

## Review

# Fructose 2,6-Bisphosphate and Plant Carbohydrate Metabolism

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### ABSTRACT

The control of the fructose 2,6-bisphosphate (Fru2,6P<sub>2</sub>) concentration and its possible role in controlling carbohydrate synthesis and degradation are discussed. This regulator metabolite is involved in the fine tuning of photosynthetic metabolism, and in controlling photosynthetic partitioning, and may also be involved in the response to hormones, wounding, and changing water relations. Study of the mechanisms controlling Fru2,6P<sub>2</sub> concentrations could reveal insights into how these responses are mediated. However, the detailed action of Fru2,6P<sub>2</sub> requires more attention, especially in respiratory metabolism where the background information about the compartmentation of metabolism between the plastid and cytosol is still inadequate, and the potential role of pyrophosphate has to be clarified.

Since its discovery at the beginning of this decade, Fru2,6P<sub>2</sub><sup>1</sup> has been the subject of active research in a wide variety of organisms and tissues (7). It is ubiquitous in eucaryotic organisms, and regulates enzymes which catalyze key control reactions in glycolysis. This pathway has probably been studied more intensively than any other metabolic sequence, and the discovery of Fru2,6P<sub>2</sub> provides a timely reminder that important control systems can be elusive. In plants, where far less is known about metabolism, the discovery of Fru2,6P<sub>2</sub> has stimulated interest in an area which had long been regarded as being of little interest and, anyway, likely to resemble animals or yeast. The aim of this review is to outline how study of Fru2,6P<sub>2</sub> has highlighted a series of basic problems in plant metabolism which are of importance for our understanding of metabolic biochemistry, and also because of their potential impact on many other areas of plant physiology including partitioning and transport, the action of hormones, and the control of water relations.

### GENERAL FEATURES OF THE Fru2,6P<sub>2</sub> SYSTEM

**Animals and Yeast.** The general features of control by Fru2,6P<sub>2</sub> can be summarized as follows: (a) Fru2,6P<sub>2</sub> is an extremely potent activator of PFK, and inhibitor of Fru1,6Pase. At micro-

molar concentrations, or lower, it alters the activity of the enzymes and changes their sensitivity to regulation by other effectors, especially adenine nucleotides. (b) Specific enzyme activities exist, which catalyze the synthesis and degradation of Fru2,6P<sub>2</sub>, termed Fru6P,2-kinase and Fru2,6P<sub>2</sub>ase. In liver, these activities reside on a single bifunctional protein. Whether this is always so in other tissues remains an open question. (c) These enzymes are regulated via metabolites, and via protein modification. As the rates of synthesis and degradation are usually changed in a reciprocal way, this system has the properties of a "cascade" and acts to amplify small changes of metabolite levels, or signals which trigger the protein phosphorylation/dephosphorylation cycle. (d) High levels of Fru2,6P<sub>2</sub> favor glycolysis, and low levels favor gluconeogenesis. Low Fru2,6P<sub>2</sub> also favors quantitative interconversion of storage and transport carbohydrates (e.g. glycogen and glucose in the liver) by preventing glycolysis from competing for the carbohydrate.

The details of this control system can be quite varied (7). In liver, for example, Fru6P,2-kinase is inhibited and Fru2,6P<sub>2</sub>ase is activated after phosphorylation by the cyclic-AMP dependent protein kinase. This is part of the response to the hormone glucagon, which leads to glycogen mobilization and release of glucose to the blood. The lowered Fru2,6P<sub>2</sub> ensures that the glycogen is not diverted toward respiration. In contrast, yeast Fru6P,2-kinase is activated by a cyclic-AMP dependent protein kinase. Here, cyclic-AMP rises when glucose becomes available, and the rise of Fru2,6P<sub>2</sub> then inhibits gluconeogenesis and stimulates glycolysis so the glucose is utilized.

