Chapter 15

Inorganic Sulfur Compounds as Electron Donors in Purple Sulfur Bacteria

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Summary

Most anoxygenic phototrophic bacteria can use inorganic sulfur compounds (e.g. sulfide, elemental sulfur, polysulfides, thiosulfate, or sulfide) as electron donors for reductive carbon dioxide fixation during photolithoautotrophic growth. In these organisms, light energy is used to transfer electrons from sulfur compounds to the level of the more highly reducing electron carriers $NAD(P)^+$ and ferredoxin. In this chapter the sulfur oxidizing capabilities of the different groups of anoxygenic phototrophic bacteria are briefly

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summarized. This chapter then focuses on the pathways of sulfur compound oxidation in purple sulfur bacteria of the families *Chromatiaceae* and *Ectothiorhodospiraceae*. A variety of enzymes catalyzing sulfur oxidation reactions have been isolated from members of this group and *Allochromatium vinosum*, a representative of the *Chromatiaceae*, has been especially well characterized also on a molecular genetic level. In this organism intracellular sulfur globules are an obligate intermediate during the oxidation of thiosulfate and sulfide to sulfate. Thiosulfate oxidation is strictly dependent on the presence of three periplasmic Sox proteins encoded by the *soxBXA* and *soxYZ* genes. Sulfide oxidation does not appear to require the presence of Sox proteins. Flavocytochrome *c* is also not essential leaving sulfide:quinone oxidoreductase as the probably most important sulfide-oxidizing enzyme. Polysulfides are intermediates en route of sulfide to stored sulfur. Sulfur is deposited in the periplasm and present as long chains probably terminated by organic residues at one or both ends. The oxidation of stored sulfur is completely dependent on the proteins encoded in the *dsr* operon. These include siroamide-containing sulfite reductase (DsrAB), a transmembrane electron-transporting complex (DsrMKJOP) and a iron–sulfur flavoprotein with NADH:acceptor oxidoreductase activity (DsrL). The last step of reduced sulfur compound oxidation in purple sulfur bacteria is the oxidation of sulfite. This can occur either via the enzymes adenosine 5′-phosphosulfate (APS) reductase and ATP sulfurylase which are non-essential in *Alc. vinsoum* or via direct oxidation to sulfate. The nature of the enzyme catalyzing the latter step is still unresolved in purple sulfur bacteria.

I. Introduction

Anoxygenic phototrophic bacteria are generally not able to use water as an electron-donating substrate for photosynthetic CO_2 reduction. The common property of these bacteria is the ability to carry out light-dependent, (bacterio)chlorophyll-mediated processes, a property shared with cyanobacteria, prochlorophytes, algae and green plants. In contrast to the latter, reduced sulfur compounds, molecular hydrogen, reduced iron or simple organic molecules typically serve as photosynthetic electrons in anoxygenic phototrophic bacteria. Bacteriochlorophylls are present not only in facultatively and obligately anaerobic anoxygenic phototrophic bacteria but also in large numbers of bacterial species that are strictly dependent on energy generation by respiratory electron transport processes. These organisms are called aerobic phototrophic bacteria (Yurkov, 2006) or "aerobic bacteriochlorophyll-containing (ABC) bacteria (Imhoff and Hiraishi, 2005).

The utilization of reduced sulfur compounds as photosynthetic electron donors is – though to a different extent – common to almost all groups of phototrophic prokaryotes. Classical in this respect are the purple (families *Chromatiaceae* and *Ectothiorhodospiraceae*) and green sulfur bacteria (family *Chlorobiaceae*) all of which utilize reduced sulfur compounds as electron donors. A number of classical purple "nonsulfur" bacteria, some members of the filamentous anoxygenic phototrophs (also termed green gliding bacteria or green non-sulfur bacteria) of the family *Chloroflexaceae*, and a few representatives of the strictly anaerobic gram-positive Heliobacteria are also able to oxidize reduced sulfur compounds during photosynthesis. Even certain species of the cyanobacteria can perform anoxygenic photosynthesis at the expense of sulfide as electron donor (rf Chapter Hauska/Shahak). Photoautotrophic growth with sulfur compounds has so far not been described for any of the ABC bacteria.

One purpose of this chapter is to briefly introduce researchers not specializing in bacterial sulfur metabolism to the sulfur-oxidizing capabilities of the various groups of anoxygenic phototrophic bacteria. Ecology and taxonomy of anoxygenic phototrophic bacteria are described in detail in the chapters by Imhoff and Overmann. It should be emphasized that some older reviews still serve as a valuable source of information especially regarding sulfur oxidation patterns by whole cells of anoxygenic phototrophic bacteria (Brune, 1989; Brune, 1995b).

Abbreviations: *Acd*. – Acidiphilium; *Alc*. – *Allochromatium*; APS – adenosine 5′-phosphosulfate; *Ect*. – *Ectothiorhodospira*; EC – extracellular; FAPs – filamentous anoxygenic phototrophs; HiPIP – high potential iron-sulfur protein; *Hlr*. – *Halorhodospira*; IC – intracellular; *Mch*. – *Marichromatium*; nd – not determined; *Pcs*. – *Paracoccus*; SQR-sulfide: quinone oxidoreductase *Rba*. – *Rhodobacter*; *Tca*. – *Thiocapsa*; *Tcs*. – *Thiocystis*

II. Sulfur Oxidation Capabilities of Anoxygenic Phototrophic Bacteria

In the following section the sulfur oxidation capabilities of the various groups of anoxygenic phototrophic bacteria will be briefly described. Sulfur oxidation capabilities in the aerobic bacteriochlorophyll-containing bacteria, the Heliobacteria and the anoxygenic filamentous phototrophs are rather limited. Information about the enzymes involved is in most cases not available. The sulfur oxidation pathways in the other groups are far more complex. Therefore, separate chapters concentrate on the biochemistry, molecular genetics, genomics and proteomics of sulfur oxidation in the green sulfur bacteria (Hanson, Frigaard). The sulfur metabolism in purple nonsulfur bacteria is reviewed in a forthcoming volume of this series (Sander and Dahl, 2008) This chapter focuses on sulfur compound oxidation in the purple sulfur bacteria of the families *Chromatiaceae* and *Ectothiorhodospiraceae*.

A. Aerobic Anoxygenic Bacteriochlorophyll-Containing Bacteria

ABC bacteria are probably very important as destructors of organic compounds in a broad range of habitats (Yurkov, 2006). Although this increasingly large group of bacteria is very heterogeneous phylogenetically, morphologically and physiologically, all share the inability to use bacteriochlorophyll for anaerobic photosynthetic growth and the presence of photochemical reactions in cells only under aerobic conditions (Hiraishi and Shimada, 2001). Furthermore they share aerobic chemoorganotrophy as the preferred mode of growth, low levels of bacteriochlorophylls and strong inhibition by light of bacteriochlorophyll synthesis under normal growth conditions. While fully active reaction center and LH1 complexes with bacteriochlorophyll are present in all species studied so far, peripheral antenna (LH2) are absent in most species (Hiraishi and Shimada, 2001).

All species of the ABC bacteria, except the β-Proteobacterium *Roseotales depolymerans*, belong to the α-Proteobacteria (class *Alphaproteobacteria*) where they do not form a homogeneous cluster but are closely interspersed with phototrophic and non-phototrophic species (Imhoff and Hiraishi, 2005). Differentiation and taxonomy of ABC bacteria is difficult to understand even for experts in the field as several species not containing bacteriochlorophyll have been placed in genera of the aerobic anoxygenic bacteria.

None of the ABC bacteria are able to grow photolithoautotrophically with sulfur compounds as electron donors. However, the ability to oxidize inorganic sulfur compounds has been described for several representatives of this group. Examples are *Roseinatronobacter thiooxidans*, a strictly aerobic obligately heterotrophic alkaliphile that can oxidize sulfide, thiosulfate, sulfite and elemental sulfur to sulfate in the presence of organic compounds (Sorokin et al., 2000). In another study, Yurkov et al. (1994) showed thiosulfate-oxidizing activity in *Erythromicrobium hydrolyticum*, strain E4(1) and *Rosoecoccus thiosulfatophilus*, strain RB-7. The most pronounced oxidative sulfur metabolism is present in species of the genus *Acidiphilium*. A number of studies have demonstrated sulfur-dependent chemolithotrophy of and sulfur oxidation by *Acd. acidophilum* (formerly *Thiobacillus acidophilus*) (Pronk et al., 1990; Meulenberg et al., 1992b; Hiraishi et al., 1998). In *Acd. acidophilum* the utilization of thiosulfate is initiated by the oxidative condensation of two molecules of thiosulfate yielding tetrathionate. This step is catalyzed by the periplasmic enzyme thiosulfate:cytochrome *c* oxidoreductase (Meulenberg et al., 1993). The details of the further oxidation of tetrathionate to sulfate are largely unclear. Meulenberg et al. (1993) obtained indications that tetrathionate oxidation takes place in the periplasm in *Acd. acidophilum*. Furthermore, a tetrathionate hydrolase (de Jong et al., 1997), a trithionate hydrolase (Meulenberg et al., 1992a) and a sulfite:cyctochrome *c* oxidoreductase (de Jong et al., 2000) have been characterized from the organism.

B. Heliobacteria

Heliobacteria are anoxygenic phototrophic bacteria that contain bacteriochlorophyll *g* as the sole chlorophyll pigment. This unique Bchl, found only in the heliobacteria, distinguishes them from all other anoxygenic phototrophic bacteria (Madigan, 2001b). They lack differentiated photosynthetic internal membranes, such as the membrane vesicles or lamellae of purple bacteria or the chlorosomes of green bacteria. Representatives of the heliobacteria mainly occur in soils and are phylogenetically

related with gram-positive bacteria, specifically the *Bacillus/Clostridium* lineage. As far as is currently known, heliobacteria are obligate anaerobes. However, they can grow both photo- and chemotrophically. Photoheterotrophic growth occurs on a restricted number of organic compounds as carbon sources. Chemotrophic growth in the dark occurs by fermentation of pyruvate or lactate. Photoautotrophic growth has not been demonstrated with any species of heliobacteria. If sulfide is added to the culture media, it is frequently oxidized to elemental sulfur that appears in the medium (Bryantseva et al., 2000; Madigan, 2001a). *Heliobacterium sulfidophilum* and *Heliobacterium undosum* are especially tolerant to sulfide (up to 2 mM at pH 7.5). Many but not all members of the *Heliobacteriaceae* can assimilate sulfate as the sole source of sulfur (Madigan, 2001a).

C. Filamentous Anoxygenic Phototrophs (Chloroflexaceae)

Chloroflexus, *Chloronema*, *Oscillochloris*, *Roseiflexus* and *Heliothrix* are well described genera of the filamentous anoxygenic phototrophs (FAPs) (Hanada and Pierson, 2002). Filamentous morphology and gliding motility are typical features of these anoxygenic phototrophic organisms. Three of the genera, *Chloroflexus*, *Chloronema* and *Oscillochloris* contain chlorosomes, structural elements that are attached to the cellular membranes and contain the light-harvesting bacteriochlorophylls *c* and *d*. All five genera contain bacteriochlorophyll *a*. The filamentous anoxygenic bacteria are not closely related to the green sulfur bacteria and belong into one of the deepest bacterial phyla (*Chloroflexi*) of the Bacteria. This phylum also harbours non-phototrophic filamentous gliding bacteria. Most but not all anoxygenic filamentous bacteria are facultatively aerobic and preferentially utilize organic substrates in their phototrophic or chemotrophic metabolism.

The biochemically best characterized member of the *Chloroflexaceae* is *Chloroflexus aurantiacus*, a thermophilic organism that prefers photoheterotrophic growth. Slow photoautrophic with hydrogen or sulfide as electron donors has been observed for some strains of the species (Madigan and Brock, 1977). Photoautotrophic growth on sulfide has also been described for *Oscillochloris trichoides* and appears to be present also in marine and hypersaline filamentous anoxygenic bacteria (Keppen et al., 1993; Hanada and Pierson, 2002). Sulfur appearing in the medium (often affixed to the cells) is the end product of sulfide oxidation. *Chloroflexus aurantiacus* is able to cover its need for sulfur for biosynthetic purposes by the assimilation of sulfate or thiosulfate, as evidenced by the presence in the genome of a gene cluster encoding proteins involved in assimilatory sulfate reduction (e.g. ATP sulfurylase CaurDraft_0193, APS kinase CaurDraft_0191, PAPS reductase CaurDraft_0192, sulfite reductase CaurDraft_0197).

