Autoregulation of heat-shock system in *Drosophila Melanogaster*

Analysis of heat-shock response in a temperature-sensitive cell-lethal mutant

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The cells of the cell-lethal mutant l(1)ts403 are characterized by an extremely low level of synthesis of heat-shock proteins (hsp) after temperature elevation. The mutation affects not only the kinetics of hsp synthesis but also the electrophoretic pattern of hsp. Studies of puffing, RNA and protein synthesis in the mutant strongly suggest that hsp are involved in the regulation of their own synthesis at the level of puff induction, transcription and translation.

Heat-shock protein *Puffing* Drosophila *ts mutation*

1. INTRODUCTION

Heat-shock genes in *Drosophila* represent a system which is now intensively studied by different means and in various aspects [1,2].

Heat shock not only induces specific puffing in salivary gland chromosomes but also leads to dramatic changes in the protein synthesis in various *Drosophila* tissue studied [3]. Heat shock brough about the appearance of a number (6–7) of new proteins (heat-shock proteins - hsp) while others synthesized under normal growth conditions are either no longer synthesized or their synthesis is substantially reduced. The functional significance of hsp however is still rather obscure. Lindquist and co-workers [4,5] studied heat-shock response and recovery in *Drosophila* tissue culture cells under a variety of induction conditions. The investigators blocked the function of the heat-shock system using cycloheximide and actinomycin or by incorporating amino acid analogs into hsp. Their data suggest that hsp are involved in regulating their own synthesis at both transcriptional and post-transcriptional levels. Along these lines, Bonner [6] studied the induction of heat-shock puffs in isolated salivary gland nuclei and concluded that hsp represent a feed-back regulatory system, i.e. hsp being the inhibitors of puffing at corresponding regions.

We were lucky to find out that the ts mutation of cell-lethal class localized by Arking [7] in the X-chromosome dramatically affects heat-shock response in *D. melanogaster* [8]. Later, mutations of this type were found in other organisms including *Dictyostelium* and *E. coli* [9,10].

The purpose of this study was to examine the recovery process after heat-shock in this mutant characterized by an extremely low level of hsp sythesis after temperature elevation.

2. MATERIALS AND METHODS

2.1. Drosophila *stocks*

Here, we used ts mutation 1(1)ts403 isolated and described by Arking [7]. By means of the twin spot test the investigator, classified the mutation as ts cell lethal one. It was localized at locus 42.0 of the X-chromosome. For this stock (1(1)ts403), the temperature-sensitive period estimated by 'shift-down' experiments covers the whole life cycle of the animals. The mutant affects both larval and imaginal tissues, including the larval fat body and

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salivary glands. The Oregon RC wild-type stock was used as control.

2.2. *Polytene chromosome analysis*

Third instar larvae were heat-shocked by placing them for 30 min in prewarmed vials in a 37°C water bath. After heat-shock salivary glands were dissected in 45% acetic acid and then transferred to a staining solution of 2% orcein (Sigma) in 50% acetic acid: 30% lactic acid. Puff width was measured at the puff's widest point and was normalized to a nearby major band: 63A for 63BC puff, 94A for 93D puff, and 87F for 87C puffs. Salivary gland permanent preparations and transcription autoradiograms were done as described [8].

2.3. *Isolation of hsp and electrophoresis*

The glands were transferred to a 10 μl drop of sodium phosphate buffer containing 10–20 μCi [^{35}S]methionine for 30 min (Amersham: spec. act. 175 Ci/mmol). Proteins were extracted as described [8]. Samples of up to 50 μl were analysed on 11% discontinuous SDS-polyacrylamide gels [11] using 1.5 mm thick slabs. The labeled proteins were detected in dried gels by autoradiography on Kodak Royal X-Omat RP-54 X-ray film.

3. RESULTS

3.1. *Heat-shock puffs and RNA synthesis in mutant and wild-type cells*

In our experiments when Oregon RC larvae were treated for 30 min at 37°C, all the heat-inducible puffs regressed almost completely after 1 h recovery at 25°C. In marked contrast in 1(1)ts403 larvae, during the course of recovery, very large puffs are seen for several hours at all major heat-inducible loci with the exception of 93D. Fig.1 summarizes the puffs measurements performed in salivary glands nuclei of wild-type (Oregon RC) and mutant 1(1)ts403 larvae after 30 min of heat shock. It is evident that the puffing pattern of heat-shock loci is quite different in mutant and normal cells. While the 87CI region is characterized by quantitatively the same degree of puffing in both cases, the size of puffs at chromosomal locus 63BC coding for hsp 84 in mutant cells significantly exceeds that of the corresponding locus in wild-type larvae. The results of these puff measurements in the mutant and normal cells in the course of recovery are presented in fig.2. These data lead us to conclude that the 63BC region preserves its maximal puffing state in the cells of the mutant much longer than in the control.

