Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 1354–1358, March 1986
Cell Biology

Heat shock causes destabilization of specific mRNAs and destruction of endoplasmic reticulum in barley aleurone cells

(gibberellic acid/α-amylase)

FAITH C. BELANGER*, MARK R. BRODL†, AND TUAN-HUA DAVID HO†‡

*Department of Plant Biology, University of Illinois, Urbana, IL 61801; and †Department of Biology, Washington University, St. Louis, MO 63130

Communicated by J. E. Varner, October 28, 1985

ABSTRACT **In response to a phytohormone, gibberellic acid, the aleurone layers of barley seeds synthesize and secrete α-amylases, which are coded by a set of stable mRNAs. When aleurone layers are subjected to heat shock treatment, the synthesis of α-amylase is suppressed while heat shock proteins are induced. The suppression of α-amylase synthesis is not the result of translational control as reported in several other systems. Rather, the sequences of α-amylase mRNA are rapidly degraded during heat shock as shown by *in vitro* translation and dot blot hybridization with a cDNA probe. Upon recovery from heat shock, the tissue resumes the synthesis of α-amylase in 2–4 hr. However, in the presence of a transcription inhibitor, cordycepin, the resumption of synthesis of α-amylase does not take place, indicating that new transcription of α-amylase genes is necessary for this recovery process. The degradation of α-amylase mRNAs correlates with the rapid destruction of endoplasmic reticulum as observed by electron microscopy, a phenomenon that has not been reported previously as a heat shock response. Since α-amylase mRNA is associated with the endoplasmic reticulum via membrane-bound polyribosomes, we suggest that the destruction of the endoplasmic reticulum during heat shock causes the destabilization and the eventual degradation of α-amylase mRNA.**

In response to rapidly increasing temperatures, many organisms synthesize a set of proteins that are commonly known as the heat shock proteins (HSP). This heat shock response has been observed in nearly all organisms and tissues so far investigated (1), although some exceptions have been reported (2–4). Except for *Xenopus* oocytes (5), the synthesis of HSP appears to be due to the increased transcription of the HSP genes (1).

How the increase in temperature is sensed by the tissue is not known. Chemical agents such as amino acid analogues, arsenite, cadmium, and ethanol have been found to induce HSP synthesis in some systems in the absence of a temperature increase (1). Although ubiquitin (6), ATP-dependent protease (7), and enolase (8) have been shown to be induced by heat shock, the exact function and identity of the remaining HSP remain unclear. Agents that induce HSP synthesis have also been found to induce thermotolerance (1). It has been suggested that a primary signal for HSP synthesis may result from a change in the cellular membranes (9, 10) and that the resulting HSP may exert a protective effect on the membranes (11).

In pursuit of this possibility, we have investigated the biochemical and ultrastructural effects of heat shock on barley aleurone layers. The barley aleurone layer is a homogeneous, nondividing tissue whose gene expression is dramatically altered in the presence of the plant hormone, gibberellic acid (GA_3, one of the gibberellins). In the presence of GA_3, the cell's metabolism is redirected toward the *de novo* synthesis and secretion of several hydrolases including α-amylase, protease, and ribonuclease (for reviews, see ref. 12). By 24 hr of treatment with GA_3, α-amylase becomes about 40% of the newly synthesized protein (12). It has been established from the use of cloned cDNA probes that the observed increase in α-amylase synthesis is due to the GA_3-induced transcription of α-amylase mRNA (13–15).

GA_3 application also induces an extensive proliferation of endoplasmic reticulum (ER), which becomes organized into stacks (16, 17). The synthesis and secretion of α-amylase have been associated with the ER (18). Thus, the GA_3-induced barley aleurone layer provides an interesting system for the study of possible membrane effects of heat shock because it is a uniform tissue whose metabolism is largely directed to the membrane-associated synthesis and secretion of α-amylase. In this paper we present biochemical and ultrastructural evidence that indicate that heat shock causes the disruption of the ER, which may lead to the destruction of α-amylase mRNA sequences that are normally associated with the ER.

MATERIALS AND METHODS

Plant Materials. Barley seeds (*Hordeum vulgare* L. var. Himalaya, 1974 harvest) were obtained from Washington State University (Pullman, WA). Sterilized embryoless half seeds were imbibed for 4 days on filter paper overlaying vermiculite, which was soaked with 20 mM sodium succinate, pH 5.0/20 mM $CaCl_2$. The aleurone layers were dissected away from the starchy endosperm under aseptic conditions and incubated as appropriate.

