Immunolocalization of solanaceous SUT1 proteins in companion cells and xylem parenchyma: New perspectives for phloem loading and transport

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SUMMARY

Leaf sucrose transporters are essential for phloem loading and long-distance partitioning of assimilates in plants that load their phloem from the apoplast. Sucrose loading into the phloem is indispensable for the generation of the osmotic potential difference that drives phloem bulk flow and is central for the long-distance movement of phloem sap compounds, including hormones and signaling molecules. In previous analyses, solanaceous SUT1 sucrose transporters from tobacco, potato and tomato were immunolocalized in plasma membranes of enucleate SEs. Here we present data that identify solanaceous SUT1 proteins with high specificity in phloem CCs. Moreover, comparisons of SUT1 localization in the abaxial and adaxial phloem revealed higher levels of SUT1 protein in the abaxial phloem of all three solanaceous species, suggesting different physiological roles for these two types of phloem. Finally, SUT1 proteins were identified in files of xylem parenchyma cells, mainly in the bicollateral veins. Together, our data provide new insight into the role of SUT1 proteins in solanaceous species.

INTRODUCTION

Unlike in animals, vascular flow in higher plants is not powered by a single, heart-like organ, but rather by numerous, molecular pumps in the plasma membranes of specialized cells (apoplastic loaders) or by a different, not yet fully understood mechanism (symplastic loaders). In the phloem of apoplastic loaders, the molecular pumps generate intracellular, osmotic driving forces that represent the basis for the mass flow from fully developed source leaves to all kinds of sink tissues and thus for long-distance allocation of photosynthates and for long-distance signaling. In many higher plants, including Brassicaceae (e.g. Arabidopsis) and Solanaceae (e.g. tomato, tobacco an potato), sucrose is the main product of
photosynthesis and, therefore, used as the primary osmoticum to generate and maintain this driving force. In other plant families, sucrose is allocated together with polyols (e.g. mannitol in Apiaceae or sorbitol in Plantaginaceae) or with raffinose family oligosaccharides (RFOs), such as raffinose, stachyose or verbascose (e.g. in Lamiaceae and Cucurbitaceae).

In sucrose and sucrose/polyol translocating plants (apoplastic loaders), these assimilates enter the sieve element-companion cell (SE-CC) complexes via plasma membrane-localized transporters (Stadler et al., 1995; Stadler and Sauer, 1996; Kühn et al., 1997; Ramsperger-Gleixner et al., 2004). In sucrose/RFO translocating plants, phloem loading appears to be entirely symplastic (Schulz, 2005; Turgeon et al., 1993; Turgeon et al., 2001; Turgeon, 2006).

In apoplastic loaders, the driving force for phloem bulk flow is well understood. Fueled by H⁺-ATPases (Giaquinta, 1979; Delrot, 1981; Parets-Soler et al., 1990; DeWitt and Sussman, 1995) and regulated by K⁺-channels (Deeken et al., 2000; Lacombe et al., 2000; Deeken et al., 2002; Philippar et al., 2003) plasma membrane-localized transporters load their substrates into the cytoplasm of the SE-CC complex. This loading generates high intracellular solute concentrations (Moing et al., 1992; Lohaus et al., 2000; Lohaus and Fischer, 2002) and thus a water potential gradient between the SE-CC complex and the surrounding tissues. This gradient drives water into the sieve tube system and an increase in turgor pressure. The differential between sucrose loading and unloading in source and sink regions drives the bulk flow through the sieve tube system and assimilate allocation (Münch in 1930).

The importance of the involved solute transporters became obvious in plants with reduced mRNA levels (Riesmeier et al., 1994; Bürkle et al., 1998) or with blocked expression of sucrose transporter genes (Gottwald et al., 2000). In these
mutants, leaves accumulated soluble carbohydrates and starch, showed reduced chlorophyll content and eventually chlorotic lesions.

Phloem bulk flow is also required for long-distance signaling by phytohormones or macromolecular factors. Biosynthetic enzymes for abscisic acid (Koiwai et al., 2004) or jasmonic acid (Hause et al., 2003) or for enzymes involved in day-length perception and flowering time regulation (An et al., 2004; Corbesier et al., 2007; Lin et al., 2007) were identified in or shown to act from phloem CCs. Moreover, different types of RNAs were shown to move through the phloem (Jorgensen et al., 1998; Yoo et al., 2004; Haywood et al., 2005; Banerjee et al., 2006; Roney et al., 2007), and plant viruses utilize phloem mass flow for the rapid spread between different plant organs (Ding et al., 1996; Horns and Jeske, 1997; Cruz et al., 1998; Lucas and Wolf, 1999).

Immunohistochemical studies on the cell-specificity of transporters involved in phloem loading yielded different results for different dicot families. Whereas the SUC2 proteins of Arabidopsis and Plantago were identified in CCs (Stadler et al., 1995; Stadler and Sauer, 1996), solanaceous transporters from tomato (Lycopersicon esculentum, LeSUT1), potato (Solanum tuberosum, StSUT1) and tobacco (Nicotiana tabacum; NtSUT1) were found exclusively in SEs (Kühn et al., 1997). Since mature SEs are enucleate and devoid of ribosomes (Esau, 1969; Evert, 1992), it was hypothesized that solanaceous SUT1 proteins may be synthesized either within the CCs and then be targeted to the SEs, or within the SEs by a so far undetected mechanism after targeted cell-to-cell trafficking of SUT1 mRNAs (Kühn et al., 1997; Lalonde et al., 2003).

Since then, three more papers were published (Barker et al., 2000; Kühn et al., 2003; Hackel et al., 2006) supporting this SE-specific localization of solanaceous SUT1 proteins. Additional evidence came from the identification of sucrose
transporter mRNAs in the phloem sap of potato (Kühn et al., 1997), barley (*Hordeum vulgare*; Doering-Saad et al., 2002), and pumpkin (*Cucurbita maxima*; Ruiz-Medrano et al., 1999; Roney et al., 2007) and in nematode-induced syncytia of Arabidopsis roots (Jürgensen et al., 2003). These syncytia are linked symplastically to phloem SEs and the connecting plasmodesmata were shown to have size exclusion limits allowing macromolecular cell-to-cell movement (Hoth et al., 2005).

To further investigate the reasons for this discrepancy between the results and models of different groups, we raised an antiserum against a 43-amino acid (aa) peptide from the highly conserved loop-region in the center of these proteins. The specificity of this antiserum was tested on Western blots of plasma membrane proteins from *StSUT1*-expressing yeast cells and by immunohistochemical analyses of SUT1 proteins on tobacco, tomato and potato phloem sections. We could not confirm the published localization of SUT1 proteins in SEs. We rather identified SUT1 with high specificity in the CCs of all three species. Another novel observation was the localization of SUT1 proteins in xylem parenchyma.

