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Actin in the *Drosophila* Embryo: Is There a Relationship to Developmental Cue Localization?

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Summary

Recent genetic manipulations have revealed that the cytoplasm of the early *Drosophila* embryo contains localized information that specifies the future embryonic axes. It is the restricted distribution or activity of particular gene products, either messenger RNA or protein, that is crucial for this specification. While some of the genes responsible for this information have been sequenced and the nature and distribution of their products examined, it is not known how this localization is established or maintained. The actin-based cytoskeleton is a likely candidate for the formation, of a cytomatrix that would allow such distributions and yet no direct evidence has yet been found that implicates actin in positional cue localization. In this review I summarize what is known about actin filament behavior in *Drosophila* embryos and compare it to the distribution of positional cues. My purpose is to juxtapose these two bodies of information such that the relationship between them may be revealed.

Introduction

In the last five years, Christiane Nusslein-Volhard and co-workers have shown by means of a genetic approach some of the genes that regulate the rudimentary body-plan that is already laid out in the *Drosophila* egg and early embryo. The localization of these gene products or their activity determines the coordinates of the embryo by means of three independent systems – one specifying the anterior–posterior axis, one specifying the dorsal–ventral axis, and one specifying the ends⁽¹⁾. The way in which these coordinates are specified is different for each, but for each coordinate, it seems to be the asymmetric distribution of the active product of at least one gene which is decisive for the establishment of that coordinate. One of these mechanisms involves the localization of a maternal mRNA, while the other two depend on a series of protein interactions, including an hypothesized localized activation of transmembrane signalling, that result in the restricted distribution or localized behavior of the morphogen.

What are the molecules that structure the egg cytoplasm, and how might they establish or maintain the lay-out of embryonic coordinates? The proteins most likely to provide a superstructure within the egg cytoplasm are the highly conserved and well-characterized cytoskeletal proteins: actin, tubulin, and intermediate filament proteins. In this essay, I will focus on actin. Although a recent review has touched upon this topic⁽²⁾, it will be covered in this review in more detail with emphasis on different aspects of the issue.

There is strong evidence that actin is involved in the redistribution and localization of positional information in other organisms. For example, in *C. elegans*, P-granules, which serve as a marker for germ cell determinants, are initially uniformly distributed in the egg, but segregate into the posterior end of the embryo after fertilization, during the formation of the 'pseudo-cleavage furrow' that accompanies the second meiotic division⁽³⁾. P-granule segregation is dependent on the integrity of actin filaments during this critical time, although by phalloidin staining, the actin filaments themselves are located in the opposite (anterior) half of the egg. Hence, it is possible that segregation is the result of a contraction in the anterior region that squeezes the granules posteriorly, or that the filaments necessary for segregation are a minor, less easily detected, structure.

A variation of this scenario occurs in Ascidian embryos during the first division, when a constriction passes down from the animal pole to the vegetal pole. Instead of squeezing the markers for determinants in the opposite direction as in *C. elegans*, the constriction in Ascidians carries with it the myoplasm, which contains determinants for posterior structures. This constriction is again dependent on intact actin filaments⁽⁴⁾, which can be seen by phalloidin staining or S1-decoration to be present in a dense network beneath the plasma membrane of the vegetal pole (see ref. 5 and references therein). Calcium waves that are stimulated by sperm-egg fusion coincide with this contraction both in time and space⁽⁶⁾. Similarly in zygotes of the brown algae, *Fucus pelvetia*, a steady inward calcium current develops at the future rhizoid, and appears to be associated with the fixation of the embryonic axis which occurs in response to light⁽⁷⁾. Both the fixation of the axis and the evolution of the calcium current are inhibited by cytochalasins. In addition, by phalloidin-staining, filamentous actin is also found more concentrated in the cortical region of the rhizoid pole. These observations have led to the idea that there is a relationship between actin filaments and calcium channels, and that these two act in concert upon cytoplasmic determinants to specify the future differentiation of the rootlet^(7,8).

