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An α-Crystallin Protein Cognate in Germ Cells of the Moth, *Plodia interpunctella*

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Previously we had reported the production of an antiserum to an antigen found primarily in germ cells of the Indianmeal moth, *Plodia interpunctella* (Zimowska *et al.*, 1991). The antigen, molecular weight 25 000 kDa, and a related protein, molecular weight 21 000 kDa, co-purified with the follicular epithelium yolk protein. Antisera to the two proteins were raised, and they both reacted with the same four small polypeptides, which had molecular weights of 20 000, 21 000, 25 000 and 28 000 kDa, that were present in the eggs throughout embryogenesis. A 30 amino acid sequence of an internal fragment of the 25 000 kDa molecular weight polypeptide showed sequence similarity with the α-crystallin A chain polypeptides from the lenses of vertebrate eyes and, to a lesser extent, with small heat shock proteins. Based on the sequence similarity with the α-crystallins, we suggest that this family of polypeptides from the germ cells of this moth be considered as cognates of the α-crystallins, and the 25 000 molecular weight polypeptide described here be given the designation *ac25*. Using immuno-gold labeling with antiserum to *ac25*, the α-crystallins were shown to be distributed throughout the cytoplasm and nucleoplasm of the oocyte and nurse cells, but not present within yolk spheres or other organelles of the oocyte or nurse cells. Immunofluorescent staining of males showed antigenic material in the sperm bundles within the testes. Oenocytes of the pupal and adult stages also contained cross-reactive material. Published by Elsevier Science Ltd

Heat shock proteins Ovary Oocyte Oenocyte Testes

INTRODUCTION

One of the fundamental questions of developmental biology centers on how germ cells differentiate separately from somatic cells. For many eukaryotes, the identity of germ cells is clearly established early in embryogenesis, but the specific nature of germ cell determinants has not been established (Mahowald, 1992). In flies, germ cells form in the posterior of the egg where polar granules are localized. In *Drosophila melanogaster*, a class of maternal genes contribute products that are localized in the posterior of the oocyte during follicular development and form the polar granules. The posterior class of genes provides information not only for specific posterior determinants that control abdominal development, but also for the formation of pole cells that are the primary germ plasm. However, none of the molecules of the posterior class acting by itself can lead to pole cell formation and germ cell development. Thus, a single determinant that specifies "germ cell" has not been identified and suggests that germ cell determination may require a group of products acting in concert.

There are several proteins that are localized in the polar granules of *D. melanogaster* and that are expressed exclusively in germ cells throughout development: e.g. *vasa* (Hay *et al.*, 1990; Lasko and Ashburner, 1990), *orb* (Lantz *et al.*, 1992) and *germ cell-less* (Jongrens *et al.*, 1992). *vasa* is an RNA ATP-dependent helicase (Hay *et al.*, 1988; Lasko and Ashburner, 1988) that contributes to the localization of the posterior determinant, *nanos*, and is expressed constitutively and exclusively in germ cells (Hay *et al.*, 1990; Lasko and Ashburner, 1990). The predicted *orb* protein has similarity with the RRM family of RNA-binding proteins and because the *orb* transcript accumulates in the posterior of the egg, the protein may mediate localization of maternal RNAs during oogenesis and early embryogenesis. Similarly, the mRNA for *germ cell-less* is localized posteriorly and is necessary for germ cell development, but the *gcl* protein localizes in the nuclear pores of germ cells (Jongrens *et al.*, 1992).

In addition to germ cell specifically expressed proteins, there are proteins that have a developmental pattern of

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expression in the germ cells that is different from their somatic expression. The *fat facets* gene produces two large unique proteins, with unknown functions, that are essential for germ cell development and delimiting the number of photoreceptors in the developing eye (Fischer-Vise *et al.*, 1992). During oogenesis, the *faf* protein is localized in the posterior pole plasm, but it is not required for localization of the posterior determinants. In addition, two small (*Hsp26* and *Hsp28*) and one large (*Hsp83*) heat shock proteins (hsps) are expressed in germ cells (Zimmerman *et al.*, 1983; Mason *et al.*, 1984; Glaser *et al.*, 1986) and maternal *Hsp83* transcripts are even localized in the posterior pole plasm of the eggs (Ding *et al.*, 1993).

In Lepidoptera, the origin of germ cells in the cellularizing egg is undetermined. In the silkworm, *Bombyx mori*, no clear evidence of pole plasm has been found in the eggs using microscopical or histological techniques (Nagy *et al.*, 1994) or by fate mapping using localized u.v.-irradiation (Myohara, 1994). In the tobacco hornworm, *Manduca sexta*, a monoclonal antibody to a neuroglian (Nardi, 1994), that is localized on the surface of the germ cells, was used to establish the earliest temporal identification of the germ cells during embryogenesis. At 10% of the embryonic developmental time, the germ cells were observed as a single loose aggregate on the midline of the embryo towards the posterior pole (Nardi, 1993). However, the neuroglian antigen was not present in earlier germ cells so that the site of germ cell formation could not be established.

In the Indianmeal moth, *Plodia interpunctella* (Hübner), antiserum to an antigen that is highly expressed in germ cells, but not somatic cells, of the ovary was used to examine the development of germ cells in the follicles of pupal and adult females (Zimowska *et al.*, 1991). This antigen has been detectable in all stages of postembryonic development (Shirk, Beckemeyer, and Zimowska, unpublished). To examine the potential of this antigen as a germ cell specific marker, we describe the isolation, characterization and identification of this protein. Based on a 30 amino acid sequence of an internal fragment, this protein shares similarity with α-crystallin A chain proteins from the lens of vertebrate eyes.

MATERIALS AND METHODS

Insects and egg preparations

The *Plodia interpunctella* colony was reared according to Silhacek and Miller (1972) in a 16 h light—8 h dark cycle at 30°C and 70% relative humidity.

All washing and homogenization procedures for the eggs were carried out on ice with buffers at 0°C. 3–5 g of 0–1 h old eggs were washed three times in deionized water and once in homogenization buffer (40 mM Tricine, pH 6.8, 150 mM NaCl, 200 mM sucrose, 1 mM $MgCl_2$, 0.1 mM phenylmethylsulfonylfluoride). The washed eggs were resuspended in 10 ml of homogenization buffer and then homogenized in a glass homogenizer with a Teflon pestle. The homogenate was centrifuged for 20 min at 10 000 ***g*** and 4°C. The infranatant between the floating lipid layer and the pellet was removed and then diluted to 20 ml with homogenization buffer. The diluted infranatant was brought to 75% ammonium sulfate saturation to precipitate the proteins. The solution was kept for 2 h at 0°C, and then centrifuged 20 min at 10 000 ***g*** and 4°C. The supernatant was removed and the pellet was dissolved in phosphate buffered saline (PBS; 50 mM $NaPO_4$, pH 7.6, 150 mM EDTA, 0.01% NaN_3). The protein solution was dialyzed (Spectrophore 15 kDa cutoff dialysis tubing) exhaustively against PBS. The dialyzed solution was used for gel permeation chromatography.