D. Green Sulfur Bacteria

All green sulfur bacteria fall into a coherent taxonomic group that forms a separate bacterial phylum, the *Chlorobi* (Garrity and Holt, 2001). Besides bacteriochlorophyll *a* in the reaction center bacteriochlorophyll *c*, *d*, or *e* and various carotenoids of the chlorobactene and isorenrieratene series are used as photosynthetic pigments. Intracytoplasmic membranes are not formed, the light harvesting complexes reside on chlorosomes. All green sulfur bacteria have similar metabolic properties. They are strictly anaerobic and obligately phototrophic and can grow with CO_2 as only carbon source. In contrast to the purple bacteria CO_2 is fixed via the reductive tricarbonic acid cycle. Sulfide is used as electron donor by almost all of these species (the iron-oxidizing *Chlorobium ferrooxidans* is the only known exception) and oxidized to sulfate with intermediary accumulation of extracellular sulfur. The chemical speciation of the deposited sulfur is discussed in section VI.E. Many species are able to grow with elemental sulfur and some species also use thiosulfate (Frigaard and Bryant, 2008). *Chlorobaculum parvum* (formerly *Chlorobium vibrioforme* subsp. *thiosulfatophilum* (Imhoff, 2003)) NCIB 8346 and a strain described by Helge Larsen as *Chlorobium thiosulfatophilum* can use tetrathionate as electron donor (Larsen, 1952; Khanna and Nicholas, 1982). Sulfite utilization has not yet been described for any green sulfur bacterium.

E. Purple Nonsulfur Bacteria

The purple "nonsulfur" bacteria are an extremely heterogeneous group of bacteria. Representatives are found within the *Alpha-* and the *Betaproteobacteria* (Imhoff et al., 2005). The species in this group

vary not only with respect to their cell morphology, the structure of intracytoplasmic membrane systems, the carotenoid composition and the carbon sources used but also with respect to the electron donors that can be used for photosynthesis. All species prefer photoheterotrophic growth under anaerobic conditions. In addition, many species can grow photoautotrophically with hydrogen or sulfide as electron donor, many of which do not oxidize sulfide completely to sulfate but form sulfur as the end product instead. However, in many other species, among them the species of the genus *Rhodovulum*, *Rhodopseudomonas palustris* or *Blastochloris sulfoviridis*, sulfate is the end product of sulfide oxidation (reviewed in Brune, 1995b; Imhoff et al., 2005). Thiosulfate is also used by many species, and oxidized either to tetrathionate (*Rhodopila globiformis* (Then and Trüper, 1981)) or completely to sulfate (e.g. *Rhodovulum* species (Brune, 1995b; Appia-Ayme et al., 2001; Imhoff et al., 2005)). Under microoxic to oxic conditions in the dark most representatives of the purple "non-sulfur" bacteria can grow chemoorganotrophically, some are also capable of chemolithoautotrophic growth. In addition, some species are able to metabolize sugars in the dark in the absence of oxygen by using nitrate, dimethyl sulfoxide or trimethylamine-N-oxide as electron acceptors.

F. Purple Sulfur Bacteria

The purple sulfur bacteria belong to the *Gammaproteobacteria* and fall in two families, the *Chromatiaceae* and the *Ectothiorhodospiraceae*. Both form coherent groups on the basis of their 16S rRNA sequences. During phototrophic growth in batch cultures with sulfide as electron donor, the oxidation of sulfide and sulfur follow each other. The most important and easily recognized distinguishing feature between the members of these two families is the site of sulfur deposition during growth on sulfide. In *Chromatiaceae* sulfur globules appear inside the cells while they are formed outside the cells in *Ectothiorhodospiraceae*. A notable exception among the *Ectothiorhodospiraceae* is *Thiorhodospira sibirica*. This organism deposits sulfur not only outside of the cell in the medium but also attached to the cells or in the periplasm (Bryantseva et al., 1999). The sulfur-metabolizing capabilities of the purple sulfur bacteria are summarized in Table 1.

1. Chromatiaceae

Generally, two physiological groups can be differentiated within the *Chromatiaceae*: The large-celled species (eg. *Chromatium okenii*, *Allochromatium warmingii* and *Isochromatium buderi*) are strictly anaerobic, obligately phototrophic and require sulfide or elemental sulfur as photosynthetic electron donors and as sources of sulfur for biosynthesis. The other group includes most of the small-celled species (eg. *Alc. vinosum*, *Allochromatium minutissimum*) which are metabolically much more versatile. In addition to sulfide and elemental sulfur these organisms use thiosulfate and some also use sulfite as electron donors (Imhoff, 2005a). Some organic sulfur compounds can also serve as electron donors for photosynthetic growth of *Chromatiaceae*: *Thiocapsa roseopersicina* splits mercaptomalate and mercaptopropionate to fumarate and H_2S and acrylate and H_2S, respectively and then uses the liberated H_2S as electron donor (Visscher and Taylor, 1993). This organism furthermore oxidizes dimethyl sulfide to dimethyl sulfoxide (Visscher and van Gemerden, 1991). Most of the small-celled representatives of the *Chromatiaceae* are able to assimilate sulfate for biosynthetic purposes, can grow photoorganoheterotrophically in the absence of reduced sulfur compounds and are able to grow as chemolithotrophs on reduced sulfur compounds. Some species can even grow as chemoorganotrophs in which case the addition of sulfide or thiosulfate as a sulfur source is required because the assimilation of sulfate is repressed under aerobic conditions (Kondratieva et al., 1981). During fermentative dark metabolism of *Chromatiaceae* sulfur compounds (elemental sulfur) can serve as acceptors of electrons liberated by the oxidation of stored carbon compounds (polyhydroxyalcanoic acid).

2. Ectothiorhodospiraceae

Almost all members of the *Ectothiorhodospiraceae* are halophilic and alkaliphilic bacteria. The family comprises four phototrophic genera (*Ectothiorhodospira, Halorhodospira, Thiorhodospira, Ectothiorhodosinus*). Formally, the genus *Ectothiorhodosinus* (Gorlenko et al., 2004) has no standing in nomenclature.

Table 1. Sulfur metabolizing capabilities of purple sulfur bacterial genera.

Genus	Sulfur substrates	Intermediates	End product	Sulfate assimilation	Chemoautotrophic growth
Chromatiaceae					
Allochromatium	Sulfide, sulfur, thiosulfate, sulfite, (latter two not in *Alc. warmingii*)	Sulfur, IC	Sulfate	+ (not in *Alc. warmingii*)	Some species
Chromatium	Sulfide, sulfur	Sulfur, IC	Sulfate	−	−
Halochromatium	Sulfide, thiosulfate, sulfur, sulfite	Sulfur, IC	Sulfate	−	+ (sulfide, thiosulfate)
Isochromatium	Sulfide, sulfur	Sulfur, IC	Sulfate	−	−
Lamprobacter	Sulfide, thiosulfate, sulfur	Sulfur, IC	S^0 and sulfate	−	+
Lamprocystis	Sulfide, thiosulfate, sulfur	Sulfur, IC	Sulfate	−/nd	+/−
Marichromatium	Sulfide, thiosulfate, sulfur, sulfite (only *Mch. gracile*)	Sulfur, IC	Sulfate	+/−	+/−
Lamprobacter	Sulfide, thiosulfate, sulfur	Sulfur, IC	S^0 and sulfate	−	+
Lamprocystis	Sulfide, thiosulfate, sulfur	Sulfur, IC	Sulfate	−/nd	+/−
Rhabdochromatium	Sulfide, thiosulfate, sulfur	Sulfur, IC	Sulfate	nd	−
Thermochromatium	Sulfide, sulfur	Sulfur, IC	Sulfate	nd	−
Thioalkalicoccus	Sulfide, sulfur	Sulfur, IC	Sulfate	nd	−
Thiobaca	Sulfide	Sulfur, IC	Sulfate	nd	nd
Thiocapsa	Sulfide, thiosulfate, sulfur, sulfite (only *Tca. litoralis* and *Tca. pendens*)	Sulfur, IC	Sulfate	+/−	+/−
Thiococcus	Sulfide, sulfur	Sulfur, IC	Sulfate	−	−
Thiocystis	Sulfide, thiosulfate (not in *Tcs. gelatinosa*), sulfur, sulfite in some strains	Sulfur, IC	Sulfate	+in some strains	+
Thiodictyon	Sulfide, sulfur	Sulfur, IC	Sulfate	nd	−
Thioflaviococcus	Sulfide, sulfur	Sulfur, IC	Sulfate	nd	−
Thiohalocapsa	Sulfide, thiosulfate, sulfur, sulfite	Sulfur, IC	Sulfate	−	+
Thiolamprovum	Sulfide, thiosulfate, sulfur	Sulfur, IC	Sulfate	−	+
Thiopedia	Sulfide, sulfur	Sulfur, IC	Sulfate	−	−
Thiorhodococcus	Sulfide, thiosulfate, sulfur	Sulfur, IC	Sulfate	−	+/−
Thiorhodovibrio	Sulfide, sulfur	Sulfur, IC	Sulfate	nd	+
Thiospirillum	Sulfide, sulfur	Sulfur, IC	Sulfate	nd	−
Ectothiorhodospiraceae					
Ectothiorhodospira	Sulfide, thiosulfate (not in *Ect. marismortui*), sulfur, sulfite (nd for some species)	Polysulfide, sulfur, EC	Sulfate	+in some species	+in some species
Halorhodospira	Sulfide, thiosulfate only in *Hlr. halophila*	Sulfur, EC	Sulfur or sulfate	+in *Hlr. halochloris*	−

(continued)

Table 1. (continued)

Genus	Sulfur substrates	Intermediates	End product	Sulfate assimilation	Chemoautotrophic growth
Thiorhodospira	Sulfide, sulfur	Sulfur, EC & IC	Sulfate	nd	–
*Ectothiorhodosinus**	Sulfide, thiosulfate	Sulfur, EC	Sulfate	nd	nd

The tabulated data were mostly taken from Imhoff (2005a; 2005b). Additional information was taken from Rees et al. (2002); Zaar et al. (2003); Gorlenko et al. (2004); Arunasri et al. (2005).

IC, intracellular; EC, extracellular; nd, not determined.

* The genus *Ectothiorhodosinus* has no standing in nomenclature.

All species of the genus *Ectothiorhdodospira* grow well under anoxic conditions in the light with reduced sulfur compounds as photosynthetic electron donors and in the presence of organic carbon sources and inorganic carbonate. Under the alkaline growth conditions which are optimal for *Ectothiorhodospira* species, polysulfides are stable intermediates during sulfide oxidation. As a result, polysulfides have been described as the first measurable oxidation products almost 25 years ago (Then and Trüper, 1983; Then and Trüper, 1984). When grown on elemental sulfur *Ect. halochloris* does not oxidize this compound to sulfate, but reduces it to sulfide and polysulfide (Then and Trüper, 1984). Several species of the genus *Ectothiorhodospira* are also able to grow chemolithotrophically on sulfur compounds (Table 1). Members of the genus *Halorhodospira* oxidize sulfide to sulfur which is further oxidized to sulfate by some species. Thiosulfate is only used by *Hlr. halophila* (Raymond and Sistrom, 1969) and poorly by *Halorhodospira neutriphila* (Hirschler-Rea et al., 2003). Sulfur can also be used by some species (Imhoff, 2005b).

III. Electron Transport in Purple Sulfur Bacteria

During photoautotrophic growth of purple sulfur bacteria reduced sulfur compounds yield electrons for the reduction of CO_2. The electrons from the sulfur compounds are transferred to CO_2 via the photosynthetic electron transport chain and NAD^+. Photosynthetic electron transport and CO_2 fixation are therefore intimately intertwined with the oxidation of reduced sulfur compounds and will be briefly presented.

Light-driven electron flow in purple sulfur bacteria is essentially cyclic and involves two membrane-embedded complexes, the reaction center and the cytochrome *bc*1 complex (Fig. 1). In most purple bacteria the reaction center is intimately associated with a tetraheme cytochrome binding two heme *c* with a relatively low redox potential (10 mV) and two heme *c* with high redox potential (330 and 360 mV, respectively) (Nitschke et al., 1993). The reaction center uses light energy to transfer electrons from a mobile periplasmic or membrane-associated donor protein with a positive redox potential to quinone in the membrane. The reduction of the quinone occurs with incorporation of two protons from the cytoplasm close to the cytoplasmic membrane surface. The cycle is complete when the electrons are transferred back to the mobile electron-carrying protein via the cytochrome bc_1 complex.

