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Tissue- and development-specific induction and turnover of *hsp70* transcripts from loci 87A and 87C after heat shock and during recovery in *Drosophila melanogaster*

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Summary

The haploid genome of *Drosophila melanogaster* normally carries at least five nearly identical copies of heat-shock-inducible *hsp70* genes, two copies at the 87A7 and three copies at the 87C1 chromosome sites. We used *in situ* hybridization of the cDNA, which hybridizes with transcripts of all five *hsp70* genes, and of two 3′ untranslated region (3′UTR; specific for the 87A7- and 87C1-type hsp70 transcripts) riboprobes to cellular RNA to examine whether all these copies were similarly induced by heat shock in different cell types of *D. melanogaster*. Our results revealed remarkable differences not only in the heat-shock-inducibility of the *hsp70* genes at the 87A7 and 87C1 loci, but also in their post-transcriptional metabolism, such as the stability of the transcripts and of their 3′UTRs in different cell types in developing embryos and in larval and adult tissues. Our results also revealed the constitutive presence of the heat-shock-inducible form of Hsp70 in a subset of late spermatogonial cells from the second-instar larval stage onwards. We suggest that the multiple copies of the stress-inducible *hsp70* genes do not exist in the genome of *D. melanogaster* only to produce large amounts of the Hsp70 rapidly and at short notice, but that they are specifically regulated in a developmental-stage-specific manner. It is likely that the cost/benefit ratio of not producing or of producing a defined amount of Hsp70 under stress conditions varies for different cell types and under different physiological conditions and, accordingly, specific regulatory mechanisms operating at the transcriptional and post-transcriptional levels have evolved.

Key words: Hsp70, hsrω, *Drosophila melanogaster*, heat shock, untranslated region, development.

Introduction

Cells of all living organisms respond to thermal and several other environmental stresses by mounting a well-orchestrated series of events that are collectively termed the heat-shock or stress response. A hallmark of the heat-shock response is the significantly elevated levels of synthesis of a set of new proteins, the heat-shock proteins or Hsps (Schlesinger et al., 1982; Feder and Hofmann, 1999). The different Hsps are grouped into several families of which the Hsp70 family is the most conserved and is also the most highly induced by cellular stresses (Feige and Polla, 1994; Macario et al., 1999).

Nearly all eukaryotic species carry multiple genes coding for Hsp70 family proteins (Gunther and Walter, 1994). The Hsp70 family in *Drosophila* spp. includes both heat-inducible (the *hsp70* family) and constitutively expressed (the *hsc70* family) members. Five different *hsc70* genes are expressed in *D. melanogaster* under normal growth conditions, with spatial and temporal variations (Craig et al., 1983; Elefant and Palter, 1999). Dipteran insects and their ancestors have undergone an evolutionary proliferation of *hsp70* genes. All species of *Drosophila* have a common organization of the inducible *hsp70* genes, with two copies being present as inverted repeats (Feder and Krebs, 1998; Konstantopoulou et al., 1998). In all species of the *melanogaster* subgroup studied, the entire locus is once again duplicated, and the two loci occupy neighbouring locations.

The stress-inducible *hsp70* genes in *D. melanogaster* occur in multiple copies at two different, but adjacent, loci (the 87A7 and 87C1 bands on polytene chromosomes) on the right arm of chromosome 3. The two clusters are separated from each other by approximately 500 kb of DNA. The 87A7 locus contains two copies arranged as inverted repeats with approximately 1.7 kb of spacer DNA between them (Goldschmidt-Clermont, 1980; Ish-Horowicz and Pinchin, 1980). The 87C1 locus contains three heat-inducible *hsp70* gene copies. Two (distal-1 and distal-2, respectively) of the three *hsp70* genes at 87C1 are in tandem and are separated from the divergently oriented third gene (the proximal *hsp70* gene) by approximately 38 kb of DNA. This 38 kb DNA contains numerous α/β repeats, producing heat-inducible non-coding RNAs with no known function. The protein-coding sequences in the five *hsp70* gene copies show approximately 97 % identity. In addition, a region extending for approximately 400 bp upstream of the transcription start point is also highly conserved in all the five *hsp70* gene copies

(Ingolia et al., 1980; Karch et al., 1981; Leigh Brown and Ish-Horowicz, 1981).

Although the protein-coding sequences and the 5′ regulatory sequences of the five *hsp70* genes show very high homology, their 3′ untranslated region (3′UTR) shows significant divergences. The 3′UTRs [approximately 250 bp upstream of the poly(A)$^+$ site] of the two *hsp70* genes at the 87A7 locus are similar to each other, but show high divergence (approximately 68 %) compared with those of the *hsp70* gene copies at the 87C1 locus. Interestingly, the 3′UTRs of the proximal and the distal-1 *hsp70* genes at the 87C1 locus show high homology, but these are moderately divergent from that of the distal-2 (57 % homology) *hsp70* gene at the same locus (Torok et al., 1982).

The biological need for the presence of five copies of a gene coding for nearly identical heat-inducible Hsp70 in the *D. melanogaster* genome is intriguing. One possibility is that the multiple copies of *hsp70* genes help produce more Hsp70 rapidly in response to heat shock or other stress (Feder and Krebs, 1998; Feder and Hofmann, 1999). In addition or alternatively, copies of *hsp70* genes at the two loci may be differentially regulated during development for some cell-specific requirement. Earlier studies in this laboratory have indicated that transcription at the 87A and 87C loci could be regulated differently under certain conditions of heat shock. For example, when new transcription at the non-coding 93D locus was inhibited during heat shock, either because of combined treatment with another inducer such as benzamide or colchicine or because of the deletion of the 93D locus, the relative transcriptional or puffing activity of the *hsp70* genes at the 87A and 87C loci in larval salivary glands was altered in a treatment-specific manner (for reviews, see Lakhotia and Sharma, 1996; Lakhotia et al., 1999). In view of the differential induction of 87A and 87C puffs under these conditions and the divergence in the 3′UTR sequences, which are known to have important roles in the stability and localization of the transcripts (Sachs, 1993; Curtis et al., 1995; Ross, 1996), it is possible that the individual members of this multigene *hsp70* family in *D. melanogaster*, although coding for similar proteins, may indeed be regulated differentially in other cell types also. The present study examines this possibility.

Transcription of the *hsp70* genes from the 87A and 87C loci in embryos, in different larval tissues and in adult testes following heat shock and during recovery was analyzed by RNA:RNA *in situ* hybridization using the non-radioactive digoxigenin-labelled coding region or 3′UTR-specific riboprobes. The results showed that, although the hsp70 transcripts were induced after heat shock in most (but not all) tissues, their quantity and properties varied. In certain cells and tissues, transcripts from the 87A and 87C loci showed differential induction and stability. Immunostaining with antibody specific for heat-shock-induced Hsp70 revealed an unexpected constitutive presence of Hsp70 in mitotically dividing spermatogonial cells. Our results clearly showed that the heat-shock-inducibility of the *hsp70* genes from the two clusters is differentially regulated in different cell types and that the induced transcripts are metabolized in a cell- and development-specific manner. It appears that the multiple copies of *hsp70* genes are not just a consequence of duplication to produce more Hsp70 during heat shock, but that the multiple copies exert functions in a cell- and developmental-stage-specific pattern.

Materials and methods

Fly stocks and rearing conditions

The Oregon R$^+$ wild-type strain of *Drosophila melanogaster* Meigen was reared at 22±1 °C on standard food containing agar, maize powder, yeast and sugar.

Treatments

Heat shock and recovery

Embryos or different tissues from late third-instar larvae or testes from different larval and adult stages were heat-shocked for 40 min at 37 °C in Poels' salt solution (PSS) (Lakhotia and Tapadia, 1998). As parallel controls, the tissues were dissected out at room temperature (22 °C) directly from unstressed larvae and flies and incubated in PSS at room temperature for 40 min. In experiments requiring recovery from heat shock, whole larvae or adult flies were heat-shocked for 40 min at 37 °C in vials with moist filter paper and then returned to normal food vials and allowed to recover at room temperature for the desired period (see Results). Following recovery, the larvae or adults were quickly dissected in PSS, and the required organs were removed for processing for *in situ* localization of hsp70 transcripts or Hsp70 (see below). Pilot experiments revealed that heat shock to isolated organs or to intact organisms resulted in comparable levels of induction of the *hsp70* genes. In one set of experiments, testes from different unstressed larval, pupal and adult stages were quickly dissected out in PSS at room temperature and immediately processed for immunostaining with Hsp70 antibody (see below).

