VvHT1 encodes a monosaccharide transporter expressed in the conducting complex of the grape berry phloem

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Abstract

The accumulation of sugars in grape berries requires the co-ordinate expression of sucrose transporters, invertases, and monosaccharide transporters. A monosaccharide transporter homologue (VvHT1, Vitis vinifera hexose transporter 1) has previously been isolated from grape berries at the véraison stage, and its expression was shown to be regulated by sugars and abscisic acid. The present work investigates the function and localization of VvHT1. Heterologous expression in yeast indicates that VvHT1 encodes a monosaccharide transporter with maximal activity at acidic pH (pH 4.5) and high affinity for glucose ($K_m = 70 \mu$M). Fructose, mannose, sorbitol, and mannitol are not transported by VvHT1. In situ hybridization shows that VvHT1 transcripts are primarily found in the phloem region of the conducting bundles. Immunofluorescence and immunogold labelling experiments localized VvHT1 in the plasma membrane of the sieve element/companion cell interface and of the flesh cells. The expression and functional properties of VvHT1 suggests that it retrieves the monosaccharides needed to provide the energy necessary for cell division and cell growth at an early stage of berry development.

Key words: Assimilate transport, grapevine, monosaccharide transport, ripening, unloading.

Introduction

The ripening of grape berries is accompanied by a large accumulation of glucose and fructose (1 M or more, total sugar concentration) in the vacuoles of the mesocarp cells. Because sugars are imported in the berries as sucrose via the phloem, the process of hexose accumulation in the vacuoles of the flesh cells must involve the activity of sucrose transporters, monosaccharide transporters, and enzymes cleaving sucrose at some point.

Three full-length cDNA clones (VvSUC11, VvSUC12, and VvSUC27) encoding homologues of sucrose transporters were isolated from grape (cv. Shiraz) berries collected 2 weeks after the initiation of ripening. In addition to the flowers and fruits, VvSUC11 is also expressed in young and mature leaves whereas VvSUC12 is expressed only in the young leaves. No expression is detected for VvSUC11 and VvSUC12 in the roots and tendrils. By contrast, VvSUC27 is strongly expressed in the roots and tendrils in addition to the reproductive organs (Davies et al., 1999). Both VvSUC11 (also called VvSUT1) and VvSUC12 were successfully expressed in yeasts and shown to mediate sucrose transport with a $K_m$ of about 1 mM (Ageorges et al., 2000; Manning et al., 2001).

A full length (VvHT1) and a partial (VvHT2) cDNA clone which are both homologous to monosaccharide transporters were isolated from grape (cv. Pinot noir) berries at the véraison stage (Fillion et al., 1999). Quantitative RT-PCR indicated that the expression of VvHT2 in the berries starts about 2 weeks before véraison, and peaks about 1 week after véraison (Fillion et al., 1999). The level of VvHT2 expression is weak compared with VvHT1. VvHT1 is expressed in the berries and in the young leaves. Quantitative RT-PCR showed that in the berries, VvHT1 expression is strong shortly after anthesis, then declines, and increases again after véraison. However, microarray
analysis suggests that the second peak of expression does not always occur (Terrier et al., 2005). The promoter of VvHT1 contains several sugar boxes (Fillion et al., 1999; Atanassova et al., 2003), and its activity is increased, both in tobacco BY2 cells and in grape cell suspension cultures, by sucrose and glucose, as well as by the non-transported, non-metabolized sucrose analogue palatinose (Atanassova et al., 2003). A one-hybrid approach using as a target a short proximal fragment of VvHT1 promoter encompassing two sugar boxes was developed to identify transcription factors controlling the expression of VvHT1. This enabled the identification of a member of the ASR (abscisic acid, stress, ripening induced) protein family as part of a transcription-regulating complex involved in the sugar and ABA-control of VvHT1 expression (Cakir et al., 2003).

In spite of the detailed information concerning the control of its expression, the exact function, and the tissue, cell, and membrane localization of VvHT1 are still unknown. Sense expression of VvHT1 in tobacco under the control of the strong constitutive cauliflower mosaic virus 35S promoter reduced expression of the endogenous tobacco monosaccharide transporter MST, decreased growth, and altered assimilate partitioning (Leterrier et al., 2003). These physiological effects resulted from post-transcriptional gene silencing of MST, due to the high sequence homology between MST and VvHT1. However, these effects might have been observed, whether VvHT1 encodes a bona fide monosaccharide transporter or a sugar sensor with high homology to a monosaccharide transporter.

