

Cell-specific expression and heat-shock induction of Hsps during spermatogenesis in *Drosophila melanogaster*

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SUMMARY

The developmental and heat-shock-induced expression of two small heat-shock proteins (Hsp23 and Hsp27) was investigated during spermatogenesis in *Drosophila melanogaster*. Both of these Hsps were expressed in unstressed and stressed male gonads as shown by immunoblotting. Immunostaining of whole-mount organs and thin sections of testes showed that an anti-Hsp23 antibody specifically decorated cells of the somatic lineage, such as the cyst cells and the epithelial cells of the testis and of the seminal vesicle. Hsp27 was expressed in some somatic cells (cyst cells and epithelial cells of the accessory glands) and, in addition, was also visible in the maturing spermatocytes of the germline. The same cell-specific pattern of expression was observed after heat shock, and cells which did not express Hsp23 and Hsp27 in the absence of stress were similarly unable to mount a heat shock response for these s-Hsps. However other Hsps such as Hsp70 and Hsp22 were induced under heat-shock conditions in testes.

Actinomycin D prevented the heat-induced accumulation of these Hsps indicating that the induction of Hsps was regulated at the transcriptional level. The heat shock transcriptional factor of *Drosophila* (DmHSF), present in significantly lower amount in testes when compared to other tissues such as the head, was shown to be required for the heat activation of Hsp22 and Hsp70. Immunostaining revealed that HSF expression was restricted to specific cells such as cyst cells, epithelial pigment cells, spermatogonia and spermatids but not the primary spermatocytes. These data show that the expression and induction of the different small Hsps is regulated in a cell-specific manner under both normal and heat shock conditions and suggest that factors other than the DmHSF are involved in this regulation in male gonads.

Key words: Spermatogenesis, Hsp27, Hsp23, Heat shock, *Drosophila*, Heat shock factor

INTRODUCTION

Exposure of cells to various environmental insults including heat shock results in the induction of a small group of conserved proteins known as the heat shock proteins (Hsps) (reviewed by Morimoto et al., 1994). In addition, some of the Hsps have been reported to be expressed in the absence of stress during embryogenesis and development in the fruitfly *Drosophila melanogaster* (Dm) (Sirotkin and Davidson, 1982). In *D. melanogaster*, the low molecular mass Hsps (s-Hsps: small heat shock proteins), Hsp27, Hsp26, Hsp23, and Hsp22, are encoded by four of seven heat-inducible genes clustered at chromosomal region 67B (Petersen et al., 1979; Ayme and Tissières, 1985). In addition to being stress-inducible, the synthesis of s-Hsps can be regulated by the molting hormone 20-hydroxyecdysone as shown in developing imaginal discs and in cultured cells (Ireland et al., 1982; Sirotkin and Davidson, 1982; Cheney and Shearn, 1983; Beaulieu et al., 1989).

While the synthesis of the four s-Hsps of *D. melanogaster* is coordinated during heat shock in cultured cells, their expression during fly development is not (reviewed by Arrigo

and Tanguay, 1991; Arrigo and Landry, 1994; Michaud et al., 1997). For example, ovarian cells of *D. melanogaster* contain mRNAs encoding Hsp27 and Hsp26 that are stored in oocytes (Graziosi et al., 1980; Zimmerman et al., 1983; Mason et al., 1984). P-element transformation studies have revealed the presence of multiple regulatory elements controlling the transcription of these small Hsp genes (Cohen and Meselson, 1985; Glaser and Lis, 1990). Using a hsp26-lacZ fusion gene, Glaser et al. (1986) showed that the hsp26 gene was expressed in the absence of stress in different tissues including gonads and neurocytes. The expression of Hsp27, Hsp26 and Hsp23 in the central nervous system and in germ lines of *D. melanogaster* has also been reported (Pauli et al., 1990; Haass et al., 1990; Marin et al., 1993, 1996; Marin and Tanguay, 1996).

In addition, each s-Hsp appears to be expressed in specific cells of some organs. In gonads of *Drosophila*, Hsp26 mRNA has been detected in nurse cells of developing ovaries, in primary spermatocytes as well as in some spermatogonia (Glaser et al., 1986). Hsp27 and Hsp26 polypeptides have been detected in specific cells of the germ line and in some somatic parts of the male reproductive system (Pauli et al., 1990; Marin et al., 1993). Hsp23 has also been reported to be expressed in

specific regions of testes but this protein has not been previously associated with any distinct structure of this organ (Marin et al., 1993), suggesting that each individual s-Hsp may have cell-specific functions during gametogenesis.

The induction of heat shock genes by elevated temperatures and other forms of physiological or chemical stress is mediated by a heat shock transcription factor (HSF) (reviewed by Wu et al. 1994). DmHSF is synthesized constitutively (Zimarino and Wu, 1987) and stored in a latent monomeric form in the nuclei of cells under normal conditions (Westwood et al., 1991; Westwood and Wu, 1993). In response to stress, the inactive monomeric form of HSF is converted to an active trimer and binds with high affinity to conserved, upstream heat shock elements (HSEs), activating or enhancing the transcription of heat shock genes (Perisic et al., 1989; Westwood and Wu, 1993).

Herein we have examined the cellular pattern of expression of Hsp23 and Hsp27 during spermatogenesis in unstressed and heat-stressed adults of *D. melanogaster*. Each of these s-Hsp exhibits selective expression in specific cells during the normal spermatogenic process. Heat shock does not appreciably affect the expression of these small Hsps and the same cell-specific pattern of expression is observed after heat shock. Cells which do not express Hsp23 and Hsp27 in the absence of stress are similarly unable to mount a heat shock response for these s-Hsps. This contrasts with two other Hsps, Hsp22 and Hsp70, whose synthesis is rapidly induced by heat shock in this organ. The only known heat shock transcriptional factor in *Drosophila* (DmHSF) is shown to be necessary for the heat activation of these proteins. Using confocal microscopy, we also show that HSF is only expressed in a specific subset of cells of the testes.

