Recovery of protein synthesis after heat shock: Prior heat treatment affects the ability of cells to translate mRNA

(thermal tolerance/translational regulation)

NANCY S. PETERSEN AND HERSCHEL K. MITCHELL

Division of Biology, California Institute of Technology, Pasadena, California 91125

ABSTRACT   A mild heat shock at 35°C, which induces heat shock gene expression, greatly enhances survival and the recovery of protein synthesis in *Drosophila* cells after a higher temperature heat shock. The 35°C treatment is also effective in preventing heat-induced developmental defects in pupae. We show here that the major larval mRNAs are present in approximately normal (25°C) concentrations after a 40.1°C heat shock whether or not the animals receive a pretreatment. This indicates that the pretreatment affects translation directly rather than messenger concentration. We also observe selective translation of heat shock messages and some 25°C messages during recovery from heat shock.

Pretreatment at a nonlethal temperature (30 min at 35°C) dramatically improves the ability of *Drosophila melanogaster* larvae, pupae, adults, and cell lines to withstand a normally lethal heat shock. This type of pretreatment also prevents developmental defects (phenocopies) induced in *Drosophila* pupae by heat shock (1). The acquisition of resistance to heat has been described in a wide variety of plant (2, 3) and animal systems, including mammalian cell lines (4–7). Understanding the molecular basis for this phenomenon is therefore of general interest. We have shown previously that the dramatic effects on survival and phenocopy prevention are paralleled by a much more rapid recovery of protein synthesis in the animals that received the 35°C pretreatment (1). We are interested in how the pretreatment improves recovery from heat shock and, in particular, in the possibility that one or more of the heat shock gene products mediates the effects on recovery through effects on regulation of RNA and protein synthesis. In this paper we show that the 35°C pretreatment does not affect concentrations of the major larval messages as has been previously suggested (1, 8), but rather it affects the ability of the cells to translate mRNA.

MATERIALS AND METHODS

Fly Culture and Labeling in Vivo. Embryos collected over a 2-hr period (9) were kept at 25°C for 3½ days. Third-instar larvae were washed and heat treated in moist vials submersed in a water bath of the temperature indicated. Larvae recovering from heat treatment were kept in vials containing a small amount of moist yeast. Larvae were injected with [^{35}S]methionine (3.0 μCi per larva; 1 Ci = 3.7 × 10^{10} becquerels) as described (10). Thirty minutes after the injection, larvae were ground in cold 10% trichloroacetic acid. Six larvae were used for each time point. The acid precipitate was washed twice with ethanol, dried, and dissolved in sample buffer. One-fifth of each sample was run on the gel.

RNA Isolation and Protein Synthesis in Vitro. Larvae, wings, or salivary glands were frozen in liquid nitrogen and ground in a glass grinder in extraction buffer (0.22 M Tris·HCl, pH 7.5/0.04 M EDTA/0.02 M NaCl/1% sodium dodecyl sulfate) plus an equal volume of phenol. One volume of chloroform was added immediately after grinding and samples were extracted (11, 12). Samples were centrifuged and reextracted twice with 2 vol of chloroform, and then precipitated with ethanol. After reprecipitation from 1 M sodium acetate the samples were dissolved in water and centrifuged 5 min at 10 × g, and the A_{260} of the supernatant was used to determine RNA content. The New England Nuclear rabbit reticulocyte lysate translation kit was used as described in the supplier's brochure except that the final volume of each translation reaction was 5 μl instead of 25 μl.

Sodium Dodecyl Sulfate Gels. Polyacrylamide (12.5%) gels were run as described (13). The gels were fixed and treated with En^{3}Hance (New England Nuclear). The gels were then dried and autoradiograms were made by using Kodak SB-5 film.

Densitometer tracings were done in a Joyce–Loebl densitometer. Care was taken to remain within the linear range of the film. Areas under each protein peak were used as a measure of amount of labeled protein present.

RESULTS

Dependence of the in Vitro Translation System on Added RNA. We wished to use the in vitro protein synthesis system described in *Materials and Methods* to measure the relative concentrations of specific mRNAs during recovery from heat shock. In order to do this we demonstrated that the amount of protein made in the in vitro system is dependent on added mRNA. In the experiment described in Fig. 1, we added increasing amounts of larval RNA to the in vitro system, and a proportionate increase in the intensity of labeling of all the proteins was observed. Two bands appeared when no RNA was added [at 25 and 44 kilodaltons (kDal)], and these also increased in intensity with added RNA. The increase is probably due to added mRNA that codes for protein of the same molecular weight as the endogenous protein. The 44-kDal endogenous protein comigrates with a major component synthesized by larvae in vivo.

