Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 6543–6547, May 1998
Plant Biology

The 3′ untranslated region of a rice α-amylase gene functions as a sugar-dependent mRNA stability determinant

MING-TSAIR CHAN* AND SU-MAY YU†

Institute of Molecular Biology, Academia Sinica, Nankang, Taipei 11529, Taiwan, Republic of China

Communicated by Shang F. Yang, Academia Sinica (Taiwan), Taipei, Taiwan (R.O.C.), March 10, 1998 (received for review March 15, 1997)

ABSTRACT **In plants, sugar feedback regulation provides a mechanism for control of carbohydrate allocation and utilization among tissues and organs. The sugar repression of α-amylase gene expression in rice provides an ideal model for studying the mechanism of sugar feedback regulation. We have shown previously that sugar repression of α-amylase gene expression in rice suspension cells involves control of both transcription rate and mRNA stability. The α-amylase mRNA is significantly more stable in sucrose-starved cells than in sucrose-provided cells. To elucidate the mechanism of sugar-dependent mRNA turnover, we have examined the effect of *αAmy3* 3′ untranslated region (UTR) on mRNA stability by functional analyses in transformed rice suspension cells. We found that the entire *αAmy3* 3′ UTR and two of its subdomains can independently mediate sugar-dependent repression of reporter mRNA accumulation. Analysis of reporter mRNA half-lives demonstrated that the entire *αAmy3* 3′ UTR and the two subdomains each functioned as a sugar-dependent destabilizing determinant in the turnover of mRNA. Nuclear run-on transcription analysis further confirmed that the *αAmy3* 3′ UTR and the two subdomains did not affect the transcription rate of promoter. The identification of sequence elements in the α-amylase mRNA that dictate the differential stability has very important implications for the study of sugar-dependent mRNA decay mechanisms.**

Sugar repression of gene expression is a fundamental and ubiquitous regulatory system for adjusting to changes in nutrient availability in both prokaryotic and eukaryotic cells. Expression of enzymes involved in carbohydrate metabolism often is feedback-regulated by excess sugar metabolites. In multicellular plants, feedback repression of gene expression by excess sugars provides an additional mechanism for maintaining an economical balance between supply (source) and demand (sink) for carbohydrate allocation and utilization among tissues and organs (1–3). Sugar depletion has been shown to enhance expression of a wide variety of genes involved in photosynthesis, reserve mobilization, and carbohydrate-export processes in plants (4); however, the molecular mechanism of sugar repression remains largely unknown.

The sugar-dependent repression of α-amylase gene expression provides an ideal model for studying the mechanism of sugar feedback regulation in plants. α-Amylases are *endo*-amylolytic enzymes that catalyze the hydrolysis of α-1,4-linked glucose polymers and play an important role in degradation of starch in higher plants. In rice germinating embryos and cultured suspension cells, expression of α-amylase genes is activated by sugar depletion and repressed by sugar provision (3, 5, 6). An increase in both the transcription rate and mRNA half-life under sucrose depletion contributes to the increase in the steady-state level of α-amylase mRNA (7, 8). Studies in transgenic rice confirmed that the α-amylase gene promoters control sugar-dependent repression of reporter gene expression (9–11).

Most studies of sugar repression in yeasts, animals, or plants have emphasized transcriptional control mechanisms and perhaps overlooked the posttranscriptional component. The glucose-sensitive, differential mRNA stability in yeast may be a global phenomenon (12). However, in *Saccharomyces cerevisiae*, mechanisms controlling the glucose-sensitive turnover of mRNA has been studied only for the mRNAs encoding the iron-protein (Ip) and flavoprotein (Fp) subunits of succinate dehydrogenase (12–14). The 5′ untranslated region was shown to play a dominant role in the glucose-dependent turnover of the Ip mRNA (13). Another example of posttranscriptional regulation that is also linked to the availability of extracellular metabolite is the iron-dependent destabilization of transferrin receptor (TfR) mRNA in mammalian cells. The stem-loop structures located in the 3′ untranslated region (3′ UTR) mediate the iron-dependent stability of the TfR mRNA (15, 16).

