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Cell-specific heat-shock induction of Hsp23 in the eye of *Drosophila melanogaster*

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Abstract The expression of two small heat shock proteins (sHsp), Hsp23 and Hsp27, was examined by immunological approaches in the eye of *Drosophila melanogaster*. Neither Hsp23 nor Hsp27 is detectable in unstressed (23 °C) eyes but both proteins are induced by heat shock (35 °C). In response to heat stress, Hsp27 is expressed in all cells of the ommatidium including the cone, pigment and photoreceptor cells. However, the heat-induced expression of Hsp23 is restricted to a single cell type of the ommatidium, the cone cells, suggesting that Hsp23 is regulated by specific mechanisms acting to inhibit the expression of this polypeptide in some ommatidial cells. The cell-specific induction of Hsp23 under stress conditions does not seem to be regulated by the *Drosophila melanogaster* heat shock transcriptional factor (DmHSF). In both unstressed and stressed conditions, DmHSF is detected in all the different types of ommatidial cells where it is found associated with the nucleus.

These observations suggest that factors, other than the heat shock transcriptional factor, are involved in regulating the expression of the *hsp23* gene under stress conditions.

INTRODUCTION

The small α-crystallin-related, heat shock-induced genes of *Drosophila melanogaster* that encode the four main small heat shock proteins (sHsps), Hsp22, Hsp23, Hsp26 and Hsp27, belong to a family of genes whose activation involves complex regulatory mechanisms. In this system, the synthesis of the sHsps is induced by heat shock (Arrigo 1980; Vincent and Tanguay 1982), by the molting hormone ecdysone (Ireland et al 1982; Sirotkin and Davidson 1982), as well as during the development of the fly, in the absence of stress. Multiple *cis*- and *trans*-regulatory transcription elements have been shown to control the expression of members of this family of genes (Cohen and Meselson 1985; Glaser and Lis 1990). Thus, the genes coding for Hsp26 and Hsp27 are expressed in nurse cells during oogenesis and in embryos (Zimmerman et al 1983; Pauli et al 1990). Glaser et al (1986) showed that the gene coding for Hsp26 was constitutively expressed in germ line cells (spermatocytes and nurse cells), as well as in neurocytes of the central nervous system and in the epithelium. Hsp27 has also been reported to be expressed in the brain and in gonads of *Drosophila* adults in the absence of stress (reviewed in Arrigo and Tanguay 1991). In addition to having their own tissue-specific pattern of expression in unstressed cells, each sHsp appears to be specifically expressed in distinct regions of the same tissue (Marin et al 1993).

Drosophila sHsps show homology with the vertebrate eye lens protein α-crystallin (Ingolia and Craig, 1982), and this has led to the suggestion that α-crystallin has evolved from a Hsp-like ancestor. The conserved α-crystallin domain, found in *Drosophila* sHsps and in mammalian Hsp27, consists of 83 residues in the second half of the protein (Southgate et al 1983). It is becoming increasingly clear that α-crystallin and the sHsps share important properties that may be relevant for their common functions as stress proteins (reviewed in De Jong et al 1993). These properties include stress-inducibility and intracellular relocalization upon stress (Arrigo and Tanguay 1991; Klemenz et al 1991; Voorter et al 1992). α-crystallin and some sHsps have also been reported to interact with

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plasma membranes and cytoskeletal elements (Maisel and Perry 1972; Miron et al 1991; Lavoie et al 1993).

A particular characteristic of the sHsps is their redistribution inside or around the nucleus during heat shock. This was first observed in *Chironomus* salivary gland cells (Vincent and Tanguay 1979), and in *Drosophila* cultured cells (Arrigo 1980; Vincent and Tanguay 1982; Beaulieu et al 1989). It has been suggested that this phenomenon is modulated by the metabolic state and the degree of thermoresistance of the cells (Rossi and Lindquist 1989; Lavoie et al 1993). Another property of the sHsps under stress conditions is their capacity to form super-aggregated structures. This has been particularly well described for mammalian Hsp27 (Mehlen and Arrigo 1994), and for α-crystallins (Klemenz et al 1991). Such a phenomenon occurs in lens cells during aging, as well as in individuals developing cataract, a pathology resulting in the opacification of the lens (Seizen et al 1978).

The function of the sHsps is still unclear, although there is mounting evidence that they are engaged in protein-protein interactions. Several studies of mammalian Hsp27 have suggested that Hsp27 may be a necessary component of a signaling pathway between mitogens and actin polymerization at the membrane (Miron et al 1991; Lavoie et al 1993). The sHsps have also been shown to be involved in cell protection against thermal shock (Landry et al 1989; Rollet et al 1992; Mehlen et al 1993; for a review, see Arrigo and Landry 1994).