The periplasmic electron carrier protein is cytochrome c_2 in several of the well studied purple nonsulfur bacteria, e.g. *Rhodobacter sphaeroides, Rhodobacter capsulatus*, and *Blastochloris viridis* (see, for example Drepper and Mathis (1997)). Surveys of photosynthetic electron transfer among other proteobacterial species, however, showed that the participation of HiPIP (high potential iron–sulfur protein), a ferredoxin-like [4Fe–4S] protein with a redox potential of +350 mV, instead of soluble cytochrome *c* is the rule rather than the exception (Menin et al., 1998). The idea has been put forward, that HiPIP is the electron carrier of choice in the purple sulfur bacteria in the families *Chromatiaceae* and *Ectothiorhodospiraceae*, but that the majority of purple nonsulfur bacteria are likely to utilize cytochrome c_2 (van Driessche et al., 2003). Other soluble cytochromes, such

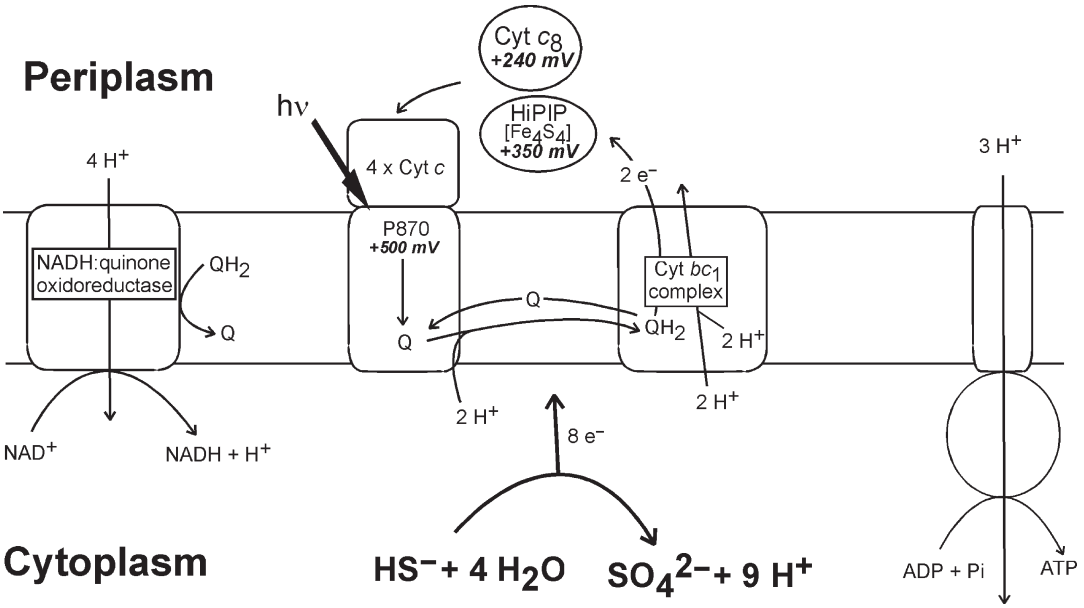

Fig. 1. Schematic representation of photosynthetic electron flow in the purple sulfur bacterium *Allochromatium vinosum*. Oxidation of sulfur compounds as the electron source for NAD^+ reduction is shown for sulfide in a simplified fashion. It should be noted that only part of the several steps involved in sulfur compound oxidation take indeed place in the cytoplasm (see Section IV).

as cytochrome c_8 or the membrane-associated cytochrome c_y can also mediate electron flow from the reaction center to the cytochrome *bc*1 complex in some species (Jenney et al., 1994; Samyn et al., 1996; Kerfeld et al., 1996). In *Allochromatium vinosum*, our model organism for the investigation sulfur oxidation pathways, both, HiPIP and cytochrome c_8 can serve as reductants of the high potential reaction center heme. In this purple sulfur bacterium the growth conditions influence the identity of the electron donor that is preferentially used. Cells grown autotrophically in the presence of sulfide and thiosulfate appear to use almost exclusively HiPIP while cytochrome c_8 is used in cells grown with organic compounds (Vermeglio et al., 2002).

The oxidation of quinol at the bc_1 complex results in the release of two protons into the periplasm. The resulting proton gradient drives ATP synthesis and the reduction of NAD^+ to NADH with quinol as the reductant. NADH and CO_2 are required for the reduction of CO_2 to carbohydrates via the Calvin cycle. Electrons drained from photosynthetic electron flow for the reduction of CO_2, are replaced by electrons released from oxidizable substrates. Taking into consideration the redox potential of the sulfur compounds used as photosynthetic electron donors by purple sulfur bacteria, the respective electrons could in principle be transferred to periplasmic *c*-type cytochromes or directly into the quinone pool. Periplasmic cytochromes, such as flavocytochrome *c* and cytochrome c_{551} (SoxA), may feed electrons from sulfide or thiosulfate (see below) into the photosynthetic pathways via the same soluble carriers as are part of the cyclic system. Sulfide:quinone oxidoreductase would directly reduce quinone with electrons from sulfide. Electrons resulting from cytoplasmic oxidation of sulfite via APS reductase may also be directly transferred to quinone. It should be noted that the dissimilatory APS reductase is an iron–sulfur flavoprotein that bares no resemblance to the APS/PAPS reductases of the assimilatory sulfate reduction pathway.

IV. Biochemistry of Sulfur Oxidation Pathways in Purple Sulfur Bacteria

The last comprehensive reviews about the biochemistry of sulfur oxidation pathways in purple sulfur bacteria were published by Daniel C Brune (Brune, 1989; Brune, 1995b). In these

excellent articles a wealth of information on sulfur oxidation patterns by whole cells of anoxygenic phototrophic bacteria was presented. In addition, the available data on enzymes potentially involved in sulfur transformations in these organisms was summarized. At that time, it was very difficult to unify the available information into a valid scheme, mostly due to the facts that a whole array of different organisms had been investigated and that molecular genetic information was essentially not present. In order to obtain a better picture of sulfur oxidation in purple sulfur bacteria we concentrated on one model bacterium, *Allochromatium vinosum* DSMZ 180^T and developed reverse genetics for this organism (Pattaragulwanit and Dahl, 1995). The following discussion on the oxidation of different reduced sulfur compounds, the properties of sulfur globules and their degradation therefore focuses on this organism. Results obtained with other purple sulfur bacteria are discussed but a complete survey of all available information is not attempted. Further relevant information is available through the genome sequence of *Halorhodospira halophila* SL1 (NZ_AAOQO1000001.1), a member of the *Ectothiorhodospiraceae*. In this organism many genes encoding proteins potentially involved in sulfur oxidation are clustered (genes Hhal1932 through Hhal1967). In Fig. 2 a comparison between the arrangement of these genes and those currently known for *Alc. vinosum* is presented. The genome sequence of *Alc. vinosum* has not yet been determined.

A. Oxidation of Thiosulfate

Thiosulfate ($S_2O_3^{2-}$) is a rather stable and environmentally abundant sulfur compound of intermediate oxidation state. It fulfils an important role in the natural sulfur cycle and is used by many phototrophic and chemotrophic sulfur oxidizers (Jørgensen, 1990; Sorokin et al., 1999). Two completely different pathways of thiosulfate oxidation appear to exist in purple sulfur bacteria. In one form tetrathionate is produced by oxidation of two thiosulfate anions via thiosulfate dehydrogenase (thiosulfate:acceptor oxidoreductase, EC 1.8.2.2). In the second form thiosulfate is completely oxidized to sulfate via several different mechanisms.

1. Thiosulfate Dehydrogenase

The formation of tetrathionate from thiosulfate has been mainly studied in chemoorganotrophic bacteria that use thiosulfate as a supplemental but not as the sole energy source (Jørgensen, 1990; Sorokin et al., 1999; Podgorsek and Imhoff, 1999). The pathway occurs only in a few purple sulfur bacteria including *Alc. vinosum* (Smith and Lascelles, 1966; Hensen et al., 2006).

In *Alc. vinosum* the ratio between tetrathionate and sulfate formed from thiosulfate is strongly pH-dependent with more tetrathionate as the product under slightly acidic conditions (Smith, 1966). In *Alc. vinosum* thiosulfate dehydrogenase is a periplasmic 30-kDa monomer with an isoelectric point of 4.2. The enzyme contains heme *c* and is reduced by thiosulfate at pH 5.0 but not at pH 7.0. In accordance, the pH optimum of the enzyme was determined to be 4.25 (Hensen et al., 2006). An examination of the kinetic properties of *Alc. vinosum* thiosulfate dehydrogenase with ferricyanide as artificial electron acceptor was initiated but interpretation of experimental results is complicated by the fact that enzymes that use two molecules of the same substrate do not follow regular Michaelis–Menten kinetics. However, some important constants could be estimated: the limiting V_{max} is about 34,000 units (mg protein)$^{-1}$ (corresponding to a k_{cat} of 1.7×10^4 s^{-1}) and the $[S]_{0.5}$ for ferricyanide is about 0.5. $[S]_{0,5}$ is the substrate concentration that yields half maximal velocity. It is important to note that it is not identical to K_m as a K_m cannot be given for reactions not following Michaelis–Menten kinetics (Segel, 1993). While thiosulfate did not display strong substrate inhibition at any of the experimental ferricyanide levels, ferricyanide did show substrate inhibition on *Alc. vinosum* thiosulfate dehydrogenase (Hensen et al., 2006). Furthermore, the enzyme was significantly inhibited by sulfite (50% inhibition at 80 µM sulfite). Under optimized assay conditions cytochrome *c* from yeast is used as electron acceptor instead of ferricyanide by the enzyme, whereas horse heart cytochrome *c* is not accepted. The properties of *Alc. vinosum* thiosulfate dehydrogenase described by Hensen et al. (2006) are compatible with older data presented by Smith (1966) and Fukumori and Yamanka (1979). In both reports a tetrathionate-forming activity with a pH optimum

