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Effect of Low-Temperature Rearing on Heat Shock Protein Synthesis and Heat Sensitivity in *Drosophila melanogaster*

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The patterns of synthesis of heat shock proteins (hsp) and heat sensitivity to elevated temperatures in larvae of *Drosophila melanogaster* reared since hatching at 20°C (warm-reared) or at 10°C (cold-reared) were compared. The pattern of hsp synthesis in salivary glands from the cold- and warm-reared late-third-instar larvae exposed for 1 hr to 33°C or to 37°C was generally similar except for remarkable differences in the 23 kd hsp and a heat-inducible 14 kd polypeptide. The hsp 23 was abundantly synthesised in control as well as heat-shocked warm-reared larval salivary glands, its synthesis in heat-shocked glands being dependent on new transcription. The synthesis of hsp 23 was much less in control glands of cold-reared larvae and was not further inducible by heat shock. The 14 kd polypeptide synthesis was greater in control as well as heat-shocked salivary glands of cold-reared larvae, whereas, in the warm-reared ones, its activity was much less. The cold-reared larvae showed greater sensitivity to elevated temperature; fewer adults eclosed when the cold-reared late-third-instar larvae were exposed to 40°C for 1 hr and also a pretreatment at 37°C for 1 hr was less effective in stopping the killing effect of a subsequent 40°C heat shock in cold-reared than in warm-reared larvae. The greater thermosensitivity of the cold-reared larvae may be correlated with the altered patterns of heat shock gene transcription and translation in cold-reared larvae.

Key words: thermotolerance, hsp 23, heat shock genes, hsr 93D, cold rearing

INTRODUCTION

It was shown in an earlier study [1] that rearing of *Drosophila melanogaster* larvae since hatching at 10°C had a profound effect on heat shock-induced puffing at the two major loci in salivary glands: The 93D heat shock puff in cold-reared larvae was nearly completely regressed in response to heat shock, and the 87C locus formed a significantly larger puff than its duplicate locus at 87A. To see what effect/s this altered heat shock puffing pattern had on the heat shock protein (hsp) synthesis, in the present study we examined the pattern of hsp synthesis in salivary glands of cold-reared larvae. Our results revealed a significant effect of the cold rearing on

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synthesis of the low-molecular-weight hsps, particularly hsp 23. Since one of the important functions of the hsps appears to be in providing thermotolerance [2–5], we also examined the thermosensitivity of the 10°C-reared larvae and found that these larvae were significantly much more sensitive to the killing effect of a brief 40°C heat shock than were the 20°C-reared (warm-reared) larvae. A preexposure to 37°C prior to the 40°C treatment also did not restore the viability of cold-reared larvae to normal level unlike that in the warm-reared larvae.

MATERIAL AND METHODS

hsp Synthesis in Cold- and Warm-Reared Larvae

Eggs of a wild type strain (Oregon R) of *D. melanogaster* were collected in food-filled Petri dishes at hourly intervals and allowed to hatch at 20°C ± 1°C. Freshly hatched larvae were immediately transferred either to an incubator maintained at 10°C ± 2°C or at 20°C ± 1°C and allowed to grow until late-third-instar stage. Since the duration of the larval period was considerably prolonged at 10°C (about 30–35 days [6]), cold- and warm-reared larvae of comparable biological age were obtained by selecting in both cases only those late-third-instar larvae that were actively crawling outside the food on the underside of the Petri dish lid. Examination of salivary gland chromosomes of these larvae confirmed their comparable biological age, since a majority of them had puffing patterns that corresponded with the PS 4–6 of Ashburner [7]. Salivary glands from the cold (10°C)- and warm (20°C)-reared larvae were excised in Poels' salt solution [8] and heat shocked at 33°C or at 37°C for 30 min followed by radiolabeling with either ^{14}C-Chlorella protein hydrolysate (act. 50 μCi/ml; sp. act. 24 mCi/mAtom carbon; BARC, Bombay, India) or with ^{35}S-methionine (act. 100 μCi/ml; sp. act. >800 Ci/mM; Amersham, Amersham, England) for 30 min at the respective temperature. Parallel control glands from both groups of larvae were incubated and labeled at 20°C in the same manner.

