MECHANISMS AND REGULATION OF MONOSACCHARIDE TRANSPORT IN SUSPENSION CULTURED CELLS OF *VITIS VINIFERA*

C. Conde[1], A. Agasse[2], S. Delrot[2], R. M. Tavares[1] and H. Gerós[1]

[1]Centro de Biologia | Departamento de Biologia | Universidade do Minho | Campus de Gualtar | Braga | Portugal

[2]Laboratoire de Physiologie et Biochimie Végétales | Bâtiment Botanique | Université de Poitiers | Poitiers | France

INTRODUCTION

The grape (*Vitis vinifera* L.) is a productive plant considered as the world's premier fruit, with nearly 9 million hectares of viticulture land in 1990. The ripening process is characterized by massive sugar import into the berries. Up to now, studies on sugar transport in *V. vinifera* have been carried out mainly using molecular biology approaches, and *VvHT1* and *VvHT2* genes associated with sugar accumulation were identified (Fillion *et al.*, 1999, Ageorges *et al.*, 2000).

Besides their role as carbon and energy sources, sugars can act as regulatory signals that affect expression levels of several genes controlling key processes and hence plant development. The ability to sense altered sugar concentrations is extremely important in the context of resource allocation, allowing the plant to tailor its metabolism in source tissues to meet the demands in sinks. In this work, *V. vinifera* heterotrophic suspension cultured cells were used as a model system to study *VvHT1* expression and sugar transport into sink cells.

RESULTS

Growth in batch cultures with sucrose

Cell suspensions were cultivated in liquid mineral medium with sucrose as sole carbon and energy source (Fig. 1). Sucrose is hydrolyzed extracellularly within 4 days and growth occurs along with glucose and fructose consumption.

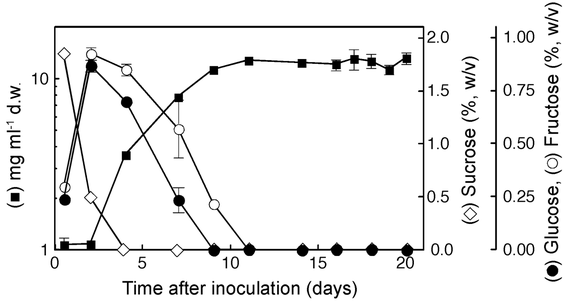

Figure 1.. Dry weight and sugars concentration in suspension cultures of *Vitis vinifera* cells grown with 2% sucrose.

Monosaccharide transport

Kinetics

Initial uptake rates of 0.02-50 mM D-[^{14}C]glucose and L-[^{14}C]glucose measured as described by Oliveira *et al*. (2002) are depicted in Figure 2. The application of a computer-assisted non-linear regression analysis (GraphPad software) to the data suggested the involvement of a saturable transport system associated with non-saturable "diffusion-like" transport. The kinetic parameters were: K_m, 0.05 ± 0.15 mM glucose and V_{max}, 1.45 ± 0.04 nmol glucose min^{-1} mg^{-1} d.w.; and for the diffusion-like component, k_d, 0.08 µl min^{-1} mg^{-1} d.w.

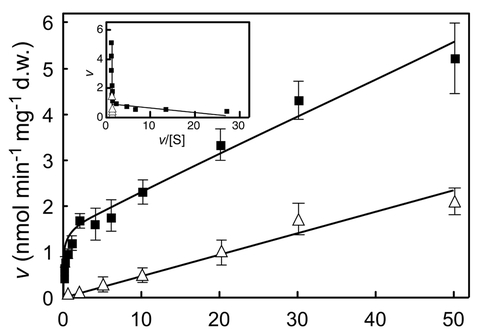

Figure 2. Glucose transport by suspension cultured cells of *V. vinifera* cultivated with 2% sucrose as in Figure 1. Initial uptake rates of D-[^{14}C]glucose (■) and L-[^{14}C]glucose (△), at pH 5.0, by cells collected at the end of exponential growth phase when total sugar concentration had fallen to around 0.1%.

