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Review

The Cinderella story of sucrose hydrolysis: Alkaline/neutral invertases, from cyanobacteria to unforeseen roles in plant cytosol and organelles

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ABSTRACT

Over the past decades, considerable advances have been made in understanding the crucial role of sucrose and the regulation of its metabolism in plant life. Recent studies in cyanobacteria and the analysis of several genomic sequences point towards an ancient origin of plant sucrose metabolism before the cyanobacterial phylogenetic radiation. In agreement with the generally accepted cyanobacterial endosymbiotic origin of plant chloroplasts, most of the cyanobacterial genes were transferred to the nucleus and their protein products were preferentially re-imported to the plant organelle. In the case of sucrose metabolism, the enzymes sucrose-phosphate synthase (SPS) and sucrose-phosphate phosphatase (SPP), responsible of the disaccharide synthesis, and sucrose synthase (SuS) and alkaline/neutral invertases (A/N-Inv), involved in sucrose cleavage, appear to have a cyanobacterial origin. However, whereas SPS and SPP are likely to be exclusively localized in the cytosol of modern plant cells, SuS and A/N-Inv isoforms are distributed between the cytosol and different subcellular locations. Particularly, A/N-Invs are the least studied proteins of sucrose catabolism. They were somewhat underestimated, and thought to play no relevant role in carbon metabolism. However, some striking recent findings about the presence of A/N-Inv forms inside plant organelles, as well as the description of novel physiological functions, led us to re-evaluate the importance of these Cinderella enzymes. The additional roles uncovered for A/N-Invs disclose new scenarios for the interconnection between the cytosol and organelles and for complex crosstalk signalling pathways.

Contents

1. Introduction

1.1. Proteins involved in plant sucrose metabolism

Sucrose (α-D-glucopyranosyl β-D-fructofuranoside), one of the most abundant products in nature, is not only the main

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photosynthesis-derived compound and the predominant molecule of carbon translocation in most plants, but also plays a central role in their functional biology and in responses to environmental stresses [1]. Moreover, it has become evident that sucrose and its hydrolysis products are important metabolic signals that modulate gene expression and regulate plant development [2–5].

Sucrose metabolism has been widely studied and considerable advances have been made in the understanding of its regulation in plants [6,7]. The pathway for sucrose biosynthesis involves the sequential action of sucrose-phosphate synthase (SPS, EC 2.4.1.14) and sucrose-phosphate phosphatase (SPP, EC 3.1.3.24) yielding free sucrose and inorganic phosphate (Pi) [8]. The hydrolysis of the intermediate, sucrose-6P, leads to an essentially irreversible synthesis pathway that provides an efficient production of sucrose, even at low concentrations of substrates.

The regulation of sucrose hydrolysis has become a central issue in plant carbon metabolism. Utilization of sucrose as a source of carbon and energy depends on the breakdown of the α1–β2-glycosidic bond, either by the action of invertases that irreversibly hydrolyse the disaccharide to glucose and fructose, or sucrose synthase (SuS, EC 2.4.1.13), a glucosyltransferase that catalyzes a readily reversible reaction [6,7]. However, SuS is usually assigned a role in sucrose cleavage under most physiological conditions. In the presence of a nucleoside diphosphate (preferentially UDP, in plants) SuS leads to the production of sugar nucleotides, which can act as glucose donor in the synthesis of cellulose and callose in most plant species [6,9–11]. To complete the picture of sucrose metabolism enzymes in the plant cell, there are two classes of invertase activity, initially differentiated by their optimum pHs *in vitro*: (i) acid invertases (Ac-Invs, EC 3.2.1.26, β-fructofuranosidases) with a characteristic optimum pH between 4.5 and 5.0 and (ii) alkaline/neutral invertases (A/N-Invs) with pH optima in the range of 6.5–8.0 [12,13].

1.2. Location of sucrose metabolizing enzymes in the plant cell

The enzymes of sucrose metabolism, with the exception of Ac-Invs, were thought to be cytosolic [6,12–14]. The different localizations exhibited by Ac-Invs granted these enzymes important physiological roles and prompted an extensive and detailed study in several plant species. It is well-accepted that plant Ac-Invs are glycoproteins evolutionarily related to invertases from yeast and bacteria, and responsible for the hydrolysis of sucrose in the intercellular space (or the cell wall), and also inside vacuoles [15]. Thus, these locations reflect their involvement in various aspects of the plant life cycle, as controlling sucrose allocation and plant development, in the response of plants to environmental stimuli, cell enlargement, responses to wounding and pathogen attack [16–18].

SPS, SuS and A/N-Invs were thought to be cytosolic proteins [19]. However, recent evidence indicates that only the sucrose biosynthesis pathway via SPS and SPP is exclusively located in the cytosol of the plant cell, since sucrose catabolism by SuS and A/N-Inv isoforms may also occur in other subcellular locations. In the present article we will focus our attention mainly on the novel localizations of A/N-Invs.

The central role of SuS enzymes in controlling the mobilization of sucrose into many important metabolic pathways prompted the investigation of the different isoforms present in a plant species [20–24]. Although SuS activity was initially described in the soluble protein fraction, more recent reports demonstrated that after phosphorylation, a significant pool of the protein is membrane-bound in association with the cellulose/callose synthase complex, supplying substrates for cell-wall biosynthesis [9,25,26]. SuS is also associated with the tonoplast in *Beta vulgaris* cells, corroborating its involvement in sucrose mobilization from the vacuole in sucrose-storing organs [27]. In sycamore (*Acer pseudoplatanus*) cells, SuS was demonstrated to be simultaneously targeted to plasmalemma and tonoplast membranes [28]. Interestingly, there was a lack of direct relationship between membrane type location and degree of phosphorylation, but the data supported the relevance of phosphorylation to SuS activity [28]. In contrast, in maize plants, the phosphorylation state of SuS does play a crucial role in anchoring the protein to the cell membranes. Two phosphorylated forms of the enzyme (SUS1 and SUS-SH1) are associated with membranes, whereas a non-phosphorylated form (SUS2) localized to the cytosol [29]. Association of SuS with the Golgi fraction has also been described in maize [30] and linked to the synthesis of cellulose polymers. Similarly, in tobacco pollen tubes, SuS isoforms occur in the soluble, plasma membrane and Golgi fractions, as well as a SuS-like protein seems to be in association with the cell wall [31]. In pollen tubes, a phosphorylated SuS form is more abundant in the cytoplasm and associated with the cell wall, and a non-phosphorylated protein is specific to the plasma membrane [31]. The three maize SuS protein sequences revealed that the SUS-SH1 protein is marked by a putative mitochondrial targeting signal at its N-terminus [32], and the SUS1 and SUS-SH1 isoforms are partly localized in mitochondria and nuclei [32,33]. *Arabidopsis thaliana* has a six-member SuS gene family, whose protein products are closely related to each other in both sequence and general kinetic properties. These genes have different spatial and temporal patterns of expression [34]. The *Arabidopsis* SUS2 seed isoform seems to be mainly localized in the plastids of the embryo [35]. Remarkably, despite the accepted importance of SuS isoforms, it was recently reported that none of the six isoforms in *Arabidopsis* is individually required for normal growth and reproduction [36]. In addition, data from a comprehensive loss-of-function study revealed that SuS isoforms are not required for cellulose synthesis in *Arabidopsis*, and that SUS6, and probably SUS5 are confined to phloem sieve elements, and are involved in callose synthesis in sieve plates [36]. These led to new questions about the precise role of sucrose catabolism by different SuS isoforms in plants.