Previously, we have shown that the mRNA half-life of one rice α-amylase gene, *αAmy3*, decreased from 6 h to 1.5 h under repressed conditions (8). By examining the transient expression of chimeric genes in rice protoplasts, the 3′ UTR of *αAmy3* mRNA was shown to mediate sugar-dependent repression of fused heterologous gene expression (17). To prove that the *αAmy3* 3′ UTR is responsible for the selective stabilization of mRNA, we transformed rice with chimeric genes carrying various domains of the *αAmy3* 3′ UTR. In this report, we show that the entire *αAmy3* 3′ UTR and two of its subdomains function as sugar-dependent destabilizing determinants in the turnover of a heterologous mRNA. This study identifies a cis-acting element in the mRNA controlling metabolite regulation.

MATERIALS AND METHODS

Plant Material. The rice variety used for transformation was *Oryzae sativa* L. cv. Tainan 5. Embryogenic calli were initiated as described by Chan *et al.* (9) except that the medium was replaced with MS medium (18) containing 1% agarose (wt/vol), 3% sucrose, and 2 μg/ml 2,4-dichlorophenoxyacetic acid (2,4-D) with pH 5.8. After 4 weeks, suspension cultures were initiated by transferring the calli into a liquid MS medium containing 3% sucrose, 2 μg/ml 2,4-D, 0.2 μg/ml kinetin, and 0.1 μg/ml GA_3. The suspension culture was shaken on a reciprocal shaker at 120 rpm and incubated at 26°C in the dark. Suspension cells were subcultured weekly before bombardment.

Transformation of Rice Suspension Cells. In a volume of 70 μl, 1.5 mg of gold particles (Bio-Rad) with an average size of 1 μm were coated with 2 μg of test plasmid and 1 μg of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: UTR, untranslated region; Ip, iron protein; ActD, actinomycin D.

*Present address: Institute of Bioagricultural Sciences, Academia Sinica, Nankang, Taipei 11529, Taiwan, Republic of China.
†To whom reprint requests should be addressed.

pTRA151 (containing hygromycin-resistant gene) (19) DNA by a $CaCl_2$/spermidine precipitation method (20). Rice suspension cells were bombarded with the coated gold particles as described by Cao *et al.* (21) by using a helium gas-retrofitted Biolistic PDS-1000 Particle Delivery System (Bio-Rad). Bombarded cells were transferred to fresh MS solid medium and incubated at 26°C in the dark for 6 days before being transferred to MS solid medium containing 50 μg/ml hygromycin. After 4 weeks, all putative transformed calli were subcultured to MS liquid medium containing 50 μg/ml hygromycin to generate suspension cell culture.

RESULTS

The *αAmy3* 3′ UTR and Two of Its Subdomains Independently Mediate Sugar-Dependent Repression of mRNA Accumulation. The effect of *αAmy3* 3′ UTR on the accumulation of a heterologous mRNA was investigated in transformed rice cell lines. Plasmids pLAm and pLN (17), which contain the entire *αAmy3* 3′ UTR and *Agrobacterium* nopaline synthase gene (*Nos*) 3′ UTR, respectively, downstream of the ubiquitin (*Ubi*) promoter and luciferase (*Luc*) coding region, were used for rice transformation. The *αAmy3* 3′ UTR was divided into three subdomains: domains I, II, and III. Plasmids pLIN, pLIIN, and pLIIIN containing these DNA domains inserted between the *Luc* coding region and *Nos* 3′ UTR were also used for rice transformation. Structures of these plasmids and nucleotide sequences of the three DNA fragments are shown in Fig. 1. Identification of transformed rice calli was confirmed by genomic DNA gel blot analysis (data not shown).

Five independently transformed cell lines for each construct were randomly selected and the levels of *Luc* mRNA were examined by RNA dot blot analysis. The ratio of mRNA levels in cells starved for sucrose to those in cells provided with sucrose are presented in Fig. 2*A*. A ratio equal to 1 (log 10^0) means that the mRNA levels under the two growth conditions were equal. Ratios higher than log 10^0 indicated that the *Luc* mRNA level increased after sucrose starvation, and ratios less than log 10^0 indicated the *Luc* mRNA level decreased after sucrose starvation. The results show that levels of *Luc* mRNA in cell lines transformed with pLAm, pLIN, and pLIIIN were increased by sucrose starvation. In contrast, levels of *Luc* mRNA in cell lines transformed with pLN and pLIIN were decreased by sucrose starvation.

