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Regulation of heat shock gene induction and expression during *Drosophila* development

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Abstract. Some heat shock genes are expressed in the absence of stress during embryogenesis and metamorphosis in the fruit fly *Drosophila melanogaster*. Their functions in these processes are unknown. During development, each of the four members of the small heat shock protein family (Hsp27, Hsp26, Hsp23 and Hsp22), which are coordinately induced in response to a heat stress, shows a specific pattern of expression in diverse tissues and cells. This expression is driven through cell-specific enhancers in the promoter regions of their genes. In addition, some of the Hsps show cell-specific induction by heat shock. Hsp23, for example, is only inducible in a single cell type (cone cells) of the eye ommatidium, while the other small Hsps are inducible in all cells of the eye unit. In germ line tissues such as testes, Hsp23 and 27 are both readily expressed in the absence of stress (albeit in distinct cell lineages) and cannot be further induced by heat shock. Hsp27 is expressed throughout oogenesis, but its intracellular localization is stage-specific, being nuclear from germarium to stage 6 and cytoplasmic from stage 8 onwards. Finally the small Hsps show tissue-specific post-translational modifications. Thus the function(s) of the small Hsps may be modulated by different cell and developmental stage-specific mechanisms operating either on their expression, their cellular localization or their structure by post-translational modifications.

Key words. Small heat shock proteins; Hsp27; Hsp23; gene regulation; development; heat shock response; oogenesis; spermatogenesis; *Drosophila melanogaster*.

Introduction

The heat shock response was first observed in the fruitfly *Drosophila* as the induction of new puffs on the polytene chromosomes of salivary glands [1, 2]. A similar response is induced upon exposure to various chemical or environmental insults. A subset of specific proteins, thereafter called heat shock proteins (Hsps), was shown to be preferentially expressed after heat shock. In *D. melanogaster*, these proteins have molecular weights of 22, 23, 26, 27, 70 and 83 kDa [3, 4]. They have been divided into three different groups on the basis of their relative molecular masses: the small heat shock proteins (sHsps), the Hsp/Hsc70 family and Hsp83.

While *D. melanogaster* is a model of choice for developmental studies, there is still little known about the significance of the developmental features of the heat shock response in this organism. The expression of the Hsp genes during development and metamorphosis in *Drosophila* has been the subject of a number of reviews [5–9]. Here we present an update on (1) the developmental, tissue- and cell-specific induction and expression of Hsps by heat shock, (2) the pattern of expression of the Hsp genes in the absence of stress, with particular emphasis on the members of the sHsp family, and (3) the developmental and tissue-specific localization and post-translational modifications of sHsps. Finally, we discuss the developmental pattern of expression of one of the largest puffs activated by heat shock (93D), which has not yet been shown to produce any defined polypeptide.

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The heat shock genes in *Drosophila melanogaster*

The sHsp genes of *D. melanogaster* are clustered within a 12-kb section of the 67B region on the left arm of the third chromosome [10–15]. Each one of these genes consists of a single open-reading frame (ORF). Sequence analysis reveals three major domains of homology between the sHsps (fig. 1). The prominent one is located in the carboxy-terminal section of all four sHsps and consists of an 80-amino acid domain homologous to a domain in mammalian α-crystallin [16]. Attached directly downstream of this region is a 25-amino acid stretch also conserved in all sHsps [13]. No particular property or function has yet been defined for this region. Finally, a very hydrophobic amino-terminal domain of 15 amino acids is found in all sHsps with the exception of Hsp22. This domain has been suggested to play a role in protein-membrane interactions [17].

The Hsp/Hsc70 family contains two major classes of proteins sharing a high degree of homology: the Hscs (heat shock cognates), Hsp68 and Hsp70. These proteins are encoded at multiple loci in the genome of *D. melanogaster*: two Hsp70 genes are found at region 87A7 [18–20], and region 87C1 contains three to five supplementary copies of *hsp70*, depending on the fly strain [20–22]. Multiple Hscs, which are proteins ex-

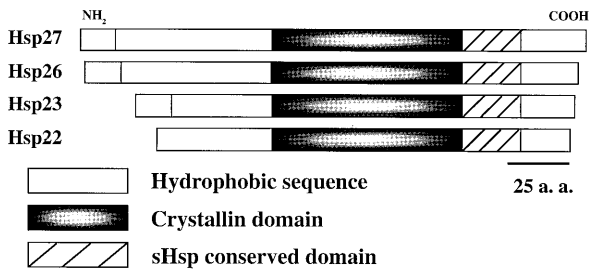

Figure 1. Structure and protein domains of the small Hsp genes of *D. melanogaster*.

