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Membrane-Transport Systems for Sucrose in Relation to Whole-Plant Carbon Partitioning

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ABSTRACT Sucrose is the principal product of photosynthesis used for the distribution of assimilated carbon in plants. Transport mechanisms and efficiency influence photosynthetic productivity by relieving product inhibition and contribute to plant vigor by controlling source/sink relationships and biomass partitioning. Sucrose is synthesized in the cytoplasm and may move cell to cell through plasmodesmata or may cross membranes to be compartmentalized or exported to the apoplasm for uptake into adjacent cells. As a relatively large polar compound, sucrose requires proteins to facilitate efficient membrane transport. Transport across the tonoplast by facilitated diffusion, antiport with protons, and symport with protons have been proposed; for transport across plasma membranes, symport with protons and a mechanism resembling facilitated diffusion are evident. Despite decades of research, only symport with protons is well established at the molecular level. This review aims to integrate recent and older studies on sucrose flux across membranes with principles of whole-plant carbon partitioning.

Key words: Carbohydrate metabolism; molecular transport; membrane proteins; phloem physiology; gene expression.

INTRODUCTION

Sucrose (Suc) is the principal product of photosynthesis used for the distribution of assimilated carbon in plants. Suc is produced in the cytoplasm, directly from the products of photosynthesis or from the utilization of storage reserves, and may follow several fates. A prominent transport fate is to move cell to cell via plasmodesmata through the collective cytoplasm of plant cells called the symplasm (*-plasm* terminology is used here in preference to *-plast*, following the arguments of Erickson (1986)). Alternatively, Suc may cross an endomembrane to enter an organelle—principally the vacuole for storage—or it may cross the plasma membrane to enter the collective cell-wall space (apoplasm) for subsequent uptake into adjacent cells. As a relatively large, polar solute, Suc requires proteins to facilitate efficient movement across membranes. Physiological studies support active transport energized by the proton motive force and facilitated diffusion across both the tonoplast and the plasma membrane. For energized transport, Suc/H^+ antiport to move Suc into vacuoles is supported by physiological studies and Suc/H^+ symport to move Suc out of vacuoles is supported by molecular evidence. Suc/H^+ symport across the plasma membrane is well established while a mechanism with characteristics of facilitated diffusion remains enigmatic. Genes and proteins catalyzing Suc/H^+ symport have been called Suc transporters (SUT) or Suc carriers (SUC), and those participating in passive transport have been called Suc facilitators (SUF). No genes or proteins catalyzing Suc/H^+ antiport are identified. In this document, SUT will be used when referring to proteins mediating Suc membrane transport in general and gene/protein names will be used when referring to specific SUTs.

Research progress on the central role of SUTs in whole-plant carbon partitioning is reflected in the history of excellent reviews. Robert Giaquinta (1983) and Daniel Bush (1993) published extensive reviews on physiological and biochemical considerations of Suc membrane transport, respectively, before molecular cloning techniques were widely applied. Sylvie Lalonde and colleagues (2004) and Norbert Sauer (2007) subsequently published extensive surveys on the *SUT* genes that were rapidly being characterized. More recently, reviews emphasizing monocot SUTs (Braun and Slewinski, 2009), the regulation of SUT activity (Kuhn and Grof, 2010), and SUT activity in relation to photosynthetic primary productivity (Ainsworth and Bush, 2011) have appeared. This review aims to integrate earlier research with more recent findings in the context of whole-plant Suc partitioning.

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BIOCHEMICAL SUMMARY OF Suc MEMBRANE TRANSPORT

Michaelis-Menton plots showing biphasic kinetics for uptake of Suc across membranes into developing cotyledons and isolated leaf disks have been available for decades (Figure 1) (Sovonick et al., 1974; Lichtner and Spanswick, 1981; Maynard and Lucas, 1982a). The first phase, evident at low concentrations, is saturable and requires a proton gradient (Giaquinta, 1977) and the second phase is linear with Suc concentration and unsaturable. The first phase is described as being mediated by a high-affinity/low-capacity mechanism and the second phase has been referred to as a low-affinity/high-capacity mechanism. The development of isolated membrane vesicles as experimental tools was fundamental to elucidating the biochemical characteristics of Suc transport across plant membranes, including $K_{0.5}$ for both H^+ and Suc, pH and membrane potential dependence, stoichiometry, electrogenicity, and specificity (Bush, 1990; see Bush (1993) for detailed review and discussion). It is clear now that the saturable phase represents thermodynamically unfavorable uptake of Suc energized by the proton-motive force and catalyzed by Suc/H^+ symporters. All SUTs isolated to date are characterized as Suc/H^+ symporters with the exception of several SUFs identified in legumes (discussed below). Despite the identification of SUFs, the linear phase is not defined at the molecular level.

The first *SUT* genes identified and sequenced were isolated from spinach (*SoSUT1*; *Spinacia oleracea*) and potato (*StSUT1*; *Solanum tuberosum*) cDNA-expression libraries that were screened in yeast. The yeast strain used was engineered to grow on Suc as the sole carbon source only if Suc were internalized via a cDNA-encoded transporter (Riesmeier et al., 1992, 1993). Subsequently, *SUT* genes were isolated from numerous species by sequence homology (Gahrtz et al., 1994; Sauer and Stolz, 1994; Burkle et al., 1998; Aoki et al., 1999) and more recently by analysis of sequenced genomes and EST libraries (Meyer et al., 2000; Aoki et al., 2003; Sauer et al., 2004; Baud et al., 2005). It is now apparent that all plants have a small family of *SUT* genes that are members of the major facilitator superfamily, with 12 membrane spanning domains and the N- and C-termini on the cytoplasmic side of the membrane.

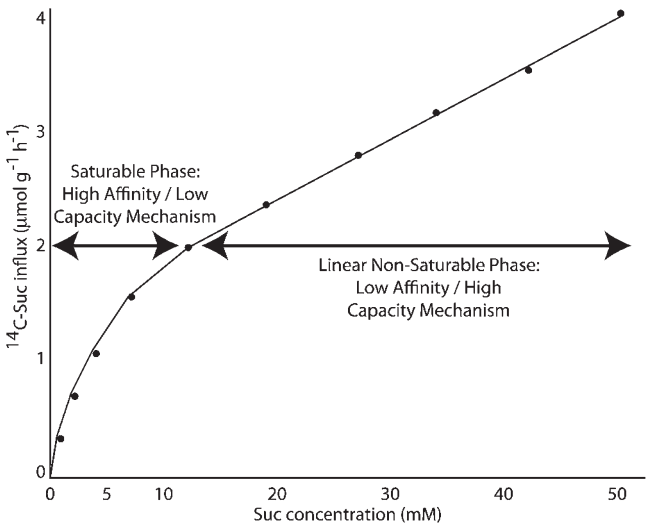

Figure 1. A Representative Plot of [^{14}C]Suc Influx into Leaf Disks Relative to External Suc Concentration.

The graph shows the saturable phase ascribed to a high-affinity/low-capacity mechanism, now known to be catalyzed by Suc/H^+ symporters, and the linear, non-saturable phase, ascribed to a low-affinity/high-capacity mechanism. Although described as having characteristic of facilitated diffusion, the mechanism remains unknown. The plot is drawn based on Maynard and Lucas (1982a).

Members of the SUT family cluster into distinct clades and these are useful in forming hypotheses on function. Aoki and colleagues identified three distinct branches (Type I, II, and III) in one of the earliest comprehensive trees (Aoki et al., 2003), as did Lalonde and colleagues (Clade I, II, and III) (Lalonde et al., 2004). As expected, these trees have the same basic structure, but the Type/Clade naming is not consistent, with Aoki's Type II and III branches corresponding to Lalonde's Clade III and II, respectively. As more sequences were identified, the number of major branches has increased from three to four (Sauer, 2007), to the current five (Braun and Slewinski, 2009; Kuhn and Grof, 2010), as the Type II (Clade III) branch has undergone further subdivision. However, inconsistency in nomenclature remains, with various authors using different conventions. Furthermore, individual genes were initially named in the order they were identified and then on homology to existing genes, such that gene names do not provide an intuitive indicator of sub-family membership. The Groups 1 through 5 convention (Braun and Slewinski, 2009) will be used in this document. Table 1 summarizes the characteristics of the five groups and provides representative members of each.

Group 1 comprises monocot-specific SUTs involved in phloem transport, Suc uptake into sink tissues including grain filling, and general retrieval from the apoplasm (Aoki et al., 1999, 2003, 2004; Scofield et al., 2007; Braun and Slewinski, 2009; Slewinski et al., 2009). All appear to localize to the plasma membrane and have moderate affinity for Suc, with $K_{0.5}$ values ranging from 3.7 mM for ZmSUT1 from maize (*Zea mays*) (Carpaneto et al., 2005) to 10.6 mM for HvSUT1 from barley (*Hordeum vulgare*) when tested in *Xenopus* oocytes by electrophysiology (Sivitz et al., 2005). Group 1 SUTs are thought to contribute to the saturable uptake kinetics in monocots and have the highest specificity for Suc among the SUT groups (Sun et al., 2010).

