Origin of sucrose metabolism in higher plants: when, how and why?

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Since the discovery of sucrose biosynthesis, considerable advances have been made in understanding its regulation and crucial role in the functional biology of plants. However, important aspects of this metabolism are still an enigma. Studies in cyanobacteria and the publication of the sequences of several complete genomes have recently significantly increased our knowledge of the structures of proteins involved in sucrose metabolism and given us new insights into their origin and further evolution.

In nature, there are few free disaccharides. Trehalose (α-D-glucopyranosyl-α-D-glucopyranoside) and sucrose (α-D-glucopyranosyl β-D-fructofuranoside) are both non-reducing sugars synthesized by similar pathways and are the most common naturally occurring disaccharides [1]. Trehalose is found in a wide range of organisms including bacteria, fungi, invertebrates and, exceptionally, in higher plants [1,2], whereas sucrose is mainly limited to oxygenic photosynthetic organisms, including unicellular algae and cyanobacteria, and is the key sugar in plant life. The function of sucrose in microorganisms has not been fully elucidated, although it is associated with environmental stress responses [3–7] and somehow fulfills similar storage and protection functions to those described for trehalose. However, it is generally accepted that, in higher plants, sucrose occupies a central position as the major product of photosynthesis and as a transport molecule in growth, development, storage, signal transduction and acclimation to environmental stress [1,8–10].

The presence of trehalose in all kingdoms led to the suggestion that it might be evolutionarily more ancient than sucrose [2]. Why, then, would sucrose, a novel disaccharide, have emerged during evolution? Although our knowledge has accumulated, the universal occurrence of sucrose in plant cells is still a mystery [8], and the question 'why sucrose?' [11] is still unanswered. The biochemical and molecular characterization of sucrose biosynthesis in prokaryotic organisms [12–19] contributed new insights into the origin and evolution of sucrose metabolism. In addition, the publication of sequences of several complete genomes has stimulated a range of new analyses of gene and protein evolution. Recent studies of the phylogenetic origin of sucrose-biosynthesis-related proteins (SBRPs), which include sucrose-phosphate synthase (SPS), sucrose synthase (SuS) and sucrose-phosphate phosphatase (SPP) [18], shed some light into 'the riddle of sucrose' but also raised new questions.

Sucrose pathway in extant organisms

In plants, triose phosphates produced in the chloroplast through the Calvin cycle are transported into the cytosol, where hexose phosphates and, subsequently, sucrose are synthesized. SBRP and invertases, the enzymes responsible for sucrose metabolism in higher plants (Box 1), and their encoding genes have been well characterized from various plant species [20,21]. Much less is known about sucrose metabolism in unicellular organisms. Studies on SBRPs from several species of Chlorophyta showed that they are similar to those of higher plants [3]. The biosynthesis of sucrose in prokaryotic organisms through the concomitant action of SPS and SPP was found in the cyanobacteria *Anabaena* sp. and *Synechocystis* sp. PCC 6803 [12,13,16,18,19]. Remarkably, SuS has been found in *Anabaena* sp. PCC7119 and seems to be restricted to filamentous nitrogen-fixing cyanobacteria [14,15]. Also, alkaline and neutral invertases were recently characterized in both unicellular and filamentous cyanobacteria (W.A. Vargas *et al.*, pers. commun.). Cyanobacterial enzymes display important biochemical and structural differences in comparison with the orthologous plant proteins. SPSs are not specific for UDP–glucose (Glc) (ADP–Glc, GDP–Glc and, to a minor extent, TDP–Glc could also be used as substrates) and both SPS and SPP are monomeric proteins with polypeptides of lower relative molecular mass than the respective plant subunits [12,13,16]. Recently, it has been shown that *Anabaena* SuS is involved in sucrose cleavage *in vivo* and in the carbon flux in the N_2-fixing filament [17]. By contrast, little is known about sucrose metabolism in non-cyanobacterial prokaryotes. In the model photosynthetic bacteria *Rhodospirillum rubrum* and *Rhodobacter capsulatus*, neither sucrose nor the presence of SRBP were found (G.L. Salerno, unpublished). The biosynthesis of sucrose has been attributed to SPS in two species of proteobacteria [7]. Additional studies are needed to understand the biochemistry and the role of sucrose in these bacteria.

