Metabolic Derepression of α-Amylase Gene Expression in Suspension-cultured Cells of Rice*

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Su-May Yu††§, Yen-Hong Kuo‡‡§, Ginger Sheu††, Yi-Jun Sheu**, and Li-Fei Liu†

From the ††Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, 11529, the ‡‡Departments of Agronomy **Botany, National Taiwan University, Taipei, Taiwan, 10764, and the †††Institute of Life Science, National Tsing-Hua University, Hsinchu, Taiwan, 30043 Republic of China

We present evidence to show that the α-amylase gene family in rice is under two different modes of regulation: 1) hormonal regulation in germinating seeds, and 2) metabolic repression in cultured cells by available carbohydrate nutrients. Expression of α-amylase genes in deembryoned rice seeds is known to be induced by exogenous gibberellic acid. On the other hand, expression of α-amylase genes in suspension-cultured cells is induced by the deprivation of carbohydrate nutrient. A lag period of 2–4 h is required for the induction of α-amylase mRNA in sucrose-depleted medium. The induction of α-amylase expression is extraordinarily high and levels of α-amylase mRNA can be increased 8–20-folds after 24 h of sucrose starvation. The synthesis and secretion of α-amylase is also dependent upon the level of carbon source. The derepression or repression of α-amylase synthesis can be readily reversed by the deprivation or replenishment of sucrose in the medium, respectively. Glucose and fructose exert a repression on the α-amylase synthesis similar to that of sucrose. A hypothesis that explains the induction of α-amylase synthesis by carbohydrate starvation is proposed. Our data have suggested a hitherto undiscovered, potentially important control mechanism of carbohydrate metabolism in higher plants.

The amylolytic breakdown of storage starch is one of the central biochemical events in the germination of cereal grain. Early in seed germination, the embryosynthesizes gibberellins which diffuse to the aleurone cells where gibberellins act as signals to activate the synthesis and secretion of α-amylases and other hydrolases. These enzymes digest the stored starch and other nutrients in the endosperm to support the growth of young seedlings. α-Amylase is the most abundant hydrolase produced in the barley aleurone tissue in response to gibberellic acid (GA₃), one of the gibberellins during seed germination (Jacobsen and Chandler, 1987). The appearance of α-amylase in germinating seeds is due to the de novo synthesis of this enzyme (Chrispeels and Varner, 1967) which is accompanied by a dramatic increase of α-amylase mRNA after GA₃ treatment of aleurone layers (Chandler et al., 1984; Deikman and Jones, 1985; Higgins et al., 1976; Muthukrishnan et al., 1983). In vitro transcription using isolated nuclei from GA₃-treated aleurone protoplasts of barley and oat showed that GA increases the rate of transcription of the α-amylase genes (Jacobson and Beach, 1985; Zwar and Hooley, 1986).

During the culture of rice suspension cells, we observed that the expression of α-amylase genes was induced as cells entered the stationary phase. A similar phenomenon was observed in the Gram-positive prokaryote Bacillus in which the expression of α-amylase genes had been shown to be subject to catabolite repression. There was a sharp rise in amylase activity as the Bacillus cells entered the stationary phase in nonrepression medium (Lsoide et al., 1989; Nicholson and Chambris, 1985) and the production of α-amylase in stationary-phase cells was repressed by the presence of glucose, and derepressed when glucose was absent (for review, see Priest, 1977). The glucose-regulated proteins found in cultured animal cells after glucose starvation have been studied extensively (for review, see Lee, 1987). Although the functions of glucose-regulated proteins are still not clear, GRP78 was shown to be identical to the immunoglobulin heavy chain-binding protein (Hendershot et al., 1988; Munro and Pelham, 1986) and therefore is suggested to be involved in regulating transport of secreted or transmembrane proteins from endoplasmic reticulum to the Golgi apparatus during glucose starvation or other stress conditions (Bole et al., 1986; Hendershot and Kearney, 1988).

In higher plants, glucose is synthesized through photosynthesis and accumulated in the form of starch in chloroplasts, or is converted to sucrose in the cytosol for export. Export of sucrose from leaf cells connects carbon assimilation at the source (photosynthetic tissues) to carbon utilization at the sink (nonphotosynthetic tissues) (Stitt, 1986). Feeding of leaves with sucrose or glucose causes a substantial increase in leaf sugar content and significant decrease in photosynthesis rate (Foyer, 1988). Therefore, sugars can regulate photosynthesis when products of photosynthesis exceed the capacity of photosynthetic utilization. Feed-back regulation of photosynthesis by sugars has recently been demonstrated at the molecular level. Using isolated maize mesophyll protoplasts and a transient expression method, transcription of seven maize photosynthetic gene promoters was shown to be repressed by the photosynthetic end products sucrose and glucose and by the exogenous carbon source acetate (Sheen, 1990). The metabolic regulation of gene expression seems to play an important role in maintaining an economical balance of supply and demand of biomolecules in higher plants.