pressed at normal temperature and not further induced by stress, have also been described [23]. The genes coding for Hsc1, Hsc2 and Hsc4 have been localized to regions 70C, 87D and 85E, respectively [24, 25]. Finally, the Hsp83 protein is encoded by a single gene located at region 63BC on polytene chromosomes. *Hsp83* is the only Hsp gene which contains an intron [26, 27].

Heat shock gene induction and transcription

The heat shock response, which results in the prominent expression of Hsps over normal proteins, is regulated at two molecular levels. First, heat shock rapidly activates or increases the transcription of Hsp genes. Second, heat shock mRNAs show a preferential translation over normal mRNA [28]. Heat shock gene induction relies on de novo transcription, which may explain why no induction of Hsps is observed in differentiated gametes or in preblastoderm embryos [29–31]. The preferential translation of Hsp mRNA is the result of two main events. Hsps mRNA contain untranslated sequences which promote preferential translation over normal mRNA [28]. Also, heat shock induces a block in splicing, which does not affect sHsp or Hsc/Hsp70 expression. However, expression of Hsp83, the only Hsp gene containing an intron, is optimum at 33–35 °C and has been shown to be inhibited at higher temperature as a consequence of this inhibition of splicing [32]. The optimal induction temperature for the sHsp genes (35 °C) is lower than the one for the optimal induction of Hsp70 (37 °C) [33], even though none of these genes possesses introns. The reasons for such differential response to heat shock by the Hsp genes remain to be identified.

Heat shock induction of Hsps relies on a particular transcription factor: the heat shock factor (HSF) (reviewed in [34]). In *D. melanogaster*, the HSF is encoded by a single gene located in region 55A. This factor is synthesized constitutively and exists as a monomer which is distributed in a diffuse manner all over chromatin [35]. As a result of heat stress, the HSF trimerizes in an activated form with a high DNA-binding affinity [36–38]. It then binds to distinct loci ($\approx$100, including the nine major HSP loci) [34]. Its binding to the promoter of Hsp genes is mediated through a conserved element, the heat shock element (HSE). The HSE was first identified as a sequence required for heat inducibility in the *Drosophila hsp70* gene [39, 40]. HSF/HSE binding activates the transcription of the different Hsp genes and allows their overexpression. Further analysis and sequence comparison of functional HSEs in *Drosophila* Hsp genes led to the definition of an HSE as a repeating array of 5-bp sequences 5′-nGAAn-3′ where each repeat is inverted relative to the immediate adjacent repeat [41–43]. Different numbers of HSEs are found in front of the different Hsp genes, and a certain number of these HSEs need to be bound by the HSF to confer heat shock-induced expression to each Hsp gene.

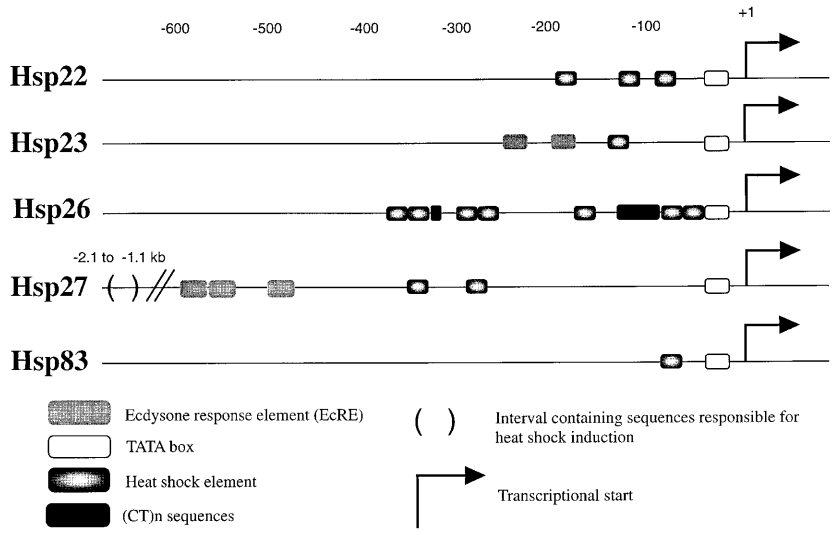

Figure 2. Schematic representation of the promoter regions of the sHsp genes in *D. melanogaster*. The structure of *hsp83* is shown for comparison.