Mechanisms for the control of sucrose metabolism have been extensively studied in higher plants [20]. Outstanding differences in regulatory properties were found for the cyanobacterial SBRPs compared with plant enzymes [12–14,16,18]. In particular, cyanobacterial SPSs are not modulated by allosteric effectors (Glc-6-phosphate and inorganic phosphate) and there is no evidence of reversible

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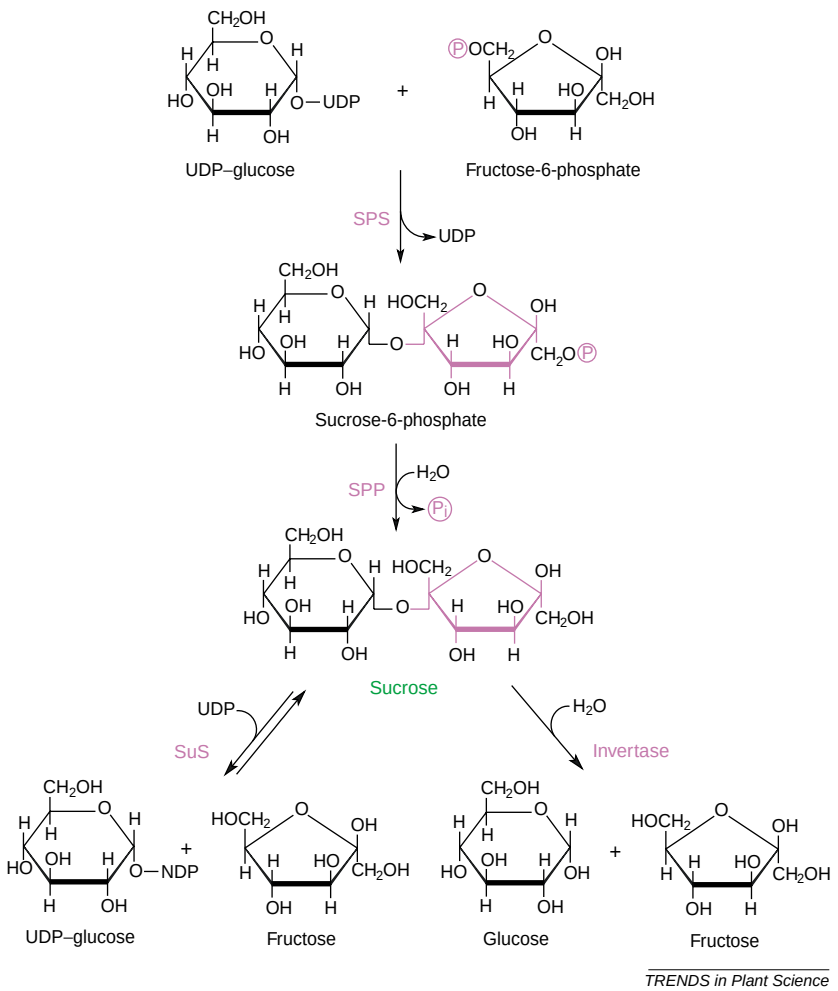

Fig. I.

Box 1. Sucrose metabolism in higher plants

The principal sucrose-biosynthesis route involves the sequential action of sucrose-phosphate synthase (SPS; UDP–glucose:D-fructose-6-phosphate 2-α-D-glucosyltransferase, EC 2.4.1.14) and sucrose-phosphate phosphatase (SPP; sucrose-6^F-phosphate-phosphohydrolase, EC 3.1.3.24) yielding free sucrose and inorganic phosphate (P_i) (Fig. I) [a]. The hydrolysis of the intermediate by SPP leads to an essentially irreversible pathway providing an efficient production of sucrose even at low substrate concentrations. Another enzyme, sucrose synthase (SuS; UDP–glucose:D-fructose 2-α-D-glucosyltransferase, EC 2.4.1.13), catalyzes a readily reversible reaction and could be involved in both the synthesis and cleavage of sucrose. However, SuS is usually assigned a role in sucrose cleavage under most physiological conditions in sucrose-using tissues, supplying sugar nucleotides, precursors in the formation of structural and storage polysaccharide [b]. By contrast, the hydrolysis of sucrose into hexoses is an irreversible reaction catalyzed by invertases (EC 3.2.1.26), which exist in several isoforms and play an important role when there is a demand for carbon and energy. In plant tissues, there are two classes of invertase activity, differentiated by their pH optima: neutral and alkaline invertases (pH optima between 6.5 and 8.0), which are localized in the cytosol, and acid invertase (pH optimum ~5.0), which are extracellular or vacuolar [b,c].

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phosphorylation of SPS or SuS, as described for the plant enzymes [20]. Thus, functional regulation of prokaryotic SBRPs and evolution of the complexity of their regulation must be addressed in future studies.