***In Vivo* Labeling and Analysis of Proteins.** Ten aleurone layers per sample were placed in 2 ml of 20 mM sodium succinate, pH 5.0/20 mM $CaCl_2$ in a 25-ml sterile flask. The flasks were incubated at the appropriate temperature in a reciprocal shaker at 120 cycles per min. Prior to pulse labeling with [^{35}S]methionine, the buffer was removed and replaced with 1 ml of fresh buffer. Samples were labeled for 30 min or 1 hr with 25–100 μCi (1 Ci = 37 GBq) of [^{35}S]methionine (specific activity > 1000 Ci/mmol) per ml. At the end of the labeling period, the buffer was removed and the layers were rinsed with 1 mM methionine. The layers were homogenized in 100 μl of 10 μM leupeptin (a thioprotease inhibitor) in the succinate buffer (pH 5.0). The homogenates were mixed with 300 μl of Laemmli (19) $NaDodSO_4$ gel loading buffer.

In vivo and *in vitro* synthesized proteins were analyzed by one-dimensional 11% $NaDodSO_4$/polyacrylamide gel electrophoresis as described by Laemmli (19).

RNA Isolation and *in Vitro* Translations. RNA was isolated from aleurone layers by using a guanidine·HCl extraction procedure (20). One to two hundred aleurone layers were

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: GA_3, gibberellic acid; HSP, heat shock protein(s); ER, endoplasmic reticulum.

‡To whom reprint requests should be addressed.

homogenized in 30 ml of 5 M guanidine·HCl/10 mM Tris·HCl, pH 8.5/5 mM EGTA/0.1% lauryl sarcosine plus 210 μl of 2-mercaptoethanol and 30 μl of Antifoam A (Sigma) in a Polytron for 1–2 min. The resulting extract was filtered through one layer of Miracloth. One-half volume of ethanol was added, and the solution was placed in the freezer overnight. The RNA precipitate was collected by centrifugation at 5,000 × *g* for 40 min and was redissolved in 15 ml of 4 M guanidine·HCl/10 mM EDTA, pH 7.0. One-half volume of ethanol was added, and the RNA was precipitated and collected as before. The RNA pellets were washed twice with 95% ethanol and redissolved in 3 ml of sterile H_2O. The pellet also contained a considerable amount of insoluble material, which was removed by centrifugation at 14,000 × *g* for 15 min. This step was repeated twice, and the supernatants were combined. The RNA was precipitated by adding 0.1 volume of 3 M sodium acetate (pH 5.0) and 3 volumes of ethanol and was recovered by centrifugation at 27,000 × *g* for 30 min.

Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography as described by Maniatis *et al.* (21) except that the binding buffer was 0.5 M LiCl/0.01 M Tris·HCl, pH 7.4/0.5% $NaDodSO_4$, and the eluting buffer was 0.01 M Tris·HCl, pH 7.4/0.05% $NaDodSO_4$.

In vitro translations were performed by using nuclease-treated rabbit reticulocyte lysate obtained from Promega Biotec (Madison, WI).

Dot-Blot Hybridization. Samples of RNA were dissolved in 10 mM Tris·HCl buffer, pH 7.5/1 mM EDTA. A series of 2-fold dilutions of RNA was made, and 5 μl of each dilution was spotted on a GeneScreen membrane (New England Nuclear). The membrane was dried and baked at 80°C for 2–3 hr. The baked membrane was soaked in 10 mM sodium phosphate buffer (pH 7.7) for 10–15 min before being UV-irradiated (1,200 μW/cm^2) for 90 sec (22). The membrane filter was prehybridized and hybridized by the procedure of Maniatis *et al.* (21). The probe used was nick-translated cDNA coding for the low-pI α-amylase isozyme (clone E). Because of the extensive sequence homology between the high- and low-pI isozymes, this probe could potentially detect gene transcripts for both isozymes (13–15).

Electron Microscopy. Tissue samples were processed in two different ways depending on their exposure to GA_3. The presence of GA_3 promotes partial cell wall degradation, permitting shorter dehydration and infiltration times than in the absence of GA_3. Tissue samples *ca.* 0.5 mm^2 were fixed in 3% glutaraldehyde/2% formaldehyde buffered with either 0.05 M cacodylate buffer (without GA_3) or 0.05 M Millonig's buffer (with GA_3) at pH 7.0 for 4 hr. The tissue was washed in buffer and then postfixed in a buffered (as above) 2% OsO_4 solution (pH 7.0) for 4 hr. Samples were again washed in buffer, dehydrated in a 50–100% graded series of acetone baths, infiltrated with "modified Ladd's" resin (23), and polymerized at 45°C under vacuum. Sections ≈60 nm thick were cut on a Microstar 35-mm diamond knife, stained with 1.5% aqueous uranyl acetate and Reynold's lead citrate, and viewed in a Hitachi H-600 electron microscope. Morphometric measurements were made with a Houston Instruments HIPAD digitizer.

