Dynamic changes in the distribution of cytoplasmic myosin during *Drosophila* embryogenesis

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Summary

Dramatic changes in the localization of conventional non-muscle myosin characterize early embryogenesis in *Drosophila melanogaster*. During cellularization, myosin is concentrated around the furrow canals that form the leading margin of the plasma membrane as it plunges inward to package each somatic nucleus into a columnar epithelial cell. During gastrulation, there is specific anti-myosin staining at the apical ends of those cells that change shape in regions of invagination.

Both of these localizations appear to result from a redistribution of a cortical store of maternal myosin. In the preblastoderm embryo, myosin is localized to the egg cortex, sub-cortical arrays of inclusions, and, diffusely, the yolk-free periplasm. At the syncytial blastoderm stage, myosin is found within cytoskeletal caps associated with the somatic nuclei at the embryonic surface. Following the final syncytial division, these myosin caps give rise to the myosin rings observed during cellularization.

These distributions are observed with both whole immune serum and affinity-purified antibodies directed against *Drosophila* non-muscle myosin heavy chain. They are not detected in embryos stained with anti-*Drosophila* muscle myosin antiserum or with preimmune serum. Although immunolocalization can only suggest possible function, these myosin localizations and the coincident changes in cell morphology are consistent with a key role for non-muscle myosin in powering cellularization and gastrulation during embryogenesis.

Key words: cytoplasmic myosin, *Drosophila* embryogenesis, cellularization, gastrulation, apical constriction, cell shape change.

Introduction

Non-muscle cytoplasmic myosins have been identified in eukaryotes throughout phylogeny. Presumably, they participate in such diverse cellular motilities as intracellular vesicle movement, cytoplasmic streaming, cell surface receptor capping and generation of cortical tension, cytokinesis, and cell shape changes (Pollard, 1981; Warrick and Spudich, 1987; Spudich, 1989; Kiehart, 1990). Indeed, both antibody microinjection studies and molecular genetic approaches have demonstrated that myosin function is required for cytokinesis (Mabuchi and Okuno, 1977; Kiehart et al. 1982; de Lozanne and Spudich, 1987; Knecht and Loomis, 1987).

We have previously identified a conventional non-muscle myosin isoform from *Drosophila* (Kiehart and Feghali, 1986), and have cloned and sequenced the genes that encode its heavy and light chains (Kiehart et al. 1989; Ketchum et al. 1990; Chang et al. unpublished data). The heavy chain gene encodes a 6 kb transcript that is expressed in a developmentally regulated fashion, with peaks of message accumulation at 4–12 h of embryogenesis, early third instar larval and early pupal stages. The gene is at polytene chromosome band 60E9, and is distinct, by polytene location, by cross hybridization and by sequence analysis, from the *Drosophila* muscle myosin heavy chain gene at 36B (Bernstein et al. 1983; Rozek and Davidson, 1983; Kiehart et al. 1989; Ketchum et al. 1990).

The first insights into non-muscle myosin localization during *Drosophila* embryogenesis were made using an antibody against Sarcophaga flight muscle myosin (Warn et al. 1979, 1980). However, interpretation of these studies is limited by the use of a probe from a heterologous species, questions about myosin isoform specificity, problems with background fluorescence, and a consideration of only those developmental stages prior to gastrulation.

Here, we focus on the distribution of non-muscle myosin at cellularization and gastrulation during *Drosophila* embryogenesis. We also examine the myosin distribution at earlier stages, in order to address how the pattern of myosin localization in these later stages develops. We use antibodies specific for *Drosophila*...
cytoplasmic myosin (Kiehart and Feghali, 1986) to establish the localization of myosin in specimens fixed at various stages of embryogenesis. We review the relationship between the distribution of myosin and other cytoskeletal components in the embryo, particularly actin and spectrin, as determined by our own colocalization studies and the staining patterns published by other investigators. Our observations suggest that cytoplasmic myosin is in the right place at the right time to contribute to force production in cellularization (cytokinesis) and various cell shape changes and movements during gastrulation that are required for successful embryogenesis. Our studies provide direct evidence for a specific myosin localization in cells undergoing an apical constriction coincident with changes in cell sheet morphogenesis.

Materials and methods

Antibodies

Preparation of rabbit anti-Drosophila cytoplasmic myosin, muscle myosin and spectrin polyclonal sera has been described previously (Kiehart and Feghali, 1986; Byers et al. 1987). Anti-actin monoclonal antibody was a gift from Dr James Lessard and has been described elsewhere (Lessard, 1988). Anti-human histone monoclonal antibody was purchased from Chemicon (Temecula, CA). Anti-tubulin monoclonal antibody was a gift from Dr L. S. B. Goldstein. Affinity-purified rhodamine-labeled goat anti-mouse IgG, rhodamine-labeled goat anti-rabbit IgG, and fluorescein-labeled goat anti-rabbit IgG were purchased from Hyclose Laboratories (Logan, Utah) and Tago Immunologicals (Burlingame, CA).