Structure of SBRPs

SBRPs in modern plants and cyanobacteria were proposed to be multiple-domain proteins with a modular architecture that might have arisen from primordial functional domains shuffled during evolution [18]. Interestingly, a comparable organization has been found in the proteins responsible for trehalose biosynthesis [2]. The characterization of *Anabaena* SPSs uncovered a 400 amino acid region shared by all SPSs and SuSs, allowing a functional glucosyl-transferase domain (GTD) to be defined (Fig. 1a). A general sequence

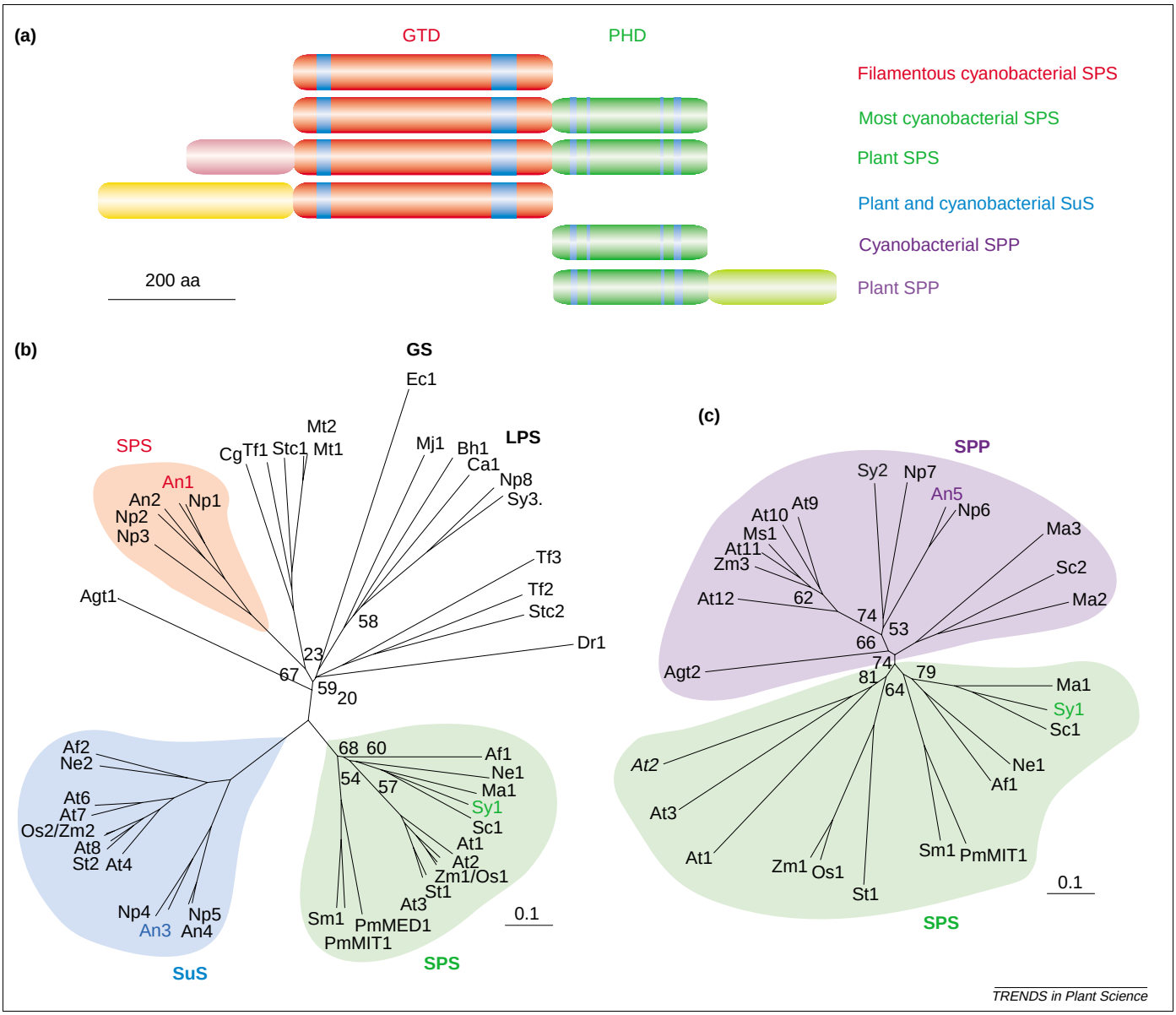

Fig. 1. Structure and phylogenetic analysis of sucrose-biosynthesis-related proteins (SBRPs). (a) The structure of SBRPs were deduced after BLASTp and CLUSTALX analysis. The glucosyl-transferase domain (GTD) is depicted as a red box, the phosphohydrolase domain (PHD) as a green box, the characteristic N-terminal extension of sucrose synthase (SuS) as a yellow box, the N-terminal extension of plant sucrose-phosphate synthase (SPS) as a pink box and the C-terminal extension of plant sucrose-phosphate phosphatase (SPP) as a light-green box. Blue boxes within the GTD and PHD indicate the positions of conserved signature motifs of SBRPs [18]. (b,c) Unrooted neighbor-joining phylograms were constructed after sequence alignment of the GTD (b) or PHD (c) using CLUSTALX with a BLOSSUM matrix and a bootstrap trial of 1000. The graphical representations of the trees were generated using TREEVIEW. Bootstrap results are not shown when values were higher than 85%. Similar tree topologies were observed by maximum parsimony and likelihood analysis (not shown). Sequences were obtained from the non-redundant protein databases of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) by BLAST searches. Open reading frames were scored as SBRP homologs for *E* values of $\leq 10^{-20}$ when compared with proteins of established biochemical function: *Anabaena* sp. PCC 7120 SPS-A, An1 (AJ302071); *Anabaena* sp. PCC 7120 SPP, An5 (AJ302073); *Anabaena* sp. PCC 7119 SuS-A, An3 (AJ010639); and *Syechocystis* sp. PCC 6803 