MATERIALS AND METHODS

Preparation of protein extracts

Testes and heads from cold-anaesthetised young male (3-5 days old) *Drosophila melanogaster* (Oregon-R stock) raised at 23°C were manually dissected in Ringer's solution (182 mM KCl, 46 mM NaCl, 3 mM CaCl₂, 10 mM Tris-HCl, pH 7.2). Organs were transferred to Eppendorf tubes and homogenised in Ringer's solution with a micro tissue grinder. SDS lysis buffer was quickly added (final 2.3% (w/v) SDS, 0.075 M Tris-HCl, pH 6.8, 5% (v/v) β-mercaptoethanol, 10% (w/v) glycerol and 0.005% (v/v) bromophenol blue) after removing a sample of homogenate for protein content determination. Samples were then heated at 95°C for 5 minutes. Protein content was determined with the Micro BCA Protein Assay Reagent Kit (Pierce). For heat shock treatments, flies were placed for 1 hour at 35°C in 50 ml Falcon tubes submerged in a thermostatted water bath.

Actinomycin D treatment

Thirty testes from unstressed males dissected in Ringer's solution were transferred to Eppendorf tubes, and pre-incubated with 15 µg of actinomycin D (Sigma) diluted in 50 µl of Ringer's solution for 15 minutes at room temperature prior to being exposed to a 35°C heat shock for 1 hour. Non-heat-shocked organs were left for 75 minutes at room temperature in the same actinomycin D-Ringer's solution. To assess the efficacy of the in vitro heat shock treatment, additional testes in Ringer's solution were concomitantly incubated at either room temperature or at 35°C for 1 hour in the absence of the inhibitor. Untreated and actinomycin D-treated testes were transferred to

Eppendorf tubes containing 50 µl of SDS lysis buffer, homogenized and heated at 95°C for 5 minutes.

Antibodies

Monoclonal antibodies specific to *Drosophila melanogaster* Hsp23 (7B12) and Hsp27 (2C8) (Marin et al., 1993) were used at a 1:100 dilution. Polyclonal antibodies to Hsp70 (antibody #799) (Tanguay et al., 1993), DmHsp22 (Tanguay, unpublished) and DmHSF (antibody #943) (Westwood et al., 1991) were used at dilutions of 1:5,000 in immunoblotting experiments. The anti-DmHSF was diluted 1:500 for the immunohistochemical assays.

Gel electrophoresis and immunoblotting

Proteins were separated on one-dimensional SDS gels as outlined by Thomas and Kornberg (1975), with modifications in the pH of the running buffer (8.5 instead of 8.8), and in the acrylamide:bis ratio (30:0.8 instead of 30:0.15) as described elsewhere (Marin et al., 1993). Equivalent amounts of proteins (20 µg) were loaded on gels for all samples. Proteins were electrophoretically transferred to Immobilon-P (Millipore, Bedford, MA) or nitrocellulose membranes (Gelman), and immunoblotted as described earlier (Marin et al., 1993). The membranes were incubated with the primary antibody for two hours at room temperature and washed several times in PBT (PBS buffer: 135 mM NaCl, 5 mM KCl, 8 mM Na₂HPO₄, 15 mM KH₂PO₄, pH 7.0) + 0.1% Tween-20). Primary antibodies were detected after incubation for 1 hour at room temperature with anti-mouse IgG or anti-rabbit IgG horseradish peroxidase-conjugated secondary antibodies (ECL, Amersham, Little Chalfont, England or Chemiluminescence Blotting Substrate, Boehringer Mannheim, Germany) diluted 1:10,000 in nonfat dry milk. Detection was done according to the manufacturer's protocol.

Whole-mount staining

Testes from 3- to 5-day-old males were dissected in PBS and fixed for 20 minutes in 4% paraformaldehyde (Polysciences, Inc., Warrington, PA) diluted in PBX (PBS + 0.1% Triton X-100). The tissues were washed three times for 10 minutes in PBX, and saturated for 90 minutes in PBXB (PBX + 1% bovine serum albumin; ICN Biochemicals, Montreal, Canada). Testes were incubated for 2 hours at room temperature with primary anti-Hsps or anti-DmHSF antibodies in PBXB at the dilutions indicated above, rinsed and washed three times for 20 minutes in PBX. Tissues were then incubated with 1:100 fluorescein isothiocyanate-conjugated goat IgG anti-mouse (FITC-GAM), FITC-conjugated horse IgG anti-rabbit (FITC-GAR) or 1:250 tetramethyl rhodamine isothiocyanate-conjugated horse IgG anti-rabbit (TRITC-GAR) secondary antibodies (Molecular Probes, Inc., Eugene, OR) in PBXB for 2 hours at room temperature. Before use, all secondary antibodies were preadsorbed on 4% paraformaldehyde-fixed *Drosophila* embryos overnight at 4°C. After washing three times for 30 minutes with PBX, stained testes were mounted in PBS-

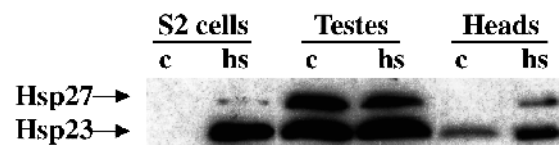


Fig. 1. Immunoblot analysis of Hsp23 and Hsp27 expression in the head and the testis of *Drosophila melanogaster*. Heads and testes from non-heat-shocked (23°C) (C) and heat-shocked (35°C, 1 hour) (HS) adult flies were dissected separately, and their proteins resolved on SDS-PAGE for immunoblotting (see Materials and Methods). The membrane was blotted with the anti-Hsp27 (2C8) and the anti-Hsp23 (7B12) antisera. Protein samples of control and heat-shocked (35°C, 1 hour) *Drosophila* S2 cells were used as a control of antibody specificity and efficiency of heat shock treatment.