On the other hand, where are A/N-Invs localized? These elusive enzymes have been barely studied in the past because of their low and unstable activity. They were described as cytosolic proteins, and somewhat underestimated, and thought to play no relevant role in carbon metabolism and plant development [16,37–43]. Since A/N-Invs are the least studied proteins of sucrose catabolism, their physiological function remained largely unknown. Some striking recent findings about the presence of functional A/N-Inv forms inside plant organelles, as well as the description of novel physiological roles, necessitate a re-evaluation of the importance of these enzymes and have revived interest in their investigation. The aim of the present review is to discuss the recently reported findings on A/N-Invs that suggest these Cinderella enzymes have novel cytosol-organelle metabolic connections and previously unforeseen roles in plant development.

2. Alkaline/neutral invertases: little known enzymes with surprising locations and roles

A/N-Invs are a group of intriguing enzymes found in oxygenic photosynthetic organisms [13,44]. Compared with the other sucrose metabolism enzymes, A/N-Invs have been "kept in the cinders", hardly taken into account in biochemical, physiological and molecular studies. Because of their generally low and labile activity, biochemical studies and purification to homogeneity of these enzymes resulted rather difficult compared with other sucrose metabolism proteins. In contrast to Ac-Invs, A/N-Invs are not glycosylated and do not belong to the β-fructofuranosidase family since they hydrolyze sucrose but not other β-fructose-containing sugars [13,41,44,45]. A/N-Inv activity was shown to be

strongly inhibited by the hydrolysis products and not affected by heavy metals, suggesting a markedly different catalytic site to that of Ac-Invs [13,37,38,40,42–44].

2.1. A/N-Inv isoforms

Formerly, A/N-Invs were classified according to their optimum pH, either close to 6.5–7 or to 7.8–8.0, and named neutral-Invs (N-Invs) or alkaline-Invs (A-Invs), respectively. Representatives of both isoforms have been purified from several plant species and from *Anabaena* (*Nostoc*) sp. PCC 7120, a filamentous nitrogen-fixing cyanobacterium, and have been biochemically characterized [37–40,42,44–46]. Since a single amino acid substitution has in Ac-Invs strong consequences on the optimum pH of the enzyme [47], it was suggested that the A/N-Inv optimum pHs may also be a property associated to the amino acids involved in the catalytic site, related to their physiological role and/or location [46]. Only the coding sequences of functionally characterized A-Inv isoforms have a conserved motif (DGE/DG), e.g. in *Anabaena* An-InvA, *A. thaliana* At-CINV1 and wheat Ta-A-Inv [46]. Interestingly, this motif is located in the region 2 of the A-Inv protein, and was suggested as the possible substrate binding site of the enzyme [45]. This difference between A-Inv and N-Inv may introduce different acid/base properties in the putative catalytic site, leading to differences in optimum pH between both isoforms [46]. Further studies, such as active site identification and 3D structure determination will help in clarifying this issue.

The first functional characterization of the complete repertoire of *A/N-Inv* genes that occur in a genome was carried with *Anabaena* sp. PCC 7120. It has two genes (*An-invA* and *An-invB*, coding for an A-Inv and a N-Inv, respectively) [44]. Plant genome analyses have expanded our knowledge of A/N-Invs and have uncovered putative multigene families encoding these proteins in the genomes of *A. thaliana* (nine genes), *Oryza sativa* (eight genes), *Populus trichocharpa* (sixteen genes), *Vitis vinifera* (nine genes) and *Lotus japonicus* (seven genes) [48–52].

Even though A/N-Invs have unique biochemical properties, their physiological functions are still not clear. They have been ascribed with rather general functions such as cellular maintenance and growth [16]. However, this concept drastically changed when novel biochemical and molecular characteristics including unexpected roles for these enzymes were found. The first breakthrough was to show that not all of them are located in the cytosol but some A/N-Inv forms are targeted to subcellular compartments, as chloroplasts, mitochondria and nuclei [49,53–55]. The A/N-Invs are crucial for the regulation of a plethora of physiological events associated with root development, reproduction, and acclimation to environmental stresses [36,46,52,54,56–59]. Furthermore, different isoforms seem to be involved in distinct molecular events controlling crucial cellular processes, including gene expression and carbohydrate balance.

2.2. A/N-Inv in the cytosol

A growing body of evidence suggests that sucrose hydrolysis through cytosolic A/N-Invs is a pivotal event involved in carbon distribution, cellular differentiation, tissue development and responses to environmental stresses [36,46,52,54,56–59].

Sucrose metabolism was largely studied in plants under environmental stresses because it is involved in adaptation to unfavourable conditions such as drought, low temperatures, or high salinity [60–66]. Curiously, in most of those reports the expression of A/N-Inv was not analyzed. However, the important role of cytosolic invertases in abiotic stresses, growth, and cell development has begun to be elucidated.

A soluble invertase (Ta-A-Inv) with alkaline optimum pH for activity is induced in fully expanded wheat leaves in response to osmotic and cold stress at the same time that sucrose biosynthesis is enhanced [46]. Similarly, *Arabidopsis* plants subjected to osmotic stress (3% mannitol) had induced expression of a cytosolic A/N-Inv form (AtCYT-INV1) with concomitant accumulation of soluble carbohydrates (sucrose, glucose, and fructose) [56]. In both cases, sucrose cycling involving SPS and cytosolic A/N-Inv activities probably occurred. Such cycling may allow higher sensitivity to the environmental conditions, modulating rates of sucrose synthesis and degradation, controlling sugar accumulation and respiration, maintaining osmotic potential, and promoting sugar signalling [67,68].

A role was found for cytosolic A/N-Inv in *Arabidopsis* root development [54,56]. The characterization of *Arabidopsis* plants impaired in the functional expression of an A/N-Inv (AtCYT-INV1) helped to find its involvement in plant development; including primary root elongation, root hair growth, reproduction, and in the inhibition of lateral root growth during osmotic stress [56]. An *Arabidopsis* T-DNA insertion knockout mutant that was deficient in the cytosolic invertase CINV1 had lower activity of both N- and Ac-Invs as well as shortened roots [54]. AtCYT-INV1 [56] and *Arabidopsis* CINV1 [54] are identical products of unique gene, formerly denoted as At-A/N-InvG [42].

The loss of two closely related cytosolic isoforms (*Arabidopsis* CINV1 and CINV2), dramatically slowed plant growth [36]. The phenotype of this double mutant had less sucrose catabolism in root cells, highly reduced root growth, loss of starch from the root cap, and enlarged cell types, which had a tendency to collapse. Importantly, this shows that soluble SuS (Cinderella step-sister) is not required for normal growth, whereas cytosolic A/N-Inv Cinderella is indispensable. Thus, cytosolic A/N-Inv may be crucial in supplying carbon from sucrose to non-photosynthetic cells in *Arabidopsis* and also to control cell development [36].

In rice, one isoform of A/N-Inv (OsCYT-INV1≡OsNIN8, a homologue to AtCYT-INV1) controls the supply of hexoses needed for root growth [57,58]. The loss of function of that cytosolic invertase had more pronounced effects in the rice mutant than in the *Arabidospsis* one having no expression of AtCYT-INV1. The mutant rice plants had shorter roots due to a reduction in size of the radical cells. The mutant also produced less seeds as a consequence of a lower pollen fertility. Similarly, in the model legume *L. japonicus*, the expression of seven A/N-Inv isoforms (LjINV1-LjINV7) was thoroughly investigated [52]. The cytosolic LjINV1, previously described as a nodule enhanced isoform [59], was expressed in all parts of the plant. However, LjINV1 is crucial for development but not essential for nodule formation or function [52]. *Lotus* plants impaired in its expression were unable to grow to maturity and produced infertile flowers due to a lack of pollen.

