

# UDP-sugar pyrophosphorylase controls the activity of proceeding sugar-1-kinases enzymes

Claudia Geserick and Raimund Tenhaken\*

University of Salzburg; Department of Cell Biology; Salzburg, Austria

**Keywords:** UDP-sugar pyrophosphorylase, glucuronokinase, salvage pathway, nucleotide sugars, coregulation

Plant cell wall synthesis requires a number of different nucleotide sugars which provide the building blocks of the different polymers. These nucleotide sugars are mainly provided by de novo synthesis but recycling pathways also contribute to the pools. The last enzyme of the recycling pathway is UDP-sugar pyrophosphorylase (USP), a single copy gene in *Arabidopsis*, of which a knockout is lethal for pollen development. Here we analyze the dependency between USP enzyme activity and the upstream glucuronokinase. Gene silencing of USP by miRNA cause a concomitant reduction of USP and of glucuronokinase activity presumably to prevent the accumulation of sugar-1-phosphates interfering with normal metabolism and depleting the phosphate pool of the cell.

The synthesis of plant cell wall polymers requires a number of different nucleotide sugars as building blocks. A complex enzymatic network is providing the different GDP- and UDP-sugars to polymer synthases in the Golgi apparatus.<sup>1</sup> Mutants in nucleotide sugar interconverting enzymes from *Arabidopsis* have shown that a reduction in one (or more) nucleotide sugars alters polymer biosynthesis of cell walls.<sup>2-4</sup> This can be a reduction in the formation of certain polymer type or changes in their substructure composition. Except for UDP-glucose most other nucleotide sugars are present in low concentrations in plant cells, often below 10  $\mu$ M in cellular extracts.<sup>5</sup> Thus a coordinated and balanced formation of nucleotide sugars is a prerequisite for normal cell wall polymer biosynthesis

During development of plants the cell wall is remodeled and some polymers are degraded or modified by hydrolases which release sugar monomers as final products. These sugars are recycled to nucleotide sugars by the action of two principle enzymatic reactions. First a number of different substrate specific sugar-1-kinases activate the monomers into the corresponding sugar-1-phosphates (Fig. 1). They are substrates for the subsequent enzyme UDP-sugar pyrophosphorylase (USP) an enzyme with broad substrate specificity (Fig. 1). USP takes at least 5 different sugar-1-phosphates as substrates and converts them into the corresponding UDP-sugars thereby releasing pyrophosphate.<sup>6-9</sup> The reaction is energetically reversible so that equilibrium between the pairs UTP plus sugar-1-phosphate and UDP-sugar plus pyrophosphate is reached. For the USP from pea, the same ratio of the metabolite pairs is reached independent on the enzymatic reaction in forward or reverse mode which is for Glc-1-phosphate plus

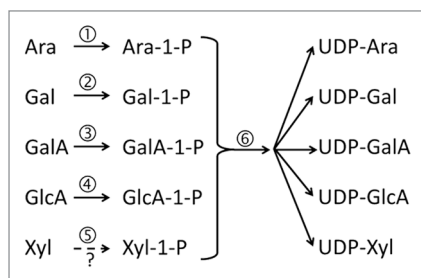
UTP about 30% UDP-glucose and 70% Glc-1-phosphate.<sup>6</sup> By adding pyrophosphatase to the reaction the same reaction results in almost 100% UDP-Glc.<sup>6</sup> In the cellular context, the hydrolysis of the energy-rich pyrophosphate by phosphatases or pyrophosphate dependent proton pumps drives the biosynthesis pathway toward the UDP-sugars.

USP is encoded by a single copy gene in *Arabidopsis* (At5g52560). Attempts to obtain homozygous knockout mutants failed and suggest an essential role of USP for the development of vital pollen.<sup>10,11</sup> Aberrant formation of the pecto-cellulosic network of the intine in pollen grains carrying a defect in the USP gene was demonstrated by.<sup>10</sup> A recent paper by Geserick and Tenhaken (2013) further analyses the function of USP for plant cells. They confirmed the lethality of homozygous knockouts in USP and developed a strong knockdown line for USP to study the role of USP for nucleotide sugar metabolism during plant development. Different genetic complementation approaches for *usp*-mutants were performed. Efforts rescuing the recycling of single but not all sugars (e.g., glucuronic acid) failed suggesting that the physiological role of USP is broader than the salvage of a single sugar. The *usp* knockdown plant accumulates arabinose and xylose suggesting that these two sugars in vivo are important substrates for USP. As shown in Figure 1, the substrates for USP are sugar-1-phosphates which were not directly measured in the study by.<sup>11</sup> Nevertheless, the finding of the accumulation of arabinose and xylose most likely in addition to arabinose-1-phosphate and xylose-1-phosphate suggests a biochemical mechanism that prevents the full conversion of e.g., arabinose into arabinose-1-phosphate. To understand and