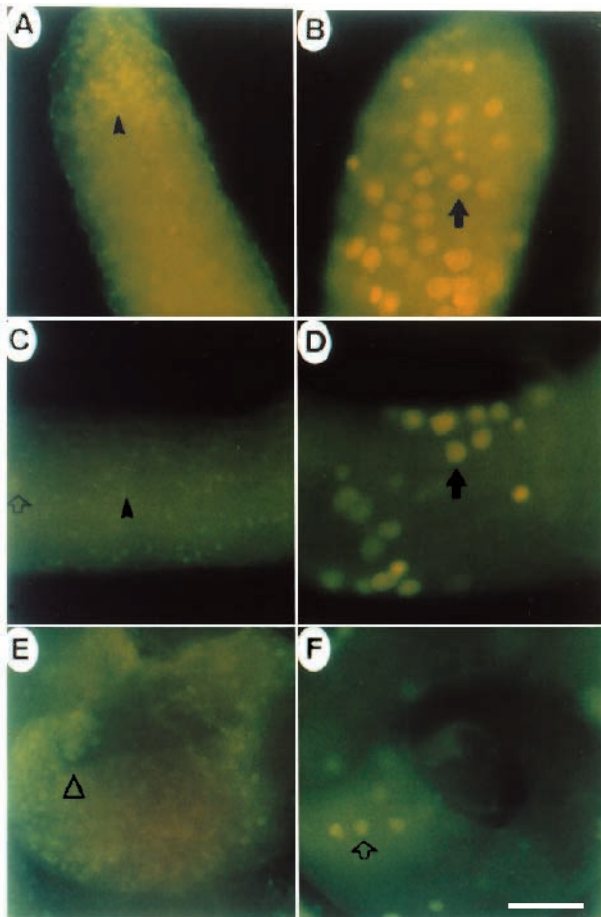


Fig. 2. Cell specific expression of Hsp23 and Hsp27 during *Drosophila* spermatogenesis. Whole-mount staining of non-heat-shocked (23°C) testes incubated with the anti-Hsp23 antibody (A,C,E) or the anti-Hsp27 antibody (B,D,F). FITC-labeled goat anti-mouse IgG was used as the secondary antibody. Different regions of the testis are represented: (A and B) apical, (C and D) elongated, and (E and F) terminal regions. The distinct testis cells are indicated: apical epithelial cells (filled arrowheads), spermatocytes (filled arrows), terminal epithelial cells (open arrowhead), and cyst cells (open arrows). Bar, 50 μ m.

glycerol (10:1) with 1 mg/ml paraphenylenediamine (Sigma). Slides were examined with a DAS Leitz Microscope equipped with epifluorescence optics and photographed with Kodak Royal 100 ASA film or with an LSM 310 laser scanning confocal microscope (Zeiss). Nuclei visualisation by confocal microscopy was obtained by adding 1 μ M Yo-Pro 1 (Molecular Probes, Inc., Eugene, OR) to the last washing solution.

Staining of tissue sections

Testes were fixed and dehydrated as previously described (Marin et al., 1993). Gonads embedded in TissuePrep (Fisher Scientific, Montreal, Canada) were cut in 5 μ m thick sections and deposited on microscope slides previously treated with 1% Bacto gelatin (Gibco, Grand Island, NY) in water. Tissue-sections were rehydrated through a decreasing ethanol series and washed in PBS prior to incubation at room temperature with the specific primary antibodies diluted 1:10 in PBB (PBS + 5 mg/ml bovine serum albumin). After washing slides three times in PBS, visualization of the primary antibody was accomplished by incubating sections for 1 hour at room temperature with the FITC-GAM antibody diluted 1:50. Slides were washed in PBS and

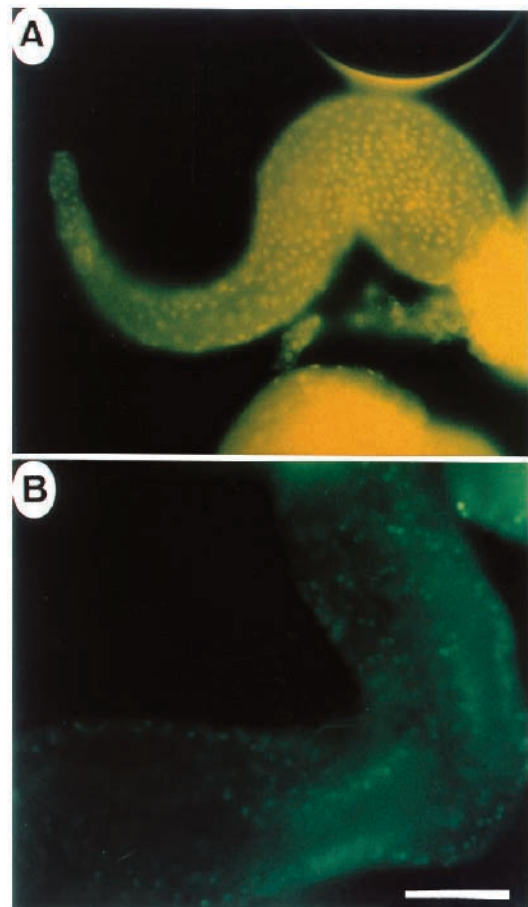


Fig. 3. Expression of Hsp23 and Hsp27 in adjacent organs of non-heat-shocked (23°C) testes. (A) Seminal vesicle stained with anti-Hsp23. (B) Accessory gland stained with anti-Hsp27. Bar, 50 μ m.

mounted in AquaPolyMount (Polysciences, Inc., Warrington, PA). No appreciable labelling was obtained when the secondary FITC-labelled antibody was incubated in the absence of primary antibodies. Detection of Hsp27 in the ommatidial eye unit was done using a biotin-labelled secondary antibody (Vectastain ABC Kit, Vector laboratories, Mississauga, Ontario) and developed as previously described (Marin et al., 1996).

