Three Distinct Distributions of F-Actin Occur during the Divisions of Polar Surface Caps to Produce Pole Cells in *Drosophila* Embryos

R. M. WARN, L. SMITH and A. WARN
School of Biology, University of East Anglia, Norwich NR4 7TT, United Kingdom

**ABSTRACT** The F-actin distribution was studied during pole cell formation in *Drosophila* embryos using the phalloidin derivative rhodaminyl-lysine-phallotoxin. Nuclei were also stained with 4′-6 diamidine-2-phenylindole dihydrochloride to correlate the pattern seen with the nuclear cycle. The precursors of the pole cells, the polar surface caps, were found to have an F-actin-rich cortex distinct from that of the rest of the embryo surface and an interior cytoplasm that was less intensely stained but brighter than the cytoplasm deeper in the embryo. They were found to divide once without forming true cells and then a second time when cells formed as a result of a meridional and a basal cleavage. Three distinct distributions of the cortical F-actin have been identified during these cleavages. It is concluded that the first division, which cleaves the polar caps but does not separate them from the embryo, involves very different processes from those that lead to the formation of the pole cells. A contractile-ring type of F-actin organization may not be present during the first cleavage but is suggested to occur during the second.

The mechanism of *Drosophila* pole cell formation is of interest to both cell and developmental biologists because the pole plasm, from which the cells are formed, is a very well-documented case of a region of an egg that contains morphogenetic determinants. These determinants have important controlling influences over the spatial organization of the cells formed and quite possibly over the determination of their fate (for reviews see references 4, 12, 19, and 23). Pole plasm forms as an area of cortical cytoplasm at the posterior tip of the growing oocyte. It is characterized by the presence of polar granules, small basophilic bodies 0.5–1.0 μm diam, of unknown significance but present in the germ cell lines of many groups. The pole cells, which are derived from this region only, have a roughly spherical shape quite distinct from the columnar morphology of the somatic blastoderm cells that make up the rest of the embryo. Once formed, they migrate to the gonads where they give rise to the germ cells.

As a first step towards understanding the controlling influences of pole plasm, it is necessary to understand how pole cells are formed and how this process is distinct from the formation of the blastoderm cells and the somatic surface caps that are their progenitors. The caps are unusual structures. They are not true cells but surface protuberances each overlying a nucleus (6, 24). During the syncytial blastoderm stage, they undergo four cycles of expansion and flattening followed by division and a rounding up of the daughter caps. Cell membranes then extend simultaneously between all the caps to form the single layered cellular blastoderm.

Fluorescent derivatives of the cyclic peptide phalloidin, derived from the mushroom *Amanita phalloides*, have proved to be specific stains for F-actin microfilaments in vitro (22, 28). By using this technique, it is possible to visualize the F-actin distribution associated with the surface caps during the syncytial and cellular blastoderm stages (25, 26). In this paper we describe the distribution of F-actin during the formation of the progenitors of the pole cells, the polar surface caps, their divisions, and pole cell formation.

**MATERIALS AND METHODS**

The techniques for embryo preparation, staining, mounting, and epifluorescence microscopy have been previously described (25, 26). To obtain embryos with precisely staged pole cells, we observed them until the correct stage was reached and then immediately pierced them with a microneedle. Staining of F-actin was done with rhodaminyl-lysine-phallotoxin (RLP)1 a chemically syn-

1 Abbreviations used in this paper: DAPI, 4′-6 diamidine-2-phenylindole dihydrochloride; RLP, rhodaminyl-lysine-phallotoxin; st, stage.
thesized derivative of phalloidin (27), at a concentration of 1 μg/ml in PBS for 20 min at 25°C. After being washed in PBS, the nuclei were stained with 0.5 μg/ml DAPI (4′-6 diamidino-2-phenylindole dihydrochloride) and mounted as described previously. 5% (wt/vol) n-propyl gallate was added to the mounting medium to reduce photobleaching. After photographing the surface caps on the sides of the embryos, we cut off the posterior pole region with fine surgical needles. This part was then stuck on a polylysine-coated coverslip, pole upwards. The coverslips were prepared by immersion in a 1 mg/ml aqueous solution of poly-L-lysine (molecular weight range, 150-300,000; Sigma Chemical Co., St. Louis, MO) for 30 min and then air drying. Over 400 embryos were examined during the course of this study. Photography was done with Ilford XPi film (Ilford Ltd., Ilford, Essex, England).