Specificity

D-glucose and D-fructose share the same monosaccharide carrier, which exhibits higher affinity for D-glucose (Fig. 3A and 3B), explaining why glucose is the first substrate to be consumed after sucrose hydrolysis (see Fig. 1). D-galactose and D-xylose are also substrates for the monosaccharide carrier. Sucrose, D-mannitol, and D-arabinose are not recognized (Fig. 3C). Glucose carrier is also able to accept 2-deoxy-D-glucose and 3-*O*-MG, but not L-glucose (Fig. 3D).

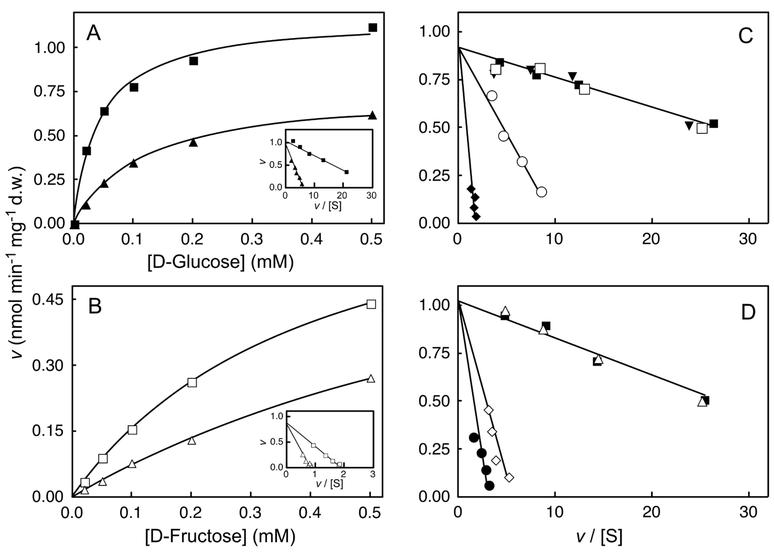

Figure 3. Specificity of *V. vinifera* monosaccharide carrier. (A) Initial uptake rates of D-[^{14}C]glucose in the absence (■) and in the presence (▲) of 20 mM fructose. (B) Initial uptake rates of D-[^{14}C]fructose in the absence (□) and in the presence (△) of 1 mM glucose. Inserts: Eadie-Hofstee plots of the initial uptake rates of D-[^{14}C]glucose and D-[^{14}C]fructose. Eadie-Hofstee plots of the initial uptake rates of D-[^{14}C]glucose in the absence of other sugars (■) and in the presence of 5 mM xylose (○), 5 mM galactose (◆), 5 mM mannitol (□) and 5 mM arabinose (▼) (C) or in the presence of the following glucose analogs: 8 mM L-glucose (△), 0.5 mM 2-deoxy-D-glucose (◇) and 0.5 mM 3-*O*-MG (●) (D). Transport was measured at pH 5.0 in cells cultivated with 2% sucrose as in Figure 1 and collected at the end of exponential growth phase as described in Figure 2.

Energetics

Data from energetic studies on glucose transport in *V. vinifera* are presented in Figure 4. Results are consistent with the involvement of a glucose/H$^+$ symporter with a stoichiometry of 1 glucose:1 H$^+$.