RESULTS

Hsp23 and Hsp27 are expressed in the absence of stress in testes of *Drosophila melanogaster*

The expression of Hsp23 and Hsp27 in testes of *D. melanogaster* adults was first estimated by immunoblotting. In *Drosophila* S2 tissue culture cells, these two Hsps are not detectable in non-heat shocked cells and accumulate after a heat shock (Fig. 1). In testes, both of these s-Hsps are expressed in unstressed flies (c: control). The expression of these small heat shock proteins does not increase significantly after heat shock, as indicated by the relative intensity of bands on the immunoblot. For comparison, protein samples from the heads of the same control and heat-shocked males were loaded on the same gel. The level of Hsp23 is lower in unstressed heads (although overexposure also reveals a weak level of Hsp27 in

control heads; data not shown) and both Hsp23 and Hsp27 are clearly heat-inducible as shown in Fig. 1. The expression of s-Hsps in the central nervous system in the absence of stress has been reported previously (Haass et al., 1990; Pauli et al., 1990; Marin et al., 1993).

Hsp23 and Hsp27 show cell-specific expression in testes

To determine if Hsp23 and Hsp27 were present in different cell types of the testis, their expression was examined by whole-mount immunocytochemistry. As shown in Fig. 2, the staining with the anti-Hsp23 antibody was associated with pigment cells of the sheath (Fig. 2A, solid arrowhead), with the cyst cells (Fig. 2C, open arrow, see also Fig. 4A and B) and with the epithelial cells at the terminal end of the male gonad that fuses with the seminal vesicle (Fig. 2E, open arrowhead). In addition, a weak fluorescence with anti-Hsp23 was noticed on a filamentous structure along the gonad likely related to elongated spermatid bundles (see Figs 4B and 5A, open arrowhead on white circle). In contrast, Hsp27 was mainly expressed in germ line cells. Hsp27-positive cells in the growth phase region were typical maturing spermatocytes, with large nuclei and prominent nucleoli (Fig. 2B and D, solid arrow). Hsp27 was also visible in somatic cyst cells (Fig. 2F, open arrow).

These two small Hsps were also present in some adjacent structures of the male gonad. Thus, Hsp23 was expressed in the cells surrounding the seminal vesicle (Fig. 3A) whereas

Table 1. Summary of the different cell types expressing Hsp23, Hsp27 and HSF in male gonads

Cell type	Hsp23		Hsp27		HSF	
	C	HS	C	HS	C	HS
Germ cells						
Spermatogonia	-	-	-	-	+	++
Primary spermatocytes	-	-	+	+	-	-
Secondary spermatocytes	-	-	-	-	-	-
Spermatids	-*	-*	-	-	+	++
Somatic cells						
Cyst cells	+	+	+	+	+	+
Pigment epithelial cells	+	+	-	-	+/-	+/-
Terminal epithelial cells	+	+	-	-	-	-
Epithelial cells of the seminal vesicle	+	+	-	-	-	-
Epithelial cells of the accessory glands	-	-	+	+	+	+

- , no expression detected; +/-, expression in only a subset of cells; +, expression; ++, high level of expression; *, note that Hsp23 has been observed in elongated tail of spermatid bundles.

Hsp27 was visible in epithelial cells of the accessory glands (Fig. 3B). In the case of the seminal vesicle, the Hsp23-stained cells were the secretory cells of the epithelium but not the content of the organ (the latter being mature sperm). An identical pattern of expression for both proteins was obtained in other immunological experiments where a biotinylated

