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Induction of Apoplastic Invertase of *Chenopodium rubrum* by D-Glucose and a Glucose Analog and Tissue-Specific Expression Suggest a Role in Sink-Source Regulation[1]

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Photoautotrophic suspension-culture cells of *Chenopodium rubrum* that were shifted to mixotrophic growth by adding glucose were used as model system to investigate the influence of the source-sink transition in higher plants on the expression and enzyme activities of intracellular and extracellular invertases. The complete cDNA coding for an extracellular invertase was cloned and sequenced from *C. rubrum*, and its identity has been proven by heterologous expression in *Saccharomyces cerevisiae*. The higher activity of extracellular invertase after preincubation in the presence of glucose was paralleled by an increased expression of the corresponding gene. The induction by glucose could be mimicked by the nonmetabolizable glucose analog 6-deoxyglucose. Both enzyme activity and mRNA level of extracellular invertase showed a sink-tissue-specific distribution in plants. The activity of neutral and acidic intracellular invertases were not affected by preincubation of autotrophic tissue cultures with sugars, nor did they show a tissue-specific distribution in plants. The data suggest that apoplastic invertase not only has an important function in phloem unloading and carbohydrate partitioning between source and sink tissues but may also have a role in establishing metabolic sinks.

It is a fundamental characteristic of plant cells to use photosynthesis as a source of energy, which results in carbohydrates as primary products. Although carbon autotrophy is the most typical feature of plant cells, whole plants represent physiological mosaics of photosynthetically active tissues such as mature leaves (source tissue) exporting carbohydrates to photosynthetically less active or inactive tissues such as stems, flowers, and roots (sink tissues). All plants develop from a purely heterotrophic embryo, and plant growth is accompanied by changes with respect to which organs represent sink or source tissue. Since the dominant metabolic pathway within a plant cell is not strictly determined, the question arises of how the observed source-sink transitions are regulated. In addition, regulatory mechanisms have to be assumed to determine the relative sink strength of plant tissues, which ultimately influences the partitioning of carbohydrates between competing sink regions.

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There is increasing evidence that sugars not only serve as the carbon source for heterotrophic growth but are also involved in the transcriptional regulation of enzymes involved in photosynthesis and heterotrophic metabolism (Farrar, 1991; Williams et al., 1992). This type of regulation by Glc, Fru, or Suc has been designated as metabolic regulation (Karrer and Rodriguez, 1992). Induction of enzymes involved in heterotrophic metabolism by sugars have been described for the green algae *Chlorella* (Hilgarth et al., 1991) and several higher plant species (Farrar 1991; Koch et al., 1992, and refs. therein). Suc is the main transport sugar in most plant species and enzymes involved in Suc metabolism in sink organs are expected to be important both in the establishment of sinks and in determining relative sink strength.

Invertases (EC 3.2.1.26; β-fructosidase) catalyze the hydrolysis of Suc to Glc and Fru. Plant invertases have been studied extensively during the past decades and invertases have been purified from a wide variety of plants and tissues. Most tissues analyzed contain multiple forms of invertases that are characterized by their different intracellular localizations, by pH optima, and by pIs (Avigad, 1982). Soluble invertases are always intracellular and differ in their pH optima; acidic invertases (with activity optima in the range of pH 3.5–5.1) are thought to function in the vacuole of the plant cell, whereas alkaline invertases (pH 7.0–7.8) are cytoplasmic. In the apoplast an invertase with an acidic pH optimum is ionically bound to the cell wall.

There is still much uncertainty as to the function of the different intracellular isoenzymes; it has been proposed that the soluble invertases participate in the regulation of hexose levels in mature leaves (Ricardo, 1974; Ricardo and Sovia, 1974) and fruits (Lingle and Dunlop, 1987), in the mobilization of Suc stored in vacuoles, in turgor regulation (Leigh et al., 1979), and in gravistimulation (Wu et al., 1993). The extracellular invertase of carrot was the first cloned plant invertase (Sturm and Chrispeels, 1990), and in recent years several partial and full-length clones of intracellular and extracellular plant invertases have been isolated and sequenced (Arai et al., 1992; Klann et al., 1992; Hedley et al., 1993; Wu et al., 1993). Several lines of physiological and genetic evidence suggest an important role of cell-wall-bound extracellular invertase in carbohydrate partitioning in plants. Transgenic plants overexpressing yeast invertase (Stitt et al., 1990; van Schaewen et al., 1990;

Dickinson et al., 1991) demonstrated sink regulation of photosynthetic metabolism in source tissue; the accumulating carbohydrates caused inhibition of photosynthesis and an increase in respiration due to the action of the heterologous invertase. Deficiency in invertase activity in the corn mutant *miniature-1* causes aberrant pedicel and endosperm development (Miller and Chourey, 1992). Genetic evidence obtained with this mutant indicates that invertase activity directly affects the metabolic and developmental stability of the maternal cells.

To study the effect of source-sink transition on the enzymatic activity and the expression of intracellular and extracellular invertases, a photoautotrophic cell-suspension culture of *Chenopodium rubrum* (Hüsemann and Barz, 1977; Hüsemann, 1981), which may be shifted to mixotrophic growth by adding Glc (Schäfer et al., 1992), was used. The use of tissue cultures has the advantage that growth conditions may be strictly controlled and experiments are not complicated by differentiation processes. In addition, the use of photoautotrophic cultures to analyze sugar effects obviates the need for a starvation period prior to the experiment because the cells are cultured in a carbohydrate-free medium; thus, effects due to energy deprivation are less likely to occur. Furthermore, the use of these cultures excludes wounding and stress syndromes observed with cut plant tissues.

In this paper, data are presented concerning the effect of sugars on the activities of intercellular and extracellular invertases in photoautotrophic suspension cultures. The results obtained with tissue cultures have been complemented by analyzing the distribution of the different invertase activities in *C. rubrum* plants. The cDNA coding for extracellular invertase of *C. rubrum* has been cloned and sequenced. Its transcriptional regulation by sugars and its tissue-specific expression in plants have been analyzed. Based on the observed induction by Glc and the sink-specific expression in plants, the possible function of apoplastic invertase in regulating source-sink transition and carbohydrate partitioning is discussed.

MATERIALS AND METHODS

Plants, Cell Cultures, and Bacterial and Yeast Strains

The photoautotrophic, hormone- and vitamin-independent suspension culture of *Chenopodium rubrum L.* (fat hen or goosefoot; Hüsemann, 1981) is a derivative of the culture established by Hüsemann and Barz (1977) and has been subcultured in our laboratory since 1991. The cells were grown as described previously (Roitsch and Tanner, 1994). Photomixotrophic growth was initiated by adding 100 mM D-Glc, Suc, or Fru to the medium.