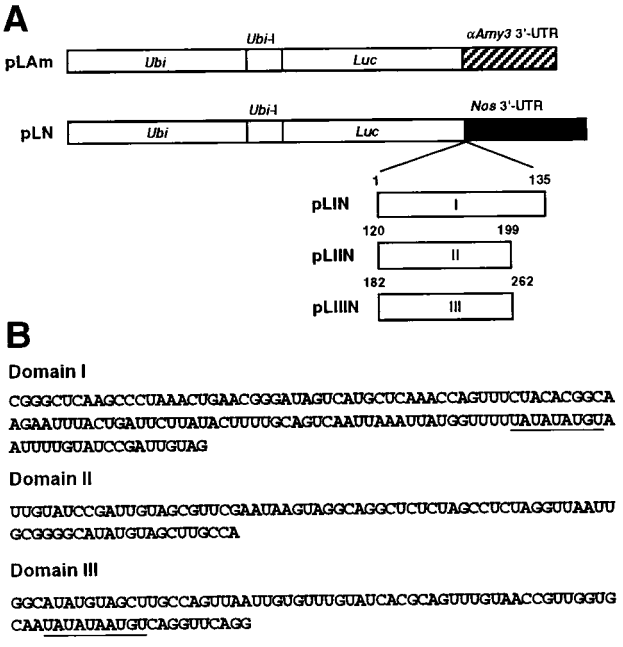

FIG. 1. Structures of chimeric genes used for rice transformation. (*A*) Construction of pLAm and pLAN were described in Chan and Yu (17). All the constructs contain *Ubi* promoter, *Ubi* first intron (*Ubi*-I), *Luc* coding region, and *αAmy3* or *Nos* 3′ UTR. Domains I, II, and III derived from *αAmy3* 3′ UTR were inserted between the *Luc* coding region and the *Nos* 3′ UTR in pLN. The nucleotide sequences are numbered from the first nucleotide after translation stop codon TGA. (*B*) Nucleotide sequences of domains I, II, and III. Underline indicates the conserved sequence between domains I and III.

