Identification of Secreted and Cytosolic Gelsolin in *Drosophila*

Maria Cristina Stella, Heike Schauerte, Kristina L. Straub, and Maria Leptin

Max-Planck-Institut für Entwicklungsbiologie, 72076 Tübingen, Federal Republic of Germany

Abstract. We have cloned the gene for *Drosophila* gelsolin. Two mRNAs are produced from this gene by differential splicing. The protein encoded by the longer mRNA has a signal peptide and its electrophoretic mobility when translated in vitro in the presence of microsomes is higher than when it is translated without microsomes. The protein translated from the shorter mRNA does not show this difference. This indicates that *Drosophila* like vertebrates has two forms of gelsolin, one secreted, the other cytoplasmic. The mRNA for both is present ubiquitously in the early embryo. Later, the cytoplasmic form is expressed in parts of the gut. The RNA for the secreted form is expressed in the fat body, and the secreted protein is abundant in extracellular fluid (hemolymph). The cytoplasmic form of gelsolin co-localizes with F-actin in the cortex of the cells in the embryo and in larval epithelia. However, during cellularization of the blastoderm it is reduced at the base of the cleavage furrow, a structure similar to the contractile ring in dividing cells.

THE actin cytoskeleton plays important roles in the behavior of cells, for example during cell migration or cell shape changes. During these processes, its structure is modulated by actin binding proteins (ABPs)[1]. One class of ABPs which regulate the state of polymerization of actin in the cell are the severing and capping proteins, also called class I F-actin capping proteins (reviewed by Pollard and Cooper, 1986; Weeds and Maciver, 1993). They are a family of proteins with extensive sequence homology, and so far, members of this family have been identified in *Dictyostelium* (severin), *Physarum* (fragmin), and vertebrates (gelsolin, villin, Mbh I) (Ampe and Vanderkerckhove, 1987; André et al., 1988; Arpin et al., 1988; Bazari et al., 1988; Prendergast and Ziff, 1991; Sakurai et al., 1991; Way and Weeds, 1988; Yu et al., 1990). Each of these proteins consists of either three (severin, fragmin, and MbhI) or six (villin and gelsolin) repeats of homologous segments of 120–130–amino acid residues long plus unique sequences at the amino or carboxy termini, like, for example, the carboxy-terminal head piece in villin. Gelsolin exists in a secreted and a cytosolic form, and the mature secreted form usually has a short (up to 25 amino acids) amino-terminal plasma extension not found in the intracellular form. It is found in blood plasma and is produced by muscles (Nodes et al., 1987).

Severing proteins can sever actin filaments, cap the fast growing end of actin and nucleate actin polymerization. Their functions are regulated by Ca^{2+} and PIP_2, suggesting a role for these proteins in the modification of the cytoskeleton in response to extracellular signals (Forscher, 1989; Lassing and Lindberg, 1988). These activities have been mapped to different parts of the molecules by analysis of the interactions of actin with different proteolytic fragments or bacterially expressed variants of severing proteins (Eichinger et al., 1991; Way et al., 1989, 1990, 1992).

However, not much is known about their functions in vivo. In strains of *Dictyostelium* that lack severin, cell motility is not visibly affected (André et al., 1989) whereas overexpression of gelsolin in mouse fibroblast cultures induces disaggregation of stress fibers and an increase in cell motility (Cunningham et al., 1991), consistent with the in vitro evidence that gelsolin regulates the state of the cytoskeleton.

We are using *Drosophila* to isolate genes for ABPs and to study their functions throughout development. In *Drosophila*, the behavior of actin and of some of its associated proteins has been extensively described (reviewed by Fyrberg and Goldstein, 1990; Kiehart, 1990; Schejter and Wieschaus, 1993). Especially the early phases of embryogenesis show dynamic changes of the actin cytoskeleton and are easily accessible to microscopic analysis (Karr and Alberts, 1986; Kellogg et al., 1988; Warn and Magrath, 1983). During the first nine nuclear cycles, actin is found in an evenly distributed punctate pattern in a thick layer below the egg surface. When the nuclei begin to migrate towards the cortex of the egg, changes in the actin organization become apparent. First, at nuclear cycle 10, a meshwork of actin (actin

Address all correspondence to M. Leptin, Institut für Genetik, Universität Köln, Weyertal 121, 50931 Köln, FRG.

1. *Abbreviations used in this paper*: ABP, actin binding protein; BS, blocking solution.

cap) is formed above each nucleus. Actin caps are typical for interphase nuclei and they appear in nuclear cycles 11, 12, and 13. During each nuclear cycle actin caps spread out and their edges become associated with the transient membrane furrows that separate the dividing nuclei. After each nuclear division actin caps reassemble over the new nuclei. During interphase 14 the egg is subdivided into cells by membranes growing inwards from the surface between the nuclei (a process with many similarities to cytokinesis; Schejter and Wieschaus, 1993). During this process, actin is localized underneath the membranes of the cellularizing blastoderm and is enriched at the advancing cleavage furrows. A number of *Drosophila* ABPs have been isolated biochemically (Dubreuil et al., 1987; Dubreuil et al., 1990; Heintzelman et al., 1993; Kiehart and Feghali, 1986; Miller and Alberts, 1989; Miller et al., 1989) and some of these proteins were found to be associated with different domains of the actin skeleton during the early stages of development.

We have cloned a *Drosophila* homologue of gelsolin, one of the best characterized members of the severing protein family. We show that like vertebrates (Kwiatkowski et al., 1986, 1988), *Drosophila* expresses two differentially spliced transcripts coding for a secreted and an intracellular gelsolin. (The cDNA for the secreted form has recently also been cloned by another group [Heintzelman et al., 1993].) The characterization and the chromosomal localization of this gene are the first steps towards obtaining mutants in gelsolin in order to understand its functions in vivo.

Materials and Methods

Flies

Flies were grown and collected under standard conditions (Ashburner, 1989; Wieschaus and Nüsslein-Volhard, 1986). We used *white* flies as wild-type stock.

DNA and RNA

Standard methods were used for the preparation of mRNA and cloned DNA (Sambrook et al., 1989). The *Drosophila* genomic DNA was prepared according to Ashburner (1989). Genomic Southerns were hybridized in 7% SDS, 5 mM EDTA, 0.5 M sodium phosphate, pH 6.8. The low stringency hybridization was performed at 42°C overnight followed by two washes, 10-min each, in 1× SSC plus 0.1% SDS at 42°C.

Oligonucleotides were synthesized in a Pharmacia gene assembler (Pharmacia Fine Chemicals, Piscataway, NJ).

Cloning of Drosophila Gelsolin

As template for PCR, single strand cDNA was synthesized from total embryonic RNA using standard protocols (Sambrook et al., 1989). To reduce the background, the cDNA preparation was digested with RNAse A before using it for the PCR. The primers were chosen from regions in the first segment of gelsolin. This is the most conserved segment among the six severing proteins whose sequences we had available (pig and human gelsolin, human and chicken villin, *Physarum* fragmin, and *Dictyostelium* severin). Within this segment we chose four regions with high homology to make primers, as listed below (I = Inosine; Recognition sites for ClaI [sense oligo] and EcoRI [antisense oligo] are shown in bold.).