Chenopodium plants were grown in the greenhouse under natural light conditions. The *Escherichia coli* strain DH5α was used for cloning into plasmids and the *E. coli* strain C600Hfl for work with the λ phage. The *Saccharomyces cerevisiae* strain SEY2102 (Emr et al., 1983) was used for heterologous expression for the extracellular invertase and the strain DBY746 as a positive control for yeast invertase assays.

Invertase Activity Assays

To assay invertase activity in plant suspension cultures, cells were washed once with Murashige-Skoog medium (Murashige and Skoog, 1962) and resuspended at 50% packed cells in homogenization buffer (200 mM Hepes, pH 7.5; 3 mM DTT; 3 mM $MgCl_2$; 1 mM EDTA; 2% glycerol). Cells were disrupted by sonicating four times for 10 s at 50 W with a Branson sonifier model B-12 (G. Heinemann, Schwäbisch Gmünd, Germany). Tissue from *Chenopodium* plants were homogenized in a small volume of homogenization buffer using a mortar and pestle. The homogenate suspensions were centrifuged at 14,000*g* for 10 min at 4°C; the supernatant (cytosolic) fraction was removed and used as the soluble enzyme preparation. The pellet (cell-wall fraction) was washed three times with distilled water and resuspended in homogenization buffer. Both enzyme preparations were dialyzed against 12.5 mM potassium phosphate buffer, pH 7.4, for 3 h at 4°C.

Invertase activity at pH 4.5 and 7.0 was tested according to the method of Sung et al. (1989), except that the samples were incubated for 30 min at 26°C. A Glc test kit (Boehringer Mannheim) was used according to the manufacturer's instructions to determine the amount of Glc liberated. Boiled extracts were used as controls. Extract protein contents were determined according to the method of Bradford (1976) with BSA as the standard protein. Specific activity of invertase is reported as micrograms of Glc produced per milligram of protein per hour. The data shown in the paper are the mean values from at least five independent experiments.

To determine intracellular and extracellular invertase activity of yeast cells, spheroplast lysate fractions were prepared essentially as described by Novick and Schekman (1979), and invertase activities were determined as described above.

Nondenaturing PAGE

To release the cell-wall-bound invertase from the cell wall the washed pellet fraction (see above) was resuspended in homogenization buffer containing 1 M NaCl and 15 mM EDTA and incubated overnight. Bound enzyme was recovered in the supernatant subsequent to centrifugation at 14,000*g* for 10 min at 4°C. This preparation was concentrated using Centricon-30 centrifugation concentrators (Amicon, Beverly, MA) according to the instructions of the supplier. The extracted invertase was separated on 7.5% native polyacrylamide gels according to the method of Reisfeld et al. (1962). Proteins were applied at the anode and electrophoresis was performed at a constant current of 30 mA for 6 h at 4°C. Invertase activity was visualized by incubating the gels in buffer (70 mM K_2PO_4/40 mM citrate) containing 7% Suc for 1 h at 30°C. The hydrolysis products were detected with 0.1% 2,3,5-triphenyltetrazolium chloride dissolved in 0.5 M NaOH. Color development was stopped with 10% acetic acid.

Isolation of RNA

For the isolation of nucleic acids, cells were harvested by centrifugation, frozen in liquid nitrogen, and ground with a mortar and pestle in the presence of liquid nitrogen. Total nucleic acids were isolated according to the method of Bell et al. (1986) and total RNA was isolated by using Nucleobond AX columns (Macherey-Nagel, Düren, Germany) according to the instructions of the supplier. Polyadenylated mRNA was isolated by affinity chromatography on two successive oligo(dT)-cellulose columns (Maniatis et al., 1982).

PCR Amplification

Degenerate oligonucleotide primers were used for PCR amplification of invertase cDNA sequences. Sequences of the two primers, used, OIN3, CCTTCAC(C/T)T(A/G/C/T)TT(C/T)TA(C/T)CA(A/G)TA(C/T)AA(C/T)CC and OIN4, CCTTTC(A/G)(A/T)A(A/G)AA(A/G)GT(C/T)TT(A/G/T)G(A/T)(A/T)GCGTA, were based on the two conserved amino acid motifs H(L/F)FYQYNP and (Y/F)YASKTF(Y/F)D in the N-terminal part of extracellular invertase from carrot (Sturm and Chrispeels, 1990) and intracellular invertase from mung bean (Arai et al., 1992) and tomato (Klann et al., 1992). To amplify cDNA sequences by PCR a cDNA first strand was synthesized as described by Sambrook et al. (1989) using 0.5 μg of mRNA and 2.5 ng/μL of statistically random hexamers as primers in a total volume of 20 μL. PCR amplification was performed in the DNA Thermal Cycler (Perkin-Elmer Cetus). The reaction mixture (100 μL) contained either 20 μL of the cDNA reaction mixture as described above or 5 μg genomic DNA, 100 pmol each of primer OIN3 and OIN4, 155 μM of deoxyribonucleotide triphosphates, 2 units of Taq DNA polymerase (Perkin-Elmer Cetus), and Taq DNA polymerase reaction buffer according to the manufacturer's instructions. Denaturation of DNA was carried out at 95°C for 6 min for the first cycle and then for 1 min. Primer annealing and primer extension reactions were carried out at 48°C for 1 min and at 72°C for 1 min, respectively. A total of 35 cycles of amplification were performed. A sequence-specific primer (OIN5, GATGTGGGTTTCATGAACGACTCG) and an oligo(dT) primer were used to carry out 3′ anchored PCR essentially as described by Frohman et al. (1988). PCR products were visualized on 1.2% agarose gels stained with ethidium bromide. The PCR products were purified by phenol extraction from low-melting-point agarose gels. PCR products were blunt-ended with Klenow polymerase, phosphorylated using T4 DNA kinase, and purified from low-melting-point agarose. PCR products were cloned into the *Sma*I site of pUC19 (Yanisch-Perron et al., 1985) using *E. coli* strain DH5α as host.

Construction of a cDNA Library and Screening

Polyadenylated RNA (5 μg) isolated from Glc-incubated suspension-culture cells was used to construct a cDNA library by means of a cDNA synthesis kit (Pharmacia) according to the instructions of the supplier except for the use of a CL-4B Sepharose column for the separation of nonlinked adapters. The cDNA was cloned into λgt10 using a commercial packaging kit (Promega). The library was screened using standard procedures (Sambrook et al., 1989) with the radiolabeled PCR fragment from plasmid pRR22/4/34 coding for a putative extracellular invertase. Positive clones were subsequently purified and the *Eco*RI insert of one clone was subcloned into pUC19, resulting in plasmid pMB3.

Sequencing

Nucleotide sequencing using the dideoxy chain-termination method (Sanger et al., 1977) was performed by using the Sequenase 2.0 kit (United States Biochemical) with subcloned fragments and custom-made oligonucleotide primers for sequencing of internal sequences. Sequence analysis was performed using the sequence analysis software package of the University of Wisconsin Genetics Computer Group (Devereux et al., 1984) on a VAX microcomputer.

