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Can Sucrose Cleavage Enzymes Serve as Markers for Sink Strength and Is Sucrose a Signal Molecule During Plant Sink Development?

Clanton C. Black,[1] Tadeusz Loboda,[1] Jin-Qiang Chen,[1] and Shi-Jean S. Sung[2]

[1]Department of Biochemistry and Molecular Biology
[2]Institute of Tree Root Biology, Southern Research Station, USDA-Forest Service
The University of Georgia, Athens, GA 30602, USA

INTRODUCTION

Sucrose cleavage is an essential reaction for higher plant cells to initiate intermediary metabolism and to direct its carbon into the host of essential compounds derived therefrom for maintaining the cells of intact plants. Indeed critical cellular events during their lifetime, such as cell division or growth, are not initiated in the absence of sucrose. As the primary form of systemically translocated carbon in plants, sucrose, via its sweetness, has attracted attention since antiquity. In addition, a large literature exists on the roles of sucrose and measurements of sucrose concentration in various plant tissues (1,19,30,51). Hence, we can readily appreciate the attention that the pioneers of sucrose biochemistry, Drs. Luis F. Leloir and Carlos E. Cardini, gave to understanding both the synthesis and the cleavage of sucrose. Though immense progress has been made subsequently in understanding the roles of sucrose in plants, their pioneering biochemical discoveries are foundation stones for all sucrose research today!

Knowing about the pivotal roles of sucrose in supporting so many activities of plants, about a decade ago we set out to study plant sucrose metabolism stimulated by the discovery of a substrate level pool of pyrophosphate (PPi) (13,36) and the identification of Fru-2,6-P_2 (11,33,48) as a potent regulator of plant intermediary metabolism particularly with the PPi-dependent phosphofructokinase (PPi-PFK) (4,5). A first focal point was to characterize the three enzyme activities that may cleave sucrose at a given cell because we reasoned

that one could not hope to know how the breakdown of sucrose occurred without knowledge of all sucrose cleavage enzymes and their isoenzymes. Simultaneously, a host of putative regulatory mechanisms plus temporal and spatial aspects unique to plant cellular and molecular development also needed to be understood. Portions of such formidable research tasks will be reported here, but many are still unknown. The research was focused further by studying plant sink strength and the roles of sucrose in changing sink strength within developing whole plants (39-44). This focus arose because the economically harvested product of most crops, e.g., seeds and fruits, is a derivative of sink strength, hence a derivative of sucrose supply and cleavage. We soon realized that sucrose was a potential signal molecule whose supply exhibited a strong developmental interplay with the expression of some enzymes of sucrose intermediary metabolism, e.g., sucrose synthase and the PPi-PFK in developing filling sinks (3,43,44,54).

Therefore, this review will focus on: the concentrations of sucrose available to plant cells; some biochemical traits of sucrose cleavage enzymes; the development of sinks and sink strength in several plants; and then, based on these data, an integrated model will be presented on sucrose as a signal molecule during plant sink cell development.

CONCENTRATIONS OF SUCROSE AVAILABLE TO PLANT CELLS

In plant biology, it is acknowledged that all plant cells can live on sucrose and many translocation studies show that sucrose is the universal carbohydrate of systemic transport. Specific plants may also translocate sugar alcohols or sucrose derivatives, i.e., raffinose and stachyose, but these plants simultaneously translocate at least an equal amount of sucrose. One way to highlight the uniqueness of sugar metabolism by higher plant cells is to compare their sucrose supply with human cells that are supplied glucose via the blood stream. Elaborate control mechanisms exist to steadily regulate the blood glucose supply to human cells near 4.4 mM (38). In sharp contrast, plant cells have notably variable amounts of sucrose available to them over their lifetime; ranging from nil up to near 800 mM in specific cell types. Table I cites a few examples of the range of sucrose concentrates that have been measured at various cellular sites within plants. Plants also rapidly transport large amounts of sucrose in short periods, e.g., day-night changes of 10-fold or greater are common (Table I). Clearly, individual plant cells are capable of metabolizing and responding to a wide range of sucrose nutrition.