From the data with *A. thaliana*, rice and *L. japonicus*, it can be concluded that cytosolic A/N-Invs play essential roles during growth and development of different plant species [36,52,54,56–58]. Contrary to previous thinking, these observations highlight the importance of A/N-Inv for cytosolic sucrose catabolism, carbon circulation within the plant cell, and the physiological processes that ensure a normal plant development.

2.3. A/N-Inv isoforms associated with the plasma membrane and the nucleus

By exploring the physiological function of phosphatidylinositol monophosphate 5-kinase (PIP5K) in *A. thaliana*, the cytosolic CINV1 isoform was shown to interact with PIP5K9, a component of phosphatidylinositol signalling pathways necessary for normal root growth [54]. The association was corroborated *in vivo* after co-immunoprecipitation of PIP5K9 and CINV1 from extracts of

Arabidopsis transgenic plants over-expressing both proteins. Then, the subcellular localization of PIP5K9 and CINV1 was also investigated *in vivo* in *Nicotiana benthamiana* leaves and in onion epidermal cells. PIP5K9 and CINV1 co-localized in membranes and the nucleus. This revealed even more interesting aspects for sucrose hydrolysis and its role in plant cells. Notably, CINV1 lacks a subcellular localization signal, but its fate seems to be determined by its association with PIP5K9. Signalling pathways regulated by inositol 1,4,5-trisphosphate (ITP) are associated with plant development, in response to several stresses, and other physiological processes. Still, the regulation of these pathways is largely unknown [69–71]. The physical association of PIP5K9 with CINV1 shows a crosstalk between ITP and sugar signalling pathways, involving this A/N-Inv form as one of the potential components [54,58]. Even though both sucrose and its hydrolysis products can fulfil this role, in many cases it is glucose the direct signalling molecule. Such a role is difficult to establish for sucrose because it can be readily converted into fructose and glucose. It is well documented that hexoses can trigger sugar responses and signalling in plants to regulate plant growth and development [5,72]. The involvement of the cytosolic CINV1 in signal transduction pathways is also supported by the sequestration of the enzyme inside the nucleus and the reduction of its activity by association with PIP5K9. These findings represent an outstanding advance in this research field, but the molecular mechanisms and the significance of CINV1 for sugar signalling and signal transduction still needs to be revealed. Remarkably, *Arabidopsis* plants impaired in the expression of the gene encoding CINV1 were less sensitive to glucose, and had defects in development and alterations in the transcription of a wide variety of genes [54]. Similar *Arabidopsis* phenotypes, including ones that are glucose insensitive during seedling development and have a defective control of glucose-dependent gene expression, are found in plants impaired in the expression of a hexokinase (HXK), which is ascribed as the glucose sensor in the mechanisms for sugar sensing and signal transduction [73].

The importance of A/N-Inv activity in sugar signalling may reside in regulating sucrose concentration, or in the production of glucose, which could be further sensed by HXK [72]. Interestingly, *A. thaliana* AtHXK1 is involved in a nuclear protein complex that directly modulates specific target genes in a glucose-dependent manner [74]. Thus, it can be speculated that the presence of CINV1 in the nucleus may be associated with the production of sucrose-derived glucose for the AtHXK1-mediated control of gene expression. Despite it was suggested that glucose binds to AtHXK1 for such regulation, the presence of glucose or sucrose inside the nucleus has not yet been described. Further research is needed to identify how a carbohydrate signal is transferred from the cytosol to the nucleus and whether CINV1 activity and the HXK1-related protein complex work synergistically in *Arabidopsis* to control sugar-dependent gene expression. It will be intriguing to gain a better understanding of the intricate molecular mechanisms that underlie ITP metabolism, sugar signalling, and the control of CINV1 activity and its subcellular localization.

2.4. A/N-Inv in chloroplasts

Chloroplasts are the hallmark plant organelles where photochemical reactions produce energy and support CO_2 fixation, sustaining plant growth and development. Photosynthesis and carbon metabolism in the whole plant are strictly coordinated by feedback regulation and are a main target of sugar signalling. Much evidence indicates that the rate of sucrose synthesis in the cytosol is coordinated with the rate of CO_2 fixation and starch synthesis in the chloroplasts. These pathways are interconnected by a rigorous control on a unidirectional flux of triose-phosphates toward the cytosol through the triose-phosphate translocator, which, in turn, imports Pi to the organelle [75]. Conversely, starch accumulation in chloroplasts depends on precise regulation of the enzymes involved in its synthesis and degradation, which were shown to be indirectly controlled by sucrose [76]. While starch degradation is regulated by the carbohydrate status of the cell, the accumulation of sucrose parallels the activation state of ADP-glucose pyrophosphorylase (AGPase), a key enzyme in starch biosynthesis that is also activated by a decrease in the Pi:3-phosphoglycerate ratio. However, the relationship between the rates of starch synthesis and sucrose content is probably more complex than a simple explanation based on an overflow model. Other levels of regulation were investigated, including the sensitivity of AGPase to the redox state of the cell and the involvement of trehalose-6-P [77–81]. Sucrose causes a 30-fold increase in trehalose-6-P levels in *Arabidopsis* leaves, which is accompanied by redox activation of AGPase and the stimulation of starch synthesis [79]. Recent evidence indicates that trehalose-6-P could signal the availability of sucrose and inhibit SnRK1 (a SNF1-related protein kinase 1) activity, promoting biosynthetic reactions and growth [80]. Additionally, a recent report unveils the function of NADP-thioredoxin reductase C in regulating AGPase and the attendant synthesis of starch in chloroplasts [81]. However, the link between sucrose and starch accumulation in the chloroplasts is not yet fully understood, requiring further exploration.

The occurrence of sucrose in plastids, specifically in chloroplasts, was severely debated for more than two decades. Even though it was known that isolated spinach chloroplasts hydrolyzed sucrose into glucose and fructose [82], neither sucrose metabolizing enzymes nor sucrose transporters had been found in the organelles. Also, since sucrose content inside the plastids had been detected at very low levels, it was suggested that sucrose may be quickly metabolized or exported out by some unknown manner. Two decades later, sucrose entering chloroplasts was indirectly inferred, from transgenic tobacco plants expressing a plastid-targeted levansucrase gene (*SacB*) [83]. Using subcellular fractionation, levan production, which should take place from sucrose, was shown to be associated with chloroplasts isolated from protoplasts of the transformed plants. Nevertheless, levan association with sucrose inside chloroplasts in leaves of tobacco and potato [83] was challenged by Cairns [84] after a thoroughly analysis of the presented data, concluding that the subcellular targeting in bacterial levan transformants has not been unequivocally established. To date, the origin and fate of plastid sucrose is still intriguing.