The present work was conducted to test the actual transport function of VvHT1 by heterologous expression in a yeast mutant, and to obtain detailed information on its localization. VvHT1 turned out to be a functional plasma membrane monosaccharide transporter, but surprisingly for this type of transporter, it was mainly expressed in the conducting complex of the grape berry phloem.

Materials and methods

Cloning and heterologous expression of VvHT1

VvHT1 cDNA was cloned by PCR from a cDNA library produced from mRNA of grape berries (cv. Ugni Blanc) at the véraison stage (Cakir et al., 2003). PCR was conducted during 40 cycles using Takara-Z-Taq polymerase (Cambrex, Belgium), with a hybridation temperature of 64 °C, and the following primers: VvHT1F: 5′-GGAGGAATTCTAGGCTGCTGGGATTGATGAG-3′; and VvHT1R: 5′-GCAGGAATTCTACATTTTCCTACAGGCTAGTTTCC-3′. The PCR product was subcoloned into the pGEMT-easy vector (Promega, France) for amplification and sequencing.

After excision by a SacII/SphI restriction, the PCR product was inserted in the pBluescript KSII (Stratagene) vector. VvHT1 cDNA was excised from this vector by a BamHI/SacII digestion, and inserted unidirectionally in the corresponding restriction sites of the pDR195 yeast shuttle vector. pDR195 (Rentsch et al., 1995) was a kind gift of Dr D Rentsch (University of Bern, Switzerland). The pDR195-VvHT1 construct was used to transform the EBY.VW4000 Saccharomyces cerevisiae strain by a polyethylene glycol based method (Dohmen et al., 1991). The EBY.VW4000 strain is completely deficient in glucose uptake due to multiple mutations on the hexose transporters (Wieczorke et al., 1999), but it can grow on a maltose medium. The growth phenotype of the transformants was tested on media containing different sugars, and their ability to absorb labelled glucose was measured as described by Noiraud et al. (2000). Unless stated otherwise, data points are means of four replicates from experiments that were repeated at least twice.

In situ hybridization

In situ hybridization experiments were carried out on several grape varieties. Tissue samples from berries, petioles, and leaves of Chardonnay, Ugni Blanc, and Pinot Noir varieties were fixed by 4% paraformaldehyde in PBS buffer (50 mM sodium phosphate, pH 7.2) and embedded in paraffin. In the case of berries from Ugni Blanc variety, some fixations were also achieved by perfusion (Diakou and Carde, 2001). Ten µm sections were prepared from the paraffin blocks and mounted on poly-L-lysine coated slides. DIGoxigenin-labelled sense and antisense riboprobes were synthesized by in vitro transcription from pKSII (Stratagene) or pGEMT-easy (Promega) vectors carrying, respectively, the cDNAs of either VvHT1 or DFR (dihydrolavonol 4-reductase), the latter being used as a control. Plasmids were linearized by XbaI/XhoI or SacI/XhoI restriction for the VvHT1 and DFR probes, respectively. DIGoxigenin-11-UTP was incorporated by either T3 or T7 polymerase according to the manufacturer’s instructions (Boehringer Mannheim). The riboprobes were partially hydrolysed in sodium carbonate buffer at 60 °C for 40 min to increase access of the probe (200 pb) to the target mRNA. After several prehybridization treatments, in situ hybridization was performed overnight at 55 °C (Bostwick et al., 1992).

Hybridization of the riboprobe was detected with anti-digoxigenin antibodies conjugated to alkaline phosphatase and visualized by colour development with 5-bromo-4-chloro-3-indolyl-phosphate and tetrazolium nitroblue (Boehringer Mannheim).

