Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development
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Sucrose cleavage is vital to multicellular plants, not only for the allocation of crucial carbon resources but also for the initiation of hexose-based sugar signals in importing structures. Only the invertase and reversible sucrose synthase reactions catalyze known paths of sucrose breakdown \textit{in vivo}. The regulation of these reactions and its consequences has therefore become a central issue in plant carbon metabolism. Primary mechanisms for this regulation involve the capacity of invertases to alter sugar signals by producing glucose rather than UDPglucose, and thus also two-fold more hexoses than are produced by sucrose synthase. In addition, vacuolar sites of cleavage by invertases could allow temporal control via compartmentalization. In addition, members of the gene families encoding either invertases or sucrose synthases respond at transcriptional and posttranscriptional levels to diverse environmental signals, including endogenous changes that reflect their own action (e.g. hexoses and hexose-responsive hormone systems such as abscisic acid [ABA] signaling). At the enzyme level, sucrose synthases can be regulated by rapid changes in sub-cellular localization, phosphorylation, and carefully modulated protein turnover. In addition to transcriptional control, invertase action can also be regulated at the enzyme level by highly localized inhibitor proteins and by a system that has the potential to initiate and terminate invertase activity in vacuoles. The extent, path, and site of sucrose metabolism are thus highly responsive to both internal and external environmental signals and can, in turn, dramatically alter development and stress acclimation.

\textbf{Introduction}

The only known enzymatic paths of sucrose cleavage in plants are catalyzed by invertases (sucrose $+$ H$_2$O $\rightarrow$ glucose $+$ fructose) and sucrose synthases (sucrose $+$ UDP $\leftrightarrow$ fructose $+$ UDPglucose) (Figure 1). Both of these paths typically degrade sucrose \textit{in vivo} but the products of their reactions differ in important ways [1,2]. Invertases produce glucose instead of UDPglucose (UDPg), and thus also form twice as many hexoses. Either of these features could give invertases a greater capacity to stimulate specific sugar sensors [3–9]. The resulting signals can alter expression of diverse genes [3,10,11], so invertases can potentially be strong effectors of widely varying processes. These include the biosynthesis and perception of hormones such as abscisic acid (ABA). In addition, both sugar and hormone signals can also affect the expression of the genes that encode sucrose synthase and invertase [1–3,5,7,12,13,14,15,16,17]. This review explores the hypothesis that sucrose metabolism lies at the heart of a sensitive, self-regulatory developmental system in plants. Its influence appears to be balanced by the capacity for sensing sucrose itself,

Abbreviations

ABA abscisic acid
CIN cytoplasmic invertase
CWIN cell wall invertase
ENOD early nodulation
PPi inorganic pyrophosphate
PPV precursor protease vesicle
SnRK SNF-related kinase
SUS sucrose synthase

UDP uridine di-phosphate
UDPG UDPglucose
VIN vacuolar invertase
VPE vacuolar processing enzyme γ

Figure 1

The sucrose-cleaving enzymes are pivotal to maintaining the balance between both sugar signals and metabolic paths. Different sensing mechanisms are activated by hexoses and by other downstream metabolites, and also by sucrose itself. The invertase path of sucrose cleavage generates free glucose and twice as much overall substrate for hexose-based sugar sensing as does the degradative action of the reversible sucrose synthase reaction (i.e. two hexoses as opposed to fructose $+$ UDPg). In addition, genes that encode sucrose-cleaving enzymes are responsive to the action of their own enzyme products. They respond to sugar signals in addition to affecting the generation of these signals.
but different systems and responses are involved [18,19*,20*,21,22*].

Additional factors in the link between sucrose metabolism and sugar signals lie in the physical path of sucrose import and sites of sucrose cleavage (Figure 2). Sucrose can move from phloem into the cytoplasm of sink cells with or without crossing the plasmamembrane or the cell wall space. This is an important distinction because points of membrane interface have been implicated in specific mechanisms of both sucrose and hexose sensing [1,4,8,22*,23]. The plasma membrane can be exposed to abundant sucrose and/or hexoses if plasmodesmatal connections between cells are absent [3,4] (or functionally limited [24]). Cell wall invertase (CWIN) can markedly increase localized hexose levels in these instances, which typify developing seeds and grains [6,25,26*] in which there is no symplastic (plasmodesmatal) continuity between maternal and seed tissues. Pathogens and other specific stimuli can also induce cell wall invertases, even when plasmodesmatal paths are intact [5,17*,27], and these instances can favor sucrose import and amplification of hexose signals if sucrose moves out into the extracellular space.