Here we show another type of metabolic regulation of gene expression in higher plants. We also present evidence to show that this is a new mode of regulation for expression of α-amylase genes in rice, which has never been reported before.
In addition to regulation by GA, in germinating seeds of rice, the expression of α-amylase genes in suspension-cultured cells of rice is regulated by the level of carbohydrate present in the culture medium. The synthesis of α-amylases and levels of their mRNA are greatly induced under sucrose starvation. An increase of α-amylase synthesis is assumed to be due to a decrease in the drosylation of cellular starch as an energy source when exogenous carbon source is depleted. Under normal growth condition with an adequate supply of sugars in the medium, the expression of α-amylase genes is subject to metabolite repression.

EXPERIMENTAL PROCEDURES

Materials—The rice variety used for cell culture was *Oryza sativa* cv. TN5. The polyclonal antibodies used to detect α-amylase by immunoblotting were obtained from Tuan-Hua David Ho (Washington University, St. Louis) and were raised in rabbit against barley aleurone α-amylases. The α-amylase genomic DNA pBAR used for Northern blot analysis was obtained from Ray Wu (Cornell University).

Cell Cultures of Rice—The embryo-containing portion of the immature (10–12 days post-anthesis) rice grain was removed by excision. The resultant half-grain was sterilized with 1% NaOCl and 1 drop of Tween 20 for 20–30 min and then washed extensively with sterile distilled water. The half-grain was then placed with scutellum side up on Murashige and Skoog (MS) agar medium (Murashige and Skoog, 1962) containing 0.8% agar (w/v), 3% sucrose, and 5 μM 2,4-D. The pH of the medium was adjusted to 5.8 before autoclaving. The embryo culture was incubated at 25 °C under 24-h white light illumination (2000 lux). Four weeks later, initiated calli were transferred to a liquid MS medium containing 3% sucrose and 10 μM 2,4-D. Approximately 500 mg fresh weight of calli were cultured in 30 ml of medium in a 125-ml Erlenmeyer flask. The suspension culture was shaken on a reciprocal shaker at 120 rpm and incubated at 25 °C under constant light (2000 lux).Established suspension-cultured cells were subcultured every 7 days by transferring about 0.5 ml (approximately 0.4 g of fresh weight cells/ml) of cells into 25 ml of fresh liquid MS medium in a 125-ml flask. The cell cultures were maintained growing logarithmically and 7-day-old cultures were used for initiating all the indicated experiments.

Sample Collection—Suspension-cultured cells were collected by filtration through a 400-mesh nylon sieve, blot-dried on paper towels, and cultured cells according to the method described by Belanger et al. (1986). RNA blot analysis was performed as described by Thomas (1983).

Western Blot Analysis—Prior to fractionation on SDS-polyacrylamide gel electrophoresis, proteins were denaturated at 100 °C for 30 s in a sample buffer containing 10% (w/v) sucrose, 2% (w/v) SDS, 5% β-mercaptoethanol, and 0.01% (w/v) bromophenol blue. Electrophoresis was performed in a 4% stacking, 10% resolving polyacrylamide gel according to a described method (Laemmli, 1970). Proteins were transferred to a nitrocellulose filter by electroblotting in a Transphor 8-mercaptoethanol, and λDNA, and 50% methanol. The protein blot was stained with the anti-α-amylase antibodies using the avidin-biotin-peroxidase system (Vectastain ABC kit, Vector Lab). Prestained protein molecular weight standards (Bethesda Research Laboratories) were used as markers.

Thin Layer Chromatography—Examination of sugars present in culture medium was performed with a modified thin layer chromatography (Chaplin, 1986). A precoated silica gel (Silica Gel-60 F254 from Merck) was used as the solid support. The solvent system used for separating sugars contains ethyl acetate, acetic acid, and H2O at a ratio of 3:3:1. The reagents used for detection of sugars include aniline, diphenylamine, acetone, and 80% H3PO4 at a ratio of 4 ml, 4 g, 200 ml, 90 ml.

