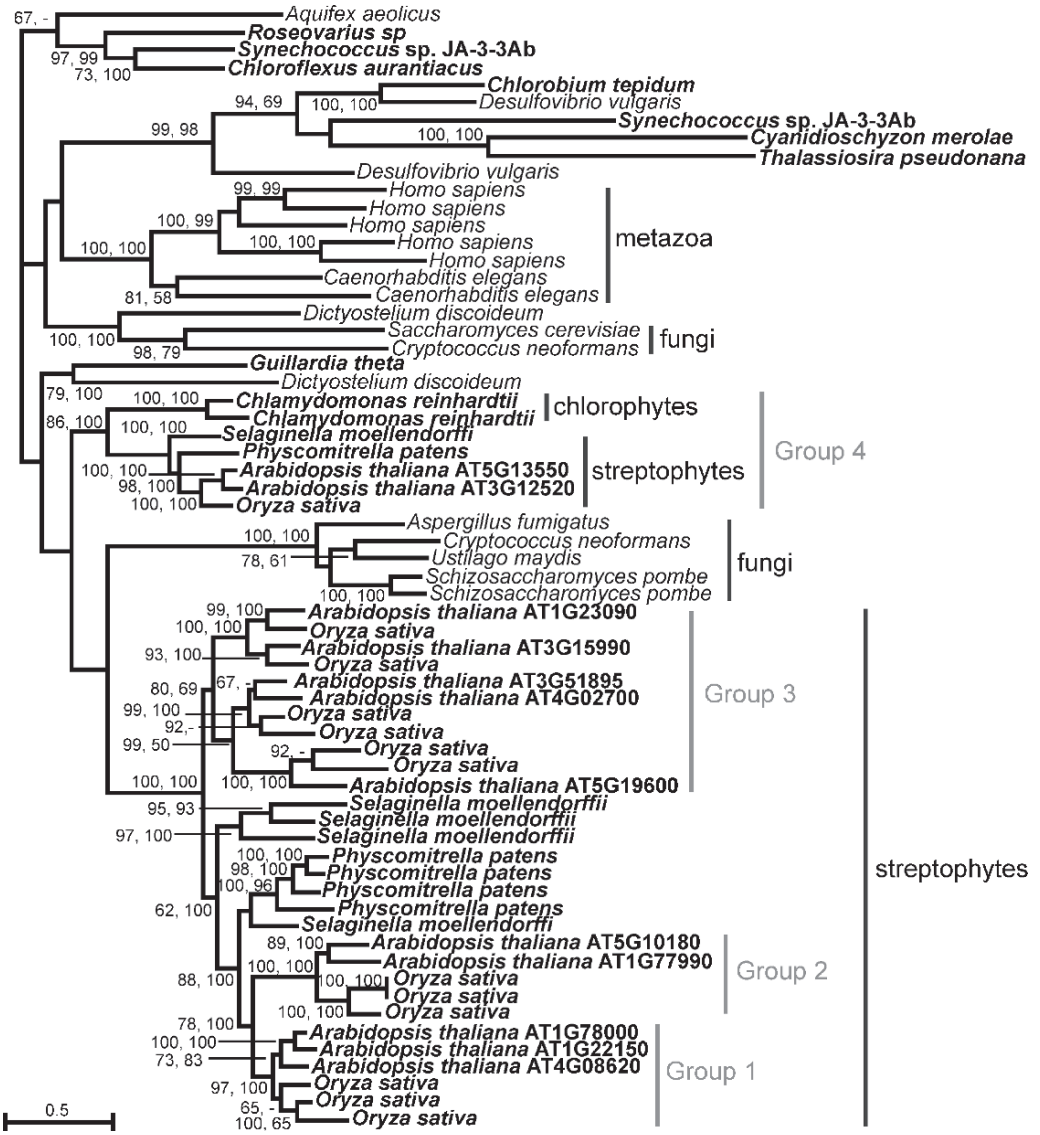

Fig. 2. Protein maximum likelihood phylogeny for plant type sulfate transporters. The tree was inferred using PhyML with nine categories of rates. Numbers at nodes indicate bootstrap support over 60 from (top to bottom or left to right) maximum likelihood, as determined by PhyML, and weighted neighbor joining. Analysis used 285 characters, the gamma-shaped parameter 1.797 and proportion of invariable sites 0.000. Photosynthetic organisms are typed in bold.

APS is an energetically very unfavorable process, which has to be driven forwards by consumption of the reaction products by subsequent reactions. APS is thus reduced to sulfite by APR or phosphorylated to PAPS by APS kinase. However, the hydrolysis of the second reaction product of ATPS, pyrophosphate, by inorganic pyrophosphatase (PPase) also contributes to shifting of reaction equilibrium towards APS synthesis. ATPS is found also in Metazoa, in which, although unable to reduce sulfate, it plays an essential role in the synthesis of PAPS as a donor of activated sulfate for various sulfotransferases (Varin et al., 1997; Honke and Taniguchi, 2002). In these species ATPS and APS kinase are fused, creating a single bifunctional protein, PAPS synthetase (Rosenthal and Leustek, 1995; Venkatachalam, 2003). Similarly, in *Mycobacteria* and *Rhizobia*

ATPS and APS kinase are essential for the synthesis of PAPS to enable the sulfation of virulence and Nod factors, respectively (Folch-Mallol et al., 1996; Mougous et al., 2006). ATPS is also required in anaerobic sulfate-reducing bacteria, where APS serves as terminal electron acceptor for respiration, and in several classes of chemolitotrophic bacteria, which use ATPS to synthesize ATP from APS and pyrophosphate (Kappler and Dahl, 2001).

2. Types of ATPS in Different Organisms

ATPS from different organisms have very different molecular structures in terms of subunits, however the basic subunit in some of them is conserved and readily alignable. Plant ATPS is a homotetramer of 52–54 kDa polypeptides (Murillo and Leustek, 1995). Activity has been detected in both chloroplasts and the cytosol (Lunn et al., 1990). In *Arabidopsis*, the overall foliar ATPS activity continually declined during plant growth. During this time the more abundant chloroplast ATPS activity decreased, while cytosolic activity grew (Rotte and Leustek, 2000). ATPS thus seems to have different functions in the two compartments: sulfate assimilation in the plastids and activation of sulfate for synthesis of sulfated compounds in the cytosol (Rotte and Leustek, 2000). ATPS is encoded by small multigene families in all plant species analyzed to date. cDNAs encoding chloroplast and cytosolic isoforms of ATPS were isolated from potato (Klonus et al., 1994). On the other hand, four isoforms of ATPS were isolated from *Arabidopsis*, all of them containing a chloroplast transit peptide (Murillo and Leustek, 1995; Hatzfeld et al., 2000a) and so activity in the cytosol is unaccounted for. No information is available for the biochemical properties of ATPS from algae. However, sequence analysis of the two ATPS isoforms found in the genome of the diatom *T. pseudonana* revealed one fused to APS kinase and PPase forming a polyprotein, which indicates an increased efficiency of catalysis compared with ATPS alone. As already discussed, metazoan ATPS is part of PAPS synthetase and functions as a single protein of 56 kDa (Venkatachalam, 2003). Most animals possess two copies of the gene. ATPS from yeast and fungi is also fused to APS kinase; however, the protein is a homohexamer of 59 to 64 kDa subunits. The APS kinase-like domain is not functional and is the site of allosteric regulation by PAPS (MacRae et al., 2001; Ullrich et al., 2001). Bacterial ATPS on the other hand, consists of four heterodimers composed from 35 kDa CysD and 53 kDa CysN subunits (Leyh et al., 1988). CysD belongs to the ATP pyrophosphatase family of proteins and is thus distantly related to APR and PAPS reductase. CysD is the catalytic subunit of bacterial ATPS. Its activity is, however, energetically dependent on hydrolysis of GTP by the CysN subunit. CysD and CysN are therefore almost invariably linked in a single operon, which often contains the APS kinase encoding gene, CysC. *Mycobacteria* and *Rhizobia*, which require PAPS for sulfation, possess multiple copies of the CysD/N operon, the latter species as NodP and NodQ on the symbiotic plasmid. Interestingly, the bacterial CysN gene originated from an archaeal or eukaryotic translation elongation factor 1α (EF-1α) by lateral gene transfer (LGT) and acquired new function (Inagaki et al., 2002). No gene homologues of CysD or CysN (apart from EF-1α) are found among eukaryotes, so that this form of ATPS is limited to prokaryotes and some Archaea, which in turn most probably acquired the sulfate assimilation operon by LGT from proteobacteria. A further ATPS can be found in the sulfate reducing bacteria where, similar to plants, it is a homooligomer of single-function subunits (Sperling et al., 1998).

3. Phylogenetic Analysis

ATPS sequences were obtained from sequenced genomes and several EST projects. Multiple isoforms were found in most plant and algal species. Maximum likelihood analysis resulted in a complex tree with many unexpected relationships (Fig. 3). Whereas the different isoforms of plant ATPS (plastidic and cytosolic) cluster together and are therefore results of relatively recent gene duplications these were, very surprisingly, unrelated to the chlorophyte sequences. Instead they are very strongly related to the ATPS part of the PAPS protein found in Metazoa.

The chlorophytes fall deep in a well-supported clade that also contains many alpha-proteobacteria, algae and protozoans with secondary-plastids and fungi. It is by no means certain, but certainly likely that the origin of these isoforms in eukaryotes is

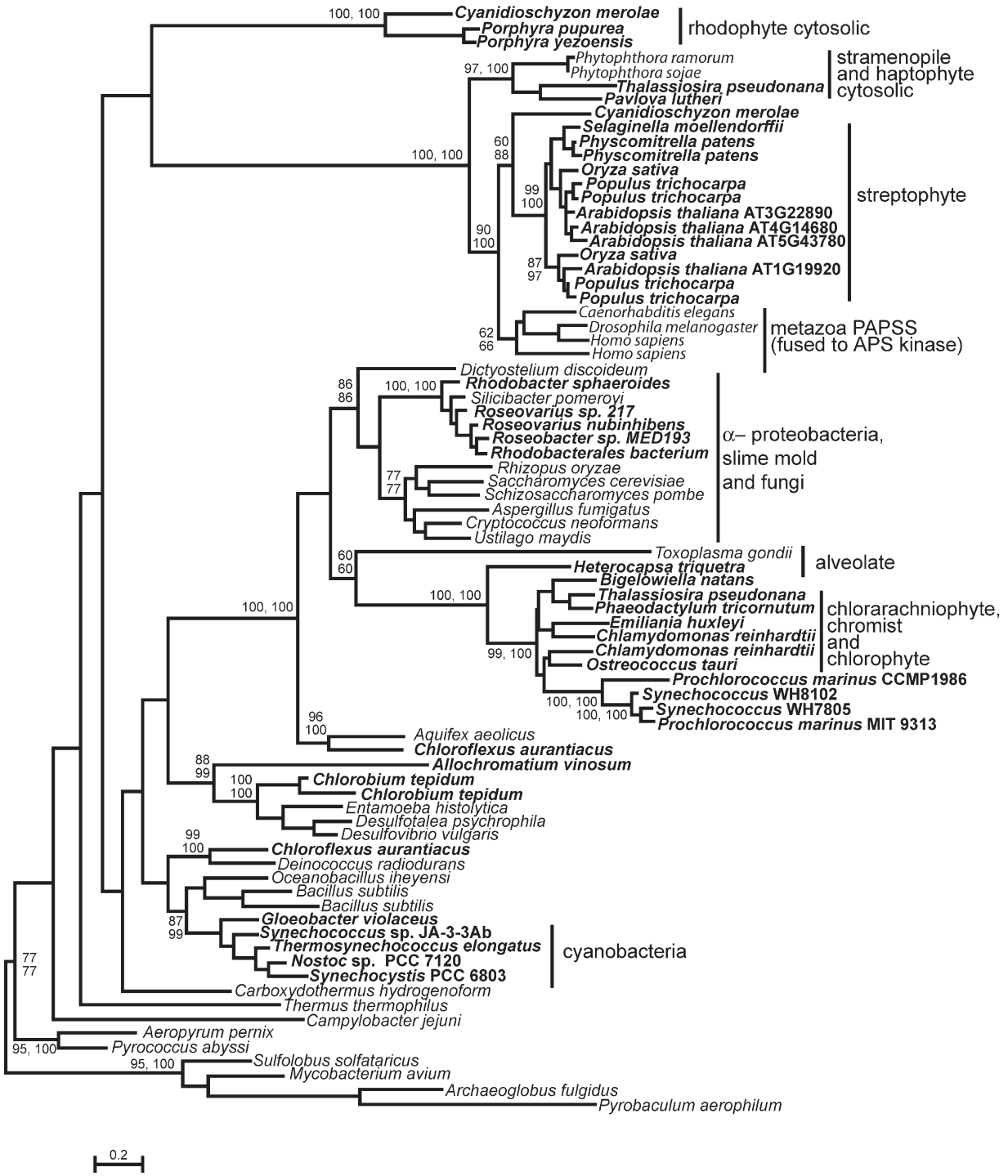

Fig. 3. Protein maximum likelihood phylogeny for ATP sulfurylase. The tree was inferred using PhyML with nine categories of rates. Numbers at nodes indicate bootstrap support over 60 from (top to bottom or left to right) maximum likelihood, as determined by PhyML, and weighted neighbor joining. Analysis used 433 characters, the gamma-shaped parameter 1.514 and proportion of invariable sites 0.059. Photosynthetic organisms are typed in bold.

the mitochondria. Also in this clade are several strains of *Prochlorococcus*/*Synechoccus*, known to exchange genetic data by LGT via phage with high frequency (Lindell et al., 2004; Sullivan et al., 2005; Coleman et al., 2006). All other cyanobacteria are elsewhere suggesting that these cyanobacteria obtained this isoform from a eukaryote.