Gel permeation chromatography

The dialyzed protein solution of the precipitated egg homogenate was layered onto an S-300 Sephacryl (Pharmacia Biotechnology; Piscataway, NJ) column (95×2.5 cm) and eluted with PBS. The column was run at 20 ml h^{-1} at 4°C, and the absorbance (280 nm) of the eluate was measured continuously using an ISCO UA-6 absorbance monitor (ISCO, Inc.; Lincoln, NE). Fractions that contained the major protein peak as determined by u.v.-absorbance and by SDS-PAGE were pooled and concentrated using a YM10 membrane in an ultrafiltration cell (Amicon, Inc.; Beverly, MA) at 4°C.

Ion exchange chromatography

The combined fractions from the S-300 gel permeation column were dialyzed exhaustively against PBS plus 5 mM KCl. The dialyzate was applied to a DEAE Sepharose C1-6B (Pharmacia Biotechnology; Piscataway, NJ) column (40×2.5 cm) and eluted with a linear 250 ml gradient from 5 to 250 mM KCl at 4°C. The absorbance was monitored continuously using an ISCO UA-6 absorbance monitor.

Polyacrylamide gel electrophoresis

Proteins were electrophoresed in denaturing conditions by 10% SDS-PAGE (Laemmli, 1970). Proteins were resolved by two-dimensional electrophoresis according to O'Farrell (1975). For Western blots, the proteins were resolved by SDS-PAGE and electroblotted to Trans-Blot membrane (BioRad; Hercules, CA), as described previously (Bean *et al.*, 1988). The immunoreactive bands were visualized with an Immun-Blot color assay (BioRad) using alkaline phosphatase-linked goat–anti-rabbit IgG as the second antibody.

Immunohistochemistry

Antiserum to the 25 000 molecular weight peptide was raised in New Zealand white rabbits. Fraction "a" from the DEAE-Sepharose chromatography was resolved by SDS-PAGE, the gels were placed in 1 M KCl to visualize the proteins, and the individual bands were cut from the gel (Bean *et al.*, 1988). Approximately 1.5 mg

of protein of the 25 000 molecular weight protein band was emulsified thoroughly in Freund's complete adjuvant (Sigma; St. Louis, MO), and the emulsion was injected subcutaneously and intramuscularly into separate rabbits. 4 weeks later, the rabbits were boosted with injections of 0.5 mg of the protein in Freund's incomplete adjuvant. The specificity of the antiserum was tested by Western blots, as described below, with bound egg homogenate proteins. The rabbits were terminally bled two weeks following the final boost.

Immunofluorescent detection of *ac25* and YP2 in paraffin sections was conducted by the following procedure. Whole pupae were fixed in aqueous Bouin's fixative, dehydrated and embedded in paraffin. 4 μm sections were made from the embedded abdomens. The sections were oxidized in 0.5% sodium borohydride in water and blocked with 1% BSA in TBS (0.5 M NaCl, 20 mM Tris, pH 7.5). The sections were reacted with *ac25* antiserum, YP2 antiserum or preimmune serum diluted 1:250 in TBS for 30 min at 22°C, and then washed three times in TBS for 5 min each. The sections were reacted with goat–anti-rabbit-FITC (Sigma; St Louis, MO) diluted 1:40 in TBS for 30 min at 22°C, and then rinsed three times in TBS for 5 min each. The sections were mounted in 10% glycerol in TBS (pH 9.0). The specimens were viewed and photographed with an Olympus BHS microscope (Olympus Corp.; Lake Success, NY) equipped with a BH2-RFC reflected light fluorescence attachment with a HQ FITC filter set (Chroma Technology Corp.; Brattleboro, VT) and a DAPI/FITC/Texas Red triple filter set (Chroma Technology Corp.).

The immuno-gold localization of *ac25* and YP2 in ultra thin sections followed procedures described previously (Zimowska *et al.*, 1994). Etched ultra thin sections were incubated with either *ac25* antiserum, YP2 antiserum (Bean *et al.*, 1988), preabsorbed antiserum, or nonreactive serum diluted 1:250. After washing, the sections were exposed to goat–anti-rabbit IgG linked with 20 nm colloidal gold (Polysciences, Inc.; Warrington, PA). Ultrastructural examination was performed on a Hitachi (H-600) transmission electron microscope operating at 75 kV.

Amino acid sequence analysis

The Protein Chemistry Core Facility, Interdisciplinary Center for Biotechnology Research, University of Florida, provided the digestion of *ac25* with endoproteinase lysine-C to generate an internal fragment and the amino acid sequence analysis of the fragment. Plugs containing *ac25* were excised from 2D-gels and then macerated in extraction buffer (0.1% SDS, 50 mM Tris, pH 8.8, 0.1 mM EDTA, 0.2 M ammonium bicarbonate). The digestion was taken to completion by reaction with 3 mU endoproteinase lysine-C μg protein^{-1} at 37°C for 12 h. The digestate was collected, concentrated, and then precipitated with nine volumes of acetone at −20°C for 12 h. The precipitate was collected by centrifugation at 14 000 ***g*** for 20 min. The pellet was redissolved in 10 mM Tris, pH 8, 2% SDS, and acetone precipitated a second time, as above. The pellet was redissolved in 10 mM Tris, pH 8, 2% SDS and brought to dryness in a SpeedVac concentrator (Savant Instruments, Inc.; Farmingdale, NY). The pellet was dissolved in water, and the proteins were resolved by SDS-PAGE and stained with silver. Fragments were cut from the gel, and the NH_2-terminal amino acid sequence of the fragments was determined by automated dansyl–Edman degradation using an on-line sequencing analyzer (Model 470A, Applied Biosystems, Foster City, CA) as described by Walker (1984). The derived amino acid sequence was used to search peptide and nucleotide sequence databases using the BLAST network service.

