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Review

Cytoskeletal mechanisms of ooplasmic segregation in annelid eggs

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Introduction

Ooplasmic segregation (or localization) is a precisely programmed reorganization of egg cytoplasm and occurs in the eggs of many organisms. The most important aspect of this event is the generation of a heterogenous spatial organization of the cytoplasm within a single egg cell. The resulting differential distribution of the cytoplasm leads to qualitative differences in blastomere cytoplasm. It has long been thought that these differences are responsible for the process of cell diversification and embryonic axis formation in early development (Wilson, 1925; Davidson, 1986; Goldstein and Freeman, 1997). The elucidation of the mechanism underlying ooplasmic segregation comprises an important step toward an understanding of how the fates of cells are established during early development.

In the present article, we review recent studies on the role of the cytoskeleton in ooplasmic segregation in annelid eggs. Ooplasmic segregation in annelid eggs represents one of the "classical" examples of cytoplasmic rearrangements and still provides an important system to investigate its underlying mechanisms and developmental significance. It is a process by which clear cytoplasm (i.e., yolk-free or -deficient cytoplasm) segregates from the yolk to form distinct ooplasmic domains. The clear cytoplasm is rich in membranous organelles, and it may also include a variety of maternal regulatory molecules (Holton *et al.*, 1994; Master *et al.*, 1996; Savage and Shankland, 1996). Modes of ooplasmic segregation (e.g., size, shape or final location of ooplasmic domains within an egg) are apparently diverse among the Annelida, but they appear to be conserved within each of three classes (i.e., Polychaeta, Oligochaeta and Hirudinida). Representative species from each of the three classes have been examined for cytoskeletal mechanisms of ooplasmic segregation. This allows us to consider some evolutionary aspects of ooplasmic segregation.

Spatial modes of ooplasmic organization in annelid eggs

In nearly all of the annelid eggs described so far (with some exceptions; see below), two cytoplasmic domains become discernible before the first cleavage: a yolky domain and a non-yolky domain. The yolky domain is filled with yolk granules and lipid droplets; the non-yolky domain is characterized by scarcity of yolk granules and richness of membranous organelles such as mito-

Abbreviations used in this paper: AC, actin cytoskeleton; MF, microfilaments; MTB, microtubules.

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chondria and endoplasmic reticulum. With respect to the distribution of these domains within an egg, four types of ooplasmic organization are seen in annelid eggs.

The first type of ooplasmic organization (which is hereafter referred to as type A) is comprised of animally localized clear cytoplasm and vegetal yolky domain, both of which often appear to be stratified along the animal-vegetal (egg) axis (Fig. 1A). This egg organization is seen in polychaetes such as *Nereis, Platynereis,* and *Diopatra* (Wilson, 1892; Huebner and Anderson, 1976; Dorresteijn, 1990; Dondua *et al.*, 1997). *Chaetopterus* eggs exhibit similar cytoplasmic organization, but the clear cytoplasmic domain is much smaller than that in the other polychaetes cited above (Jeffery and Wilson, 1983; Eckberg and Anderson, 1995).

The second type of ooplasmic organization (type B) is also composed of two stratified ooplasmic domains, but in contrast to type A, the non-yolky domain is localized at the vegetal pole side and the yolky domain at the animal pole side (Fig. 1B). This type has been reported only in the polychaete *Pseudopolydora* (Myohara, 1979, 1980). Eggs of this animal form polar lobes at the first and second cleavages; the vegetal clear cytoplasm is segregated to the polar lobe. The subsequent fate of the clear cytoplasm, however, is not known.

The third type of egg organization (type C) is characterized by simultaneous formation of clear cytoplasm domains at the animal and vegetal poles of the egg (Fig. 1C). Eggs of this type are seen in clitellates (i.e., oligochaetes and hirudinidans) but not in polychaetes (Whitman, 1878; Schleip, 1914; Penners, 1922; Devries, 1973). There have not been reports so far of any oligochaetes or hirudinidans that produce eggs of type A or B.

Additionally, there appears to be some oligochaete and branchiobdellid species that undergo development without forming any domains of clear cytoplasm (Salensky, 1887; Tannreuther, 1915; Penners, 1930). This type of egg organization (type D) is characterized by uniform distribution of yolk granules throughout the egg. Eggs of this type do not show any sign of reorganization of visible ooplasmic constituents at least before first cleavage. Although details of early development of these animals remain to be explored, these eggs will provide a useful comparison (a "natural" experiment) for studying ooplasmic segregation in eggs of other types.

