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Update on Metabolic Regulation

Metabolite Signals Regulate Gene Expression and Source/Sink Relations in Cereal Seedlings

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Our knowledge of the genetic structures and regulatory mechanisms active during cereal seedling development has increased dramatically in recent years. For this reason, a more integrated view of the molecular aspects of seedling development and physiology is needed. This review presents the Carbon Metabolite Signal Hypothesis, which attempts to explain how regulatory interactions between metabolite signals and hormonal signals participate in controlling gene expression, growth, and metabolism of the cereal seedling.

SOURCE/SINK RELATIONS AND α-AMYLASE ISOZYMES IN CEREAL SEEDLINGS

The cereal seedling scutellum, a modified seedling leaf, together with the endosperm and aleurone, functions as a nonphotosynthetic source during seedling development. These seedling tissues serve a role analogous to that of the photosynthetic leaf during vegetative development. Starch in the endosperm is broken down into sugars by α-amylase and other hydrolytic enzymes secreted from the aleurone and scutellum. These sugars are taken up into the scutellum, converted into Suc, loaded into the phloem, and transported to the embryo axis. Suc synthase and invertase are the enzymes responsible for initiating the metabolism of Suc within the embryo axis sink tissues. Suc provides the carbon and energy for biosynthesis in the sinks. The intracellular osmotic strength necessary to maintain turgor pressure and drive tissue expansion in the developing seedling also depends, in part, on metabolites derived from Suc.

Studies on the α-amylase genes have provided insights into the molecular biology of cereal seedling development. The cereal α-amylase genes have been classified into three subfamilies, Amy1, Amy2, and Amy3, with at least one gene from each subfamily present in the genome of every cereal species studied (Huang et al., 1992). The complete rice α-amylase gene family has been cloned and characterized (Yu et al., 1992; Thomas et al., 1994). The expression of different α-amylase genes is developmentally regulated throughout rice plant development (Table I). The process of seedling development can be divided into three stages: imbibition, germination, and seedling elongation. The relatively low level of *Amy3D* gene expression comprises the majority of the α-amylase gene expression in the rice scutellum during the seedling germination stage (Fig. 1) (Karrer et al., 1991; Ranjhan et al., 1992). *Amy3D* gene expression ceases around the time of the developmental transition when root and shoot meristems begin to expand and emerge through the seed coat. During the subsequent seedling elongation stage, the *Amy1A* gene is expressed at a high level in the rice aleurone, whereas the *Amy3B*, *Amy3C*, and *Amy3E* genes are expressed at moderate levels. There is little or no expression of *Amy1B*, *Amy1C*, *Amy2A*, or *Amy3A* in the developing rice seedling.

The early expression of *Amy3D* followed by a set of later isozymes (*Amy1A*, *Amy3B*, *Amy3C*, *Amy3E*) may be an important part of the transition from the germination stage to the seedling elongation stage in rice. Distinct catalytic roles of different α-amylase isozymes were observed in a study of recombinant yeast strains that were engineered to express and secrete the Amy1A and Amy3D isozymes from rice. The Amy3D isozyme preferentially attacks low mol wt starch, which may be accessible at the start of the germination stage, but has very little activity against intact starch granules. The Amy1A isozyme, which is expressed during the seedling elongation stage, attacks starch granules and has less activity toward maltosaccharides than does the Amy3D isozyme (M. Terashima, S. Katoh, R.L. Rodriguez, unpublished data). During this period the breakdown of low mol wt soluble starch may be catalyzed primarily by β-amylase, α-glucosidase, or by other isozymes of α-amylase. Similarly, among the α-amylase isozymes expressed in wheat during the seedling elongation stage, α-amylase group I isozymes attack starch granules, whereas α-amylase group II isozymes act primarily against soluble starch (Sargeant, 1979).