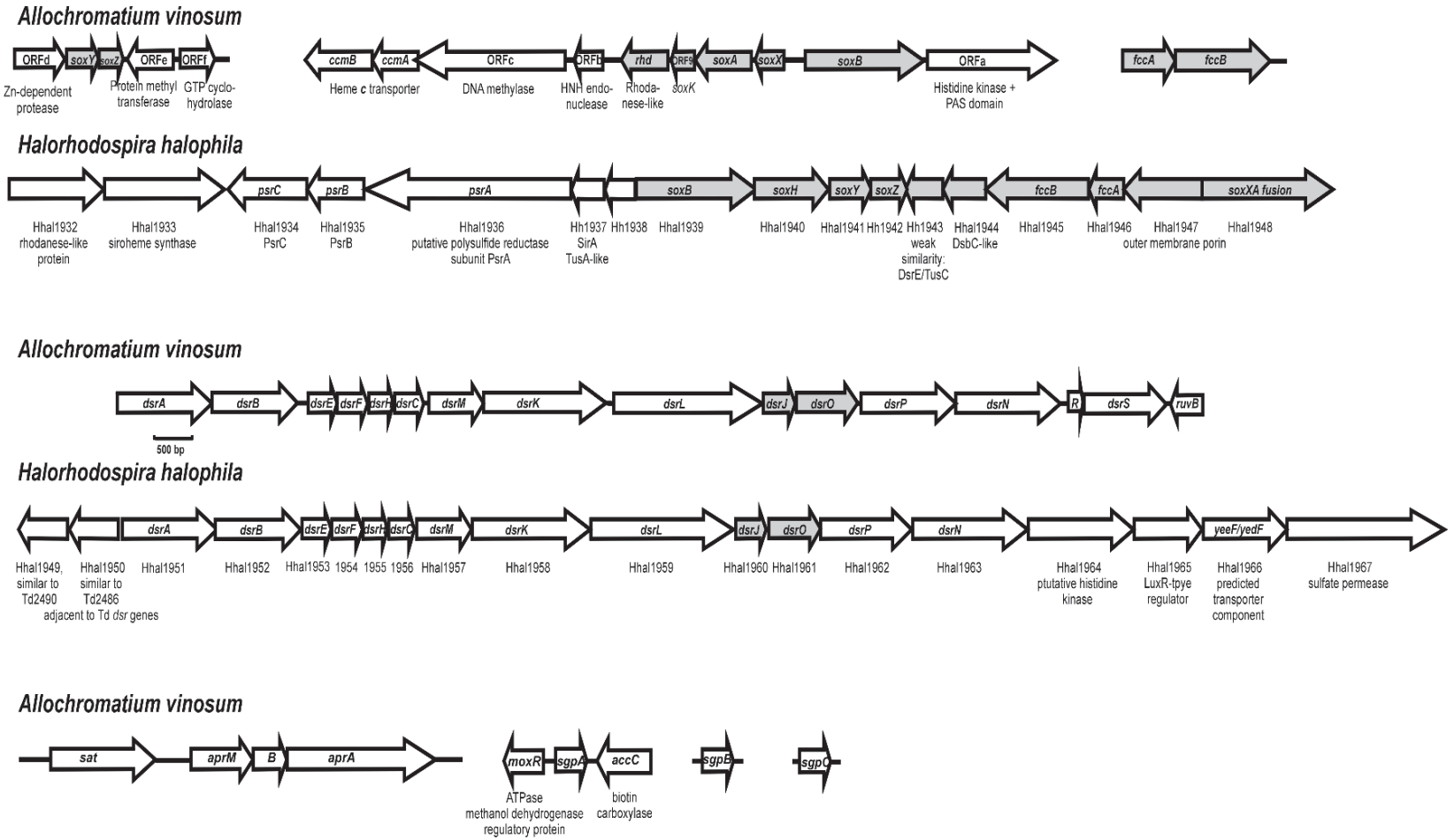

Fig. 2. Comparison of the established sulfur oxidation genes in *Allochromatium vinosum* DSMZ 180^T and the genes of the sulfur oxidation cluster in the genome of *Halorhodospira halophila* SL1. Related genes/gene clusters are immediately compared where possible. It should be noted that the *Hlr. halophila* genes are all connected, while in *Alc. vinosum sox* genes are found in two independent loci and the *dsr*, *fcc* and *sat-apr* genes each form independent entities. The three *Alc. vinosum sgp* genes also represent independent transcriptional units. The *Allochromatium sat-apr* and *sgp* genes do not have equivalents in the *Halorhodospira* genome. Genes encoding proteins predicted to be localized in the periplasm are highlighted in grey.

in the acidic range was described. Fukumori and Yamanaka (1979) found that *Alc. vinosum* thiosulfate dehydrogenase used HiPIP isolated from the same organism as an efficient electron acceptor. This is in complete agreement with the fact that both, thiosulfate dehydrogenase and HiPIP are located in the periplasm of *Alc. vinosum* (Brüser et al., 1997) where HiPIP is photooxidized by the reaction center (van Driessche et al., 2003).

With our current analysis we cannot confirm the presence of any tetrathionate-forming enzyme operating at pH 8.0 in *Alc. vinosum* as has been claimed earlier (Schmitt et al., 1981; Knobloch et al., 1981). Of the tetrathionate-forming enzymes characterized so far, thiosulfate dehydrogenase from *Acidithiobacillus thiooxidans* (Nakamura et al., 2001) most closely resembles the enzyme from *Alc. vinosum*. Both species belong to the *Gammaproteobacteria*. The protein from *Acidithiobacillus* has been described as a monomeric 27.9-kDa *c*-type cytochrome with a pH optimum at 3.5. Thiosulfate dehydrogenases from other sources show remarkable heterogeneity with respect to structural properties and catalytic characteristics (Kusai and Yamanaka, 1973; Then and Trüper, 1981; Visser et al., 1996) which has been interpreted as indicating convergent rather than divergent evolution (Visser et al., 1996). A gene sequence encoding a heme-containing thiosulfate dehydrogenase has not yet been reported. A Blast search with the amino-terminal sequence of the enzyme from *Alc. vinosum* yielded only one significantly related sequence, a hypothetical *c*-type cytochrome from *Cupriavidus (Ralstonia*, *Wautersia*) *metallidurans* (Hensen et al., 2006).

2. Oxidation of Thiosulfate to Sulfate

Many purple sulfur bacteria can oxidize thiosulfate completely to sulfate (Table 1). In batch cultures of purple sulfur bacteria growing on thiosulfate the formation of sulfur globules is sometimes – but not always – observed. It is therefore very important to note, that the formation of sulfur globules is known to be an obligatory step during the oxidation of thiosulfate to sulfate in *Alc. vinosum* and probably also in other purple sulfur bacteria. Two independent lines of evidence prove that sulfur formation is an essential step: (1) An *Alc. vinosum* mutant unable to form sulfur globules due to the lack of sulfur globule proteins cannot grow on thiosulfate (Prange et al., 2004) and (2) *Alc. vinosum* mutants blocked in sulfur oxidation form intracellular sulfur globules from thiosulfate as a dead end product (Pott and Dahl, 1998). In addition, studies with radioactively labelled thiosulfate demonstrated very clearly that the more reduced sulfane and the more oxidized sulfone sulfur atoms are processed differently in purple sulfur bacteria (Smith and Lascelles, 1966; Trüper and Pfennig, 1966). Only the sulfane sulfur accumulates as stored sulfur [S^0] before further oxidation, whereas the sulfone sulfur is rapidly converted into sulfate and excreted. The formation of sulfur as an intermediate in purple sulfur bacteria is different from the thiosulfate-oxidizing pathway (Sox pathway) that occurs in a wide range of facultatively chemo- or photolithotrophic bacteria like *Paracoccus pantotrophus* or *Rhodovulum sulfidophilum* (Appia-Ayme et al., 2001; Friedrich et al., 2001). In the latter, both sulfur atoms of thiosulfate are oxidized to sulfate without the appearance of sulfur deposits as intermediates.

In spite of this fundamental difference similar proteins appear to be essential for thiosulfate oxidation to sulfate in organisms forming sulfur as an intermediate and those not producing sulfur. Gene inactivation and complementation studies clearly showed that the *soxBXA* and *soxYZ* genes, located in two independent gene regions (Fig. 2), are essential for thiosulfate oxidation in *Alc. vinosum* (Hensen et al., 2006). Three periplasmic Sox proteins were purified from *Alc. vinosum*: the heterodimeric *c*-type cytochrome SoxXA (SoxX 11 kDa, SoxA 29 kDa; one covalently bound heme is present in each subunit), the heterodimeric SoxYZ (SoxY 12.7 kDa, SoxZ 11.2 kDa) and the monomeric SoxB (62 kDa, predicted to bind two manganese atoms) (Hensen et al., 2006).

In *Alc. vinosum* the genes *soxB* and *soxXA* are transcribed divergently. Upstream of *soxB* a gene encoding a potential regulator protein is located and immediately downstream of *soxA* two further interesting genes are found: The first (ORF9) encodes a hypothetical 12.2-kDa (9.2 kDa after processing) protein with a signal peptide. A homologous gene (*orf1020* or *soxK*) is present in all currently known *sox* gene clusters of thiosulfate-oxidizing green sulfur bacteria (Frigaard and Bryant, 2008), however, a homolog is not

present in the *Hlr. halophila* sulfur gene cluster (Fig. 2). The second (*rhd*) encodes a putative periplasmic protein (22.2 kDa after processing) containing a conserved domain typical for rhodaneses. A homologous gene is neither found close to *sox* genes of green sulfur bacteria nor in *Hlr. halophila. In vitro*, rhodaneses (thiosulfate:sulfur transferases) can catalyze the transfer of the sulfane sulfur atom of thiosulfate to cyanide yielding thiocyanate (rhodanide, SCN^-) and sulfite. This is, however, not the physiological role in most cases. In the past, the detection of rhodanese and thiosulfate reductase activity in phototrophic sulfur bacteria led to the assumption that thiosulfate would be cleaved into sulfite and sulfide in the presence of suitable reduced thiol acceptors like glutathione and dihydrolipoic acid, and that the H_2S formed during the proposed reaction would be immediately oxidized to sulfur stored in sulfur globules (Brune, 1989; Brune, 1995b; Dahl, 1999). However, gene inactivation showed that the *Alc. vinosum rhd* product does not play such a vital role and is dispensable for thiosulfate oxidation (Hensen et al., 2006). The physiological role of the *rhd*-encoded protein remains to be elucidated. The deduced properties of other genes encoded in immediate vicinity of the *Alc. vinosum sox* genes were described in detail by Hensen et al. (2006). A function in oxidative sulfur metabolism of these hypothetical proteins is not obvious.

In *Hlr. halophila* putative *sox* genes are clustered but not organized in a single operon (Fig. 2). The gene *soxH* which is apparently not present close to the sequenced *sox* genes in *Alc. vinosum* is not required for lithotrophic growth on thiosulfate in *Pcs. pantotrophus* (Rother et al., 2001). In *Hlr. halophila soxBHYZ* appear to be co-transcribed. They are separated from a gene encoding a fusion of SoxXA by a divergently oriented cluster of four genes, among them *fccAB* possibly encoding a flavocytochrome *c* (sulfide dehydrogenase). The derived FccB polypeptide also shows similarity to SoxF, an important though not essential component of the *Pcs. pantotrophus* Sox system (Bardischewsky et al., 2006). However, the similarity is significantly lower than that to the flavoprotein subunit FccB of *Alc. vinosum* flavocytochrome *c* (Dolata et al., 1993; Reinartz et al., 1998). The gene immediately upstream of *fccB* in *Hlr. halophila* is clearly related to *fccA* encoding the cytochrome *c* subunit of *Alc. vinosum* flavocytochrome *c* while similarity to *soxE* from *Pcs. pantotrophus* is below detection limits in searches using the BLAST algorithm (Altschul et al., 1990).

Occurrence and arrangement of *sox* genes in both purple sulfur bacteria is different from that in *Pcs. pantotrophus* in which the *sox* gene cluster comprises 15 genes organized into three transcriptional units, *soxRS*, *soxVW* and *sox XYZABCDEFGH*. In this organism the periplasmic proteins SoxXA, SoxYZ, SoxB and $Sox(CD)_2$ are essential for thiosulfate oxidation *in vivo* and *in vitro*. Currently, a model has been proposed that SoxXA initiates oxidation and covalent attachment of thiosulfate to a conserved cysteine (residue 138) in SoxY of the SoxYZ complex. SoxB would then hydrolytically release sulfate leaving a cysteine-138-persulfide in SoxYZ, which is proposed to be oxidized by the hemomolybdoenzyme $Sox(CD)_2$ yielding a cysteine-S-sulfonate. In the final step SoxB would again release sulfate and thereby recycle SoxYZ. In green sulfur bacteria, a *orf1015(soxJ)-soxXYZA-orf1020(soxK)-soxBW* genomic arrangement is generally found (Frigaard and Bryant, 2008). We were not able to detect the genes *soxCD* in *Alc. vinosum* and they are also not present in the genome of *Hlr. halophila*. Furthermore, these genes are absent in the magnetotactic *Magnetococcus* sp. MC1 and *Thiobacillus denitrificans. Alc. vinosum* and the latter two organisms have in common that they form sulfur as intermediate during thiosulfate oxidation, either as globules or as finely dispersed membrane-associated sulfur (Schedel and Trüper, 1980; Williams et al., 2006). The genomes of thiosulfate-oxidizing green sulfur bacteria (Frigaard and Bryant, 2008) also do not contain *soxCD*. Sulfur formation during thiosulfate oxidation has been described for one of these species, *Chlorobaculum parvum* DSM 263 (Steinmetz and Fischer, 1982). Sulfur may be an intermediate also in the other green sulfur bacteria, though may not be detectable due to a high turnover rate. Polysulfides have also been suggested as intermediates occurring in the periplasm of green sulfur bacteria during thiosulfate oxidation (Frigaard and Bryant, 2008). Summarizing the observation that the lack of *soxCD* appears to correlate with the formation of sulfur or possibly polysulfides as metabolic intermediates we suggested the

following model (Fig. 3) (Hensen et al., 2006): The initial oxidation and covalent binding of thiosulfate to SoxYZ would be brought about by SoxXA and sulfate would then be hydrolytically released by SoxB just as proposed for *Pcs. pantotrophus* (Friedrich et al., 2001). However, in organisms like *Alc. vinosum* that lack "sulfur dehydrogenase" the sulfane sulfur atom linked to SoxY cannot be directly further oxidized. We suggest that the sulfur is instead transferred to growing sulfur globules (or polysulfide). Such a suggestion is feasible because the sulfur globules in *Alc. vinosum* and in many if not all other organisms forming intracellular sulfur deposits reside in the bacterial periplasm (Pattaragulwanit et al., 1998; Dahl and Prange, 2006) (see also below) and therefore in the same cellular compartment as the Sox proteins (Hensen et al., 2006). Such a mechanism would require the transfer of SoxY-bound sulfur to the sulfur globules, a process that is currently unclear. The sulfur transferase encoded by the *rhd* gene has the capacity to play such a role however its inactivation did not lead to a detectable phenotype. Possibly, other sulfur transferases present in the cells function as a back up system.