Cytoskeletal mechanisms of ooplasmic segregation

As summarized above, ooplasmic organization of types A and B is seen in polychaetes but not in clitellates. Conversely, type C is found in clitellate eggs but not in polychaete eggs. Experimental analyses of ooplasmic segregation, which leads to the generation of clear cytoplasmic domains, have so far been done on *Nereis, Platynereis* and *Chaetopterus* for polychaetes, *Tubifex* for oligochaetes, and *Helobdella* and *Theromyzon* for hirudinidans. To date, however, no data are available for polychaete eggs that generate ooplasmic organization of type B. In this article, ooplasmic organization of types A and B is referred to as "unipolar" organization, and that of type C as "bipolar" organization.

Polychaeta: Nereis, Platynereis and Chaetopterus

Ooplasmic segregation in *Nereis* and *Platynereis* consists of two steps (Okada, 1988; Dorresteijn, 1990; Dorresteijn and Kluge, 1990; Dondua *et al.*, 1997). The first step begins with breakdown of the germinal vesicle upon fertilization. Nucleoplasm derived from the germinal vesicle intermingles with the surrounding clear cytoplasm, moves up to the animal pole, and forms a yolk-free cytoplasmic domain at the animal pole (Fig. 2A). Meiotic spindle assembly occurs in this domain; there is no significant alteration in distribution of the yolk-free cytoplasm while the egg undergoes polar body formation twice. The second step of ooplasmic segregation is the spread of the animally located clear cytoplasm toward the egg equator (Fig. 2A). This spread begins shortly after the second meiosis and appears to occur along the surface of the animal hemisphere; it is accompanied by rapid migration of yolk granules toward the vegetal pole. Thus, shortly before the first cleavage, two cytoplasmic domains that are stratified along the egg axis are generated: that of clear cytoplasm occupying the animal hemisphere and that of yolk and lipid in the rest of the egg (i.e., the equator and the vegetal hemisphere).

Experiments with cytoskeleton inhibitors suggest that the translocation of clear cytoplasm toward the animal pole during the first step is mediated by microtubules. It is likely that microtubules comprising the meiotic apparatus are responsible for directing the movement of the yolk-free cytoplasm toward the animal pole; it is highly probable that as in *Chaetopterus* eggs, the animal pole cortex of nereid eggs is provided with a specialized property that makes it possible to interact with spindle poles (see Lutz *et al.*, 1988). Aster-mediated segregation of ooplasm has also been reported in cleavage-arrested *Chaetopterus* eggs. In this animal, when fertilized eggs are treated with cytochalasin B, cell divisions are blocked, but relocalization of the yolky endoplasm to the egg's center, which is sensitive to colchicine, takes place "normally"

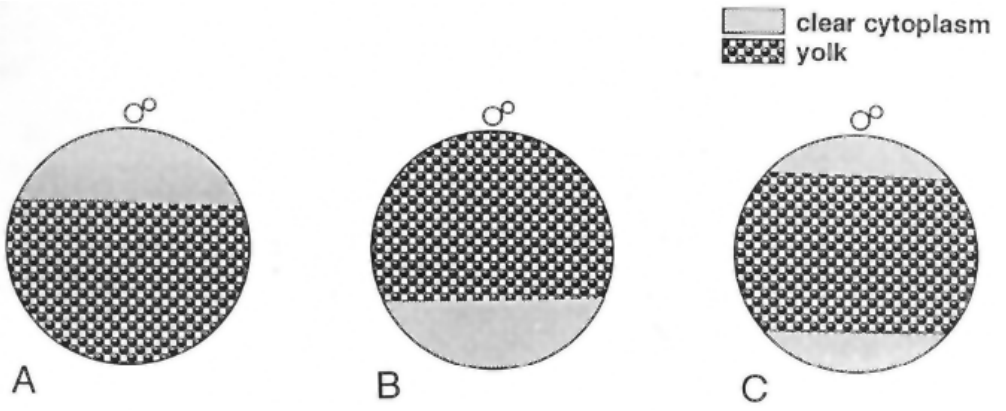

Fig. 1. Three types of ooplasmic organization in annelid eggs before first cleavage. *All eggs are viewed from the side; the animal pole with polar bodies (open circles) is on top.* **(A)** *Type A. Clear cytoplasm is localized at the animal pole.* **(B)** *Type B. Clear cytoplasm is localized at the vegetal pole.* **(C)** *Type C. Two separate pools of clear cytoplasm are formed at the poles of the egg.*