In other sets, salivary glands from warm-reared larvae were heat shocked at 33°C or at 37°C for 30 min in the presence of actinomycind D (AMD; 10 μg/ml) and then labeled at the respective temperature with ^{35}S-methionine (as above) in the presence of AMD. Parallel control glands were incubated and labeled at 33°C or 37°C in the absence of AMD. The radiolabeled proteins of salivary glands were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) using 10–18% linear gradient gels in the discontinuous buffer system of Laemli [9]. The gels were stained with Coomassie brilliant blue (CBB) and autofluorographed by the method of Laskey and Mills [10]. A quantitative estimation of the relative rates of synthesis of different hsps in cold- and warm-reared salivary glands was obtained by densitometry of the fluorograms using the gel scanner attachment of a "Kontron" UV spectrophotometer ("UVIKON 810").

Thermotolerance of Cold- and Warm-Reared Larvae

Cold- and warm-reared late-third-instar larvae (selected as above) were transferred to fresh glass vials containing moistened filter papers and exposed in parallel water baths maintained at 20°C (control), at 37°C, or at 40°C for 1 hr; another set of cold- and warm-reared larvae was first exposed to 37°C for 1 hr and then to 40°C for 1 hr. After the treatments, larvae were transferred to fresh food vials and allowed to continue their development at 20°C until eclosion. In each set, 40–50 larvae were

taken for each treatment, and four such sets were run. The numbers of flies eclosing in each vial were recorded and expressed as percent of the initial numbers of larvae treated in the given case to indicate viability following each treatment.

RESULTS

Synthesis of hsps in Salivary Gland Cells of Cold- and Warm-Reared Larvae

Typical examples of CBB-stained gels and their fluorograms to reveal the patterns of synthesis of hsps at 20°C, 33°C, and 37°C in salivary glands from cold- and warm-reared larvae are presented in Figure 1. The synthesis of the high-molecular-weight (HMW) hsps (82, 70, 68, and 36 kd) at 33°C and 37°C was more or less comparable in the two groups of larvae (see Fig. 1). Among the low-molecular-weight (LMW) hsps, the synthesis of hsp 23 was remarkably different in the two groups: hsp 23 was abundantly synthesized in control as well as heat-shocked glands of warm-reared larvae. On the other hand, hsp 23 was synthesised at a very low level in control glands of cold-reared larvae, and heat shock did not appreciably induce its synthesis, neither at 33°C nor at 37°C (see Fig. 1, lanes 10–12). Analysis of the densitometric scans of fluorograms revealed that synthesis of hsp 23 in control as well as heat-shocked salivary glands of warm-reared larvae was about two and one-half to three times greater than in corresponding samples from the cold-reared larvae (detailed data not presented). Comparison of the CBB-stained gels also showed a clear difference in the amount of protein at the 23-kd position in the lanes from warm- and cold-reared larvae, respectively (compare lanes 1–3 with lanes 4–6 in Fig. 1). Synthesis of the other LMW hsps (27, 26, and 22 kd) was more or less similar in both groups of larvae at 33°C and 37°C. When the warm-reared larval salivary glands were heat shocked and labeled at 33°C in the presence of AMD, none of the hsps, except hsp 23, were seen to be labeled; hsp 23 was also much less labeled than in corresponding glands heat shocked and labeled without the AMD (see Fig. 2, lanes 8 and 11). Glands heat shocked and labeled at 37°C in the presence of AMD did not show appreciable label in any polypeptide, including hsp 23 (Fig. 2, lanes 9 and 12).