**Control of the Fru2,6P<sub>2</sub> Concentration in Plants.** Research in several laboratories has confirmed that the basic elements of the Fru2,6P<sub>2</sub> system are present in plants. Fru2,6P<sub>2</sub> is widely distributed in photosynthetic and nonphotosynthetic plant tissues (1, 2, 7, 8, 13) as are the enzymes responsible for its synthesis and degradation (4, 8). The plant Fru6P,2-kinase shows many similarities to its counterpart in liver and yeast in its sensitivity to regulation by metabolites. All these enzymes are stimulated by Fru6P and P<sub>i</sub>, and inhibited by 3-carbon metabolites (4, 7, 13). In plants, the most effective 3-carbon metabolites are PGA and dihydroxyacetone-P, whereas compounds like glycerol-3-P and lactate are more effective in liver. However, this may just reflect the differing starting points for gluconeogenesis in these tissues (see below).

It remains unclear whether protein phosphorylation contributes to the control of Fru6P,2-kinase in plants. Although early studies clearly showed that the liver cyclic-AMP dependent protein kinase did not significantly alter the activity of spinach leaf Fru6P,2-kinase, it remained an open question whether other, endogenous, protein kinases might be more effective. Recently, diurnal changes of Fru6P,2-kinase and Fru2,6P<sub>2</sub>ase have been found in spinach leaves (13) and Fru6P,2-kinase activity also rises after treating carrots or potatoes with ethylene (12). Several laboratories are currently reinvestigating whether such changes

<sup>1</sup> Abbreviations: Fru1,6P<sub>2</sub>, fructose 1,6-bisphosphate; Fru1,6Pase, fructose 1,6-bisphosphatase; Fru2,6P<sub>2</sub>, fructose 2,6-bisphosphate; Fru2,6Pase, fructose 2,6-bisphosphatase; Fru6P, fructose 6-phosphate; Fru6P,2-kinase, fructose 6-phosphate, 2-kinase; Glc6P, glucose 6-phosphate; hexose-P, sum of glucose 6-phosphate, fructose 6-phosphate, and glucose 1-phosphate; PFK, ATP-phosphofructokinase; PFP, pyrophosphate; fructose 6-phosphate phosphotransferase; PGA, 3-phosphoglycerate; triose-P, sum of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate.

are due to phosphorylation, or some other form of protein modification.

These studies should also reveal whether the plant Fru6P,2-kinase is a bifunctional protein, or whether Fru6P,2-kinase and Fru2,6P<sub>2</sub>ase are separate proteins in plants. Although Fru6P,2-kinase and Fru2,6P<sub>2</sub>ase activities co-purified during a partial purification (4), two more recent studies suggest plants may contain a low affinity Fru2,6P<sub>2</sub>ase ( $K_m$  of about 100  $\mu\text{M}$  compared to 0.1  $\mu\text{M}$  in the previously characterized enzyme), and which does not contain any Fru6P,2-kinase activity (S Morrell, CC Black, personal communication; F MacDonald, C Cseke, BB Buchanan, personal communication). More studies are needed to establish whether the low affinity enzyme is also involved in controlling Fru2,6P<sub>2</sub> concentrations *in vivo*, and to clarify its relation to Fru6P,2-kinase.

**Target Enzymes for Fru-2,6P<sub>2</sub> in Plants.** In mammals and yeast, Fru2,6P<sub>2</sub> regulates the enzymes which catalyze the inter-conversion of hexose-P and triose-P. A similar site of action is found in plants, with two important differences. First, ATP-dependent PFK is not regulated by Fru2,6P<sub>2</sub>. This finding has been corroborated in many laboratories (see Huber [8] for references). Instead, plants contain a novel enzyme called PFP which catalyzes a reversible phosphorylation of Fru6P using PP<sub>i</sub> as the phosphoryl donor (1, 2, 8). PFP is widely distributed in plant tissues and is often present at activities which resemble or exceed that of the ATP-phosphofructokinase. Fru2,6P<sub>2</sub> activates PFP when it is catalyzing the PP<sub>i</sub>-consuming (glycolytic) reaction as well as the PP<sub>i</sub>-generating (gluconeogenic) reaction. A second major difference lies in the compartmentation of Fru2,6P<sub>2</sub>. As will be discussed later in more detail, plant carbohydrate metabolism is compartmented between the cytosol and plastid. In leaves, nonaqueous density gradient centrifugation showed Fru2,6P<sub>2</sub> is restricted to the cytosol, as is PFP (14).