SPS, Sy1(*srl*0045). The other sequences used are: Af1/2 (TIGR920/Contig:10034:a ferroxidans); Agt1 (NC003062); Agt2 (NP531360); An2 (AJ302072); An4 (AJ316584); At1 (AL049487); At2 (AL391222); At3 (AC004809); At4 (AB0016872); At5 (AB17068); At6 (AL353871); At7 (AF075597); At8 (AC012396); At9 (AL132972); At10 (AC0224261); At11 (AL132957); At12 (AC007017); Bh1 (NP242281); Ca (NC003030); Cg (NP599648); Dr (NP294949); Ec1 (NP417887); Ma1/2 (DOE156889/Contig437.revised.gene2 and 3.protein); Mj1 (NP127136); Me1 (AF283566); Mt1 (NP215000); Mt2 (NP334913); Ne1/2 (DOE915/Contig476.revised.gene194 and 195.protein); Np1 (AJ316587); Np2 (AJ316594); Np3 (AJ316588); Np4 (AJ316590); Np5 (AJ316590); Np6 (AJ316585); Np7 (AJ316586); Np8 (DOE63737/Contig603.revised.gene8.protein); Os1 (T04103); Os2 (X64770); PmMED1 (AJ316591); PmMIT1 (AJ316592); Sc1/2 (jmarq32049/Contig051302–307); Sm1 (AJ316594); St1 (Q43845); St2 (U24087); Stc1 (NP628379); Stc2 (NP630288); Sy2 (srl0953); Sy3 (NP440720); Tf1 (DOE2021/Contig61.revised.gene291.protein); Tf2 (DOE2021/Contig58.revised.gene69.protein); Tf3 (DOE2021/Contig63.revised.gene327.protein); Zm1 (P31927); Zm2 (X02400); Zm3 (AF283564). Abbreviations: Af, *Acidothiobacillus ferroxidans*; Agt, *Agrobacterium thumefaciens*; An, *Anabaena* sp. PCC 7120; At, *Arabidopsis thaliana*; Bh, *Bacillus halodurans*; Ca, *Clostridium acetibutylicum*; Cg, *Corynebacterium glutamicum*; Ma, *Magnetococcus* sp. MC1; Me, *Medicago truncatula*; Mj, *Methanococcus jannaschii*; Mt, *Mycobacterium tuberculosis*; Ne, *Nitrosomonas europaea*; Np, *Nostoc punctiforme*; Os, *Oryza sativa*; PmMED, *Prochlorococcus marinus* MED4; PmMIT, *P. marinus* MIT9313; Sc, *Synechococcus* sp. PCC 7002; Sm, *Synechococcus marinus* WH8102; St, *Solanum tuberosum*; Stc, *Streptomyces coelicolor*; Sy, *Synechocystis* sp. PCC 6803; Tf, *Thermobifida fusca*; Zm, *Zea mays*; aa, amino acids; GS, glycogen synthase; LPS, lipopolysaccharide glycosyltransferase. Sequence clusters that include biochemically characterized proteins [An-SPS (An1), red; Sy-SPS (Sy1), green; An-SuS (An3), blue; An-SPP (An5), purple] are shaded.