Another remarkable difference in protein synthesis in cold- and warm-reared salivary glands was related to a 14-kd polypeptide that was synthesized at much higher levels in control glands of cold-reared larvae, and this level was further elevated by heat shock (Fig. 1). In warm-reared larvae, however, the control level of synthesis of the 14-kd polypeptide was low and was only slightly elevated by heat shock (Fig. 1). Densitometric scans of fluorograms showed that incorporation of label in the 14-kd polypeptide was about one and one-half times (33°C) to four times (37°C) greater in heat-shocked glands of cold-reared than of warm-reared larvae.

Thermotolerance of Cold- and Warm-Reared Larvae

The percent adult emergence values for the control and differently heat shocked cold- and warm-reared larvae are presented in Figure 3. It was seen that cold- and warm-reared larvae showed comparable viability (in terms of adult emergence) in control and 37°C heat-shocked groups. A severe heat shock at 40°C greatly reduced the viability so that fewer adults eclosed: The cold-reared larvae were nearly two times more sensitive to the 40°C treatment than the warm-reared ones. A 1-hr pretreatment at 37°C prior to the 40°C severe shock considerably reduced the

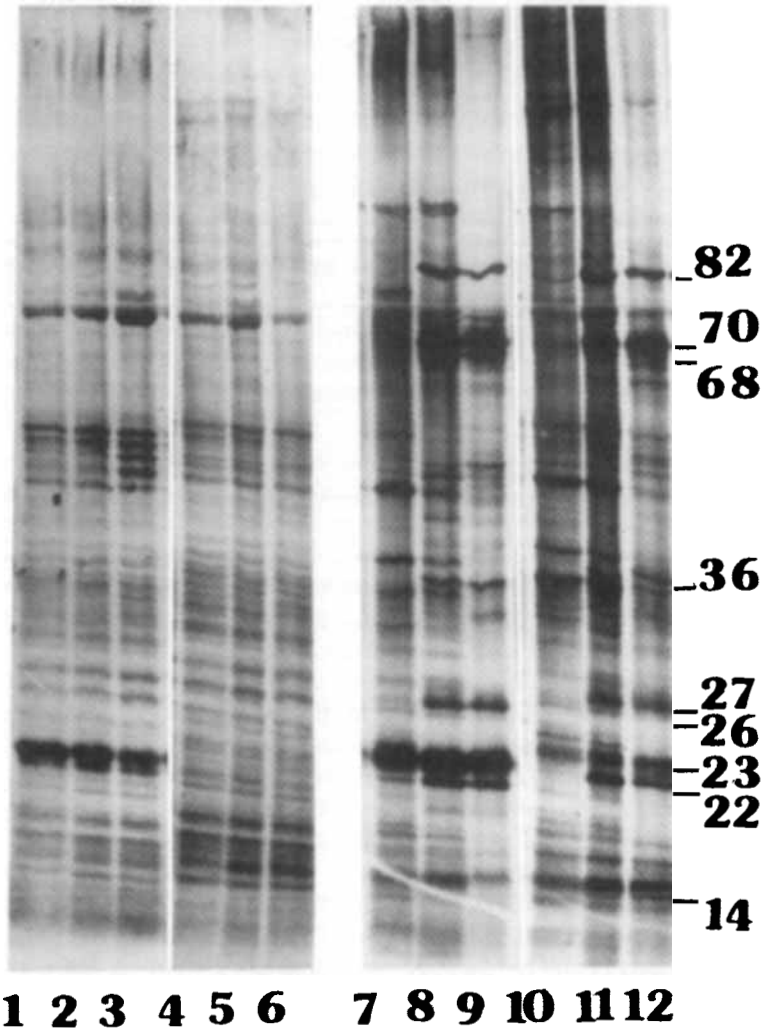

Fig. 1. Patterns of protein synthesis in salivary glands of warm-reared **(lanes 1–3** and **7–9)** or cold-reared **(lanes 4–6** and **10–12)** larvae after in vitro incubation for 30 min at 24°C (lanes 1,7 and 4,10) or at 33°C (lanes 2,8 and 5,11) or at 37°C (lanes 3,9 and 6,12). Lanes 1–6 show the CBB-staining patterns; lanes 7–12 show corresponding autofluorograms (^{35}S-methionine labeled). The hsps are indicated by their molecular weights (kd).

