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Sulfur: From Acquisition to Assimilation

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INTRODUCTION

Sulfur (S) is an essential element present in proteins, lipids, and important metabolites (Meister and Anderson, 1983; Gupta et al., 1990; Grossman and Takahashi, 2001), including signaling molecules (Schultze et al., 1992). S can be limiting in the environment and also can limit plant productivity



in certain agricultural settings (Mahler and Maples, 1986, 1987; Warman and Sampson, 1994). In recent history many soils have accumulated high S levels, resulting either from administration of fertilizers contaminated with SO_4^{2-} salts, or exposure to pollutants present in acid rain (Cole and Johnson, 1977; Johnson et al., 1982; David et al., 1988; MacDonald et al., 1991). However, most organisms are unable to store S, making growth and development dependent on external S sources. Therefore, with increased fertilizer purity and decreased occurrence of acid rains, low levels of available S in diverse ecosystems can limit growth and development of plants (Marschner, 1995) and microbes. This chapter describes the pathways associated with SO_4^{2-} assimilation in *Chlamydomonas*, the multiplicity of ways in which photosynthesis in eukaryotes can acclimate to limiting S conditions, and the regulatory elements associated with this acclimation process. It also uses *Chlamydomonas* genomic (Merchant et al., 2007) and experimental information to highlight the similarities and differences of assimilation and acclimation processes between *Chlamydomonas* and vascular plants.

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I. SO_4^{2-} ACQUISITION AND ASSIMILATION

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A. Overview

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The SO_4^{2-} anion, usually the most abundant S form in soils, can be taken up by plants and microbes, activated, and used for sulfation of molecules such as lipids, polysaccharides and proteins, or incorporated into various compounds, including cysteine and methionine, by reductive assimilation (Bick and Leustek, 1998; Leustek and Saito, 1999; Saito, 2000; Smith et al., 2000). A summary of pathways used in the assimilation of SO_4^{2-} , the genes associated with assimilation, and the acclimation of *Chlamydomonas* to S-limiting conditions, are given in Figure 5.1 and Table 5.1.

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B. Hydrolysis

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The majority of SO_4^{2-} may not be bioavailable for most organisms in some soils. This is a result of leaching of SO_4^{2-} through the soil matrix and its tight adsorption onto the surface of soil particles. Furthermore, a large proportion of soil SO_4^{2-} may be covalently bonded to organic molecules in the form of sulfate esters and sulfonates (C–S bonds). Finally, plants and microbes release a variety of S-containing organic compounds (e.g. proteins, lipids, polysaccharides, dimethylsulfoniopropionate) when they die, which are usually rapidly cycled into the available S pool through the action of hydrolytic enzymes in the soil: these enzymes are mostly released by microorganisms.

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Many microorganisms synthesize and secrete aryl- and alkyl-sulfatases and sulfonatases, which can cleave SO_4^{2-} from organic S compounds in the



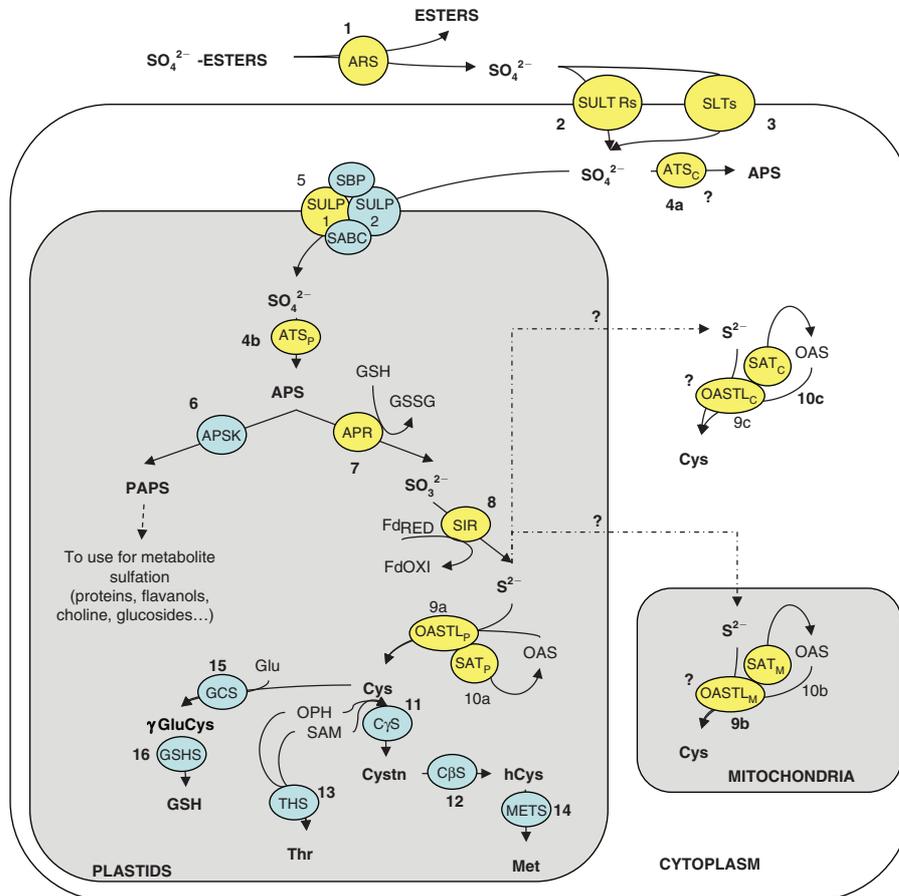


FIGURE 5.1 SO₄²⁻ assimilation pathway. **1**, arylsulfatase; **2**, H⁺/SO₄²⁻ transporter family; **3**, Na⁺/SO₄²⁻ transporter family; **4**, ATP sulfurylase, with two hypothetical localizations: the cytoplasm (ATSc) and plastid (ATSp); **5**, chloroplast SO₄²⁻ transport system; **6**, APS kinase; **7**, APS sulfotransferase; **8**, sulfite reductase; **9**, acetylserine(thiol)lyase, with three possible localizations as indicated by subscripts: cytoplasm (c), plastid (p), or mitochondrion (m); **10**, serine acetyltransferase, with three possible localizations as indicated by subscripts: cytoplasm (c), plastid (p), or mitochondrion (m); **11**, Cystathionine γ-synthase; **12**, Cystathionine β-synthase; **13**, threonine synthase; **14**, methionine synthase; **15**, γ-glutamylcysteine synthetase; **16**, glutathione synthetase. Proteins encoded by genes that have been shown to be up-regulated under S starvation conditions in *Chlamydomonas* are shown in yellow. Protein designations along with their abbreviations are presented in Table 5.1. APS, adenosine 5'-phosphosulfate; PAPS, 3'-phosphoadenosine 5'-phosphosulfate; GSH, reduced glutathione; GSSG, oxidized glutathione; S²⁻, sulfide; OAS, O-acetylserine; Cystn, cystathionine; hCys, homocysteine; SAM, S-adenosylmethionine; OPH, O-phosphohomoserine; γGluCys, γ-glutamylcysteine.

soil. Sulfatases hydrolyze esterified sulfate from organic molecules, which promotes S cycling. The synthesis of sulfatases is often regulated by SO₄²⁻ availability. *Chlamydomonas* makes a prominent, extracellular, cell-wall-associated arylsulfatase (ARS) in response to S limitation (Schreiner et al.,

Table 5.1 Genes involved in S assimilation and those up-regulated during S deprivation