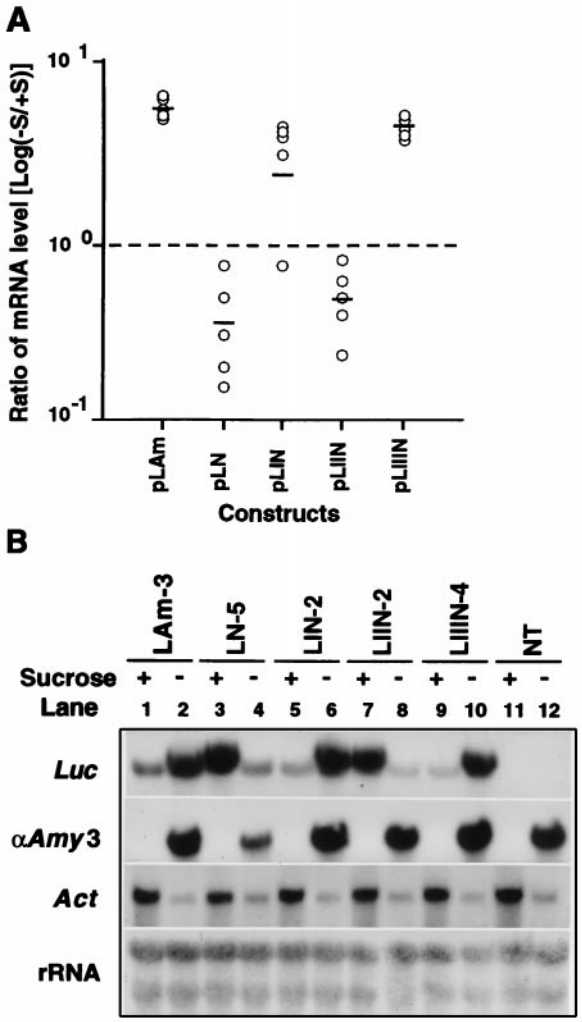

FIG. 2. The *αAmy3* 3′ UTR and its subdomains independently mediate sugar-dependent repression of heterologous mRNA accumulation in transformed rice suspension cells. Rice suspension cells were transfected with plasmids shown in Fig. 1 and cultured as described in *Materials and Methods*. Four weeks after transfection, the transformed suspension cells were cultured in sucrose-containing or sucrose-free medium for 2 days and total RNA was purified. (*A*) Levels of *Luc* mRNA were analyzed with RNA dot blot analysis by using the *Luc* coding region as a probe. Radioactive signal representing the amount of *Luc* mRNA in each dot was quantified by using a PhosphorImager (Molecular Dynamics). For each construct, the ratio of mRNA level was determined by dividing the level of *Luc* mRNA in cells starved for sucrose (−S) by that in cells provided with sucrose (+S). Logarithm value of the ratio (open circle) was then used to plot the graph. Bars represent the average value for each construct. (*B*) Gel blot analysis of mRNA from one representative transformed cell line of each construct. NT indicates nontransformed control. Five micrograms of total RNA was loaded in each lane. The same blot was stripped and rehybridized with indicated probes as described previously (8). The probes were *Luc*, *Act1* (22), and rRNA (23) cDNAs and *αAmy3*-specific DNA (8). The *Luc* and *αAmy3* probes hybridized to a same blot, and the *Act* and rRNA probes hybridized to another parallel-prepared blot.

One representative transformed cell line for each construct was chosen for RNA gel blot analysis. In all the cell lines tested, levels of the *αAmy3* mRNA increased, but levels of the actin (*Act*) mRNA decreased, after sucrose starvation (Fig. 2*B*). Levels of the *Luc* mRNA in cells transformed with pLAm, pLIN, and pLIIIN increased after sucrose starvation (Fig. 2*B*, lanes 2, 6, and 10), but *Luc* mRNA in cells transformed with pLN and pLIIN decreased after sucrose starvation (Fig. 2*B*, lanes 4 and 8). These results demonstrated that the entire *αAmy3* 3′ UTR and its two subdomains specifically mediate sugar-dependent repression of *Luc* mRNA accumulation.

The *αAmy3* 3′ UTR and Its Subdomains Function Independently as Sugar-Dependent mRNA Stability Determinants. To determine whether the low abundance of the *Luc-αAmy3* mRNA in sucrose-provided cells was a result of a rapid decay of this mRNA, after inhibition of transcription of the pLAm-transformed cells, the decay pattern of mRNA was analyzed by RNA gel blot analysis. We have shown previously that treatment of suspension cells with 10 μg/ml actinomycin D (ActD) for at least 12 h was required to inhibit more than 95% of total RNA transcription (7). The results showed that levels of *Luc-αAmy3* and *αAmy3* mRNAs were low or undetectable in