Group 2 comprises dicot-specific SUTs that are also involved in phloem loading, uptake of Suc into various sink tissues, and general retrieval from the apoplasm. This group has received the greatest attention because they were the first SUTs identified, are active in species amenable to manipulation with genetic and molecular techniques, and because members have a clear function in phloem loading and carbon partitioning. They localize to plasma membranes and mediate high-affinity saturable uptake with $K_{0.5}$ for Suc ranging from 0.066 mM for

Table 1. Summary of SUT Family Type/Clade/Group Classifications and Apparent Functions of Representative Members.

Type[1]/clade[2]	Group[3]/ clade[4]	Distribution, function	Affinity ($K_{0.5}$ mM) in yeast or (oocytes)	Representative members	Location, apparent physiological function	Antisense/mutant phenotype	Reference[5]
Type I/Clade I	Group 2/ SUT1	Dicot, high-affinity uptake	0.5 0.77 (1.44) 0.5 (0.066) 1.0 (0.5) 99.8	AtSUC1 AtSUC2 AtSUC9 Solanaceae SUT1 PsSUF1	Numerous sinks, uptake Phloem, loading/retrieval Broadly, high-affinity retrieval Phloem and sinks, loading/retrieval Bidirectional Suc facilitator	Pollen defects Stunted, accumulate carbohydrate Early flowering Stunted, accumulate carbohydrate Unknown	(Feuerstein et al., 2010) (Srivastava et al., 2008) (Sivitz et al., 2007) (Schmitt et al., 2008) (Zhou et al., 2007)
Type II/Clade III	Group 1/ SUT3	Monocot, high affinity uptake	(7.5) (3.7)	OsSUT1 ZmSUT1	Broadly, seed filling, not loading Phloem & sinks, loading	Shriveled seeds Stunted, accumulate carbohydrate	(Scofield et al., 2002) (Slewinski et al., 2009)
	Group 3/ SUT2	Monocot and Dicot, low affinity	Not active 1.9	Solanaceae SUT2 AtSUC3/ AtSUT2	Sink organs, pollen tube growth Sinks and wounded tissue	Reduced pollen growth None apparent	(Hackel et al., 2006) (Meyer et al., 2004)
	Group 5/ SUT5	Monocot, high-affinity uptake	(2.3)	OsSUT5	Broadly	Unknown	(Sun et al., 2010)
Type III/Clade II	Group 4/ SUT4	Dicot and monocot, low affinity	Unknown (16.0) 6.0 37.8	HvSUT2 LjSUT4 StSUT4 PsSUF4	Mesophyll, tonoplast transport Nodules, tonoplast transport Broadly, plasma membrane Bidirectional Suc facilitator	Unknown Unknown Early flowering and tuberization Unknown	(Endler et al., 2006) (Reinders et al., 2008) (Chincinska et al., 2008) (Zhou et al., 2007)

[1] Based on Aoki et al. (2003).
[2] Based on Lalonde et al. (2004).
[3] Based on Braun and Slewinski (2009).
[4] Based on Kuhn and Grof (2010).
[5] Only the most recent or thorough reference is provided here; see text for more thorough descriptions and citations.

Arabidopsis AtSUC9 (in oocytes; 0.5 mM in yeast) (Sivitz et al., 2007) to 1.44 mM for AtSUC2 (Chandran et al., 2003). Group 2 SUTs have broader substrate specificity than Group 1 and can transport a variety of glucosides when expressed in oocytes, some of which are naturally occurring secondary metabolites in plants (Sivitz et al., 2007). AtSUC5 also transports the structurally unrelated compound, biotin (vitamin H) (Ludwig et al., 2000).

The Group 3 transporters are found in both monocots and dicots and have additional amino acids at both termini and in loop 4, which extends into the cytoplasm. SlSUT2 of tomato (*Solanum lycopersicum*) was originally described as a potential sucrose sensor without symporter activity (Barker et al., 2000) but orthologs from other species are active (Meyer et al., 2000; Barth et al., 2003).

Group 4 SUTs are roughly 47% similar to Group 2 SUTs and have approximately 10-fold lower affinity for Suc (Weise et al., 2000; Reinders et al., 2008). Because of this, they were described as low-affinity/high-capacity transporters and possible mediators of the non-saturable, first-order kinetics represented by the linear phase of Suc uptake experiments (Figure 1). Group 4 SUTs will transport a greater variety of substrates than Group 1 SUTs, but are more specific than those in Group 2 (Reinders et al., 2008). Particularly intriguing about this group is the demonstration that members localize to the tonoplast in some species and to the plasma membrane in others (see below).

Group 5 is monocot-specific and was split from Group 1 (Braun and Slewinski, 2009). OsSUT5 of rice, the only member of this group to be biochemically analyzed, has higher affinity for Suc than Group 1 OsSUT1 (OsSUT1 $K_{0.5}$ = 7.5 mM; OsSUT5 $K_{0.5}$ = 2.3 mM; both at pH 5.6), less pH dependence for activity, and broader substrate specificity (Sun et al., 2010). These characteristics support the division of Group 5 from Group 1.

Group 1 and Group 2 transporters, despite their evolutionary divergence and differences in affinity for Suc and other substrates, appear to be functionally orthologous. This raises questions about the phylogentic origins of SUTs and how they were adopted for use in different plant lineages. Unfortunately, the only sequences outside of the angiosperms are four genes from the moss *Physcomittrela patens* that cluster together closest to Group 2 (Kuhn and Grof, 2010), and their activities are not published. It will be informative to obtain sequences from more basal angiosperms to understand how/why monocots and dicots use different SUTs for fundamental aspects of carbon partitioning, and it will also be very informative to get sequences from phyla between mosses and angiosperms to learn how SUTs adapted during the evolution of land plants in general and vascular plants specifically.

Recently, a SUT from a plant-pathogenic fungus, UmSrt1, was characterized and shown to be essential for effective infection. Hyphae of the corn-smut fungus *Ustilago maydis* penetrate the plant-cell wall to associate closely with the plant plasma membrane and be well suited for uptake of apoplasmic organic carbon (Wahl et al., 2010). Most biotrophic fungi hydrolyze Suc to monosaccharides, which can trigger a defense response in the host plant. By catalyzing Suc uptake directly, UmSrt1 may help avoid this response. UmSrt1 $K_{0.5}$ for Suc is 26 μM in yeast, which is 20-fold lower than that established for AtSUC9 in yeast, and UmSrt1 is more specific for Suc than plant SUTs. UmSRT1 has 12 membrane-spanning domains typical of the major facilitator super family but has less than 30% sequence similarity to plant and fungal hexose transporters, which are the closest characterized proteins in databases. *UmSrt1* is expressed only after infection is established and is an essential gene for virulence symptoms. Interestingly, pathogenicity of *U. maydis* with *UmSrt1* deleted was restored when transformed with *AtSUC9*. The high affinity and specificity of UmSrt1 contrast with the characteristics of SUT1 from the fungus *Schizosaccharomyces cerevisiae*, which has higher affinity for maltose ($K_{0.5}$ = 6.5 ± 0.4 mM) than for Suc ($K_{0.5}$ = 36.3 ± 9.7 mM) (Reinders and Ward, 2001). Biotrophs or symbionts that take nutrients from the apoplasm represent promising tools for further assessing Suc membrane transport, since they have evolved mechanisms to exploit the host's pattern of carbon partitioning.

ENDOMEMBRANE TRANSPORT OF Suc

Suc in the cytoplasm, whether synthesized or imported, can be compartmentalized to vacuoles and other organelles (Figure 2). In photoautotrophic cells, vacuoles store excess Suc during the day and export it at night (Martinoia et al., 2000; Neuhaus, 2007). In the leaves of C3 and CAM plants, Suc partitioning between vacuoles and cytoplasm appears to be by facilitated diffusion driven by the concentration gradient between the two compartments. In heterotrophic storage tissue, such as the tuber of sugar beet, Suc accumulates in the vacuole for long-term storage by an apparent Suc/H^+ antiport system at the expense of the acidic pH of the lumen. Neither of these systems is characterized at the molecular level. Sucrose binding proteins (SBP) are 62–64-kDa proteins associated with tissues involved in Suc transport, and may mediate non-saturable Suc transport (Overvoorde et al., 1996), but results are not consistent with sequences from different species (Zhang et al., 2007). Recently, mung bean (*Vigna radiata*) VrSBP1 was localized to the tonoplast (Wang et al., 2009), suggesting that SBPs may participate in facilitated diffusion into vacuoles. Despite claims of transport activity, there is also evidence that SBPs are storage proteins that bind Suc and localize to the protein-storage vacuole in cotyledons (Elmer et al., 2003). More work on SBPs is required to fully appreciate their role in Suc partitioning.

