

A novel set of heat shock polypeptides in malpighian tubules of *Drosophila melanogaster*

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Abstract. In contrast to the general notion of induction of a common set of heat shock polypeptides (HSP) in all cell types, heat shocked malpighian tubules (MT) of *Drosophila melanogaster* larvae did not synthesize the common set of HSP induced in salivary glands or brain ganglia of larvae and in gonads (testis or ovary) of adult flies. Instead, heat shocked MT of late 3rd instar larvae and freshly eclosed adults synthesized a novel set of polypeptides (MT-specific HSP) with a major induced band at 58 kd. Surprisingly, the MT of older flies synthesized both the MT-specific as well as the common set of HSP in response to heat shock. Fusion genes with hsp70 or hsp26 promoter linked to the lac-Z (beta galactosidase) or to ADH (alcohol dehydrogenase) reporter gene were also not induced in larval MT but showed good induction in the MT of older flies. This unexpected finding raises intriguing issues regarding the nature of MT-specific HSP, their genes and the cell type dependent induction of the two sets of HSP.

Keywords. Heat shock response; HSP; *D. melanogaster*; 58 kd HSP; malpighian tubules.

1. Introduction

Heat shock response is an important parameter for studies on gene expression and its regulation. The universality and general uniformity of this response in diverse cell types of all organisms is one of the most remarkable aspects of this cellular response to stress. Generally, three major classes, the 84 kd, the 70 kd and the small molecular weight families of HSP are induced by heat shock in most cell types of diverse organisms that have been examined so far (Burdon 1988; Pardue 1988). During the course of our studies on heat shock response in different cell types and developmental stages of *Drosophila*, we noted a very unusual response in the malpighian tubules (MT) of larvae and adults: unlike in other tissues, none of the usual HSP were induced by heat shock in the MT of the late 3rd instar larvae or freshly eclosed flies of *D. melanogaster*. Rather, a novel set of polypeptides was induced by the stress in these cells. More intriguingly, MT from older flies responded to heat shock by induction of both the common as well as the MT-specific sets of HSP.

2. Materials and methods

2.1 *Drosophila* stocks

In addition to a wild type strain (Oregon R⁺) of *Drosophila melanogaster*, transformed stocks of *D. melanogaster* containing HSP70 or HSP26 promoters

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fused with the alcohol dehydrogenase (ADH) or beta-galactosidase (Lac-Z) reporter gene were also used. The HSP70-ADH fusion gene stock was as described by Bonner *et al.* (1984) in which the fusion gene is inserted at the polytene band 61C of the 3rd chromosome (named *Adh^{hsp61C}*). The HSP70-Lac-Z and the HSP26-Lac-Z promoter fusion stocks were described by Lis *et al.* (1983) and Glaser *et al.* (1986) and were named *Bg9* (the fusion gene inserted in the polytene region 9 of X-chromosome) and *26Z-84D* (the fusion gene inserted in the polytene band 84D of chromosome 3), respectively. The above HSP promoter-reporter fusion genes are known to be heat-inducible and their transcripts are also known to be efficiently translated at elevated temperatures like the normal heat shock transcripts (see Lis *et al.* 1983, Bonner *et al.* 1984, Glaser *et al.* 1986). All flies and larvae were reared on standard *Drosophila* food at 20°C.

2.2 Heat shock induced protein synthesis in different tissues

Malpighian tubules of wild type (Oregon R⁺) late 3rd instar larvae and young or older flies (within 6 h of eclosion or more than a week old, respectively), and other tissues like salivary glands and brain ganglia (from larvae) and gonads (testis and ovary) of freshly eclosed or older flies, were removed from the same groups of animals, heat shocked *in vitro* at 37°C for 30 min and then labelled with ³⁵S-methionine at 37°C as described earlier (Singh and Lakhotia 1988). Parallel controls were incubated and labelled at 24°C for equivalent periods. For MT samples, tissue from about 50 individuals was used per lane while for other samples, tissues from 8–10 individuals were taken for each lane of the 10–18% gradient slab gel. Conditions for SDS-polyacrylamide gel electrophoresis and subsequent fluorography were as described earlier (Singh and Lakhotia 1988). As judged by Coomassie brilliant blue staining of gels, equivalent amounts of proteins were present in control and heat shock lanes of different tissues. In some experiments, the larval MT and salivary glands were heat-shocked either at 37°C for 60 or 90 min or at 41°C for 30 min prior to labelling and electrophoresis of proteins as above.