Northern Blot Analysis

Northern blot hybridizations were performed as described by Sauer and Tanner (1989). Isolated DNA fragments used as hybridization probes were with [α-^{32}P]dATP by oligolabeling (Feinberg and Vogelstein, 1983).

RESULTS

Activity of Intracellular and Extracellular Invertases of Suspension-Culture Cells after Preincubation with Sugars

The effect of mixotrophic growth on the activity of the different types of invertases in *C. rubrum* suspension-culture cells was tested. A photoautotrophic culture was split into two parallel cultures in the late logarithmic growth phase. To one of the cultures 100 mM D-Glc was added and incubation was continued for 48 h. The second culture was maintained under photoautotrophic conditions and used as a control. The suspension-culture cells were homogenized by sonication and the homogenate was separated into a cytosolic supernatant fraction and a cell-wall fraction. The activity of acidic and neutral invertases was determined in the different fractions and is shown in Table I. The activity of both neutral and acidic invertases in the cytosolic fraction was very low (0.31–0.44 μg Glc h^{-1} mg^{-1} protein) and was not affected by preincubation with D-Glc. In the cell-wall fractions only an acidic invertase could be detected, and the activity of the cell-wall-bound acidic invertase was 5.7 times higher than that of the soluble acidic invertase in the corresponding cytosolic fraction. Preincubation in the presence of 100 mM D-Glc resulted in significantly increased activity of the apoplastic invertase; the activity was 2.2 times higher than in the photoautotrophic control cells. In further experiments we tested the effect of preincubation with the substrate Suc. The activity was 3.5 times higher than in photoautotrophically maintained control cells and thus exceeded the stimulation by Glc. To rule out nonspecific induction due to the osmotic effect of the sugars applied, the suspension-culture cells

Table I. *Activity of intracellular and extracellular invertases of C. rubrum suspension-culture cells and of different tissues of C. rubrum plants*

The results shown are the means of at least five independent experiments; SDs are given in parentheses. n.d., Not determined.

Cell Type	Specific Invertase Activity		
	Intracellular		Extracellular
	pH 4.5	pH 7.0	
	μg Glc h^{-1} mg^{-1} protein		
Suspension culture cells			
Autotrophic	0.401 (0.039)	0.331 (0.028)	1.902 (0.189)
Glc[a]	0.442 (0.041)	0.313 (0.027)	4.184 (0.411)
Suc[a]	n.d.	n.d.	6.651 (0.614)
Mannitol[a]	n.d.	n.d.	2.205 (0.199)
Plants			
Source leaves	0.078 (0.007)	0.062 (0.005)	0.115 (0.012)
Stems	0.032 (0.003)	0.060 (0.006)	0.221 (0.021)
Roots	0.078 (0.006)	0.098 (0.009)	3.420 (0.287)

[a] The cells were incubated for 36 h with 100 mM of D-Glc, Suc, or mannitol, respectively.

were incubated in the presence of mannitol; Table I shows that the addition of 100 mM mannitol did not result in a significantly increased invertase activity.

To further characterize the cell-wall-associated invertase from *C. rubrum* the cell-wall extract was treated with 1 M NaCl and 15 mM EDTA. By this incubation 85 to 95% of the activity could be released from the cell wall. The solubilized extracellular invertase was separated on a native polyacrylamide gel and visualized by an activity stain (data not shown). Under the experimental conditions used, the protein migrated to the anode, indicating that it has a pI above 8.0. The higher invertase activity after preincubation with Suc compared to photoautotrophically grown cells also was visible on the activity gel.

Activity of Intracellular and Extracellular Invertase in Different Tissues of *C. rubrum* Plants

Since the activity of extracellular invertase of photoautotrophic suspension-culture cells was affected by a shift to mixotrophic growth, we tested the activities of intracellular and extracellular invertases in source and sink tissues of *C. rubrum* plants. Fresh tissue was homogenized by grinding with a mortar and pestle, and the homogenate was separated into a cytosolic and a cell-wall fraction as described for the suspension-culture cells.

Table I shows that the activity of both neutral and acidic invertases in the cytosolic fraction was low (0.032–0.098 μg Glc h^{-1} mg^{-1} protein) and no significant difference was found in the different tissues analyzed. In the cell-wall fraction, only an acidic invertase could be detected, which thus showed a tissue-specific distribution. Whereas the activity was very low in source leaves, 0.115 μg Glc h^{-1} mg^{-1} protein, the activity was 1.9 times higher in stems and 30 times higher in roots.

cDNA Cloning of an Extracellular Invertase from *C. rubrum* and Sequence Analysis

Degenerate oligonucleotide primers based on the two conserved amino acid motifs H(L/F)FYQYNP and (Y/F)ASKTF(Y/F)D in the N-terminal part of extracellular invertase of carrot (Sturm and Chrispeels, 1990) and the intracellular invertases of mung bean (Arai et al., 1992) and tomato (Klann et al., 1992) were used to amplify cDNA sequences from *C. rubrum* suspension-culture cells. Amplification by PCR resulted in the expected product of about 700 bp. The PCR products were subcloned into pUC19 and preliminary characterization by restriction enzyme analysis using 4-bp-cutter enzymes revealed heterogeneity in the amplification product. Sequence analysis revealed three different types of sequences. Two of the nucleic acid sequences were highly homologous (52% identity) and showed a high homology to cloned invertase cDNA sequences. One of the nucleotide sequences (clone pRR22/4/34) showed a higher homology to the extracellular carrot invertase (62% identity); the encoded protein is henceforth referred to as CIN1. The other sequence (clone pRR22.25) had a higher homology to the two intracellular sequences (66 and 72%), and the encoded protein is referred to as CIN2. The third type of PCR product showed no significant homology to any sequence in the nucleic acid sequence data bases.

The PCR fragment with the higher homology to extracellular invertase was used to screen a λgt10 cDNA library of *C. rubrum*. Of 10 positive clones, the largest cDNA insert was subcloned into pUC19 and sequenced. Since the 1.8-kb insert of the cDNA clone contained no poly(A) tail, the missing 3′ noncoding sequences (16 bp) were cloned by anchored PCR using a cDNA-specific primer and an oligo(dT) primer with cDNA as substrate as described in "Materials and Methods."