Affinity-purification of anti-cytoplasmic myosin polyclonal serum

Rabbit anti-Drosophila cytoplasmic myosin antibody was purified from polyclonal serum by elution from cytoplasmic myosin absorbed to nitrocellulose using Pollard’s (1984) modification of the method developed by Olmsted (1981), or on columns of Drosophila cytoplasmic myosin by modifications of standard methods (Cuatrecasas and Anfinsen, 1971; Lutz and Kiehart, unpublished data). The concentration of affinity-purified antibody was estimated by a dye binding assay (Smith et al. 1985) using bovine serum albumin as a standard.

Rhodamine-phalloidin

A working solution was made by drying, under vacuum, 5 μl of a 3.3 μM stock of rhodamine phalloidin (Molecular Probes, Eugene, Oregon) in methanol and resuspending it in 500 μl phosphate-buffered saline (PBS; prepared as described by Karr and Alberts, 1986).

Preparation of embryos for immunofluorescence

Embryos from Drosophila melanogaster (Canton-S) flies were collected at 25°C on grape juice/agar plates at timed intervals (Elgin and Miller, 1978), rinsed off plates and dechorionated by standard methods (Karr and Alberts, 1986). All subsequent fixation and immunostaining steps were performed at room temperature by modification of a number of previously described methods (Zalokar and Erk, 1977; Mitchison and Sedat, 1983; Karr and Alberts, 1986; Wieschaus and Nusslein-Volhard, 1986).

To verify patterns of contractile and cytoskeletal protein localization, various fixatives, based on previously published methods, were used. All gave similar results. They included various formaldehyde concentrations (2.5, 3.7, 8% formaldehyde; Karr and Alberts, 1986); a mixture of 4 ml 95% EtOH, 1 ml 50% acetic acid, 1 ml formalin (from Zalokar and Erk, 1977); and 90% methanol (Warn and Warn, 1986). Embryos were fixed for 3–5 min in all cases. Exposure to fixatives, particularly formaldehyde-based fixatives, for longer periods of time resulted in severely diminished immunofluorescent signal. The inclusion of 50 mM EGTA, pH 7.5 in fixative solutions and in subsequent antibody incubations gave improved immunofluorescent images.

Cryostat sectioning of embryos

Fixed embryos were prepared for sectioning as described previously (Pescacreta et al. 1989). Sections were cut at a thickness of 5 μm on a Minotome (IEC, Needham Heights, MA) cryostat. Staining solutions and times of incubation were as detailed below for whole-mount embryos.

Antibody staining

Embryos were blocked for 30 min–1 h in incubation solution (PBS+10 mg ml⁻¹ bovine serum albumin+0.4% Triton X-100). This concentration of Triton X improved staining and antibody penetration.

The solution was replaced with 1 ml fresh incubation solution containing an appropriate dilution of the primary antiserum (1:1000), affinity-purified antibody (30 μg ml⁻¹), rhodamine phalloidin (0.33 μM), anti-tubulin monoclonal supernatant (1:100), anti-histone monoclonal supernatant (1:1500) or Hoechst stain (1 μg ml⁻¹). Embryos were stained with each solution for 1 h.

Embryos were washed by resuspension in fresh incubation solution for 1 h with three changes of fresh solution; incubated for 1 h in secondary antibody diluted 1:1000 with incubation solution; and then washed again for at least 1 h with three changes of fresh solution.

Observation of embryos and analysis of myosin localization

Incubation solution was replaced with a mounting solution of 50% glycerol in PBS. Embryos were allowed to equilibrate in this solution at least 30 min prior to mounting. Embryos were placed on a microscope slide under a coverglass edged with silicone grease to control the extent to which embryos were flattened. Confoocal (MRC-6000, Biorad, Cambridge, MA) imaging was performed on a Zeiss Axioplan microscope, using a 63× objective (NA 1.40). Other images were obtained through a Zeiss IM35 inverted microscope under Nomarski DIC optics with an epifluorescence attachment, using 16× (NA 0.35), 40× (NA 0.75), and 63× (NA 1.4) lenses. Photobleaching of rhodamine-stained specimens was not significant for brief periods of observation. Photographs were taken with an Olympus OM-2 camera mounted on the 35 mm port of the IM35 microscope using Kodak Tri-X film processed with Diafine developer (Acufine, Inc., Chicago, IL). Prints of confocal images were obtained using a Sony video printer (Park Ridge, New Jersey).

Prior to cellularization, Hoechst or anti-histone staining was used to identify the developmental stage of each embryo, as well as to determine the mitotic phase and number of nuclei per unit area at the embryonic surface. The number of nuclei unambiguously defined the number of mitotic cycles that had occurred during development.

In all experiments, the pattern of specific antibody staining was compared directly to specimens stained with appropriate
controls of preimmune or non-immune sera. To avoid repetition, we only include the control data for the non-muscle myosin distribution during the cellular blastoderm stage. Levels of preimmune and non-immune staining remained at a comparably low level for all stages analyzed.