All promoter regions of the sHsp genes contain HSEs as well as other sequences which are specifically necessary for heat-induced expression (fig. 2). Important regions for heat activation of *hsp27* are found up to 2.1–1.1 kb upstream of the gene itself; sequences allowing milder heat shock induction can also be found in the −986/−227 region [44]. These secondary sequences were further refined to the −455/−227 interval [45].

The *hsp26* gene possesses seven different HSEs, only three of which are necessary for full heat shock induction of the protein: HSE1 and 2 (located at −60) and HSE6 (located at −350) [46–48]. Other sequences beside the HSEs have been identified as important to ensure the capacity of heat shock inducibility of this gene. Thus two sequences containing (CT)n*(GA)n repeats are critical to maintain chromatin structure and ensure accessibility of the HSE to the HSF. Deletion of one of these sequences (located at −135/−85) is correlated with a decrease in transcriptional activity, even when the TATA box and the HSEs are present [49, 50]. *Hsp23* requires both a sequence located between −145/−132 [51] and an element upstream of −186 [52] for heat-related expression. In the case of the *hsp22* gene, two control elements with different functions have been characterized in the untranslated leader region of its mRNA. One of these is responsible for the transcriptional activation of *hsp22* after heat shock, while the other is responsible for allowing translation of this mRNA under heat shock conditions [28]. Three HSEs are also found at 26, 46 and 147 bp upstream of the *hsp22* TATA box [53].

As shown in figure 2, *hsp83* with a single HSE from position −88 to −49 may have a simpler heat shock regulation [54] compared with other Hsps such as *hsp26*, which possesses in its promoter region seven HSEs, three of which are necessary for heat induction.

Heat shock induction of Hsps shows cell and tissue specificity

In *Drosophila*, as well as in many other biological systems, all cells of an organism are capable of mounting a heat shock response at most stages of development. A notable exception is early embryogenesis, when preblastoderm embryos do not respond to heat shock [30, 55]. In the case of the small Hsp genes of *D. melanogaster*, it has been generally accepted that the four main sHsps are induced coordinately in response to heat shock. Recently, however, some sHsps have been shown to be induced in a cell-specific manner by heat shock.

A first example of cell-specific response to heat shock is that of the eye of *Drosophila*, which consists of a large number of ommatidia, each one including different specialized cell types such as photoreceptor, cone and pigment cells. After heat shock, Hsp23 is expressed exclusively in a single cell type, the cone cells, while Hsp27 is expressed in all cell types of the ommatidium [56]. Hsp26 shows a response similar to Hsp27 and responds in all cells of the ommatidium (fig. 3). The ubiquitous expression of Hsp27 and Hsp26 in response to heat shock eliminates the possibility that the heat shock per se may be inefficient in certain cell types of the ommatidia. The absence of Hsp23 induction in photoreceptor and pigment cells cannot be explained by the absence of the transcriptional factor, as the *Dm* HSF is equally present in all cells of the eye unit [56]. The lack of induction may be due to the presence of other factors repressing this Hsp at the transcriptional or post-transcriptional levels. Whether Hsp23 is repressed by factors operating at the level of chromatin, such as the absence of binding of the GAGA factor on the *hsp23* promoter, or through other interfering factors is presently unknown.

Another case of cell-specific response is the testes, where Hsp23 and Hsp27 are not induced by heat shock, while Hsp22 and Hsp70 are strongly induced in the same organ (R. Marin, S. Michaud, J. T. Westwood et al., unpublished). Figure 4 shows an immunoblot of Hsp23 and Hsp27 expression in different organism of adult flies before or after heat shock. Hsp23 and Hsp27 show a cell-specific pattern of expression in the absence of stress and after heat stimulation (see below). Their expression is not upregulated by heat shock, in contrast to the situation in other tissues such as the head (fig. 4). While very little HSF is detectable in testes, this does not seem to explain the absence of response of Hsp23 and Hsp27, as other *hsp* genes do respond rapidly to heat induction. There is at present no evidene for other HSFs in *D. melanogaster*. One possibility is that these small Hsps exert an autoregulatory control over their own synthesis.