In contrast, plasmodesmatal continuity remains intact and provides the predominant transfer path for sucrose throughout most maternal sinks (i.e. roots, shoots, fruit flesh and so on) ([3–5]; Figure 2). In these tissues, sucrose can be imported with minimal effect on known signaling mechanisms [3,4]. Once inside the importing cells, sucrose metabolism again becomes important to sugar sensing, this time to endogenous hexose- and possibly sucrose-signaling systems [4,5,10,11,22*,28]. The products of sucrose synthase (SUS) action initiate the fewest hexose-based signals [3,6] (which is advantageous under many conditions [8,17*,29–31]), and cytoplasmic invertases (CINs) are minimally active in most systems [2]. However, cytoplasmic sucrose is frequently transported into vacuoles for cleavage.

Vacuolar invertases (VINs), like their cell wall counterparts, generate abundant hexoses and hexose-based sugar signals [5,9,27,32]. The VINs also mediate the primary path of sucrose cleavage in expanding tissues [1,2], and thus contribute to considerable hexose flux across the tonoplast and to the entry of hexoses into cytoplasmic metabolism. Temporal control of both processes is further facilitated by vacuolar compartmentalization, which could integrate the import and signaling functions of VIN with its osmotic role in expansion.

Sucrose-cleaving enzymes can alter plant development through sugar signals

In addition to their production of metabolic substrates, each site and path of sucrose cleavage described above can initiate a distinctive profile of sugar signals, which in turn can have profound developmental effects (Figure 3). In general, hexoses favor cell division and expansion, whereas sucrose favors differentiation and maturation [6,26*,33,34]. These effects, together with information from analyses of numerous systems, has led to an invertase/sucrose-synthase control hypothesis for transitions through the prominent stages of development [6,26*,33,34]. According to this hypothesis, invertases mediate the initiation and expansion of many new sink structures [5,9,35], often with vacuolar activity preceding that in cell walls [17*,36*]. The action of cell wall invertase coincides with the elevated expression of hexose transporters in some systems [23]. Later transition to storage and maturation phases is facilitated by changes in the hexose/sucrose ratio (i.e. the cellular ‘sugar state’ [6,26*,33,34]), and by shifts from invertase to sucrose-synthase paths of sucrose cleavage. In some highly localized regions, elevated levels of cell wall invertase persist during maturation [17*,37]. Sucrose synthase, too, can be active in localized sites during the early phases of development (e.g. in the phloem and at sites of rapid cell wall...
Sucrose metabolism

Developmental progression

Vascular invertases:
- Sink initiation and expansion
- Maximal generation of vascular hexoses
- Expansion (linked to photosynthate availability)
- Respiration
- Hexose-based sugar sensing
  (cell division and initial differentiation)

Cell wall invertases:
- Continued sink initiation and expansion
- Maximal generation of cell wall hexoses
- Reduction of turgor-based transport inhibition, especially as volume increases to near maximum
- Turgor reduction in zones of phloem unloading
  (localized action often in much of development)
- Respiration
- Hexose-based sugar sensing
  (cell division and related gene expression)

Sucrose synthases:
- Storage and maturation
- Generation of UDPG instead of glucose
- Direct shuttling of UDPG to biosynthesis
- ATP-conserving respiratory path
- Minimization of hexose-based sugar sensing
  (enhanced expression of genes for storage and maturation)