**Quantitative immunoblots**

The quantitative immunoblot procedure used for the estimation of relative levels of cytoplasmic myosin present in embryos at different developmental stages was as described previously (Pesacreta et al. 1989). Immunoblots were performed as described by Kiehart and Feghali (1986) but dilutions of sera were in Tween buffer (150 mM NaCl, 20 mM Tris–HCl, pH 7.5, 0.1% Tween-20, and 0.01% thimerosal, with a final concentration of 5% goat serum included for blocking purposes).

**Nomenclature**

'Non-muscle' and 'cytoplasmic' are used interchangeably throughout the text to describe the conventional (myosin-II), *Drosophila* non-muscle myosin isoform.

We assume the reader has a basic knowledge of early *Drosophila* development. For detailed descriptions of *Drosophila* embryogenesis, please consult Sonnenblick, 1950 and Campos-Ortega and Hartenstein, 1985.

**Results**

The distribution of non-muscle myosin changes dramatically as the *Drosophila* embryo develops. The changes are most dramatic during cellularization and gastrulation.

**Cytoplasmic myosin is at the furrow canals during cellularization**

During cellularization, cytoplasmic myosin is localized at the leading edge of the furrow canals. This distribution is consistent with a key role in generating the changes in cell shape that transform the syncytial blastoderm into the columnar epithelium of the cellular blastoderm.

As cellularization begins, myosin pervades the cortex of the embryo and is concentrated at high levels in a ring that encircles each nucleus (Fig. 1). These rings appear to interconnect and form a network that encompasses the entire periphery of the *Drosophila* embryo. Sagittal optical sections that bisect these rings and the nuclei localize myosin to a dot on either side of each somatic nucleus (Fig. 1A, sagittal section, arrowheads).

As cellularization proceeds, membrane extends deeper and deeper between adjacent nuclei as the furrow canals are displaced towards the embryo interior (Fullilove and Jacobson, 1971). Fluorescent images confirm that the polygonal rings of myosin are precisely coincident with the location of the furrow canal (Fig. 1). Little or no myosin associates with the membrane that trails outward from the furrow canal to the embryonic surface.

Once the furrow canals have reached the base of the nuclei, they become more triangular or wedge-shaped, as the dots of myosin staining are transformed into teardrops (Fig. 1G, arrowheads). Their form suggests that the furrow canals on either side of a given nucleus are being drawn closer together and that the myosin rings have contracted to become smaller than when they surrounded each nucleus. In *en face* views, the polygonal rings of myosin coincide with the furrow canals have become distinct circles of myosin staining (Fig. 1H, I). Interestingly, the circles have begun to detach from one another, and in some cases no longer contact each other directly, but rather are interconnected by a meshwork of myosin (Fig. 1H, I). We consider it likely that the nearly isomorphic contraction of this network assists in drawing the furrow canals deeper and deeper into the embryo.

Once the furrow canals invaginate to their maximum extent (approx. 30 µm), myosin is associated with the basal and immediately adjacent lateral aspects of each newly forming cell (Fig. 1J, arrowheads). The myosin circles contract further within the surrounding myosin meshwork (Fig. 1K, L), and surround the bridge of cytoplasm that connects each forming cell with the yolk mass. These small connections persist into early gastrulation (Rickoll, 1976). Thus, during this final phase of cellularization, non-muscle myosin is displaced both in the direction of, and perpendicular to, the advancement of the furrow canals.

Actin is localized in a similar fashion during cellularization (Warn, 1986; Pesacreta et al. 1989; Young et al. unpublished results). Double-stained preparations show that the region of highest actin concentration at the furrow canal is coincident with the most concentrated region of myosin. However, in contrast to myosin, actin is also localized along the lateral borders of the forming cells. Interestingly, spectrin also stains the lateral margins of the cells but fails to localize to the leading edge of the furrow canals (Pesacreta et al. 1989). Instead, spectrin lags slightly behind the furrow canals.

**Specificity of anti-cytoplasmic myosin staining**

Preimmune serum fails to detect any antigens when used to stain immunoblots of whole embryo homogenates (Fig. 2A, lane 1). In contrast, affinity-purified antibodies (data not shown) and anti-cytoplasmic myosin antiserum (Fig. 2A, lane 2) both recognize a single 205 × 10^3 M, band when used to stain similar immunoblots. Quantitative analysis (Fig. 2B) indicates that there is a fairly uniform level of non-muscle myosin present in embryos through early gastrulation, at or near the level observed in unfertilized eggs. About four hours following fertilization, there is a significant increase in the level of myosin, coincident with an increase in the level of transcript accumulation from the non-muscle myosin gene (Kiehart et al. 1989).

