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Update on Biochemistry

Invertases. Primary Structures, Functions, and Roles in Plant Development and Sucrose Partitioning

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One of the key features of plants is their ability to reduce carbon dioxide in the presence of sunlight and water to sugars, and the subsequent transport of assimilated carbon to the nonphotosynthetic tissues (sink tissues). In most plants, the transported sugar is Suc, a nonreducing disaccharide, in which Glc and Fru are linked ($\alpha 1 \rightarrow \beta 2$). Depending on the physiological activities and the biochemical needs of the heterotrophic tissues, Suc is channeled into various pathways in different subcellular compartments. It may enter glycolysis and the tricarboxylic acid cycle for the production of ATP and NADH. The carbon of the disaccharide may also be used for the biosynthesis of primary metabolites important for tissue growth and development. Likewise, Suc may become converted into polymers such as starch, triacyl glycerides, or polypeptides for long-term storage, or into secondary compounds, enabling plants to cope with predators and pests or other environmental challenges. Many of these processes may take place in the same cell at the same time and, thus, allocation of Suc to these different processes requires efficient and precise control mechanisms.

Utilization of Suc as a source of carbon and energy depends on its cleavage into hexoses, and in plants either Suc synthase (EC 2.4.1.13) or invertase (EC 3.2.1.26) catalyzes this reaction. Suc synthase is a glycosyl transferase, which converts Suc in the presence of UDP into UDP-Glc and Fru. Invertase is a hydrolase, cleaving Suc into the two monosaccharides. Suc synthase is a cytoplasmic enzyme and, in most plants, two closely related isoforms have been identified (for summary, see Sturm et al., 1999). Invertase exists in several isoforms with different biochemical properties and subcellular locations (Sturm, 1996; Tymowska-Lalanne and Kreis, 1998). The specific functions of the different invertase isoforms are not clear, but they appear to regulate the entry of Suc into the different utilization pathways. Because sugars in plants are not only nutrients but also important regulators of gene expression (Koch, 1996), invertases may be indirectly involved in the control of cell differentiation and plant development.

PLANT CELLS HAVE VACUOLAR, EXTRACELLULAR (CELL WALL), AND CYTOSOLIC INVERTASES

Most plant species contain at least two isoforms of vacuolar invertase, which accumulate as soluble proteins (soluble acid invertases) in the lumen of this acidic compartment. Likewise, several isoforms of extracellular invertase (cell wall invertases) that are ionically bound to the cell wall have been detected. Vacuolar and cell wall invertases share some biochemical properties, e.g. they cleave Suc most efficiently between pH 4.5 and 5.0 and attack the disaccharide from the Fru residue. Thus, these so-called acid invertases are β-fructofuranosidases and also hydrolyze other β-Fru-containing oligosaccharides such as raffinose and stachiose. Additionally, plants have at least two isoforms of cytoplasmic invertase with pH optima for Suc cleavage in the neutral or slightly alkaline range. Neutral and alkaline invertases are less well characterized but, in contrast to the acid invertases, these enzymes appear to be Suc specific.

VACUOLAR AND EXTRACELLULAR INVERTASES HAVE SIMILAR ENZYMATIC PROPERTIES

Acid invertases have been purified from several plant species (for summary, see Unger et al., 1992). The enzymes have a K_m for Suc in the low-millimolar range. Activity is inhibited by heavy metal ions such as Hg^{2+} and Ag^{+}, suggesting the presence of a sulfhydryl group at the catalytic site. Acid invertases are also inhibited by their reaction products, with Glc acting as a non-competitive inhibitor and Fru as a competitive inhibitor. The mature polypeptides are *N*-glycosylated and the majority have molecular masses between 55 and 70 kD. Analysis of some of the purified proteins on denaturing SDS gels under reducing conditions revealed the presence of proteolytic fragments. A 70-kD monomeric form of vacuolar invertase of mung bean hypocotyls was found to be split into a 30-kD N-terminal and a 38-kD C-terminal fragment (Arai et al., 1991). Likewise, the 68-kD monomer of isoform I of vacuolar invertase from carrot was fragmented into N- and C-terminal polypeptides of 43 and 25 kD, respectively (Unger et al., 1992, 1994). Under native conditions, these fragments appear to be tightly associated and, in a complex, possess enzyme activity. Fragmentation does not appear to be an artifact of protein purification, but instead seems to be under developmental control. For example, the full-length proteins predominate in very young hypocotyls of bean and seedlings of carrot, whereas with increasing hypocotyl and seedling age the fragments were more abundant. Whether fragmentation has a physiological function is not clear.