A common developmental profile of the contributions of sucrose- cleaving enzymes to sequential stages of sink initiation, expansion, and storage/maturation. Both vascular and cell wall invertases maximize hexose production, which in turn enhances respiration and the generation of hexose-based signals. These hexose-based signals upregulate diverse early development genes, including those involved in cell division. Vascular invertases concurrently favor cell expansion through their two-for-one enhancement of osmotic solutes in the vacuoles, as well as by linking the initial volume increase to photosynthate availability. As cell expansion slows, the role of cell wall inverterase becomes increasingly important in preventing the turgor-based inhibition of symplastic transfer, and also in reducing turgor in the zones of phloem unloading. As the cell division and expansion phases shift to those of storage and maturation, sucrose synthases become more important, and contribute in at least three overall areas. These are their capacity to direct carbon to an ATP-conserving respiratory path that is advantageous in many sink tissues, to facilitate the shuttling of UDPG to biosynthetic products or other special functions, and to minimize the production of hexoses and thus hexose-based signals during specific stages of sink development.

deposition [17*,38–40]). Overall, however, diverse evidence supports the contention that the balance between inverterase and sucrose synthase activity can alter plant development through differential effects on sugar signaling systems.

Invertase-derived hexose signals can also markedly alter the expression of genes for both the biosynthesis and sensing of specific hormones (e.g. ABA). Genes for both processes can be regulated at transcriptional and posttranscriptional levels by hexose signals [11,41,42]. In some instances, effects on one hormone system may indirectly influence others (e.g. antagonisms between ABA and ethylene or auxin [11,41–43]). Nonetheless, the influence of sugar-sensing systems extends from cytokinins [11,42] to gibberellins [42,44], and from auxin [11] to ethylene [10,41–43,45]. Some thorough and striking studies of the sugar–ABA interface have been carried out recently. Here, hexose-based signals (originating from sucrose cleavage) are implicated in regulation of ABA biosynthetic genes [45], in the early steps of ABA sensing [46], and in influencing downstream elements in this signal transduction system [46,47].

The influence of sucrose cleavage products on hormone systems combined with the regulation of sucrose metabolism by those hormone systems together comprise a means of integrating individual cellular responses into those of the multicellular organism.

Roles and regulation of sucrose synthases

Essential roles of sucrose synthases

Recent developments indicate that the role of sucrose synthase in sucrose import may involve a dual capacity to direct carbon toward both polysaccharide biosynthesis and an adenylate-conserving path of respiration. A key function of sucrose synthase in biosynthetic processes is supported by evidence of its contribution to cell wall formation, which is inhibited in maize sucrose synthase knock-outs (K Koch et al., unpublished), maize mutants [2,48], anti-sense carrot plants [5], and anti-sense cotton seeds [40*]. The work on cotton [40*] showed that high-cellulose fibers do not form if sucrose synthase activity has been significantly decreased; and where antisense effects extend from epidermis to the seed interior, embryo and endosperm development are also inhibited. Additional analysis of mutant and antisense plants that had reductions in sucrose synthase (compiled in [9]) also showed that these plants had markedly less starch in storage organs (e.g. carrot roots and potato tubers). The UDPG product of sucrose synthase has been implicated in the formation of the starch [49] and in the synthesis of callose [39,50] and diverse cell wall polysaccharides [51,52].

The centrality of sucrose synthase to sucrose import by many structures may relate to the typically moderate, but widespread, reductions of oxygen levels within certain
structures. Recent data indicate that endogenous oxygen levels are generally reduced to varying degrees in active sinks such as potato tubers [53*] and developing seeds [54*], and also in the phloem [55*]. Sucrose synthase can operate effectively under these conditions [7], and can even reduce the extent of oxygen depletion [53*], but invertases typically do not operate well under these conditions [7]. The activity of invertases can also exacerbate the problem of oxygen depletion [53*]. Evidence from transgenic potatoes shows that the normal developmental shift to sucrose cleavage by sucrose synthase in wildtype tubers improves not only their adenylate balance, starch biosynthesis, and respiratory costs, but also their endogenous oxygen levels relative to those of tubers that overexpress alternate sucrose breakdown enzymes (i.e. invertase or sucrose phosphorylase) [53*]. Sucrose synthase is one of only a few genes to be upregulated under low-oxygen conditions [8,30]; its essentiality under these conditions is indicated by the capacity of wildtype plants but not sucrose-synthase-deficient mutants to survive flooding [56,57]. Under pronounced low-oxygen conditions, sucrose synthase responds rapidly to early rises in cytosolic Ca<sup>2+</sup> [31,50] and can support considerable biosynthesis (of cellulose and callose) [50,51]. The functional significance of sucrose synthase, as opposed to invertase, is likely to be particularly important in low-oxygen conditions where it can aid the respiratory conservation of adenylates through the production of UDPG instead of alternate hexoses (which would each require an ATP for entry into glycolysis).