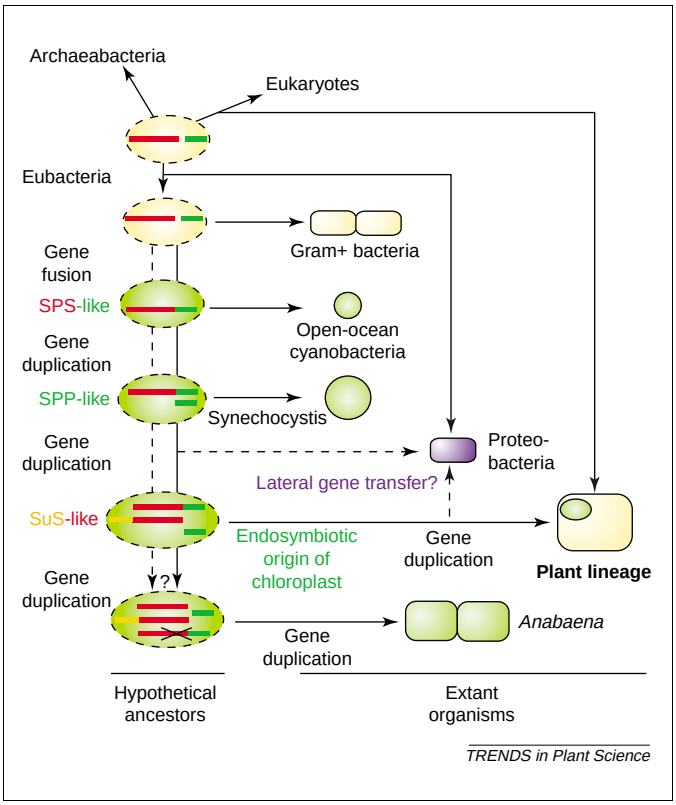

Fig. 2. Hypothetical evolutionary pathway from a two-domain common ancestral sucrose-phosphate synthase (SPS)-like gene to modern cyanobacterial and plant sucrose-biosynthesis-related proteins (SBRPs). The phylogenetic relationships among species are depicted according to rRNA sequence analysis [35]. A gene fusion of a glucosyl-transferase domain (GTD)-like and a phosphohydrolase domain (PHD)-like primordial domains (common to organisms from all kingdoms) might have given rise to an hypothetical common-ancestral SPS gene. Duplications of the PHD and GTD during cyanobacterial diversification might have produced sucrose-phosphate phosphatase (SPP) and sucrose synthase (SuS) genes, respectively. Plant sucrose metabolism has been acquired during the endosymbiotic origin of the chloroplast at the time of the cyanobacterial phylogenetic radiation. Independent gene duplications seemed to have been responsible for the expansion of the SBRP family in plants and filamentous heterocyst-forming cyanobacteria. A few proteobacteria are likely to have acquired SBRPs laterally from cyanobacteria and/or plants. SPSs of filamentous heterocyst-forming cyanobacteria might have arisen twice during cyanobacterial evolution, but their phylogenetic origin is still unclear.

signature [(DE)xGGQxxY(VIL)x(DE)$x_{300-430}$ExFGxxx ExxxxxxPxxA(TS)xGG] has been suggested for SPS and SuS proteins, where x represents any amino acid and residues in parentheses are alternatives at that position [18]. Similarly, the characterization of *Anabaena* SPP [16] defined a phosphohydrolase domain (PHD) of SBRPs sharing conserved residues with other phosphohydrolases [DxDxTx_{27}Tx_{119}Kx_{24}DxxxD] [16,18,19]. Three different domain arrangements have been described for SPSs [18]: (1) the minimal SPS unit (GTD), such as *Anabaena* SPSs; (2) the two-domain SPS prototype (GTD–PHD), such as *Synechocystis* SPS; and (3) plant SPSs (N-terminal-regulatory-extension–GTD–PHD) (Fig. 1a). Other particular structural features of SBRP include the N-terminal extension at the GTD of all SuSs, the N-terminal extension at the GTD of plant SPSs and the C-terminal extension at the PHD of plant SPPs. All these SBRP extensions, which have no obvious relationship with other known protein domains by sequence analysis, have been suggested to represent quaternary structure determinants because cyanobacterial and plant SuSs, and plant SPSs and SPPs are oligomeric proteins [12,14,16]. No monomeric SuS has yet been described, so identifying such a protein in extant organisms would be an interesting challenge.