Based on these observations and after intensive controls we conclude that solanaceous species execute their phloem loading and retrieval process(es) from the CCs. Moreover, differences in the amount of SUT1 proteins identified in the abaxial and adaxial phloem of Solanaceae provide new insight into the different physiological roles of these two types of phloem.

**RESULTS**

**Production and test of a new antiserum**

A complete *NtSUT1* open reading frame (ORF) was amplified from RNA of tobacco leaves (cv. Xanthii) with primers designed according to the published *NtSUT1a* sequence (accession: X82276; Bürkle et al., 1998). A second pair of
NtSUT1a-derived primers was used to amplify a truncated, internal fragment encoding the predicted, cytoplasmic loop between transmembrane helices VI and VII (aa residues 239 to 280 in the published sequence; Fig. 1A). This 2nd PCR introduced a stop-codon at the 3’-end of the fragment that was fused to the 3’-end of the ORF of the Escherichia coli maltose-binding protein (MBP). The fusion was used to immunize two rabbits. In previous publications (Lemoine et al., 1996; Kühn et al., 1997), shorter peptides from the same region (Fig. 1A) were used to raise antisera that identified solanaceous SUT1 proteins in protein fractions from SUT1-expressing yeast cells and in immunohistochemical analyses of plant tissue.

The initially obtained NtSUT1 ORF differed slightly (2 additional plus 7 different aa) from the published NtSUT1a sequence (Bürkle et al., 1998; Fig. S1). Most of these differences were conserved in tomato and potato SUT1 proteins (Fig. S1). The corresponding gene was named NtSUT1x (x for Xanthii) and deposited in the EMBL database (accession: AM491605).

To test, if the observed differences are cultivar-specific [cv. Xanthi (this paper) versus cv. Samsun (Bürkle et al., 1998)] we amplified and sequenced the complete NtSUT1 ORF also from cv. Samsun. However, several independently analyzed sequences from cv. Samsun turned out to be also NtSUT1x (accession: FM164640; 99.02% identity on the amino acid level with NtSUT1x from cv. Xanthii; Fig. 1A and Fig. S1).

During further attempts to find the published NtSUT1a sequence in the tobacco cultivars Xanthii and Samsun, a second NtSUT1 sequence was identified in both cultivars [97.8% (Xanthii) and 98.2% (Samsun) identity on the amino acids level with the NtSUT1x protein from the same cultivar]. These sequences encode 100% identical proteins in both cultivars and were named NtSUT1y (accession for NtSUT1y from cv. Xanthii: FM164638; accession for NtSUT1y from cv. Samsun: FM164639).
Under no conditions, even with primers that were designed to amplify specifically the published \textit{NtSUT1a} sequence (Bürkle et al., 1998) we were able to find \textit{NtSUT1a} sequences.

The 43-aa, \textit{NtSUT1x}-derived peptide that was eventually used to raise new antisera shared 93.0\% identity with the corresponding peptides of the published \textit{NtSUT1a} and the newly identified \textit{NtSUT1y} sequences, and 88.4\% identity with the corresponding peptides from \textit{LeSUT1} (X82275) and \textit{StSUT1} (X69165).

After affinity-purification of the new anti-solanaceous SUT1 antiserum (\textit{\alpha SolSUT1}), it was tested on Western blots of plasma membrane proteins from \textit{StSUT1}-expressing yeast cells. \textit{\alpha SolSUT1} recognized a single, 47-kDa polypeptide but no band in controls (Fig. 1B). This demonstrated that \textit{\alpha SolSUT1} that had been raised against an \textit{NtSUT1} peptide recognized the highly related \textit{StSUT1} protein.

\textbf{Immunolocalization analyses with \textit{\alpha SolSUT1} in tobacco}

To confirm the specificity of affinity-purified \textit{\alpha SolSUT1} antiserum also in fixed and embedded plant tissue, immunofluorescence analyses were performed with tobacco source leaves. Tobacco leaves possess five vein classes (Avery, 1933), with class I veins representing the midrib, class II the veins arising as single branches from this midrib, and class III the veins that form “islands” between adjacent class II veins. The smallest veins found within these “islands” are called minor veins or - depending on their developmental stage - class IV and class V veins. Minor veins and class III veins are built from collateral vascular bundles with a single, so-called abaxial phloem below their xylem. Class I and class II veins possess an additional adaxial phloem that is located above the xylem vessels in the leaves and inside the xylem ring of the stem.

As expected, \textit{\alpha SolSUT1}-derived fluorescence was detected in the abaxial and
adaxial phloem of midribs from tobacco source leaves (Fig. 2A and 2B). Unexpectedly, however, and in contrast to published data (Kühn et al., 1997) the fluorescence in the adaxial phloem was much weaker. The identity of the labeled cells was determined by simultaneous staining with \(\alpha\)SolSUT1/2\textsuperscript{nd} antibody and 4’,6’-diamidino-2-phenyl-indole (DAPI). Based on previous publications (Kühn et al., 1997) and because mature SEs are enucleate, \(\alpha\)SolSUT1-dependent fluorescence (green) and DAPI-derived fluorescence (blue) were expected in different cells. However, in cross (Fig. 2C) and longitudinal sections (Fig. 2D) of tobacco source leaf phloem many of the \(\alpha\)SolSUT1-labeled cells were nucleate. This finding, the shape of the labeled cells (long with small diameter) and the fact that the \(\alpha\)SolSUT1-labeled cells typically ended next to sieve plates (Fig. 2D) suggested that \(\alpha\)SolSUT1 labels CCs and not SEs.