These differences highlight aspects of plant metabolism which, presumably, have a general significance for their regulatory strategies. However, they also make it more difficult to assess the contribution of Fru2,6P<sub>2</sub> in controlling plant metabolism, because an adequate answer to this question will require a characterization of the subcellular organization of plant metabolism, as well as the role of PP<sub>i</sub> and PFP. This makes evaluation of the role of Fru2,6P<sub>2</sub> during respiratory metabolism particularly difficult, because PFP is often high in these tissues and our knowledge of the intracellular transport processes in nonphotosynthetic carbon metabolism is effectively nonexistent. I shall therefore first consider the role of Fru2,6P<sub>2</sub> in controlling cytosolic metabolism during photosynthesis, where our background knowledge is slightly more advanced.

### CONTROL OF PHOTOSYNTHETIC SUCROSE SYNTHESIS

To establish that Fru2,6P<sub>2</sub> is acting to control metabolism, it is necessary to show (a) a potential target enzyme is present, (b) a change in the flux is accompanied by an appropriate change of Fru2,6P<sub>2</sub>, and (c) the substrates and products of the target enzyme change in a way consistent with regulation of this enzyme being an important factor in causing the change of flux. In addition, a complete account will require an explanation of why the Fru2,6P<sub>2</sub> concentration has changed.

**Coordination of Sucrose Synthesis and the Availability of Photosynthate.** During photosynthesis, chloroplasts convert CO<sub>2</sub> and P<sub>i</sub> into triose-P which are exported to the cytosol and converted to sucrose and P<sub>i</sub>. The P<sub>i</sub> returns to the chloroplast in a strict counter exchange for more triose-P, catalyzed by the phosphate translocator. Clearly photosynthesis will be inhibited unless the rate of sucrose synthesis is controlled to ensure that enough P<sub>i</sub> is released to maintain the stromal P<sub>i</sub> concentration. However, photosynthesis will also be inhibited if triose-P are

withdrawn too rapidly, because this leads to a depletion of the Benson-Calvin cycle pools (13, 14).

The first irreversible reaction leading to sucrose in the cytosol is catalyzed by the cytosolic Fru1,6Pase, and this enzyme is inhibited by Fru2,6P<sub>2</sub> (13, 14). As photosynthesis increases, a progressive decrease of Fru2,6P<sub>2</sub> activates this enzyme and allows triose-P to be removed for sucrose synthesis. This decline of Fru2,6P<sub>2</sub> can be attributed to inhibition of Fru6P,2-kinase by rising levels of triose-P and PGA as well as a stimulation of Fru2,6P<sub>2</sub>ase and inhibition of Fru6P,2-kinase as the cytosolic P<sub>i</sub> declines (13).

The stimulation of the cytosolic Fru1,6Pase by falling Fru2,6P<sub>2</sub> is amplified by changes of other metabolites, including AMP, P<sub>i</sub>, and Fru1,6P<sub>2</sub> (14). For example, as triose-P levels rise there will be an increase of Fru1,6P<sub>2</sub> because these metabolites are linked by reactions which are at, or near, equilibrium. A model which has been developed on the basis of the *in vivo* metabolite levels and fluxes and the properties of the partially purified enzyme predicts that the cytosolic Fru1,6Pase remains inactive until a critical, or "threshold" concentration of triose-P is reached. Once this is passed, the enzyme is strongly activated by further changes of triose-P as these lead to a reciprocal increase of substrate and decrease of Fru2,6P<sub>2</sub>. This provides a way of ensuring that sucrose synthesis is turned off to protect the metabolite levels in the Benson-Calvin cycle if these are in danger of falling too far, while allowing sucrose synthesis and the recycling of P<sub>i</sub> to be strongly activated as photosynthate becomes available (13).