RNA:RNA in situ *hybridization (RISH) and immunostaining*

Three recombinant clones, i.e. *pPW18* (containing the 5′UTR and the coding region of *hsp70* but not the 3′UTR) (Sharma and Lakhotia, 1995), *pVZ-70-3′#1* (containing the 3′UTR sequence of the proximal *hsp70* gene at the 87A7 locus) (Dellavalle et al., 1994) and *pVZ-70-3′* (containing the 3′UTR sequence of the proximal *hsp70* gene at the 87C1 locus) (Dellavalle et al., 1994), were used to generate digoxigenin (DIG)-labelled antisense riboprobes. *pPW18* was linearized with *Hin*dIII, while *pVZ-70-3′#1* and *pVZ-70-3′* UTR clones were linearized with *Sal*I. The linearized plasmids were transcribed *in vitro* using DIG-UTP (Roche, Germany) as the labelled substrate, following the manufacturer's instructions, to generate anti-sense riboprobes. The *pPW18* riboprobe detects all the hsp70 transcripts from either of the two clusters, whether with or without the 3′UTRs. In contrast, the *pVZ-70-3′#1* riboprobe detects only the 3′UTR-carrying hsp70 transcripts from either of two *hsp70* gene copies at the 87A7 site, while the *pVZ-70-3′* riboprobe detects the 3′UTR-carrying

hsp70 transcripts from the 87C1 locus (excluding the distal-2 *hsp70* gene). The three probes will be referred to as cDNA, 87A3′UTR and 87C3′UTR, respectively, in the following. The specificity of each of the riboprobes was checked by RISH with polytene chromosome preparations from mildly heat-shocked salivary glands of wild-type late third-instar larvae.

The desired riboprobes were hybridized *in situ* to cellular RNA in embryos and tissues from larvae, pupae and adults, as described previously (Prasanth et al., 2000; Lakhotia et al., 2001).

The 7Fb rat monoclonal antibody, which detects only the heat-inducible form of Hsp70 in *D. melanogaster* (Velazquez and Lindquist, 1984), was used at a dilution of 1:400 for immunostaining of different tissues, as described previously (Prasanth et al., 2000; Lakhotia et al., 2001). In some cases, wild-type embryos were immunostained with the nervous-system-specific antibody BP104 (Hortsch et al., 1990) to identify the neural cells. In each case, antibody binding was detected colorimetrically using appropriate secondary antibodies conjugated with alkaline phosphatase, as described previously (Prasanth et al., 2000; Lakhotia et al., 2001).

Microscopy and analysis

All RISH and immunostained preparations were examined under a Nikon E800 microscope, and the images were assembled using Adobe Photoshop 5.0 software.

Results

To analyze the heat-shock inducibility of *hsp70* genes from the 87A and 87C loci and the localization and turnover of their transcripts in different cell types from embryo to adult, RISH was carried out using DIG-labelled antisense riboprobes derived either from the 3′UTRs of *hsp70* genes from loci 87A and 87C, which specifically detect the transcripts of the respective locus, or from cDNA without the 3′UTR, which detects transcripts of all five *hsp70* genes. To confirm the specificity of these probes, RISH was performed on mildly heat-shocked wild-type larval salivary gland polytene chromosomes using the three probes separately. As expected, the cDNA probe hybridized to hsp70 transcripts at both the 87A and 87C loci (Fig. 1A). The 87A3′UTR probe hybridized only to hsp70 transcripts at the 87A locus, but not at the 87C locus (Fig. 1B). The 87C3′UTR probe hybridized only to the 87C locus (Fig. 1C) and, as expected, gave two hybridization bands at the 87C1 locus since the 3′UTR of the third gene at this site is considerably different (Torok et al., 1982). None of the probes hybridized to any other chromosomal sites.

Differential expression and stability of heat-shock-inducible transcripts from hsp70 *genes at the 87A and 87C loci in different cell types*

Embryonic cells

Unstressed embryos did not show detectable hybridization with any of the three riboprobes (not shown). Fig. 2 shows examples of RISH patterns with the two 3′UTR riboprobes in heat-shocked embryos of different developmental stages. The RISH patterns with the cDNA probe were similar in all cases to the sum of the two 3′UTR probe patterns in embryos of corresponding stages (not shown). RISH in heat-shocked embryos revealed that the *hsp70* genes responded to heat shock only after stage 4 (nuclear cycle 12–13) onwards. At stage 5, the whole of the embryo, except the pole cells (the germ cell precursors), showed intense staining with all the probes (Fig. 2A,B). None of the three riboprobes hybridized to the pole cells in heat-shocked embryos (Fig. 2A,B).

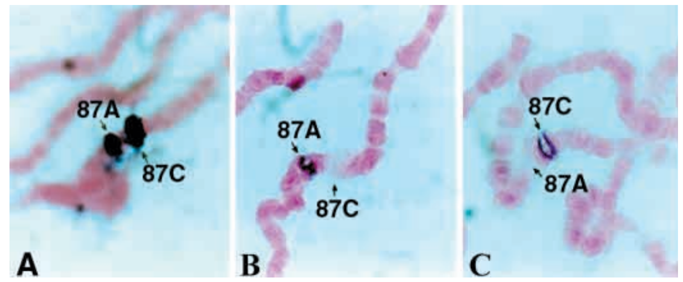

Fig. 1. RNA:RNA *in situ* hybridization in wild-type larval salivary gland polytene chromosome spreads using digoxigenin-labelled *hsp70* cDNA without the 3′UTR (A), 87A3′UTR (B) or 87C3′UTR (C) riboprobes. The arrows indicate the 87A and 87C loci. Note the absence of any cross-hybridization of the 3′UTR probes.

The *hsp70* genes from both loci showed uniform induction in all regions of stage 6–7 embryos, with slightly more intense hybridization in the ventral furrow area (Fig. 2C,D). During stage 9–10 of embryonic development, the neuroblast cells (the precursors of the embryonic nervous system which are distributed in multiple rows in the ventral neurogenic region on both sides of the dorso-ventral furrow) showed more intense hybridization with all the probes (Fig. 2E,F). The neuroblasts in the procephalic region, which later differentiates into brain, also showed strong induction of 87A as well as 87C *hsp70* genes (Fig. 2E,F). The remainder of the somatic cells showed nearly uniform induction of *hsp70* genes from both loci, but the hybridization signal was always weaker than in the neuronal cells. At stage 11, the neuronal precursors strongly expressed *hsp70* genes from both loci after heat shock (Fig. 2G–J). However, from stage 11 onwards, the 87A3′UTR showed markedly stronger hybridization in the proneural clusters, the precursors of the peripheral nervous system, while the 87C3′UTR probe hybridized only weakly in these cells (compare Fig. 2G,I with Fig. 2H,J). This difference in the induction of *hsp70* genes from the two clusters was accentuated as development proceeded. From stage 13 onwards, cells of the central and the peripheral nervous system showed a strong induction of 87A genes only (Fig. 2K,N,Q,T); the hybridization signal with the 87C3′UTR probe in these cells of heat-shocked embryos was very weak (Fig. 2O,R,U). The glial cells in the ventral nerve cord, however, showed hybridization with both 3′UTR probes (Fig. 2K–U). The BP104 antibody, which specifically stains embryonic central and peripheral nervous systems (Hortsch et al., 1990), was used as a marker to identify the neuronal cells in different