RESULTS

Protein isolation and antibody characterization

During purification of the yolk proteins from embryos, a peak from the ion exchange column was identified that contains small polypeptides in addition to follicular epithelium yolk protein (FEYP) (Fig. 1 (A),(B)). The polypeptides had molecular weights of between 21 000 and 25 000 (Fig. 1 (B)). Rabbit polyclonal antisera were raised against the two major polypeptide bands. Antiserum to the 25 000 molecular weight polypeptide reacted with four egg polypeptides in the molecular weight range of 20 000–28 000 (Fig. 1 (C)). Antiserum to the 21 000 molecular weight polypeptide also reacted with the same four polypeptides (data not shown). Polypeptides that reacted with the antiserum to the 25 000 molecular weight polypeptide were present throughout embryonic development, but progressively decreased in

TABLE 1. Amino acid composition (mol/1000 mol) of two α-crystallin cognates from *P. interpunctella* eggs

Amino acid[a]	*ac25*	*ac21*
Cys	10	5
Asp/Asn	93	98
Thr	43	50
Ser	79	50
Glu/Gln	131	110
Pro	65	68
Gly	73	81
Ala	71	84
Val	67	55
Met	1	8
Ile	40	46
Leu	89	85
Try	41	41
Phe	44	35
His	27	30
Lys	71	61
Arg	67	66

The results from a typical analysis.
[a]Tryptophan was not determined.

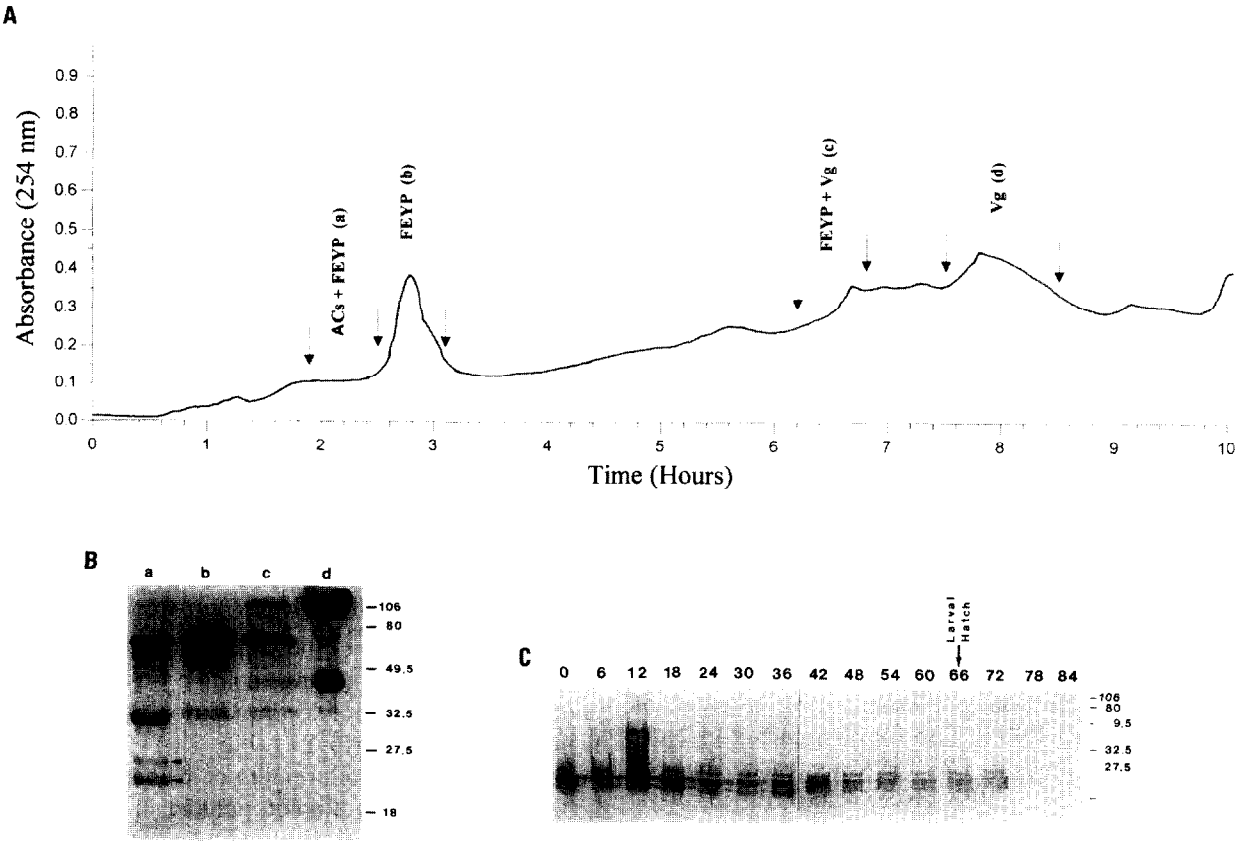

FIGURE 1. Purification of α-crystallin cognate proteins from embryos of *P. interpunctella*. (A) Egg proteins from embryos were resolved by ion exchange chromatography on DEAE Sepharose as described in Materials and Methods. (B) Combined fractions, as bracketed by arrow heads, from the ion exchange column were resolved by SDS-PAGE and stained with Coomassie blue. Lane designations: a, combined fraction (a) from ion exchange chromatography that included FEYP subunits and *acs*; b, combined fraction (b) from ion exchange chromatography that included FEYP subunits; c, combined fraction (c) from ion exchange chromatography that included FEYP and vitellin subunits; d, combined fraction (d) from ion exchange chromatography that included vitellin subunits. The *acs* (arrow heads) were present in the combined fraction (a) that also contained the FEYP subunits YP2 and YP4. (C) As shown in a Western blot, antiserum raised against the 25 000 molecular weight peptide reacted with four peptides of molecular weights from approximately 20 000 to 28 000. The Western blot also shows that the *acs* were present in embryos throughout embryogenesis and into the first larval instar. Lane designation indicates time after laying. The migration distance of the molecular weight markers ($\times 10^{-3}$) are indicated on the right margin of (B) and (C).

amount after hatching of the first instar larvae to an undetectable level (Fig. 1 (C)).

Amino acid composition and sequence analysis

The amino acid composition of the 25 000 and 21 000 molecular weight polypeptides were determined (Table 1.) There were no significant differences in the composition between the two polypeptides. However, compared with many other proteins, the relative amount of the aspartate/asparagine residues was low.

