Tissue-specific Expression of the Heat Shock Protein HSP27 during *Drosophila melanogaster* Development

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Abstract. The alpha-crystallin-related heat shock (stress) protein hsp27 is expressed in absence of heat shock during *Drosophila melanogaster* development. Here, we describe the tissue distribution of this protein using an immunoaffinity-purified antibody. In embryos, hsp27 translated from maternal RNA is uniformly distributed, except in the yolk. During the first, second, and early third larval stages, hsp27 expression is restricted to the brain and the gonads. These tissues are characterized by a high level of proliferating cells. In late third instar larvae and early pupae, in addition to the central nervous system and the gonads, all the imaginal discs synthesize hsp27. The disc expression seems restricted to the beginning of their differentiation since it disappears during the second half of the pupal stage: no more hsp27 is observed in the disc-derived adult organs. In adults, hsp27 is still present in some regions of the central nervous system, and is also expressed in the male and female germ lines where it accumulates in mature sperm and oocytes. The transcript and the protein accumulate in oocytes since the onset of vitellogenesis with a uniform distribution similar to that found in embryos. The adult germ lines transcribe hsp27 gene while no transcript is detected in the late pupal and adult brain. These results suggest multiple roles of hsp27 during *Drosophila* development which may be related to both the proliferative and differentiated states of the tissues.

THE heat shock or stress response results in the preferential synthesis at elevated temperatures of a few polypeptides, the heat shock proteins (hsps)[1] (reviewed in Schlesinger et al., 1982; Nover, 1984; Lindquist, 1986). In *Drosophila melanogaster*, the genes encoding several of the major hsps are also expressed during fly development in the absence of external stress (Sirotkin and Davidson, 1982; Mason et al., 1984; and recently reviewed by Bond and Schlesinger, 1987; Pauli and Tissières, 1989). The major exception is hsp70 whose expression is almost completely dependent on a heat shock (Velazquez et al., 1983; Mason et al., 1984). However, at least three closely related genes are active during several stages of the *Drosophila* development (Ingolia and Craig, 1982*a*; Craig et al., 1983; Palter et al., 1986).

The low molecular weight heat shock proteins hsp22, hsp23, hsp26, and hsp27 are encoded by four of the seven heat-inducible transcription units clustered within 15 kb of DNA at the chromosomal locus 67B (Petersen et al., 1979; Craig and McCarthy, 1980; Corces et al., 1980; Wadsworth et al., 1980; Voellmy et al., 1981; Ayme and Tissières, 1985). The protein products of the three other genes called genes 1, 2, and 3 (Southgate et al., 1985) have not so far been identified. The RNA transcribed from these genes were first observed at particular stages of development (Sirotkin and Davidson, 1982). The seven genes can be separated in two families according to their structural properties. Six of them compose the first group: hsp22, 23, 26, 27, and genes 1 and 3. These genes have a conserved domain of ~50–80 amino acids which is 35–50% homologous to a region of mammalian lens proteins, the alpha-crystallins (Ingolia and Craig, 1982*b*; Southgate et al., 1983; Ayme and Tissières, 1985; Wistow, 1985; Pauli and Tonka, 1987). Gene 2, the only member of the second family, does not resemble any of the other genes of this locus and is also the least heat inducible of the seven genes of locus 67B (Pauli et al., 1988).

Several studies describe the developmental expression of the low molecular heat shock genes (Sirotkin and Davidson, 1982; Cheney and Shearn, 1983; Mason et al., 1984; Ayme and Tissières, 1985; Arrigo, 1987; Arrigo and Pauli, 1988; Pauli et al., 1989). It was shown that the amounts of transcripts which accumulate are extremely different from one gene to another. For instance, at the beginning of the pupal stage, the hsp23 mRNA is very abundant, while the hsp22 message is almost undetectable; the five other genes are expressed at intermediate levels. It has been proposed that the molting hormone β-ecdysone is directly responsible for most of their developmental expression since the hormonal treatment of tissue culture cells or isolated imaginal discs induces the transcription of these genes (Ireland and Berger, 1982;

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1. *Abbreviation used in this paper*: hsp, heat shock protein.

Ireland et al., 1982; Vitek and Berger, 1984). The involvement of β-ecdysone is also supported by genetic analyses (Thomas and Lengyel, 1986; Dubrovsky and Zhimulev, 1988). Recently, in a study comparing the accumulation of hsp23 and hsp27 mRNAs and polypeptides, we observed that the maximal abundance of these hsps occurred when their corresponding mRNAs had almost completely disappeared (Pauli et al., 1989). These observations suggest a complex regulation of the expression of the small hsps during *Drosophila* development.

Only two studies have addressed the question of the tissue specificity of the developmental expression of the low molecular weight hsps. Using in situ hybridization to tissue sections, the expression of hsp26 and 27 has been shown in the ovarian nurse cells, with the transcripts accumulating in the developing oocyte (Zimmerman et al., 1983). With both in situ hybridization and P-element transformation of a fusion gene, hsp26 expression was found in spermatocytes, nurse cells, and during the third larval and early pupal stages in epithelium, imaginal discs, proventriculus, and neurocytes (Glaser et al., 1986). Here, we report the spatial and temporal expression of hsp27 during *Drosophila* development analyzed by immunohistology and in situ hybridization.

Materials and Methods

Expression of hsp27 in Bacteria

Plasmid 17955 (Southgate and Voellmy, 1983) was digested with Sma I and Sal I. The 1.2-kb fragment containing the hsp27 coding region plus 3′ sequence was isolated by gel electrophoresis and cloned into plasmid pEx1 (Stanley and Luzio, 1984) digested by Sma I and Sal I. The whole hsp27 coding region except the 9 NH_2-terminal amino acids was cloned in phase with a cro-beta-galactosidase gene which is controlled by the PR promoter of bacteriophage lambda (Stanley and Luzio, 1984). The resulting plasmid was transformed in *Escherichia coli* strain pop2136 which contains a thermolabile lambda repressor (Genofit, Geneva, Switzerland). To avoid the expression of the hybrid protein, growth was at 30°C. Large scale preparations of the hybrid protein were done as follows. 1 ml of overnight culture was added to 100 ml L-Broth containing 100 μg/ml ampicillin, and grown at 30°C to an optical density of 0.2 at 550 nm (~5 h). To induce the expression of the hybrid protein, the culture was shifted to 42°C for 1.5–2 h. Bacteria were collected by centrifugation, resuspended in 10 ml cold solution A (1.5% sucrose, 0.05 M Tris-HCl, pH 8.5, 0.05 M EDTA), and centrifuged again. The pellet was resuspended in 3 ml solution A and 0.3 ml of 10 mg/ml lysozyme in solution A was added. After 15–30 min on ice, 3 ml of solution B (0.1% Triton X-100, 0.05 M Tris-HCl, pH 8.5, 0.05 M EDTA) was added, mixed by inversion, and incubation was continued for 15 min on ice. After centrifugation (10 min, 10,000 rpm, rotor HB-4 [Sorvall Instruments Div., Newton, CT], 4°C), the pellet was resuspended in 3 ml cold solution C (0.15 M NaCl, 0.01 M Tris-HCl, pH 7.5). The DNA was broken by sonication (until the viscosity had strongly decreased) and the solution was centrifuged again. The resuspension, sonication, and centrifugation were repeated three times with 3 ml solution C containing an increasing concentration of urea:2, 4, and 5 M. The pellet of the 5 M step contained most of the hybrid protein, which was ~95% pure as judged by Coomassie blue staining of SDS-polyacrylamide gels. The 5 M pellet was resuspended by sonication in 0.5 ml solution C containing 7 M urea, and 0.15 ml of 5× sample buffer (10% glycerol, 15% SDS, 62.5 mM Tris-HCl, pH 6.8, 25% 2-mercaptoethanol, 0.3% bromophenol blue) was added. After heating 5 min in boiling water, the preparation was stored at −20°C.