Similarly, preimmune serum produces no specific staining patterns in immunofluorescence on fixed embryo specimens (Fig. 3A). Affinity-purified antibodies (Fig. 3B) and whole anti-cytoplasmic myosin antiserum (Fig. 3C) produce localization patterns that are nearly identical to each other, although there is a slight increase in the level of diffuse staining observed
Fig. 1. Myosin is localized at the leading edge of the furrow canals during cellularization. Confocal fluorescent micrographs of whole-mount embryos, fixed and stained with anti-cytoplasmic myosin antiserum at different times during cellularization, are shown. Panels A, D, G, and J represent optical sagittal sections of different embryos, fixed at progressively later times during cellularization. (A, D) Slow phase of cellularization; (G, J) rapid phase. Arrowheads in the photographs point out specific alterations in the staining pattern: in A, ‘dots’ on either side of each nucleus; in G, ‘teardrops’; in J, basal and immediately adjacent lateral aspects of each forming cell. Panels B, E, H, and K are optical en face sections of the embryos depicted in panels A, D, G, and J, respectively, at a depth of focus coincident with the location of the furrow canals. Through-focal series verify that these sections are at the peak of staining intensity. Panels C, F, I, and L are higher magnification images of panels B, E, H, and K. Scale bar in A is 10 μm, and is for panels A, B, D, E, G, H, J, and K. Scale bar in C is 5 μm and is for panels C, F, I, and L.
when using whole serum. Anti-\textit{Drosophila} muscle myosin antiserum fails to duplicate the localizations observed in embryos stained with non-muscle myosin antibodies (data not shown). Controls were performed for all developmental stages with comparable results, to avoid repetition, control data are presented only for cellularization stage embryos (Fig. 3). These controls demonstrate that the myosin patterns observed with both anti-\textit{Drosophila} cytoplasmic myosin antiserum and affinity-purified antibodies accurately reflect the distribution of cytoplasmic myosin in developing embryos.

\textbf{Myosin is in invaginating furrows during gastrulation}

Myosin accumulates along the apical margins of cells that invaginate during gastrulation. We have focused on the myosin distribution during the formation of the amnioproctodeal invagination (the invagination of the posterior midgut rudiment and the pole cells; Figs 4, 5) because this has been particularly amenable to time-lapse video analysis of living embryos (Kiehart \textit{et al.} 1990). We observe similar apical accumulations of myosin in other furrows and invaginations that also originate during early gastrulation (data not shown).

Those cells whose apical boundaries narrow or constrict during cell sheet morphogenesis accumulate myosin at their apical ends (Fig. 5A, B sagittal section, arrowheads). In surface views, this concentration of apical myosin appears as a bright region of staining within a field of rather uniformly staining cells (Fig. 4A–C). Optical sections indicate that the cells that lack myosin staining at their apices are at the margins of the cell sheet furrows (Fig. 5A, B) and in regions of the embryo that bulge outward (Fig. 5C). In all cases where apical staining of myosin is observed, the morphology of the embryo is consistent with regions undergoing or having recently undergone a shape

Fig. 3. Immune serum is specific for \textit{Drosophila} cytoplasmic myosin. Panel A is a confocal fluorescent micrograph of a cellularizing embryo, fixed and stained with preimmune serum at a time when the furrow canals have reached the base of the nuclei. No staining coincident with the furrow canals is seen with preimmune serum (see arrowhead). Panel B is a similar embryo, fixed and stained with affinity-purified anti-cytoplasmic myosin antibodies. Panel C is a similar embryo, fixed and stained with whole anti-cytoplasmic myosin antiserum. Myosin staining is coincident with the furrow canals in B and C, as documented in Fig. 1. There is a slight increase in the level of diffuse periplasmic staining observed using whole immune serum, as compared to affinity-purified antibodies. Raw images were collected, merged side by side on a single frame, then processed to ensure that image manipulation by digital processing was identical for all panels. Scale bar is for all panels and is 10 \textmu m.
Fig. 4. Myosin concentrates in the pole cells and cell sheet furrows during gastrulation. Panels A, B, and C reveal myosin localization in whole-mount embryos, fixed and stained for anti-cytoplasmic myosin at progressively later times during amnioproctodeal invagination. (A) Pole cells have begun to be carried onto the dorsal surface of the embryo. Pole cells and the underlying plate of cells stain brightly for cytoplasmic myosin. Arrowhead indicates region of increased fluorescence along pole cell membrane. (B) In a slightly older embryo, cells have begun to invaginate. Pole cells still stain brightly, as does the region of invagination. There is a particularly bright line of staining at the anterior margin of the depression. (C) The pole cells have disappeared into the embryo interior. Myosin concentrates in the anterior margins of the amnioproctodeal invagination, with decreased levels at posterior regions. Scale bar is for all panels and is 10 μm.

change. Thus, there is a tight correlation between myosin redistribution and the specific cell shape changes that have been proposed to initiate the infolding of furrows and drive cell sheet shape changes (see Discussion).

Cytoplasmic myosin at earlier stages
We have localized myosin at earlier developmental stages in order to understand how myosin gets positioned to participate in cellularization and gastrulation.

Preblastoderm embryos
The egg cortex of the preblastoderm embryo is enriched in cytoplasmic myosin. There is a well-defined zone of myosin approximately 1–2 μm thick that extends completely around the egg periphery (Fig. 6A; arrowhead). Warn et al. (1979) observed a similar cortical distribution of myosin in preblastoderm embryos with an antibody against Sarcophaga muscle myosin. The antisera that we raised against Drosophila muscle myosin fails to detect this cortical layer of myosin.