This was confirmed by immunolocalization analyses with cross sections of tobacco minor veins (Fig. 2E and 2F). Again the \(\alpha\)SolSUT1-labeled cells were unambiguously identified as CCs due to their large size and their well-conserved positions. None of the analyzed sections showed fluorescence in the tiny SEs in the center (Fig. 2E and 2F). This minor vein-typical ratio in cell diameters (CCs larger than SEs) that is inverse in midribs (CCs smaller than SEs) has been described (Esau, 1969). In fact, in tobacco midribs, \(\alpha\)SolSUT1 labeled only the smallest cells, which again identified these cells as CCs (Fig. 2G). Tobacco sink leaves showed no \(\alpha\)SolSUT1-labeling (Fig. 2H). This complies with previous reports on very low \textit{SUT1} mRNA levels in sink leaves (Riesmeier et al., 1993; Bürkle et al., 1998) and with analyses of \textit{LeSUT1-promoter/GUS} plants that demonstrated that the activity of the \textit{LeSUT1} promoter follows the sink-to-source transition (Kühn et al., 2003).
αSolSUT1-specific localization of NtSUT1 was observed in all vein classes connecting the midrib and the minor veins. Figures 2I and 2K show labeling of CCs in class II and class III veins of a tobacco source leaf. Typically, class II veins (Fig. 2I) are already bicollateral, whereas class III veins (Fig. 2K) are still collateral and do not yet possess adaxial phloem. As in class I veins (Fig. 2B), αSolSUT1 treatment showed no or only weak labeling of CCs in the adaxial phloem of class II veins. In contrast, CCs were nicely labeled in the abaxial phloem of class II and III veins.

Finally, NtSUT1 localization was studied in tobacco stem sections (Fig. 2L and 2M). Typically, these sections showed more or less equal labeling of the CCs in the two types of phloem (Fig. 2M). In sections of younger stem regions, however, that were from near the top of the respective plant labeling was occasionally seen preferentially in the abaxial phloem (Fig. 2L).

To our surprise, αSolSUT1-specific fluorescence was not restricted to the phloem. Mainly stem sections (Fig. 2M), but (with decreasing intensity) also leaf-derived sections of class I (Fig. 2B), class II (Fig. 2I) and class III veins (Fig. 2K) showed a clear αSolSUT1-dependent labeling of xylem parenchyma cells. This labeling was observed primarily in parenchyma cells showing direct contact to lignified xylem vessels (Fig. 2B, 2K, 2I, 2M and 2N). However, less intense staining was also seen in cell files extending into the younger, not yet or hardly lignified xylem (Fig. 2M). In none of the previous reports on SUT1 localization in Solanaceae labeling of xylem parenchyma had been observed.

Immunolocalization analyses with αSolSUT1 in tomato and potato

So far, our analyses demonstrated that αSolSUT1, which was raised against a region conserved in solanaceous SUT1 proteins, labels potato StSUT1 on Western
blots (Fig. 1B) and tobacco NtSUT1 in immunohistochemical analyses. In contrast to previous reports, (i) αSolSUT1 localized NtSUT1 to phloem CCs and not to phloem SEs, (ii) αSolSUT1-labeling differed between abaxial and adaxial phloem, and (iii) αSolSUT1-labeling was observed also in xylem parenchyma.

For further analyses of the cell-specificity SUT1 proteins, similar analyses were performed with tomato (Fig. 3A to 3C) and potato (Fig. 3D to 3F). As in tobacco, αSolSUT1 labeled individual cells in the phloem of tomato (Fig. 3A) and potato midribs (Fig. 3F), and again the labeling was stronger in the abaxial phloem. As in tobacco, the labeled cells were characterized as CCs based on the presence of nuclei (Fig. 3D), on their small diameter in midribs (Fig. 3C) and their large diameter and position in minor veins (Fig. 3B and 3E). No labeling was detected in the tiny, central SEs of minor veins (Fig. 3B and 3E) or in the larger SEs of midrib phloem (Fig. 3C). Finally, αSolSUT1 labeled cells of the xylem parenchyma both in tomato (Fig. 3A) and, although only weakly, in potato (Fig. 3F).

*Immunolocalization controls*

So far, immunohistochemical analyses were presented only for solanaceous species known to share high degrees of sequence homology (about 90% identity) in those parts of their SUT1 proteins that correspond to the NtSUT1 peptide used for immunization. To test the possibility of unspecific binding of αSolSUT1 to an unknown epitope, we performed immunolocalizations also with less closely related solanaceous species. In none of these [habanero chili (*Capsicum chinense*, Fig. 4D), bell pepper (*Capsicum annuum*, not shown), petunia (*Petunia hybrida*, not shown), physalis (*Physalis peruviana*, Fig. 4C) and blue potato bush (*Lycianthes rantonnetii*, not shown)] SUT1 sequences have been determined. As further controls, we
included non-solanaceous species with known sucrose transporter sequences [plantain (*Plantago major*, Fig. 4B) and Arabidopsis (Fig. 4A)]. The sequence identity between the tobacco SUT1 peptide used to raise αSolSUT1 (Fig. 1A) and the corresponding peptides from the plantain PmSUC2 protein (accession: X75764) or the Arabidopsis AtSUC2 protein (accession: Q39231) are only 44.2 and 46.5%, and immunodetection of AtSUC2 or PmSUC2 by αSolSUT1 should not occur.

In none of these species αSolSUT1-specific signals were detected, although fluorescence labeling was obtained in parallel experiments with tobacco (not shown). This makes it unlikely that affinity-purified αSolSUT1 binds unspecifically to non-SUT1 epitopes in CCs or xylem parenchyma.

**Controls for αSolSUT1 purification and comparative controls with αSolSUT1 and P1-antiStSUT1**

Another explanation for the difference between our and the published localization data might be that the observed αSolSUT1-dependent fluorescence (Figs. 2 and 3) does not result from αSolSUT1-binding to SUT1 proteins at all but rather from potentially co-enriched antibodies that bind to an unknown, non-SUT1 epitope. This co-purification might result from unspecific binding of antibodies to components of the blocking buffer (e.g. milk proteins) or to the MBP-portion of the MBP-NtSUT1x fusion protein during the purification procedure. To exclude this possibility two sets of controls were performed.

For the first control, two parallel affinity purifications of raw αSolSUT1 antiserum were performed. One was done with the MBP-NtSUT1x fusion protein, the other with unfused MBP. These two purifications differed only in the presence or absence of the 43 aa of NtSUT1x, and potentially unspecifically bound antibodies should be purified
in both cases. The obtained, affinity purified antisera (αSolSUT1 from MBP-NtSUT1x and MBP-αSolSUT1 from MBP) were analyzed in parallel and images were taken under identical conditions. Whereas αSolSUT1 yielded the expected CC-specific signals (Fig. 5B), no fluorescence was seen with MBP-αSolSUT1 (Fig. 5A). For the second control, tobacco sections were treated with affinity-purified preimmune serum. Again, these analyses showed no labeling (supplemental data; Fig. S2).

The peptides used to raise StSUT1-specific antisera in previous analyses (Kühn et al., 1997) were 13 or 15 aa long and represented fragments of the 43-aa peptide used to raise αSolSUT1 (Fig. 1A). In principle, all of these sera should recognize the same epitope and, consequently, immunohistochemical analyses should yield the identical localization.