The spread of the Viridiplantae suggests that there were ancestrally two isoforms in the ancestor of green algae and plants, one from an endosymbiont and one from the host. The plant lineage kept the host copy while the green algal lineage kept the plastid copy. Since there is little sequence data from the basal chlorophyte lineages, from before the divergence of plants, this cannot be proven at present.

Algae in the red plastid lineage also encoded multiple isoforms of ATPS of differing evolutionary origins. The dissimilatory ATPS of anaerobic sulfate reducing and sulfur oxidizing bacteria cluster with genes from some Gram positive bacteria, e.g., *Bacillus subtilis*, which does not possess the CysD/N system. Another surprising finding is that the ATPS from *Entamoeba histolytica* clustered among the bacterial taxa. The role for ATPS in *Entamoeba* is not obvious, since this eukaryote does not possess APS or PAPS reductase and is thus not able to reduce sulfate. However, *Entamoeba* produces monoethyl sulfate and 3-cholesteryl sulfate (Bakker-Grunwald and Geilhorn, 1992), therefore, the ATPS, which is fused to APS kinase, is probably responsible for production of the activated sulfate for these sulfations.

C. APS Kinase

1. Biochemical Properties

APS kinase (EC 2.7.1.25) catalyzes the transfer of phosphate from ATP to APS to form PAPS. PAPS is an important metabolite, not only as a form of activated sulfate for reduction in fungi and some heterotrophic bacteria but also for the sulfation of various metabolites. Although APS kinase does not participate in the pathway of sulfate assimilation in plants and algae, it interacts with the pathway by competing for APS with APR. The enzyme from *Penicillium chrysogenum* was crystallized as homodimer of 24 kDa subunits (MacRae et al., 2000). In plants, APS kinase is localized in the chloroplast and the cytosol. Four genes encoding APS kinase are found in the *Arabidopsis* genome, all located on different chromosomes. Three of these genes code for proteins with chloroplast transit peptides, the forth, located on chromosome 3, likely encodes the isoform responsible for cytosolic activity. Very little is known about the biochemical properties and functions of the individual plant APS kinases.

APS kinase belongs to the group of P-loop-fold proteins that hydrolyze or bind nucleoside triphosphates, such as kinases and sulfotransferases (Leipe et al., 2003). Within this large group of proteins it belongs more specifically to the DxD group of kinases together with gluconate kinase, shikimate kinase, and dephosphocoenzyme A kinase. APS kinases from different organisms are well conserved. APS kinase is often found fused to ATP sulfurylase, which catalyzes the synthesis of its substrate. These fusion proteins contain the APS kinase domain at N-terminus (Metazoa) or at C-terminus (some bacteria and filamentous fungi) and can even be fused with yet another protein, as in *T. pseudonana*. Whereas in Metazoa the APS kinase is active in the synthesis of PAPS, in filamentous fungi the APS kinase domain is inactive and functions in regulation of ATPS activity (MacRae et al., 1998). The PAPS in fungi such as *P. chrysogenum*, is synthesized from APS by a second APS kinase paralogue.

2. Phylogenetic Analysis

Analysis of APS kinase sequences revealed that the multiple isoforms found in plants and green algae are specifically related, the duplications having occurred at the base of the tracheophytes (Fig. 4). The position of the clade, however, is entirely unsupported, as is much of the backbone of the tree and so it is not clear if the ancestry is from the plastid (cyanobacterial) or from the host. Likewise the relationships between the fungal, metazoan and various proteobacterial lineage, though being individually supported, cannot be resolved at present.

D. APS and PAPS Reductase

1. APS and PAPS Dependent Sulfate Assimilation – an Historical Overview

Since the sulfate assimilation pathway was first resolved in the enteric bacteria, *Escherichia coli*

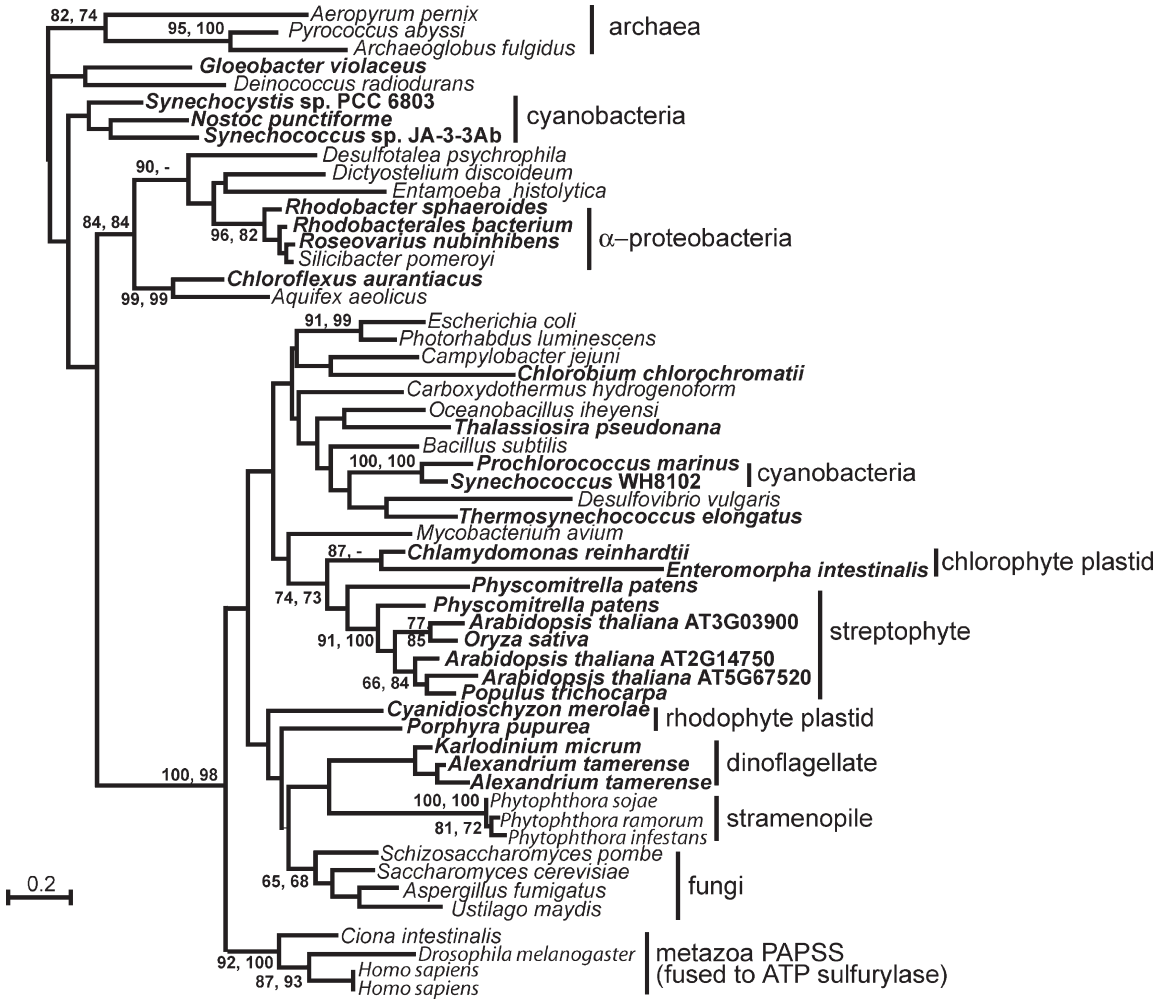

Fig. 4. Protein maximum likelihood phylogeny for APS kinases. The tree was inferred using PhyML with nine categories of rates. Numbers at nodes indicate bootstrap support over 60 from (top to bottom or left to right) maximum likelihood, as determined by PhyML, and weighted neighbor joining. Analysis used 151 characters, the gamma-shaped parameter 1.211 and proportion of invariable sites 0.086. Photosynthetic organisms are typed in bold.

and *Salmonella typhimurium* (Kredich, 1971), it was thought that both activation steps, adenylation of sulfate to APS with subsequent phosphorylation to PAPS, are generally required for sulfate reduction. However, studies with the green alga *Chlorella* revealed that APS can be directly utilized to form sulfite bound to a thiol carrier and the corresponding enzyme was named APS sulfotransferase (Tsang et al., 1971; Schmidt, 1972). The APS sulfotransferase activity was detected in a variety of plants and photosynthetic bacteria (Schmidt, 1975; Schmidt and Trüper, 1977); therefore it was considered to represent the major sulfate reducing enzyme in photosynthetic organisms in contrast to enteric bacteria and yeast, which seemed to utilize PAPS for reduction (Brunold, 1990, Schmidt and Jäger, 1992). Since APS and PAPS are very similar substrates and the reaction conditions for their reduction are also very similar, complementation of *E. coli* mutants deficient in *cysH* was chosen as a method to clone plant APS and/or PAPS reducing enzyme(s). Three homologous cDNA clones were obtained from *A. thaliana* encoding a protein similar to *E. coli* PAPS reductase with a C-terminal thioredoxin-like extension (Gutierrez-Marcos et al., 1996;

Setya et al., 1996). Since the enzyme produced sulfite from APS but not from PAPS, it was named APS reductase (Setya et al., 1996). The cDNA was shown to correspond to the previously characterized APS sulfotransferase but biochemical analysis of its reaction products revealed that it produces free sulfite and is therefore a reductase (Suter et al., 2000). In attempts to show that APR is the only plant enzyme responsible for sulfate reduction to sulfite the single copy gene encoding APR was disrupted in the moss *Physcomitrella patens* by homologous recombination (Koprivova et al., 2002). This resulted in loss of APR activity but not the ability of such plants to grow on sulfate as the sole sulfur source and thus led to cloning of putative PAPS reductase from this moss species (Koprivova et al., 2002). On the other hand, phylogenetic analysis found that many *cysH* genes, from various bacterial lineages, are more related to plant APR than to PAPS reductase from *E. coli*. These observations led to the discovery of bacterial assimilatory APS reductase, which lacks the thioredoxin-like domain and requires thioredoxin for activity (Abola et al., 1999; Bick et al., 2000; Kopriva et al., 2002; Williams et al., 2002).

2. Biochemical Properties of APS and PAPS Reductases

APR catalyzes a thiol-dependent two-electron reduction of APS to sulfite (Suter et al., 2000). In higher plants it is localized exclusively in the plastids (Koprivova et al., 2001). The enzyme was first purified from the green alga *Chlorella* as a protein of molecular weight greater than 300 kDa (Schmidt, 1972). Li and Schiff (1991) purified APR from *Euglena gracilis* as a tetramer of 25 kDa subunits, whereas APR from *Porphyra yezoensis* consisted of 43 kDa subunits (Kanno et al., 1996). The molecular mass of native *Lemna minor* APR (Suter et al., 2000) and recombinant APR2 from *A. thaliana* (Kopriva and Koprivova, 2004) was estimated to be 91 kDa, revealing that plant APR is a dimer of ca. 45 kDa subunits. Assimilatory APR from several bacteria is either a dimer or monomer of approximately 30 kDa subunits (Kim et al., 2004; Carroll et al., 2005a). Both plant and bacterial assimilatory APR possess an atypical diamagnetic and asymmetric [Fe_4S_4] cluster (Kopriva et al., 2001, 2002; Carroll et al., 2005a). APR reactions can be divided into three steps. In the first the N-terminal part of the protein reacts with APS resulting in a stable reaction intermediate with sulfite bound to the only conserved Cys residue between APS and PAPS reductases (Weber et al., 2000). In the second step the intermediate is released by the C-terminal thioredoxin-like domain in plant APR or by free thioredoxin in case of bacterial APR. Finally, the thioredoxin or thioredoxin-like domain are reduced by thioredoxin reductase or GSH (Weber et al., 2000; Kopriva and Koprivova, 2004; Carroll et al., 2005b).

PAPS reductase is comprised of two 28 kDa subunits devoid of any prosthetic groups. It contains a single conserved cysteine residue which is responsible both for the dimerisation and for enzyme activity. In the first step of the reaction mechanism a reduced PAPS reductase binds PAPS, reduces it to sulfite and is oxidized in the process. The return to reduced state is achieved by reaction with reduced thioredoxin or glutaredoxin (Lillig et al., 1999).

APS reductase is found also in dissimilatory sulfate reducers and sulfur oxidizing bacteria and Archaea. The enzyme is a heterodimer of a 75 kDa FAD containing α-subunit and a 20 kDa β-subunit binding two [Fe_4S_4] clusters (Fritz et al., 2002). Again, there is no sequence similarity to the assimilatory APR.