To obtain additional structural information about the 25 000 molecular weight polypeptide, purified polypeptide was subjected to NH_2-terminal amino acid sequencing. The initial trial resulted in no sequence suggesting that the polypeptide contained a blocked amino acid residue at the amino terminal position. In order to obtain a sequence for verification of the cloned DNA sequences, the 25 000 molecular weight polypeptide was purified to a single spot by two-dimensional gel electrophoresis (Fig. 2 (A)), and the purified polypeptide was subjected to digestion with endoproteinase lysine-C to generate an unblocked internal fragment of the polypeptide. Two fragments with molecular weights of approximately 12 500 and 10 500 were selected and sequenced (Fig. 2 (B)). The consensus sequence of the first 30 amino acids of the internal fragments are shown in Fig. 3. The amino acid sequence derived from the two fragments differed only by the presence of the first five amino acids identified in the 12 500 molecular weight fragment, but which were not present in the 10 500 molecular weight fragment.

The amino acid sequence of the internal fragment of the 25 000 molecular weight polypeptide was compared with known protein sequences. The fragment showed considerable similarity with α-crystallin chain A polypeptides from lenses of the eyes of vertebrates. The greatest sequence similarity with the 30 amino acid internal fragment of the 25 000 molecular weight polypeptide was observed with the α-crystallin chain A polypeptide from the spiny dogfish (de Jong *et al.*, 1988) at 77%, which included 17 identical amino acids plus six conservative substitutions (Fig. 3). This degree of sequence

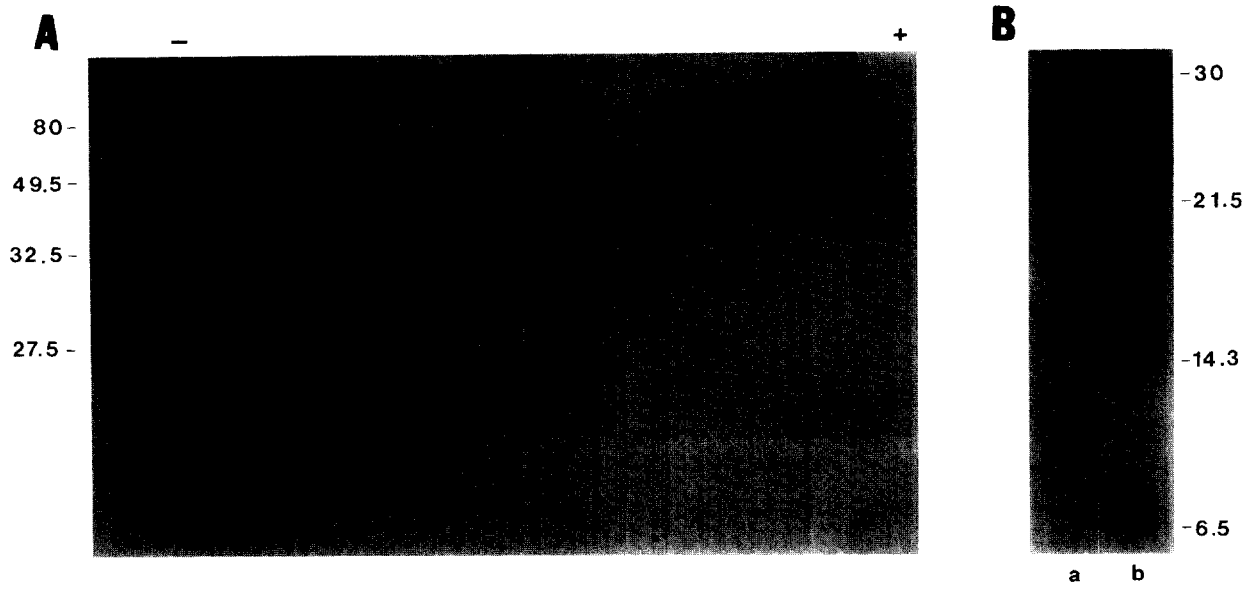

FIGURE 2. Purification and endoproteolytic cleavage of *ac25*. (A) Two dimensional electrophoresis of the combined fraction (a) resolved *ac25* into a single spot. (B) The *ac25* spot was cut from the 2D gels and subjected to digestion with endopeptidase Lysine-C. Undigested *ac25* (arrow) was present with the fragments from the proteolytic digestion. Fragments with molecular weights of 12 500 and 10 000 (arrow heads, respectively) were subjected to amino acid sequencing. Lane designations: a, endoproteinase Lysine-C digest of *ac25*; b, molecular weight standards. The molecular weights ($\times 10^{-3}$) of the standards are marked on the left of (A) and on the right of (B).

		Identities (%)	With Conservative Substitutions (%)	Smallest Sum Probability
Piac25[a]	ADK-DKLQINLDVQHFSPEEISVKTADGFVV			
Saac[b]	se.-.rFM...n.K.....l...IV.Dy.E	57	77	$4.3xe^{-7}$
Amac[c]	s.r-..FT.M...K.....dl...II.D..E	60	73	$5.9xe^{-7}$
Ggac[d]	s.r-..FT.M...K.....dl...II.D..E	60	73	$5.9xe^{-7}$
Hsac[e]	s.r-..FV.F...K.....dl...VQ.D..E	60	73	$9.2xe^{-7}$
Hshsp27[f]	RHTA.rWRvs...N..a.d.lt...K..V.E	47	70	$2.3xe^{-5}$
Dmhsp23[g]	IG.-.GFQvCm..S..K.S.lV..VQ.NS.l	40	53	0.0037
Dmhsp27[g]	VG.-.GFQvCm..Sq.K.n.lt..VV.NT..	40	60	0.0054
Dmhsp26[g]	VG.-.GFQvCm..Aq.K.S.ln..VV.aS..	40	60	0.036

FIGURE 3. Amino acid sequence of an internal fragment of *ac25* and comparison with proteins from selected species. Amino acid sequence similarity comparisons were generated by a BLAST search. Abbreviations: ".", identity; "–", insertion; "lower case", conservative substitutions; "upper case", non-identity. [a]Piac25=*P. interpunctella ac25*. The sequence is a consensus between the sequences derived from the 12 500 and 10 500 molecular weight internal fragments of *P. interpunctella ac25*; [b]Saac=Spiny dog fish α-crystallin chain A protein; de Jong *et al.*, 1988; [c]Amac=alligator α-crystallin chain A protein; de Jong *et al.*, 1985; [d]Ggac=chicken α-crystallin chain A protein; de Jong *et al.*, 1984; Thompson *et al.*, 1987; [e]Hsac=human α-crystallin chain A protein; McDevitt *et al.*, 1986; Jaworski and Piatigorsky, 1989; [f]Hshsp27=human heat shock protein 27; Hickey *et al.*, 1986; Fuqua *et al.*, 1989; Carper *et al.*, 1990; [g]Dmhsp27, 26, 23=*D. melanogaster* heat shock proteins; Ingolia and Craig, 1982; Southgate *et al.*, 1983; Ayme and Tissières, 1985.

similarity was shared with many other α-crystallin chain A polypeptides from numerous other vertebrates. The closest sequence similarity with proteins from invertebrates was with the small heat- shock proteins, *hsp27*, *hsp26* and *hsp23* from *D. melanogaster*. There were only 12 identical amino acids between the *hsps* of *D. melanogaster* and the 30 amino acid internal fragment of the 25 000 molecular weight polypeptide from *P. interpunctella*.