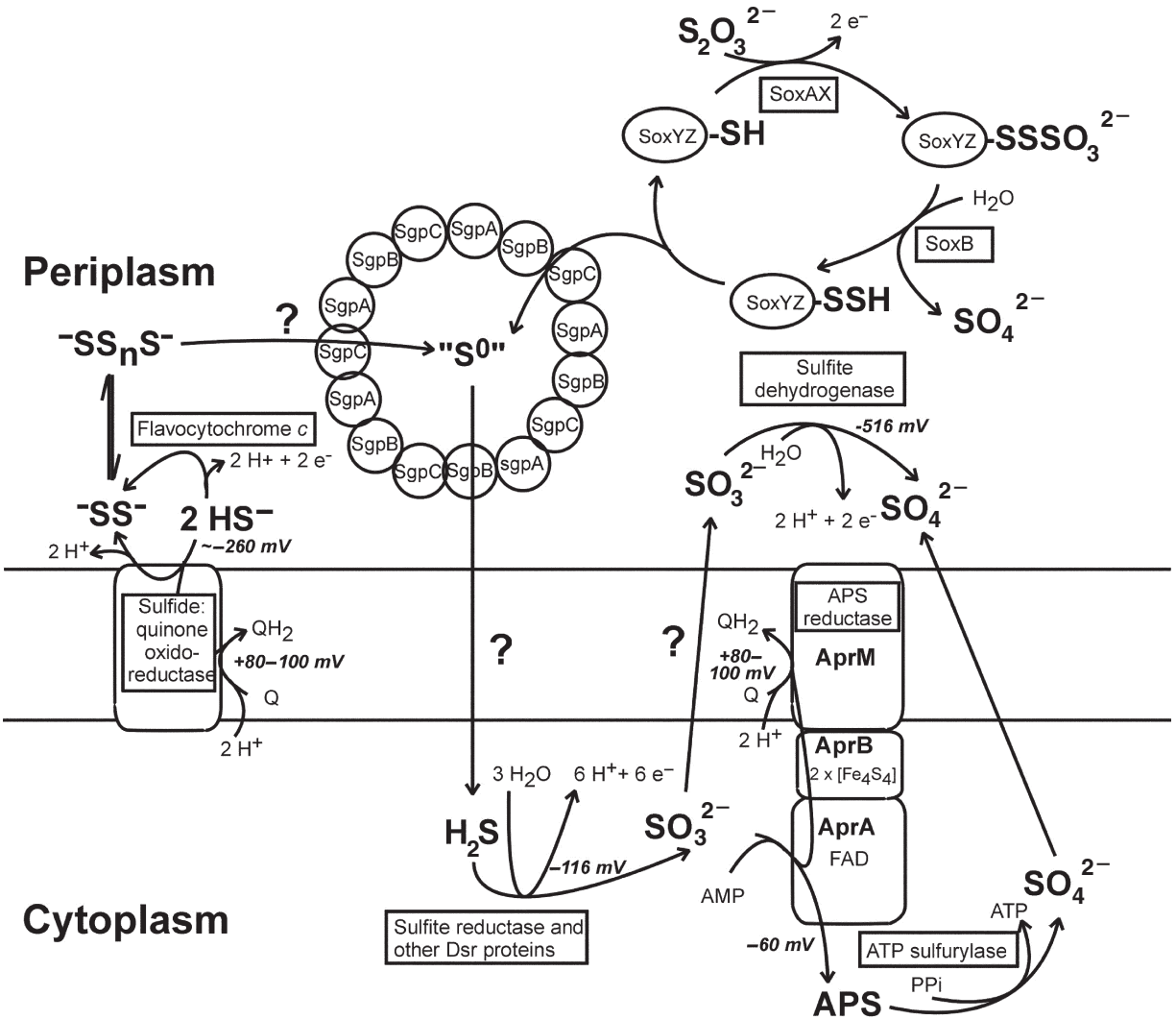

Fig. 3. Model of the sulfur oxidation pathway in *Allochromatium vinosum*. Direct oxidation of sulfite to sulfate is hypothesized to occur periplasmically by a classical sulfite dehydrogenase. However, as elaborated in the text, the possibility of direct cytoplasmic oxidation of sulfite cannot be excluded. *APS*, adenosine 5′-phosphosulfate.

B. Oxidation of Sulfide to "Elemental" Sulfur

In purple sulfur bacteria, the main enzymes that have been discussed as catalyzing the oxidation of sulfide are the periplasmic FAD-containing flavocytochrome *c* and the membrane-bound sulfide: quinone oxidoreductase (SQR) (Brune, 1995b; Reinartz et al., 1998) (Fig. 3).

The distribution of flavocytochrome *c* among the anoxygenic phototrophic bacteria and its chemical and catalytic properties have been discussed in detail elsewhere (Brune, 1989; Brune, 1995b; Frigaard and Bryant, 2008). The protein is located in the periplasm, consists of a FAD-binding (FccB, 46–47 kDa) and a smaller heme *c*-binding subunit (FccA, 21 kDa, two heme *c* in *Alc. vinosum* (van Beeumen et al., 1991)). The genome of *Hlr. halophila* contains three copies of potential *fccAB* genes (Hhal1945 and 1946, Hhal1162 and 1163, Hhal1330 and 1331). In vitro, flavocytochromes can efficiently catalyze electron transfer from sulfide to a variety of small *c*-type cytochromes (e.g. cytochrome c_{550} from *Alc. vinosum* (Davidson et al., 1985)) that may then donate electrons to the photosynthetic reaction center. However, the *in vivo* role of flavocytochrome *c* is unclear. It occurs in many purple and green sulfur bacteria but there are also many species that lack this protein. Moreover, an *Alc. vinosum* mutant deficient in flavocytochrome *c* exhibits sulfide oxidation rates similar to those of the wild type (Reinartz et al., 1998).

As an alternative to sulfide oxidation via flavocytochrome *c*, the transfer of electrons from sulfide primarily into the quinone pool was proposed, based on energetic considerations as well as on the inhibitory effect of rotenone, CCCP, and antimycin A on NAD photoreduction by sulfide (Brune and Trüper, 1986; Brune, 1989). Sulfide: quinone oxidoreductase (SQR) activity has in the meantime been described for many phototrophic organisms including the cyanobacterium *Oscillatoria limnetica* (Arieli et al., 1994), the purple nonsulfur bacterium *Rhodobacter capsulatus* (Schütz et al., 1997), green sulfur bacteria (Shahak et al., 1992) and also *Alc. vinosum* (Reinartz et al., 1998). The properties of this enzyme from diverse sources are described in detail in the chapter by Hauska and Shahak. Although *Alc. vinosum* membranes exhibit SQR activity, my laboratory has so far neither been able to detect a *sqr*-related gene via Southern hybridization with heterologous probes or heterologous PCR nor could we detect the protein with antibodies directed against the *Rba. capsulatus* protein (M. Reinartz and C. Dahl, unpublished). We therefore hypothesize that the enzyme from *Alc. vinosum* and possibly other purple sulfur bacteria has properties distinct from those of characterized SQRs. In accordance, the *Hlr. halophila* genome contains one only distantly related homolog (Hhal1665) of the biochemically well characterized SQR from *Rhodobacter capsulatus* (Schütz et al., 1999; Griesbeck et al., 2002).

In *Rba. capsulatus*, SQR is a peripherally membrane-bound flavoprotein with its active site located in the periplasm (Schütz et al., 1999). The primary product of the SQR reaction is soluble polysulfide whereas elemental sulfur does not appear to be formed *in vitro* (Griesbeck et al., 2002). Very probably, disulfide (or possibly a longer chain polysulfide) is the initial product of sulfide oxidation, which is released from the enzyme. Polysulfide anions of different chain lengths are in equilibrium with each other and longer-chain polysulfides can be formed by disproportionation reactions from the initial disulfide (Steudel, 1996). When whole cells of *Rba. capsulatus* grow with sulfide, elemental sulfur is formed as the final product. In principle, elemental sulfur can form spontaneously from polysulfides (Steudel, 1996).) In experiments using isolated spheroplasts from *Chlorobium vibrioforme* and *Allochromatium minutissimum*, soluble polysulfides have been detected as the product of sulfide oxidation (Blöthe and Fischer, 2000). Polysulfides were also detected as primary products of sulfide oxidation by whole cells of *Alc. vinosum* (Prange et al., 2004) and have been reported as intermediates of the oxidation of sulfide to extracellular sulfur by species of the purple sulfur bacterial family *Ectothiorhodospiraceae* (Trüper, 1978; Then and Trüper, 1983). While transient formation of polysulfide by the latter organism species has originally been attributed to chemical reaction between H_2S and elemental sulfur promoted by the alkaline culture medium (Trüper, 1978), it now appears more likely that they present biochemically generated intermediates.

It remains difficult to assign any function to flavocytochrome *c*, a protein that is constitutive in *Alc. vinosum* (Bartsch, 1978). Based on the large difference of redox potential between flavocytochrome *c* and the photosynthetic reaction center, Brune (1995b) suggested that flavocytochrome *c* may represent a high affinity system for sulfide oxidation that might be of advantage for the cells especially at very low sulfide concentrations. At present such a function cannot be excluded and flavocytochrome *c* could indeed supplement the energetically more efficient system involving electron transfer from sulfide to quinone via SQR.

In *Alc.vinosum*, sulfite reductase operating in reverse, i.e. in the direction of sulfite formation, has also been discussed to be involved in sulfide oxidation (Schedel et al., 1979). However, we have shown that this protein is not essential for sulfide oxidation but rather absolutely required for oxidation of intracellularly stored sulfur (Pott and Dahl, 1998). In the purple nonsulfur bacterium *Rhodovulum sulfidophilum* the Sox enzyme system that catalyzes the oxidation of thiosulfate to sulfate (see above), is also indispensable for the oxidation of sulfide in vivo (Appia-Ayme et al., 2001). However, in *Alc. vinosum* mutants deficient of either flavocytochrome *c* (Reinartz et al., 1998), *sox* genes or both (D. Hensen, B. Franz and C. Dahl, unpublished) sulfide oxidation proceeds with wild-type rates indicating that that SQR plays the main role in sulfide oxidation in this organism.

It should be noted that cytochromes without flavin groups have also been proposed to mediate electron transfer from sulfide to the reaction center in some purple sulfur bacteria (Fischer, 1984; Brune, 1989; Leguijt, 1993).

C. Oxidation of Polysulfides

As outlined above, polysulfides appear to be the primary product of the oxidation of sulfide in purple sulfur bacteria. It is therefore not astonishing that those members of the *Chromatiaceae* that have been studied with respect to the utilization of externally added polysulfides with an average chain length of 3–4 sulfur atoms (*Alc. vinosum* and *Tca. roseopersicina*) readily used these compounds as photosynthetic electron donors (van Gemerden, 1987; Steudel et al., 1990; Visscher et al., 1990). It is currently unknown how polysulfides are converted into sulfur globules. Theoretically this could be a purely chemical, spontaneous process as longer polysulfides are in equilibrium with elemental sulfur (Steudel et al., 1990). However, we have shown that *Alc. vinosum* sulfur globules do not contain major amounts of sulfur rings but probably consist of long-chains of sulfur with organic residues at one or both ends (Prange et al., 1999; Prange et al., 2002a). Such organylsulfanes must eventually be formed by an unknown (enzymatic) mechanism.

D. Uptake of External Sulfur

Very many purple sulfur bacteria including *Alc. vinosum* are able to oxidize externally supplied solid, virtually insoluble elemental sulfur (Table 1). This step – although very important in the global sulfur cycle – is hardly understood.