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VACUOLAR AND EXTRACELLULAR INVERTASES HAVE SOME COMMON MOLECULAR FEATURES BUT DIFFER IN IMPORTANT RESPECTS

Like plant invertase, yeast invertase exists in different isoforms with discrete subcellular locations (Carlson and Botstein, 1982). A secreted form of the enzyme is highly *N*-glycosylated and its expression is regulated by Glc repression. Cytoplasmic yeast invertase is a nonglycosylated polypeptide that is constitutively expressed at low levels. The two isoforms are encoded by the same gene and the two transcripts originate from differential splicing of a heteronuclear mRNA. In contrast, cDNA cloning of plant acid invertases revealed that each isoform is encoded by a different gene (Sturm, 1996; Tymowska-Lalanne and Kreis, 1998). The polypeptides encoded by these genes can be divided into two main classes with different properties. One class consists of cell wall invertase polypeptides with a basic pI, and the second class comprises vacuolar invertase polypeptides with an acidic pI.

The first cloned plant acid invertase was cell wall invertase from carrot (Sturm and Chrispeels, 1990). Since its isolation in 1990, more than 20 sequences of cDNAs for acid invertases from mainly dicot species have been deposited into the databases (Tymowska-Lalanne and Kreis, 1998). The cDNA-derived amino acid sequences all belong to a large protein family and are related to the sequences of invertases from yeast and bacteria. Common features are the pentapeptide NDPNG (βF-motif), which is close to the N terminus of the mature proteins, and a Cys residue and its neighboring amino acids, which are located closer to the C terminus (Fig. 1). It can be assumed that these conserved sequences are important constituents of the catalytic domain of acid invertases.

Comparison of the amino acid sequences derived from the cDNAs for acid invertases with the N-terminal sequences of the mature polypeptides revealed the presence of N-terminal domains up to 100 amino acid residues long, which most likely consist of a signal peptide and an N-terminal propeptide (Fig. 1) (Sturm and Chrispeels, 1990; Unger et al., 1994). The function of the putative propeptides is not clear, but in analogy to other "pro-

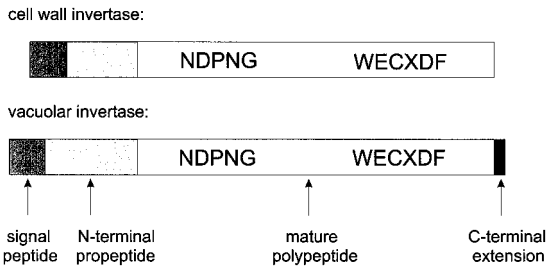

Figure 1. Schematic comparison of the amino acid sequences deduced from cDNAs of carrot cell wall invertase and isoenzymes I and II of carrot vacuolar invertase (Unger et al., 1994). The mature proteins share 64% similarity and 46% identity. The amino acid sequence NDPNG is a hallmark of plant acid invertases (βF-motif; Sturm and Chrispeels, 1990). The sequence WECXDF indicates a highly conserved peptide domain consisting of a Cys residue and a few neighboring amino acids (Sturm and Chrispeels, 1990).

proenzymes," they may play a role in protein folding (Klionsky et al., 1988), protein targeting (Klionsky et al., 1988), or in the control of enzyme activity (Hasilik and Tanner, 1987).

In comparison with the sequences of plant cell wall invertases, those of the vacuolar invertases appear to have short C-terminal extensions (Fig. 1) (Unger et al., 1994). The corresponding domains of isoform I and II of vacuolar invertase of carrot contain short hydrophobic amino acid stretches, which, in analogy to studies on barley lectin and tobacco chitinase, may form the critical cores of vacuolar sorting signals (Bednarek and Raikhel, 1992).

THE BIOCHEMICAL PROPERTIES OF NEUTRAL AND ALKALINE INVERTASES CLEARLY DIFFER FROM THOSE OF THE ACID INVERTASES

Because the plant invertases with pH optima between 7.0 and 7.8 are extremely labile and enzyme activity is rapidly lost after tissue homogenization, their purification turned out to be very difficult. Only in a few cases were polypeptides of apparent electrophoretic homogeneity obtained (for summary, see Ross et al., 1996). With the exception of alkaline invertase from carrot (Lee and Sturm, 1996), the native polypeptides are homotetramers composed of subunits with a molecular mass of 54 to 65 kD. The polypeptides are not *N*-glycosylated and preferentially hydrolyze Suc with a K_m of 10 mM. They are strongly inhibited by Glc, Fru, and Tris, but not by heavy metal ions, suggesting marked differences between the catalytic sites of neutral/alkaline and acid invertases.

NEUTRAL AND ALKALINE INVERTASES ARE UNIQUE TO PLANTS AND PHOTOSYNTHETIC BACTERIA

Recently, a cDNA was obtained from poison rye grass (*Lolium temulentum*) that codes for a polypeptide with neutral/alkaline invertase activity (Gallagher and Pollock, 1998). The clone was isolated by a functional assay in which *Escherichia coli* cells were transformed with an *L. temulentum* cDNA library and then screened for cells capable of growth on minimal medium supplemented with Suc as the sole carbon source. In extracts of these cells, Suc was cleaved into Glc and Fru, with maximal activity in the pH range of 7.0 to 7.5 and a K_m of approximately 18 mM. The enzyme is not a β-fructofuranosidase and was inhibited by Tris and Fru. The cDNA clone codes for a protein of 571 amino acids with an estimated molecular mass of approximately 63 kD.