Affinity Purification of the Antiserum, Immunoprecipitation, and Immunoblotting

The hybrid protein of a 100-ml culture was loaded in a single well (10 cm large) on SDS-7.5% polyacrylamide gels. Electrophoresis was performed for twice the time necessary for the bromophenol blue to migrate out of the gel. The position of the hybrid protein was identified by the white precipitate obtained after immersion of the gel in cold 1 M potassium acetate. The band was cut and transferred by electrophoresis onto nitrocellulose filter. The filter was then washed with PBS (135 mM NaCl, 5 mM KCl, 8 mM Na_2HPO_4, 15 mM KH_2PO_4, pH 7.0) containing 0.5% BSA, cut in small squares, and transferred into a test tube (Eppendorf, Hamburg, West Germany). After several washings with PBS, the nitrocellulose pieces were incubated with 0.5 ml of crude hsp27 antiserum (Arrigo and Pauli, 1988) at 4°C with occasional agitation for at least 6 h. The serum was removed and the filters were washed with 1 ml PBS 6–10 times for 5 min at room temperature. Elution of the bound antibodies was done by incubation of the nitrocellulose fragments with 0.4 ml of 0.2 M glycine, pH 3.0, during 1 min (vortex), followed by neutralization with 24 μl of 1 M Tris-HCl, pH 8.8. The eluate was transferred to a new Eppendorf tube and fragments were washed with 0.1 ml PBS which was then pooled with the eluate. To stabilize the purified immunoglobulins, BSA was added to 0.1% final concentration. The nitrocellulose pieces were stored at −20°C and reused one to two times. The specificity of the purified antibody was tested by immunoblotting (dilution 1:100) as described previously (Arrigo and Pauli, 1988).

For immunoprecipitation, KC tissue culture cells (Echalier and Ohanessian, 1970) were heat treated for 1 h at 37°C and then labeled for 5 h at 20°C with [^{35}S]methionine (300–500 Ci/mmol; Amersham International, UK). This procedure allowed most of the hsps to concentrate in the cytoplasm after the heat shock. The cells were lysed in a buffer containing 10 mM Tris-HCl, pH 7.4, 50 mM NaCl, 1 mM $MgCl_2$, and 0.1% NP-40 and the cytoplasmic fraction was incubated with 10 μl of either immune or nonimmune sera and then with 100 μl of protein A–Sepharose (Pharmacia Inc., Uppsala, Sweden) as already described (Arrigo et al., 1985).

Embryos, larvae, pupae, and adult flies were lysed in Laemmli sample buffer. Glass beads (0.5 mm in diameter) were used to pulverize the larvae, pupae, and adult flies. After thorough homogenization (no debris of insect visible), the lysate was heated to 100°C for 3 min. Pools of more than 10 individuals of the different stages of the developing insect were collected. Electrophoresis was performed in SDS-polyacrylamide gels containing 12% acrylamide and 0.3% bis-acrylamide as already described (Arrigo, 1987). In each case equal amount of protein, corresponding to a fraction of the pooled insects, were loaded per lane of the gel. Immunoblot analysis was performed as already described (Bowen et al., 1980; Zeller et al., 1983). Proteins were electrophoretically transferred for 18 h at 50 V. Affinity-purified hsp27 antiserum (Arrigo and Pauli, 1988) was used at a dilution of 1:100 in PBS containing 1% BSA. Detection of the primary antibody was performed with 5 μCi/ml of [^{125}I] protein A (Amersham International, 37 mCi/mg). After washing the membranes with 0.5 M or 1 M LiCl, autoradiographs were exposed at −70°C on Fuji X-ray film with the aid of fast tungstate intensifying screens (Ilford Ltd., Basildon, Essex, England).

Immunohistology

Whole embryos were prepared as follows. A 24-h collection at 24°C was dechorionated and washed as described by Karr and Alberts (1986). About 500 embryos were transferred in a 5-ml glass test tube containing 0.9 ml of 100 mM Pipes, pH 6.9; 2 mM $MgSO_4$; 1 mM EGTA. After addition of 0.1 ml 37% formaldehyde and 2 ml heptane, embryos were shaken gently during 1 min and then incubated for 20 min at room temperature with gentle rotation. Fixed embryos were at the interface. The lower aqueous phase was removed with a Pasteur pipette and 2 ml methanol was added. Embryos were shaken vigorously during 30–60 s. Devitellinized embryos sank. The heptane and methanol was pipetted off and embryos were washed first with 3 ml methanol, then twice with BBT (55 mM NaCl, 40 mM KCl, 15 mM $MgSO_4$, 5 mM $CaCl_2$, 10 mM Tricine, 20 mM glucose, 5 mM sucrose, 0.05% azide, 0.1% BSA, 0.1% Triton X-100, pH 7.0), and finally incubated three times for 15 min in BBT with gentle rotation. They were incubated overnight at 4°C with rotation in 0.4 ml of a 1:25 dilution in BBT of the purified antibody. They were then rinsed twice in PBT (PBS containing 0.1% BSA and 0.1% Triton X-100) and washed in PBT four to five times for 30 min at room temperature. Long washings are necessary to fully remove the azide. They were then incubated for 6–8 h at 4°C in 0.5 ml of a 1:1,500 dilution of a goat anti-rabbit IgG coupled to peroxidase (Amersham International). After four washings of 30 min each in PBT, embryos were stained for 5–10 min at room temperature with 0.5 ml PBT containing 0.5 mg/ml 3,3′ diaminobenzidine (Sigma Chemical Co., St. Louis, MO) and 0.03% H_2O_2. Progress of the staining was followed under a binocular microscope. The reaction was stopped by dilution and washing with PBT. Embryos can be stored in PBT for several weeks at 4°C. They were mounted under coverslips and examined with a Leitz orthoplan microscope (magnifications 125× and 200×). If the first or the second antibody was omitted

or if preimmune serum was used, no staining was observed even after a 30-min reaction (not shown).

8-μm frozen tissue sections were prepared essentially as described by Hafen and Levine (1986) except that the slides were coated with poly-L-lysine. Sections were conserved in ethanol at 4°C. For immunohistology, slides were washed in jars with BBT three to four times for 15 min. 50 μl of 1:25 dilution of antibody in BBT were spotted on the sections and covered with a coverslip. Slides were incubated overnight at 4°C in a moist chamber. Washings were as described for whole embryos. The second antibody was added in 75 μl under a coverslip and peroxidase staining was with 100 μl spotted onto the sections. Histological coloration was performed with 5% Giemsa in 10 mM phosphate buffer, pH 6.8, for 1–2 min as described by Braun et al. (1989), and sections were mounted under coverslips with a drop of Entellan (Merck A. G., Darmstadt, West Germany). The peroxidase reaction gives a red-brown coloration which is darkened in those cells strongly stained by the Giemsa tissue dye.

In Situ Hybridization

Sections were prepared as above, dehydrated, air-dried, and pretreated for hybridization at room temperature by two incubations of 2 min in 2× SSC, and acetylated 10 min in 225 ml 0.1 M Triethanoamine, pH 8.0, containing 560 μl of anhydrid acetic. They were washed in 2× SSC and then PBS, dehydrated, and air-dried. Antisense ^{35}S-RNA probe specific for hsp27 was synthesized from an Sma I–Ava II (position +27–+232; Southgate and Voellmy, 1983) clone in pBS+ (Stratagene Cloning Systems, La Jolla, CA) according to the supplier's instructions and its size was reduced to 50–100 nucleotides at pH 10.2 as described by Cox et al. (1984). Hybridization was performed overnight at 50°C in 50% formamide, 0.3 M NaCl, 20 mM Tris-HCl, pH 8.0, 5 mM EDTA, 10 mM DTT, 1× Denhardt's, 0.5 mg/ml tRNA, and 8–10% dextran sulfate. 15 μl of hybridization buffer containing 500,000 cpm were used per slide. Several rinsings at room temperature in 2× SSC were followed by RNase A digestion (Cox et al., 1984) and two or three washings at 60°C in 0.2× SSC for 30 min each. Autoradiography was as described by Hafen and Levine (1986). A control sense RNA gave no signal (not shown).