Sucrose synthases may also be central to the efficacy of at least some symbioses (e.g. nitrogen-fixing nodules [58,59]) and have been implicated in the maintenance of others (e.g. mycorrhizae). A rugf<sup>l</sup>-a (rugosus meaning wrinkled) mutation that reduces sucrose synthase levels in pea seeds and nodules inhibits nitrogen-fixation [58]; conversely, if soybean nodules harbor symbionts that are unable to fix nitrogen, then sucrose synthase is not induced [59]. Sucrose synthase and VIN are also induced specifically in root cells that have mycorrhizal arbuscules [60] (and sometimes also in adjacent cells [61]), and this upregulation occurs early in development [62].

Further roles for sucrose synthase in development and sucrose import by diverse sinks have been implicated. The sucrose synthase gene is one of the first to show elevated expression as leaf primordia differentiate from the apical meristem [63]. The leaves and roots of carrot plants in which sucrose synthase has been reduced transgenically are also ultimately smaller than those of wildtype plants [5], and similar tomato transgenics showed reduced fruit set from the earliest flowers [64]. A sucrose synthase path of sucrose cleavage also typically predominates over those involving invertases during the storage and maturation stages of organ development [65,66]. At least some of these developmental effects are likely to stem from the capacity of sucrose synthase to minimize hexose-based sugar signals, particularly during periods when these signals could be detrimental to differentiation and/or maturation [6].

It is also worth noting that sucrose synthase may be significant to human nutrition in a context not previously appreciated: the amino acid content of this protein, together with its abundance in mature grains, make it an important contributor to nutritionally limiting lysine levels in maize kernels [67]. Approximately 75% of total kernel lysine is contributed by sucrose synthase and two other cytoskeleton proteins (UDPG starch glucosyl transferase and fructose 1,6-bisphosphate aldolase).

**Sucrose synthase’s role in phloem sieve elements**

Recent work has immunolocalized sucrose synthase to sieve-tube elements as well as to companion cells [17*], thus linking sucrose cleavage to sieve-tube function more directly than previously recognized (Figure 4). Earlier studies had localized sucrose synthase to adjacent companion cells [68], a site compatible with the results of metabolite analysis that indicated activity in or near the transport path [69]. Later work also showed that inorganic pyrophosphate (PPI) is essential for phloem function, and implicated a sucrose-synthase-based path of respiration, probably in companion cells [70]. However, recent results move sucrose synthase into close physical proximity with the sieve-tube plasma membrane and a phloem-specific ATPase that is believed to aid sucrose transport and compartmentalization [17*]. This sieve-tube locale could also facilitate sucrose synthase’s probable role in directly supplying UDPG for the rapid wound-induced biosynthesis of callose plugs. Further advantages of sucrose synthase in both sucrose transport and callose biosynthesis are its capacities to favor the cycling of PPI as opposed to ATP [29,55*,69] and to function effectively under low oxygen conditions [30,56]. Both PPI cycling and low oxygen conditions are typical of the phloem [55*,69]. The actin and/or membrane association of sucrose synthase [2,71,72,73*] may also provide an important physical anchor for its action within the phloem transport stream.

**Transcriptional control of sucrose synthases**

Several advances have arisen from attempts to unravel the basis of the differential expression of sucrose synthase genes, particularly in response to oxygen and sugar availability. Those sucrose synthase genes that are upregulated by carbohydrate deprivation are also the most strongly induced by low-oxygen, and show narrow patterns of expression that could confer sucrose import or survival priority for key tissues [3,8,30]. The starvation-inducible maize sucrose synthase (Shrunken1 [Sh1]) also respond rapidly to low O<sub>2</sub>, with marked increases in both its mRNA levels and enzyme activity, especially under modest oxygen depletion (i.e. 3% O<sub>2</sub>) [30]. Additional
Sucrose metabolism in phloem sieve-tube elements. (a) Sucrose synthase localization extends to the sieve-tube element, where it visually co-localizes with phloem-specific membrane ATPases [17] and lies in close proximity to sucrose transporters and sites of rapid wound-induced callose formation. The sieve-tube lumen is open to solute flow, but detectable levels of sucrose synthase do not move within it [69]. The capacity of sucrose synthase to bind actin [72] and membranes [2,52,73,84,85,90*], and/or to localize in specific regions of the cytoplasm [39] is compatible with its minimal mobility and highly localized presence on or near the sieve-tube plasmamembrane [17].