To check for nonspecific staining, embryos were incubated with a large excess of unlabeled phalloidin (5 mg/ml) before staining them with RLP. As seen in Fig. 9a there was no specific staining of the posterior pole or elsewhere. Only a slight increase in background fluorescence was noted. Controls, which demonstrated the blocking of phalloidin staining by F-actin but not G-actin, using both mammalian and insect actin, have been previously described (25).

RESULTS

During stage 9 (29) small, highly folded protrusions were found in the posterior pole region above each nucleus (Figs. 1, a-c, and 9b). Associated with these forming caps were numerous F-actin-rich microprojections and the whole surface around the caps was highly convoluted. While the caps were forming, the nuclei were in interphase. The caps were found to grow rapidly in size, forming irregular oval domes with a cortical F-actin layer in close association with the plasmalemma (Fig. 9c). The average number of polar caps formed (sample of 16 embryos) was approximately seven (range, three to nine).

Figs. 2, a–c, and 9d show polar caps in an early stage of the process of division. As caps entered division they were found to flatten to shallow protrusions with a slight depression in the center. At both ends the cortical layer was thrown up into many small projections that stained strongly with RLP. The region between did not stain as strongly but some microprojections were present. The cap interior stained quite positively for F-actin but less so than the cortex. In the interior a central region, comparatively depleted in F-actin, became progressively more apparent as cleavage proceeded. This region extended towards the sides of the caps and also up towards the top surface. The nuclei were in metaphase or sometimes anaphase. When in metaphase the nuclei were located in the center of the F-actin depleted region. During anaphase they moved towards the ends. Daughter caps were then found to bulge out from both ends as shallow domes each demarcated by a highly folded cortical actin layer (Figs. 3, a–c, and 9,e and f). As the new caps pushed out, a region of plasmalemma not included in the caps formed a break region between them. Beneath, the F-actin of the cap interiors

![Figures 1-4](image-url)
rounded up under the newly demarcated cortices. The region of cleavage showed only weaker, more diffuse staining. As the final stages of cleavage were reached, there was also some loss of RLP staining within the cytoplasm of the cap interiors. This can be seen by comparing Figs. 2 b and 3 b. This reduction in staining was however significantly less than that between forming daughter caps. Quite conspicuous microprojections were present between the polar surface caps, their cores being rich in F-actin. The nuclei were in anaphase or telophase.

A second round of polar surface cap swelling then occurred. Fig. 9g shows an early stage in the process and Fig. 9h shows a later step when the caps were quite rounded and had reached their maximum height. Fig. 4, a–c are of caps approximately the same size as those in Fig. 9h. Two distinct domains were evident as in the previous cap generation. Underlying the plasmalemma was the brightly staining F-actin cortical layer. As before, this had an irregular texture and followed a surface thrown up into small microprojections. Beneath the cortex the cytoplasm within the caps also stained positively with RLP. At the level where the polar caps joined the embryo surface, irregular darker ovals were present surrounded by fluorescent “haloes,” which represented the junctions of the cap membrane with that of the embryo surface proper. The areas of fluorescence included the basal region of the caps and the embryo plasmalemma in this region. The darker ovals marked the less strongly staining egg cytoplasm that lay below. Nuclei were in interphase from soon after the start of the

