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The gelsolin-related *flightless I* protein is required for actin distribution during cellularisation in *Drosophila*

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SUMMARY

We have analysed the developmental defects in *Drosophila* embryos lacking a gelsolin-related protein encoded by the gene *flightless I*. Such embryos have previously been reported to gastrulate abnormally. We now show that the most dramatic defects are seen earlier, in actin-dependent events during cellularisation of the syncytial blastoderm, a process with similarities to cytokinesis. The blastoderm nuclei migrate to the periphery of the egg normally but lose their precise cortical positioning during cellularisation. Cleavage membranes are initially formed, but invaginate irregularly and often fail to close at the basal end of the newly formed cells. The association of actin with the cellularisation membranes is irregular, suggesting a role for *flightless I* in the delivery of actin to the actin network, or in its stabilisation.

Key words: Cytoskeleton, Morphogenesis, Cytokinesis, Gastrulation

INTRODUCTION

Many cellular and developmental processes depend on the modulation of the actin cytoskeleton, controlled by a large number of actin binding proteins. One of these proteins is gelsolin, a member of the group of F-actin capping proteins which are involved in regulating the degree of polymerisation of actin. Gelsolin has been found in vertebrates as well as in *Drosophila*, and there are closely related proteins in slime moulds (Heintzelmann et al., 1993; Stella et al., 1994; references in Weeds and Maciver, 1993). The gene for a new, gelsolin-like protein, called *flightless I*, with the same six-segment structure as gelsolin, but with an additional N-terminal part containing leucine rich repeats has been identified in humans, *Caenorhabditis elegans* and *Drosophila* (Campbell et al., 1993). The interactions of gelsolin with actin and the effects of gelsolin on actin filaments in vitro are well characterized (Way et al., 1992; McLaughlin et al., 1993), but its role in vivo is less well understood (for a review see Weeds and Maciver, 1993) although results from a gelsolin knock-out mouse are beginning to generate new insights (Witke et al., 1995). A number of mutations in the *flightless I* gene are known in *Drosophila*, which allows the function of this gelsolin-related protein to be analyzed in vivo. Flies homozygous for viable mutant alleles show flight defects, while lethal alleles lead to late larval or pupal death (Perrimon et al., 1989). In eggs lacking maternally supplied wild-type *flightless I* gene product (which, for simplicity, we will call *flightless I* embryos), development is disrupted during early embryogenesis. We show here that this disruption is due to defects in cellularisation of the blastoderm.

In the *Drosophila* embryo the first cells are formed after the nucleus of the zygote has undergone 13 synchronous cleavages in the absence of cytokinesis. After the eighth cleavage the majority of the nuclei migrate to the cortex of the egg, where they continue to divide, eventually forming a monolayer of about 6,000 nuclei, regularly aligned in an approximately hexagonal array. This array is divided into cells by membranes which invaginate from the surface of the egg between the nuclei and then close the nuclei off towards the central egg cytoplasm and yolk mass. An exception to this is the formation of the germ line cells, which arise 5 cleavage cycles earlier, when a subset of nuclei reach the posterior pole of the egg and are enclosed by membranes. The process of cellularisation can be divided into two phases, an initial slow phase of invagination and a second fast phase during which the membranes reach their final depth of about 30 μm and which is concluded by the invaginating membranes constricting below the nuclei to form the basal cell membrane.

All of the above processes depend on an intact actin cytoskeleton as shown by experiments in which embryos were injected with cytochalasin (reviewed by Schejter and Wieschaus, 1993b). If embryos are treated early, the nuclei fail to migrate from the center to the cortex of the egg. In embryos treated after the nuclei have reached the cortex, the blastoderm nuclei lose their regular alignment under the surface of the egg and cellularisation is completely inhibited.

Just prior to cellularisation, filamentous actin is concentrated

in cap-like structures between the egg cortex and the underlying nuclei (Warn and Magrath, 1982). These caps expand and as their edges reach each other a hexagonal network of actin filaments is formed that prefigures the sites of membrane invaginations. Actin remains associated with the newly formed cell membranes and the advancing cleavage furrow while different actin-binding proteins associate with various domains of the subcortical actin network (Miller et al., 1989; Thomas and Kiehart, 1994; Young et al., 1991). Myosin accumulates at the leading edge of the invaginating membrane furrows (Young et al., 1991). The localization of these cytoskeletal components to the cleavage furrows during cellularisation is reminiscent of the actin-myosin contractile ring that separates animal cells during cytokinesis. Based on this similarity, it has been proposed that contraction of the actin-myosin hexagonal network might provide the force to pull down membranes between the nuclei during cellularisation (Warn and Magrath, 1983; Schejter and Wieschaus, 1993b). Disruption of actin filaments by cytochalasin or interfering with myosin function by injecting antibodies inhibits cellularisation (Katoh and Ishikawa, 1989; Young et al., 1991).

Three genes (*nullo*, *serendipity-α* and *bottleneck*) have been identified that are expressed zygotically and are required for cellularisation (Schejter and Wieschaus, 1993a; Rose and Wieschaus, 1992; Schweisguth et al., 1990). They affect different aspects of cellularisation. *nullo* and *serendipity-a* are required for maintaining the hexagonal network of actin and membranes during the early phase of cellularisation while *bottleneck* protein is needed to ensure that the final constriction of the membranes occurs at the correct time. These genes are transcribed during a very narrow time window beginning just before cellularisation, and they, along with two others (Merrill et al., 1988), are the only genes required in the zygote for cellularisation (Schejter and Wieschaus, 1993a). This means that all the other components involved in the process of cellularisation must be provided maternally, as is already known to be the case for actin and myosin. Several maternal effect genes required for cellularisation have been found (Rice and Garen, 1975). We show here that the maternally synthesized gelsolin-like protein, *flightless I*, is required for the proper distribution of actin along the membrane network and for cellularisation to proceed correctly.