Correspondence to: Raimund Tenhaken; Email: Raimund.tenhaken@sbg.ac.at

Submitted: 05/31/13; Revised: 06/19/13; Accepted: 06/20/13

Citation: Geserick C, Tenhaken R. UDP-sugar pyrophosphorylase controls the activity of proceeding sugar-1-kinases enzymes. Plant Signal Behav 2013; 8:e25478  
<http://dx.doi.org/10.4161/psb.25478>



**Figure 1.** Schematic pathway of recycling of nucleotide sugars. The sugar monomers are phosphorylated at the 1-position by substrate specific sugar-1-kinases. The second step is catalyzed by UDP-sugar pyrophosphorylase, which accepts several sugar-1-phosphates as substrates. 1) Arabinokinase; 2) Galactokinase; 3) Galacturonokinase; 4) Glucuronokinase; 5) putative Xylokinnase; 6) UDP-sugar pyrophosphorylase.

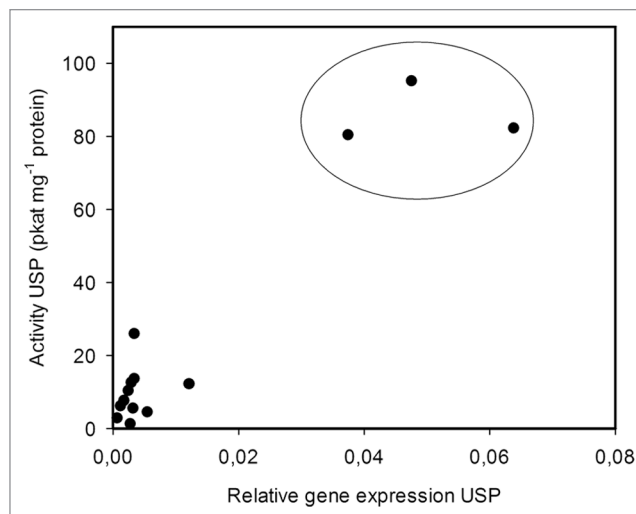
dissect this observation we analyzed the activity of glucuronokinase<sup>12</sup> as a representative enzyme of the sugar-1-kinase group and of USP in transgenic lines showing a downregulation of *USP* by expression of a miRNA targeted against the *USP* gene.

**Figure 2** shows the activity of USP in correlation with the amount of transcripts for the enzyme. It is evident that a close correlation exists between the amount of *USP* mRNA and USP activity. The 3 wild type samples are grouped and circled in **Figures 2 and 3**. The wild type cluster is strongly separated from the group of silenced plants. The same transgenic lines were used to measure the activity of glucuronokinase. In **Figure 3A** the activity of glucuronokinase is plotted against the activity of USP. Surprisingly, the miRNA lines with low USP activity have also a low glucuronokinase activity, indicating a link between both enzymes. A residual activity of glucuronokinase remains in plants which is independent of the USP activity. The transcripts for glucuronokinase are lower in silenced *USP* plants compared with wild type plants (**Fig. 3B**). This suggests that at least part of the reduction of glucuronokinase activity, observed in *USP* silenced plants, is due to lower transcription of the glucuronokinase gene. However most of the reduction of glucuronokinase activity is not explained by the transcript level suggesting a post-transcriptional modification of the enzyme.

How USP activity controls the amount of glucuronokinase is currently unknown. The direct inhibition of either enzyme by metabolites is rather unlikely in our measurements because all protein extracts were desalted on Sephadex to remove low molecular weight compounds including sugar-1-phosphates.