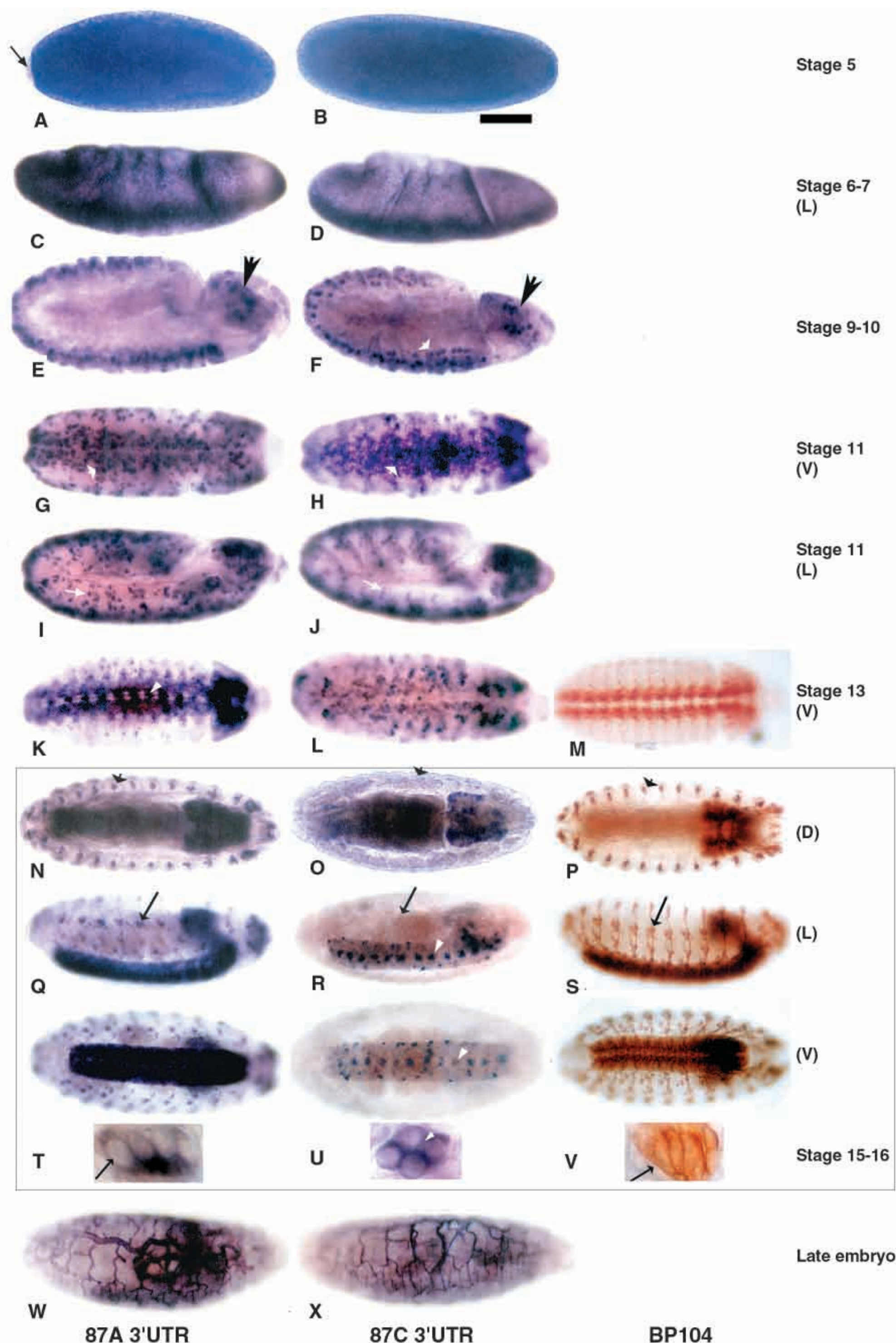

Fig. 2. Heat-shock-induced expression of *hsp70* genes from the 87A and 87C loci in wild-type embryos of developmental stages 5–16 as revealed by RNA:RNA *in situ* hybridization (RISH) using 3′UTR riboprobes of *hsp70* genes from the 87A (A,C,E,G,I,K,N,Q,T,W) or the 87C (B,D,F,H,J,L,O,R,U,X) locus. Stages of the embryos are indicated on the right (D, L and V in this column refer to the dorsal, lateral or ventral views, respectively, of the embryo). The arrow in A points to the pole cells. The black arrowheads in E and F and the small white arrowheads in F, G and H indicate the neuroblasts in the procephalic region and ventral nerve cord, respectively. The white arrows in I and J indicate the proneural clusters in stage 11 embryos. The white arrowheads in K, R and U refer to the glial cells. The small black arrows in N, O and P point to the tracheal pits, while the long black arrows in Q, R, S and T show the peripheral nervous system cells. Insets in T, U and V show the clusters of neuroblasts (T and V) and glial cells (U) from the respective embryos. Embryos in M, P, S and V are immunostained with the BP104 antibody to show the embryonic central and peripheral nervous systems for comparison with RISH patterns in K–U. Scale bar (applicable to all parts of the figure, except the insets in T, U and V, which are at higher magnification), 200 µm.

embryonic stages (Fig. 2M,P,S,V). In very late embryos (Fig. 2W,X), the tracheal system also showed strong hybridization with all three riboprobes. The central nervous system continued to show stronger hybridization with the 87A3′UTR (Fig. 2W). The non-neuronal somatic cells in late embryonic stages showed uniform induction of transcripts from both the loci after heat shock but at a much lower level than in the neural cells and tracheal network (Fig. 2W,X). RISH with the cDNA probe generated a more-or-less uniform signal throughout all embryonic stages, with stronger hybridization in the nervous system (not shown).

In all cells of the various embryonic stages examined, hsp70 transcripts were localized in the cytoplasm (see insets in Fig. 2T,U).

RISH patterns in embryos recovering from heat shock were not examined.

Larval gut

No hsp70 transcripts were detectable in any of the gut cells in unstressed late third-instar larvae. After heat shock, the hsp70 transcripts were localized in the cytoplasm of larval gut cells. It is interesting to note that the transcripts were mostly restricted to the area immediately adjacent to the outer face of the nuclear envelope (see insets 1, 2 and 3 in Fig. 3A,B).

Proventriculus

Cells in the proventriculus did not show any appreciable hybridization with any of the three riboprobes, either immediately after heat shock or during recovery (Fig. 3).

Gastric caeca

Cells in the gastric caeca (two pairs of finger-like projections from the anterior midgut or ‘stomach’) showed strong induction of *hsp70* genes from both loci after heat shock (Fig. 3A,B). After 2 h of recovery, both the 87A and the 87C transcripts continued to be present in these cells (Fig. 3C–E), but no hsp70 transcripts were detectable in the gastric caeca after 4 h of recovery (Fig. 3F–H).

Anterior part of the midgut (‘stomach’)

Soon after heat shock, cells in the enlarged anterior part of the midgut or ‘stomach’ exhibited intense expression of *hsp70* genes from both the loci (Fig. 3A,B). After 2 h of recovery, cells in the ‘stomach’ region showed positive hybridization only with 87C3′UTR (Fig. 3D) and cDNA (Fig. 3E), but not with the 87A 3′UTR riboprobe (Fig. 3C). After 4 h of recovery, none the 3′UTR probes showed any hybridization (Fig. 3F,G), but a group of cells in the anteriormost enlarged part of the ‘stomach’ continued to show hybridization with the cDNA probe (Fig. 3H).

Immediately after the heat shock, the narrower looped segment that joins the anterior and middle parts of the midgut showed weak hybridization with the 87A3′UTR riboprobe but moderate hybridization with the 87C3′UTR riboprobe (Fig. 3A,B). After 2 h of recovery, all three riboprobes gave a weak signal, but none showed any detectable hybridization in this part of the midgut after 4 h of recovery (Fig. 3F,G).