2.3 Expression of ADH or beta-galactosidase after heat shock in the promoter-fusion stocks

Healthy late 3rd instar larvae and old flies of *Adh^{hsp61C}*, *Bg9* and *26Z-84D* stocks were heat-shocked at 37°C for 1 h, allowed to recover at 24°C for 0, 1, 2 or 3 h following which the internal organs, mainly the gut and associated tissues, were dissected in Poels' salt solution (Lakhotia and Mukherjee 1980), and stained for ADH (*Adh^{hsp61C}*) or beta-galactosidase (*Bg9* or *26Z-84D*) activity, essentially as described by Bonner *et al.* (1984) and O'Kane and Gehring (1987), respectively. Briefly, for ADH staining the dissected tissues were fixed for 5 min at 24°C in 3.7% formaldehyde in 0.05 M sodium phosphate buffer (pH 7.5), washed in the same buffer and transferred to the ADH activity stain (see table 1 for composition). For beta-galactosidase staining the dissected tissues were fixed in 5% glutaraldehyde in citric-phosphate buffer (10 mg KH₂PO₄ and 2.54 mg monohydrate citric acid in 10 ml distilled water, pH 7.2) for 5 min, washed in the same buffer and transferred to the beta-galactosidase activity stain (table 1). Following the staining for ADH or beta-galactosidase, the tissues were briefly fixed in acetic acid-methanol (1:3) and

Table 1. Composition of ADH and beta-galactosidase activity stains.

<i>A. ADH activity stain</i>	
0.05 M sodium phosphate buffer (pH 7.5)	2 ml
Nitroblue tetrazolium salt (NBT, 5 mg/ml)	0.5 ml
Nicotinamide adenine dinucleotide (NAD, 5 mg/ml)	0.1 ml
2-Butanol	0.1 ml
Phenazine methosulphate (PMS, 1 mg/ml)	0.05 ml
Staining done in the dark at room temperature for 15–30 min	
<i>B. Beta-galactosidase activity stain</i>	
1% X-gal in dimethyl formamide	0.2 ml
100 mM potassium ferricyanide	0.05 ml
100 mM potassium ferrocyanide	0.05 ml
Citric-phosphate buffer (pH 7.2)	0.7 ml
Staining done overnight in the dark at 37°C	

mounted on slides in 50% glycerol. Parallel batches of control larvae or flies were kept at 24°C and their tissues also stained for respective enzyme activity.

3. Results

3.1 *A different set of heat shock polypeptides is synthesized in MT*

It is well known that in response to heat shock, cells of *D. melanogaster* synthesize three families of HSP: (i) the 82 kd hsp, (ii) the hsp70 family comprising the hsp70 and hsp68 and (iii) the hsp20 family, comprising hsp27, hsp26, hsp23 and hsp22 (Tissiers *et al.* 1974; Ashburner and Bonner 1979; Burdon 1988; Pardue 1988; Singh and Lakhota 1988). The same set of HSP was found to be induced by a 30 min heat shock in larval salivary glands and brain ganglia, and in gonads (testes and ovaries) of young or older flies (see figure 1) and in agreement with earlier studies, hsp70 was the most abundantly labelled polypeptide in heat-shocked samples of all these tissues. Surprisingly, however, MT from larvae or young adults did not show induction of any of these hsps when heat-shocked for 30 min; as seen in figure 1 (lanes 4 and 7), no labelled bands were seen in heat-shocked larval and young adult MT at positions corresponding to any members of the hsp70 or the hsp20 families although a labelled band was present at hsp82 position in control as well as heat-shocked MT lanes. Thus while the common HSP were not induced by heat shock in larval and young adult MT, labelling of certain other polypeptides in this tissue was noticeably increased after heat shock, the most prominent of these being a band at the 58 kd position. Besides the 58 kd, synthesis of polypeptides migrating to 74, 47, 45, 33, 32, 15, 13 and 12 kd band positions was either newly induced or was increased in heat-shocked MT when compared to their respective controls. It may be noted that some of these later polypeptides were also labelled in heat-shocked samples of other tissues but when compared to their respective controls (compare lanes 1 and 2, and 3 and 4 in figure 1a), it was clear that in MT their labelling was increased by heat shock, while in other tissues their labelling was reduced after the heat shock. Thus, since among the tissues examined, in only MT the synthesis of polypeptides migrating with apparent molecular masses of 74, 58, 47, 45, 33, 32, 15,