Left (Sense) Primers
Region I: Amino acid residues 66–72 in human plasma gelsolin: PGLQIWR; Residues G, I, W, and R are present in all six proteins. (62–68 in *Drosophila* plasma gelsolin; PGLEIWR).
Oligonucleotide 1 (degeneracy of 72):

cgatcgat C C I G G I (G/T/C) T I (C/A) A (G/A) A T (C/T/A) T G G (C/A) G

Region II: amino acid residues 89–95 in human plasma gelsolin: FFTGDAY; Residues F, G, D, and Y are present in all six proteins. (85–91 in *Drosophila* plasma gelsolin; FYTGDSF).
Oligonucleotide 2 (degeneracy of 256):

taatcgat C (T/C) T T (A/T) (T/C) T (G/A) (A/G) I G T I (T/C) G A (G/A) (T/G) T C I T A

Right (Antisense) Primers
Region III: amino acid residues 145–151 in human plasma gelsolin: QHREVQG; Residues Q, R, Q, and G are present in all six proteins. (142–148 in *Drosophila* plasma gelsolin; QHREVQD).
Oligonucleotide 3 (degeneracy of 64):

taggaattcc C (T/C) T G I A C (T/C) T C I C (G/T) (A/G) T (A/G) (T/C) T G

Region IV amino acid residues 170–176 in human plasma gelsolin: GGVASGF; Residues G, G, V, G, and F are present in all six proteins. (167–173 in *Drosophila* plasma gelsolin; GGVGTGF).
Oligonucleotide 4 (degeneracy of 8):

taggaattcc A (T/A) I C C I G (T/A) I (G/T) C I A C I C C I C C

The only successful combination was 1 and 4. The PCR was performed according to Sambrook et al. (1989), using the following conditions: 94°C 1 min, 43°C 1.5 min, 72°C 2 min. After the first 30 cycles, another 2 U of Taq polymerase (Pharmacia Fine Chemicals) were added and the reaction was carried on for a further 30 cycles. When the reaction products were run on a 2% agarose gel, seven bands in the range of 70–600 bp were visible. A Southern blot of such a gel was hybridized with a probe for human gelsolin at low stringency and a signal was found over a band of ~300-bp, the size expected for the fragment between the primers 1 and 4. To clone this band, Klenow polymerase fragment (2 U, 1 h at 37°C) was added to end repair the products of the amplification after 60 PCR cycles. The PCR products were phenol extracted, ethanol precipitated, and resuspended in water, followed by overnight digestion with ClaI and EcoRI. The two enzymes were inactivated by heating, and then the PCR products were kinased and gel purified. The band identified with the Southern blot was isolated and ligated to phosphatased pBluescript SKII+ (Stratagene, La Jolla, CA), previously digested with EcoRI and ClaI. 121 recombinant clones were sequenced; 30% of them were identical and showed homology with gelsolin. None of the other clones had any obvious similarity with genes for actin binding proteins. The insert with gelsolin homology was used as a probe for screening embryonic cDNA libraries (1 to 4 h and 12 to 24 h of development) (Brown and Kafatos, 1988) and for further experiments.

Sequencing

All sequencing reactions were performed using the Sequenase II kit (US Biochemicals, Cleveland, OH) according to the instructions of the manufacturers. The cloned PCR fragments were sequenced using SK and KS primers (Stratagene). The cDNA clone A was sequenced by the shot gun method (Sambrook et al., 1989). The 5′ ends of the other cDNA clones were sequenced directly in the pNB40 vector using the SP6 primer (Promega Biotec, Madison, WI). Sequences were analyzed using the GCG package programs.

Identification of the Splice Sites

The PCR amplified fragment was used as a probe for screening a genomic library (gift of J. Tamkun, UCSC, Santa Cruz, CA). Cosmids were isolated, digested with HindIII and a 5.2-kb band containing most of the *Drosophila* gelsolin gene was subcloned in pBluescript SKII+ (Stratagene). The oligonucleotides used as primers to determine the junctions between introns and exons were the following:

A T A C G C T A T T T G C G A C T	(clone A)
A T A A G T A C T C A G T T C C T G	(clone B)
T T C G C C G G A T T G T A C G G G	(region common to A and B)

The sequencing reactions were performed using as template the cloned genomic fragment and the cDNAs.

In Vitro Translation

The template plasmids were linearized with NotI, phenol extracted, precipitated, and resuspended in 0.1% DEPC-treated H_2O. An in vitro transcrip-

tion was performed according to Melton et al. (1984) with the modification of Driever et al. (1990), using 1 μg of linearized plasmid in each reaction. For the in vitro translation, 20 μl of rabbit reticulocyte lysate (Amersham Corp., Arlington Heights IL), 2 μl of [^{35}S]methionine (Amersham Corp.) and 500 ng of in vitro synthesized RNA were incubated at 30°C for 1 h, with or without canine microsome membranes (Promega Biotec). At the end of the reaction, Laemmli sample buffer was added and the samples were boiled for 3 min, centrifuged, and loaded on a 7.5% polyacrylamide gel. The gels were run at 35 mA, fixed in H_2O/acetic acid/isopropanol (1:6:2.5), and dried. The signal was detected using Amersham Hyperfilm MD.

Western Blot

Embryonic extracts and immunoblots were done as described by Sprenger and Nüsslein-Volhard (1992). The hemolymph was prepared by puncturing the thoraces of flies and spinning them in an Eppendorf tube on a cushion of glasswool. The hemolymph can then be collected from the tip of the tube (Kambysellis, 1984). The membranes (BA 85 Nitrocellulose; Schleicher & Schuell, Keene, NH) were blocked for 1 h at room temperature in blocking solution (BS: 10% low fat dry milk in phosphate-buffered saline containing 0.2% Triton X-100) and then incubated with the first antibody over night at 4°C. The antibody against β-tubulin was from Amersham Corp. and it was used at a dilution of 1:1,000; the antibody against gelsolin was used at a dilution of 1:200. The membranes were washed three times for 20 min in BS and were incubated with peroxidase-conjugated anti-rabbit antibody (Dianova GmbH, Hamburg, Germany; used at a dilution of 1:5,000). After washing three times for 10 min with BS and one time with phosphate buffered saline, the blots were developed using the ECL System (Amersham Corp.) and Amersham Hyperfilm MD to detect the signal.

Production of Antibodies

The antiserum against gelsolin was prepared by immunizing rabbits with a bacterially expressed fusion protein. The fusion protein was obtained by cloning the NsiI-HindIII fragment (nucleotides 487–2503) of *Drosophila* gelsolin in the expression vector pQE9 (Qiagen, Chatsworth, CA) (pQE9-gel). The *Escherichia coli* strain M15[pREP4] was transformed with the pQE9-gel construct and protein production was induced by adding 1 mM IPTG. The bacterial extract was later purified on a Ni^{2+} column (NTA resin; Qiagen) according to the instrucions of the manufacturers. The immune serum was affinity purified using an Affigel 15 (Bio-Rad Laboratories, Cambridge, MA) column to which the purified bacterial protein had been conjugated.

Staining and In Situ Hybridization of Embryos

Staged embryos were dechorionated with bleach, fixed in 4% formaldehyde in PBS, and devitellinized by hand. The embryos were blocked in blocking solution (PBS, 1% BSA, 1% Triton X-100, 1% goat serum) for 1 h, and incubated in gelsolin antibody (at a dilution of 1:200 in BS) for 2 h. The embryos were then washed three times in BS, 20-min each, and the secondary antibody (FITC-conjugated goat anti-rabbit; Dianova) was added at a dilution of 1:1,000 in BS. After 2 h the embryos were washed and mounted in Mowiol.

For frozen sections, dechorionated embryos were fixed in 18% formaldehyde in PBS for 20 min and were oriented for transverse sectioning in OCT embedding medium, frozen, sectioned on a cryostat, and mounted on gelatin-coated microscope slides. The staining was performed in the same way as for whole-mount embryos. Stained sections were mounted in Mowiol.

Rhodamin-conjugated phalloidin (Sigma Chemical Co., St. Louis, MO) was used at a concentration of 1 μg/ml and DAPI (Sigma Chemical Co.) was used at the concentration of 2 μg/ml. Stained embryos were observed with oil immersion lenses on a Zeiss Axiophot fluorescence microscope and photographed on Kodak technical pan film at 400 ASA.

In Situ Hybridizations

In situ hybridizations on whole mount embryos were performed as described (Tautz and Pfeifle, 1989). The specific probe for clone A was made from the gel-purified BglI-HindIII fragment (nucleotides 1–330 plus 83 nucleotides from the vector) from clone A, the specific probe B from the ScaI-HindIII fragment (nucleotides 1–56 plus 74 nucleotides from the vector) from clone B. The common probe was the NsiI-HindIII fragment (nucleotides 487-2503) from clone A.

Results

Cloning of the Drosophila Gelsolin Gene

To clone severing protein genes in *Drosophila melanogaster*, we performed reverse PCR using embryonic poly(A)$^+$ mRNA as template (see Materials and Methods). We obtained a fragment that showed significant sequence homology with fragmin, villin, severin, and gelsolin genes.

On Southern blots the PCR fragment hybridized to a single 5.2-kb Hind III band at high stringency and to an additional 2.8-kb band at lower stringency (Fig. 1). The low stringency signal does not represent other homologous fragments of the same gene (e.g., segment 4), since it is not seen when the blot is hybridized with a full-length cDNA at high stringency. A full-length cDNA hybridized to the 5.2-kb Hind III and weakly to a 2.6-kb band at high stringency (not shown; see Heintzelmann et al., 1993). The same probe hybridized to two further bands at low stringency (not shown). None of the low stringency signals are due to a cross-hybridization with the gene *quail*, which codes for a villin-related protein of *Drosophila*, since quail probes do not hybridize to these bands (Marajan-Miklos, S., and L. Cooley, personal communication). They therefore may represent genes for other severing proteins.