Therefore, comparative immunohistochemical studies were performed in yeast cells (StSUT1-expressing and control strains) with αSolSUT1 and with a published antiStSUT1 antiserum (P1; Kühn et al., 1997). P1-antiStSUT1 was used to successfully label SEs in potato and tobacco, and was raised against the StSUT1 peptide RENELPEKDEQEIDE of the predicted central loop of SUT1 proteins (Fig. 1A). The yeast strains were identical to those that had already been used for the Western blot shown in Fig. 1B. Cells were fixed, embedded and sectioned using the identical protocol that had been applied for all plant tissues shown so far. Figures 5D and 5F demonstrate that fluorescence was detected with both antisera exclusively in sections of the SUT1-expressing strain. No fluorescence was seen in the strain harboring the empty vector (Fig. 5C and 5E).

This result demonstrated that αSolSUT1 and P1-antiStSUT1 recognize the same protein. Therefore, we repeated the immunolocalization of NtSUT1 and StSUT1 (Kühn et al., 1997) in parallel with αSolSUT1 and P1-antiStSUT1. With
αSolSUT1 we obtained the already described labeling of CCs in all sections (not shown). With P1-antiStSUT1, however, we obtained two totally unexpected results: Firstly, in our hands purified P1-antiStSUT1 did not label SEs, and secondly, at sufficiently high concentration (1:50 to 1:250 dilution), P1-antiStSUT1 antiserum decorated clearly and specifically CCs of tobacco (Fig. 5G) and potato source leaves (Fig. 5H and 5I). Moreover, we observed P1-antiStSUT1-dependent fluorescence in xylem parenchyma cells (data not shown). Interestingly, the fluorescence signals obtained in potato were much brighter than the signal obtained in tobacco, which might result from a minor difference in the NtSUT1 sequence and the peptide originally used to raise P1-antiStSUT1. When we used the P1-antiStSUT1 antiserum at higher dilutions (up to 1:5000) no fluorescence was detected (not shown).

In previous papers that used P1-antiStSUT1 antiserum for immunodetection of SUT1 proteins, tissue fixation was typically performed with different chemicals (0.1% glutaraldehyde and 6% formaldehyde; Barker et al., 2000; Kühn et al., 2003; Hackel et al., 2005). To test the potential influence of this fixation technique we also used the glutaraldehyde/formaldehyde fixation protocol. In our hands, no or only very weak signals were detected with this fixation protocol, but these weak signals were also restricted to CCs (not shown). This suggested that the glutaraldehyde/formaldehyde fixation destroys or masks at least part of the antigenic epitope of the SUT1 proteins analyzed.

What is the reason for the observed discrepancy in SUT1 localization?

The results obtained in our comparative analyses with αSolSUT1 (this paper) and the previously published P1-antiStSUT1 (Kühn et al., 1997) were both clarifying and puzzling. They were clarifying, because two antisera that were raised using different approaches [peptide coupled to a carrier protein (Kühn et al., 1997) versus
C-terminal fusion protein (this paper) against slightly different peptides of a conserved region of plant sucrose transporters, and that were affinity-purified with different techniques [peptide attached to a column (Kühn et al., 1997) versus fusion protein bound to nitrocellulose (this paper)] labeled the same protein on Western blots and in immunohistochemical analyses of tissue sections. They were puzzling, however, because the very same antiserum labeled SEs and not CCs when used by different groups. To find a possible answer for this discrepancy, we performed additional tests.

Firstly, immunohistochemical analyses were performed with unpurified αSolSUT1 serum. We observed strong labeling of SEs in all sections from tobacco (Fig. 6A and 6B), potato and tomato (not shown) with unpurified αSolSUT1. No or only weak labeling was seen in CCs under these conditions. However, in several independently performed affinity purifications of raw αSolSUT1 serum and after enrichment of the affinity-purified antiserum (see METHODS section) (i) the strong labeling of SE was removed, (ii) most of the label in the adaxial phloem disappeared and (iii) specific labeling of CCs was obtained (Fig. 2 and 3).

Secondly, control analyses were performed with the preimmune serum of the rabbit that had been used to raise the αSolSUT1 antiserum. As with unpurified αSolSUT1, this labeling yielded strong and specific fluorescence of tobacco (Fig. 6C and 6D), potato or tomato (not shown) SEs, but no labeling of CCs. Moreover, the preimmune serum showed the previously described (Kühn et al., 1997) equal labeling of abaxial and adaxial phloem (Fig. 6C).

Based on these results, we analyzed preimmune sera of almost 50 rabbits on cross sections of tobacco midribs (not shown). With about 80% of these preimmune sera we observed selective labeling of SEs. This finding suggested that antibodies directed against an unknown, SE-specific epitope should be found with a reasonably
high frequency in antisera that had been raised against non-SE proteins. In fact, when we used a serum that had been raised by another group against the Arabidopsis chloroplast protein AtCSP41B [At1g09340 (Raab et al., 2006)], we could label SEs of solanaceous plants (Fig. 6E). Similar results were obtained with other antisera (not shown). In summary, these results suggest that the previous localization of solanaceous SUT1 proteins in SEs (Kühn et al., 1997; Barker et al., 2000; Kühn et al., 2003; Hackel et al., 2006) may result from an inherent background of SE-recognizing antibodies frequently present in rabbit sera. We suppose that preimmune sera controls were performed in all previous reports on SUT1 immunolocalization (Kühn et al., 1997 and others).

**DISCUSSION**

**CCs mediate phloem loading also in solanaceous species**

The presented data immunolocalize the well-characterized solanaceous sucrose transporters LeSUT1, StSUT1 and NtSUT1 to the CCs of tomato, potato and tobacco. This contradicts previous reports on the immunolocalization of these proteins in SEs (Kühn et al., 1997; Barker et al., 2000; Kühn et al., 2003; Hackel et al., 2006). It is in agreement with localization data published for sucrose transporters of other dicot families, such as *Arabidopsis* (Brassicaceae; Stadler and Sauer, 1996) and *Plantago* (Plantaginaceae; Stadler et al., 1995), where the respective proteins were localized to CCs. In summary, these data suggest that Solanaceae, and potentially all apoplastic loading dicots, execute their loading and retrieval process(es) from the CCs and that species-specific differences for this essential step may not exist.