THE GA SIGNAL STIMULATES CEREAL SEEDLING SOURCE METABOLISM

The hormone GA plays a major role in control of cereal seedling development. In the aleurone layer, the GA signal induces the transcription of genes encoding α-amylase and many other hydrolytic enzymes responsible for mobilizing endosperm reserves (Fincher, 1989). Induction of α-amylase gene expression is controlled through the interaction of regulatory proteins with *cis*-acting elements in the promoters of the α-amylase genes. Nucleotide sequence comparisons of several rice, barley, and wheat α-amylase genes revealed a highly conserved sequence element (TAACRRA) in the promoters of these genes (Huang et al., 1990b). (DNA sequence ambiguity code: R = A or G.) This element is required for GA induction of α-amylase gene expression, hence the name Gibberellic Acid Response Element or GARE box (Rogers et

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Table I. *α-Amylase gene expression in rice tissues*

The nine α-amylase gene clones represent all members of the gene family found in rice cv IR36 (indica-type). There is some evidence for a 10th α-amylase gene in rice cv M202 (japonica-type) (Ranjhan et al., 1991), but no clone for this putative 10th member of the gene family has been isolated to date. Naming of the rice α-amylase genes has been adapted to conform to the recommendations of the Commission on Plant Gene Nomenclature (Bairoch et al., 1993).

Gene Name	Germinated Seedlings	Cultured Cells	Root	Leaf	Developing Seeds
All genes	100[a]	65	1	3	4
Amy1A	++++[b]	++	++++	++++	+++
Amy1B	−	+	−	−	−
Amy1C	−[c]	NA[d]	NA	NA	NA
Amy2A	+	+	+	+	+
Amy3A	+	++	−	−	−
Amy3B	++	NA	NA	NA	NA
Amy3C	++	NA	NA	NA	NA
Amy3D	++	++++	++	++	−
Amy3E	+++	+++	+++	+++	++++

[a] Relative levels of total α-amylase mRNA in different tissues of rice are normalized to the level of expression observed in germinated seedlings; based on northern hybridization data (Karrer et al., 1991). [b] Relative levels of mRNA for each gene relative to the other genes expressed in that tissue as estimated from northern blot hybridization or RNA-PCR experiments (Huang et al., 1990a; Karrer et al., 1991). Comparison of gene expression from one tissue to another must factor in the total amylase mRNA levels at the top of each column. The amount of gene expression is indicated from the highest (++++) to the lowest (+). Minus signs (−) indicate that no gene expression was observed. [c] Lack of expression based on restriction digest of RNA-PCR products (Karrer et al., 1993). [d] NA, Not available.

al., 1994). In the DNA sequences for the rice α-amylase genes *Amy1A* and *Amy1B*, GARE boxes are located approximately 200 bases to the 5′ side of the ATG translation start codon (Huang et al., 1990a). Nuclear proteins that bind to the GARE box and other α-amylase promoter *cis* elements have been identified (Sutliff et al., 1993), including one DNA-binding protein whose production is induced by GA (Goldman et al., 1994).

As more is discovered about the structure and function of the cereal α-amylase genes, it has become increasingly difficult to completely describe their regulation using only the GA regulatory model. In aleurone half-seeds of barley cv Himalaya produced on plants grown at cool temperatures, α-amylase gene expression during seed germination is highly dependent on the presence of GA. In contrast, aleurones of many cereal species, including other barley cultivars, exhibit considerable α-amylase gene expression in the absence of added GA (Fincher, 1989). The gene corresponding to barley cDNA Clone E was expressed at equally high levels over a wide range of GA concentrations from 1 nM to 1 μM (Huang et al., 1984). Regulation of Clone E was not tested in the absence of GA; thus, we cannot distinguish the alternative hypotheses that expression is fully stimulated at an exceedingly low GA concentration or that expression is independent of GA. The level of *Amy3D* expression in the rice scutellum does not respond to addition of either GA or ABA (Karrer and Rodriguez, 1992). The expression of *Amy3D* and *Amy3E* in rice cell cultures is also unaffected by GA or by an inhibitor of GA biosynthesis (Simmons and Rodriguez, 1989; Thomas et al., 1994). GA-insensitive production of α-amylase was also detected in the scutellum tissue of sorghum (Aisien and Palmer, 1983) and barley (MacGregor and Marchylo, 1986). Finally, no GARE boxes were found in the DNA sequences from the promoters of the *Amy3D* and *Amy3E* genes (Huang et al., 1990a). Thus, GA may only accelerate α-amylase gene expression, whereas another regulatory mechanism is required to initiate α-amylase gene expression in the aleurone (Fincher, 1989). Expression of α-amylase in the scutellum is clearly regulated in a different manner than that in the aleurone. Indeed, considering the complexity of the α-amylase gene family, the critical role of α-amylase in initiating the breakdown of starch, and the multiple roles of starch-derived sugar as nutritional, osmotic, and regulatory molecules, it is not surprising that multiple regulatory mechanisms contribute to the control of α-amylase gene expression.