The transcriptional control of *Drosophila* heat shock genes under stress conditions is mediated by a heat shock transcriptional factor, HSF. The heat-inducible binding of HSF to heat shock elements (HSEs) found upstream of all heat shock genes appears to be a major regulatory step in the pathway leading to heat shock gene activation (for a review see Wu et al 1994). A single HSF has been described in *Drosophila melanogaster* (DmHSF; Wu et al 1987). DmHSF is found as an inactive monomer in normal cells, and is activated through a conformational change to a trimeric structure in response to stress by a still unknown mechanism (Westwood et al 1991; Westwood and Wu 1993).

Here we report on the heat-induced patterns of expression of Hsp27 and Hsp23 in the eye of *Drosophila* adults after heat shock. It has been generally accepted that the four main sHsps of *Drosophila* are coordinately induced in response to heat shock. However, we have observed that the heat shock induction of Hsp23 is restricted to a single type of ommatidial cells (the cone cells) while the expression of Hsp27 is induced in all cell types of the eye (cone, pigment and photoreceptor cells). The absence of expression of Hsp23 in response to heat shock does not seem to be related to the HS transcription factor as DmHSF is shown to be present in all cells of the ommatidial unit where it associates with nuclei under stress conditions. This suggests that the induction of Hsp23 during heat shock may be regulated by cell-specific mechanisms operating independently of, or in addition to, HSF activation.

RESULTS AND DISCUSSION

The *Drosophila* compound eye consists of some 800 unit eyes (facets or ommatidia), arranged in a precise hexagonal array (Ready et al 1976; reviewed in Dickson and Hafen 1993; and in Wolff and Ready 1993). Each ommatidium includes a fixed number of cells: eight photoreceptors (numbered R1 through R8, in a trapezoid pattern), four cone cells, and six pigment cells that optically insulate the unit. Each photoreceptor has a rhabdomere produced by multiple unfolding of the cell membrane. The arrangement of the pigment cells is also highly regular: two primary pigment cells around the four cone cells, six secondary pigment cells surrounding the photoreceptor group, and a tertiary pigment cell at the posterior end of each horizontal face (see schematic illustration in Fig. 2).

Hsp23 and Hsp27 are induced by heat shock in the eye of *Drosophila*

The expression of Hsp23 and Hsp27 in the eye of *Drosophila* was tested by immunoblotting using monoclonal antibodies. As shown in Figure 1, both of these sHsps are expressed in the brain of unstressed flies. After a heat shock, the level of expression of Hsp27 increases while Hsp23 only shows a slight induction. In the eye, neither of these sHsps is expressed in the absence of heat shock. However, following 1 h of heat shock at 35 °C, Hsp23 and Hsp27 are induced as shown in this immunoblot.

Hsp23 and Hsp27 show distinct cell-specific expression in the ommatidium

Hsp27, Hsp26 and Hsp23 have previously been reported to be expressed in some regions of the brain in the absence of stress. To see if the heat-inducible expression of Hsp23 and Hsp27 was uniform within the eye structure, longitudinal sections of fixed eyes from non-heat-shocked (23 °C) and heat-shocked (35 °C, 1 h), flies were immunostained with the anti-Hsp23 and the anti-Hsp27 antibodies. As shown in the immunohistochemical results of Figure 2A, no staining was observed with the anti-Hsp27 or with the anti-Hsp23 antibody on non-heat-shocked eyes, confirming the immunoblotting results of Figure 1. Surprisingly, after heat shock, these two sHsps showed a cell-specific pattern of expression in the eye. Thus, Hsp23