RESULTS

When barley aleurone layers were treated with 1 μM GA_3, the *de novo* synthesis of α-amylase was induced (Fig. 1). By 16 hr of GA_3 treatment, α-amylase became the predominant newly synthesized protein (compare lanes 1 and 5 in Fig. 1). When barley aleurone layers without previous hormone treatment were exposed to elevated temperature, the synthesis of several new proteins was observed. In the heat-shocked GA_3-induced aleurone layers, there was also a dramatic yet selective reduction in α-amylase synthesis in addition to the synthesis of the new proteins (Fig. 1). In both the presence and absence of GA_3 at least 11 proteins showed increased synthesis in response to increasing temperature (Fig. 1). These proteins had apparent molecular masses of 105, 101, 87, 84, 76, 74, 71, 34, 19, 18, and 17 kDa, which were similar to those reported for other plant species (24, 25). HSP synthesis first became apparent at 35°C (Fig. 1, lanes 3 and 7), with the optimum temperature being 40°C (Fig. 1, lanes 4 and 8). Exposure to 45°C appeared to be lethal to barley aleurone layers in that protein synthesis was eliminated.

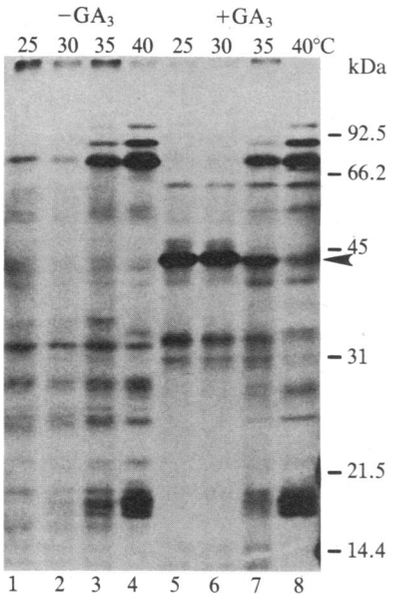

FIG. 1. Effect of temperature on the pattern of protein synthesis in aleurone layers. Aleurone layers were incubated at 25°C in the absence (lanes 1–4) and presence (lanes 5–8) of 1 μM GA_3 for 16 hr prior to a 3-hr exposure at the temperatures indicated on top of the gel. Labeling with 25 μCi of [^{35}S]methionine per ml took place during the final hour of incubation. The proteins were separated on an 11% $NaDodSO_4$ gel and visualized by fluorography. The molecular mass standards in kDa are shown to the right of the gel. The arrow indicates the portion of α-amylase (44 kDa).

When GA_3-treated aleurone layers were subjected to the heat shock temperature of 40°C, there was a fast induction of HSP, with a concomitant decrease in the synthesis of α-amylase reaching a minimum of 3 hr (Fig. 2, lane 7). Upon prolonged heat shock treatment, there was a slight recovery in α-amylase synthesis at 12–24 hr (Fig. 2, lanes 10 and 11), but the level of α-amylase synthesis did not return to that of the controls (Fig. 2, lanes 3 and 12).

Regulation of Protein Synthesis in Heat-Shocked Tissue. To investigate at what level the altered protein synthesis is regulated in heat-shocked aleurone cells, we performed *in vitro* translations and dot-blot hybridizations to measure the activity and number of sequences of specific mRNA. Fig. 3 shows the *in vitro* translation products of heat-shocked and non-heat-shocked tissues as well as the corresponding *in vivo* labeled samples. Total RNA, poly(A)$^+$ RNA and poly(A)$^-$ RNA "(unbound)" fractions from heat-shocked tissues coded for several proteins not seen in the translation products of RNA from non-heat-shocked tissues. In addition, these proteins had mobilities essentially identical to the HSP induced *in vivo* (Fig. 3).

It has been established that *in vitro* α-amylase is synthesized as a 46-kDa precursor (13) (compare lanes 7 and 10 in Fig. 3). In the GA_3-treated aleurone layers, heat treatment resulted in a reduction in α-amylase synthesis both *in vivo* (Fig. 3, lane 11) and *in vitro* (Fig. 3, lanes 5 and 8). This