Evidence that A/N-Inv isoforms could be directed into rice plastids [49] and then, the identification of chloroplast-specific A/N-Inv in *Arabidopsis* and in spinach [53], disclosed a novel point of control in the regulation of leaf carbon partitioning between the cytosol and chloroplasts. Undoubtedly, the demonstration of an A/N-Inv isoform (At-A/N-InvE) inside the chloroplast adds a new proof on the possible fate of sucrose inside the organelles. Moreover, reverse genetic experiments suggested that the production of the chloroplast-localized *Arabidopsis* A/N-Inv is involved in the control of carbon balance between cytosol and chloroplasts. The phenotype of the *Arabidopsis At-A/N-InvE* knock-out plants suggested a novel scenario for carbon movement within the photosynthetic cell, where the activity of an A/N-Inv seems to be linked to starch accumulation [53]. The scheme in Fig. 1 depicts how monosaccharides from sucrose hydrolysis may contribute to starch accumulation, suggesting a novel connection between sucrose and chloroplast starch metabolism. It also highlighted the possible entry of sucrose into different metabolic pathways in mitochondria, plasma membrane and nucleus, and novel locations for A/N-Inv and SuS proteins.

Further understanding of the function of sucrose hydrolysis inside chloroplasts may help to comprehend how sucrose mediates

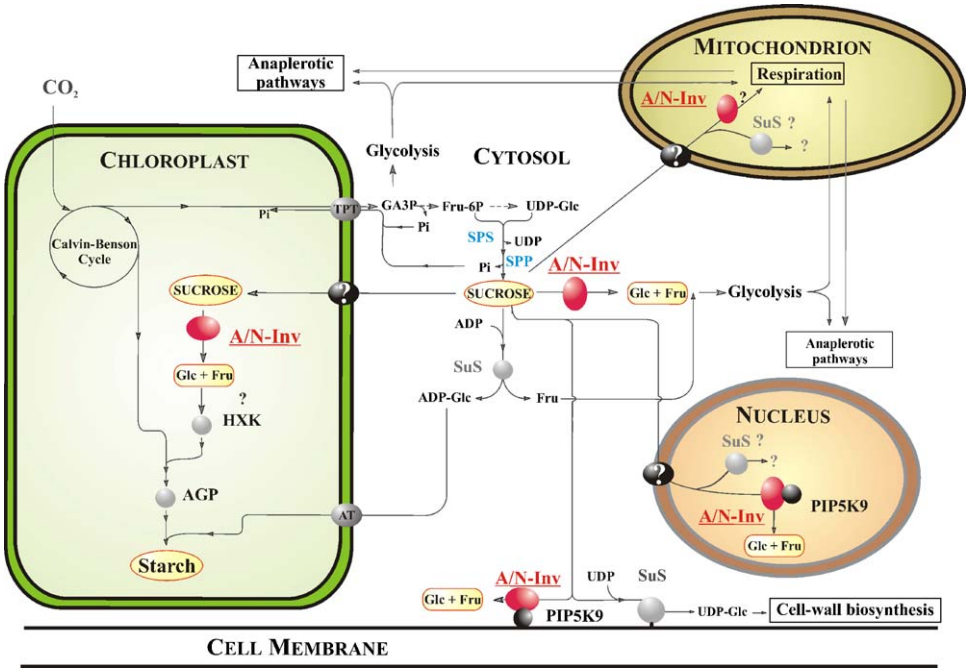

Fig. 1. Proposed model for intracellular carbon trafficking in a photosynthetic cell, based on the results reviewed in this report. The schematic illustration represents a photosynthetic plant cell where A/N-Invs are included in the cytosol, inside the chloroplast, mitochondria and nucleus. The scheme also illustrates SuS in the cytosol or associated with membranes. In this model we also indicate SuS with question marks inside the mitochondria and nucleus because its presence in such organelles was only demonstrated in heterotrophic tissues but not in photosynthetic cells. SPS and SPP, enzymes responsible of sucrose synthesis are also indicated. ADP-Glc, ADP-glucose; AGP, ADP-Glc pyrophosphorylase; AT, ADP-Glc translocator; Fru, fructose; Fru-6P, fructose-6-phosphate; Glc, glucose; GA3P, glyceraldehyde-3-phosphate; HXK, hexokinase; Pi, inorganic phosphate; TPT, triose-phosphate translocator; UDP-Glc, UDP-glucose.

starch level. Whether sucrose, or its hydrolysis products, modulate enzyme activities related to plastid starch synthesis remains to be elucidated. In addition, sucrose-derived hexose signalling pathways may also be involved in starch biosynthesis as HXKs also occurs inside chloroplasts [7,85]. Regulatory mechanisms integrating also trehalose-6-P signalling pathway, AGPase redox activation, protein–protein interactions, such as the case of PIP5K9–CINV1 interaction or with other regulatory proteins, should also be taken into account in future studies.

2.5. A/N-Inv in mitochondria

Oxidative respiration in mitochondria is an important fate of hexoses, product of sucrose hydrolysis. However, recent findings that glucolytic enzymes are associated with the outer mitochondrial membrane in a respiration-dependant manner, together with the presence of A/N-Inv forms in the mitochondrial matrix point to a more close relationship between mitochondria and carbohydrate metabolism. Consequently, novel physiological functions could be envisaged for mitochondrial A/N-Inv [49,55,86].

Transient expression of fusion proteins combining the amino terminal region of rice OsNIN1 with GFP demonstrated that OsNIN1::GFP fusion protein was targeted to mitochondria [49]. There are putative mitochondrial targeting signals in A/N-Inv isozymes from carrot and *Lolium temulentum* [53], as well as for some *Arabidopsis* A/N-Inv forms (W.A. Vargas and G.L. Salerno, unpublished). Next, the presence of A/N-Inv was confirmed in the matrix of mitochondria isolated form Jerusalem artichoke tubers [55].

As already mentioned, SuS is also targeted to maize mitochondria but a novel and non-catalytic function was assigned for this isoform, due to its poor activity and low affinity for sucrose [32]. However, A/N-Inv from Jerusalem-artichoke tuber mitochondria was highly active, with kinetic properties similar to other well-characterized A/N-Invs, suggesting that sucrose could be hydrolyzed in the matrix of this organelle [55]. The presence of sugar transporters in the mitochondrial inner membrane was also investigated. Thus, it was proposed that the invertase system was composed of an enzyme in the organelle matrix and the corresponding sugar transporter [55]. *Zymomonas mobilis* copes with osmotic stress by regulating the sucrose/glucose + fructose ratio to increase the osmolarity in the cytosol. It was suggested that plant mitochondrial A/N-Inv isozyme may similarly be involved in osmotic stress adaptation mechanisms for the organelle and the whole cell. This comparison is risky due to distinct differences in the metabolic and physiological complexity between plants and bacteria. The extent of glycolytic enzymes associated with the surface of mitochondria is dependent on the respiration rate in both *Arabidopsis* and potato tuber cells [86]. Still, it is not clear how the mitochondrial invertase system may be integrated into intermediary metabolism and what is its role inside the organelle. It remains to be demonstrated whether it represents a functional link between respiration and sucrose metabolism, providing a metabolic connection between carbon photoassimilation and mitochondrial respiration. Even though A/N-Inv activity yields glucose and fructose that can be used for energy production [16], no direct connection between these two processes has been presented [87]. To date, we are only aware that A/N-Invs are also targeted to mitochondria, which increases the complexity of carbon partitioning between cytosol and organelles. This finding is of greater interest for plant biologists studying plant energetics and the metabolic coordination in photosynthetic cells. However, further work is needed to elucidate the role of sucrose hydrolyzing SuS and A/N-Inv in plant mitochondria.