The formal valence of elemental sulfur is zero. Elemental sulfur tends to catenate and to form chains with various lengths (polymeric sulfur) or ring sizes (Steudel and Eckert, 2003). All sulfur and allotropes are hydrophobic, not wetted by water and hardly dissolvable in water (Steudel, 1989). The most stable form of elemental sulfur at ambient pressure and temperature is cyclic, orthorhombic α-sulfur (α-S_8) (Steudel, 2000). Polymeric sulfur consists mainly of chain-like macromolecules but the presence of large S_n rings with n>50 is likely (Steudel and Eckert, 2003). Commercially available elemental sulfur sublimed at ambient temperature ("flowers of sulfur") consists of S_8 rings, traces of S_7 rings which are responsible for the yellow colour and varying amounts of polymeric sulfur. The bonding energy between S–S bonds in polymeric sulfur is 2.4 kJ mol^{-1} weaker than in *cyclo*-octasulfur (Steudel and Eckert, 2003) and it might therefore be more accessible for sulfur-oxidizing bacteria (Franz et al., 2006).

Enzymes catalyzing the uptake and oxidation of externally added elemental sulfur have not yet been isolated from any species of phototrophic sulfur bacteria. The process must include binding and/or activation of the sulfur as well as transport inside of the cells. In principle, two different strategies would be possible: physical contact of the cells to their insoluble substrate and direct electron transfer from the cell envelope to the substrate via outer membrane proteins (Myers

and Myers, 2001) or excretion of reducing substances, e.g. low molecular weight thiols that can act on substrate distant from the cells. Both possibilities are discussed in detail in the chapter by Hanson. Generally, little information is available about adhesion to and attack of extracellular sulfur. Leaching sulfur-oxidizing bacteria like *Acidithiobacillus ferrooxidans* appear to follow the first pathway and attach to sulfur by extracellular polymeric substances, specifically, lipopolysaccharides (Gehrke et al., 1998). Structures, attached to the cell wall (the so-called "spinae") have been postulated to mediate adhesion of a green sulfur bacterium to extracellularly deposited sulfur (Pibernat and Abella, 1996). In all cases so far, a reaction activating elemental sulfur prior to its oxidation is postulated, due to the stability and low water solubility of the substrate. In case of *cyclo*-octasulfur this activation reaction could be an opening of the S_8 ring by nucleophilic reagents, resulting in the formation of linear inorganic or organic polysulfanes. In addition, the reduction of elemental sulfur to water-soluble sulfide is discussed. Both reactions could be carried out by thiol groups of cysteine residues. Along this line, it was proposed for *Acidithibacillus* and *Acidiphilium* that extracellular elemental sulfur is mobilized by thiol groups of special outer membrane proteins and transported into the periplasmic space as persulfide sulfur (Rohwerder and Sand, 2003). Experimetal evidence for the existence of an outer membrane protein involved in cell-sulfur adhesion in this organism was obtained by Ramírez et al. (2004). In this respect it might be interesting to note that a gene encoding a potential outer membrane porin is found in the sulfur gene cluster of *Hlr. halophila* where it is situated immediately upstream of genes encoding a potential flavocytochrome *c* (Fig. 2). For *Alc. vinosum* we recently obtained first experimental evidence that an intimate physical cell-sulfur contact is indeed a prerequisite for uptake of elemental sulfur (Franz et al., 2006).

In our model organism *Alc. vinosum* the first step during oxidation of externally supplied sulfur is the accumulation of sulfur in intracellular sulfur globules which are then further oxidized to sulfate. XANES measurements provided evidence that *Alc. vinosum* uses only or at least strongly prefers the polymeric sulfur (sulfur chains) fraction of commercially available elemental sulfur and is probably unable to take up and form sulfur globules from *cyclo*-octasulfur (Franz et al., 2006). We did not find evidence for the formation of intermediates like sulfide or polysulfides during uptake of elemental sulfur. One might speculate that "sulfur chains" rather than the more stable "sulfur rings" are the microbiologically preferred form of elemental sulfur also for other sulfur-oxidizing bacteria.

E. Sulfur Globules and Their Properties

In anoxygenic phototrophic sulfur bacteria, sulfur appears to be generally deposited outside of the cytoplasm. Green sulfur bacteria and purple sulfur bacteria of the family *Ectothiorhodospiraceae* form extracellular sulfur globules while the globules are located in the periplasmic space in members of the family *Chromatiaceae* (Pattaragulwanit et al., 1998).

Despite the different site of deposition (outside or inside the confines of the cell) the sulfur appears to be of a similar speciation in the different groups of phototrophic sulfur bacteria: The exact chemical nature of the "elemental sulfur" in bacterial sulfur globules has been a matter of debate for many years (for a detailed historical account consult Dahl and Prange (2006). In most investigations, methods were used that required extraction of the sulfur globules from the cells prior to analysis (e.g. X-ray diffraction, (Hageage et al., 1970)) which causes changes in the chemical structure of the sulfur (Prange et al., 2002a). Only recently, X-ray absorption near-edge structure (XANES) spectroscopy at the sulfur K-edge using synchrotron radiation was introduced as an in situ approach to investigate the sulfur speciation in intact bacterial cells (Prange et al., 1999; Pickering et al., 2001; Prange et al., 2002a). A detailed description of these methods is given in the chapter by Prange et al. XANES spectroscopy yielded the following results for phototrophic sulfur bacteria: irrespective of whether the sulfur is accumulated in globules inside or outside the cells, it mainly consists of long sulfur chains very probably terminated by organic residues (mono-/bis-organyl polysulfanes) in purple and also in green sulfur bacteria. Most probably, the organic residue at the end of the sulfur chains present in the sulfur globules is glutathione or very similar to glutathione (Prange et al., 2002a). This

hydrophilic residue could be responsible for maintaining the sulfur in a "liquid" state at ambient pressure and temperature. Earlier speculations and proposals that reduced glutathione (probably in its amidated form) could act as a carrier molecule of sulfur to and from the globules (Bartsch et al., 1996; Pott and Dahl, 1998) are supported by the XANES spectroscopy results (Prange et al., 2002a). Furthermore, XANES spectroscopy yielded evidence that the sulfur chains in globules of *Alc.vinosum* are gradually shortened during oxidation of intracellularly stored sulfur to sulfate (Prange et al., 2002b). It should be mentioned that some controversy has arisen about the interpretation of data acquired by XANES spectroscopy: Investigations of phototrophic sulfur bacteria by two different groups (Pickering et al., 2001; Prange et al., 2002a) yielded partly comparable experimental data but were interpreted in quite a different way. Pickering et al. (2001) concluded on the basis of theoretical considerations that the sulfur is "simply solid S_8".The discrepancies are mainly based on the measurement mode (George et al., 2002; Prange et al., 2002c). The model for the sulfur globules of *Alc.vinosum* that corresponds best with the available experimental data consists of long sulfur chains terminated by organic groups as was suggested by Prange et al. (Kleinjan et al., 2003). Sulfur of sulfur globules isolated in the presence of oxygen from anaerobically grown *Alc.vinosum* was found as S_8 rings (Prange et al., 2002a), indicating the influence of oxygen and the necessity of in situ methods like XANES spectroscopy that can be applied to avoid destruction of the original sulfur environment.

While sulfur globules appear to be more or less evenly distributed in many species of the *Chromatiaceae*, they can have very special and conspicuous localizations in other species. In *Allochromatium warmigii* for example, globules are predominantly located at the two poles of the cell. Dividing cells form additional sulfur globules near the central division plane. In *Lamprobacter modestohalophilus* the sulfur globules appear in the center of cells, while they are found in the peripheral part of the cells that is free of gas vesicles in species of the genera *Lamprocystis* and *Thiodictyon*. Sulfur globules are also found in the cell periphery in *Thiopedia rosea* (Imhoff, 2005a). For *Thiorhodovibrio winogradskyi* a formation of up to ten small sulfur globules in a row along the long cell axis has been reported (Overmann et al., 1992). The specialized arrangement of sulfur inclusions suggests an important structure function relationship.

The sulfur globules in the *Chromatiaceae* are enclosed by a protein envelope, a feature shared by most if not all of the chemotrophic sulfur-oxidizing bacteria that form intracellular sulfur globules (Brune, 1995a; Dahl, 1999; Dahl and Prange, 2006). In *Alc. vinosum* this envelope is a monolayer of 2–5 nm consisting of three different hydrophobic "sulfur globule proteins" (Sgps) of 10.5 kDa, 10.6 kDa (SgpA and SgpB) and 8.5 kDa (SgpC), while that of the related *Thiocapsa roseopersicina* contains only two proteins of 10.7 and 8.7 kDa (Brune, 1995a; Pattaragulwanit et al., 1998). In *Alc. vinosum* the sulfur globule proteins are synthesized with cleavable amino-terminal signal sequences implying Sec-dependent transport across the cytoplasmic membrane and finally a periplasmic localization of the proteins and therefore the whole sulfur globules. The targeting process was experimentally verified with *phoA* fusions in *E. coli* (Pattaragulwanit et al., 1998) and also in *Alc. vinosum* (Prange et al., 2004). Electron micrographs of two other species of the family *Chromatiaceae* (*Thiocystis violaceae* and *Tca. roseopersicina*) provided further support for an extracytoplasmic localization of the sulfur globules (Pattaragulwanit et al., 1998).

The two larger sulfur globule proteins (SgpA and SgpB) of *Alc.vinosum* are homologous to each other and to the larger protein of *Tca. roseopersicina*. The smaller sulfur globule proteins (SgpC) in *Alc. vinosum* and *Tca. roseopersicina* are also homologous, indicating that these proteins are highly conserved between different species of the family *Chromatiaceae*. Interestingly, all three sulfur globule proteins are rich in glycine and aromatic amino acids, particularly tyrosine. The amino acid sequences contain tandem repeats typically found in cytoskeletal keratin or plant cell wall proteins suggesting that they are structural proteins rather than enzymes involved in sulfur metabolism (Brune, 1995a). A direct/covalent attachment of chains of stored sulfur to the proteins enclosing the globules is unlikely as none of the Sgp proteins sequenced so far contains cysteine residues.

Little is known about the function of the sulfur globule proteins. Proteinaceous envelopes have never been reported for extracellular sulfur globules. Consistent with this observation, neither the complete genome sequences of several green sulfur bacteria (Frigaard and Bryant, 2008) nor the *Hlr. halophila* genome contain homologues of *Alc. vinosum sgp* genes. As outlined above, the sulfur speciation in sulfur globules of anoxygenic phototrophic bacteria is nearly identical irrespective whether it is accumulated in globules inside or outside the cells. It therefore appears that the Sgp proteins themselves are not responsible for keeping the sulfur in a certain chemical structure. Ideas have been promoted, that the protein envelope serves as a barrier to separate the sulfur from other cellular constituents (Shively et al., 1989) and/or that it provides binding sites for sulfur-metabolizing enzymes (Schmidt et al., 1971). In *Alc. vinosum* mutants SgpA and SgpB can replace each other in the presence of SgpC (Pattaragulwanit et al., 1998; Prange et al., 2004). A mutant possessing SgpA and SgpB but lacking SgpC can grow on sulfide and thiosulfate. This mutant forms significantly smaller sulfur globules. SgpC therefore probably plays an important role in sulfur globule expansion. SgpA and SgpB are not fully competent to replace each other as sulfur globule formation is not possible in mutants possessing solely SgpA or SgpB. Experiments with a *sgpBC*$^{-}$ double mutant clearly showed that an envelope is indispensable for the formation and deposition of intracellular sulfur. Neither sulfide nor thiosulfate is oxidized by this mutant (Prange et al., 2004). In *Alc. vinosum* cell survival is absolutely dependent on the presence of at least SgpA even under conditions that do not allow sulfur globule formation (Prange et al., 2004). All three *sgp* genes of *Alc.vinosum* form separate transcriptional units (Pattaragulwanit et al., 1998). All are constitutively expressed, however, the expression of *sgpB* and *sgpC* is significantly enhanced under photolithoautotrophic compared to photoorganoheterotrophic conditions. The *sgpB* gene is expressed ten times less than *sgpA* and *sgpC* implying that SgpA and SgpC are the "main proteins" of the sulfur globule envelope (Prange et al., 2004).

Sulfur globules can also serve as an electron acceptor reserve that allows a rudimentary anaerobic respiration with sulfur. Under anoxic conditions in the absence of light purple sulfur bacteria like *Alc. vinosum* can reduce stored sulfur back to sulfide (van Gemerden, 1968; Trüper, 1978). Nothing is known about the enzymatic mechanisms underlying these processes.