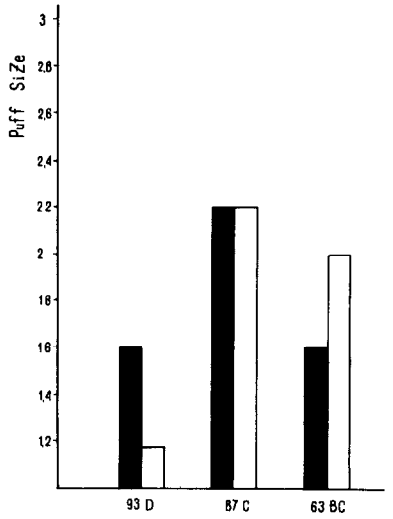

Fig.1. Heat-shock puffs induction in mutant 1(1)ts403 (□) and Oregon RC (■) salivary gland chromosomes. The larvae were incubated for 30 min at 37°C.

The [^{3}H]uridine incorporation pattern closely correlates with heat-shock puff induction; the

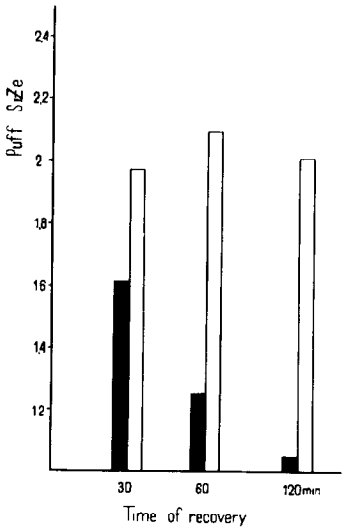

Fig.2. Puff size measurements for 63BC:63A in mutant 1(1)ts403 (□) and Oregon RC wild-tipe larvae (■) in the course of recovery after heat-shock treatment.

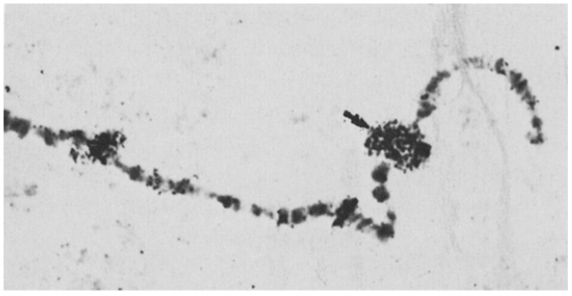

Fig.3. Labeling pattern of the distal end of 3L-chromosome of 1(1)ts403 larvae after 2 h recovery. The heavily labeled 63BC puff is indicated by the arrow. Exposure time was 5 days.

63BC locus forming a large puff is also one of the most heavily labeled sites (fig.3). It is interesting to mention the weak induction of the 93D puff in the mutant cells and its low level of [^{3}H]uridine incorporation. Another very interesting observation that may also be related to the regulation of the heat-shock system is the very slow recovery process at the level of transcription in the mutant cells. Thus, for at least 2 h after heat shock, RNA synthesis is confined to the heat-shock loci (fig.4).

3.2. *Protein synthesis in the mutant and wild-type cells in the process of recovery after heat-shock*

Previously, when comparing the synthesis of hsp in mutant 1(1)ts403 cells and normal cells, we discovered a drastic drop of label incorporation in the former case after heat-shock treatment. Fig.5 demonstrates the process of recovery in terms of protein synthesis in mutant cells after heat-shock. It is evident that almost complete quantitative restoration of the level of protein synthesis takes place in the mutant cells after 4 h recovery at 25°C. This indicates that the low level of hsp synthesis observed in mutant tissues is of reversible nature and not simply due to cell death after high-temperature treatment.