In several control experiments we were able to demonstrate that SE-specific antibodies are frequently found in rabbit preimmune sera (Fig. 6C and 6D) and that
SE-specific labeling can be obtained with antisera raised against non-SE proteins (Fig. 6E). This may contribute to the previously published SE-specific localization of SUT1 proteins. Another reason for the observed discrepancy may be the use of different fixation protocols. In our hands, the previously published P1-antiStSUT1 antiserum (Kühn et al., 1997) labels CCs but not SEs (Fig. 5G to 5I) after tissue fixation with ethanol/acidic acid. On glutaraldehyde/formaldehyde fixed tissue (Kühn et al., 1997), however, the signals detected with P1-antiStSUT1 in CCs were only very weak. This may indicate that in contrast to the fixation with ethanol/acidic acid, glutaraldehyde/formaldehyde fixation reduces the antigenicity of SUT1 proteins.

Indirect evidence for a possible CC-specific localization of solanaceous SUT1 was obtained in previous analyses with tobacco plants expressing a LeSUT1/green-fluorescent protein (GFP) fusion under the control of the RolC promoter from Agrobacteria (Lalonde et al., 2003). In these analyses, the LeSUT1-GFP fusion was detected only in CCs, but based on the reported localization of SUT1 in SEs it was concluded that the C-terminal GFP fusion blocks cell-to-cell movement of LeSUT1.

SE-specific localization (Barth et al., 2003, PmSUC3 in Plantago major; Meyer et al., 2004, AtSUC3 in Arabidopsis) or dual localization in SEs plus CCs (Knop et al., 2004, AmSUT1 in Alonsoa meridionalis) has also been described for sucrose transporters of other dicot species. However, AtSUC3 and PmSUC3 belong to a different subgroup of sucrose transporters that does not mediate phloem loading (Barth et al., 2003; Hackel et al., 2006), and for A. meridionalis sequence information about potentially cross-reacting SUC3-type sucrose transporters is lacking. Similarly, SE-localized sucrose transporters were described in wheat (Triticum aestivum L.; TaSUT1; Aoki et al., 2004) and rice (Oryza sativa L.; OsSUT1; Scofield et al., 2007b). However, in both species the identity of the labeled protein is not absolutely clear, because cross-reactions of the used antiserum with other sucrose transporters...
could not be excluded (Furbank et al., 2001; Scofield et al., 2007a). Obviously, these
data demonstrate the presence of SE sucrose transporters, but most of these
transporters are discussed to be involved either in the release of sucrose from SEs or
in sucrose retrieval.

**N. tabacum has two NtSUT1 genes**

In both tobacco cultivars that were used to amplify NtSUT1 cDNAs (cv. Xanthii and
cv. Samsun) we found two almost identical SUT1 sequences that were named
NtSUT1x and NtSUT1y. Under no condition and with no set of primers tested (see
Materials and Methods) we were able to amplify the previously published NtSUT1a
sequence (Bürkle et al., 1998). NtSUT1x and NtSUT1y are likely to represent
orthologous SUT1 genes and to be encoded by the two subgenomes of the
allotetraploid species *N. tabacum* that is known to have formed from the diploid
progenitors *N. sylvestris* and *N. tomentosiformis* (Kenton et al., 1993; Kitamura et al.,
2000). Comparison of NtSUT1a (Bürkle et al., 1998), NtSUT1x and NtSUT1y (this
paper) protein sequences and of a partial tobacco SUT1 sequence from a tobacco
hybrid (*N. langsdorffii* x *N. sanderae*; NlxsSUT1; assembled from accessions
ABF06450 and ABF06446; Fig. S1) demonstrates that the NtSUT1a sequence quite
likely was obtained from an NtSUT1x mRNA but contains sequencing errors.

Obviously, one could speculate that αSolSUT1 might discriminate between the
two NtSUT1 proteins and label only NtSUT1x, which was used to raise the
antiserum, but not NtSUT1y. This can be excluded. Between the 43-aa peptide used
to raise αSolSUT1 and the respective peptide in NtSUT1y there are only two
differences: His250 in NtSUT1x is Gln250 in NtSUT1y, and Gly257 in NtSUT1x is Ala257
in NtSUT1y. In StSUT1 (Fig. 1A) the respective residues (Glu256 and Ala263) are
identical to those in NtSUT1y. Nevertheless, αSolSUT1 labels StSUT1 both on Western blots (Fig. 1B) and in fixed leaf sections from potato (Fig. 3D to 3F).

**Different abundance of SUT1 proteins in abaxial and adaxial phloem suggests physiological roles for these tissues**

The abaxial phloem of the different vein classes in Solanaceae corresponds to the sole phloem in Brassicaceae and other plant families. In contrast to Kühn et al. (1997), who described equal labeling in both the abaxial and the adaxial phloem, we always observed stronger αSolSUT1 signals in the abaxial than in the adaxial phloem of all vein classes with bicollateral phloem (Fig. 2B, 2I, 3A and 3F). This may be taken as novel molecular evidence for different physiological roles of these two types of phloem. In contrast to the primary phloem loading, i.e. the loading of newly synthesized sucrose that has just been released from the mesophyll, which is generally accepted to occur in the single (abaxial) phloem of minor veins, (i) it seems likely that the transporters in the midrib and in other large veins are rather retrieving sucrose that has leaked out of the phloem. (ii) Especially the adaxial phloem of bicollateral veins might not or hardly be engaged in primary phloem loading, which might be the reason for fewer SUT1 proteins in this part of the phloem.

In fact, different physiological roles for abaxial and adaxial phloem were previously suggested by flux analyses of $^{14}$C-labelled assimilates in stems of tomato plants (Bonnemain, 1968). In petioles of $^{14}$CO$_2$-exposed leaves more label was detected in the abaxial than in the adaxial phloem. Based on this difference it was concluded that the primary or even exclusive role of the abaxial phloem in tomato stems is the long-distance transport of assimilates to the root system, whereas the primary or exclusive role of the adaxial phloem is the assimilate transport to aerial sinks (Bonnemain, 1968).
SUT1 or SUT1-type transporters are present in xylem parenchyma

Unexpectedly, our analyses with αSolSUT1 identified SUT1 proteins also in the xylem parenchyma of bicollateral leaf veins and with higher intensity in stem sections (Fig. 2B, 2I, 3A and 3F). Again, this result disagreed with previous analyses from Solanaceae. However, it is consistent with reports from other species.

Decourteix and coworkers (2006) identified a SUT1-type sucrose transporter, JrSUT1, in walnut (Juglans regia) xylem parenchyma, which is thought to regulate changes in sucrose concentrations during winter (Améglio et al., 2004). Moreover, a SUT1-type sucrose transporter (DcSUT2) was found in the xylem parenchyma of carrot (Daucus carota) tap roots (Shakya and Sturm, 1998). It was speculated that sucrose might be the primary carbon source for the xylem parenchyma in these developing sinks.