3. Distribution of APS and PAPS Reductases in Different Organisms

Biochemical data from various APS and PAPS reductases pointed to a clear association of the ability to reduce APS in the presence of an FeS cluster (for details see Kopriva and Koprivova, 2004). Most importantly, a bifunctional APS/PAPS reductase from *Bacillus subtillis* possesses the FeS cluster. After chemical removal of the cofactor APS, but not PAPS, reduction is abolished (Berndt et al., 2004), which strongly corroborates the functional link between the cluster and APS reduction. The cofactor is bound to the protein by three or four cysteine residues, which are found as two invariant Cys pairs in almost all APR proteins (Kopriva et al., 2002; Kim et al., 2004; Kopriva and Koprivova, 2004; Carroll et al., 2005a). These cysteine pairs thus serve as a sequence marker to distinguish APS from PAPS reductases (Kopriva et al., 2002; Kopriva and

Koprivova, 2004). Analysis of 599 sequenced prokaryotic genomes revealed that, based on the presence of the two Cys pairs, 202 species (or strains) possess APS reductase whereas 98 contain PAPS reductase, the bifunctional APS/PAPS reductase from *B. subtillis*, and possibly other Firmicutes, being counted among the APRs. The few Archaea capable of assimilatory sulfate reduction contain an APR. On the other hand, fungi encode PAPS reductase exclusively. PAPS reductase is confined to only two bacterial lineages: γ-proteobacteria and cyanobacteria, both lineages, however, also contain species with APR. Species of cyanobacteria, such as *Synechocystis* and *Synechococcus*, have long been known to reduce PAPS, but certain species, e.g., *Plectonema*, are known to be APS reducing (Schmidt, 1977). Most of the sequenced cyanobacteria possess the PAPS reductase gene, however, our recent analysis of several cyanobacteria revealed that the reduction of APS is much more common in these species than would seem from the genomic sequences (Kopriva S, Wiedemann G, unpublished). This is very important when considering the origin of the APS reductase in the plastids of algae and plants.

The putative PAPS reductase from the moss *P. patens*, which also contains an APS reductase, is more similar to fungal and bacterial PAPS reductases than to plant APS reductases and does not possess the two Cys pairs. It also lacks the C-terminal thioredoxin-like domain. Surprisingly, a detailed biochemical analysis revealed that although the enzyme reduces PAPS, it is far more active with APS. Since this protein does not bind an FeS cluster, it represents a novel form of APS reductase (Kopriva et al., 2007). This novel 'APR-B', although able to complement the loss of 'normal' APR in *P. patens* APR knock-outs (Koprivova et al., 2002), is substantially less catalytically efficient *in vitro* than the FeS cluster possessing APR. Orthologs of this novel APS reductase were found in a further lower plant lineage, in the spike-mosses *Selaginella lepidophylla* and *S. moellendorffii*, suggesting that its presence in *Physcomitrella* is not a result of a recent horizontal gene transfer to that species specifically. In *P. patens* there is evidence of expression of both APR and APR-B, but in *S. lepidophylla* transcripts have only been found for APR-B. The genomic copy of APR in the related *S. moellendorffii* contains several base changes in the active site suggesting that the protein may be inactive (Kopriva S, unpublished). Other lower plants, such as *Equisetum* or ferns, possess an APS reductase (Kopriva S, unpublished) and all chlorophyte sequences to date support the presence of an APS reductase only.

The diatom *T. pseudonana* reduces APS at a very high rate, up to 100-fold higher than plants (Gao et al., 2000; Kopriva S, unpublished). Nevertheless, the only two genes homologous to APS reductase identified in the complete genome are more similar to PAPS reductase (or the novel APR-B from lower plants) and lack the FeS binding Cys pairs. However, like the APS reductase sequences of plants, they also contain C-terminal thioredoxin-like domains (Kopriva S, unpublished). Similar genes were found in several other algae, the haptophyte *Emiliania huxlei*, the chlorarachniophyte *Bigelowiella natans*, the diatom *Fragilariopsis cylindrus*, and the dinoflagellate *Heterocapsa triquetra*. Although the enzymatic activity of these proteins has not yet been confirmed *in vitro*, like *T. pseudonana*, many of these species have been shown to reduce APS (Gao et al., 2000; Kopriva S, unpublished), and it seems that they also belong to the new class B of APS reductase. Without the two cysteine pairs an FeS cluster cannot be bound to these proteins and so this novel class of APR probably does not require the cofactor.

4. Phylogenetic Analysis

In Fig. 5 the combined APR and PAPR phylogenetic tree is presented without species names and only the major lineages are indicated to show the relationship and distribution of these isoforms. However, a greater number of characters can be included with separate analyses of the two enzymes and so the two classes were also analyzed independently (Figs. 6 and 7). Because the new APR-B enzyme found in some algae and lower plants is more related to the PAPR enzymes, these are included in the analysis of this enzyme. Although previous analyses resolved a split between APS and PAPS reductases (Kopriva et al., 2002; Kopriva and Koprivova, 2004), this is not the case in the maximum likelihood analysis. Both the combined tree and the individual APR and PAPR trees are rooted with three Archaeal genes of

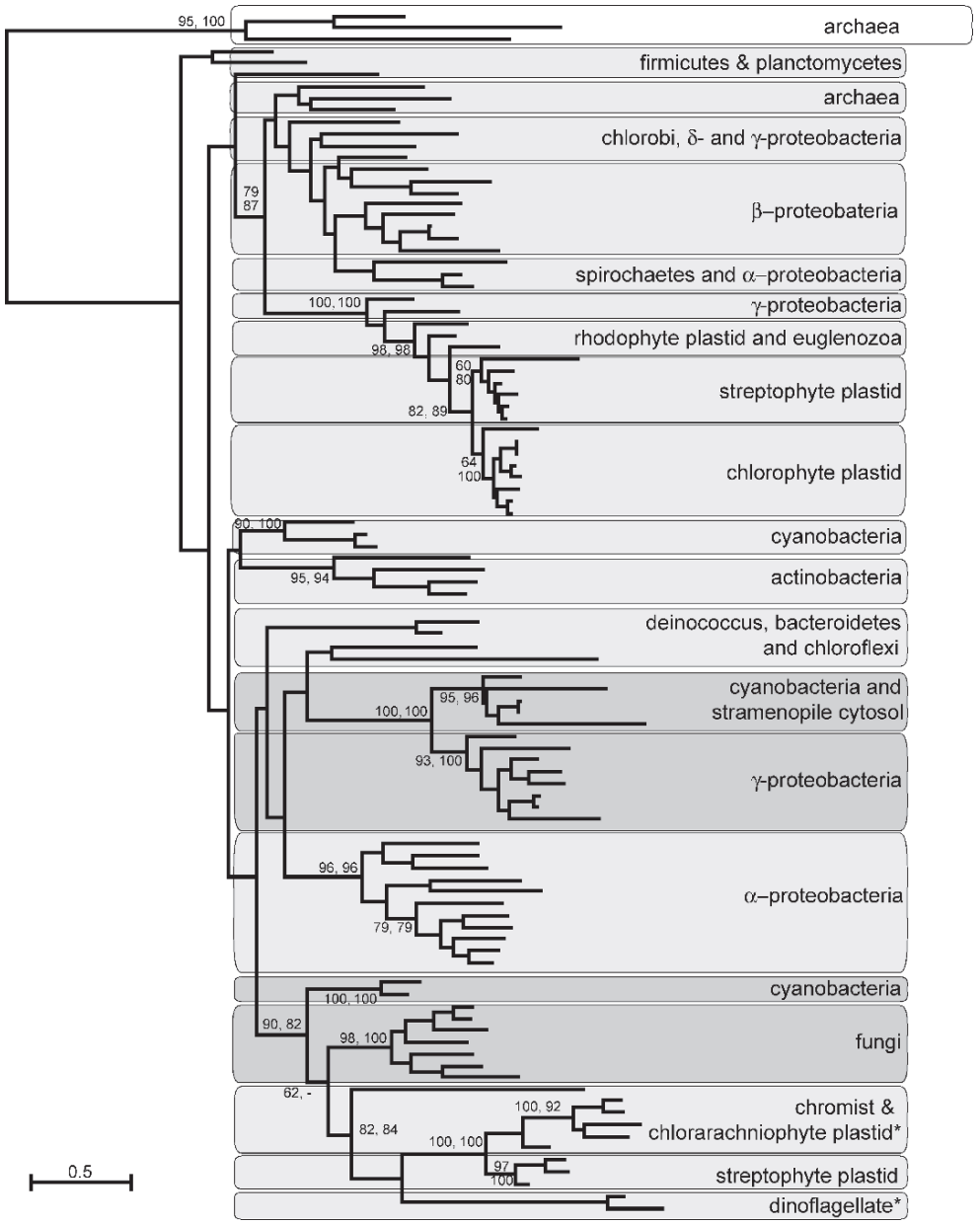

Fig. 5. Protein maximum likelihood phylogeny for APS and PAPS reductases. The tree was inferred using PhyML with nine categories of rates. Numbers at nodes indicate bootstrap support over 60 from (top to bottom or left to right) maximum likelihood, as determined by PhyML, and weighted neighbor joining. Analysis used 184 characters, the gamma-shaped parameter 1.27 and proportion of invariable sites 0.014. Photosynthetic organisms are typed in bold. Light and dark shading marks APS and PAPS reductases, respectively.

unconfirmed function that show weak similarity to both APS reductase and also to the bacterial CysD subunit of ATPS.

In the APR tree, all plants and algae with primary plastids are specifically related (Fig. 6). Related to these is the APR found in the mitochondria of *E. gracilis*. It is possible that this species, which contains a secondary plastid of green algal origin, relocated the sulfate-reducing pathway, which it likely obtained from this endosymbiont, to the mitochondria.

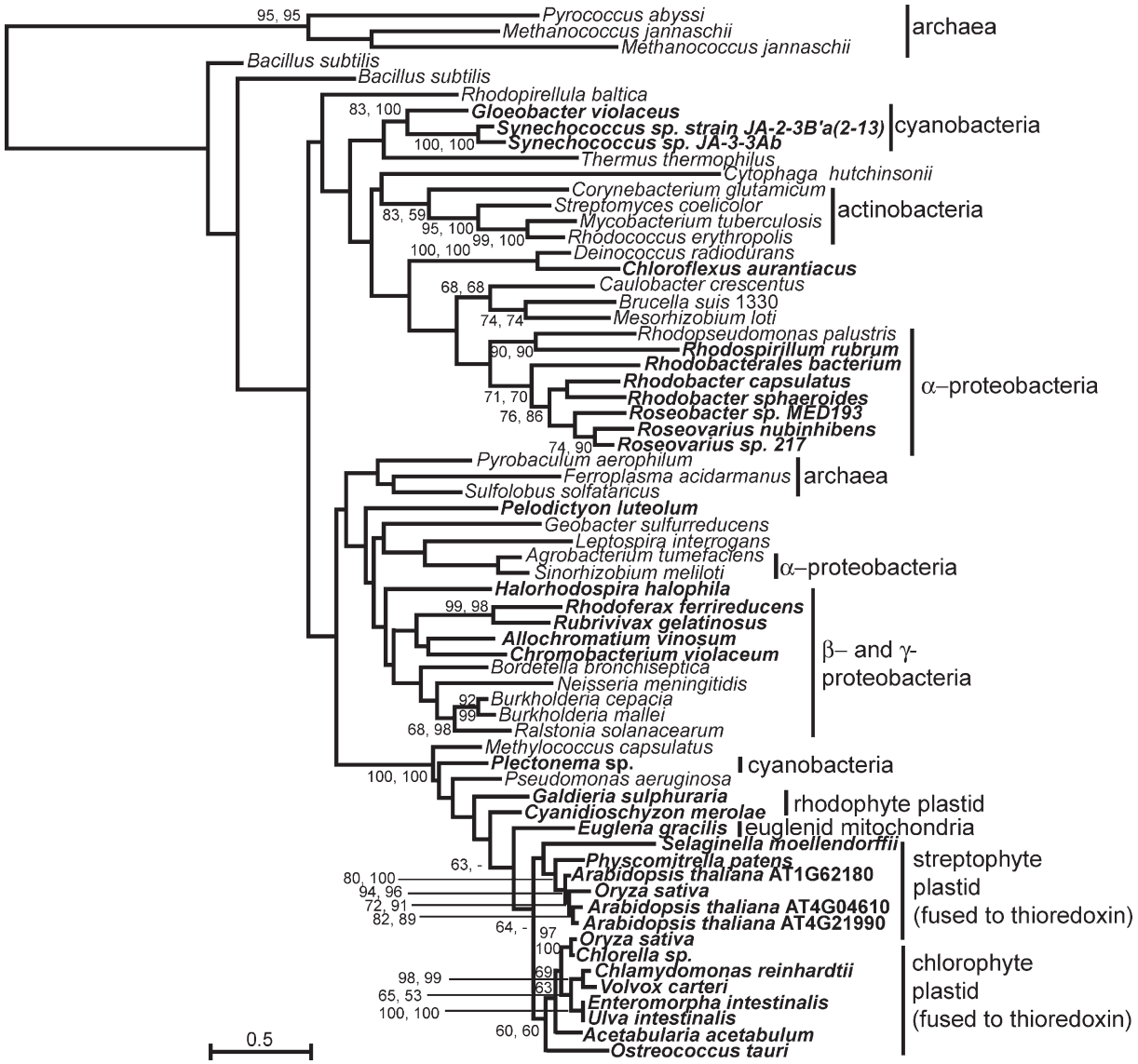

Fig. 6. Protein maximum likelihood phylogeny for APS reductases. The tree was inferred using PhyML with nine categories of rates. Numbers at nodes indicate bootstrap support over 60 from (top to bottom or left to right) maximum likelihood, as determined by PhyML, and weighted neighbor joining. Analysis used 149 characters, the gamma-shaped parameter 1.159 and proportion of invariable sites 0.018. Photosynthetic organisms are typed in bold.