On Northern blots the PCR fragment hybridized to a broad band at about 2.7 kb, which was later shown also to hybridize with full length cDNA clones (not shown). This broad band is probably a doublet representing two splice forms of the gelsolin gene (see below). The PCR amplified fragment was used as a probe for screening two embryonic cDNA libraries (see Materials and Methods). We analyzed seven clones which by restriction digests fell into two classes, A and B. The gene represented by these cDNAs is localized on the right arm of the third chromosome, in the region 81F-82A (not shown; see also Heintzelman et al., 1993).

We used the longest clone from each class (called clone A and clone B; 2,888 and 2,651 bp, respectively) for further analysis. At least clone A (which is 89-bp longer at the 5′ end than the previously published cDNA for secreted gelsolin) is a full length cDNA, since it has a G as the first nucleotide in the cDNA which is absent in the genomic sequence. This is usually found in the full length clones from this library

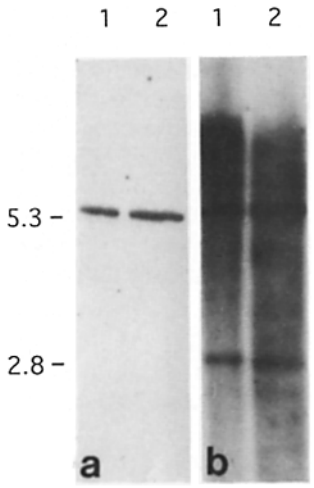

Figure 1. Southern blots hybridized with the same gelsolin probe (the original PCR amplified and cloned fragment from segment 1) at high and low stringency hybridization conditions. Genomic fly DNA (5 μg in each lane) was digested with BamHI (lanes *1*) and with BamHI plus HindIII (lanes *2*). *a* shows the autoradiography of a Southern blot hybridized and washed at high stringency, *b* the result using a twin filter at low stringency (see Materials and Methods for hybridization and washing conditions).

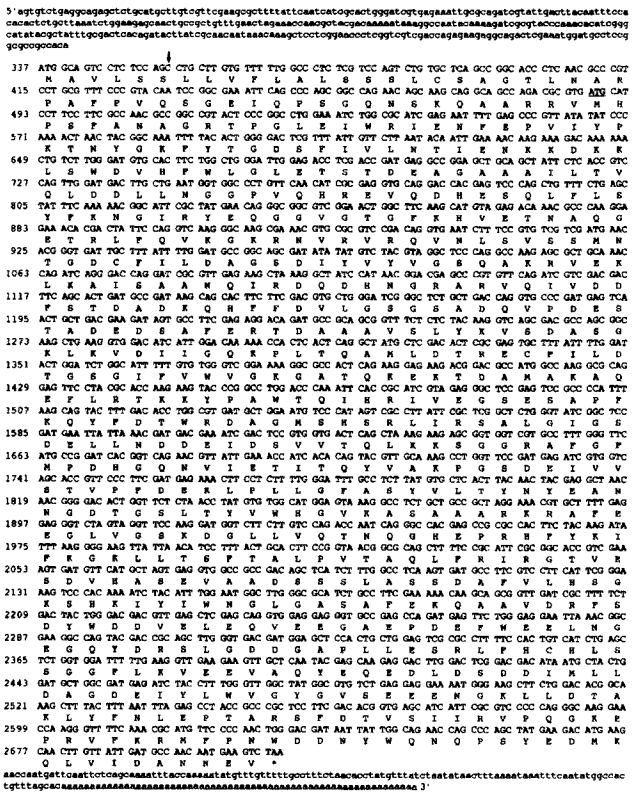

Figure 2. Nucleotide and amino acid sequence of clone A. The untranslated regions are shown in lower case; the open reading frame is shown in upper case. The arrow marks the nucleotide where the sequences of clone A and clone B become identical. The translation start site of clone B is underlined. The sequence data for secreted gelsolin are available from EMBL/GenBank/DDBJ under accession number X75630; the data for cytoplasmic gelsolin have the accession number X75629.

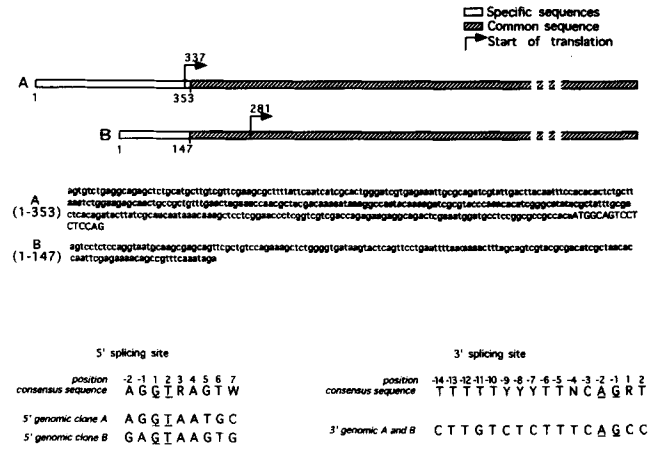

Figure 3. (*a*) Sequences and diagrammatic representation of the difference in the 5′ ends of clones A and B. The diagram shows that the first 353 nucleotides from clone A and the first 147 nucleotides from clone B differ. From nucleotide 354 in clone A and nucleotide 148 in clone B the two cDNAs are identical. The first translated nucleotide of the cytoplasmic form is nucleotide 281 in clone B. Capital letters in the sequence are translated nucleotides. (*b*) Genomic sequences around the splice sites at position 353 in cDNA A and position 147 in cDNA B. The sequences are compared with the 5′ and 3′ consensus splice sites in flies. The underlined nucleotides represent residues that are conserved in all intron/exon boundaries.

(Brown et al., 1989). We sequenced clone A completely (Fig. 2), and clone B partially. The two clones have open reading frames of 2,373 and 2,223 bp, respectively. They each code for a protein with the six segment structure typical of gelsolin and without the villin headpiece.

Clone B has 148 bp at the 5′ end that are not present in clone A. They replace the first 353 bp at the 5′ end of the clone A (Fig. 3). Probes specific for the 5′ ends of the two cDNAs hybridized to the same 2.6-kb EcoRI-HindIII fragment both in Southern blots of genomic DNA and of cosmids covering this region (data not shown). We subcloned and partially sequenced the genomic region that contains the gelsolin gene. Fig. 3 shows the sequences of the splice sites at the 3′ exon/intron boundary of the specific exons of clones A and B and at the 5′ end of the first common exon. The nucleotide residues that match the splice consensus sequences (Jackson, 1991; Mount et al., 1992) are underlined. These results, together with the identity of the 3′ ends of clone A and B show that the two mRNAs arise by differential splicing from the same gene.

Protein Products of Clone A and Clone B

Clone A codes for a polypeptide of predicted 87 kD containing a putative signal peptide (von Heijne, 1986, 1990). The sequence coding for the signal peptide and for a further 50 amino acids is not present in clone B. Clone B contains a stop codon in position 15, and the first ATG that can be used for initiation of translation is in position 281, in the part of the sequence that is in common with A (position 487 of clone A). This suggests, that, surprisingly, *Drosophila*, like vertebrates, also has a secreted and a cytoplasmic form of gelsolin. To test if the putative signal peptide present in A is functional we performed an in vitro translation assay in the presence of canine microsomal membranes. If the putative signal peptide is functional, the microsome membranes should process the protein to a mature form of lower molecular weight. Fig. 4 *b* shows the result of this experiment using clone B and clone A as templates. The protein runs at a higher molecular mass than predicted from the primary sequence, at about 102 kD. As expected, the presence of microsomes does not affect the mobility of B (Fig. 4 *b*, compare lanes *3* and *4*), whereas the protein translated from clone A migrates faster if translated in the presence of microsomal membranes (compare lanes *1* and *2*; sometimes, addition of a higher concentration of microsomes results in a further reduction in the size of the translation product to the size of the protein from clone B). This result strongly suggests that the protein product of clone A is secreted.