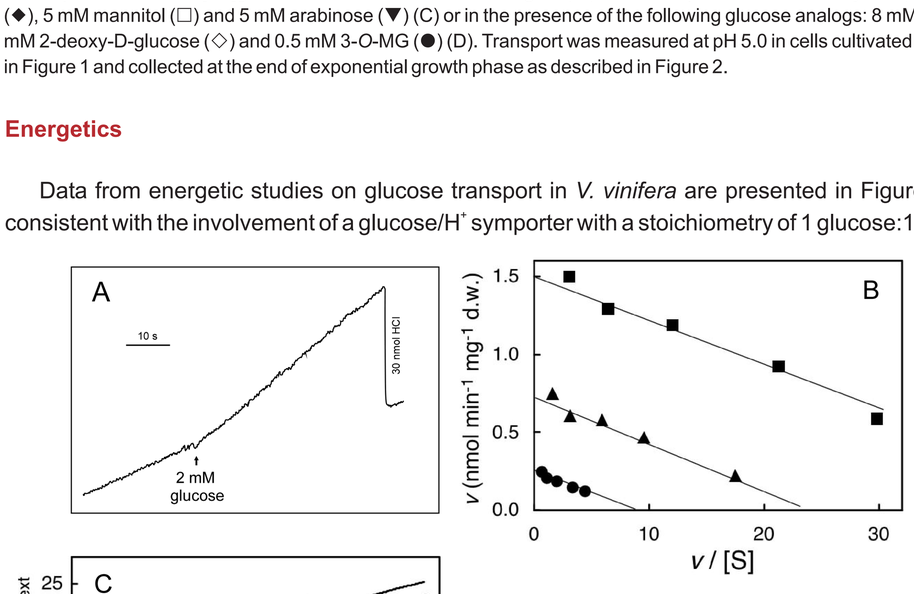

Figure 4. Energetics of *V. vinifera* monosaccharide carrier. (A) Proton uptake, at pH 5.0, associated with the addition of 1mM glucose to a weakly buffered cell suspension. (B) Eadie-Hofstee plots of the initial uptake rates of D-[^{14}C]glucose, at pH 5.0, in the absence (■) or in the presence of 0.05 mM CCCP (●) and 10 mM TPP$^+$ (▲). (C) Accumulation of labeled 3-*O*-MG at pH 7.0 (○) and pH 5.0 in the absence (●) and in the presence of 0.05 mM CCCP (▲). Cells were cultivated with 2% sucrose as in Figure 1 and collected at the end of exponential growth phase as described in Figure 2. Initial extracellular concentration of labeled 3-*O*-MG: 0.1 mM.

Sugar regulation of monosaccharide transport and VvHT1 expression

Monosaccharide transport activity and expression analysis of *VvHT1* along sugar depletion in the medium are presented in figures 5A and 5B. The parallel between *VvHT1* transcripts and V_{max} of glucose transport suggests that expression of the carrier is controlled at the transcriptional level, although others levels of regulation cannot be ruled out. *VvHT2* transcripts were not detected, suggesting that it is not expressed in this sink cell model.