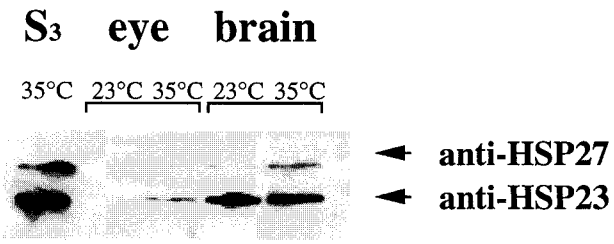

Fig. 1 Expression of Hsp23 and Hsp27 in the eye and brain of *Drosophila* adults. The eyes and brains from cold-anesthetized flies of the Oregon-R stock of *Drosophila melanogaster* raised at 23 °C were manually dissected in phosphate buffer saline (PBS) (135 mM NaCl, 5 mM KCl, 8 mM Na_2HPO_4, 15 mM KH_2PO_4 pH 7.0). For heat shock treatments, flies were exposed for 1 h at 35 °C in an Eppendorf tube. Dissected tissues from 15 non-heat-shocked or heat-shocked flies were homogenized directly in 30 µl of sodium dodecyl sulfate (SDS) lysis buffer (0.075 Tris-HCl (pH 6.8), 2.3% (w/v) SDS, 5% (v/v) bromophenol blue), and heated at 95 °C for 5 min. Protein samples from heat shocked *Drosophila* S3 cells were prepared as previously described for Kc cells in Marin et al (1993), and used as a control of the specificity of the anti-sHsp antibodies. Proteins were separated on one-dimensional SDS polyacrylamide 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). These conditions resulted in a better resolution in the region of the sHsps. The proteins were then electrophoretically transferred to Immobilon-P membranes (Millipore, Bedford, MA). For immunoblotting, monoclonal antibodies against *Drosophila* Hsp83 (3E6, Carbajal et al 1990), Hsp27 (2C8), and Hsp23 (7B12, Marin et al 1993), and a polyclonal anti-*Drosophila* HSF (943bb) antibody (Westwood et al 1991), were diluted in non-fat dry milk (Johnson et al 1984) at dilutions of 1 : 20 000, 1 : 200, 1 : 100, and 1 : 5000, respectively. Primary antibodies were detected by incubation of the membrane with a horseradish peroxidase-conjugated secondary anti-mouse or anti-rabbit IgG (ECL, Amersham, UK) diluted 1 : 5000 in non-fat dry milk, and processed for detection according to the manufacturer's protocol.

was only detected in cone cells (Fig. 2B, outlined arrow), whereas Hsp27 was observed to be expressed in all cell types of the eye (Fig: 2C and C'). Hsp27 was also expressed in the eight photoreceptors (R1-R8) of the ommatidial unit (some of these are visible in Fig. 2C and C', the basal nuclei of R8 cells not being visible at this level of sectioning). Both Hsps seemed to be associated with the nuclei of cells.

Traditionally, the induction of the main sHsp genes of *Drosophila* has been considered to be coordinated in response to heat shock. The present observation that heat shock produces a differential response of the sHsp genes in some cells within the eye was unexpected and raises a number of interesting questions about the regulation of expression of the members of this family of genes. It is interesting to note that while the expression of Hsp27 is exclusively related to stress conditions in the eye of adults, this polypeptide accumulates during the development of the fly in the absence of stress. Thus, Hsp27 has been observed in the different ommatidial cells of unstressed late pupae, when imaginal eye disc cells of this tissue stop dividing and begin to differentiate, but it is not further detected after complete differentiation of the disc (Pauli et al 1990). These observations suggest that different mechanisms control the synthesis of the sHsps throughout fly development.

Unexpectedly, Hsp23 was found to be heat shock-induced exclusively in cone cells. In other immunohistochemical experiments in which flies were previously heat-shocked for 1 h at 35 °C and left to recover for 2 h at room temperature, the same cone-cell-specific pattern of expression of Hsp23 in the ommatidium was observed (data not shown), indicating that the absence of this sHsp from photoreceptor and pigment cells is not due to a late expression of the *hsp23* gene in these two types of cells. In heat-shocked brains and gonads, Hsp23 has been found to be present in two distinct post-transcriptional modified forms both of which are recognized by the anti-Hsp23 antibody (Marin et al 1996). Thus, the possibility that a modified form of Hsp23 expressed in these cells is not recognized by this antibody is unlikely. In addition, no traces of this polypeptide are visible in pigment or photoreceptor cells even after long periods of incubation with the antibody. The absence of Hsp23 staining on these two ommatidial cell types was also obtained when a FITC-conjugated secondary antibody was used to detect the anti-Hsp23 antiserum (data not shown).