An alteration of the threshold for activating sucrose synthesis could allow the metabolic conditions to be modified in response to differing environmental or internal requirements. For example, the substrate affinity of the cytosolic Fru1,6Pase is increased 10-fold in the mesophyll cells of the C-4 species, *Zea mays* (14). This allows higher concentrations of triose-P be maintained, and could be important in ensuring that there is a large concentration gradient available to drive diffusion back into the bundle sheath cells, as is required during photosynthesis in this species.

**Control of Partitioning between Sucrose and Starch.** Evidence is also accumulating that Fru2,6P<sub>2</sub> is involved in the control of partitioning between sucrose and starch. Fru2,6P<sub>2</sub> rises 2- to 3-fold as sucrose accumulates in leaves during the photoperiod in whole spinach plants, or in detached leaves, or when leaf discs are floated on sugar solutions (13, 14). The increased Fru2,6P<sub>2</sub> restricts sucrose synthesis leading to an increased triose-P level in the cytosol, and more photosynthate is retained in the chloroplast for conversion to starch (13).

While these findings provide a framework for understanding how photosynthetic partitioning is controlled, many questions remain. When the supply of P<sub>i</sub> to isolated chloroplasts is decreased, there is a stimulation of starch synthesis, because ADP glucose pyrophosphorylase is stimulated by a rising PGA/P<sub>i</sub> ratio (13, 14). An analogous mechanism could operate in leaves, but conclusive evidence is lacking. While a restriction of sucrose synthesis would be expected to lead to a lowering of the stromal P<sub>i</sub>, this is, technically, difficult to measure. There is also no clear evidence that PGA increases in leaves in conditions where high Fru2,6P<sub>2</sub> is leading to rapid starch accumulation (13). We may need to consider whether additional, maybe longer-term, regulation allows ADP glucose pyrophosphorylase to be activated without marked changes of the stromal metabolites. Such mechanisms might not have been seen in experiments with isolated chloroplasts which are restricted to a short time span.

The mechanisms which cause Fru2,6P<sub>2</sub> to increase also require more attention. At least two factors may be involved. One is an increase of the Fru6P,2-kinase: Fru2,6P<sub>2</sub>ase ratio (see above) during the photoperiod in spinach leaves (13). Regulation of sucrose-P synthase also plays a crucial role in controlling partitioning (8, 14). Huber (8) and associates have established that

sucrose-P synthase activity varies in parallel with sucrose synthesis in a wide range of conditions and species. When sucrose-P synthase activity decreases during the photoperiod in spinach, there is an accumulation of hexose-P in the cytosol (13). This will lead to an increase of Fru2,6P<sub>2</sub> because Fru6P activates Fru6P,2-kinase and inhibits Fru2,6P<sub>2</sub>ase (4, 13).

We do not know what triggers these alterations in the activity of Fru6P,2-kinase and sucrose-P synthase. While it is possible that accumulating sucrose could directly inhibit its own synthesis, we also need to consider the possibility of a parallel control mechanism, which does not necessarily require changes of the leaf sucrose level (14). Nonaqueous fractionation of spinach leaves suggests the cytosolic sucrose remains relatively constant during the photoperiod, and that the increase of sucrose in the leaf is due to accumulation in the vacuole (13). Of course, there are technical problems in measuring the sucrose concentration at its site of synthesis in leaves, and in separating this from the sucrose in the transport pathways of the vascular tissue.