## References

1. Bar-Peled M, O'Neill MA. Plant nucleotide sugar formation, interconversion, and salvage by sugar recycling. *Annu Rev Plant Biol* 2011; 62:127-55; PMID:21370975; <http://dx.doi.org/10.1146/annurev-arplant-042110-103918>
2. Burget E-G, Verma R, Mølhøj M, Reiter W-D. The biosynthesis of L-arabinose in plants: molecular cloning and characterization of a Golgi-localized UDP-D-xylose 4-epimerase encoded by the MUR4 gene of *Arabidopsis*. *Plant Cell* 2003; 15:523-31; PMID:12566589; <http://dx.doi.org/10.1105/tpc.008425>
3. Reboul R, Geserick C, Pabst M, Frey B, Wittmann D, Lütz-Meindl U, et al. Down-regulation of UDP-glucuronic acid biosynthesis leads to swollen plant cell walls and severe developmental defects associated with changes in pectic polysaccharides. *J Biol Chem* 2011; 286:39982-92; PMID:21949134; <http://dx.doi.org/10.1074/jbc.M111.255695>
4. Zablackis E, York WS, Pauly M, Hantus S, Reiter WD, Chapple CC, et al. Substitution of L-fucose by L-galactose in cell walls of *Arabidopsis mur1*. *Science* 1996; 272:1808-10; PMID:8650583; <http://dx.doi.org/10.1126/science.272.5269.1808>
5. Alonso AP, Piasecki RJ, Wang Y, LaClair RW, Shachar-Hill Y. Quantifying the labeling and the levels of plant cell wall precursors using ion chromatography tandem mass spectrometry. *Plant Physiol* 2010; 153:915-24; PMID:20442274; <http://dx.doi.org/10.1104/pp.110.155713>
6. Kotake T, Yamaguchi D, Ohzono H, Hojo S, Kaneko S, Ishida HK, et al. UDP-sugar pyrophosphorylase with broad substrate specificity toward various monosaccharide 1-phosphates from pea sprouts. *J Biol Chem* 2004; 279:45728-36; PMID:15326166; <http://dx.doi.org/10.1074/jbc.M408716200>



**Figure 2.** Enzymatic activity of USP in *USP*-miRNA silenced plants. The activity of USP is widely correlated with the amount of transcripts for *USP*, which were silenced by the expression of a targeted miRNA against the *USP* gene. A miRNA 319 targeting *USP* was designed according to (<http://wmd3.weigelworld.org/cgi-bin/webapp.cgi>). The USP enzyme activity and transcript levels was measured according to<sup>11</sup>

The downregulation of the sugar-1-kinase is of physiological importance as otherwise the cells may deplete the phosphate pool to an undesired extent. Furthermore, sugar-1-phosphates may interfere with regular cellular pathways causing undesired side effects. Given that glucuronokinase and USP act sequentially to convert glucuronic acid into UDP-GlcA it would also make sense that both enzymes interact or stabilize each other to allow efficient substrate conversion. During evolution, the genes of some of the sugar-1-kinases (e.g. for fucose) have merged with appropriate pyrophosphorylases resulting in two-domain fusion proteins.<sup>13</sup>

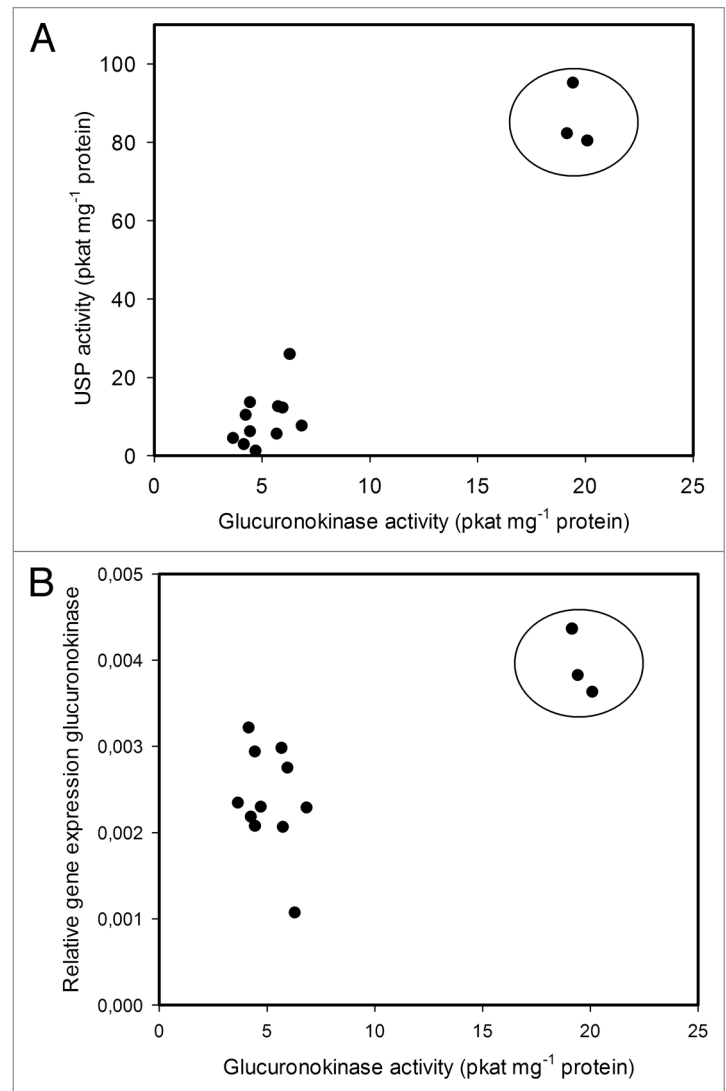
## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Acknowledgment