The complete cDNA sequence and the derived amino acid sequence are shown in Figure 1. The cDNA sequence contains one open reading frame starting from nucleotide 26 with an ATG start codon and ending at nucleotide 1743 with a UUA stop codon. The putative translational start site is the only Met codon between a stop codon (UUA) close to the 5′ end of the cDNA and the DNA sequence. In addition the −4 and −5 positions with respect to the Met codon are identical with the proposed consensus sequence

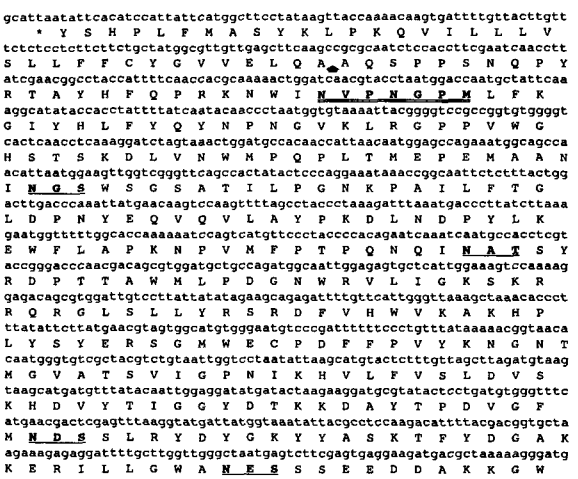

Figure 1. The nucleotide and deduced amino acid sequence of a cDNA of acidic invertase from *C. rubrum*. Deduced amino acids are shown in the one-letter code. The arrow shows a possible signal sequence cleavage site. The β-fructosidase motif (NVPNGPM) is underlined twice, and potential sites of *N*-glycosylation are underlined by a single line.

of start Mets in plants (Lütcke et al., 1987). The open reading frame encodes a polypeptide chain of 573 residues with a calculated molecular mass of 64.6 kD.

In addition to the open reading frame, the cDNA also contains 25 bp of 5′ untranslated and 210 bp of 3′ untranslated sequence with a poly(A) tail. The consensus signal for polyadenylation AATAAA, which is usually located 10 to 30 nucleotides upstream of the poly(A) tail, is lacking in the cDNA sequence. It might be replaced by the sequence AATTAAT, located 28 bp upstream of the poly(A) tail. Such a deviation from the consensus sequence theme has also been shown for several other plant mRNAs (Heidecker and Messing, 1986). The N terminus of the deduced amino acid sequence shows typical characteristics of a cleavable signal sequence with a putative cleavage site at position 28 (von Heijne, 1983). The protein sequence contains four potential glycosylation sites, and the calculated pI is 9.9.

The deduced protein sequence of CIN1 has a high homology to published invertase sequences. The dendrogram shown in Figure 2 shows that CIN1 is more closely related to the known extracellular invertases than to intracellular invertases. The identity with the extracellular invertase of carrot is 50.6% (66.4% homology; Sturm and Chrispeels, 1990) and with the partial sequence of a putative extracellular invertase of potato it is 51.9% (69.2%; Hedley et al., 1993). The homology to cloned intercellular invertases is much lower: the derived amino acid sequence of the cloned cDNA shows 40.8% identity (59.1% homology) with the intracellular invertase from tomato (Klann et al., 1992) and 44.9% identity (61.8% homology) with a soluble invertase from mung bean (Arai et al., 1992).

A comparison of two of the highly conserved amino acid sequences among invertases is shown in Figure 3. CIN1 contains a modified β-fructosidase motif (NVPNGPM, Fig. 3A) present in most of the cloned Suc-cleaving enzymes (Sturm and Chrispeels, 1990). The second highly conserved box is shown in Figure 3B. The thio group of the conserved Cys has been proposed to be important for the catalytic function of invertases (Hedley et al., 1993) and the amino acid that follows it seems to be characteristic for extracellular and intracellular invertases. All known extracellular invertases, including the CIN1 sequence, possess a Pro residue at this position, whereas in the plant intracellular invertases the prolin residue is substituted by a Val.

Heterologous Expression of the Extracellular Invertase of *C. rubrum*

To prove definitely that the cloned cDNA codes for an extracellular invertase, the invertase protein CIN1 was expressed in the bakers' yeast *S. cerevisiae*. The cDNA coding for extracellular invertase was cloned in both sense and antisense orientations downstream of the plasma membrane ATPase promoter of *S. cerevisiae* in the yeast expression vector pNEV-E (Sauer and Stolz, 1994), resulting in plasmids pRR32.02 and pRR32.05, respectively. The yeast strain SEY2102 (Emr et al., 1983), which carries a mutation in the endogenous invertase gene, was transformed with the different plasmid constructs and both intracellular and

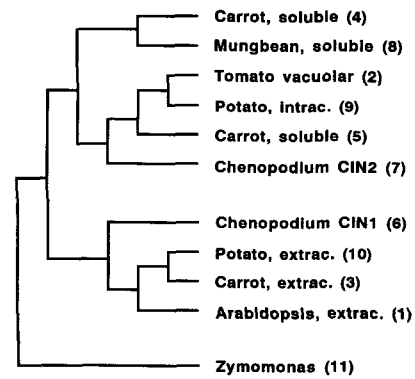

Figure 2. Phylogenetic relation of CIN1 and CIN2 to other cloned plant invertases and the invertase from *Zymomonas*. The dendrogram was generated by comparison of the deduced amino acid sequences by the PILEUP program of the University of Wisconsin Genetics Computer Group sequence analysis software package (Devereux et al., 1984). Sources: 1, Schwebel-Dugue et al., 1994; 2, Elliot et al., 1993; 3, Sturm and Chrispeels, 1990; 4, Unger et al., 1994; 5, EMBL accession No. X67163; 6, this work, EMBL accession No. X81792; 7, this work, EMBL accession No. X81793; 8, Arai et al., 1992; 9, EMBL accession No. X70368; 10, Hedley et al., 1993; 11, Yanase, 1991.

A

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                                                    88
Arabidopsis, extrac. (1) ...R..TGFH FQPPKNW.M N DPNG PMIYKG
Arabidopsis, extrac. (2) .....QKHYI YDCM.QYS. K IATA PMLYKG
Carrot, extrac. (3)      .....RTGYH FQPKQNW.I N DPNG PMYYKG
Carrot, soluble (4)      SWQR..TSFH FQPQENW.M N DPNG PLFHMG
Carrot, soluble (5)      ..WQRS.SFH FQPNQNW.M N DPNG PLFYKG
Chenopodium CIN1 (6)     .....RTAYH FQPRKNW.I N VPNG PMLFKG
Chenopodium CIN2 (7)     .C......LH CDSR..... . ..GS PL....
Mungbean, soluble (8)    SWQR..TSFH FQPEKNW.W N DPNG PMYYKG
Potato, intrac. (9)      ..WQRT.AYH FQPQKNW.M N DPNG PLYHKG
Potato, extrac. (10)     ......TGYH FQPPKNW.I N DPNA PMYYNG
Potato, extrac. (11)     ......TGYH FQPPKNW.I N DPNG PMYYNG
Tomato, vacuolar (12)    W.QR..TAYH FQPQKNW.M N DPNG PLYHKG
Zymomonas, soluble (13)  SSEWY.PGFH VTPLTGW.M N DPNG LIFFKG
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B

