Sulfate Transport in Plants: A Personal Perspective

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Abstract Early key research milestones for sulfate transport in plants include the first description of kinetics of sulfate uptake into plant roots (Leggett and Epstein, Plant Physiol 31:222–226, 1956), nutritionally regulated sulfate uptake into plants (Clarkson et al., J Exp Bot 34:1463–1483, 1983), and the first gene for a plant sulfate transporter (Smith et al., Proc Natl Acad Sci U S A 92:9373–9377, 1995a). Since then a well-described gene family encoding putative sulfate transporters has been characterized in multiple species, initially most notably in Arabidopsis but subsequently for a number of other models or important crops (examples: Brassica, wheat, rice, poplar and Medicago, see Buchner et al., Genome 47:526–534, 2004a; Buchner et al., Plant Physiol 136:3396–3408, 2004b; Buchner et al., Mol Plant 3:374–389, 2010; Kumar et al., Plant Signal Behav 10:e990843, 2015; Dürr et al., Plant Mol Biol 72:499–517, 2010; Gao et al., Planta 239:79–96, 2014). Regulation of expression has been well documented and this regulation is both a useful marker of sulfur-nutritional status and a model for the elucidation of control pathways. The complexity of the gene family in relation to functional, regulatory and spatial distribution indicates an apparent whole plant management system for sulfur, coordinated with growth and demand and interacting with nutrient availability. In addition to sulfate, there is direct involvement of this transporter family in the uptake and accumulation of both selenate and molybdate, with clear consequences for nutritional quality. Is the story now complete almost 60 years since the first transport description and 20 years since the first sulfate transporter gene isolation, and a plethora of research projects and publications? Do we know how sulfur is acquired and appropriately distributed within the plant? Do we know the critical signals that control these processes? Are we even sure that these processes are coordinated? This review documents research progress and assesses to what extent the key questions have been addressed.

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Introduction

All plants require sulfur for growth and for land plants is most is acquired from the external environment as sulfate. For land plants this from the soil via the roots. Typically concentrations are low and often extremely variable. Thus, transport needs to be active to facilitate uptake against a concentration gradient, specific for sulfate and regulated to optimize uptake to growth and ensure optimal energy utilization in this process. For vascular plants transport is not only across a single membrane at the soil-root interface but also across many other plasma membranes to facilitate distribution, across the chloroplast membrane to the site of reduction and also across the tonoplast to allow transport in and out of the vacuole for the transient storage of excess sulfate taken up.

Progress on the understanding of plant sulfate transporters has been substantial and reported in successive volumes of the Sulfur Workshop series, with key landmark papers from a number of groups being published throughout this period. Some early key milestones in the development of the plant sulfate transporter research field are illustrated as a timeline in Fig. 1.

The first suggestion for active absorption was in an analysis of whole plant uptake of sulfate into barley roots. An enzyme based description of affinities and competition by selenate but not nitrate or phosphate unequivocally demonstrated the activity of a transmembrane ion transporter (Leggett and Epstein 1956). It would be 40 years before the molecular components would be identified in plants (Smith et al. 1995b). Prior to this key elements of regulation by de-repression (that is induction upon starvation) were described in a topical legume (Clarkson et al. 1983) and suggestion for involvement of a metabolite linking N and S metabolism, namely *O*-acetylserine (OAS), was described in maize (Clarkson et al. 1999). The importance of OAS as a regulator of gene expression for a cluster of genes has been described, separating S-related and other regulation (Hubberten et al. 2012, 2015). Mechanistic evidence for transport being driven by proton gradients was obtained in a duckweed (Lass and Ullrich-Eberius 1984).

The first substantial progress on the identification of sulfate transporters genes was inevitably for bacteria (Ohta et al. 1971; Sirko et al. 1990), fungi and yeast (Ketter et al. 1991; Smith et al. 1995b; Cherest et al. 1997), mammalian systems (Schweinfest et al. 1993; Hästbacka et al. 1994; Silberg et al. 1995) and finally in plants (Kouchi and Hata 1993; Smith et al. 1995a, 1997 ; Takahashi et al. 1996). Similarities in the sequence of many of the genes, some not identified as sulfate transporters was first noted by Sandal and Marcker (1994). Much of this work has been reviewed in previous volumes in this series: Kredich 1993 (bacteria); Thomas et al. 1997 (yeast); Davidian et al. 2000, Hawkesford et al. 2003, Buchner et al. 2010, Hawkesford 2012 (plants) and elsewhere (Markovich 2001) for mammalian transporters. These transporters are now recognised to be part of a large family of transmembrane ion transporters known as SulP (see also Price et al. 2004).