MATERIALS AND METHODS

Fly stocks

The *flightless I* stocks fli^{DA534}, $fli^{HC183}$and fli^{W2} (of which fli^{DA534}, fli^{HC183} have now been lost from all stock collections we are aware of) were obtained from the Umea stock centre. fli^{W2} produces a slightly stronger phenotype than the other alleles: earlier zygotic lethality (Perrimon et al., 1989), and stronger maternal effect (our own observations). Its phenotype is as strong as that of the allele *D44* (not shown) which has been shown to be complete null allele due to a small deletion in the promoter region of the *flightless I* gene (deCouet et al., 1995). *ovoD1* was from the Tübingen stock collection.

Antibodies and phalloidin

The following antibodies were used: monoclonal mouse anti-lamin, provided by H. Saumweber, used at 2 μg/ml (Risau et al., 1981); monoclonal mouse anti-phosphotyrosine, provided by G. Steinhilber, used at 4 μg/ml (Steinhilber et al., 1990); mouse anti-β-galactosidase, diluted 1/10,000 (Sigma); rabbit anti-twist serum, diluted 1/500 (Roth et al., 1989); secondary antibodies were Texas Red-conjugated or biotinylated goat antisera against mouse or rabbit IgG, all used at 1/1,000 (all from Dianova). All incubations were carried out in PBS + 0.1% Tween-20 (PBST). Before adding the first antibody, the embryos were incubated for 30 minutes in PBST + 1% BSA, 5% normal goat serum. Typically, antibody staining was performed by incubating 5-50 μl embryos in 50-500 μl of primary antibody for 2 hours at room temperature or overnight at 4°C. The embryos were then washed in several changes of PBST over at least 30 minutes, incubated in secondary antibody (same volume as above) and washed again. Fluorescently stained embryos were then mounted in glycerol containing anti-bleaching reagent (DABCO). Biotinylated secondary antibodies were visualized using the Vectastain ABC kit from Vector labs using DAB as substrate. Embryos treated in this way were embedded in Araldite and photographed as wholemounts or processed for sectioning as previously described (Leptin and Grunewald, 1990). To visualize actin filaments we used rhodamine-labelled phalloidin (Sigma) at 1 μg/ml in PBS.

Mitotic recombination

Germ line clones were induced by mitotic recombination as described by Wieschaus and Noell (1986). Mitotic recombination was induced by X-irradiation (1,000 rad) of 40-48 hour old larvae transheterozygous for a *flightless I* allele and the dominant female sterile *ovoD1* mutation. Germ line cells developing in females treated in this way fall into three classes. First, progeny of cells in which no mitotic recombination occurs (the vast majority) remain heterozygous for both mutations and die due to the effect of the *ovoD1* mutation. Second, cells in which a recombination distal to *ovoD1* occurs generate one daughter that has lost the *ovoD1* mutation, but not gained the *fli* mutation and is therefore wild type in both genes. These cells produce wild-type eggs. The other daughter dies due to the presence of the *ovoD1* mutation. Third, cells in which a proximal recombination occurs produces one daughter that is homozygous for *ovoD1* and therefore dies while the other daughter is wild type for *ovoD1* and homozygous for *fli*. These cells develop as *fli* mutant germ line clones.

Emerging females were set up in individual crosses to identify flies with appropriate recombinant germ lines: 90% of the females were sterile (no recombinant germ line clones); 10% of the females had recombinant germ line clones; of these, 80% had proximal recombinations producing germ line clones lacking wild-type *flightless* product, while 20% had distal recombinations which led to the production of wild-type eggs and wild-type, viable embryos. Females with the appropriate germ line clones were pooled and set up for egg collections.

To study the effects of *flightless* on oogenesis, all three categories of females were analysed. Flies that did not produce embryos also showed no sign of developing oocytes, consistent with the notion that the whole ovary was phenotypically *ovoD1*. Ovaries from both classes of fertile flies, those producing wild type and those producing mutant embryos, were normal and showed no defects.

Collection, processing and photography of eggs

Eggs for live observation and fixation were collected and dechorionated according to standard protocols (Wieschaus and Nüsslein-Volhard, 1986). For live observation they were mounted on glue-covered microscope coverslips and photographed with transmitted light on a Zeiss Axiovert at 20× magnification using Agfa APX25 Film. For antibody staining they were fixed in freshly prepared 3.7% formaldehyde dissolved in PBS and devitellinised by shaking on a heptane/methanol interface, unless they were to be used for cytoskeletal staining, in which case they were devitellinised by hand. Stained embryos were photographed on a Zeiss Axiophot Microscope using either Kodak Technical Pan Film at 100 ASA (histochemical stains) or at 400 ASA (immunofluorescence). Fluorescently labelled embryos were also analysed on a Bio-Rad MRC600 Confocal microscope.