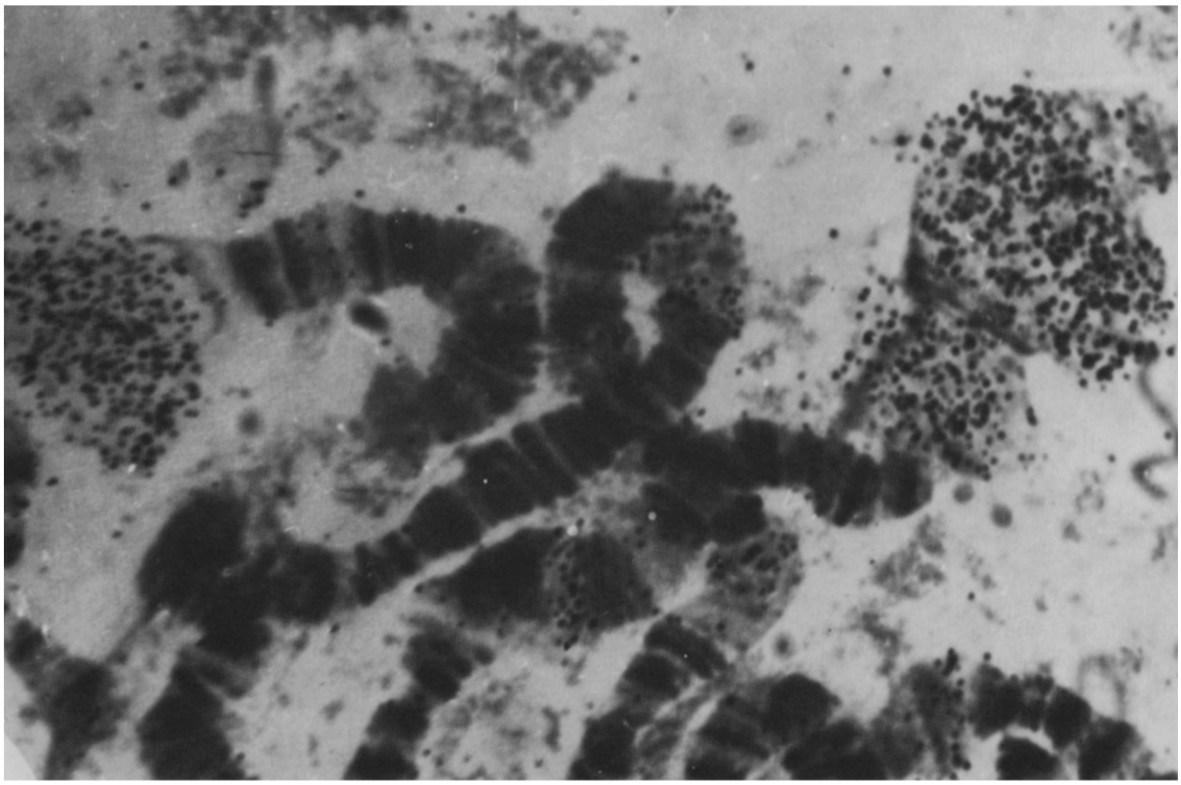

Fig.4. A typical labeling pattern of 1(1)ts403 salivary gland chromosomes after 2 h recovery after heat shock. Note the very weak labeling of the 93D site. Exposure time was 6 days.

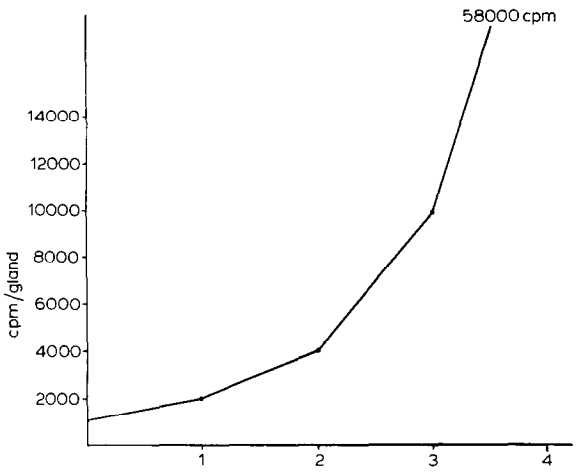

Fig.5. Restoration of total protein synthesis in the mutant larvae 1(1)ts403 in the course of recovery after heat shock. The incorporation of [^{35}S]methionine into proteins in the mutant before heat shock was 65 000 cpm/gland.

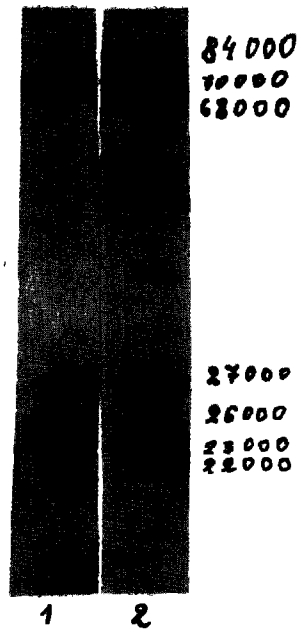

Fig.6. The pattern of heat-induced protein synthesis in salivary glands of Oregon RC (2) and 1(1)ts403 (1) larvae immediately after heat shock. The positions of the major hsps are indicated on the right.