There is a more diffuse pool of cytoplasmic myosin in the egg periplasm (Fig. 6A, bracket). The level of staining is clearly above the background. Immunological probes directed against other cytoskeletal components (e.g. actin, spectrin and tubulin) also give a similar level of diffuse periplasmic labeling that is above that observed with preimmune or non-immune control sera (see Warn et al. 1984; Karr and Alberts, 1986; Pesacreta et al. 1989; also Young et al. unpublished results). The yolk mass is relatively devoid of cytoplasmic myosin staining (Fig. 6A), but there is some staining of the cytoplasm that surrounds the embryonic nuclei as they migrate towards the embryonic periphery (data not shown).

Cytoplasmic myosin is also concentrated in arrays of inclusions confined to the cortex and subjacent egg periplasm (Fig. 6B, C; arrows). These arrays are longitudinal distributions of particles that radiate outward from either or both of the embryonic poles, and span the entire length of the embryo. Each array contains two types of stained inclusions: points of staining 1–2 μm across and spheres of staining approx. 4 μm in diameter. These arrays of inclusions disappear from the region of the somatic nuclei during the syncytial blastoderm stage. However, they are still observed at the posterior embryonic pole in the region of pole bud formation, at times when they are no longer visible across the rest of the embryo. Antisera against other cytoskeletal components fail to detect these same arrays. For example, punctate actin staining occurs uniformly across the entire embryonic surface and is not organized in the longitudinal arrays seen with antomyosin (Karr and Alberts, 1986; Warn, 1986; Pesacreta et al. 1989).

Syncytial blastoderm
Cytoplasmic myosin redistributes as the nuclei approach the embryonic surface between the end of nuclear cycle 9 and interphase of nuclear cycle 10. The longitudinal arrays of inclusions begin to disappear,
the uniform layer of cortical myosin becomes disrupted, and myosin becomes concentrated into planar, disk-like caps near the plasma membrane, centered with respect to each interphase nucleus (Fig. 7A, face view; Fig. 7B, C, sagittal section). This suggests that the cortex between each somatic nucleus and the plasma membrane becomes a new focus for myosin organization. These cytoskeletal caps also contain actin and spectrin (Warn et al. 1984; Karr and Alberts, 1986; Pesacreta et al. 1989). In double-staining experiments, we have never observed the presence of myosin in the caps without actin, or vice versa. We conclude that myosin and actin are recruited to the caps simultaneously. Spectrin, in contrast, enters the caps later (Pesacreta et al. 1989).

Following the formation of the cytoskeletal cap structures (and perhaps as a consequence), a cytoplasmic protrusion or bulge forms over each somatic nucleus (Turner and Mahowald, 1976). During these changes, myosin is in tight apposition to the plasma membrane (Fig. 8A). Thus, the planar myosin caps become somewhat stretched and curved as the domed cortex of each surface cap bends in concert with the formation of the bulges. This is evident in face views at focal planes that transect surface cap domes as a bright ring of staining that encircles and is concentric with each nucleus (Fig. 8B). Regions of the embryonic surface that are not included within a surface cap are relatively devoid of myosin.

The general dynamics of the myosin distribution during the divisions of the syncytial blastoderm nuclei and the surface caps closely parallel the description in the literature for actin (see Warn et al. 1984; Karr and Alberts, 1986). Double-staining experiments suggest that the positions of the myosin caps are determined by the centrosomes of the mitotic spindle (data not shown). Interestingly, myosin, like actin, fails to accumulate in the region between the two separating poles of a given spindle, as would be expected for a contractile ring that marks the equatorial plane of division between newly forming surface caps.

**Pole bud and pole cell formation**

Cytoplasmic myosin localizes to the pole buds in a pattern that differs from that observed for the somatic caps. Myosin is distributed diffusely in the pole bud cytoplasm at levels markedly higher than that observed in all other regions of the embryo at this time (Fig. 9A, arrow). The pole cell cytoplasm includes this high level of myosin until the start of cytokinesis at the end of nuclear cycle 13 (pole cells again display high levels of cytoplasmic myosin during the early stages of gastrulation; Fig. 4A, B). There are small accumulations of cytoplasmic myosin along the pole cell margins, in tight apposition to the plasma membrane (Fig. 9B, arrowheads), particularly where adjacent pole cells are in contact with one another. These observations contrast those that we have previously documented for spectrin (see Fig. 5 of Pesacreta et al. 1989) where there is minimal cytoplasmic spectrin staining and a decrease in the amount of spectrin near the pole cell plasma.