It's also germaine to note that plants do not use glucose as a major translocated carbon source. Indeed, plants do not directly synthesize glucose nor fructose from hexose phosphates as do animals. Rather, these free sugars in plants are released from polysaccharides, beginning with disaccharides. As

Table I. *Some Measured Sucrose Concentrations in Higher Plants*

Tissue or cellular site	Concentration mM		References
Sugar beet roots,			
immature root:	.14		16
mature root:	208		16
vacuoles:	180 to 200		--
Numerous plant vacuoles:	100 to 250		1,25,51
Spinach leaves,	Day	Night	
cytosol:	75-103	13	15,31
vacuole:	45	1.1	--
Developing wheat grain,	Light	Dark	
sieve tube sap:	540	270	14
endosperm cavity sap:	65	--	
Developing rice grain endosperm:	64	--	--
Sugarcane stalk,	Apoplast	Symplast	
immature node:	102	110	49
mature node:	548	668	49
Phloem of numerous plants:	300 to 800		31,45,41

evidence for this, there is no compelling evidence that plant cells express the hexose phosphatases, e.g., Glc-6-P phosphatase which is a prominent enzyme in human cells (38). We conclude that changes in the availability of sucrose must be integrated within a plant so that plant cells can make rather drastic but vital changes in, e.g., whether or not to grow or to divide or to support a specific cellular function such as a set of sink strorage reactions and that plant cells must have unknown signal mechanisms to ascertain the wide variation in sucrose nutrition they will experience (Table I).

TRAITS OF PLANT SUCROSE CLEAVAGE ENZYMES

The prominence of sucrose in plants is immediately evident upon realizing that each plant cell has three separate enzymes plus their isoenzymes for cleaving sucrose, namely, sucrose synthases, acid and alkaline invertases. Furthermore, each intact plant cell has three separate routes for translocated sugar to enter the cell, i.e., via plasmodesmata, or in the plasma membrane either by carrier proteins or by diffusion; these are in addition to the intracellular synthesis and storage of sucrose. Historically, it seems likely that Leloir and Cardini thought of sucrose synthase as an enzyme primarily for sucrose synthesis because PPi, which is needed to propose a major cleavage role (30,47), was unknown in plants then. A host of researchers have studied the enzymes of sucrose metabolism through much of this century. Table II was collated from the literature to compare the biochemical characteristics of the three prominent sucrose cleavage enzyme activities in plants. Much uncertainty exists about the detailed biochemistry of acid invertases which we hope this meeting volume will help resolve. Even so, some of the characteristics cited in Table II begin to help us understand the roles of multiple sucrose cleavage enzymes acting on the same substrate for a single plant cell. First, when the three enzymes are compared, we can realize the necessity of their roles in cleaving the highly variable concentrations of sucrose. For example, all of their initial kinetic responses to increasing sucrose are hyperbolic (Table II). The sucrose Km values (calculated for 0 to 150 mM sucrose) for acid invertase generally are near 1 to 3 mM; alkaline invertase are near 10 to 15 mM; and sucrose synthase are near 40 to 60 mM (Table II).

Then, the general activity responses of the three enzymes to increasing sucrose up to 700 mM (Fig. 1), near the highest sucrose concentration found in plants (Table I), show that two of the activities, sucrose synthase and alkaline invertase, exhibit a hyperbolic response without a strong indication of substrate inhibition (Fig. 1A and 1C). In contrast, acid invertase may be markedly inhibited at sucrose concentrations above about 200 mM (Fig. 1B). There seems to be two general inhibition responses by acid invertases to high sucrose above about 200 mM (Fig. 1B). Many of the acid invertases were progressively inhibited up to about 500 mM, but retained about 20% of their activity even at 700 mM. But two activities, one from potato stems and one from bush bean pods, were only moderately inhibited, about 25%, even at 700 mM sucrose. Perhaps their insensitivity is because both of these isoenzymes are localized near phloem cells with their high sucrose contents (Table I). Acid invertases have the lowest Km values, 1 to 3 mM, of the three cleavage activities and are substrate saturated over much of the range of sucrose contents in plant tissues (Table I).

Table II. *Some Biochemical Characteristics of Purified Sucrose Cleavage Enzymes from Higher Plants*[a]

Characteristic	Sucrose synthases	Acid invertases	Alkaline invertases
Substrate(s)	sucrose plus UDP	sucrose raffinose, stachyose	sucrose
pH optimum	7.0-7.5	5.0	7.0-7.5
Kinetics	hyperbolic	hyperbolic	hyperbolic
Reaction	reversible	irreversible	irreversible
Km, sucrose	40-60 mM	--	--
UDP	0.01-2 mM	--	--
MW, native enzyme	360 kD	50-400 kD	240 kD
Subunit	89 kD	30-80 kD	58
Isoenzymes	yes	yes	?
Subunits	tetramer	2,4,6 or 8	tetramer
Subcellular location	cytosol	vacuole, cell wall	cytosol
Glycoprotein	no	yes	no

[a]Collated from many references including (1,6,10,19,27,30) concentrating on highly purified enzymes. A much wider range of Km and MW values can be found in literature studies with crude preparations and much contradictory evidence exists on the acid invertaes (1,10,19,37).

