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Minireview

Cytosolic fructose-1,6-bisphosphatase: A key enzyme in the sucrose biosynthetic pathway

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Abstract

Fructose-1,6 bisphosphatase (FBPase) is a ubiquitous enzyme controlling a key reaction. In non-photosynthetic tissues, it regulates the rate of gluconeogenesis. In photosynthetic tissues, two FBPase isozymes (chloroplastic and cytosolic) play key roles in carbon assimilation and metabolism. The cytosolic FBPase is one of the regulatory enzymes in the sucrose biosynthetic pathway – its activity is regulated by both fine and coarse control mechanisms. Kinetic and allosteric properties of the plant cytosolic FBPase are remarkably similar to the mammalian and yeast FBPase, but differ greatly from those of the chloroplastic FBPase. Cytosolic FBPase is relatively conserved among various organisms both at amino acid and nucleotide sequence levels. There is slightly higher similarity between mammalian FBPase and plant cytosolic FBPase than there is between the two plant FBPases. Expression of plant cytosolic FBPase gene is developmentally regulated and appears to be coordinated with the expression of Rubisco and other carbon metabolism enzymes. Similar to the gluconeogenic FBPase, relatively rapid end product repression of FBPase gene occurs in plant. However, unlike the gluconeogenic FBPase, a concurrent decline in plant FBPase activity does not occur in response to increased end product levels. The physiological significance of FBPase gene repression, therefore, remains unclear in plants. Both expression and activity of the cytosolic FBPase are regulated by environmental factors such as light and drought conditions. Light-dependent modulation of FBPase activity in plants appears to involve some type of posttranslational modification. In addition to elucidating the exact nature of the presumed posttranslational modification, cloning of genomic and upstream sequences is needed before we fully understand the molecular regulation of the cytosolic FBPase in plants. Use of transgenic plants with altered rates of FBPase activity offers potential for enhanced crop productivity.

Abbreviations: FBPase – fructose-1,6-bisphosphatase; F-2,6-BP – fructose-2,6-bisphosphate; RuBP – ribulose-1,5-bisphosphate; F6P – fructose-6-phosphate; PEPC – phosphoenolpyruvate; triose-P – triose phosphate

I. Introduction

The presence of a specific phosphatase that catalyzed the hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate and inorganic phosphate was first reported in the mammalian liver and kidney tissues by Gomori (1943). This ubiquitous enzyme, fructose-1,6-bisphosphatase (FBPase; D-fructose-1,6-bisphosphate 1-phosphohydrolase, EC 3.1.3.11) plays an important regulatory role in carbohydrate metabolism and utilization in all organisms by controlling a highly specific and irreversible reaction (Marcus 1981). In non-photosynthetic tissues, FBPase controls the rate of fructose-6-phosphate production in the gluconeogenic pathway. In *Escherichia coli* and *Saccharomyces cerevisiae* it is essential for growth on glycerol, succinate and acetate. In bumble bee flight muscle, the reaction

results in heat liberation.

In photosynthetic tissues FBPase plays a key role in both the carbon reduction pathway (Calvin cycle) in the chloroplast and the sucrose biosynthetic pathway in the cytosol. At least two FBPase isozymes have been identified in photosynthetic tissues: chloroplastic and cytosolic, both of which are considered regulatory enzymes in their respective pathways. The chloroplastic FBPase is involved in the regeneration of ribulose bisphosphate (RuBP) as well as in the formation of precursors for chloroplastic starch synthesis. The cytosolic FBPase located in the cytosol of the mesophyll cells is a key enzyme in the sucrose biosynthetic pathway, catalyzing the first irreversible reaction in the conversion of triose-phosphate (triose-P) to sucrose (Huber 1986).

The catalytic and regulatory properties of FBPases isolated from gluconeogenic tissues (Marcus 1981, Tejwani 1983, Benkovic and deMaine 1982) and the chloroplastic FBPases (Buchanan 1980 and references therein, Zimmermann et al. 1976, Kelly et al. 1982) have been extensively studied. Much less, however, is known about the plant cytosolic FBPase, despite its pivotal role in sucrose synthesis. In this review, I summarize the current state of knowledge of this important enzyme, especially recent advances in the past few years resulting in the cloning of the gene encoding the enzyme and its molecular regulation.