The sequence from the cyanobacteria *Plectonema*, although related with strong support to plants and primary algae, does unambiguously fall at the base of the plastid clade and, though it is likely, cannot be confirmed as the source of the plastidial APR. The other lineages of bacteria form monophyletic clades, although these are, for the most part, unsupported. The position of the Archaea with APR, which is suggested to have arisen by lateral transfer, although being nested within the proteobacteria is unsupported and not related to any particular group. The origin of the enzyme in this lineage is also unresolved at present.

Within the PAPR tree the eukaryote sequences from fungi and algae with secondary plastids, as well as those from the mosses and spike mosses cluster together with the exception of a couple of divergent sequences from *G. theta* and *T. pseudo-*

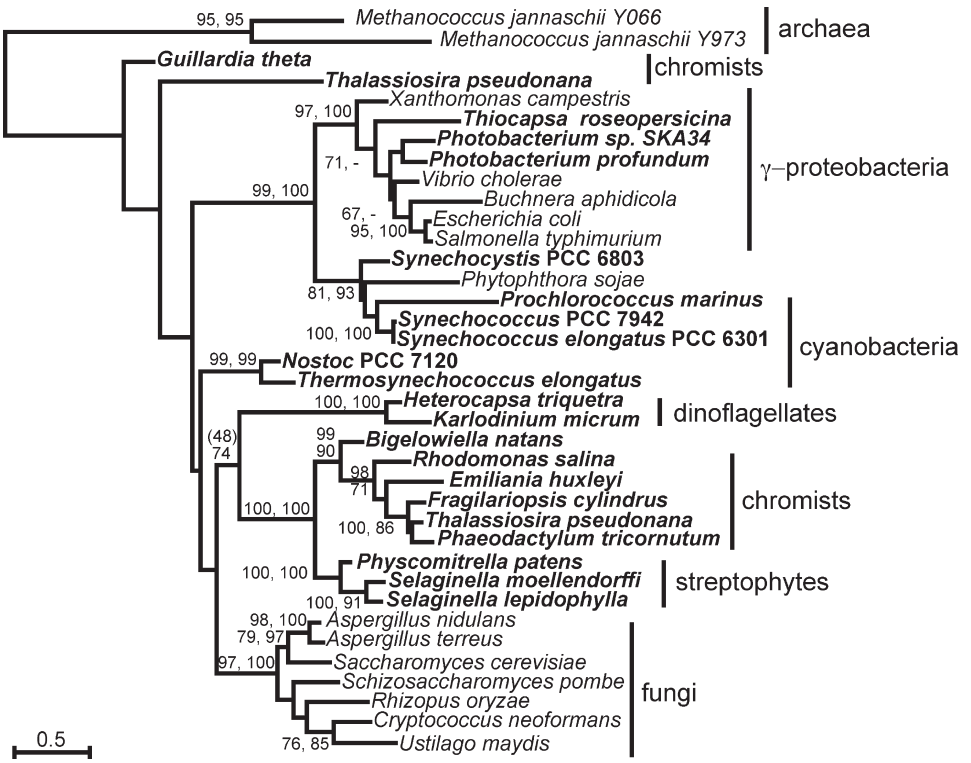

Fig. 7. Protein maximum likelihood phylogeny for PAPS reductases. The tree was inferred using PhyML with nine categories of rates. Numbers at nodes indicate bootstrap support over 60 from (top to bottom or left to right) maximum likelihood, as determined by PhyML, and weighted neighbor joining. Analysis used 149 characters, the gamma-shaped parameter 1.559 and proportion of invariable sites 0.030. Photosynthetic organisms are typed in bold.

nana (Fig. 7). The relationship with the fungi is not supported but the chromist and alveolate algae relationship is moderately supported. Within this larger 'algal' clade, the chromists are strongly related to the chlorarachniophyte *B. natans*, and also to the streptophyte isoforms. While *B. natans* is known to contain multiple enzymes more related to the red- than green-algal lineage (Archibald et al., 2003), this is extremely interesting. The possibility that the green lineage, after the divergence of streptophytes, obtained an APR-B from a chromist alga cannot be excluded.

The cyanobacteria are related to the γ-proteobacteria, to the exclusion of all eukaryotes and so the origin of the algal APR-B is not plastidial (unsurprising since primary plastids contain APR) and they are likely of host (eukaryotic) origin. It is evident however, that the new APS reductase isoform evolved from a PAPS reductase and changed the substrate specificity to APS while retaining the FeS independent reaction mechanism. This seems to be the most efficient way of reducing activated sulfate, as the energy costs for building the cofactor are eliminated as well as the necessity of a second activation step by ATP, which is the price organisms possessing PAPS reductase pay for saving the cluster. It seems that during the evolution of plants, at least for some time, two genes were present but the APR-B was lost soon after branching of the lineage leading to spike-mosses. Since all chlorophyte sequences to date support the presence of an APS reductase only, it cannot be ruled out that the PAPS reductase, which gave rise to APR-B was introduced to the plant lineage by LGT before the divergence of bryophytes and then lost again after the divergence of the lycopodiophytes.

E. Sulfite Reductase

1. Biochemical Properties

Sulfite reductase catalyzes the six electron reduction of sulfite to sulfide. The electron donor for

the reduction is ferredoxin (Fd) in plants (EC 1.8.7.1) or NADPH in bacteria (EC 1.8.1.2). Plant SiR contains one siroheme and one [4Fe–4S] cluster per monomer as cofactors (Krueger and Siegel, 1982). The SiR protein structure and sequence is very similar to Fd-nitrite reductase, which catalyzes an equivalent step, a six electron reduction of nitrite to ammonia, in nitrate assimilation. The similarity of the enzymes is corroborated by the fact that both enzymes can reduce sulfite and nitrite, albeit with different efficiency (Krueger and Siegel, 1982). Indeed, in *Arabidopsis* SiR contains 19% identical amino acids with nitrite reductase indicating that these genes may have the same evolutionary origin. SiR activity was localized exclusively to plastids both in photosynthetic and non-photosynthetic organs (Brunold and Suter, 1989). Interestingly, SiR is abundant in the nucleoids of pea chloroplasts and is able to compact chloroplast DNA (Sato et al., 2001), which might represent a new function apart from sulfate assimilation.

2. Types of SiR in Different Organisms

The reaction mechanism of sulfite reduction requires siroheme, therefore this cofactor, as well as the iron sulfur cluster, are found in SiRs from all sources. The overall structure of the SiR enzyme, however, differs substantially in various species. Plant SiR is a monomeric protein of 65 kDa containing no prosthetic groups other than the siroheme and FeS cluster (Nakayama et al., 2000). SiR is encoded by a single copy gene in *Arabidopsis*, in contrast to other enzymes of the pathway (Bork et al., 1998). However, two or more SiR isoforms are present in other plant species (Yonekura-Sakakibara et al., 1998). In contrast, the bacterial NADPH-SiR is an oligomer of eight 66 kDa flavoprotein subunits (CysJ) and four 64 kDa siroheme and [4Fe–4S] cluster binding hemoproteins (CysI) (Crane et al., 1995). SiR in yeasts and other fungi are again different, composed from α and β subunit of 116 and 167 kDa respectively. Similar to bacterial SiR, the fungal enzyme requires siroheme, FAD and FMN (Kobayashi and Yoshimoto, 1982).

In addition to the assimilatory sulfite reductase, a dissimilatory sulfite reductase (Dsr) (EC 1.8.99.1) is present in organisms reducing sulfite to sulfide during anaerobic respiration, such as *Desulfovibrio* species and *Archaeoglobus fulgidus* (Dahl et al., 1993). Dsr is also present in the sulfur oxidizing bacteria *Thiobacillus denitrificans* and *Allochromatium vinosum*, where it catalyzes the reverse reaction, oxidation of sulfide to sulfite (Stahl et al., 2002). The enzyme is a tetramer of two α and two β subunits with a molecular mass between 40 and 45 kDa, again, containing a siroheme linked to a [4Fe–4S] cluster. Dsr from *Desulfovibrio* and some other species contains a third polypeptide of ca. 12 kDa but its function is not known (Pierik et al., 1992). There is no sequence similarity between the Dsr subunits and any of the assimilatory SiR forms.

3. Phylogenetic Analysis

Since they all are readily alignable, we compared sulfite reductase from plants and algae together with the siroheme binding SiR subunits from bacteria and fungi with several nitrite reductase (NiR) sequences from different sources (Fig. 8). The analysis shows that the two enzymes (SiR and NiR) arose from an ancient gene-duplication in Eubacteria, before the primary endosymbiosis that gave rise to plastids. A relationship to cyanobacteria in both the SiR and NiR branches supports an endosymbiont origin for both SiR and NiR in all plastids; both primary and secondary, red and green. The non-photosynthetic stramenopile, *Phytophthora*, however, is more related to the fungi SiR and likely encodes a protein of host origin. Like the fungi *Phytophthora* possesses only homologues of SiR, NiR in this lineage being of an independent, unrelated origin. The SiR is closely related to proteobacteria but shows no supported relationship to α-proteobacteria and so a mitochondrial origin cannot be assumed. The photosynthetic bacteria encode a SiR protein that is only marginally related to other photosynthetic organisms and may represent an ancient form of assimilatory SiR.

F. Serine Acetyltransferase

1. Biochemical Properties

Serine acetyltransferase (SAT, EC 2.3.1.30) catalyzes the acetylation of L-serine with acetyl-CoA to form *O*-acetylserine in the first step of L-cysteine synthesis. SAT forms a complex called cysteine synthase with the subsequent enzyme, OASTL, which is essential for its enzymatic activity

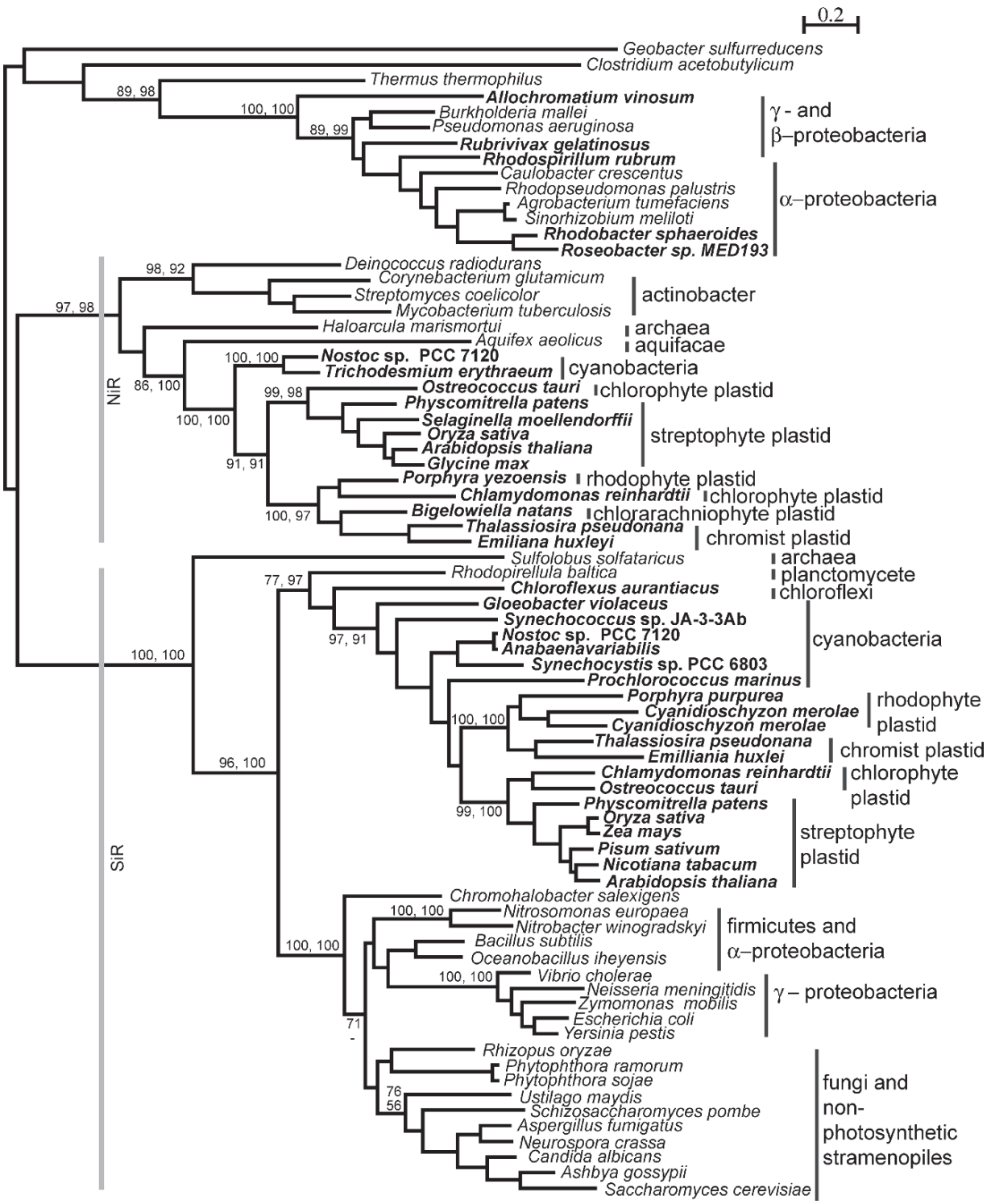

Fig. 8. Protein maximum likelihood phylogeny for sulfite and nitrite reductases. The tree was inferred using PhyML with nine categories of rates. Numbers at nodes indicate bootstrap support over 60 from (top to bottom or left to right) maximum likelihood, as determined by PhyML, and weighted neighbor joining. Analysis used 379 characters, the gamma-shaped parameter 1.58 and proportion of invariable sites 0.006. Photosynthetic organisms are typed in bold.