	Activity	Genes	Proteins	References	Accession number
1	Arylsulfatase	ARS1 ARS2	ARS1 ARS2	[1], [2], [3], [4] [3]	EDP04612 EDP04462
2	Sulfate transporter (H ⁺ /SO ₄ ²⁻ family)	<i>SULTR1</i> SULTR2 <i>SULTR3</i> <i>SULTR4</i>	SULTR1 SULTR2 SULTR3 SULTR4	unpublished	[no accession #] EDP00477 EDP00036 EDO99355
3	Sulfate transporter (Na ⁺ /SO ₄ ²⁻ family)	SLT1 SLT2 <i>SLT3</i>	SLT1 SLT2 SLT3	unpublished [4]	EDP05421 EDP05750 EDP05749
4	ATP sulfurylase	ATS1 ATS2	ATS1 ATS2	[3], [4], [5] [4], [6]	EDP03369 EDP07432
5	Chloroplast sulfate transporter (subunits)	SULP1 <i>SULP2</i> <i>SABC</i> <i>SBP1</i>	SULP1 SULP2 SABC SBP1	[7], [8] [9] [9] [9]	EDP03937 EDP10015 EDP08170 EDP08595
6	APS kinase	<i>APK1, AKN</i>	APK	[4]	EDP01296
7	APS sulfotransferase	APR, MET16	APR	[3], [4]	EDP00776
8	Sulfite reductase	SIR1 <i>SIR2</i> <i>SIR3</i>	SIR1 SIR2 SIR3	[4] [4]	EDO98734 [no accession #] EDP05929
9	O-acetylserine(thiol)-lyase	<i>ASL1, OASTL1</i> <i>ASL2, OASTL2</i> <i>ASL3, OASTL3</i> ASL4, OASTL4, Cys1A	OASTL1 OASTL2 OASTL3 OASTL4	[3], [4], [10]	EDP01088 EDO97451 EDP05983 EDP04425, EDP04426*
10	Serine acetyltransferase	SERAT1, SAT1 <i>SERAT2, SAT2</i>	SAT1 SAT2	[3], [4]	EDP07949, EDP07950*# EDO98094
11	Cystathionine γ -synthase	<i>CGS1, METB</i>	METB		EDP01529
12	Cystathionine β -lyase	<i>CBS, METC</i>	METC		EDO98869
13	Threonine synthase	<i>THS</i>	THS		EDP08010
14	Methionine synthase	<i>METH</i> <i>METE</i>	METH METE	[11] [11]	EDP08397 EDO96787
15	γ -Glutamylcysteine synthetase	<i>GSH1</i>	GSH1		EDP06622
16	GSH synthetase	<i>GSH2</i>	GSH2		EDP04651
–	Extracellular polypeptides	ECP76 ECP88	ECP76 ECP88	[4], [12] [12]	EDP01767 EDP02984
–	Selenium binding protein	SPD1, SBDP	SBDP	[4]	EDP06040
–	Positive regulatory protein	<i>SAC1</i>	SAC1	[3], [4], [13]	EDP00156
–	Negative regulatory protein	<i>SAC3</i>	SAC3	[3], [14], [15]	EDO96994

The numbers in the first column correspond to those used in Figure 5.1. For those genes that have been shown to be up-regulated during S deprivation, the gene designations are in bold. The symbol * indicates that more than one transcript appears to be encoded by the gene. ([1] de Hostos et al. (1989); [2] Davies et al. (1994); [3] Ravina et al. (2002); [4] Zhang et al. (2004); [5] Yildiz et al. (1996); [6] Allmer et al. (2006); [7] Chen et al. (2003); [8] Chen and Melis (2004); [9] Melis and Chen (2005); [10] Ravina et al. (1999); [11] Croft et al. (2006); [12] Takahashi et al. (2001); [13] Davies et al. (1996); [14] Davies et al. (1999); [15] Irihimovitch and Stern (2006).

1975; de Hostos et al., 1988, 1989). The protein has at least three O-linked oligosaccharides, is stable in the extracellular space, and is translated as a precursor protein with a signal sequence that is cleaved as it is exported from the cell (de Hostos et al., 1988). The extracellular location of ARS allows it to hydrolyze soluble sulfate esters in the medium, releasing free SO₄²⁻ for assimilation. Identification of the ARS polypeptide (de Hostos et al., 1988) led to the cloning and characterization of two ARS genes, *ARS1* and *ARS2* (de Hostos et al., 1989; Ravina et al., 2002), that are contiguous on the genome and arranged in a tail-to-tail orientation. Transcripts from both of these genes increase in response to S deprivation (de Hostos et al., 1989; Ravina et al., 2002).

p0050 Examination of the *Chlamydomonas* genome has revealed the presence of 18 putative ARS genes, many of which are physically linked in gene clusters (Figure 5.2A; the linked genes often show high sequence similarity (Figure 5.2B). This organization suggests a common evolutionary origin and the potential for similar regulatory features. It is not clear if the different ARS genes are distinct with respect to substrate specificity, subcellular localization or catalytic properties, and it is not known how most of the individual genes (other than *ARS1* and *ARS2*) respond during exposure of the cells to S deprivation. While all of the ARS genes have the canonical sulfatase signature, the ARS1 and ARS2 polypeptides are the most similar (98.5% identity), which might reflect a recent gene duplication.

s0050 C. Transport

s0060 1. Overview

p0060 Initial studies with *Chlamydomonas* demonstrated that the maximum velocity (V_{\max}) and the substrate concentration at which SO₄²⁻ transport was at half-maximum velocity ($K_{1/2}$) were altered when S-replete cells were transferred to medium devoid of S; the V_{\max} for SO₄²⁻ increased by approximately 10-fold while the $K_{1/2}$ decreased by roughly 7-fold (Yildiz et al., 1994). Higher affinity SO₄²⁻ transport could be detected within an hour of S deprivation. The development of enhanced SO₄²⁻ transport activity following S starvation was blocked by the addition of cycloheximide (an inhibitor of cytosolic translation) to cell cultures, but not by the addition of chloramphenicol (an inhibitor of chloroplast translation). These results demonstrate the requirement for cytosolic protein synthesis during the development of new transport activities following S deprivation. Moreover, SO₄²⁻ transport in *Chlamydomonas* is an energy-dependent process, and may be driven by a proton gradient generated by a plasma membrane ATPase (Yildiz et al., 1994).

p0070 Based on whole genome analysis and EST information, seven putative eukaryotic-type SO₄²⁻ transporter genes have been identified in *Chlamydomonas*. Four of these are of the plant type (H⁺/SO₄²⁻ co-transporters) and are designated *SULTR1*, *SULTR2*, *SULTR3*, and *SULTR4*. The

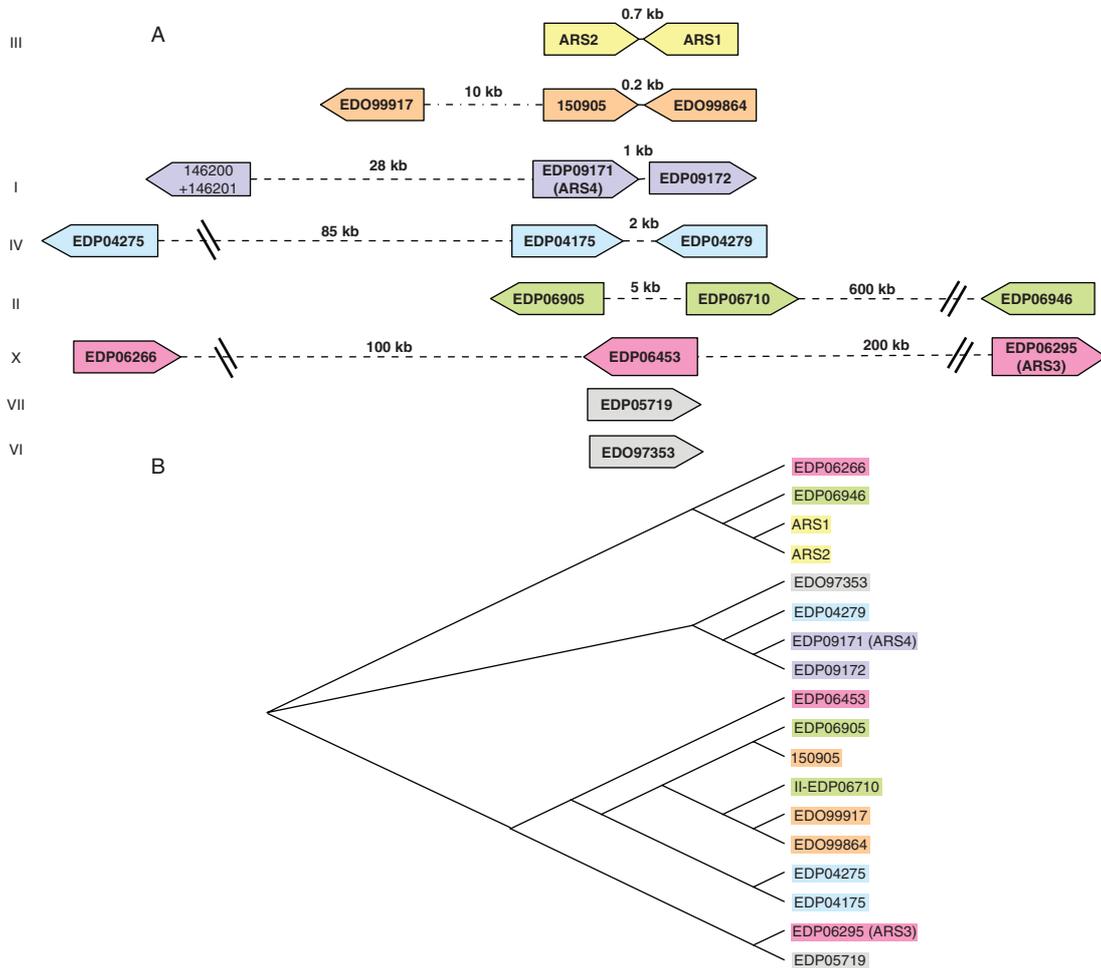


FIGURE 5.2 The ARS gene family. (A) ARS and ARS-like genes on the *Chlamydomonas* genome. Blocks represent ARS genes and show their orientations. The distances between genes are indicated in kb. (B) Phylogenetic tree of the ARS-like genes (BioEdit 7.0.5.3), showing GenBank accession numbers for the proteins predicted from the genomic sequence.