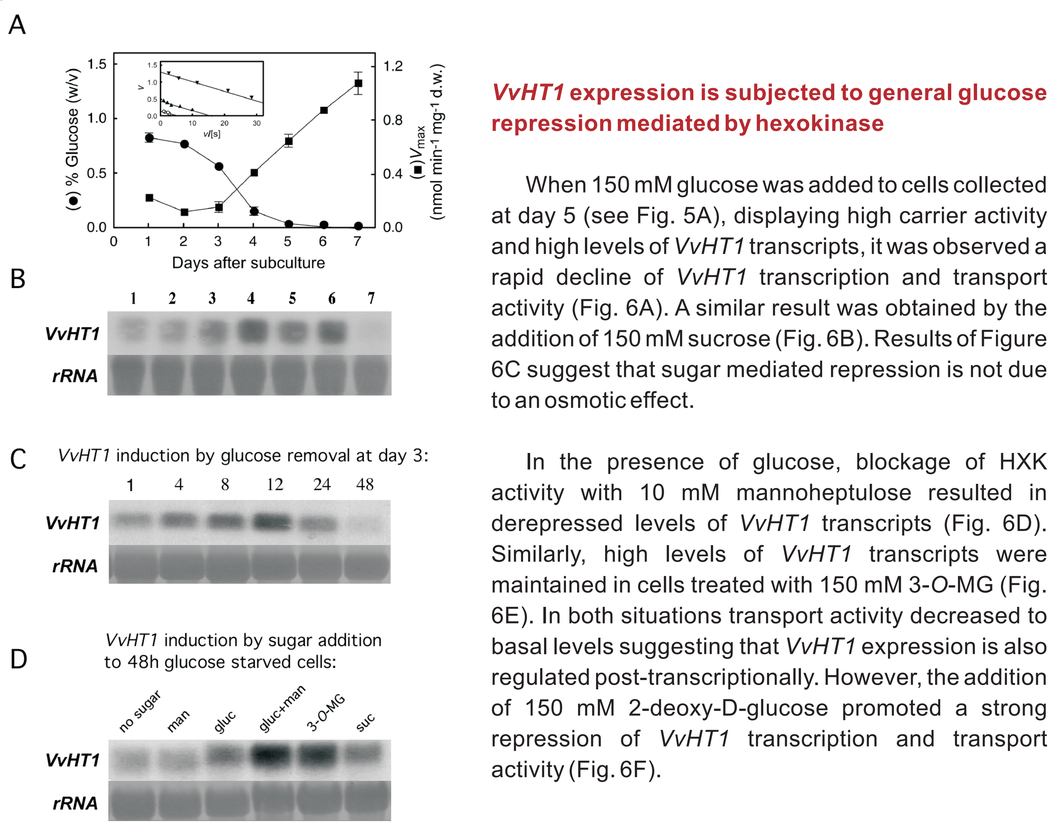

Figure 5. Monosaccharide carrier activity and northern analysis of *VvHT1* in suspension cultured cells of *V. vinifera*. (A) Activity of the monosaccharide/H$^+$ symporter (■) and glucose concentration in the culture medium (●). Insert: Eadie-Hofstee plots of the initial uptake rates of D-[^{14}C]glucose, at pH 5.0, in cell aliquots harvested from the culture at day 1 (○), day 4 (▲) and day 7 (▼). (B) *VvHT1* expression in cells grown as indicated in A. (C) Effect of glucose removal on *VvHT1* expression. Cells were grown as indicated in A and transferred at day 3 to the same medium without glucose. (D) *VvHT1* expression 12 hours after addition of 10 mM mannoheptulose (man), 150 mM glucose (gluc), 150 mM glucose + 10 mM manoheptulose (gluc + man), 150 mM 3-*O*-methyl-D-glucose, and 150 mM sucrose (suc) to cells cultivated during 48 h in the absence of glucose as indicated in C.

VvHT1 expression is subjected to general glucose repression mediated by hexokinase

When 150 mM glucose was added to cells collected at day 5 (see Fig. 5A), displaying high carrier activity and high levels of *VvHT1* transcripts, it was observed a rapid decline of *VvHT1* transcription and transport activity (Fig. 6A). A similar result was obtained by the addition of 150 mM sucrose (Fig. 6B). Results of Figure 6C suggest that sugar mediated repression is not due to an osmotic effect.

In the presence of glucose, blockage of HXK activity with 10 mM mannoheptulose resulted in derepressed levels of *VvHT1* transcripts (Fig. 6D). Similarly, high levels of *VvHT1* transcripts were maintained in cells treated with 150 mM 3-*O*-MG (Fig. 6E). In both situations transport activity decreased to basal levels suggesting that *VvHT1* expression is also regulated post-transcriptionally. However, the addition of 150 mM 2-deoxy-D-glucose promoted a strong repression of *VvHT1* transcription and transport activity (Fig. 6F).

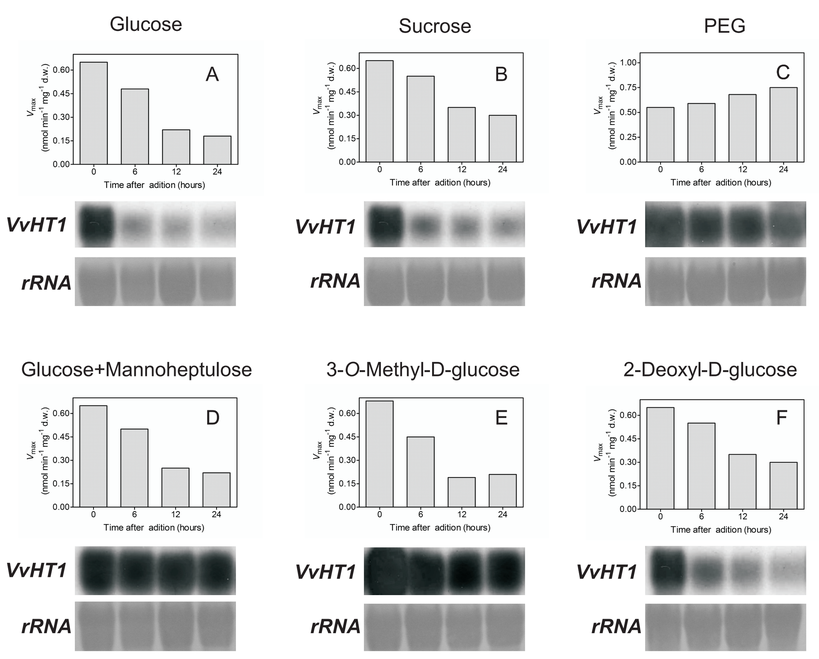

Figure 6. Repression of *V. vinifera* monosaccharide/H$^+$ symporter by different sugars. D-[^{14}C]glucose uptake and *VvHT1* transcripts were measured in cell aliquots at time periods indicated after the addition of 150 mM glucose, 150 mM sucrose, 150 mM PEG, 150 mM glucose plus 10 mM mannoheptulose, 150 mM 3-*O*-MG, and 150 mM 2-deoxyglucose to cultures at day 5 (see Figure 5.A).