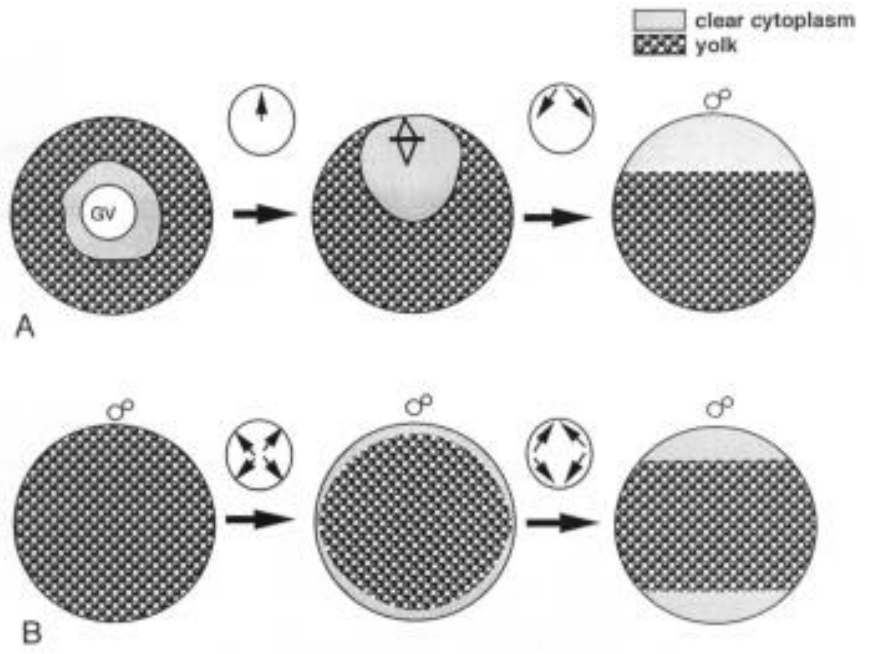

Fig. 2. Diagrammatic summary of ooplasmic segregation in polychaetes (A) and clitellates (B). *All are viewed from the side; the animal pole is on top.* **(A)** *Unipolar ooplasmic segregation. Upon breakdown of the germinal vesicle (GV), nucleoplasm and the surrounding cytoplasm mix to form a clear cytoplasmic domain at the egg's center. As a meiotic apparatus forms in this domain, the clear cytoplasm as a whole moves up to the animal pole and becomes localized there (first step). Following the extrusion of the second polar body, the clear cytoplasm spreads along the surface toward the equator; at the same time, yolk granules also move toward the vegetal pole (second step). As a result of these movements, clear cytoplasm and the yolk are stratified along the egg axis.* **(B)** *Bipolar ooplasmic segregation. Shortly after the second meiosis, clear cytoplasm migrates outward from the inner endoplasmic region and forms a subcortical layer devoid of yolk granules (first step). This subcortical ooplasm then translocates along the surface toward the animal and vegetal poles in the animal and vegetal hemispheres, respectively (second step). Thus, three cytoplasmic domains are generated in the egg: clear cytoplasmic domains at both poles and a yolky domain in between.*

(Eckberg, 1981).

The second step of ooplasmic segregation in the nereid eggs is impaired by cytochalasin-treatments, suggesting the involvement of microfilaments (Dorresteijn and Kluge, 1990; Dondua *et al*., 1997). At present, it is not known whether the microfilament system in these eggs drives the movement of clear cytoplasm, yolk granules, or both. In *Platynereis*, in addition to microfilaments, microtubules may also play a role in the second step (Dorresteijn and Kluge, 1990).

Oligochaeta: Tubifex

Ooplasmic segregation in eggs of the freshwater oligochaete *Tubifex* is a process of accumulation of yolk-free ooplasm called pole plasm to the animal and vegetal poles, and it occurs following the second meiosis (Penners, 1922; Lehmann, 1956; Hess, 1959; Henzen, 1966). This process consists of two steps (Fig. 2B; Shimizu, 1982b). First, mitochondria and endoplasmic reticulum, which are major membranous organelles of the pole plasm, migrate from the inner endoplasmic region outward, and they form a subcortical layer devoid of lipid droplets and yolk granules. The second step is the translocation of this subcortical ooplasm along the surface toward the pole; this poleward movement results in the localization of pole plasms at both poles of the egg (Figs. 3 and 4A).