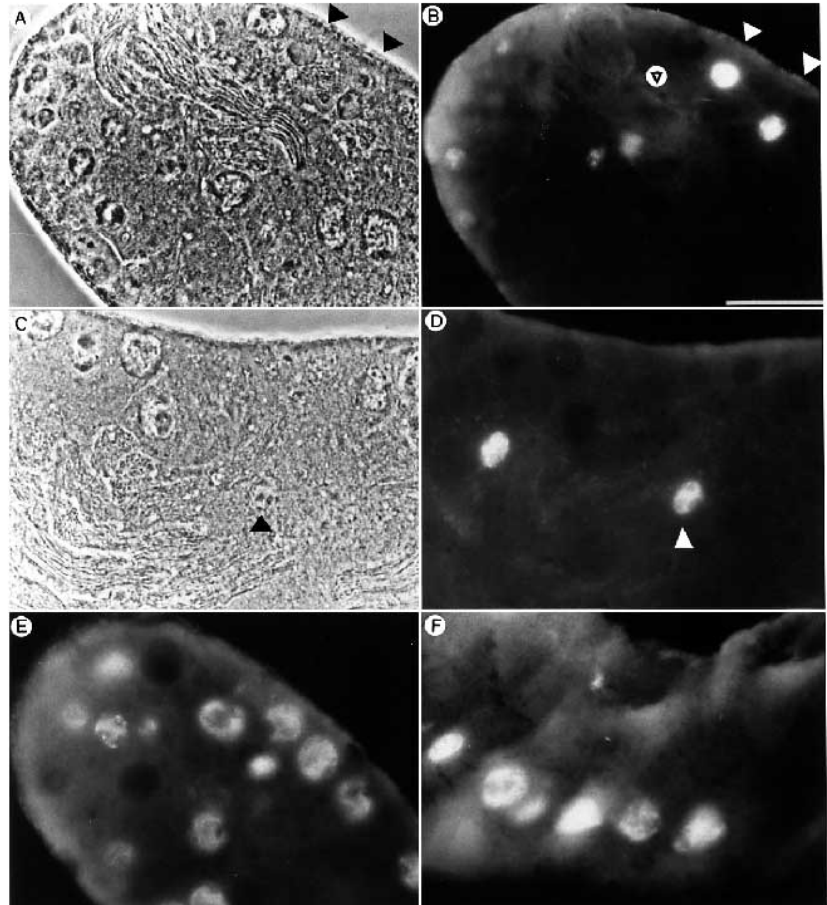


Fig. 4. Immunolocalization of Hsp23 and Hsp27 in the nuclei of testis cells. 5 μ m thin sections from non-heat-shocked adult males testes were stained with the anti-Hsp23 (B and D) and the anti-Hsp27 (E and F) antisera. Nuclei of cyst cells and spermatocytes display reactions with the two different antisera. A and C correspond to the phase contrast photographs of B and D, respectively. (B,D,F) Immunofluorescence photographs. Arrowheads point to identical positions of cyst nuclei in the phase-contrast and fluorescence pictures. The open arrowhead on white circle in B points to the spermatid bundles stained with the anti-Hsp23 antibody. Bar, 50 μ m.

secondary antibody was used to detect the primary antibody labelling (data not shown). The distinct patterns of expression of these two small Hsps are summarized in Table 1.

The whole-mount experiments revealed the presence of both Hsp23 and Hsp27 in the nuclei of the different cells of this organ. To further confirm these observations, 5 μm thin transverse sections of fixed non-heat-shocked (23°C) testes were also immunostained with the anti-Hsp23 and the anti-Hsp27 antibodies. Both proteins were detected at the nuclei of distinct testis cell types. Hsp23 was associated with the nuclei of somatic cyst cells (Fig. 4B and D, white arrowheads) and epithelial pigment cells (not visible in this section). Hsp27 showed a typical nuclear staining in both primary spermatocytes and cyst cells (Fig. 4E and F).

Hsp23 and Hsp27 retain cell-specific expression after heat shock

The experiments described above show that the constitutive expression of these small Hsps is associated with specific groups of cells, Hsp23 being expressed in somatic cells whereas Hsp27 is mostly observed in germline cells. In previous work on *Drosophila* male gonads (Bonner et al., 1984), a cell-specific heat induction was observed for a hsp70-ADH (alcohol dehydrogenase) fusion gene. Therefore, we examined if the synthesis of Hsp23 and Hsp27 was also restricted to specific spermatogenic cells after a heat stress.

Thin-sections of testes from heat-shocked (35°C, 1 hour) flies were analyzed by immunohistochemistry, using the anti-Hsp23 and anti-Hsp27 antisera. Immunostaining showed that the patterns of expression observed for both of these small Hsps in unstressed conditions also persisted in testes from

heat-stressed flies. Again, the only cells showing Hsp23 expression were cyst (Fig. 5A, white arrowhead) and epithelial cells, and those expressing Hsp27 were immature spermatocytes (Fig. 5B, arrow) and cyst cells. The same immunostaining results were obtained when flies were submitted to longer heat shock and recovery periods (1 hour, 35°C + 2 hours, 23°C; 2 hours, 35°C + 2 hours, 23°C) and no staining was observed for Hsp27 in epithelial cells or for Hsp23 in germ cells (data not shown).

To eliminate the possibility that these results might be due to an ineffective heat shock treatment, longitudinal eye-sections from the same heat-shocked males were simultaneously examined. In the eye, Hsp23 and Hsp27 are not expressed constitutively but are induced by heat-shock in a cell-specific manner (Marin et al., 1996). Hsp27 was expressed in all the different ommatidial cells following heat shock, and no expression of Hsp27 was observed in non-heat-shocked eyes (Fig. 5C and D). This confirms that the absence of induction of Hsp23 and Hsp27 is specific to testis cells.

Hsp70 and Hsp22 are heat-shock inducible in testes

As the immunoblot and staining data indicated an absence of response of Hsp23 and Hsp27 to a heat shock, we looked if the expression of other Hsps was heat-inducible in testes. As shown in Fig. 7 (lanes 3 to 6), Hsp70 was constitutively expressed in testes and was induced by heat shock (lanes 3-4). The low level of expression of this Hsp in control heads was also increased by the heat treatment (lanes 5-6). Hsp22, another member of the small Hsp family, showed no detectable expression in control testes but was clearly induced under stress conditions. Thus, the heat-shock induction of these two