The specificity of hybridization was tested by hybridizing either the sense or the antisense digoxigenin-labelled VvHT1 riboprobes with 50 mg mRNA of the different grape berry monosaccharide transporters that are available in this laboratory: VvHT1 (AJ001061), VvHT2 (AY663846), VvHTE (AY861386), VvHT7 (AY854146), and pGLT (AY608701). The mRNAs were synthesized from the corresponding full-length cDNAs cloned in pGEMT-easy, except for VvHT2 which was cloned in pKSII. The RNAs were spotted (50 ng each in 0.5 µl) onto a Hybond N nylon membrane (Amersham France), and fixed by heating for 30 min at 80 °C. Hybridization with the digoxigenin-labelled probes was carried out for 3.5 h at 55 °C. After hybridization, the membranes were washed in 2× SSC (two times 15 min), 1× SSC (10 min), 0.5× SSC (5 min), and for 5 min in maleic acid buffer (0.1 M maleic acid, 0.15 M NaCl, pH 7.5). The membranes were blocked for 30 min in 1% blocking reagent (Roche) and incubated for 30 min in anti-digoxigenin antiserum (1/10 000 in 1% blocking reagent). The membranes were then washed twice for 15 min in maleic acid buffer containing 0.3% Tween 20, and detection buffer (0.1 M Tris/HCl, pH 7.5, 0.1 M NaCl). They were revealed with a chemiluminescence reagent (CDP-star, Roche) and exposed on an ECL film (Amersham France).

Antiserum preparation and characterization

A rabbit polyclonal antibody directed against the C-terminal part of VvHT1 was prepared and purified as described in Zhang et al. (2004). The specificity of the serum was checked by Western blot against a plasma membrane fraction prepared from a grape cell culture. The embryogenic cell culture (41B) was maintained as described in Coutos-Thévenot et al. (1992). Frozen cells (30 g) were powdered in a Waring blender in 200 ml of buffer (0.1 M TRIS-MES,
pH 8.0; 0.2 M EDTA, pH 8.0; 0.5 M sucrose; 10 mM dithiothreitol; 100 μM PMSF; 0.6% PVPP). A microsomal fraction was prepared by centrifugation and resuspended in 5 mM potassium phosphate, pH 7.8; 0.25 M sucrose, 1 mM dithiothreitol. A fraction enriched in plasma membrane was prepared by phase partition (Larsson et al., 1984) in a medium containing 1 mM KCl and 6.4% Dextran T500 and polyethylene glycol 4000.

Denaturing gel electrophoresis of the plasma membrane proteins, transfer, and incubation with the serum were conducted as described by Sakr et al. (1997). The sections were incubated overnight in the VvHT1 antiserum (1/25 dilution) at room temperature. Controls were prepared by either omitting the serum or saturating the serum with the purified peptide used as the antigen.

Semi-thin sections were washed for 10 min in PBS then in PBS-BSA 1% (w/v), and incubated for 3 h in the FITC-labelled secondary antibody (GAR-FITC F-0382 Sigma), 1/200 dilution. Tissue autofluorescence was inhibited by Evans blue (1/10 000 diluted in PBS) and sections were mounted in glycerol/PBS (50/50, v/v) added with antifade agent (Vectashield, Vector Laboratories). Observations were made under blue light (excitation: 495 nm; emission: 535 nm) using a Zeiss Axioplan microscope.

Immunogold reaction was also performed on thin sections, using 15 nm gold particle-labelled goat anti-rabbit IgG (Biocell) 1/50 diluted. Samples were observed with a Jeol (1010) microscope operated at 80 kV.

Results

A full-length VvHT1 cDNA clone was isolated by PCR from a grape (cv. Ugni Blanc) cDNA library using the VvHT1F and VvHT1R primers. The PCR product (1.6 kb) was subcloned into the pGEMT-easy vector, for amplification in E. coli (DH5-α) and sequencing. Sequencing indicated that the sequence of the VvHT1 cDNA clone
matched the sequence of the genomic clone isolated from a Ugni Blanc genomic library by Fillion et al. (1999). VvHT1 cDNA was excised by a SacII/SpeI restriction, and inserted in the pKSII vector before insertion in the pDR195 yeast shuttle vector as described in the Materials and methods. The pDR195-VvHT1 construct was checked by PCR and sequencing, and used to transform the EBY.VW4000 yeast strain which is deficient in glucose transport. The EBY.VW 4000 strain is able to grow on maltose, but not on glucose or fructose (Wieczorke et al., 1999). Complementation by pDR195-VvHT1 restores growth on 100 μM glucose (Fig. 1A). Uptake experiments with labelled sugars showed that VvHT1 mediates the uptake of [14C] glucose with high affinity, whereas no glucose uptake was detected in the strain complemented by the empty vector (Fig. 1B). The optimal pH for glucose uptake was below 4.5 (Fig. 1C).