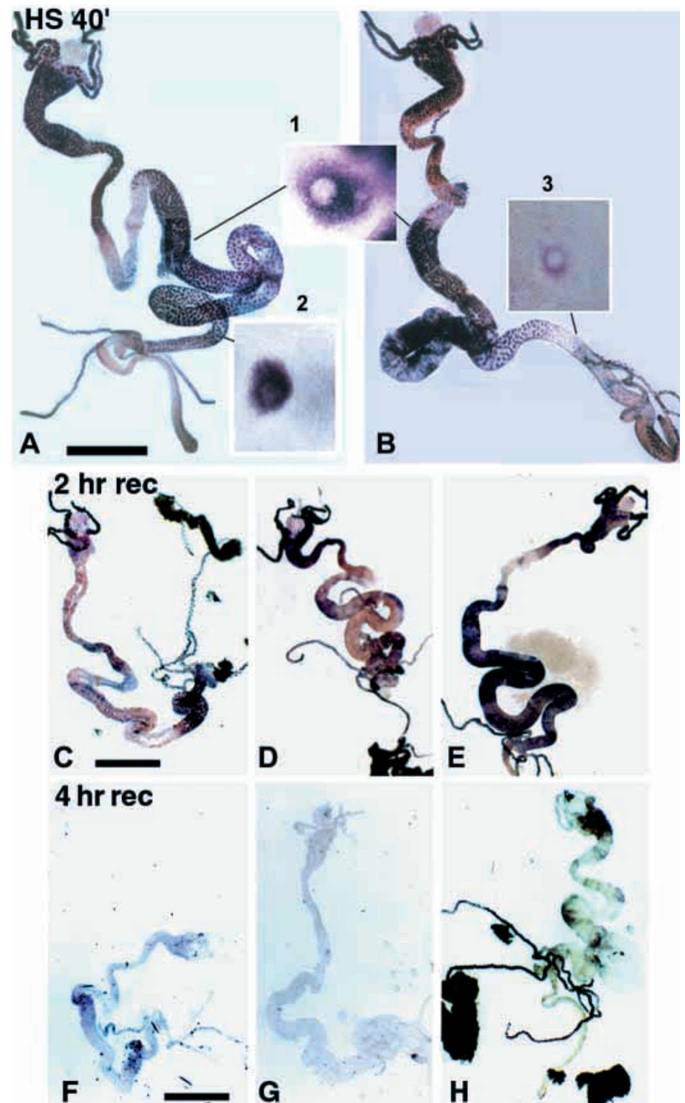

Fig. 3. Differential expression of *hsp70* genes in larval gut as revealed by RNA:RNA *in situ* hybridization (RISH) using the 87A3′UTR (A,C,F), the 87C3′UTR (B,D,G) or the cDNA (E,H) riboprobe after 40 min of heat shock (HS) (A,B) or after 2 h (C,D,E) or 4 h (F,G H) of recovery (rec) from a 40 min heat shock. Insets in A and B show cells from the gut region at a higher magnification: inset 1 corresponds to cells from the middle part of the midgut (the two 3′UTR probes give a similar perinuclear signal in the cytoplasm); inset 2 shows a cell from the distal part of the midgut with strong 87A3′UTR hybridization; inset 3 is a cell from a similar position to that in inset 2 showing weak hybridization with the 87C3′UTR riboprobe. Scale bars (which apply to all images in a given row, except the insets), 200 μm.

Middle part of the midgut

Cells in the long coiled middle part of the midgut, which includes the region with larger polytene cells, showed pronounced hybridization with all the probes soon after heat

shock (Fig. 3A,B and inset 1). By 2 h of recovery, the staining in these cells had weakened with both the 3′UTR probes (Fig. 3C,D), but the cDNA probe continued to show strong hybridization (Fig. 3E). The cDNA signal also became weak after 4 h of recovery (Fig. 3H).

Posterior part of the midgut

Cells in the posterior part of the midgut (a relatively small, straight region that adjoins the hindgut) expressed the 87A genes relatively more strongly (Fig. 3A and inset 2) than the 87C genes (Fig. 3B and inset 3). These cells continued to show moderate hybridization with the 87A3′UTR riboprobe after 2 h of recovery (Fig. 3C), but were devoid of any hybridization after 4 h of recovery (Fig. 3F–H).

Hindgut

Cells of the hindgut showed low levels of expression of *hsp70* genes from both loci after heat shock (Fig. 3A,B). However, after 2 h of recovery, these cells became devoid of the transcripts of both loci (Fig. 3C–E).

Larval imaginal discs and brain

RISH in late third-instar larval imaginal discs and brain with the *hsp70* riboprobes revealed the absence of any hsp70 transcripts in unstressed tissues (not shown). After 40 min of heat shock, the eye-antennal (Fig. 4A,G) and other (not shown) imaginal discs showed comparable patterns of intense hybridization with all three riboprobes. The RISH patterns with the 87C3′UTR were similar to those with the 87A3′UTR riboprobe, and only the latter are therefore illustrated in Fig. 4. In certain areas of these tissues, all the riboprobes gave a weak or barely detectable hybridization signal (Fig. 4).

After 30 min of recovery, the eye discs continued to show enhanced hybridization with the cDNA probe, but hybridization in the antennal disc became weak (Fig. 4H). Regions on either side of the morphogenetic furrow of the eye disc continued to show strong hybridization with the cDNA riboprobe after 30 min and 1 h of recovery (Fig. 4H,I). After 30 min of recovery from heat shock, unlike the cDNA probe, the 3′UTR riboprobes (both 87A and 87C) showed hybridization only in a specific subset of imaginal disc cells (Fig. 4B,D,E). In the eye disc, these cells were mostly localized on both sides of the progressing morphogenetic furrow (Fig. 4B). In the wing (Fig. 4D) and leg (Fig. 4E) discs, cells of similar appearance exhibited intense hybridization with the 3′UTR riboprobes. In the optic lobes of the larval brain recovering from heat shock, a comparable cell-specific hybridization with the 87A or the 87C 3′UTR riboprobes was observed (Fig. 4F). In all these cells (discs and brain), hsp70 transcripts were distributed in the cytoplasm (see insets in Fig. 4D,F). After 1 h of recovery, the 3′UTR riboprobe signal in these clusters of cells became weak (Fig. 4C). After longer periods of recovery, the 3′UTR riboprobes did not show any hybridization in imaginal discs and brain ganglia (not shown).

The unstressed larval imaginal discs gave no immunostaining with the 7Fb antibody (not shown). Heat shock for 40 min resulted in an enhanced signal in most areas of all the discs (Fig. 4J). Certain areas of all the discs showed stronger immunostaining, and this correlated with the greater

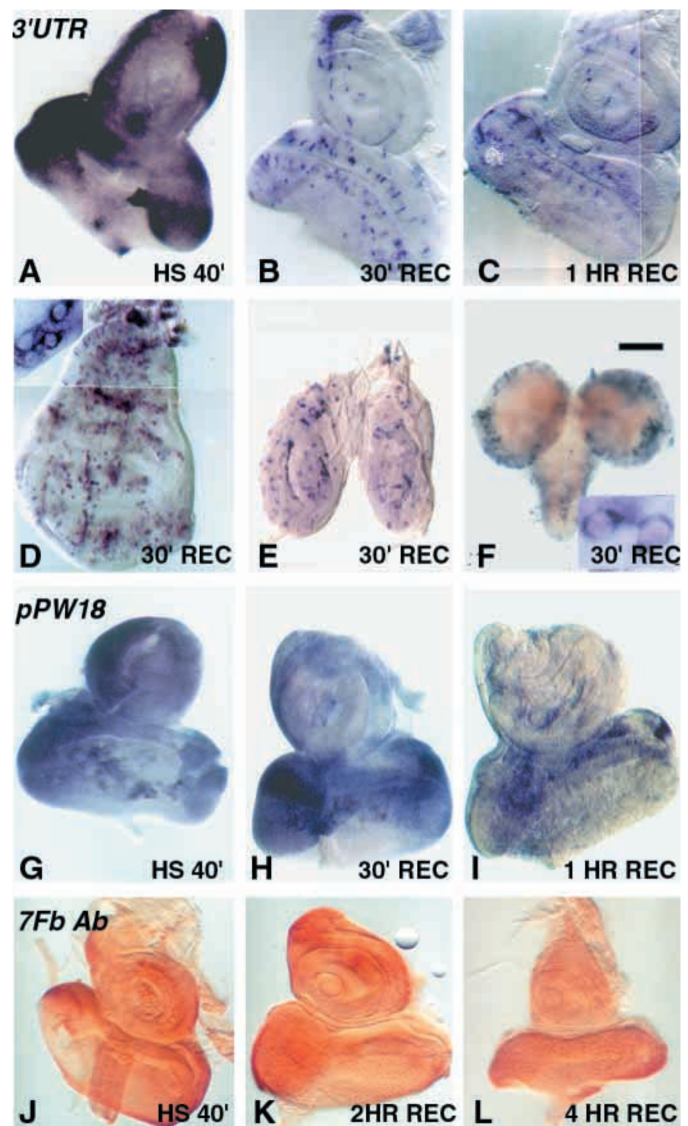

Fig. 4. (A–I) Expression of *hsp70* genes following heat shock (HS) (A,G) or after 30 min (B,D–F,H) or 1 h (C,I) of recovery (REC) from heat shock in late third-instar larval eye-antennal (A–C,G–I), wing (D) or leg (E) imaginal discs or in brain ganglia (F) as revealed by *in situ* hybridization with the 87A3′UTR (A–F) or the cDNA (*pPW18*) (G–I) riboprobe. *In situ* hybridization patterns with the 87C3′UTR riboprobe were similar to those seen with the 87A3′UTR riboprobe. Note the specific hybridization of the 3′UTR riboprobe with clusters of dividing cells during recovery from heat shock (B–F) in all the imaginal discs and the brain. Insets in D and F show clusters of cells in the wing disc and the optic lobes of brain ganglia, respectively, at higher magnification, demonstrating perinuclear cytoplasmic hybridization with the 3′UTR riboprobe. (J–L) Eye discs of late third-instar larvae immunostained with the 7Fb antibody to show the distribution of Hsp70 in heat-shocked (J) discs and in those allowed to recover from heat shock for 2 h (K) or 4 h (L). Scale bar, 200 μm.

levels of hsp70 transcripts in these regions (e.g. compare Fig. 4A,G,J). The levels of Hsp70 in various imaginal discs declined as the recovery period increased (Fig. 4K,L).