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                                                    269
Arabidopsis, extrac. (1) ... .......G.M WECPD FFPVT RFG
Arabidopsis, extrac. (2) ... .......G.M WECPD FFPVS VTDKKNRLD.
Carrot, extrac. (3)      ... .......G.M WECPD FFPVS LKGL......
Carrot, soluble (4)      ... .......G.M WECVD FYPVS VTGS......
Carrot, soluble (5)      ... .......G.M WECVD FYPVS KFG.......
Chenopodium CIN1 (6)     PLY SYERS..G.M WECPD FFPVY KNG.......
Chenopodium CIN2 (7)     ... .......G.M WECVD FYPVS TTG.......
Mungbean, soluble (8)    ... .....GTG.M WECVD FFPVS KKN.......
Potato, intrac. (9)      ... .......G.M WECVD FYPV. ....S.....
Potato, extrac. (10)     ... .......G.. WECPD FFPVL LHG.......
Potato, extrac. (11)     ... .......GN. WECPD FFPV. ....S.....
Tomato, vacuolar (12)    ... .......G.M WECVD FYPVS TK........
Zymomonas, soluble (13)  ... .........M WECPD FF..S L.........
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Figure 3. Comparison of highly conserved regions of the deduced amino acid sequence of CIN1 and CIN2 with those of other plant invertases and an invertase of *Zymomonas*. The amino acid sequences are in the one-letter code and have been aligned by introducing gaps (. . .) to maximize identity using the LINEUP and PRETTY program of the University of Wisconsin Genetics Computer Group sequence analysis software package (Devereux et al., 1984). References for the various invertase sequences are given in the legend of Figure 2. The numbers refer to the positions of the corresponding amino acids in the sequence of cell-wall invertase of carrot (Sturm and Chrispeels, 1990). A, Region containing the β-fructosidase motif (boxed). B, Region containing a Cys residue important for catalytic activity of invertases (Sturm and Chrispeels, 1990). The highly conserved region is boxed and the position following the Cys residue, conserved within the extracellular and intracellular invertases, is marked by an arrow. extrac., Extracellular; intrac., intracellular.

extracellular invertase activities of the transformed yeast cells were determined. Yeast strain DBY 746, expressing intracellular and extracellular yeast invertase, was used as a positive control. Table II shows that in the yeast strain SEY2102, when the yeast was transformed with plasmid pRR3202 but not with plasmids pRR3205 and pNEV-E, both an intracellular and extracellular invertase activity could be measured. The measured extracellular invertase activity was significantly lower. It remains to be determined whether the signal sequence of the plant extracellular invertase does not function properly in yeast or whether the heterologous protein is trapped within the yeast secretory pathway.

Expression of Extracellular Invertase under Autotrophic and Mixotrophic Culture Conditions

To address the question of whether the higher activity of extracellular invertase after preincubation with D-Glc or Suc is due to an induction of the corresponding gene, we carried out a northern blot analysis. Total RNA was isolated from a photoautotrophic culture and a culture that was preincubated in the presence of 100 mM of D-Glc, Fru, or Suc for 48 h. The RNA was probed with the radiolabeled 1.8-kb cDNA insert of pMB3. After preincubation in the presence of 100 mM D-Glc, Fru, or Suc, a transcript of about 2.0 kb could be demonstrated (Fig. 4A). In contrast, in the autotrophic culture, only a very low level of this specific transcript could be detected (visible only after overexposure of the x-ray film).

To control for the effects of osmotica on extracellular invertase expression, suspension-culture cells were incubated in equivalent molarities of mannitol. Preincubation in the presence of 100 mM mannitol induces expression of extracellular invertase, although induction by Glc, Fru, or Suc is much higher (Fig. 4A).

Characterization of the Induction of Extracellular Invertase by Sugars

In further experiments, the nature of induction of extracellular invertase by D-Glc was determined more precisely. To study the kinetics of induction by Glc, 100 mM Glc was added to photoautotrophically grown cells, and samples were removed at different time intervals. Expression of extracellular invertase could already be detected after 1 h and increased up to 48 h (Fig. 4B).

The minimal Glc concentration necessary to induce expression of intracellular invertase was determined by incubating the suspension-culture cells in the presence of increasing concentrations of D-Glc. The cells were incubated for 12 h, which was shown to be sufficient time to

Table II. *Expression of extracellular invertase from C. rubrum in S. cerevisiae*

Strain	Specific Invertase Activity	
	Intracellular	Extracellular
	μg Glc h^{-1} mg^{-1} protein	
SEY2102(pRR32.02)	1.61 (0.08)	0.63 (0.03)
SEY2102(pRR32.05)	0.01 (0.00)	0.0 (0.0)
SEY2102(pNEV-E)	0.00 (0.00)	0.0 (0.0)
DBY746	1.16 (0.05)	0.95 (0.04)
DBY746, derepressed[a]	1.46 (0.07)	290.1 (14.3)

[a] Strain DBY was derepressed for expression of extracellular yeast invertase as described by Schauer et al. (1985). The results shown are the means of three independent experiments; SDs are given in parentheses.

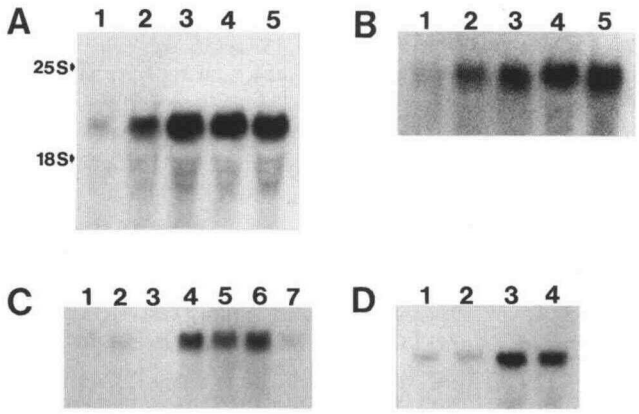

Figure 4. Northern blot analysis of total RNA from suspension-culture cells of *C. rubrum*. RNA (25 μg) was separated by formaldehyde-agarose gel electrophoresis, blotted onto nitrocellulose filters, and probed with radiolabeled cDNA coding for extracellular invertase (CIN1) from *C. rubrum*. A, Effect of different sugars and mannitol on CIN1 expression. The cells were grown photoautotrophically (1) or incubated for 48 h with 100 mM mannitol (2), D-Glc (3), Fru (4), or Suc (5). B, Time course of CIN1 expression after addition of 100 mM D-Glc. Samples were removed after 0 h (1), 1 h (2), 12 h (3), 24 h (4), and 48 h (5). C, Influence of different Glc concentrations and mannitol on CIN1 expression. The cells were grown photoautotrophically (1) or incubated for 12 h with 5 mM (2), 10 mM (3), 20 mM (4), 50 mM (5), or 100 mM (6) D-Glc or 20 mM mannitol (7). D, Effect of 6-deoxyglucose on the expression of CIN1. Cells were grown photoautotrophically (1) or incubated for 24 h with 30 mM mannitol (2), 6-deoxyglucose (3), or D-Glc (4).

induce expression of extracellular invertase. As shown in Figure 4C, 20 mM Glc is sufficient to induce the gene for extracellular invertase and increasing concentrations of Glc do not give rise to a higher mRNA level. Incubation with 20 mM mannitol for 12 h had no effect on the expression of extracellular invertase.