Cytological localization of ac25

Using immunofluorescent staining, polypeptides that reacted with the *ac25* antiserum were present in the oocytes and nurse cells of vitellogenic follicles from pharate adult females, but not in the follicular epithelial cells (Fig. 4 (A)). Staining for *ac25* was also apparent in the germinal vesicle of the oocyte and the nuclei of the nurse cells. As a control, similar preparations were stained with antiserum to YP2, a subunit to the follicular

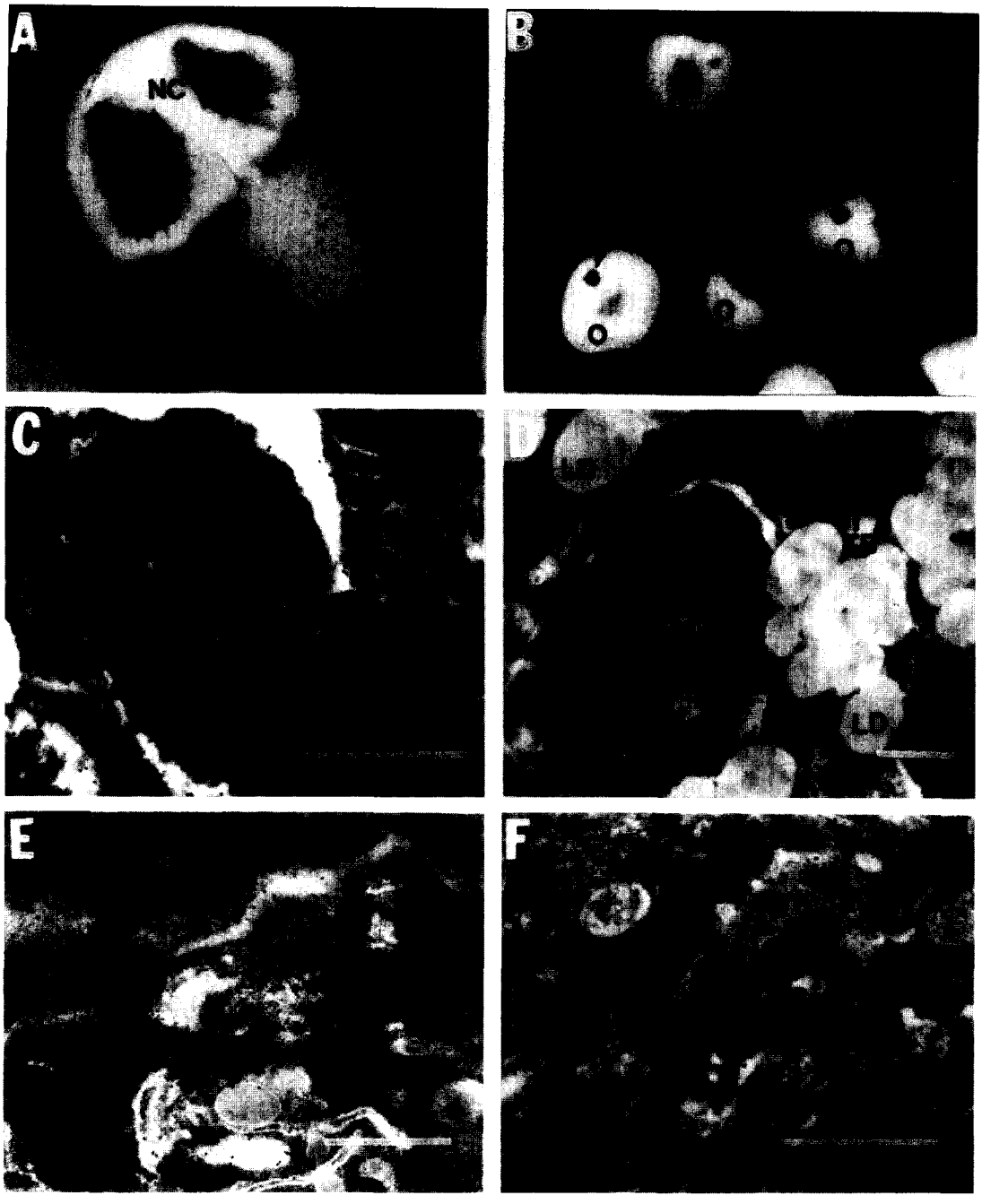

FIGURE 4. Immuno-localization of *acs* in oocytes and nurse cells of *P. interpunctella*. The *acs* were present in oocytes and nurse cells but not the follicular epithelial cells of vitellogenic oocytes as seen in paraffin sections (A). Reduced levels of staining for *acs* were present in the germinal vesicle of the oocyte and nuclei of the nurse cells in addition to the cytoplasm. When stained with antiserum to YP2 (B), the thick sections showed that the protein was restricted to the oocyte. YP2 reactive material was not observed in the germinal vesicles of the oocyte nor in the nurse cells. Ultrathin sections of ovarian follicles from late pharate adult females were immuno-gold labeled with antiserum to *ac25* (C). α-Crystallin reactive material was observed in the cytoplasm of the oocyte but not in the yolk spheres (YS), tubular bodies (TB), or mitochondria of the oocytes. Ultrathin sections of follicles were immuno-gold labeled with antiserum to YP2 (D) and YP2 reactive material was restricted to the yolk spheres, but was not observed in the cytoplasm of oocytes. Ultrathin sections of nurse cells immuno-gold labeled with antiserum to *ac25* (E) showed reactive material in the nucleoplasm as well as the cytoplasm of the nurse cells. The *ac25* reactive material was present in the cytoplasm of the nurse cells but was not found within the organelles (F). Abbreviations: FC, follicle cells; GV, germinal vesicle; LD, lipid droplet; NC, nurse cell; N, nucleus; O, oocyte; SB, spherical body; TB, tubular body; YS, yolk spheres. (A) 1240× magnification; (B) 420× magnification; scale bars=0.5 μm (C), (D), (E) and (F).