In ovaries, the response of Hsp27 to heat shock is cell- and stage-specific. In nurse cells, Hsp27 expression and localization (see below) do not seem to be altered by heat treatment. However, Hsp27 is strongly induced in follicle cells [57]. One interesting observation in this specific subset of ovarian cells is that the induced Hsp27 shows the same stage-specific localization pattern seen in the nurse cells (see below). The mechanism(s) underlying this peculiar cell- and stage-specific regulation of Hsp27 in ovaries are still unknown.

Hsps show a stage- and cell-specific pattern of expression in the absence of stress

Early studies suggested that Hsps were expressed not only under stress conditions but could also be found at lower levels at normal temperatures [31, 58]. Three members of the sHsp family, Hsp27, Hsp26 and Hsp23, have been reported to be expressed under normal conditions. However, in contrast to their coordinated synthesis following heat shock, each sHsp displays a distinct

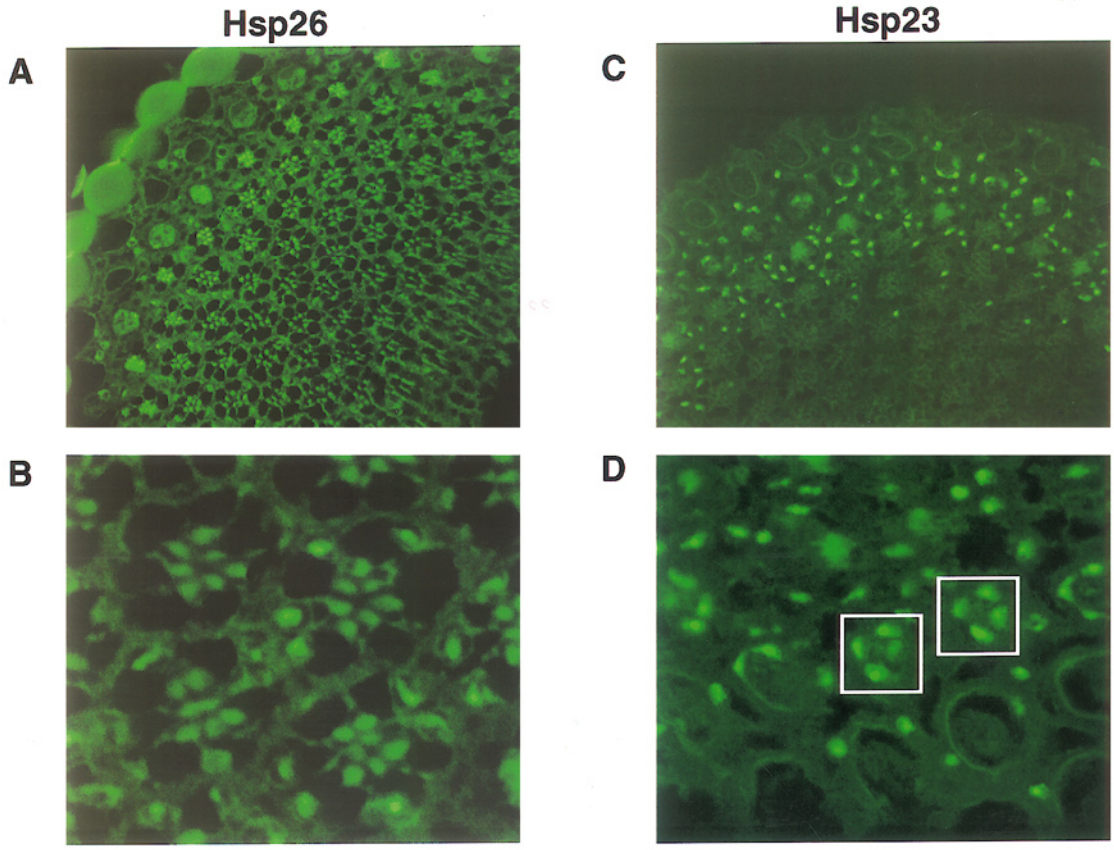

Figure 3. Localization of Hsp23 and Hsp26 in the eye of *Drosophila* by immunofluorescence microscopy using specific monoclonal antibodies. The confocal microscopy images show sections of the eye of a fly heat-shocked at 35 °C (*B* and *D* being of higher magnification than *A* and *C*). Hsp26 is seen in all photoreceptor cells and in other accessory cells of the ommatidial unit (*A* and *B*). In contrast, Hsp23 is only expressed in cone cells of the eye (*C* and *D*). Individual ommatidia exhibiting clear expression of Hsp23 in each of their four cone cells are surrounded by a white box (*D*).