A role of the xylem parenchyma-localized SUT1 proteins in the frost tolerance of Solanaceae seems unlikely. Moreover, our data do not support a possible role in sucrose exchange between the two types of phloem, because a direct connection of the xylem parenchyma cell files especially to the adaxial phloem was not observed. Typically, the innermost, αSolSUT1-labeled xylem parenchyma cells are immediately adjacent to lignified xylem vessels but separated by 2 to 4 large parenchymatic cells from the cell clusters of the adaxial phloem. We speculate that the files of αSolSUT1-labeled xylem (or ray) parenchyma cells either mediate the supply of sucrose from the abaxial phloem to the xylem parenchyma cells, and possibly to cells involved in cellulose production and lignification. Alternatively, the identified sucrose transporters may have retrieval functions similar to those discussed for sucrose transporters in the phloem (Maynard and Lucas, 1982; Stadler et al., 1995; Hafke et al., 2005) and their physiological function might be to keep the xylem sucrose free. A direct release of
$^{14}$C-labeled assimilates from the abaxial or adaxial phloem into the xylem sap has not been observed in previous analyses (Bonnemain, 1968).

**What are the consequences of these new localization data?**

Based on the initial report on the localization of SUT1 proteins in enucleate SEs (Kühn et al., 1997) a cell-to-cell transport mechanism from CCs into SEs had to be postulated either for SUT1 proteins or for SUT1 mRNAs. In fact, evidence for mRNA trafficking was provided by in situ localization analyses that revealed accumulation of StSUT1 mRNA at the orifices of the plasmodesmata between CCs and SEs and that detected StSUT1 mRNA within potato SEs. However, it was shown before (Anderson and Cronshaw, 1969) that severance of vascular tissue frequently results in pressure release and in an increased hydrostatic flow that sweeps ruptured organelles, starch grains or proteins into the sieve pores of SEs. Most likely, this pressure drop extends into the intimately connected CCs, and their cellular content - including SUT1 mRNAs - may, therefore, be forced towards the connecting plasmodesmata. This may explain the accumulation of SUT1 mRNA at the orifices of the plasmodesmata connecting CCs and SEs (Kühn et al., 1997).

Clearly, the absence of SUT1 proteins from SEs renders discussions on SUT1 mRNA translation in ribosome-free SEs unnecessary. Nevertheless, sucrose transporter mRNAs were found in the phloem sap of several plants (Ruiz-Medrano et al., 1999; Doering-Saad et al., 2002; Roney et al., 2007), and in symplastically linked, nematode-induced syncytia of Arabidopsis (AtSUC2; Jürgensen et al., 2003; Hoth et al., 2005). However, despite this presence of AtSUC2 mRNA, AtSUC2 protein has neither been found in Arabidopsis SEs nor in nematode-induced Arabidopsis syncytia (Stadler et al., 1996; Hoth et al., 2005). Nevertheless, the finding of SUC2 and SUT1 mRNAs in phloem sap plus the identification of various other types of RNAs in the
phloem sap (Jorgensen et al., 1998; Yoo et al., 2004; Haywood et al., 2005; Banerjee et al., 2006) show, that CC-synthesized mRNAs and other types of RNAs do enter the SEs. It has been shown repeatedly that phloem movement of RNAs is part of a so far not fully understood signaling cascade (Ruiz-Medrano et al., 1999; Yoo et al., 2004).

After the localization of solanaceous SUT1 proteins in the plasma membranes of SEs (Kühn et al., 1997) also sucrose transporters of the SUT2 (Barker et al., 2000) and SUT4 type (Weise et al., 2000) were localized in SE plasma membranes of solanaceous species and SUT4 was even discussed as a low affinity/high capacity (LAHC) phloem loader. This co-localization (Reinders et al., 2002a) initiated studies on the physical interaction of these 3 types of proteins. In fact, co-expression in yeast seemed to support this hypothesis (Reinders et al., 2002a; Reinders et al., 2002b; Schulze et al., 2003). However, after the localization of SUT1-type proteins in CCs (this paper) and after the recent localization of SUT4-type transporters in vacuoles (Endler et al., 2006) the model of regulatory SUT1, SUT2 and SUT4 interactions is quite unlikely.

Finally, the identification of SE-specific antibodies in numerous rabbit preimmune sera demonstrates that special care has to be taken in immuno-labelings of higher plant vasculature. For results suggesting SE-specific localization of a protein, careful controls will be needed.

MATERIALS AND METHODS

Strains and growth conditions

Tomato (Solanum esculentum cv. Moneymaker), potato (Solanum tuberosum cv. Solara) and tobacco plants (Nicotiana tabacum cv. Xanthii and cv. Samsun) were grown in potting soil in the greenhouse under ambient conditions. Leaf material of
other species (Capsicum chinense, Capsicum annuum, Petunia hybrida, Physalis peruviana, and Solanum rantonnetti) was from field-grown plants. Cloning was performed in Escherichia coli strain DH5α (Hanahan, 1983).

**Fusion protein isolation and immunohistochemistry**

cDNA sequences representing complete NtSUT1 coding sequences were amplified from cDNAs derived from total RNA from N. tabacum cv. Xanthii or N. tabacum cv. Samsun leaf tissue using the following primers [(NtSUT1-5’) GAA TTC AAG CTT GTA AAA GAA ATG GAG AAT GGT ACC AAA AAA CTT and (NtSUT1-3’) GAA TTC AAT GAT GGT GAT GGT GAT GGA AAC CGC CCA TTG TGG TGG CTG GTT TGG CA]. The fragment encoding the NtSUT1x 43-aa peptide was amplified [primers: (NtSUT1x-5’) GAA TTC CGC GAA AAC GAG CTC CCC GAA and (NtSUT1x-3’) GGA TCC TCA CCA CAT GGG TCT TGG TAA ATC], sequenced and cloned into the E. coli expression vector pMal2c (New England Biolabs, Frankfurt/Main, Germany). Immunizations were performed by Dr. Pineda Antibody Service (Berlin, Germany).

Attempts to identify NtSUT1a in N. tabacum cv. Xanthii and cv. Samsun were also performed with the primers cNtSUT1537f (CAT CAA ACG CCT TCT TCT CC) and cNtSUT1987r (AGT TCA ACA ACA GCC CCA AA) that should amplify NtSUT1x, NtSUT1y and NtSUT1a, and the PCR products were sequenced directly. To enforce amplification of NtSUT1a, PCRs were eventually performed with the primers NtSUT1aREF480f (GGC TGA TCT GTC CGG CGG AAA AGC G) and NtSUT1aREF770r (AAG AAC GGG ACT TTT GAT TTT CGC G). The 3’-ends of these primers were designed to span the predicted gaps after positions 168 and 256 in the NtSUT1a protein sequence (Fig. S1).