Testes

RISH analysis in the larval testes also showed a locus-specific differential expression of the *hsp70* genes. The unstressed larval testes did not show detectable hybridization with any of the three *hsp70* riboprobes (Fig. 5A). After a 40 min heat shock, the spermatogonial cells, near the anterior tip of the testis, showed strong induction of 87A *hsp70* genes (Fig. 5C), whereas the 87C transcripts showed a homogeneous expression throughout the testis (Fig. 5D). The cDNA probe also hybridized uniformly throughout the testis (Fig. 5E). Previous RISH analysis and reporter studies in *hsp70-lacZ* transgenic lines revealed that only spermatogonia and developing spermatids, but not spermatocytes, showed heat-shock induction of *hsp70* (Bendena et al., 1991). Therefore, the uniform hybridization of the 87C3′UTR riboprobe throughout the testis seen in the present study may correspond to the induction of 87C *hsp70* genes in somatic sheath cells of larval testis (Fig. 5B).

The spermatogonial cells continued strongly to show the presence of 87A *hsp70* gene transcripts even 2 h after recovery from heat shock (Fig. 5I,K), but after 4 h of recovery, hybridization in these cells was undetectable (Fig. 5N). The 87C3′UTR probe showed uniform hybridization throughout the testis after 30 min (Fig. 5H) and 1 h (Fig. 5J) of recovery. The signal was weaker after 2 h of recovery (Fig. 5L), and this further weakened after 4 h of recovery (Fig. 5O). Interestingly, after 4 h of recovery from heat shock, the testes continued to show homogeneous hybridization with the cDNA riboprobe (Fig. 5P).

Immunostaining of unstressed larval testes with the Hsp70-specific 7Fb antibody revealed the distinct presence of Hsp70 in spermatogonial cells (Fig. 5B). The somatic hub cells at the anterior tip of the testis and all other cell types were devoid of any staining. Heat shock for 40 min resulted in much stronger staining of spermatogonia and an overall enhanced staining in all other areas of the larval testis (Fig. 5F), which continued until 2 h of recovery (Fig. 5M). After 4 h of recovery, the overall staining became weak, but the spermatogonial cells showed a distinctly stronger signal (Fig. 5Q).

RISH in adult testes using the cDNA probe revealed cell-type-specific induction and stability of hsp70 transcripts. No detectable signal was observed in the unstressed control testis (Fig. 6A). Even the spermatogonial cells showed no detectable hybridization (see inset to Fig. 6A). Heat shock for 40 min resulted in induction of *hsp70* genes, particularly strongly in the spermatogonial cells (Fig. 6B and inset), with the transcripts being localized in the cytoplasm. Unlike the larval testis, the hsp70 transcripts disappeared from the spermatogonial cells in adult testis within 2 h of recovery (Fig. 6C,D). For reasons that are unclear, neither of the 3′UTR riboprobes generated distinct RISH staining in adult testis.

The pair of somatic cyst cells, which remain associated with every cyst of growing germ cells until sperm maturation (Fuller, 1993), showed a delayed induction of *hsp70* genes. Hsp70 transcripts were not detectable in these cells immediately after heat shock (Fig. 6B), but intense expression was seen after 2 h of recovery from heat shock (Fig. 6C); the transcripts were distributed in the cytoplasm as well as in the nucleus (inset in Fig. 6C). Cyst cells continued to show positive staining even after 4 h of recovery (Fig. 6D). At this time, the transcripts were mostly localized in the cyst cell cytoplasm (inset in Fig. 6D).

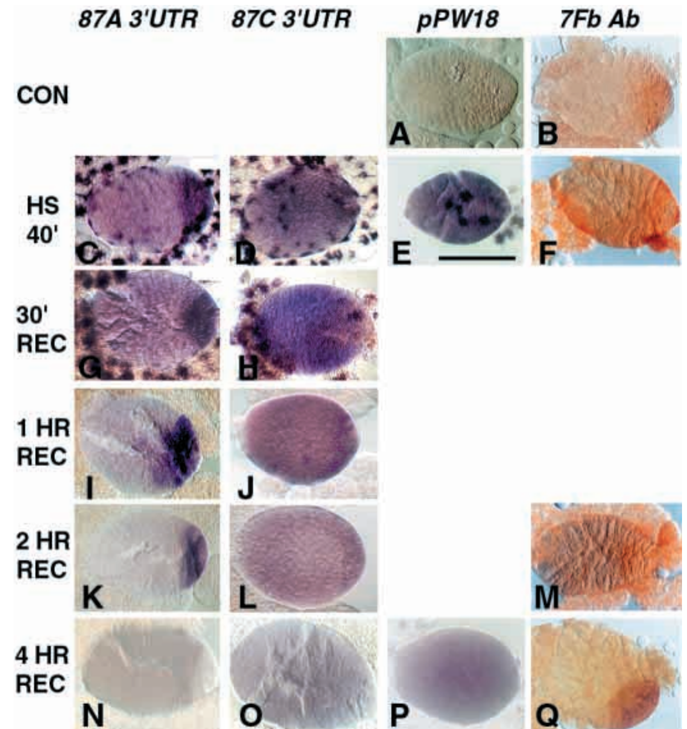

Fig. 5. Expression of *hsp70* genes in larval testes as revealed by RNA:RNA *in situ* hybridization using the cDNA (*pPW18*) (A,E,P) 87A3′UTR (C,G,I,K,N) or 87C3′UTR (D,H,J,L,O) riboprobe and the *in situ* profile of Hsp70 as revealed by immunostaining with the 7Fb antibody (*7Fb Ab*) (B,F,M,Q) in unstressed control (CON) (A,B) or heat-shocked (HS) (C–F) testes or those allowed to recover for 30 min (G,H), 1 h (I,J), 2 h (K–M) or 4 h (N–Q) at room temperature (22 °C) after a 40 min heat shock. Note the constitutive presence of Hsp70 in the anterior region (spermatogonial cells) in unstressed testes and also the greater induction of the locus 87A *hsp70* genes in these cells following heat shock. Scale bar, 500 µm.

Cells in the proximal region of the testis, connecting with the seminal vesicle, showed a strong induction soon after heat shock (Fig. 6J). The somatic sheath cells on the seminal vesicle also exhibited intense heat-shock-induced expression of *hsp70* genes (Fig. 6J). After 2 and 4 h of recovery, the signal in the proximal part of the testis and in the seminal vesicle became weak, but the cyst cells and some other unidentified cells in the seminal vesicle showed delayed but enhanced expression (Fig. 6K,L and insets).

Immunostaining of the unstressed adult testis with the 7Fb

antibody showed the constitutive presence of Hsp70 protein in a subset of late gonial cells (Fig. 6E). Heat shock resulted in an overall increase in the level of Hsp70 throughout the testis (Fig. 6F,N). Enhanced levels of Hsp70 were observed in the somatic tip cells, in spermatogonia, in unidentified cells in the proximal part of the testis and in the seminal vesicle. Although 7Fb staining in other cell types became weak after 2 h of recovery, the spermatogonia and cells in the proximal part of the testis continued to show higher levels of Hsp70 (Fig. 6G,O). However, a distinctive staining in the cyst cells, which showed a delayed induction of hsp70 transcripts (see above), was not detectable in testes recovering from heat shock. By 4 h of recovery, the signal in all cell types, other than the spermatogonia, was weaker (Fig. 6H,P).