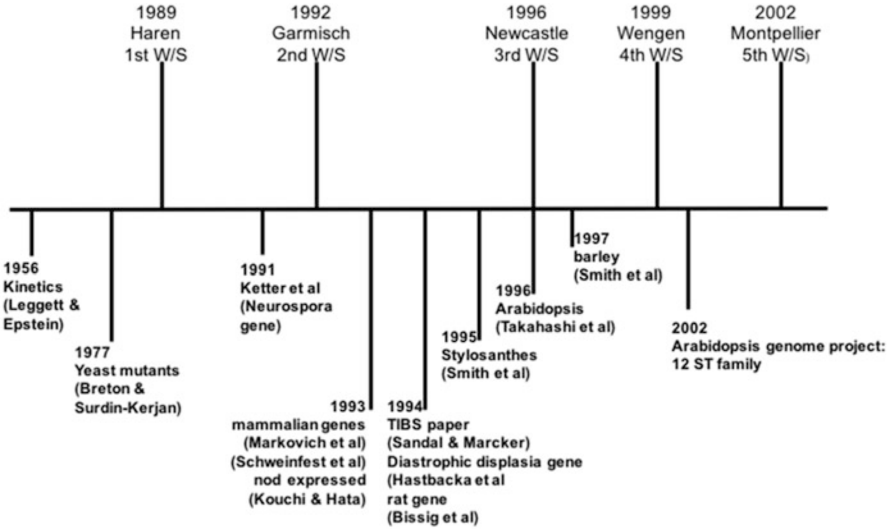

Fig. 1 Key early milestones in the development of the understanding of sulfate transport in plants placed in relation to the first 5 Sulfur Workshops

A Family of Sulfate Transporters

In a series of papers predominantly from the Takahashi group but with notable contributions from a number of others including the Davidian group it became apparent that a gene family of up to 14 genes encoded a group of related proteins in Arabidopsis (Takahashi et al. 1996, 1997; Vidmar et al. 2000). Similar gene families were subsequently identified in Brassica (Buchner et al. 2004b), in rice (Kumar et al. 2015), poplar (Dürr et al. 2010), Medicago (Gao et al. 2014) and in wheat (Buchner et al. 2004a).

Phylogenetic analysis of plant sulfate transporter sequences indicates discrete clades within the family (Fig. 2) and it has been proposed that these align with discrete functions and that within clades there may be some functional redundancy (Hawkesford 2003). In summary, Group 1 represents high affinity types responsible for up take into the cell, particularly in the roots, and are subject to nutritional regulation. Group 2 are lower affinity, less regulated and distributed throughout the plant. Group 3 are somewhat more enigmatic (see below), Group 4 are uniquely tonoplast located and responsible to vacuolar efflux. Group 5 are the most distantly related to the rest of the family and the 2 members are quite distinct from each other, lack a STAS domain and remain something of a puzzle. They seem to be involved in Mo accumulation, perhaps transport and as such have been name mot1 and mot2 (Tomatsu et al. 2007; Baxter et al. 2008; Gasber et al. 2011).