Concomitant with the cloning of *L. temulentum* cDNA, a carrot cDNA was isolated by using an internal tryptic peptide sequence of the purified neutral invertase (Sturm et al., 1999). The deduced sequence shares 71% similarity (63% identity) with the sequence encoded by the *L. temulentum* cDNA, suggesting that the two clones code for related polypeptides with possibly similar roles in Suc metabolism. The N termini of the sequences differ markedly, whereas a domain of approximately 450 amino acid residues is highly conserved. Four amino acid residues (NDPN) of the pentapeptide NDPNG, which is a hallmark

of the acid β-fructofuranosidases (Sturm and Chrispeels, 1990), are conserved in the *L. temulentum* sequence but were not found in the sequence of the carrot clone. Thus, it is unlikely that this sequence is essential for the function of cytoplasmic plant invertases.

The function of the carrot protein encoded by the cDNA was confirmed by its expression in *E. coli*. The deduced amino acid sequence lacks an N-terminal signal peptide and shares no similarity with the sequences of the acid invertases. The protein is Cys-rich, and homologous sequences were only detected in the genomes of photosynthetic bacteria and plants. Therefore, the protein must have evolved independently of other Suc-cleaving enzymes. Unlike acid invertase, neutral invertase is not a β-fructofuranosidase and Suc appears to be its sole substrate. Transcripts for neutral invertase were detected in all plant organs at all developmental stages analyzed, with the highest levels in rapidly developing tissues, suggesting a more general and possibly growth-related function of the enzyme in carrot Suc metabolism.

GENES FOR ACID INVERTASES ARE REGULATED DEVELOPMENTALLY AND BY SUGARS, WOUNDING, AND PATHOGENS

Genes for acid invertases have been isolated from tomato (Elliott et al., 1993), Arabidopsis (for summary, see Tymowska-Lalanne and Kreis, 1998), maize (Xu et al., 1995), and carrot (Ramloch-Lorenz et al., 1993; Lorenz et al., 1995; Sturm, 1996). Their structures are fairly similar and contain six to eight exons (Tymowska-Lalanne and Kreis, 1998). With the exception of the gene for the main form of carrot cell wall invertase (Ramloch-Lorenz et al., 1993), all of the other genes contain an extremely small exon (exon 2) that codes only for the core tripeptide (DPN) of the conserved βF-motif NDPNG. In potato, the mini exon of one of the genes was susceptible to alternative splicing (Bourney et al., 1996). No aberrant post-transcriptional processing was observed during normal invertase gene expression in potato. In contrast, RNA processing was perturbed by cold stress, resulting in the deletion of the mini exon from some transcripts. It is not known whether the aberrant splicing of the invertase gene has a physiological role.

From carrot, five genes for acid invertases have been isolated, including the genes for the main form of the cell wall enzyme and the two isoforms of vacuolar invertase (Fig. 2; Sturm, 1996). Comparison of their 5′ upstream regions showed no common sequence elements, suggesting independent modes of regulation. Analysis of isoform-specific steady-state transcript levels confirmed this finding and showed markedly different organ- and development-stage-specific expression patterns (Sturm et al., 1995). Similar results were obtained in a study of the expression of genes for one vacuolar and four cell wall invertase isoforms from tomato (Godt and Roitsch, 1997). Interestingly, both carrot (Lorenz et al., 1995) and tomato (Godt and Roitsch, 1997) contain a flower-specific gene for an acid invertase. Together, these findings suggest that plants have evolved a small family of acid invertase genes that are expressed independently at specific times and places during plant development.

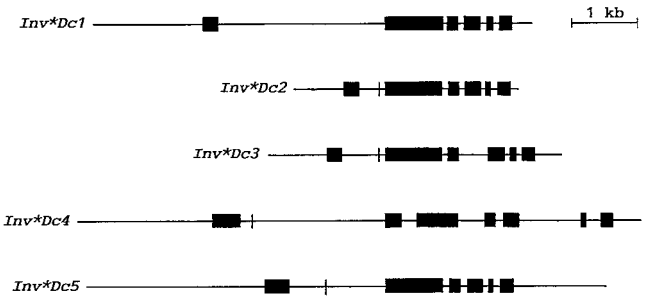

Figure 2. Structural maps of genomic clones for cell wall invertase and vacuolar invertase from carrot. *Inv*Dc1*, *Inv*Dc2*, and *Inv*Dc3* code for cell wall invertase and related enzymes (Lorenz et al., 1995). *Inv*Dc4* and *Inv*Dc5* code for isoenzyme I and II of vacuolar invertase (M. Hardegger and A. Sturm, unpublished results). The coding regions are represented as black boxes, with intervening and non-coding sequences shown as lines.