To analyze whether Glc or some metabolite of Glc induces the expression of extracellular invertase we preincubated autotrophic suspension-culture cells with the nonmetabolizable Glc analog 6-deoxyglucose. Figure 4D shows that 6-deoxyglucose, which cannot be phosphorylated, induces expression of extracellular invertase to a level comparable to the induction observed in the presence of D-Glc. The control incubation with mannitol rules out a nonspecific osmotic effect on CIN1 expression.

Tissue-Specific Expression of Extracellular Invertase

Expression of extracellular invertase (CIN1) mRNA in different tissues from *C. rubrum* was compared on a northern blot of total cellular RNA (Fig. 5). Probing of the blot with the radioactively labeled insert of cDNA clone pMB3 revealed that the transcript for extracellular invertase could be detected only in roots and not in mature leaves and stems.

DISCUSSION

The complete cDNA coding for a putative extracellular invertase (CIN1) from *C. rubrum* has been cloned and sequenced. The derived protein sequence has a higher homology to cloned extracellular invertases than to intracellular invertases. Other characteristic properties of extracellular invertases are a leader sequence, specific sequence elements, and a high pI. The structural homology was proven by functional expression of the cDNA in *S. cerevisiae*. In addition, the expression pattern of the corresponding gene correlates only with the activity of the extracellular enzyme in the two different systems analyzed, sugar-treated suspension-culture cells and different tissues of intact plants. The extracellular invertase activity of *C. rubrum* could be related to a protein with a high pI ionically bound to the cell wall. These observations strongly suggest that the cloned cDNA encodes the extracellular invertase of *C. rubrum*.

Photoautotrophic suspension cultures were used as a model system to analyze the effect of mixotrophic growth on the expression of extracellular invertase. The higher enzyme activity of cell-wall-bound extracellular invertase in the presence of 100 mM Glc and Suc was paralleled by an increased expression of the corresponding gene. Control experiments using mannitol ruled out a nonspecific induction due to the osmotic effect of the sugars applied. The observed induction of extracellular invertase suggests that the sugars are not only the carbon source for heterotrophic growth but also function as inducers of enzymes of heterotrophic metabolism. This supports the increasing evidence for a central role of hexoses or Suc in the control of metabolic enzymes in algae (Hilgarth et al., 1991) and higher plants (Williams et al., 1992). Whereas photosynthetic enzymes and Chl synthesis are repressed (Sheen, 1990; Krapp et al., 1991; Schäfer et al., 1992), cytoplasmic enzymes involved in sink metabolism were shown to be induced in response to sugars. Induction of metabolic enzymes by hexoses or Suc has been reported for Suc synthase (Salanoubat and Belliard, 1989), ADP-Glc pyrophosphorylase (Müller-Röber et al., 1990), and granule-bound starch synthase (Visser et al., 1991). To our knowledge, this is the first

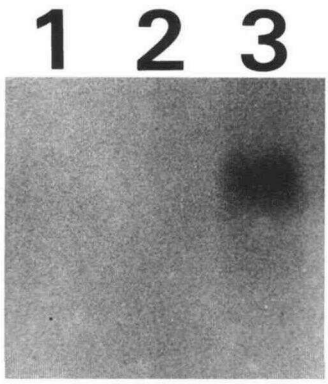

Figure 5. Northern blot analysis of total RNA from different tissues from *C. rubrum* plants. RNA (25 μg) was separated by formaldehyde-agarose gel electrophoresis, blotted onto nitrocellulose filters, and probed with radiolabeled cDNA coding for extracellular invertase from *C. rubrum*. RNA was isolated from source leaves (1), stems (2), and roots (3).

report that shows that extracellular invertase is also a member of the family of enzymes of heterotrophic metabolism that are coordinately induced by sugars. It has been reported that heterotrophically grown carrot suspension cultures contain high levels of mRNA for extracellular invertase (Sturm and Chrispeels, 1990), which is in agreement with our data that Glc, Fru, and Suc induce the corresponding gene in *C. rubrum*. The incubation of photoautotrophic cells in the presence of 20 mM Glc for 12 h was shown to be sufficient to induce the expression of extracellular invertase. The finding that the level of transcription is not further elevated by higher Glc concentrations may indicate that sink metabolism is fully induced above a certain sugar concentration. The concentration of 20 mM is the approximate sugar concentration measured in the apoplast of bean stems (3–100 mM, Patrick, 1990), which supports the physiological role of the observed induction of extracellular invertase by sugars.

The activities of both neutral and acidic intracellular invertases were not affected by preincubation of autotrophic cultures with sugars, nor did they show a tissue-specific distribution in plants. The activities were considerably lower than the activity of the extracellular invertase. The observed differential regulation of the activity of the two acidic invertases supports protein data (Fahrendorf and Beck, 1990) that the intracellular form is not the precursor of the cell-wall-bound extracellular isoenzyme.

The effect of induction of extracellular invertase by Glc could be mimicked by the nonmetabolizable Glc analog 6-deoxyglucose. This observation indicates that Glc and not one of its metabolites is the primary signal that interacts with a putative receptor involved in signal transduction and ultimately results in transcriptional activation of the corresponding gene. Although induction by a Glc analog has not yet been reported for any other enzyme under metabolic control, our finding that a Glc-induced Suc synthase from *C. rubrum* is also induced by 6-deoxyglucose supports the proposed model (D.E. Godt and T. Roitsch, unpublished observations). These data also suggest that the strong effects of Suc on enzyme activity and expression level result from hydrolysis of Suc and transcriptional activation by the cleavage product Glc. This is supported by measurements of the Glc concentration in the medium after the addition of Suc. We have determined a Glc concentration of 20 and 50 mM after 12 and 48 h, respectively, which was shown to be sufficient for transcriptional activation of extracellular invertase expression. Since most plant tissues possess highly active extracellular invertases, Suc is usually cleaved in the apoplast. Thus, hexoses, rather than Suc, also may be the actual signal in systems in which induction by Suc has been reported (Salanoubat and Belliard, 1989; Müller-Röber et al., 1990). Incubation in the presence of Suc resulted in a markedly greater effect on enzyme activity compared to hexose, whereas the resulting mRNA levels were the same. This observation indicates that, in addition to the transcriptional activation via the cleavage product Glc as discussed above, a positive effect of the invertase substrate, Suc, on enzyme activity has to be assumed.

In addition to the specific induction by Glc, the expression of extracellular invertase is nonspecifically induced by high mannitol concentrations, although to a much lower extent. Whereas the incubation in the presence of 20 mM mannitol for 12 h did not result in higher mRNA levels, a significant induction was observed when using 100 mM mannitol for 48 h. A possible explanation is a nonspecific effect of the high osmotic value. Alternatively, the effect may be related to uptake of this sugar alcohol. For other plant species a slow mannitol uptake has been demonstrated (Cram, 1984), which could explain the effect at high concentrations and long incubation times.