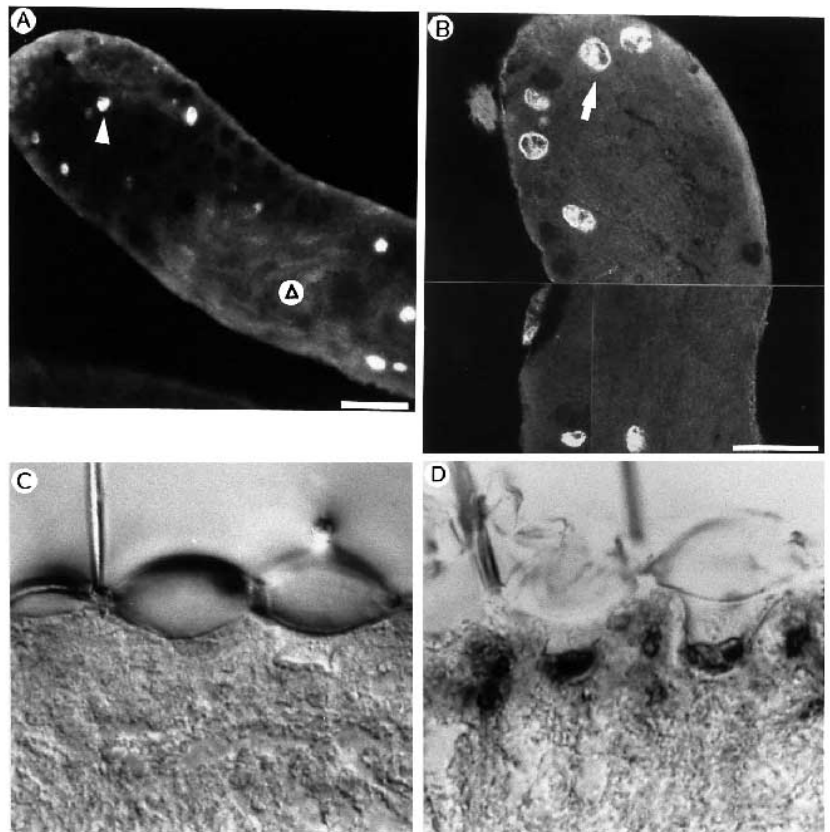


Fig. 5. Cell-specific expression of Hsp23 and Hsp27 in eyes and testes of stressed *Drosophila*. (A and B) 5 μm thin sections from heat-shocked (35°C, 1 hour) testes incubated with anti-Hsp23 (A) and anti-Hsp27 (B) antisera. The arrowhead points to cyst cells of the apical region and the arrow to spermatocytes. Note also the staining in spermatid bundles (open arrowhead on white circle in A). The epithelial cells of the sheath are not visible at this focal plane. (C and D) Longitudinal sections of non-heat-shocked (23°C) (C) and heat-shocked (35°C, 1 hour) (D) eyes of the same animal incubated with the anti-Hsp27 antiserum and stained with a Vectastain biotinylated secondary antibody. Bars, 50 μm .

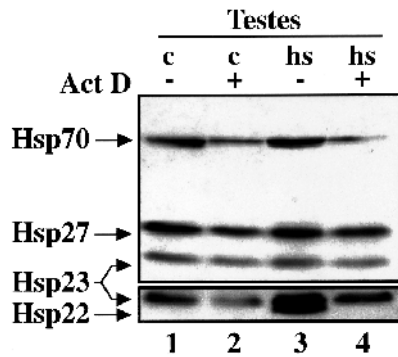


Fig. 6. Effects of actinomycin D on Hsp synthesis. Dissected testes incubated in vitro with actinomycin D (lanes 2 and 4) were either left at room temperature for 1 hour (lane 2) or submitted to a 35°C, 1 hour heat shock (lane 4), and blotted with the anti-Hsp70, anti-Hsp27 and anti-Hsp22 antibodies. Two other protein samples from non-heat-shocked (lane 1) and heat-shocked (lane 3) testes incubated in the absence of actinomycin D were also run as a control of heat shock treatments.

Hsps not only attests the efficiency of the heat shock treatment at 35°C but also shows that testes can respond to a heat stress.

To see whether the heat-induced expression of Hsp70 and Hsp22 in testes was regulated transcriptionally by de novo synthesis of Hsp mRNAs or through translational activation of stored inactive heat shock mRNAs, we examined the effects of a transcriptional inhibitor (actinomycin D) on the induction and synthesis of Hsps. As shown in Fig. 6, Hsp70 and Hsp22 were induced by a heat shock in the absence of actinomycin D (lane 3). Pre-incubation with actinomycin D prevented their induction as no increase in Hsp70 level could be observed and no Hsp22 band was detected on the western blot (lane 4). The expression of Hsp27 and Hsp23 was only lightly affected by actinomycin D, a finding consistent with the fact that these Hsps are expressed at a high level in this tissue prior to heat shock. This suggests that the heat-induced expression of Hsp70 and Hsp22 in testes is regulated at the transcriptional level.

***Drosophila melanogaster* HSF (DmHSF) is required for the heat induction of Hsp22 and Hsp70**

In *Drosophila melanogaster*, induction of heat shock proteins under stress conditions is believed to be transcriptionally regulated by a single known heat shock transcription factor (DmHSF) (Clos et al., 1990). Since Hsp23 and Hsp27 did not respond to heat shock in testes, we examined the expression of DmHSF in male gonads by probing with an antibody specifically directed against DmHSF (Westwood et al., 1991) (Fig. 7). DmHSF was clearly present in all samples of normal flies (lanes 3-6) although its level was significantly reduced in testes as compared to heads. Semi-quantitative blotting indicates that there is approximately 4 times more HSF in heads than in testes on an equal protein basis (data not shown). In all tissues, we observed a light increase in the amount of HSF immunoreactivity combined with a small decrease in the mobility of the protein after heat shock, likely due to post-translational modification.

In order to verify if this DmHSF was the factor directly responsible for the heat activation of Hsp22 and Hsp70, a mutant strain of *D. melanogaster* (HSF⁴) carrying a tempera-

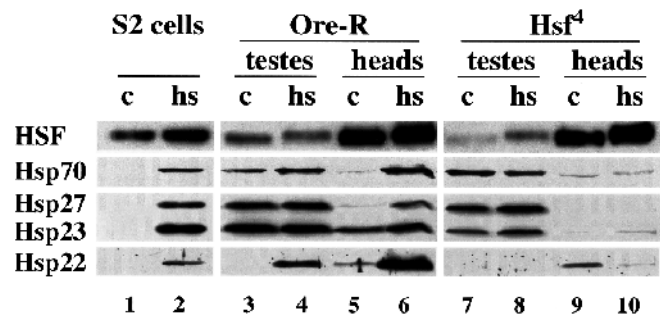


Fig. 7. Requirement of a functional HSF for Hsp22 and Hsp70 heat induction. Western blots of dissected head and testes proteins from non-heat-shocked (c) and heat-shocked (hs) adult flies (Ore-R (lane 3-6) and Hsf⁴ (lane 7-10) strains) were, respectively, probed with (i) anti-Hsp23 and anti-Hsp27, (ii) anti-Hsp22, and anti-Hsp70, (iii) anti-Hsp70 and anti-HSF (#943). The data obtained are here summarized for better visualization. Protein samples from *Drosophila* S2 cells (control and heat shock/lane 1-2) serve as immunoblotting control.

ture-sensitive form of the HSF was used (Jedlicka et al., 1997). As shown in Fig. 7, no increase in Hsp22 or Hsp70 could be seen in any samples after heat shock in this ts-mutant (lanes 7 to 10). The heat induced synthesis of Hsp23 and Hsp27 seen in heads of wild-type Ore-R flies (lane 5 versus 6) was also inhibited in HSF⁴ head samples (lane 9 versus 10). However, levels of Hsp23 and Hsp27 remain stable in all testes samples. These results confirm that the activation of Hsp22 and Hsp70 in testes and heads necessitates the presence of a functional DmHSF.