epithelium yolk protein that has previously been shown to be restricted to the yolk spheres of the oocyte (Zimowska *et al.*, 1994). YP2 was present only in the oocyte with no apparent stained material in the germinal vesicle or nurse cells of the vitellogenic follicles (Fig. 4 (B)). Immuno-gold labeling of ultrathin sections from vitellogenic follicles with antiserum to *ac25* showed the presence of labeled material in the cytoplasm of oocytes

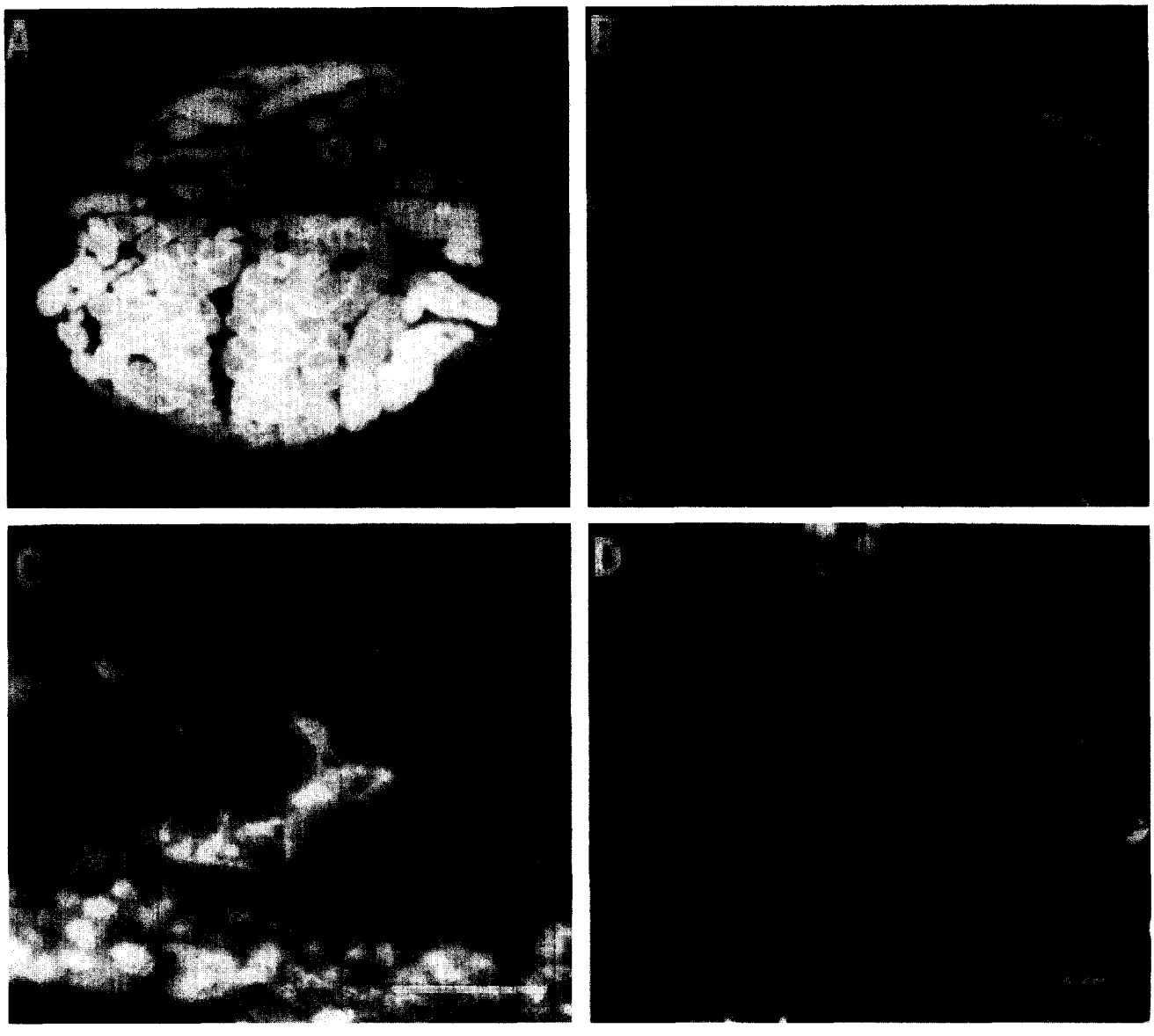

FIGURE 5. Immuno-localization of *acs* in testes and oenocytes of *P. interpunctella*. Paraffin sections of male pupae were immunofluorescently stained with antiserum to *ac25* as the primary antiserum and with goat anti-rabbit IgG-FITC as the secondary antibody (A). *acs* reactive material was observed in the sperm bundles of testes from pharate adult males. Paraffin sections of male pupae immunofluorescently stained with pre-immune serum as the primary antiserum and with goat anti-rabbit IgG-FITC as the second antiserum (B) showed little non-specific reactive material. In addition to the testes and ovaries, *acs* reactive material was also present in oenocytes of mid pharate adult males (C). Paraffin sections of pupae were immunofluorescently stained with antiserum to *ac25* as the primary antiserum and with goat anti-rabbit IgG-Texas Red as the secondary antibody. The nuclei of the cells were stained with DAPI. Paraffin sections of the male pupae immunofluorescently stained with pre-immune serum as the primary antiserum and with goat anti-rabbit IgG-Texas Red as the secondary antiserum (D) showed little non-specific reactive material. As in (C), the nuclei of the cells were stained with DAPI. Abbreviations: FB, fat body; E, epidermis; M, muscle; Oe, oenocyte; S, sperm bundle. Scale bars=0.1 mm (A) and (B); 50 μm (C) and (D).

(Fig. 4 (C)), but not within the yolk spheres or other organelles (mitochondria, tubular bodies, lipid droplets) within the oocyte. Again using YP2 as a control, the YP2 labeled material was restricted to the yolk spheres within the oocytes and was not observed associated with any other oocyte organelle (Fig. 4 (D)). Examination of nurse cells from ultrathin sections labeled with antiserum to *ac25* showed that the reactive material was restricted to the nucleoplasm and cytoplasm (Fig. 4 (E), (F)), but not in any of the organelles within the cells. Using immunofluorescent staining of paraffin sections, the polypeptides that reacted to the *ac25* antiserum were also observed in sperm bundles within testes from late pharate adult males (Fig. 5 (A)). In the pupal and adult stages, cross-reacting material was also detected in oenocytes (Fig. 5 (C)). Reaction of sections with preimmune serum showed the levels of background fluorescence for each of the preparations (Fig. 5 (B),(D)).