When the extracellular invertase is active, Suc is hydrolyzed into the hexose monomers. Thus, the transmembrane Suc gradient between phloem sap and cell wall is increased to drive phloem unloading of Suc into the apoplast. A high extracellular invertase activity increases carbohydrate transport to sink organs and thus may increase sink strength, as has also been proposed by other authors (Morris, 1982; Ho, 1984). According to the proposed mechanism, the observed specific expression in sink organs of *C. rubrum* plants suggests that extracellular invertase may contribute to carbohydrate partitioning between source and sink tissue. Both enzyme activity and level of mRNA were higher in roots than in leaves and stems. The identification of a transcriptionally regulated apoplastic invertase in *C. rubrum* agrees with the model of Eschrich (1980, 1989) for phloem unloading. It has been proposed that a nonregulated Glc uptake system in addition to a regulated invertase is responsible for apoplastic phloem unloading. The data show that extracellular invertase is under dual control; the expression of the corresponding gene is regulated by sugars, as shown above, and the enzyme activity is regulated by the pH of the apoplast fluid, as originally proposed. The hexose monomers will then be taken up into the cytoplasm of the sink cells by a family of high-affinity Glc uptake proteins, which are tissue-specifically expressed (Sauer and Tanner, 1993) and not regulated by sugars (Roitsch and Tanner, 1994). The observed induction of extracellular invertase by sugars suggests that the higher hexose concentration due to Suc hydrolysis further induces invertase expression in the sink cells (feed-forward regulation).

Based on the finding that mRNA levels were elevated as early as 1 h after the photoautotrophic cultures were shifted to mixotrophic growth, it may be proposed that extracellular invertase is involved in the process of source to sink transition. The assumption that cell-wall-bound invertase may have a role in establishing metabolic sinks is further supported by the following facts: (a) Gradients of cell-wall-bound invertase are found around tumors induced by *Agrobacterium tumefaciens* (Weil and Rausch, 1990); (b) the increased hexose contents due to overexpression of the heterologous yeast invertase in transgenic tobacco, Arabidopsis, and tomato (van Schaewen et al., 1990; Dickinson et al., 1991) resulted in the induction of sink metabolism in source leaves (Stitt et al., 1990); (c) in the systems with apoplastic phloem unloading, invertase ac-

tion is the first enzymatic step of metabolism of incoming Suc in sink organs.

In plants sink to source transitions usually occur, whereas in the experiments reported the transition in the opposite direction has been analyzed. However, the data presented in this paper show that the photoautotrophic suspension cultures of *C. rubrum* shifted to mixotrophic growth by adding Glc are an appropriate model system in which to analyze the regulation of source-sink transitions in higher plants. The stimulation of expression of CIN1 after a shift to mixotrophic growth in the suspension cultures is in agreement with its specific expression in sink tissues. In addition, observations from other laboratories also support the use of *C. rubrum* suspension-culture cells to study sink-source regulation (Schäfer et al., 1992; Krapp et al., 1993).

Although there is growing evidence that a number of enzymes involved in sink metabolism are coordinately regulated by hexoses, it remains to be determined which of the enzymes under metabolic control is limiting sink strength. Overproduction of the limiting enzyme under control of the appropriate tissue-specific promoters could be applied to specifically increase sink strength of storage sinks such as fruits or taproots and thus increase the yield of harvestable sink organs.

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The EMBL accession numbers for the sequences reported in this article are X81792 and X81793 for CIN1 and CIN2, respectively.

LITERATURE CITED

Arai M, Mori H, Imaseki H (1992) Cloning and sequence of cDNAs for an intracellular invertase from etiolated hypocotyl of mung bean and expression of the gene during growth of seedlings. Plant Cell Physiol **33:** 245–252

Avigad G (1982) Sucrose and disaccharides. *In* FA Lowus, W Tanner, eds, Encyclopedia of Plant Physilogy, New Series, Vol 13A. Springer Verlag, Berlin, pp 217–347

Bell JN, Ryder TB, Wingate UPM, Bailey JA, Lamb CJ (1986) Differential accumulation of plant defense gene transcripts in a compatible and incompatible plant-pathogen interaction. Mol Cell Biol **6:** 1615–1623

Bradford MM (1976) A rapid and sensitive method for the determination of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72:** 248–254

Cram WJ (1984) Mannitol transport and suitability as an osmoticum in root cells. Physiol Plant **61:** 396–404

Devereux J, Haeberli P, Smithies O (1984) A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res **12:** 387–395

Dickinson CD, Altabella T, Chrispeels MJ (1991) Slow-growth phenotype of transgenic tomato expressing apoplastic invertase. Plant Physiol **95:** 420–425

Elliot 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

Emr SS, Scheckman R, Flessel MC, Thorner J (1983) An *MFalpha1-SUC2* (alpha-factor-invertase) gene fusion for study of protein localization and gene expression in yeast. Proc Natl Acad Sci USA **80:** 7080–7084

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

Eschrich W (1989) Phloem unloading of photoassimilates. *In* DA Baker, JA Milburn, eds, Transport of Photoassimilates. Longman Scientific and Technical, Essex, UK, pp 206–263

Fahrendorf T, Beck E (1990) Cytosolic and cell wall bound invertases from leaves of *Urtica diotica L.*: a comparison. Planta **180:** 237–244

Farrar JF (1991) Starch turnover: its role in source-sink relations and a comparison with the role of sucrose. *In* JL Bonnemain, S Delrot, WJ Lucas, J Dainty, eds, Recent Advances in Phloem Transport and Assimilate Compartmentation. Ouest Editions, Nantes, France, pp 213–223

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction fragments to high specific activity. Anal Biochem **132:** 6–12

Frohman MA, Dush MK, Martin GR (1988) Rapid production of full length cDNAs from rare transcripts: amplification using single gene-specific primer. Proc Natl Acad Sci USA **85:** 8998–9002

Hedley PE, Machray GC, Davies HV, Burch L, Waugh R (1993) cDNA cloning and expression of a potato (*Solanum tuberosum*) invertase. Plant Mol Biol **22:** 917–922

Heidecker G, Messing J (1986) Structural analysis of plant genes. Annu Rev Plant Physiol **37:** 439–466

Hilgarth C, Sauer N, Tanner W (1991) Glucose increase the expression of the ATP/ADP translocator and the glyceraldehyde-3-phosphate dehydrogenase genes in *Chlorella*. J Biol Chem **266:** 24044–24047

Ho LC (1984) Partitioning of assimilates in fruiting tomato plants. Plant Growth Regul **2:** 277–285