13 and 12 kd. was always (58 kd) or often (others) induced or increased by heat shock, we consider them to be MT-specific HSP. A band at the 14 kd position was often induced by heat shock in most tissues: we presume this to correspond to a histone variant reported earlier (Sanders 1981; Singh and Lakhotia 1988).

Unlike in the larva or the young adult, the MT from older flies responded to heat shock by synthesizing both the common as well as the MT-specific HSP; as seen in lane 9 of figure 1b, in addition to the 58 kd and several other MT-specific HSP, all the three families of common HSP were also well-induced in MT of older flies. As in other tissues, hsp70 was the most abundant in heat-shocked MT from older flies. To check if lack of induction of the common set of HSP in larval and young adult MT was due to different threshold time or temperature requirements, the MT from late 3rd instar larvae were heat-shocked at 37°C for 60 or 90 min or at 41°C for 30 min prior to labelling with ³⁵S-methionine for 30 min. Prolonged heat shock (60 or 90 min) also did not induce the common set of HSP in larval MT. Even after a 41°C shock for 30 min, only the MT-specific HSP were noticeable in fluorograms although the rate of protein synthesis was severely inhibited at this temperature (not shown). Longer autoradiographic exposure or loading of more labelled protein sample per lane also did not reveal synthesis of hsp70 or the other usual HSP in larval MT.

3.2 Heat shock promoters are not inducible in larval MT

To know if the heat shock elements or promoters (Bienz and Pelham 1987) of the common set of HSP are at all inducible in larval MT, we used transformed stocks of *D. melanogaster* in which the ADH or Lac-Z genes act as reporters under the control of HSP70 or HSP26 promoters (see Lis *et al.* 1983, Bonner *et al.* 1984, Glaser *et al.* 1986, O'Kane and Gehring 1987). Tissues of larvae or flies after recovery from heat shock (0 to 3 h recovery) or of controls were stained for ADH or beta-galactosidase activity, as required. As shown in figure 2, the larval MT of neither the *Bg9* nor the *26Z-84D* genotype showed any appreciable beta-galactosidase activity after heat shock (upto 3 h of recovery) although in other tissues of the same animal, good staining was apparent even immediately after the 1 h shock. On the other hand, MT from heat-shocked older flies of these genotypes displayed good beta-galactosidase activity like other tissues (not shown). Comparable results were seen with ADH staining in the *Adh^{hsp70C}* stock (not shown). MT from the control batches of larvae also did not show any detectable enzyme (ADH or beta-galactosidase) activity.

4. Discussion

It is clear from present data that heat-shocked MT from larvae and young adults of *D. melanogaster* fail to synthesize the common set of HSP which are ubiquitously induced in all other cell types tested so far (Tissiers *et al.* 1974; Ashburner and Bonner 1979; Bienz and Pelham 1987; Singh and Lakhotia 1988). In a preliminary study (our laboratory, unpublished) we looked for hsp70 transcripts in control and heat-shocked larval and adult MT by dot-blot hybridization of total cell lysates with a probe for hsp70 mRNA (pPW229, see Livak *et al.* 1978); larval MT did not show any appreciable levels of hsp70 transcripts even after heat shock while in the adult