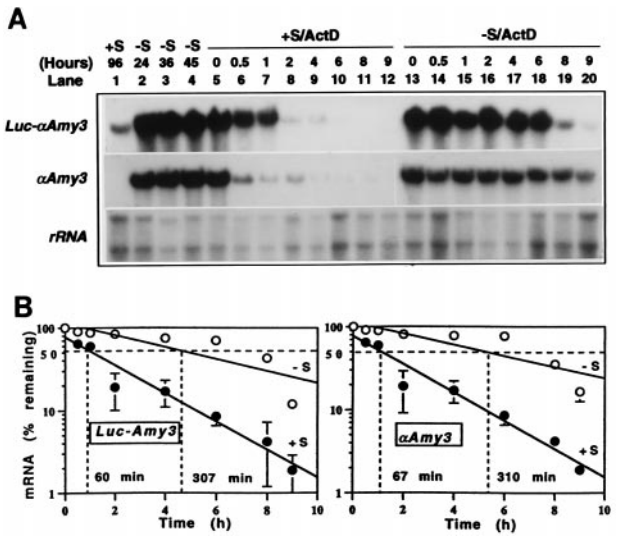

FIG. 3. The *αAmy3* 3′ UTR functions as a sugar-dependent mRNA stability determinant. (*A*) Transformed cell line LAm-3 was suspension cultured in sucrose-containing (+S) medium for 96 h (RNA in lane 1) and transferred to sucrose-free (−S) medium for 24 h (RNA in lane 2). ActD was added to the medium to a final concentration of 10 μg/ml. Cells were incubated in the −S medium containing ActD for another 12 h and then divided in half. Half the cells were transferred to a +S medium containing ActD (RNAs in lanes 5–12). The other half were transferred to a −S medium containing ActD (RNAs in lanes 13–20). Cells were collected after 0.5–9 h, and RNAs were purified. RNA gel blot analysis was performed by using various probes used in the experiment described in Fig. 2. The *Luc* and *αAmy3* probes hybridized to the same blot, and the rRNA probe hybridized to another parallel-prepared blot. Five micrograms of total RNA was loaded in each lane. Lanes 3 and 4, cells incubated in −S medium lacking ActD for 36 and 45 h, respectively. (*B*) Levels of mRNA shown in lanes 5–20 of *A* were quantified by using the PhosphorImager. Each of the α-amylase mRNA levels of lanes 5–12 and 13–20 was first normalized to the rRNA level for each time point. The relative α-amylase mRNA levels then were determined by dividing the mRNA levels by levels of lane 5 or lane 13. The logarithm values of *Luc-αAmy3* (*Left*) and *αAmy3* (*Right*) mRNA levels were subjected to linear regression analysis and plotted as a function of time. The open and solid circles indicate mRNA from cells grown in −S and +S medium, respectively. Error bar indicates the SE of mRNA levels from three independently transformed cell lines. The error bars for mRNA levels from cells grown in −S medium are too short to be shown on the graph. The dashed line indicates the time at which 50% of mRNA remained. The half-life of mRNA is shown at the bottom of the graph.

cells provided with sucrose (Fig. 3*A*, lane 1), but increased significantly after sucrose starvation for 24, 36, and 45 h (Fig. 3*A*, lanes 2–4). After cells were starved for 24 h and then pretreated with ActD for 12 h (Fig. 3*A*, lane 5 or 13), both the *Luc-αAmy3* and *αAmy3* mRNAs also accumulated. These cells were then divided into two halves: one half was grown with sucrose and the other half was grown without sucrose. The two mRNAs decayed more slowly in cells grown without sucrose (Fig. 3*A*, lanes 14–20) than in cells provided with sucrose (Fig. 3*A*, lanes 6–12). Fluctuation in the levels of rRNA was a result of variation in the amount of RNA loaded. Half-lives of the two mRNAs were determined with three independently transformed cell lines, and the results are shown in Fig. 3*B* and Table 1. The half-lives of *Luc-αAmy3* and *αAmy3* mRNAs were 60 and 67 min, respectively, in cells provided with sucrose, but increased to 307 and 310 min, respectively, in cells starved for sucrose. The increase in mRNA half-life induced by sucrose was approximately 4- to 5-fold, which was consistent with our previous study (8). These results demonstrated that both the *Luc-αAmy3* and *αAmy3* mRNAs were more stable in cells starved of sucrose.

Because insertion of domain I or domain III between the *Luc* coding region and the *Nos* 3′ UTR conferred sugar-dependent repression of mRNA accumulation (Fig. 2), we sought to determine whether the two sequences confer differential stability to the *Luc-Nos* mRNA. In all the transformants carrying different constructs, the *αAmy3* mRNA decayed more rapidly in cells grown with sucrose than without sucrose (Fig. 4), which was consistent with the result of Fig. 3. In the pLN- and pLIIN-transformed cells, the *Luc-Nos* and *Luc*-II-*Nos* mRNAs decayed more slowly in cells grown with sucrose (Fig. 4*A*, lanes 5–12) than without sucrose (Fig. 4*A*, lanes 13–20). In contrast, in the pLIN- and pLIIIN-transformed cells, the *Luc*-I-*Nos* and *Luc*-III-*Nos* mRNAs decayed more rapidly in cells grown with sucrose (Fig. 4*B*, lanes 5–12) than without sucrose (Fig. 4*B*, lanes 13–20). These results demonstrated that insertion of domains I and III, but not domain II, enhanced the stability of *Luc-Nos* mRNA in the starved cells.

Half-lives of *Luc-Nos* mRNA inserted with various subdomains of *αAmy3* 3′ UTR were also determined with three independently transformed cell lines for each construct, and the results are summarized in Table 1. In the absence of sucrose, the half-life of *Luc-Nos* mRNA was 60 min, which was similar to that of the *Luc*-II-*Nos* mRNA (52 min) but less than that of the *Luc*-I-*Nos* mRNA (125 min) and *Luc*-III-*Nos* mRNA (190 min). In the presence of sucrose, the half-life of *Luc-Nos* mRNA was 150 min, which was significantly higher than that of the *Luc*-I-*Nos* mRNA (50 min) but only slightly higher than that of the *Luc*-II-*Nos* mRNA (100 min) and the *Luc*-III-*Nos* mRNA (100 min). These results demonstrate that insertion of domains I and III enhanced the stability of the *Luc-Nos* mRNA in cells starved of sucrose and reduced the stability of this mRNA in cells grown with sucrose.