lethality. However, although the warm-reared larvae given 37°C and 40°C treatments consecutively showed about 78% viability (adult emergence), the viability of the similarly treated cold-reared larvae was only about 40% (Fig. 3). Thus, although the 37°C pretreatment nearly doubled the survival following 40°C treatment in both sets of larvae, the cold-reared larvae continued to be much more sensitive to the killing effect of 40°C than the warm-reared larvae.

DISCUSSION

As was mentioned in the Introduction, the present study was undertaken to examine the consequences of the altered heat shock puffing at the 93D (no induction)

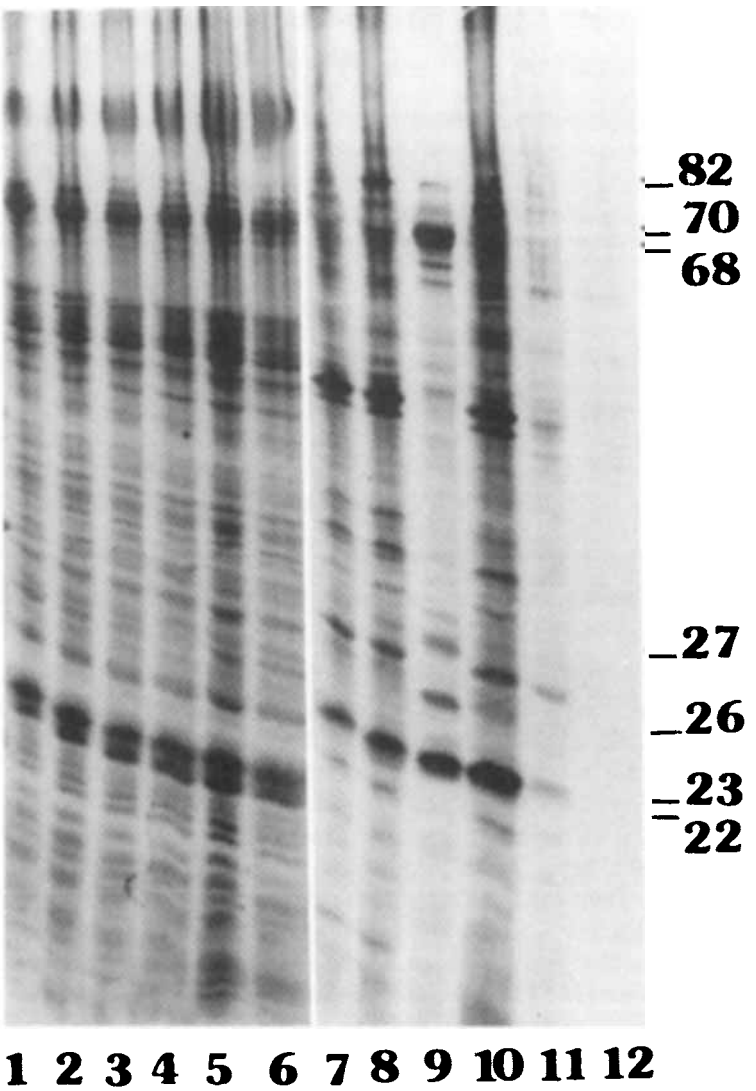

Fig. 2. Effect of AMD on heat shock-induced protein synthesis in salivary glands of warm-reared larvae. **Lanes 1–6** show the CBB, staining pattern. **Lanes 7–12** show corresponding autofluorograms. The different lanes are: control (24°C) without (lanes 1,7) and with AMD (lanes 4,10) treatment; 33°C heat shock without (lanes 2,8) and with AMD (lanes 5,11) treatment; and 37°C heat shock without (lanes 3,9) and with AMD (lanes 6,12) treatment. The hsps are indicated by their molecular weights (kd).