For affinity-purification of αSolSUT1, nitrocellulose filters soaked with maltose-
binding protein (MBP)-NtSUT1x-fusion peptide (1 mg peptide ml\(^{-1}\)) and blocked with skim milk-containing buffer [50 mM Tris (pH7.5), 150 mM NaCl, 0.1% Triton-X-100, 1% skim milk powder] were incubated in raw antiserum for at least 60 min at 4°C, washed, and bound antibodies were released as described (Sauer and Stadler, 1993). Antibodies against the MBP were removed from this solution in a second round using nitrocellulose filters that had been soaked with unfused MBP.

**Preparation of yeast plasma membranes, gel electrophoresis and Western blots**

Plasma membrane preparation and protein extraction was as described (Stolz et al., 1994) and separated proteins (Laemmli, 1970) were transferred to nitrocellulose filters as published (Dunn, 1986).

**Tissue sectioning, fixation and embedding**

Immunolocalizations in free-hand sections of non-embedded leaf tissue or in microtome sections of methacrylate-embedded tissue as well as DAPI stainings were performed as described (Stadler and Sauer, 1996) using ethanol/acetic acid (3:1) for fixation. For selected controls, fixations were performed with 0.1% glutaraldehyde and 4% formaldehyde instead. Antibody detection was by anti-rabbit IgG-Cy3 (red fluorescence, Dianova, Hamburg, Germany) or by IgG-Cy2 conjugates (green fluorescence, Dianova, Hamburg, Germany) on microtome and hand-cut sections.

**Confocal microscopy**

Antibody-decorated sections were imaged using a confocal laser scanning microscope (Leica TCS SP II; Leica Microsystems, Bensheim Germany). CY2 antibody conjugate was excited by 488-nm light, fluorescence was observed using a
detection window from 495 to 530 nm. CY3 antibody conjugate was excited by 543 nm light, fluorescence was observed in a detection window from 552 to 617 nm. Cell wall autofluorescence was excited by 488-nm light and detected in a window of 560 to 640 nm. DAPI-fluorescence was excited using a 405 nm diode and observed using a detection window of 425 to 475 nm.

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FIGURE LEGENDS

Figure 1. Comparison of the central regions of different SUT1 proteins and characterization of αSolSUT1.

A, alignment of 138 aa of published solanaceous SUT1 proteins (NtSUT1a: X82276; LeSUT1: X82275; StSUT1: X69165) and the new tobacco sequences NtSUT1x (AM491605) and NtSUT1y (FM164638). Transmembrane helices V, VI and VII are underlined, the central cytoplasmic-loop region of NtSUT1x used for antibody production is highlighted, and potato sequences previously used (Kühn et al., 1997) for the production of the anti-StSUT1 antisera P1 (RENELPEKDEQEIDE) and P2 (EIDEKLAGAGKSK) are labeled with asterisks. Differences in the NtSUT1a, NtSUT1x and NtSUT1y sequences are boxed (including the corresponding amino acids in LeSUT1 and StSUT1).

B, Coomassie-stained gel (left) and immunoblot analysis (right) of plasma membrane proteins (10 µg lane⁻¹) from yeast cells expressing StSUT1 or from control cells. Arrows show the additional band of recombinant StSUT1 protein at an apparent molecular weight (MWapp) of 47 kDa and the corresponding signal on the immunoblot (dilution of affinity-purified αSolSUT1: 1:40).

Figure 2. Localization of NtSUT1 in tobacco by fluorescence detection with αSolSUT1. Yellow and orange staining shows autofluorescence of phenolics in lignified cells, green staining shows NtSUT1 localization. White light images and confocal images were superimposed in A, C to E and G to L and N.

A, transverse section of the midrib (class I vein) of a source leaf. B, transverse section of the midrib of a source leaf. C and D, histochemical staining of nuclei with DAPI (0.2 µg ml⁻¹) in cross C and longitudinal sections D of the midrib of a source leaf. E, NtSUT1 protein in a transverse section of a source leaf minor vein (class V).
**F**, schematic drawing of image **E** highlighting the regular arrangement of cells. **G**, localization of NtSUT1 in the smallest cells of the abaxial phloem in a midrib; transverse section of a source leaf. **H**, absence of NtSUT1 from sink leaves (transverse section). **I**, localization of NtSUT1 in the CCs of the abaxial phloem in a class II vein. No labeling is seen in the adaxial phloem. **K**, localization of NtSUT1 in the CCs of the abaxial phloem in a class III vein. **L**, localization of NtSUT1 in the abaxial phloem of a stem section. No or only weak labeling is seen in the adaxial phloem. **M**, cross section through the bottom part of a mature stem showing NtSUT1 in the abaxial and adaxial phloem and in files of xylem parenchyma cells. **N**, enlarged region of a stem cross-section showing NtSUT1 in xylem parenchyma cells (green).

Abbreviations: abp, abaxial phloem; adp, adaxial phloem; cc, companion cell; ccn, companion cell nucleus; le, lower epidermis; palp, palisade parenchyma; pp, phloem parenchyma; se, sieve element; sp, sieve plate; spop, spongy parenchyma; ue, upper epidermis; vb, vascular bundle; xp, xylem parenchyma; xv, xylem vessel(s); xy, xylem. Antibody detection was by anti-rabbit IgG-Cy2 conjugate on 5-µm-thick microtome sections in **E** and **H**, or on hand-cut sections (all others). Bars are 5 µm in **C**, **E** and **G**, 20 µm in **K** and **N**, 25 µm in **D** and **H**, 50 µm in **I**, 100 µm in **L** and **M** and 200 µm in **A** and **B**.

**Figure 3.** Immunohistochemical localization of SUT1 proteins in tomato, **A** to **C**, and potato, **D** to **F**, by fluorescence detection with αSolSUT1. Orange stainings in **A**, **E** and **F** show autofluorescence of phenolics in lignified cells, green staining shows SUT1 localization. White light images and confocal images were superimposed in **B** to **E**.

