UDP-Glucose Pyrophosphorylase. An Old Protein with New Tricks[1]

Leszek A. Kleczkowski*, Matt Geisler, Iwona Ciereszko, and Henrik Johansson

Department of Plant Physiology, Umeå Plant Science Centre, Umeå University, 901 87 Umeå, Sweden (L.A.K., M.G., H.J.); and Institute of Biology, University of Bialystok, Swierkowa 20b, 15–950 Bialystok, Poland (I.C.)

Sugars are central to a plant's raison d'etre as products of photosynthesis. They are the ultimate source of energy and carbon skeletons for all biomolecules, and they provide the material out of which a plant builds its cell walls, fibers, and wood. Thus, regulation of any activity involved in biosynthesis of sugars, especially Suc (the major transport form of carbon in plants), is of utmost interest in understanding the growth and development strategies of a plant. Sugars are also potent regulators of gene expression, via e.g. a hexokinase (HXK) transduction mechanism that senses hexoses, or via Suc-specific or osmoticum transduction pathways, further underlying the importance of sugars in plant homeostasis.

WHY STUDY UDP-GLC (UDPG) PYROPHOSPHORYLASE (UGPASE)?

UGPase represents an important activity in carbohydrate metabolism, catalyzing a reversible production of UDPG and pyrophosphate (PPi) from Glc-1-P and UTP. In young and mature leaves, UGPase is primarily involved in the Suc biosynthesis pathway, providing UDPG for Suc phosphate synthetase (SPS), whereas in other tissues, including immature apical leaves, which to some extent depend on imported carbon, UGPase may take part in Suc breakdown, using UDPG produced by Suc synthase (SuSy; ap Rees, 1992; Winter and Huber, 2000). In certain sink tissues, e.g. cereal seed endosperm, the reverse reaction (use of UDPG) is coupled to the activity of cytosolic ADP-Glc pyrophosphorylase, resulting in an equimolar production of ADP-Glc from UDPG (Kleczkowski, 1994), the former being the sole immediate precursor of starch in all plants. This assures close interaction of the reactions of Suc metabolism and starch synthesis in this tissue.

Despite its critical positioning at the crossroads of Suc synthesis/breakdown, UGPase has received less attention than other enzymes of Suc metabolism. One of the reasons is that the enzyme is much more active than other activities in the Suc pathways and thus, as the argument goes, it is probably not regulating the flow of carbon to/from Suc. Moreover, antisense study of potato (*Solanum tuberosum*) plants found that even a 96% decrease of UGPase activity in tubers had no effect on sugar levels (Zrenner et al., 1993). However, recently, several lines of evidence point toward regulation of UGPase at gene and protein levels, and questions have been raised whether, under certain conditions, the enzyme is rate limiting. Also, crystallization of human UDP-*N*-acetylglucosamine pyrophosphorylase (AGX; Peneff et al., 2001), which is related to UGPase, has provided a basis for interpreting earlier function/structure studies on UGPase. The present review will cover recent advances in our knowledge on UGPase. More detailed information on earlier work can be found in Kleczkowski (1994).

HOW MANY UGPASE GENES IN PLANTS?

It has been assumed that UGPase is represented by a single *UGP* gene in plants. A systematic screening for *UGP* cDNAs in barley (*Hordeum vulgare*) has yielded only one type of transcript in leaves, embryos, and endosperm (Eimert et al., 1996). Also, small differences in cDNA sequence of UGPases in potato have previously been explained as resulting from allelic polymorphism (Sowokinos et al., 1997). However, from the Arabidopsis genomic database, it is now evident that there are two highly homologous *UGP* genes in this species (*At3g03250* and *At5g17310*, located on chromosomes 3 and 5, respectively). Both genes are expressed, and the corresponding cDNAs are found in expressed sequence tag libraries (e.g. accession nos. T44798 and T43261, respectively). Two homologous *UGP* cDNAs were also found in poplar (*Populus* spp.) and rice (*Oryza sativa*), and in the slime mold *Dictyostelium discoideum*; in the latter, the genes were proposed to have different roles under different developmental stages, based on analyses of viable mutants (Bishop et al., 2002).