Results

Affinity Purification of hsp27 Antibody

The preparation and characterization of a polyclonal antiserum recognizing *Drosophila* hsp27 has already been described (Arrigo and Pauli, 1988; Beaulieu et al., 1989). However, in late larvae and pupae, this antiserum recognized three other polypeptides of 33, 85, and 120 kD which could be related to hsp27. Consequently, this crude antiserum was affinity purified to remove the cross-reactivity against these other polypeptides before use for the determination of the spatial and temporal expression of hsp27 during fly development. The purification was achieved by immunoaffinity using purified β-galactosidase-hsp27 hybrid protein. A DNA fragment consisting of the whole but the nine first residues of hsp27 plus several hundred base pairs of 3′ untranslated sequence was cloned in phase to the carboxy terminus of β-galactosidase. The fusion gene was expressed in bacteria and used to affinity purify hsp27 antiserum as described in Materials and Methods. As seen in Fig. 1 *A*, this affinity-purified antiserum immunoprecipitated only hsp27 from the cytoplasm of [^{35}S]methionine-labeled KC cells recovering from a heat shock treatment. In addition, a protein blot of four *Drosophila* developmental stages probed with the purified antibody shows that in all stages hsp27 was the only detectable protein (Fig. 1 *B*). In particular, we note the absence of the 33-kD band in pupae, in which the crude antiserum gave a signal as strong as that of hsp27 (not shown, see Arrigo and Pauli, 1988). The affinity-purified antibody was used for immunohistological experiments. It was detected

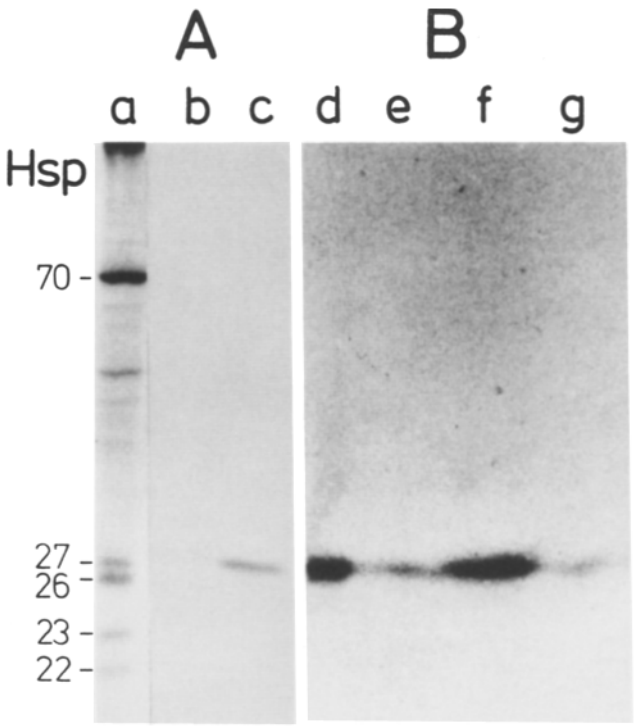

Figure 1. Characterization of an affinity-purified antiserum recognizing hsp27. (*A*) KC tissue culture cells were heat shock treated for 1 h at 37°C and subsequently labeled with [^{35}S]methionine for 5 h at normal temperature. The cells were lysed and prepared for immunoprecipitation as described in Materials and Methods. (*a*) Total cell extract; (*b* and *c*) immunoprecipitation either using preimmune serum (*b*) or affinity-purified anti-hsp27 serum (*c*). An autoradiography of the gel is presented. The affinity-purified anti-hsp27 serum was also used to probe a protein blot containing four *Drosophila* developmental stages (*B*). The same amount of protein was analyzed in each developmental stage as described in Materials and Methods. The blot was revealed with 5 μCi/ml of [^{125}I] protein-A. After washing the membranes with 0.5 M LiCl, autoradiographs were exposed at −70°C on Fuji X-ray film with the aid of Ilford fast tungstate intensifying screens. (*d*) 1-h-old embryo; (*e*) third instar larvae; (*f*) mid-pupae; and (*g*) 2-d-old adult.

with a second antibody coupled to peroxidase as described in Materials and Methods.

hsp27 Is Uniformly Distributed in Embryos

We have already demonstrated that the hsp27 protein is present during the entire embryogenesis, although its amount decreases by the end of this period and is hardly detectable in first instar larvae (Arrigo and Pauli, 1988). The immunological staining of whole embryos at different stages is consistent with this result. hsp27 appeared to be uniformly distributed in early embryos (Fig. 2 *A*). The presence of a high level of hsp27 from the very beginning of embryogenesis (preblastoderm stages) is in good agreement with the staining observed in ovaries (see below, Fig. 5, *A*, *C–E*). In the absence of hsp27 antiserum no peroxidase coloration was seen (not shown). However, to rule out the possibility that this uniform labeling was unspecific, we analyzed the effect of a heat shock before fixation of the embryos. Several studies have shown, in yeast, plants, *Drosophila*, and mammals, that the low molecular weight hsps are localized inside