3. Evolution and phylogenetic relationships of A/N-Invs

The analysis of the deduced amino-acid sequences from A/N-Inv encoding genes retrieved from completely sequenced genomes of plants and microorganisms, provided a comprehensive view of

Table 1
Number of *A/N-Inv* homologous genes occurred in completely sequenced genomes, and subcellular targeting of the corresponding gene products.

Species	Total number of genes	Number of A/N Inv isoforms expected in different cell locations					Reference
		Mitochondrion	Plastid	Nucleus	Cytosol	Membrane	
A. thaliana[a]	9	2	2	1	5	1	[51,53,54]
O. sativa	8	2	2	Nd	4	Nd	[49,51,53]
L. japonicus[b]	7	3	2	Nd	2	Nd	[52]
V. vinifera[b]	9	2	2	Nd	5	Nd	[51]
P. trichocharpa[b]	16	4	3	Nd	9	Nd	[50,51]

Nd: not determined.
[a] Total gene number is nine. However, one gene product (CINV1 isoform) was detected in cytosol, cell membrane and nucleus [54].
[b] The number of mitochondrial or chloroplastic isoforms was predicted based on sequence clustering with *A. thaliana* or *O. sativa* A/N-Inv targeted to mitochondria or chloroplasts, respectively.

their origin and evolution. Homologs to *A/N-Inv* genes had been reported in plant and cyanobacterial genomes [44]. Particularly, one or two homologous sequences could be retrieved from genomes of unicellular or filamentous nitrogen-fixing cyanobacterial strains, respectively, but no homologs could be found in unicellular alga genomes, rising new questions about sucrose hydrolysis in those organisms ([44], W.A. Vargas and G.L. Salerno, unpublished). The presence of homologs in open-ocean strains, phylogenetically located at the base of the cyanobacterial radiation, suggests that A/N-Invs originated from an ancestral A/N-Inv like gene about 2–3.5 billion years ago [44], supporting the proposed origin of sucrose metabolism based on phylogenetic analyses of SPS and SPP encoding genes [88]. Since no homologs for SuS genes had been identified in open-ocean strains, it was suggested that sucrose hydrolysis by A/N-Inv-like proteins might be associated with the origin of sucrose metabolism [44]. Further gene duplications during cyanobacterial diversification might have originated the two different A/N-Invs in filamentous nitrogen-fixing strains.

In agreement with the generally accepted cyanobacterial endosymbiotic origin of plant chloroplasts, most of the cyanobacterial genes were transferred to the nucleus but their protein products were preferentially re-imported to the organelle [89,90]. Genes involved in sucrose metabolism seem to be efficiently lost from the chloroplasts because no homolog has been identified in sequenced plastid genomes and, initially, it was believed that their protein products were exclusively localized in the cytosol of plant cells [19].

The discovery of plant SuS and A/N-Inv isoforms distributed between the cytosol and different subcellular locations allows us to propose a new picture for carbohydrate metabolism in the photosynthetic cells presenting an intricate crosstalk and regulation between sucrose and most metabolic pathways in contemporary plants (Fig. 1).

Genome analyses have expanded our knowledge of A/N-Invs by identifying multigene families in *A. thaliana*, *O. sativa*, *P. trichocharpa*, *V. vinifera* and *L. japonicus* [48–52] (Table 1). Phylogenetic analyses using deduced amino-acid sequences of A/N-Invs from cyanobacteria and plants confirmed that all plant A/N-Invs grouped together in two sister clades representing two separated families [48–53]. The sequences carrying subcellular targeting peptides (either to mitochondria or to chloroplasts) group together in a single cluster (clade α) apart from the sequences lacking any subcellular targeting signal (clade β) [48–53]. Similar results were also obtained when the phylogenetic reconstruction was performed after removing the signal peptide suggesting different evolutionary histories for both clades (W.A. Vargas and G.L. Salerno, unpublished). *P. trichocharpa* has 16 PtNIN homologous sequences, ascribed as cytosolic A/N-Inv isoforms [50]. However, several of those sequences correspond to truncated forms, preventing a proper prediction of their subcellular targeting. The phylogenetic reconstruction shows that six sequences (PtNIN1 to PtNIN6) group with organelle-targeted isoforms from *Arabidopsis* (belonging to clade α) [48,50,53] and the remaining ten sequences group with cytosolic forms, located in the clade β [48,50]. Similarly, some A/N-Inv isoforms from *V. vinifera* and *L. japonicus* [51,52] group in the clade α, suggesting that those isoforms also might be targeted either to mitochondria or chloroplasts (Table 1). However, only the analysis of the full length sequences and the functional characterization of the different genes will truly confirm the subcellular fate of those gene products.

The differential clustering, even when different species are analyzed simultaneously, suggests that both A/N-Inv families might have diverged at early stages in the plant lineage. After the endosymbiotic origin of vascular plant chloroplasts, both families might have branched after proteins within clade α acquired signal peptides for subcellular localizations, and afterwards both families followed independent evolutionary paths.Genomic duplications in different plants may have led to a big A/N-Inv gene family where genetic redundancy, a very common phenomenon in plants, could be speculated [44,48,51,53]. The little knowledge about relevance of the different A/N-Inv encoding genes makes rather difficult to conclude about redundancy classes (full, partial, and unequal redundancy [91]). In agreement with the limited information, mainly based on phenotypes of a few *Arabidopsis* mutants, it can be suggested that the A/N-Inv encoding genes do not present full genetic redundancy. The strong phenotypes observed in single mutants, indicate unique functions for those mutated genes. Further investigations, such as comprehensive gene expression assays and studies on single and multiple A/N-Inv mutants, will be required to determine the relevance of each member of this gene family in different plant species, and the occurrence of genetic redundancy.

4. Conclusions and future directions

Tremendous progress has been made over the past three years on plant A/N-Invs. These enzymes have risen from a state of invisibility to being seen as essential proteins in plant life. Localization experiments and reverse genetic strategies allowed their re-evaluation and revealed additional novel functions. Evidence in *Arabidopsis* suggests that A/N-Invs even surpass the importance of SuS in normal growth and reproduction.

The fact that A/N-Inv are proteins with multiple locations in the plant cell (cytosol, chloroplasts, mitochondria, nuclei) makes them excellent candidates for the coordination of metabolic processes that take place in the different compartments. Sucrose hydrolysis by an A/N-Inv inside the chloroplast probably participates in controlling chloroplast-cytosolic carbon partitioning. The molecular mechanisms and signalling pathways by which sucrose hydrolysis inside the chloroplast mediates the regulation of carbon exchange and starch accumulation are still enigmatic.

The physical interaction between PIP5K9 and an A/N-Inv form in *Arabidopsis* links the phosphatidylinositol signalling pathways

to sugar metabolism in controlling several developmental processes in plants. This level of regulation through a protein–protein interaction connecting two central signalling pathways may lead to unexpected mechanisms of crosstalk regulation. However, basic questions regarding the function of A/N-Inv isoforms targeted to mitochondria and nuclei still remain unanswered.

We believe that an exciting scenario is emerging, but further efforts are needed to fully ascertain the physiological function of A/N-Inv isoforms and the regulatory mechanisms that govern sucrose flux to the different subcellular compartments. Yet, there is a glaring lack of understanding about the simultaneous expression of the different A/N-Inv genes in a single cell/tissue, and the interplay of the other actors of sucrose metabolism. Additional investigations should include the characterization of sucrose transporters to the organelles and a comprehensive study of the expression of each A/N-Inv-encoding gene. The analysis of the consequences due to suppression or over-expression of organellar A/N-Invs in transgenic plants will help in clarifying the importance of sucrose hydrolysis in the different cell-compartments.