When analyzed at the derived amino acid level, there is 59% to 96% identity among plant UGPases (Katsube et al., 1990; Eimert et al., 1996; Pua et al., 2000; Abe et al., 2002) and approximately 55% identity with the corresponding proteins from slime mold (Bishop et al. 2002), mammalian liver (Konishi et al.,

1993; Chang et al., 1996), and yeast (*Saccharomyces cerevisiae*; Daran et al., 1995). Eukaryotic UGPases are significantly divergent from those of bacterial origin, with very little or no amino acid sequence identity (approximately 8%; Fig. 1). Plant UGPases share 13% to 15% identity with AGXs from plants and animals, but have over 40% similarity when allowing for functional aa substitutions. Similarity is a better measure of a common function, whereas percentage of identity is a more useful measure of evolutionary relatedness (randomly aligned Arabidopsis proteins have approximately 5% identity and 10% similarity). There is also some relatedness (10%–16% identity and 20%–30% similarity) between plant UGPase and SPS (Eimert et al., 1996), suggesting a common early ancestor for those proteins. Interestingly, bacterial UGPase appears to be more related to SuSy (approximately 15% identity) than to plant UGPase (approximately 8% identity) or SPS (approximately 10% identity; Fig. 1), further underlying a complex evolutionary path for those enzymes.

SUC-SPECIFIC REGULATION OF *UGP* EXPRESSION

The expression of *UGP* from Arabidopsis leaves was strongly up-regulated by Suc, but not by Glc or osmoticum, and the Suc effect was, to a large extent, mimicked by exposing leaves to the light; the light effect was probably at least partly mediated by Suc formed during photosynthesis (Ciereszko et al.,
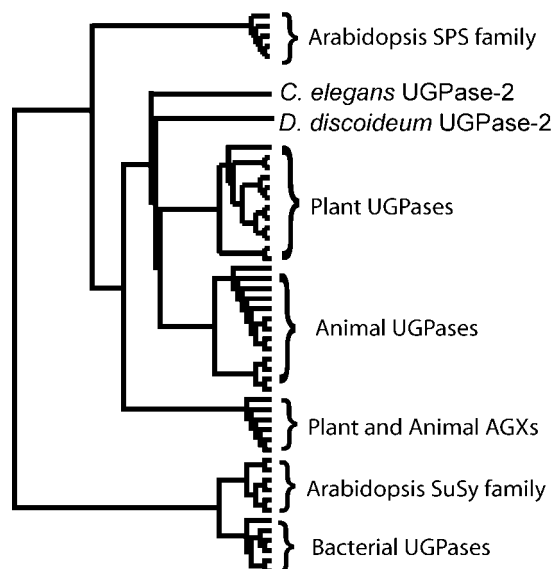

Figure 2. OKA-sensitive regulation of *UGP* and *SUS1* genes. The boxes and arrows represent hypothetical pathways for Suc, osmoticum, and Glc signaling. Double arrowheads represent multiple or unknown steps. An unknown phosphorylated intermediate (X) antagonistically regulates *SUS1* or *UGP* gene expression depending on phosphorylation status. OKA inhibits PP1 and 2A, and prevents dephosphorylation of X. This probably causes accumulation of X-P and depletion of X, which in turn strongly up-regulates *SUS1*, and suppresses expression of *UGP*. The enzymatic interconvertability of Glc and Suc is indicated, both contributing also to osmotic status, similarly to some unmetabolizable compounds (e.g. mannitol). For simplicity, the same PP is indicated as important for the expression of *UGP* and *SUS1*; in fact, we cannot exclude that the two genes are regulated by distinct OKA-sensitive PPs.