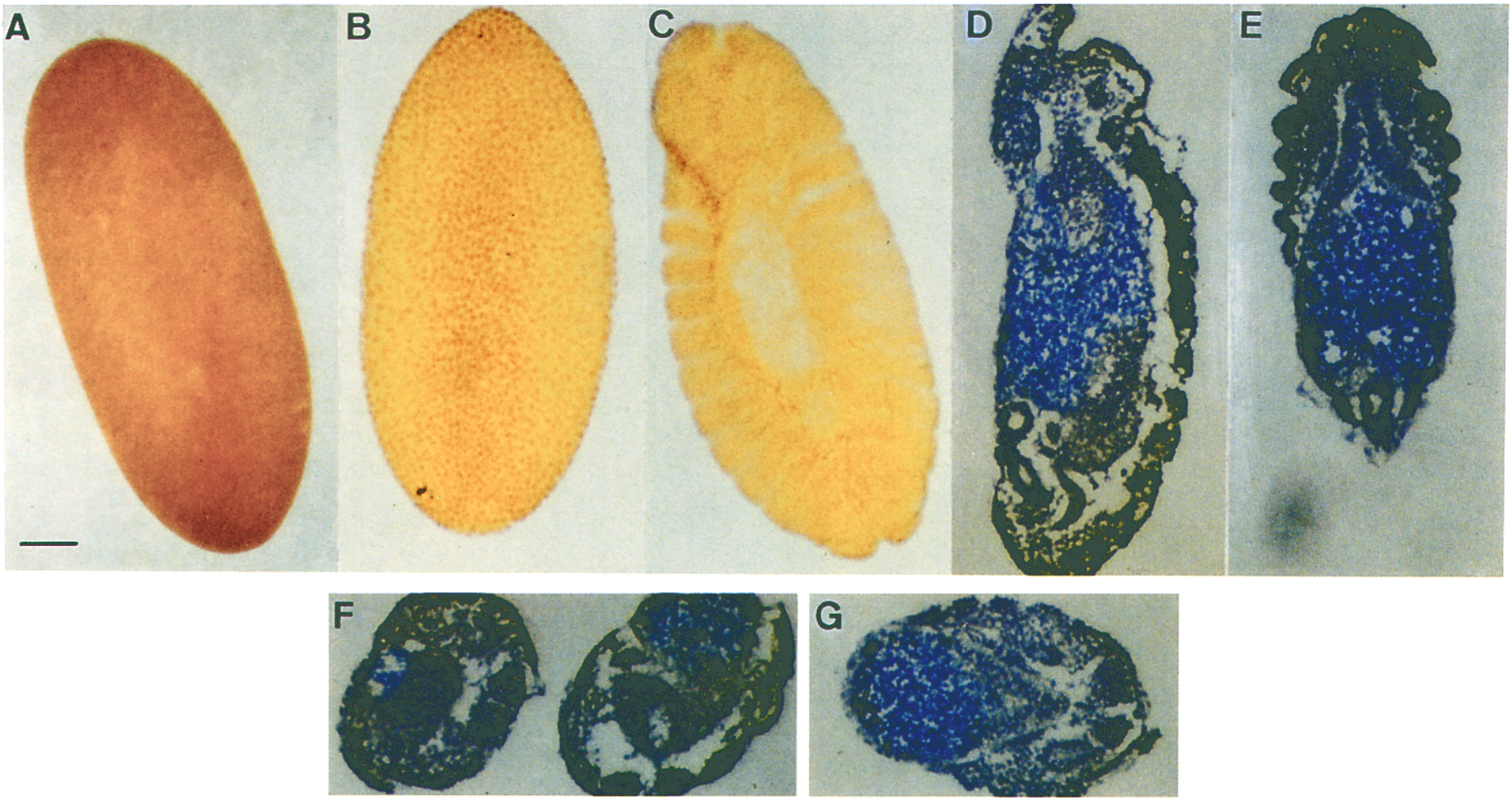

Figure 2. Immunolocalization of hsp27 during embryogenesis. Whole embryos (*A–C*) or embryonic sections (*D–G*) were processed for immunohistology using the affinity-purified hsp27 antiserum and goat antirabbit antiserum coupled to peroxidase as described in Materials and Methods. (*A* and *B*) 1-h-old embryo kept either at 20°C (*A*) or analyzed after a 15-min heat shock treatment performed at 37.5°C (*B*); (*C*) 10-h-old embryo; (*D–G*) thin sections of 10-h-old embryo, stage 14 (Campos-Ortega and Hardenstein, 1985); (*D*) sagital section; (*E*) ventral section; (*F* and *G*) transversal sections. A dark brown coloration indicates the presence of hsp27. The only tissue which does not contain hsp27 is the yolk stained in blue. Bar, 50 μm.

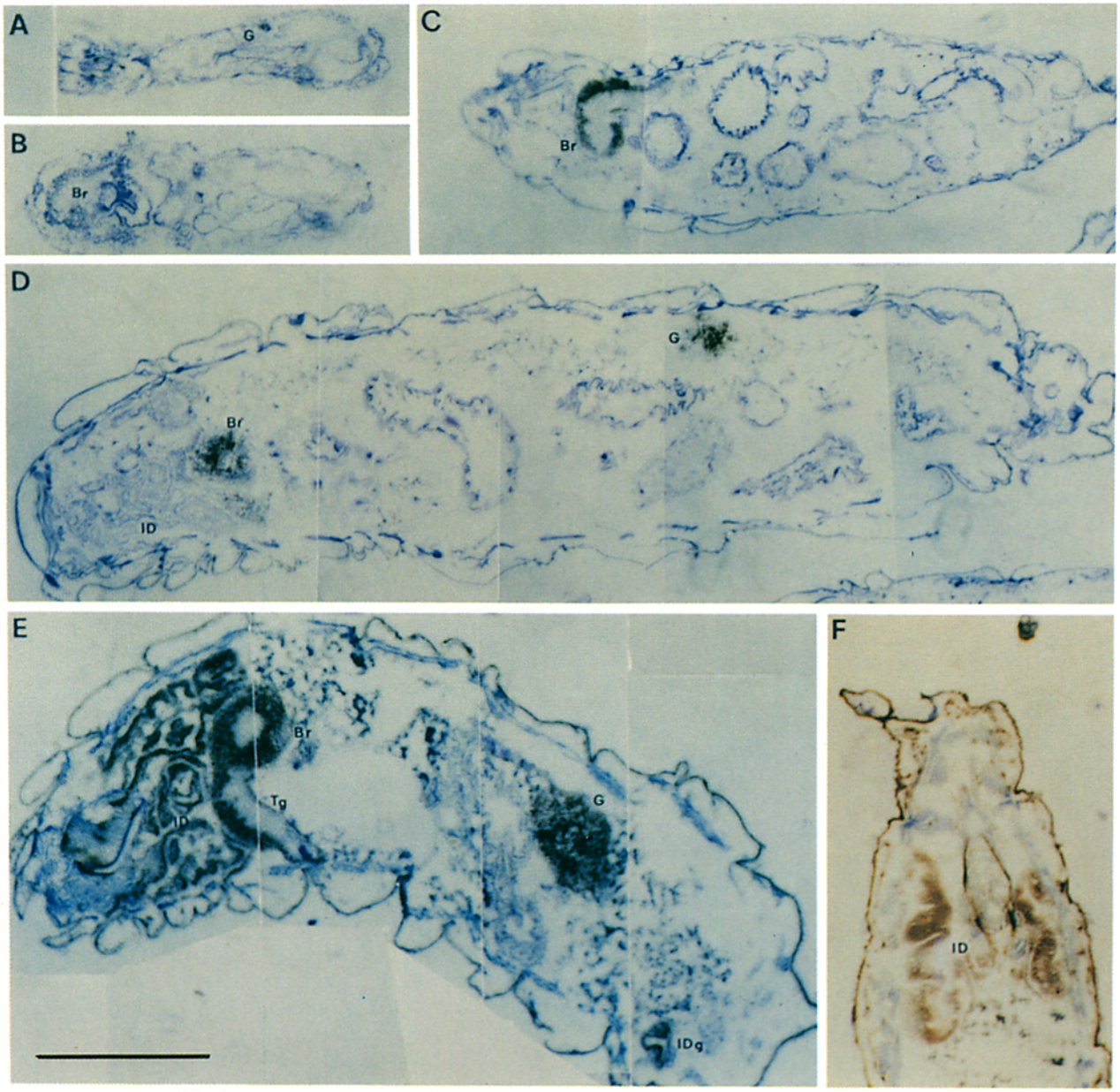

Figure 3. Immunolocalization of hsp27 during the larval stages. Thin sections of developing larvae were processed for immunohistology using affinity-purified hsp27 antiserum as described in Fig. 2 and Materials and Methods. (*A* and *B*) first instar larvae, longitudinal *A* and sagital *B* sections. A weak staining was found in the brain and the gonads. (*C*) Second instar larva, sagital section; (*D*) early third instar larvae, longitudinal section; (*E*) late third instar larva, sagital section; (*F*) young pupa, longitudinal section. Tissues of interest are indicated by the following abbreviations: *G*, gonad (testis); *Br*, brain; *ID*, imaginal discs; *IDg*, genital imaginal disc; *Tg*, thoracic ganglion. The brown coloration reveals the presence of hsp27. The cuticular staining of the pupae is unspecific. Bar, 500 μm.

the nucleus during a thermal stress (Arrigo et al., 1980; Arrigo and Ahmad-Zadeh, 1981; Vincent and Tanguay, 1982; Collier and Schlesinger, 1986; Arrigo, 1987; Arrigo and Welch, 1987; Collier et al., 1988; Beaulieu et al., 1989; Rossi and Lindquist, 1989), where they are recovered as very large aggregates (>$2 \cdot 10^6$ d) (Arrigo et al., 1988). Embryos were subjected to a heat shock at 37.5°C for 15 min which did not allow a large de novo synthesis of hsps. In heat-treated preblastoderm (Fig. 2 *B*) and blastoderm embryos (not shown), hsp27 was only detected at the level of the nuclei. In later stages, it is difficult to estimate the precise localization of hsp27 due to the excessive number of nuclei (not shown). Together with the protein blot of Fig. 1, the heat shock effect on the embryonic staining confirmed the specificity of the affinity-purified antiserum.