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**Fig. 5.** Cytoplasmic myosin is localized to the apical ends of cells specifically in regions of cell sheet invagination. Panels A, B, and C are different confocal sagittal sections of the same embryo during early gastrulation, fixed and stained for cytoplasmic myosin. All views are at the posterior end, in the region of the amnioproctodeal invagination and are directly comparable to the whole mount shown in panel A of Fig. 4. Panels A and B illustrate the accumulation of myosin in the apical ends of cells undergoing an apical constriction (see arrowheads). Panel C is provided for comparison to the absence of apical staining in other regions of the embryo. Scale bar is for all panels and is 10μm.
membrane. Our observations of living embryos (Kiehart, Young and Inoué, unpublished observations and Kiehart et al. 1990) indicate that these staining patterns are coincident with a period of increased movement and pole cell surface activity.

During pole cell formation, there is an increase in the level of cytoplasmic myosin around the base of each pole bud, as it pinches off from the bulk of the embryo (Fig. 9A, arrowhead). Warn and colleagues (1985) document a comparable localization of actin during pole cell formation. The co-localization of actin and myosin in this region strongly suggests that these proteins play a role in the cytokinetic event that separates the pole cells from the bulk of the syncytial blastoderm.

Myosin at later stages
We have not investigated the myosin distribution in detail at stages later than early gastrulation. A diffuse cytoplasmic distribution with some additional concentration in the cortex appears to characterize virtually all cells. Interestingly, appreciable amounts of myosin accumulate in the developing nervous system (Fig. 10) to levels above those seen for surrounding tissues.

Discussion
Our data demonstrate that non-muscle myosin is in the right place at the right time to contribute to cell shape changes that drive cellularization and gastrulation of the Drosophila embryo. We speculate that conventional non-muscle myosin is a key motor for these movements. The data establish the distribution of cytoplasmic myosin in developing Drosophila embryos from egg deposition to early gastrulation. We obtain similar patterns of localization in whole-mount and sectioned embryos and in specimens prepared by a variety of different protocols with both formaldehyde- and alcohol-based fixatives (see Materials and methods for details). Immunoblot analysis (Fig. 2B) and developmental Northerns (Kiehart et al. 1989) suggest that early changes in localization involve a reorganization of a maternally derived myosin pool. After early gastrulation (approx. 4h), zygotic transcription and translation contribute new myosin to the existing pool.

The distributions that we document through the cellular blastoderm stage are consistent with preliminary studies by Warn and his colleagues (Warn et al. 1979, 1980). They used high concentrations of an antiserum against a muscle myosin isoform from a heterologous species. Anti-Drosophila muscle myosin antibodies that strongly label the developing musculature of fixed Drosophila embryos fail to duplicate the patterns that we have documented using our antibodies.
Non-muscle myosin in Drosophila development

Fig. 7. Myosin is reorganized in the cortex when nuclei arrive during cycle 10. (A) Fixed whole-mount nuclear cycle 10 embryo stained with affinity-purified anti-cytoplasmic myosin antibody, face view. Myosin caps have formed within the cortex and overlie each somatic nucleus. (B) Optical, sagittal section of fixed whole-mount embryo at nuclear cycle 10, stained with anti-cytoplasmic myosin antiserum. There is an accumulation of myosin in the cortex above each nucleus (arrowheads), as verified in C where the embryo is double-labeled with Hoechst to identify nuclear position. Scale bar is for all panels and is 20 µm.

against Drosophila cytoplasmic myosin (data not shown). We interpret the staining patterns seen by Warn et al. (1979, 1980) as resulting from a low level of antibodies directed against one or more of the small number of epitopes shared by muscle and cytoplasmic isoforms of Drosophila myosin (for cross-reactivity of the anti-cytoplasmic and anti-muscle myosin sera used in this study, see Fig. 7 of Kiehart and Feghali, 1986; for sequence similarities between cytoplasmic and muscle myosin isoforms, see George et al. 1989 and Ketchum et al. 1990).

Myosin in cellularizing embryos

The dramatic reorganization of myosin during cellularization is consistent with an active role for this chemomechanical force producer in the shape changes that transform the syncytial blastoderm into the cellular blastoderm. The localization of myosin around the invaginating furrow canals, the coexistence of actin at the furrow canals that we have observed and that has been described by others (Warn et al. 1984; Warn, 1986; Pesacreta et al. 1989; Warn et al. 1990), and experiments that show anti-myosin injection blocks cellularization (Lutz and Kiehart, 1987; and unpublished data), are consistent with a role for myosin in force production for cellularization. The changes in distribution parallel the recruitment of myosin to the contractile ring as evidenced by antibody localization studies in dividing cells over a wide range of phyla from slime molds (Dictyostelium; Yumura and Fukui, 1985) to mammals (Fujiwara and Pollard, 1976; Nunnally et al. 1980; reviewed in Conrad and Schroeder, 1990). In cultured cells, such studies have been confirmed by elegant, fluorescent myosin light chain microinjection studies that allow the changing distribution of myosin to be followed in living cells (Mittal et al. 1987).