Uptake kinetics run at pH 4.5 indicated that glucose uptake mediated by VvHT1 in yeast obeys a single saturation phase with an apparent $K_m$ of 70 μM and a $V_{max}$ of about 14 nmol glucose mg$^{-1}$ protein min$^{-1}$ (Fig. 2B). By contrast, fructose uptake occurred at a very low rate and did not show any sign of saturation in the concentration range studied (Fig. 2A).

Uptake of radioactive glucose (150 μM) was challenged by an excess (1500 μM) of unlabelled galactose and mannose (Fig. 3). An excess of unlabelled glucose, used as an internal control, also strongly inhibited the uptake of radioactive glucose. These data suggest that galactose and mannose may compete with glucose for uptake, whereas sucrose and fructose do not (Fig. 3). However, direct measurements of the uptake of radiolabelled sugars showed that mannose and mannitol were not significantly taken up by the EBY.VW4000 strain expressing the pDR195-VvHT1 construct, and that fructose and sorbitol uptake were very small (Table 1). Thus, mannose may compete with glucose for the active site of the transporter at the external side of the plasma membrane, but it is not transported inside the cell.

Table 1. Uptake of various labelled sugars (100 μM) by the EBY.VW4000 yeast strain carrying either the empty vector pDR195 or the pDR195-VvHT1 construct

<table>
<thead>
<tr>
<th>Labelled sugar</th>
<th>Sugar uptake (nmol mg$^{-1}$ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pDR195</td>
<td>pDR195-VvHT1</td>
</tr>
<tr>
<td>β-Glucose</td>
<td>1.05±0.31</td>
</tr>
<tr>
<td>β-Fructose</td>
<td>1.05±0.20</td>
</tr>
<tr>
<td>β-Mannose</td>
<td>0.95±0.18</td>
</tr>
<tr>
<td>β-Sorbitol</td>
<td>0.95±0.12</td>
</tr>
<tr>
<td>β-Mannitol</td>
<td>1.25±0.29</td>
</tr>
</tbody>
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transcripts were localized by in situ hybridization in the petiole, leaf blade, and berries of Chardonnay and Ugni Blanc (two white varieties) and Pinot Noir (a black variety). The specificity of hybridization was first tested by dot blot (Fig. 4). An excess digoxigenin-labelled sense or antisense full length VvHT1 riboprobe was hybridized with VvHT1, VvHT2, VvHT6, VvHT7, and pGLT RNAs. At the nucleotide level, the similarities between VvHT1 and the other transporters tested are 44.4%, 21.7%, 47.1%, and 26.7%, respectively, for VvHT2, VvHT6, VvHT7, and pGLT. The similarities between VvHT1 and VvHT3, VvHT4, and VvHT5 which have been cloned in the laboratory of I Dry (Adelaide, Australia) are 47.2%, 47.5%, and 47.9% (M Hayes and I Dry, personal communication).

The VvHT1 antisense riboprobe hybridized only with the corresponding sense RNA (Fig. 4, upper panel), and the sense VvHT1 riboprobe did not detect any sense RNA among those that were tested (Fig. 4, lower panel). Given that VvHT3, VvHT4, VvHT5, and VvHT7 have about the same (and highest) similarity with VvHT1 (47%), the lack of hybridization of VvHT1 with VvHT7 (Fig. 4) suggests that the VvHT1 antisense probe is specific for this transporter.

In both white varieties (Fig. 5) and in the black variety (data not shown), the highest amounts of transcripts hybridizing with the digoxigenin-labelled antisense VvHT1 probe were detected in the phloem region of the conducting bundles of the leaf (Fig. 5A1), petiole (Fig. 5B1), and berries (Fig. 5C1). The specificity of detection was checked using the sense probe (Fig. 5A2, B2, C2). Phloem cells may appear to contain more label than the other cells because of their small size. To check that this was not the case, in situ hybridization was also performed with a probe expected to cross-react with several types of cells in the berries. For this purpose, a dihydroflavonol 4-reductase (DFR) probe was used. DFR, which is involved in the flavonoid pathway, is the last enzyme common to the synthesis of anthocyanidins and flavan-3-ols or tannins. The digoxigenin-labelled antisense DFR probe was mostly detected in the collenchyma cells and phloem region of the conducting bundles in the petiole, and more weakly in the parenchyma cells (Fig. 5D1). No signal was detected with the sense DFR probe (Fig. 5D2).