Figure 1. Cladogram of UGPase and related proteins. Plant and animal UGPases form separate clades, related to AGXs and, to a lesser degree, to SPS. There is little or no homology between eukaryotic and prokaryotic UGPases, but the latter share significant homology with the Arabidopsis SuSy family. UGPase-2 is the second divergent UGPase protein in *C. elegans* and slime mold, whereas UGPases-1 from both organisms fall within the animal UGPase clade. This figure was generated with Clustal-X (v 1.83) alignment and Tree-view (v 1.66).
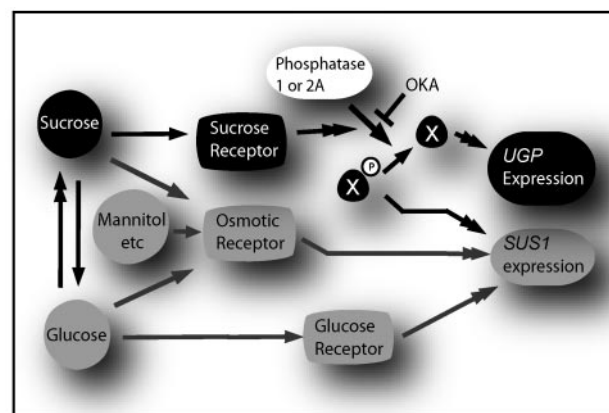
2001b). Suc up-regulation was also observed for *UGP* from potato tubers (Spychalla et al., 1994). Suc-specific signaling may have different components from HXK-mediated Glc (hexose) signaling or osmoticum signal pathways (Rook et al. 1998, Loreti et al., 2001). Glc is likely to be a cell autonomous signal as it is not usually exported to other cells, whereas Suc is readily transported via the phloem.

In Arabidopsis, the Suc effect on *UGP* was independent of HXK status, and was completely blocked by okadaic acid (OKA), a potent inhibitor of protein phosphatases (PP) 1 and 2A (Ciereszko et al., 2001b). This was accompanied by a marked OKA-dependent up-regulation of a SuSy (*SUS1*) gene that is prone to hexose (via HXK) and osmoticum regulation (Déjardin et al., 1999; Ciereszko and Kleczkowski, 2002). The OKA effects indicate that a phosphoprotein (X-P) that serves as a substrate for PP1 and/or PP2A is mediating the up-regulating signal for *SUS1*, whereas up-regulation of *UGP* requires dephosphorylation of X-P or some other phosphoprotein that acts as a substrate for PP1 and/or PP2A (Fig. 2). Thus, hexose (and/or osmoticum) and Suc-signaling pathways may intersect antagonistically, at least for *SUS1* and *UGP*. The presence of distinct signaling pathways for *UGP* and *SUS1* genes may represent a mechanism where UDPG is assured to be produced even if one of the pathways is inactive or blocked.

STRESS REGULATION OF *UGP* EXPRESSION

In Arabidopsis, *UGP* expression and UGPase activity/protein content were strongly up-regulated by conditions resulting in phosphorus deficiency. The approaches included the use of mutants affected in inorganic phosphate (Pi) status, growth of wild-type plants on liquid media lacking Pi, and the feeding of Man to leaves to decrease internal content of Pi (Ciereszko et al., 2001a). The Pi stress did not result in significant increase in Suc in *pho1* mutant (Pi deficient), suggesting distinct signaling mechanisms for transmitting P deficiency and Suc effects on *UGP* expression in this species. In pea (*Pisum sativum*) roots, UGPase protein increased after cadmium-excess stress, possibly reflecting Cd-induced Pi deficiency effects at the *UGP* expression level (Repetto et al., 2003). The UGPase step may constitute an important mechanism increasing Pi availability during P stress, at least in source tissues where UGPase is involved in Suc synthesis. The PPi released in the synthesis of UDPG by UGPase is hydrolyzed to Pi by pyrophosphatase(s), thereby increasing the availability of Pi for P-deprived plants.

