Mad moves of the building blocks – nucleotide sugars find unexpected paths into cell walls

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A raft of recent studies, including a new paper by Zhao et al. (2018), has shed new light on the importance of cellular topology in controlling the flux of nucleotide sugars to their destinations. It is an exciting time, as the rapidly progressing elucidation of the convoluted flux of carbon bodies through nucleotide sugar metabolism and inter-organellar transport means we are approaching an understanding of the control principles of plant cell wall polysaccharide biosynthesis.

Plant science is approaching the vision of cell walls constructed ‘fit for purpose’ (Johnson et al., 2017). The assembly of carbohydrates from monosaccharides encompasses the stereospecific transfer of specific (di)phosphonucleotide-activated sugars onto specific acceptor molecules by glycosyltransferases estimated to be encoded by well over 500 Arabidopsis loci (Hansen et al., 2012). At least 16 nucleotide sugars are generated in the nucleotide sugar interconversion network and many of the enzymes involved have now been cloned and characterized (Reiter and Vanzin, 2001; Seifert, 2004; Reiter, 2008; Bar-Peled and O’Neill, 2011). More recently, Golgi-localized nucleotide sugar transporters have been identified (Temple et al., 2016). But how does nucleotide sugar metabolism and transport align with carbohydrate biosynthesis in the living plant?

Regulation of substrate flux

Most nucleotide sugars are made from UDP-D-glucose (UDP-Glc) in the cytosol (Box 1). This central carbohydrate precursor is converted to UDP-L-rhamnose, UDP-galactose and UDP-D-glucuronic acid (UDP-GlcA), the latter being converted to UDP-D-apiose and UDP-D-xylose (UDP-Xyl). Only the enzyme that generates UDP-D-galacturonic acid (GalA) from UDP-GlcA is exclusively Golgi localized. By contrast, two UDP-sugar interconverting enzymes exist in two forms localized in topologically separated compartments – one form in the cytosol and another one in the Golgi. The reason for this plant-specific duality is not immediately apparent.

The first example is the biosynthesis of UDP-Xyl, a precursor for xylans (York and O’Neill, 2008), xyloglucan, rhamnogalacturonan II (RG II) and xylogalacturonan (Caffall and Mohnen, 2009). UDP-Xyl synthase (UXS) catalyses the decarboxylation of UDP-GlcA to UDP-Xyl with the UXS3, -5 and -6 genes encoding cytosolic UXS and the UXS1, -2 and -4 loci encoding the Golgi-localized isofoms. Because UDP-Xyl is exclusively required inside the Golgi, the three cytosolic UXS isofoms appear redundant. More crucially, to give the cytosolic pool of UDP-Xyl access to the site of carbohydrate biosynthesis, a Golgi-localized UDP-Xyl transporter is needed. Indeed, three UDP-Xyl transporters (UXT1–3) were ingeniously identified using a biochemical assay and the uxt1 mutant showed a defect in glucuronoxylan (GX) structure and abundance. This phenotype suggested that polysaccharide biosynthesis somehow depended on cytosolic UDP-Xyl despite the presence of functional UXS in the Golgi. However, there was only a relatively minor reduction of total cell wall xylose in uxt1 and no carbohydrate other than GX was affected in the mutant. Moreover, the uxt2 uxt3 double mutant was phenomenally normal (Ebert et al., 2015).

This key paper by Ebert et al. (2015) opened important questions for understanding how substrate flux is regulated in planta. Does the GX-specific defect in uxt1 reflect substrate channelling from UXT1 to GX-specific xylosyltransferases? And vice versa, is the relatively subtle overall phenotype of uxt1 due to genetic redundancy in UDP-xylose transport or the action of Golgi-localized UXS? Zhao et al. (2018) address these questions, showing that in the uxt1 uxt2 uxt3 triple mutant, plant growth and secondary cell wall thickening are severely affected and, in addition to GAX, xyloglucan is also defective. Although not contradicting the substrate channelling hypothesis their paper conclusively demonstrates the crucial importance of the cytosol to Golgi transport of UDP-Xyl for multiple xylose-containing carbohydrates. This further corroborates previous triple-mutant studies comparing the two forms of UXS, which showed that cytosolic UXS, but not the Golgi-localized enzyme, affected the incorporation of xylose into cell wall polysaccharides (Kuang et al., 2016; Zhong et al., 2017).

Mechanistic basis of separation

What might be the mechanistic basis for this apparent separation of metabolite fluxes (Box 1)? A common feature

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of both UXS and UDP-Glc dehydrogenase (UGD), which generates UDP-GlcA, is that both enzymes are inhibited by UDP-Xyl (Harper and Bar-Peled, 2002; Pattathil et al., 2005; Klinghammer and Tenhaken, 2007). Therefore, developmental up-regulation of cytosolic UXS at the onset of secondary cell wall formation would increase cytosolic UDP-Xyl, thereby feedback-inhibiting UGD. The inhibition of UGD would down-regulate the biosynthesis of both UDP-GalA and, via Golgi-localized UDP-Xyl biosynthesis, UDP-L-arabinose (UDP-Ara), which are mainly needed during primary cell wall biosynthesis. But if xylose-containing carbohydrates receive UDP-Xyl from cytosolic UXS, what is the biological function of the Golgi form? Two genetic studies failed to establish a clear role for UXS1, -2 and -4 (Kuang et al., 2016; Zhong et al., 2017). However, as the loci are primarily expressed in tissues that undergo primary cell wall formation (Kuang et al., 2016), it would be interesting to further investigate the uxs1 uxs2 uxs4 carbohydrate-chemical phenotype in actively expanding tissues.

Indirect evidence for the importance of Golgi-localized UXS in providing the substrate for UXE and ultimately for arabinosyltransferases came from the finding that a transporter for UDP-GlcA, called UUAT1, was important for the arabinose content of cell walls (Saez-Aguayo et al., 2017). Once inside the Golgi, UDP-GlcA is either converted to UDP-GalA by UDP-glucuronic acid 4-epimerase (GAE) or to UDP-Xyl by UXS. From UDP-Xyl, UDP-Xyl 4-epimerase (UXE) generates UDP-Ara and, crucially, the uxe1 mutant showed a dramatic reduction in cell wall arabinose content (Burget and Reiter, 1999; Burget et al., 2003).

**Pentagonal arabinofuranose, hexagonal pyranose**

Another crucial aspect of UDP-Ara biosynthesis is that the pentagonal arabinofuranose (Araf) but not the hexagonal pyranose (Arap) form of L-arabinose is found in most cell wall
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