DISCUSSION

We have identified a group of small polypeptides that are constitutively expressed in germ cells of *P. inter-*

punctella. At least four polypeptides were detected on immunoblots of oocyte proteins from *P. interpunctella* using the antiserum to one of the polypeptides, *ac25*, which demonstrates that this group shares antigenic similarity. Although we observed four polypeptides, the total number of members of this protein family and their relatedness will require molecular cloning of the genes.

We previously showed that within the pupal and adult ovaries of *P. interpunctella*, the *acs* were present in the germ cells, i.e. nurse cells and oocytes, of all follicles (Zimowska *et al.*, 1991). In this report, we showed that the *acs* were localized within the cytoplasm of the oocytes and nurse cells, but were not within the organelles such as yolk spheres, mitochondria, tubular bodies or lipid droplets. These proteins are constitutively present in germ cells in all stages of larval, pupal and adult stages (Shirk, Beckemeyer and Zimowska, unpublished). In the testes, the *acs* were localized within the sperm bundles. In addition to the germ cells, the cytoplasm of oenocytes from pupae and adults also contained *acs* reactive material. It is not known whether the reactive material in the oenocytes is from the *acs* or is cross-reactive material from related proteins, such as small heat shock proteins. This will be determined when molecular probes for the *ac25* transcripts can be used to determine the level of gene expression in this tissue. The presence of the *acs* in germ cells of *P. interpunctella* was similar to the finding of constitutively expressed small heat shock proteins (*hsps*) in germ cells of *D. melanogaster*, described previously by others (Zimmerman *et al.*, 1983; Mason *et al.*, 1984; Glaser *et al.*, 1986; Hoffman *et al.*, 1987).

A 30 amino acid internal fragment was sequenced from *ac25*, and it was found to share significant similarity with the α-crystallin proteins that are a major component of the lens of the vertebrate eye. This sequence corresponds to a highly conserved region that is shared among the α-crystallin proteins from all species of vertebrates examined. The conserved sequence between *ac25* and the α-crystallin proteins also corresponds to the same conserved region that is shared between the α-crystallin proteins and the small *hsps* of *D. melanogaster* (Ingolia and Craig, 1982; Southgate *et al.*, 1983; Ayme and Tissières, 1985). The internal fragment of *ac25* aligns with the beginning of a conserved region that is shared in the seven small *hsps* of *D. melanogaster* (Ayme and Tissières, 1985). Even though there is some sequence similarity between the internal fragment of *ac25* from *P. interpunctella* and the small *hsps* of *D. melanogaster*, it is significant that the *ac25* fragment has greater similarity with the α-crystallin proteins than with the small *hsps* from *D. melanogaster*. Over the length of this sequence, *ac25* from *P. interpunctella* has 17–18 of the 30 amino acids identical with α-crystallin proteins, while only 12 were identical with the *D. melanogaster* small *hsps*. On the basis of the similarity between the α-crystallin proteins and this polypeptide for the amino acid sequence and the cDNA sequence (Shirk, Broza and Hapner, unpublished), we tentatively designate this group of proteins the α-crystallin cognates (*acs*) in *P. interpunctella*.

While the significance of the structural similarity between *P. interpunctella ac25* and the α-crystallins of vertebrates and the small *hsps* of *Drosophila* remains uncertain, it may suggest functional similarity. The amino acid region from approximately 75 to 125 of the α-crystallins is highly conserved with a region of the small *hsps*, and may be responsible for the formation of the large aggregates that have been reported for these proteins (for reviews see Wistow and Piatigorsky, 1988; de Jong *et al.*, 1993; Ingolia and Craig, 1982). This region is also highly conserved with the 30 amino acids sequenced from the *P. interpunctella ac25*. In addition to forming aggregates, the small *hsps* of *Drosophila* have been found in association with the nucleoskeleton (Sinibaldi and Morris, 1981), prosomes (Arrigo *et al.*, 1985), cytoskeleton (Leicht *et al.*, 1986), and have been identified as chaperones that influence protein refolding following heat stress (Jakob *et al.*, 1993). The properties of the *P. interpunctella ac25* will be examined to determine whether it also shares similar activities with these related proteins.

REFERENCES

Arrigo A.-P., Darlix J.-L., Khandjian E. W., Simon M. and Spahr P.-F. (1985) Characterization of the prosome from *Drosophila* and its similarity to the cytoplasmic structures formed by the low molecular weight heat shock proteins. *EMBO J.* **4,** 399–406.

Ayme A. and Tissières A. (1985) Locus 67B of *Drosophila melanogaster* contains seven, not four, closely related heat shock genes. *EMBO J.* **4,** 2949–2954.

Bean D. W., Shirk P. D. and Brookes V. J. (1988) Characterization of yolk proteins from the eggs of the Indianmeal moth, *Plodia interpunctella*. *Insect Biochem.* **18,** 199–210.

Carper S. W., Rocheleau T. A. and Storm F. K. (1990) cDNA sequence of a human heat shock protein HSP27. *Nucl. Acids Res.* **18,** 6457.

De Jong W. W., Leunissen J. A. M., Leenen P. J. M., Zweers A. and Versteeg M. (1988) Dogfish α-crystallin sequences: comparison with small heat shock proteins and *Schistosoma* egg antigen. *J. Biol. Chem.* **263,** 5141–5149.

De Jong W. W., Leunissen J. A. M. and Voorter C. E. M. (1993) Evolution of the α-crystallin/small heat-shock protein family. *Molec. Biol. Evol.* **10,** 103–126.

De Jong W. W., Zweers A., Versteeg M., Dessauer H. C. and Goodman M. (1985) α-Crystallin A sequences of *Alligator mississippiensis* and the lizard *Tupinambis teguixin*: molecular evolution and reptilian phylogeny. *Molec. Biol. Evol.* **2,** 484–493.

De Jong W. W., Zweers A., Versteeg M. and Nuy-Terwindt E. C. (1984) Primary structures of the α-crystallin A chains of twenty-eight mammalian species, chicken and frog. *Eur. J. Biochem.* **141,** 131–140.

Ding D., Parkhurst S. M., Halsell S. R. and Lipshitz H. D. (1993) Dynamic *Hsp83* RNA localization during *Drosophila* oogenesis and embryogenesis. *Molec. Cell. Biol.* **13,** 3773–3781.