In addition to the organ- and development-specific regulation of gene expression, alterations in sugar composition and concentration markedly affect some of the acid invertase genes, e.g. those for vacuolar invertases from maize (Xu et al., 1996). Two gene classes with contrasting sugar responses were identified. One class is up-regulated by increasing carbohydrate supply, whereas a second class in the same gene family is repressed by sugars and up-regulated by depletion of this resource. Responses to Glc, Suc, and other metabolizable sugars were similar. In contrast, in cells of *Chenopodium rubrum*, the expression of genes for soluble acid invertase was not affected by sugars, whereas the expression of the gene for cell wall invertase was enhanced (Roitsch et al., 1995). Interestingly, in plants such as carrot, no sugar regulation of acid invertase genes was found. Why these fundamental differences exist in the responsiveness of acid invertase genes to sugars in different plant species is not clear. One possibility is that in plants such as carrot, which store high concentrations of sugars, regulation of acid invertase genes by sugars may not be beneficial and, therefore, either did not develop or has been lost during evolution. In these plants, short-term physiological changes only lead to minor sugar concentration changes, which may not be large enough to efficiently alter regulation of gene expression.

An increase in acid invertase activity in response to hormones such as auxins (Morris and Arthur, 1984), GAs (Wu et al., 1993), or cytokinins (Ehness and Roitsch, 1997) was observed in several plant species. It is not clear whether these effects are due to direct regulation of invertase genes by plant hormones or via stimulated cell proliferation creating new sinks for Suc. In cultured cells of *C. rubrum*, the increase in cell wall invertase mRNA by cytokinin was paralleled by an increase in transcripts for a Glc transporter (Ehness and Roitsch, 1997), most likely resulting in a higher carbohydrate supply of hormone-stimulated cells.

Genes for acid invertases have also been found to be regulated by wounding. For example, the activity of solu-

ble acid invertase was markedly increased in aging slices of sweet potato tuber (Matsushita and Uritani, 1974), rapidly reached a maximum at 18 h, and decreased thereafter. Furthermore, expression of a gene for carrot cell wall invertase was markedly altered by mechanical wounding of tap roots (Sturm and Chrispeels, 1990). A transient increase of the steady-state mRNA level with a maximum of 12 h after the treatment was followed by an increase in enzyme activity. Induction of gene expression was not systemic but was restricted to the wound site.

A correlation between increased acid invertase activity and infection of plants with various pathogens has been reported several times (for summary, see Sturm and Chrispeels, 1990). In carrot tap roots, the response to infection with the bacterial pathogen *Erwinia carotovora* was extremely fast and transient (Sturm and Chrispeels, 1990). Maximal levels of transcripts were reached 1 h after first contact with the pathogen and rapidly declined thereafter. Again, induction of gene expression by pathogen infection appears not to be systemic, but restricted to the site of infection (Benhamou et al., 1991).

ACID INVERTASES ARE REGULATED BY A PROTEINOUS INHIBITOR

During the purification of cell wall invertase from a suspension culture of tobacco, a small polypeptide of 17 kD that inhibits enzyme activity in a pH-dependent manner was identified (Weil et al., 1994). The highest inhibition was found at pH 4.5, the optimal pH for cell wall invertase activity. The inhibitor polypeptide was shown to colocalize with the enzyme in the cell wall, suggesting an important physiological role in the regulation of invertase activity. However, the finding that binding of the inhibitor to invertase is inhibited by fairly low concentrations of Suc (half-maximum activity at 1.3 mM) is puzzling and raises questions about its postulated function.

A cDNA clone containing partial sequences of the purified inhibitor protein was isolated (Greiner et al., 1998). Its deduced amino acid sequence has no homology to a protein with known function, but several related sequences were identified in the genomes of other plant species, including Arabidopsis. A *His*-tagged recombinant fusion protein was shown to inhibit the activities of several cell wall and vacuolar plant invertases in vitro but no inhibitory activity was found toward invertases from fungi (Greiner et al., 1998). The cDNA for the inhibitor was also used for expression studies, and a comparison with the steady-state mRNA levels for cell wall invertase revealed that the expression of both proteins is not always coordinate.