RESULTS

Effect of GA on the Expression of α-Amylase Genes in Rice—Previously, we have observed that GA induced the expression of α-amylase genes in deembryoed rice seed. Accumulation of α-amylase mRNA in the GA3-treated seeds as a function of time after GA3 addition was determined by Northern blot analysis (Fig. 1A, lanes 7–12). No α-amylase mRNA was detected in the deembryoed seeds without GA3 treatment (Fig. 1A, lanes 1–6). We were interested to know whether GA also increases the α-amylase mRNA level in the suspension-cultured cells of rice. To analyze this phenomenon, 7-day-old cells were transferred to the regular MS medium with or without 1.0 μM GA3, and the appearance of α-amylase mRNA was monitored at different times. Levels of α-amylase mRNA remained low 1–8 days after the cells were transferred to both medium (Fig. 1B, lanes 2–4 and 6–8). Surprisingly, a dramatic increase of α-amylase mRNA level was observed at day 12 regardless of the presence or absence of GA3 (Fig. 1B, lanes 5 and 9). The results show that the expression of α-amylase genes was not significantly altered with the addition of GA3 in medium; however, the expression was induced at later growth stages of the suspension culture whether or not the medium contained GA3.

Metabolic Repression of α-Amylase Gene Expression by Sucrose—Since the end product of α-amylase action on its substrate is sugar (glucose), we suspected that the induction of α-amylase gene expression might be related to the sugar

![Fig. 1. Effect of GA3 on the expression of α-amylase genes in rice. A, deembryoed rice seeds were treated with (+) or without (−) 10 μM GA3 for various days and collected. B, cultured rice cells were grown in sucrose-containing medium with (+) or without (−) GA3 for various days and collected. Total RNA was then purified from the treated seeds or cells, and the α-amylase mRNA was detected by Northern blot analysis using 32P-labeled pBAR as probe. Five μg of RNA was applied to each lane.](image)
present in medium. Therefore, we further investigated the relationship between the expression of α-amylase genes, the sugar level in medium, and the growth curve of cells (Fig. 2A). Although the carbon source added into the culture medium was sucrose, a small amount of sucrose was found to be hydrolyzed to glucose and fructose during autoclaving as determined by thin layer chromatography. We also found that sucrose in the medium was hydrolyzed to glucose and fructose during culture of cells (Fig. 6A). Therefore, the sugar detected in medium is actually a mixture of sucrose, glucose, and fructose. The anthrone-positive sugars decreased dramatically at the onset of the stationary-phase and were depleted to almost undetectable levels at day 12. When sugars were still present, the level of total α-amylase mRNA remained low. But a substantial increase in α-amylase mRNA became apparent at day 12 and reached a maximum at day 14 concomitant with sugar depletion. The results suggest that depletion of sugars in the medium is very likely related to the onset of the stationary-phase and expression of the α-amylase genes.

**Regulation and the Expression of α-Amylase Genes in Suspension-Cultured Cells**

To further prove that the expression of α-amylase genes is regulated by available sugars, 7-day-old cells were transferred to sucrose-containing or sucrose-free medium, and the appearance of α-amylase mRNA was monitored at different times. The level of α-amylase mRNA remained low 1 and 3 days after the cells were transferred to a fresh medium containing sucrose (Fig. 2B, lanes 2 and 3). In contrast, the α-amylase mRNA level was greatly increased only 1 day after the cells were transferred to the sucrose-free medium (Fig. 2B, lane 4) and remained at high levels for at least another 2 days (Fig. 2B, lanes 5 and 6). The results confirm the notion that the presence of sucrose in the medium repressed the expression of α-amylase genes in the cells.

**Kinetics of Accumulation of α-Amylase mRNA during Sucrose Starvation**—To analyze this phenomenon, 5-day-old (early log-phase) cells were transferred from sucrose-containing medium to sucrose-free medium. Relative mRNA level as a function of incubation time in the sucrose-free medium is presented (Fig. 3). There was an initial decrease of α-amylase mRNA level within 30 min after the transfer of culture, then followed by a lag period of approximately 2 h before the rapid increase of α-amylase mRNA. The α-amylase mRNA levels after 4 and 24 h increased 2- and 8-fold, respectively. If 7-9-day-old (late log-phase) cells were transferred from sucrose-containing to sucrose-free medium, up to 40-fold increase of α-amylase mRNA level can be observed after 24 h (Fig. 2B).