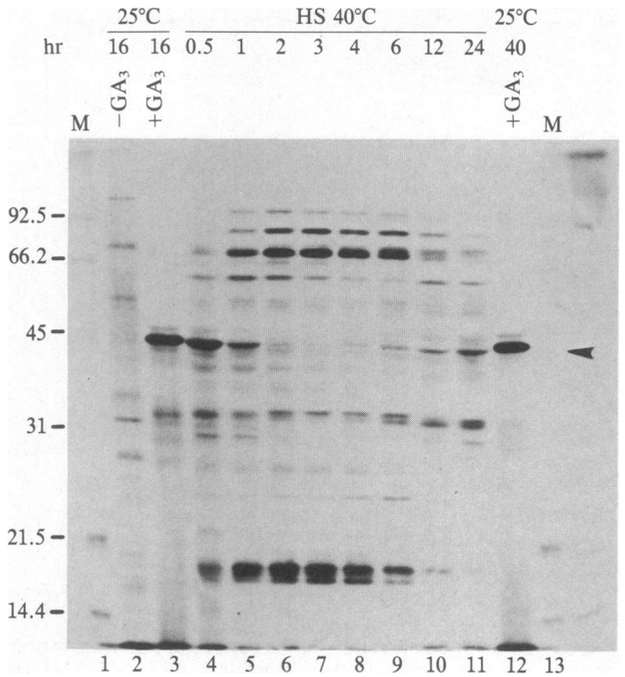

FIG. 2. Time course of the heat shock response. Aleurone layers were incubated at 25°C in the absence (lane 2) or presence (lane 3) of GA_3 for 16 hr prior to incubation at 40°C for the indicated hours (lanes 4–11). GA_3 was present throughout the 40°C heat shock (HS) treatment. Layers were also incubated at 25°C for 40 hr in the presence of GA_3 (lane 12). Labeling with 100 μCi of [^{35}S]methionine per ml took place during the final 30 min of incubation. The proteins were separated on an 11% $NaDodSO_4$ gel and visualized by fluorography. Molecular size markers (lanes M) are shown in kDa; the arrow indicates the position of α-amylase (44 kDa).

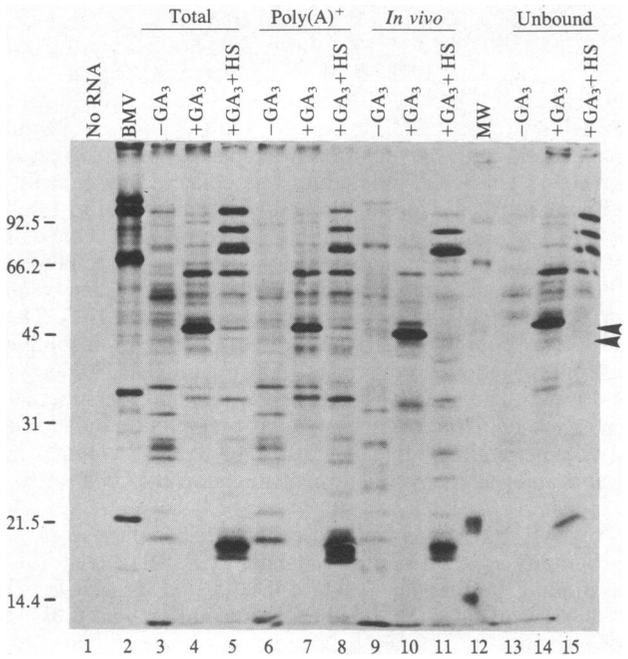

FIG. 3. Comparison of *in vivo* and *in vitro* protein synthesis in heat-shocked (+HS) and non-heat-shocked aleurone layers. Aleurone layers were incubated at 25°C in the absence ($-GA_3$) or presence ($+GA_3$) of 1 μM GA_3 prior to a 3-hr incubation at 40°C. For the *in vitro* samples, total RNA (lanes 3–5), poly(A)$^+$ RNA (lanes 6–8), and poly(A)$^-$ RNA (lanes 13–15) were isolated and translated in the rabbit reticulocyte system. *In vivo* labeling (lanes 9–11) and sample processing were described for Fig. 1. BMV (lane 2) is mosaic virus mRNA used as a control. Equal amounts of total acid-precipitable radioactivity were applied to the gel. The top arrowhead points to the *in vitro* synthesized α-amylase precursor and the lower one points to the *in vivo* synthesized α-amylase. Sizes are shown in kDa.

observation indicates that there was a loss of functional α-amylase mRNA during the heat shock. It can be seen that the reduction in *in vitro* α-amylase synthesis occurred with total RNA as well as with the poly(A)$^+$ RNA. Thus, the decrease was not due to a loss in ability to bind to oligo(dT)-cellulose, as previously reported for some 25°C mRNAs in *Drosophila* (27). Dot-blot hybridization experiments using a cloned α-amylase cDNA probe were also carried out to measure the level of α-amylase mRNA sequences. RNA was isolated from aleurone layers incubated for 19 hr at 25°C in the absence or presence of 1 μM GA_3 and from layers incubated in the presence of GA_3 for 16 hr at 25°C, followed by a 3-hr incubation at 40°C. There was little or no hybridization between the probe and the RNA isolated from tissue incubated in the absence of GA_3 (Fig. 4). By visually comparing the extent of hybridization in the GA_3-treated samples with or without heat shock, there appeared to be an 8-fold decrease in hybridization in the heat-shocked samples. This implies that >85% of the α-amylase mRNA sequences were lost in the heat-shocked tissue.

