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Journal
Trends in Plant Science, 25(6)

ISSN
1360-1385

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Publication Date
2020-06-01

DOI
10.1016/j.tplants.2020.03.007

Peer reviewed

Article submission number: PLANTS-D-20-00012

Synthesis and function of complex sphingolipid glycosylation

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Keywords

GIPC, glycosylinositol phosphorylceramide, plasma membrane, phytoglycolipid

Abstract

Glycosylinositol phosphorylceramides (GIPCs) constitute an important class of plasma membrane lipids in plants. The complex glycan headgroup of GIPCs varies between plant species and tissues. Recent studies have shown that the structure of the glycan headgroup is important for development, abiotic stress tolerance, and interactions with pathogenic and symbiotic microorganisms.

Main text

Glycosylinositol phosphorylceramides (GIPCs) are the major class of sphingolipids in plants. They are thought to be found primarily in the outer leaflet of the plasma membrane, where they are estimated to make up more than half of the total lipid. They consist of a complex polar glycan headgroup, which protrudes into the apoplast, linked to a ceramide (Figure 1, 2). Sphingolipids were first identified in mammals in 1884 by Johann Thudichum, and it is claimed that he named them after the riddle of the Sphinx, due to their enigmatic nature. GIPCs were first identified in plants in the 1950s ("phytoglycolipids"). However, research on them essentially ceased in the 1970s, in part due to their insolubility in standard lipid-extraction protocols. New analytical techniques developed in 2006 allowed them to be "rediscovered" [1]. GIPCs are unique to plants, fungi and some protozoans. While animal sphingolipids also have a ceramide group, the polar headgroup is different from the glycosylinositol group in plants.

A recent review described important roles of GIPCs in programmed cell death and response to both abiotic and biotic stress in plants [2]. The review by Huby et al. focused on mutants affected in the biosynthesis of the ceramide part of GIPCs, which are generally severely impacted in their ability to synthesize GIPCs. However, several recent studies have shown that modification to the glycan headgroup of GIPCs can cause severe growth defects or lethality, depending on which sugars are lost, even if ceramides are synthesized and accumulate normally. Following synthesis of the ceramide in the ER, it moves to the Golgi where the inositol phosphate ceramide synthases (IPCSs) add the inositol phosphate group [3]. We and our collaborators then identified the proteins responsible for three enzymatic activities in the subsequent glycosylation pathway (and

showed their importance in arabidopsis (*Arabidopsis thaliana*], rice (*Oryza sativa*) and *Medicago truncatula*) [4–7]. We also identified three Golgi-localized transporters required for delivering substrates to the GIPC glycosyltransferases [8–10]. Knock-out mutations in the *IPUT1* gene encoding the glucuronosyltransferase that adds the first sugar in the glycan headgroup cause lethality, even though the precursor inositol phosphorylceramide accumulates in the plants [6]. Mutant pollen tubes grow at normal rates but are severely impacted in their ability to reach the ovule and cause fertilization [5]. However, rescue of the pollen function results in plants that are severely affected in vegetative development, showing that GIPC glycosylation is also important for other stages of development. While all plant GIPCs have glucuronic acid as the first sugar, the subsequent sugars vary between tissues and species [11]. In arabidopsis, mannose is the predominant second sugar residue, and plants deficient in adding mannose to glucuronic acid are strongly inhibited in growth [4,9,10]. The first mutants found to be deficient in mannosylated GIPCs were mutated in a gene encoding the Golgi-localized GDP-mannose transporter, GONST1 [9]. The only known function of this transporter and its homolog GONST2 [10] is to provide GDP-mannose for the GIPC mannosyltransferase GMT1. This transferase was identified in a mutant with a phenotype similar to *gonst1* (including a cell wall with reduced cellulose content, and a constitutive defense response), and was shown to belong to glycosyltransferase family GT64 [4]. Another member of this family, GINT1, was shown to add *N*-acetylglucosamine (GlcNAc) rather than mannose to glucuronic acid of GIPCs [7]. In rice the second sugar is always GlcN[Ac] and *Osgint1* mutants exhibit seedling lethality. Arabidopsis has GlcN[Ac] GIPCs as a minor form in seeds, but this form is not essential for seed development. In *Medicago truncatula* roots, the mannose form is

the major form present, while the GlcN[Ac] form is a minor component. However, the *GINT1* GlcNAc transferase gene is highly upregulated in root nodules and in roots infected with arbuscular mycorrhizal fungi. Downregulation of *GINT1* has no apparent impact on root growth in the absence of symbionts but strongly inhibits the development of functional root nodules and arbuscules (Moore, Mortimer and Scheller, in review). Notably, arabidopsis has a third member of the GT64 family with so far unknown function. It seems likely that it is involved in GIPC biosynthesis, perhaps in a specific molecular form that has not been clearly identified.