A proteomic approach on vacuoles purified from barley mesophyll cells (*Hordeum vulgare*) identified HvSUT2 in the tonoplast fraction (Endler et al., 2006). Transient expression of GFP fusions to HvSUT2 and its closest *Arabidopsis* homolog, AtSUC4, in onion epidermal cells and *Arabidopsis* leaves localized fluorescence to the tonoplast. When fused to GFP, the *Lotus japonicus* homolog, LjSUT4, similarly localized to

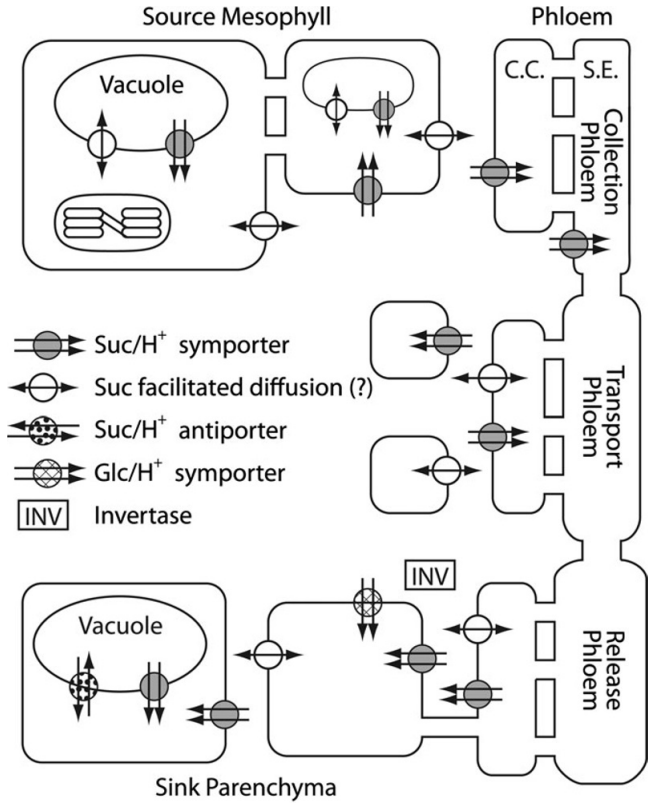

Figure 2. Diagram of Suc Membrane Transport in Different Parts of the Plant.

Top: In mesophyll cells, vacuolar influx/efflux is driven by concentration gradients characteristic of facilitated diffusion, and the presence of Suc/H^+ symporters implies energized efflux. Efflux across the plasma membrane occurs by an unknown mechanism and retrieval by SUTs may be a general phenomenon; efflux may be greater in the vicinity of the sieve-element/companion-cell complex. Uptake of Suc from the apoplasm into the phloem (i.e. phloem loading) occurs in the collection phloem and is catalyzed by SUTs.

Middle: Suc is released from the transport phloem to enter transient storage reserves and nurture flanking tissues. Suc released from transient storage reserves are most likely retrieved into the phloem by the same SUTs involved in phloem loading.

Bottom: Suc and other nutrients are distributed from the release phloem symplasmically through plasmodesmata or across membranes to the apoplasm. Suc in the apoplasm is recovered into recipient cells directly by SUTs or is hydrolyzed by cell wall invertases and recovered by monosaccharide transporters. Suc accumulation in vacuoles appears to be catalyzed by Suc/H^+ antiport and Suc/H^+ symporters on the tonoplast imply energized efflux from vacuoles. Refer to text for further details and citations.

tonoplasts in *Medicago truncatula* roots and nodules and to tonoplasts in *Arabidopsis* (Reinders et al., 2008). All three genes were characterized as Suc/H^+ symporters in either yeast or oocytes, where the proteins apparently mis-localized to the plasma-membrane to allow Suc uptake from the surrounding solutions (Weise et al., 2000; Reinders et al., 2008). These results are significant because a Suc/H^+ symporter on the tonoplast implies energized efflux from the vacuole, which is not predicted from physiological or biochemical studies. The ortholog from potato, StSUT4, however, immunolocalized to enucleate sieve elements (Weise et al., 2000); was found only on the plasma membrane fraction in Western blots from potato leaves; and StSUT4–GFP fusions localized to the plasma membrane and perinuclear membrane of tobacco and potato epidermal cells (Chincinska et al., 2008). Group 4 proteins may therefore localize to different membranes in different species. The most abundantly expressed SUT in poplar (*Populus tremula*×*alba*) is the group 4 *PtaSUT4*, and the protein was localized to the tonoplast by fusion with GFP and expression in *Nicotiana benthamania* protoplasts (Payyavula et al., 2011) but to the plasma membrane by proteomics (Nilsson et al., 2010). PsSUF4, a potential Suc facilitator from pea (*Pisum sativum*), supported bi-directional, pH- and energy-independent Suc transport in yeast, characteristic of facilitated diffusion (Zhou et al., 2007). *PsSUF4* clusters closely with *LjSUT4* in Group 4, raising the possibility that it may localize to the tonoplast.

EFFLUX OF Suc ACROSS THE PLASMA MEMBRANE

Suc in the apoplasm is typically measured in the range of 1–5 mM (Lohaus et al., 1995; Ayre et al., 2003; Nadwodnik and Lohaus, 2008), but has also been estimated as high as 20 mM (Sovonick et al., 1974). It must be noted, however, that apoplastic Suc cannot be measured with high resolution, and local concentrations may vary substantially. Notwithstanding, these values are low relative to those in the cytoplasm and passive methods would, in principle, be sufficient for Suc transfer from the symplasm to the apoplasm. Conversely, if exogenous Suc is present at sufficient levels, passive transport from the apoplasm to the symplasm would be thermodynamically favorable. In Suc-uptake experiments (Figure 1), the non-saturable, linear component of the biphasic kinetics has characteristics of facilitated diffusion, but it is argued that this interpretation is too simple because, in part, linear uptake is inhibited by anoxia and maltose (Maynard and Lucas, 1982b). However, Suc is well known to escape to the apoplasm and, where measured, linear efflux kinetics are observed (Huber and Moreland, 1980; Anderson, 1983). It is appealing to generalize that non-saturable uptake and linear efflux are not independent, but are components of the same mechanism working in reverse.

In whole plants, most attention on Suc in the apoplasm has focused on efflux from mesophyll cells in preparation for phloem loading; efflux from the length of the phloem network; and efflux from the symplasm of the post-phloem pathway in sink organs (Figure 2). However, efflux to the apoplasm is likely a general phenomenon from all cells with differences in rates controlled by unknown factors (possibly turgor—see below). It is currently not possible to monitor Suc in intact tissues with sufficient resolution to determine rates of efflux from specific cell types. Rather, best-guest inferences are made based on the physiology and anatomy of the local tissues and

cells. For example, a scarcity of plasmodesmata indicates symplasmic disjunction and a necessary apoplasmic transport step, and transfer-cell morphology may represent a proliferation of membrane to accommodate an abundance of proteins involved in efflux or influx (Pugh et al., 2010). There is also a general assumption that efflux to the apoplasm will be in close proximity to sites of retrieval (see, e.g. Giaquinta, 1983; Haritatos et al., 2000).

Efflux in Source Leaves

The predominant fate of Suc produced in photoautotrophic cells is long-distance transport to heterotrophic sinks via the phloem (Figure 2). Based on its role in nutrient distribution and its location in the plant, the phloem network is commonly divided into the collection phloem, the transport phloem, and the release phloem (van Bel, 1996). In preparation for long-distance transport, Suc produced in mesophyll cells moves towards the collection phloem in minor veins where phloem loading occurs. Phloem loading is the accumulation of solute, predominantly sugars, into the phloem to generate the high hydrostatic pressures that facilitate long-distance transport. Three mechanisms of phloem loading have been proposed for different species and multiple mechanisms can operate in the same species: (1) phloem loading of Suc (or other transport sugar) from the apoplasm; (2) polymer trapping of Suc that diffuses into the phloem through specialized, highly branched plasmodesmata; and (3) diffusion without polymer trapping, where the highest concentrations of solute are in the mesophyll cells and these enter the phloem passively through regular plasmodesmata (Rennie and Turgeon, 2009; Slewinski and Braun, 2010a). The latter process is therefore not true loading, since there is not a solute concentrating step, but has been referred to as passive loading (Rennie and Turgeon, 2009; Slewinski and Braun, 2010a).