Electron microscopy

Embryos were fixed according to the method of Zalokar and Erk (1977). They were then washed in PBS, refixed for 90 minutes in 1% OsO_4 in PBS at 5°C, washed in water, incubated for 1 hour in 1% uranylacetate in water at 5°C and dehyadrated in ethanol followed by propylene oxide. They were embedded in Epon (Roth), sectioned (70 nm), and stained with uranyl acetate and lead citrate.

RESULTS AND DISCUSSION

Early stages of development in *flightless I* embryos

Embryos that are homozygous mutant for *flightless I* alleles show no defects since the maternal contribution of the wild-type gene product is sufficient to allow development up to late larval stages (Perrimon et al., 1989). Embryos devoid of wild-type maternal product can be derived from homozygous mutant germ line clones created in heterozygous mothers by X-ray induced mitotic recombination. We investigated the embryonic *flightless I* phenotype in embryos from such germ line clones (which we will call *flightless I* embryos) by time-lapse video-recording of live embryos, and by immunohistochemical analysis of cytoskeletal elements, cell membranes and nuclei. Until the beginning of cellularisation (stage 5; stages according to Campos-Ortega and Hartenstein, 1985) *flightless I* embryos are indistinguishable from wild-type embryos in all aspects we analysed. They show normal periodic contractions of the cytoplasm during each round of nuclear division and form a normal syncytial blastoderm and pole cells; at the beginning of the cellularisation phase the cortical cytoplasm is cleared of yolk and lipid particles, and the syncytial nuclei elongate (Fig. 1). *flightless I* embryos have regular mitotic planes and the distribution of actin and tubulin appears to be normal. Furthermore, gene expression along the dorsal-ventral axis of the embryo is normal (Fig. 1), showing that the gastrulation defects observed previously (Perrimon et al., 1989), and see below) are not due to defects in the dorsal-ventral patterning system that sets up the fates of ventral cells.