(Kredich, 1996; Bogdanova and Hell, 1997; Droux et al., 1998). The complex, however, does not facilitate the reaction by substrate channeling, because the OASTL in complex is not functional and the synthesis of cysteine is accomplished by a free OASTL (Droux et al., 1998; Wirtz et al., 2001). SAT is a hexamer of 29 kDa subunits. It is a member of the hexapeptide acyltransferase family of enzymes, folding in a characteristic left-handed parallel β-helix domain (Olsen et al., 2004). SAT is an important regulatory step in sulfate assimilation as it undergoes a feedback regulation by cysteine. Its product, OAS, directly, or after spontaneous isomerization to N-acetylserine, is a potent transcriptional regulator of sulfate assimilation genes (Kredich, 1996; Leustek et al., 2000). Since in plants cysteine synthesis takes place in all three compartments capable of protein synthesis isoforms of SAT and OASTL are found in the plastids, cytosol, and mitochondria. Consequently, SAT is encoded in multigene families of 3–5 members (Kawashima et al., 2005).

However, not all organisms assimilating sulfate possess a functional SAT. The amino acid acceptor of sulfide in budding yeast is O-acetylhomoserine, which is synthesized by homoserine transacetylase (Met2; Born et al., 2000). Although catalyzing a very similar reaction, there is no sequence similarity between Met2 and SAT. Surprisingly however, despite there being no SAT homologue encoded in the *S. cerevisiae* genome, SAT activity was clearly detected *in vitro* (Takagi et al., 2003). Since expression of *E. coli* SAT, but not OASTL, was capable of reverting cysteine auxotrophs it seems that the physiological role of this activity (if it is not an artifact) is other than in cysteine synthesis (Takagi et al., 2003). SAT is, however, found in *Schizosaccharomyces pombe*, which thus seems to possess both pathways of cysteine synthesis, and in the parasites *Entamoeba histolytica* and *E. dispar*. The biochemical and molecular properties of SAT and OASTL are described in detail in Chapter 4.

2. Phylogenetic Analysis

All plastid containing organisms are contained in a well-supported clade, distinct from the cyanobacteria (Fig. 9). SAT in these organisms can be confirmed to be of host, rather than cyanobacterial endosymbiont origin. This clade, however, also contains proteobacteria. The α-proteobacteria do not branch at the base of the eukaryotes, most of the internal branches of this clade are not well supported and the monophyly of streptophytes is not recovered. A mitochondrial origin, and subsequent re-targeting to plastids in lineages that obtained plastids, cannot be, therefore, ruled out for eukaryotes.

Outside of the main eukaryotic clade, the non-photosynthetic protists *Entamoeba* and *Trypanosoma* show a specific relationship to δ-proteobacteria. Neither group is well sampled, however, and this relationship may not hold with more taxa.

G. O-Acetylserine (thiol)lyase

1. Biochemical Properties

O-acetylserine (thiol)lyase (EC 4.2.99.8) catalyzes the formation of cysteine from OAS and sulfide. The enzyme belongs to the family of β-replacement enzymes utilizing pyridoxal-5′-phosphate as cofactor. OASTL is a homodimer of 35 kDa subunits found inactive in the cysteine synthase complex modulating the activity of SAT or, in the active form, as a free enzyme (Droux et al., 1998; Wirtz et al., 2001). In bacteria, OASTL is encoded by two genes *cysK* and *cysM*. Whereas CysK is specific for *O*-acetylserine and is predominantly expressed under aerobic growth, CysM has a broader substrate specificity comprising many different β-substituted amino acids (Maier, 2003) and is highly expressed at anaerobic conditions and in presence of thiosulfate (Kredich, 1996). In plants and algae isoforms of OASTL, similar to SAT, are localized in the cytosol, mitochondria, and plastids in all organs (see Chapter 4). Accordingly, at least three genes are present in genomes of higher plants (Jost et al., 2000). Since sulfate assimilation is specific to plastids, the mitochondrial and cytosolic SAT and OASTL may have alternative functions. Indeed, the primary function for the mitochondrial OASTL was proposed to be detoxification of cyanide, acting as a β-cyanoalanine synthase (Hatzfeld et al., 2000b; Maruyama et al., 2000; Warrilow and Hawkesford, 2000).

In yeast, Metazoa, and protozoan parasites, cysteine is synthesized by transsulfuration from homocysteine in two steps catalyzed by cystathionine β-synthase (CBS) and cystathionine γ-lyase

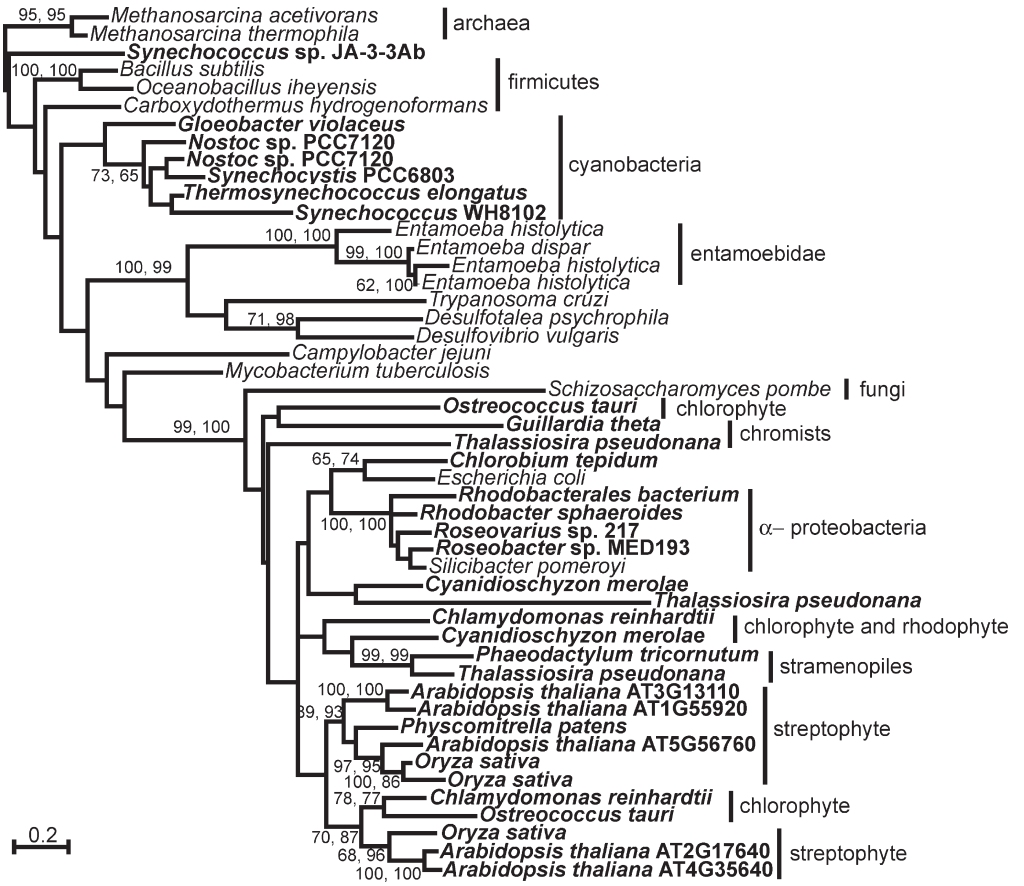

Fig. 9. Protein maximum likelihood phylogeny for serine acetyltransferases. The tree was inferred using PhyML with nine categories of rates. Numbers at nodes indicate bootstrap support over 60 from (top to bottom or left to right) maximum likelihood, as determined by PhyML, and weighted neighbor joining. Analysis used 157 characters, the gamma-shaped parameter 1.125 and proportion of invariable sites 0.055. Photosynthetic organisms are typed in bold.

(Ono et al., 1999; Nozaki et al., 2005). Homocysteine can be produced from methionine, but in yeast it is the primary product of sulfate assimilation formed by O-acetylhomoserine thiollyase (OAHSTL; Ono et al., 1999). OAHSTL is very similar to OASTL and is also able to catalyze cysteine synthesis from OAS and sulfide. But since the SAT homologues in yeast are unable to synthesize OAS, the OAHSTL does not play any direct role in Cys synthesis (Takagi et al., 2003). CBS is therefore found in most eukaryotes, including all fungi, and all metazoa except nematodes. It is however, lost in the Viridaeplantae lineage. Surprisingly, rather than CBS, OASTL were found in the nematodes *Caenorhabditis elegans* and *C. briggsae* and also in *Dictyostelium discoideum*. No other genes of sulfate assimilation are present in these genomes. On the other hand, genes encoding ATPS, SAT and OASTL were found in the parasite *Entamoeba histolytica*, which is not capable of sulfate assimilation since it is lacking the reducing enzymes (P)APR and SiR. Since it is impossible to distinguish between CBS and OASTL at the primary sequence level, both were included in the phylogenetic analysis. A more detailed description of cysteine synthesis and its regulation is given in Chapter 4.

2. Phylogenetic Analysis

OASTL proteins of Viridaeplantae and rhodophytes are all related (Fig. 10). However they are part of a large and unsupported clade that also contains many prokaryotes. A cyanobacterial

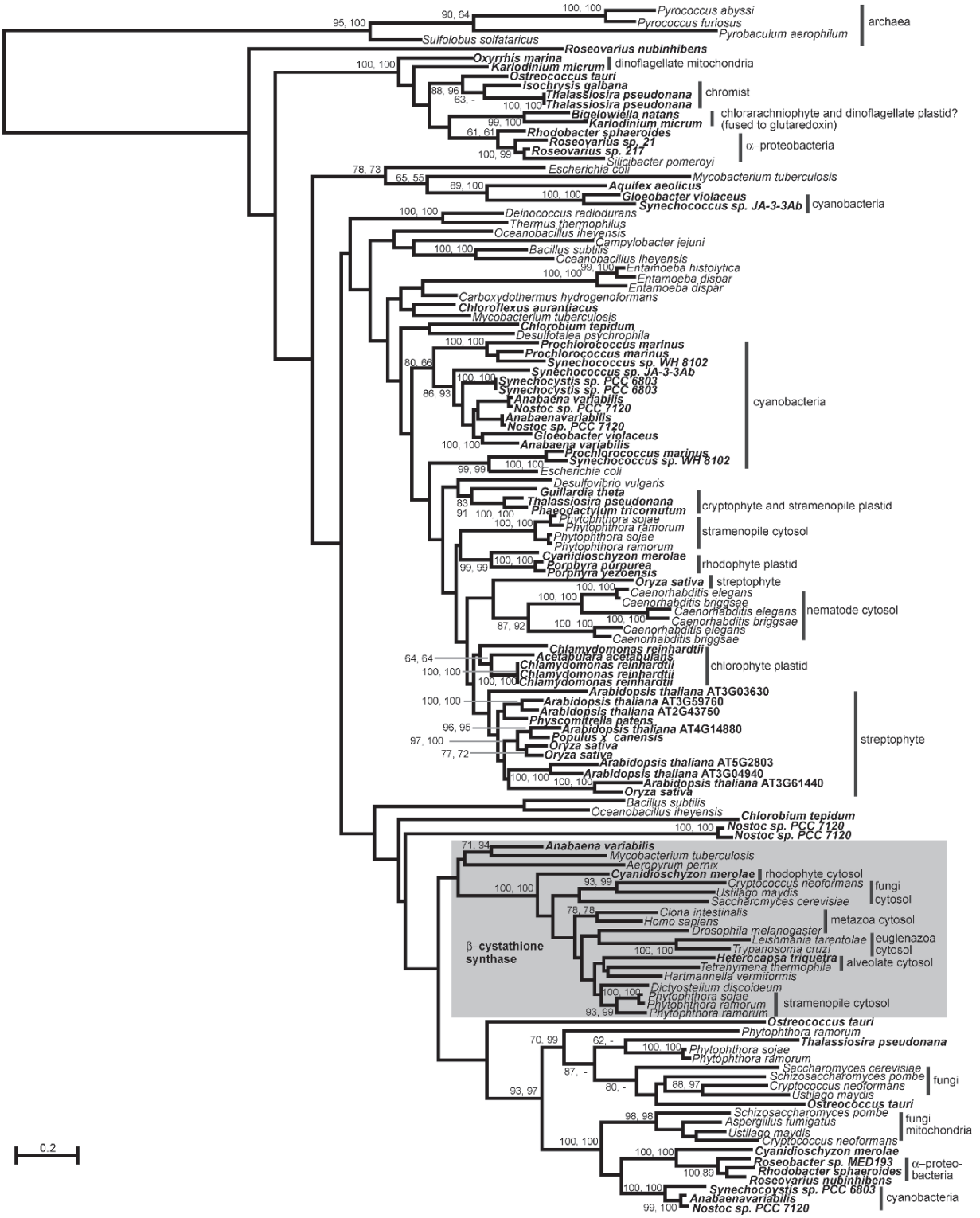

Fig. 10. Protein maximum likelihood phylogeny for O-acetylserine (thiol)lyases and cystathionine β-synthases. The tree was inferred using PhyML with nine categories of rates. Numbers at nodes indicate bootstrap support over 60 from (top to bottom or left to right) maximum likelihood, as determined by PhyML, and weighted neighbor joining. Analysis used 359 characters, the gamma-shaped parameter 1.088 and proportion of invariable sites 0.006. Photosynthetic organisms are typed in bold.