Phloem loading from the apoplasm requires Suc to enter the cell-wall space before energized accumulation into the phloem. Although Suc is present throughout the apoplasm, with efflux likely occurring from all mesophyll cells, the prevailing model is that Suc for long-distance transport enters the apoplasm in the immediate vicinity of the minor-vein phloem, with the phloem parenchyma commonly cited as the cells most likely responsible for release (Giaquinta, 1983). Movement through the mesophyll symplasm via plasmodesmata is calculated to be more efficient than efflux to the apoplasm with diffusion through the cell-wall space. In addition, efflux in the immediate vicinity of the phloem solves the potential problem of water entering from the xylem and hindering apoplasmic Suc from reaching the phloem. As anatomical evidence, *Arabidopsis* phloem parenchyma cells have transfer-cell wall ingrowths where they abut sieve elements and may be specialized for Suc efflux (Haritatos et al., 2000). Notwithstanding, there is little experimental evidence supporting or refuting preferential Suc release in the vicinity of the phloem and the site of Suc efflux remains ambiguous.

In both polymer trapping and diffusion, Suc efflux to the apoplasm would be detrimental to sugars entering the phloem. However, Suc is present in the apoplasm and sugar/H^+ symporters are identified in vascular tissues of *Alonsoa meridionalis*, which is a model plant for the polymer trap mechanism (Knop et al., 2004). The role of these SUTs was initially not clear and contributed to uncertainty of the polymer trapping mechanism. *A. meridionalis* has two structurally distinct types of phloem cells within the same veins: one with specialized intermediary cells for polymer trapping and the other with ordinary companion cells. It was found that a gene involved in polymer trapping, *AmSTS1*, encoding stachyose synthase, expresses only in the intermediary cells and the AmSUT1 Suc/H^+ symporter localizes only to ordinary companion cells (Voitsekhovskaja et al., 2009). These findings show that two types of phloem loading can occur in the same vein and demonstrate that Suc in the apoplasm, and SUTs in the minor veins, are compatible with polymer trapping. Melon plants (*Cucumis melo*) also phloem load by polymer trapping, but, during infection by Cucumber Mosaic Virus (CMV), expression levels of *CmSUT1* increase in vascular bundles and there is more Suc in the phloem sap (Gil et al., 2011). The authors of this study propose that melon contains the machinery for both polymer trapping and apoplasmic loading and that CVM infection triggers a defense response that alters the mode by which photoassimilates are loaded for long-distance transport. *Verbascum phoeniceum* transports raffinose and stachyose and is also a model species for polymer trapping (McCaskill and Turgeon, 2007). Down-regulating *VpSUT1* by RNAi nearly abolished saturable uptake of [^{14}C]Suc in leaf disks, but plants were normal with respect to growth rate, photosynthesis, and the ability to clear starch during the night (Zhang and Turgeon, 2009). These results argue that Suc efflux to the apoplasm has little impact on phloem transport in this species and that saturable [^{14}C]Suc uptake is primarily for retrieval of leaked Suc.

Efflux along the Phloem Path and in Sink Organs

Once loaded into the phloem, Suc moves along hydrostatic pressure gradients by bulk flow through the transport phloem. The transport phloem connects source and sink tissues and represents the longest contiguous stretch of phloem in the long-distance-transport pathway. Phloem anatomy and studies with fluorescent dyes show that the transport phloem is symplasmically isolated from surrounding tissues (Kempers and van Bel, 1997; Kempers et al., 1998), but lateral tissues require nutrients and also act as transient storage reserves in stems and roots. These anatomical data, as well as physiological measure of sugar loss and retrieval in the phloem, argue that nutrient exchange with flanking tissues is through the apoplasm (Minchin et al., 1984; Minchin and Thorpe, 1987) and imply passive efflux coupled to energized retrieval (Figure 2). Electrophysiology on intact stems supports a model for controlled Suc distribution based on thermodynamically

favorable electrochemical gradients between the phloem and flanking cells (Hafke et al., 2005).

In terminal sink tissues (i.e. those at or very near the end of the phloem network), Suc unloading from the release phloem occurs through the symplasm or to the apoplasm with subsequent uptake into recipient cells (Figure 2) (Patrick, 1997; Lalonde et al., 2003; Zhang et al., 2007). Phloem unloading of dyes and fluorescent proteins demonstrate that symplasmic unloading dominates in strong sinks such as root tips, developing fruits, integuments around developing seeds, and in young sink leaves, and that the plasmodesmata that enable this movement have size exclusion limits sufficient to allow transfer of proteins up to 67 kDa (Ruan and Patrick, 1995; Oparka et al., 1999; Stadler et al., 2005a, 2005b). These regions of the phloem have been called unloading domains and further transport through post-phloem symplasmic domains can be extensive. Free GFP, for example, moves readily through the root tip when unloaded from the phloem, whereas larger proteins are restricted to a narrow region surrounding the phloem (Stadler et al., 2005b). Suc and other nutrients would be able to unload and move through such post-phloem symplasmic domains more readily than proteins.

However, Suc efflux must occur to reach symplasmically isolated sink tissues, such as where high levels of osmotically active solute are required in the recipient cells, or between maternal and filial tissues in developing seeds (Patrick, 1997; Lalonde et al., 2003; Zhang et al., 2007). The unloading strategy in sinks may switch between symplasmic and apoplasmic routes based on the physiological need of the tissues. For example, unloading in potato stolons is initially to the apoplasm but switches to transport through the symplasm during starch accumulation in tubers (Viola et al., 2001). Conversely, unloading in developing tomato and grape berry is through the symplasm and switches to the apoplasm during ripening when starch is converted to sugar (Ruan and Patrick, 1995; Zhang et al., 2006). A similar phenomenon occurs in developing cotton fibers, which are single cells that grow on the seed coat. While cotton fibers are rapidly elongating, they require high solute concentrations for turgor pressure to drive expansion. During this stage, they are disengaged from the symplasmic domain of the seed coat, necessitating efflux to the apoplasm prior to uptake into fibers. In later development, when large quantities of photoassimilate are required for growth of the extensive secondary cell wall, the fiber, seed coat, and phloem form a contiguous symplasm to permit diffusion through plasmodesmata (Ruan et al., 2001).

Most studies on *trans*-membrane Suc flux are conducted with ^{14}C-labeled sugars, which are subject to metabolism, compartmentalization, and rapid diffusion through aqueous environments. Current methods for detection lack the resolution required to establish the sites of [^{14}C]Suc influx or efflux from intact tissues. Recently, fluorescence (or Förster) resonance energy transfer (FRET) nanosensors were used to measure Suc and glucose (Glc) uptake into root tips and provided evidence for facilitated diffusion across the plasma membrane (Chaudhuri et al., 2008). FRET nanosensors are engineered proteins that make use of ligand-dependent changes in protein confirmation to alter interaction between FRET donor and acceptor fluorophores. The ratio of emission from the donor relative to the acceptor is used as a proxy for measuring analyte concentrations. By using cytoplasmic Glc and Suc-specific nanosensors, FRET responses to perfusion of root tips with either compound were detected within 10 s and were fully reversible in 10–180 s. Elimination of Glc and Suc could have been by efflux to the apoplasm or metabolism, but preloading the tissues with substrate did not affect the results, arguing against transport across an endomembrane and sequestration in an organelle. The response was independent of protonophores, K^+ ions, and pH gradients, indicating that both influx and efflux may be mediated by proton-independent transport systems (Chaudhuri et al., 2008). *Arabidopsis* root tips are particularly amenable to FRET because of their small size, simple architecture, and transparent nature. Similar findings in aerial portions of the plant would not be surprising.

WHAT PROTEINS MEDIATE EFFLUX?

Because Suc efflux to the apoplasm is essential for plant growth and development, identifying, characterizing, and localizing the enzymes that facilitate this transfer is widely recognized as one of the great challenges in the field of whole-plant carbon partitioning. Early work with radiotracers suggested that efflux could be inhibited by anoxia, protonophores, or with *para*-chloromercuribenzene sulfonic acid (PCMBS), which is a non-permeable inhibitor of Suc/H^+ symporters (Huber and Moreland, 1981; Anderson, 1983; Aloni et al., 1986; Hayes et al., 1987). These and similar studies indicated that unloading was energy dependent and perhaps mediated by the same proteins involved in uptake. As Suc/H^+ symporter expression patterns were determined, it was found that those in the phloem of leaves were also in the phloem of sink organs, further suggesting a role in unloading (Truernit and Sauer, 1995; Kuhn et al., 2003). Frommer and colleagues localized Solanaceae SUT1, SUT2, and SUT4 proteins to sieve elements and showed that these proteins can form homo- and hetero-dimers or oligomers in yeast (Reinders et al., 2002; Kuhn et al., 2003), as could SUTs from *Arabidopsis* (Schulze et al., 2003). They speculated that Suc/H^+ symporters could form specific interactions with each other or with other cellular components to promote efflux across the membrane, possibly as uniporters (Reinders et al., 2002; Kuhn et al., 2003). More recently, it was shown that StSUT1 forms homo-dimers in plants and that, in yeast, oligomerization is stimulated by oxidizing agents (Krugel et al., 2008, 2009).