To detect possible subtle differences in the localization of hsp27, thin sections of developing embryos (stage 14) were also analyzed (Fig. 2, *D–G*). Except for the yolk which was completely negative, hsp27 staining was observed in every tissue, with a possible accumulation in the ventral nervous system. The embryonic synthesis of hsp27 must take place entirely during preblastoderm stages since the maternal transcript disappears after 2–3 h of development (Zimmerman et al., 1983; Pauli et al., 1989). We conclude that the

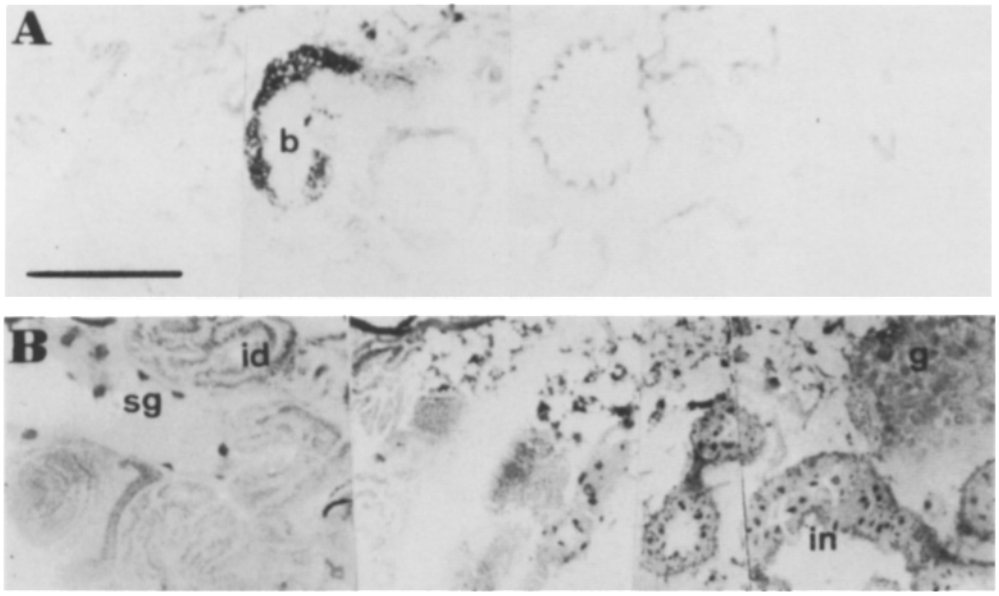

Figure 4. Presence of hsp27 in most of the larval tissues after heat shock. Early third instar larva were either kept at normal temperature (*A*) or exposed to a heat stress of 1 h at 37°C and allowed to recover for 2 h at 20°C (*B*). Thin sagital sections of the larvae were processed for immunohistology using affinity-purified hsp27 antiserum as described above but without Giemsa staining. Tissues of interest are indicated by the following abbreviations: *g*, gonad (testis); *b*, brain; *id*, imaginal discs; *sg*, salivary glands; *in*, intestine. The dark coloration reveals the presence of hsp27. Note the strong nuclear accumulation of hsp27 in the cells of most of the tissues of the heat treated larvae. Bar, 500 μm.

hsp27 protein synthesized during oogenesis (see below) and in preblastoderm embryos becomes distributed during blastoderm cellularization into all the embryonic cells.

Larval and Pupal Expression

Tissue sections were prepared and stained as described in Materials and Methods for the first, second, early and late third larval stages, white prepupae, 5–10 h early pupae, and late pupae (<10 h before eclosion). Contrasting with the rather uniform staining of early embryos, hsp27 was restricted to a few tissues during subsequent developmental stages, and even to a small number of cells in a particular organ. During the three larval stages, except at the end of the third, a significant staining was found in only two tissues: the CNS (cortex of the brain and thoracic and abdominal ganglia) and the male gonads (Fig. 3, *A–D*). We have not detected any expression in the female gonads, but due to the very small size of the larval ovaries, it is difficult to ascertain whether hsp27 was expressed or not in these tissues. No expression of hsp27 was observed at the level of the polytenized tissues, including the salivary glands, fat cells, and hypoderm. A similar result was observed when the staining with Giemsa was omitted (Fig. 4 *A*), and no peroxidase coloration was observed in the absence of anti-hsp27 serum (not shown). As a control, third instar larvae were heat treated (37°C for 1 h, followed by a 2-h recovery period at 20°C). In those larvae, hsp27 was now detected in most of the tissues and its localization was predominantly nuclear (Fig. 4 *B*).

At the end of the third instar larval period, in addition to the gonads and the central nervous system, a third group of tissues began to be stained by anti-hsp27 antibody: the imaginal discs, including the genital disc (*IDg*) located at the posterior part of the animal (Fig. 3 *E*). A similar staining of the discs was observed in young pupae (Fig. 3 *F*). Visual quantification of hsp27 staining, although not quantitative, suggests that the maximal abundance of hsp27 in the imaginal discs is reached during the first 10 h of the pupal stage. This is particularly evident for the imaginal discs of the eyes which are easily recognizable on each side of the insect body (Fig. 3, *E* and *F*).

Adult Expression of hsp27

In the adult, hsp27 was localized in two different tissues: the germ lines and the CNS. In the female germ line hsp27 accumulated in the ovarian nurse cells during early vitellogenesis, stage 8–10 (Fig. 5, *A*, *C–E*). See the review by Mahowald and Kambysellis (1980) for a precise description of these stages. Little staining of the growing oocyte was observed during this period. hsp27 was then massively transferred into the oocyte during the breakage of the nurse cells at stage 11 (Fig. 5, *C–E*). Hsp27 was uniformly distributed in the mature unfertilized oocyte, similarly to its distribution in young embryos. The accumulation of the hsp27 polypeptide in mature gametes was not restricted to female cells. The strong staining of elongated spermatid bundles (Fig. 5, *B* and *F*) suggests that this is also true for the sperm. It is also interesting to note the intense staining of the vasa deferent cells, particularly at the level of the nucleus (Fig. 5, *G* and *G'*). This indicates that besides the germ line, some somatic parts of the male reproductive system also express hsp27.

In the adult, the neural expression was limited to a few clusters of cells in the brain and in the thoracic ganglion (Fig. 5, *A* and *B* and Fig. 6, *E–G*) while a more general staining of the nervous system was observed in larvae and pupae (Fig. 6, *A–D*). In larvae and pupae, the neurocyte (body of the neuron) was stained but not the neuropile (axons). However, in adult flies, a weak staining of the neuropile in some parts of the brain was also observed (Fig. 6, *E* and *F*). Hsp27 did not seem to be restricted to one type of neurons, small ganglion cell, large ganglion cell or giant cell of Hertweck, described by Miller (1950). Note that the adult eyes were devoid of any hsp27 staining (Figs. 5, *A* and *B* and 6, *E–G*). However, in late pupae, the almost completely differentiated eyes were still slightly positive for hsp27, particularly in the cells local-