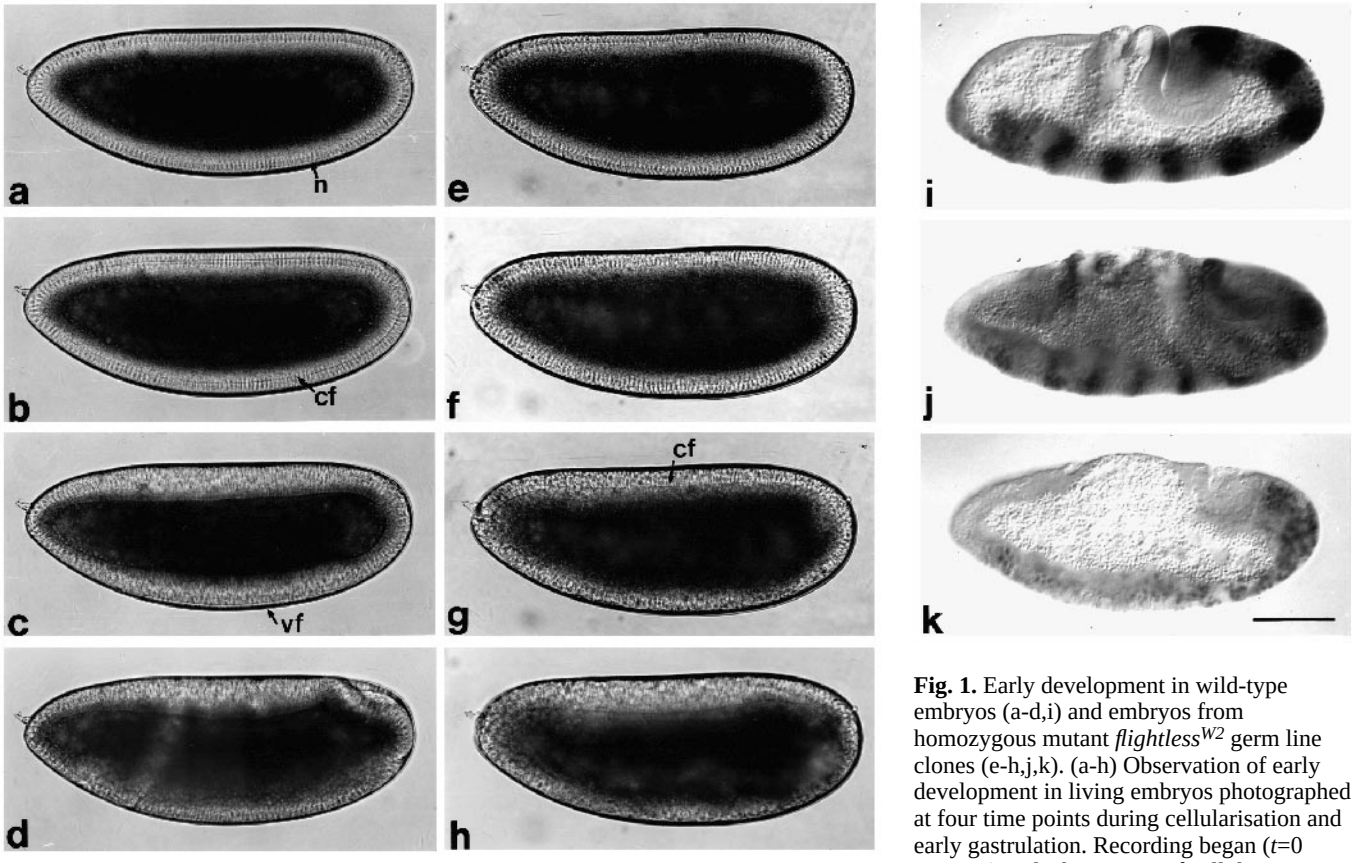

Fig. 1. Early development in wild-type embryos (a-d,i) and embryos from homozygous mutant *flightless*^{W2} germ line clones (e-h,j,k). (a-h) Observation of early development in living embryos photographed at four time points during cellularisation and early gastrulation. Recording began (*t*=0 minutes) at the beginning of cellularisation. Time points shown are *t*=9 minutes (a,e), *t*=27 minutes (b,f), *t*=54 minutes (c,g) and *t*=63 minutes (d,h). Nuclei are initially aligned in a regular layer in the cortical cytoplasm of both wild-type and mutant embryos (a,e). When cellularisation begins, the contour of the cleavage furrows (the front of the ingrowing cleavage membranes) is only clearly visible in wild-type embryos (b), but appears only in small patches in older mutant embryos (g). As gastrulation begins, the epithelia of the mutant embryo begin to desintegrate (h). (i-k) Contribution of the paternal wild-type chromosome. Embryos from wild-type females (i) or from females with *flightless I* mutant germ lines (j,k) that were mated with males carrying an X chromosome marked with a transgene expressing lacZ in seven stripes in the early embryo. This allows the identification of mutant embryos that have inherited the paternal X chromosome, and therefore a wild-type copy of the *flightless I* gene, from those with the paternal Y chromosome, which does not contain the *flightless I* gene. The defects in embryos with a paternal wild-type copy of the *flightless I* gene are slightly less severe than those in embryos without, showing a mild rescue by the paternal gene. n, syncytial layer of nuclei; cf, leading edge of cleavage furrows; vf, ventral furrow. Bar, 100 μm.

Observation of live embryos

The first defects in *flightless I* embryos become visible during cellularisation of the syncytial blastoderm. Fig. 1 shows photographs of living wild-type and *flightless I* embryos at four time points during the development of the cellular blastoderm and early gastrulation. Before cellularisation begins, the nuclei are aligned and the level of their basal ends is visible as a contour in both wild-type and *flightless I* live embryos, although the nuclear layer looks slightly less regular in *flightless I* embryos (Fig. 1e). When the nuclei then become separated by the cleavage furrows invaginating from the surface of the egg, this process occurs synchronously within the whole wild-type egg, such that the advancing fronts of the membranes are also visible as a contour (Fig. 1b). In *flightless I* embryos no membrane contour is visible initially (Fig. 1f), and later only occasional patches of this contour are seen (Fig. 1g). In addition, the cortex of *flightless I* embryos looks generally less regular.

In spite of the cellularisation defect, the mutant embryos begin to gastrulate (Fig. 1h). A ventral furrow and the head fold are formed (though abnormal looking), the posterior midgut primordium moves dorsally and invaginates, carrying the pole cells with it, and the germ band begins to extend. However, the germ band never reaches its final extension and after approximately 5 hours of embryonic development (at 25°C) the mutant embryos begin to desintegrate (Fig. 1k).

We observed some variability in the phenotype and tested whether this was due to the effect of the paternally provided wild-type copy of the *flightless I* gene (the *flightless I* gene is on the X-chromosome, so that half of the embryos receive an X-chromosome with a wild-type *flightless I* gene from the father, while the other half receive the Y chromosome, which does not carry the *flightless I* gene). We mated the females with males in which the X-chromosome was marked with a transgene that expressed β-galactosidase under control of the *eve* promotor and then stained the progeny with antibodies against β-galactosidase. Those embryos receiving the paternal X-chromosome, recognizable by seven stripes of β-galactosidase expression (Fig. 1j), generally showed less severe defects (more intact cells, larger patches of apparently undisrupted epithelium) than embryos without any wild-type copies of the *flightless I* gene (Fig. 1k). This shows that there is a weak rescue of the phenotype by the paternal genome.

Cellularisation defects

The observations of live embryos suggest that two aspects of development, nuclear positioning and cell membrane formation, might be defective in *flightless I* embryos. To study these defects in more detail, we stained *flightless I* embryos with antibodies that visualize nuclei and membranes (monoclonal antibodies against nuclear lamin and against phosphotyrosine; Risau et al., 1981; Steinhilber et al., 1990) and analysed transverse sections at higher magnification. While the elongated nuclei in the wild-type cellularising blastoderm are regularly aligned at the periphery of the egg (Fig. 2c), in the mutant they are less regular, with some nuclei losing their position and sinking towards the center of the egg (Fig. 2d). Note, however, that the nuclei have elongated, and that at earlier stages their positioning at the periphery of the egg is normal (Fig. 2a,b). This is in contrast to the situation in cytochalasin-treated embryos in which nuclear positioning is also affected at earlier stages (Edgar et al., 1987). Thus, the transition from *flightless I*-independent to *flightless I*-dependent nuclear positioning marks a transition in the role of the actin cytoskeleton or in the stringency of nuclear positioning.