The responses of both sucrose synthases and alkaline invertases to increases in sucrose (Fig. 1A and 1C) would allow these cytosolic enzymes to increase activity, without new enzyme synthesis, over much of the range of physiological sucrose concentration (Table I). Indeed, an informative analogy exists between these two sucrose cleavage enzymes and the metabolism of glucose in the human

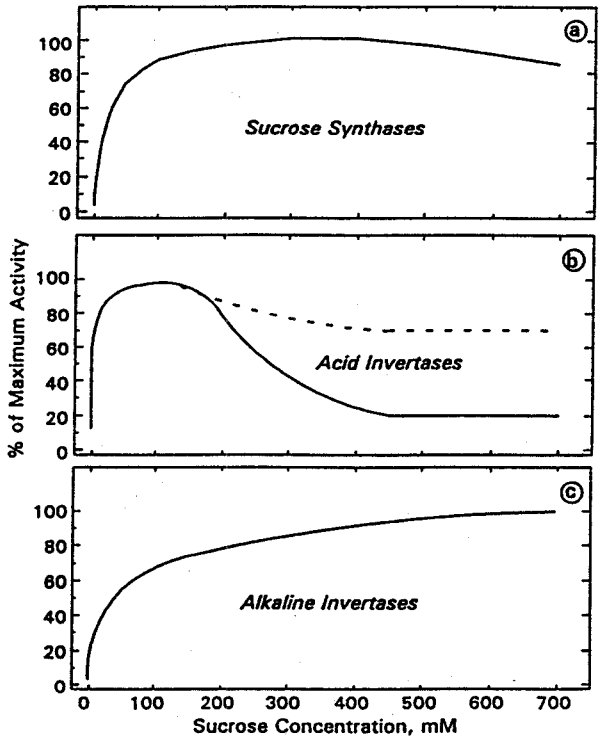

Figure 1. Composite responses for three groups of plant sucrose cleavage enzymes to the measured range of sucrose concentration found in plants. These curves do not account for specific responses of isoenzymes. The sucrose synthase plot is for sugarbeet root, bush bean seeds, potato tubers, and wild garlic bulb enzymes. Two responses of acid invertases are given, the dashed curve is for a potato stem and a bush bean pod enzyme; the solid line is for mung bean seeds, Lamium leaf, corn root, and tomato fruit enzymes. The alkaline invertase plot is for red beet root, carrot root, bush bean pod, corn root, tomato fruit, mung bean pod, and soybean hypocotyl enzymes.

liver to regulate blood glucose levels. In the liver, the glucokinase has a Km value of 10 mM which is higher than the blood glucose supply even after a bigmeal which may rise momentarily to 6.6 mM. And the glucokinase is not inhibited by Glc-6-P (38). These two plant enzymes have sucrose Km values (Table II) near or above many cytosolic sucrose values (Table I) and are not inhibited by much higher sucrose concentrations (Fig. 1A and 1C). So both

sucrose synthase and alkaline invertase can readily increase their activity over a wide physiological range of sucrose concentrations, without new protein synthesis, nor a need for regulation, similar to the liver glucokinase.

DEVELOPMENT OF PLANT SINKS AND SINK STRENGTH

In biology, one expects organismal growth curves to be sigmoidal, but the temporal growth of a plant part or individual sinks are neither necessarily sigmoidal nor linear. In fact, sink growth can pause and then resume, sometimes repeatedly, yielding various growth curves (2,42). When we measured sucrose cleavage enzymes in various sinks, we also found several temporal behavioral patterns for enzyme activities, expressed as total activity per sink or on a protein basis. Figure 2 illustrates some temporal patterns, e.g., the sucrose synthase pattern for lima bean seeds was sigmoidal initially (not shown) and then bell shaped (53), a bush bean seed pattern was double peaked (42), and a whole tomato fruit pattern was an inverted U-shape (39). Thus, some plant sinks do not follow sigmoidal curves either for growth or for enzyme activities. However, sink growth and sucrose synthase activities followed similar behavioral patterns. In contrast acid invertase and alkaline invertase activities did not resemble the growth curves. In Figure 2, acid invertase activity did not change in concert with seed weight or age or with fruit growth. Note alkaline invertases also were measured for each example in Figure 2 but it's activities were lower than acid invertase and did not show any detectable change with sink growth (not shown) (39,42,53). The only plant tissues in which acid invertases correlate with growth are tissues that have enlarging, particularly elongating cells, (1,2,10,37) as confirmed in Figure 2c with the bush bean pod. These growing sinks were importing sucrose and sucrose synthase was the only cleavage activity that followed the same pattern.