![Fig. 2. Effect of sugars in medium on the expression of α-amylase genes in suspension-cultured cells of rice. A, the relationship between cell growth, medium sugar level, and the appearance of α-amylase mRNA in the suspension-cultured cells. Cells were grown in normal medium containing 3% sucrose. At 2-day intervals, cells were collected, and the media were removed for analysis of sugar concentration. Cells were weighed and subsequently used for RNA purification. Open boxes indicate fresh weight of cultured cells; closed boxes indicate sugar level of the medium. B, effect of sucrose starvation on the appearance of α-amylase mRNA in the suspension-cultured cells. Cells were grown in sucrose-containing (+ sucrose) or sucrose-free (− sucrose) medium for various times and collected. Total RNA was purified from cells collected in A and B, and the α-amylase mRNA was detected by Northern blot analysis using 32P-labeled pBAR as probe. Five μg of RNA was applied to each lane.](image-url)

![Fig. 3. Accumulation of α-amylase mRNA during sucrose starvation. Cells were grown in sucrose-free medium, and total RNA was purified from the cultured cells at various times. Five μg of RNA was applied to each lane. α-Amylase was detected by Northern blot analysis using 32P-labeled pBAR as probe. The autoradiogram was quantified by densitometry to obtain the relative level of α-amylase mRNA at various times after incubation in the sucrose-free medium.](image-url)
α-Amylase has long been known as an extracellular secretory protein in germinating cereal seeds (Akazawa and Hara-Nishimura, 1985). The rice seed callus was also found to synthesize and secrete high levels of α-amylase (Simmons and Rodriguez, 1989). Our rice suspension-cultured cells were originally derived from the scutellum which has been shown to be an important tissue for the biosynthesis of α-amylase in germinating rice seeds (Okamoto and Akazawa, 1979). It would be interesting to know whether α-amylase is secretory in these suspension-cultured cells and whether or not its accumulation in the culture medium also responds to sucrose starvation. Therefore, media from the same experiment as described in Fig. 4A were collected and assayed for the appearance of α-amylase by Western blot analysis. The 44-kDa α-amylase was not detected in the sucrose-containing medium throughout the 48-h incubation period (Fig. 4B, lanes 1–5). In contrast, α-amylase was detectable in 12 h in the sucrose-free mannitol-containing media either in the absence (Fig. 4B, lane 6) or presence (Fig. 4B, lane 10) of starch. Furthermore, α-amylase increased with incubation time in a similar manner as in Fig. 4A. We also detected the elevated α-amylase activities which parallel the increase of α-amylase detected by Western blot analysis (data not shown). Another test for α-amylase secretion is the ability of cultured cells to utilize extracellular starch. Because the plasma membrane is impermeable to starch, α-amylase has to be secreted so that cells can utilize starch as the carbon source. Cells grown in the sucrose-free medium containing starch showed a linear increase of growth. Cells grown in the sucrose-free medium without starch, however, showed a rapidly declined of growth 2–3 days after sucrose starvation (data not shown). The above results indicate that α-amylase was secreted from cells into the medium and its accumulation was regulated by sucrose.

Two other proteins with molecular masses of approximately 85 and 196 kDa were also detected in the rice cell extract but not in the medium by α-amylase antibodies in Western blot analysis. We do not know what these two proteins are at this moment as the barley α-amylase polyclonal antibodies employed in our Western blot analysis were raised against purified α-amylases. The levels of these two proteins remained unchanged in different medium throughout the entire incubation period (Fig. 4A). Consequently, these proteins serve as an internal control for the quantitative protein loading.

Reversibility of the Metabolic Repression and Derepression of α-Amylase Synthesis—To analyze this phenomenon, cells were alternately grown in sucrose-free and sucrose-containing media for two cycles. The incubation time of culture in different medium in each cycle was 48 h. Cells were washed in fresh sucrose-free or sucrose-containing medium prior to transfer to the indicated medium. Cells were collected at 0, 24, and 48 h, and α-amylase was extracted and detected by Western blot analysis. In the first cycle, levels of α-amylase increased with time in the sucrose-free medium (Fig. 5, lanes 1–3), then decreased with time as the cells were transferred to sucrose-containing medium (Fig. 5, lanes 3–5). In the second cycle, α-amylase increased again in the sucrose-free medium (Fig. 5, lanes 5–7) and decreased as the cells were transferred to sucrose-containing medium (Fig. 5, lanes 7–9), although the signal on the Western blot does not seem to decrease very much in the second cycle. α-Amylase levels remained low and unchanged in a control experiment in which the cells from the same starter culture as in Fig. 5 were transferred to a fresh sucrose-containing medium at the end of each 48-h incubation period (data not shown). The results indicate that the repression or derepression of α-amylase synthesis is reversible and mediated by the presence or ab-