Both the first and second steps are blocked by cytochalasin treatments, suggesting the involvement of the actin cytoskeleton (AC). In contrast, microtubule inhibitors such as colchicine and nocodazole fail to exert any inhibitory effects on ooplasmic segregation in the *Tubifex* egg (Shimizu, 1982b and unpublished results). The possibility that ooplasmic segregation in the *Tubifex* egg occurs independently of microtubules has also been confirmed in an experiment using another inhibitor, tubulazole C (see Astrow *et al*., 1989).

Tubifex eggs contain two configurations of AC: one is a thin sheet of densely packed actin filaments located at the cortex, and the other is a loose network of actin filaments located in deeper parts (Shimizu, 1984). (These two organizations are hereafter referred to as cortical and endoplasmic AC, respectively.) Several lines of evidence strongly suggest that the first step of ooplasmic segregation in the *Tubifex* egg is caused by the endoplasmic AC, whereas the second step is driven by the cortical AC.

When *Tubifex* eggs are injected with botulinum C3 exoenzyme (ADP-ribosyltransferase), the cortical AC, but not the endoplasmic AC, is selectively disrupted through inactivation of rho proteins (Shimizu, 1996). In such C3-injected eggs, outward migration of ooplasm occurs, although migrating ooplasm is organized into patches rather than a continuous layer as observed in intact eggs. This ooplasmic movement is inhibited by cytochalasin D treatments. These results suggest that the endoplasmic AC is able to move ooplasm centrifugally in the absence of the cortical AC. During the first step, membranous organelles that are undergoing outward migration are organized in aggregates with short actin filaments. These aggregates are often found to be strung up from the surface inward (Shimizu, 1984). It is conceivable that the domain of organelle aggregates contracts, giving rise to their centrifugal movements, because this domain contains actin filaments and anchors to the egg surface. Since the centrifugal migration of organelles occurs even in the absence of the cortical AC, as seen in C3-injected eggs, it seems likely that cytoskeletal elements other than the AC might be responsible for anchorage of organelle aggregates to the egg surface.

At the end of the first step or the beginning of the second step of ooplasmic segregation, three AC domains become discernible: cortical AC, an elaborate network of actin filaments in the subcortical cytoplasm (which is to undergo poleward migration), and the underlying yolky region with actin bundles linking yolk granules (Shimizu, 1984). There is also evidence for actin filaments linked structurally between the cortical AC and the subcortical AC. Several lines of evidence suggest that among these AC domains, the cortical AC plays a key role in driving the underlying cytoplasm (Shimizu, 1986, 1996). First, the

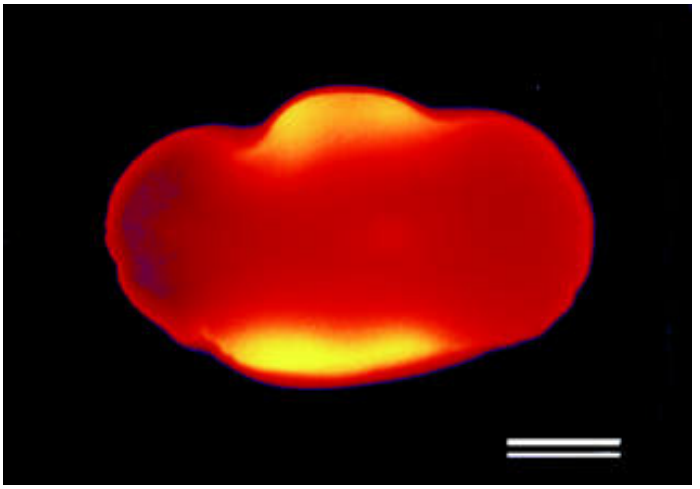

Fig. 3. Localization of F-actin (yellow) in the pole plasms located at both poles of the *Tubifex* egg. *Side view; the animal pole is on top. An egg undergoing the second step of ooplasmic segregation was fixed with formaldehyde, stained with rhodamine phalloidin, and photographed by epifluorescence microscopy. Bar, 100 μm.*

endoplasmic AC alone is unable to cause poleward movements of the subcortical cytoplasm. Second, the egg surface moves together with the underlying cytoplasm in both the animal and vegetal hemispheres of the egg. This suggests that the cortex contracts in the same direction as the ooplasmic movement. Third, cortical actin filaments reorganize and move toward the pole in both hemispheres of the egg. This is unambiguous morphological evidence for poleward contraction of the cortical actin lattice. Fourth, the cortex can contract toward the pole independently of the underlying cytoplasm, which is movable and stratified by centrifugal force (e.g., 1700g). This suggests that the force and directionality of the cortical contraction are derived from the cortex itself and not the inner cytoplasm. Lastly, the subcortical cytoplasm is physically connected to the cortex; this connection is resistant to a centrifugal force of up to 650g.