### CONTROL OF PLANT RESPIRATORY METABOLISM

**Compartmentation between the Plastid and Cytosol.** In tissues like liver, muscle, or yeast, the interconversion of hexose-P and triose-P is clearly an important branch point in metabolism. Hexose-P are the immediate product of carbohydrate mobilization and, although they are the starting point for respiration, they can also be directly used to resynthesize further carbohydrates. In contrast, triose-P already represent a commitment to carbohydrate breakdown for respiration and/or growth. By acting on this interconversion of hexose-P and triose-P, changes of Fru2,6P<sub>2</sub> can direct carbohydrate toward respiratory breakdown, or resynthesis of other carbohydrates for storage or export.

In plants, this picture is complicated by the compartmentation of primary metabolism between the plastid and the cytosol (3). Plant cells have parallel pathways in the cytosol and in the plastid for glycolysis, the oxidative pentose phosphate pathway, as well as for the reversal of these pathways via Fru1,6-Pase. This duplication is probably closely linked to the role of starch and sucrose as major storage and transport carbohydrates, respectively. Thus, while starch is synthesized and stored exclusively in the plastid, the synthesis of sucrose is restricted to the cytosol.

The route by which these carbohydrates are interconverted and its interaction with growth and respiration will depend upon the transport properties of the plastid envelope membrane. Studies by Heldt and associates have established there are two major transport systems in photosynthetically competent chloroplasts (3). The phosphate translocator catalyzes exchange of P<sub>i</sub>, triose-P, and PGA, and plays a dominant, if not exclusive, role during photosynthesis (see above). A second, less well characterized, system allows glucose and other sugars (but not sucrose) to be moved across the envelope membrane. The glucose transporter has a lower activity than the phosphate translocator, but is active enough to play a substantial role during the degradation of chloroplast starch during the night (14). It is unclear what contribution these, or other, transporters make in nonphotosynthetic tissues because the transport properties of plastids from these tissues has not yet been studied. This means, effectively, that we do not know the metabolic pathway between sucrose and starch in any of the storage tissues which are our major food sources.

This impasse can be illustrated by considering how Fru2,6P<sub>2</sub> would impact on starch-sucrose interconversions in tissues where one, or the other, of these transport systems is dominant. In tissues where the major flux between sucrose and starch occurs via transport of 6-carbon units (e.g. hexose sugars), changes of Fru2,6P<sub>2</sub> would be able to act in a way analogous to that in liver (see above) and allow a quantitative interconversion of storage and transport carbohydrates, without this necessarily leading to an increased rate of respiration. In contrast, if the phosphate

translocator were the dominant transport system, the key sites for controlling the removal of carbon for respiration and growth would be at the reactions catalyzed by pyruvate kinase and P-enolpyruvate carboxylase. In such tissues, Fru2,6P<sub>2</sub> would control the distribution of carbon between sucrose and starch, by acting on the rate at which triose-P are made available or removed in the cytosol. Indeed (see above) this is precisely what Fru2,6P<sub>2</sub> does during photosynthetic metabolism.

**PP<sub>i</sub> as Potential Energy Source in Sucrose Metabolism.** Another fascinating aspect of plant metabolism is the potential role of PP<sub>i</sub> in cytosolic metabolism. The 'textbook' view has been that PP<sub>i</sub> is hydrolyzed to provide a driving force for many biosynthetic reactions. One example would be starch synthesis in the plastid, where an active pyrophosphatase (3, 5, 14) hydrolyzes the PP<sub>i</sub> produced in the reaction catalyzed by ADP-glucose pyrophosphorylase.