The different localizations found for VvHT1 and DFR transcripts indicate that the preferential phloem localization of VvHT1 transcripts is not artefactual. Tannin vacuoles were also observed in phloem cells (asterisks in Fig. 5A–C).

Various immunolocalization experiments were conducted to identify the cellular localization of VvHT1 precisely. Because VvHT1 is mainly expressed before véraison, and fixation procedures are much more troublesome with ripe berries, this work was carried out with young berries (5 mm diameter). In these berries, flesh cells surrounding the external vascular traces are characterized by a narrow wall, a thin layer of cytoplasm and a large central vacuole with tannin clusters next to the tonoplast (Fig. 6A). The flesh cells underlying the epidermis contain many plasmodesmatal groups in the cell walls and large amounts of vacuolar tannins (Fig. 6B). The sieve elements and the companion cells have approximately the same size, and the phloem parenchyma cells also contain tannins (Fig. 6C). Lateral interconnections of sieve elements (SE) are characterized by many pores (Fig. 6D).

A rabbit polyclonal serum directed against the C-terminal of VvHT1 (Zhang et al., 2004) was used to localize the VvHT1 protein in the berry tissues. First, the specificity of the serum was checked by Western blot with a purified plasma membrane fraction prepared by phase partitioning from a grape cell suspension. The serum recognized a major band at 52 kDa, and a faint band at 30 kDa (Fig. 7A). Immunofluorescence observations were made after incubation of semi-thin sections from young grape berries (5 mm diameter) with this antiserum. Extensive fluorescence was observed in the plasma membrane of both flesh cells (Fig. 7B) and phloem cells (Fig. 7C). No significant fluorescence was observed when the serum was first saturated with the purified antigenic peptide (C terminal part of VvHT1) (Fig. 7D).

Immunogold labelling performed with the same anti-VvHT1 serum on thin sections from grape berries (5 mm diameter) allowed a more precise localization of the glucose transporter. In flesh cells, gold particles were found both in the plasma membrane, and in underneath vesicles that may correspond to exocytic vesicles (Fig. 8A). In the conducting complex, a high density of gold particles was found in the plasma membrane of the sieve element/companion cell interface (Fig. 8C).

Discussion

Although several monosaccharide transporters have been functionally characterized and localized at the cell level in Arabidopsis thaliana (AtSTP1 in guard cells, Stadler et al., 2003; AtSTP2, AtSTP6, and AtSTP9 in pollen; Truemert et al., 1999; Schneiderheit et al., 2003; Scholz-Starke et al., 2004) and in grape berries (AtSTP1 in guard cells, Stadler et al., 2003; AtSTP2, AtSTP6, and AtSTP9 in pollen; Truemert et al., 1999; Schneiderheit et al., 2003; Scholz-Starke et al., 2004).
very few monosaccharide transporters have been cloned and characterized in perennial fruits that are important crops (Gao et al., 2003, in sour cherry; Gear et al., 2000 in tomato fruit).

In grape berries, which import sucrose and accumulate high concentrations of glucose and fructose, both sucrose transporters and monosaccharide transporters must play an important role in the accumulation process. To understand completely sucrose unloading and monosaccharide accumulation, it is necessary to identify, map, and characterize all the sucrose transporters, the invertases, and the monosaccharide transporters that are expressed in the berries.