The hypothesis has been suggested in a number of recent publications that in *Drosophila* embryos a similar association exists between the actin cytoskeleton and positional information^(9,10), but so far direct evidence for this is scant. One of the purposes of this essay is to juxtapose what is known about actin filament distribution and behavior in fly embryos against what is known about the localization of positional cues to see if there is any validity to such an hypothesis. In addition, information regarding the mechanisms of localization of subcellular constituents in non-embryonic cells will be used to generate hypothetical scenarios for such mechanisms in embryos. At this time there is a difficulty in distinguishing between those putative cytoskeletal elements that directly effect positional information and those that have more global cellular actions, which effect development indirectly. Obviously, the first step in establishing criteria for distinguishing between direct and indirect effectors is the acquisition of a detailed picture of the behavior of the cytoskeleton in wild-type normal embryos. Certainly, those elements that, when disrupted, change the normal cytoskeletal behavior might be considered indirect, while those cytoskeletally-associated proteins that disturb developmental fields but not the cytoskeleton are likely candidates for having a more intimate role in localizing positional information. Since each of the coordinates is specified by a different mechanism, one might suspect that

actin filaments would not be intimately required for all of them, but rather that they are instrumental in only some specific steps, which result in their establishment or enactment.

Despite the present lack of direct evidence, there are still reasonable grounds for pursuing the idea that actin filaments are involved in the localization of positional information. My hope is that the juxtaposition presented in this essay might serve as an inspiration for the design of experiments to test for direct interactions between the cytoskeleton and positional information in *Drosophila*. Certainly *Drosophila*, with its strong genetics, would be a wonderful system in which to pursue this topic.

The Organization of Actin in the Early Embryo

In *Drosophila*, a loose network of actin filaments is found in the cortex of eggs and early embryos, concentrated within 10 microns of the surface membrane⁽¹¹⁾. These filaments are uniformly distributed in the cortex during the first eight nuclear divisions, which occur deep in the yolk mass. After this division, the nuclei begin to migrate to the embryonic surface, where they form an evenly-spaced syncytial monolayer by the tenth nuclear division. Nuclei may be able to migrate to the surface in the absence of actin filaments, but regularity in the distribution of nuclei, both in the distance from the embryonic surface and the distance between nuclei, is not maintained without actin⁽¹²⁾, suggesting a structural role for actin filaments in the maintenance of nuclear position.

There are six actin genes in *Drosophila*⁽¹³⁾, at least one of which encodes a cytoplasmic actin. Different length transcripts of this gene are produced by differential splicing in a developmentally regulated pattern⁽¹⁴⁾. Thus far a pattern has not been described either for oogenesis nor prior to gastrulation; hence the individual contributions of different isoforms to the events occurring during these early developmental stages has not been explored.

When the nuclei reach the cortical cytoplasm, the surface of the embryo reorganizes such that clusters of irregular microvilli appear over each nucleus during interphase, and disappear during metaphase⁽¹⁵⁾. Actin filaments reorganize during the nuclear cycle in concert with the surface shape changes, alternatively being concentrated in caps over each interphase nucleus, and congregating in a zone that surrounds and demarcates each spindle at metaphase during cycles 11 to 13^(11,16–18). This band has been referred to as a ‘pseudocleavage furrow’, because it does not form at the metaphase plate nor separate the daughter nuclei but appears instead to separate the spindle poles laterally. Thus, if one wished to profit from *Drosophila* genetics for a dissection of the genes necessary for cleavage furrow formation, it would be more appropriate to focus on the true cleavage furrows occurring in cycle 14, which result in cellularization rather on these pseudo-furrows of early nuclear divisions⁽¹⁹⁾.