II. Role in photosynthetic sucrose production

In a large majority of plants, sucrose and starch are the two stable end products of photosynthesis, both of which are made from triose-P (in some species, sugar alcohols or raffinose sugar series are synthesized in addition to sucrose). A schematic representation of carbon flow from the chloroplast to the cytosol of a mesophyll cell is shown in Fig. 1. In the cytosol of mesophyll cells, triose-P is converted to sucrose by a series of enzymatic reactions (Daie 1988, Stitt and Quick 1989, Huber and Huber 1992). As depicted in the figure, triose-P exits from the chloroplast via the triose-P/Pi translocator which has a 1:1 exchange stoichiometry (Flugge and Heldt 1984, Flugge 1987). Approximately 80% of the total triose-P is utilized for RuBP regeneration and the remaining 20% for end product synthesis via the two FBPase isozymes – portions of the latter will be utilized in other processes such as photorespiration and amino acid synthesis.

Rates of sucrose synthesis are primarily regulated by two enzymes; the cytosolic FBPase and sucrose-phosphate synthase (SPS). Both enzymes catalyze irreversible reactions and have tight allosteric regulations by cytosolic metabolites and intermediates in vivo (see discussion for FBPase in III). The rate of sucrose synthesis is also controlled via molecular regulation by changes in the amount of these regulatory enzyme proteins and/or posttranslational modification of the preexisting enzyme (Walker and Huber 1989, Huber and Huber 1992, Harn and Daie 1992b, Khayat et al. 1993).

Huber and Huber (1992) discussed the relative importance of SPS regulation in sucrose synthesis. Data from mutants lacking cytosolic FBPase activity (Sharkey et al. 1992) clearly indicate that partitioning of carbon into sucrose is significantly reduced. Although control of any metabolic pathway involves interactions between many enzymes, in the case of SPS and FBPase, distribution of the control appears to depend upon the prevailing conditions. Under exactly which conditions SPS or FBPase are rate-limiting is not well understood. For example, SPS is presumed to be dominant under conditions of high photosynthesis, high CO_2 and light (Stitt and Quick 1989, Huber and Huber 1992). Molecular approaches should help to shed some light on these questions.

Due to its strategic location in the sucrose biosynthetic pathway, cytosolic FBPase is ideally situated to control the rate of triose-P withdrawal from the chloroplast. Therefore, changes in the activity of this enzyme may coordinate rates of sucrose synthesis with rates of photosynthesis. When triose-P supply limits sucrose synthesis, down regulation of the cytosolic FBPase interrupts the conversion of F-6-P to hexose-P, leading to SPS inhibition due to limited substrates (Stitt et al. 1983), and so preventing excessive depletion of the stromal metabolites. This feed forward control mechanism provides a means for linking the rate of photosynthetic carbon fixation with sucrose synthesis. On the other hand, when sink demand declines, a feedback inhibition of sucrose synthesis is initiated to adjust the rate of triose-P export to the lower demand (Stitt 1990). In this case, SPS activity is inhibited (at least in some species; tobacco, pea and