This strongly suggests that the cortex in the *Tubifex* egg generates not only motive force for movement of subcortical cytoplasm but also determines its direction. Involvement of an actomyosin force-generating mechanism in this process is suggested by the fact that contractile activities of isolated cortices, which are readily induced by addition of ATP, are completely inhibited by their preincubation with N-ethylmaleimide-modified heavy meromyosin (Shimizu, 1985). On the other hand, it appears that directionality of ooplasmic movement is ascribable to the polarized organization of the cortical AC. In both hemispheres of the egg, cortical actin filaments are distributed in a gradient increasing from the equator to the polar region of the egg (Shimizu, 1984, 1986), suggesting that the contraction of the cortical AC is stronger in the polar region than in the equatorial region. The bipolar cortex of the *Tubifex* egg thus forms two focal points for ooplasmic segregation. It should be noted that the bipolar organization of the cortical AC does not originate from oogenesis, but is generated *de novo* during the second meiosis via biochemical pathways involving protein kinase C (Shimizu, 1997).

Hirudinida: Helobdella and Theromyzon

Ooplasmic segregation in leech eggs is very similar to that in oligochaete eggs. It takes place after the second meiosis and results in localization of yolk-free cytoplasm (called pole plasm or teloplasm) at both poles of the egg (Fig. 2B). So far, two glossiphoniid leeches, *Helobdella* and *Theromyzon*, have been subjected to experimental analyses of mechanisms for ooplasmic segregation. Interestingly, these two leeches have been shown to have distinct cytoskeletal mechanisms for ooplasmic segregation (Astrow *et al.*, 1989; Fernandez *et al.*, 1998).

In *Helobdella*, one of the earliest signs of ooplasmic segregation is aggregation of mitochondria located near the egg surface. These mitochondrial aggregates then migrate toward the poles along the surface, giving rise to their localization at both poles (Astrow *et al.*, 1989). At present, it is not known whether these mitochondria originate from the inner cytoplasmic region of the *Helobdella* egg.

Microtubules play an important role in driving yolk-free cytoplasm including mitochondrial aggregates toward the poles of the *Helobdella* egg, as demonstrated by inhibitor studies (Astrow *et al.*, 1989). Treatment with tubulazole C or nocodazole blocks ooplasmic movement or teloplasm formation completely. However, microfilament inhibitors fail to do so; teloplasm formation proceeds normally in cytochalasin-injected eggs.

In support of these results, microtubules that run parallel to the egg surface are present in the egg cortex, and during teloplasm accumulation, microtubule networks become concentrated in the animal and vegetal cortex relative to the equatorial cortex (Astrow *et al.*, 1989). It is conceivable that these cortical microtubules are responsible for teloplasm migration along the surface. Details of the mechanism for the microtubule-mediated ooplasmic translocation, however, remain to be elucidated.

Ooplasmic segregation in *Theromyzon* has been more extensively examined by Fernandez *et al.* (1998). These authors have proposed that ooplasmic segregation (teloplasm formation) in this animal consists of three steps. The first step is the development of a subcortical ooplasmic layer that results from selective outward migration of membranous organelles. This process may correspond to the first step of ooplasmic segregation in the *Tubifex* egg, but unlike the latter, it is a microtubule-dependent process (Fernandez *et al.*, 1998). Microtubules extending from a microtubule-organizing center located at the egg's center may be involved in this process (Fernandez and Olea, 1995). The second step is the redistribution of subcortical mitochondria into latitudinal rings located at both poles and interlinking meridional bands. This process is sensitive to cytochalasin treatments, suggesting the involvement of actin microfilaments. The last step is bipolar translocation of polar rings and meridional bands of mitochondria, which are finally localized at both poles of the egg. Fernandez *et al.* (1998) showed that even in colchicine-injected eggs, teloplasm accumulates at both poles of the egg, though the volume of accumulated teloplasm appears to be smaller than that in control eggs. This suggests that cytoskeletal elements other than microtubules can drive poleward ooplasmic movements during the third step. It is probable that such cytoskeletal elements include microfilaments. Judging from the fact that both the second and third steps of ooplasmic segregation in *Theromyzon* occur along the egg's surface and involve actin cytoskeleton, it seems more reasonable to regard the second step as an earlier part of the third step.