DmHSF is expressed in specific cells of the testes

The localization of HSF under control and heat shock condition was next examined in order to see if the expression of this factor was restricted to specific cells of the testes. Under control condition, HSF was observed in spermatogonial cells (pre-meiotic stage) (data not shown) and in some epithelial pigment cells (Fig. 8A, open arrowhead). Cyst cells (Fig. 8A, open arrow) and post-meiotic spermatids (Fig. 8B, filled arrow) showed a higher expression of the HSF. After heat shock, an increase in the signal of HSF was seen in spermatogonial cells (Fig. 8C-E, filled arrowhead) and expression of the HSF is maintained in cyst cells (Fig. 8C-E, open arrow) and spermatids (Fig. 8F, filled arrow). HSF was not observed in primary spermatocytes. These data show that the relative amount of HSF varies in the different cell types of the *Drosophila* testes.

DISCUSSION

The genes encoding *D. melanogaster* Hsp23 and Hsp27 have been reported to be activated under stress conditions as well as at different developmental stages during metamorphosis (Sirotkin and Davidson, 1982; Ireland et al., 1982). As shown here, Hsp23 and Hsp27 are expressed in unstressed and stressed male gonads of *D. melanogaster*. Hsp27 is found in primary spermatocytes and somatic cyst cells whereas the expression of Hsp23 is restricted to the somatic cell lineage. The cellular distribution observed for Hsp27 in testes is similar

to that of Hsp26, with the latter reported to be present in the cytoplasm of developing spermatocytes (Glaser et al., 1986; Marin et al., 1993). Promoter analysis using a reporter gene showed the presence of spermatocyte-specific promoter regions for Hsp26 (Glaser et al., 1986; Glaser and Lis, 1990). Pauli et al. (1990) also detected expression of Hsp27 in post-meiotic spermatid bundles, suggesting that this expression was likely of spermatocyte origin. In addition to cell specific promoters, some regulatory factors may also be needed during spermatogenesis to modulate the expression of each individual small Hsp gene as was shown for the *hsp26* gene during oogenesis (Frank et al., 1992).

The expression of Hsp23 and Hsp27 in distinct cell types may indicate that these small Hsps perform different functions during spermatogenesis. It is interesting to note that the presence of Hsp27 in gametogenic cells is restricted to those cells that have reached meiotic divisions (maturing spermatocytes). Hsp27 could be implicated in some intracellular changes taking place in spermatocytes to differentiate in mature gametes. Alternatively, the *hsp27* gene product may play a role in the control of the abundant RNA synthesis occurring in these cells early during spermatogenesis (Gould-Somero and Holland, 1974). Besides the germ line, the somatic cyst cells also express Hsp23 and Hsp27 indicating that these two proteins could be functioning coordinately in somatic cell lines. Other possible roles played by these Hsps may relate to cellular stability and protection, as suggested by the presence of the two s-Hsps in other structures of the male reproductive system: Hsp27 in epithelial cells of the accessory glands, and Hsp23 in those of the seminal vesicle. In addition, Hsp23 has been shown to be part of a filamentous structure in spermatid

bundles (Marin et al., 1993, and this work), suggesting that this protein could be associated with specific elements of the cytoskeleton.

It has been traditionally accepted that the small Hsps of *Drosophila* are coordinately induced at elevated temperatures, resulting in the accumulation in the cell of all these polypeptides (Mirault et al., 1978). However, as shown here, when adult testes are submitted to heat shock treatment only cells that express Hsp23 and Hsp27 constitutively (i.e. in the absence of stress) conserve an active pattern of expression, and no significant *de novo* synthesis of these two Hsps is observed. The absence of response of Hsp23 and Hsp27 to heat shock is not due to a delayed transcription of the genes in these cells, as the same immunostaining results were obtained with longer heat shock and/or recovery period treatments of this organ (data not shown). In early embryonic cells and in pupae, heat shock has also been observed to have little effect on the synthesis of s-Hsps (Graziosi et al., 1980; Arrigo, 1987).

One possible explanation for the absence of heat induction is that the presence of small hsp may inhibit heat induction of the *hsp23* and *hsp27* genes. In human cells, heat activation of the HSF is inhibited in cells overexpressing Hsp27 (Fuqua et al., 1994). However, such an autoregulatory loop mechanism appears unlikely here as it would not explain the lack of induction of Hsp23 and Hsp27 in cells expressing HSF such as spermatogonia and spermatids, nor the heat inducibility of the other hsp like Hsp22 (see below). A second possibility is that in cells constitutively expressing HSF and both Hsp23 and Hsp27 (cyst cells), the expression is at or near maximal and the activation of heat shock gene expression by stress does not appreciably increase the synthesis of these two genes.