and 87C (increased puffing) loci in salivary glands of cold-reared larvae of *D. melanogaster* [1]. The 93D locus does not appear to code for any hsp [11–13], so the nonpuffing of this locus may not be directly discernible in protein gels. The 87A and 87C loci carry multiple copies of the hsp 70-coding sequences [14]. Our present results failed to detect any increased synthesis of the hsp 70 in cold-reared larval glands which could be correlated to the increased puffing of the 87C locus in these larvae. This lack of correlation between altered puffing of 87A/87C loci and the level

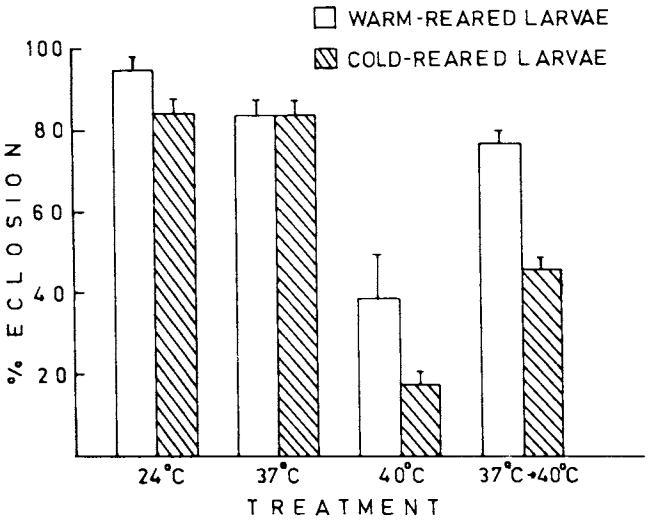

Fig. 3. Comparison of thermotolerance in warm- and cold-reared larvae of *D. melanogaster*. Exposure to different treatments is indicated on abscissa and the coordinate indicates the mean percent eclosion of flies in relation to the numbers of larvae treated. Vertical bars indicate SE.

of hsp 70 synthesis in cold-reared larvae may be due either to the known presence of multiple copies of the hsp 70 sequences at each of the duplicate loci so that altered transcription of one is compensated by the others or to the earlier-suggested idea that the increased puffing of 87C locus under certain conditions of heat shock is related to altered transcription or turnover pattern of transcripts of the heat-inducible but noncoding alpha-beta repeat sequences located at the 87C locus [15].

A result not immediately anticipated on the basis of earlier puffing and transcription studies [1] was the very low level of synthesis of the hsp 23 in cold-reared larvae. The genes for hsps 27, 26, 23, and 22 are closely spaced at the 67B heat shock locus [16, 17] and the heat shock-induced puffing of this locus was found to be similar in cold- and warm-reared larvae [1]. All the LMW hsps are also ecdysone-inducible, and, among these, hsp 23, being more stable, accumulates in appreciable quantities in salivary glands and other tissues of late-third-instar larvae [18–22]. Accordingly, we found prominent CBB staining and labeling of the 23-kd polypeptide in control salivary glands of the warm-reared larvae, and as expected the synthesis of hsp 23 was further stimulated by heat and shock. Our AMD experiments revealed that the high levels of synthesis of hsp 23 seen in control and heat-shocked glands of warm-reared larvae were dependent on different transcripts, since the inhibition of new heat shock RNA synthesis by AMD in 37°C-shocked glands led to complete disappearance of newly synthesised hsp 23 along with all other hsps. The persistent synthesis of hsp 23 in glands shocked at 33°C in the presence of AMD apparently was due to incomplete inhibition of translation of normal cellular transcripts, which included ecdysone-induced hsp 23 messages. Thus it is important to note that, even though the control and heat-shocked glands of warm-reared larvae show more or less equally high level synthesis of hsp 23, heat shock causes abundant induction of a fresh set of transcripts that alone are translated at elevated tempera-