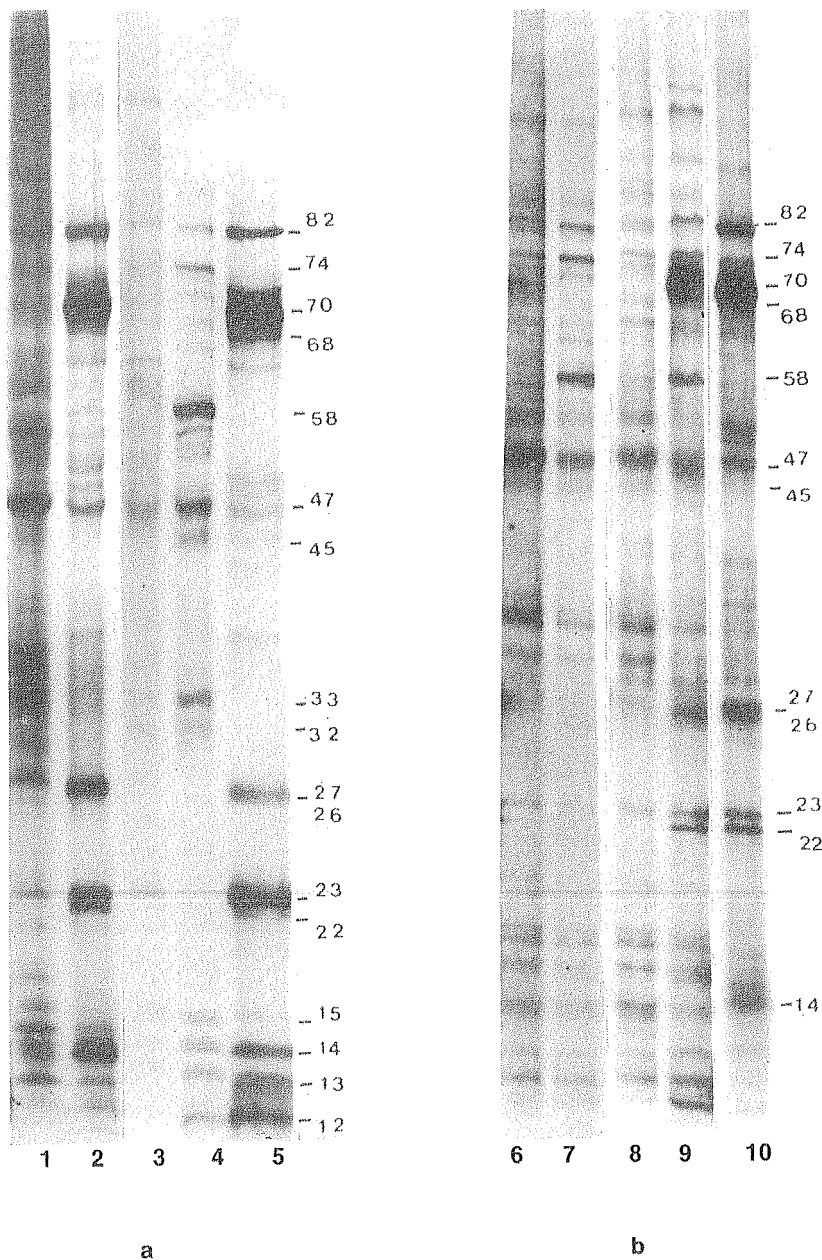


Figure 1. Heat shock induced protein synthesis in larval (a) and adult (b) tissues of *D. melanogaster*. Lanes 1 and 2, brain ganglia, lanes 3 and 4, malpighian tubules and lane 5, salivary glands of late 3rd instar larvae in a; lanes 2, 4 and 5 are after 30 min heat shock at 37°C while lanes 1 and 3 are control samples. Lanes 6-9 are malpighian tubules from young (lane 6: control, lane 7: heat shocked) and old (lane 8: control, lane 9: heat shocked) flies; lane 10 is heat shocked testes from young flies. The molecular sizes (in kd) of the common as well as the MT-specific HSP are marked.