DmHSF is expressed in all cells of the ommatidium

It is generally accepted that, in *Drosophila*, heat-inducible transcription is mediated by an unique HSF that binds to the HSEs, and activates transcription of heat shock genes in vitro (Amin et al 1988). Binding of the HSF to DNA is low, or undetectable, under normal conditions, and the HSF is localized in the nucleus as an inactive oligomer. A heat shock stimulus is required to convert the HSF to an active multimer form that binds with high affinity to the HSE in the nucleus of cells (reviewed in Wu et al 1994). The heat shock induction of Hsp23 in a restricted number of cells of the ommatidia was unexpected, as most Hsps are believed to be coordinately induced after heat shock. We, therefore, looked for the presence of the HSF in non-heat-shocked (23 °C) and heat-shocked (35 °C, 1 h) *Drosophila* eyes and brains, using an anti-HSF antibody (Westwood et al 1991). In the immunoblot, the anti-HSF antibody recognized a 110 kDa band in both protein extracts (Fig. 3). This band, which corresponds to the apparent mass (110 kDa) of the HSF natural protein (Wu et al 1987), was present in both unstressed and stressed eyes and brains. After heat shock, the HSF migrated on gels at a slightly higher molecular weight than in unstressed ones. This difference in migration may be due to post-translational modifications of the natural protein occurring under stress conditions. To test if the 35 °C heat shock on the flies was effective, an anti-Hsp83 antibody was also used in these experiments (Fig. 3,

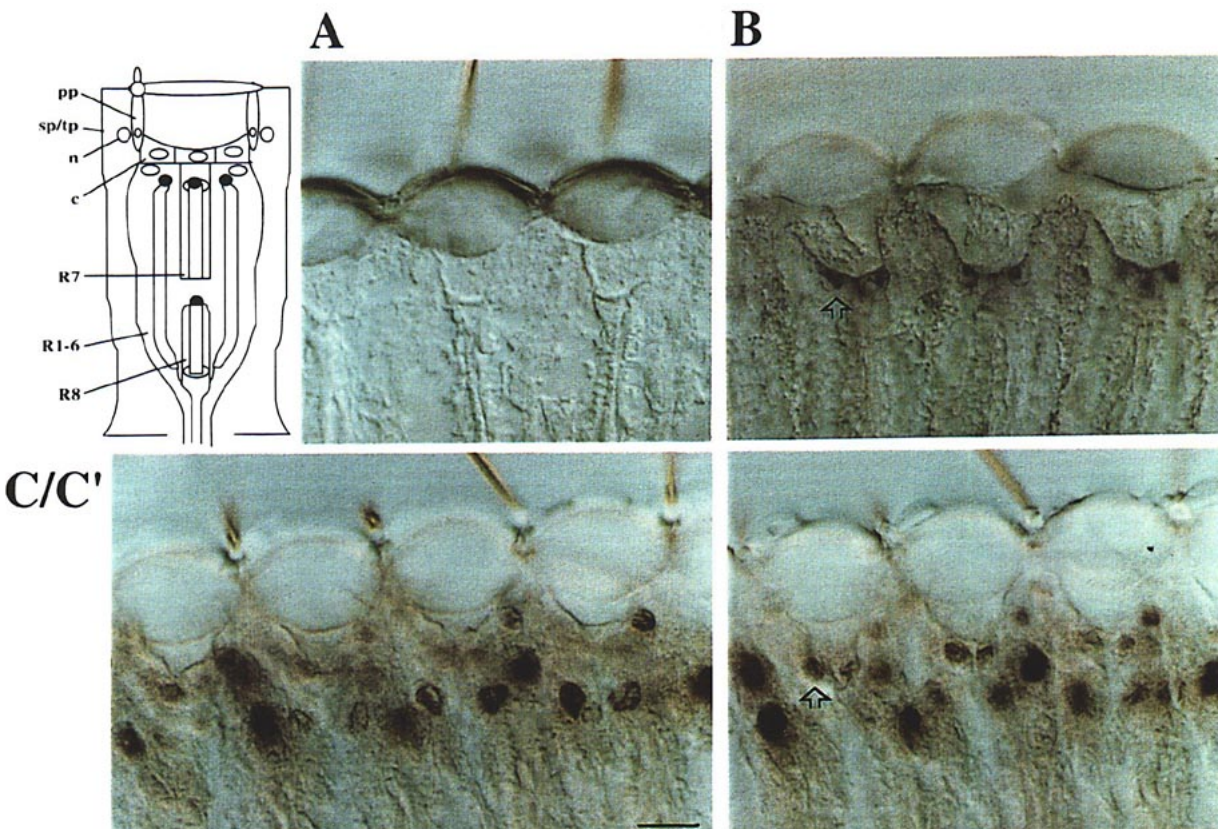

Fig. 2

bottom). *Drosophila* Hsp83 has previously been shown to be either constitutively expressed or heat shock-induced in cells.