three remaining SO_4^{2-} transporters are of the animal type ($\text{Na}^+/\text{SO}_4^{2-}$ co-transporters) and are designated *SLT1*, *SLT2*, and *SLT3*. Neither functionality nor cellular localizations for these transporters have been demonstrated using biochemical and/or genetic approaches.

2. Plant type $\text{H}^+/\text{SO}_4^{2-}$ co-transporters

The putative plasma membrane $\text{H}^+/\text{SO}_4^{2-}$ transporters of *Chlamydomonas* encode polypeptides with strong sequence similarity to the SLC26A anion family of polypeptides. Similar proteins are required for the transport of SO_4^{2-} across the plasma membrane and tonoplast membranes in vascular

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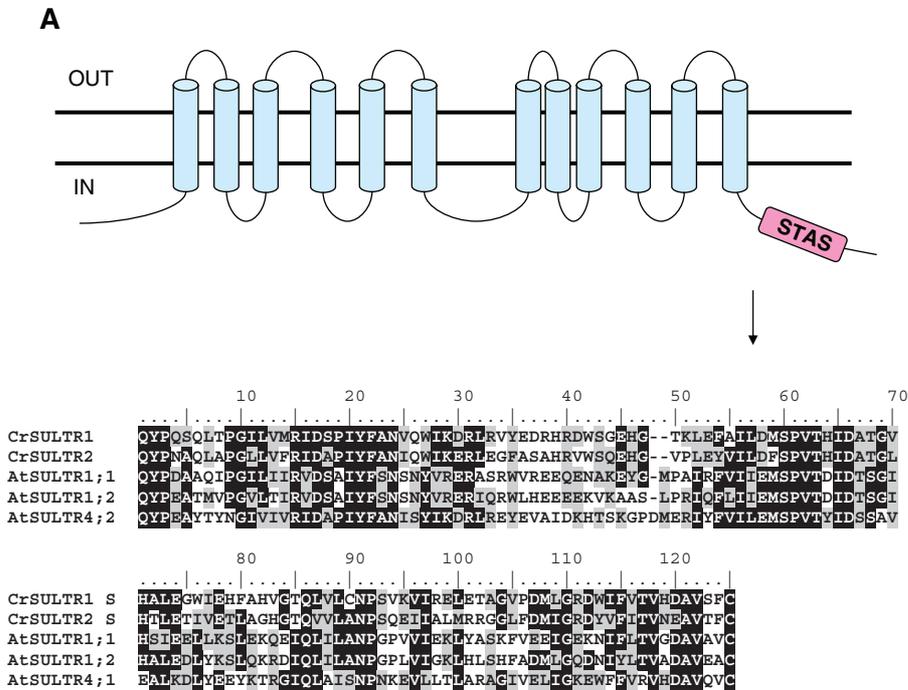


FIGURE 5.3 SO₄²⁻ transporter structure. (A) Representation of the structure of a H⁺/SO₄²⁻ transporter in the membrane, showing 12 transmembrane domains and the carboxy-terminal STAS domain that protrudes into the cytoplasm. (B) ClustalW alignment of STAS domains present in SULTR1 and SULTR2 of *Chlamydomonas*, and SULTR1;1, SULTR 1;2, and SULTR 4;1 of *Arabidopsis*. The vertical arrow indicates the putative phosphorylation site in the STAS domain. The alignment was performed using BioEdit v.7.0.5.3.

plants, while the chloroplast appears to use a bacterial-type transport system. Genes encoding H⁺/SO₄²⁻ transporters from numerous plants have been identified (Smith et al., 1997; Takahashi et al., 2000; Howarth et al., 2003). Many of these have been associated with SO₄²⁻ transport function based on rescue of the methionine-dependent phenotype of a *Saccharomyces cerevisiae* (yeast) null mutant for the major SO₄²⁻ transporters, *Sul1* and *Sul2*. Furthermore, many of these transporters have been localized to specific subcellular compartments, and show increased expression (Takahashi et al., 2006) when plant growth is limited by the SO₄²⁻ concentration. In *Chlamydomonas* only the *SULTR2* transcript increases in respond to S deprivation (unpublished).

Like most other nutrient transporters in eukaryotes, the H⁺/SO₄²⁻ transporters have between 10 and 14 predicted transmembrane domains (TMD) that can be divided into two symmetrical sets of similar TMDs oriented in opposite directions along the polypeptide chain (e.g. in Figure 5.3A, TMD 1–6 have duplicated to form TMD 7–12, but they are in the inverse orientation). The H⁺/SO₄²⁻ transporters also contain a cytosolic

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carboxy-terminal sequence that has been designated the STAS domain (Aravind and Koonin, 2000; Figure 5.3B). This domain is attached to the catalytic moiety of the transporter through a region of low conservation and of highly variable length, designated the linker (L) domain. The STAS domain has sequence (Duncan et al., 1996) and structural (Rost et al., 1997; based on the PHD-POPITS program) similarity to the bacterial anti-sigma factor antagonist SpoIIAA, although STAS domains are generally not highly conserved. The SpoIIAA polypeptide interacts with SpoIIAB and undergoes a cycle of phosphorylation–dephosphorylation that controls the heterologous protein–protein interaction, which in turn serves to regulate sporulation in *Bacillus subtilis* (Ho et al., 2003). *Chlamydomonas* SULTR3 and SULTR4 are the most diverged from plant-type transporters, with the latter lacking the STAS domain. However, these observations should be viewed as preliminary since the gene models for SULTR3 and SULTR4 are not supported by EST data.

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The STAS domain is critical for normal function of many anion transporters, and mutations that cause diastrophic dysplasia and Pendred's syndrome in humans map to STAS domains (Everett and Green, 1999). Furthermore, like the SpoIIAA, the STAS domain may bind nucleotide triphosphates (Najafi et al., 1996) and be modulated in its function via a phosphorylation–dephosphorylation cycle. These features suggest that the STAS domain serves a general regulatory function. Studies of plant SO_4^{2-} transporters also demonstrated a STAS domain requirement for transport function (Rouached et al., 2005; Shibagaki and Grossman, 2004, 2006).

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Based on structural analyses, the STAS domain is thought to consist of four β -strands forming a β -sheet, surrounded by five α -helices. The β -sheet, in association with hydrophobic surfaces of the α -helices, forms a hydrophobic core that is not readily accessible to the external medium. In contrast, the externally exposed surfaces of the α -helices and the loops between α -helices and β -strands are predicted to be available for interactions with other molecules (Rouached et al., 2005). Random mutations introduced into the STAS and L domains of SULTR1;2 of *Arabidopsis* demonstrated that mutations that affect the β -strands often alter the stability and/or biogenesis of the transporter, whereas lesions introduced into specific α -helices often affect transport activity without affecting protein abundance (Shibagaki and Grossman, 2006). Furthermore, mutations in the L domain of SULTR1;2 mainly alter transporter activity, although some mutations can affect biogenesis. For the SLC26A family of transport proteins, the STAS domain appears to participate in some aspects of homeostatic regulation by influencing transport-dependent/associated processes. For example, the DRA Cl^- - HCO_3^- antiporter STAS domain can directly interact with the Cystic Fibrosis Transmembrane Conductance Regulator, which is a cAMP-regulated Cl^- channel, and this interaction modulates the activity of both the transporter and the channel (Ko et al., 2004).