However, the nice development patterns for sucrose synthase activity seen in Figure 2 were essentially absent when we studied individual developing potato tubers (43) and more recently the vascular cambium zone (40) of individual roots on pine trees (Fig. 3). These scattergraph responses were confusing initially until we realized that these intact plants were selectively feeding sucrose into competing sinks, i.e. different tubers or roots, on the same plant. We came to realize that sink strength was changing in the same plant and in turn the growth of each sink was not sigmoidal. Fven so, acid invertase and alkaline invertase activities had no relationship with growth of individual tubers or roots. In a temporal fashion, sink strength moved from each tuber or root without relationship to its spatial position on the plant. But, when a tuber or root was growing and importing sucrose, sucrose synthase was the enzyme that marked it as an active sink. Therefore, after we had measured sucrose cleavage enzyme

activities in a variety of individual sinks, we proposed that sucrose synthase can be used as a marker for sink strength (2,39,41,43).

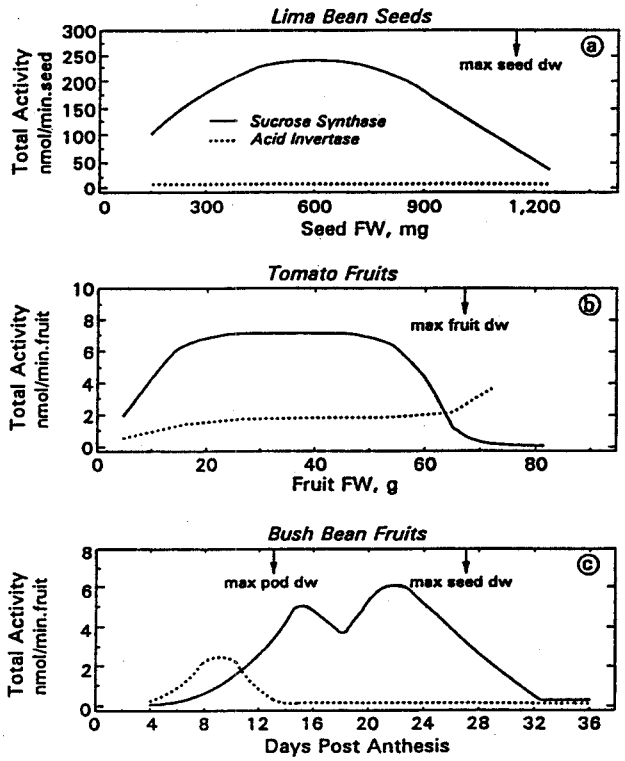

Figure 2. Examples of changes in sucrose synthase and acid invertase activities during the development of plant sinks. These graphs were drawn from published data on lima bean seeds (53), tomato fruits (39), and bush bean fruits (42). In the bush bean data, the acid invertase tracks pod elongation whereas sucrose synthase primarily tracks seed growth; these fruit data are for one pod and five seeds (42).