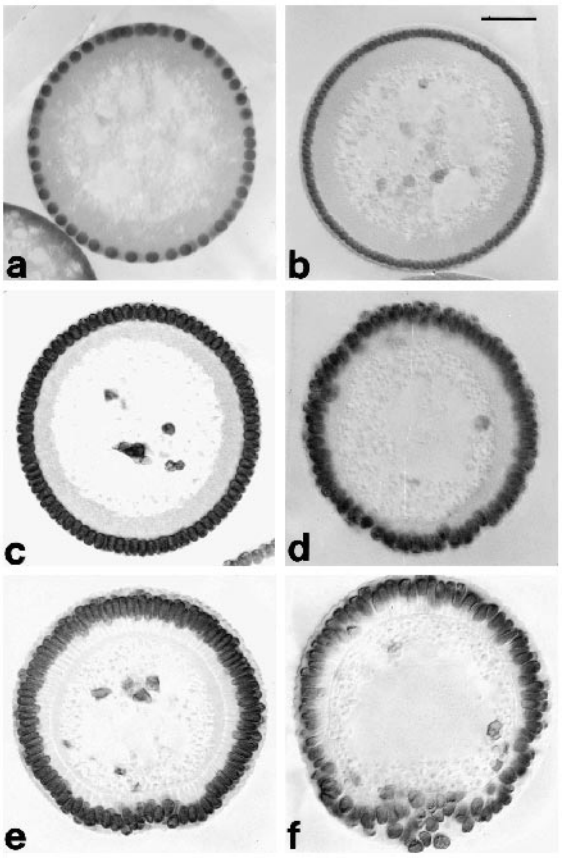

Fig. 2. Defects in nuclear positioning seen in cross-sections of embryos stained with antibodies against nuclear lamin. (a,c,e) Wild type; (b,d,f) mutant. No differences between wild-type and *flightless I* eggs are visible during syncytial blastoderm stages (a, interphase 12; b, interphase 14). Soon after the nuclei have begun to elongate and cellularisation has begun (c), the layer of nuclei in mutant eggs begins to look disorganized (d). When gastrulation begins, nuclei in ventral cells move away from the apical cell surface (facing the outside of the embryo) (e). In mutant embryos, they move out of the incompletely cellularized peripheral layer of cytoplasm into the inside of the egg (f). Bar, 50 μm.

When gastrulation begins, nuclei in ventral cells move away from the apical cell surface (facing the outside of the embryo) (Fig. 2e). In mutant embryos which have not completed cellularisation, this leads to the migration of these nuclei out of the peripheral layer of cytoplasm into the inside of the egg (Fig. 2f).

The defects in cell membrane formation result in an irregular appearance of the blastoderm (Fig. 3b). They are visible as soon as the cleavage furrows appear. Instead of advancing synchronously and in parallel (Fig. 3c,e), the cleavage furrows in *flightless I* embryos reach deeper between some nuclei than between others and they are not always perpendicular to the surface of the egg (Fig. 3d,f). Electron microscopy shows that the advancing membranes in *flightless I* embryos are less straight