The results discussed above, generated from studies of both plants and animal systems, would suggest that the L-STAS regions of the *Chlamydomonas* SULTR proteins are involved in transporter function and regulation and/or the responses of the alga to S deprivation.

s0080 **3. Animal type Na⁺/SO₄²⁻ co-transporters**

p0120 *Chlamydomonas* contains three genes encoding proteins with strong sequence similarity to mammalian Na⁺/SO₄²⁻ transporters (*SLT* genes). Two of these, *SLT2* and *SLT3*, are arranged in tandem and in the same orientation. The transcripts from *SLT1* and *SLT2* increase when the cells are deprived of depleted (Zhang et al., 2004). The putative SLT transporters have significant sequence similarity to a regulatory element that controls *Chlamydomonas* responses to S deprivation, designated SAC1 (Davies et al., 1996) (see below), as shown in Figure 5.4A. The Na⁺/SO₄²⁻ transporters have no apparent STAS domain but do contain TrkA-C domains, which have been associated with potassium transport and which may bind NAD⁺, but as yet have no defined physiological or regulatory function (Tucker and Fadool, 2002; Kraegeloh et al., 2005). The arrangement of the TrkA-C domains in the *Chlamydomonas* Na⁺/SO₄²⁻ transporters and SAC1 are shown in Figure 5.4B.

s0090 **4. Other SO₄²⁻ transporters**

p0130 Once in the cytoplasm, SO₄²⁻ enters the plastid where it is reduced. Initially, a single *Chlamydomonas* gene encoding a subunit of a putative SO₄²⁻ transporter associated with chloroplasts was isolated and designated *SULP1* (Chen et al., 2003; Chen and Melis, 2004). The *SULP1* protein is predicted to be transmembrane, with strong similarity to the hydrophobic permease-associated ABC transporter subunit CysT (characteristic of bacterial transporters). *SULP1* mRNA and protein increase upon S starvation and a *sulp* antisense strain exhibited reduced SO₄²⁻ uptake capacity, lower levels of photosynthesis (as measured by light-saturated rates of O₂ evolution), low levels of Rubisco and the Photosystem II (PS II) reaction center polypeptide D1, and signs of S deficiency, even when a moderate concentration of SO₄²⁻ was included in the medium (Chen and Melis, 2004; Chen et al., 2005). Failure to isolate antisense transformants with a large reduction in the *SULP1* transcript level suggests that a complete loss of gene function may be lethal and that this transporter defines the major path for SO₄²⁻ import into *Chlamydomonas* plastids.

p0140 Other components of the chloroplast SO₄²⁻ transporter were subsequently identified (Melis and Chen, 2005), including a second transmembrane protein (*SULP2*), a stromal-targeted nucleotide binding protein (SABC) and the substrate binding protein (SBP). The mature *SULP1* and *SULP2* polypeptides contain seven transmembrane domains and one or two large hydrophilic loops that protrude into the cytosol. The nuclear

A

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SAC1 -----MIRGVVDAIVCLFAASTLLLRG--ITQARDPAGLANDSIVSIALMMMIACLESSCALPVEPELVLGRSREWVGQL
SLT1 MAALSWQGIVAVPTALAFVMAADWVGPDITFTVLLAFLTAPDGOIVTVAKAAAGYNTGLLTVFLYVVAEGITQTGGLEIMNVILGRSSVHWALV
SLT2 -MGFQWQGSVSTPTALAFVMAADWVGPDITFTVLLAFLTAPDGOIVTVAKAAAGYNTGLLTVFLYVVAEGITQTGGLEIMNVILGRSSVHWALV
SLT3 MAALSWQGIVAVPTALAFVMAADWVGPDITFTVLLAFLTAPDGOIVTVAKAAAGYNTGLLTVFLYVVAEGITQTGGLEIMNVILGRSSVHWALV

SAC1 RHHIAVASVAVNNTPLVAVMIFVVEVSWCRNNNHPSRFMMPLSYSAALGGCLTIGTSTNLHARGLAQDDDK
SLT1 RSMFPVMVLSAFLNNTPCVTFMIFILISWGRRCGVP IKKLLIPLSYAAVLGGTCTSIGTSTNLVTVGLQDARYAKSKVQDAKFOI-----FD
SLT2 RSMFPVMVLSAFLNNTPCVTFMIFILISWGRRCGVP IKKLLIPLSYAAVLGGTCTSIGTSTNLVTVGLQDARYAKSKVQDAKFOI-----FD
SLT3 RSMFPVMVLSAFLNNTPCVTFMIFILISWGRRCGVP IKKLLIPLSYAAVLGGTCTSIGTSTNLVTVGLQDARYAKSKVQDAKFOI-----FD

SAC1 -----LKI PFVEVCHIGLELTA
SLT1 -----YGVPPYALWGFVFIILACGFLLPGNSSRYAKDLLIAVRVLPSSSVVKKKLDKSGLLQOQSGFVITATYRAGOLIKISDPSIIVLGGDILYVS
SLT2 MIPCTRLPMHVPYALWGFVFIILACGFLLPGNSSRYAKDLLIAVRVLPSSSVVKKKLDKSGLLQOQSGFVITATYRAGOLIKISDPSIIVLGGDILYVA
SLT3 -----YGVPPYALWGFVFIILACGFLLPGNSSRYAKDLLIAVRVLPSSSVVKKKLDKSGLLQOQSGFVITATYRAGOLIKISDPSIIVLGGDILYVA

SAC1 -----V
SLT1 GELDVEFEVGEELGLALVNOGCE--DAERPPGSGEEAVFSANGAAPYKLVQAKLSKTSDDLIGRTVREVSWQGRFGLIPVAIQNGREDGRLSDVVLA
SLT2 GELDVEFEVGEELGLALVNOGCE--TSAERPPGSGEEAVFSANGAAPYKLVQAKLSKTSDDLIGRTVREVSWQGRFGLIPVAIQNGREDGRLNDVVLA
SLT3 GELDVEFEVGEELGLALVNOGCE--TDGQAVGDSAPAFHDIGASPYKLVQAKLSKTSDDLIGRTVREVSWQGRFGLIPVAIQNGREDGRLNDVVLA

SAC1 AGSIYVLFSPILLRKR-----TMMAAVVDAPRPPVSVVVDARFAHICETESAGLRHLRGLADLQCGATVSPPTTIIIGLQKQTEKAGD
SLT1 AGDVLIDTTPFYDEREDKRNNEKAVKDGAAKEFVIGVVKRSEVVGKTVSAAGLRGIPGLFVLSVDADGGSVSDSSDYLKIQDDTTWAAAD
SLT2 AGDVLIDTTPFYDEREDKRNNEKAVKDGAAKEFVIGVVKRSEVVKNTVSAAGLRGIPGLFVLSVDADGGSVSDSSDYLKIQDDTTWAAAD
SLT3 AGDVLIDTTPFYDEREDKRNNEKAVKDGAAKEFVIGVVKRSEVVKNTVSAAGLRGIPGLFVLSVDADGGSVSDSSDYLKIQDDTTWAAAD

SAC1 IQGWHLLGLPGLPISSADLAADLSETVAGSPSSRIMVAVVGLSSPTCMTLHPSPPSPFPCVAVLAVHRCGPRAGLGDIVVTCGDMLEAGPD
SLT1 VAVGFLSKFPGLELVQ-----GQVDKTCSTILYRHLVQAAVSHKGPVIGKTVDRVFRFTLYNAAVAVHRCGPRAGLGDIVVTCGDMLEAGPD
SLT2 VAVGFLSKFPGLELVQ-----GQVDKTCSTILYRHLVQAAVSHKGPVIGKTVDRVFRFTLYNAAVAVHRCGPRAGLGDIVVTCGDMLEAGPD
SLT3 VAVGFLSKFPGLELVQ-----GQVDKTCSTILYRHLVQAAVSHKGPVIGKTVDRVFRFTLYNAAVAVHRCGPRAGLGDIVVTCGDMLEAGPD

SAC1 FLQKVFSTEWALAVDAFRVTLFPRD--PLALFMSLGFPIALIV--FNSMDVLFSTITLVCLFAYVPTGVLTVTSQCRATPSSILTAAGGFGVAKMT
SLT1 WADEHRHDKSVLVQVPESSPPKRSRMIGVLLATGMVLTQIGGLKSEKRYTHLWPAAVLTAALMLTGCNNADQARKAIMVDVLTAAAGVSAALE
SLT2 WADEHRHDKSVLVQVPESSPPKRSRMIGVLLATGMVLTQIGGLKSEKRYTHLWPAAVLTAALMLTGCNNADQARKAIMVDVLTAAAGVSAALE
SLT3 WADEHRHDKSVLVQVPESSPPKRSRMIGVLLATGMVLTQIGGLKSEKRYTHLWPAAVLTAALMLTGCNNADQARKAIMVDVLTAAAGVSAALE