**Figures 5–8** (Fig. 5, a–d) St 10 polar caps showing flattening. (a) Top surface; (b) field of focus at center of caps to show interior cytoplasm; (c) field close to cap bases; (d) nuclei in prophase. Levels of focus the same in Figs. 6–8. (Fig. 6, a–d) St 10 polar caps immediately prior to cleavage. Nuclei in prophase. (Fig. 7, a–d) St 10 polar caps showing meridional cleavage. Nuclei in late telophase or interphase. (Fig. 8, a–d) St 11 polar caps showing cleavage at base. Nuclei in interphase. x 400.
bulging process. Counts of the number of polar surface caps present at stage 10 gave an average of 12 (range 8–16, 16 embryos examined). This was a slight underestimate as on occasion some caps were lateral to the posterior pole and could not be counted. During the second round of polar cap swelling, the somatic caps were found to appear over the rest of the surface of the embryo. This can be noted by comparing the sides of the embryos in Fig 9, b and g. In Fig. 9 b the lateral surfaces were smooth, whereas in Fig. 9 g the somatic caps were present.

Once the polar caps were fully formed they were found to flatten somewhat while enlarging further laterally (Figs. 5, a–d, and 9 f). The caps came almost to touch each other and most of the surface in the posterior pole region became occupied by caps. The nuclei were in prophase or metaphase. A change then occurred in the F-actin distribution (Figs. 6, a–d, and 9 j). The cortical F-actin showed somewhat increased folding at both ends. F-actin was also found to be somewhat depleted relative to the ends in the central interior cytoplasm. Distinct oval fluorescent halos were still present at the bases of the caps. By this point in the cycle the nuclei were in metaphase or anaphase.

![Figure 9](image_url)
A hoop of RLP staining then became apparent midway across the surface of the polar caps and was associated with meridional cleavage (Figs. 7 a-d and 9 k). Fig. 7 a shows the top cortical surface of such a cap in division. The cortical F-actin layer extended across the surface to the center where it was pulled inwards. As can be seen, the cap surfaces rounded up during cleavage. Fig. 7 b was at a lower level of focus at the plane of the furrow base. A distinct band of RLP fluorescence was present in the region of the central furrow. Fluorescence of a similar intensity was also associated with the cortical F-actin all around the forming cells. In Fig. 7 c, a lower level of focus again is shown, that immediately below the furrow and at the level of the embryo surface. The junction between the cap and embryo was visible as a dark oval surrounded by a broad band of fluorescence. The furrow was close to the junction and about to bisect it at the end of the downward movement. It can be seen as an indistinct band across the darker oval at the base. The nuclei were in telophase or interphase.

The arrowed cell in Fig. 8 a-d shows the last stage of pole cell formation, the pinching off of the cell at its base from the embryo surface. In Fig. 8 c, a small dark circle was present at the base of this cell, visible against a bright background. The background fluorescence came from the cortical F-actin of the embryo and from the adjacent surface of the newly formed pole cells. Only under this cell was the dark cytoplasm of the embryo interior still visible. Fig. 8 a-c also illustrates the brightly staining layer of cortical F-actin associated with the newly formed cells. Fig. 9 l shows a stage 11 embryo where the pole cells had fully formed. Some of them were detached from the embryo surface and made contact only with other pole cells. Counts of the number of newly formed cells gave an average of 24 (range 18–30 from 10 embryos).

DISCUSSION
The finding of Foe and Alberts (6) that the polar surface caps initially protrude from the surface at stage 9, one nuclear cycle ahead of those of the rest of the embryo, is confirmed here. This had not been clearly described before, although it had been noted that caps formed first at the posterior pole (9). We also confirm that the polar surface caps go through two division cycles before being cleaved off from the embryo. This kind of mechanism has already been used to explain the partial cleavage of the Loligo (1, 2), Stomatoca (16), and Aequorea (21) eggs.

A problem with such a hypothesis is that the hoops of RLP staining are not significantly brighter or otherwise different from other regions of the cap cortex at this stage. But the cortex of forming and newly formed pole cells is rich in F-actin.