In contrast, degradation of sucrose via sucrose synthase leads to the production of UDP-glucose, and UDP-glucose pyrophosphorylase will need to operate in the opposite (PP<sub>i</sub>-consuming) direction. This route is likely to occur in tissues which are importing sucrose for cell wall synthesis or starch synthesis. This potential need for PP<sub>i</sub> in the cytosol has received fresh attention following the discovery of PFP (see above). Three studies have now shown that plants contain significant amounts of PP<sub>i</sub> (1, 2, 9). In view of the pyrophosphatase activities in the plastid, this PP<sub>i</sub> is likely to be located in the cytosol (5). This has recently been confirmed by nonaqueous fractionation of spinach leaves (H Weiner, M Stütt, unpublished data). It might be speculated that the compartmentation of plant metabolism is linked to the role of PP<sub>i</sub> in sucrose mobilization in the cytosol, and that this is separated from many biosynthetic reactions including starch and fat synthesis by restricting these to the plastid.

The potential importance of PP<sub>i</sub> is also underlined by the discovery of a PP<sub>i</sub>-dependent proton pump on the tonoplast membrane of plant vacuoles (16). The role of this pump is unknown, but one possibility would be that simultaneous operation of ATP- and PP<sub>i</sub>-dependent proton pumps could actually lead to generation of PP<sub>i</sub>. This opens the possibility that cytosolic pH, or transport across the tonoplast, might interact with control of the PP<sub>i</sub> level and carbohydrate metabolism. The finding that Fru2,6P<sub>2</sub> rises during K<sup>+</sup>-induced swelling of guard cells provides a hint that such interactions could actually occur (6).

**Changes of Fru2,6P<sub>2</sub> during Respiratory Metabolism.** In analogy with animals and yeast, we might expect Fru2,6P<sub>2</sub> to stimulate glycolysis in plants. However, we are dealing with a more complicated situation and need to consider several related questions: (a) does Fru2,6P<sub>2</sub> increase in plant tissues when respiration and/or sucrose mobilization is increased, (b) would this increase act to stimulate the glycolytic or the gluconeogenic (PP<sub>i</sub>-generating) reaction of PFP, and (c) are these increases related to metabolism of sucrose and/or starch.

Several cases are now known in which manipulation of plant material to increase carbohydrate breakdown and glycolysis leads to increased Fru2,6P<sub>2</sub>. These include wounding-induced respiration in Jerusalem artichokes (7), potatoes, and carrots (15), supplying ethylene to carrot or potato storage tissue (12), supplying high exogenous sugars to leaf tissue (14, 15), and inducing rapid malate synthesis during stomatal opening (6). It may be significant that most of these treatments lead to large scale biosynthesis of proteins, secondary products, or the generation of anions to maintain ionic and osmotic relations.

It should be noted that Fru2,6P<sub>2</sub> is not always a dominant factor in controlling glycolysis in animals and yeast, especially in conditions where changes of the energy supply are involved, such as the working muscle, or in the Pasteur effect. An example in plants where respiration rises without a change of Fru2,6P<sub>2</sub> is thermogenesis in the *Arum maculatum* spadix (1).

**The Role of PFP.** Since Fru2,6P<sub>2</sub> stimulates both the glycolytic

and the  $PP_i$ -generating reaction of PFP, it could be acting by stimulating glycolysis, or by providing more  $PP_i$  for carbohydrate mobilization. I do not believe this problem can be solved at present, but I will discuss some lines of evidence which bear on this point, to highlight the kind of information which will be required to reach an answer. The answer could well vary depending on the tissue.

One approach is ask whether the activity of PFP found in tissues correlates with their rates of respiration, or the route of sucrose mobilization. Such studies suggest PFP could be involved in generating  $PP_i$ , as its activity does not correlate with respiration but does resemble the distribution of sucrose synthase (1). This idea has been extended in a novel pathway for sucrose mobilization in which PFP and UDP glucose pyrophosphorylase act in concert to provide UTP which is used to phosphorylate fructose (8). However, these results also support an alternative interpretation. If there were to be another way of generating  $PP_i$ , the simultaneous presence of high PFP and sucrose synthase activities could indicate that sucrose mobilization and glycolysis are both being driven by  $PP_i$ . Also, as previously pointed out (1), the absence of a correlation between respiratory  $CO_2$  release and PFP activity could be due to the large scale use of the products of glycolysis for biosynthesis. Clearly, studies of enzyme activities need to be accompanied by detailed quantitative analyses of fluxes and  $PP_i$  levels to distinguish these possibilities.