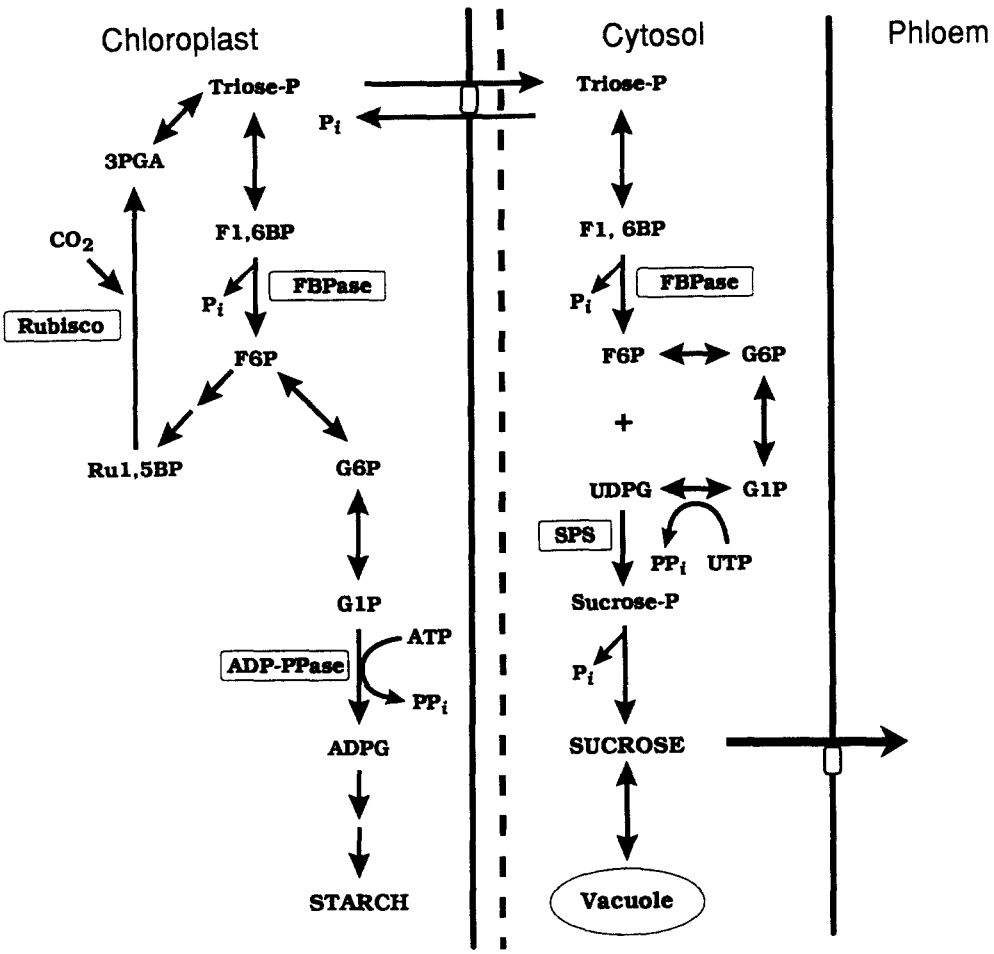

Fig 1. Schematic representation of carbon flow from chloroplast to cytosol of a mesophyll cell. FBPase, fructose-1,6-bisphosphatase; F-2,6-BP, fructose-2,6-bisphosphate; RuBP, ribulose-1,5-bisphosphate; F6P, fructose-6-phosphate; PEPC, phosphoenolpyruvate carboxylase; triose-P, triose phosphate.

peanut), leading to a chain reaction (including accumulation of F-6-P and F-2,6-BP, then decreased FBPase activity, resulting in low triose-P export), ultimately resulting in increased starch synthesis in the chloroplast (Pi is now recycled within the stroma). At the organismal level, a tightly controlled balance between feed forward and feedback mechanisms would ensure optimal rates of photosynthesis based on a genetically determined capacity which is subject to environmental conditions. Further discussion of the coordination of the two pathways at the molecular level is presented later (V. A).

III. Kinetic properties and fine control

The cytosolic FBPase of both spinach (Kelly et al. 1982) and sugar beet (Khayat et al. 1993) are tetramers of approximately 37 kD. In other eucaryotic organisms and depending on the tissue, the molecular mass of the tetrameric holoenzyme is between 140 to 160 kD. The molecular mass of FBPase in various microorganisms ranges from 100–500 kD consisting of two or six subunits (Tejwani 1983). Our understanding of the kinetic properties of the plant cytosolic FBPase is based mostly on

studies with the enzyme from spinach leaf (Zimmermann et al. 1978, Kelly et al. 1982) and to some extent castor bean endosperm (Kruger and Beever 1984).