The very unusual modes of cleavage of the polar caps during cycle 9 and the somatic caps during cycles 10–13 (cf. reference 26) are rather similar. In both cases the caps swell out and then flatten. During the nuclear cycle, F-actin builds up on either side of a central region comparatively depleted in F-actin. The nucleus is present in the center of this region until mitosis. Cleavage then occurs as the result of a bulging out of daughter caps from a shallow protrusion coupled with the appearance of a small region of unincluded plasmalemma in the central break region. Under this region of unincluded plasmalemma, the RLP staining becomes progressively reduced and comes to be identical to that of the rest of the embryo surface not associated with caps. The F-actin organization associated with each daughter progressively rounds up as the new caps form. This pattern of F-actin distribution would seem to be very different from that which occurs in regular cell cleavage during which a ring of microfilaments underlies the cleavage furrow and constriction of the ring divides the cell in two (for reviews see references 3, 17, and 18).

At the tenth cleavage the formation of the pole cells is marked by a pattern of F-actin distribution unique to the polar caps. Fig. 10 is a schematic diagram illustrating the quite different organization of the polar caps during cleavage cycles 9 and 10. Towards the end of cycle 10, distinct hoops of RLP staining appear in the center of the polar cap surfaces and move down as the caps are cleaved. The pairs of forming pole cells are quite close together, separated only by the cleavage furrow region. This is in distinct contrast to the previous division where the daughter caps are comparatively far apart and bubble out from the ends of the old cap. As a working hypothesis, we propose that the RLP staining hoops of cycle 10 contain contractile microfilaments that shorten, thereby extending the cell membranes and causing cleavage. This kind of mechanism has already been used to explain the partial cleavage of the Loligo (1, 2), Stomatoca (16), and Aequorea (21) eggs.
actin and it may not be possible to distinguish contractile F-actin filaments in the cleavage region from those elsewhere simply on the basis of RLP fluorescence. Thus, some additional means will be required to unambiguously identify actively contracting microfilaments, e.g., myosin distribution (7). Although the mode of polar cap cleavage during cycles 9 and 10 are clearly very different, prior to this the caps in both cycles go through similar phases of swelling and subsequent flattening.

The second phase of pole cell formation occurs at right angles to the first and involves the cleavage of the cells at their bases from the embryo surface. We hypothesize that a contractile ring acts at the junction between the pole cells and the embryo surface. In this case it would form as a circle and progressively constrict the pole cell base until the cell was cleaved from the embryo. A similar mode of function has been postulated for polar lobe formation in fertilized eggs of Ilyanassa obsoleta. Stimpson. Am. Zool. 13:961-980.

We thank Professor Th. Wieland for generous gifts of rhodaminyl-l-seryl phallotoxin, J. Lamb for discussions and comments on the paper, M. Symmons for drawing diagram 10, and S. Ward for putting the paper on disk.

Received for publication 18 June 1984, and in revised form 18 October 1984.

REFERENCES

1. Arnold, J. M. 1969. Cleavage furrow formation in a teolecithal egg (Ilyanassa obsoleta). I. Filaments in early furrow formation. J. Cell Biol. 41:904-904.

2. Arnold, J. M. 1971. Cleavage furrow formation in a teolecithal egg (Ilyanassa obsoleta). II. Direct evidence for a contraction of the cleavage furrow base. J. Exp. Zool. 176:73-86.

3. Aushin, E. J. 1981. Immunofluorescence studies of cytoskeletal proteins during cell division. In Mitosis/Cytokinesis. A. M. Zimmerman and A. Forer, editors. Academic Press, Inc., New York. 231-248.

4. Bownes M. 1982. Embryogenesis. In A Handbook of Drosophila Development. R. Ransom, editor. Elsevier/North Holland Biomedical Press, Amsterdam. 67-94.

5. Conrad, G. W. 1973. Control of polar lobe formation in fertilized eggs of Ilyanassa obsoleta. Stimpson. Am. Zool. 13:961-980.

6. Fox, V. E., and B. M. Alberts. 1983. Studies of nuclear and cytoplasmic behaviour during the five mitotic cycles that precede gastrulation in Drosophila embryos. J. Cell Sci. 61:51-70.

7. Fujisawa, K., and T. D. Pollard. 1976. Fusophism antibody localization of myosin in the cytoplasm, cleavage furrow and mitotic spindle of human cells. J. Cell Biol. 71:847-875.