A second approach involves studying the *in vitro* properties of PFP, in an attempt to predict how it would respond *in vivo* (2, 8, 10). This approach is hampered by a lack of information about cytosolic metabolite concentrations during respiration and the free concentration of Fru2,6P<sub>2</sub> *in vivo* (much may be bound on enzymes), and also by the complex way in which many ions and metabolites act to alter the sensitivity of PFP to Fru2,6P<sub>2</sub> (10). Thus, although the glycolytic and gluconeogenic reactions respond to different Fru2,6P<sub>2</sub> concentration ranges, it is difficult to relate these to the changes occurring *in vivo*. In addition, it is emerging that Fru2,6P<sub>2</sub> not only modifies catalysis, but may also affect oligomerization which, in turn, affects the ratio of the two reaction directions (2). A working hypothesis has been advanced that increased Fru2,6P<sub>2</sub> favors glycolysis by increasing the oligomerization of PFP (2), but we still need more information about the detailed molecular properties of this enzyme.

A third approach is to ask whether the cytosolic hexose-P concentration declines as Fru2,6P<sub>2</sub> rises. This would be consistent with an activation of PFP in the glycolytic direction. In agreement, the increase of Fru2,6P<sub>2</sub> in swelling guard cell protoplasts is accompanied by a decline of the hexose-P (6). However, comparable data are not available in other systems, and subcellular fractionation techniques still have to be developed for respiratory tissues. This approach is also complicated by the possibility that a coordinate activation of the ATP-dependent PFK would lead to a similar decline of hexose-P.

**Sucrose and Starch.** Fru2,6P<sub>2</sub> increases when carbohydrate breakdown and/or respiration are stimulated in starch-containing (potato, guard cells) and sucrose-containing (carrot) tissues (see above), which suggests that Fru2,6P<sub>2</sub> can stimulate respiratory use of both these carbohydrates. This would imply that the products of starch degradation are exported to the cytosol as C-6 units. More studies of the behavior of the subcellular metabolite pools as well as the transport properties of amyloplasts will be needed to provide direct evidence for these suggestions.

The available evidence is also consistent with low Fru2,6P<sub>2</sub> promoting the interconversion of starch and sucrose. For example, Fru2,6P<sub>2</sub> does not rise during starch accumulation in *Arum spadix* (1) nor in potato tubers (11, 15), and Fru2,6P<sub>2</sub> remains low or even decreases during the conversion of starch to sucrose during cold-sweetening of potatoes (11). Clearly, if the interconversion occurred via transport of hexose units across the envelope membrane, Fru2,6P<sub>2</sub> could be acting to restrict glycolysis in a

way analogous to liver. However, other scenarios are also possible, and a clear conclusion will be possible only when far more data are available about the subcellular metabolite pools and the transport properties of amyloplasts.

## CONCLUSIONS

Two major conclusions can be drawn from the research on Fru2,6P<sub>2</sub> in plants. First, Fru2,6P<sub>2</sub> clearly plays an important role in controlling plant metabolism. It is a central factor in the coordination of chloroplast and cytosolic processes during photosynthesis, the control of photosynthetic partitioning, and it is emerging how it may be involved during the response to hormones and wounding, and to osmotic or ionic adjustment at least in stomata. Clearly, research into the factors which control the Fru2,6P<sub>2</sub> level during these responses could uncover important targets for the action of 'higher level' control in plants and lead to a more integrated understanding of many aspects of plant physiology. However, our second conclusion must be that research into Fru2,6P<sub>2</sub> in plants is severely hampered by an inadequate understanding of many aspects of plant carbohydrate metabolism, including the subcellular compartmentation of metabolism and the transport between the cytosol and plastid in nonphotosynthetic tissues. Hopefully, the research activity generated by investigation of Fru2,6P<sub>2</sub> will also lead to a stimulation of research into what, perhaps, appear to be more mundane problems, as these processes may be central in determining the yield in at least some of our most important agricultural crops.