SAC1 VTGDLHRLCSLLNVFSWMCRAQ--PVAATVASTLTAIINSNAAVTLNMYPTARDLAKQVGSHEGPLYLUNIGASSTFSTHCVQTNLMVSGFGGVRFL
SLT1 GTGVAAKFANAITSIGKSGAGCGAALTAIYIATALLSELLTNNAGAIMYPTAAAGDALKITPKDTSVAVMLGASAGFINPFSYQTNLMVYAGNYSVR
SLT2 GTGVAAKFANAITSIGKSGAGCGAALTAIYIATALLSELLTNNAGAIMYPTAAAGDALKITPKDTSVAVMLGASAGFINPFSYQTNLMVYAGNYSVR
SLT3 NTGVAKFWANAITSIGKSGAGCGAALTAIYIATALLSELLTNNAGAIMYPTAAAGDALKITPKDTSVAVMLGASAGFINPFSYQTNLMVYAGNYSVR

SAC1 DFRFGPLCFVAALITVPCWLVFSPRT
SLT1 EFAIVGAPFOIWLMIVAGFLIVRNQHWQVWIVSWICTAGIVLLPALYFLLPRTRIKHKIDGFFRTAVLNPKAALERRRSRRO-----VSHFR
SLT2 EFAIVGAPFOIWLMIVAGFLIVRNQHWQVWIVSWICTAGIVLLPALYFLLPRTRIKHKIDGFFRTAVLNPKAALERRRSRRO-----VSHFR
SLT3 EFAIVGAPFOIWLMIVAGFLIVRNQHWQVWIVSWICTAGIVLLPALYFLLPRTRIKHKIDGFFRTAVLNPKAALERRRSRRO-----VSHFR

SAC1 -----
SLT1 TDLSGSSGSP--LPAKPIVA-
SLT2 SDGSGSSDSPR-ALGVPRVITA
SLT3 TDGSSSTEDVALTFIEMPRMGVR
    
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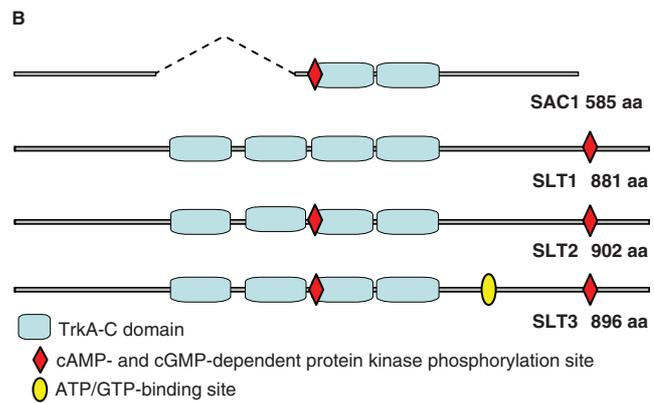


FIGURE 5.4 Features of the SLT or SAC1-like transporters. (A) *ClustalW* alignment of SLT SO_4^{2-} transporters. The alignment was performed using BioEdit v.7.0.5.3. (B) Schematic representation of the TrkA-C domains in SAC1 and SLT proteins. SAC1 is physically smaller than the SLT polypeptides; it has two TrkA-C domain repeats (the broken line represents a gap in the alignment) while SLT1, SLT2, and SLT3 have four. Other domains with possible functional relevance in the activity/regulation of these proteins are also depicted.

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genome of the diatom *Thalassiosira pseudonana* also encodes subunits of a bacterial-type SO₄²⁻ transporter (Armbrust et al., 2004) while in the red alga *Cyanidioschyzon merolae* they are present in both the nuclear and plastid genomes (Ohta et al., 2003). Bacterial-type SO₄²⁻ transporter gene subunits have been noted to be on the chloroplast genomes of many algae. These findings suggest that the algal chloroplast SO₄²⁻ transport system resembles that of prokaryotes, and that some genes encoding these polypeptides may be transferred from the original endosymbiont genome to the nuclear genome of the host organism. The distribution of these transporter genes between the nuclear and plastid genomes varies, and studies of these genes are likely to be enlightening from biochemical and evolutionary perspectives, and reveal ways in which the expression of the different transporter subunits are coordinately regulated.

s0100 **D. Incorporation into cysteine and methionine**

p0150 SO₄²⁻ that enters the cell may be activated by ATP sulfurylase (ATS) to form adenosine 5'-phosphosulfate (APS). ATS isoforms are located in both the plastid and cytosol of plant cells (Rotte, 1998). In contrast to *Arabidopsis* in which there are at least three plastidic and one putative cytosolic ATS (Rotte and Leustek, 2000), *Chlamydomonas* has two genes, *ATS1* and *ATS2* (Yildiz et al., 1996; Allmer et al. 2006), both encoding a protein with a potential chloroplast transit peptides. These finding suggest that in *Chlamydomonas* all activation of SO₄²⁻ occurs in the chloroplast. However, biochemical work is required to verify subcellular locations of the ATS isozymes. *ATS1* and *ATS2* transcripts have been shown to increase during S starvation (Yildiz et al., 1996; Ravina et al., 2002; Zhang et al., 2004).

p0160 The APS generated by the ATS reaction can serve as substrate for SO₄²⁻ reduction or be further phosphorylated by APS kinase (phosphosulfate kinase), encoded in *Chlamydomonas* by a single, intronless gene, to yield 3'-phosphoadenosine 5'-phosphosulfate (PAPS) (Arz et al., 1994; Lee and Leustek, 1998). PAPS can be used by sulfotransferases to catalyze sulfation of various metabolites including flavanols, choline, and glucosides (Varin et al., 1997). APS can also be reduced to sulfite by APS sulfotransferase (APS reductase; gene *APR1* or *MET16*; Gutierrez-Marcos et al., 1996; Setya et al., 1996; Ravina et al., 2002). APS sulfotransferases are in the plastid (Rotte, 1998), and the source of reductant for the enzyme is probably reduced glutathione (Bick et al., 1998; Prior et al., 1999). The mRNA and activity for APR increase during S starvation, suggesting that a key juncture for control of S assimilation occurs at the point at which APS interacts with either APS kinase or APR (Gutierrez-Marcos et al., 1996; Takahashi et al., 1997); the relative flux through the two paths would likely depend on the demand for sulfur-containing amino acids for protein synthesis. In *Chlamydomonas*, both the *APR1* transcript and APR activity increase during S starvation (Ravina et al., 2002; Zhang et al., 2004).



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In plants the SO_3^{2-} generated by the APR reaction is reduced to sulfide by the plastid sulfite reductase (SIR), which uses electrons from reduced ferredoxin (Yonekura-Sakakibara et al., 2000). In *Chlamydomonas* there are two ferredoxin-type SIR genes (*SIR1* and *SIR2*) and one bacterial-type (*SIR3*). *SIR1* and *SIR2* expression increase in response to S deprivation (Zhang et al., 2004). The sulfide formed in the SIR reaction combines with O-acetylserine (OAS) to generate cysteine, a reaction catalyzed by O-acetylserine(thiol)lyase (OASTL). OASTL is present in the cytosol, chloroplast, and mitochondrion in *Arabidopsis* (Lunn et al., 1990). Genes encoding different OASTL isoforms have been isolated from various plants (Sirko et al., 2004). The one *ASL* gene characterized from *Chlamydomonas* exhibits an increase in expression during S deprivation (Ravina et al., 1999; Zhang et al., 2004), but three additional putative *ASL* genes on the *Chlamydomonas* genome have not been characterized.

p0180

Serine acetyltransferase (SAT) catalyzes the formation of OAS in the cytosol, chloroplast, and mitochondrion (Ruffet et al., 1995). Cytosolic SAT is feedback inhibited at micromolar concentrations of cysteine (Saito et al., 1995; Noji et al., 1998, 2001; Noji and Saito, 2002). SAT is associated with OASTL and four molecules of pyridoxal 5'-phosphate in the cysteine synthase complex (Bogdanova and Hell, 1997), but the isolated complex is inefficient in synthesizing cysteine, and complex formation/dissociation may represent a way in which SAT and OASTL activities are regulated (Droux et al., 1998). *Arabidopsis* has five *SAT* (also known as *SERAT*) genes encoding proteins that differ in their expression levels, and changes in the abundance of the *SAT* transcripts occur in response to S starvation. Furthermore, the *Arabidopsis* SAT proteins differ in L-cysteine sensitivity and subcellular location (Kawashima et al., 2005). In *Chlamydomonas* the *SAT1* gene has been characterized, and its transcript level and protein activity increase in response to S starvation (Ravina et al., 2002; Zhang et al., 2004). The genome also encodes a second gene, *SAT2*, which like the first, has a putative chloroplast transit peptide.