Evolution of sucrose metabolism

Phylogenetic analysis of both the GTD and the PHD pointed towards an ancient origin of plant sucrose metabolism, before the cyanobacterial phylogenetic radiation (2 billion to 3.5 billion years ago) [18]. A gene fusion of a GTD and a PHD-like primordial domain was proposed to have produced a two-domain common ancestral SPS-like gene. This hypothesis was strongly supported by the presence of *Synechocystis* SPS-homologous sequences in the ancient cluster of open-ocean cyanobacteria, phylogenetically located at the base of the cyanobacterial radiation (Fig. 1b,c). It has been suggested that duplications of the PHD in a two-domain SPS-like gene might have evolved into SPP. The splitting of SPS and SPP into two different polypeptides might have provided a new level of regulation that allowed protein–protein interactions and the channeling of the intermediate product (sucrose-6-phosphate), as has been proposed for plant SPS and SPP. Based on the presence of SuS genes in the most-recently radiated cyanobacterial species, it has been also proposed that a more-recent gene duplication of the GTD in an SPS-like gene might have given rise to SuS [18]. In another recent report, it has been suggested that SPS might have originated from a joining of SuS and SPP [22]. Because the second of these hypotheses would entail parallel loss of SuS genes in most cyanobacterial lineages, it seems less probable than the model presented in Fig. 2. The assumption that plant sucrose metabolism was acquired during the endosymbiotic origin of the chloroplast at the time of the cyanobacterial phylogenetic radiation [18] is strongly supported by the fact that plant SBRPs share a common branch with their corresponding cyanobacterial homologs in a protein phylogeny (Fig. 1b,c).

Based on the presence of some homologs to genes for SBRPs in three proteobacteria (*Acidithiobacillus ferrooxidans*, *Magnetococcus* sp. MC1 and *Nitrosomonas europaea*), it has recently been suggested that sucrose synthesis originated in this lineage or in a common ancestor of proteobacteria and cyanobacteria [19]. However, this interpretation seems to be unlikely given (1) a phylogenetic analysis that shows that proteobacterial SPS and SPP branch close to the *Synechocystis* proteins within, and not before, the cyanobacterial phylogenetic radiation (Fig. 1b,c). (2) Genes for SBRPs are present in every cyanobacterial genome sequenced to date (and there is also evidence of sucrose biosynthesis in many other cyanobacteria [4–6,15]; G.L. Salerno, unpublished), in contrast to proteobacterial SBRP-gene homologs, which could only be retrieved from three out of 72 sequenced genomes (at the time of writing). (3) Proteobacterial SuS homologs are more closely related to the plant proteins than to the cyanobacterial SuSs. Accordingly, proteobacteria are likely to have acquired genes for SBRPs from cyanobacteria and plants through lateral gene transfer (LGT). It has been pointed out that LGT between prokaryotes after the origins of organelles could, together with parallel gene