Hüsemann W (1981) Growth characteristics of hormone and vitamin independent photoautotrophic cell suspension cultures from *Chenopodium rubrum*. Protoplasma **10:** 415–431

Hüsemann W, Barz W (1977) Photoautotrophic growth and photosynthesis in cell suspension cultures of *Chenopodium rubrum*. Physiol Plant **40:** 77–81

Karrer EE, Rodriguez RL (1992) Metabolic regulation of rice α-amylase and sucrose synthase genes in planta. Plant J **2:** 517–523

Klann E, Yelle S, Bennett AB (1992) Tomato fruit acid invertase complementary DNA. Plant Physiol **99:** 351–353

Koch KE, Nolte KD, McCarty DR, Avigne WT (1992) Sugar levels modulate differential expression of maize sucrose synthase genes. Plant Cell **4:** 59–69

Krapp A, Hofmann B, Schäfer C, Stitt M (1993) Regulation of the expression of rbcS and other photosynthetic genes by carbohydrates: a mechanism for the "sink regulation" of photosynthesis? Plant J **3:** 817–828

Krapp A, Quick WP, Stitt M (1991) Ribulose-1,5-bisphosphate carboxylase-oxygenase, other Calvin-cycle enzymes, and chlorophyll decrease when glucose is supplied to mature spinach leaves via the transpiration stream. Planta **186:** 58–69

Lütcke H, Chow K, Mickel F, Moss K, Kern H, Scheele G (1987) Selection of AUG initiation codons differ in plants and animals. EMBO J **6:** 43–48

Leigh RA, ap Rees T, Fuller WA, Banfield J (1979) The location of invertase activity and sucrose in the vacuoles of storage roots of beetroot (*Beta vulgaris*). Biochem J **178:** 539–547

Lingle SE, Dunlap JR (1987) Sucrose metabolism in netted muskmelon fruit during development. Plant Physiol **84:** 386–389

Maniatis T, Fritsch DF, Sambrook J (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Miller EM, 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 (1982) Hormonal regulation of sink invertase activity: implications for the control of assimilate partitioning. *In* PF Wareing, ed, Plant Growth Substances. Academic Press, New York, pp 659–668

Müller-Röber BT, Koßmann J, Hannah LC, Willmitzer L, Sonnewald U (1990) One of two different ADP-glucose pyrophosphorylase genes from potato responds strongly to elevated levels of sucrose. Mol Gen Genet **224:** 136–146

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco cultures. Physiol Plant **15:** 473–479

Novick P, Schekman R (1979) Secretion and cell-surface growth are blocked in a temperature-sensitive mutant of *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA **76:** 1856–1862

Patrick JW (1990) Sieve element unloading, cellular pathway, mechanism and control. Physiol Plant **78:** 298–308

Reisfeld RA, Lewis UJ, Williams DE (1962) Disk electrophoresis of basic proteins and peptides on polyacrylamide gels. Nature **195:** 281–283

Ricardo CPP (1974) Alkaline β-fructosidase of tuberous roots: possible physiological function. Planta **118:** 333–343

Ricardo CPP, Sovia D (1974) Development of tuberous roots and sugar accumulation as related to invertase activity and mineral nutrition. Planta **118:** 43–55

Roitsch T, Tanner W (1994) Expression of a sugar-transporter gene family in a photoautotrophic suspension culture of *Chenopodium rubrum* L. Planta **193:** 365–371

Salanoubat M, Belliard G (1989) The steady-state level of potato sucrose synthase mRNA is dependent on wounding, anaerobiosis and sucrose concentration. Gene **84:** 181–185

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA **74:** 5463–5467

Sauer N, Stolz J (1994) SUC1 and SUC2: two sucrose transporters from *Arabidopsis thaliana*. Expression and characterisation in bakers' yeast and identification of the histidine tagged protein. Plant J **6:** 67–77

Sauer N, Tanner W (1989) The hexose carrier from *Chlorella*. C-DNA cloning of a eucaryotic H^+-cotransporter. FEBS Lett **259:** 43–46

Sauer N, Tanner W (1993) Molecular biology of sugar transporters in plants. Bot Acta **106:** 277–287

Schäfer C, Simper H, Hofmann B (1992) Glucose feeding results in coordinate changes of chlorophyll content, ribulose-1,5-bisphosphate carboxylase-oxygenase activity and photosynthetic potential in photoautotrophic suspension cultured cells of *Chenopodium rubrum*. Plant Cell Environ **15:** 343–350

Schauer I, Emr S, Coleman G, Schekman R (1985) Invertase signal and mature sequence substitutions that delay intercompartmental transport of active enzyme. J Cell Biol **100:** 1664–1675

Sheen J (1990) Metabolic repression of transcription in higher plants. Plant Cell **2:** 1027–1038

Stitt M, von Schaewen A, Willmitzer L (1990) "Sink" regulation of photosynthetic metabolism in transgenic tobacco plants expressing yeast invertase in their cell wall involves a decrease of the Calvin-cycle enzymes and an increase of glycolytic enzymes. Planta **183:** 40–50

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

Schwebel-Dugue N, Mitili NM, Krivitzky M, Jean-Jacques I, Williams JHH, Thoma M, Kreis M, Lecharny A (1994) *Arabidopsis* gene and cDNA encoding cell-wall invertase. Plant Physiol **104:** 809–819

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

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

Visser RGF, Stolte A, Jacobsen E (1991) Expression of a chimaeric granule-bound starch synthase-GUS gene in transgenic potato plants. Plant Mol Biol **17:** 691–699

von Heijne G (1983) Patterns of amino acids near signal-sequence cleavage sites. Eur J Biochem **133:** 17–21

von Schaewen A, Stitt M, Schmidt R, Sonnewald U, Willmitzer L (1990) Expression of a yeast-derived invertase in the cell wall of tobacco and *Arabidopsis* leads to accumulation of carbohydrate and inhibition of photosynthesis and strongly influences growth and phenotype of transgenic tobacco plants. EMBO J **9:** 3033–3034

Weil M, Rausch T (1990) Cell wall invertase in tobacco crown gall cells. Plant Physiol **94:** 1575–1581

Williams JHH, Winters AL, Farrar JF (1992) Sucrose: a novel plant growth regulator. *In* H Lambers, LHW van der Plas, eds, Molecular, Biochemical and Physiological Aspects of Plant Respiration. SPB Academic Publishers, The Hague, pp 1–7

Wu L-L, Song I, Karuppiah N, Kaufman PB (1993) Kinetic induction of oat shoot pulvinus invertase mRNA by gravistimulation and partial cDNA cloning by the polymerase chain reaction. Plant Mol Biol **21:** 1175–1179

Yanase M (1991) Cloning, sequencing and characterization of the intracellular invertase gene from *Zymomonas mobilis*. Agric Biol Chem **55:** 1383–1390

Yanisch-Perron C, Vieira J, Messing J (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequence of the M13 mp18 and pUC19 vectors. Gene **33:** 103–119