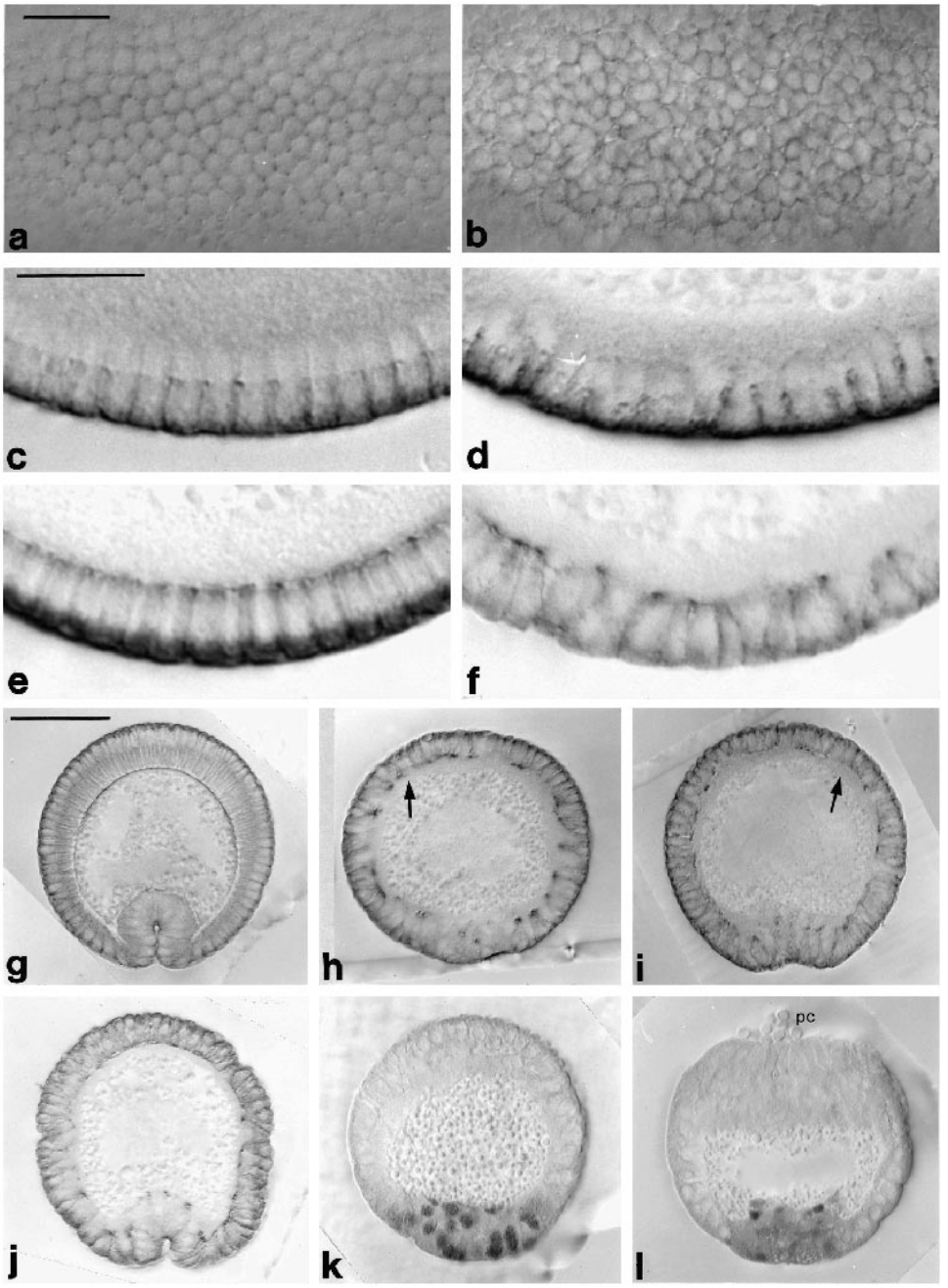

Fig. 3. Cellularisation and gastrulation defects in *flightless I* embryos. Surface views (a,b) and cross sections (c-i) of embryos stained with anti-phosphotyrosine (a-j) and *twist* (k,l) antibodies. (a,c,e,g) Wild type; (b,d,f,h-l) *flightless I.* While a surface view of a wild-type cellular blastoderm (a) shows the typical regular polygonal pattern, this pattern is much less regular in *flightless I* mutants but most cells are close to normal in size (b). While all membranes reach equally far into the wild-type embryo and grow perpendicular to the surface (c,e), they reach varying depths in *flightless I* embryos (d,f). (g-j) Wild-type (g) and *flightless I* embryos of the same age. The mesoderm anlage in the wild-type embryo has invaginated and formed a tube and all of the peripheral cytoplasm has been incorporated into cells. In the mutant embryos, a rim of unincorporated cytoplasm is visible (arrow). The mutant embryos have succeeded to varying degrees in making a ventral furrow. In the slightly older embryos (k,l) stained with *twist* antibodies the ventral nuclei are seen to have moved towards the center of the egg. (l) The ventral nuclei are dividing and *twist* protein is found in the cytoplasm. Two nuclei have completed mitosis and reformed. pc, pole cells. Note that, once cellularisation is completed in wild-type embryos, the lateral and basal cell membranes show only weak phosphotyrosine staining, whereas staining tends to remain strong in *flightless I* embryos. We do not know whether the strong staining in late *flightless I* embryos reflects the persistence of a phosphotyrosine (or a cross-reacting epitope), or whether it is due to the local accumulation of abnormal quantities of cell membranes. Bars: (a-f) 10 μm; (g-l) 100 μm.

and do not end in the typical tear-drop-shaped sections of the furrow canals which mark the leading edge of the cleavage furrows (Fig. 4). Instead, they are sometimes branched and their ends appear collapsed. In *flightless I* embryos, membrane vesicles are associated with the invaginating cleavage membranes that are not found in the wild type (Fig. 4). The furrow canals in wild-type embryos widen when they have reached their final depth and eventually fuse to give rise to the yolk membrane. In *flightless I* embryos, this widening does not occur normally (Fig. 3h,i) and aggregates of membrane appear at the bases of some cells, but they do not form a continuous basal cell membrane. As a result of these defects, the uniform blastoderm cell layer is not formed and in many positions the peripheral cytoplasm remains open towards the yolk.

Since the defects in nuclear positioning and cellularisation might be causally related, we used double-labelling experiments to determine whether a spatial correlation or a temporal order of these events could be established. However, they occur too close in time and the defects are often too subtle to allow a reliable assessment of their correlation in space and, therefore, any speculations on a potential causal relationship.

Consistent with the postulated role of *flightless I* as an actin binding protein, the defects observed in mutant embryos lacking wild-type *flightless I* protein resemble some of the effects of cytochalasin-induced actin-depolymerization (Edgar et al., 1987). However, not all of the actin-dependent processes in the early embryo are affected by the lack of wild-type *flightless I* protein (early cytoplasmic contraction waves, nuclear