The *αAmy3* 3′ UTR and Its Subdomains Do Not Affect Transcription. To determine whether fusion of the *αAmy3* 3′ UTR or its subdomains downstream of the *Ubi-Luc* chimeric gene affected the transcription rate of the *Ubi* promoter that

Table 1. Effect of sucrose on half-lives of *αAmy3* mRNA and *Luc* mRNAs carrying various domains of *αAmy3* 3′ UTR

Transformed cell lines	Half-life		Fold of change in half-life (−S/+S)
	−S, min	+S, min	
Amy3	300 ± 10	65 ± 3	4.6
Luc-Amy3	307 ± 6	60 ± 17	5.0
Luc-Nos	60 ± 3	150 ± 25	0.4
Luc-I-Nos	125 ± 36	50 ± 15	2.5
Luc-II-Nos	52 ± 3	100 ± 0	0.5
Luc-III-Nos	190 ± 4	100 ± 10	1.9

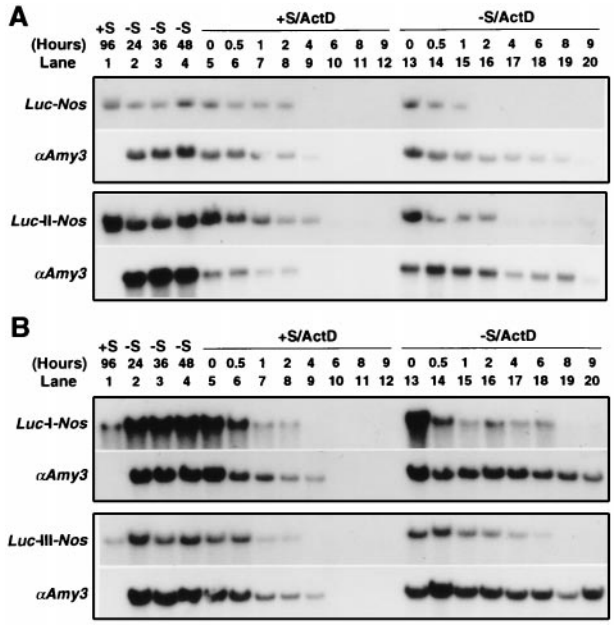

FIG. 4. Two subdomains of *αAmy3* 3′ UTR independently mediate sugar-dependent decay of heterologous mRNA. Cell culture, treatment of cells with ActD, and RNA gel blot analysis all were performed as described in the legend of Fig. 3*A*. (*A*) Decay pattern of *Luc* mRNA from transformed cell lines LN-5 (*Upper*) and LIIN-2 (*Lower*). (*B*) Decay pattern of *Luc* mRNA from transformed cell lines LIN-2 (*Upper*) and LIIIN-4 (*Lower*).

led to change of the mRNA abundance, nuclear run-on transcription analysis was performed, and the results are shown in Fig. 5. The reason for not using *αAmy3* in the analysis was because the mRNAs of endogenous *αAmy3* and chimeric *Ubi-Luc-αAmy3* genes share the same 3′ UTR and the labeled *Luc-αAmy3* cDNA derived from cellular RNA might cross-hybridize to the *αAmy3* DNA immobilized on the membrane. As shown in Fig. 5, when transcription rates of the rRNA gene in cells grown with or without sucrose were kept at the same level (dots 1 and 6), the transcription rate of another α-amylase gene, *αAmy8*, was higher in cells grown without sucrose (dot 8) than with sucrose (dot 3) and that of *Act* was similar regardless of whether cells were grown with or without sucrose