The integration of the recently published knowledge about A/N-Inv in various plant species in situations where sucrose metabolism is fundamental for the plant physiology, such as plant–microbe interactions, and responses to abiotic stress or plant hormones, will provide further insights into A/N-Inv roles. Functional and expression studies of isoforms present in other photosynthetic organisms, filing the gap between cyanobacteria and vascular plant A/N-Invs, will contribute to a better understanding of A/N-Inv evolution. It would not be surprising if additional roles for A/N-Invs are uncovered in the near future.

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References

[1] G. Avigad, P.M. Dey, Carbohydrate metabolism: storage carbohydrates, in: P.M. Dey, J.B. Harborne (Eds.), Plant Biochemistry, Academic Press, 1997, pp. 143–204.
[2] K.E. Koch, Y. Wu, J. Xu, Sugar and metabolic regulation of genes for sucrose metabolism: potential influence of maize sucrose synthase and soluble invertase response on carbon partitioning and sugar sensing, J. Exp. Bot. 47 (1996) 1179–1185.
[3] S. Smeekens, Sugar-induced signal transduction in plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 51 (2000) 49–81.
[4] F. Rolland, B. Moore, J. Sheen, Sugar sensing and signaling in plants, Plant Cell 14 (Suppl.) (2002) S185–S205.
[5] M. Hummel, F. Rahmani, S. Smeekens, J. Hanson, Sucrose-mediated translational control, Ann. Bot. 104 (2009) 1–7.
[6] H. Winter, S.C. Huber, Regulation of sucrose metabolism in higher plants: localization and regulation of activity of key enzymes, Crit. Rev. Plant Sci. 19 (2000) 31–67.
[7] K.E. Koch, Sucrose metabolism regulatory mechanisms and pivotal roles in sugar sensing and plant development, Curr. Opin. Plant Biol. 7 (2004) 235–246.
[8] S.C. Huber, J.L. Huber, Role and regulation of sucrose-phosphate synthase in higher plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 47 (1996) 431–444.
[9] Y. Amor, C. Haigler, S. Johnson, M. Wainscott, D.P. Delmer, A membrane-associated form of sucrose synthase and its potential role in synthesis of cellulose and callose in plants, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 9353–9357.
[10] Y.-L. Ruan, D.J. Llewellyn, R.T. Furbank, Suppression of sucrose synthase gene expression represses cotton fiber cell initiation, elongation, and seed development, Plant Cell 15 (2003) 952–964.
[11] H.D. Coleman, J. Yan, S.D. Mansfield, Sucrose synthase affects carbon partitioning to increase cellulose production and altered cell wall ultrastructure, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 13118–13123.
[12] Z. Tymowska-Lalanne, M. Kreis, The plant invertases: physiology, biochemistry and molecular biology, Adv. Bot. Res. 28 (1998) 71–117.
[13] A. Sturm, Invertases. Primary structures, functions and roles in plant development and sucrose partitioning, Plant Physiol. 121 (1999) 1–7.
[14] F. Keller, M. Frehner, A. Wiemken, Sucrose synthase, a cytosolic enzyme in protoplasts of Jerusalem artichoke tubers (*Helianthus tuberosus* L.), Plant Physiol. 88 (1988) 239–241.
[15] A. Sturm, M.J. Chrispeels, cDNA cloning of carrot extracellular β-fructosidase and its expression in response to wounding and bacterial infection, Plant Cell 2 (1990) 1107–1119.
[16] T. Roitsch, M.-C. González, Function and regulation of plant invertases: sweet sensations, Trends Plant Sci. 9 (2004) 606–613.
[17] L.I. Sergeeva, J.J. Keurentjes, L. Bentsink, J. Vonk, L.H. van der Plas, M. Koornneef, D. Vreugdenhil, Vacuolar invertase regulates elongation of *Arabidopsis thaliana* roots as revealed by QTL and mutant analysis, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 2994–2999.
[18] P.A. Swarbrick, P. Schulze-Lefert, J.D. Scholes, Metabolic consequences of susceptibility and resistance (race-specific and broad-spectrum) in barley leaves challenged with powdery mildew, Plant Cell Environ. 29 (2006) 1061–1076.
[19] G.L. Salerno, L. Curatti, Origin of sucrose metabolism in higher plants: when, how and why? Trends Plant Sci. 8 (2003) 63–69.
[20] C.S. Echt, P.S. Chourey, A comparison of two sucrose synthetase isozymes from normal and shrunken-1 maize, Plant Physiol. 79 (1985) 530–536.
[21] S. Chopra, J. Del-Favero, R. Dolferus, M. Jacobs, Sucrose synthase of *Arabidopsis*: genomic cloning and sequence characterization, Plant Mol. Biol. 18 (1992) 131–134.
[22] H. Fu, W.D. Park, Sink- and vascular-associated sucrose synthase functions are encoded by different gene classes in potato, Plant Cell 7 (1995) 1369–1385.
[23] P.S. Chourey, E.W. Taliercio, S.J. Carlson, Y.L. Ruan, Genetic evidence that the two isozymes of sucrose synthase present in developing maize endosperm are critical, one for cell wall integrity and the other for starch biosynthesis, Mol. Gen. Genet. 259 (1998) 88–96.
[24] D.H.P. Barratt, L. Barber, N.J. Kruger, A.M. Smith, T.L. Wang, C. Martin, Multiple, distinct isoforms of sucrose synthase in pea, Plant Physiol. 127 (2001) 655–664.
[25] H. Winter, J. Huber, S.C. Huber, Membrane association of sucrose synthase: changes during the graviresponse and possible control by protein phosphorylation, FEBS Lett. 420 (1997) 151–155.
[26] C.C. Subbaiah, M.M. Sachs, Altered patterns of sucrose synthase phosphorylation and localization precede callose induction and root tip death in anoxic maize seedlings, Plant Physiol. 125 (2001) 585–594.
[27] E. Etxeberria, P. Gonzalez, Evidence for a tonoplast-associated form of sucrose synthase and its potential involvement in sucrose mobilization from the vacuole, J. Exp. Bot. 54 (2003) 1407–1414.
[28] J. Pozueta-Romero, D. Pozueta-Romero, P. Gonzalez, E. Etxeberria, Activity of membrane-associated sucrose synthase is regulated by its phosphorylation status in cultured cells of sycamore (*Acer pseudoplatanus*), Physiol. Plant 122 (2004) 275–280.
[29] K.A. Duncan, S.C. Hardin, S.C. Huber, The three maize sucrose synthase isoforms differ in distribution, localization, and phosphorylation, Plant Cell Physiol. 47 (2006) 959–971.
[30] M.S. Buckeridge, C.E. Vergara, N.C. Carpita, The mechanism of synthesis of a mixed-linkage (1→3),(1→4)beta-D-glucan in maize: evidence for multiple sites of glucosyl transfer in the synthase complex, Plant Physiol. 120 (1999) 1105–1116.
[31] D. Persia, G. Cai, C. Del Casino, C. Faleri, M.T.M. Willemse, M. Cresti, Sucrose synthase is associated with the cell wall of tobacco pollen tubes, Plant Physiol. 147 (2008) 1603–1618.
[32] C.C. Subbaiah, A. Palaniappan, K. Duncan, D.M. Rhoads, S.C. Huber, M.M. Sachs, Mitochondrial localization and putative signaling function of sucrose synthase in maize, J. Biol. Chem. 281 (2006) 15625–15635.
[33] C.C. Subbaiah, S.C. Huber, M.M. Sachs, D.M. Rhoads, Sucrose synthase: expanding protein function, Plant Signal. Behav. 2 (2007) 28–29.
[34] Z. Bieniawska, D.H.P. Barratt, A.P. Garlick, V. Thole, N.J. Kruger, C. Martin, R. Zrenner, A.M. Smith, Analysis of the sucrose synthase gene family in *Arabidopsis*, Plant J. 49 (2007) 810–828.
[35] J.G.A. Núñez, J. Kronenberger, S. Wuillème, L. Lepiniec, C. Rochat, Study of AtSUS2 localization in seeds reveals a strong association with plastids, Plant Cell Physiol. 49 (2008) 1621–1626.
[36] D.H.P. Barratt, P. Derbyshire, K. Findlay, M. Pike, N. Wellner, J. Lunn, R. Feil, C. Simpson, A.J. Maule, A.M. Smith, Normal growth of *Arabidopsis* requires cytosolic invertase but not sucrose synthase, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 13124–13129.
[37] J.Q. Chen, C.C. Black, Biochemical and immunological properties of alkaline invertase isolated from sprouting soybean hypocotyls, Arch. Biochem. Biophys. 295 (1992) 61–69.
[38] W. Van den Ende, A. Van Laere, Purification and properties of a neutral invertase from the roots of *Cichorium intybus*, Physiol. Plant. 93 (1995) 241–248.
[39] H.A. Ross, D. McRae, H.V. Davies, Sucrolytic enzyme activities in cotyledons of the faba bean (developmental changes and purification of alkaline invertase), Plant Physiol. 111 (1996) 329–338.
[40] H.S. Lee, A. Sturm, Purification and characterization of neutral and alkaline invertase from carrot, Plant Physiol. 112 (1996) 1513–1522.
[41] R.P. Walker, A.L. Winters, C.J. Pollock, Purification and characterization of invertases from leaves of *Lolium temulentum*, New Phytol. 135 (1997) 259–266.
[42] D.J. Vorster, F.C. Botha, Partial purification and characterisation of sugarcane neutral invertase, Phytochemistry 49 (1998) 651–655.
[43] J.A. Gallagher, C.J. Pollock, Isolation and characterization of a cDNA clone from *Lolium temulentum* encoding for a sucrose hydrolytic enzyme which shows alkaline/neutral invertase activity, J. Exp. Bot. 49 (1998) 789–795.
[44] W. Vargas, A. Cumino, G.L. Salerno, Cyanobacterial alkaline/neutral invertases. Origin of sucrose hydrolysis in the plant cytosol? Planta 216 (2003) 951–960.