pattern of expression at specific stages of development in *Drosophila* [58, 59]. The only Hsp not expressed in early development, Hsp22, sees its mRNA increase in thoraxes of old flies [60]. Whether or not this expression is driven by internal damages that accumulate during aging remains to be determined.

The expression of the sHsps has been determined either by immunological methods involving the use of antibodies recognizing specific members of the sHsp family [57, 61–63] or by promoter-driven reporter gene expression in transgenic flies [47, 64]. Hsp23 is expressed during embryogenesis in specific glial cells, tentatively identified as midline glial cells [6, 61, 65]. Immunofluorescence studies on salivary glands of *D. melanogaster* suggested that Hsp23 may be present at normal temperatures in the cytoplasm of cells from this organ [66]. Cheney and Shearn [59] confirmed the presence of Hsp23 during development by demonstrating that it was synthesized in late third instar larvae, at a time when an increase in the endogenous level of β-ecdysone hormone is observed. A hint suggesting the importance of β-ecdysone in the induction of sHsps was the observation of the strong synthesis of these proteins in tissue-culture cells or imaginal discs treated with this hormone [67–70]. It was shown that this hormonal induction is regulated by the binding of a specific transcription factor, the ecdysone receptor, to specific sequences found upstream of some of the sHsp genes (see fig. 2) [52, 71, 72]. Two such sequences found at regions

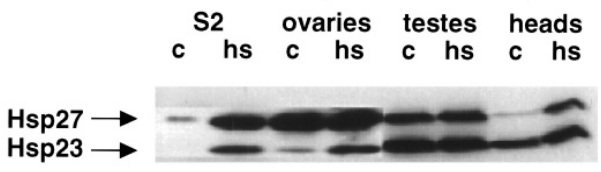

Figure 4. Immunoblot showing the expression of Hsp23 and Hsp27 prior to or after a heat shock in different tissues. Hsp27 is present under control conditions in ovaries and testes and is not further induced by heat shock. Hsp23 is induced by heat shock in ovaries but not in testes, where its level of expression remains the same. A clear induction of these two Hsps is seen in heat-shocked fly heads and S2 culture cells.

−242/−218 and −200/−181 have been identified in the promoter region of the *hsp23* gene [52]. Hsp23 continues to accumulate in pupae, where it peaks at 120 h, several days after the maximal accumulation of its mRNA [73], but is almost totally absent from 1-week-old flies [74]. In addition to sequences allowing hormonal regulation, the sHsps appear to be under the control of other *cis*-regulatory elements found upstream of these genes [46, 47, 53, 64, 75].

In young adults, Hsp23 is still present in gonads, neurocytes and glial cells of the central nervous system (CNS) [62]. In male gonads, Hsp23 is expressed constitutively in specific cells of the somatic lineage such as the cyst cells, terminal epithelial cells and epithelial cells of the seminal vesicle. However, Hsp23 also seems to be associated with filamentous structures probably related to spermatid bundles (R. Marin, S. Michaud, J. T. Westwood et al., unpublished).

Hsp26, just as Hsp23, seems to have clearly defined domains of expression during fly development. Using germline transformation with an *hsp26-lacz* fusion gene, Glaser et al. [47] identified the sites of expression of the Hsp26 fusion protein in numerous tissues (spermatocytes, nurse cells and epithelium) of larvae, pupae and adults. In situ hybridization confirmed these sites of expression of *hsp26*. Since spermatocytes consistently expressed *hsp26* during all of development, Glaser and Lis [64] further examined the promoter and identified three regions involved in spermatocyte-specific expression (−251 to −135, −135 to −85 and +11 to +632). *hsp26* mRNA was also found in nurse cells and developing oocytes of females gonads [31]. Sequences from −500 to −350 of the *hsp26* promoter were found to contain the element(s) important for female germline expression [46]. Other tissues expressing Hsp26 during development are the epithelium, proventriculus, larval brain and ventral ganglion. Hsp26 level is low in brain, but is abundant in gonads throughout larval and pupal development [62].