We used densitometer tracings from the autoradiogram shown in Fig. 1 to measure the increase in labeling of three proteins. Fig 2 shows that, for three different proteins, the amount of protein synthesized increases linearly with added RNA over the concentration range used. Higher RNA concentrations do not further stimulate protein synthesis and in fact can be inhibitory.

The increase in protein synthesis with added RNA could be due to nonspecific stimulation of the translation of endogenous

Abbreviation: kDal, kilodaltons(s).

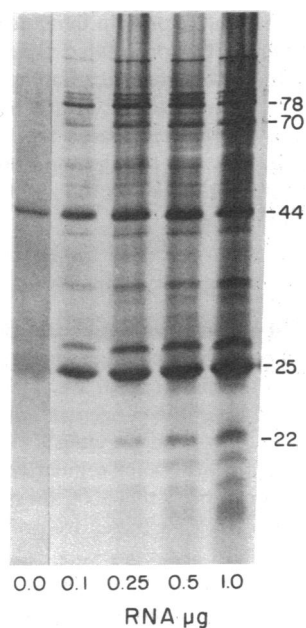Cell Biology: Petersen and Mitchell

Proc. Natl. Acad. Sci. USA 78 (1981)   1709

FIG 1. Dependence of the *in vitro* translation system on added RNA. Autoradiogram of 12.5% polyacrylamide/NaDodSO₄ gel. Each lane shows [^{35}S]methionine incorporated into protein in one 5-μl reaction mix containing the amount of total larval RNA indicated. The RNA used in this concentration series was extracted from 3½-day larvae heated 30 min at 35°C and 20 min at 40.2°C, followed by 1 hr at 25°C. Molecular masses of the proteins (kDal) are indicated on the right.
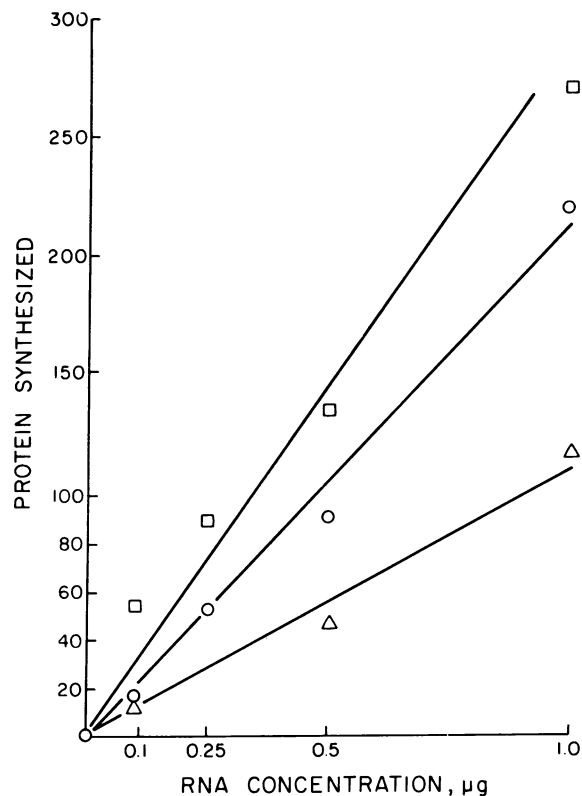

FIG. 2. Amount of protein synthesized *in vitro* as a function of larval RNA concentration. Densitometer tracings of the autoradiogram in Fig. 1 were used to quantitate the relative amounts of three different proteins synthesized (arbitrary units). □, 78-kDal protein (nonheat shock); ○, 70-kDal heat shock protein; △, 22-kDal heat shock protein.

message. In order to show that this is not the case, we translated RNA isolated from several different sources. Fig. 3 shows translation products of RNA isolated from salivary glands (heat shocked and not), from 36-hr pupal wings and whole 36-hr pupae. The protein patterns are clearly different in all of these cases. In particular, the translation of heat shock message into the 83-, 70-, 68-, and 22-kDal heat shock proteins is evident in the heat-shocked salivaries.