(b) A model for the regulation of sucrose use within sieve tubes integrates recent information on the cellular and sub-cellular localization of this sugar with the results of analyses of metabolic changes in the phloem sap [66,70] and internal environment [55*]. Two of the greatest demands for sucrose use are likely to be callose biosynthesis, which is massive during defensive plugging, and ATP production for the maintenance of steep sucrose gradients across the plasma membrane. Both processes could be readily supported by products of the sucrose synthase reaction with minimal input from local mitochondria, especially if PPI levels favor the activity of UDPG pyrophosphorylase (UGPase). The resulting uridine tri-phosphate (UTP) can contribute either directly or indirectly to glycolysis and ATP formation. The alternative demands made by callose biosynthesis work on sugar-inducible sucrose synthases in potato has indicated that this upregulation requires SNF-related kinase (SnRK), a SNF1 ortholog, because the response was abolished at the mRNA level in SnRK-antisense plants [74–76]. Although data from analogs that cannot be metabolized suggest little or no hexokinase involvement in the induction of rice Rsus1 [77], separate roles for both hexokinase-dependent and -independent sugar-sensing mechanisms are implicated in the responses of an Arabidopsis Rsus1 [78].

One of the strongest known plant enhancers of gene expression is the first intron of the Sh1 maize sucrose synthase, which is particularly effective when introduced into the 5′ region of a gene or construct. Recently, a 145-bp portion of the 1028 bp Sh1 intron was found to induce gene expression by 20–50-fold. Most of this induction was mediated by the T-richness of a 35-bp motif rather than its specific sequence [79].

Translational control of sucrose synthase

A key contributor to sustaining elevated levels of sucrose synthase mRNA and protein under low oxygen appears to be the preferential loading of sucrose synthase mRNAs onto large polysomes [80], where their translation is enhanced by upregulation of both initiation and elongation [80]. Sucrose synthase activity increases rapidly but to varying degrees under low oxygen [30], but a still-greater capacity for activity increase after stress release is indicated by the high levels of accumulated mRNA. Such disparities between regulation at transcriptional and posttranscriptional levels may have specific physiological significance.

Regulation of sucrose synthase by sub-cellular localization

Recent developments in determining how the membrane localization of sucrose synthase is controlled are presaged by earlier work on the enzyme’s apparent association with rosettes of the plasma membrane cellulose synthase complex (Figure 5). Such a relationship could allow UDPG from sucrose synthase to feed directly into cellulose formation, and essential UDP to be recycled readily [81]. Evidence indicates that the involvement of sucrose synthase in this rapidly changing structure [38,52] might also extend to the analogous formation of mixed-linkage poly-glycans [82], and to the production of callose near the phragmoplast or in localized ‘exoplastic zones’ [39,52,83].

Recent work indicates a still-broader role for the transient association of sucrose synthase with membranes, and further, that the control of this localization might
Recent studies and/or systems, however, such that the free cytoplasmic enzyme can be hypophosphorylated by [72,73], hyper- [72,73], or equally phosphorylated [89] relative to membrane- or actin-bound forms. Emerging data indicate that developmental status can influence this relationship [85], possibly because of shifts in membrane type or composition.