CARBON METABOLITE SIGNALS REPRESS α-AMYLASE GENE EXPRESSION IN THE CEREAL SEEDLING SOURCE

Metabolic regulation of gene expression, in which the excess or deficiency of certain metabolites effects changes in gene expression, is an important mechanism in the control of source/sink relations in plants (Farrar, 1991). Treatment with Suc, Glc, or acetate represses expression of many photosynthetic genes in the vegetative source leaves of maize (Sheen,

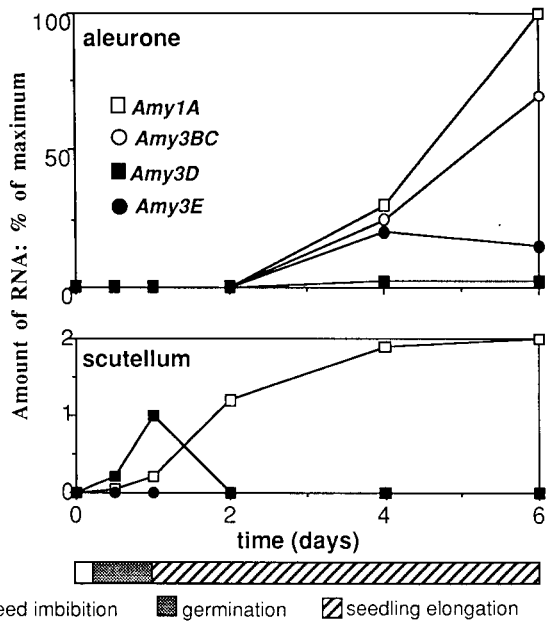

Figure 1. α-Amylase gene expression during rice seedling development. Dry rice seeds were moistened starting at time zero. RNA amounts are represented as percent maximum expression of *Amy1A* in aleurone (Karrer et al., 1991; Ranjhan et al., 1992).

1990). Glc represses photosynthetic gene expression in leaves of *Chenopodium*, spinach, tobacco, and potato (Krapp et al., 1993). When the sinks cannot absorb the photosynthate as fast as it is produced, the excess sugar accumulating in the source may be the signal for sink regulation of photosynthetic rate (Krapp et al., 1993).

A similar mechanism of metabolic regulation may function in the nonphotosynthetic tissue of the cereal seedling source. Various sugars and the organic acid butyrate regulate expression of the α-amylase genes in cereal seedlings. Butyrate, or some metabolite derived from butyrate, inhibits α-amylase gene expression in the barley aleurone (Kumar et al., 1985). The level of *Amy3D* mRNA in the scutellum tissue of the rice seedling is reduced when sugars such as Suc, Glc, Fru, or maltose accumulate in the tissue (Karrer and Rodriguez, 1992). Suc also represses the expression of both the *Amy3D* and *Amy3E* genes in rice cultured cells (Huang et al., 1993), so it is likely that *Amy3E* gene expression in aleurone is also metabolically regulated. A Glc concentration of 100 mM reduces *Amy3D* mRNA levels by 50%, whereas at 250 mM Glc the *Amy3D* mRNA level is near zero. Equivalent concentrations of mannitol or sorbitol have no effect on *Amy3D* mRNA accumulation, indicating that the change in gene expression is not due to osmotic effects (Karrer and Rodriguez, 1992). Induction of a Suc synthase gene at Glc concentrations that repress the *Amy3D* gene (see below) shows that lack of *Amy3D* expression is not due to a general inhibition of gene expression and demonstrates the specificity of the metabolic regulation mechanism. The metabolic regulation of *Amy3D* and *Amy3E* is controlled at the levels of both transcription and mRNA stability (Huang et al., 1993; Sheu et al., 1994). Osmotic stress causes a general inhibition of protein translation in cereal seedlings (Chrispeels, 1973). *Amy3D* mRNA accumulation is completely repressed at osmotic concentrations at which total protein synthesis is only partially inhibited, further demonstrating that these effects are under the control of different mechanisms.