p0190

Methionine is synthesized from cysteine and O-phosphohomoserine (OPH) through three consecutive reactions catalyzed by cystathionine γ -synthase, cystathionine β -lyase, and methionine synthase (Ravanel et al., 1998a). Cystathionine γ -synthase, localized in chloroplasts, catalyzes cystathionine formation (Wallsgrave et al., 1983; Ravanel et al., 1995a,b) and is controlled by availability of OPH and S-adenosylmethionine (SAM). However, OPH can serve as substrate for both cystathionine γ -synthase and threonine synthase. The relative activities of these enzymes are controlled by the S status of cells. Threonine synthase is most active during S-replete growth when levels of SAM are high (Curien et al., 1998). When SAM levels drop, threonine synthase activity declines and a greater proportion of the OPH is converted to methionine via cystathionine γ -synthase (Ravanel et al., 1998a). *Chlamydomonas* has a single cystathionine γ -synthase gene.



In *Arabidopsis*, cystathionine γ -synthase mRNA is controlled at the level of stability; destabilization of the mRNA is triggered either by methionine or a methionine metabolite (Chiba et al., 1999).

p0200 Cystathionine β -lyase generates homocysteine from cystathionine (Wallsgrave et al., 1983; Droux et al., 1995). The gene is single-copy in *Arabidopsis* and the enzyme, which has properties similar to those of the bacterial enzyme (Droux et al., 1995; Ravel et al., 1995b, 1996, 1998b), is likely chloroplast targeted. A single gene for cystathionine β -lyase has been identified in *Chlamydomonas*. Methionine formation is catalyzed by methionine synthase via the methylation of homocysteine. *E. coli* has two methionine synthase genes, one encoding a cobalamin (vitamin B₁₂)-dependent and the other a cobalamin-independent enzyme, while animals only have the cobalamin-dependent form and plants only have the cobalamin-independent form. *Chlamydomonas* has both enzyme types, and expression of the cobalamin-independent form may be controlled by a riboswitch mechanism in which cobalamin would bind the RNA and arrest translation (Croft et al., 2005; Grossman et al., 2007).

s0110 E. Glutathione synthesis

p0210 Glutathione (GSH = γ -Glu-Cys-Gly) is a dominant non-protein thiol in plants (Rennenberg, 1982); it plays a role in regulating the uptake of SO₄²⁻ by plant roots (Herschbach and Rennenberg, 1994; Lappartient and Touraine, 1996) and serves as a major antioxidant. GSH is a substrate for GSH-S-transferases, which are involved in detoxification of xenobiotics (Marrs, 1996). GSH is also the precursor of phytochelatins, peptides that help plants cope with heavy metals in the environment (Rauser, 1987; Scheller et al., 1987; Grill et al., 1989) and serve in redox buffering (Foyer and Halliwell, 1976; Law et al., 1983; Kunert and Foyer, 1993; Meister, 1994). GSH synthesis occurs in plastids and is catalyzed by γ -glutamylcysteine synthetase and GSH synthetase. *Arabidopsis* γ -glutamylcysteine synthetase and GSH synthetase cDNAs were isolated by complementation of *E. coli* mutants (May and Leaver, 1994; Rawlins et al., 1995); at least one gene encoding GSH synthetase is present in *Chlamydomonas*.

s0120 II. S STARVATION RESPONSES

s0130 A. Overview

p0220 Typically, organisms exhibit a suite of responses when their growth becomes nutrient-limited. These responses have been described as "general" and "specific." General responses are those associated with deprivation for any essential nutrient and include the cessation of cell growth and division, accumulation of storage carbohydrates and modulation of metabolic processes, including a decrease in photosynthesis. Of all of these responses,



that of photosynthesis has been the most extensively studied. During nutrient limitation in *Chlamydomonas* (S and phosphorus), PS II activity declines through various mechanisms (Wykoff et al., 1998). Furthermore, mutant strains unable to shut down photosynthetic electron transport during nutrient limitation die rapidly (Davies et al., 1996), suggesting that reduction of PS II activity is critical for survival during nutrient limitation. On the other hand, a decline in photosynthetic activity (while maintaining respiratory rates) causes the cultures to become anaerobic (Wykoff et al., 1998), which can trigger fermentation metabolism and a concomitant increase in expression of two iron-only hydrogenases that can catalyze light-driven H₂ production (Forestier et al., 2003; Mus, et al., 2007). This finding is being exploited by several researchers for biocatalytic H₂ production in S-depleted *Chlamydomonas* cultures (Fedorov et al., 2005; Fouchard et al., 2005). A better understanding of S deprivation responses may provide insights into metabolisms that facilitate the generation of H₂ (see Chapter 7).

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The specific responses are those that are associated only with deprivation for a single nutrient. In the case of S deprivation, they are mostly associated with activities that promote scavenging of extracellular S and the recycling of intracellular S. These processes include the increased synthesis of SO₄²⁻ transporters and hydrolytic enzymes that facilitate the use of esterified sulfate, the recycling of proteins and lipids (Ferreira and Teixeira, 1992), and the replacement of proteins rich in S amino acids with functionally similar proteins that use few S amino acids (e.g. the IRL protein of maize [Petrucco et al., 1996], the ECPs of *Chlamydomonas* [Takahashi et al., 2001], and the β-conglycinin storage protein of soybean [Naito et al., 1994; Kim et al., 1999]). In general, high cysteine and glutathione levels repress SO₄²⁻ assimilation, while S starvation causes elevated activities of key enzymes in the assimilatory pathway. Administration of high concentrations of cysteine and glutathione to plant roots causes reduction in mRNA accumulation for SO₄²⁻ transporters, ATP sulfurylase and APS sulfotransferase (Bolchi et al., 1999; Lappartient et al., 1999; Lee and Leustek, 1999). Glutathione may function in repressing genes encoding key enzymes in the S assimilation pathway (Lappartient et al., 1999) while OAS, the substrate for cysteine synthesis, is a positive effector of the CYSB transcription factor in bacteria (Kredich, 1992), and also acts as a positive effector in plants. Roots subjected to exogenous OAS exhibit increased accumulation of mRNAs encoding the SO₄²⁻ transporters (Smith et al., 1997) and APS sulfotransferase (Koprivova et al., 2000).

s0140

B. Genes responsive to S deprivation

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There are numerous genes in *Chlamydomonas* that respond to S deprivation (see Table 5.1 and Figure 5.1). For example, S deprivation activates



genes involved in hydrolyzing SO_4^{2-} from organic compounds (*ARS1*, *ARS2*), transporting SO_4^{2-} across the plasma membrane (*SULTR2*, *SLT1*, and *SLT2*) and incorporating SO_4^{2-} into amino acids (*ATS*, *APR*, *ASL*, and *SAT*) (Davies et al., 1994; Ravina et al., 2002; Zhang et al., 2004; Eberhard et al., 2006). In addition, two prominent extracellular polypeptides, ECP76 (76 kD) and ECP88 (88 kD) are synthesized in response to S deprivation (Takahashi et al., 2001). The *ECP76* and *ECP88* genes are rapidly activated following elimination of S from the growth medium, and their mRNAs are rapidly degraded (half-life of <10 minutes) once S-deprived cells are provided with adequate levels of SO_4^{2-} (Takahashi et al., 2001). ECP76 and ECP88 have features of cell wall proteins, but the mature polypeptides together possess only a single S-containing amino acid. Hence, highly regulated processes likely tailor the protein-rich *Chlamydomonas* cell wall under S deprivation in ways that conserve S for other cellular activities. The new cell wall material may also accommodate conditions in which the cells are no longer elongating and dividing (e.g. changes in extensibility and elasticity).

p0250 Microarray analyses using an array representing ~3,000 unique genes demonstrated that the levels of transcripts from over 140 genes in wild-type cells increased by fourfold or more following elimination of S from the medium (Zhang et al., 2004). Approximately 40 transcripts decreased to below 25% of the level present in nutrient-replete cells. Many transcripts that increased during S deprivation were previously characterized as encoding S-deprivation-induced proteins (e.g. ARS, ECP76, proteins important for SO_4^{2-} assimilation). Others, however, encoded novel polypeptides of the light harvesting family (e.g. LHCSR2, see below), enzymes involved in scavenging reactive oxygen species, and putative regulatory elements.