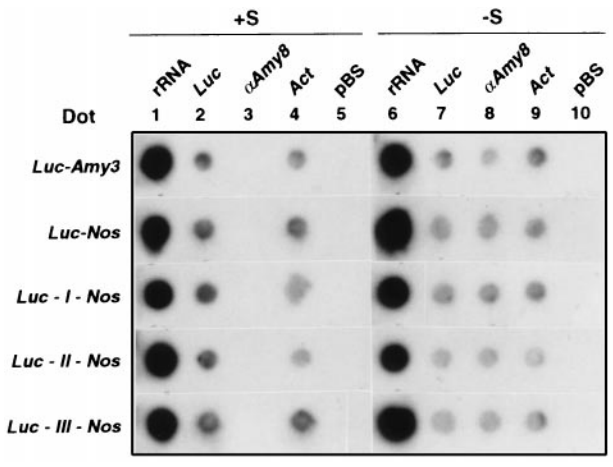

FIG. 5. The *αAmy3* 3′ UTR and its subdomains do not affect transcription. Transformed rice suspension cells (same as Fig. 4) were cultured in sucrose-containing or sucrose-free medium for 12 h. Nuclei were isolated and nuclear run-on transcription analysis was performed as described previously (7). Five micrograms each of rRNA, *Act1*, and *Luc* cDNAs and *αAmy8*-specific DNA (8) was dot-blotted on the membrane. pBS, pBluescript DNA (Stratagene).

(compare dot 4 with dot 9). This result is consistent with that of our previous study (8). Under this physiological condition, the transcription rates of *Ubi-Luc* carrying the entire or subdomains of the *αAmy3* 3′ UTR in cells starved of sucrose were not higher than that in cells grown with sucrose (Fig. 5, compare dot 2 with dot 7). In fact, the transcription rate of *Ubi-Luc* was usually 2-fold higher in cells grown with sucrose than in cells grown without sucrose. This study suggests that the *αAmy3* 3′ UTR and its subdomains did not affect the transcription rate of the *Ubi* promoter.

DISCUSSION

Our finding that mRNA stability plays an important role in the sugar-dependent expression of α-amylase genes has added a new dimension to the study of the mechanism of sugar repression in higher plants. In this study, we demonstrate that the *αAmy3* 3′ UTR can target the *Luc* mRNA for rapid decay, but the *Nos* 3′ UTR delays the decay of the *Luc* mRNA, in cells provided with sucrose. This indicates that the *αAmy3* 3′ UTR is specifically recognized as a mRNA instability determinant in cells provided with sucrose. The similar half-lives of the endogenous *αAmy3* and the *Luc-αAmy3* mRNAs (Fig. 3) suggest that the *αAmy3* 3′ UTR is a major, if not the only, determinant for the sugar-dependent instability of α-amylase mRNA.

In comparison to the glucose-dependent turnover of the Ip mRNA in *S. cerevisiae*, difference in the processes underlying mRNA degradation has been observed. Cereghino *et al.* (13) showed that sequence elements in the 5′UTR, but not in the coding region or 3′ UTR, were the major determinants for differential turnover of Ip mRNA in response to glucose provision. They also showed that degradation of Ip mRNA started at the 5′ end and that the 5′ exonuclease XRN1 was involved. Currently we are testing whether the mRNA degradation process starts at the 5′ or 3′ end of *αAmy3* mRNA. One similar phenomenon observed between the two mRNAs is that the translation inhibitor cycloheximide slows down the degradation of Ip mRNA in yeast provided with glucose (13) and also enhances the accumulation of *αAmy3* mRNA in rice suspension cells provided with sucrose (8).

An important step toward understanding the mechanism governing the stability of mRNA is the identification of cis-acting sequences. The finding that the *αAmy3* 3′ UTR contains two subdomains that can each independently stabilize the *Luc-Nos* mRNA in the starved cells is interesting. In the starved cells, insertion of domain II did not affect the half-life of *Luc-Nos* mRNA (Table 1), suggesting that the increase in *Luc-Nos* mRNA half-life by insertion of domain I or domain III was conferred specifically by the cis-acting sequences residing within the latter two RNA domains. On the other hand, in cells grown with sucrose, insertion of each domain more or less reduced the half-life of *Luc-Nos* mRNA, suggesting that insertion of a random sequence may affect the stability of this mRNA. It is not known whether a positive factor slows down the turnover of *αAmy3* mRNA and *Luc* mRNAs carrying various domains of the *αAmy3* 3′ UTR in the starved cells, or whether a negative factor speeds up the turnover of these mRNAs in cells grown with sucrose. We found that sucrose significantly enhanced the rate of poly(A) removal from *αAmy3* mRNA (unpublished result), which may facilitate an early event in the process of mRNA degradation. However, we have not yet determined whether the *αAmy3* 3′ UTR or any of the inserts affects polyadenylation or other anomalous processing of the *Luc* mRNA.