Low temperature had a strong up-regulating effect on *UGP* from potato tubers (Spychalla et al., 1994; Borovkov et al., 1996) and leaves of Arabidopsis (Ciereszko et al., 2001b). In Arabidopsis, the cold signal was transmitted via an ABA-independent pathway, as found using *aba* mutants. Cold treatment frequently leads to sugar accumulation and, in potato, UGPase has been the main marker for processes leading to "cold sweetening", i.e. accumulation of sugars when tubers are stored in the cold (Sowokinos et al., 1997). With UGPase being intrinsically involved in Suc metabolism, low temperature up-regulation of *UGP* may have direct effects on the "cold-sweetening" phenomenon. The *UGP* was also down-regulated by drought and flooding conditions (Ciereszko et al., 2001b). The latter strongly induced *SUS2* gene from Arabidopsis leaves, with the concomitant increase in SuSy protein (Déjardin et al., 1999). Thus, it appears that flooding (O$_2$ deprivation) may exert differential effects on UGPase and SuSy, suggesting that the two enzymes are not metabolically linked via UDPG under these conditions.

THE PREDICTED THREE-DIMENSIONAL STRUCTURE OF UGPASE AGREES WITH MUTANT FUNCTION DATA

A major recent advance in studies on pyrophosphorylases has been the crystallization of human AGX1 (Peneff et al. 2001). This protein is functionally related and has approximately 40% protein sequence similarity to UGPases. A predicted structure for the UGPase consensus sequence (reference no. KOG2638) can be found in the National Center for Biotechnology Information conserved domain database (http://www3.ncbi.nlm.nih.gov/) and visualized with the cn3d program (available at www.ncbi.nlm.nih.gov/Structure/cn3d/). Swissmodel (http://swissmodel.expasy.org/) enables easy template-homology modeling and visualization using their DeepView program (http://swissmodel.expasy.org/spdbv/). The primary sequence for barley UGPase (GenBank accession no. X91347) was homology modeled using the crystal structure for AGX1 (Expasy file name IJV1.pdb) to produce a predicted three-dimensional structure in Figure 3. The visualization programs
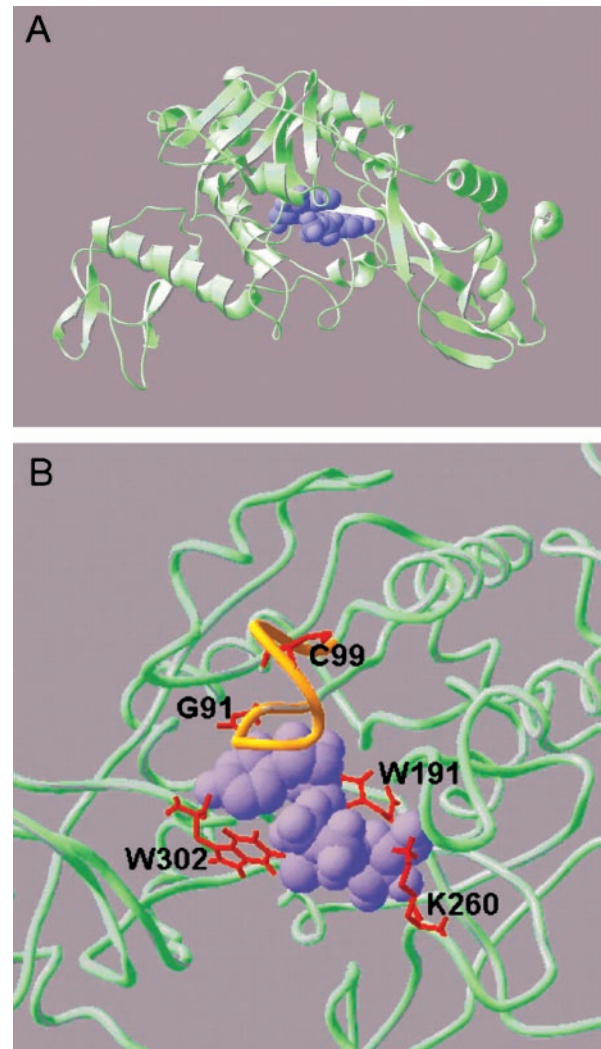