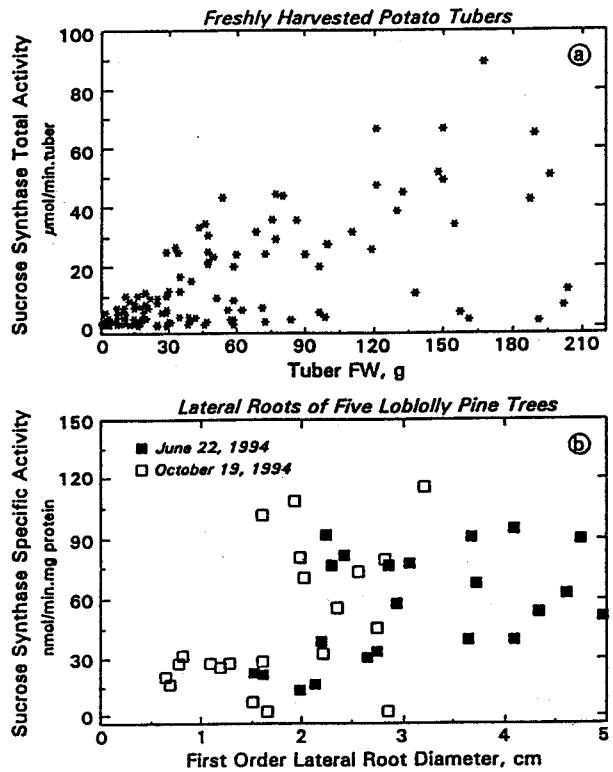

Figure 3. Sucrose synthase activities during the development of potato tubers (43) and with loblolly pine tree roots. The pine trees were from a 9-year-old plantation. We excavated the entire root system and chose different diameter roots to assay their vascular cambium root zones (40). Note that in both plant tissues, acid and alkaline invertases were very low, near the limits of detection.

A MODEL ON SUCROSE AS A CELL SIGNAL MOLECULE DURING SINK DEVELOPMENT

From data such as those collected in Table I both on the range of sucrose concentrations and on the rapid daily fluxes that can occur, we propose that plant cells must have molecular mechanisms for reacting to sucrose concentration other than mass action effects on intermediary metabolism. One

can easily imagine that it would be disastrous, even fatal, for plant cells to initiate many vital processes without a supply of sucrose!

Since most organisms can live off simple sugars, some of these sugar detection mechanisms have been studied thoroughly. Some examples of the case for detecting simple sugars which act as "signal molecules" are the lactose system in *E. coli*, the glucose-repressed genes in the yeast *S. cerevisiae*, and a very informative series of studies on sucrose metabolism with the sucrose-dependent gene regulation in *Bacillus subtilis* and other bacteria. The well known textbook level insulin/glucagon tandem for responding to the level of glucose in human blood is given here to contrast with plants since its clear that human blood "buffers" its simple sugar supply by glucose uptake and glycogen synthesis processes. But, in plants, the translocated sucrose supply is not closely regulated, rather it varies over orders or magnitude to various cells (Table I and earlier text). Therefore, we have postulated that each plant cell must be capable of quantitatively detecting and responding to sucrose, i.e., sucrose is a signal molecule that plants can detect and transduce into gene expression (41).

There is a strong history of plant sink/source type studies showing that sugars can either induce or repress various enzymes, i.e., plants can detect sugars! For example, Claussen et al. (8,9) reported a close relationship between sucrose synthase activities and sink tissues. They changed a sucrose exporting eggplant leaf into a sucrose sink leaf and observed an increase in the sucrose synthase activity with no change in the invertase activities. Furthermore, incubation of detached eggplant leaves in increasing level of sucrose solution resulted in a corresponding increase of sucrose synthase activity, but the invertase activity did not change. Similar data were presented with sucrose synthase for a variety of sucrose importing active plant sinks (2,12,16,24, 26,28,35,41) and elevated sucrose levels induced the expression of other genes e.g., patatin I (32,50), ADP-Glc pyrophosphorylase (29), sweet potato storage protein (18), proteinase inhibitor II (22,23), chalcone synthase (46), nitrate reductase (7), soybean leaf vegetative storage proteins (34), and lipoxygenase A (34). The repression of plant genes particularly by free hexoses, glucose and fructose, also has been reported for photosynthetic enzymes (21) and for two glyoxylate cycle enzymes (17). However, how plant cells sense sugars and transduce the signal into specific genes is unknown.

In our work, two enzymes were exceptionally responsive to their sucrose supply, sucrose synthase and the PPi-PFK (3,41,53). Both enzymes seemed to track the sucrose supply with activity going up as sucrose increased and down as sucrose decreased. It was this reversible aspect of gene expression that lead us to develop the following model for how sucrose could be detected and induce these enzymes as sink strength increased and decreased. Figure 4 is a model to illustrate imported sucrose serving as a signal molecule to induce gene