**Regulation of sucrose synthase protein turnover**

Despite the ‘extreme stability’ of sucrose synthase protein under some conditions [77], it is becoming apparent that several mechanisms contribute to tightly control its turnover (Figure 6). First, the phosphorylation that activates the enzyme (at the S15 site or its equivalent) [86,87] is also implicated in predisposing sucrose synthase to phosphorylation at a second site (S170 or its equivalent) [90]. This second phosphorylation, in turn, targets the protein for ubiquitin-mediated degradation via the proteasome [90]. Phosphorylation at the first site can be linked to sugar availability and/or to other signals through the catalysis of this step by both Ca2+-dependent protein kinases (CDPKs) [87,90,91] and/or a sugar-responsive Ca2+-independent SnRK [75,88,89,90]. The second phosphorylation can be catalyzed by CDPKs but not SnRks [90]. Second, this avenue of sucrose synthase breakdown can be inhibited if the second phosphorylation site (S170 or equivalent) is blocked by the binding of ENOD40 proteins (which were initially named for their role in early nodule development but which are now known to have diverse functions throughout the plant) [90,92,93]. The protective association of ENOD40s with sucrose synthase has been implicated in the control of vascular function, phloem loading/unloading, and influence the balance between sucrose synthase’s role in respiration and special functions in biosynthesis or compartmentalization (Figure 5c). The enzyme is typically soluble in the cytoplasm and can contribute readily to an ATP-conserving path of respiration (Figure 4). However, it can also move rapidly on and off membrane or cytoskeletal locations, indicating that it might also have a number of special functions. These include activities at plasma membrane and Golgi sites for cell wall biosynthesis [39,51,81–83], a possible role at the tonoplast that is related to the use and/or storage of vacuolar sucrose [84], and an activity at points on actin that could facilitate starch formation through plastid proximity and/or other metabolic shuttlings via ‘metabolons’ [2,71,72].

Sugars and other signals can affect the localization of sucrose synthase, providing a potential means of fine-tuning the balance between respiration and biosynthesis [2,71,85]. Although sucrose synthase has a non-specific affinity for membranes [85], early work indicated that the phosphorylation status of the enzyme is involved in regulating this association [57,72]. This is further supported by the sensitivity of candidate kinases to cellular signals [49,75,86–89]. The extent of sucrose synthase phosphorylation in different fractions has varied with studies and/or systems, however, such that the free cytoplasmic enzyme can be hypophosphorylated by [72,73], hyper- [72,73], or equally phosphorylated [89] relative to membrane- or actin-bound forms. Emerging data indicate that developmental status can influence this relationship [85], possibly because of shifts in membrane type or composition.
Figure 6

Current model for SUS regulation through protein turnover. (a) Stable forms of SUS include those activated by phosphorylation at an S\(^{15}\) site (or equivalent) \[49,73^*,86,87]\, especially if a second phosphorylation site is blocked by the binding of ENOD proteins \[90\]. (b) If the protective ENOD protein is absent, however, the initial phosphorylation event predisposes SUS to a degradative process that begins with a second phosphorylation at the S\(^{170}\) site (or its equivalent). This targets SUS for ubiquitin-mediated degradation by the proteosome \[90\]. The protective association of ENOD40s with sucrose synthase has been implicated in control of vascular function, phloem loading/unloading, and assimilate import \[92,93^*,94]\, P, phosphate; S, serine.

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assimilate import \[90^*,92,94^*\]. ENOD40 mRNAs are elevated, for example, at sites of high sink activity and at points of rapid unloading in phloem.

Roles and regulation of invertases

Roles of vacuolar invertases

It is now becoming evident that vacuolar invertases contribute prominently to both sucrose import \[5,17^*,36^*\]; P Commuri et al., unpublished data) and sugar signals \[9,27,32\], particularly during the expansion growth of diverse sink structures \[1,5,9,17^*,26^*,36^*,95\]. These roles for vacuolar invertases are additional to previously recognized functions including turgor regulation and the control of sugar balance in fruit tissues and mature tubers \[5,9,96\]. The sucrose import and potential signaling influences of vacuolar invertases arise from their role in the primary path of cleavage for sucrose entering growing/expanding sink tissues. The transfer of sucrose to vacuoles for initial metabolism has a dual advantage in, first, giving a two-for-one return on the cost of transporting osmotically active solutes into the vacuole for expansion and, second, adjusting organ expansion relative to carbohydrate supply. Such contributions by vacuolar invertase depend, however, on continued capacity for cell wall expansion. If turgor exceeds this potential, then sucrose cleavage by these invertases could have a minimal or even a negative effect on sucrose import \[24\]. During sink initiation and the initial expansion growth of many sinks, however, vacuolar invertases appear to have a key role \[5,9,35,36^*,95\].