INVERTASES FUNCTION IN METABOLISM AND OSMOREGULATION

In connection with the various roles Suc plays in plants (nutrient, osmoticum, and signal molecule), invertases may have several different functions (Fig. 3). Most likely, invertases cleave Suc into hexoses to provide cells with fuel for respiration and with carbon and energy for the synthesis of

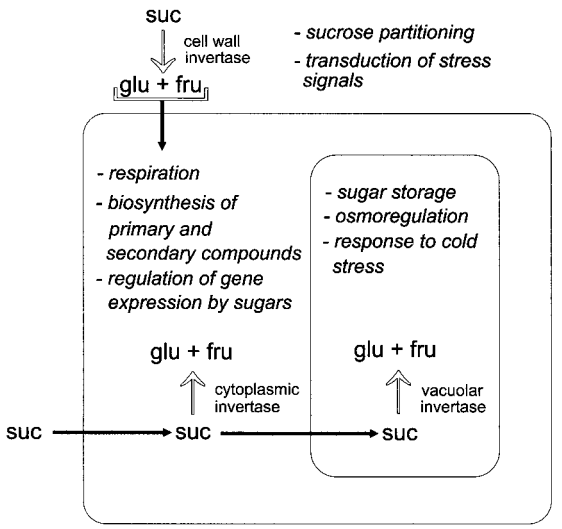

Figure 3. Subcellular locations and proposed functions of plant invertases. Plant cells may accumulate different invertase isoforms in the apoplast, cytoplasm, and vacuole. Hexoses generated by the activities of the different enzymes most likely have different fates and functions (a few important examples are indicated in italics).

numerous different compounds. Invertases may also be involved in the long-distance transport of Suc by generating the necessary Suc concentration gradient between sites of phloem loading and unloading (Eschrich, 1980). Cleavage of Suc into Glc and Fru could greatly increase the osmotic pressure of cells, suggesting a possible function of invertases in cell elongation and plant growth (Gibeaut et al., 1990).

In numerous studies, invertase functions were deduced from a correlation of invertase activity with physiological processes such as tissue growth or utilization and storage of sugars in sink organs. High acid invertase activity was found in rapidly growing tissues such as developing roots of carrot (Ricardo and Ap Rees, 1970) or elongating stems of bean (Morris and Arthur, 1985), in which the Suc content was low or declining rapidly. Thus, the function of acid invertases in these tissues is to hydrolyze Suc under conditions where there is a high demand for hexoses.

Tissue growth not only requires hexoses as a source of energy and carbon, but is also dependent on a driving force for cell elongation, namely the relative maintenance of cell osmotic pressure and an increase in cell wall extensibility. Indirect evidence for the function of soluble acid invertase in osmoregulation was provided by analysis of the upper and lower halves of the leaf sheet pulvinus during the graviresponse (Gibeaut et al., 1990). In the lower half, invertase activity was increased 3-fold, and this correlated with an osmotic potential gradient as the driving factor for growth. A study of cell elongation in developing sunflower hypocotyls showed a close correlation between the rate of cell elongation and soluble acid invertase activity (Pfeiffer and Kutschera, 1995). The authors suggested that the maintenance of cell turgor was due to enhanced hydrolysis of imported Suc. Likewise, Woodson and Wang (1987) interpreted high soluble acid invertase activity and elevated

levels of reducing sugars in opening carnation flowers as important factors in the regulation of carnation petal growth, which is mainly caused by cell enlargement. The source of these reducing sugars was thought to be the import and subsequent rapid hydrolysis of Suc.

In sugar-storing sink organs such as fruit, high acid invertase activity appears to be correlated with the accumulation of hexoses. In the Suc-storing fruit of wild tomato, including *Lycopersicon chmielewskii*, invertase activity is greatly reduced, whereas in the hexose-storing fruit of domestic tomato *(Lycopersicon esculentum*), invertase activity increases at the onset of fruit ripening (Klann et al., 1993). The reduction of invertase activity by an antisense approach (Klann et al., 1996) or gene suppression (Ohyama et al., 1995) led to the conversion of hexose-storing into Suc-storing fruit, demonstrating that in tomato a soluble invertase controls sugar composition. Similar conclusions were drawn from a study of sugar accumulation during cold storage of mature potato tubers. Reduction of soluble acid invertase activity by an antisense approach was correlated with a decrease in the hexose to Suc ratio but did not alter the total amount of released sugars (Zrenner et al., 1996). Thus, a soluble acid invertase functions in this postharvest process as a regulator of sugar composition.

INVERTASES AND SUC ALLOCATION

A more controversial question is whether invertases are involved in Suc metabolism in actively filling sink organs such as seeds, tubers, or roots. In developing seeds of lima bean and tubers of potato, Suc synthase was found to be the prominent Suc breakdown activity, and sucrolysis via invertase was low and secondary (Sung et al., 1989). This conclusion is supported by a study of endosperm development in wheat (Riffkin et al., 1995). The authors reported that Suc synthase had consistently higher activity than invertase throughout endosperm development, and that invertase activity did not change appreciably. Weber et al. (1995) studied the molecular physiology of photosynthate unloading and partitioning during seed development of fava bean and reached the opposite conclusion. During the prestorage phase, they found that high levels of hexoses in the cotyledons and the apoplastic endospermal space were correlated with high levels of cell wall-bound invertase in the seed coat. They proposed a model for an invertase-mediated unloading process during early seed development, in which cell wall invertase contributes to establishing sink strength in young seeds. A similar conclusion was reached by Miller and Chourey (1992), who studied the genetic defect in a natural mutant of maize called *Miniature-1* (*Mn1*). In this mutant, the seeds have only one-fifth of the normal weight. The authors showed that the *Mn1* seed locus encodes an endosperm-specific isozyme of cell wall invertase. The lack of invertase activity in the mutant causes an early degeneration and withdrawal of maternal cells from the endosperm, and thereby an interruption of the transport of photoassimilates into the developing kernel.