s0150 C. Identification of genes controlling S deprivation responses

p0260 The responses displayed by *Chlamydomonas* during S starvation require an efficient mechanism for sensing S availability and activating a signaling pathway(s) that modulates structural, metabolic and physiological processes, leading to a new cellular homeostasis. Little is known about regulatory elements that control S acclimation processes in plants, although recently a transcription factor was isolated that appears to bind the promoters of genes that become active when S availability declines (Maruyama-Nakashita et al., 2006).

p0270 In the case of *Chlamydomonas*, the screening of thousands of insertional mutants has enabled identification of several proteins involved in the acclimation of cells to S deprivation (Davies et al., 1994; Pollock et al., 2005). Transformants of interest were identified based on their inability to synthesize ARS (Davies et al., 1996) in response to S deprivation, or because they constitutively expressed ARS activity under S-replete conditions. Potential mutants were examined for co-segregation of the mutant phenotype with



the inserted “tag,” survival during S-deprivation conditions and changes in the abundance of transcripts previously shown to be responsive to S levels.

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The first mutant identified using this strategy was *sac1* (sulfur acclimation). The SAC1 protein plays a central role in controlling S deprivation responses (Davies et al., 1996; Zhang et al., 2004). The mutant strain is unable to synthesize ARS and exhibits abnormal SO_4^{2-} uptake in response to S deprivation. Essentially no induction of any other gene associated with SO_4^{2-} acquisition and assimilation was observed in a *sac1* mutant (Yildiz et al., 1996; Takahashi et al., 2001; Ravina et al., 2002; Zhang et al., 2004). Furthermore, transcripts from several genes associated with photosynthetic electron transport and the amelioration of damaging effects elicited by the accumulation of reactive oxygen species rise in response to S deprivation, and appear to be under SAC1 control. With respect to light harvesting and electron transport, the *LHCSR2* transcript accumulates to high levels in response to S deprivation, while transcripts encoding many other LHC polypeptides decline (Zhang et al., 2004). These changes in transcript levels are Sac1-dependent.

p0290

Transcripts from other genes show extreme accumulation in the *sac1* mutant; among them are those encoding two small chaperones thought to be associated with chloroplasts, HSP22E and HSP22F (see Chapter 19). An increase in these chaperones may reflect an extreme stress response because of the inability of mutant cells to acclimate; the cells die within 2 days of initiation of S deprivation (Davies et al., 1996). This death has been linked to electron flow out of PS II. Hence, modification of photosynthetic electron transport during S deprivation appears to be critical for cell survival. Mutants unable to perform these modifications would exhibit hyper-reduction of the electron transport chain (e.g. PQ pool), which could adversely affect cellular metabolism, leading to the accumulation of reactive oxygen species and extensive cellular damage (Davies et al., 1996).

p0300

The *SAC1* gene product is predicted to be similar to $\text{Na}^+/\text{SO}_4^{2-}$ transporters (*SLT* genes; Davies and Grossman, 1998). Its deduced polypeptide sequence and the *sac1* mutant phenotype suggest a similarity with the yeast Snf3p system. Snf3p is a yeast “transporter-like” regulatory protein that governs expression of genes involved in hexose utilization (Ozcan et al., 1996, 1998), but also interacts with other stress-related processes (Sanz, 2003). The similarity between SAC1 and Snf3p raises the possibility that polypeptides whose original function was to bind and transport various substrates into cells may have evolved into regulatory elements that sense extracellular or intracellular nutrient concentrations. This information would then be communicated to a signal transduction pathway involved in modulating the efficiency of nutrient acquisition processes and tuning cellular metabolism to the potential for cell growth. Two TrkA-C domains present in the central region of the SAC1 protein could play a role in sensing and transduction mechanisms. The function of the TrkA-C domains (also



known as RCK-C) is not well understood. They are present in many proton channels as one or two tandem repeats, and are thought to generate homodimer interactions that produce a cleft between two lobes of the transport complex (Anantharaman et al., 2001; Dong et al., 2005). Similar to SAC1, the other SAC1-like proteins (SLTs) also have TrkA-C domains, but they have four tandem repeats rather than two (Figure 5.4B). Both in SAC1 and the SLTs, the TrkA-C domains are positioned in a predicted cytosolic hydrophilic loop that separates two sets of transmembrane helices.

p0310 Another mutant, designated *sac3*, exhibits low-level constitutive ARS activity in S-replete medium, but like wild-type cells shows an increase in ARS activity following transfer of cells to S-depleted medium. Hence, SAC3 functions either directly or indirectly in maintaining repression of ARS activity when S is readily available; other S-starvation-induced genes may also be negatively regulated by SAC3 (Ravina et al., 2002). SAC3 seems to positively regulate the activity of SO_4^{2-} transporters since while their mRNAs still increase during S deprivation of the *sac3* mutant, there is little increase in the capacity of mutant cells to transport SO_4^{2-} (no change in the V_{max} for SO_4^{2-} ; Davies et al., 1999). In addition, unlike wild-type cells, *sac3* does not exhibit a decrease in chloroplast transcriptional activity (Irihimovitch and Stern, 2006) during S starvation, suggesting that SAC3 is required to inactivate the chloroplast RNA polymerase sigma factor Sig1 when the cells are starved for S. This inactivation may involve proteolytic activities that are phosphorylation-dependent, since SAC3 encodes a putative serine-threonine kinase of the plant-specific SNRK2 family. The protein targets of SAC3 remain to be identified.

p0320 Recently we identified 49 other mutants (*ars* mutants) that do not properly acclimate to S deprivation (Pollock et al., 2005). The *Chlamydomonas* sequences flanking the insertion were determined in 80% of these strains and the genes disrupted by the insertions in some key mutants are given in Table 5.2. Not all of the mutants have been characterized for many of the responses associated with S starvation, but some strains that have been examined in some detail are described below.

p0330 Two allelic mutants, *ars11* and *ars44*, possess insertions in a gene encoding another member of the serine-threonine kinase family; like SAC3, the encoded polypeptide is similar to SNRK2 family proteins (Pollock et al., 2005). These mutants have a *sac1*-like phenotype, that is no ARS activity in S-depleted medium, defective SO_4^{2-} uptake, and little decrease in the rate of photosynthetic O_2 evolution during the first day of S deprivation. Some members of the *Arabidopsis* SNRK2 kinase family appear to function in controlling S-limitation responses. *Arabidopsis* plants with T-DNA insertions in *SNRK2.3* exhibit a decreased induction of *SULTR2;2*, which encodes a low-affinity SO_4^{2-} transporter, and also accumulate OAS (Kimura et al., 2006). Overall, five of the ten *Arabidopsis* SNRK2 genes show increased expression following S starvation.

p0340

Another mutant with essentially no induction of ARS activity during S starvation is interrupted for a gene encoding a putative guanylyl cyclase (*ars401*), one of more than 50 putative proteins of this family encoded by the *Chlamydomonas* genome (Merchant et al., 2007). It will be interesting to elucidate the specificity of these cyclases and the biological processes to which they are linked. Finally, a mutant affected in a member of the E3 ubiquitin ligase complex (SCF (Skp1/Cullin/F-box protein)-type, SGT-1-like gene), which is involved in protein degradation, also makes little active ARS when transferred to medium lacking S.

p0350

The phenotypes of all of the mutants discussed above can be rescued by introduction of a wild-type gene, although for most other mutants presented in Table 5.2, both linkage and complementation analyses are still required. In addition, double mutant analyses will help determine possible epigenetic relationships among the different genes identified by the screen. While the genetic screens performed thus far have identified a number of key proteins involved in the acclimation of *Chlamydomonas* to S deprivation, extending our knowledge of this process considerably, only a few of the mutants are represented by multiple alleles, which suggests that other genes that are important for normal S-deprivation responses in *Chlamydomonas* remain to be identified.