We are thankful to Anja Geretschläger for advice on the glucuronokinase enzyme and to Doris Wittmann for technical help during the analysis. This work was funded by a grant from the Austrian science foundation (FWF, P20297) to RT.

7. Litterer LA, Schnurr JA, Plaisance KL, Storey KK, Gronwald JW, Somers DA. Characterization and expression of Arabidopsis UDP-sugar pyrophosphorylase. *Plant Physiol Biochem* 2006; 44:171-80; PMID:16757173; <http://dx.doi.org/10.1016/j.plaphy.2006.04.004>
8. Kotake T, Hojo S, Yamaguchi D, Aohara T, Konishi T, Tsumuraya Y. Properties and physiological functions of UDP-sugar pyrophosphorylase in Arabidopsis. *Biosci Biotechnol Biochem* 2007; 71:761-71; PMID:17341835; <http://dx.doi.org/10.1271/bbb.60605>
9. Ohashi T, Cramer N, Ishimizu T, Hase S. Preparation of UDP-galacturonic acid using UDP-sugar pyrophosphorylase. *Anal Biochem* 2006; 352:182-7; PMID:16581011; <http://dx.doi.org/10.1016/j.ab.2006.02.026>
10. Schnurr JA, Storey KK, Jung HJ, Somers DA, Gronwald JW. UDP-sugar pyrophosphorylase is essential for pollen development in Arabidopsis. *Planta* 2006; 224:520-32; PMID:16557401; <http://dx.doi.org/10.1007/s00425-006-0240-1>
11. Geserick C, Tenhaken R. UDP-sugar pyrophosphorylase is essential for arabinose and xylose recycling, and is required during vegetative and reproductive growth in Arabidopsis. *Plant J* 2013; 74:239-47; PMID:23373795; <http://dx.doi.org/10.1111/tpj.12116>
12. Pieslinger AM, Hoepflinger MC, Tenhaken R. Cloning of Glucuronokinase from Arabidopsis thaliana, the last missing enzyme of the myo-inositol oxygenase pathway to nucleotide sugars. *J Biol Chem* 2010; 285:2902-10; PMID:19951951; <http://dx.doi.org/10.1074/jbc.M109.069369>
13. Kotake T, Hojo S, Tajima N, Matsuoka K, Koyama T, Tsumuraya Y. A bifunctional enzyme with L-fucokinase and GDP-L-fucose pyrophosphorylase activities salvages free L-fucose in Arabidopsis. *J Biol Chem* 2008; 283:8125-35; PMID:18199744; <http://dx.doi.org/10.1074/jbc.M710078200>
14. Pieslinger AM, Hoepflinger MC, Tenhaken R. Nonradioactive enzyme measurement by high-performance liquid chromatography of partially purified sugar-1-kinase (glucuronokinase) from pollen of *Lilium longiflorum*. *Anal Biochem* 2009; 388:254-9; PMID:19272347; <http://dx.doi.org/10.1016/j.ab.2009.03.002>



**Figure 3. (A)** Glucuronokinase activity plotted against USP activity. **(B)** Glucuronokinase activity plotted against mRNA for the gene. The glucuronokinase enzyme activity was measured according to <sup>14</sup>C transcripts for glucuronokinase were measured by SybrGreen qPCR using primers GACCATCTCC TGAATCGTTT G and ACCATCTTTG CCGAACCATA C and EF1 $\alpha$  as a reference gene.