Figure 3. Predicted three-dimensional structure of barley UGPase. The overall UGPase structure (A) was predicted using Swissmodel first approach method using human AGX-1 as a template fold and barley UGPase protein sequence. The model shows segments of UGPase as α-helices (curls), β-sheets (ribbons with arrows), and coils (wire tubes). The predicted location of the substrate (UDPG) is shown as a purple space-filled model. In the enlargement of the active site, shown in wireframe view (B), the nucleotide binding (NB) loop conserved in all pyrophosphorylases is colored orange. Residues shown by mutation to be critical to activity are shown in red and labeled in amino acid unicode (e.g. W = Trp, K = Lys) along with their position in the barley UGPase protein sequence. Illustrations were generated by Swissprotein Deepview (v 3.7).

are very user friendly, and we recommend down-loading and viewing the key mutant residues in three-dimensional images rather than relying on the two-dimensional images we present here.

The overall putative three-dimensional structure of UGPase monomer is bowl-like, with an active site positioned in a central groove (Fig. 3A). This shape is common for AGX and UGPase, and perhaps for all pyrophosphorylase-like proteins (Peneff et al. 2001). The active site of UGPase contains several amino acid residues that were shown to be important for sub-strate binding and catalysis of the enzyme (Fig. 3B). For instance, Trp-191, Trp-302, and Lys-260 (based on a barley UGPase amino acid sequence) were sug-gested to be involved in UDPG binding for potato and liver UGPase (Kazuta et al., 1991; Chang et al., 1996). In the derived structure of barley UGPase (Fig. 3B), the positive charge of Lys-260 may well stabilize the negative charge of the two phosphates of UDPG. In other studies, Cys-99 was implicated in PPi bind-ing for the liver and barley enzyme (Chang et al., 1996; Martz et al., 2002), whereas in hamster lung fibroblasts, a mutation in a Gly residue correspond-ing to Gly-91 of barley UGPase resulted in a persis-tently low UDPG level (Flores-Diaz et al., 1997), con-sistent with the positioning of Gly-91 at the active site of the protein. Gly-91 and Cys-99 lie within what Peneff et al. (2001) refer to as the NB loop (Fig. 3B, labeled in orange), a conserved domain common for pyrophosphorylases. Cys-99 sits in the middle of this loop, and lies above the two phosphates of UDPG, an ideal position for binding and stabilization of the pyrophosphate bridge. On the other hand, Gly-91 sits on the edge of the NB loop, interacting with the uracil residue of UDPG.

OLIGOMERIZATION AS GENERAL REGULATORY MECHANISM FOR PYROPHOSPHORYLASES?

Recent work with barley UGPase has established that the protein readily oligomerizes and that only monomeric form of the enzyme is active. This was based on activity staining for forward and reverse reactions of UGPase (after native PAGE). Based on mutant studies, the deoligomerization step (forma-tion of monomers) was found as rate limiting (Martz et al., 2002). A monomer is also the only active form of human AGX; a dimer of this enzyme was pro-posed to dissociate to monomers under assay condi-tions (Peneff et al., 2001). The dimerization process apparently modifies the structural environment of the active site, which is open in the monomer and occluded at the dimer interface. Oligomerization was also demonstrated as an important regulatory mech-anism for ADP-Glc pyrophosphorylase from several species (Hendriks et al., 2003). Because a change in quaternary state of those distinct but functionally related proteins is coupled with changes in enzy-matic activity, it is tempting to propose that the

oligomerization phenomenon is a common regula-tory feature of the pyrophosphorylase family.