If there is a secreted form of gelsolin in *Drosophila*, then it should be possible to find it in extracellular fluids. We therefore isolated hemolymph from adult flies and analyzed it by Western blotting using the antibody described below. Fig. 4 *c* shows that hemolymph contains a protein that is strongly labeled by the gelsolin antibody (see below for a discussion of the size of the protein), while a control staining of the same blot with anti-β-tubulin antibodies gives a hardly detectable signal. Therefore the gelsolin signal cannot be ascribed to cellular contaminants in the hemolymph (which

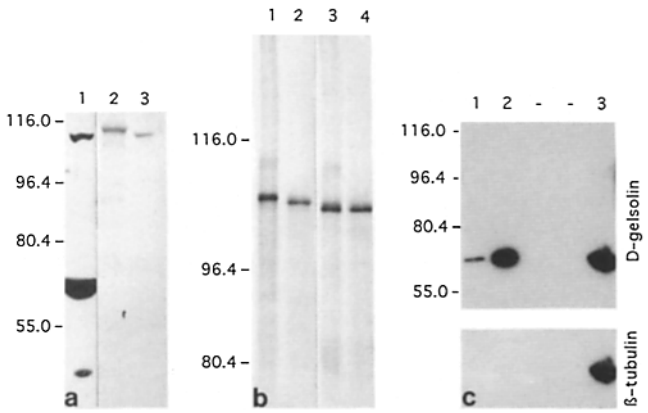

Figure 4. (*a*) Western blot analysis of in vivo and in vitro translated gelsolin. Lysate from five flies (lane *1*) was run on a gel in parallel with in vitro translated [^{35}S]Methionine-labeled protein derived from clone A (lane *2*) and from clone B (lane *3*). A blot was made from the gel and lane *1* was stained with the antibody against gelsolin while the protein in lanes *2* and *3* was detected by autoradiography. (*b*) In vitro translation products derived from clone A (lanes *1* and *2*) and clone B (lanes *3* and *4*), in the absence (lanes *1* and *3*) of microsomal membranes, or with 0.9 μl of microsomal membranes (lanes *2* and *4*) added. At higher concentrations of membranes (1.8 μl) the product of clone A sometimes runs at the same size as the product of clone B. To maximize the resolution in the 116-kD region, 7.5% polyacrylamide gels were used and these gels were run for a very long time until everything below the 80.4-kD marker band had run off the gel (*a*, compare lanes *2* and *3*, for a whole gel of an in vitro translation experiment). (*c*) Western blot showing gelsolin in adult fly lysate and in the hemolymph. In lane *3*, the extract of three flies was loaded. Lanes *1* and *2* contain the hemolymph from 3 and from 50 flies, respectively. The lower panel shows the same blot stained with an antibody against β-tubulin.

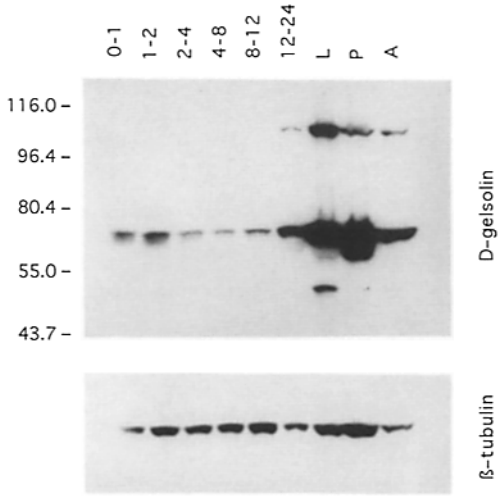

Figure 5. Western blot showing gelsolin expression during development. A Western blot of lysates from embryos of different ages (indicated in hours above the lanes) as well as larvae (*L*), pupae (*P*), and adult flies (*A*) was stained with the antibody against gelsolin (*top*). The lower panel shows the same blot stained with an antibody against β-tubulin.

are responsible for the very weak tubulin signal), since in the total protein extracts from flies (lane *3*) the intensity of the gelsolin and tubulin signals are equal.

Expression of Gelsolin Protein

The anti-gelsolin antiserum recognizes two major bands at 102 and 63 kD. The band of 102 kD has the same apparent molecular weight as the in vitro translated gelsolin (Fig. 4 *a*) and is probably the full gelsolin protein. The band of 63 kD is also present in Coomassie blue-stained gels of purified gelsolin produced in bacteria. We think that this band is a degradation product, since antibodies affinity purified on the larger band recognize the smaller band and vice versa, and gelsolin has a tendency to break down very easily (P. McLaughlin, personal communication). The relative amount of the larger band is highest when embryos are frozen very quickly in liquid nitrogen and then immediately lysed in Laemmli sample buffer, but we have found no way of preparing embryonic lysates in which the lower band was completely absent. This suggests that it is produced almost instantaneously when the embryos are lysed. We cannot exclude that it might even exist in vivo. The fact that we find only the lower band in hemolymph might be a reflection of the way the hemolymph is harvested (see Materials and Methods). Fig. 5 shows a Western blot of different developmental stages incubated with anti-gelsolin antibody and, as a control, with a polyclonal antibody against β-tubulin. Gelsolin is present throughout development, although its abundance is variable.

Expression Patterns of Clone A and Clone B

To test if both forms of gelsolin were expressed in all cells, or in different tissues, we performed in situ hybridizations with probes specific for each clone and with a probe from the region common to both clones. The common probe hybridizes strongly to two tissues in the embryo, the fat body (a mesodermal organ which secretes many of the proteins of the hemolymph) and the gut. The gut consists of two layers, the endodermal gut epithelium and the mesodermal visceral musculature surrounding this epithelium, and it is in this layer that gelsolin is expressed. When the specific probes are used for in situ hybridization, we find that the expression pattern seen with the common probe is the sum of the two nonoverlappig expression patterns of clone A and clone B. Secreted gelsolin (clone A) is expressed only in the fat body, while cytosolic gelsolin (clone B) is expressed in the gut musculature (Fig. 6). In very young embryos (0–4 h) both mRNAs are present ubiquitously at low levels (not shown). They are probably provided maternally.

Distribution of Gelsolin in the Embryo

We analyzed the distribution of gelsolin in syncytial and cellularizing embryos using the affinity purified antibody. The *Drosophila* actin cytoskeleton undergoes dramatic changes during the syncytial stages of development. From the earliest stages until nuclear cycle 9, while the dividing nuclei populate the interior of the egg, F-actin and gelsolin are localized subcortically in a layer that extends to a depth of 2–4-μm below the plasma membrane. A surface view of this stage shows the same ubiquitous punctate staining for both F-actin and *Drosophila* gelsolin (Fig. 7).

By nuclear cycle 10 the nuclei have migrated to the periphery of the embryo where their associated spindles induce the