Vacuolar invertase expression is also sensitive to an array of signals, including sugars, hormones, and environmental stimuli \[3,13,14^*,17^*,96\]. Hence, these enzymes are in a position to modulate responses to diverse inputs. Among these are the influence of gravity and indole acetic acid (IAA) on bending stems \[13\], cytokinins and IAA on the formation and expansion of tumors \[17^*\], drought and ABA on hexose levels in leaves \[14^*\], and cold on the sweetening of tubers \[96\]. The apparent contribution of vacuolar invertase in the adjustment of reproductive load under stress and resource limitation, when the early abortion of some fruits or seeds can allow the survival of others, is also significant \[36^*\].

Roles of cell wall invertases

Cell wall invertases are central to phloem unloading in some, but not all, sucrose-importing structures. Their significance is most prominent in sinks in which an apoplastic (cell wall) step is involved because of a gap in plasmodesmatal connections between cells \[9,25\]. This occurs in developing seeds and pollen, where an unloading role for cell wall invertases is consistent with results from *Vicia faba* \[6\], barley \[26^*\], and maize \[37\]. Stress to ovaries during early development can also induce abortion, which is preceded by rapid changes in first vacuolar then cell wall invertases \((36^*); P\) Commuri *et al.*, pers. comm.; J Mullin, pers. comm.). Cell wall invertase contributes predominantly to the development of pollen (another apoplastically isolated sink structure), and localized antisense reductions in cell wall invertases can be used to manipulate male fertility \[16\]. Cell wall invertases can also influence sinks in which plasmodesmatal connections remain intact, if at least some sucrose moves across the cell wall space. There is evidence in support of such a role for cell wall invertases in developing carrot roots \[5\], potato tubers \[48\], and in response to signals from some biotic \[17^*,27\] and abiotic stresses \[5\].

The highly responsive transcriptional regulation of invertases

Invertase genes respond to diverse signals, including those generated by direct and indirect effects of their...
own expression [3,15,16]. Invertases are also repressed by low oxygen, strongly and rapidly enough to serve as potential markers of endogenous oxygen availability [7]. In addition, invertases respond to a full spectrum of plant hormones, and to a wide range of environmental and pathogenic signals [13,14*,15,16]. Different family members can also show contrasting responses to sugars or plant hormones [3,14*,36*], and the expression of the same gene can differ markedly with tissue and/or conditions. The maize fcr2 vacuolar invertase gene, for example, is upregulated in the leaves of drought-stressed plants [14*] but downregulated in ovaries and young kernels under similar conditions [36*]. The effects of stress signals, including ABA ([14*]; K Koch, unpublished), are modified by other developmental signals. Collectively, these signals facilitate the contributions of invertase to the survival and acclimation of leaves, yet restrict reproductive investment to a limited number of offspring that will have a greater chance of support to maturity.

**Regulation of targeting and turnover for vacuolar invertase**
The regulation of vacuolar invertase at the protein level involves a previously unrecognized means of controlling both compartmentalization and breakdown, the precursor protease vesicle (PPV) and vacuolar processing enzyme (VPE\(\gamma\)) system (197*; Figure 6). Analysis of an *Arabidopsis* vacuolar invertase (AtFRUCT4 [At1g12240]) shows that this protein can be compartmentalized for extended periods in the PPVs, which are distinctive spindle-shaped endomembrane vesicles that lie between ribosomes and vacuoles [97*]. This storage of invertases can apparently delay delivery to vacuoles, thus imposing an additional level of control beyond that of mRNA-, protein-, or total-enzyme activity levels. In addition, these PPV compartments house an inactive form of VPE\(\gamma\) protease (At4g32940) that can be released into vacuoles together with the invertase, and that can subsequently target the invertase for degradation [97*]. The VPE\(\gamma\) auto-activates upon entry into the acidic vacuole, and includes at least one vacuolar invertase among its specific substrates. The PPV compartmentalization of a vacuolar invertase could thus regulate both the time at which its activity in vacuoles begins and its vulnerability to subsequent turnover by the VPE\(\gamma\) protease (Figure 7).