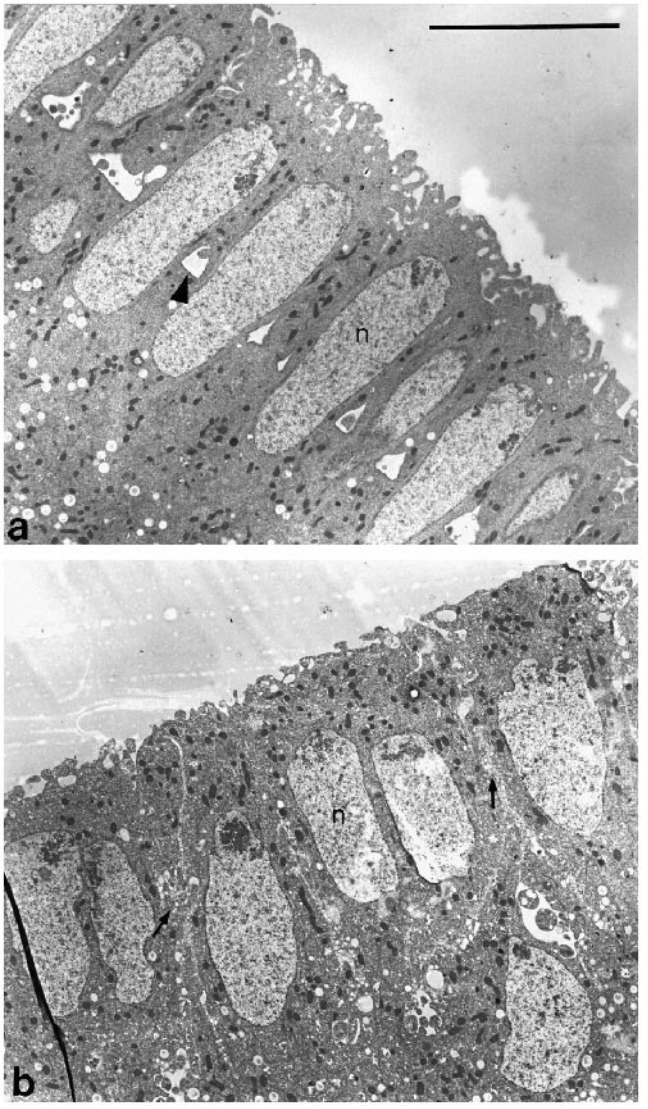

Fig. 4. Electronmicrographs of cellularisation in a wild type (a) and *flightless I* (b) embryo. The cleavage furrows in the wildtype end in a wide canal (the furrow canal; arrowhead), which appears tear-drop shaped in cross-section and is not seen in the mutant. The cleavage membranes in the mutant are irregular and surrounded by many small vesicles (arrows; these vesicles are much smaller than the lipid vesicles, L, found below the layer of nuclei in both the wild-type and the mutant embryo). n, nucleus. Bar, 10 μm.

migration). Furthermore, it is worth noting that *flightless I* is not essential for the actin-dependent processes occurring during the development of the oocyte, since normal-looking and fertilizable eggs are produced from mutant germlines (data not shown; see Materials and Methods). Since *flightless I* is provided maternally, and therefore present in the early egg, the absence of defects in the early actin-dependent processes means that *flightless I* is either not used early during normal development (but just stored for later use), or that it is normally used, but in its absence another protein can take over its function. A candidate for this other protein might be the true gelsolin homolog, which is also present in the early egg (Stella et al., 1994). However, in view of the rather severe cellularisation phenotype it is not easy to imagine how an early *flightless I* function could be taken over completely by another protein. More likely, *flightless I*, like the other cellularisation genes, may indeed have a function specific for cellularisation.

Cytokinesis in flightless I mutants

Since cellularisation corresponds functionally to cytokinesis we analysed whether lack of *flightless* led to defects in cytokinesis at other stages of development. However, we did not observe any. In zygotically mutant embryos from heterozygous germ lines, which survive until late larval stages, when their maternally provided wild-type protein runs out, no defects in cytokinesis were observed even in rapidly proliferating tissues (imaginal discs). Furthermore, *flightless I* is not needed for cell divisions in the mutant germ line clones, since these germ lines produce normal eggs. Thus, *flightless I* is not essential for normal cytokinesis. Again, this may be because it does not participate in normal cytokinesis at all, or because its function can be completely replaced by another protein. The requirements for normal cytokinesis during the division of a small cell might be much less stringent than those for making the cellularisation of the blastoderm occur reliably, perfectly and quickly. Finally, the cellularisation of the pole cells also does not require *flightless I*. However, this is less surprising, since many mutations are known that either perturb pole cell formation or somatic cellularisation (Niki and Okada, 1981; Merrill et al., 1988; Rickoll and Counce, 1981), but not both, suggesting major differences in the two processes.

Gastrulation in incompletely cellularised embryos

In view of the cellularisation defects it is not surprising that gastrulation in *flightless I* embryos is perturbed. On the contrary, it is interesting that in spite of these defects some aspects of gastrulation proceed normally. Gastrulation movements normally begin as soon as cellularisation is complete. In the wild-type embryo, ventral cells flatten, then constrict on their apical sides, and their nuclei move basally. These events also occur in *flightless I* embryos (Figs 2, 3) showing that the completion of cell formation is neither a signal for the subcellular rearrangements associated with epithelial invagination, nor a prerequisite for them to occur. In many embryos the ventral epithelium disintegrates completely, probably as a result of nuclei moving away from the apical end towards the basal end in the incompletely closed ventral cells. In the most severely affected embryos, the ventral nuclei move into the interior of the egg and no ventral furrow is formed (the previously reported gastrulation defect; Fig. 3k,l). In less severely affected embryos (probably including those receiving a wild-type copy of the *flightless I* gene from the father), the ventral epithelium makes an invagination (Fig. 3j).