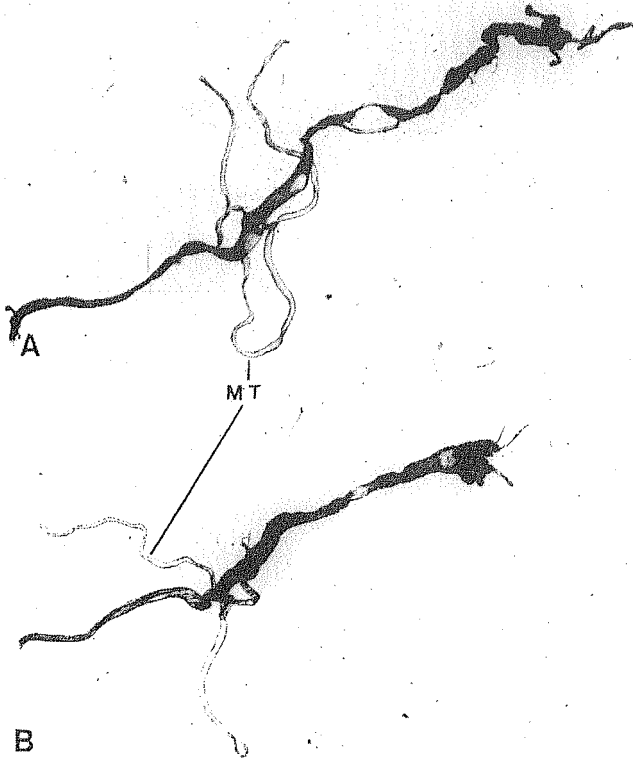


Figure 2. Beta-galactosidase staining of MT and the gut of fusion gene-carrying late 3rd instar larvae after 2 h recovery from heat shock at 37°C for 1 h: (A) from *Bg9* larvae (*hsp70* promoter-lac z fusion gene) and (B) from *26Z-74D* larvae (*hsp26* promoter-lac z fusion gene). Note the complete absence of staining of malpighian tubules. Beta-galactosidase activity in tissues from non-heat shocked control larvae of both types was negligible (not shown).

MT, a significant increase in *hsp70* transcripts was detectable after heat shock. Presumably, therefore, the larval MT also do not accumulate the other common heat shock transcripts. This is confirmed by observations on the non-inducibility of beta-galactosidase or ADH activity in MT of larvae carrying the appropriate reporter gene under the control of *hsp70* or *hsp26* promoter. This shows that the HSP promoters, whether endogenous or the additional ones, do not respond to the usual heat shock signal in MT from larvae. Spermatocytes and early cleavage cells in certain organisms also do not show heat shock induced protein synthesis (Pardue 1988). However, unlike these, the MT of *Drosophila* respond to heat shock by synthesizing a novel set of MT-specific HSP. Such dramatic tissue-specific differences in HSP synthesis contrasts with the prevailing evidence for a generally uniform response to heat shock by all cell types of an organism (Bienz and Pelham 1987; Burdon 1988; Pardue 1988). In one of the earliest studies on heat shock protein synthesis in *Drosophila*, Tissiers *et al.* (1974) reported induction of the same common set of HSP in salivary glands, brain ganglia, imaginal disks and MT of late larvae and early pupae. We do not know the reasons for this fundamental difference