Fig. 5. Localization of \( VvHT1 \) transcripts in grape tissues. The \( VvHT1 \) transcripts localization was performed in the leaves (small veins, A), petioles (B), and berries (external vascular bundle, C) of Chardonnay grapes. Cross-sections were probed with digoxigenin-labelled \( VvHT1 \) antisense RNA (A1, B1, C1). Controls (A2, B2, C2) were performed with digoxigenin-labelled \( VvHT1 \) sense RNA. For comparison, the localization of \( DFR \) transcripts was performed in Pinot Noir petioles (D). Cross-sections were probed with digoxigenin-labelled \( DFR \) antisense RNA (D1) or with digoxigenin-labelled \( DFR \) sense RNA (D2). The \( VvHT1 \) transcripts (stained in blue in A1, B1, C1) are localized in the phloem tissue (arrows), and the \( DFR \) transcripts (arrows in D1) in the collenchyma (Co), parenchyma (PC), and phloem cells (PH). In controls (A2, B2, C2, D2), the arrows show the absence of transcript detected with digoxigenin-labelled \( VvHT1 \) or \( DFR \) sense RNA probes. Tannins in large vacuoles are indicated by asterisks. X, xylem; f, phloem fibre; v, vessels. Scale bars, 100 µm.
during their development. Although three sucrose transporters were cloned (Davies et al., 1999), and two were functionally expressed in yeasts (Ageorges et al., 2000; Manning et al., 2001), no grape monosaccharide transporter has yet been characterized from a functional standpoint. The present work provides a functional characterization and a precise localization of VvHT1, a monosaccharide transporter homologue that had been cloned from grape berries at the véraison stage (Fillion et al., 1999).

In spite of numerous initial attempts in this laboratory and in the laboratory of N Sauer (Erlangen, Germany), it was impossible to observe transport after complementation of yeast mutants with the VvHT1 cDNA isolated by PCR from the Pinot Noir library originally used by Fillion et al. (1999). It turned out that the cDNA isolated in this way always contained a stop codon in position 1163, truncating the protein at about two-thirds of the sequence. Whether this was due to the presence of a pseudogene in the Pinot Noir genome or to an artefact in the library is not known. Since a genomic clone highly homologous to VvHT1 had also been isolated from a Ugni Blanc genomic library (Fillion et al., 1999), cloning was attempted by PCR on a Ugni Blanc cDNA library that had been previously

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**Fig. 6.** Ultrastructural features of grape (Ugni Blanc) berries (5 mm in diameter). (A) Flesh cells surrounding the external vascular traces are characterized by a narrow wall, a thin layer of cytoplasm (white arrow), and a large central vacuole (V) with tannin clusters (asterisk) next to the tonoplast. (B1) Flesh cells underlying the epidermis contain tannins (asterisk) that fill the vacuole and many plasmodesmata groups in narrow areas (white open arrow) of the wall (W). (B2) Detail of a plasmodesmata group (white arrowhead); plasmalemma, white triangle. (C) Sieve elements (SE) and companion cells (CC) of the same size and phloem parenchyma cells (PA) with tannins (asterisk). (D) Lateral interconnections of sieve elements (SE) are characterized by numerous pores (arrows) limited by a callose deposit (triangles); IS, intercellular space; M, mitochondrion; N, nucleus; P, plastid; Pp, P-proteins; V, vacuole; W, wall. Scale bar, 2 μm.
Sequencing verified that the Ugni Blanc VvHT1 cDNA obtained in this way matches the sequence predicted from the Ugni Blanc genomic DNA described by Fillion et al. (1999) and encodes a full-length monosaccharide transporter homologue.

Expression of this clone in a yeast mutant restores its ability to absorb glucose (Fig. 1). Radioactivity measurements indicate that VvHT1 encodes a monosaccharide transporter exhibiting a high affinity for glucose (Fig. 3). Mannose and mannitol are not significantly transported by VvHT1, and sorbitol and fructose are only very poorly transported, at least in the concentration range tested (Fig. 2; Table 1). Immunofluorescence (Fig. 7) and immunolabelling (Fig. 8) data show that VvHT1 localizes to the plasma membrane and not the tonoplast.