It is not clear whether the behavior of actin during the last four nuclear divisions before cellularization (nuclear cycles 10–14) contributes to the segregation of maternal positional information, although they appear to affect the localization of zygotic transcripts⁽¹²⁾. The formation of these pseudocleavage furrows also appears to be necessary for the nuclei to remain intact and diploid, and this is essential for the next steps of development. This requirement is probably more likely to be an indirect one⁽²⁰⁾. In *daughterless* *abo-like*

mutant embryos, cleavage furrows do not form, and microtubules from centrosomes associated with one nucleus will invade the spindle of a neighboring nucleus, creating mono-polar and tri-polar metaphase spindles and defects in chromosome segregation, such that chromosomes from different nuclei are distributed to the same centrosome.

Contribution of the Centrosome to the Organization of Cortical Actin

The centrosome alone can migrate to the cortex without its attendant nucleus and once there induce the formation of the pole cells and the reorganization of the actin network that normally accompanies nuclear migration^(11,21–23). In embryos treated with aphidicholine, which blocks DNA replication, nuclear migration to the cortex does not occur. However, centrosomes appear evenly distributed in the cortex at the proper time, actin caps form over these independent centrosomes, and the posterior pole pinches off into a-nucleate blebs resembling pole cells.

How the centrosome might mediate this reorganization is not known, but by drawing some analogies to other systems, we can generate an hypothesis: perhaps the centrosome, through its nucleation of microtubules, directs the distribution of membrane-associated actin-binding proteins to the plasma membrane *via* microtubule-based organelle transport, and these membrane components in turn influence the actin distribution.

Some evidence can be cited to support this hypothesis: the actin-binding proteins spectrin and myosin have been found in centrosomes, both bio-chemically and by immunocytochemistry^(24,25). While neither of these proteins has been described as being present in the centrosomal region of *Drosophila* embryos, their distribution correlates with that of actin during the last four cleavages of the syncytial blastoderm^(17,26,27). Furthermore, the centrosome acts as a way-station for the recycling of small vesicles containing integral membrane proteins⁽²⁸⁾, as well as for the transport of membranous organelles from the Golgi apparatus⁽²⁹⁾. Thus, it is conceivable that peripheral membrane proteins, such as spectrin, might piggy-back on such vesicles to arrive at the apical or lateral membrane.

Actin-associated Proteins

The distribution of actin filaments depends upon the activities of a large number of actin-associated proteins which regulate the ratio of polymer to monomer, the site and orientation of filaments, and crosslink or bundle filaments into more complex structures⁽³⁰⁾. In order to understand how the distribution of actin filaments is regulated in the embryo to generate a cytoskeletal superstructure upon which to hang determinants, it is necessary to know what these regulatory proteins are. Actin and many of its associated proteins have been highly conserved during evolution. Thus, some actin-binding proteins in *Drosophila* can be identified by their similarity in biochemical behavior or in sequence to the actin-binding proteins identified in other organisms^(27,31–33). However, those proteins involved in activities unique to the embryo or not yet characterized in other systems must be identified by some other means. One way to do this is biochemical, using immobilized actin filaments as an affinity matrix. By this approach, a large number of actin-associated proteins have been found in *Drosophila* embryos. Immunofluorescence using antibodies to 14 of these proteins demonstrated that they are associated with the actin cap or the cleavage furrow or

both during the nuclear cycle. None of those that stained the submembranous cytoskeleton stained deeper in the embryo. Nor were any distributed in a pattern suggestive of a relationship to known positional cues, or to developmental fields in the pre-gastrulation embryo⁽³⁶⁾. The molecular weights of several of these proteins suggest that they are novel, rather than homologues of known proteins. Some of these novel proteins could be used by many types of cells for cleavage furrow formation, or be unique to the embryo.

A genetic approach to the identification of actin-associated proteins involved in positional cue localization has failed as yet to produce results^(35,36), perhaps because they have overlapping functions⁽³⁷⁾, or because there may be several gene copies, or because they are not unique to the embryo (in which case their mutants would be general lethals).