The actin cytoskeleton in flightless I embryos

In embryos mutant for certain other genes required for cellularisation (*nullo* or *serendipity-α*) the membrane-associated actin network dissolves in many places such that the membranes fail to form or disappear and multinucleate cells are formed (Schweisguth et al., 1990; Rose and Wieschaus, 1992). This defect was not observed in *flightless I* embryos (Fig. 5). Neither the cell membranes nor the actin cytoskeleton ever disappear completely between adjacent nuclei, and multinucleate cells are not observed. Cytoplasmic myosin, which is

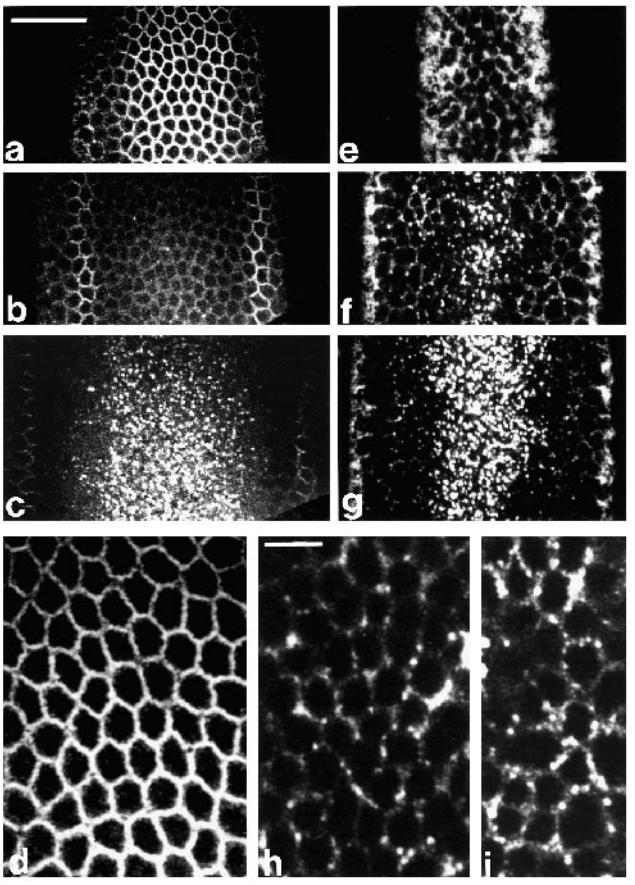

Fig. 5. Optical sections of the actin cytoskeleton (visualized with fluorescent phalloidin) in a wild-type (a-d) and a mutant (e-h) embryo. Optical sections near the surface (a,e), at the level of the basal end of the blastoderm cells (b,f) and below the cell layer (c,f) of blastoderm stage embryos. In the wild type, the punctate actin staining is confined to the region below the hexagonal membrane and actin network (b,c), whereas it invades the cellularised region in the mutant (f). Higher magnification shows that actin is not evenly distributed along the membranes of the mutant embryo. Bars: (a,b,c,e,f,g) 10 μm; (d,h,j) 20 μm.

located at the base of the cleavage furrows and is thought to produce the force for their advancement, is localized appropriately in *flightless I* embryos (not shown). However, the actin cytoskeleton is irregular and, like the cleavage membranes, it reaches to varying depths in the peripheral cytoplasm. In addition, actin is unevenly distributed along the membranes (Fig. 5h,i). It does not appear to lie in straight filaments between the apices of the hexagonal network but accumulates in grains along the membranes. Furthermore, the particulate staining in the center of the embryo, normally confined to the region below the cortical cytoplasm and membrane network (Fig. 5d) invades the cellularising region in *flightless I* embryos.

Conclusion

The irregular distribution of actin along the blastoderm membranes indicates that *flightless I* may regulate the state of the actin cytoskeleton, rather than only being involved in attaching something to or moving something along actin filaments (a role that might be fulfilled by its N-terminal leucine-rich domain). The irregularity of the actin network may arise from the network being expanded by the contractile apparatus without new actin being integrated. The vesicles we observe near the invaginating membranes may be consistent with this, since similar vesicles were seen in embryos mutant for another celluarisation gene, *shibire*. Swanson and Poodry (1981) assumed that these vesicles contained material which could not be integrated into the growing membranes. Thus, both *shibire*, which codes for dynamin (Chen et al., 1991; van der Bliek and Meyerowitz, 1991), and *flightless I* might be involved in aspects of delivering membrane material and/or actin to the invaginating cleavage furrows, but use different parts of the cytoskeleton.

flightless I is one of several known genes needed for cellularisation of the blastoderm. Mutations in other genes also lead to defects, but not to a complete disruption of cellularisation. This might seem surprising, but there are good reasons why many proteins with overlapping functions might be deployed to ensure that this process always occurs rapidly and efficiently, even under adverse conditions. Cellularisation is essential for the further development of the embryo. Gastrulation begins on the ventral side exactly at the time point when ventral cells have completed cellularisation, and the failure of these cells to complete cellurisation by this time has disastrous consequences (see Fig. 3). It is therefore important for the organism to have backup mechanisms that protect this process from being disrupted. On the other hand cellularisation is an impressive phenomenon in its regularity, speed and perfection (about 40 minutes to create a tall columnar epithelium of 6,000 perfectly regular, identical looking cells), a major task for the acto-myosin system to perform and probably far more sensitive to potential perturbations than normal cytokinesis. Cellularisation may thus be a process that is uniquely suited for studying proteins with partially overlapping functions that would not be revealed in a genetic analysis of normal cytokinesis.

It will be interesting to see to what degree the function of gelsolin in *Drosophila* is similar to that of *flightless I*, and conversely, in which cells of the vertebrate body the vertebrate homolog of *flightless I* is expressed and which roles it performs in these cells. The defects described here may help to guide the search for functions of *flightless I* in vertebrates.

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