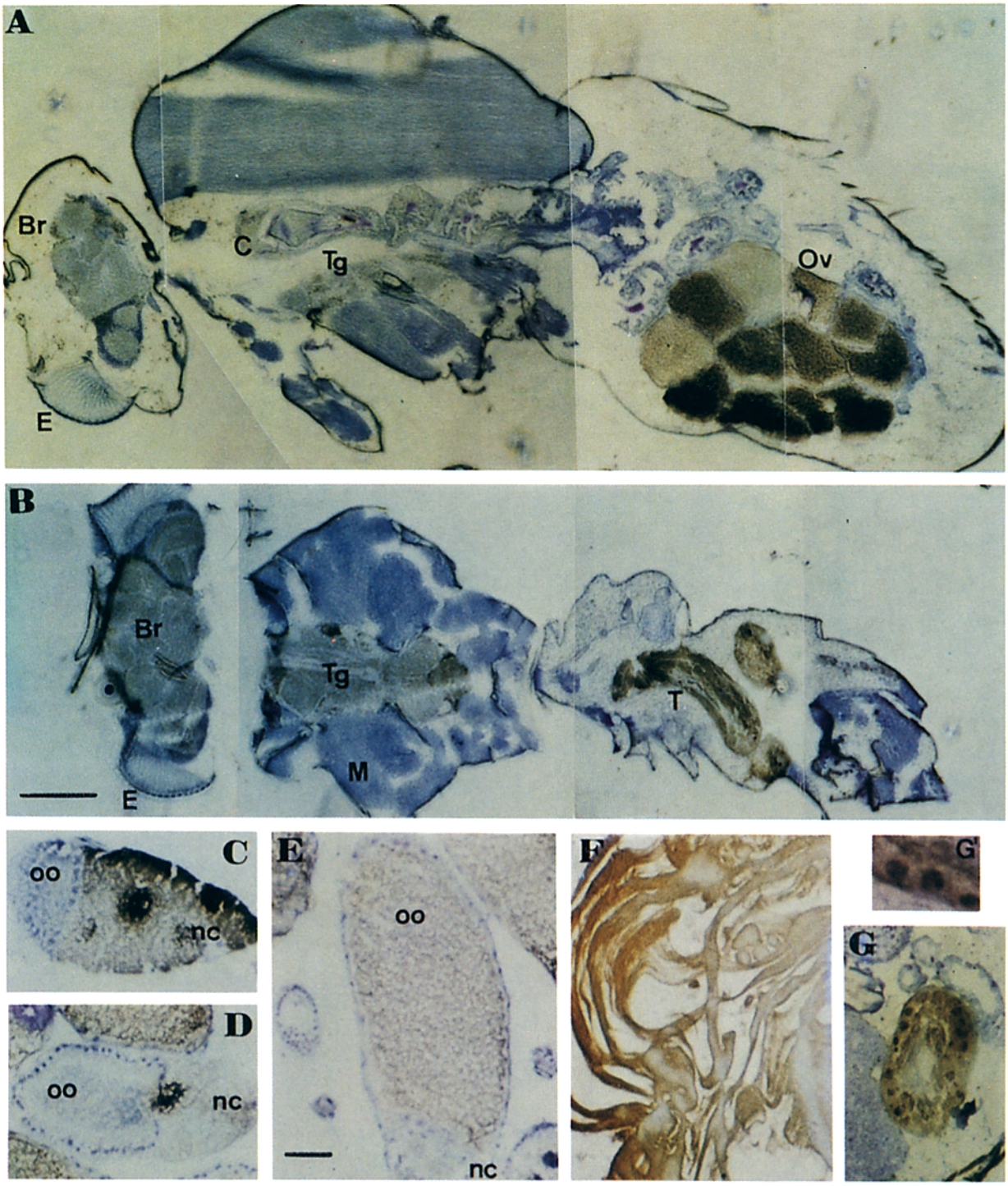

Figure 5. Immunolocalization of hsp27 in adult flies. Thin sections of adult flies were used for the immunohistological detection of hsp27, as described in Figs. 2 and 3. (*A*) Sagital section of 4-d-old female fly; (*B*) longitudinal section of a newborn male fly; (*C–E*) sections of ovaries showing the localization of hsp27 during the maturation of the oocyte; note that the nurse cells synthesize hsp27 which is then transferred to the maturing oocyte; (*F*) squash of dissected testis; (*G*) enlargement of the abdomen from a section of a male fly showing a vasa deferent duct; (*G'*) enlargement of G showing the predominantly nuclear localization of hsp27. Tissues of interest are indicated by the following abbreviations: *T*, testis; *Br*, brain; *Tg*, thoracic ganglion; *Ov*, ovary; *M*, muscle; *E*, eye; *C*, cardia, *oo*, oocyte; *nc*, nurse cells. Brown coloration indicates the presence of hsp27. Bars: (A and B) 200 μm; (C–G) 50 μm.

ized at the top portion of the ommatidia (Fig. 6 *C*). This result suggests that hsp27 is no more expressed after the complete differentiation of the imaginal discs into the corresponding adult organs.

In Situ Hybridization

To further analyze the tissue specific expression of hsp27, the level of the corresponding mRNA transcripts was analyzed

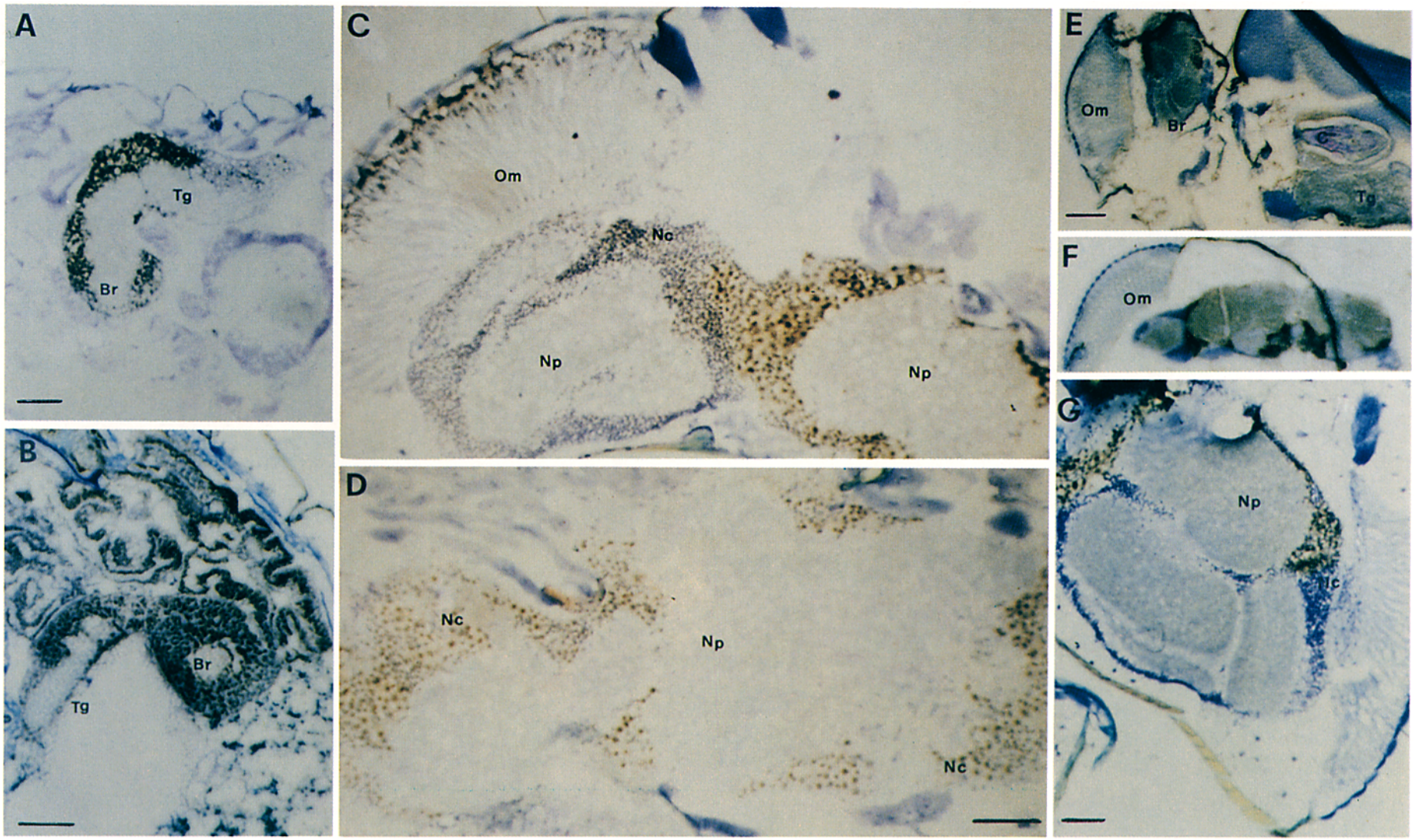

Figure 6. Immunolocalization of hsp27 in the CNS during the larval to adult development. A comparative analysis of the immunohistological localization of hsp27 in the CNS in second instar larvae, late third instar larvae, late pupae, and young adult flies is presented. Thin sections of the developing insect were processed as described above. Enlargement of the regions of the insect showing the CNS is presented. (*A*) Second instar larva, sagital section; (*B*) late third instar larva, sagital section; (*C*) partial view of the head of a late pupa, longitudinal section, note the hsp27 staining at the top of the omatidies; (*D*) thoracic ganglion of late pupa, longitudinal section; (*E–G*) sections of the head an adult fly. (*E*) sagital section; (*F*) longitudinal section; (*G*) enlargement of a longitudinal section. Tissues of interest are indicated by the following abbreviations: *Br*, brain; *Tg*, thoracic ganglion; *Nc*, neurocyte; *Np*, neuropile; *Om*, ommatidia. Brown coloration indicates the presence of hsp27. Bars: (B, E, F) 100 μM; (A, C, D, G) 50 μm.