We next examined the distribution pattern of the HSF in the different cells of the ommatidial unit. Figure 4 shows tangential eye sections from heat-shocked flies stained with the anti-DmHSF antibody. The *Drosophila* HS transcription factor was present in the nuclei of all

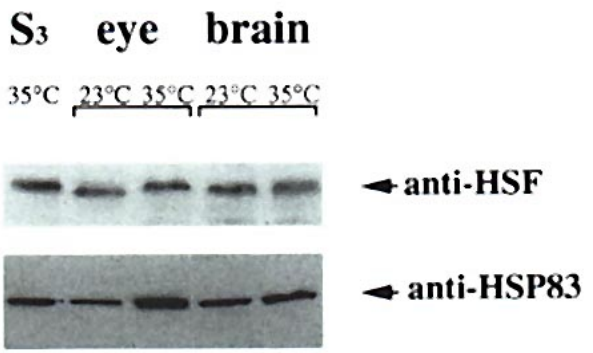

Fig. 3 Expression of *Drosophila* HSF in the eye and the brain of flies. Eyes and brains proteins from unstressed (23 °C) and stressed (35 °C) adult flies, and heat-shocked *Drosophila* S3 cells separated on SDS-PAGE were blotted with a polyclonal anti-*Drosophila* HSF antibody. A monoclonal anti-Hsp83 antibody was also used as an internal control for the heat shock treatment (see also legend of Fig. 2).

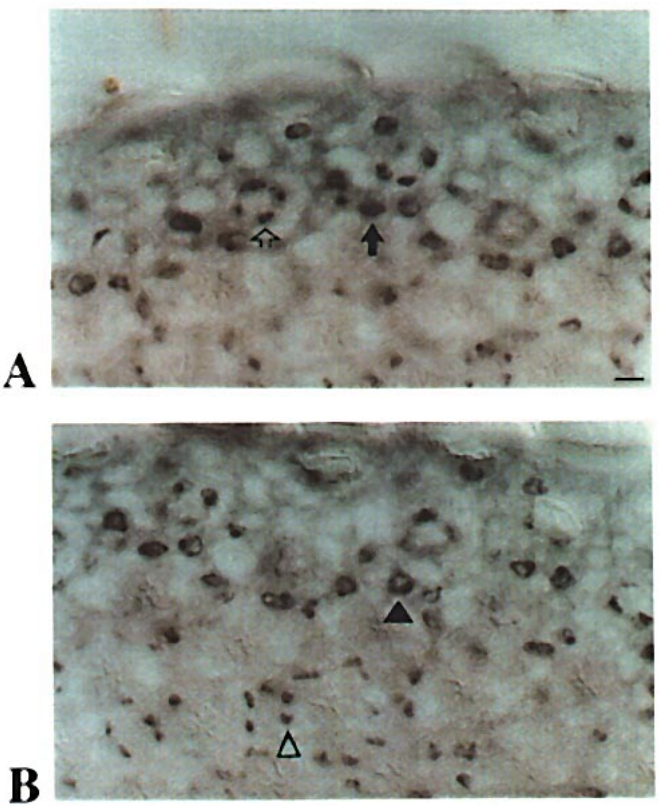

Fig. 4

cells of the ommatidium including the cone (Fig. 4A, outlined arrow), photoreceptor (Fig. 4B, outlined arrowhead) as well as pigment (Fig. 4A, solid arrow; Fig. 4B, solid arrowhead) cells. In unstressed flies, the HSF was also observed to be associated with the nuclei of all the different ommatidial cells (data not shown). These results make unlikely the possibility that the absence of Hsp23 from pigment and photoreceptor cells could be related to the relative amounts of HSF present in each type of ommatidial cells.

Thus, the absence of Hsp23 expression in some specific cells of the eye may be due to the presence of other factors repressing this sHsp at the transcriptional or post-transcriptional level. Different scenarios may account for these observations. First, at the transcriptional level, specific events involving the lack of binding of the HSF to the Hsp23 promoter could be taking place. Previous studies on the Hsp23 promoter have provided evidence for the presence of at least four distinct regions containing functional HSEs located 5′ upstream of the promoter of the *hsp23* gene (Mestril et al 1986). Several of these HSEs, dispersed in some hundred base pairs, are necessary for the complete expression of the *hsp23* (Pauli et al 1986). For some reason, the sequences necessary for the heat shock expression of this polypeptide may be blocked in photoreceptor and pigment cells of the eye, preventing the binding of the HSF.