in our and Tissier *et al.*'s (1974) results with MT. However, in our experiments, we consistently observed not only the synthesis of the novel set of HSP in larval MT but also the absence of the usual set of HSP that are induced in other tissues of the same animal after heat shock. In view of the uniqueness of our present results, certain aspects of methodology may be considered here. It is unlikely that the MT in our samples did not suffer the heat stress since, first, other tissues were heat-shocked simultaneously under identical conditions; second, similarly heat-shocked MT from older flies did show the induction of the typical as well as the other HSP, and third, a longer heat shock at 37°C or a more severe shock at 41°C also failed to induce the typical hsp's in larval MT. The co-induction of the usual as well as the MT-specific sets of HSP in older fly MT also suggests that the MT-specific HSP are not degradation products of the usual set. We consider it also unlikely that the absence of beta-galactosidase staining in MT could possibly be due to interference in uptake of the X-gal substrate since similar results were seen with ADH staining which requires butanol as a substrate and which being a smaller molecule is not expected to be not available to ADH in MT, if induced. Moreover, since we did see good staining for either enzyme in heat-shocked MT of older flies, the absence in larval MT seems more likely to be due to absence of the enzyme activity itself rather than to problems related to availability of substrate etc. It is possible that the MT cells are sluggish in new RNA synthesis and this may perhaps be responsible for the absence of induction of the usual set of HSP in this tissue. However, the significant point to note is that *synthesis of a new set of polypeptides was readily induced by heat shock in larval MT while the usual HSP or the reporter enzymes (in transformed stocks) were not detectable even later*. Whether the synthesis of MT-specific HSP is dependent upon new transcription or not remains to be examined. Since transcription of heat shock genes is autoregulated (Didomenico *et al.* 1982), a possible reason for the lack of induction of the usual set of HSP in larval MT could be that these proteins normally pre-exist in MT. However, the absence of ADH or beta-galactosidase activity in MT of non-heat shocked larvae carrying the appropriate promoter-reporter fusion gene shows that this tissue does not constitutively express or accumulate heat-shock gene products. In view of all these considerations, we believe that the lack of induction of the usual set of HSP in the MT of larvae or young adults is not an artifact.

The universality of the heat-shock response in stressed cells is due to interaction of the stress-activated heat-shock transcription factor (HSTF), ubiquitously present in all cells, with 5' consensus heat shock promoter or regulatory elements (HSE) present on all heat shock genes examined so far (Bienz and Pelham 1987; Wu *et al.* 1987; Burdon 1988; Pardue 1988). Our observations on beta-galactosidase or ADH activity in fusion gene carrying stocks show that the absence of the common set of HSP in larval MT is most likely due to transcriptional regulation since these promoter-reporter hybrid genes carry the required heat shock transcription and translation regulatory sequences (Lis *et al.* 1983; Bonner *et al.* 1984; Glaser *et al.* 1986). Obviously the interaction between HSE and HSTF is somehow modified in MT cells. Co-induction of the common as well as the MT-specific HSP in MT of older flies reveals another aspect of the complex regulation of the diverse heat shock genes in this tissue. This developmental regulation of heat shock protein synthesis in MT of *D. melanogaster* is apparently a different phenomenon than the reported (Fleming *et al.* 1988) altered patterns of HSP synthesis in aged *D. melanogaster* flies.

The MT-specific set of HSP does not appear to be unique to *D. melanogaster* since we have noted a comparable situation in MT of *D. hydei* also: in this case too a 58 kd polypeptide was most abundantly induced in heat-shocked larval MT while the usual set of HSP was not induced (data not presented). Another recent study in our laboratory (Nath and Lakhotia 1989) on the heat shock response in MT and other tissues at different developmental stages of *Chironomus* also revealed certain MT-specific HSP. However, unlike the present situation in *Drosophila*, heat shocked MT in *Chironomus* always synthesized the common set of HSP as well. The unique heat shock response in MT of *Drosophila* may be related to their specialized functions in osmoregulation and excretion.

As far as we are aware, a 58 kd HSP has so far not been reported for *Drosophila*. However, this MT-specific abundant HSP seen in our present study may not be an altogether novel HSP since in recent years a 58 kd minor HSP, located in mitochondria, has been reported in cells from man, *Xenopus*, *Tetrahymena*, yeast etc. (McMullin and Hallberg 1988). The 58 kd HSP in these cells is homologous to the *groEl* HSP of *E. coli*. It is therefore possible that the 58 kd HSP seen by us only in MT of *Drosophila* is homologous to this highly conserved but normally minor HSP.

In summary, our results have revealed an unexpected aspect of the heat shock response. The identity and nature of the genes induced by heat shock in MT remains to be examined. What makes the common set of heat shock genes refractory to induction in stressed MT and what keeps the MT-specific genes from expression in other tissues are some very interesting and intriguing questions. Further studies on these aspects and the heat shock response in other specialized cell types are expected to reveal exciting answers.

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