[45] A. Sturm, D. Hess, H.S. Lee, S. Lienhard, Neutral invertase is a novel type of sucrose-cleaving enzyme, Physiol. Plant. 107 (1999) 159–165.
[46] W.A. Vargas, H.G. Pontis, G.L. Salerno, Differential expression of alkaline and neutral invertases in response to environmental stresses: characterization of an alkaline isoform as a stress-response enzyme in wheat leaves, Planta 226 (2007) 1535–1545.
[47] M. Goetz, T. Roitsch, The different pH optima and substrate specificities of extracellular and vacuolar invertases from plants are determined by a single amino-acid substitution, Plant J. 20 (1999) 707–711.
[48] X. Ji, W. Van den Ende, A. Van Laere, S. Cheng, J. Bennett, Structure, evolution, and expression of the two invertase gene families of rice, J. Mol. Evol. 60 (2005) 615–634.
[49] S. Murayama, H. Handa, Genes for alkaline/neutral invertase in rice: alkaline/neutral invertases are located in plant mitochondria and also in plastids, Planta 225 (2007) 1193–1203.
[50] P.N. Bocock, A.M. Morse, C. Dervinis, J.M. Davis, Evolution and diversity of invertase genes in *Populus trichocarpa*, Planta 227 (2008) 565–576.
[51] A. Nonis, B. Ruperti, A. Pierasco, A. Canaguier, A.-F. Adam-Blondon, G. Di Gaspero, G. Vizzotto, Neutral invertases in grapevine and comparative analysis with *Arabidopsis*, poplar and rice, Planta 229 (2008) 129–142.
[52] T. Welham, J. Pike, I. Horst, E. Flemetakis, P. Katinakis, T. Kaneko, S. Sato, S. Tabata, J. Perry, M. Parniske, T.L. Wang, A cytosolic invertase is required for normal growth and cell development in the model legume, *Lotus japonicus*, J. Exp. Bot. 60 (2009) 3353–3365.
[53] W. Vargas, H.G. Pontis, G.L. Salerno, New insights on sucrose metabolism: evidence for an active A/N Inv in chloroplasts uncovers a novel component of the intracellular carbon trafficking, Planta 227 (2008) 795–807.
[54] Y. Lou, J.Y. Gou, H.W. Xue, PIP5K9, an *Arabidopsis* phosphatidylinositol monophosphate kinase, interacts with a cytosolic invertase to negatively regulate sugar mediated root growth, Plant Cell 19 (2007) 163–181.
[55] A. Szarka, N. Horemans, S. Passarella, A. Tarcsay, F. Örsi, A. Salgó, G. Bánhegyi, Demonstration of an intramitochondrial invertase activity and the corresponding sugar transporters of the inner mitochondrial membrane in Jerusalem artichoke (*Helianthus tuberosus* L.) tubers, Planta 228 (2008) 765–775.
[56] X. Qi, Z. Wu, J. Li, X. Mo, S. Wu, J. Chu, P. Wu, AtCYT-INV1, a neutral invertase, is involved in osmotic stress-induced inhibition on lateral root growth in *Arabidopsis*, Plant Mol. Biol. 64 (2007) 575–587.
[57] L. Jia, B. Zhang, C. Mao, J. Li, Y. Wu, P. Wu, Z. Wu, OsCYT-INV1 for alkaline/neutral invertase is involved in root cell development and reproductivity in rice (*Oryza sativa* L.), Planta 288 (2008) 51–59.
[58] S.-G. Yao, R. Kodama, H. Wang, M. Ichii, S. Taketa, H. Yoshida, Analysis of the rice SHORT-ROOT5 gene revealed functional diversification of plant neutral/alkaline invertase family, Plant Sci. 176 (2009) 627–634.
[59] E. Flemetakis, R.C. Efrose, T. Ott, C. Stedel, G. Aivalakis, M.K. Udvardi, P. Katinakis, Spatial and temporal organization of sucrose metabolism in *Lotus japonicus* nitrogen-fixing nodules suggests a role for the elusive alkaline/neutral invertase, Plant Mol. Biol. 62 (2006) 53–69.
[60] J.A. Tognetti, G.L. Salerno, M.D. Crespi, H.G. Pontis, Sucrose and fructan metabolism of different wheat cultivars at chilling temperatures, Physiol. Plant. 78 (1990) 554–559.
[61] C.L. Guy, J.L.A. Huber, S.C. Huber, Sucrose phosphate synthase and sucrose accumulation at low temperature, Plant Physiol. 100 (1992) 502–508.
[62] M.E. Balibrea, J. Dell'Amico, M.C. Bolarin, F. Perez-Alfocea, Carbon partitioning and sucrose metabolism in tomato plants growing under salinity, Physiol. Plant. 110 (2000) 503–511.
[63] F.M. Fernandes, M.C. Arrabaça, L.M.M. Carbalho, Sucrose metabolism in *Lupinus albus* L. under salt stress, Biol. Plant. 48 (2004) 317–319.
[64] I. Niedzwiedz-Siegen, R. Bogatek-Leszczynska, D. Côme, F. Corbineau, Effects of drying rate on dehydration sensitivity of excised wheat seedling shoots as related to sucrose metabolism and antioxidant enzyme activities, Plant Sci. 167 (2004) 879–888.
[65] R. Valluru, W. Van den Ende, Plant fructans in stress environments: emerging concepts and future prospects, J. Exp. Bot. 59 (2008) 2905–2916.
[66] G.P. Xue, C.L. McIntyre, D. Glassop, R. Shorter, Use of expression analysis to dissect alterations in carbohydrate metabolism in wheat leaves during drought stress, Plant Mol. Biol. 67 (2008) 197–214.
[67] J.M. Rohwer, F.C. Botha, Analysis of sucrose accumulation in the sugar cane culm on the basis of in vitro kinetic data, Biochem. J. 358 (2001) 437–445.