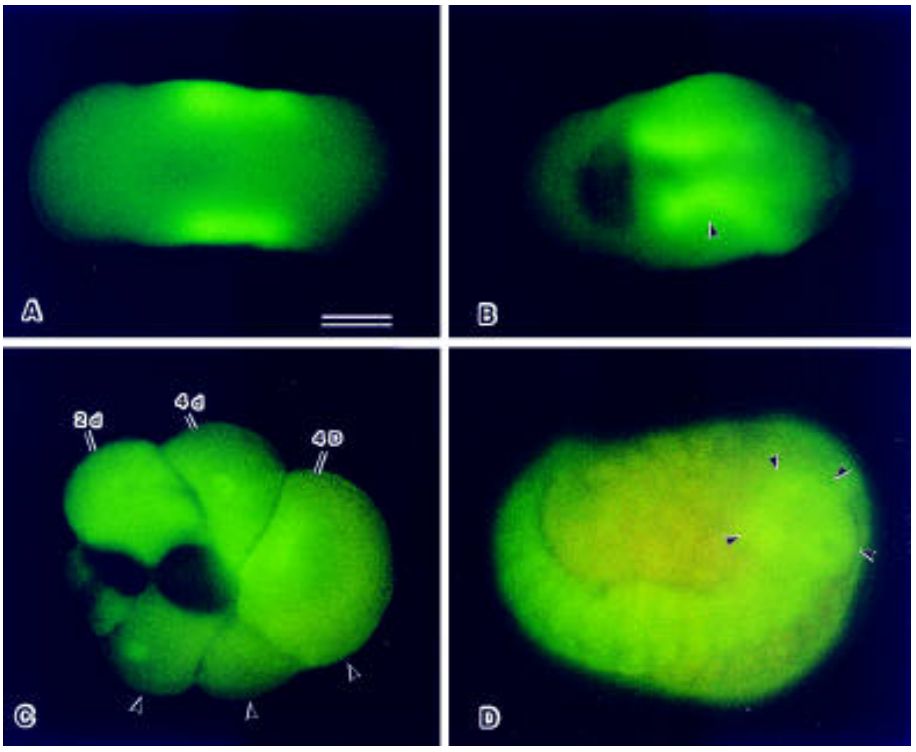

Fig. 4. Fluorescence micrographs showing the distribution of green autofluorescence for mitochondria in *Tubifex* embryos during early cleavages. (A-C) *Sections of embryos bisected along the plane including the animal-vegetal and anteroposterior axes. The animal pole is on top; the anterior end of the embryo is on the left.* **(A)** *One-cell stage. Pole plasms indicated by mitochondrial fluorescence are localized at both poles of the egg.* **(B)** *Four-cell stage. The vegetal pole plasm (arrowhead) is undergoing movement directed toward the animal pole.* **(C)** *Twenty-four-cell stage. The bulk of pole plasms is segregated to the second (2d) and fourth (4d) micromeres of the D cell line, though a trace of pole plasms remains in the 4D-cell. Note that there is no trace of pole plasm at the vegetal side of macromeres (arrowheads).* **(D)** *A living 4-day embryo. Mitochondrial fluorescence is localized to ectodermal teloblasts (which are derived from the cell 2d; arrowheads) and germ bands extending from teloblasts to the anterior end. Bar, 100 μm.*

Fate of ooplasmic domains during early development

It has long been noticed that in many annelid embryos, ooplasmic compositions are different between blastomeres at the animal and vegetal sides. At the sixth cleavage, for example, vegetally located macromeres (A-D) are filled with yolk granules and contain only a trace of clear cytoplasm (Fig. 4C). In contrast, more animally located blastomeres (i.e., micromeres) are filled with clear cytoplasm; even if yolk granules are included in these cells, their amount relative to the blastomere's volume is very low. Apparently, embryos of clitellates as well as polychaetes generate a "unipolar" organization with respect to the distribution of clear cytoplasm along the embryo's animal-vegetal axis.