Throughout the larval stages, Hsp27 expression is mainly restricted to the CNS and gonads. Like Hsp23, Hsp27 can also be found in the imaginal discs of third instar larvae, correlating with a peak in ecdysone production. The minimal promoter region of *hsp27* necessary for correct spatiotemporal expression during development includes region −58/+87. Sequences upstream of this region are also necessary to increase transcription three- to fivefold. One particular element necessary for this increase in transcription has been further defined to be in the −553/−327 region [72]. In late pupae, Hsp27 is present at the top of the eye ommatidial unit but has disappeared from the eye of the newborn fly. Once again, in the adult, the protein becomes not only tissue-specific but also cell-specific within some tissues. Hsp27 is limited to a few clusters of cells in the brain and thoracic ganglion, where it must be very stable since no mRNA for Hsp27 is detected in the CNS of the adult [76].

Hsp27 is also abundant in nurse cells of ovaries of unstressed flies. Interestingly, Hsp27 is also expressed during stages 8 to 10 in central follicle cells in the posterior of the egg chamber. Subsequent to stage 10, no Hsp27 is visible in follicle cells [57]. Important regions of the *hsp27* gene directing ovarian expression were shown by Hoffman et al. [75] to be located between −986/−227 and +87/+148. In testes, Hsp27 shows prominent expression in spermatocytes but is also present in somatic cyst cells and in cells of the accessory glands (R. Marin, S. Michaud, J. T. Westwood et al., unpublished).

Tissue and developmental stage-specific intracellular localization and modifications of sHsps

A nuclear accumulation of Hsps after heat shock has been reported for many of the Hsps in different cellular systems [5]. In *D. melanogaster*, each of the small Hsps shows a distinct intracellular localization in the absence of heat stress. Hsp22 is localized in mitochondria (Tanguay et al., unpublished), while Hsp23 and Hsp26 are mainly found in the cytoplasm although in different structures (R. M. Tanguay, unpublished). Hsp27 is the only sHsp showing a nuclear localization in cultured cells both after heat shock or after induction by ecdysterone in the absence of heat stress [67, 77]. This nuclear localization can also be seen after heat shock during development [8, 17]. In contrast with its intracellular localization in cultured cells, Hsp27 is mainly cytoplasmic during development. Recently, this small Hsp was shown to have a stage-dependent localization during normal oogenesis [57]. From germarium to stage 6 of ovarian development, Hsp27 is localized in the nuclei of all cells of the germline cyst. From stage 8 and onward, Hsp27 changes its localization, becoming perinuclear and cytoplasmic. This stage-dependent intracellular localization is also observed after heat shock both in nurse cells as well as in follicle cells.

The stage-specific nuclear accumulation of Hsp27 during oogenesis is particularly intriguing. The determinants of protein shuttling betweeen the nuclear and cytoplasmic compartments are not fully clear. As no nuclear localization signals (NLS) have been defined for this Hsp, one possibility is that Hsp27 may be bound to certain nuclear proteins at these stages. A second possibility is that stage-specific post-translational modifications of this Hsp may influence its cellular localization. Phosphorylation is known to determine the karyophilic behaviour of certain proteins such as the transcription factor NFκB [78]. Hsp27 is a phosphoprotein, and its phosphorylation can be affected by the steroid hormone ecdysterone [79], by heat shock and during development [17, 62]. However, the possibility that the change in localization of Hsp27 is related to its phosphorylation state seems unlikely, as the same isoforms of this Hsp are found in early- and late-stage egg chambers [57].

The differential localization of Hsp27 during biological processes such as oogenesis suggests functional significance of the localization. We are currently examining this issue by looking at protein partners with which Hsp27 interacts at different stages of development.

Finally, the Hsps of *D. melanogaster* have also been shown to have tissue-specific post-translational modifications in the absence of heat shock [80]. Four isoforms of Hsp27 were found to be expressed in the head and testes, while only two forms were seen in ovaries. In the case of Hsp23, two isoforms were expressed in head and testes and only one in ovaries. While the nature of the post-translational modification of Hsp23 is still unknown, the distinct isoforms of Hsp27 probably result from phosphorylation as shown by their susceptibility to phosphatase and by phosphorylation of recombinant *Dm* Hsp27 by a mammalian Hsp27 kinase, MAPKAP-K2 [80]. The *Drosophila* homologue for this kinase has recently been cloned [81], but it is still unknown if this homologue is the kinase interacting with Hsp27 in vivo. Heat shock also affects the sHsp isoform distribution within the tissues. These data suggest that tissue- and Hsp-specific post-translational modifications may modulate the functions of these proteins in various cell types.