Transcription Dependency of Resumption of α-Amylase Synthesis During Recovery from Heat Shock. If, as appeared from the data presented above, there was a loss in α-amylase mRNA sequences during heat shock, then new RNA synthesis should be required for recovery of α-amylase synthesis. This possibility was investigated as follows. Aleurone layers were incubated for 16 hr at 25°C in the presence of GA_3, then subjected to a 3-hr heat shock at 40°C. At the end of the heat-shock treatment, cordycepin, an effective transcription inhibitor in barley aleurone cells (27), was added to a final concentration of 0.1 mM to prevent new RNA synthesis. Samples were returned to 25°C for various times to allow recovery from the heat shock. The synthesis of α-amylase was resumed during the recovery from the heat shock only in the absence of cordycepin (e.g., Fig. 5, lanes 13 and 14). By comparing the recovery in the absence and presence of cordycepin, it is clear that new RNA synthesis was required for recovery of α-amylase synthesis. Our results with cordycepin have confirmed the notion that α-amylase mRNA is degraded in heat-shocked tissue, and the resumption of α-amylase synthesis during recovery is dependent upon new transcription of α-amylase genes.

Ultrastructural Effects of Heat Shock on Aleurone Layers. To investigate the possible cause of α-amylase mRNA degradation during heat shock, we examined the effect of heat shock on the barley aleurone layer at the ultrastructural level. Aleurone layers were incubated for 16 hr at 25°C in the absence or presence of GA_3, followed by a 4-hr heat shock at 40°C. The ultrastructure of aleurone cells in the absence and

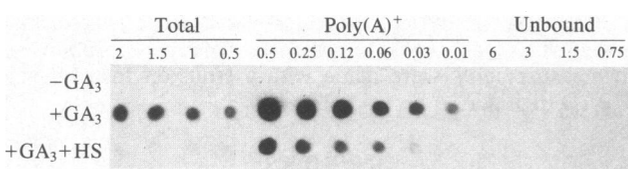

FIG. 4. Direct determination of α-amylase message level in heat-shocked tissues (HS) by dot-blot hybridization. RNAs were isolated from aleurone layers incubated for 19 hr at 25°C in the absence ($-GA_3$) and presence ($+GA_3$) of 1 μM GA_3 and from layers incubated in the presence of GA_3 for 16 hr at 25°C and then were given a 3-hr incubation at 40°C. The numbers above each lane indicate different amounts (μg) of total RNA, poly(A)$^+$ RNA, and poly(A)$^-$ RNA (unbound) applied to a GeneScreen membrane. The membrane was probed with a ^{32}P-labeled α-amylase cDNA (clone E).

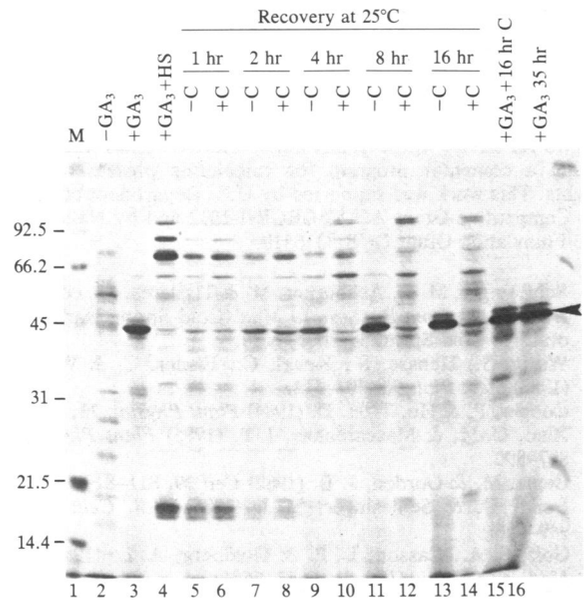

FIG. 5. Effect of cordycepin on the recovery of α-amylase synthesis after a heat shock (HS). Aleurone layers were incubated at 25°C for 16 hr in the absence (lane 2) or presence (lane 3) of 1 μM GA_3 (+GA_3) prior to a 3-hr incubation at 40°C (lane 4). Samples were then returned to 25°C in the presence (+C) or absence (−C) of 0.1 mM cordycepin for the indicated times (lanes 5–14). Non-heat-shocked layers were also incubated with GA_3 at 25°C for 35 hr (lane 16) and with GA_3 at 25°C for 16 hr followed by 16 hr at 25°C with C (lane 15). Samples were labeled and processed as described for Fig. 1. The arrow indicates the position of α-amylase.