In the absence of effector molecules, the Km of the cytosolic FBPase for the substrate, F-1,6-BP, is about 4–6 μM (Stitt et al. 1985). The enzyme requires Mg^{++} and is subject to inhibition by P_i, F-6-P, AMP (mild inhibition; Ki = 120 μM) and strong inhibition by F-2,6-BP (Ki = 0.07 μM [Stitt 1990]). AMP inhibition is noncompetitive (allosteric) with F-1,6-BP and competitive with Mg^{++}. The potent inhibitor, F-2,6-BP increases the enzyme's sensitivity to AMP inhibition and dramatically decreases the substrate affinity (100-fold), resulting in sigmoidal saturation curves (Herzog et al. 1984). F-2,6-BP exerts its strongest inhibition at low substrate concentrations and acts synergistically with AMP (Herzog et al. 1984). In the presence of F-2,6-BP, product inhibition by P_i is increased, while inhibition by F-6-P does not occur (Stitt et al. 1985). There has been debate whether F-2,6-BP is an allosteric inhibitor (Van Schaftingen 1987), a simple competitive inhibitor of the cytosolic FBPase (Pilkis et al. 1987) or both (Meek and Nimmo 1983). Based on crystallography studies, Ke et al. (1990) reported that F-2,6-BP was a competitive inhibitor of the pig kidney FBPase. From crystal structure of pig kidney FBPase complexed with fructose-2,6-bisphosphate, Liang et al. (1992) recently reported that in addition to binding to the active site, F-2,6-BP shows minor binding to AMP sites. For the plant cytosolic FBPase, Huber (1986) had suggested that F-2,6-BP may bind to a site other than the catalytic site. Kinetic properties of the cytosolic FBPase are also altered in the presence of various ions. For example, K^+ and Rb^+ increase and Li^+ decreases its sensitivity to F-2,6-BP (Stitt and Schreiber 1988). On the other hand, depending on substrate concentration and presence of other divalent cations, Ca^{++} can act either as an inhibitor or activator of the enzyme (Prado et al. 1991).

F-2,6-BP is known to accumulate in drought-stressed leaves, as a result of which in vitro inhibition of the cytosolic FBPase occurs (Stitt 1990). Recently, Harn and Daie (1992b) reported that the activity of partially purified FBPase decreased in drought-stressed sugar beet leaves. The decline in the activity could not have been due to F-2,6-BP, because F-2,6-BP had been removed during the partial purification. These authors also showed that both FBPase protein and transcript were stable under moderate drought stress but declined in severely stressed sugar beet leaves. However, considerable protein and transcript were present in the leaf even under very severe stress. Apparently, in addition to fine control by F-2,6-BP, coarse control mechanisms are involved in the regulation of the plant FBPase activity (Harn and Daie 1992b, Daie and Harn 1992 – also see 'Sec. V.C.' for additional discussion).

The allosteric properties of the plant cytosolic FBPase are remarkably similar to the mammalian and yeast FBPase (Van Schaftingen 1987), but differ greatly from those of the chloroplastic FBPase. Kinetic properties of the two plant isozymes are compared in Table 1. Chloroplastic FBPase is not inhibited by AMP or F-2,6-BP, and has very low Mg^{++} requirement (Zimmermann et al. 1976, Stitt et al. 1985). A major difference between the two isozymes is their regulation in light. Considerable evidence indicates that the chloroplastic FBPase is activated in the light by changes in the tertiary structure of the enzyme induced by reduction via the thioredoxin/ferredoxin system (Buchanan 1980). Although it is believed that all the disulfide bonds present in the chloroplastic FBPase are intra-subunit, it is not known which cysteines are oxidized, nor is it known which of those might be involved in light regulation. This mechanism is presumably absent in the cytosolic FBPase.

IV. Structure and characteristics of FBPase genes

Full length cDNA sequences encoding the gluconeogenic enzyme have been isolated from several sources including rat liver (El-Maghrabi et al. 1988), pig kidney (Williams and Kantrowitz 1992), yeast (Rogers et al. 1988) and *E. coli* (Hamilton et al.