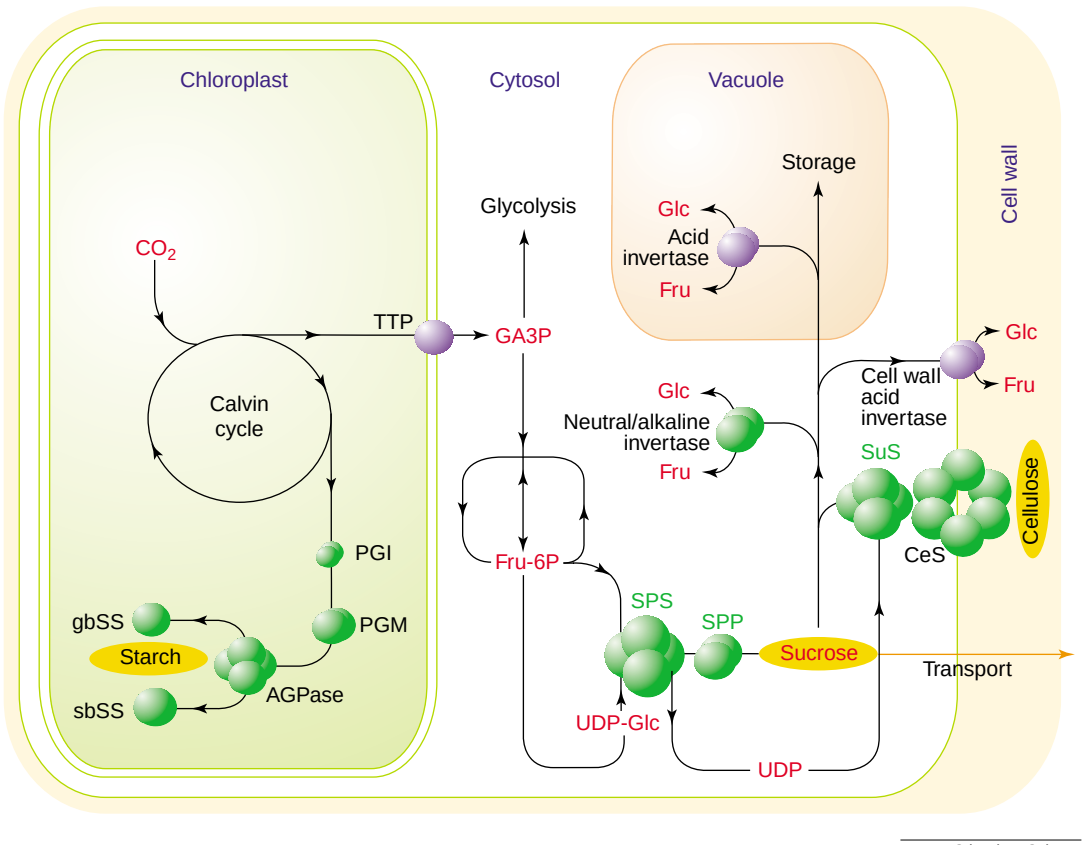

Fig. 3. Subcellular compartmentalization of endosymbiotically acquired carbohydrate-metabolism gene products in higher plants. Proteins derived from cyanobacteria are shown in green. Phylogenetic relationships were taken from the literature [18,28,36,37] (W.A. Vargas *et al.*, pers. commun.). During the endosymbiotic origin of plant chloroplasts, most of the cyanobacterial genes were transferred to the nucleus but their products have different fates. Those proteins involved in cyanobacterium-specific functions (such as those of the Calvin cycle) are preferentially reimported to the organelle, where they do not interfere with the host cytoplasmic metabolism, whereas others replaced host proteins in the cytosol because of functional redundancy by selection or merely chance [28]. Interestingly, the endosymbiotically inherited sucrose-biosynthesis-related proteins gave rise to a novel and successful cytoplasmic pathway in the plant lineage. Chloroplasts have not retained the polysaccharide-rich cell wall of cyanobacteria. Plant cell-wall polysaccharide genes (such as SuS and CeS) might have also originated from cyanobacteria during the endosymbiosis event. Abbreviations: ADPase, ADP–glucose pyrophosphorylase; CeS, cellulose synthase; gbSS, granule-bound starch synthase; PGI, phosphoglucoisomerase; PGM, phosphoglucomutase; sbSS, soluble starch synthase. The number of balls corresponds to the quaternary structure of the native enzyme. Ball size does not reflect relative molecular mass.

losses, lead to erroneous inferences of gene origin [23–25]. Further genome sequencing of both proteobacteria and cyanobacteria would help to clarify this issue.

The proposed model (Fig. 2) indicates that SBRP evolution might have been more intricate than previously suggested [18,19]. Thus, it is not clear how *Anabaena* SPSs have arisen. How can the particular similarity of these proteins with a family of known and putative glycosyl transferases (including glycogen synthase, lipopolysaccharide glycosyl transferase and galactosyl and mannosyl transferase) present in both eubacteria and archaea (Fig. 1b) be explained? Should it be ascribed to LTG or to an independent evolution of SPSs (i.e. a polyphyletic origin)? A three-dimensional model of GTD should help to elucidate the underlying phylogenetic relationships.

In spite of the claimed biochemical similarities between sucrose and trehalose metabolisms [2], sequence and structural analyses grouped SPS and trehalose-phosphate synthase in different glycosyl-transferase families [26], indicating that the two metabolic pathways are not close phylogenetic relatives. Neither SBRP is phylogenetically related to sucrose phosphorylases present in other bacteria (such as *Pseudomonas saccharophila*).

New cytoplasmic pathway in plant cells

According to the generally accepted cyanobacterial endosymbiotic origin of plant chloroplasts, most of the cyanobacterial genes were transferred to the nucleus [23] but their products were preferentially reimported to the organelle [27], where they do not interfere with the host's cytoplasmic metabolism [28]. Genes for SBRPs seem to be lost efficiently from the chloroplasts because no homolog has been identified in sequenced plastid genomes. However, chloroplasts did not retain the sucrose biochemistry of their free-living ancestor, in contrast to the proteins related to starch metabolism, suggesting that chloroplasts (unlike free-living cyanobacteria) cannot metabolize sucrose. It has recently been shown that sucrose could enter chloroplasts efficiently [29], but whether this transport entails a physiological role for sucrose inside the organelle is still unknown.