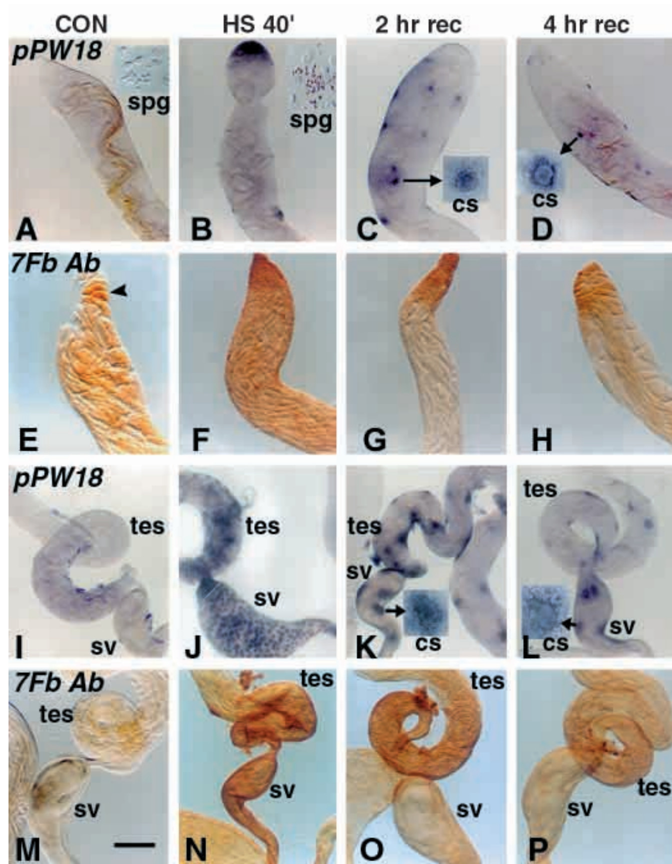

Fig. 6. The *in situ* expression of *hsp70* genes revealed by RNA:RNA *in situ* hybridization using cDNA (*pPW18*) riboprobe (A–D and I–L) and the *in situ* profile of Hsp70 seen after immunostaining with the 7Fb antibody (*7Fb Ab*) (E–H and M–P) in the distal (A–H) and proximal (I–P) parts of adult testes (tes) and seminal vesicles (sv) in unstressed (A,E,I,M) controls (CON) or after 40 min of heat shock (B,F,J,M) and in tissues allowed to recover for 2 h (C,G,K,O) or 4 h (D,H,L,P) at room temperature (22 °C) after a 40 min heat shock. The insets in A and B show the expression of *hsp70* in spermatogonial cells (spg), while those in C and D show the cyst cells (cs) at higher magnification. The arrowhead in E shows the constitutive presence of Hsp70 in the spermatogonial cells. Insets in K and L show the expression of *hsp70* in cyst cells (cs) in the seminal vesicles. Scale bar (applicable to all images, except to insets), 200 µm.

Since the spermatogonial cells of third-instar larval and adult testes showed the constitutive presence of Hsp70 (Figs 5B, 6E), unstressed testes from second-instar larval, pupal and adult stages were immunostained with the 7Fb antibody to investigate the status of constitutive expression of Hsp70 in spermatogonia during development. No detectable signal was observed in the testis of 26-h-old (post hatching) early second-instar larvae (Fig. 7A), but weak staining was seen in spermatogonia in testes from 44-h-old second-instar larvae (Fig. 7B) and 52-h-old early third-instar larvae (Fig. 7C). Spermatogonial staining continued to be prominent at all subsequent stages until the adult stage (Fig. 7D–M). In these dividing spermatogonial cells, Hsp70 was localized mostly in the cytoplasm (Fig. 7L). RISH analysis was also carried out in these testes with the cDNA probe to analyze the level of hsp70 mRNA. Surprisingly, apart from the pupal testes (160 h after hatching, see Fig. 7N), no other stages displayed any detectable RISH signal.

Discussion

Several studies have examined the constitutive and/or induced expression of *hsp70* genes in relation to development and differentiation (Krone et al., 1997; Heikkilla et al., 1997; Rallu et al., 1997; Chandolia et al., 1999; Luft and Dix, 1999; Feder and Hofmann, 1999; Elefant and Palter, 1999). Most of these studies have focused on the differential expression of different Hsc70 and Hsp70 isoforms during normal development. Very few studies have examined whether the different cell types show differences in the stress-induced synthesis of Hsps and still fewer studies have focused on the differential activation of the different stress-inducible *hsp70* genes in response to cellular stress. Our earlier studies (for a review, see Lakhotia and Sharma, 1996) and a report by Hochstrasser (1987) in cells with large polytene chromosomes revealed a differential responsiveness to stress of *hsp70* genes at the 87A and 87C loci in *D. melanogaster*. A comparable analysis with other cell types without the cytologically analyzable polytene chromosomes has not yet been made.

In cells with large polytene chromosomes, differential puffing of the 87A and 87C loci provides a simple assay for any differences in transcriptional activity of the two clusters of *hsp70* genes; this is not possible in other cell types. Nevertheless, in view of the very high conservation of the coding and the upstream regulatory regions in the five *hsp70* genes, their uniform heat-shock-inducibility in various cell types seems to have generally been taken for granted. In the present study, we exploited the differences in the 3′UTRs of the different sets of *hsp70* genes in *D. melanogaster* to examine the inducibility of the different *hsp70* genes by heat shock in cell types of *D. melanogaster* that lack the analyzable polytene chromosomes. Our present analysis does not provide information on the transcriptional activation of the distal-2 *hsp70* gene at the 87C1 locus since its 3′UTR, being different again (Torok et al., 1982), is not recognized by any of the two 3′UTR riboprobes used here.

The expected patterns of hybridization of the three riboprobes with heat-shocked polytene chromosomes (Fig. 1) confirmed their specificity. The 95D region did not hybridize with any of the riboprobes, although the *hsp68* gene present at this site shares approximately 85 % DNA sequence homology with the *hsp70* genes (Holmgren et al., 1979). This established that the stringency of our RISH conditions was fairly high so that the 3′UTR riboprobes could hybridize only to hsp70 mRNAs derived from the corresponding locus.

Induction of the hsp70 *genes at the 87A and 87C clusters*

The most significant finding of the present RISH analysis with locus-specific hsp70 3′UTR riboprobes was that the *hsp70* genes in different cell types do not respond to heat shock in a similar manner. The more significant differences in induction and the subsequent metabolism of hsp70 transcripts in the different cell types examined in the present study are summarized in Tables 1 and 2. As may be noted from these tables, the differences relate not only to little or no or delayed transcriptional induction of the *hsp70* genes in some cell types but, more importantly, to differential transcriptional activation of copies of the *hsp70* genes at the 87A and 87C loci and to differential persistence of hsp70 mRNAs during recovery in certain cell types. In addition, the survival of 3′UTRs on the induced hsp70 mRNAs during recovery from heat shock also varied.

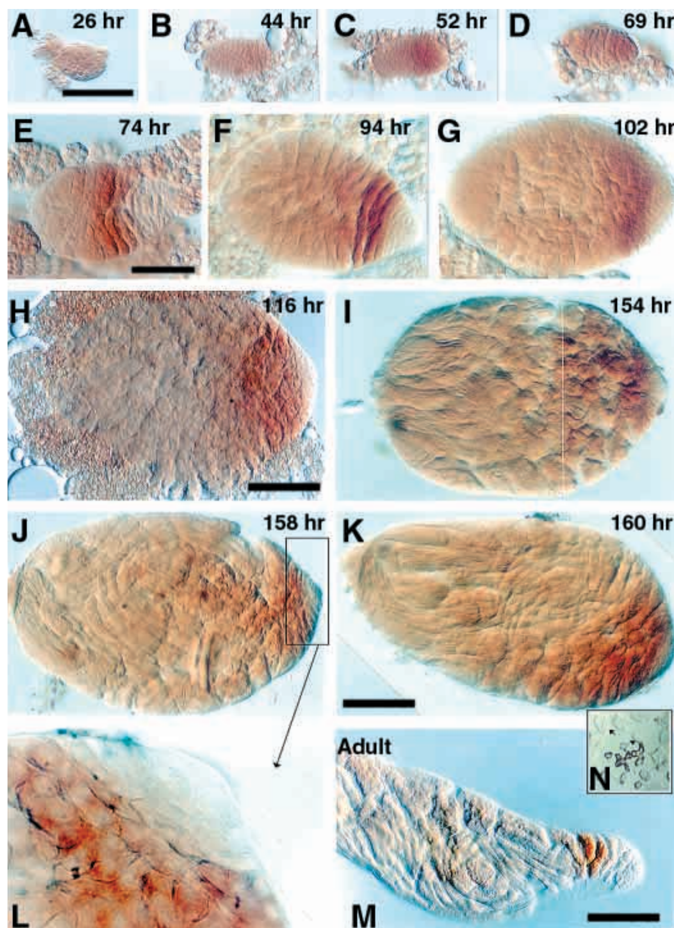

Fig. 7. The *in situ* profile of Hsp70 in unstressed testes of wild-type larvae (A–H) or pupae (I–L) of different ages (marked on each panel in hours after egg hatching) and in an adult (M) revealed by immunostaining with the 7Fb antibody. A higher-magnification image of the boxed area in J is shown in L. Expression of *hsp70* genes in the spermatogonial cells of the unstressed pupal testis detected by RNA:RNA *in situ* hybridization using the cDNA riboprobe is shown in N. Scale bars (which apply to all images in a given row, except L and N), 200 μm.