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Footnote:

2 S.-M. Yu, unpublished results.
Metabolic Repression of $\alpha$-Amylase Synthesis by Different Sugars—Sucrose is hydrolyzed to glucose and fructose prior to uptake by suspension cultured cells of rice (Amino and Tazawa, 1988). By thin layer chromatography, we had also determined that the filter-sterilized sucrose in medium was hydrolyzed to glucose and fructose during the culture of cells (Fig. 6A). The level of glucose and fructose increased inversely with the decrease of sucrose as cells grew and sucrose in the medium was eventually completely hydrolyzed to glucose and fructose. To investigate whether fructose and glucose directly exert metabolic repression on the $\alpha$-amylase synthesis, cells were transferred to medium containing fructose, glucose, sucrose, or no sugar and incubated for 2 days. In this experiment, all the sugars were filter-sterilized and then added into autoclaved MS medium. Proteins were collected directly from the medium without further purification and the $\alpha$-amylases were detected by Western blot analysis. Levels of $\alpha$-amylases in all the media containing sugars were low (Fig. 6B, lanes 1–3). In contrast, the accumulation of $\alpha$-amylase in the sugar-free medium was relatively high (Fig. 6B, lane 4). This result indicates that sugars such as glucose, fructose, and sucrose which are readily utilisable by cells can suppress the synthesis of $\alpha$-amylase.

**DISCUSSION**

Because of the central role $\alpha$-amylase plays in the starchy endosperm mobilization in germinating cereal grains, a major research effort has been focused on the regulation of this enzyme during seed germination. Since we have observed that GA$_3$ induced $\alpha$-amylase gene expression in deembryoed rice seed, we also examined the effects of GA$_3$ on $\alpha$-amylase gene expression in the suspension-cultured cells of rice. Unlike the response from the deembryoed seeds to exogenous GA$_3$, application of GA$_3$ to the cultured cells of rice did not significantly increase the level of $\alpha$-amylase mRNA (Fig. 1). The level of $\alpha$-amylase mRNA in deembryoed rice seeds was significantly increased after GA$_3$ treatment, compared to untreated seeds, indicating that GA$_3$ is biologically active. These results are in agreement with a similar observation on $\alpha$-amylase synthesis in rice callus (Simmons and Rodriguez, 1989). Although the cultured cells were originally derived from the scutellum which has been shown to be a tissue for biosynthesis of $\alpha$-amylases in response to GA$_3$ (Okamoto and Aka-zawa, 1979), we do not know whether the cultured cells are receptive to exogenous GA$_3$ at this moment. Nevertheless, we found that expression of $\alpha$-amylase genes in the cultured cells is induced under conditions of carbohydrate depletion. The extent to which metabolic repression of $\alpha$-amylase gene expression is relieved correlates with the degree of growth limitation of the carbon source (Fig. 2). Therefore, expression of the $\alpha$-amylase gene family in rice appears to be under two different modes of regulation: 1) hormonal regulation in germinating seeds, and 2) metabolic repression in cultured cells by carbohydrate nutrients. Whether these two modes of regulation operated through a same or different molecular mechanism is not known. Molecular mechanisms underlying the two different modes of regulation and interactions between them will be the focus for further studies.

Questions were raised on the in vivo role of $\alpha$-amylase in
the carbohydrate-starved culture cells. We have observed many starch granule-containing amyloplasts in the normally cultured cells of rice. Therefore we assume that as the cells sense a shortage of carbohydrate supply, they are responding by synthesizing and secreting large amounts of α-amylases in order to digest intra- or extracellular starch as an energy source. Consequently, the synthesis of α-amylase is crucial for cell survival. Under normal growth conditions with an adequate supply of readily metabolizable carbon source, the rate of α-amylase synthesis is low and subject to metabolic repression. A lag period of approximately 2 h is required before the induction of α-amylase mRNA (Fig. 3). During the lag period the cells were likely adapting to the loss of sucrose as the primary carbon and energy source and turning on the α-amylase genes in response to this nutritional stress. It would be interesting to know whether the expression of other genes is modulated simultaneously or before the induction of α-amylase mRNA during this lag period.