Actin and the Initial Body-plan

A simple model to explain the localization of bicoid mRNA in the anterior region of the embryo might be that it interacts with actin filaments, such that filaments at the anterior end of the oocyte might trap the bicoid mRNA as it is pumped into the oocyte from the nurse cells. However, thus far, actin filaments have only been observed concentrated in the cortex within 10 microns of the surface, whereas bicoid transcripts are localized at the anterior pole, throughout the full thickness of the egg – both at the cortex and deeper^(1,9). Hence, unless actin filaments are present deeper in the yolk mass, it seems unlikely that they could directly mediate the regionalization of this determinant. The confocal microscope may aid in revealing the nature of such cytoskeletal elements deep in the yolk mass – indeed, actomyosin aggregates have been found and well may have additional functions than storage⁽³⁸⁾.

Mutations besides *bicoid* have been identified that disturb the normal distribution of bicoid message. For example, *exuperantia* and *swallow* mutants have a reduced concentration of bicoid message in the anterior third of the egg, and consequently have abnormal head formation^(39–41). The gene products for these mutations have not yet been fully described, and their relationship to known cytoskeletal proteins is not understood. Unlike mutations that disrupt cleavage or pseudo-cleavage furrows, these mutations are likely to be direct effectors of the developmental plan that may have cytoskeletal associations.

In contrast to *bicoid* localization, there is some circumstantial evidence linking the cytoskeleton to localization of the posterior group gene products. Many of the mutations that affect the establishment of the posterior axis also affect the formation of the cleavage furrows during cellularization, suggesting that these genes have an effect on cytoskeletal functioning (reviewed in ref. 2).

Actin and the Specification of the Dorsal–ventral Axis

A complex set of events involving signal transduction reminiscent of classical induction generates the dorsal–ventral axes. During this process, the *dorsal* (*dl*) gene product redistributes from the cytoplasm to the nucleus in the ventral region^(42–44). This results in the formation of ventral structures, such as mesoderm and neurectoderm, which are lacking

in *dl*⁻ mutants. The pathway that produces this redistribution is partly known and involves extracellular serine proteases⁽⁴⁵⁾ and a trans-membrane protein: the *Toll* gene product⁽⁴⁶⁾.

Since actin filaments are concentrated at the cortex, it seems likely that they might be involved in changes in the cytomatrix involving transmembrane receptors. Consistent with this idea is the fact that the *Toll* gene is homologous in some regions to a transmembrane receptor that is the membrane anchor for the actin-rich membrane-skeleton in human blood platelets, glyco-protein Ib (GPIb)⁽⁴⁶⁾ and to the interleukin I receptor. The cytoplasmic domain of GPIb is attached to actin filaments through actin-binding protein⁽⁴⁷⁾. The extracellular domain has a low affinity binding site for thrombin, a serine protease which does not cleave GPIb. Thrombin stimulates a reorganization of the membrane-associated actin filaments⁽⁴⁸⁾, as well as a dramatic polymerization of actin, producing long filopods. That some such reorganization of actin filaments also occurs in *Drosophila* embryos is suggested by the presence at nuclear cycle 14 of enhanced surface protrusions over the ventral-most regions of the embryo⁽⁴⁹⁾, the site of activity of the Toll protein. How Toll is activated locally is not known, but one hypothesis is that there is a localized concentration of an activating ligand. An interesting corollary to add to these is the fact that TGF- β is activated by the serine protease, plasmin⁽⁵⁰⁾. The receptor for TGF- β is not known, but one of the members of this family of growth factors appear to be responsible for establishing the dorsal axis in *Xenopus* embryos⁽⁵¹⁾, while another, *decapentaplegic* gene product, is active later in the establishment of the dorsal axis in *Drosophila*^(52,53).