Fig.6 is a typical radioautograph showing the proteins in salivary glands from mutant and normal larvae immediately after heat-shock. It is clear that the patterns of protein synthesis differ considerably in the mutant and wild-type cells. Thus, hsp 84 is not synthesized after heat shock in 1(1)ts403 at 37°C. Another interesting feature of the hsp pattern in the mutant cell immediately after heat shock is the comparatively low level of synthesis of hsp 70, this protein being the main polypeptide synthesized in normal *Drosophila* cells after temperature elevation.

As can be seen in fig.7 illustrating the process of recovery in terms of protein synthesis, induction of hsp 84 could be clearly detected only after 2 h recovery (sample 3). In general one may conclude

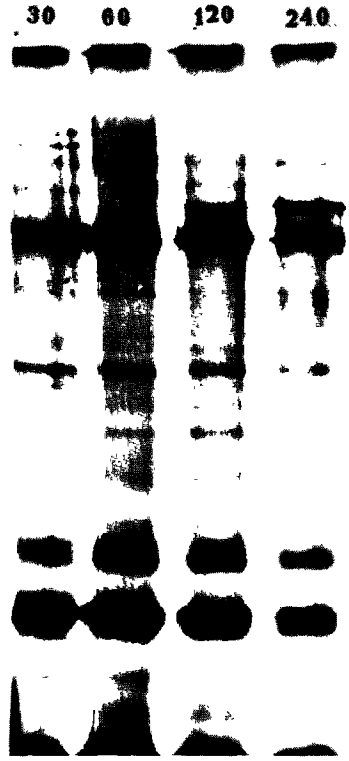

Fig.7. Recovery of protein synthesis after heat shock in the mutant 1(1)ts403 and wild-type cells. Larvae heat treated at 37°C for 30 min were returned to 25°C and their salivary glands were pulse labeled with [^{35}S]-methionine in the course of recovery. The numbers above each lane indicate the beginning of the labeling period in min relative to the point when cells were returned to 25°C.

that after a couple of hours, the synthesis of normal cellular proteins is restored in cells of wild-type larvae (sample 7). In marked contrast, the mutant cells exposed to high temperature (37°C), first exhibit the abnormal ('mutant') pattern of hsp synthesis (samples 1, 2), but after approx. 2 h recovery reveal the normal pattern of hsp including 84 hsp and 70 hsp, and subsequently continue to synthesize hsp for several hours at normal temperature (samples 3, 4).

4. DISCUSSION

The experiments by Lindquist et al. [4,5] and Bonner [6] mentioned above enabled them to suggest an autoregulatory role of hsp. However, Lindquist and co-workers used different inhibitors of protein and RNA synthesis and amino acid analogs in their studies, which obviously require a lot of rather complicated control experiments.

The present experiments have many obvious advantages for investigation of heat-shock regulation. For example, we did not apply any chemical interference to the cellular metabolism, the low level of hsp synthesis being the result of point mutation in the presumptive regulatory gene. The changed pattern of protein synthesis in the 1(1)ts403 larvae characterized by the absence of hsp 84 and a small induction of hsp 70 offers an opportunity to study not only the mechanism of induction, but also the function of individual hsp in the process of recovery. Furthermore, in our system we are able to investigate the heat-shock response at both the cytological level (puff induction) and at the level of transcription and protein synthesis.

Our experiments demonstrate that in the mutant studied all the major heat-shock puffs with the exception of the 93D puff are normally formed after temperature elevation. Moreover, the puffs in the mutant preserve their size and continue to synthesize RNA for several hours during the course of the recovery process at 25°C. As for puff 93D, it is necessary to bear in mind that it does not code for and hsp and the role of this locus in the heat-shock response is still unclear [12].

It is interesting that in the mutant puff 63BC coding for hsp 84 reaches an extraordinary large size after about 1 h recovery at 25°C and is characterized by an exceptionally high level of [^{3}H]uridine incorporation. The simplest, but not the only explanation of this fact is that each hsp directly inhibits its own gene.

Previously, using the methods described by Velasquez et al. [13], we showed that while small quantities of heat-induced proteins were synthesized in the mutant cells immediately after temperature elevation, these proteins apparently lost the ability to enter the nucleus and bind to chromatin, thus imitating the behaviour of hsp synthesized in the presence of amino acid analogs [14]. One may suggest that in the mutant defective hsp do not fulfill their protective function to cells exposed to elevated temperatures and thus this 'heat-shock state' of the cells will be preserved for some time after temperature elevation. In fact, in our experiments, we observed the persistence of heat-inducible puffs and intensive synthesis of hsp long after initial heat shock in the cells of the mutant.

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