Biochemical and functional analysis of GONST3 and 4, nucleotide-sugar transporters of *Arabidopsis thaliana*.

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For the synthesis and/or modification of glycoproteins and polysaccharides in the lumen of the Golgi apparatus, specific transporter proteins are required for the import of nucleotide-sugars synthesised in the cytosol. We have identified GONST3 and 4 from *Arabidopsis thaliana*, which possess the molecular characteristics of nucleotide-sugar transporters (NSTs) and phylogenetic analysis suggests that GONST3 and GONST4 arose early in the evolution of NSTs. Our work is focussed on determining their substrate specificity and on analysing their role *in planta*. To achieve these aims, GONST3 and 4 were fused to epitope tags and using *Agrobacterium*, tobacco leaves were transiently transformed and GONST4-His and GONST4-GFP were localised to the Golgi apparatus.

A possible substrate of GONST3 and 4 is GDP-L-galactose which is unavailable commercially. Therefore, GDP-L-galactose was synthesised enzymatically from GDP-D-mannose for use in transport assays. After subcellular fractionation, GONST4-His and GONST4-GFP were capable of transporting GDP-L- galactose and GDP-L-fucose, but not GDP-D-mannose or UDP-D-glucose, into the lumen of this organelle. The specificity of GONST3 is currently being determined.

In addition, to determine their function *in vivo*, *GONST3* and *4* expression levels were reduced by post-transcriptional gene silencing. Using *Arabidopsis* lines transformed with promoter-GUS fusion constructs, both NSTs are highly expressed in floral organs and roots. Therefore, the morphology and composition of L-fucose- and L-galactose-containing glycoproteins and polysaccharides is being studied in depth in these organs. GONST4 is thus the first known NST capable of transporting GDP-L-galactose and the only known polysaccharide requiring this substrate is the pectin, rhamnogalacturonan II.

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