Recently, a new class of SUT—Suc facilitators (SUF)—were described and shown to express broadly in pea (*Pisum sativum*) and bean (*Phaseolus vulgaris*) (Zhou et al., 2007). In yeast, SUF proteins supported bi-directional transport of Suc independently of pH and energy in a fashion characteristic of

facilitated diffusion. Sequence analysis placed SUFs within the known SUT family: *PsSUF1* and *PvSUF1* clustered with Group 2 transporters and *PsSUF4* clustered with Group 4. This implies that modest changes to SUT structure can convert an energized transporter to a passive facilitator. All members of the SUT family in *Arabidopsis* are characterized as Suc/H^+ symporters and not facilitators, and SUFs may thus have limited distribution. In species that do not encode SUFs, the conformational changes that convert SUTs to SUFs could be mediated by protein–protein interactions or other post-translational modification, as suggested by Frommer and colleagues (Reinders et al., 2002; Kuhn et al., 2003).

Further supporting the possibility that SUTs can catalyze efflux as well as influx is the finding that ZmSUT1 in oocytes is reversible if the chemical and electrochemical gradients across the membrane are favorable (Carpaneto et al., 2005). In the transport phloem of maize, the measured values of 850 mM Suc in the phloem, 1 mM in the surrounding apoplasm, and a pH gradient of 1 would favor Suc efflux at membrane potentials more positive than –115 mV. Since the membrane potential in this region is in the range of –60 mV, ZmSUT1-mediated efflux is feasible (Carpaneto et al., 2005). However, favorable conditions are unlikely in the post-phloem symplasmic domains that provide Suc to isolated organs, since the symplasmic Suc concentrations are much lower and apoplasmic Suc levels are higher (Zhang et al., 2007). Notwithstanding, these results show mechanistically that at least one SUT can move Suc in either direction based on prevailing conditions.

If the same transporters involved in uptake are also involved in efflux, altering their expression should influence carbon partitioning in predictable ways. AtSUC2 is the predominant SUT involved in phloem loading in *Arabidopsis* (Gottwald et al., 2000) and is also expressed strongly along the transport phloem, where it most likely engages in retrieval of leaked Suc, but could also have a role in efflux (Truernit and Sauer, 1995). To test the function of AtSUC2 in the transport phloem, Srivastava and colleagues complemented a homozygous *Atsuc2-4* knockout mutant with an *AtSUC2* cDNA expressed only in the collection phloem. This permitted phloem loading in minor veins but *AtSUC2* expression was absent along the remainder of the phloem network. Relative to wild-type, higher levels of sugar and starch were measured in tissues around the transport phloem. This was interpreted as evidence that AtSUC2 did not play a major role in efflux but that its retrieval function was missing along the transport phloem (Srivastava et al., 2008).

Are there candidates for Suc efflux carriers outside the SUT family? As described above, FRET nanosensors indicated rapid influx and possibly efflux of Glc and Suc from *Arabidopsis* root tips. High-resolution expression mapping of root tips showed that only the monosaccharide transporter *STP1* and the SUT *AtSUT2/SUC3* are significantly expressed in the outer cell layers (Chaudhuri et al., 2008). However, the authors point out that the *Arabidopsis* genome contains many transporters that do not group with characterized families (i.e. transporters of unknown function); that many of the 53 members of the hexose transporter family in *Arabidopsis* have not been characterized and may function as uniporters; and that one of the 38 members of the aquaporin family, which are now recognized to move compounds other than water, could facilitate Suc transport (Chaudhuri et al., 2008). The finding that a Suc/H^+ symporter from a fungus clusters most closely to monosaccharide transporters, but is still only 30% similar (Wahl et al., 2010), is relevant here because it emphasizes that SUTs may have sequences quite distant from canonical plant Suc/H^+ symporters.

ENERGIZED INFLUX OF SUCROSE ACROSS THE PLASMA MEMBRANE

Once released to the apoplasm, Suc may be retrieved directly by Suc/H^+ symporters or may be hydrolyzed by cell-wall invertase and the hexose retrieved by monosaccharide transporters (Figure 2). Evidence suggests that both pathways are used in combination or at different stages of development (Ruan and Patrick, 1995; Godt and Roitsch, 2006; Zhang et al., 2006; McCurdy et al., 2010). The Suc concentration gradient from apoplasm to symplasm requires that Suc uptake is energized in most, if not all, cells under physiological conditions and, mechanistically, all Suc/H^+ symporters do the same thing (transport Suc at the expense of the proton-motive force), irrespective of where they express and localize. It is not surprising therefore that the same transporter may be expressed in both source and sink organs, and that some cells may express multiple genes. In most species, SUT expression and/or localization is not assessed with sufficient resolution to determine function based on the cell type. For example, expression in source leaves determined by qRT–PCR or Northern or Western blot could represent a role in phloem loading, general retrieval of leaked Suc, both, or neither. Discussion here is therefore biased toward systems with higher-resolution analysis.

Influx in Source Organs

SUT expression patterns in the non-phloem cells of the leaf illuminate the fate of Suc that enters the apoplasm but is not exported. SUTs belonging to Groups 2 and 3 express in guard cells and may contribute to the hydrostatic potential that controls stomatal opening (Meyer et al., 2004; Weise et al., 2008). Transpiration from stomatal pores can concentrate apoplasmic Suc to 150 mM around the guard cells (Kang et al., 2007). Transporters from two different groups may help optimize Suc partitioning in this dynamic environment. Group 2 and 3 SUTs also express in leaf trichomes.

Energized accumulation of Suc in the minor veins for translocation was established decades ago (Figure 2) (Sovonick et al., 1974). It was also established decades ago that the proton motive force provided the energy for phloem loading (Giaquinta, 1977, 1979). Once SUT encoding genes were functionally identified by complementation in yeast, evidence for their role in phloem loading was inferred by localizing

expression to phloem cells (Riesmeier et al., 1993; Truernit and Sauer, 1995). Definitive evidence for a role of specific Group 1 and Group 2 SUTs in phloem loading came from gene repression of *SUT1* in Solanaceae species (Riesmeier et al., 1994; Kuhn et al., 1996; Lemoine et al., 1996; Burkle et al., 1998; Hackel et al., 2006) and insertional mutagenesis of *AtSUC2* in *Arabidopsis* (Gottwald et al., 2000) and *ZmSUT1* in maize (Slewinski et al., 2009). All of these have characteristics typical of phloem loading defects and impaired carbohydrate transport, including dramatically stunted stature, source leaves that hyperaccumulate sugar and starch, increased levels of anthocyanin, and premature leaf chlorosis followed by necrosis. The accumulation of carbohydrate in leaves feedback inhibits photosynthesis (Stitt et al., 2010) such that these plants also have lower primary productivity (Kuhn et al., 1996; Burkle et al., 1998). Anthocyanin accumulation is promoted by Suc and contributes to photoprotection in vegetative tissues under conditions of high light (Steyn et al., 2002; Solfanelli et al., 2006). Since photosynthesis is feedback-inhibited in plants with defects in phloem transport, the photochemistry that normally converts light energy to chemical energy is curtailed and the leaves are more susceptible to photo-oxidative damage. The accumulation of anthocyanin in these transport-inhibited leaves is therefore analogous to a photo-protective response to excessive illumination.

Several T-DNA insertion alleles are described for *AtSUC2* (*Atsuc2-1* through *Atsuc2-4*) that are considered to be knockouts, since full-length transcript is not detected and any potential proteins resulting from sequences upstream of the T-DNA insertion are not active (Gottwald et al., 2000; Srivastava et al., 2009a). These mutants are unique because gene repression by antisense-RNA is not 100% and the *Mutator1* transposon in *Zmsut1* results in a new transcript with an out-of-frame ATG translational start site upstream of the natural start site, such that a protein with SUT activity is possible. Production of viable seed is reported for the *Atsuc2-4* allele, indicating that a degree of phloem transport continues in the absence of AtSUC2 activity (Srivastava et al., 2009a). Considering the importance of phloem loading to nutrient transport, it is perhaps surprising that *Atsuc2* mutants show any growth. In the absence of AtSUC2 activity, overlapping function of other SUTs or compensatory activity of hexose transporters may permit photoassimilate transport to sink tissues (Gottwald et al., 2000). *Arabidopsis* has nine Suc/H^+ symporters (Sauer, 2007) and the monosaccharide transporter superfamily has 53 putative members (Buttner, 2007). However, it is not evident that the other SUTs have characteristics that could substitute for *AtSUC2* (Sauer, 2007; Sivitz et al., 2007, 2008). AtSUT4 and AtSUT2/SUC3 proteins localize to leaf veins but expression is weak and these may have their major role in sink organs (Barker et al., 2000; Schulze et al., 2003; Meyer et al., 2004; Chincinska et al., 2008). Alternatively, K^+ ions, amino acids, and other sugars may contribute sufficiently to phloem hydrostatic pressure to promote phloem transport in *Atsuc2* mutants (Srivastava et al., 2009a). Another possibility is that phloem loading from the apoplasm is beneficial, but not absolutely essential, for transport in *Arabidopsis*. As described above, many species have their highest solute concentrations in mesophyll cells and plasmodesmata between mesophyll and companion cells permits diffusion directly into the phloem for long-distance transport (Rennie and Turgeon, 2009). Mature leaves of *Atsuc2-4* plants accumulate ~18-fold higher levels of Suc than wild-type (Srivastava et al., 2008), which would facilitate diffusion into the phloem for long-distance transport without an energized concentrating step. The companion cells of *Arabidopsis* minor veins share more plasmodesmata with mesophyll cells than tobacco (an archetype for Suc loading from the apoplasm) but fewer than the abundant connections of true 'passive loaders' like willow and apple (Turgeon and Medville, 1998; Haritatos et al., 2000; Reidel et al., 2009). *Arabidopsis* leaf-vein morphology is therefore compatible with—but not optimized for—source-to-sink transport driven by hydrostatic pressure gradients initiating in mesophyll cells. As with any phloem-transport mechanism, bulk flow will occur through the sieve tubes if the hydrostatic pressure gradient between source and sink is greater than the resistance imposed by the sieve-plate pores.