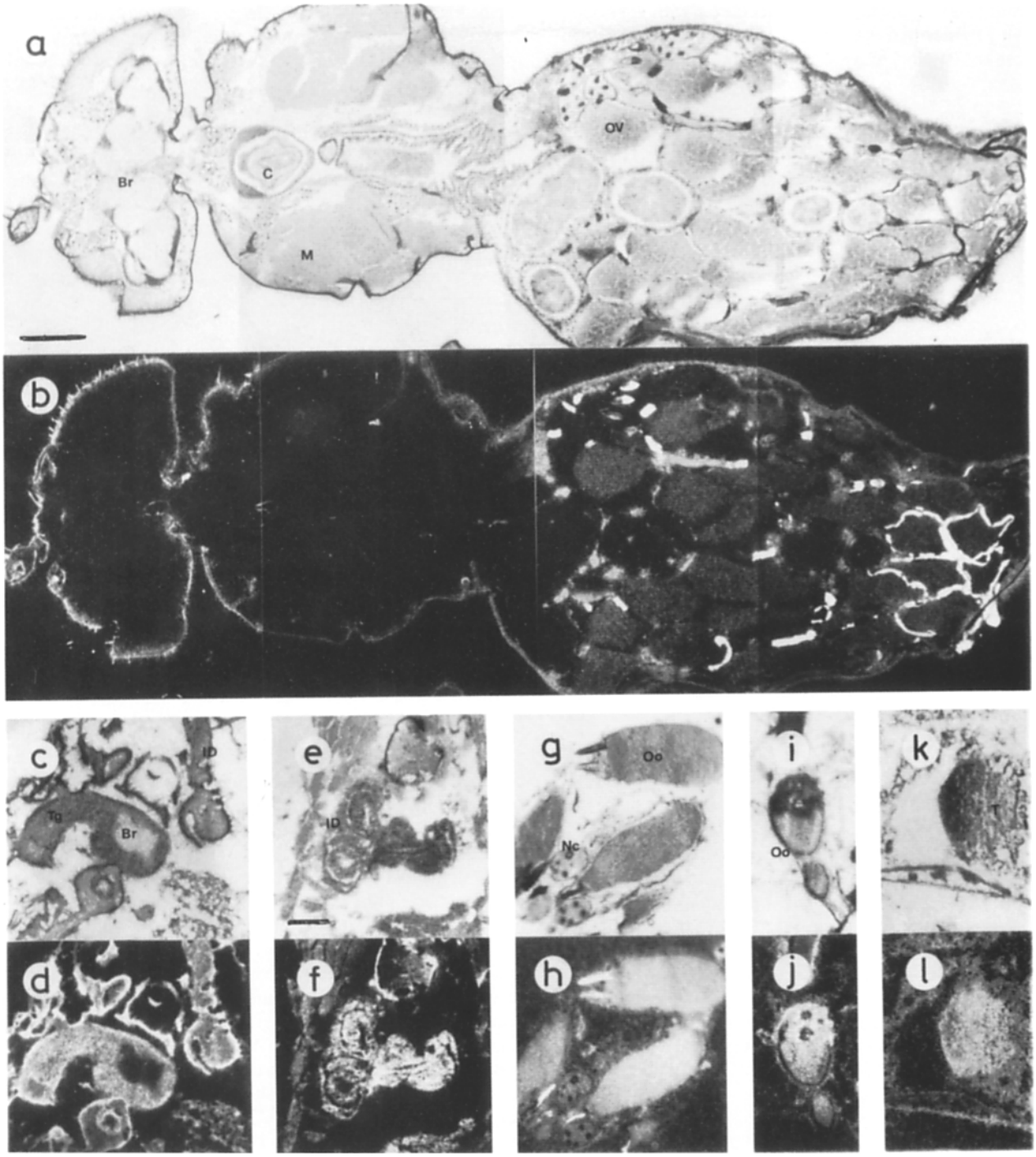

Figure 7. Distribution of hsp27 mRNA transcripts by in situ hybridization. Thin sections of the developing insect as well as sections of dissected organs were used for in situ detection of hsp27 mRNA transcripts as described in Materials and Methods. Phase contrasts and dark-field illuminations as presented. (*a* and *b*) Section of 4-d-old female; (*c* and *d*) sagital section of a late third instar larva showing the brain and imaginal discs; (*e* and *f*) as *c* and *d* but predominantly imaginal discs; (*g–j*) section of dissected ovaries; (*k–l*) section of third instar larvae showing the testis. Tissues of interest are indicated by the following abbreviations: *Br*, brain; *C*, cardia; *OV*, ovary; *ID*, imaginal discs; *Oo*, oocyte; *M*, muscle; *Tg*, thoracic ganglion; *Nc*, nurse cells; *T*, testis. (*a*, *c*, *e*, *g*, *i*, *k*) Phase contrasts; (*b*, *d*, *f*, *h*, *j*, *l*) dark-field illuminations. Bars: 150 μm (*A* and *B*); 100 μM (*C–L*).

by in situ hybridization as described in Materials and Methods. Surprisingly, in the central nervous system, no hsp27 transcripts was detected in the adult fly (Fig. 7, *a* and *b*), while a strong signal was observed in third instar larvae (Fig. 7, *c* and *d*). This suggests that the hsp27 polypeptide present in the adult neurons was synthesized during the larval and pupal stages and has therefore a very long half life. This possibility is strengthened by the fact that most of the larval central nervous system contained hsp27 and more neurocytes appeared to be stained in the pupal brain than in the

adult brain (Figs. 3, 5, and 6). In agreements with the observations of Zimmerman et al. (1983), the adult ovaries showed an intense expression of hsp27 transcripts (Fig. 7, *a* and *b*, *g–j*). As expected from the protein staining shown in Fig. 3, transcription of hsp27 gene was observed in larval testes (Fig. 7, *k–l*). A high level of hsp27 transcripts was also detected in imaginal discs of late third instar larvae (Fig. 7, *c–f*). Therefore, the larval and pupal expression of hsp27 is due to de novo transcription, as expected since the maternal hsp27 mRNA disappears during early embryogenesis (Zimmerman et al., 1983).

Discussion

Using immunological detection with an affinity-purified antiserum, we have found a complex pattern of accumulation of hsp27 during *Drosophila* development. Hsp27 was uniformly distributed in preblastoderm embryos. During gastrulation, this protein was still present in every tissue, with the exception of the yolk. In first, second, and early third instar larvae, this hsp was expressed only in the CNS and the male gonad. In addition to these tissues, all the imaginal discs strongly accumulated hsp27 at the end of the third instar larval stage and during the first part of the pupal period. In the newborn adult fly, hsp27 was still detected in the gonads and the CNS. Based on their pattern of expression, it is tempting to classify the tissues that express hsp27 in two groups: one expresses hsp27 rather transiently while the other shows a relatively constant activity. The first group would contain the imaginal discs and the embryonic tissues. In embryos, besides the polypeptide already accumulated during oogenesis, the translation of hsp27 mRNA had to take place during the first 2–3 h of embryogenesis, since the RNA was no more detectable after this period (Zimmerman et al., 1983). The protein was then slowly degraded over the entire embryonic period (Arrigo and Pauli, 1988). In late embryos, tissue-specific degradation of hsp27 may explain the slightly higher level of staining observed at the level of the nervous ventral cord. Although, we cannot exclude a weak de novo synthesis of hsp27 in this tissue. For the imaginal discs the transcription and the translation of hsp27 roughly coincided with the beginning of their differentiation. In the developing discs, the mRNA encoding hsp27 disappeared before the middle of the pupal period (Pauli et al., 1989), while the protein was more progressively degraded over the pupal stage. The disc-derived adult tissues did not contain hsp27 (see for instance the eye, Fig. 6 *C*). The second group of tissues that expresses hsp27 during a rather long period of development would contain the CNS and the germ lines. The CNS transcribed hsp27 during the three larval stages while no more transcripts were detected in the adult. However, the protein was still detectable in some adult neurones. This observation indicates that hsp27 found in the CNS of pupae and adult flies was probably synthesized during the larval stages. In the male germ line, hsp27 was expressed since the first instar stage and accumulated in mature sperm. Because of the very small size of the larval ovaries, we were not able to determine without ambiguity whether this protein was also present in these tissues. However, hsp27 was detected in nurse cells during vitellogenic stages of oogenesis and in mature gametes of the adult. Contrasting with the observation described above for the CNS, hsp27 transcription was very strong in the adult ovaries and testes. It is also interesting to mention that, in whole pupae, we have observed a lag of several days between the maximal expression of hsp27 mRNA and the maximal expression of the corresponding polypeptide (Pauli et al., 1989). Taken together, these results suggest that modulations in the stability of hsp27 polypeptides may also regulate the tissue-specific level of this polypeptide during development. Interestingly, hsp27 as well as the other small hsps are in the form of aggregates which are of similar size to those formed by alphaAB-crystallin (Arrigo et al., 1988). Therefore, the stability of these proteins may be modulated by their state of aggregation.