Table 1. Comparison of the kinetic properties of spinach cytosolic and chloroplastic FBPases

	Cytosolic	Chloroplastic
Mol. Wt. (Kd)	37 000	44 000
Km for substrate (μM)	2–4	33
Km for Mg^{++} (mM)	0.26	2
Vmax (U/mg)	120	154
pH optima	7.5–8.2	8.8 (ox); 7.5–8.2 (red)
Ki for f-2,6-BP(μM)	0.07	Very high
Ki for AMP (μM)	120	No effect
Activation by DTT	No	Yes

1988). The use of these probes has allowed the development of a good information base regarding molecular regulation of this enzyme in the respective organisms. However, data on the molecular regulation and coarse control of the cytosolic FBPase in plants have been non-existent until recently, mostly due to lack of antibodies and/or nucleic acid probes. To date, cDNA sequences encoding several chloroplastic and cytosolic FBPases have been obtained: wheat chloroplastic (Raines et al. 1988), Arabidopsis chloroplastic (Horsnell and Raines 1991), potato chloroplastic (Kossmann et al. 1992), sugar beet cytosolic (Harn and Daie 1992a), spinach cytosolic (Hur et al. 1992) and potato cytosolic (U. Sonnewald, personal communication). To date, no genomic clones of the plant cytosolic or chloroplastic FBPases have been isolated and characterized. With the availability of both antibody (Khayat et al. 1993) and/or cDNA probes for several plant species, a better understanding of the role of coarse control and molecular regulation of cytosolic FBPase in the photosynthetic carbon metabolism is being developed in several laboratories.

Cytosolic FBPase is relatively conserved among various organisms both at the amino acid and nucleotide sequence levels and share several important common properties at the level of the protein. The degree of homology at the amino acid level among several FBPases is presented in Table 2. Homology among the chloroplastic FBPases of wheat, potato, arabidopsis and spinach is 79–83% and homology between the two known plant cytosolic FBPases is more than 90%. There is slightly higher similarity between the mammalian FBPase and the plant cytosolic FBPase (up to 56.4%) than there is between the two plant isozymes (as low as 48.3%; also see caption of Table 2 regarding pea chloroplastic FBPase). Divergence between the two plant isozymes is as great as it is between the plant cytosolic enzyme and other gluconeogenic FBPases, so that the chloroplastic FBPases have a slightly lower degree of identity to the gluconeogenic enzymes (40–45%) than do the plant cytosolic isozymes (47–56%). Since the plant cytosolic FBPase exhibits similar regulatory properties common to all other gluconeogenic FBPases, and because the identity between cytosolic and chloroplastic isozymes is about 50%, it is likely that both plant FBPases shared a common origin; the chloroplastic enzyme having lost the AMP binding site and gaining the site for light regulation during the course of evolution.

Although localized in different compartments, plastidic and cytosolic FBPases are both nuclear encoded enzymes by single but distinct genes (Chueca et al. 1984). Interestingly, despite many similarities between the two isozymes including catalyzing of identical reactions, they differ in

Table 2. Percent amino acid similarity among FBPases from a wide range of tissue sources. Reproduced from Ladror et al. (1990) with modifications and additions

Source[a]	SL	RL	Sc	Sp	Ec	Scyt	Schol	Wchl	SBcyt
H									56.4
PK	91.0	85.5	48.5	46.6	41.3	53.4	45.5	45.2	55.6
SL		82.7	46.7	46.0	40.7	54.2	45.5	45.2	54.2
RL			46.7	45.1	41.5	52.8	44.0	43.7	50.2
Sc				62.7	41.3	49.7	40.0	40.6	51.1
Sp					44.1	46.9	43.5	42.8	50.5
Ec						43.8	46.5	46.6	46.3
Scyt							52.5	50.0	92.7
Schl								81.1	52.3
Wchl								—	48.3
Achl								79.3	51.5
Pchl								83.0	51.0[b,c]

[a] H, human; Pk, pig kidney; SL, sheep liver; RL, rat liver; Sc, *Saccharomyces servisiae*; Sp, *Saccharomyce pombe*; Ec, *E. coli*: SB sugar beet; S, spinach; W, wheat; A, *Arabidopsis*; P, pea; cyt, cytosolic; chl, chloroplastic.