Developmental expression of the other Hsp genes

Specific members of the *hsp70* family, the Hscs, are expressed in the absence of stress at normal temperatures and show a high level of expression throughout development. Studies on *hsc4* revealed that this gene is constantly transcribed at high level in the embryo, in larvae and in adults [82, 83]. Using in situ hybridization, Perkins et al. [84] showed that *hsc4* transcripts were particularly enriched in cells undergoing rapid growth as well as in cells of tissues active in endocytosis, like the garland gland.

Contrary to the high level of Hscs under normal conditions, the *hsp70* mRNA level in noninduced state is approximately one-thousandth of the level after heat shock induction [85]. Such a low level of expression may indicate that Hsp70 does not play a crucial role in the development of the fly. The only situation where Hsp70 is detected at high level without heat shock is in the indirect flight and leg muscles of old flies [60].

Like the sHsps and the Hscs, Hsp83 has been shown to be expressed in the absence of stress both in cell culture [4, 27, 86] and in animals [87]. Hsp83 was first shown to be expressed during normal embryogenesis [55]. During metamorphosis, this expression was shown to corroborate with peaks in ecdysone titer [88]. Hsp83 was also found in pupae and young adults, but was reduced in old males [6]. The presence of *hsp83* transcripts in mature females is due to its expression in the nurse cells of the developing ovaries [31]. Elements necessary for the developmental expression of *hsp83* are found in the promoter region spanning the $-880/-170$ interval [54].

A recent interesting study also characterized the localization of *hsp83* mRNA during oogenesis and embryogenesis [89]. During oogenesis, *hsp83* mRNA is first detected in regions 2 and 3 of the germarium in nuclei of all 16 cells of the germline cyst. This expression continues until the end of stage 5, when *hsp83* mRNA is degraded. Stages 6 to 8 are totally devoid of mRNA for Hsp83, but a high level of transcription for this gene is seen in nuclei of nurse cells from stages 9 to 11. Stage 10B marks the beginning of transfer of maternal mRNA from nurse cells to the oocyte, where *hsp83* mRNA is stabilized. This maternal mRNA is then distributed throughout the early embryo from nuclear divisions 1 to 5. During cleavage divisions 6 to 8, maternal *hsp83* mRNA is concentrated at the posterior pole via a combination of generalized degradation associated with a localized protection at the posterior pole. *Hsp83* mRNA is then taken up by the pole cells. High levels of *hsp83* mRNA are present in pole cells during their migration and in the gonads of embryos, larvae and adults [31, 54]. *Hsp83* is also transcribed zygotically in the anterior third of the embryo commencing at the syncytial blastoderm stage. This expression is missing in *bicoid*$^-$ mutants, indicating that this polarity determinant may play a direct role in promoting *hsp83* developmental expression. It will be of interest to see whether the temporal pattern of expression of the Hsp83 protein is similar to its mRNA. Studies which should help understand the function(s) of this Hsp are in progress.

Developmental expression of the product of the 93D locus: Hsr ω

Locus 93D, among those activated by heat shock, stands apart due to its unique and intriguing features. It is located at 93D6-7 on chromosome 3R. The gene spans over 10 kb and includes two exons and one intron followed by a long series of tandem arrays of repeat units [90]. *Hsr* ω (heat shock RNA omega) encodes for three major mRNAs, among which two seem to be untranslated [91–93]. One of the transcripts ($\omega 1$) is nuclear, whereas the two others ($\omega 2$ and $\omega 3$) are found in the cytoplasm. Although sequence data indicate the presence of a small ORF, no protein product of the 93D locus has yet been found. Like other Hsp genes, the 93D locus exhibits a developmental regulation as well as a heat shock regulation [94, 95]. This locus can further be induced by several different agents, such as benzamide [96] and colchicine [98]. These specific agents can induce *hsr* ω transcription without inducing Hsps; it was also shown that specific situations can induce a general heat shock response without the induction of *hsr* ω (reviewed in [98]). These properties of induction clearly demonstrate that the regulation of this locus is also under elaborate mechanisms.