SUT activity in leaves also contributes to mechanisms that govern the onset of flowering and tuberization, presumably by regulating Suc levels and Suc signaling. *AtSUC9* expresses broadly through the plant, including the lamina of leaves, and is particularly well suited to retrieving apoplasmic Suc at low concentrations. *Atsuc9* mutants flower early in short days (Sivitz et al., 2007) in which *Arabidopsis* flowering is controlled by the so-called autonomous pathway. A link between sucrose and flowering is well known from physiological experiments (Bernier, 1988) and AtSUC9 therefore appears to participate in delaying Suc-mediated floral induction via the autonomous pathway. Because *AtSUC9* is broadly expressed, it is not clear whether the Suc-mediated signal originates in leaves or at the meristem. However, flowering and tuber production are photoperiodically controlled in some potato accessions, and RNAi-mediated inhibition of *StSUT4* promoted both processes (Chincinska et al., 2008). Grafting and defoliation experiments showed clearly that *StSUT4*-repressed source leaves contribute significantly to the onset of flowering and tuberization in sink organs (Chincinska et al., 2008).

Influx along the Path Phloem and in Sink Organs

Along the transport phloem, energized uptake of Suc from the apoplasm must occur into flanking tissues for metabolism, growth, and storage, and Suc must also be retrieved back into the phloem to maintain phloem content and hydrostatic pressure (Figure 2) (Hafke et al., 2005). A SUT has not been defined for uptake into lateral tissues of *Arabidopsis*, but one with broad expression would be a candidate (e.g. *AtSUC9*). Solanaceae SUT1 was immunolocalized to xylem parenchyma cells in leaves and stems, and may be involved in uptake of Suc for cellulose and lignin in tracheids and vessels elements, and also to keep Suc out of the xylem stream (Schmitt et al., 2008).

ShSUT1 of sugarcane immunolocalized to the inner bundle sheath (mestome sheath) in the major veins of leaves and to cells surrounding the vascular bundles in stems. This localization indicates that ShSUT1 may keep Suc out of the xylem stream or retrieve Suc leaking from the storage parenchyma. An antibody against rice OsSUT1 did not cross-react with ShSUT1 but did bind to phloem cells, indicating that another SUT is involved in phloem loading and retrieval in sugarcane. A Group 2 transporter, *HbSUT3*, from rubber tree (*Hevea brasiliensis*) expresses strongly in the latex producing laticifers (Tang et al., 2010). Tapping the trees to harvest the latex increases laticifer sink-strength and causes marked increases in *HbSUT3* expression, which, in turn, correlates positively with latex yield. *HbSUT3* therefore seems to be involved in uptake of Suc that is released from the transport phloem.

Suc retrieval into the transport phloem is better characterized than uptake into flanking tissues. In bean stems, for example, Suc efflux from the transport phloem was 6% cm^{-1} and retrieval was 3% cm^{-1} (Minchin and Thorpe, 1987). Retrieval was mediated by SUTs as indicated by treating stems with PCMBS. Based on expression pattern, retrieval in the transport phloem is most likely catalyzed by the same symporters as those that catalyze loading in the collection phloem (Truernit and Sauer, 1995; Kuhn et al., 2003). This is functionally supported by experiments described above, in which *AtSUC2* cDNA was expressed in the collection phloem, but not in the transport phloem of homozygous *Atsuc2-4* plants: the accumulation of soluble sugar and starch in tissues surrounding the transport phloem was interpreted as evidence that retrieval into the phloem was diminished in the absence of the *AtSUC2* expression (Srivastava et al., 2008).

The majority of SUTs identified function in various terminal sink tissues. For example, all characterized Group 1 SUTs from monocots and all five *SUT* genes in rice (spanning Groups 1, 3, 4, and 5) express in developing seeds and other reproductive structures (Aoki et al., 1999, 2002, 2003); all three solanaceae SUTs are found in fruits or tubers; and, in *Arabidopsis*, where characterization is greatest, all SUTs are found in various sinks, including *AtSUC2*, which expresses in the transport phloem of roots and siliques (Truernit and Sauer, 1995).

Repression of the Group 1 OsSUT1 in rice resulted in shriveled grains but had little impact on phloem transport (Scofield et al., 2002). This is the only finding so far of a single Group 1 SUT being a dominant contributor to seed biomass specifically.

Of the Group 2 SUTs in *Arabidopsis*, AtSUC1 is characterized as expressing in trichomes, roots, and male and female reproductive organs, including pollen (where it is essential for microgametophyte germination and growth), anthers, funiculus epidermis, and the megagametophyte (Stadler et al., 1999; Sivitz et al., 2008; Feuerstein et al., 2010). Curiously, *AtSUC1* has different expression patterns in different *Arabidopsis* accessions (Feuerstein et al., 2010). The implications of this from a carbon-partitioning standpoint are (1) that AtSUC1 activity is not essential in these sinks, (2) that other transport systems are able to fill the role of AtSUC1, and (3) that the benefit of *AtSUC1* expression in those tissues is highly plastic. The authors reported different levels of *AtSUC1* expression in the pollen and funicular epidermis of different ecotypes and speculated that these differences may influence pollen tube growth and fertility between ecotypes (Feuerstein et al., 2010). In addition, *AtSUC6* and *AtSUC7* have numerous ecotype-specific substitutions that, in some cases, introduce stop codons, and *AtSUC7* undergoes alternative splicing, with only ecotype WS predicted to produce a functional protein (Sauer et al., 2004). *AtSUC6* and *AtSUC7* were therefore characterized as pseudogenes, but this should now be reexamined, and it has been suggested that *AtSUC7* may have ecotype-specific functions in flowering (Feuerstein et al., 2010). AtSUC8 is weakly active in the placenta, and no phenotypes were reported for T-DNA insertion mutants (Sauer et al., 2004). *AtSUT5* expresses in the endosperm of developing seeds (Baud et al., 2005) and is thus involved in nutrient acquisition by the filial tissue along with *AtSUC1* and *AtSUC3*, which expresses in the seed coat and suspensor (Meyer et al., 2004). Knockout alleles of *AtSUC5* are slightly delayed in embryo development and have a significant but transient reduction in fatty acid concentration, suggesting that an alteration in carbohydrate partitioning is manifest in downstream products of metabolism (Baud et al., 2005). However, AtSUC5 is also associated with biotin transport and thus the observed phenotypes may stem from a biotin deficiency (Ludwig et al., 2000).

Group 3 SUTs in general have broad but low levels of expression in numerous sink cells, including wounded tissues, pollen, guard cells, trichomes, root tips, the suspensor, seed coats (Meyer et al., 2004), and in *Plantago major*, the embryo (Barth et al., 2003). Group 3 SUTs are transcribed in companion cells but the proteins immunolocalize to enucleate sieve elements in *Arabidopsis*, *Plantago major*, and Solanaceae species (Barker et al., 2000; Barth et al., 2003; Meyer et al., 2004) (see Kuhn and Grof (2010) for further discussion). A visible phenotype for *AtSUT2/SUC3* mutants in *Arabidopsis* is not described, but antisense inhibition of *SlSUT2* in tomato affected fruit and seed development due to defects in pollen tube germination and growth (Hackel et al., 2006).

Group 4 SUTs were discussed above in the 'Endomembrane Transport of Suc' section and may localize to the tonoplast or plasma membrane and perinuclear region in a species-dependent manner, and may also contribute to the Suc-mediated flowering and tuberization (Endler et al., 2006; Chincinska et al., 2008). One member of Group 5, OsSUT5, was analyzed biochemically (Sun et al., 2010), but, apart from being expressed broadly (Aoki et al., 2003), nothing is known about the physiological role of this group.