8. Gishol, H., and J. W. Sedat. 1982. Fluorescence microscopy; reduced photobleaching of rhodamine and fluorescein protein conjugates by n-propyl gallate. Science (Wash. DC). 217:125-1255.

9. Hauther, A. F. 1923. The origin of the germ cells in Drosophila melanogaster. J. Morphol. 37:385-423.

10. Ilmensee, K., A. P. Mahowald, and M. R. Loomis. 1976. The ontogeny of germ plasm during oogenesis in Drosophila. Dev. Biol. 49:40-65.

11. Imazumi, T. 1954. Recherches sur l'expression des facteurs liés heréditaire chez l'embryon de la drosophile. I. La variation du volume de l'embryon pendant la première période du developpent. Protelasma 44:1-10.

12. Mahowald, A. P. 1977. The germ plasm of Drosophila: an experimental system for the analysis of determination. Ann. Zool. 17:351-363.

13. Nakada, M. 1982. Loss of the ability to form pole cells in Drosophila embryos with artificially delayed nuclear arrival at the posterior pole. In Embryonic Development, Part A: Genetic Aspects. Alan R. Liss, Inc., New York. 363-372.

14. Rahonowicz, M. 1941. Studies on the cortical and early embryology of the egg of Drosophila melanogaster. J. Morphol. 89:1-49.

15. Ricukott, W. L. 1976. Cytosplastis continuity between embryonic cells and primitive cells during early gastrulation in Drosophila melanogaster. Dev. Biol. 49:304-410.

16. Schroeder, T. E. 1968. Cytoskeletal filament distribution in the cleavage furrow. Exp. Cell Res. 58:272-276.

17. Schroeder, T. E. 1975. Dynamics of the contractile ring. In Molecules and Cell Movement. S. Inoue and R. E. Stephens, editors. Raven Press, New York. 305-314.

18. Schroeder, T. E. 1982. Interrelations between the cell surface and the cytoskeleton in cleaving sea urchin eggs. Cell 29:170-176.

19. Sonnenblick, B. P. 1930. The early embryology of Drosophila melanogaster. In Biology of Drosophila. M. Demesec, editor. John Wiley and Sons, Inc., New York. 62-167.

20. Swaasam, M. M., and C. A. Poody. 1980. Pole cell formation in Drosophila melanogaster. Dev. Biol. 75:419-430.

21. Takeda, D. 1970. Cortical cytosplastic filaments of cleaving eggs: a structural element corresponding to the contractile ring. J. Cell Biol. 44:192-209.

22. Verderame, M., D. Alcorta, M. Egnor, K. Smith, and R. Pollack. 1980. Cytoskeletal F-actin patterns quantitated with fluorescein isothiocyanate-phalloidin in normal and transformed cells. Proc. Natl. Acad. Sci. USA 77:6624-6628.

23. Wanz, R. M. 1979. The polen plasm of Drosophila. In Maternal Effects in Development. D. R. Newby and M. Balls, editors. Cambridge University Press, Cambridge. 199-219.

24. Warn, R. M., and R. Magrath. 1983. Observations by a novel method of surface changes during the syncytial blastoderm stage of the Drosophila embryo. Dev. Biol. 89:540-548.

25. 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.

26. Warn, R. M., R. Magrath, and S. Webb. 1984. Distribution of F-actin during cleavage of the Drosophila syncytial blastoderm. J. Cell Biol. 98:158-162.

27. Wieland, T., Miura, T., and A. Seeliger. 1983. Analogs of phalloidin. In Mitosis/Cytokinesis. A. M. Zimmerman and A. Forer, editors. Academic Press, Inc., New York. 231-248.

28. Wulf, E., A. Deboren, F. A. Bautz, H. Faulstich, and T. Wieland. 1979. Fluorescent dyes for filament visualization. Exp. Cell Res. 98:158-162.

29. Zaks, M., and J. Erk. 1976. Division and migration of nuclei during early embryogenesis of Drosophila melanogaster. J. Microsc. Biol. Cell. 25:97-106.