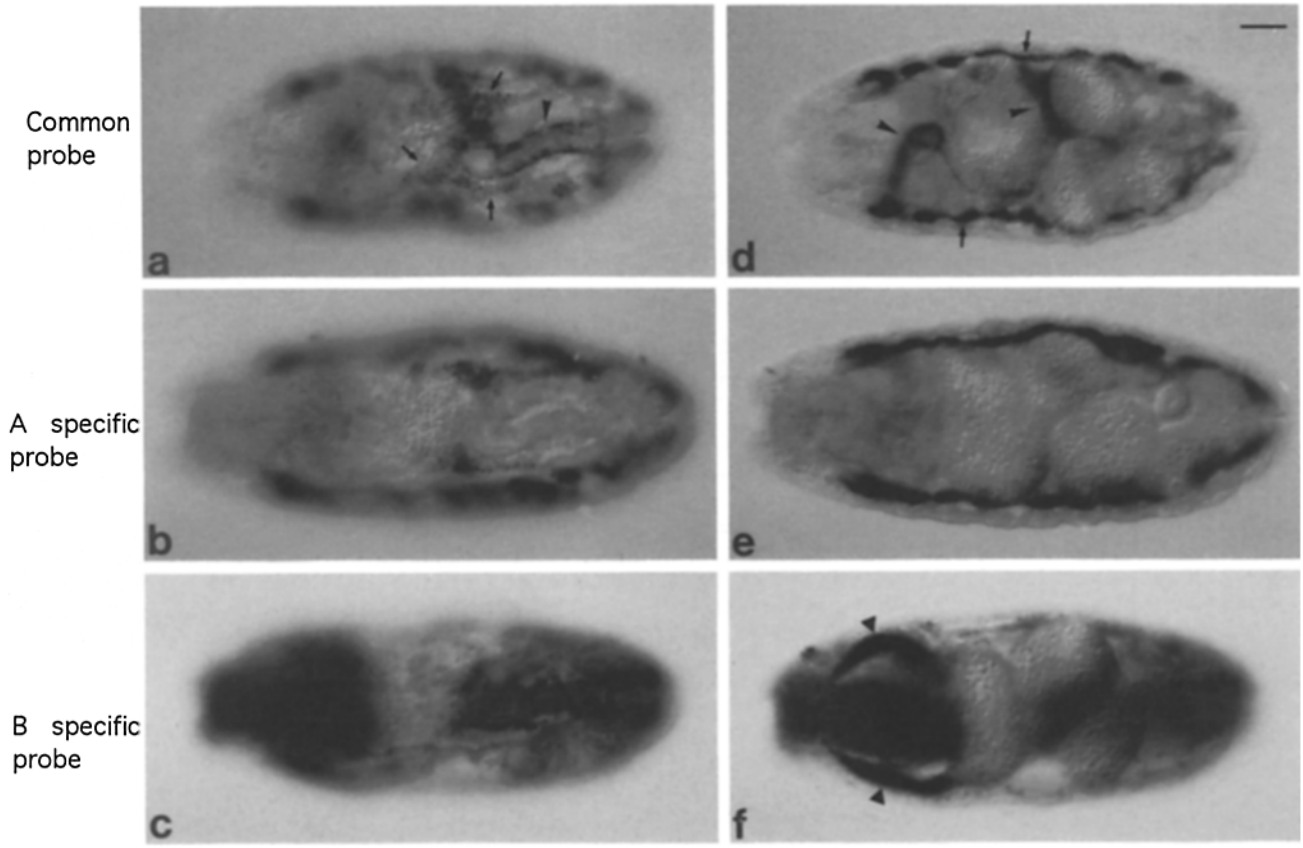

Figure 6. Expression of gelsolin in stage 16/17 embryos (dorsal views). Optical sections near the dorsal surface (*a–c*) and in the middle (*d–f*) of stage 16/17 embryos. The embryos were hybridized with probes specific for the 5 ends of the secreted (*b* and *e*; clone A) and cytosolic (*c* and *f*; clone B) forms, and with a common probe (*a* and *d*). The common probe detects transcripts in the gut (*arrowheads*) and the fat body (*arrows*). The hindgut is visible in sections near the dorsal surface (*left*), while midgut and foregut are visible in the lower focal plane shown on the right. The expression pattern seen with the common probe is the sum of the patterns seen with the specific probes. Probe A hybridizes only to the fat body, probe B only to the gut. The embryos hybridized with the two specific probes were stained for much longer to make sure that no signals other than those in the tissues mentioned would appear. The signal in the salivary glands (marked by triangles in *f*) is an artefact, probably due to enzyme trapped in the lumen of the salivary gland. Bar, 15 μm.

first F-actin rearrangement. Microfilaments condense above each nucleus to form a caplike structure. During nuclear cleavage cycles 10 to 13 F-actin rearranges from subcortical caps over interphase nuclei to a polygonal network separating mitotic spindles, and then back to the interphase caps of the next cycle. During these changes gelsolin colocalizes with F-actin (Fig. 8).

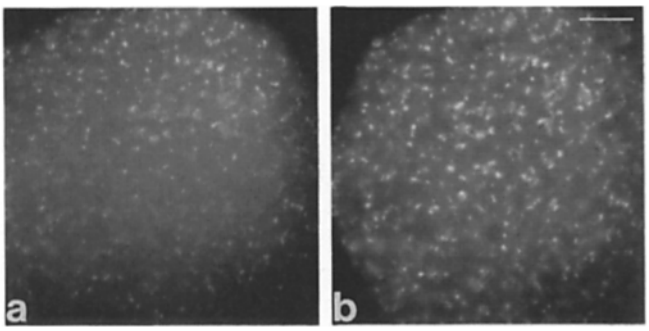

Figure 7. Surface view of part of an early embryo stained with rhodamin-phalloidin to visualize F-actin (*a*) and with gelsolin antibodies (*b*). Bar, 10 μm.

During interphase 14 the syncytial layer of nuclei is divided into cells by membranes growing inwards from the surface of the embryo. During this process F-actin is localized beneath the membranes and is enriched at the advancing edge of the furrow (the furrow canal). Gelsolin is also associated with the invaginating membranes, but it is not enriched at the furrow canal (Fig. 9). When the cleavage furrows reach a depth of about 20 μm, the furrow canals widen and form the basal plasma membranes of the cells. Actin is abundant at these membranes, while gelsolin is initially less concentrated at the basal membranes compared to lateral membranes. A few minutes after cellularization is complete and gastrulation begins, and from then on throughout the rest of embryogenesis, gelsolin is found associated with the whole outline of each cell.

Discussion

The *Drosophila* gene we have cloned codes for a member of the class of actin severing proteins, which by its structure is most closely related to gelsolin. The results we present here confirm and extend the findings of Heintzelman et al. (1993). The *Drosophila* secreted form of gelsolin shows the typical

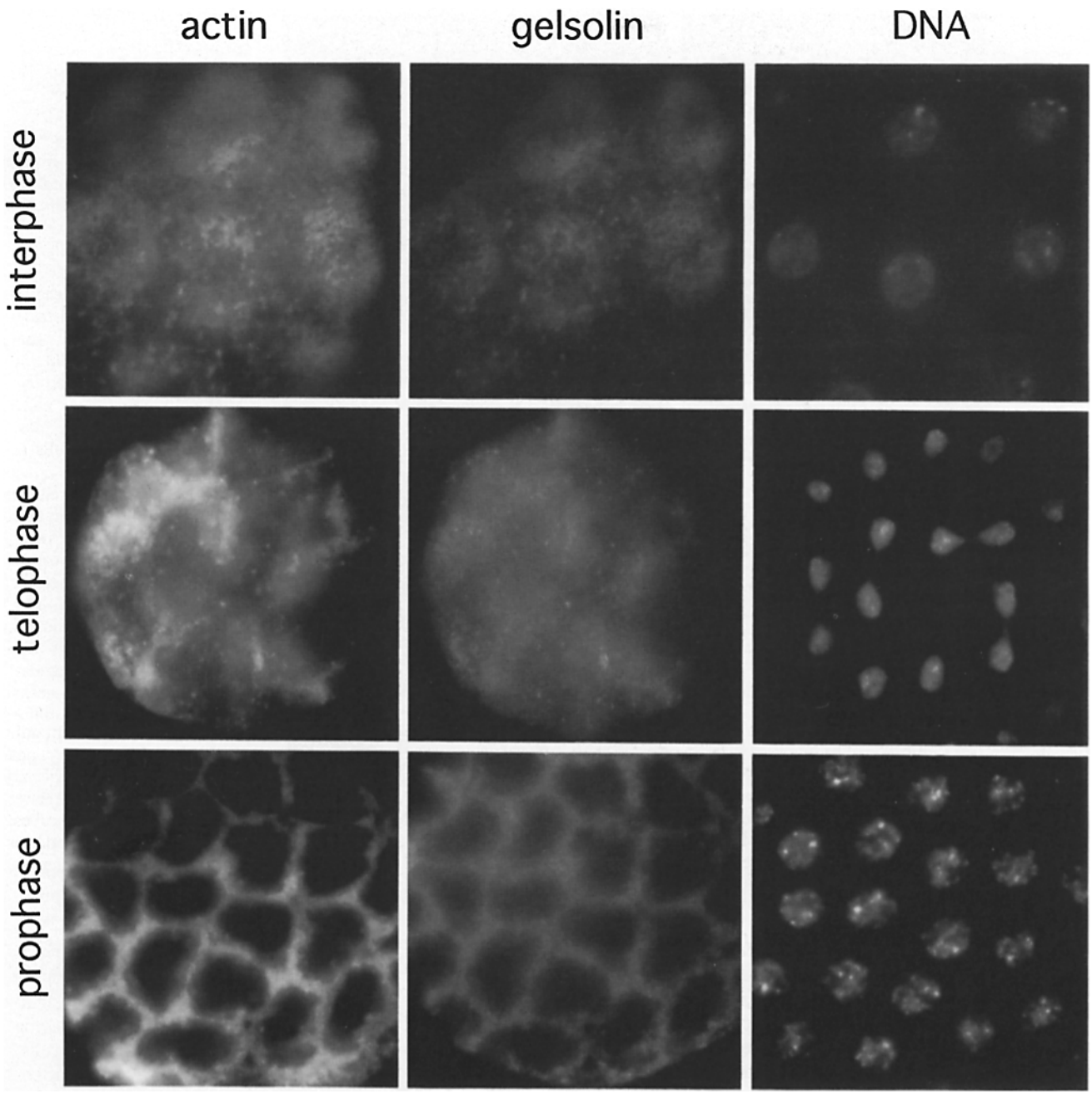

Figure 8. Triple staining of the same embryos for actin (first column), gelsolin (second column), and for nuclei (third column) of different early cleavage stages. The first row shows part of an embryo during interphase of cycle 10. The second and the third rows show telophase of cycle 11 and prophase of cycle 12. Bar, 10 μm.