Recognition of the cis-acting sequences within *αAmy3* 3′ UTR as mRNA stability determinants most likely involves sequence- or structure-specific RNA-binding factors or RNases. Examination of the nucleotide sequences reveals that domains I and III each contain a stretch of a 9-bp AU-rich

conserved sequence (Fig. 1*B*). RNA structure prediction of the entire *αAmy3* 3′ UTR by computer analysis revealed extensive regions of putative duplex formation, and regions encompassing domains I, II, and III each contained a putative stem-loop structure. Interestingly, the 9-bp conserved sequence is located in the loop regions of both domains I and III (17). Further detailed analyses are required to reveal the structure–function or sequence–function relationships of the *αAmy3* 3′ UTR. Biochemical investigation of cellular factors that interact with the cis-acting elements in *αAmy3* 3′ UTR also should facilitate understanding of the regulatory mechanisms of sugar-dependent RNA degradation in plants.

We thank Dr. Ching-Chung Wang for critical review of this manuscript and Ms. Lin-Tze Yu for technical assistance. This work was supported by grants from Academia Sinica and the National Science Council (NSC 85-2311-B-001-050) of the Republic of China.

1. Sheen, J. (1994) *Photosynth. Res.* **39,** 427–438.
2. Thomas, B. R. & Rodriguez, R. L. (1994) *Plant Physiol.* **106,** 1235–1239.
3. Yu, S.-M., Lee, Y.-C., Fang, S.-C., Chan, M.-T., Hwa, S.-F. & Liu, L.-F. (1996) *Plant Mol. Biol.* **30,** 1277–1289.
4. Koch, K. E. (1996) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47,** 509–540.
5. Yu, S.-M., Kuo, Y.-H., Sheu, G., Sheu, Y.-J. & Liu, L.-F. (1991) *J. Biol. Chem.* **266**, 21131–21137.
6. Yu, S.-M., Tzou, W.-S., Lo, W.-S., Kuo, Y.-H., Lee, H.-T. & Wu, R. (1992) *Gene* **122,** 247–253.
7. Sheu, J.-J, Jan, S.-P., Lee, H.-T. & Yu, S.-M. (1994) *Plant J.* **5,** 655–664.
8. Sheu, J.-J., Yu, T.-S., Tong, W.-F. & Yu, S.-M. (1996) *J. Biol. Chem.* **271,** 26998–27004.
9. Chan, M.-T., Chang, H.-H., Ho, S.-L., Tong, W.-F. & Yu, S.-M. (1993) *Plant Mol. Biol.* **22,** 491–506.
10. Chan, M.-T., Chao, Y.-C. & Yu, S.-M. (1994) *J. Biol. Chem.* **269,** 17635–17641.
11. Huang, N., Chandler, J., Thomas, B. R., Koizumi, N. & Rodriguez, R. L. (1993) *Plant Mol. Biol.* **23,** 737–747.
12. Cereghino, G. P. & Scheffler, I. E. (1996) *EMBO J.* **15,** 363–374.
13. Cereghino, G. P., Atencio, D. P., Saghbini, M., Beiner, J. & Scheffler, I. E. (1995) *Mol. Biol. Cell* **6,** 1125–1143.
14. Lombardo, A., Cereghino, G. P. & Scheffler, I. E. (1992) *Mol. Cell. Biol.* **12,** 2941–2948.
15. Müllner, E. W. & Kühn, L. C. (1988) *Cell* **53,** 815–825.
16. Casey, J. L., Koeller, D. M., Ramin, V. C., Klausner, R. D. & Harford, J. B. (1989) *EMBO J.* **8,** 3693–3699.
17. Chan, M.-T. & Yu, S.-M. (1998) *Plant J.*, in press.
18. Murashige, T. & Skoog, F. (1962) *Physiol. Plant* **15,** 473–497.
19. Zheng, A., Hayashimoto, A., Li, Z. & Murai, N. (1991) *Plant Physiol.* **97,** 832–835.
20. Cao J., Wang, Y. C., Klein, T. K., Sanford, J. & Wu, R. (1990) in *Plant Gene Transfer*, eds. Lamb, C. J. & Beachy, R. N. (Wiley, New York), pp. 21–33.
21. Cao, J., Duan, X., McElroy, D. & Wu, R. (1992) *Plant Cell Rep.* **11,** 586–591.
22. McElroy, D., Rothenberg, M. & Wu, R. (1990) *Plant Mol. Biol.* **14,** 163–171.
23. Sano, Y. & Sano, R. (1990) *Genome* **33,** 209–218.