Review

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Intracellular and cell-to-apoplast compartmentation of carbohydrate metabolism

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In most plants, carbohydrates represent the major energy store as well as providing the building blocks for essential structural polymers. Although the major pathways for carbohydrate biosynthesis, degradation, and transport are well characterized, several key steps have only recently been discovered. In addition, several novel minor metabolic routes have been uncovered in the past few years. Here we review current studies of plant carbohydrate metabolism detailing the expanding compendium of functionally characterized transport proteins as well as our deeper comprehension of more minor and conditionally activated metabolic pathways. We additionally explore the pertinent questions that will allow us to enhance our understanding of the response of both major and minor carbohydrate fluxes to changing cellular circumstances.

Major pathways of carbohydrate metabolism

Although the major pathways of carbohydrate metabolism are long established in plants, our understanding of their compartmentation remains far from complete [1]. Indeed, the recent development of the first compartmented genome scale model for *Arabidopsis thaliana* [2] required that the authors included more transport processes than are described at the molecular level to date (for recent reviews see [3–5]). It is only very recently that key carbohydrate-related transporters have been identified, including the SWEET family transporters [6,7] and the plastidial glycolate glycerate transporter [8]. Although these transporters bear high fluxes, recent years have also led to a better understanding of the structure, biosynthesis, degradation, and transport of less abundant carbohydrates and their derivatives, such as heteroglycans and sugar alcohols. Here we review current knowledge of the pathways of: (i) export and/or import of carbon from the plastids; (ii) the impact of cytosolic heteroglycans; and (iii) novel insights on the stoichiometry of the cellular and/or apoplastic carbohydrate network concerning both apoplastic carbohydrate metabolism and the SWEET transporter family. We also describe how these networks are functionally regulated by developmental, organ-specific, and environmental cues that modulate the relative abundances of pool sizes of both metabolites that are well characterized at a functional level and those for which the physiological role is currently less clear. Finally, we provide our perspective on crucial approaches to further integrate our understanding of the importance of the subcellular compartmentation and functionality of carbohydrate molecules in land plants. For the purposes of the review we largely focus our discussion of photosynthetic metabolism on examples from *Arabidopsis* and that of heterotrophic metabolism on the potato (*Solanum tuberosum*) tuber.

Import and export of carbon to and from plastids

Plastids are the major sources and sinks of carbohydrates. In photosynthetically active chloroplasts, the Calvin–Benson cycle generates the basic carbohydrates for further metabolism (Figure 1). By contrast, amyloplasts are the dominant sinks for storage of carbohydrates in the form of storage starch. During light-dependent carbon fixation in the chloroplast, triose phosphates and finally fructose 6-phosphate are generated. Triose phosphate transporters are well documented to export photosynthate from the chloroplast into the cytosol, thereby driving sucrose formation in the light [9]. The fructose 6-phosphate is subsequently converted into glucose 6-phosphate by the plastidial phosphoglucoisomerase and further into glucose 1-phosphate by the action of the plastidial phosphoglucomutase. Glucose 1-phosphate is the starting point for starch synthesis via the ADP-glucose pyrophosphorylase pathway (Figure 1). The formation of this complex polymer requires the action of starch synthases and branching and debranching enzymes [10–12]. These transitory starch reserves are essential for the plant to overcome limitations of carbon and energy in the dark, when photosynthesis is nonfunctional, and must be managed prudently to optimize growth and reproductive success [13,14]. This includes metabolic adjustments in response to changes in the light regimen [15,16] and temperature [17].