The levels of *hsr ω* transcripts are specifically regulated at different embryonic and larval stages [99]. Embryonic stages show fluctuating *hsr ω* mRNA levels, while second-stage larvae exhibit low transcript levels. Third instar larvae and pupae show much higher levels of the three transcripts, suggesting a role for ecdysone in the regulation of this locus as seen for other Hsps [98]. Sequences with close homology to the known EcRE (ecdysone response element) are found at −710 and −483 in the promoter region of this gene [100], while three HSEs are present at −466, −250 and −57 of the promoter region. Furthermore, two GAGA sequences usually responsible for maintaining chromatin conformation through GAGA factor binding are found at −496 and −68 [101]. *Hsr ω* mRNA is also present in restricted regions or cell types in the gonads of adult flies. In the ovaries, nurse cells exhibit a strong expression, while follicle cells and the oocyte do not seem to express *hsr ω*. In testes, *hsr ω* is mainly in the middle section of this organ. The promoter region responsible for this protein expression in ovarioles was identified in −346/0; expression in the rest of the body necessitates the presence of the −844/−346 promoter interval [100].

The exact role of the *hsr ω* locus in development and under heat shock conditions is still unknown. It was reported that the 93D locus affected synthesis and/or turnover of Hsp70 [102]; furthermore, it was suggested that the role of the cytoplasmic transcript was to monitor the transcriptional machinery, while the nuclear trnscripts may be involved in synthesis and turnover/transport of other transcripts [90]. These potential roles must be crucial, because even though this locus is dispensable for Hsps induction, it is necessary for fly survival after heat shock [92]. In addition, the fact that homologues of this gene have been found in all *Drosophila* species observed so far points toward an important function for this gene.

Multiple functions(s) for the Hsps during development or under stress?

Do Hsps perform identical function(s) in unstressed and stressed cells? The chaperone functions of the Hsp70, Hsp60 and Hsp90 members are now well documented in yeast and in mammalian systems. The major role of Hsp/Hsc70 is defined by their chaperoning activity both under normal conditions as well as during stress (reviewed in refs 103, 104). Importance of Hsp70 in thermotolerance was also demonstrated in living flies [104]. However, the expression of Hsp70 can be detrimental to growth and/or cellular division [105]. Sequestration of Hsp70 (to allow growth to continue) is made by aggregation of Hsp70 into large granules. The efficiency of this process varies at different stages of development [104].

The roles played by the members of the sHsp family are less clear. Mammalian Hsp27 has been shown to protect cells during stress [106, 107]. The protection mechanisms have been suggested to operate either in protection of signal transduction pathways or alternatively through the chaperone activity of these Hsps (reviewed in ref. 5). In *Drosophila*, the selective induction of the sHsps by ecdysterone in a hormone-sensitive cell line was found to bring about the thermotolerant phenotype in the absence of heat shock [108]. Thermotolerance was also acquired in the Chinese hamster cell line O23 transfected with *Drosophila* Hsp27 [109]. Furthermore, protection against oxidative stress was observed in COS and L929 cells expressing *Drosophila* Hsp27 [110, 111]. This protection was shown in stably transfected L929 or in NIH-3T3 cells transiently transfected with *Drosophila* Hsp27 to be mediated by an intracellular increase in glutathione levels [112].

Whether the sHsps expressed in the absence of stress perform identical functions during stress is uncertain. Although the tissue, cell and developmental specificity of expression of the small Hsps argues for cell-specific functions, it cannot be excluded that they perform more general function(s) related to either the cell cycling activity or the state of differentiation. The tissue-specific post-translational modifications of some of the sHsps and their different intracellular localization will also have to be taken into account in trying to evaluate their functions.

Finally, many questions on the induction of heat shock genes in the absence of stress remain unsolved. Whether unique or multiple control elements operate in different tissues or at different developmental stages remains largely unknown. Additional interacting proteins may be involved in activation or repression of heat shock genes in certain cell types. Factors favouring the interaction of HSF with the HSE elements may be present in a tissue or developmental stage-specific manner, altering the response of the various sHsp genes. Elucidation of these mechanisms will certainly be helpful in finding the exact function(s) of these proteins during development.

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