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## LITERATURE CITED

1. AP REES T, S MORELL, J EDWARDS, PM WILSON, JM GREEN 1985 Pyrophosphate and the glycolysis of sucrose in higher plants. *In* RL Heath, J Preiss, eds, Regulation of Carbon Partitioning in Photosynthetic Tissue. American Society of Plant Physiologists, Rockville, MD, pp 76-92
2. BLACK CC, NW CARNAL, N PAZ 1985 Roles of pyrophosphate and fructose-2,6-bisphosphate in regulating plant sugar metabolism. *In* RL Heath, J Preiss, eds, Regulation of Carbon Partitioning in Photosynthetic Tissue. American Society of Plant Physiologists, Rockville, MD, pp 45-62
3. DENNIS DT 1986 The Biochemistry of Energy Utilisation in Plants. Blackie, Glasgow
4. CSEKE C, BB BUCHANAN 1983 An enzyme synthesising fructose-2,6-bisphosphate occurs in leaves and is regulated by metabolite effectors. *FEBS Lett* 155: 139-142
5. GROSS P, T AP REES 1986 Alkaline inorganic pyrophosphatase and starch synthesis in amyloplasts. *Planta* 167: 140-145
6. HEDRICH R, K RASCHKE, M STITT 1985 A role for fructose-2,6-bisphosphate in regulating carbohydrate metabolism in guard cells. *Plant Physiol* 79: 977-982
7. HERS HG, J FRANCOIS, E VAN SCHAFTINGEN 1985 Fructose-2,6-bisphosphate versus cyclic AMP in liver and in lower eucaryotic cells. *Curr Top Cell Regul* 27: 399-410
8. HUBER SC 1986 Fructose-2,6-bisphosphate as a regulatory metabolite in plants. *Annu Rev Plant Physiol* 37: 233-246
9. HUBER SC, T AKAZAWA 1986 A novel sucrose synthase pathway for sucrose degradation in cultured sycamore cells. *Plant Physiol* 81: 1008-1013
10. KOMBRINK E, NJ KRUGER 1984 Inhibition by metabolic intermediates of pyrophosphate: fructose 6-phosphate phosphotransferase from germinating castor bean endosperm. *Z Pflanzenphysiol* 114: 443-453
11. MORRELL S, T AP REES 1986 Sugar metabolism in developing tubers of *Solanum tuberosum*. *Phytochemistry* 7: 1579-1585
12. STITT M, C CSEKE, BB BUCHANAN 1986 Ethylene induced increase in fructose-2,6-bisphosphate in plant storage tissues. *Plant Physiol* 80: 246-248
13. STITT M, R GERHARDT, I WILKE, HW HELDT 1987 The contribution of fructose-2,6-bisphosphate to the regulation of photosynthetic sucrose synthase. *Physiol Plant* 69: 377-386
14. STITT M, SC HUBER, P KERR 1987 Regulation of photosynthetic sucrose synthesis. *In* MD Hatch, NK Boardman, eds, The Biochemistry of Plants, Vol. 8. Academic Press, New York. In press
15. STITT M, C CSEKE 1987 Alterations of fructose 2,6-bisphosphate during plant respiratory metabolism. *In* M Gibbs, ed, Hungarian-USA Binational Symposium on Photosynthesis, Salve Regina College, August 15-18, 1986
16. WANG Y, RA LEIGH, KH KAESTNER, H SZE 1986 Electrogenic H<sup>+</sup>-pumping pyrophosphatase in tonoplast vesicles of oat roots. *Plant Physiol* 81: 497-502