[68] C. Roby, S. Cortes, M. Gromova, J.L. Le Bail, J.K. Roberts, Sucrose cycling in heterotrophic plant cell metabolism: first step towards an experimental model, Mol. Biol. Rep. 29 (2002) 145–149.
[69] O. Krinke, Z. Novotna, O. Valentova, J. Martinec, Inositol trisphosphate receptor in higher plants: is it real? J. Exp. Bot. 58 (2007) 361–376.
[70] R.H. Tang, S. Han, H. Zheng, C.W. Cook, C.S. Choi, T.E. Woerner, R.B. Jackson, Z.M. Pei, Coupling diurnal cytosolic Ca^{2+} oscillations to the CASIP3 pathway in *Arabidopsis*, Science 315 (2007) 1423–1426.
[71] E.A. Ananieva, G.E. Gillaspy, A. Ely, R.N. Burnette, F.L. Erickson, Interaction of the WD40 domain of a myoinositol polyphosphate 5-phosphatase with SnRK1 links inositol, sugar, and stress signalling, Plant Physiol. 148 (2008) 1868–1882.
[72] F. Rolland, E. Baena-Gonzalez, J. Sheen, Sugar sensing and signaling in plants: conserved and novel mechanisms, Ann. Rev. Plant Biol. 57 (2006) 675–709.
[73] B. Moore, L. Zhou, F. Rolland, Q. Hall, W.H. Cheng, Y.X. Liu, I. Hwang, T. Jones, J. Sheen, Role of the *Arabidopsis* glucose sensor HXK1 in nutrient, light, and hormonal signalling, Science 300 (2003) 332–336.
[74] Y.-H. Cho, S.-D. Yoo, J. Sheen, Regulatory functions of nuclear hexokinase1 complex in glucose signalling, Cell 127 (2006) 579–589.
[75] E.A. MacRae, J.E. Lunn, Control of sucrose biosynthesis, in: W. Plaxton, M. Mc Manus (Eds.), Advances in Plant Research, Control of Primary Metabolism in Plants, Blackwell, 2006, pp. 234–257.
[76] A.M. Smith, M. Stitt, Coordination of carbon supply and plant growth, Plant Cell Environ. 30 (2007) 1126–1149.
[77] J.H.M. Hendriks, A. Kolbe, Y. Gibon, M. Stitt, P. Geigenberger, ADP-glucose pyrophosphorylase is activated by posttranslational redox-modification in response to light and to sugars in leaves of *Arabidopsis* and other plant species, Plant Physiol. 133 (2003) 838–849.
[78] A. Kolbe, A. Tiessen, H. Schluepmann, M. Paul, S. Ulrich, P. Geigenberger, Trehalose 6-phosphate regulates starch synthesis via posttranslational redox activation of ADP-glucose pyrophosphorylase, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 11118–11123.
[79] J.E. Lunn, R. Feil, J.H.M. Hendriks, Y. Gibon, R. Morcuende, D. Osuna, W.R. Scheible, P. Carillo, M.R. Hajirezaei, M. Stitt, Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADP-glucose pyrophosphorylase and higher rates of starch synthesis in *Arabidopsis thaliana*, Biochem. J. 397 (2006) 139–148.
[80] Y. Zhang, L.F. Primavesi, D. Jhurreea, P.J. Andralojc, R.A. Mitchell, S.J. Powers, H. Schluepmann, T. Delatte, A. Wingler, M.J. Paul, Inhibition of SNF1-related protein kinase1 activity and regulation of metabolic pathways by trehalose-6-phosphate, Plant Physiol. 149 (2009) 1860–1871.
[81] J. Michalska, H. Zauber, B.B. Buchanan, F.J. Cejudo, P. Geigenberger, NTRC links built-in thioredoxin to light and sucrose in regulating starch synthesis in chloroplasts and amyloplasts, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 9908–9913.
[82] M. Stitt, H.W. Heldt, Physiological rates of starch breakdown in isolated intact spinach chloroplasts, Plant Physiol. 68 (1981) 755–761.
[83] N. Gerrits, S.C.H.J. Turk, K.P.M. van Dun, S.H.D. Hulleman, R.G.F. Visser, P.J. Weisbeek, S.C.M. Smeekens, Sucrose metabolism in plastids, Plant Physiol. 125 (2001) 926–934.
[84] A.J. Cairns, Fructan biosynthesis in transgenic plants, J. Exp. Bot. 54 (2003) 549–567.
[85] J.O. Giese, K. Herbers, M. Hoffmann, R.B. Klösgen, U. Sonnewald, Isolation and functional characterization of a novel plastidic hexokinase from *Nicotiana tabacum*, FEBS Lett. 579 (2005) 827–831.
[86] J.W. Graham, T.C. Williams, M. Morgan, A.R. Fernie, R.G. Ratcliffe, L.J. Sweetlove, Glycolytic enzymes associate dynamically with mitochondria in response to respiratory demand and support substrate channelling, Plant Cell 19 (2007) 3723–3738.
[87] A.H. Millar, I.D. Small, D.A. Day, J. Whelan, Mitochondrial biogenesis and function in *Arabidopsis*, in: The Arabidopsis Book, American Society of Plant Biologists, Rockville, MD, July 2008 , doi:10.1199/tab.0111 http://www.aspb.org/publications/arabidopsis/.
[88] A. Cumino, L. Curatti, L. Giarrocco, G.L. Salerno, Sucrose metabolism: *Anabaena* sucrose-phosphate synthase and sucrose-phosphate phosphatase define minimal functional domains shuffled during evolution, FEBS Lett. 517 (2002) 19–23.
[89] F. Abdallah, F. Salamini, D. Leister, A prediction of the size and evolutionary origin of the proteome of chloroplasts of *Arabidopsis*, Trends Plant Sci. 5 (2000) 141–142.
[90] T. Rujan, W. Martin, How many genes in *Arabidopsis* come from cyanobacteria? An estimate from 386 protein phylogenies, Trends Genet. 17 (2001) 113–120.
[91] G.C. Briggs, K.S. Osmont, C. Shindo, R. Sibout, C.S. Hardtke, Unequal genetic redundancies in *Arabidopsis*—a neglected phenomenon? Trends Plant Sci. 11 (2006) 492–498.