Thus, sugar or some related metabolite that accumulates in the seed during germination acts as a regulatory signal to repress expression of the *Amy3D* and *Amy3E* genes. This regulatory mechanism may act to control the rate of starch breakdown in the source to match the rate of sugar export to the sink tissues (Fig. 2). Genes encoding other enzymes of the starch breakdown pathway have not been tested for metabolic regulation of gene expression, so it is not yet clear if the α-amylase genes are a focal point of regulation or if perhaps the entire pathway is coordinately regulated by this mechanism. Note that seeds of rice cv M202 germinate 24 h after the start of imbibition, which precedes the time of expression of the GA-induced α-amylase gene *Amy1A* (Fig. 1). Metabolic regulation of genes such as *Amy3D* and Suc synthase *Sus1* may be the critical control mechanism during the germination period, with GA regulation playing its role later during the seedling elongation period.

SUGAR SIGNAL(S) REGULATE CEREAL SEEDLING SINK METABOLISM AND SUPPRESS THE GA SIGNAL

Translocation of Suc from the source to the sink provides both nutrition and regulatory signal(s) needed to regulate

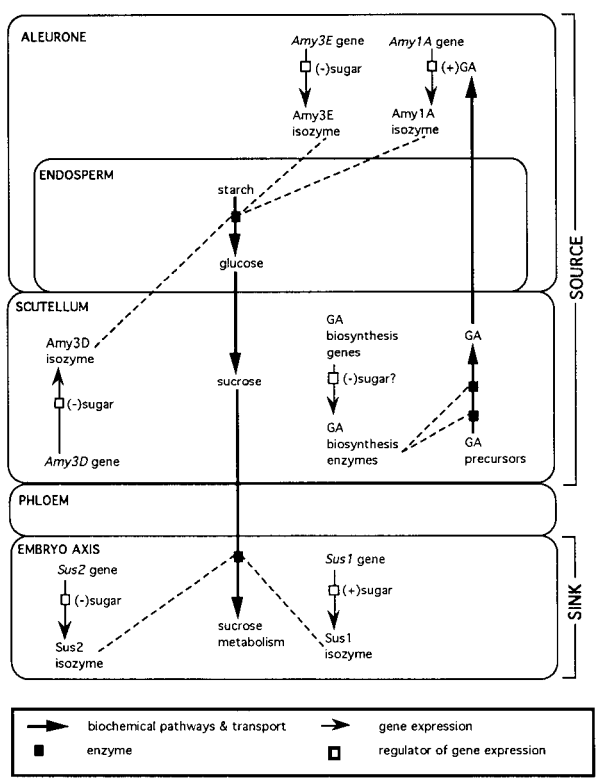

Figure 2. The Carbon Metabolite Signal Hypothesis. The diagram illustrates how carbon metabolite signals interact with the GA signal for control of gene expression, starch mobilization, and source/sink relations in the cereal seedling.

sink metabolism (Farrar and Williams, 1991). Solute concentration has been proposed to regulate the rate of phloem unloading in sink tissues (Patrick, 1990). The activity of Suc synthase in certain sink tissues has also been related to the rate of sink filling (Sung et al., 1989). Sugar regulation of gene expression provides a specific mechanism that may participate in the control of these processes. Cereal species have two types of Suc synthase gene for metabolism of Suc in the sink tissues, one that is metabolically induced by sugar and one that is metabolically repressed by sugar. Suc synthase gene nomenclature recommended by the Commission on Plant Gene Nomenclature is adopted below (Hannah et al., 1994).