Glucose positively regulates expression of *VvHT1*

The removal of glucose from 3 days old *V. vinifera* culture promoted *VvHT1* transcription within 12 hours. However, the prolonged absence of glucose resulted in the decrease of *VvHT1* transcription to levels lower than those detected in glucose repressing conditions (Fig. 5C).

The addition of glucose to 48 h starved cells promoted the induction of *VvHT1* transcription to levels observed in repressing conditions. In cells treated either with glucose plus mannoheptulose or 3-*O*-MG a stronger accumulation of *VvHT1* transcripts was observed, possibly as a result of HXK impairment in mediating the repression signal (Fig. 5D).This expression profile is in agreement with previously reported data from Atanassova *et al* (2003).

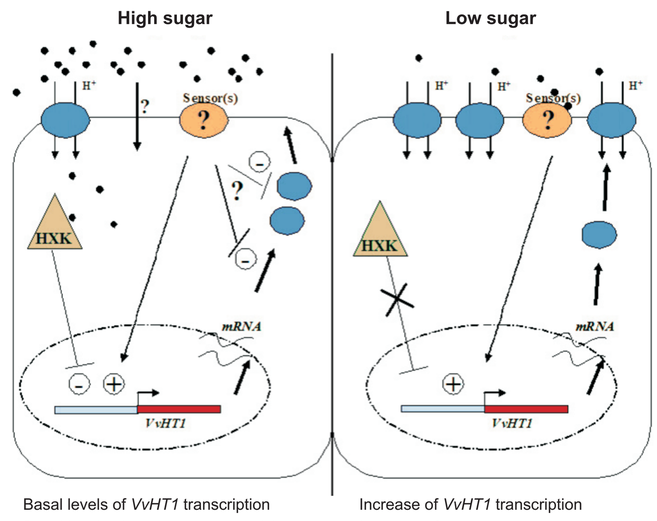

Figure 7. Regulation of monosaccharide transport by sugar in *V. vinifera* cultured cells.

Concluding remarks

- *V. vinifera* cultured cells are able to growth in mineral media with sucrose after the disaccharide had been hydrolysed by an extracellular invertase.
- Cells displayed activity for a monosaccharide/H$^+$ symporter with a stoichiometry of 1 glucose:1 H$^+$ and K_m = 0.05 mM glucose, encoded by *VvHT1.*
- Glucose repressed carrier activity and *VvHT1* transcription mediated by a hexokinase signalling pathway.
- In addition to repressing at high concentrations, glucose is required for induction of *VvHT1* expression.

References

-Ageorges, A., Issaly, N., Picaud, S. and Delrot. S. (2000). Characterization of an active sucrose transporter gene expressed during the ripening of grape berry (*Vitis vinifera* L.). Plant Physiol. Biochem. 38: 177-185.

Atanassova, R., Leterrier, M., Gaillard, C., Agasse, A., Sagot, E., Coutos-Thévenot, P., and Delrot, S. (2003). Sugar-regulated expression of a putative hexose transport gene in grape. Plant Physiol. 131: 326-334.

-Fillion, L., Ageorges, A., Picaud, S., Coutos-Thévenot, P., Lemoine, R., Romieu, C. and Delrot, S. (1999) Cloning and expression of a hexose transporter gene expressed during the ripening of grape berry. Plant Physiol. 120: 1083-1093.

-Oliveira, J., Tavares, R.M., Gerós, H. (2002). Utilization and transport of glucose in *Olea europaea* cell suspensions. Plant Cell Physiol. 43: 1510-1517.

Acknowledgements: Carlos Conde is supported by FCT, grant ref. SFRH/BD/10689/2002