In polychaetes such as *Platynereis* and *Nereis*, it is expected that localization of clear cytoplasm to animally located blastomeres could be generated by simple cutting-up of the "unipolar" ooplasmic organization established before the first cleavage. Precise morphometric analyses by Dorresteijn (1990) suggest that this is the case for *Platynereis dumerilii*. The animally located clear cytoplasm in *Platynereis* eggs is partitioned by four macromeres, A-D, in rough proportion to their entire volume, during the first two unequal divisions that are meridional. The largest D cell inherits 60% of the egg's total amount of clear cytoplasm. During subsequent divisions, the D quadrant produces micromeres 1d-4d at the animal side. At each of these divisions, clear cytoplasm, which is located at the animal side of the D quadrant, is constricted off and allotted to the emerging micromere. In this way, nearly all of the clear cytoplasm of the D quadrant is segregated into the animally located micromeres (1d-4d); the resulting 4D macromere finally contains only a trace of clear cytoplasm (for developmental fates of these cells, see Wilson, 1892; Okada, 1988). A similar localization pattern of clear cytoplasm has also been demonstrated in embryos of *P. massiliensis* (Schneider *et al.*, 1992).

As in polychaetes, early cleavages in embryos of clitellates are principally a process of cutting-up of the preexisting ooplasmic organization. To achieve segregation of the entire yolk-free cytoplasm to animally located blastomeres, however, an additional mechanism must operate to relocate the vegetal pole plasm (teloplasm) toward the animal side of the embryo. In fact, it has been demonstrated in *Tubifex* and *Helobdella* that this mechanism operates during the third cleavage (Shimizu, 1988; Holton *et al.*, 1989). The vegetal pole plasm (teloplasm) redistributes toward the animal pole in the D cell (Fig. 4B) and is unified with the animal ooplasmic pool. In *Tubifex*, this redistribution is directed to the mitotic apparatus, which is localized at the animal pole, suggesting the involvement of the mitotic apparatus or microtubules. In fact, this redistribution is blocked by microtubule inhibitors (Shimizu, 1988, 1989). Although it is presently uncertain to what extent the two pools of ooplasm are mixed at the animal pole of the D cell, it appears that as in polychaetes, unified pools of ooplasm are cut up by cleavage planes and partitioned to D-cell line micromeres, especially 2d and 4d (Fig. 4C). The pole plasms (teloplasms) are then inherited by teloblasts and their progenies, blast cells (Fig. 4D; for further development of blast cells, see Shimizu, 1982a; Shankland, 1991; Weisblat, 1994).

At present, it is not known whether similar aster-mediated ooplasmic rearrangements occur within polychaete blastomeres. Since the localization of the mitotic apparatus to the animal side during the third cleavage is an event that has been conserved throughout the Annelida and since the translocation of cytoplasmic constituents along astral microtubules is a general feature of animal cells (Rebhun, 1972; Hamaguchi *et al.*, 1986; Kobayakawa,

1988), however, it is thought that a mechanism that potentially mediates polarized ooplasmic rearrangements within a blastomere may have been preserved throughout the Annelida. The ooplasmic redistribution in blastomeres of *Helobdella* and *Tubifex* apparently represents a case where this mechanism generates large-scale ooplasmic rearrangements. In polychaetes such as *Platynereis*, this mechanism presumably operates to "refine" segregation of clear cytoplasm and yolk granules in blastomeres (see Dorresteijn, 1990).

Evolutionary aspects of bipolar ooplasmic segregation

As described earlier, polychaetes and clitellates begin their embryonic development with distinct ooplasmic organizations. Nevertheless, both generate, through early cleavages, homologous embryonic organization that is expressed unipolarly along the egg axis. It appears that evolution has operated to conserve this "unipolar" embryonic organization in spite of diverse initial egg organization. On the other hand, the bipolar segregation that results in the animal and vegetal pools of clear cytoplasm has clearly been preserved in the Clitellata. An innovation leading to bipolar segregation must have occurred in the clitellate (oligochaete) lineage but not in the polychaete lineage. Presumably, development of cellular structures or cues that give the egg bipolarity may have occurred early in the clitellate lineage. These structures or cues could reside in the egg cortex, as seen in modern oligochaete eggs, and direct bipolar organization of the cytoskeleton. The evolutionary divergence in cytoskeletal mechanisms for bipolar ooplasmic segregation may have proceeded through species in which two cytoskeletal mechanisms operated in parallel with some degree of redundancy (Nelson and Weisblat, 1992).