Comparison of Protein Synthesis in Vivo and in Vitro During Recovery from Heat Shock. In Fig. 4 we look first at the recovery of larval protein synthesis *in vivo* after a 40.1°C heat shock both with and without a 35°C pretreatment. Second, we look at mRNA populations in the same animals in order to determine whether the pronounced effects of heat shock on protein synthesis are due to changes in mRNA concentration or to regulatory events affecting the translation of mRNA.

Fig. 4 *Left* shows *in vivo* protein synthesis during recovery at 25°C from a 20-min 40.1°C heat shock. Larvae either were heated 30 min at 35°C (P) or remained at room temperature before the 40.1°C shock (O). Larvae were injected with [^{35}S]methionine and labeled *in vivo* for 30 min immediately after the heat shock and 1, 3, and 6 hr later. For comparison the two left lanes show patterns of protein synthesis at 25°C and at 35°C. It can be seen that at 35°C larvae are making all the normal 25°C proteins as well as heat shock proteins of 83, 70, 26 and 22 kDal. Immediately after the 40.1°C shock, there was virtually no protein synthesis in nonpretreated animals, whereas pretreated animals synthesized mostly heat shock proteins. After 3 hr, the pretreated animals made many 25°C proteins, whereas the nontreated animals still mostly synthesized heat shock proteins. After 6 l r, the synthesis of 25°C proteins was nearly normal in pretreated animals, whereas the protein synthesis in the nontreated animals resembled that in the pretreated animals 3 hr after heat shock. Thus, the pretreatment

affects the time of recovery of both heat shock protein synthesis and normal protein synthesis. The synthesis of one 49-kDal protein is less affected than the rest by the 40.1°C treatment. Synthesis of this protein seems not to be affected by the pretreatment.

In Fig. 4 *Right* we show *in vitro* translation of RNA made from larvae recovering from the heat shock described for 4 *Left*. It is immediately evident that there is excellent correspondence in molecular weights between the proteins made *in vivo* and those made in the *in vitro* protein synthesis system. The two left-hand lanes show translation of RNA made from animals at 25°C and 35°C. The 35°C lane shows heat shock proteins along with 25°C proteins, indicating that heat shock mRNA is made during the 35°C pretreatment. The striking fact about the *in vitro* translation of the message from the 40.1°C treated animals is that all of the 25°C messages are present throughout the recovery period in about the same concentrations whether or not the animals were pretreated at 35°C. The heat shock messages are present in all of the pretreated animals. In the nontreated animals, the message for the 70-kDal heat shock protein appears rapidly and the messages for the 22-kDal and 84-kDal heat shock proteins appear more slowly.

The fact that mRNA concentrations are relatively constant after heat shock indicates that the dramatic effects on protein synthesis are due to selective translation of mRNA. The 70kDal heat shock protein provides a particularly striking example of the effects of the 40.1°C treatment and the 35°C pretreatment on protein synthesis. The concentration of mRNA for the 70-kDal heat shock protein is nearly the same for pretreated and nonpretreated animals 1 hr after the 40.1°C shock. (We have run concentration experiments like those in Figs. 2 and 3 to confirm this fact.) However, *in vivo* 1 hr after the 40.1°C shock,
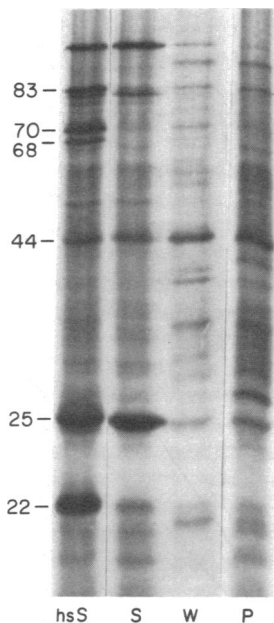
FIG. 3. Comparison of *in vitro* translation products made by using RNA from different sources. Autoradiogram of a gel showing [35S]methionine-labeled products of *in vitro* translation. RNA was isolated from: hsS, heat-shocked salivary glands; S, 25°C salivary glands; W, 36-hr pupal wings; P, whole 36-hr pupae. The positions of five heat shock proteins—83-, 70-, 68-, 25-, and 22-kDal—and the endogenous 44-kDal band are indicated. On these gels the 25- to 28-kDal heat shock proteins are not separated from a normal salivary gland protein. The 22-kDal heat shock protein is actually a multiple band that resolves into three major components on two-dimensional gels (unpublished data).

very little 70-kDal heat shock protein is made in the nonpretreated animals, whereas the pretreated animals make large amounts of this protein. Densitometer tracings show that the pretreated animals make more than 10 times as much 70-kDal heat shock protein as the nonpretreated animals. This shows that the 40.1°C treatment severely inhibits protein synthesis and the 35°C pretreatment enhances the ability of the cells to translate heat shock message.