sixfold segmental repeat structure plus a leader peptide and plasma extension at the NH_2 terminus. For segment 1 of human gelsolin the crystal structure has been determined (McLaughlin et al., 1993) and those residues shown to be core residues as well as the hydrophobic residues forming the center of the actin-binding patch (F72, I127, V130, and F173) are identical between human and *Drosophila* gelsolin. Of the other eight residues contacting actin, five are identical (H52, Q131, D134, Q142, and H143) and two are conservative substitutions (Y114/F11 and D77/E73). Thus the structure and the actin binding properties of vertebrate and *Drosophila* gelsolin are probably very similar. Also the Ca^{2+} complexing residues (E121, D134, and V169) and some of the residues thought to be involved in the interaction with PIP_2 (residues 160–193) (Janmey et al., 1992; Yu et al., 1992) are conserved. The conservation of these key residues suggests that gelsolin in *Drosophila* responds to the same types of regulatory signals as it does in vertebrates, and that its cellular functions are likely to be the same. However, it is worth noticing that the similarity between the first and fourth segment of *Drosophila* gelsolin is much lower than the similarity between segment 1 and segment 4 of vertebrate gelsolin.

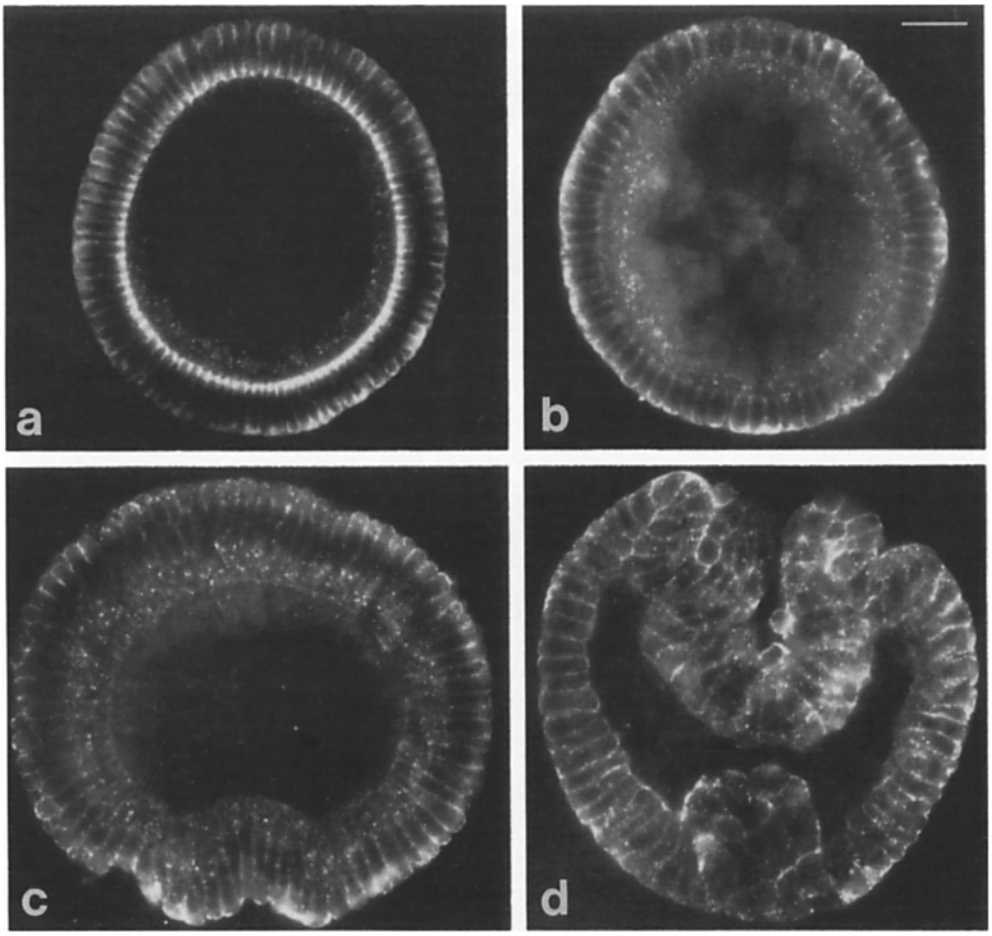

Figure 9. Transverse frozen sections of embryos stained with rhodamin-phalloidin (*a*) and antibodies against gelsolin (*b–d*). During cellularization of the blastoderm, actin is highly enriched at the leading edge of the membrane furrows (*a*), while gelsolin is more evenly associated with the whole plasma membrane (*b*). After cellularization is complete, gelsolin lines the plasma membrane of all cells (*c* and *d*). The embryo in *c* has just begun to form a ventral furrow, seen as an indentation of the epithelium in the lower part of the figure. This furrow has invaginated completely in the embryo shown in *d*. The deep invagination in the dorsal side of this embryo is the posterior midgut invagination, which carries the germ line cells into the inside of the embryo. Two of these cells are seen in the lumen of the invagination. Bar, 25 μm.

If, as suggested, these segments are used for actin nucleation (McLaughlin et al., 1993), this might suggest that *Drosophila* gelsolin has different actin-nucleation properties.

Expression of Secreted and Cytosolic Gelsolin mRNAs

The mRNAs for secreted and cytosolic gelsolin are expressed in different tissues in the embryo, in the fat body and in the gut, respectively. It is important to point out that this does not mean that there is no cytosolic gelsolin in other tissues. In embryos stained with gelsolin antibodies gelsolin is seen in all cells. Since gelsolin mRNA is present from the beginning of embryogenesis, it is likely that the protein we see is produced from this early, probably maternally produced mRNA and that it is very stable. Furthermore, the maternally provided protein seen in the early embryo might also be partitioned into the cells of the embryo and remain in these cells throughout embryogenesis.

We do not know why certain parts of the gut musculature transcribe high levels of cytosolic gelsolin mRNA, or why other parts of the embryo do not. The regions in the midgut that express cytosolic gelsolin are the regions where the gut forms constrictions. These constrictions initiate the process that gives the gut its final, tubelike form and are imposed on the gut by the visceral mesoderm. As these constrictions form, the cytoskeleton in the mesodermal cells in this regions undergoes major rearrangements (Reuter and Scott, 1990), and it is possible that gelsolin may be involved in this process.

The expression of the secreted gelsolin mRNA in fat body is consistent with the presence of the secreted protein in hemolymph, into which the fat body secretes various products. It is interesting that the two major tissues expressing gelsolin are mesodermal, since also vertebrate gelsolin is expressed in mesodermally derived tissues (secreted gelsolin in muscles, cytosolic gelsolin in macrophages) (Hartwig et al., 1989; Nodes et al., 1987).

Subcellular Localization and Possible Functions of Cytosolic Gelsolin

The cytosolic form of *Drosophila* gelsolin is associated with F-actin, as seen in embryos stained with gelsolin antibodies. During the early nuclear cleavage cycles gelsolin colocalizes with F-actin. It shows a punctate distribution in the layer of cortical cytoplasm, as F-actin does. The function of these local actin concentrations is not known, but they might be sites of actin storage, as has been proposed for actin granules in the sea urchin egg cortex, which also have an associated severing protein (Wang and Spudich, 1984). It will be interesting to see if these granules are affected in gelsolin mutants.