REGULATION OF TRANSPORTERS

Suc transport is a dynamic process that must be controlled relative to the environmental and physiological needs of the plant. Tissue-specific expression is mediated by DNA

sequences in the canonical promoters as well as throughout the genes. As examples, the broad extent of *AtSUC1* and *AtSUC9* expression was not appreciated until whole-gene translational fusions with reporter genes were tested (Sivitz et al., 2007). Whole-gene fusions were also necessary for expression analysis of *LeSUT1*: expression in companion cells and guard cells was ascribed to intron 2, while intron 3 was necessary for expression in trichomes (Weise et al., 2008).

SUT genes with a clear role in phloem loading are induced in the minor veins in a pattern consistent with the sink-to-source transition: expression initiates at the distal tip of the leaf and progresses toward the basal end as the leaf matures (Riesmeier et al., 1993; Truernit and Sauer, 1995; Aoki et al., 1999). The *AtSUC2* promoter requires light stimulation for this induction in minor veins, as determined in transgenic tobacco plants (Wright et al., 2003), and it is reasonable to speculate that the endogenous *NtSUT1* does as well. In addition, *ZmSUT1*, *StSUT1*, and all three SUTs from tomato (*SlSUT1*, *2*, and *4*) are diurnally regulated (Kuhn et al., 1997; Aoki et al., 1999; Chincinska et al., 2008).

Hormones also modulate SUT expression. Ethephon, which releases ethylene, and gibberellins enhance *StSUT4* expression in potato (Chincinska et al., 2008), and Ethrel, another ethylene liberator, enhances *HbSUT3* expression in the laticifers of rubber tree (Tang et al., 2010). Exogenous hormone application impacts many gene-expression cascades and it is not known whether these are direct or indirect effects. However, *AtSUC1* has an abscisic acid-responsive element in its promoter that contributes directly to gene regulation (Hoth et al., 2010). Post-transcriptional regulation of SUT activity is also described. SUT proteins undergo rapid turnover and protein levels can be quickly altered (Kuhn et al., 1997; Vaughn et al., 2002). A recent model proposes that SUT localization to sterol-enriched membrane rafts promotes actin-dependent endocytosis, after which SUT-containing vesicles may be targeted to the lytic vesicle for degradation or recycled to the plasma membrane (Liesche et al., 2010). As discussed above ('What Proteins Mediate Efflux?'), Group 2, 3, and 4 SUT proteins show a potential to form homo and heterdimers in yeast (Reinders et al., 2002; Schulze et al., 2003), and StSUT1 homodimers in plants were recently confirmed by biochemical and imaging techniques (Krugel et al., 2008, 2009). Furthermore, in both plants and yeast, an oxidative environment increases the proportion of dimer relative to monomer (Krugel et al., 2008, 2009). The significance of SUT oligomerization is yet to be fully appreciated in plants, but may influence activity.

SUT proteins also interact with other proteins to modulate activity. Apple (*Malus domestica*) transports both sucrose and sorbitol and evidence was recently presented that MdSUT1 and a sorbitol transporter, MdSOT6, both of which localize to the plasma membrane, interact physically with a cytochrome b5, MdCYB5-A, which is anchored in the endoplasmic reticulum (Ma et al., 2009). In yeast, MdCYB5-A, or its *Arabidopsis* ortholog AtCYB5-A, can interact with either symporter and increase affinity for the respective sugars. Mutations that disrupt these interactions without significantly altering transporter activity abolish this affinity increase. The authors proposed that these interactions occur in response to low sugar levels in the cytosol and increase symporter affinity to help maintain internal sugar homeostasis (Ma et al., 2009). This intriguing work was conducted in yeast and protoplasts, and support for the model in whole plants would be a tremendous breakthrough for our understanding of transporter regulation.

Several mutations in maize have recently been described that have characteristics of blocked transport, including reduced stature, hyper-accumulation of carbohydrate in leaves, and anthocyanin increases correlating with elevated carbohydrates. These are *Tie-dyed1*, *Tie-dyed2*, and *Psychedelic* (Braun et al., 2006; Baker and Braun, 2008; Slewinski and Braun, 2010b). *Tie-dyed1* was recently cloned and encodes a novel transmembrane protein found only in grasses, but two conserved subdomains are present in monocots and dicots (Ma et al., 2009). Possible regulation of maize *SUT* genes and/or proteins was discussed, but further work is required to unravel the function of these genes in carbon partitioning.

A physiological trigger regulating sucrose accumulation in the phloem appears to be hydrostatic (turgor) pressure. For example, bathing a test system in hypertonic solutions of sorbitol to draw water out and reduce turgor pressure enhances sucrose uptake and acidification of the bathing solution, suggesting that both SUTs and ATPases are stimulated (Smith and Milburn, 1980; Aloni et al., 1986; Daie, 1996). Turgor-regulated SUT activity in the phloem is consistent with findings that drought stress sufficient to impact photosynthesis has relatively little effect on translocation, since osmotic adjustment maintains pressure and transport (Sung and Krieg, 1979). In *Arabidopsis*, microarray experiments show modest increases (twofold) in *AtSUC2* expression in response to drought, abscisic acid (a drought-induced hormone), or turgor stimulation (Grennan, 2006). Also, more effective sucrose transport during drought is implicated as an effective drought-tolerance mechanism in drought-resistant bean cultivars (Cuellar-Ortiz et al., 2008).

SUGAR LEVELS, SUTS, AND HYDROSTATIC PRESSURE MAY INTEGRATE WHOLE-PLANT CARBON PARTITIONING

Sugar signaling is important to SUT expression and, in combination with the associated changes in hydrostatic pressure, provides a mechanism for whole-plant integration of carbon status (Figure 3). SUTs respond to Suc differently. Rice *OsSUT1* in germinating seeds and *Arabidopsis AtSUC1* are induced by exogenous Suc (Matsukura et al., 2000), whereas *BvSUT1* in the phloem of sugar beet (*Beta vulgaris*) leaves (Chiou and Bush, 1998; Vaughn et al., 2002) and *PsSUT1* in pea cotyledons (Zhang et al., 2007) are repressed by exogenous Suc. *AtSUC2* is similarly repressed in *Arabidopsis* seedlings grown on media with increasing Suc concentrations (K. Dasgupta and B.G. Ayre,

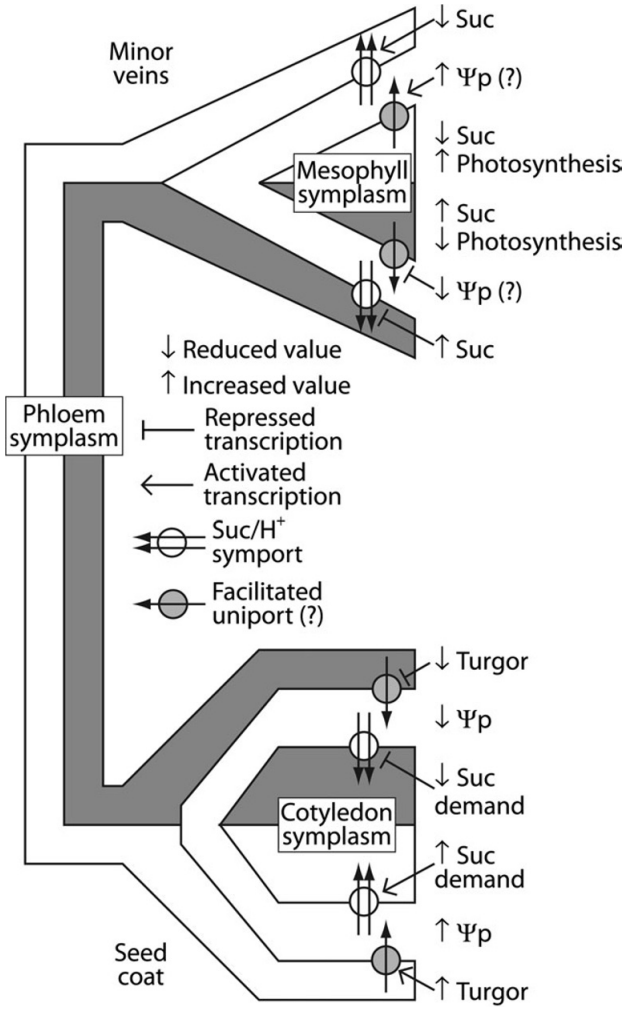

Figure 3. Integration of Carbohydrate Demand in Sink Organs with Photosynthesis in Source Leaves.

White Path: (Bottom) Suc starvation in developing cotyledons activates expression of SUTs that remove Suc from the seed apoplasm to increase apoplasmic solute potential (Ψp). Higher Ψp in the apoplasm promotes osmosis into seed-coat cells to increase hydrostatic pressure and activate nutrient release from the post-phloem symplasmic domain of the seed coat. (Top) Reduced Suc in the phloem relieves inhibition on SUT expression in source-leaf phloem to increase activity. Phloem loading increases apoplasmic Ψp, and may increase nutrient release from the mesophyll symplasm (possibly from phloem parenchyma cells) in a fashion analogous to release from the seed coats; may operate through a Suc-specific signaling pathway; or release may be controlled solely by the enhanced Suc-concentration gradient between the phloem apoplasm and the mesophyll symplasm. Regulation at this step is speculative, as indicated by (Ψp (?)). Efficient Suc transport out of the mesophyll stimulates photosynthesis.