Endosymbiotically inherited genes for SBRPs have given rise to a novel and successful cytosolic pathway in

the plant lineage (Fig. 3). The intricate cross-talk between sucrose and most metabolic pathways in contemporary plants might have been adjusted early after the endosymbiotic origin of chloroplasts. The appearance of sucrose synthesis in the primitive plant cytosol from central metabolic intermediates (fructose-6-phosphate and a sugar–nucleotide) might have contributed to a rapid evolution and diversification of carbohydrate metabolism genes. Might this ancient interaction between sucrose and other metabolic pathways have been the basis of the contemporary role of sucrose in the regulation and coordination of key plant functions? It would be of great interest to find prokaryotic models, probably less complex, of sucrose signal transduction pathway to provide insights into the plant pathways.

Why sucrose?

In the proposed phylogenetic model (Fig. 2), trehalose and sucrose metabolism might have faced each other several times during evolution: (1) during the cyanobacterial phylogenetic radiation, resulting in the loss of the trehalose pathway in some cyanobacteria; (2) after the endosymbiotic origin of chloroplast in the plant lineage; and (3) as a consequence of LGT.

What might have been the selective advantages of sucrose that led to such an evolutionary choice in the cyanobacterial lineage? Might the evolution of photosynthesis towards an oxygenic process have been related to the emergence of sucrose for enhanced carbon-fixation efficiency and the biosynthesis of novel polysaccharides? No doubt the properties of the sucrose molecule could have been crucial, even though several other factors might also have contributed to the appearance and success of this disaccharide. It is well established that the β-D-fructofuranoside nature of sucrose is something of rarity and an obvious difference from trehalose. In addition to its higher free energy of hydrolysis than trehalose, conversion of the liberated D-fructose from the furanose form to an equilibrium mixture of pyranose and furanose [30] provides extra free energy. Thus, sucrose (and not trehalose) could act as a donor of glucosyl or fructosyl residues and can be effective as a precursor molecule for the synthesis of polysaccharides and sugar nucleotides. This might have been the driving force for the evolution of an ancestral SBRP towards SuS, an enzyme that can directly link sucrose metabolism with biosynthetic processes [31]. Moreover, no freely reversible reaction similar to that catalyzed by SuS is known in trehalose metabolism.

Sucrose seems not to be essential for cyanobacterial survival, because mutants with a disrupted SPS could be isolated from *Synechocystis* [13]. By contrast, given the central role of sucrose in plants, once compared to that of glucose in the animal world [8], a plant without sucrose is not conceivable. The lack of plant mutants that do not synthesize sucrose could support the essential role of sucrose for plant viability.

Most plants are not considered to accumulate trehalose [32]. The identification of a plethora of trehalose-related biosynthesis genes in *Arabidopsis* remains an enigma in our understanding of the role of trehalose in plants [32,33]. The relative abundance of trehalose over sucrose metabolism genes in *Arabidopsis* markedly contrasts their relative abundance of transcripts in expressed-sequence-tag databases. Is there any bias towards the silencing of trehalose-related biosynthesis genes in the plant lineage? Might trehalose metabolism in higher plants be evolving to provide different functions than those of a stress and storage molecule?

What might have been the selective advantage of acquiring SBRPs by LTG? It is worth considering the case of *Escherichia coli*. This organism lacks SBRP homologs, as do most proteobacteria, but its survival under adverse environmental conditions is enhanced ~10 000-fold upon transformation with *Synechocystis* SPS [34]. Could the acquisition of SBRP be just a matter of finding the appropriate endosymbiont or gene donor under an environmental constraint?

Final remarks

Even if portions of the hypothesis and model described above are somewhat speculative, the suggested answers to 'when, how and why sucrose' are intended to provide a framework for future studies. With the availability of the *Arabidopsis* genome, it will become possible to characterize all genes for SBRPs functionally by the analysis of knockout or gain-of-function mutants. Also, it would be interesting to address biochemical and molecular studies in less-complex organisms (e.g. open-ocean cyanobacteria, proteobacteria and lower plants) to get new perspectives on plant sucrose metabolism. Another important approach will be the structural analysis of enzymes. The crystal structure of SBRPs will offer mechanistic insights into their catalytic and regulatory properties, and into their phylogenetic relationships. An important contribution to an integrated view of sucrose metabolism will be a simultaneous study of sucrose transporters that are responsible for sucrose uptake and distribution over cellular compartments, in conjunction with sucrose sensing and signaling.

We hope that the experimental lines of research proposed above will help us to gain not only relevant and fundamental knowledge about plant biology, but also powerful tools to use in crop engineering in the near future.

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