INVERTASES AND DEVELOPMENT

Marked changes in the activity of acid and alkaline invertases appear to be intimately related to the process of cell differentiation in carrot tissue cultures (Silva and Ricardo, 1992). Somatic embryogenesis was characterized by an increase in alkaline invertase and a decrease in the acid enzyme. Nonembryogenic cell lines, in contrast to embryogenic cell lines, maintained very high acid invertase activities. The authors concluded that if Suc participates in plant cell morphogenesis, the persistence of high acid invertase activity at certain developmental stages could affect differentiation by reducing the level of Suc. Thus, the concerted action of two invertases in the cell may contribute to establishing the appropriate levels of sugars, which, by interaction with other components, participate in the regulation of development.

To study the functions of acid invertases in carrot, the antisense technique was used to generate transgenic carrot plants with reduced enzyme levels (Tang et al., 1999). Phenotypic alterations appeared at very early stages of development. In somatic embryos expressing antisense mRNA for cell wall invertase, the cotyledons failed to separate. In contrast, embryos expressing antisense mRNA for vacuolar invertase had quite large cotyledons but stunted hypocotyls and roots. At the stage when control plantlets had two to three foliage leaves and one primary root, shoots of transgenic plantlets expressing antisense mRNA for either cell wall invertase or vacuolar invertase were not separated into individual leaves but consisted of several stunted, interconnected green structures.

When transgenic plantlets were grown on a mixture of Suc, Glc, and Fru instead of only Suc, the malformation was clearly alleviated and plantlets looked more or less normal. Plantlets from hexose-containing media or from transgenic lines with less severe phenotypes produced mature plants when transferred to soil. Such plants in soil expressing antisense mRNA for cell wall invertase had a bushy appearance due to the development of extra leaves. Simultaneously, tap root development was markedly delayed and reduced. Compared with control plants, the leaf to root dry-weight ratio of antisense cell wall invertase plants was shifted from 1:3 to 17:1. Plants expressing antisense mRNA for vacuolar invertase also had more leaves than control plants, but the tap root developed normally though smaller, with a leaf to root dry-weight ratio of 1.5:1. The data suggest that invertases have multiple functions. In the early stages, invertase appears to play a role in plant development, most likely via control of sugar composition and metabolic fluxes. Later, when reduction of cell wall invertase or vacuolar invertase activity have shifted development in favor of leaves, both isoenzymes appear to have important functions in Suc partitioning.

FUTURE PROSPECTS

During the past decade, great progress has been made in the characterization and functional analysis of plant invertases. Despite these accomplishments, numerous important questions still need to be answered. Why are there inver-

tases with different properties in different subcellular compartments and how do these enzymes cooperate? What is the function of neutral/alkaline invertase and why does it exist only in photosynthetic organisms? Under what conditions do the products of Suc hydrolysis regulate invertase gene expression and enzyme activity? What other metabolites or effector molecules modulate invertase expression? What is the role of the proteinaceous invertase inhibitor (Greiner et al., 1998)? What is the significance of the N-terminal propeptides of the acid invertases and the fragmentation of the mature polypeptides? Where are the regulatory elements for gene activity in the 5′, 3′, and introns of the genes (Fu et al., 1995)? What is the evolutionary and regulatory significance of the intron that encodes the DNP tripeptide? What is the physiological significance of the wound and pathogen response of gene activity? Why does Suc needs to be cleaved when plants are wounded or attacked by pathogens and are the hexoses released involved in stress signaling? And, finally, what is the evolutionary link between β-fructosidases and fructosyltransferases, enzymes that share more than 50% amino acid sequence identity (Sprenger et al., 1995; Vijn and Smeekens, 1999)?

Answers to these questions may only come from multidisciplinary approaches, and most likely will include combined usage of physiological, biochemical, and molecular techniques. The knowledge gained will fill the gaps in our understanding of one of the most fundamental processes in plants, which may allow the successful manipulation of carbohydrate metabolism and partitioning in our future crop plants.