DISCUSSION

We have shown that pretreating animals at 35°C for 30 min directly affects the recovery of both heat shock protein synthesis and normal protein synthesis after a 40.1°C heat treatment. The pretreatment does not affect the concentrations of major larval messages as had been suggested previously (1, 8). Our results also show a great deal of translational regulation after heat shock and agree with previous reports that 25°C messages are present in the cell, while heat shock messages are preferentially translated (6, 14–17).

The defect in translation that shuts down protein synthesis at high temperatures could be an initiation defect. Several lines of evidence suggest that this is the case. First, polysomes decay at high temperatures in cell lines and reform on selected mRNAs (18, 19, 20). The speed with which polysomes decay indicates that polypeptide chain elongation and termination are not affected by the heat treatment. Second, the fact that there is selective translation of certain messages indicates either that the accessibility of certain messages to the translational machinery is altered or that an initiation factor is altered.

The effect of the 35°C pretreatment on recovery of protein synthesis leads us to suggest that a heat shock gene product may
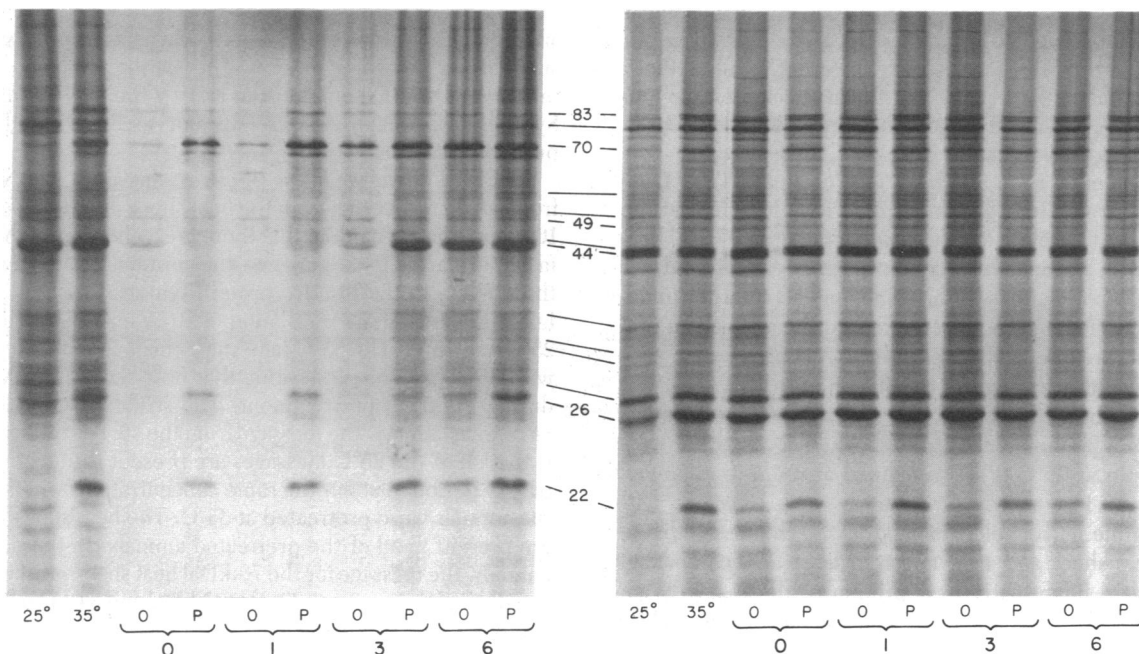