Compared with those of polychaetes, eggs of clitellates are significantly large and heavily yolky (see Table 1). Presumably, the evolution of large, yolky eggs, together with other characteristics such as hermaphroditic reproductive systems and the clitellum, freed the early oligochaetes from the marine environment and gave rise to their successful exploitation of freshwater and land habitats (Brusca and Brusca, 1990). The emergence of bipolar ooplasmic segregation in clitellate annelid eggs may be related to the enlargement of eggs. For large yolky eggs, it is apparent that a bipolar mode of ooplasmic segregation has advantages over a unipolar mode in reducing the time required for ooplasmic localization, which would become longer in proportion to enlargement of egg size, to be completed.

Concluding remarks

Ooplasmic segregation in annelid eggs consists of two successive stages: centrifugal movement of clear cytoplasm toward the egg periphery and its migration along the surface. Three cytoskeletal mechanisms that involve actin cytoskeleton, microtubules and astral microtubules, respectively, operate in these processes. Annelid eggs accomplish ooplasmic rearrangements through various combinations of these mechanisms (Table 1). None of these mechanisms is unique to annelids, but the mechanisms are found in a variety of phyla (Freeman, 1978; Elinson and Houliston, 1990; Sardet *et al*., 1994). If one considers the versatility of the cytoskeleton (Amos and Amos, 1991), it is not surprising that the same kind of cytoskeletal elements is involved in diverse processes of ooplasmic rearrangements. On the other hand, annelid eggs may present a unique case in which a homologous process is brought about by distinct cytoskeletal elements, as seen in oligochaetes and leeches. Further comparative studies on these animals would provide an insight into the evolution of cytoskeletal mechanisms for ooplasmic localization.

The origin, spatial organization and fate of cytoskeletal elements responsible for ooplasmic segregation are developmentally important issues, but all of them remain to be explored in most annelid species. Furthermore, almost nothing is known about what part of the egg polarizes the cytoskeletal organization and thereby the ooplasmic movement. In this connection, it is of interest to explore this issue in relation to intermediate filaments, which appear to be present, but have not been studied extensively, in annelid eggs (Eckberg and Anderson, 1995).

Summary

Annelid embryos are comprised of yolk-deficient animal and yolk-filled vegetal blastomeres. This "unipolar" organization along the animal-vegetal axis (in terms of ooplasmic distribution) is generated via selective segregation of yolk-free, clear cytoplasm to the animal blastomeres. The pathway that leads to the unipolar organization is different between polychaetes and clitellates (i.e.,

TABLE 1

CYTOSKELETAL ELEMENTS CONTRIBUTING TO OOPLASMIC SEGREGATION IN ANNELID EGGS

	1st step (centrifugal move.)	2nd step (move. along the surface)	Ooplasmic organization	Egg diameter (μm).
Polychaeta				
Platynereis [1]	MTB (astral ?)	Actin MF	Unipolar	200
Nereis [2]	MTB (astral ?)	Actin MF	Unipolar	200
Oligochaeta				
Tubifex [3]	Actin MF	Actin MF	Bipolar	450
Hirudinida				
Helobdella [4]	MTB (?)	MTB	Bipolar	400
Theromyzon [5]	MTB	Actin MF	Bipolar	800

Abbreviations: MF, microfilaments; MTB, microtubules.
References: 1. Dorresteijn and Kluge (1990); 2. Dondua *et al.* (1997); 3. Shimizu (1982b); 4. Astrow *et al.* (1989); 5. Fernandez *et al.* (1998).

oligochaetes and hirudinidans). In polychaetes, the clear cytoplasm domain, which is established through ooplasmic segregation at the animal side of the egg, is simply cut up by unequal equatorial cleavage. In clitellates, localization of clear cytoplasm to animal blastomeres is preceded by unification of the initially separated polar domains of clear cytoplasm, which result from bipolar ooplasmic segregation. In this article, I have reviewed recent studies on cytoskeletal mechanisms for ooplasmic localization during early annelid development. Annelid eggs accomplish ooplasmic rearrangements through various combinations of three cytoskeletal mechanisms, which are mediated by actin microfilaments, microtubules and mitotic asters, respectively. One of the unique features of annelid eggs is that a homologous process is driven by distinct cytoskeletal elements. Annelid eggs may provide an intriguing system to investigate not only mechanical aspects of ooplasmic segregation but also evolutionary divergence of cytoskeletal mechanisms that operate in a homologous process.

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