t0020

Table 5.2 Sulfur acclimation/arylsulfatase mutants

Mutant	ARS %	Tagged	Accession number	Protein prediction
<i>ars5</i>	2	YES	EDP00051	Similar to PH081
<i>ars11</i>	5	YES	EDP06815	Similar to <i>SAC3</i> : Possible Ser/Thr/Tyr kinase
<i>ars20</i>	10	n.a	EDP00621	Paf1: Putative RNA polymerase II regulator
<i>ars44</i>	10	YES	EDP06815	Similar to <i>SAC3</i> : Possible Ser/Thr/Tyr kinase
<i>ars53</i>	10	n.a	EDP03402	HydA1-iron hydrogenase precursor
<i>ars63</i>	2	n.a	EDP09374	SEC24-like protein
<i>ars75</i>	2	n.a	EDP00156	Probably affects <i>SAC1</i>
<i>ars76</i>	2	YES	EDP05314	APPLE domain: mediates dimer formation
<i>ars107</i>	8	n.a	EDP09374	SEC24-like protein
<i>ars122</i>	2	YES	EDO96714	SGT-1-like (SCF-mediated ubiquitination)
<i>ars124</i>	10	n.a	EDO95880	Adenylate kinase
<i>ars401</i>	2	YES	EDP08005*	Guanylate cyclase-like

ARS activity (ARS%) is as a percentage relative to the activity measured for the parental strain under S deprivation conditions. Co-segregation of the marker and phenotype (tagged mutant) was determined by genetic crosses. n.a: not analyzed. The symbol * indicates that the guanylyl cyclase JGI model is fused with a 3'-5' exonuclease. Accession numbers are given for the wild-type protein sequences predicted to be affected by the mutations.

D. Model for acclimation of *Chlamydomonas* to S deprivation

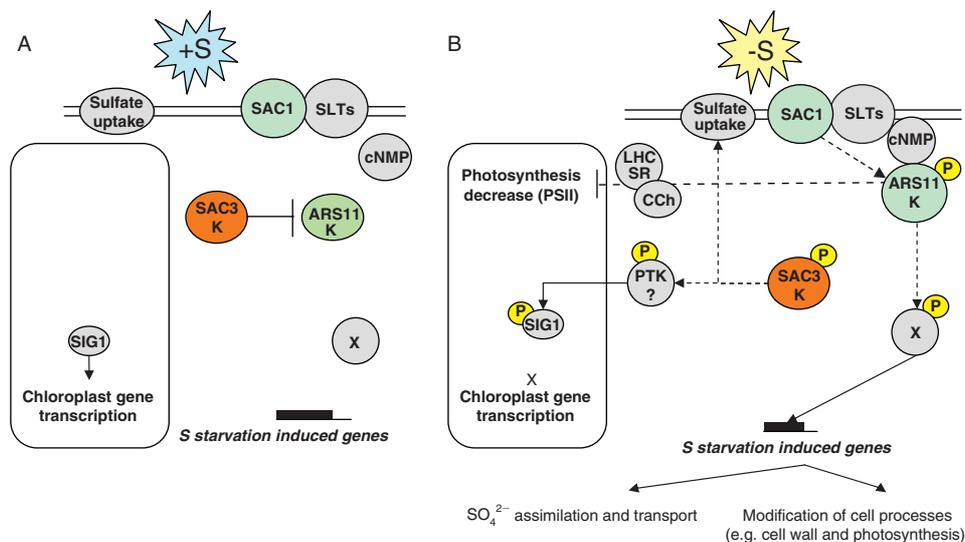
s0160

p0360

In Figure 5.5 we depict a model of the regulatory processes associated with S deprivation responses. This model is not supported at this point by protein–protein interaction evidence, so its tenets rely on the analyses of mutants and how the lesions in the mutants affect ARS and SO_4^{2-} uptake activities, the expression patterns of some S deprivation-induced genes, and some physiological responses of the mutant strains. However, the model helps put the information into a context that may guide future experimentation.

p0370

It is known that SAC1 positively enhances expression of many S deprivation-regulated genes when *Chlamydomonas* is transferred to medium devoid of S. On the other hand, the SAC3 kinase has a negative effect on some S starvation-induced genes. A double mutant *sac1 sac3*, like the *sac3* single mutant, exhibits low-level constitutive ARS activity in S-replete medium, but during S deprivation, unlike *sac1*, there is some increase in ARS expression, although lower than in either wild-type cells or the *sac3* mutant (Davies and Grossman, 1994). This finding suggests that



f0050

FIGURE 5.5 Model for S-dependent signal transduction pathways in *Chlamydomonas* during S-replete and S deprivation conditions. (A) Under S-replete condition ARS11 is not active. (B) Activation of ARS11 under S deprivation is elicited by phosphorylation through SAC1 in a process involving a guanylyl cyclase (cNMP). Other proteins such as the SLT and/or SULTR transporters may interact with SAC1 in the plasma membrane to control both transport and signaling through ARS11. Once ARS11 is activated, it may phosphorylate transcription factor X, which then elicits elevated or depressed activity from S-responsive genes, including those encoding enzymes involved in the assimilation of SO_4^{2-} , restructuring of the cell wall and modification of the photosynthetic apparatus. However, the activity of ARS11 may not be completely SAC1-dependent. SAC3 may inhibit ARS11 during S-replete conditions (e.g. through a second phosphorylation of a specific site on Ars11), which keeps the S-responsive genes completely off. In addition, during S-starvation conditions SAC3 might signal to a putative plastid kinase which in turn activates a system involved in proteolysis of SIG1 and also either directly or indirectly modulate SO_4^{2-} transport activity through post-transcriptional processes.



the relationship between *SAC1* and *SAC3* is non-epistatic, and that proteins in addition to *SAC1* and *SAC3* may be involved in controlling *ARS* expression. *SAC1*, which has strong similarity to the SLT family of proteins, may act as a sensor that resides on the plasma membrane. The TrkA-C domains present in *SAC1* and SLT transporters could be involved in protein-protein interactions and ligand binding. We hypothesize that *SAC1* interacts with the SLT proteins and under S deprivation conditions, triggers a phosphorylation cascade that modulates expression of S-regulated genes.

p0380

The kinase encoded by *ARS11* (Pollock et al., 2005) was shown to be required for *ARS* activity and is a key regulatory component that is likely part of the same phosphorylation cascade as *SAC1*. In this scenario, SO_4^{2-} availability sensed by *SAC1*, initiates a phosphorylation cascade through *ARS11* that tunes the activity of S-responsive genes, including those encoding proteins involved in SO_4^{2-} assimilation and modification of the photosynthetic apparatus (e.g. *LHCSR* genes and some specific small chloroplast chaperones), through an undefined transcription activator designated X. This process may also be controlled by a guanylyl/adenyl cyclase via the production of cGMP/cAMP, which may serve to amplify the signal through the pathway. Severe S deprivation conditions would elicit full activation of the cascade, triggering high level expression from downstream target genes.

p0390

Nevertheless, *ARS11* activation is not completely *SAC1*-dependent since the *sac1 sac3* double mutant still possesses low *ARS* activity in S-replete and S-depleted conditions. We postulate that *SAC3* may be critical for keeping *ARS11* in a completely inactive form, perhaps via a direct phosphorylation. Because there is no change in the V_{max} for SO_4^{2-} transport in S-deprived *sac3* cells (Davies and Grossman, 1994), even though SO_4^{2-} transporter mRNAs increase, it appears that *SAC3* is required for full activation of SO_4^{2-} uptake, probably at the post-transcriptional level. *SAC3* is also involved in controlling plastid gene expression through proteolytic degradation of *SIG1*, which causes down regulation of chloroplast transcription (Irihimovitch and Stern, 2006). Previous work in mustard showed that a nucleus-encoded plastid kinase, cpCK2a, modulates the chloroplast eubacterial RNA polymerase activity in response to redox poise (Orgzewalla et al., 2002). The potential interaction between *SAC3* and a putative chloroplast-localized kinase that modulates transcription in *Chlamydomonas*, remains to be established.

s0170

III. PERSPECTIVES

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Numerous polypeptides that control S deprivation responses in *Chlamydomonas* have been discovered, but it will take significantly more work to understand the exact interactions of those regulatory elements and details of the associated phosphorylation cascade. It is also critical to identify



and characterize the full complement of regulators associated with S deprivation responses (e.g. Table 5.2). Furthermore, P and S metabolism in vascular plants (Wang et al., 2002), yeast (O'Connell and Baker, 1992) and *Chlamydomonas* (Moseley et al., 2006) appear to intersect, suggesting that dynamic regulatory networks integrate nutrient stress responses, and most likely responses to other conditions that limit cell growth. Establishing these links and the specific factors that facilitate this integration would be important for understanding the dynamic homeostasis of the cell as it experiences fluctuating environmental conditions.

s0180

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p0410

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