origin seems most likely since the bulk of non-photosynthetic eukaryotes are in other, better supported, clades. Within this clade, quite separate to other Metazoa, are the OASTL homologues encoded by nematodes. These are within the plastid-lineage and, although their position in unsupported, an LGT origin cannot be ruled out. The only other non-photosynthetic eukaryotes are Phytophtora, these proteins do not show any supported relationship to their photosynthetic stramenopile relatives and so the origin of this isoform is also unclear.

There is an entirely separate and highly supported clade consisting of both auto- and heterotrophic chromalveolates as well as the tiny green alga *Ostreococcus* and the chlorarchniophyte *B. natans*. There is no experimental information for any of these isoforms and so they may have a novel function. Interestingly two, entirely unrelated members of this clade, the tertiary plastid-containing dinoflagellate *K. micrum* and also B. *natans* have a glutaredoxin moiety fused to the C terminus of the molecule.

The eukaryotic clade of CBS is well supported although its position within the tree is not, and so the root of the divergence cannot be known. It is however present in very basal eukaryotes such as the Lobosea and Mycetazoa.

The final well-supported clade consists primarily of stramenopiles and fungi, but also α-proteobacteria and a few cyanobacteria, the latter of which are quite separate from the larger cluster of their relatives. Again there is no specific relationship between the eukaryotes and the α-proteobacteria but, given that there is some evidence that this isoform is mitochondrial in fungi, an endosymbiont origin remains a possibility.

IV. Protein Complexes and Fusions

Sulfate assimilation seems to be especially prone to tinkering with domain and protein fusions. Its central role in core metabolism across the kingdoms of life is also demonstrated by ability of structurally unrelated proteins to catalyze the same reaction, indicating parallel evolution in different lineages. This was clearly demonstrated during the discussion of individual sulfate assimilation proteins (see section III.B–D). Altogether at least three unrelated proteins are capable of the adenylation of sulfate, three major APS/PAPS reducing enzymes exist, and five completely different enzymes reduce sulfite to sulfide. On top of this, many subtle variations in domain structure and fusions result in a great variety of sulfur assimilation genes and enzymes. The greatest part of this subtle variation was unraveled only very recently due to the progress in algal genomics. It was long known that ATPS is often fused with APS kinase, as in animal PAPS synthetase or in the rhizobial NodP/Q system (see section III.B). The finding of ATPS fusion with inorganic pyrophosphatase in several algae is, however, a very interesting variation in attempt to increase efficiency of this enzyme. A very special case is the fusion between ATPS and APR in the dinoflagellate *Heterocapsa triquetra*. This protein likely catalyzes the reduction of sulfate to sulfite, since no other paralogues of ATPS or APR have been detected to date. This protein fusion supports the idea that in other organisms, such as plants, the sulfate assimilation enzymes form a multienzyme complex. Clearly, cysteine synthase is such a complex (see Chapter 4), but it is possible that also ATPS, APR, and SiR form a complex, preferably associated with thylakoid membranes. Association of sulfate reducing enzymes, perhaps including also cysteine synthase, would increase the efficiency of the individual reactions by substrate channeling. This is particularly important for ATPS which is very inefficient in the forward reaction. In addition the channeling would prevent the escape of the toxic intermediates sulfite and/or sulfide as well as facilitating the transfer of reducing equivalents from photosynthesis. Indeed, immunogold localization of APR in several species of *Flaveria* plants showed a frequent association of the label with the thylakoids (Koprivova et al., 2001). On the other hand, by Western blot analysis APR was shown to be present in stroma fraction of pea chloroplasts (Prior et al., 1999). The sulfate assimilation metabolon, however, remains to be demonstrated.

The APR found in the green and red algae (except *C. merolae*) and plants is clearly a fusion between an APS/PAPS reductase and a thioredoxin. The APR from *Euglena*, which is otherwise similar, and specifically related to the APR from chlorophytes, does not contain the thioredoxin extension. Although the sequence of the C-terminal domain is more related to thioredoxin, the domain clearly functions as a glutaredoxin which is compatible with GSH being the

most probable *in vivo* electron donor (Bick et al., 1998; Kopriva and Koprivova, 2004). The fusion between ancestral APR and thioredoxin leading to the 'plant-type' APR thus brought the enzyme and its cofactor together on one polypeptide. The event most likely occurred before the divergence of red and green algae, with subsequent loss of the domain in the lineage leading to *C. merolae* (although another Cyanidale, *G. sulfuraria*, retained the fusion), and independently after the secondary endosymbiosis event gave rise to *Euglena*. Another piece into the fusion mosaic is the novel form of OASTL from *K. micrum* and *B. natans*, which are fused at their C-terminus to glutaredoxin (Patron et al., 2006). Since cysteine synthesis does not require electron transport, the function of this fusion is unclear. It might be however be involved in redox regulation of the enzyme (Patron et al., 2006).

V. Conclusions

A. Origin of Plant Sulfate Assimilation

With a single exception in *Euglena*, sulfate assimilation is localized in plastids. As with other plastidic pathways, e.g., the Calvin cycle (Martin and Schnarrenberger, 1997), or heme biosynthesis (Obornik and Green, 2005), cyanobacterial origin of the genes involved can be anticipated. However, surprisingly, most of the genes of sulfate assimilation cannot be related to cyanobacteria. The only genes for which symbiotic origin can be proposed with confidence are sulfite reductase and OASTL. Serine acetyltransferase, on the other hand, seems to be derived from the host. For other genes the evolutionary history cannot be reliably reconstructed. Great difficulty by interpretation of the data is caused by a frequent lack of monophyly of well defined taxa, e.g., cyanobacteria in ATPS and PAPS reductase trees. Plant APS reductase is most closely related to several γ-proteobacteria but also to a cyanobacterium *Plectonema*. Existence of two distinct isoforms of APR in several lower plants shows that at some point in their evolution plants possessed both genes, but it is not possible to assign one of them to the host and the other to symbionts with confidence. Interestingly, ATPS is probably the first plant gene identified which is more related to Metazoa than to Chlorophytes. The origin of plant sulfate assimilation thus probably will remain an unsolved question.

B. Sulfate Assimilation in Secondary/Tertiary Symbionts

Inclusion of the secondary and tertiary symbionts in the analysis revealed a great number of unique genes and gene variants. Among them the fusions of APS kinase, ATPS, and PPase and of OASTL with glutaredoxin, as well as the new form of APS reductase are of great interest and potential use for improving sulfur use efficiency of crop plants. As expected, the algae possessed genes from different origins, reflecting their evolutionary history. Often both gene copies from the host and the symbionts are retained and possibly the encoded proteins are differentially localized. Our understanding of sulfate assimilation in these species, although improved by this phylogenetic analysis, still requires biochemical studies of the organisms and the respective enzymes.

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References

Abola AP, Willits MG, Wang RC and Long SR (1999) Reduction of adenosine-5′-phosphosulfate instead of 3′-phosphoadenosine-5′-phosphosulfate in cysteine biosynthesis by *Rhizobium meliloti* and other members of the family *Rhizobiaceae*. J Bacteriol 181: 5280–5287

Archibald JM and Keeling PJ (2002) Recycled plastids: a green movement in eukaryotic evolution. Trends Genet 18: 577–584

Archibald JM, Rogers MB, Toop M, Ishida K and Keeling PJ (2003) Lateral gene transfer and the evolution of plastid-targeted proteins in the secondary plastid-containing alga *Bigelowiella natans*. Proc Natl Acad Sci USA 100: 7678–7683

Armbrust EV et al. (2004) The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. Science 306: 79–86

Bakker-Grunwald T and Geilhorn B (1992) Sulfate metabolism in *Entamoeba histolytica*. Mol Biochem Parasitol 53: 71–78

Berndt C, Lillig CH, Wollenberg M, Bill E, Mansilla MC, de Mendoza D, Seidler A and Schwenn JD (2004) Characterization and reconstitution of a 4Fe–4S adenylyl sulfate/phosphoadenylyl sulfate reductase from *Bacillus subtilis*. J Biol Chem 279: 7850–7855

Bhattacharya D, Yoon HS and Hackett JD (2004) Photosynthetic eukaryotes unite: endosymbiosis connects the dots. Bioessays 26: 50–60

Bick JA, Aslund F, Chen Y and Leustek T (1998) Glutaredoxin function for the carboxyl-terminal domain of the 'plant-type' 5′-adenylylsulfate reductase. Proc Natl Acad Sci USA 95: 8404–8409

Bick JA, Dennis JJ, Zylstra GJ, Nowack J and Leustek T (2000) Identification of a new class of 5′-adenylylsulfate (APS) reductases from sulfate-assimilating bacteria. J Bacteriol 182: 135–142

Bodyl A (2004) Evolutionary origin of a preprotein translocase in the periplastid membrane of complex plastids: a hypothesis. Plant Biol 6: 513–518

Bogdanova N and Hell R (1997) Cysteine synthesis in plants: protein–protein interactions of serine acetyltransferase from *Arabidopsis thaliana*. Plant J 11: 251–262

Bork C, Schwenn JD and Hell R (1998) Isolation and characterization of a gene for assimilatory sulfite reductase from *Arabidopsis thaliana*. Gene 212: 147–153

Born TL, Franklin M and Blanchard JS (2000) Enzyme-catalyzed acylation of homoserine: mechanistic characterization of the *Haemophilus influenzae met2*-encoded homoserine transacetylase. Biochemistry 39: 8556–8564

Brunold C (1990) Reduction of sulfate to sulfide. In: Rennenberg H, Brunold C, De Kok LJ and Stulen I (eds) Sulphur nutrition and sulphur assimilation in higher plants, pp 13–31. SPB Academic Publishing, The Hague, The Netherlands

Brunold C and Schiff JA (1976) Studies of sulfate utilization by algae.15. Enzymes of assimilatory sulfate reduction in *Euglena* and their cellular localization. Plant Physiol 57: 430–436

Brunold C and Suter M (1989) Localization of enzymes of assimilatory sulfate reduction in pea roots. Planta 179: 228–234

Buchner P, Takahashi H and Hawkesford MJ (2004) Plant sulphate transporters: co-ordination of uptake, intracellular and long-distance transport. J Exp Bot 55: 1765–1773

Carroll KS, Gao H, Chen H, Leary JA and Bertozzi CR (2005a) Investigation of the iron–sulfur cluster in *Mycobacterium tuberculosis* APS reductase: implications for substrate binding and catalysis. Biochemistry 44: 14647–14657

Carroll KS, Gao H, Chen H, Stout CD, Leary JA and Bertozzi CR (2005b) A conserved mechanism for sulfonucleotide reduction. PLoS Biol 3: e250

Cavalier-Smith T (2002) Chloroplast evolution: secondary symbiogenesis and multiple losses. Curr Biol 12: R62–R64

Cavalier-Smith T (2003) Genomic reduction and evolution of novel genetic membranes and protein-targeting machinery in eukaryote–eukaryote chimeras (meta-algae). Philos Trans R Soc Lond B Biol Sci 358: 109–134

Cherest H, Davidian JC, Thomas D, Benes V, Ansorge W and Surdin-Kerjan Y (1997) Molecular characterization of two high affinity sulfate transporters in *Saccharomyces cerevisiae*. Genetics 145: 627–635