It is generally accepted that myosin plays a key functional role in powering cytokinesis (Mabuchi and Okuno, 1977; Kiehart et al. 1982; de Lozanne and...
Fig. 9. Elevated levels of myosin are present in the pole cells and region of pole cell formation. (A) Cryostat section at posterior pole of fixed embryo at nuclear cycle 12, stained for cytoplasmic myosin. There is appreciable myosin in the pole cell cytoplasm (arrow), at levels higher than that observed within the surface cap cytoplasm. An accumulation of myosin is also detected at the base of this pole cell (arrowhead), where it is pinching off from the rest of the embryo. (B) Face view at posterior pole of fixed whole-mount embryo, nuclear cycle 12, stained for cytoplasmic myosin. Cytoplasmic myosin accumulates at the margins of some pole cells, particularly in regions of contact (arrowhead). Scale bar is for both panels and is 10 μm.

Spudich, 1987; Knecht and Loomis, 1987) and that myosin, with actin, is localized in a band of thick and thin filaments known as the contractile ring. Contractile ring function in animal cell cleavage is believed to be the consequence of the sliding of actin filaments, anchored to the membrane or cortex and mediated by bipolar myosin filaments. Thus, the shortening contractile ring behaves as a purse-string, serving to constrict the cell at the furrow in order to partition the cytoplasm following mitosis. However, the ultrastructural organization, assembly, regulation, function and disassembly of the contractile ring are not understood in detail. Therefore, a complete understanding of the mechanism of myosin function in force production during cellularization, specifically, and cytokinesis, in general, remains elusive.

Cytokinesis of the cellular blastoderm is topologically complex. However, all the elements of contractility appear to be present and it is likely that the overall mechanism is comparable to cytokinesis in other cells. Indeed in Drosophila, a network of some 6000 contractile rings are organized so that they form a continuous ring around the entire embryo. The radial constrictive force of each ring, being opposed by adjacent rings, results in movement directed toward the embryo interior. Thus it is formally possible that a similar, purse string-like mechanism, that works in concert with a contracting actomyosin meshwork, serves to mediate cytokinesis during cellularization. We speculate that this complex arrangement of actomyosin, in rings connected by a meshwork, is required for the simultaneous cytokinesis of the 6000 cells of the cellular blastoderm. As in other systems, it is likely that the rings and meshwork form numerous attachments to the cell surface so that localized contractile function can effect cellularization in part of the embryo, despite disruption of the contractile machinery in other regions.

A major feature of cellularization that distinguishes it from cytokinesis in simpler cells is that the net displacement of membrane occurs in two directions, orthogonal to one another. First, the furrow canals must move some 30–40 μm into the embryo, thereby laterally segregating each nucleus from its adjacent neighbors. Then, towards the end of this movement, the contractile ring at the base of each forming cell must constrict in a direction orthogonal to its original movement and parallel to the surface of the embryo, so as to form the basal margins of each columnar cell. Our data suggest that this transition in the direction of movement begins at the end of the slow phase of cellularization (Mahowald, 1963), when the furrow canals have reached the base of the nuclei. En face images of embryos, at a depth of focus coincident with the furrow canals, indicate a transition in the myosin network that occurs roughly when the furrow canals reach the base of the nuclei (see Fig. 1). The
interlocking rings of myosin that encircle each nucleus no longer make direct contact with each other, but rather appear separated from one another, held together in a meshwork of more diffuse myosin staining. During the fast phase of cellularization that follows, these rings decrease in diameter, suggesting a constriction that could serve to pinch off the newly forming cells.

The striking change in the speed of progress of the furrow canals at the slow phase–fast phase transition is consistent with a change in the direction or generation of force within the embryo at this time. We believe that the coincident rearrangement of the actomyosin network supports its direct involvement in cellularization; however, the interactions between the various elements of the contractile apparatus that allow these complicated changes to occur synchronously and in precise spatial register remain a mystery (see Merrill et al. 1985 and Wieschaus and Sweeton, 1988 for mutagenesis studies that begin to address how cytoskeletal and cellular elements may interact during cellularization).

Myosin in gastrulating embryos

We observe an accumulation of myosin at the apical ends of cells within furrows in cell sheets during the gastrulation of the Drosophila embryo. We speculate that myosin is recruited to the apical region of these cells and contracts in a purse-string fashion to initiate cell shape changes that lead to the displacement of the cell sheets. Such changes are similar to those observed during amphibian gastrulation, in which it is thought that apical constriction results in the bending of epithelial sheets (e.g. Hardin and Keller, 1988), and support computer-generated models (e.g. Odell et al. 1981) that demonstrate that an apical constriction can drive the infolding of cell sheets. Treatment of embryos of another Diperan species with microfilament-destabilizing drugs results in a complete arrest of all gastrulation movements (Kaiser and Went, 1987).