F. Oxidation of Stored Sulfur to Sulfite

The oxidative degradation of these sulfur deposits is one of the most poorly understood areas of sulfur metabolism. In the case of extracellularly deposited sulfur, this process does not only involve oxidation of the sulfur but must include binding, activation and transport into the cells (see above).

The only gene region known so far to be essential for oxidation of stored sulfur was localized by interposon mutagenesis in *Alc. vinosum* (Pott and Dahl, 1998; Dahl et al., 2005). Fifteen open reading frames, designated *dsrABEFHCMKLJOPNRS*, were identified (Figs. 2 and 3). A very similar gene cluster is found in *Hlr. halophila* (Fig. 2), which contains in addition, genes encoding putative regulatory proteins and proteins possibly involved in sulfate transport downstream of *dsrN*. In *Alc. vinosum*, the *dsrAB* products form the cytoplasmic $\alpha_2\beta_2$-structured sulfite reductase. This protein is closely related to the dissimilatory sulfite reductases from sulfate-reducing bacteria and archaea (Hipp et al., 1997). The prosthetic group of DsrAB is siroamide-[Fe_4S_4] with siroamide being an amidated form of the classical siroheme (Lübbe et al., 2006). The *dsrN*-encoded protein resembles cobyrinic acid *a, c* diamide synthases and catalyzes the glutamine-dependent amidation of siroheme. A Δ*dsrN* mutant showed a reduced sulfur oxidation rate. *Alc. vinosum* is apparently able to incorporate siroheme instead of siroamide into sulfite reductase, thereby retaining some function of the enzyme (Lübbe et al., 2006). Adjacent to *dsrAB* the *dsrEFH* genes are located. The products of these three genes show significant similarity to each other. DsrEFH were purified from the soluble fraction and constitute a soluble $\alpha_2\beta_2\gamma_2$-structured 75-kDa holoprotein (Dahl et al., 2005). DsrC is a small soluble cytoplasmatic protein with a highly conserved C-terminus including two conserved cysteine residues. Proteins closely related to DsrEFH and DsrC have recently been shown to act as parts of a sulfur relay system involved

in thiouridine biosynthesis at tRNA wobble positions in *E. coli* (Numata et al., 2006; Ikeuchi et al., 2006). The *dsrM*-encoded protein is predicted to be a membrane-bound *b*-type cytochrome and shows similarities to a subunit of heterodisulfide reductases from methanogenic archaea. The cytoplasmic iron–sulfur protein DsrK exhibits relevant similarity to the catalytic subunit of heterodisulfide reductases. DsrK is predicted to reside in the cytoplasm. DsrP is another integral membrane protein. The periplasmic proteins DsrJ and DsrO are a triheme *c*-type cytochrome and an iron–sulfur protein, respectively. DsrKJO were co-purified from membranes pointing at the presence of a transmembrane electron-transporting complex consisting of DsrMKJOP (Dahl et al., 2005). Individual in frame deletions of the *dsrMKJOP* genes lead to the complete inability of the mutants to oxidize stored sulfur (Sander et al., 2006). In accordance with the suggestion that related complexes from dissimilatory sulfate reducers transfer electrons to sulfite reductase (Pires et al., 2006), the *Alc. vinosum* Dsr complex is co-purified with sulfite reductase, DsrEFH and DsrC (Dahl et al., 2005). DsrL is a cytoplasmic iron–sulfur flavoprotein with NADH: acceptor oxidoreductase activity (Y. Lübbe and C. Dahl, unpublished). *In frame* deletion of *dsrL* completely inhibited the oxidation of stored sulfur (Lübbe et al., 2006). DsrR and DsrS are soluble cytoplasmic proteins of unknown function. The *dsr* genes, with the exception of the constitutively expressed *dsrC*, are expressed and the encoded proteins are formed at a low basic level even in the absence of sulfur compounds. An increased production of all Dsr proteins is induced by sulfide and/or stored sulfur (Dahl et al., 2005).

The mechanism by which the periplasmically stored sulfur is made available to the cytoplasmic sulfite reductase is unclear. In sulfate-reducing bacteria dissimilatory sulfite reductase catalyzes the six electron reduction of sulfite to sulfide. It has therefore been proposed that the sulfur is reductively activated, transported to and further oxidized in the cytoplasm by sulfite reductase operating in reverse. Different models have been suggested to explain the roles of the *dsr*-encoded proteins in such a scenario (Dahl et al., 2005; Pott and Dahl 1998). A modified model is shown in Fig. 4. Here, the NADH: acceptor oxidoreductase activity of DsrL is taken into account. Interestingly, the protein carries a thioredoxin motif CysXX-Cys immediately preceding the carboxy-terminal iron–sulfur cluster binding sites. This indicates a potential disulfide reductase activity. Therefore, the possibility exists that DsrL uses NADH as electron donor for reduction of a di- or persulfidic compound. DsrL could be involved in the reductive release of sulfide from a carrier molecule – probably an organic perthiol – that may transport sulfur from the periplasmic sulfur globules to the cytoplasm where it is further metabolized by Dsr proteins (Dahl et al., 2005). Glutathione amide is a likely candidate for carrying sulfur from the periplasm to the cytoplasm. Glutathione amide bears an amide group at the glycyl moiety of glutathione and is especially resistant to autoxidation. The compound was found to be largely in the persulfidic state when *Alc. vinosum* was cultured photoautotrophically on sulfide (Bartsch et al., 1996). Recently, transporters have been characterized in *E. coli* mediating export (Pittman et al., 2005) and import (Suzuki et al., 2005) of glutathione. Shuttling of glutathione amide between cytoplasm and periplasm in purple sulfur bacteria like *Alc. vinosum*, therefore also appears feasible. DsrL, being an essential protein for sulfur oxidation, is co-purified with the sulfite reductase (Y. Lübbe and C. Dahl, unpublished). Sulfide released from the perthiol could therefore be directly passed to *dsrAB*-encoded sulfite reductase thereby reducing losses caused by evaporation of gaseous H_2S. Obviously, *Alc. vinosum* sulfite reductase specifically interacts with the soluble protein DsrL on one hand and with membrane-bound Dsr proteins and DsrEFHC on the other hand. Electrons released from the oxidation of sulfide by sulfite reductase may be fed into photosynthetic electron transport via DsrC and DsrMKJOP, which would be analogous to the pathway postulated for sulfate reducers, operating in the reverse direction. DsrM could operate as a quinone reductase, DsrP as a quinol oxidase and finally the *c*-type cytochrome DsrJ would be reduced (Dahl et al., 2005). From here, electrons could be transferred to HiPIP, the primary electron donor to the photosynthetic reaction center (Vermeglio et al., 2002). The function of DsrEFH remains unclear, but as it occurs exclusively in sulfur oxidizers and shows some interaction with DsrC, it may be important for the pathway to operate in the sulfide oxidizing direction. On the

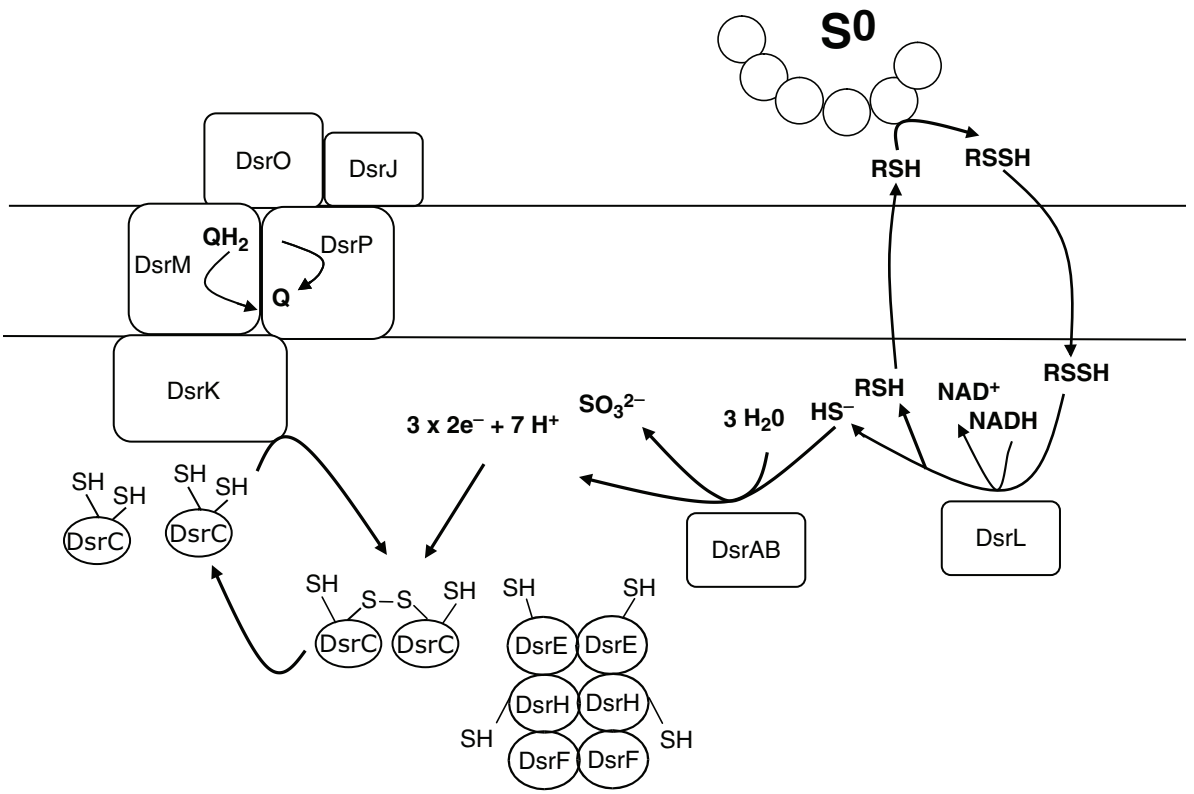

Fig. 4. Schematic presentation of Dsr proteins from *Allochromatium vinosum*. The scheme is based on sequence analysis of the encoding genes and on biochemical information where available. The products of the *dsrS* and *dsrR* genes are not shown for clarity because biochemical information is not available and possible functions cannot be predicted on the basis of sequence homologies. Both proteins are predicted to be soluble and to reside in the cytoplasm. DsrN is also not shown as it does not participate in redox or sulfur transfer reactions but is involved in biosynthesis of siroamide. Siroamide-[4Fe–4S] is a prosthetic group of sulfite reductase.

other hand, sulfur transfer reactions as performed by the related TusBCD and TusE proteins in *E. coli* (Ikeuchi et al., 2006) could be important for the Dsr-catalyzed sulfite formation pathway. As there is no experimental evidence available in this direction so far, this possibility is not taken into account in the model presented in Fig. 4.

G. Oxidation of Sulfite to Sulfate

In the final step of sulfur compound oxidation in purple sulfur bacteria, sulfite is oxidized to sulfate. Some purple sulfur bacteria can also grow on externally supplied sulfite (Table 1). As evident from Fig. 4 sulfite arising from the oxidation of more reduced sulfur compounds is generated in the bacterial cytoplasm. Two fundamentally different pathways for sulfite oxidation have been rather well characterized in a number of chemotrophic and phototrophic sulfur oxidizers (Kappler and Dahl, 2001): (a) direct oxidation by a, probably molybdenum-containing, sulfite dehydrogenase (EC 1.8.2.1); and (b) indirect, AMP-dependent oxidation via the intermediate adenylylsulfate (adenosine 5′-phosphosulfate, APS).

The simultaneous presence of both enzymatic activities has been established for a number of chemo- and photolithotrophic sulfur oxidizers belonging to the β- and γ-Proteobacteria (e.g. *Thiobacillus denitrificans, Thiobacillus thioparus*, *Allochromatium vinosum*, strains of *Thiocapsa roseopersicina*) and green sulfur bacteria (Trüper and Fischer, 1982; Brune, 1995b; Kappler and Dahl, 2001). So far, there is no evidence for an occurrence of the sulfite-oxidizing form of the

APS reductase pathway in *Alphaproteobacteria* or in *Ectothiorhodospiraceae*. In accordance, potential APS reductase genes (*aprBA*, see below) are not found in the genome of *Hlr. halophila*. It has to be kept in mind that in some cases (*Beggiatoa*, *Chromatiaceae*, green sulfur bacteria) the occurrence of one or both sulfite oxidation pathways can vary between different strains of the same genus or between genera of the same family (Kappler and Dahl, 2001; Frigaard and Bryant, 2008).