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LITERATURE CITED

Arai M, Mori H, Imaseki H (1991) Roles of sucrose-metabolizing enzymes in growth of seedlings: purification of acid invertase from growing hypocotyls of mung bean seedlings. Plant Cell Physiol **32:** 1291–1298

Bednarek SY, Raikhel NV (1992) Intracellular trafficking of secretory proteins. Plant Mol Biol **20:** 133–150

Benhamou N, Genier J, Chrispeels MJ (1991) Accumulation of β-fructosidase in the cell walls of tomato roots following infection by a fungal wilt pathogen. Plant Physiol **97:** 739–750

Bourney AS, Hedley PE, Maddison A, Waugh R, Machray GC (1996) Exon skipping induced by cold stress in a potato invertase gene transcript. Nucleic Acids Res **24:** 2347–2351

Carlson M, Botstein D (1982) Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. Cell **28:** 145–154

Ehness R, Roitsch T (1997) Co-ordinated induction of mRNAs for extracellular invertase and a glucose transporter in *Chenopodium rubrum* by cytokinins. Plant J **11:** 539–548

Elliott KJ, Butler WO, Dickinson CD, Konno Y, Vedvick TS, Fitzmaurice L, Mirkov TE (1993) Isolation and characterization of fruit vacuolar invertase genes from two tomato species and temporal differences in mRNA levels during fruit ripening. Plant Mol Biol **21:** 515–524

Eschrich W (1980) Free space invertase, its possible role in phloem unloading. Ber Dtsch Bot Ges **93:** 363–378

Fu H, Kim Y, Park WD (1995) High-level tuber expression and sucrose inducibility of a potato *Sus4* sucrose synthase gene require 5′ and 3′ flanking sequences and the leader intron. Plant Cell **7:** 1387–1394

Gallagher JA, Pollock CJ (1998) Isolation and characterization of a cDNA clone from *Lolium temulentum* L. encoding for a sucrose hydrolytic enzyme which shows alkaline/neutral invertase activity. J Exp Bot **49:** 789–795

Gibeaut DM, Karuppiah N, Chang S-R, Brock TG, Vadlamudi B, Kim D, Ghosheh NS, Rayle DL, Carpita NC, Kaufman PB (1990) Cell wall and enzyme changes during graviresponse of the leaf-sheat pulvinus of oat (*Avena sativa*). Plant Physiol **94:** 411–416

Godt DE, Roitsch T (1997) Regulation and tissue-specific distribution of mRNAs for three extracellular invertase isoenzymes of tomato suggest an important function in establishing and maintaining sink metabolism. Plant Physiol **115:** 273–282

Greiner S, Krausgrill S, Rausch T (1998) Cloning of a tobacco apoplasmic invertase inhibitor. Plant Physiol **116:** 733–742

Hasilik A, Tanner W (1987) Carbohydrate moiety of carboxypeptidase Y and pertubation of its biosynthesis. Eur J Biochem **91:** 567–575

Klann EM, Chetelat RT, Bennett AB (1993) Expression of acid invertase gene controls sugar composition in tomato (*Lycopersicon*) fruit. Plant Physiol **103:** 863–870

Klann EM, Hall B, Bennett AB (1996) Antisense acid invertase (TIV1) gene alters soluble sugar composition and size in transgenic tomato fruit. Plant Physiol **112:** 1321–1330

Klionsky DJ, Banta LM, Emr SD (1988) Intracellular sorting and processing of a yeast vacuolar hydrolase: proteinase A propeptide contains vacuolar targeting information. Mol Cell Biol **8:** 2105–2116

Koch KE (1996) Carbohydrate-modulated gene expression in plants. Annu Rev Plant Physiol Plant Mol Biol **47:** 509–540

Lee H-S, Sturm A (1996) Purification and characterization of neutral and alkaline invertase from carrot. Plant Physiol **112:** 1513–1522

Lorenz K, Lienhard S, Sturm A (1995) Structural organization and differential expression of carrot β-fructofuranosidase genes: identification of a gene coding for a flower bud-specific isozyme. Plant Mol Biol **28:** 189–194

Matsushita K, Uritani I (1974) Change in invertase activity of sweet potato in response to wounding and purification and properties of its invertases. Plant Physiol **54:** 60–66

Miller ME, Chourey PS (1992) The maize invertase-deficient *miniature-1* seed mutation is associated with aberrant pedicel and endosperm development. Plant Cell **4:** 297–305

Morris DA, Arthur ED (1984) Invertase and auxin-induced elongation in internodal segments of *Phaseolus vulgaris*. Phytochemistry **23:** 2163–2167

Morris DA, Arthur ED (1985) Invertase activity, carbohydrate metabolism and cell expansion in the stem of *Phaseolus vulgaris* L. J Exp Bot **36:** 623–633

Ohyama A, Ito H, Sato T, Nishimura S, Imai T, Hirai M (1995) Suppression of acid invertase activity by antisense RNA modifies the sugar composition of tomato fruit. Plant Cell Physiol **36:** 369–376

Pfeiffer I, Kutschera U (1995) Sucrose metabolism and cell elongation in developing sunflower hypocotyls. J Exp Bot **46:** 631–638