In the syncytial blastoderm and during cellularization gelsolin becomes associated with membranes. While the association of vertebrate gelsolin with membranes might normally be regulated by extracellular signals (Hartwig et al., 1989), in the case of the cellularization of the blastoderm it is more likely that the signals for gelsolin relocalization

come from within the syncytium, since the egg is not in communication with other cells at this stage, and since the signals for cellularization in general are thought to be intracellular (Schejter and Wieschaus, 1993). Also at later stages, the main subcellular location of gelsolin seems to be at the cortex of cells, as has also been described for some vertebrate cells (Carron et al., 1986). We do not know why gelsolin is absent from the base of the cleavage furrows during cellularization. These structures contain high levels of F-actin and cytoplasmic myosin (Young et al., 1991). Perhaps gelsolin would interfere with the contractile forces that are thought to cause the invagination of the plasma membranes.

Since our antibody was made against the part of gelsolin that is common to the secreted and the cytoplasmic form it might appear surprising that we see only cytoplasmic staining in the embryo. Various factors probably contribute to this. It has been notoriously difficult to stain *Drosophila* embryos for secreted proteins. In addition, our embryos were fixed by a method that is optimal for preserving the actin cytoskeleton, which is a lighter fixation than normally used and might allow secreted proteins to be lost. Finally, F-actin–associated proteins are very easy to see, because they form a sharp, easily recognizable pattern, whereas a secreted protein would be expected to give a more diffuse staining which would be more difficult to distinguish from background fluorescence. A very careful analysis using different fixation protocols and specific antibodies will be necessary to study the expression of the secreted form.

Comparison of Human and Drosophila Secreted Gelsolin

The identity of the gene as a true gelsolin homolog is further supported by the existence of a secreted and an intracellular form. Both in humans and in *Drosophila* the secreted form is the same as the cytosolic form with an extra 52 (humans) or 51 (*Drosophila*) amino acids at the NH_2 terminus. However, the splicing that generates the two transcripts differs between the two species. In humans, the first common exon begins nine nucleotides upstream of the start codon for the cytoplasmic form, i.e., in the plasma extension of the secreted form (Kwiatkowski et al., 1988). There is no splice site in this position in the *Drosophila* gene (Irion, U., M. C. Stella, and M. Leptin, unpublished data). In *Drosophila* the first common exon begins 133-nucleotides upstream of the cytoplasmic start codon, within the region of the leader peptide of the secreted form. There is no splice site at this position in the human gene. Thus, the splice sites in the 5′ end of the gene are not conserved.

Nevertheless, the length of the leader peptide plus plasma extension are almost identical in humans and flies. With amino acid 20 as the most likely signal peptidase cleavage site (according to von Heijne, 1986) the *Drosophila* plasma extension has a length of 30 amino acids. The conservation in the plasma extension appears much lower than in the rest of the molecule (25% for the plasma extension compared to 42% for the whole molecule, and to 58% for the first segment) suggesting that there is not much constraint on the sequence of the plasma extension. It might just serve as a linker to the signal peptide sequence. This view is supported by the fact that in pigs the plasma extension is secondarily cleaved to a shorter form of nine amino acid residues (Way and Weeds, 1988) and in chicken it is absent altogether (Nodes et al., 1987).

While the existence of a cytoplasmic gelsolin in *Drosophila* was expected (since functions of the cytoskeleton are highly conserved throughout the plant and animal kingdoms) it was not obvious that there would also be a secreted form of gelsolin in flies. The secreted gelsolin in vertebrates is found in the blood and might have evolved to serve some function specific to the vertebrate blood system. Our finding that flies also have secreted gelsolin indicates that this may not be the case. Although it is not impossible that the common ancestor of invertebrates and vertebrates had only cytoplasmic gelsolin, and the plasma extension and leader sequence were acquired independently in insects and vertebrates, it is more likely that the common ancestor already had both forms.

If secreted gelsolin did arise in the common ancestor, it must have an ancient function that has been conserved both in invertebrates and vertebrates. It has been proposed that the role of plasma gelsolin is to clear F-actin released from damaged cells (Lee and Galbraith, 1992). However, secreted gelsolin has not necessarily always had only this function. First, it is not known whether even in vertebrates there might not be other functions for secreted gelsolin. Second, it is unlikely that the ancestral species had a defined blood system that could be considered as the ancestor of insect and vertebrate blood systems. One might therefore speculate that secreted gelsolin also has a more general function common to metazoans. Secreted gelsolin can also bind to fibrin (Smith et al., 1987) which suggests other possible functions like general regulation of the viscosity of extracellular fluids, or participation in aspects of wound healing. Whether *Drosophila* secreted gelsolin indeed has any of these functions or has the same function as that proposed for its vertebrate counterpart will be easily testable once we obtain gelsolin mutants.

We thank members of the Cambridge gelsolin group (especially Brian Pope, Michael Way, Sutherland Maciver, Paul McLaughlin, and Alan Weeds) for reagents and discussions, Martin Bähler, José Casal, and Rolf Reuter for comments on the manuscript, José Casal for suggestions and help with the diagram, Barbara Grunewald for preparing the frozen sections, Shalina Marajan-Miklos and Lynn Cooley for communicating unpublished results, David Stein for allowing us to screen his copy of the genomic library (a gift from J. Tamkun), Nick Brown for the cDNA libraries, M. Schorpp for looking after the rabbits, and Uwe Irion for discussions and cloned DNA.

Received for publication 9 November 1993 and in revised form 27 January 1994.

References

Ampe, C., and J. Vanderkerckhove. 1987. The F-actin capping proteins of *Physarum polycephalum*: cap42(a) is very similar, if not identical, to fragmin and is structurally and functionally very homologous to gelsolin; cap42(b) is *Physarum* actin. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:4149–4157.

André, E., M. Brink, G. Gerisch, G. Isenberg, A. Noegel, M. Schleicher, J. E. Segall, and E. Wallraff. 1989. A *Dictyostelium* mutant deficient in severin, an F-actin protein, shows normal motility and chemotaxis. *J. Cell Biol.* 108:985–995.

André, E., F. Lottspeich, M. Schleicher, and A. Noegel. 1988. Severin, gelsolin, and villin share a homologous sequence in regions presumed to contain F-actin severing domains. *J. Biol. Chem.* 263:722–728.

Arpin, M., E. Pringault, J. Finidori, A. Garcia, J.-M. Jeltsch, J. Vandekerckhove, and D. Louvard. 1988. Sequence of human villin: a large duplicated domain homologous with other actin-severing proteins and a unique small carboxy-terminal domain related to villin specificity. *J. Cell Biol.* 107: 1759–1766.