tures. In this context, the much lower level of hsp 23 in cold-reared larval salivary glands is remarkable. On the basis of their polytene puffing (data not presented) the cold- and warm-reared larvae were of comparable biological age and, therefore, the salivary glands of cold-reared larvae were expected to show, as in the warm-reared ones, significant levels of ecdysone-induced hsp 23. This was not seen. Also, even the heat shock, both at 33°C and at 37°C, failed to elevate significantly the synthesis of hsp 23 in cold-reared larvae, although the other smaller hsps were typically induced. These observations show that the low-temperature rearing causes the synthesis and accumulation of hsp 23 to be refractory to both ecdysone and heat stimuli without simultaneously affecting the other LMW hsps. Unlike with the other LMW hsps, the regulatory sequences required for ecdysone and heat inducibility of the hsp 23 gene are known to be intermingled with each other [23]. It has been suggested [23] that some transcription factors are commonly involved in both responses of the hsp 23 gene, perhaps as general stabilizers of transcription complexes. Rearing of larvae at low temperature may affect the commonly involved transcription factor or its binding to the regulatory sequences so that both ecdysone as well as heat shock fail to induce hsp 23. The biological reasons for noninducibility of hsp 23 by ecdysone as well as heat in cold-reared larvae are not known. Whether the noninducibility of 93D locus in these larvae [1] has any effect on the hsp 23 synthesis is also not known. These remain interesting issues for further studies.

Our present results also showed that the cold-reared larvae were more sensitive than the warm-reared larvae to heat shock at 40°C. A preadaptive exposure to 37°C also was not as effective in protecting the cold-reared larvae from the lethal effect of 40°C as in the case of warm-reared larvae. The causes for the greater thermosensitivity of the cold-reared larvae may be related to any one, or more, of the following: 1) absence of 93D induction in response to heat shock in cold-reared larvae [1], 2) altered transcription/turnover of transcripts at the 87C locus [1], and 3) low levels of hsp 23 in cold-reared larvae.

In spite of the altered puffing of 87C locus, the induction of hsp 70 in cold-reared larvae was found to be comparable to that in the warm-reared larvae, so hsp 70 is unlikely to be involved in the greater thermosensitivity of the cold-reared larvae. The roles of 93D and the alpha-beta transcripts in heat shock response and thermotolerance are not known, although there is some evidence that a genetic deficiency of the 93D locus makes the larvae more thermosensitive [13]. Thus it remains possible that the greater sensitivity of the cold-reared larvae is, at least in part, related to the noninducibility of 93D heat shock locus in these larvae.

Several earlier studies [18–20] have implicated hsp 23 in providing thermotolerance, and, accordingly, the warm-reared late-third-instar larvae that accumulate ecdysone-induced hsp 23 are reported to be more thermotolerant than at earlier stages. Therefore, the relatively low abundance of hsp 23 in cold-reared larvae, both before and after heat shock, may also be an important factor in their greater thermosensitivity.

The nature and significance of the 14-kd polypeptide noted in cold-reared larvae is not known. It was found to comigrate with calf thymus H2b (data not presented), and, in view of the earlier results that there is a heat-inducible H2b in *D. melanogaster* [24,25], it is possible that this 14-kd polypeptide corresponds to an H2b species. However, the present limited data do not permit any definitive statement. Our earlier [1] and the present results thus show that the developmental conditions can

diversely affect the regulation of individual heat-shock genes, and some or all of these alterations result in modified thermotolerance. These observations provide yet more examples to emphasize the complexity of regulation and biological roles of the heat-shock response.

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