Fischer-Vise J. A., Rubin G. and Lehman R. (1992) The *fat facets* gene is required for *Drosophila* eye and embryo development. *Development* **116,** 985–1000.

Fuqua S. A. W., Blum-Salingaros M. and McGuire W. L. (1989) Induction of the estrogen-regulated "24K" protein by heat shock. *Cancer Res.* **49,** 4126–4129.

Glaser R. L., Wolfner M. and Lis J. T. (1986) Spatial and temporal pattern of *hsp26* expression during normal development. *EMBO J.* **5,** 747–754.

Hay B., Jan L. Y. and Jan Y. N. (1988) A protein component of *Drosophila* polar granules is encoded by *vasa* and has extensive sequence similarity to ATP-dependent helicases. *Cell* **55,** 577–587.

Hay B., Jan L. Y. and Jan Y. N. (1990) Localization of *vasa*, a component of *Drosophila* polar granules, in maternal-effect mutants that alter embryonic anterioposterior polarity. *Development* **109,** 425–433.

Hickey E., Brandon S. E., Potter R., Stein G., Stein J. and Weber L. A. (1986) Sequence and organization of genes encoding the human 27 kDa heat shock protein. *Nucl. Acids Res.* **14,** 4127–4145.

Hoffman E. P., Gerring S. L. and Corces V. G. (1987) The ovarian, ecdysterone, and heat-shock-responsive promoters of the *Drosophila melanogaster* hsp27 gene react very differently to perturbations of DNA sequence. *Molec. Cell. Biol.* **7,** 973–981.

Ingolia T. D. and Craig E. A. (1982) Four small *Drosophila* heat shock proteins are related to each other and to mammalian α-crystallin. *Proc. Nat. Acad. Sci. U.S.A.* **79,** 2360–2364.

Jakob U., Gaestel M., Engel K. and Buchner J. (1993) Small heat shock proteins are molecular chaperones. *J. Biol. Chem.* **268,** 1517–1520.

Jaworski C. J. and Piatigorsky J. (1989) A pseudo-exon in the functional human α-crystallin gene. *Nature* **337,** 752–754.

Jongrens T. A., Hay B., Jan L. Y. and Jan Y. N. (1992) The *germ cell-less* gene product: a posteriorly localized component necessary for gem cell development in *Drosophila*. *Cell* **70,** 569–584.

Laemmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227,** 680–685.

Lantz V., Ambrosio L. and Schedl P. (1992) The *Drosophila orb* gene is predicted to encode sex-specific germline RNA-binding proteins and has localized transcripts in ovaries and early embryos. *Development* **115,** 75–88.

Lasko P. F. and Ashburner M. (1988) The product of the *Drosophila* gene *vasa* is very similar to eukaryotic initiation factor-4A. *Nature* **335,** 611–617.

Lasko P. F. and Ashburner M. (1990) Posterior localization of *vasa* protein correlates with, but is not sufficient for, pole cell development. *Genes Develop.* **4,** 905–921.

Leicht B. G., Biessmann H., Palter K. B. and Bonner J. J. (1986) Small heat shock proteins of *Drosophila* associate with the cytoskeleton. *Proc. Nat. Acad. Sci. U.S.A.* **83,** 90–94.

Mahowald A. P. (1992) Germ plasm revisited and illuminated. *Science* **255,** 1216–1217.

Mason P. J., Hall L. M. C. and Gauz J. (1984) The expression of heat shock genes during normal development in *Drosophila melanogaster*. *Molec. Gen. Genet.* **194,** 73–78.

McDevitt D. S., Hawkins J. W., Jaworski C. J. and Piatigorsky J. (1986) Isolation and partial characterization of the human αA-crystallin gene. *Exp. Eye Res.* **43,** 285–291.

Myohara M. (1994) Fate mapping of the silkworm, *Bombyx mori*, using localized UV irradiation of the egg at fertilization. *Development* **120,** 2869–2877.

Nardi J. (1993) Modulated expression of a surface epitope on migrating germ cells of *Manduca sexta* embryos. *Development* **118,** 967–975.

Nardi J. (1994) Rearrangement of epithelial cell types in an insect wing monolayer is accompanied by differential expression of a cell surface protein. *Develop. Dynam.* **199,** 315–325.

Nagy L., Riddiford L. and Kiguchi K. (1994) Morphogenesis in the early embryo of the lepidopteran *Bombyx mori*. *Develop. Biol.* **165,** 137–151.

O'Farrell P. H. (1975) High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250,** 4007–4021.

Silhacek D. L. and Miller G. L. (1972) Growth and development of the Indian meal moth *Plodia interpunctella* (Lepidoptera: Phycitidae), under laboratory mass-rearing conditions. *Ann. Entomol. Soc. Amer.* **65,** 1084–1087.

Sinibaldi R. M. and Morris P. W. (1981) Putative function of *Drosophila melanogaster* heat shock proteins in the nucleoskeleton. *J. Biol. Chem.* **256,** 10735–10738.

Southgate R., Ayme A. and Voellmy R. (1983) Nucleotide sequence analysis of the *Drosophila* small heat shock gene cluster at locus 67B. *J. Molec. Biol.* **165,** 35–57.

Thompson M. A., Hawkins J. W. and Piatigorsky J. (1987) Complete nucleotide sequence of the chicken αA-crystallin gene and its 5′ flanking region. *Gene* **56,** 173–184.

Walker J. M. (1984) The dansyl–Edman method for peptide sequencing. In *Methods in Molecular Biology: Proteins* (Edited by Walker J. M.), pp. 213–219. Humana Press, Clifton.

Wistow G. J. and Piatigorsky J. (1988) Lens crystallins: the evolution and expression of proteins for a highly specialized tissue. *Ann. Rev. Biochem.* **57,** 479–504.

Zimmerman J. L., Petri W. and Meselson M. (1983) Accumulation of a specific subset of *D. melanogaster* heat shock mRNAs in normal development without heat shock. *Cell* **32,** 1161–1170.

Zimowska G., Silhacek D. L., Shaaya E. and Shirk P. D. (1991) Immunofluorescent analysis of follicular growth and development in whole ovaries of the Indianmeal moth. *J. Morphol.* **209,** 215–228.

Zimowska G., Shirk P. D., Silhacek D. L. and Shaaya E. (1994) Yolk sphere formation is initiated in oocytes before development of patency in follicles of the moth, *Plodia interpunctella*. *Roux's Arch. Develop. Biol.* **203,** 215–226.

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