In addition to this strict linear metabolic flux toward the production of starch, the glucose phosphates are also transported over the plastidial membranes (Figure 1). Thus, two isoforms of the glucose 6-phosphate/phosphate

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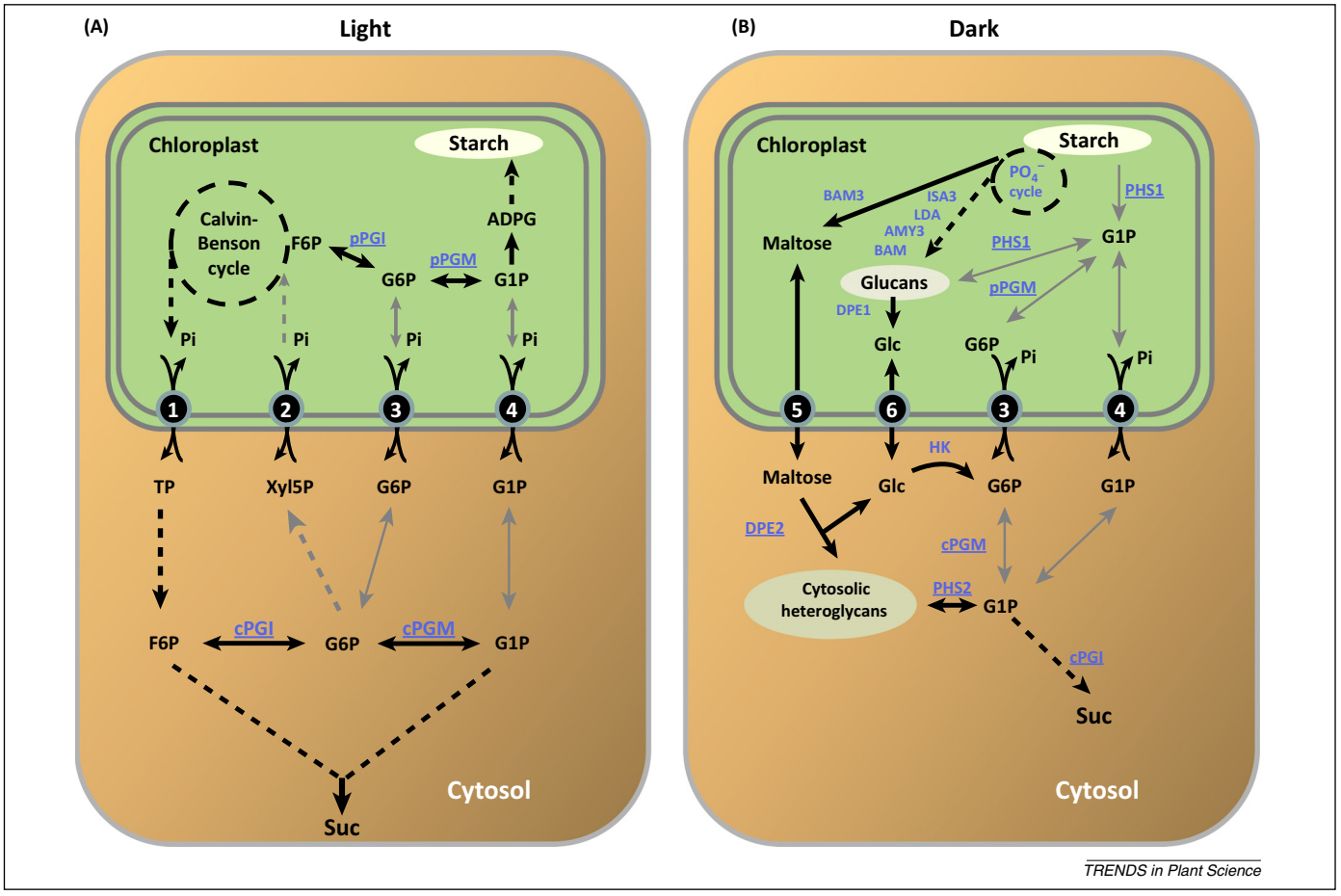