FIG. 4. Comparison of protein synthesis *in vivo* and *in vitro* in larvae recovering from a 40.1°C heat shock. (*Left*) Larval proteins made *in vivo*. Third-instar larvae were either kept at 25°C or heated 30 min at 35°C. They were then heat shocked for 20 min at 40.1°C and allowed to recover at 25°C. Larvae were removed and labeled for 30 min by injection with [35S]methionine. Controls marked 25°C and 35°C were kept at 25°C or labeled at 25°C immediately after a 30-min 35°C treatment. Lanes marked 0, 1, 3, and 6 were kept 0, 1, 3, and 6 hr at 25°C after the 40.1°C shock before labeling. O and P for each recovery time indicate treatment prior to the 40.1°C shock: O, larvae kept at 25°C; P, larvae preheated 30 min at 35°C. (*Right*) *In vitro* protein synthesis from larval RNA. Larvae were heated as in *Left*, but instead of being injected with methionine they were frozen in liquid nitrogen at various times during the recovery period and the RNA was extracted and translated *in vitro*. Each lane represents one translation using 1 μg of the appropriate RNA. Larvae that were not used for RNA and protein synthesis in these experiments were kept. Among the pretreated larvae 84% (64/76) eclosed as adults, whereas most of the animals that were not pretreated eventually died as larvae, and only 18% (24/134) eclosed. Death occurred over a period of 24 hr and only live animals were selected for the experiments.

be involved in the regulation of initiation of protein synthesis following heat shock. The synthesis of heat shock proteins has been correlated with the acquisition of heat resistance, and in yeast it has been shown that heat resistance is not acquired in the absence of protein synthesis (2).

Most heat shock proteins in *Drosophila* are also synthesized at various times in normal development (ref. 18; unpublished data). They are synthesized in response to many different chemical and environmental treatments (21, 22). The fact that "heat shock" proteins are also synthesized in a wide variety of organisms (23–25) suggests that they may be stress response proteins with functions related to cell survival. One of these functions may be to regulate recovery of protein synthesis after heat shock.

1. Mitchell, H. K., Moller, G., Petersen, N. S. & Lipps-Sarmiento, L. (1979) *Dev. Genet.* 1, 181–192.
2. McAlister, L. & Finkelstein, D. B. (1980) *Biochem. Biophys. Res. Commun.* 93, 819–824.
3. Weidner, M. & Combrink, G. (1979) *Plant Physiol.* 64, 144–149.
4. Gerner, E. W. & Schneider, M. J. (1975) *Nature (London)* 256, 500–502.
5. Sapareto, S. A., Hopwood, L. E., Dewey, W. C., Raju, M. R. & Gray, J. W. (1978) *Cancer Res.* 38, 393–400.
6. Henle, K. J., Karamz, J. E. & Leeper, D. B. (1978) *Cancer Res.* 38, 570–574.
7. Li, G. & Hahn, G. (1980) *Cancer Res.* 40, 4501–4508.
8. Lindquist, S. L. (1980) *Dev. Biol.* 77, 463–479.
9. Mitchell, H. K. & Mitchell, A. V. (1964) *Drosophila Inf. Serv.* 39, 135.
10. Mitchell, H. K. (1978) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Wright, T. R. F. (Academic, London), Vol. 2a, pp. 155–158.
11. Avis, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* 69, 1408–1412.
12. Palmiter, R. D. (1973) *J. Biol. Chem.* 248, 2095–2106.
13. Tissières, A., Mitchell, H. K. & Tracy, U. M. (1974) *J. Mol. Biol.* 84, 389–398.
14. Mitchell, H. K. & Lipps, L. S. (1978) *Cell* 15, 907–918.
15. Spradling, A., Pardue, M. L. & Penman, S. (1977) *J. Mol. Biol.* 109, 559–587.
16. Mirault, M.-E., Goldschmidt-Clermont, M., Moran, L., Arrigo, A. P. & Tissières, A. (1978) *Cold Spring Harbor Symp. Quant. Biol.* 42, 819–827.
17. Scott, M. P., Storti, R. V., Pardue, M. L. & Rich, A. (1979) *Biochemistry* 18, 1588–1594.
18. Scott, M. P., Storti, R. V. & Pardue, M. L. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 39, 1810 (abstr.).
19. McKenzie, S. L., Heinikoff, S. & Meselson, M. (1975) *Proc. Natl. Acad. Sci. USA* 72, 1117–1121.
20. McCormick, W. & Penman, S. (1969) *J. Mol. Biol.* 39, 315–333.
21. Ashburner, M. & Bonner, J. (1979) *Cell* 17, 241–254.
22. Ashburner, M. (1970) *Chromosoma* 31, 356–376.
23. Kelley, P. M. & Schlesinger, M. J. (1978) *Cell* 15, 1277–1286.
24. Miller, M. J., Xuong, N.-H. & Geiduschek, E. P. (1979) *Proc. Natl. Acad. Sci. USA* 76, 5222–5225.
25. Walsh, C. (1980) *J. Biol. Chem.* 255, 2629–2632.