presence of GA_3 for 20 hr at 25°C is shown in Fig. 6 *A* and *B*. As reported earlier (16), incubation with GA_3 induced the extensive proliferation of ER. When tissue incubated in the absence of GA_3 was given a heat shock, there was no apparent effect at the ultrastructural level (data not shown). However, when GA_3-treated layers were given a heat shock, there was a dramatic effect at the ultrastructural level. The extensive stacks of ER were no longer apparent after a heat shock, although the morphology of other organelles remained basically unchanged (Fig. 6*C*). Morphometric measurements indicated that heat shock treatments caused ≈75% reduction in the content of ER membranes as compared to the control.

DISCUSSION

Alterations of the profile of newly synthesized proteins are readily observable phenomena in many heat-shocked tissues. Three types of proteins are present in barley aleurone layers; they are induced, suppressed, or unaffected by heat shock. Besides the induction of HSP, we also have observed a drastic reduction of the synthesis of GA_3-induced α-amylase. This appears to be the consequence of destruction of mRNA sequences coding for α-amylase, as demonstrated by both *in vitro* translation and dot-blot hybridization with an α-amylase cDNA probe. The resumption of α-amylase synthesis during the recovery from heat shock is dependent on the transcription of α-amylase genes, since it does not take place in the presence of the transcription inhibitor cordycepin. Our observation is apparently different from the heat shock responses in *Drosophila*, where mRNA coding for the normal proteins is sequestered during heat shock and only HSP mRNA is translated (26). Upon recovery from heat shock in *Drosophila*, the synthesis of normal proteins is resumed even in the absence of new transcription. This type of translational control does not seem to operate in the heat-shocked barley aleurone layers. Destruction of some mRNA coding for normal proteins also has been reported in yeast under heat shock (28), where the mRNA half-life is short and the mRNA