Chesnick JM, Kooistra W, Wellbrock U and Medlin LK (1997) Ribosomal RNA analysis indicates a benthic pennate diatom ancestry for the endosymbionts of the dinoflagellates *Peridinium foliaceum* and *Peridinium balticum* (Pyrrhophyta). J Euk Microbiol 44: 314–320

Chu KH, Qi J, Yu ZG and Anh V (2004) Origin and phylogeny of chloroplasts revealed by a simple correlation analysis of complete genomes. Mol Biol Evol 21: 200–206

Coleman ML, Sullivan MB, Martiny AC, Steglich C, Barry K, DeLong EF and Chisholm SW (2006) Genomic islands and the ecology and evolution of *Prochlorococcus*. Science 311: 1768–1770

Crane BR, Siegel LM and Getzoff ED (1995) Sulfite reductase structure at 1.6 A: evolution and catalysis for reduction of inorganic anions. Science 270: 59–67

Dahl C, Kredich NM, Deutzmann R and Trüper HG (1993) Dissimilatory sulphite reductase from *Archaeoglobus fulgidus*: physico-chemical properties of the enzyme and cloning, sequencing and analysis of the reductase genes. J Gen Microbiol 139: 1817–1828

Delsuc F, Brinkmann H and Philippe H (2005) Phylogenomics and the reconstruction of the tree of life. Nat Rev Genet 6: 361–375

Droux M, Ruffet ML, Douce R and Job D (1998) Interactions between serine acetyltransferase and O-acetylserine (thiol) lyase in higher plants – structural and kinetic properties of the free and bound enzymes. Eur J Biochem 255: 235–245

Fast NM, Kissinger JC, Roos DS and Keeling PJ (2001) Nuclear-encoded, plastid-targeted genes suggest a single common origin for apicomplexan and dinoflagellate plastids. Mol Biol Evol 18: 418–426

Fleischmann RD et al. (1995) Whole-genome random sequencing and assembly of *Haemophilus influenzae Rd*. Science 269: 496–512

Folch-Mallol JL, Marroqui S, Sousa C, Manyani H, Lopez-Lara IM, van der Drift KM, Haverkamp J, Quinto C, Gil-Serrano A, Thomas-Oates J, Spaink HP and Megias M (1996) Characterization of *Rhizobium tropici* CIAT899 nodulation factors: the role of nodH and nodPQ genes in their sulfation. Mol Plant Microbe Interact 9: 151–163

Fritz G, Roth A, Schiffer A, Buchert T, Bourenkov G, Bartunik HD, Huber H, Stetter KO, Kroneck PM and Ermler U (2002) Structure of adenylylsulfate reductase from the hyperthermophilic *Archaeoglobus fulgidus* at 1.6-A resolution. Proc Natl Acad Sci USA 99: 1836–1841

Fulton JD and Grant PT (1956) The sulphur requirements of the erythrocytic form of *Plasmodium knowlesi*. Biochem J 63: 274–282

Gao Y, Schofield OM and Leustek T (2000) Characterization of sulfate assimilation in marine algae focusing on the enzyme 5′-adenylylsulfate reductase. Plant Physiol 123: 1087–1096

Goff SA et al. (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). Science 296: 92–100

Grossman AR (2005) Paths toward algal genomics. Plant Physiol 137: 410–427

Gutierrez-Marcos JF, Roberts MA, Campbell EI and Wray JL (1996) Three members of a novel small gene-family from *Arabidopsis thaliana* able to complement functionally an *Escherichia coli* mutant defective in PAPS reductase activity encode proteins with a thioredoxin-like domain and"APS reductase" activity. Proc Natl Acad Sci USA 93: 13377–13382

Hansen TA (1994) Metabolism of sulfate-reducing prokaryotes. Antonie Van Leeuwenhoek 66: 165–185

Harper JT and Keeling PJ (2003) Nucleus-encoded, plastid-targeted glyceraldehyde-3-phosphate dehydrogenase (GAPDH) indicates a single origin for chromalveolate plastids. Mol Biol Evol 20: 1730–1735

Hatzfeld Y, Lee S, Lee M, Leustek T and Saito K (2000a) Functional characterization of a gene encoding a fourth ATP sulfurylase isoform from *Arabidopsis thaliana*. Gene 248: 51–58

Hatzfeld Y, Maruyama A, Schmidt A, Noji M, Ishizawa K and Saito K (2000b) beta-cyanoalanine synthase is a mitochondrial cysteine synthase-like protein in spinach and Arabidopsis. Plant Physiol 123: 1163–1171

Hawkesford MJ and Wray JL (2000) Molecular genetics of sulphate assimilation. Adv Bot Res 33: 159–223

Hawkesford MJ, Davidian J-C and Grignon C (1993) Sulfate proton cotransport in plasma vesicles isolated from roots of *Brassica napus* L. Increased transport in membranes isolated from sulphur starved plants. Planta 190: 297–304

Honke K and Taniguchi N (2002) Sulfotransferases and sulfated oligosaccharides. Med Res Rev 22: 637–654

Imhoff JF (1992) Taxonomy, phylogeny and general ecology of anoxygenic phototrophic bacteria. In: Carr NG and Mann NH (eds) Biotechnology handbook: photosynthetic prokaryotes, pp 53–92. Plenum Press, London, New York

Inagaki Y, Doolittle WF, Baldauf SL and Roger AJ (2002) Lateral transfer of an EF-1 alpha gene: Origin and evolution of the large subunit of ATP sulfurylase in eubacteria. Curr Biol 12: 772–776

Jost R, Berkowitz O, Wirtz M, Hopkins L, Hawkesford MJ and Hell R (2000) Genomic and functional characterization of the oas gene family encoding O-acetylserine (thiol) lyases, enzymes catalysing the final step in cysteine biosynthesis in *Arabidopsis thaliana*. Gene 253: 237–247

Kanno N, Nagahaisa E, Sato M and Sato Y (1996) Adenosine 5′-phosphosulfate sulfotransferase from the marine macroalga *Porphyra yezoensis* Ueda (Rhodophyta): stabilization, purification, and properties. Planta 198: 440–446

Kappler U and Dahl C (2001) Enzymology and molecular biology of prokaryotic sulfite oxidation. FEMS Microbiol Lett 203: 1–9

Kawashima CG, Berkowitz O, Hell R, Noji M and Saito K (2005) Characterization and expression analysis of a serine acetyltransferase gene family involved in a key step of the sulfur assimilation pathway in Arabidopsis. Plant Physiol 137: 220–230

Kertesz MA (2001) Bacterial transporters for sulfate and organosulfur compounds. Res Microbiol 152: 279–290

Kim SK, Rahman A, Bick JA, Conover RC, Johnson MK, Mason JT, Hirasawa M, Leustek T and Knaff DB (2004) Properties of the cysteine residues and iron–sulfur cluster of the assimilatory 5′-adenylyl sulfate reductase from *Pseudomonas aeruginosa*. Biochemistry 43: 13478–13486

Klonus D, Hofgen R, Willmitzer L and Riesmeier JW (1994) Isolation and characterization of two cDNA clones encoding ATP-sulfurylases from potato by complementation of a yeast mutant. Plant J 6: 105–112

Kobayashi K and Yoshimoto A (1982) Studies on yeast sulfite reductase. IV. Structure and steady-state kinetics. Biochim Biophys Acta 705: 348–356

Kopriva S (2006) Regulation of sulfate assimilation in Arabidopsis and beyond. Ann Bot 97: 479–495

Kopriva S and Koprivova A (2004) Plant adenosine 5′-phosphosulphate reductase: the past, the present, and the future. J Exp Bot 55: 1775–1783

Kopriva S and Rennenberg H (2004) Control of sulphate assimilation and glutathione synthesis: interaction with N and C metabolism. J Exp Bot 55: 1831–1842

Kopriva S, Büchert T, Fritz G, Weber M, Suter M, Benda R, Schaller J, Feller U, Schürmann P, Schünemann V, Trautwein AX, Kroneck PMH and Brunold C (2001) Plant adenosine 5′-phosphosulfate reductase is a novel iron–sulfur protein. J Biol Chem 276: 42881–42886

Kopriva S, Büchert T, Fritz G, Suter M, Benda R, Schünemann V, Koprivova A, Schürmann P, Trautwein AX, Kroneck PMH and Brunold C (2002). The presence of an iron–sulfur cluster in adenosine 5′-phosphosulfate reductase separates organisms utilising adenosine 5′-phosphosulfate and phosphoadenosine 5′-phosphosulfate for sulfate assimilation. J Biol Chem 277: 21786–21791

Kopriva S, Fritzemeier K, Wiedemann G and Reski R (2007) The putative moss 3′phosphoadenosine 5′phosphosulfate reductase is a novel form of adenosine 5′phosphosulfate reductase without iron sulfur cluster. J Biol Chem 282: 22930–22938

Koprivova A, Melzer M, von Ballmoos P, Mandel T, Brunold C and Kopriva S (2001) Assimilatory sulfate reduction in C_3, C_3–C_4, and C_4 species of *Flaveria*. Plant Physiol 127: 543–550

Koprivova A, Meyer A, Schween G, Herschbach C, Reski R and Kopriva S (2002) Functional knockout of the adenosine 5′phosphosulfate reductase gene in *Physcomitrella patens* revives an old route of sulfate assimilation. J Biol Chem 277: 32195–32201

Kredich NM (1971) Regulation of L-cysteine biosynthesis in *Salmonella typhimurium*. J Biol Chem 246: 3474–3484

Kredich NM (1996) Biosynthesis of cysteine. In: Neidhart FC et al. (eds) *Escherichia coli* and *Salmonella typhimurium*. Cellular and molecular biology, pp 514–527. ASM Press, Washington, DC

Krueger RJ and Siegel LM (1982) Spinach siroheme enzymes: isolation and characterization of ferredoxin-sulfite reductase and comparison of properties with ferredoxin-nitrite reductase. Biochemistry 21: 2892–2904

Laudenbach DE and Grossman AR (1991) Characterization and mutagenesis of sulfur-regulated genes in a cyanobacterium: evidence for function in sulfate transport. J Bacteriol 173: 2739–2750

Leipe DD, Koonin EV and Aravind L (2003) Evolution and classification of P-loop kinases and related proteins. J Mol Biol 333: 781–815

Leustek T, Martin MN, Bick JA and Davies JP (2000) Pathways and regulation of sulfur metabolism revealed through molecular and genetic studies. Annu Rev Plant Physiol Plant Mol Biol 51: 141–165

Leyh TS, Taylor JC and Markham GD (1988) The sulfate activation locus of *Escherichia coli* K12: cloning, genetic, and enzymatic characterization. J Biol Chem 263: 2409–2416

Li JY and Schiff JA (1991) Purification and properties of adenosine 5′-phosphosulphate sulphotransferase from *Euglena*. Biochem J 274: 355–360

Lillig CH, Prior A, Schwenn JD, Åslund F, Ritz D, Vlamis-Gardikas A and Holmgren A (1999) New thioredoxins and glutaredoxins as electron donors of 3′-phosphoadenylylsulfate reductase. J Biol Chem 274: 7695–7698

Lindell D, Sullivan MB, Johnson ZI, Tolonen AC, Rohwer F and Chisholm SW (2004) Transfer of photosynthesis genes to and from *Prochlorococcus* viruses. Proc Natl Acad Sci USA 101: 11013–11018

Lunn J, Droux M, Martin J and Douce R (1990) Localization of ATP sulfurylase and O-acetylserine (thiol)lyase in spinach leaves. Plant Physiol 94: 1345–1352

MacRae IJ, Rose AB and Segel IH (1998) Adenosine 5′-phosphosulfate kinase from *Penicillium chrysogenum* site-directed mutagenesis at putative phosphoryl-accepting and ATP P-loop residues. J Biol Chem 273: 28583–28589

MacRae IJ, Segel IH and Fisher AJ (2000) Crystal structure of adenosine 5′-phosphosulfate kinase from *Penicillium chrysogenum*. Biochemistry 39: 1613–1621

MacRae IJ, Segel IH and Fisher AJ (2001) Crystal structure of ATP sulfurylase from *Penicillium chrysogenum*: insights into the allosteric regulation of sulfate assimilation. Biochemistry 40: 6795–6804

Maier TH (2003) Semisynthetic production of unnatural L-alpha-amino acids by metabolic engineering of the cysteine-biosynthetic pathway. Nat Biotechnol 21: 422–427

Markovich D (2001) Physiological roles and regulation of mammalian sulfate transporters. Physiol Rev 81: 1499–1533

Martin W and Schnarrenberger C (1997) The evolution of the Calvin cycle from prokaryotic to eukaryotic chromosomes: a case study of functional redundancy in ancient pathways through endosymbiosis. Curr Genet 32: 1–18

Martin W, Rujan T, Richly E, Hansen A, Cornelsen S, Lins T, Leister D, Stoebe B, Hasegawa M and Penny D (2002) Evolutionary analysis Arabidopsis, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. Proc Natl Acad Sci USA 99: 12246–12251

Maruyama A, Ishizawa K and Takagi T (2000) Purification and characterization of beta-cyanoalanine synthase and cysteine synthases from potato tubers: are beta-cyanoalanine synthase and mitochondrial cysteine synthase same enzyme? Plant Cell Physiol 41: 200–208