[b] Partial line up of highest identity of a 204 amino acid overlap region. Significantly lower percent identity is obtained if entire sequences are lined up.

[c] Accession # X68826 from JL Carrasco, A Chueca, R Hermoso, JJ Lazaro, JL Ramos, M Sahrawy, F Prado and J Lopez Gorge (unpublished).

molecular structure (primary), kinetics and therefore, regulation (Weeden 1983). For example, a unique sequence insert of 7–15 amino acid residues on the chloroplastic isozyme encodes a domain involved in the light regulation (distinct from the active site) of the plastidic enzyme (Raines et al. 1988, Marcus et al. 1988). The absence of this sequence insert on the gluconeogenic and plant cytosolic FBPases had led to the conclusion that the cytosolic isozyme is not light regulated (Ladror et al. 1990). Recent results by Khayat et al. (1993) and Daie and Harn (1992), however, indicated that the activity of the cytosolic FBPase of sugar beet is modulated by light, most likely by some form of posttranslational modification, rather than by the thioredoxin/ferredoxin system of the plastidic FBPase (see below for additional discussion).

V. Regulation of gene expression and coarse control

A. Developmental regulation

Developmental regulation of expression has been documented for many genes. For example, transcript of several photosynthetic genes accumulate during leaf development (Tobin and Silverstone 1985, Sheen and Bogorad 1986, Klaff and Gruissem 1991, Wanner and Gruissem 1991). In animals and microorganisms, FBPase is highly regulated both at the level of enzyme activity and gene transcription. It is becoming increasingly evident that the same is true for the plant cytosolic FBPase. Recently, we examined dynamics of expression of genes encoding several carbon metabolism enzymes in developing leaves of sugar beet (Harn et al. 1993). While transcript level of several regulatory enzymes including FBPase, SPS and Rubisco small subunit increased as leaf transition from sink to source progressed, that of the cytosolic aldolase, a non-regulatory enzyme in the sucrose biosynthetic pathway did not. In particular FBPase activity, protein and transcript were low in immature leaves (sink), and increased in mature (source) photosynthetically active leaves. Similar patterns of expression were observed within a single transitional leaf in which the base, middle and the tip portions of the leaf function as sink, transitional and source, respectively (Pitcher and Daie 1991). Significantly, the coordinated pattern of gene expression in various leaf stages correlated highly with leaf photosynthetic rates and sucrose content, indicating molecular coordination of sucrose synthesis and photosynthesis.

B. End product repression of gene expression

Repression of gene expression is a common mechanism for coarse control of key enzymes in a pathway when metabolic reorientation is necessary. In both mammalians and microorganisms, FBPase is highly regulated at the transcriptional level. Expression of the rat liver FBPase is repressed by insulin (El-Maghrabi et al. 1988). FBPase transcription in yeast is subject to glucose repression. The regulation is under the control of a *Ras*-independent, cAMP-dependent signal transduction pathway (El-Maghrabi et al. 1988). Funayama et al. (1980) reported that yeast FBPase was completely degraded after 1 hour exposure to glucose. In addition, synthesis of yeast FBPase is suppressed by sugars and its activity declines in the presence of glucose (Mazon et al. 1982). Recent data (unpublished results from our lab), suggest that like other FBPases, sugar repression of FBPase gene expression also occurs in higher plants.

Reports from various labs indicate that in addition to biochemical inhibition of activity, end products repress transcription of genes encoding regulatory enzymes of photosynthesis and sucrose synthesis (both plastidic and nuclear encoded genes). Sugar-repressed photosynthetic genes include Rubisco (Sheen 1990), plastidic FBPase (Krapp et al. 1991), Kossmann et al. 1992, cytosolic FBPase and SPS (unpublished results from our lab). In our lab, we monitored expression of Rubisco, FBPases, SPS and aldolase in sugar beet leaves. Our preliminary results indicate that incubation of detached leaves or leaf discs with sucrose, glucose and fructose leads to a significant decline in the transcripts of the regulatory enzymes. The observed repression of gene expression does not appear to be osmotically related, because incubation with mannitol does not lead to major changes in transcript level. While FBPase transcript declined in the presence of sugars, its activity did not. In microorganisms and mammalians both FBPase transcript and activity decline in response to high glucose levels (to control the rate of gluconeogenesis). The physiological significance of FBPase gene repression, therefore, remains unclear in plants. It is, however, conceivable that