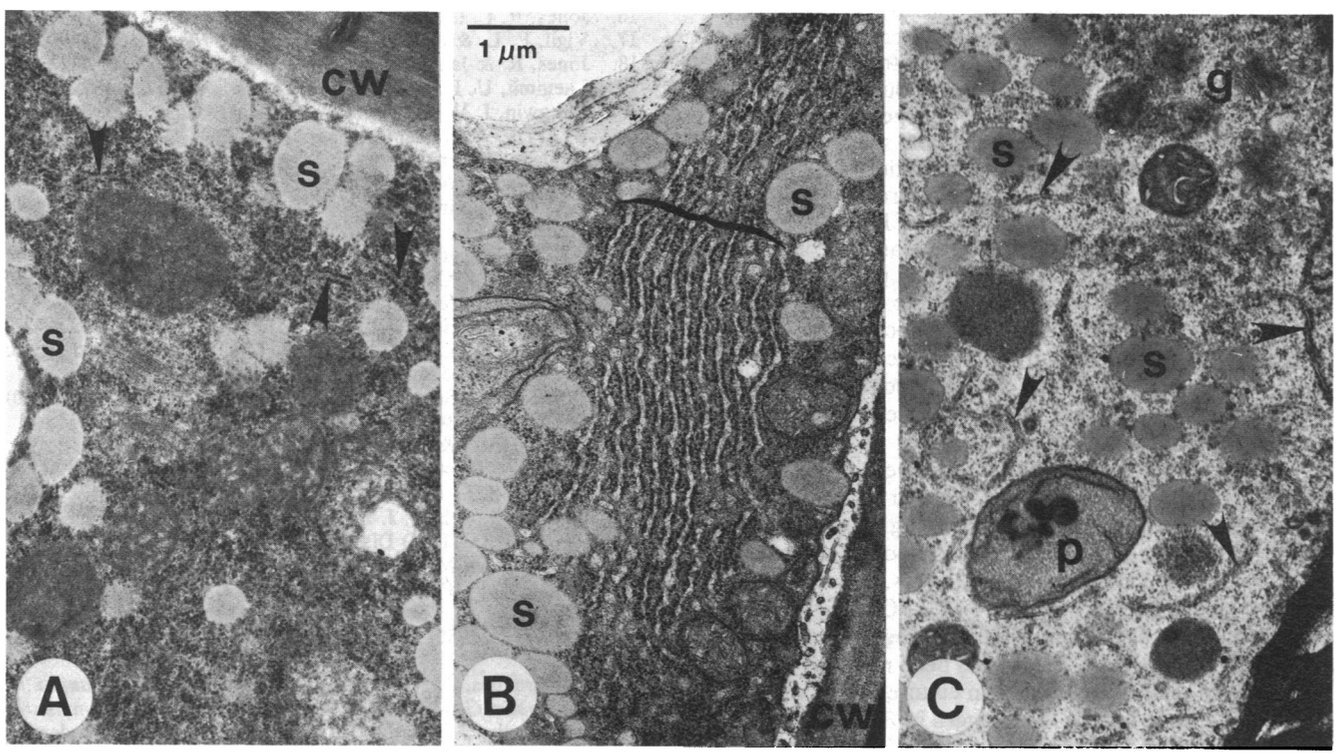

FIG. 6. Electron micrographs of barley aleurone cells showing the disappearance of GA_3-stimulated ER lamellae upon heat shock. (*A*) Without both GA_3 and heat shock; control cell lacks ER lamellae. (*B*) GA_3-treated but not heat-shocked. This sample shows prominent ER lamellae. (*C*) GA_3-treated for 19 hr, the last three being at 40°C. Heat causes a disruption and disappearance of ER lamellae. g, Golgi; cw, cell wall; p, proplastid; s, spherosome; arrowheads, ER fragments; brace, ER lamellae. All micrographs are at equivalent magnifications.

undergoes normal decay without resynthesis. In soybean cell cultures, mRNAs for the small subunits of ribulose-1,5-bisphosphate carboxylase decrease during heat shock (29). Since heat shock treatment inhibits the transcription of genes encoding normal proteins, this observation is probably also the consequence of normal mRNA decay in the absence of new synthesis. Thus, the above cases are in contrast to the situation with α-amylase in barley aleurone layers, where a normally long-lived mRNA is specifically destroyed during heat shock.

Another heat shock response in barley aleurone layers is the disruption of the normally abundant ER lamellae. To our knowledge this phenomenon has not been reported before in any cell type. The timing of ER disruption correlates well with the destruction of α-amylase mRNA (unpublished data). It has been shown that α-amylase mRNA is extremely stable in unstressed barley aleurone layers. After the mRNA reaches the maximal level, the synthesis of α-amylase continues even though the formation of new mRNA is blocked by transcription inhibitors (27). It has been estimated that the half-life of α-amylase mRNA is at least 100 hr (30). However, this stable mRNA species is rapidly destroyed during heat shock. Within 3 hr of heat shock, >85% of the α-amylase mRNA sequences are destroyed—i.e., the half-life of this mRNA has decreased to <2 hr. Being a secretory protein, α-amylase is synthesized by ER-bound polysomes. Therefore, the presence of ER is probably essential for the synthesis and posttranslational processing of this enzyme. We suggest that the stability of α-amylase mRNA is the consequence of its association with ER. Once ER is disrupted during heat shock treatment, this mRNA is no longer stable and is subjected to degradation by ribonuclease. Upon recovery from heat shock, new α-amylase gene transcripts are made and at the same time ER is reformed, which stabilizes the α-amylase mRNA—hence the resumption of α-amylase synthesis. Is this a phenomenon unique to α-amylase in aleurone cells or applicable to other secretory proteins in other cells? We have already shown that the synthesis of several other secretory proteins in barley aleurone layers is affected by heat shock treatment similarly to α-amylase (M.R.B. and T.D.H., unpublished data). In *Xenopus* liver the synthesis of vitellogenin, a secretory protein, is preferentially inhibited by heat shock (31). Therefore, the effect of heat shock on the disruption of ER may lead to a reduction of the synthesis of secretory proteins in general. A similar effect of heat shock on ER-directed protein synthesis has been observed also in at least one other plant tissue, corn roots (32). In this tissue total protein synthesis, as measured by the incorporation into acid-precipitable materials, is not affected by heat shock; however, the radioactivity associated with ER is reduced. On the other hand, it has been reported that the content of mRNA encoding soybean storage proteins increases during heat shock (33). Whether the inhibitory effect of heat shock on ER-directed protein synthesis as described in this work is applicable to other systems awaits further study.

The biochemical mechanisms underlying the destruction of ER during heat shock are not yet understood. Because of the physicochemical nature of lipid bilayers, membranes are sensitive to temperature perturbations. Changes of membrane properties have been observed frequently in freeze-injured plant tissues (34). It also has been reported that heat stress causes electrolyte leakage in cultured plant cells (35). In barley aleurone layers, the changes in ER could serve as one of the sensing mechanisms that allows the cells to recognize the presence of heat stress. The consequence of this initial response is to reduce the synthesis of secretory proteins and make the synthetic capacities available for the synthesis of HSP that are not affected by the disappearance of ER.