More recently, GIPC glycosylation has been identified as a receptor for a toxin from the plant pathogen *Phytophthora infestans* [12] and a salt sensor for the plasma membrane Ca^{2+} influx channel [13]. Many plant pathogens produce necrosis and ethylene-inducing peptide 1-like (NLP)-proteins, which can aid infection. NLPs from oomycetes were shown to bind to GIPCs (but not other plant lipids), as well as to oligosaccharides that correspond to GIPC headgroups, although with lower affinity than to the GIPCs [12]. Only eudicots are sensitive to NLPs, whereas monocots are resistant, with the exception of the monocots *Phalaenopsis amabilis*. Monocot GIPC pools tend to be dominated by headgroups with three sugar residues on the inositol phosphorylceramide (IPC) core, whereas dicots tend to have two-sugar headgroups. Interestingly, *P. amabilis* also has a mixture of 2- and 3-sugar GIPCs, implying that the number of sugar units could be an important determinant of pathogenicity. Testing of glycan headgroup mutants for NLP sensitivity could help to confirm this. In another recent study, a new *iput1* allele was identified in a forward genetic screen for arabidopsis mutants defective in salt-dependent calcium influxes, *moca1* [13]. *moca1* has a 4 amino acid deletion in IPUT1, but still retains

~10% of wild-type GIPCs, and it grows normally under standard conditions. Previously, the *gint1* mutant had been shown to be less sensitive to the effects of salinity on germination rates [7], supporting a role for GIPC glycosylation in salt sensing. Jiang et al. used isothermal titration calorimetry to demonstrate that GIPCs can bind Na^+, and then proposed a model in which this depolarizes the cell surface potential and gates the Ca^{2+} channel [13]. Multiple other processes that center around plasma membrane-spanning proteins are also disrupted in GIPC-glycan headgroup mutants, including cellulose synthesis [4] and signal transduction [4,5]. Taken together, these studies show that subtle changes to the molecular structure of the GIPC headgroup can have dramatic effects on function.

Future work will require better tools for GIPC analysis. Given the condition/cell-specific nature of the phenotypes described above, tools that support live cell imaging such as GIPC-specific dyes, and pharmacological inhibitors which can specifically inhibit synthesis, are particularly critical. Identification of a gene encoding a GIPC-specific phospholipase (such as that reported from white cabbage (*Brassica oleracea*) in [14]) would enable facile release of the glycan headgroup, and support detailed analysis of sugar identity and linkage. Finally, the discovery of additional glycosyltransferases responsible for the headgroup synthesis could be combined with synthetic biology tools, and potentially deliver crops with altered plant-microbe interactions, such as resistance to pathogens or enhanced arbuscular mycorrhizal symbiosis.

Acknowledgements

JCM and HVS are funded as part of the DOE Joint BioEnergy Institute (http://www.jbei.org) supported by the US Department of Energy, Office of Science, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the US Department of Energy.

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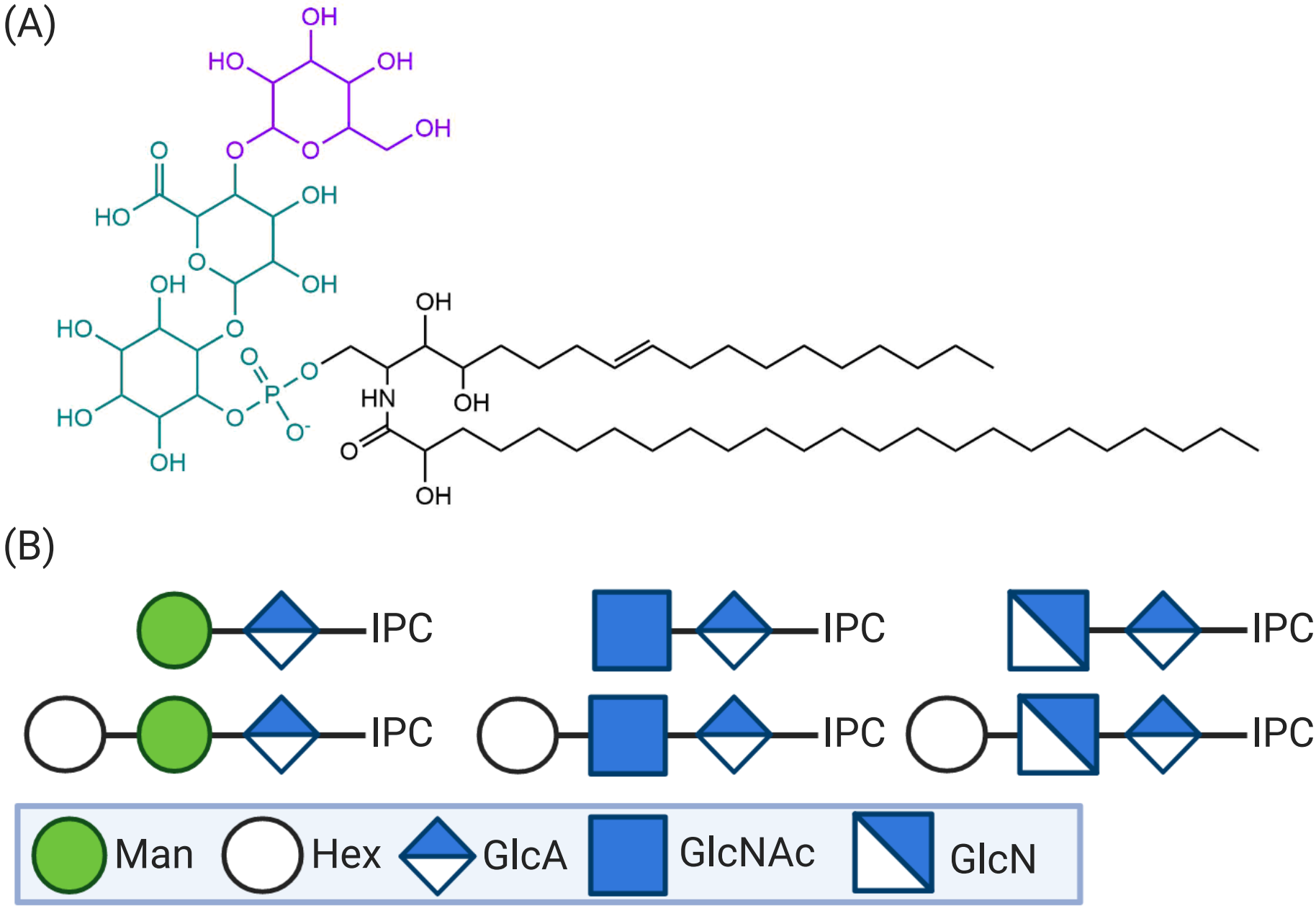
Figures legends