The first type of cereal Suc synthase gene is represented by the *Sus1* gene of maize, which has gene expression in roots that is induced by Suc, Glc, or Fru (Koch et al., 1992). A *Sus1* gene hybridization probe was used to demonstrate induction of Suc synthase gene expression by Suc, Glc, Fru, or maltose in germinating rice embryos (Karrer and Rodriguez, 1992). The tissues within the rice embryo in which this Suc synthase gene expression is occurring have not yet been defined. Metabolic induction of Suc synthase gene expression in the growing tissues of the cereal embryo and root may serve to stimulate metabolism of incoming Suc to support a rapid rate of growth (Fig. 2).

The second type of cereal Suc synthase gene is represented by the *Sus2* (= *Sh1*) gene of maize, which has gene expression in sink tissues where starch synthesis is occurring (Chen and Chourey, 1989). Expression of the maize Suc synthase gene *Sus2* is repressed by Suc in protoplasts derived from cultured cells (Maas et al., 1990) and in roots (Koch et al., 1992). The sugar repression of *Sus2* gene expression may serve to ensure that the metabolism of incoming Suc does not greatly exceed the rate at which sugar can be incorporated into starch.

A sugar metabolite signal may also regulate the GA signal in cereal seedlings. Treatment with Suc, Glc, or maltose prevents GA production/release from the barley scutellum, whereas sugar depletion permits GA release (Radley, 1969). This effect could involve allosteric changes in GA biosynthetic enzymes or metabolic regulation of gene expression. According to the hypothesis by Briggs (1992), depletion of sugar in the barley scutellum during the germination stage leads to stimulation of GA synthesis/release. GA then diffuses to the aleurone, where it stimulates increased production of hydrolytic enzymes, which leads to production of more sugar via an increased rate of starch breakdown. This sugar then inhibits the further production/release of GA (Fig. 2). The continuous presence of a GA signal may not be required to maintain the high level of α-amylase mRNA that is observed in cereal seedlings because α-amylase mRNA is quite stable (Ho and Varner, 1974).

CONCLUSIONS

Metabolite regulation of gene expression is gaining recognition as a fundamental and ubiquitous mechanism for controlling plant metabolism, during both vegetative and seedling development. The Carbon Metabolite Signal Hypothesis (Fig. 2) describes a system of regulatory interactions in which carbon metabolite signals and GA signals control carbon metabolism and source/sink relations in cereal seedlings. The cereal seedling source is autoregulated, with α-amylase gene expression controlled to produce a balance between the rate of starch breakdown and the rate at which the resultant sugars are exported to the sinks. Metabolite signals and GA signals in cereal seedlings regulate both the total amount of α-amylase gene expression and the expression of different α-amylase genes encoding isozymes with distinct substrate specificities. The GA signal functions to stimulate nutrient mobilization from the endosperm, with transport of Suc providing both nutrition and regulatory signals for the sink tissues. Metabolite induction of a Suc synthase gene activates Suc breakdown and stimulates growth of sink tissues in cereal seedlings. Metabolite repression of GA synthesis or release during cereal seedling development acts as a signal that nutrient mobilization from the endosperm has been initiated. Thus, metabolite signals and the GA signal play significant roles in control of source/sink relations and growth of the cereal seedling.

The Carbon Metabolite Signal Hypothesis may stimulate new research initiatives on gene expression during cereal seedling development to extend beyond the strictly hormonal control mechanisms of the past. The components of the metabolite regulation signal transduction pathways must be defined and their interactions characterized. Many genes in animals are regulated by cross-talk between different signal transduction pathways (Sharma, 1993) and regulatory interactions of metabolite and GA signals in plants may function by a similar mechanism. Physiological and biochemical studies are needed to measure the effects that the changes in gene expression have on the flux of metabolites through various pathways and on the rate of plant growth. Regulation of sugar transport steps in the source/sink pathway of cereal seeds has also been neglected, but it is of interest to determine whether there is any similarity between the cereal system and the mammalian systems in which expression of a Glc transporter gene is metabolically regulated (Ismail-Beigi, 1993). With the rapid progress being made in plant molecular biology and physiology, we anticipate that the answers to many of these questions will be available soon.

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