since all FBPases share ancestral, sugar-responsive, regulatory elements, gene repression does occur in plants, but without any physiological significance, since protein level and activity are not sensitive to sugar levels. In similar experiments Krapp et al. (1991) fed detached spinach leaves with glucose for up to 7 days and reported a gradual decline in photosynthesis and Rubisco activity and protein level and in the activity of the chloroplastic FBPase activity. Decline was also observed in the activity of several other photosynthetic enzymes photosynthesis-related parameters such as chlorophyll content.

Related but indirect observations were also made by Harn and Daie (1992b) showing a correlation between endogenous end product levels and expression of FBPase. When sugar beet plants were subjected to slow-developing drought conditions, sucrose content of stressed leaves (source leaves in that study) increased after 4 days. Coinciding with sucrose accumulation, a declining trend in the level of the cytosolic FBPase transcript occurred thereafter. Moreover, in studies with developing leaves of sugar beet (Harn et al. 1993), lowest FBPase transcript levels were measured in youngest leaves which contained very high levels of glucose. It is, however, difficult at this time to make firm conclusions on direct links between the level of endogenous sugars and specific transcripts, since these observations were made under in vivo conditions, where many other alterations occur.

Sheen (1990) suggested that metabolic repression of gene expression overrides other types of regulation. Support for the overriding control of metabolites has come from other studies as well. Kossmann et al. (1992) showed that despite the fact that expression of the chloroplastic FBPase is light-induced, treatment of detached potato leaves with high concentration of sucrose for 24 h in continuous light resulted in a significant decline in the gene's transcript.

C. Regulation by light

Photo-induced transcription is one of the most well studied aspects of regulation of gene expression in plants (Tobin and Silverstone 1985). Raines et al. (1988) and Lloyd et al. (1991) first reported that expression of the chloroplastic FBPase of wheat was regulated by light and specific to photosynthetically active tissue. Kossmann et al. (1992) later made similar conclusions about the plastidic FBPase of potato. The first evidence regarding regulation of expression of the cytosolic FBPase by light was reported by Khayat and Daie (1991) and Khayat et al. (1993). They also showed that the activity of cytosolic FBPase increased in the light. This was surprising, because based on structural difference between the two FBPase isozymes, it had been assumed that the cytosolic enzyme was not responsive to light.

Khayat et al. (1993) reported that in sugar beet leaves both the activity and expression of the gene encoding the cytosolic FBPase were light-modulated during a normal light/dark cycle or an extended dark period. Interestingly, no changes occurred in the amount of the FBPase protein, suggesting some type of posttranslational modification. These light-dependent changes were observed both in crude extracts and in partially purified cytosolic FBPase (separated from the chloroplastic FBPase). Cheikh and Brenner (1992) also reported increased FBPase activity in light in the leaves of growth chamber-grown but not in the leaves of field grown soybeans. However, their results were based on activity in a crude extract only which may or may not have included chloroplastic FBPase activity.

A rather unusual aspect of this light response is its relative sluggishness i.e., increase in the activity and mRNA levels occurred at least one to two hours after the onset of light, eliminating the possibility of a phytochrome-mediated response. Because of the sluggishness, Khayat et al. (1993) invoked an indirect light effect and/or presence of a cascade of reactions, rather than a direct effect of light on FBPase itself. They referred to their observations (diurnal changes in the activity and expression of the cytosolic FBPase) as 'light-dependent'and not 'light-regulated' modulations. The sluggishness may be related to the availability of some photosynthetic intermediate(s), which would then activate (directly or indirectly) the transcription of the gene (an assumption compatible with the conclusion made by Sharkey et al. (1988) suggesting that regulation of the cytosolic FBPase was more related to metabolic state of the plant). Clearly, additional work is warranted to understand the nature of the modulation and mechanism of the presumed post-translational modification of the plant cytosolic FBPase.