Grey Path: (Bottom) High levels of Suc in the developing seeds, indicating low sink demand, repress SUT expression. Apoplasmic Ψp is reduced because nutrients are not absorbed and osmosis to the apoplasm relieves hydrostatic pressure in the cells of the seed coats, leading to reduced nutrient release. (Top) Increased Suc in the phloem represses SUT expression and reduces the rate of phloem loading. Apoplasmic Suc increases and, through an unknown mechanism (Ψp (?)), reduces Suc efflux from the mesophyll symplasm to promote feedback inhibition on photosynthesis.

unpublished). *AtSUC2* also responds Glc as a metabolic indicator of carbon status (Wingenter et al., 2010). Overexpression of the tonoplast monosaccharide transporter *TMT1* results in Glc accumulation in vacuoles and reduced levels in the cytosol and correlates with increased *AtSUC2* expression, enhanced carbohydrate exudation from leaves, and increased biomass (Wingenter et al., 2010).

Bush and colleagues demonstrated that *BvSUT1* is repressed when sugar beet leaves are fed Suc via the xylem (Chiou and Bush, 1998; Vaughn et al., 2002) and proposed a model in which phloem SUT expression integrates the needs of sink tissues with photosynthetic output of source leaves (Figure 3) (Vaughn et al., 2002). This model proposes that changes in sink strength alter the rate of Suc unloading and impact Suc content in the phloem, which, in turn, modulates *AtSUC2* expression. If sink strength is reduced, Suc levels increase throughout the phloem symplasm and repress SUT expression in companion cells. Repressed SUT expression in companion cells leads to reduced uptake from the apoplasm and ultimately to increased carbohydrate in mesophyll cells and the well-documented feedback inhibition on photosynthesis (Stitt et al., 2010). Phosphorylation cascades are implicated in the Suc-mediated control of *BvSUT1* expression (Ransom-Hodgkins et al., 2003).

Intracellular Suc levels also regulate SUT expression in developing pea seeds and function as the link between the carbon needs in the cotyledons and nutrient release from the post-phloem symplasmic domain of the seed coat (Figure 3) (Zhang et al., 2007; Zhou et al., 2009). Suc influx into pea seeds correlates positively with *PsSUT1* expression, implying that it is the SUT primarily involved in uptake, whereas expression of two sucrose facilitators, *PsSUF1* and *PsSUF4*, negatively correlate with Suc uptake. *PsSUT1* expression is inhibited with increasing internal Suc levels, while *PsSUF1* is unaffected and *PsSUF4* is enhanced. These findings indicate that intracellular Suc is the input for metabolic demand and transcriptional control of *PsSUT1* is the output (Zhou et al., 2009).

Suc uptake by the pea cotyledons in turn controls efflux from the seed coat symplasm by a turgor-gated mechanism (Zhang et al., 2007). In this component of the model, uptake of Suc into the cotyledons from the apoplasm increases hydrostatic pressure in the cells of the seed coat (i.e. the solute potential in the apoplasm increases, making more water available for uptake into cells). The increased turgor pressure in the seed-coat cells activates carriers involved in nutrient release. As nutrients enter the seed apoplasm, the apoplasmic solute potential drops, causing the turgor pressure in the seed coat to be relieved and the release carriers to be deactivated. Pressure in the seed coat thus acts as a feedback-regulated homeostat to control nutrient release to growing embryos (Zhou et al., 2009).

The basic mechanisms proposed for Suc-mediated control of influx and efflux in developing seeds and Suc-mediated control of phloem loading thus appear analogous. Turgor pressure in the seed apoplasm is thought to control efflux from

the seed-coat symplasm and it is reasonable to speculate that turgor may similarly influence Suc release in source leaves. Alternatively, the Suc-concentration gradient between the mesophyll symplasm and the phloem apoplasm may be large enough to drive efflux when phloem SUTs are active and may be sufficiently small to limit efflux when phloem SUTs are repressed, such that a turgor-gated mechanism similar to that proposed in developing seeds is unnecessary. In either case, combining turgor-gated control in developing seeds with Suc-mediated control in source leaves provides a model for whole-plant carbon partitioning, in which the Suc needs of the filial symplasm is transmitted through the phloem to the mesophyll symplasm via the activation or repression of Suc transporters/carriers and facilitators (Figure 3).

FUTURE DIRECTIONS

Suc is the predominant photoassimilate transported short and long distances through the plant and membrane transport is central to this distribution. Efficient Suc transport not only provides sinks with biomass to increase yield, but also promotes photosynthesis for greater primary productivity (Ainsworth and Bush, 2011). Understanding Suc transport and compartmentalization is therefore fundamental to our understanding of plant biology, but also has central importance to biotechnology. Strategic manipulation of Suc transport may lead to more productive plants and enable specific organs to be targeted for enhanced biomass partitioning. Initial evidence suggests that manipulating SUT expression in specific cells and tissues can be used to manipulate sink/source relationships. In potato, constitutive overexpression of spinach *SoSUT1* from the CaMV 35S promoter was used in an effort to increase transport to tubers (Leggewie et al., 2003). This resulted in higher levels of starch and lower levels of sugar in leaves, but had little impact on tuber yields. These effects were likely caused by redundant cycling, in which Suc released to the apoplasm was recovered by mesophyll cells. Srivastava and colleagues expressed *AtSUC2* cDNA from a strong, phloem-specific promoter in a homozygous *Atsuc2-4* background (Srivastava et al., 2009b). The promoter was derived from Commelina Yellow Mottle Virus (Matsuda et al., 2002), which was shown by semi-quantitative RT–PCR and reporter-gene analysis to be stronger than the natural *AtSUC2* promoter and is also subject to different regulatory cascades (Srivastava et al., 2009b). This T-DNA construct effectively complemented the *Atsut2-4* defect to near wild-type levels and it was proposed that foreign promoters may be an effective means of maintaining high levels of SUT activity in conditions under which expression might normally be repressed. Ectopic expression of *StSUT1* specifically in the storage parenchyma of pea cotyledons during seed development enhanced Suc influx in a *StSUT1*-dependent fashion (roughly twofold) and also increased biomass and cotyledon growth rates in intact seeds (Rosche et al., 2002). This work also demonstrated that the location of SUT activity in the intact seed is important: since most Suc is naturally absorbed at the cotyledon epidermis, the full effect of overexpressing *StSUT1* throughout the storage parenchyma was not realized in whole seedlings (Rosche et al., 2002). As another example of manipulating SUT activity for biotechnology, enhanced SUT expression in wheat grains increased levels of storage protein, showing that enhanced Suc transport has beneficial impacts beyond carbohydrate alone (Weichert et al., 2010).

What is clear from these studies is that fundamental principles of Suc flux across membranes and transport through symplasmic and apoplasmic compartments need to be strategically applied for improved plant characteristics. Of the three modes of membrane transport proposed for Suc, facilitated diffusion, Suc/H^+ antiport, and Suc/H^+ symport, only the latter is well characterized at the molecular level. Despite advances in our understanding of Suc uptake from the apoplasm, the necessary efflux steps are poorly resolved, as is transport across the tonoplast. Key technological breakthroughs would be those that allow high-resolution analysis of Suc concentrations in intact tissues, preferably in real time. Although techniques such as laser microdissection and fluorescence-activated cell sorting allow cell-specific analysis of transcripts and proteins, they have limited utility for small metabolites because of high solubility and rapid turnover (Okumoto, 2010). Nuclear magnetic resonance imaging has been applied to whole plants, mostly in the context of water relations (Van As et al., 2009) and imaging mass spectrometry using matrix-assisted laser desorption/ionization (MALDI–IMS) is an emerging technology for spatial metabolomics in tissue sections (Zaima et al., 2010). Both techniques currently have limited resolution and, since the latter method is destructive, the tissue needs to be suitably fixed to overcome the problems of diffusion and metabolism. FRET nanosensors are a particularly promising technology and their continued improvement and deployment to apoplasmic, cytosolic, and vacuolar compartments will likely provide significant information on Suc flux across the major membranes (Okumoto, 2010). Spectral variants that allow these compartments to be analyzed simultaneously would be particularly valuable. Once the sites of Suc efflux and influx are better resolved and with greater understanding of the conditions that stimulate changes in efflux and influx, high-resolution genomic approaches, such as those demonstrated in root tips (Chaudhuri et al., 2008), can be used to identify and characterize the genes that regulate these processes. The potential of these approaches were recently demonstrated by identifying a family of Glc uniporters, called SWEETs, that are conserved from plants to animals (Chen et al., 2010).

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