Figure 1. Hypothetical pathways of carbohydrate transport and starch metabolism in *Arabidopsis* leaves. (**A**) Light phase. The Calvin–Benson cycle is driving the carbon flux and the resulting sugar and sugar derivatives are either transported into the cytosol via the triose phosphate/phosphate transporter (1), the xylulose 5-phosphate/phosphate transporter (2), the glucose 6-phosphate/phosphate transporter (3), or an as-yet-unidentified glucose 1-phosphate transporter (4) or within the chloroplast directed toward starch synthesis. The alternative and/or minor pathways (grey lines) allow metabolic flexibility and provide partial compensation for missing reactions in the corresponding mutants. (**B**) Dark phase. Starch degradation is initiated at the granule surface by reversible phosphorylation of a few glucosyl residues within the amylopectin molecule (PO_4^- cycle). Phosphorylation favors the action of hydrolytic enzymes such as β-amylase (BAM) and debranching enzyme isoamylase 3 (ISA3). Together with α-amylase (AMY3) and limit dextrinase (LDA) (also known as pullulanase) the starch is metabolized to maltose and short glucans (maltodextrins). Maltodextrins are metabolized by plastidial disproportionating isozyme 1 (DPE1) resulting in release of glucose. The major starch degradation products, maltose and glucose, are transported via their corresponding transporters MEX1 (5) and GlcT (6). In the cytosol the maltose is further metabolized via DPE2. DPE2 transfers the non-reducing glucosyl residue of maltose to a non-reducing end of the cytosolic heteroglycan and releases the other glucosyl residue as free glucose. The glucose is activated by hexokinases (HKs) resulting in the formation of glucose 6-phosphate. The cytosolic heteroglycan-bound glucosyl residues are released by the action of the cytosolic phosphorylase (PHS2) in the form of glucose 1-phosphate. Glucose 1-phosphate and glucose 6-phosphate are further used for sucrose synthesis and various cellular metabolic processes. The glucose phosphate pools are connected via the cytosolic phosphoglucomutase (cPGM) and furthermore are also connected via their corresponding transporters (3 and 4) to the chloroplastidial pools. In the chloroplast, glucose 1-phosphate is released from the pool of soluble glucans (maltodextrins) or directly from starch. Similar to the situation in the cytosol, the two glucose phosphate pools are connected via the plastidial phosphoglucomutase (pPGM). Metabolic pathways including several enzymatic actions are depicted by broken lines. Abbreviations: cPGI, cytosolic phosphoglucoisomerase; pPGI, plastidial phosphoglucoisomerase.

translocator (GPT1 and GPT2) were characterized, belonging to the transporter subfamily of phosphate translocators (PTs), located in the plastidial inner envelope membrane. In *Arabidopsis* six functional members of this subfamily are described, namely two glucose 6-phosphate/PTs, a triose phosphate/PT, two phospho*enol*pyruvate/PTs, and a xylulose 5-phosphate/PT [18]. The proposed role of GPT is providing non-green plastids with glucose 6-phosphate for biosynthesis of storage starch and for the oxidative pentose phosphate pathway [19,20]. GPT1, rather than GPT2, meets that expected function, as knockout mutants reveal interrupted pollen and embryo sac development [21]. By contrast, GPT2 reveals a significant contribution to glucose 6-phosphate transport in chloroplasts of mutant plants impaired in transitory starch synthesis. Thus, mutation of the plastidial phosphoglucoisomerase results in a lack of glucose 6-phosphate generation, which can be overcome by the import of glucose 6-phosphate formed in the cytosol driven by the export of triose phosphates from the chloroplast [18]. Furthermore, GPT2 is reported to direct metabolic acclimation to variations in light intensities as well as to modulate seed development [22]. A similar flexibility is afforded to glucose 1-phosphate. As mentioned above, glucose 1-phosphate is essential for the formation of the glucosyl donor for starch synthase, ADP-glucose. In mutants lacking the plastidial phosphoglucomutase, normal transitory starch synthesis is interrupted [23]. Surprisingly, however, the mutant still contains tiny amounts of transitory starch that could not be explained by the transport of glucose 6-phosphate [24,25]. The necessary transporter is currently unknown, but it was clearly shown that direct import of glucose 1-phosphate into chloroplasts is possible and that the glucose 1-phosphate in the chloroplast is integrated in the ADP-glucose pathway toward starch, explaining the observed transitory starch content [24] (Figure 1A). Both alternative transport processes