Ashburner, M. 1989. Drosophila: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Bazari, W., P. Matsudaira, M. Wallek, T. Smeal, R. Jakes, and Y. Ahmed. 1988. Villin sequence and peptide map identify six homologous domains. *Proc. Natl. Acad. Sci. USA.* 85:4986–4990.
Brown, N. H., and F. C. Kafatos. 1988. Functional cDNA libraries from *Drosophila* embryos. *J. Mol. Biol.* 203:425–437.
Brown, N. H., D. L. King, M. Wilcox, and F. C. Kafatos. 1989. Developmentally regulated alternative splicing for *Drosophila* integrin PS2α transcripts. *Cell.* 59:185–195.
Carron, C. P., S. Hwo, J. Dingus, D. M. Benson, I. Meza, and J. Bryan. 1986. A re-evaluation of cytoplasmic gelsolin localization. *J. Cell Biol.* 102: 237–245.
Cunningham, C., T. Stossel, and D. Kwiatkowski. 1991. Enhanced motility in NIH 3T3 fibroblasts that overexpress gelsolin. *Science (Wash. DC).* 251:1233–1236.
Driever, W., V. Siegel, and C. Nüsslein-Volhard. 1990. Autonomous determination of anterior structures in the early *Drosophila* embryo by the bicoid morphogen. *Development.* 109:811–820.
Dubreuil, R. R., T. J. Byers, D. Branton, L. S. Goldstein, and D. P. Kiehart. 1987. Drosophila spectrin.–I. Characterization of the purified protein. *J. Cell Biol.* 105:2095–2102.
Dubreuil, R. R., T. J. Byers, C. T. Stewart, and D. P. Kiehart. 1990. A beta-spectrin isoform from *Drosophila* (beta H) is similar in size to vertebrate dystrophin. *J. Cell Biol.* 111:1849–1858.
Eichinger, L., A. Noegel, and M. Schleicher. 1991. Domain structure in actin-binding proteins: expression and functional characterization of truncated severin. *J. Cell Biol.* 112:665–676.
Forscher, P. 1989. Calcium and polyphosphoinositide control of cytoskeletal dynamics. *Trends Neurosci.* 12:468–474.
Fyrberg, E. A., and L. S. B. Goldstein. 1990. The *Drosophila* cytoskeleton. *Annu. Rev. Cell Biol.* 6:559–596.
Hartwig, J. H., K. A. Chambers, and T. P. Stossel. 1989. Association of gelsolin with actin filaments and cell membranes of macrophages and platelets. *J. Cell Biol.* 108:467–479.
Heintzelman, M., A. Stewart, S. Artavanis-Tsakonas, and M. Mooseker. 1993. Cloning of a secretory gelsolin from *Drosophila melanogaster. J. Mol. Biol.* 230:709–716.
Jackson, I. 1991. A reappraisal of non-consensus mRNA splice sites. *Nucleic Acids Res.* 19:3795–3798.
Janmey, P., J. Lamb, P. Allen, and P. Matsudaira. 1992. Phosphoinositide-binding peptides derived from the sequences of gelsolin and villin. *J. Biol. Chem.* 267:11818–11823.
Kambysellis, M. P. 1984. A highly efficient method for collection of hemolymph, hemocytes or blood-borne organisms from *Drosophila* and other small insects. *Dros. Inf. Ser.* 60:219–220.
Karr, T. L., and B. M. Alberts. 1986. Organization of the cytoskeleton in early *Drosophila* embryos. *J. Cell Biol.* 102:1494–1509.
Kellogg, D. R., T. J. Mitchison, and B. M. Alberts. 1988. Behaviour of microtubules and actin filaments in living *Drosophila* embryos. *Development.* 103:675–686.
Kiehart, D. P. 1990. The actin membrane skeleton in *Drosophila* development. *Sem. Cell Biol.* 1:325–339.
Kiehart, D. P., and R. Feghali. 1986. Cytoplasmic myosin from *Drosophila melanogaster. J. Cell Biol.* 103:1517–1525.
Kwiatkowski, D. J., R. Mehl, and H. L. Yin. 1988. Genomic organization and biosynthesis of secreted and cytoplasmic forms of gelsolin. *J. Cell Biol.* 106:375–384.
Kwiatkowski, D. J., T. P. Stossel, S. H. Orkin, J. E. Mole, H. R. Colten, and H. L. Yin. 1986. Plasma and cytoplasmic gelsolins are encoded by a single gene and contain a duplicated actin-binding domain. *Nature.* 323:455–458.
Lassing, I., and U. Lindberg. 1988. Evidence that the phosphatidylinositol cycle is linked to cell motility. *Exp. Cell Res.* 174:1–15.
Lee, W. M., and R. M. Galbraith. 1992. The extracellular actin-scavenger system and actin toxicity. *New Eng. J. Med.* 326:1335–1341.
McLaughlin, P. J., J. T. Gooch, H.-G. Mannherz, and A. G. Weeds. 1993. Structure of gelsolin segment 1-actin complex and the mechanism of filament severing. *Nature (Lond.).* 364:685–692.
Melton, D. A., P. A. Krieg, M. R. Rebagliate, T. Maniatis, K. Zinn, and M. R. Green. 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* 12:7035–7070.
Miller, K. G., and B. M. Alberts. 1989. F-actin affinity chromatography: technique for isolating previously unidentified actin-binding proteins. *Proc. Natl. Acad. Sci. USA.* 86:4808–4812.
Miller, K. G., C. M. Field, and B. M. Alberts. 1989. Actin-binding proteins from *Drosophila* embryos: a complex network of interacting proteins detected by F-actin affinity chromatography. *J. Cell Biol.* 109:2963–2975.
Mount, S., C. Burks, G. Hertz, D. Stormo, O. White, and C. Fields. 1992. Splicing signals in *Drosophila*: intron size, information content, and consensus sequences. *Nucleic Acids Res.* 20:4255–4262.
Nodes, B. R., J. E. Shackelford, and H. Lebherz. 1987. Synthesis and secretion of serum gelsolin by smooth muscle tissue. *J. Biol. Chem.* 262:5422–5427.
Pollard, D. P., and J. A. Cooper. 1986. Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. *Annu. Rev. Biochem.* 55:987–1035.
Prendergast, G., and E. Ziff. 1991. Mbh1: a novel gelsolin/severin-related protein which binds actin *in vitro* and exhibits nuclear localization *in vivo. EMBO (Eur. Mol. Biol. Organ.) J.* 10:757–766.
Reuter, R., and M. P. Scott. 1990. Expression and function of the homoeotic genes *Antennapedia* and *Sex combs reduced* in the embryonic midgut of *Drosophila. Development.* 109:289–303.
Sakurai, T., H. Kurokawa, and Y. Nonomura. 1991. The CA^{2+}-dependent actin filaments-severing activity of 74-kDa protein (adseverin) resides in its NH_2-terminal half. *J. Biol. Chem.* 266:4581–4585.
Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor.
Schejter, E., and E. Wieschaus. 1993. Functional elements of the cytoskeleton in the early *Drosophila* embryo. *Annu. Rev. Cell Biol.* 9:67–99.
Smith, D. B., P. A. Janmey, T. J. Herbert, and S. E. Lind. 1987. Quantitative measurement of plasma gelsolin and its incorporation into fibrin clots. *J. Lab. Clin. Med.* 110:189–195.
Sprenger, F., and C. Nüsslein-Volhard. 1992. Torso receptor activity is regulated by a diffusible ligand produced at the extracellular terminal region of the *Drosophila* egg. *Cell.* 71:987–1001.
Tautz, D., and C. Pfeifle. 1989. A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene hunchback. *Chromosome.* 98:81–85.
von Heijne, G. 1986. A new method for predicting signal sequence cleavage sites. *Nucleic Acids Res.* 14:4683–4690.
von Heijne, G. 1990. The signal peptide. *J. Membr. Biol.* 115:195–201.
Wang, L.-L., and J. A. Spudich. 1984. A 45,000-mol wt protein from unfertilized sea urchin eggs severs actin filaments in a calcium-dependent manner and increase the steady-state concentration of nonfilamentous actin. *J. Cell Biol.* 99:844–851.
Warn, R. M., and R. Magrath. 1983. F-actin distribution during the cellularization of the *Drosophila* embryo visualized with FL-phalloidin. *Exp. Cell Res.* 143:103–114.
Way, M., and A. Weeds. 1988. Nucleotide sequence of pig plasma gelsolin. Comparison of protein sequence with human gelsolin and other actin-severing proteins shows strong homologies and evidence for large internal repeats. *J. Mol. Biol.* 203:1127–1133.
Way, M., J. Gooch, G. Pope, and A. Weeds. 1989. Expression of human plasma gelsolin in *Escherichia coli* and dissection of actin binding sites by segmental deletion mutagenesis. *J. Cell Biol.* 109:593–605.
Way, M., B. Pope, J. Gooch, M. Hawkins, and A. Weeds. 1990. Identification of a region in segment one of gelsolin critical for actin binding. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:4103–4109.
Way, M., B. Pope, and A. Weeds. 1992. Are the conserved sequences in segment 1 of gelsolin important for binding actin? *J. Cell Biol.* 116:1135–1143.
Weeds, A., and S. Maciver. 1993. F-actin capping proteins. *Curr. Opin. Cell Biol.* 5:63–69.
Wieschaus, E., and C. Nüsslein-Volhard. 1986. Looking at embryos. *In Drosophila*: A Practical Approach. D. B. Roberts, editor. IRL Press, Oxford. 199–228.
Young, P. E., T. C. Pesacreta, and D. P. Kiehart. 1991. Dynamic changes in the distribution of cytoplasmic myosin during *Drosophila* embryogenesis. *Development.* 111:1–14.
Yu, F. X., P. A. Johnston, T. Südhof, and H. L. Yin. 1990. gCAP39, a calcium ion- and polyphosphoinositide-regulated actin capping protein. *Science (Wash. DC).* 250:1413–1415.
Yu, F., H. Sun, P. Janmey, and H. Yin. 1992. Identification of a polyphosphoinositide-binding sequence in an actin monomer-binding domain of gelsolin. *J. Biol. Chem.* 267:14616–14621.