Alternatively, the absence of expression of Hsp23 could be related to the binding of the GAGA factor (GAF) (Wu 1984; Thomas and Elgin 1988; Soeller et al 1993) to the *hsp23* promoter. GAF is known to bind to specific GAGA elements in the promoter, this event being critical for heat shock inducibility (reviewed in Fernandes et al 1994). At least in the case of the *Drosophila hsp26* promoter, GAGA boxes reside adjacent to the HSEs, and are flanking the only nucleosome positioned in this promoter (Wall et al 1995). The absence of GAF in the promoter region of some ommatidial cells could inhibit the chromatin rearrangements necessary for the accessibility of the HSEs to the HSF, resulting in the inhibition of Hsp23 synthesis.

A third possibility is that other putative HSFs specific for Hsp23 may be acting in the induction of this polypeptide upon stress conditions, as in other eukaryotes a family of HSFs has been characterized (two for mouse and humans, three for chicken) (reviewed by Wu et al 1994). Concerning this hypothesis, the absence of Hsp23 in pigment cells and photoreceptors could be explained by the inactivation of this potential *hsp23*-specific HSF in these two cell lines. Finally, additional factors for the modulation of Hsp23 expression may be acting at the post-transcriptional level.

The role of Hsp27 in stressed cells of the ommatidial unit may be to preserve the original shape of the cells, thereby preventing the cytoplasm and the nuclear material along with it from flowing, for example, into the axons of photoreceptors or into the basal regions of pigment cells. On the other hand, Hsp23 may have functions specific to heat-shocked cone cells. As these cells are responsible for secreting the lens system, Hsp23 could be implicated in the protection against the formation of aggregates that could damage the lens under stress conditions. This phenomenon has been previously observed in humans, where the super-aggregated structures of α-crystallins produce cataracts (Seizen et al 1978).

Future studies should bring further understanding of the mechanisms regulating cell-specific expression of the different sHsps, and their role during development as well as under stress conditions. It is possible that other HS transcription factors or other mechanisms operating at the post-transcriptional level may be important in the control of the expression of specific *Drosophila* sHsp.

Fig. 2 Immunolocalization of Hsp23 and Hsp27 in the different cells of the ommatidium. Immunostaining of longitudinal eye sections from non-heat-shocked (A, control for both Hsp23 and Hsp27), and heat-shocked (B, C and C') *Drosophila* adults. B shows the four cone cells of the ommatidium stained with the anti-Hsp23 antibody, and C and C' represent the same eye section but stained with the anti-Hsp27 antibody and viewed at two different focus positions. The nuclei of some primary and secondary pigment cells, and R1 to R6 photoreceptor cells are visible. The different ommatidial cells are represented in the schematic eye illustration: cone cells (c, marked with outlined arrows in B and C'); primary (pp), secondary (sp), and tertiary (tp) pigment cells; photoreceptor cells (R1-R8). Bar: 2 μm.

For immunohistochemical staining, *Drosophila* heads were fixed and dehydrated as previously described (Marin et al 1993). Heads embedded in Tissue Prep (Fisher Scientific) were cut longitudinally in 8-μm-thin sections with a microtome knife (Leitz Wetzlar GMBH, Wetzlar, Germany), and the sections deposited on microscope slides previously treated with 1% Bacto Gelatin (GIBCO, Grand Island, NY) in water. Sections were rehydrated through a decreasing ethanol series and washed in phosphate buffer saline (PBS) prior to incubation at room temperature with the specific primary antibodies (7B12, 2C8 and 943) diluted 1 : 2, 1 : 5 and 1 : 100 respectively 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 with a biotin-labeled secondary antibody (Vectastain Kit, Vector laboratories, Mississauga, Ontario) diluted 1 : 200 in PBB. The incubation was carried out for 1 h at room temperature. The staining reactions were developed with 0.4 mg/ml of diaminobenzidine (DAB) and 0.03% hydrogen peroxide to get a brown staining. For a blue staining, 8% (w/v) $NiCl_2$ was also added to the reaction. The sections were examined using a Leitz Microscope. No staining was detected when primary antibodies were omitted.

Fig. 4 Expression of HSF in *Drosophila* stressed eyes. Pattern of intracellular localization of the HSF in stressed (35 °C, 1 h) cells of the ommatidium. Tangential sections of the retinal array stained with the 943 (HSF) antibody. Two different focal planes are shown to visualize the different ommatidial cells (indicated with arrows). Top: cone (outlined arrow) and primary pigment (solid arrow) cells. Bottom: photoreceptors (outlined arrowhead) surrounded by the secondary pigment cells (solid arrowhead). Bar: 2 μm.

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