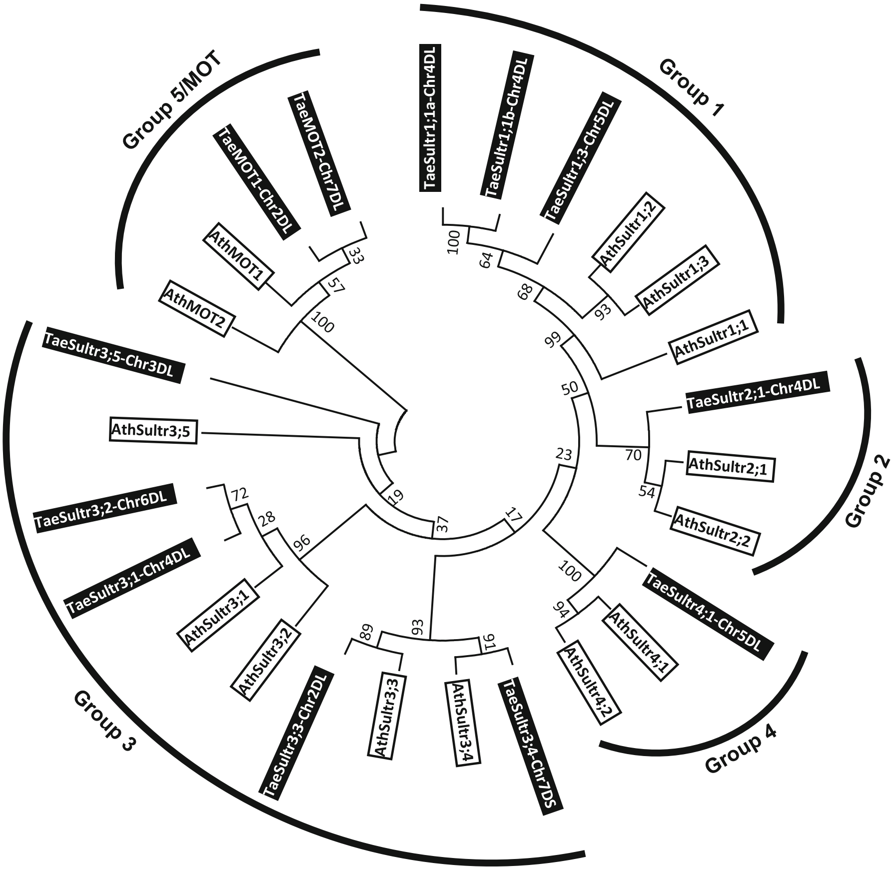

Fig. 2 Phylogenetic relationship of the wheat and Arabidopsis sulfate transporter gene families: Neighbour-Joining Tree (Mega 6, Tamura et al. 2013) from Multiple Alignment (ClustalX V.2.1, Larkin et al. 2007) of coding cDNAs of the *Triticum aestivum* cv. Chinese spring D-genome (*white bold – black highlighted*) and *Arabidopsis thaliana* (*square framed white highlighted*) sulfate transporter gene family. The bootstrap values, expressed as percentage, were obtained from 1000 replicate trees (Courtesy of Peter Buchner)

The Transporter Itself: Recent Structural Insights

Early analysis of the amino acid sequences of the transporter was suggestive of 12 transmembrane domains, based on hydrophobicity plots and occurrence of charged amino acids (Clarkson et al. 1993; Smith et al. 1995a; Takahashi et al. 1996). More recent analysis of transporters in the same superfamily (SulP/SLC26 family) combining both topology mapping of for example the BicA transporter (see Price and Howitt 2014) and for prestin, homology modelling, molecular dynamics simulations and cysteine accessibility scanning are strongly supportive of a complex 14 transmembrane model (Gorbunov et al. 2014). In this analysis a

3-dimensional model has been derived which also proposes a central cavity as the substrate-binding site, midway in an anion permeation channel. Features of this cavity are almost certainly involved in substrate specificity and could potentially be modified to further increase selectivity, for example between sulfate and selenate, opening up the potential for designer crops.

An additional feature of members of this family is the STAS domain (Aravind and Koonin 2000; Rouached et al. 2005). Activity is totally dependent upon its presence and it is strongly suggested that is it involved in protein:protein interaction regulating activity, probably involving phosphorylation of a threonine residue.

The question of whether the transport acts as a monomer or oligomer is of interest and it has been suggested that heterodimers are required for activity or may have an import regulatory role. Maximal sulfate uptake and growth were obtained when a Group 3 transporter was co-expressed with a Group 2 transporter from Arabidopsis in yeast complementation approach, suggestive of the activity of a heterodimer (Kataoka et al. 2004a). No activity of the Group 3 when expressed alone was seen in this study. In contrast Group 3 transporter isolated from Lotus root nodules was able to complement a yeast mutant when expressed by itself (Krussell et al. 2005) indicating some variability for this oligomer requirement.