UGPASE AND CELL WALL BIOSYNTHESIS

Any consideration of the role of UGPase should take into account that UDPG, the substrate/product of the enzyme, is a key metabolite for carbohydrate metabolism in photosynthetic and nonphotosyn-thetic tissues. In addition to being used in Suc path-ways, UDPG is used, directly or indirectly, in the biosynthesis of cell wall polysaccharides, reflecting the key role of UDPG as a precursor for cell wall biogenesis (Gibeaut, 2000). Among UDPG's other roles is its involvement in the synthesis of carbohy-drate moiety of glycolipids and glycoproteins (Flores-Diaz et al., 1997; Bishop et al., 2002).

The prevailing view is that UGPase is localized mainly in the cytosol. Immunogold labeling of rice cells has revealed that most of the UGPase is located in the cytosol, but also to some extent in the amylo-plast and Golgi (Kimura et al., 1992). Fractionation of rice and tobacco (Nicotiana tabacum) cells yielded some UGPase activity in the microsomes (Mikami et al., 2001), whereas in barley, high UGPase activities were found in a membrane fraction (Becker et al., 1995). The membrane association of UGPase is rem-iniscent of properties of SuSy, which has been pro-posed to bind to plasmalemma and to directly pro-vide UDPG to cellulose synthase (Amor et al., 1995). Phosphorylation/dephosphorylation events are es-sential for binding of SuSy to the plasmalemma (Win-ter and Huber, 2000); the phosphorylated enzyme is fully soluble, and dephosphorylation promotes bind-ing of SuSy to the membrane. Whereas there is no doubt that SuSy contributes strongly to cell wall biosynthesis (Amor et al., 1995; Ruan et al., 2003), the possible role of UGPase in this process still needs to be considered, especially in source tissues. Mature leaves under normal physiological conditions gener-ally contain low, if any, SuSy protein (Déjardin et al., 1999), whereas UGPase activity in leaf extracts is well in excess of photosynthetic fluxes (Igamberdiev and Kleczkowski, 2000). In leaves, the overall carbon flow overwhelmingly favors production of UDPG from fixed carbon via the UGPase reaction (ap Rees, 1992; Kleczkowski, 1994; Fig. 4). On the other hand, for UGPase to be effective in making UDPG in sink tissues, there must be an effective supply of Glc-1-P. This may arise indirectly from Fru, a product of the SuSy reaction, which releases one Fru for every UDPG made during Suc hydrolysis, or from the in-vertase reaction (Fig. 4).

Cellulose and callose are produced from UDPG supplied directly to the plasmalemma, whereas other polysaccharides (e.g. hemicelluloses and pectins) are made in Golgi bodies and are then transported out-side the plasmalemma via vesicular transport (Gibeaut, 2000). Activities providing UDPG for the
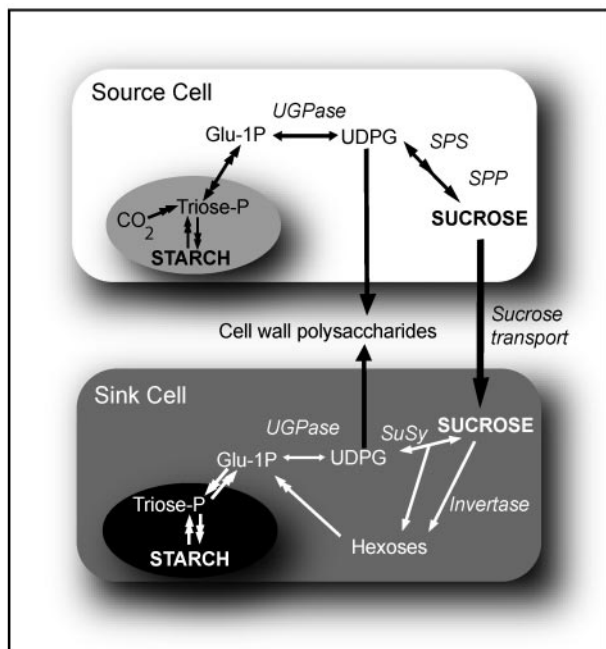
Figure 4. Possible involvement of UGPase in cell wall synthesis in source and sink tissues. UGPase in photosynthetic (source) tissues generates UDPG, and works in conjunction with SPS and Suc-P phosphatase (SPP) to produce Suc. In a sink (e.g. roots), UGPase can work in conjunction with SuSy and/or invertases to break down Suc, but can also remobilize Glc-1-P back to UDPG. Please note that UDPG is used for cell wall polysaccharide synthesis in both source and sink tissues.