Mutations in another of the dorsal group genes, *fs(1) gastrulation defective (gd)*, produce embryos that have no ventral furrow⁽⁵⁴⁾. Unlike mutations in other genes in this pathway, dorsal structures do not replace the absent ventral structures in *gd* mutants. Thus, the *gd* gene may encode the protein responsible for stimulating the cell shape changes upon which ventral furrow formation depends, which must be an actin filament-based process. It might be deduced from this that both transcriptional regulation and cell shape change contribute to ultimate differentiation, since ventral mesoderm is not formed in *gd* mutants, despite evidence suggesting that the *dorsal* gene product is active. Conversely, the cell shape change may be necessary in these cells (though probably not sufficient) for changes in transcriptional activity. A careful study of shape changes occurring in these cells during furrow formation in wild-type and mutant flies has revealed that the simple model of apical contraction producing furrowing is unlikely to be correct⁽⁵⁵⁾.

It is possible that the same stimulus that induces *dorsal* protein to be transported into ventral nuclei could act in a parallel pathway to produce actin reorganizations and cell shape change. If *dorsal* protein were maintained in the cytoplasm⁽⁵⁶⁾ by associations with actin filaments, then reorganizations of these filaments could release it, permitting it to be transported into the nucleus. In support of this speculation is a recent report of the sequence of a *dorsal*-related protein, KBF1, which contains in its carboxy terminus a sequence reminiscent of the repeats in a spectrin-binding protein, ankyrin⁽⁵⁷⁾.

It is possible that actin-dependent events similar to those occurring after fertilization in *C. elegans* and Ascidians also happen in *Drosophila* zygotes and result in the initial activation of the dorsal-ventral axis which is ultimately expressed by the nuclear translocation of the

dorsal gene product. For instance, a localized calcium current could provoke exocytosis of secretory granules containing the serine proteases which then activate the Toll transmembrane protein. This process could be accompanied by a transient asymmetry of actin filaments or a contraction of the cortex which is no longer detected at the time of egg laying. Although *Drosophila* eggs are fertilized internally, it is possible to ‘activate’ them experimentally and perhaps test this hypothesis through direct observation.

Other Ways to Structure the Cytoplasm

Could there be other cytoskeletal proteins, either unique to *Drosophila*, or specific for the egg and early embryo, that create the sort of cytoplasmic matrix that seems necessary for the stabilized location of the various positional cues? While intermediate filaments may be involved in such stabilization in other systems, their presence in *Drosophila* is controversial^(58,59).

Candidates for formation of a novel cytomatrix include the *bicaudal* gene product, which plays a role in localization *nanos* to the posterior pole⁽¹⁰⁾. This gene encodes a protein with homology to the tail of myosin II, which is the region of that protein that is involved in thick filament formation, and does not bind to actin. Another idea, which may seem far fetched at first, is that long RNA polymers might create a very dense but rapidly degradable cytoplasmic matrix, which would attach maternal message by sequence homology and helical annealing (ref. 60 and references therein). Or perhaps there are other proteins not yet identified that create a novel cytoplasmic structure, such as the major sperm protein of nematode sperm⁽⁶¹⁾, which appears to be entirely different from actin, yet plays a very similar function in the motility of these migratory sperm⁽⁶²⁾.

Conclusion

Actin filaments and a large number of actin-binding proteins are associated with the cortical cytoplasm in eggs and early embryos. Actin filaments have not yet been observed deeper in the egg yolk mass of whole mounted embryos where *bicoid* message is localized. However, it is possible that with the power of the confocal microscope, cellular structures relating to *bicoid* message distribution might be identified. Reorganizations of actin filaments are most probably part of signal-transduction pathways that establish the second axis, that of dorsal–ventral polarity, and certainly play a role in its enactment (formation of the ventral furrow). Actin filaments are also necessary for the formation of ‘pseudocleavage’ furrows, which separate adjacent dividing nuclei. The many roles of actin in the *Drosophila* egg and early embryo – both before and during gastrulation – represent an exciting area for future study.

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