This, and previous data from this laboratory (Atanassova et al., 2003; Cakir et al., 2003), clearly indicate that during grape berry ripening, a plasma membrane monosaccharide transporter which may be controlled by sucrose, monosaccharides, and abscisic acid is expressed. This provides an original example of sugar and hormonal control of a monosaccharide transporter gene in a higher plant. The inducibility of the HUP monosaccharide transporters by glucose and some glucose analogues in the unicellular green algae Chlorella kessleri has been known for a long time (Sauer and Tanner, 1989). In Chenopodium rubrum cell suspensions, the expression of the monosaccharide transporters is not sensitive to sugars (Roitsch and Tanner, 1994). However, zeatin is able to co-induce both invertase and a monosaccharide transporter in this system (Ehness and Roitsch, 1997). Monosaccharide transporters have also long been known to be induced by elicitors or pathogen attack. Thus AtSTP4 is strongly induced by fungal infection, as well as by chitin and an elicitor from Pseudomonas syringae (Truernit et al., 1996). More recently, it was shown that the fungal biotroph Erysiphe cichoracearum induced both the monosaccharide transporter AtSTP4 and the cell wall invertase in Arabidopsis (Fotopoulos et al., 2003). On the other hand, the elicitor cryptogeen from Phytophthora cryptogea completely and rapidly blocks glucose uptake by a calcium-dependent phosphorylation process (Bourque et al., 2002). This, and other data, clearly suggest interactions between sugar metabolism, transport, and attacks by phytopathogens. In this context, VvHT1 expression was shown to be strongly induced by the bacterial sugar palatinose, which is not transported nor metabolized by plant cells (Atanassova et al., 2003). However, whereas another monosaccharide transporter...
called VvHT4 is induced by powdery mildew infection in grape, VvHT1 is not (M Hayes and I Dry, personal communication).

Maximal transport activity of VvHT1 was found at pH 4.5 (Fig. 1), as for the sucrose transporters cloned from grape berries (Manning et al., 2001). Although the $K_m$ of VvHT1 for glucose is somewhat higher than that found for other monosaccharide transporters from A. thaliana and tomato which are in the range of 20–40 $\mu$M (Büttner and Sauer, 2000; Gear et al., 2000), this high affinity is somewhat unexpected in an organ that contains such a high hexose concentration as the grape berry. However, given its pattern of expression, VvHT1 is not directly involved in the phase of active sugar accumulation occurring after véraison.

Western blot experiments indicated that anti-VvHT1 serum recognized a major band at 52 kDa in plasma membranes from grape cell suspension cultures (Fig. 7A), as they do in apple fruit cells (Zhang et al., 2004). Likewise, the protein recognized by the serum is mainly localized in the cells of the conducting complex, and to a less extent in the flesh parenchyma cells of both species (Fig. 7 of the present work, and Fig. 6 in Zhang et al., 2004). The immunolocalization data are strengthened by in situ hybridization experiments showing that VvHT1 transcripts mainly concentrate in the phloem region (Fig. 5). These experiments failed to detect significant amounts of VvHT1 transcripts in flesh cells, suggesting that VvHT1 is poorly expressed in these cells and/or that the detection was not sensitive enough.

Localization of the VvHT1 monosaccharide transporter in the plasma membrane of the cells of the conducting complex is somewhat surprising because the phloem sap is often thought to transport sucrose only. However, small amounts of monosaccharides must be present in the phloem sap since sucrose synthase activity and glycolysis are active in the conducting complex (Geigenberger et al., 1993). Immunolocalization of VvHT1 in the flesh cells (Fig. 8A) was expected because those cells concentrate high amounts of glucose and fructose after véraison. However, the localization experiments reported here were made with berries collected before véraison, because fixation is very difficult with post-véraison berries that contain a lot of water.

In grape, the early expression of VvHT1, its high affinity for glucose, and its localization suggest that it may be involved in the retrieval of monosaccharides from the conducting complex and from flesh cells, at a time where energy is needed to complete growth and differentiation. Data from this laboratory and from others (M Hayes and I Dry, personal communication) indicate that at least seven monosaccharide transporters are expressed during grape berry ripening. The transporters playing a major role in monosaccharide accumulation in the flesh cells still remain to be identified.

In conclusion, this work shows that VvHT1, which was previously shown to be controlled by sugars and ABA through a member of the ASR (abscisic acid, stress, ripening induced) family acting as a transcriptional regulator (Atanassova et al., 2003; Cakir et al., 2003) actually encodes a plasma membrane transporter exhibiting high affinity for glucose, but not fructose. It also gives the first evidence that a glucose transporter functionally characterized may be present in the plasma membrane of the cells of the conducting complex. VvHT1 is more likely involved in the retrieval of monosaccharides needed for cell growth and differentiation at the early stages of berry development than in the active accumulation of glucose and fructose that occurs after véraison.

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