latter processes must be Golgi associated or there should be some UDPG transporters present to allow transport of UDPG from cytosol through the Golgi membrane. In pea stems, UDPG was shown to be transported to Golgi vesicles in exchange for UMP and there was rapid incorporation of the Glc moiety of UDPG into endogenous acceptors (Neckelmann and Orellana, 1998). There is also a turnover of Glc in Golgi vesicles (Gibeaut, 2000) and likely a reassimilation into UDPG, which may be carried out via a Golgi-associated UGPase. The involvement of UGPase in cell wall synthesis was directly demonstrated in a yeast mutant where a 10-fold-reduced UGPase activity led to a decreased β-glucan content in cell walls (Daran et al., 1995). Also, cellulose-negative mutants of *Acetobacter xylinum* were deficient in UGPase (Valla et al., 1989). Those organisms do not contain SuSy activity, therefore the strong effects of UGPase deficiency on cell wall synthesis are probably not surprising. However, overexpression of *UGP* and *SUS* genes (single and double transformations) in tobacco resulted in a significant increase in height and an increased cellulose content in several single transformed lines and in all double transformants (Coleman et al., 2003). This suggested a correlation between the up-regulation of both genes and overall growth in tobacco. In DNA microarray studies on xylogenesis in the cambial region of

poplar stems, a gene for UGPase was found to be up-regulated during the late cell expansion and secondary cell wall formation phases (Hertzberg et al., 2001). Also, in tissues undergoing heartwood formation in black walnut (*Juglans nigra*) trunks, UGPase activity was correlated with increased Suc breakdown occurring from late summer until early winter (Magel et al., 2001).

IS UGPASE A RATE-LIMITING STEP IN METABOLISM?

There is some confusion in the literature as to whether changes in UGPase content/activity bring about subsequent changes in metabolite flux. Zrenner et al. (1993) showed that UGPase activity in developing potato tubers could be reduced (by an antisense approach) by as much as 96% with no effect on carbohydrate metabolism. On the other hand, Spychalla et al. (1994) and Borovkov et al. (1996) failed to produce transgenic potatoes with an inhibition of enzyme activity greater than 30% and 50%, respectively, but this level of inhibition was sufficient to decrease sugar content in stored tubers. In Arabidopsis, a relatively small inhibition (30%) of UGPase activity in leaves of antisense plants led to a decrease in sugar content and, to some extent, to a decrease in starch level (Johansson, 2003), suggesting that the enzyme may play a rate-limiting role in carbohydrate synthesis. Given the presence of two *UGP* genes in Arabidopsis, the possibility cannot be excluded that the apparently conflicting results of antisense studies in potato may in fact reflect differential inhibition of *UGP* genes.

The extractable UGPase activity, especially in sink tissues (but also in leaves), is far in excess of carbon flow in plants (Igamberdiev and Kleczkowski, 2000), underlying the potential of UGPase for maintaining substantial fluxes, even if regulated in vivo. However, it is quite possible that under in vivo conditions, the UGPase activity is much lower and, in fact, is limiting to the metabolic pathway(s) that are connected to soluble sugar formation/degradation. For instance, the enzyme undergoes reversible oligomerization, with the deoligomerization step shown as rate limiting (Martz et al., 2002). Because only the monomer of UGPase is active, any process stimulating formation of dimers and higher order oligomers (e.g. binding to other proteins during in vivo metabolite channeling) may affect its enzymatic efficiency. Bacterial UGPase interacts with a GalF protein that modulates its activity in vivo, especially during stress conditions (Marolda and Valvano, 1996). In yeast, UGPase is regulated by phosphorylation by a Ser/Thr kinase, with the phosphorylation resulting in lower UGPase activity (Rutter et al., 2002). A similar observation was made for UGPase from spinach (*Spinacia oleracea*), but there the efficiency of enzyme phosphorylation was also modulated by binding to a