Ramloch-Lorenz K, Knudsen S, Sturm A (1993) Molecular characterization of the gene for carrot cell wall β-fructosidase. Plant J **4:** 545–554

Ricardo CPP, Ap Rees T (1970) Invertase activity during the development of carrot roots. Phytochemistry **9:** 239–247

Riffkin HL, Duffus CM, Bridges IC (1995) Sucrose metabolism during endosperm development in wheat (*Triticum aestivum*). Physiol Plant **93:** 123–131

Roitsch T, Bittner M, Godt DE (1995) Induction of apoplastic invertase of *Chenopodium rubrum* by D-glucose and a D-glucose analog and tissue-specific expression suggest a role in sink-source regulation. Plant Physiol **108:** 285–294

Ross HA, McRae D, Davies HV (1996) Sucrolytic enzyme activities in cotyledons of the faba bean. Plant Physiol **111:** 329–338

Silva MP, Ricardo CPP (1992) β-Fructosidases and in vitro dedifferentiation-redifferentiation of carrot cells. Phytochemistry **31:** 1507–1511

Sprenger N, Bortlik K, Brandt A, Boller T, Wiemken A (1995) Purification, cloning, and functional expression of sucrose:fructan 6-fructosyltransferase, a key enzyme of fructan synthesis in barley. Proc Natl Acad Sci USA **92:** 11625–11656

Sturm A (1996) Molecular characterization and functional analysis of sucrose-cleaving enzymes in carrot (*Daucus carota* L.). J Exp Bot **47:** 1187–1192

Sturm A, Chrispeels MJ (1990) cDNA cloning of carrot extracellular β-fructosidase and its expression in response to wounding and bacterial infection. Plant Cell **2:** 1107–1119

Sturm A, Hess D, Lee H-S, Lienhard S (1999) Neutral invertase is a novel type of sucrose-cleaving enzyme. Physiol Plant (in press)

Sturm A, Lienhard S, Schatt S, Hardegger M (1999) Tissue-specific expression of two genes for sucrose synthase in carrot (*Daucus carota* L.). Plant Mol Biol **39:** 349–360

Sturm A, Šebková V, Lorenz K, Hardegger M, Lienhard S, Unger C (1995) Development- and organ-specific expression of the genes for sucrose synthase and three isoenzymes of acid β-fructofuranosidase in carrot. Planta **195:** 601–610

Sung S-JS, Xu D-P, Black CC (1989) Identification of actively filling sucrose sinks. Plant Physiol **89:** 1117–1121

Tang G-Q, Lüscher M, Sturm A (1999) Antisense repression of vacuolar and cell wall invertase in transgenic carrot alters early plant development and sucrose partitioning. Plant Cell **11:** 177–189

Tymowska-Lalanne Z, Kreis M (1998) The plant invertases: physiology, biochemistry and molecular biology. Adv Bot Res **28:** 71–117

Unger C, Hardegger M, Lienhard S, Sturm A (1994) Soluble acid β-fructofuranosidase and comparison with the cell wall isoenzyme. Plant Physiol **104:** 1351–1357

Unger C, Hofsteenge J, Sturm A (1992) Purification and characterization of a soluble β-fructofuranosidase from *Daucus carota*. Eur J Biochem **204:** 915–921

Vijn I, Smeekens S (1999) Fructan: more than a reserve carbohydrate? Plant Physiol **120:** 351–359

Weber H, Borisjuk L, Heim U, Buchner P, Wobus U (1995) Seed coat-associated invertases of fava bean control both unloading and storage functions: cloning of cDNAs and cell type-specific expression. Plant Cell **7:** 1835–1846

Weil M, Krausgrill S, Schuster A, Rausch T (1994) A 17-kDa *Nicotiana tabacum* cell-wall peptide acts as an in-vitro inhibitor of the cell-wall isoform of acid invertase. Planta **193:** 438–445

Woodson WR, Wang H (1987) Invertases of carnation petals: partial purification, characterization and changes in activity during petal growth. Physiol Plant **71:** 224–228

Wu L-L, Mitchell JP, Cohen NS, Kaufman PB (1993) Gibberellin (GA_3) enhances cell wall invertase activity and mRNA levels in elongating dwarf pea (*Pisum sativum*) shoots. Int J Plant Sci **154:** 280–289

Xu J, Avigne WT, McCarty DR, Koch K (1996) A similar dichotomy of sugar modulation and develpomental expression affects both path of sucrose metabolism: evidence from a maize invertase gene family. Plant Cell **8:** 1209–1220

Xu J, Pemberton GH, Almira EC, McCarty DR, Koch KE (1995) The *Ivr1* gene for invertase from maize. Plant Physiol **108:** 1293–1294

Zrenner R, Schüler K, Sonnewald U (1996) Soluble acid invertase determines the hexose-to-sucrose ratio in cold-stored potato tubers. Planta **198:** 246–252