Synthesis of α-amylases is suppressed in cells grown in media containing sugars, whereas, it is not repressed in those cells grown in sucrose-free medium containing raw starch (Fig. 4). We found that only raw starch powder, but not the 80 °C heated and gelatinized starch, elevated the synthesis of α-amylase (data not shown). It implies that raw starch in the medium could not be immediately utilized by the starved cells. However, cells can rely on starch as the sole carbon source for growth. It suggests that α-amylases must be synthesized and secreted into medium to digest the extracellular starch after carbohydrate starvation. Secreted α-amylase, probably with the aid of other amylolytic or sugar-hydrolytic enzymes, can digest starch in the medium and result in the liberation of glucose.

The half-life of α-amylase mRNA from barley aleurone has been estimated to be greater than 100 h (Ho et al., 1987). However, it is not known whether α-amylase mRNA in the cultured cells becomes less stable after replenishment of sucrose. Reduction of α-amylase accumulation was detected 24-48 h after replenishment of sucrose to the starved cells (Fig. 5A). The rate-limiting step of α-amylase accumulation can be at the transcriptional and/or translational levels. Although the strong induction of transcripts after sugar deprivation (Fig. 2) would suggest transcriptional control, future experiment on the nuclear run-on transcription should determine the effect of sugar deprivation on the rate of transcription and better reveal the mechanism of α-amylase gene regulation.

Sucrose is most commonly used in plant cell cultures as a carbon source. In suspension-cultured cells of rice, sucrose in the medium is rapidly hydrolyzed extracellularly prior to uptake by cells. The hydrolysis of sucrose was shown to be due to cell wall-associated acid invertase which generated almost equal amounts of glucose and fructose (Amino and Tazawa, 1988). The observation that fructose and glucose exerted repression of α-amylase synthesis similar to that of sucrose (Fig. 6B) and assays with thin layer chromatography confirmed the hydrolysis of sucrose into glucose and fructose during culture of cells (Fig. 6A), suggests that sucrose is first hydrolyzed to fructose and glucose which then enters the glycolytic pathway. Since the regulatory mechanisms of the metabolic repression must include sensory and signaling mechanisms for monitoring sugar availability in the medium, it would be interesting to know whether fructose or glucose is likely to serve as the signal molecule to initiate a signal transduction cascade leading to the repression of α-amylase gene expression.

The accumulation and subsequent utilization of reserve substances are fundamental processes of living cells which enable them to maintain metabolic activities when exogenous sources are not available. Under certain environmental conditions, e. g. during night or annual resting seasons, photosynthesis is not operative and plants have to rely on internal carbon sources to maintain metabolic activities. In addition, during plant ontogenesis, active growth processes and metabolic activities would rely to a major extent on the utilization of endogenous reserve carbohydrates until the photosynthetic apparatus has been developed. Discussion on the physiology and biochemistry of starch degradation on these respects is available (Steup, 1988), however, the regulatory mechanisms of starch degradation still remain unknown. Plant tissues with reduced chloroplast density (e.g. petals and stems) or function (i.e. senescent leaves and leaves darkened for prolonged periods) have been shown to possess significantly higher α-amylase activity than leaf tissues with totally competent chloroplasts (Saeed and Duke, 1990). However, it is not clear whether or not the density and/or function of the plastids directly related to the α-amylase activity. We believe that signals must arise from certain physiological processes that lead to the elevated α-amylase activities. Our current work reveals new insights into the regulatory aspects of this process. The dynamic translocation of carbohydrates between source and sink and signal-mediated interactions among different cells and tissues of a plant makes it difficult to study how the regulation of starch mobilization is controlled in vivo. The multicellular nature of higher plants also creates difficulties for the study of metabolic regulation. In contrast, the derepression of α-amylase gene expression in the cultured cells of rice by carbohydrate starvation would provide an ideal model to study the regulation of starch immobilization and the in vivo role of α-amylases in a carbohydrate-starved cell and a photosynthetic plant at the molecular level. In contrast to being widely used as a model system for studying hormonally and developmentally regulated gene expression in plants, the metabolic derepression of α-amylase gene expression in cultured cells of rice also provides a good system for investigating the sensory, transduction, and response mechanisms used by plants to interact with their environment. Future studies on the regulatory mechanisms pertinent to α-amylase synthesis and starch immobilization at the cellular level would lead to an understanding of the physiological significance of α-amylases in the carbohydrate metabolism of whole plant.

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Regulation of Rice α-Amylase Gene

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