**A**, LeSUT1 in a transverse section of a midrib of a source leaf. **B**, LeSUT1 protein in a transverse section of a minor vein of a source leaf. **C**, higher magnification of the
abaxial phloem of a source leaf (similar to A). LeSUT1 is localized only in the smallest cells (= CCs). D, StSUT1 plus histochemical staining of a nucleus with DAPI (blue) in a cross section of a midrib of a source leaf. E, StSUT1 in a transverse section of a minor vein of a source leaf. F, StSUT1 in a transverse section of a midrib of a source leaf.

Antibody detection was by anti-rabbit IgG-Cy2 conjugate on 5-µm-thick microtome sections in B and E, or on hand-cut sections (all others). Bars are 5 µm in B, D and E, 10 µm in C, 200 µm in A, and 100 µm in F.

Figure 4. Immunohistochemical analyses with αSolSUT1 in control plants. Yellowish and orange stainings shows autofluorescence of phenolics in lignified cells. Transmission light images and confocal images were superimposed. A, cross section through the minor vein in an Arabidopsis leaf. B, cross section through the minor vein of a leaf from plantain. C, cross section through the petiole of a leaf from physalis. D, cross section through the petiole of a leaf from habanero chili.

Abbreviations: abp, abaxial phloem; adp, adaxial phloem; ph, phloem; xp, xylem parenchyma; xv, xylem vessel(s); xy, xylem. Antibody detection was by anti-rabbit IgG-Cy2 conjugate on 5-µm microtome sections in A and B, or on free-hand sections in C and D. Bars are 10 µm in A and B, and 100 µm in C and D.

Figure 5. Comparative labeling of sections from yeast, C to F, tobacco, A, B, and G, and potato, H and I, with purified αSolSUT1, B to D, with P1-antiStSUT1, E to I, or with control-purified MBP-αSolSUT1, A. Transmission light images and confocal images were superimposed in A and B and in G to I.

A, MBP-αSolSUT1 on a cross section through a tobacco source leaf vein (class I). B, αSolSUT1 on a similar section as in A. C, αSolSUT1 on yeast control. D, αSolSUT1
on StSUT1-expressing yeast. E, P1-antiStSUT1 on yeast control. F, P1-antiStSUT1 on StSUT1-expressing yeast. G, P1-antiStSUT1 on a similar section as in A. H, P1-antiStSUT1 on a cross section through a potato source leaf with two minor veins (class V). I, P1-antiStSUT1 on a minor vein (class IV) from a potato source leaf.

Abbreviations: abp, abaxial phloem; adp, adaxial phloem; cc, companion cell; se, sieve element; ue, upper epidermis; xp, xylem parenchyma; xv, xylem vessel(s); xy, xylem. Antibody detection was by anti-rabbit IgG-Cy2 conjugate on 1-µm microtome sections in C to F, on hand-cut sections A, B and G, or on 5-µm microtome sections in H and I. Bars are 2 µm in C to F, 10 µm in I, 25 µm in G, 50 µm in H, and 100 µm in A and B.

Figure 6. Labeling of tobacco SEs by fluorescence detection with unpurified αSolSUT1, A and B, with preimmuneserum, C and D, or with an antiserum raised against an Arabidopsis chloroplast protein, E. Transmission light images and confocal images were superimposed.

A, preferential labeling of SEs (red) in a transverse section of a source leaf minor vein treated with non-purified αSolSUT1. B, labeling of SEs (red) in a transverse section of midrib phloem from a source leaf treated with non-purified αSolSUT1. C, transverse section of the midrib of a source leaf treated with pre-immune serum. Yellow and orange staining shows autofluorescence of phenolics in lignified cells. D, higher magnification of abaxial phloem labeled with pre-immune serum as in C. E, labeling of SEs in a transverse section of midrib phloem from a source leaf treated with antiserum raised against the Arabidopsis chloroplast protein At1g09340.

Abbreviations: abp, abaxial phloem; adp, adaxial phloem; cc, companion cell; se, sieve element; xv, xylem vessel(s). Antibody detection was by anti-rabbit IgG-Cy3 conjugate A and B or by anti-rabbit IgG-Cy2 conjugate (all others) on 5-µm-thick
microtome sections in A or on hand-cut sections (all others). Bars are 5 μm in A, B, D and E and 50 μm in C.
A

| Protein  | Sequence   | Position |
|----------|------------|----------|
| NtSUT1a  | 160 LADLSGGKAG RMRTSNAFFS FFMAVGNVLG YAAGSYSRLY KIPFFSKTPA | 209 |
| NtSUT1x  | 160 LADLSGGKA . RMRTSNAFFS FFMAVGNVLG YAAGSYSRLY KIPFFSKTPA | 209 |
| NtSUT1y  | 160 LADLSGGKAG RMRTANAFFS FFMAVGNVLG YAAGSYSRLY KIPFFSKTPA | 209 |
| StSUT1   | 166 LADLSGGKSG RMRTANAFFS FFMAVGNILG YAAGSYSHIF KIPFFSKTKA | 215 |
| LeSUT1   | 162 LADLSGGKSG KMRTANAFFS FFKAVGNILG YAAGSYSRLY KIPFFSKTKA | 211 |

NtSUT1a 209 CDYICANLKS CFFIAVPLL SLTIILTLLV RENELPEKEE HEIDEKA.GA 257
NtSUT1x 210 CDYICANLKS CFFIAVPLL SLTIILTLLV RENELPEKEE HEIDEKA.GG 259
NtSUT1y 210 CDYICANLKS CFFIAVPLL SLTIILTLLV RENELPEKEE QEIDEKA.GG 259
StSUT1   216 CDYICANLKS CFFIAFPLL SLTIILTVLV RENELPEKEE QEIDEKA.GG 265
LeSUT1   212 CDYICANLKS CFFIAFPLL SLTIILTVLV RENELPEKEE LEIDEKA.GG 261

NtSUT1a 258 RSKVPPFFGE IFGALKDLPF PMWILLTVICS LNWIAFPFFP LYTDMWMAKE 307
NtSUT1x 260 GSKVPPFFGE IFGALKDLPF PMWILLTVICS LNWIAFPFFP LYTDMWMAKE 309
NtSUT1y 260 GSKVPPFFGE IFGALKDLPF PMWILLTVICS LNWIAFPFFP LYTDMWMAKE 309
StSUT1   266 GSKVPPFFGE IFGALKDLPF PMWILLTVICS LNWIAFPFFP LYTDMWMAKE 315
LeSUT1   262 GSKVPPFFGE IFGALKDLPF PMWILLTVICS LNWIAFPFFP LYTDMWMAKE 311

B

![Coomassie and Western Blot Images](image_url)

| MW<sub>app</sub> (kDa) |
|-------------------------|
| 66                      |
| 43                      |
| 29                      |