Our localization of myosin to the apical ends of cells during early gastrulation is novel. The coexistence of actin at the cell apices in these regions in Drosophila (Callaini, 1989) is consistent with the involvement of an actomyosin network in producing an apical constriction in regions of invagination. Other studies have localized myosin (e.g. Lee et al. 1983) and other cytoskeletal proteins (actin, e.g. Sadler et al. 1982; α-actinin, e.g. Lash et al. 1985) to the apical ends of cells during vertebrate neurulation. Although actin often shows a far more uniform accumulation to the apical ends of cells across the embryo, myosin is fairly restricted to the apical ends of cells in regions undergoing an apical constriction, and subsequent cell sheet infolding. However, the data from these studies do not permit precise correlation of myosin localization with individual cell shape changes in the region of infolding. Our results unambiguously demonstrate that an apical myosin localization is restricted to those cells that are undergoing or have just undergone apical constriction. Indeed, the tight temporal correlation between cell shape change and myosin localization fuels our speculation that non-muscle myosin plays an active role in force production for these movements. We attribute the clarity of our results to our use of high-affinity antibodies against Drosophila cytoplasmic myosin, our fixation conditions, and the powerful optical sectioning capabilities of the confocal microscope.

Origins and regulation of spatial heterogeneity of myosin during cellularization and gastrulation

Changes in myosin localization during cellularization and gastrulation likely reflect reorganization of myosin within the cells participating in a given movement. It is likely that complex regulatory cascades dictate the precise changes in myosin localization and function within regions of invagination (for review of myosin regulation, see Sellers and Adelstein, 1987). For example, the solubility of non-muscle myosin in vitro, and potentially the subcellular localization in vivo, is influenced by the phosphorylation state of myosin regulatory light chain. At least three kinases can phosphorylate the light chains and influence their function. The activity of these kinases is in turn regulated by a variety of soluble factors, including cAMP, free Ca²⁺ concentration and the stage of the cell cycle. Thus, such post-translational regulation of myosin function is likely to be quite complex. Of course, we cannot rule out additional regulation of myosin distribution due to changes in myosin transcription or translation in specific regions of the embryo.

Myosin in earlier embryos

We speculate that the subcortical inclusions that stain with anti-myosin in preblastoderm embryos represent a storage form of myosin that is recruited during myosin cap formation at nuclear cycle 10. In Drosophila and other organisms, similar inclusions have been observed that contain other cytoskeletal proteins (Strome, 1986; Warn, 1986; Weisenberg et al. 1987) and may also represent a storage form of these proteins. An alternate interpretation of these staining patterns is that they represent an association of myosin with vesicular inclusions, as data point towards association of both conventional and mini-myosins to membranes and vesicular inclusions in other systems (Adams and Pollard, 1986; Berrios and Fisher, 1986; Grollig et al. 1988; Adams and Pollard, 1989; Tang et al. 1989).

The cortex of the early Drosophila embryo is enriched in cytoplasmic myosin, as it is for a number of cytoskeletal components including actin and spectrin (Warn et al. 1984; Karr and Alberts, 1986; Pesacreta et al. 1989). However, in contrast to actin, myosin does not localize to discrete foci believed to be surface microvilli, a distribution consistent with the absence of conventional, non-muscle myosins from microvilli in other systems (reviewed by Mooseker, 1985).

We speculate that the incorporation of myosin into the cytoskeletal caps associated with each somatic nucleus of the syncytial blastoderm represents recruitment of myosin already present in the egg cortex, periplasm and, potentially, the subcortical arrays of
inclusions. The steady state levels of myosin are constant at this time, and, because little or no myosin heavy chain message is present before 4–12 h of development, we think it is unlikely that new protein synthesis contributes to the pattern of localization. Such recruitment parallels the redistribution of actin and spectrin. Signals that presumably originate in the centrosomes (Rappaport, 1986; Raff and Glover, 1989) appear to play a key role in organizing the actin network, but at this time remain a mystery.

The localization of myosin in the cortex of the Drosophila embryo prior to the onset of cellularization contrasts the lack of myosin in the cortex of other interphase cells where myosin is apparently recruited just seconds before its function in cytokinesis (e.g. Fujiwara and Pollard, 1976; Mittal et al., 1987; Schroeder, 1987; Schroeder and Otto, 1988). Perhaps myosin plays additional roles in maintaining cortical structure and organization in the Drosophila embryo that it does not play in other species. For example, this cortical myosin may contribute to the establishment and maintenance of nuclear positioning at the embryonic surface. Experiments designed to disrupt myosin function or to disrupt microfilaments in Drosophila embryos during syncytial blastoderm (by the microinjection of antibodies or microfilament-destabilizing drugs) result in the loss of nuclear positioning at the embryo surface (Zalokar and Erk, 1976; Foe and Alberts, 1983; Edgar et al., 1987; Lutz and Kiehart, 1987 and unpublished data).

**Myosin at later stages of development**

This paper does not address in detail the distribution of non-muscle myosin in embryos following early gastrulation. However, if myosin indeed plays an active role in the cell shape changes that drive gastrulation, we believe that later stages of development will be characterized by specific myosin distributions associated with other cell sheet rearrangements. For example, the strong localization of non-muscle myosin to the nervous system, and similar localizations of myosin and actin in the growth cones of neurons of various organisms in primary culture (Kuczynska and Rosenberg, 1979; Bridgman and Dailey, 1989; Forscher and Smith, 1988), are consistent with a role for an actomyosin contractile network in neurite growth and extension.

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