D. Posttranslational modification of FBPase

Coarse control by covalent modification results in rapid and dramatic changes in the activity of many enzymes. Phosphorylation/dephosphorylation is a very common form of covalent modification for many regulatory enzymes including some FBPases. Phosphorylation of the rat liver FBPase is by the cyclic AMP-dependent protein kinase (Hosey and Marcus 1981, Meek and Nimmo 1984) at a C-terminal extension site (Hosey and Marcus 1981, Rittenhouse et al. 1983) and occurs as one phosphate per subunit, resulting in a two-fold decrease in the Km of the enzyme, without altering its allosteric responses to Mg^{++}, F-2,6B-P and AMP (Meek and Nimmo 1984). Yeast FBPase is reversibly inactivated by phosphorylation (stoichiometry of 1:1) with the phosphate bound to a serine residue (Lenz and Holzer 1980, Mazon et al. 1982). Mazon et al. (1982) speculated that phosphorylation of yeast FBPase 'tags' the enzyme for a subsequent irreversible inactivation by proteolysis. These authors reported that activation of the enzyme was achieved by incubating the in vivo-inactivated enzyme with 5 mM $MgCl_2$. Surprisingly, the reactivation took about two hours to occur in vivo. This is unlike other well-studied cases in which phosphorylation/dephosphorylation achieves rapid activation/deactivation of the enzyme. Therefore, questions remain in terms of the physiological importance of this mechanism for FBPase activity in yeast. It should be noted that phosphorylation/dephosphorylation is not universal for all FBPases. For example, the mouse and rabbit liver FBPases as well as pig kidney FBPase are not phosphorylated (Meek and Nimmo 1984). Moreover, while both the rat liver and yeast FBPase have been reported to be regulated by this mechanism, the activity of the rat liver FBPase is increased but that of the yeast is decreased by phosphorylation (El-Maghrabi et al. 1988).

At least two photosynthesis and carbon metabolism-related enzymes: PEPC (Jiao et al. 1991) and SPS (Weiner et al. 1992) are reversibly activated in the light by phosphorylation/dephosphorylation. Little is known about the molecular regulation of the plant cytosolic FBPase, but similar to other regulatory enzymes of carbon metabolism, the cytosolic FBPase is possibly regulated by some form of posttranslational modification mechanism. Recent evidence suggests that the sugar beet cytosolic FBPase may be posttranslationally modified (Harn and Daie 1992b, Khayat et al. 1993). This conclusion is based on data indicating that in response to environmental cues such as long-term drought stress (Harn and Daie 1992b) or short-term light/dark changes (Khayat et al. 1993), the activity of the cytosolic FBPase in sugar beet leaves changes but the amount of the protein remains stable. Our preliminary results in this regard are not conclusive. Therefore, direct and equivocal evidence for the precise mechanism of the presumed posttranslational modification of plant cytosolic FBPase is yet to be determined.

VI. Future directions

While our knowledge base on the plant cytosolic FBPase is expanding, several critical questions remain unanswered. Much of future efforts will have to continue to focus on the coarse regulation of the enzyme under various metabolic, developmental and environmental conditions. Clearly, much more work is required in order to develop a good understanding of the light modulation of the cytosolic FBPase activity and expression. Studies must also be directed toward elucidating the mechanism of the presumed posttranslational modification.

As one of the two key enzymes regulating the sucrose biosynthetic pathway, we need to establish the role of FBPase vis a vis SPS in the shared control of the in vivo rate of sucrose synthesis, i.e., which enzyme controls carbon flux through the pathway. In addition to mutants, transgenic plants with various degrees of down/up regulation of either enzymes will be needed.

In order to fully understand molecular regulation of the gene encoding the enzyme, especially in response to high endogenous sugars, characterization of the promoter and other upstream regulatory elements is essential which would require cloning of genomic sequences. Last but not least, and to best put the pieces of a complex puzzle together, the importance of integrated, broadly-based studies relying on several disciplines must not be underestimated.

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