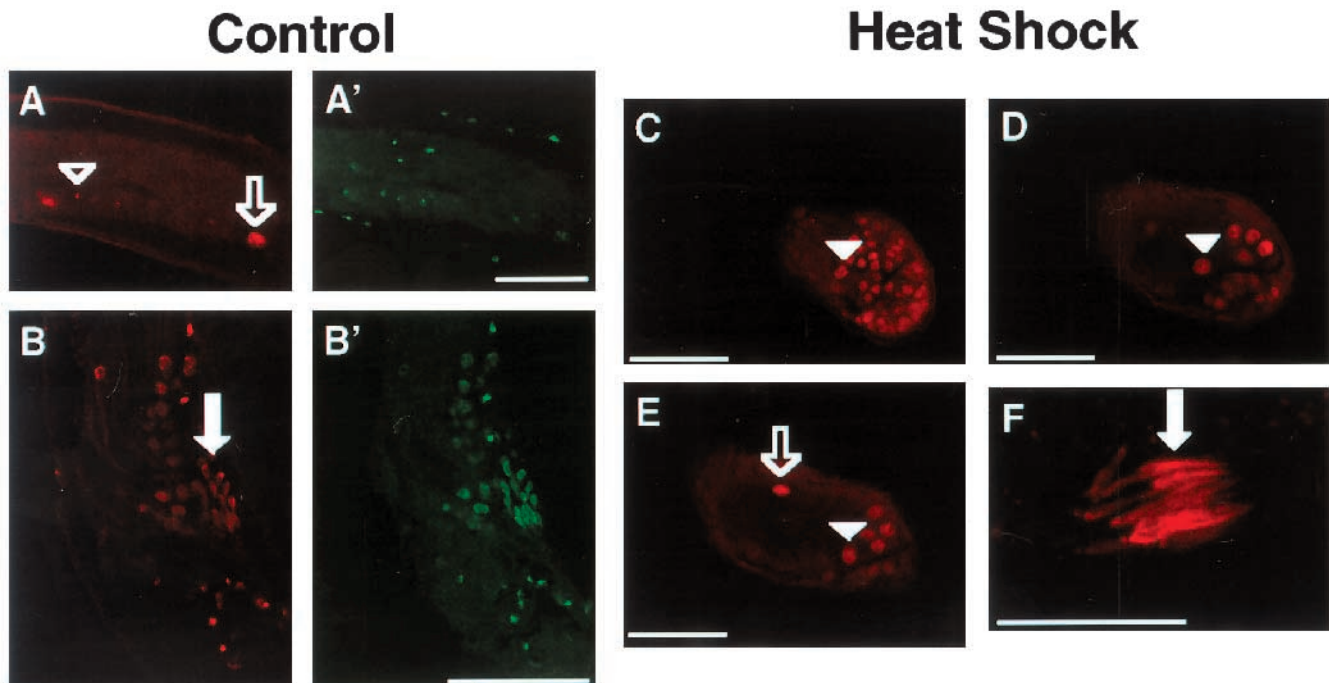


Fig. 8. Localization of HSF in specific cells of the testes. Confocal images of whole-mount non-heat-shocked (A and B) and heat shocked (C-F) testes immunostained with an anti-DmHSF antibody. In A' and B', the fluorochrome Yo-Pro 1 was used for nuclear localization. (A,B,F) Images of the elongated region of the testes, (C to E) images of the apical region. The distinct cell types are indicated: epithelial cells (open arrowhead), cyst cells (open arrows), spermatogonia (pre-meiotic cells) (filled arrowheads) and spermatid (post meiotic cells) (filled arrows). Bars: 50 μ m (A to E); 25 μ m (F).

However, this would not explain how specific cell lineages only express a subset of Hsps nor the lack of heat shock induction of Hsp23 and Hsp27 in spermatogonia and spermatids. An attractive hypothesis would be that transcription factors other than HSF are turned on in different cell lineages, modulating the expression s-Hsps either by promoting or inhibiting it at the transcriptional level.

The observation that actinomycin D inhibits the heat-shock induction of Hsp70 and Hsp22 also shows that these genes are regulated at the transcriptional level rather than by activation of pre-existent inactive mRNAs by factors acting at the translational level. To verify if the single known HSF in *D. melanogaster* was involved in the induction of Hsps in testes, flies carrying a novel temperature-sensitive mutation (Hsf⁴) which inactivates HSF activity at stress temperatures (Jedlicka et al., 1997) were submitted to heat shock. In the ts-mutant, induction of Hsp22 and Hsp70 was inhibited in heads and testes demonstrating that the heat induction of these proteins is dependent on the presence of a functional HSF (Fig. 7).

The induction of Hsp22 and Hsp70 in testes combined with the immunolocalisation results for the HSF imply that it is not the level of HSF but its cell-specific distribution that may be the limiting factor in the heat shock induction process. Varying HSF expression domains in different tissues might be a mechanism by which the organism modulates the heat shock response locally in different parts of the body to react and/or cope to stress conditions differently. It is noteworthy to point out that the expression of the HSF after heat shock (in every cell type of the testes with the clear exception of primary spermatocytes) corresponds to the pattern of heat-induced Hsp70 mRNA expression previously determined by Bendena et al. (1991). Although no clear immunostaining results could be obtained for Hsp22 or Hsp70, the present results concerning HSF localization supports previous observations suggesting that primary spermatocytes are unable to mount a heat shock response either during their maturation or during the meiotic process (Bonner et al., 1984; Bendena et al., 1991). Furthermore, the heat induced increase in mRNA for Hsp70 and hsr- ω in spermatids (Bendena et al., 1991) and the absence of Hsp23 or Hsp27 in the presence of HSF demonstrate that there is a selective heat shock response in the post-meiotic stage of spermatogenesis.

In summary, the present data suggest that factors, other than DmHSF, may be involved in the cell-specific expression of sHsps in testes. Future studies should bring further understanding of the mechanisms regulating the expression as well as the heat-shock induction of the different Hsps, and of the implications of these regulators in the functions of these proteins during gametogenesis.

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