Specificity for Sulfate, Selenate and Molybdate

The non-specificity of the transporter was exploited in early studies, particularly with yeast, to obtain sulfate transporter-less mutants by harassing the toxic nature of oxyanion analogues of sulfate, particularly selenate but also chromate (Breton and Surdin-Kerjan 1977; Smith et al. 1995b). Selenate has also been applied as a selection agent for the isolation of Arabidopsis mutants by several groups (see for example, Shibagaki et al. 2002).

As the anions sulfate, selenate and molybdate are all transported by the same transporters, it is not surprising that their respectively accumulations in plant tissues are connected. Analysis of what grain from mildly sulfate deficient plots at Rothamsted showed a remarkable accumulation of Se and Mo (Shinmachi et al. 2010; Stroud et al. 2010). The S-deficient plots had a reduced grain yield and reduced grain S-content, both decreasing by about 10%, but several-fold increases in Se and Mo content. This could be partially explained by the observed induction of sulfate transporters in the roots of these field-grown plants, increasing uptake, and a more favourable ration of selenate and molybdate relative to sulfate in the soil solution. Whilst Se generally flows the distribution of sulfate in term of redistribution during grain filling and in relation to storage protein distribution in the grain, some enriched sub-cellular regions were indicative of specific accumulation on non-protein Se, possibly in vacuoles (Moore et al. 2010). Mo was less efficiently remobilized to the grain than Se during grain filling indicating either a fixation of the mineral in the vegetative tissue or a limitation to its later transportation (Shinmachi et al. 2010; Stroud et al. 2010).

Where Now?

Much has been determined about the nature of sulfate transporters in plants, not only in model species but also in crops. A knowledge of the regulation and properties of the transporters helps explain many physiological phenomena and some agronomic responses of crops. The question remains of how may this aid in breeding better genotypes or in informing agronomic treatments.

A previously stated ideotype for optimum S use involves uptake and storage during fluctuating supply, effective remobilization upon demand and appropriate partitioning to ensure healthy and nutritious crops (Hawkesford 2012). Breeding or biotechnology may help deliver such germplasm and the acquired knowledge is an essential prerequisite for such developments. Sulfur will always be required for crop growth so effective capture and utilization are worthy targets.

Acquisition is an important issue. Certainly the adaptation of de-repression will aid scavenging, but only in conjunction with root proliferation. Prospects for improving efficiency of uptake are limited, although constitutive uptake and over-accumulation, followed by storage and effective remobilization remains one key strategy. In relation to this strategy, challenges still exist in the understanding of movement of sulfate within the plant from organ to organ, distribution within specific tissues and finally within individual cells between organelles. It is still unclear as to how S moves into and out of the chloroplast, the key point of entry into the biosynthetic pathway. One reports indicated a chloroplast localizing isoform of the family (Takahashi et al. 1999) but this remains to be corroborated. Clearer is the involvement of Group 3 transporters in release of sulfate from vacuoles, a key storage site (Kataoka et al. 2004b).

David Clarkson proposed the idea of a black box (Fig. 3) in a foreword to the proceedings of the 3rd Workshop (Clarkson 1997). Substantial progress has been made in determining detail within this box since then, but the question of how to improve sulfur nutrient use efficiency remains. Sulfur is required for growth and health, for resistance to biotic and abiotic stresses, and contributes to nutritional properties of food and feed. Decreasing requirements is unlikely to be an option, optimizing agronomic inputs remains the key practical approach, although in the future this may be complemented with plants optimized genetically for specific qualities. Some investigations into natural variation in Arabidopsis have been made

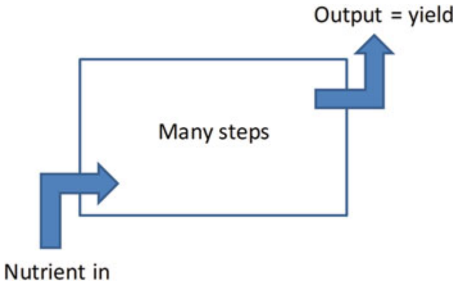

Fig. 3 Clarkson's Black Box (Clarkson 1997). A modified representation of the simple representation of nutrient use efficiency, but inevitably complex in the detail

(Loudet et al. 2007) but there has been little investigation in crop plants and this is a key area for development.

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