**Regulation of invertases by inhibitor proteins**
Inhibitor proteins are gaining increasing recognition as a potentially important means of *in-vivo* control for invertase activity. The presence of these relatively small protein inhibitors has been well documented in various systems, and they inhibit both vacuolar and cell wall invertases when present in extracts. However, the sites of action and functional significance of these proteins *in vivo* have not been fully defined. Although most inhibitor proteins appear to be cell wall proteins, a putative vacuolar homolog of a cell wall form from tobacco has been identified and introduced into transgenic potatoes [96]. The tubers of these plants had reduced capacity for the cold-induced sweetening that typically results from hexose accumulation in vacuoles. A probable effect within the vacuole was indicated for this transgenic system. In addition, the recent work of Bate et al. [98*] demonstrated

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**Figure 7**

Regulation of vacuolar invertase (VIN) at the protein level. The transfer, protective retention, and protein turnover of VIN are potentially modulated by the precursor protease vesicle (PPV) and vacuolar processing enzyme (VPE\(\gamma\)) system. At least some of the newly translated VIN enters the PPV, where it can be retained for extended periods. It is presumably protected from proteolytic degradation in this compartment, but its functional role remains unclear until it enters the vacuole. The PPV also stores a VPE\(\gamma\) protease, which remains inactive until it too moves into the vacuole. Once activated, VPE\(\gamma\) targets specific substrates that include a VIN. This invertase may thus be targeted for degradation as soon as it enters the site of its presumed action in vivo. The PPV and VPE\(\gamma\) system therefore provides a potential mechanism for modifying both the timing and the duration of at least some VIN activity.
that a maize invertase inhibitor, ZmINVINH1, is export- to the apoplast, where it could interact with invertases during early kernel development (i.e. 4–7 days after pollination). These researchers further defined the most prominent site of ZmINVINH1 expression as the embryo surrounding region (ESR), which they suggest may help to preserve crucial differences in the extracellular sugar environments of the embryo and endosperm during early development. Greater hexose levels in the endosperm apoplast may favor more rapid cell division and minimal differentiation relative to those in embryos [6].

Conclusions
Sucrose-cleaving enzymes lie at the heart of mechanisms for the distribution and use of sucrose within multicellular plants. They also occupy a pivotal position in the balance between the different sugar signals generated by imported sucrose. Their regulation has thus become the focus of considerable interest, and involves diverse and highly integrated mechanisms operating at transcriptional and posttranscriptional levels.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


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Developmental changes in the expression and localization of invertases and hexose transporters are appraised relative to the potential for hexoses produced by invertases to affect sugar sensing and cellular sugar status at different locales.


Direct probe analyzes indicate that low oxygen is common in the phloem environment and has direct implications for phloem function. Data indicate that this localization is closely controlled by phosphorylation.

Soybean nodule sucrose synthases are shown to be tightly associated with membranes. Data indicate that this localization is closely controlled by phosphorylation.

Suicide metabolism

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The authors describe a previously unrecognized phosphorylation site of sucrose synthase and its potential role in controlling the turnover of the sucrose synthase protein.


A combination of *in-vitro* translation and western blotting indicates that two small ENOD40 peptides are produced from overlapping regions at the 5′ end of the same mRNA. Further work demonstrates that these peptides bind specifically to sucrose synthase. This work, together with data from other studies [90],[94], implicates these ENOD40 peptides in enhancing the activity and longevity of sucrose synthase in diverse systems.


In-situ localization of clover ENOD40 mRNAs showed them to be present in vascular regions, especially at sites of extensive sucrose transfer. Together with data from [90],[94], this work indicates a role for ENOD40 in stabilizing functional sucrose synthase at these sites.


The authors describe a mechanism for the protective storage of vacuolar invertase and a possible delay in its vacuolar action. A system for the inactivation of this enzyme after its release into the vacuole is also described (and involves proteolysis by a specific VPEγ).


Several lines of evidence indicate that a localized expression of a cell wall invertase inhibitor from maize may have implications for early embryo development.