Matsuzaki M, et al. (2004) Genome sequence of the ultrasmall unicellular red alga *Cyanidioschyzon merolae* 10D. Nature 428: 653–657

McFadden GI (2001) Primary and secondary endosymbiosis and the origin of plastids. J Phycol 37: 951–959

Mougous JD, Senaratne RH, Petzold CJ, Jain M, Lee DH, Schelle MW, Leavell MD, Cox JS, Leary JA, Riley LW and Bertozzi CR (2006) A sulfated metabolite produced by stf3 negatively regulates the virulence of *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA 103: 4258–4263

Murillo M and Leustek T (1995) Adenosine-5′-triphosphate-sulfurylase from *Arabidopsis thaliana* and *Escherichia coli* are functionally equivalent but structurally and kinetically divergent: nucleotide sequence of two adenosine-5′-triphosphate-sulfurylase cDNAs from *Arabidopsis thaliana* and analysis of a recombinant enzyme. Arch Biochem Biophys 323: 195–204

Nakayama M, Akashi T and Hase T (2000) Plant sulfite reductase: molecular structure, catalytic function and interaction with ferredoxin. J Inorg Biochem 82: 27–32

Nelissen B, Van de Peer Y, Wilmotte A, and De Wachter R (1995) An early origin of plastids within the cyanobacterial divergence is suggested by evolutionary trees based on complete 16S rRNA sequences. Mol Biol Evol 12: 1166–1173

Nozaki T, Ali V and Tokoro M (2005) Sulfur-containing amino acid metabolism in parasitic protozoa. Adv Parasitol 60: 1–99

Obornik M and Green BR (2005) Mosaic origin of the heme biosynthesis pathway in photosynthetic eukaryotes. Mol Biol Evol 22: 2343–2353

Olsen LR, Huang B, Vetting MW and Roderick SL (2004) Structure of serine acetyltransferase in complexes with

CoA and its cysteine feedback inhibitor. Biochemistry 43: 6013–6019

Ono BI, Hazu T, Yoshida S, Kawato T, Shinoda S, Brzvwczy J and Paszewski A (1999) Cysteine biosynthesis in *Saccharomyces cerevisiae*: a new outlook on pathway and regulation. Yeast 15: 1365–1375

Patron NJ, Rogers MB and Keeling PJ (2004) Gene replacement of fructose-1,6-bisphosphate aldolase supports the hypothesis of a single photosynthetic ancestor of chromalveolates. Eukaryot Cell 3: 1169–1175

Patron NJ, Waller RF, Archibald JM and Keeling PJ (2005) Complex protein targeting to dinoflagellate plastids. J Mol Biol 348: 1015–1024

Patron NJ, Waller RF and Keeling PJ (2006) A tertiary plastid uses genes from two endosymbionts. J Mol Biol 357: 1373–1382

Payne SH and Loomis WF (2006) Retention and loss of amino acid biosynthetic pathways based on analysis of whole-genome sequences. Eukaryot Cell 5: 272–276

Perez-Jimenez JR and Kerkhof LJ (2005) Phylogeography of sulfate-reducing bacteria among disturbed sediments, disclosed by analysis of the dissimilatory sulfite reductase genes (dsrAB). Appl Environ Microbiol 71: 1004–1011

Pierik AJ, Duyvis MG, van Helvoort JMLM, Wolbert RBG and Hagen WR (1992) The third subunit of desulfoviridin-type dissimilatory sulfite reductases. Eur J Biochem 205: 111–115

Prior A, Uhrig JF, Heins L, Wiesmann A, Lillig CH, Stoltze C, Soll J and Schwenn JD (1999) Structural and kinetic properties of adenylyl sulfate reductase from *Catharanthus roseus* cell cultures. Biochim Biophys Acta 1430: 25–38

Rausch T and Wachter A (2005) Sulfur metabolism: a versatile platform for launching defence operations. Trends Plant Sci 10: 503–509

Rodriguez-Ezpeleta N, Brinkmann H, Burey SC, Roure B, Burger G, Loffelhardt W, Bohnert HJ, Philippe H and Lang BF (2005) Monophyly of primary photosynthetic eukaryotes: green plants, red algae, and glaucophytes. Curr Biol 15: 1325–1330

Rosenthal E and Leustek T (1995) A multifunctional *Urechis caupo* protein, PAPS synthetase, has both ATP sulfurylase and APS kinase activities. Gene 165: 243–248

Rotte C and Leustek T (2000) Differential subcellular localization and expression of ATP sulfurylase and 5′-adenylylsulfate reductase during ontogenesis of Arabidopsis leaves indicates that cytosolic and plastid forms of ATP sulfurylase may have specialised functions. Plant Physiol 124: 715–724

Saidha T, Na SQ, Li JY and Schiff JA (1988) A sulphate metabolising centre in *Euglena* mitochondria. Biochem J 253: 533–539

Saito K (2004) Sulfur assimilatory metabolism. The long and smelling road. Plant Physiol 136: 2443–2450

Sakharkar KR, Dhar PK and Chow VT (2004) Genome reduction in prokaryotic obligatory intracellular parasites of humans: a comparative analysis. Int J Syst Evol Microbiol 54: 1937–1941

Sánchez Puerta MW, Bachvaroff TV and Delwiche CF (2005) The complete plastid genome sequence of the haptophyte *Emiliania huxleyi*: a comparison to other plastid genomes DNA Res 12: 151–156

Sato N, Nakayama M and Hase T (2001) The 70-kDa major DNA-compacting protein of the chloroplast nucleoid is sulfite reductase. FEBS Lett 487: 347–350

Schmidt A (1972) On the mechanism of photosynthetic sulfate reduction. An APS-sulfotransferase from *Chlorella*. Arch Microbiol 84: 77–86

Schmidt A (1975) Distribution of the APS-sulfotransferase activity among higher plants. Plant Sci Lett 5: 407–415

Schmidt A (1977) Assimilatory sulfate reduction via 3′-phosphoadenosine-5′-phosphosulfate (PAPS) and adenosine-5′-phosphosulfate (APS) in blue-green algae. FEMS Microbiol Lett 1: 137–140

Schmidt A and Jäger K (1992) Open questions about sulfur metabolism in plants. Annu Rev Plant Physiol Plant Mol Biol 43: 325–349

Schmidt A and Trüper HG (1977) Reduction of adenylylsulfate and 3′-phosphoadenylylsulfate in phototrophic bacteria. Experientia 33: 1008–1009

Schwartzbach SD, Osafune T and Loffelhardt W (1998) Protein import into cyanelles and complex chloroplasts. Plant Mol Biol 38: 247–263

Setya A, Murillo M and Leustek T (1996) Sulfate reduction in higher plants: molecular evidence for a novel 5′-adenylsulfate reductase. Proc Natl Acad Sci USA 93: 13383–13388

Sirko A, Hryniewicz M, Hulanicka D and Bock A (1990) Sulfate and thiosulfate transport in *Escherichia coli* K-12: nucleotide sequence and expression of the cysTWAM gene cluster. J Bacteriol 172: 3351–3357

Sperling D, Kappler U, Wynen A, Dahl C and Truper HG (1998) Dissimilatory ATP sulfurylase from the hyperthermophilic sulfate reducer *Archaeoglobus fulgidus* belongs to the group of homo-oligomeric ATP sulfurylases. FEMS Microbiol Lett 162: 257–264

Sulli C, Fang Z, Muchhal U and Schwartzbach SD (1999) Topology of *Euglena* chloroplast protein precursors within endoplasmic reticulum to Golgi to chloroplast transport vesicles. J Biol Chem 274: 457–463

Sullivan MB, Coleman ML, Weigele P, Rohwer F and Chisholm SW (2005) Three prochlorococcus cyanophage genomes: signature features and ecological interpretations. PLoS Biology 3: 790–806

Suter M, von Ballmoos P, Kopriva S, Op den Camp R, Schaller J, Kuhlemeier C, Schürmann P and Brunold C (2000) Adenosine 5′-phosphosulfate sulfotransferase and adenosine 5′-phosphosulfate reductase are identical enzymes. J Biol Chem 275: 930–936

Stafleu FA, Bonner CEB, McVaugh R, Meikle RD, Rollins RC, Ross R and Voss EG (eds) (1972) International Code of Botanical Nomenclature. A. Oosthoek, Utrecht, The Netherlands

Stahl DA, Fishbain S, Klein M, Baker BJ and Wagner M (2002) Origins and diversification of sulfate-respiring microorganisms. Antonie Van Leeuwenhoek 81: 189–195

Stipanuk MH (2004) Sulfur amino acid metabolism: pathways for production and removal of homocysteine and cysteine. Annu Rev Nutr 24: 539–577

Takagi H, Yoshioka K, Awano N, Nakamori S and Ono B (2003) Role of *Saccharomyces cerevisiae* serine O-acetyltransferase in cysteine biosynthesis. FEMS Microbiol Lett 218: 291–297

The Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408: 796–815

Tsang ML, Goldschmidt EE and Schiff JA (1971) Adenosine-5′-phosphosulfate (APS^{35}) as an intermediate in the conversion of adenosine-3′-phosphate-5′-phosphosulfate ($PAPS^{35}$) to acid volatile radioactivity. Plant Physiol 47 Suppl 20

Ullrich TC, Blaesse M and Huber R (2001) Crystal structure of ATP sulfurylase from *Saccharomyces cerevisiae*, a key enzyme in sulfate activation. EMBO J 20: 316–329

Van de Kamp M, Schuurs TA, Vos A, Van der Lende TR, Konings WN and Driessen AJM (2000) Sulfur regulation of the sulfate transporter genes sutA and sutB in *Penicillium chrysogenum*. Appl Environ Microbiol 66: 4536–4538

van Dooren GG, Schwartzbach SD, Osafune T and McFadden GI (2001) Translocation of proteins across the multiple membranes of complex plastids. Biochim Biophys Acta 1541: 34–53

Varin L, Marsolais F, Richard M and Rouleau M (1997) Sulfation and sulfotransferases 6: biochemistry and molecular biology of plant sulfotransferases. FASEB J 11: 517–525

Venkatachalam KV (2003) Human 3′-phosphoadenosine 5′-phosphosulfate (PAPS) synthase: biochemistry, molecular biology and genetic deficiency. IUBMB Life 55: 1–11

Waller RF, Patron NJ and Keeling PJ (2006) Phylogenetic history of plastid-targeted proteins in the peridinin-containing dinoflagellate *Heterocapsa triquetra*. Int J Syst Evol Microbiol 56: 1439–1447

Waller RF, Reed MB, Cowman AF and McFadden GI (2000) Protein trafficking to the plastid of *Plasmodium falciparum* is via the secretory pathway. EMBO J 19: 1794–1802

Warrilow AGS and Hawkesford MJ (2000) Cysteine synthase (O-acetylserine (thiol) lyase) substrate specificities classify the mitochondrial isoform as a cyanoalanine synthase. J Exp Bot 51: 985–993

Waterbury JB (2001) The cyanobacteria—isolation, purification, and identification. In: Dworkin M et al. (eds) The prokaryotes: an evolving electronic resource for the microbiological community, 3rd edition, release 3.7, November 2, 2001, Springer, New York, http://link.springer-ny.com/link/service/books/10125/

Weber M, Suter M, Brunold C and Kopriva S (2000) Sulfate assimilation in higher plants: characterization of a stable intermediate in the adenosine 5′-phosphosulfate reductase reaction. Eur J Biochem 267: 3647–3653

Williams SJ, Senaratne RH, Mougous JD, Riley LW and Bertozzi CR (2002) 5′-Adenosinephosphosulfate lies at a metabolic branch point in mycobacteria. J Biol Chem 277: 32606–32615

Wirtz M, Berkowitz O, Droux M and Hell R (2001) The cysteine synthase complex from plants. Mitochondrial serine acetyltransferase from *Arabidopsis thaliana* carries a bifunctional domain for catalysis and protein–protein interaction. Eur J Biochem 268: 686–693

Wirtz M, Droux M and Hell R (2004) O-acetylserine (thiol) lyase: an enigmatic enzyme of plant cysteine biosynthesis revisited in *Arabidopsis thaliana*. J Exp Bot 55: 1785–1798

Yonekura-Sakakibara K, Ashikari T, Tanaka Y, Kusumi T and Hase T. (1998) Molecular characterization of tobacco sulfite reductase: enzyme purification, gene cloning, and gene expression analysis. J Biochem 124: 615–621

Yoon HS, Hackett JD, Pinto G and Bhattacharya D (2002a) The single, ancient origin of chromist plastids. Proc Natl Acad Sci USA 99: 15507–15512

Yoon HS, Hackett JD and Bhattacharya D (2002b) A single origin of the peridinin- and fucoxanthin-containing plastids in dinoflagellates through tertiary endosymbiosis. Proc Natl Acad Sci USA 99: 11724–11729

Yu J et al. (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). Science 296: 79–92

Yurkov VV and Beatty JT (1998). Aerobic anoxygenic phototrophic bacteria. Microbiol Molec Biol Rev 62: 695–724