In agreement with Wang and Lindquist (1999), we also found that none of the *hsp70* genes is inducible until nuclear division cycle 12 in early *Drosophila melanogaster* embryos. Wang and Lindquist (1999) showed that the cytoplasmic localization of the heat-shock factor in the preblastoderm embryos of *D. melanogaster* up to nuclear cycle 11 was responsible for the inability of *hsp70* genes to respond to heat shock in preblastoderm embryos. However, unlike the reported induction of hsp70 transcripts in some pole cells (Wang and Lindquist, 1999), we found no detectable hybridization signal in pole cells with any of the three *hsp70* riboprobes, although transcripts of the *hsp70* genes from both loci were easily detectable at stage 5 (nuclear division 13–14) in all other regions of heat-shocked embryos. In embryos from an *hsp70-LacZ* reporter gene fusion transgenic line, *Bg9* (Lis et al., 1983), we also failed to find heat-shock-induced β-galactosidase activity in the pole cells (K. V. Prasanth and S. C. Lakhotia, unpublished observations). Furthermore, *hsr*ω transcripts are also not inducible by heat shock in pole cells at this stage of embryonic development (Lakhotia et al., 2001). Thus, the absence of a typical heat-shock response in the pole cells of *Drosophila melanogaster* may be a general phenomenon.

The strong and differential induction of the *hsp70* genes in embryonic neuronal cells is remarkable. In the *Bg9* transgenic line, in which the promoter and the 5′UTR and 3′UTR sequences are derived from the proximal *hsp70* gene of the 87C1 locus (Lis et al., 1983), the β-galactosidase activity patterns in heat-shocked embryos were closely similar to the RISH patterns with the 87C3′UTR riboprobes but not to those with the 87A3′UTR riboprobe (not shown). This further confirms the differential activation of the two clusters of *hsp70* genes in the embryonic nervous system following heat shock.

Our present observations on the differential induction of the 87A and 87C *hsp70* genes in parts of the larval gut are comparable with the earlier observations of Hochstrasser (1987). Exploiting the large polytene cells in the midgut polytene cells, Hochstrasser (1987) demonstrated that, when larvae grown at room temperature were heat-shocked, the 87A locus showed a stronger puffing than the 87C locus, while in larvae reared at 16 °C, the puffing at 87C was more pronounced than that at 87A after heat shock. Hochstrasser's results thus showed that developmental conditions modified the stress-inducibility of the *hsp70* genes at the 87A and 87C loci in midgut cells. It is likely that digestive physiology, which is strongly subject to growth conditions, has a

Table 1. *Examples of differential activation of* hsp70 *genes at 87A and 87C loci following heat shock*

Pattern of transcriptional activation of *hsp70* genes following heat shock	Cell/tissue type
No induction of any of the *hsp70* genes	Stage 1–3 embryos Pole cells in stage 4–5 embryos (Fig. 2A,B) Proventriculus in late third-instar larval gut (Fig. 3)
Stronger induction of 87A as well as 87C *hsp70* genes in certain cells/cell types in a given tissue	Embryonic neuroblasts (Fig. 2E–X) Several regions in most tissues examined
Stronger induction of 87A but little or no induction of 87C *hsp70* genes	Proneural clusters in stage 11 embryos (Fig. 2G,H) Embryonic central nervous system (other than the glial cells), peripheral nervous system and tracheal pits from stage 13 onwards (Fig. 2K–X) Posterior part of larval midgut (Fig. 3B,C) Spermatogonial cells of larval (and adult?) testes (Figs 5C,D, 6B)
Stronger induction of 87C but little or no induction of 87A *hsp70* genes	Narrow segment joining the anterior and middle parts of larval midgut (Fig. 3A,B)
Delayed induction of *hsp70* genes (during recovery)	Cyst cells in adult testes (Fig. 6A–D)

Table 2. *Examples of differential metabolism of hsp70 transcripts from 87A and 87C loci during recovery from heat shock*

Pattern of hybridization of 87A3′UTR and 87C3′UTR riboprobes during recovery from heat shock	Cell/tissue type
Stronger hybridization of 87C3′UTR but little or no signal with 87A3′UTR	'Stomach' in larval gut after 2 h of recovery Sheath cells in larval testes after 1 h or longer of recovery (Fig. 5G–L,N,O)
Stronger hybridization of 87A3′UTR but little or no signal with 87C3′UTR	Posterior part of larval midgut (Fig. 3B,C) Spermatogonial cells in larval testes (Fig. 5G–L)
The 3′UTRs and the coding region decay at different times in different cells of a tissue	Many regions of larval gut (Fig. 3C–H) Non-dividing cells in larval imaginal discs and brain ganglia remove all the 3′UTRs by 30 min of recovery but the dividing cells retain these longer (Fig. 4B–I) Spermatogonial cells in larval testes retain 87A3′UTR until 2 h of recovery but the sheath cells do not show their presence after 1 h of recovery (Fig. 4G,I,K)

UTR, untranslated region.

significant effect on *hsp70* gene activation in gut cells in response to stress. This modulation of *hsp70* inducibility in gut cells is expected to be relevant to adaptability in nature.

The significant delay (after 2 h of recovery from heat shock) in transcriptional activation of the *hsp70* genes in cyst cells, which remain associated with the bundles of differentiating germ cells (Fuller, 1993), is most unusual. We are not aware of any other cell type in *Drosophila melanogaster* that shows a comparable delayed transcriptional activation of the *hsp70* genes following cellular stress. The larval Malpighian tubules are known to synthesize Hsp70 only during recovery but not immediately after heat shock (Lakhotia and Singh, 1989; Krebs and Feder, 1997b); however, in this tissue, the transcriptional activation of the *hsp70* genes at both the 87A and 87C loci still occurs within 10 min of heat shock (S. C. Lakhotia, P. Srivastava and K. V. Prasanth, in preparation). Thus, the reasons for the delayed appearance of Hsp70 in larval Malpighian tubules and in adult cyst cells are different. Further molecular studies on the regulation of the *hsp70* genes (and possibly other heat-shock genes) in cyst cells are required to understand the basis and functional significance of the delayed transcriptional activation of *hsp70* genes. It is interesting to note that, unlike the delayed induction of *hsp70* genes, heat-shocked cyst cells immediately display the characteristic changes in intranuclear distribution of the transcripts of *hsr*ω, a major non-protein-coding heat-shock gene of *D. melanogaster* (Rajendra et al., 2001).

Differential metabolism of the 3′UTRs in different cell types recovering from heat shock

The summary in Table 2 shows that, besides the above-noted differential activation of the *hsp70* genes at the 87A and 87C loci by heat shock, their transcripts are also differently metabolized in certain cells during recovery from heat stress. The absence of hybridization with the 87A or the 87C3′UTR

probe during recovery in certain cell types (Table 2) could be due either to the loss of the 3′UTR sequence alone or to complete degradation of the mRNA from the particular locus. The present study does not allow a distinction between these possibilities.

In several instances, different cell types in the same tissue showed differences in the time of removal of the 3′UTRs on hsp70 mRNAs. Thus, in larval imaginal discs and brain, while the cDNA probe continued to give an intense signal in most cells after 30 min of recovery from heat shock, the two 3′UTR riboprobes hybridized only to a subset of cells that were always in characteristically organized clusters. The distribution of these clusters of cells matched that of the actively proliferating cells detected by anti-phospho-histone antibody (Hendzel et al., 1997; Brodsky et al., 2000). It appears, therefore, that, while the actively dividing cells in these tissues retained the 3′UTRs, other cells quickly removed them. This persistence of the 3′UTRs on the hsp70 mRNA may be related to the need for rapid degradation of these transcripts in actively dividing cells since high levels of Hsps, especially Hsp70, have been reported to be detrimental to rapidly dividing cells (Feder et al., 1992; Feder and Hofmann, 1999). The 3′UTRs of hsp70 transcripts contain AU-rich instability elements that cause their rapid degradation during recovery (Yost et al., 1990; Sachs, 1993). Although, in larval spermatogonial cells, the 3′UTRs were also removed sooner than the coding region, it is intriguing that the dividing spermatogonial cells continued to show the strong presence of Hsp70 4 h after heat shock and even in the absence of thermal stress (see below). Apparently, the presence of Hsp70 does not interfere with the division process (Feder et al., 1992) in spermatogonia.