We thank Dr. John Rogers for his gift of an α-amylase cDNA clone, L. S. Lin for preparing the ^{32}P-labeled cDNA insert, Dr. David Shapiro for advice on RNA dot blots, and Dr. James Mattheis for writing a computer program for calculating protein molecular weights. This work was supported by U.S. Department of Agriculture Competitive Grant AG 82-CRCR-1-1012 and by National Science Foundation Grant DCB-8316319.

1. Schlesinger, M. J., Ashburner, M. & Tissieres, A., eds. (1982) *Heat Shock from Bacteria to Man* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
2. Wittig, S., Hensse, S., Keitel, C., Elsner, C. & Wittig, B. (1983) *Dev. Biol.* **96**, 507–514.
3. Cooper, P. & Ho, T.-H. D. (1984) *Plant Physiol.* **71**, 215–222.
4. Xiao, C.-M. & Mascarenhas, J. P. (1985) *Plant Physiol.* **78**, 887–890.
5. Bienz, M. & Gordon, J. B. (1982) *Cell* **29**, 811–819.
6. Bond, U. & Schlesinger, M. J. (1985) *Mol. Cell. Biol.* **5**, 949–956.
7. Goff, S. A., Casson, L. P. & Goldberg, A. L. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6647–6651.
8. Iida, H. & Yahara, I. (1985) *Nature (London)* **315**, 688–670.
9. Kelley, P. M. & Schlesinger, M. J. (1978) *Cell* **15**, 1277–1286.
10. Li, G. C., Shiu, E. C. & Hahn, G. M. (1980) *Radiat. Res.* **82**, 257–268.
11. Li, G. C., Shrieve, D. C. & Werb, Z. (1982) in *Heat Shock from Bacteria to Man*, eds. Schlesinger, M. J., Ashburner, M. & Tissieres, A. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 395–404.
12. Varner, J. E. & Ho, D. T.-H. (1976) in *The Molecular Biology of Hormone Action*, ed. Papaconstantinou, J. (Academic, New York), pp. 173–194.
13. Higgins, T. J. V., Jacobsen, J. V. & Zwar, J. A. (1982) *Plant Mol. Biol.* **1**, 191–215.
14. Muthukrishnan, S., Chandra, G. R. & Maxwell, E. S. (1983) *J. Biol. Chem.* **258**, 2370–2375.
15. Rogers, J. C. & Milliman, C. (1983) *J. Biol. Chem.* **258**, 8169–8174.
16. Jones, R. L. (1969) *Planta* **88**, 73–86.
17. Vigil, E. L. & Ruddat, M. (1973) *Plant Physiol.* **51**, 549–558.
18. Jones, R. & Jacobsen, J. V. (1982) *Planta* **156**, 421–432.
19. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
20. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 197–198.
22. Church, G. M. & Gilbert, W. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1991–1995.
23. Ringo, D. L., Cota-Robles, E. H. & Humphrey, B. J. (1979) *Proc. Annu. Meet. Electron Microsc. Soc. Am.* **37**, 348–349.
24. Cooper, P. & Ho, T.-H. D. (1983) *Plant Physiol.* **71**, 215–222.
25. Key, J. L., Lin, C. Y. & Chen, Y. M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3526–3530.
26. Storti, R. V., Scott, M. P., Rich, A. & Pardue, M. L. (1980) *Cell* **22**, 825–834.
27. Ho, T.-H. D. & Varner, J. E. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4783–4786.
28. Linquist, S. L. (1981) *Nature (London)* **293**, 311–314.
29. Vierling, E. & Key, J. L. (1985) *Plant Physiol.* **78**, 155–162.
30. Ho, T.-H. D. (1976) Dissertation (Michigan State University, East Lansing, MI).
31. Wolffe, A. P., Perlman, A. J. & Tata, J. R. (1984) *EMBO J.* **3**, 2763–2770.
32. Cooper, P. (1985) Dissertation (University of Illinois, Urbana, IL).
33. Mascarenhas, J. P. & Altschuler, M. (1985) in *Changes in Eukaryotic Gene Expression in Response to Environmental Stress*, eds. Atkinson, B. G. & Walden, D. B. (Academic, New York), pp. 315–326.
34. Steponkus, P. L. (1984) *Annu. Rev. Plant Physiol.* **35**, 543–584.
35. Wu, M.-T. & Wallner, S. J. (1983) *Plant Physiol.* **72**, 817–820.