Figure 1: Structure of GIPCs. (A) Example structure of a GIPC showing the ceramide (black), inositol phosphate and glucuronic acid (GlcA) (green), and mannose (purple). The inositol phosphorylceramide (IPC) linked to a GlcA is conserved in higher plants. The additional sugars vary based on species and tissue type. (B) Common GIPC structures discussed in this review. The shown structures have only two or three sugar

residues, but GIPCs with longer glycan groups also exist. The Symbol Nomenclature for Glycans (SNFG) is used to represent the structures. This figure was created using BioRender (https://biorender.com/).

Figure 2: A schematic of our current view of the plant plasma membrane. The figure highlights the asymmetry between the inner and outer leaflet, and how the glycan headgroups can be tightly packed on the surface. Examples of classes of membrane proteins are shown (not to scale), the functions of which have been proposed to be affected by GIPC headgroup structure. The Cellulose Synthase Complex is shown with examples of proteins which affect cellulose synthesis, including GPI-anchored proteins (e.g. COBRA) and transmembrane proteins (e.g. KORRIGAN). This figure was created using BioRender (https://biorender.com/).



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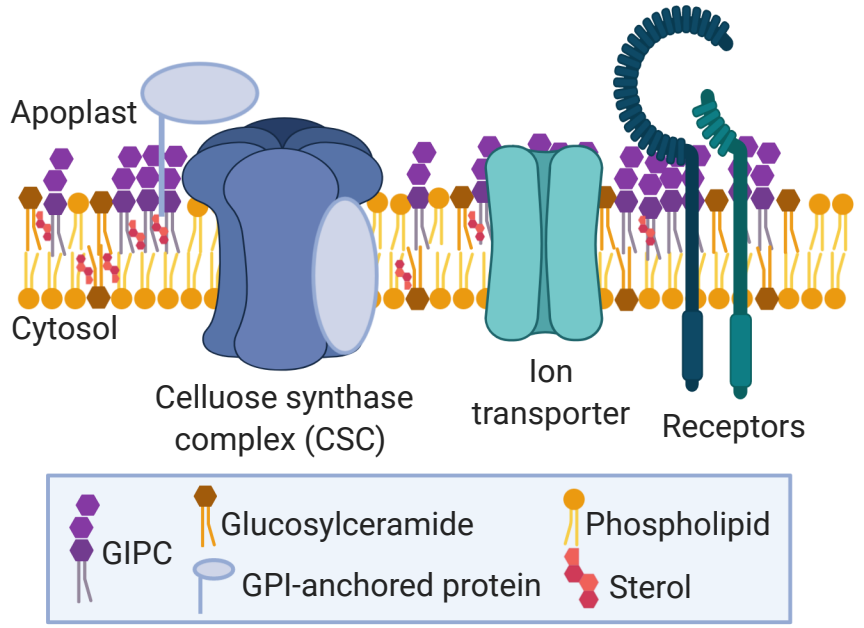

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