expressions that enhance metabolism at the beginning of intermediary metabolism in the cytoplasm of plant cells. In addition, we propose that the internal site for the reversible interconversion of Fru-6-P with Fru-1,6-P_2, by the PPi-PFK, influences the level of other signal molecules that regulate gene expression. The PPi-PFK has some key biochemical traits, e.g., this reversible enzyme is the only plant kinase, or phosphotransferase, that phosphorylates a substrate and also produces Pi while it consumes PPi. In essence, it "balances" the PPi:Pi ratio, as in a "buffer", in the cytoplasm (3,44). In Figure 4, PPi is decreasing by two enzymes and Pi is increasing. In addition, the PPi-PFK is most active as a tetramer and that enzyme dimer is formed when Fru-2,6-P_2 increases (52). For many years, it has been acknowledged that Pi turnovers rapidly in plant cells and, when it was learned that chloroplasts export triose phosphates, it soon becomes clear that cellular Pi levels are a strong metabolic control point. We combined our work and these traits with published data on other sucrose responsive proteins to prepare the integrated model in Figure 4. Last year it was learned that Pi modulates the expression of Vsp's, Inh II, Lox A, Chs, and patatin I and that PPi inhibits the expression of patatin I (34). Currently, developing work also indicates another means for regulating gene expression is via protein dimerization (20) as with the PPi-PFK or alternatively it is known that the enzymes regulating Fru-2,6-P_2 levels are multisubunit proteins that could regulate gene expression. In brief, we propose that sucrose, Pi and PPi, along with protein dimerization, can modulate gene expression in plant sink tissues. The levels of PPi, that may inhibit gene expression (34), and Pi can be balanced by the PPi-PFK and other enzymes internal to intermediary metabolism (Fig. 4). How such factors are regulated in the cytoplasm or interact in the nucleus to modulate gene expression currently are hypothetical. Even so, the model raises a host of unanswered questions for future work with plant sinks that have clear implications and benefits as we improve our crops and increase crop sink yields.

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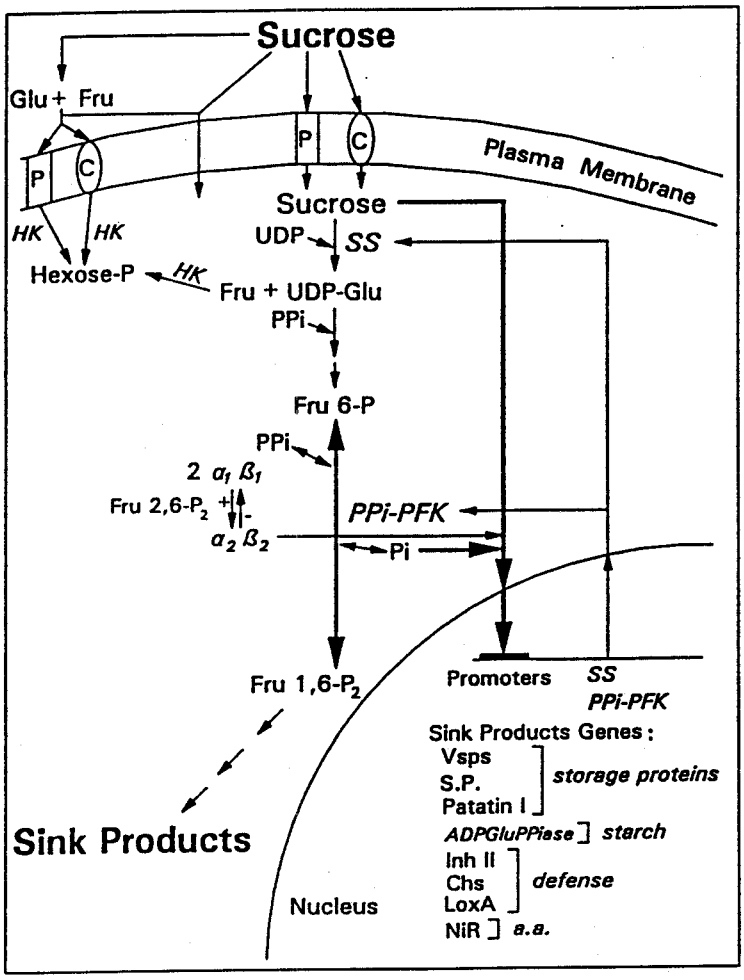

Figure 4. Model for imported sucrose as a signal to induce gene expression that increases cytoplasmic sink strength during plant development. Importing sucrose results in decreasing PPi, increasing Pi, increasing Fru-2,6-P_2, and increasing protein dimerization as with the PPi-PFK. The actions of these factors likely are through the responsive domains in the respective promoters of the sucrose responsive genes, no doubt, via yet to be discovered mechanisms. P = plasmadesmata; C = carrier proteins; HK = hexose kinases.

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