Interestingly, the tissue-specific expression of hsp27 resembles that described for a hsp26 fusion gene product (Glaser et al., 1986; Glaser and Lis, 1990). However, there are some minor differences. In particular, hsp26 expression has been found in the epithelium of third instar larvae and in early pupae as well as in the adult CNS at very low levels (Glaser et al., 1986). It is not known whether a similar pattern of developmental expression exists for the two other *Drosophila* low molecular weight hsps (hsp22 and 23).

In preblastoderm and blastoderm embryos, hsp27 was cytoplasmic as well as nuclear, while after heat shock this hsp was only detected at the level of the nuclei. A predominant nuclear localization of hsp27 was also observed in several tissues of heat-treated larvae. We were not able to localize hsp27 in neuronal and imaginal disc cells of the larvae, but in adult, high levels of this protein were detected in the nucleus of vasa-deferent, nurse, and neuronal cells. However, in most of these cells, detectable levels of hsp27 were also observed in the cytoplasm. It is intriguing that in KC tissue culture cells exposed to the molting hormone β-ecdysone an apparent nuclear localization of hsp27 is also observed (Beaulieu et al., 1989). In those cells, hsp27 was easily extracted from the nucleus by nonionic detergents while after a heat stress this was no more the case. Heat-induced nuclear insolubilization of the small hsps has been observed in several organisms (Arrigo et al., 1980; Vincent and Tanguay, 1982; Collier and Schlesinger, 1986; Arrigo, 1987; Collier et al., 1988; Arrigo and Welch, 1987; Arrigo et al., 1988; Beaulieu et al., 1989; Rossi and Lindquist, 1989). In addition, the metabolic state (Rossi and Lindquist, 1989) and the degree of thermotolerance (Arrigo, 1987; Arrigo et al., 1988) of the cell appears to modulate the nuclear localization of the small hsps during heat shock. Our finding of an apparent nuclear localization of hsp27 in some specific tissues during *Drosophila* development suggests that this polypeptide may play a role inside or at the periphery of the nucleus. Therefore, several factors may regulate the intracellular locale and, probably the function, of the low molecular weight hsps.

What are the signals involved in the expression of hsp27 during development? β-ecdysone is probably one of the triggers involved in the expression of hsp27 because this hsp is known to accumulate in response to this hormone in tissue culture cells and isolated imaginal discs (Ireland and Berger, 1982; Ireland et al., 1982). Moreover, in late third instar larvae-young pupae, the expression of hsp27 in imaginal discs corresponds to a peak of accumulation of β-ecdysone (Handler, 1982; Thomas and Lengyel, 1986). A steroid receptor binding sequence has been found upstream of hsp27

gene (Riddihough and Pelham, 1986) indicating that ecdysone probably regulates hsp27 expression. Estrogens have also been found to strongly induce hsp28 in mammals (Edwards et al., 1980; Moretti-Rojas et al., 1988; Arrigo, unpublished observations). Nevertheless, it is unlikely that β-ecdysone regulates the expression of *Drosophila* hsp27 in other tissues than imaginal discs. Particularly, it has been found that the coarse regulatory sequences of several hsp genes, when known, differ from one tissue, or one stage to another (Cohen and Meselson, 1985; Glaser et al., 1986; Klemenz and Gehring, 1986; Hofman et al., 1987; Glaser and Lis; 1990). Half life of hsp27 mRNA may also vary between tissues. β-Ecdysone may be as important for mRNA stability as for the activation of gene transcription (Pauli et al., 1989).

What are the functions of the low molecular weight hsps during *Drosophila* development? No *Drosophila* mutants of these hsps have yet been isolated, in spite of extensive mutagenesis of the 67 AD region (Leicht and Bonner, 1988). A P-element insertion upstream of hsp27 coding sequence which partially disrupts the transcription of the gene is nevertheless viable (Eissenberg and Elgin, 1987). In *Dictyostelium*, a mutant with an altered pattern of expression of the low molecular weight hsps is not viable at elevated temperatures (Loomis and Wheeler, 1982). This observation contrasts with the deletion of the yeast hsp26 gene which does not result in any altered phenotype (Petko and Lindquist, 1986). It has been suggested that the low molecular weight hsps may play a role in the development of thermotolerance (Berger and Woodward, 1983). Intriguingly, elevated levels of the mammalian hsp28 polypeptide appears sufficient to confer a partial thermal resistance to cultured cells (Landry et al., 1989).

The developmental profile of hsp27 is complex and suggests that this protein has multiple roles during development. During embryonic and larval development, hsp27 expression was predominantly observed in tissues that contained highly proliferating cells (early embryo, ventral cord of stage 14 embryos, CNS, and gonads of larvae; see Campos-Ortega and Hartenstein, 1985; Bryant, 1987; Lehner and O'Farell, 1989). There was one exception: the imaginal discs of the early larvae which contained highly dividing cells but no hsp27. However, in late third instar larvae hsp27 rapidly accumulated in these tissues when they began to differentiate and when their cellular proliferation slowed down and stopped (Bryant, 1987; Lehner and O'Farell, 1989). The disc expression occurred mainly at the beginning of their differentiation since it disappeared during the second half of the pupal stage: the disc-derived adult organs were devoid of hsp27. Similarly, after transplantation in mice, Ehrlich ascites tumor cells stop to divide concomitantly with the accumulation of a protein highly related to the low molecular weight hsps (Bielka et al., 1988; Gaestel et al., 1989). In both cases (*Drosophila* imaginal discs and Ehrlich ascites tumor cells), these related proteins are expressed in cells arrested in late S/G2 phase (Bryant, 1987; Gaestel et al., 1989). Interestingly, the pattern of expression of hsp27 during *Drosophila* development resembles (with the probable exception of the imaginal discs of the early larvae) that of Dras, the *Drosophila* homolog of the ras oncogen (Segal and Shila, 1986). Moreover, it should also be noted that hsp27 is a phosphoprotein (Rollet and Best-Belpomme, 1986). During *Drosophila* development, hsp27 is resolved in several isoforms in two-dimensional gel electrophoresis suggesting a high level of phosphorylation of this polypeptide (Arrigo and Pauli, 1988). In mammals, an increased phosphorylation of the corresponding stress protein, hsp28, rapidly occurs after treatment of tissue culture cells with agents which either modulate cell division (fresh serum, mitogens), cell differentiation (phorbol-12-myristate-13-acetate) (Welch, 1985; Regazzi et al., 1988; Arrigo, 1990), or the immune response (tumor necrosis factor) (Arrigo, 1990). Taken together, these observations suggest that the putative function(s) of hsp27 may be related to both the proliferative and differentiated states of the tissues of the developing insect.

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