1. Indirect Pathway via Adenylylsulfate (APS)

During indirect sulfite oxidation, APS is formed from sulfite and AMP by APS reductase (EC 1.8.99.2). In a second step the AMP moiety of APS is transferred either to pyrophosphate by ATP sulfurylase (ATP:sulfate adenylyltransferase, EC 2.7.7.4), or to phosphate by adenylylsulfate:phosphate adenylyltransferase (APAT, formerly ADP sulfurylase (Brüser et al., 2000)), resulting in the formation of ATP or ADP, respectively. Since ADP can be converted to ATP and AMP by adenylate kinase, both sulfate-liberating enzymes catalyze substrate phosphorylations, which are of energetic importance, especially in chemolithoautotrophic bacteria (Peck, 1968). The APS pathway can also function in sulfate reduction, serving assimilatory and dissimilatory purposes. While the APS reductases from dissimilatory sulfate reducers resemble the enzymes found in sulfur oxidizers (Hipp et al., 1997), the APS reductases functioning in assimilatory sulfate reduction studied so far are completely different enzymes related to 3′-phosphoadenosine-5′-phosphosulfate (PAPS) reductases (Bick et al., 2000; Kopriva et al. 2001). Concerning this topic, consult also the chapter by Kopriva et al. in this book.

Indirect AMP-dependent oxidation of sulfite to sulfate via APS (Fig. 3) occurs in the bacterial cytoplasm with APS reductase being membrane-bound (e.g. in many *Chromatiaceae*) or soluble, and ATP sulfurylase and APAT being soluble enzymes (Brune, 1995a; Brüser et al., 2000). In *Alc. vinosum* the genes for ATP sulfurylase (*sat*) and APS reductase (*aprMBA*, with *aprM* encoding a putative membrane anchor) form an operon ((Hipp et al., 1997), A. Wynen, H. G. Trüper, C. Dahl, unpublished, GenBank No. U84759, Fig. 2). In the genomes of four green sulfur bacteria the genes for ATP sulfurylase and APS reductase are located directly adjacent to each other (Frigaard and Bryant, 2008). Genes related with *aprM* are not present. Instead, the green sulfur bacterial APS reductase and ATP sulfurylase genes are always clustered with genes encoding a Qmo complex (*qmoABC*). A closely related complex was biochemically characterized from the sulfate reducer *Desulfovibrio vulgaris*, shown to have quinol-oxidizing activity and proposed to deliver electrons form membrane-bound quinols to APS reductase (Pires et al., 2003). In phototrophic sulfur oxidizers containing *qmo* genes, the situation could just be opposite and the Qmo complex could accept electrons from APS reductase operating in the sulfite-oxidizing direction. We propose that the membrane protein AprM serves an analogous function in *Alc. vinosum*.

APS reductase activity is usually measured as AMP-dependent sulfite oxidation with ferricyanide or *c*-type cytochromes. Substrate inhibition by AMP is characteristic for APS reductases (Taylor, 1994; Hagen and Nelson, 1997). All investigated dissimilatory APS reductases irrespective of metabolic type have been characterized as heterodimers with one α-subunit of 70–75-kDa (1 FAD) and one β-subunit of 18–23 kDa (2 [4Fe-4S] centers) (Fritz et al., 2000). Additional subunits mediating membrane association may be present (Hipp et al., 1997). The heme groups originally reported for the enzyme from the purple sulfur bacterium *Thiocapsa roseopersicina* were due to a contaminating protein (Brune, 1995b). A catalytic mechanism has been proposed in which sulfite initially forms a complex with the flavin (Brune (1995b) and references therein). This then reacts with AMP to yield APS, releasing two electrons that are transferred via the flavin to the iron–sulfur centers.

The best characterized ATP sulfurylase (Sat) from a sulfur-oxidizing bacterium is the enzyme from the endosymbiont of the hydrothermal vent worm *Riftia pachyptila* (Renosto et al., 1991; Beynon et al., 2001). Like all other ATP sulfurylases the enzyme is strictly Mg^{2+}-dependent. The V_{max} of ATP synthesis is seven times higher than that of molybdolysis, the assay used for measuring the APS-producing reaction. The *Riftia* symbiont enzyme also has a higher k_{cat} for the ATP synthesis direction (257 s^{-1} compared to 64 s^{-1}

for the assimilatory enzyme from *Penicillium chrysogenum* that works in the sulfate activating direction (Renosto et al., 1991)). The native enzyme appears to be a dimer (MW 90 kDa) composed of identical size subunits (396 residues). The ATP sulfurylase from *Alc. vinosum* is isolated as a monomer with an apparent molecular mass of 45 kDa (A. Wynen, C., Dahl, H. G. Trüper, unpublished). More information is available for ATP sulfurylases from sulfate-assimilating or sulfate-reducing organisms in which the activation of the chemically extremely inert sulfate by adenylylation is the relevant reaction. Two completely different, unrelated types of ATP sulfurylase can be distinguished: The heterodimeric CysDN type occurs exclusively in sulfate-assimilating prokaryotes, e.g., *E. coli* (Leyh, 1993). The other ATP sulfurylases characterized in sufficient detail are monomers or homo-oligomers of 41–69 kDa (Sperling et al., 1998; Gavel et al., 1998; Yu et al., 2007). Size variations are due to APS kinase or PAPS-binding allosteric domains residing on the same polypeptide in some cases. Five highly conserved regions are present, two of which are rich in basic amino acids, suggesting that they may participate in binding of $MgATP^{2-}$ and SO_4^{2-}.

The existence of APAT as an independent entity has been questioned for a long time. In 2000 the enzyme was finally purified from *Thiobacillus denitrificans* (Brüser et al., 2000): The enzyme is a homodimer of 41.4-kDa subunits. The K_M values for APS and phosphate are 300 µM and 12 mM, respectively. The pH optimum is 8.5–9.0. Catalysis is strictly unidirectional and occurs by a Ping-Pong mechanism with a covalently bound AMP as intermediate. Histidine modification suggested a histidine as the nucleotide binding residue. APAT is related to galactose-1-phosphate uridylyltransferase and diadenosine 5′, 5‴-P^1, P^4-tetraphosphate (Ap_4A) phosphorylase. Ap_4′A phosphorylase from yeast also has APAT activity while APAT from *Thiobacillus denitrificans* does not exhibit Ap_4A phosphorylase activity. The *in vivo* function of the latter enzyme may therefore indeed be the formation of ADP and sulfate from phosphate and APS. However, genetic evidence for this assumption is currently missing. The in vivo role of APAT is especially difficult to assign because all organisms with significant APAT activity (> 100 mU mg^{-1} in crude extracts) also contain ATP sulfurylase. It has been hypothesized that APAT may serve to ensure a high turnover of APS under pyrophosphate limiting conditions as this enzyme is independent of the energy-rich pyrophosphate molecule (Brüser et al., 2000). In *Alc. vinosum* APAT does not appear to be present while significant activity was found in strains of *Tca. roseopersicina* (Dahl and Trüper, 1989).

2. Direct Pathway

Two types of enzymes catalyzing direct oxidation of sulfite to sulfate are well characterized, the sulfite oxidases that can transfer electrons to oxygen, ferricyanide and sometime cytochrome *c* and the sulfite dehydrogenases that can use one or both of the latter electron acceptors but not oxygen (Kappler and Dahl, 2001; Kappler, 2007). The oxygen-dependent enzymes are not relevant in anoxygenic phototrophic bacteria.

All sulfite dehydrogenases characterized to date belong to the sulfite oxidase family of molybdoenzymes comprising established sulfite-oxidizing enzymes and proteins related to these as well as assimilatory nitrate reductases from plants (Hille, 1996). The active site is formed by a single molydopterin cofactor. Additional redox active centers may be present. The best characterized sulfite-oxidizing enzymes from the sulfite oxidase family are those from avian and mammalian sources (Kisker et al., 1997) that are homodimers containing heme *b* and molybdenum coordinated via an MPT-type molybdenum pterin cofactor, a conserved cysteine residue from the enzyme and two oxo groups. The SorAB protein from *Starkeya novella* (formerly *Thiobacillus novellus*) was the first true bacterial sulfite-oxidizing enzyme to be characterized in detail (Kappler et al., 2000; Feng et al., 2003; Kappler and Bailey, 2005; Raitsimring et al., 2005; Doonan et al., 2006). It is a periplasmic heterodimer of a large MoCo-dimer domain (40.2 kDa) and a small cytochrome *c* subunit (8.8 kDa). Its molybdenum pterin cofactor is of the MPT-type with a 1:1 ratio between Mo and MPT. During catalysis, electrons are sequentially transferred to a single heme c_{552} ($E_{m8.0}$ = +280 mV) located on the smaller subunit and passed on from there to a cytochrome c_{550} from the same organism, thought to be the enzyme's natural electron acceptor. The enzyme exhibits the Ping–Pong mechanism that is also found in eukaryotic sulfite oxidases and is non-competitively inhibited by

sulfate. It is encoded by the *sorAB* genes, which appear to form an operon by themselves. Characterized related proteins also appear to be localized in the periplasm and to contain a heme *c*-binding subunit (Myers and Kelly, 2005).

Biochemical studies and most importantly the sequencing of a large number of bacterial genomes in the past few years revealed that many bacterial genes exist that encode proteins belonging into the sulfite oxidase family (Kappler, 2007). While the well-characterized bacterial sulfite dehydrogenases are soluble proteins, membrane-bound bacterial sulfite-oxidizing enzymes have also been reported in the literature (reviewed in Kappler and Dahl, 2001; Kappler, 2007). Most of the established or predicted soluble members of the sulfite oxidase family are periplasmic enzymes, however, some of the proteins belonging to this group (however without a biochemically characterized function) are predicted to reside in the bacterial cytoplasm (Kappler, 2007). Direct oxidation of sulfite to sulfate in the bacterial cytoplasm can, therefore, not generally be excluded.

3. Sulfite Oxidation in Purple Sulfur Bacteria: an Unresolved Question

Although enzymes participating in the indirect sulfite oxidation pathway in purple sulfur bacteria have been studied for more than 30 years (Trüper and Rogers, 1971) their in vivo role is still questionable. In *Alc. vinosum* APS reductase is clearly dispensable (Dahl, 1996): The growth rates of the wild type and an APS-reductase-deficient mutant show little differences under light-limiting conditions. A difference is observed only at saturating irradiances. Under these conditions, the wild type grows considerably faster, indicating that the presence of a second pathway of sulfite oxidation allows a higher rate of supply of reducing power (Sanchez et al., 2001).

Experiments with cultures grown in the presence of the molybdate antagonist tungstate indicated that APS reductase-independent sulfite oxidation in *Alc. vinosum* is catalyzed by a molybdenum-containing enzyme. Sulfite oxidation was severely inhibited by tungstate in an APS-reductase deficient mutant, suggesting the involvement of a classical molydopterin-containing enzyme of the sulfite oxidase family (Dahl, 1996). However, it should be noted that genes related to *sorAB* cannot be detected in *Alc. vinosum* nor have the proteins been detected using antibodies (e.g. against SorAB from *Starkeya novella*, U. Kappler and C. Dahl, unpublished). This finding appears even more interesting when we realize that a gene homologous to those encoding proteins of the sulfite oxidase family is neither present in the genome of *Hlr. halophila* nor in any of the green sulfur bacterial genome sequences. As *Hlr. halophila* and some of the green sulfur bacteria do not possess genes encoding for the APS pathway, they must have a different means for sulfite oxidation. Frigaard and Bryant (2008) present the very attractive speculation that a potential protein encoded by three genes resembling those for polysulfide reductase from *Wolinella succinogenes* (Krafft et al., 1992) could play this role. In this regard, it appears rather conspicuous that three related genes (Hhal1934, 1935 and 1936) are also found in the sulfur gene cluster of *Hlr. halophila* (Fig. 2). Similar to the situation in green sulfur bacteria, the molydopterin-binding putative active site-bearing subunit (PsrA) would be localized in the cytoplasm. On the other hand, we have some indications that a *soxY*-deficient mutant of *Alc. vinosum* is severely impaired in the oxidation of sulfite (D. Hensen, B. Franz and C. Dahl, unpublished). Clearly, the question of sulfite oxidation in phototrophic sulfur bacteria will require special attention in the future.

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