14:3:3 protein (Huber et al., 2000). Mammalian UGPase apparently undergoes *O*-glycosylation (Wells et al., 2003), but the significance of this modification is not clear at the moment. Plant UGPases contain several putative glycosylation and phosphorylation sites (Eimert et al., 1996). Although those motifs are not ultimate proofs that the posttranslational modification occurs in vivo, it seems possible that plant UGPase is prone to undergo posttranslational modifications that may affect its activation state before and after extraction, so the activity measured in leaf extracts overestimates that existing in vivo.

PROSPECTS

The *UGP* from Arabidopsis is one of a limited number of genes where Suc-specific regulation has been documented (Ciereszko et al., 2001b), and the transduction is likely to occur via a different pathway from those for hexose- or osmoticum-sensitive genes (Rook et al., 1998; Loreti et al., 2001; Moore et al., 2003; see also Fig. 2). The *UGP* genes can serve as useful tools for identifying mutants in transduction of the Suc signaling, using e.g. a negative selection marker fused to the *UGP* promoter. Based on observation that Suc up-regulation of *UGP* expression is disrupted by OKA (an inhibitor of PP1/2A; Ciereszko et al., 2001b), T-DNA mutagenized lines impaired in PP1/2A can be screened to identify plants where *UGP* expression is specifically abolished. DNA microarrays performed on selected plants that are impaired in Suc sensing or in specific PP1/2A involved in Suc signaling should reveal a global perspective concerning Suc sensing/signaling, i.e. allowing the identification of genes that are regulated by Suc and those involved in Suc signaling. The same concerns the possible use of *UGP* promoters to study P deficiency and low temperature transduction pathways.

The resolution of the crystal structure of AGX (Peneff et al., 2001) and its relatedness to plant UGPase (Fig. 3) should dramatically facilitate studies on structure/function properties of UGPase. We have now a model to test, e.g. via site-directed mutagenesis, to verify details of the plant enzyme catalysis and substrate binding, as well as oligomerization processes. Another milestone should be studies on the role of specific *UGP* genes, using e.g. gene-specific antisense or RNA interference technologies. This should resolve the long-standing question of the importance of UGPase for overall carbon flux in plants and whether it is rate limiting (Zrenner et al., 1993, Spychalla et al., 1994, Borovkov et al. 1996). Also, whereas the involvement of UGPase in cell wall biosynthesis in source tissues is rather clear (Fig. 4), its possible role in sink tissues (along with SuSy) needs more experimental evidence. Finally, the knowledge of posttranslational regulation of yeast

and mammalian UGPases (Rutter et al., 2002; Wells et al., 2003) should allow more studies on similar regulatory mechanisms for plant UGPase.

CONCLUSIONS

Considerable progress has been made in studies on plant UGPase, one of key enzymes of Suc synthesis/breakdown. The UGPase genes are specifically up-regulated by Suc, involving an OKA-sensitive transduction component(s), and by a variety of abiotic stresses, including cold and P deficiency. The predicted protein structure of the UGPase monomer closely resembles a recently resolved crystal structure of a bowl-shaped AGX, with an active site in the central groove that includes several amino acids earlier shown as critical for catalysis/substrate binding. The UGPase is now recognized as existing as at least two isozymes, regulated by oligomerization and, possibly, phosphorylation. In source tissues, the enzyme provides UDPG for Suc and cell wall biosynthesis. Although present at high activity in plant extracts, the UGPase may represent a rate-limiting step in sugar metabolism in vivo.

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