The reasons for our failure to detect hybridization of 3′UTR probes with cellular RNA in adult testes, when the cDNA probe readily hybridized, are not clear. Whether this was a technical failure or whether the 3′UTRs are not transcribed or are very quickly removed from the adult spermatogonial cells is not known. In the absence of hybridization of the 3′UTR riboprobes, we cannot define the origin of the hsp70 transcripts in heat-shocked spermatogonial cells in adult testes. However, in view of the results with larval testes, it is tempting to speculate that these transcripts in the adult testes are also derived from the 87A *hsp70* genes.

Constitutive presence of Hsp70 in spermatogonia

A novel observation of the present study was the constitutive presence of Hsp70 in spermatogonia from the second-instar larval stage onwards. The 7Fb antibody is well known to react only with the heat-inducible form of *Drosophila melanogaster* Hsp70 and does not recognize any of the Hsc70 proteins or the heat-inducible Hsp68 (Velazquez and Lindquist, 1984). That the presence of Hsp70 in spermatogonial cells was not due to inadvertent stress is supported by the following considerations. The somatic hub cells at the tip of the testis, which showed elevated levels of Hsp70 soon after heat shock, displayed no constitutive immunostaining. In the unstressed spermatogonia, Hsp70 was present in the cytoplasm rather than in the nucleus, as seen in stressed cells (Velazquez and Lindquist, 1984).

In the context of the constitutive presence of Hsp70 in spermatogonia, the absence of detectable hybridization of the cDNA riboprobe in most of the unstressed testes, except in some pupal testis samples, is intriguing. Perhaps some or all of the *hsp70* genes in unstressed spermatogonia are transcribed only intermittently and/or at a very low level so that RISH fails to detect these transcripts in most cases. Alternatively or in addition, the Hsp70 in spermatogonial cells may be more stable than in other cell types so that, once synthesized, it persists even in the absence of fresh transcription. This may find support in our observation that, after 2 or 4 h of recovery from heat shock, Hsp70 was abundantly present in the spermatogonial cells of the testes, although the riboprobes showed no hybridization at this time (compare Fig. 6C,D,G,H). Further studies are needed to determine whether the other Hsps also show a comparable constitutive presence in the spermatogonial cells and if this involves the heat-shock transcription factor.

The present observation on the constitutive presence of Hsp70 in spermatogonia is the first report of the developmental expression of heat-inducible Hsp70 in *Drosophila melanogaster*. Developmental expression of Hsp70 family proteins in mammalian male germ cells is well documented (Eddy, 1999). Two members of the Hsp70 family, HSP70-2 and HSC70T, are regulated to express specifically in meiotic and post-meiotic speramatogenic cells in mice (Matsumoto and Fujimoto, 1990; Dix et al., 1997). Interestingly, homologues of Hsp70-2 are reported in the testes of many other animals (Eddy, 1999), suggesting that such spermatogenic cell chaperones are conserved across phyla. However, unlike the situation in the mouse, the meiotic stages in *Drosophila melanogaster* testes do not express Hsp70 constitutively nor is this protein heat-shock-inducible at this stage. Thus, the constitutively present Hsp70 in spermatogonial cells in *Drosophila melanogaster* may have different function(s). This needs further analysis.

Multiple levels of regulation of the hsp70 genes in Drosophila melanogaster

The strong conservation of the 5′UTRs and the immediately upstream regulatory regions in the five *hsp70* genes in *D. melanogaster* (see Introduction) have generally been accepted as the cause for the more-or-less uniform induction of Hsp70 observed in most previous studies utilizing [^{35}S]methionine labelling and/or western blotting or immunostaining. However, the present in-depth *in situ* analysis of induction of individual groups of *hsp70* genes has revealed remarkable differences in the induction of the different *hsp70* genes by heat shock. It is obvious from the summaries in Tables 1 and 2 that the heat-shock (and presumably other stress)-induced expression of Hsp70 is regulated not only at the level of transcription (no activation *versus* varying levels of activation, and selective activation of only some of the *hsp70* genes or delayed

activation) but also at the post-transcriptional (export from the nucleus) and translational (e.g. delayed translation and turnover of the hsp70 mRNAs) levels.

The mechanisms underlying this differential regulation are not known. The 3′UTRs, being different in the two sets of genes (Torok et al., 1982), obviously have a role in post-transcriptional regulation since these regions are known to affect the stability and transport of the mRNAs (Lakhotia, 1995; Whittaker et al., 1999; Lipshitz and Smibert, 2000). Our results imply that widely accepted ideas, e.g. that the *hsp70* gene promoter remains in an 'open' conformation in readiness for immediate and rapid transcriptional activation following cellular stress (Parsell and Lindquist, 1994; Feder and Hofmann, 1999; Wang and Lindquist, 1999) and that this gene is autoregulated (DiDomenico et al., 1982), may be true for most but not every cell type of *Drosophila melanogaster*. Regulation of the *hsp70* genes, the key players in the cellular stress response in *Drosophila melanogaster*, is obviously much more complex, and many additional features of their regulation are yet to be understood. Recent studies (Sorensen et al., 1999; Andrulis et al., 2000; Tang et al., 2000; Lerman and Feder, 2001; Marchler and Wu, 2001; Wu et al., 2001) are, in fact, uncovering some of these features.

The significance of the differential regulation of the five *hsp70* genes is not clear, especially since the amino acid sequences encoded by these five gene copies show very little divergence and since not all species of *Drosophila* contain duplicated sets of *hsp70* genes. However, we believe that the cell-type-specific patterns of activation of the different *hsp70* genes observed by us have functional and evolutionary significance. The fact that this evolutionary duplication occurred in the *melanogaster* group of species (Feder and Krebs, 1998; Konstantopoulou et al., 1998), and has been retained through the species diversification, itself suggests its adaptive value. In this context, two other possibilities also need to be examined further: (i) that the few amino acid differences in the Hsp70 produced by these duplicated sets of genes have a significant effect on chaperoning activity and, therefore, their differential inducibility may ensure fine-tuning of the stress response, and (ii) that the differences in the 3′UTRs provide differential targeting of the hsp70 mRNAs to subcellular compartments and that this again is relevant for specific cellular needs.

It is apparent that the multiple copies of the *hsp70* genes in *Drosophila* spp. are not just to provide abundant Hsp70 at a short notice. Studies of *hsp70* genes in natural populations of *D. melanogaster* (Krebs and Feder, 1997a; Feder and Krebs, 1998; Feder and Hofmann, 1999; Lansing et al., 2000) have also revealed significant variations in Hsp70 expression and their adaptive significance. It appears that the specific physiology of a cell/tissue type affects the cost/benefit ratio of the Hsp70 under stress conditions and, therefore, that elaborate regulatory mechanisms have evolved to fine-tune the production and level of Hsp70 in a given cell. It is interesting that much of the polymorphism in the *hsp70* genes in *D. melanogaster* populations seems to be in the UTR and promoter sequences (M. E. Feder, personal communication). More intensive studies on the induction of the *hsp70* genes in different tissues of natural populations of *Drosophila* spp. would be instructive.

The present observations on the tissue/stage-specific response of the different *hsp70* genes to heat shock have implications for many developmental genetic studies that utilize the heat-shock promoter to drive a reporter or some other gene activity in *Drosophila* spp. The *hsp70* promoter has been widely used by the fly community to conditionally express any gene in all cell types for experimental purposes. On the basis of our present results, we point out that, depending upon the specific *hsp70* gene promoter and the UTR sequences used in the construction of the transgene that is desired to be expressed in response to heat shock, its expression may vary qualitatively and quantitatively in different tissues. This needs to be taken into consideration in interpreting the experimental results.

Studies on the heat-shock response have mostly been restricted to a few easily amenable cell/tissue types in model systems maintained under controlled laboratory conditions, and this has led to the development of paradigms that do not reflect the dynamically different requirements of heat-shock gene activities under different ecological and physiological conditions (Lakhotia, 2001; Feder, 2001). Further studies on the regulation of heat-shock genes in more diverse cell types and under different environmental conditions in natural populations would provide interesting and exciting information on the complex regulation and biological relevance of this most ancient gene programme.

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