
EXPERIMENTAL EMBRYOLOGY

Ooplasmic Segregation and Axis Formation in the Polychaete *Nereis virens* Embryo

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Abstract—Ooplasmic segregation is of great importance in the development of Annelida. The mechanisms of this process are very diverse in different groups of polychaetes, oligochaetes, and leeches (Fernandez *et al.*, 1998). Ooplasmic segregation in *Nereis virens* is connected with the first meiotic spindle formation and animal-vegetative axis appearance. Spherical polyaxial symmetry of the oocyte transforms into radial stratified symmetry in the course of ooplasmic segregation. There are two main steps of ooplasmic segregation in *Nereis virens*. The first step begins after the cortical reaction when the central clear cytoplasm reaches the surface of the oocyte. The movement of the cytoplasm is sensitive to nocodazole, colchicine, and cytochalasin B and appears to be mediated by microtubules and, partly, by microfilaments. The second step is not sensitive to the microtubule inhibitors and is mediated mainly by actin filaments. Ooplasmic segregation in *Nereis virens* may be considered as a primitive form of ooplasmic segregation in Annelida.

Key words: oocyte, meiotic division, ooplasmic segregation, formation of embryonic morphological axes, polychaetes

The spiral type of cleavage is characterized by an early specification of some cell clones which occupy a strictly determined position in the developing embryo. This developmental feature is believed to be due to segregation of the corresponding cytoplasmic determinants, which come to different blastomeres/precursors of clones: ectodermal and mesodermal somatoblasts, neuroblasts, nephroblasts, trochoblasts, etc. Simultaneously with ooplasmic segregation, which is initiated by fertilization and takes place during meiotic divisions of the oocytes and their preparation for the first cleavage division, the promorphology of embryos arises, which is related to the formation of its axes.

Shimizu (1999) believes that three main types of organization of the egg cells arise in annelids as a result of ooplasmic segregation before the beginning of cleavage. The polychaetes are characterized by the unipolar type of organization, in which the clear cytoplasm devoid of inclusions is concentrated at the animal or vegetal pole, while the oligochaetes and leeches have bipolar organization, in which two polar domains of clear cytoplasm, animal and vegetal, are formed in the egg.

The concentration of the clear cytoplasm in the animal part of the egg was described in *Platynereis dumerilii* (Dorresteijn and Kluge, 1990; Dondua *et al.*, 1997), *P. massiliensis* (Schneider *et al.*, 1992), *Tylorhynchus heterochaetus* (Osanai, 1978), and *Tomopteris helgolandica* (Akesson and Melander, 1967).

According to Dorresteijn and Kluge (1990), the ellipsoid egg of *P. dumerilii* assumes a spherical shape after fertilization. The clear cytoplasm, which was located in the central part of the oocyte, is elongated towards the periphery, so that a small zone (up to 35 μm in diameter) appears on its surface whose cytoplasm does not contain yolk inclusions. This point becomes the animal pole of the embryo: here, the first meiotic spindle contacts with the egg cortex and the polar bodies are extruded. Within 20 min after the completion of meiotic divisions, the yolk grains start to move along the egg periphery towards the vegetal pole. The clear cytoplasm is concentrated in the animal hemisphere. Ooplasmic segregation is completed a short time before the first cleavage division (Kluge, 1990; Dorresteijn and Kluge, 1990).

Ooplasmic segregation assumes a peculiar pattern in the polychaetes *Myzostomium ambiguum* (Kato, 1952), *Chaetopterus pergamentaceus* (Lillie, 1906), *Sabellaria vulgaris* (Novikoff, 1940), *Autolytis fasciatus* (Allen, 1964), and others. In these, like in some molluscs, the clear cytoplasm is concentrated in the vegetal part of the egg, where it forms a peculiar polar lobe whose material finally lands in blastomere 4d (Wilson, 1904a, 1904b; Clement, 1952; Dohmen and Verdonk, 1974; Dohmen and Lok, 1975; Henry, 1986).

In the oligochaete *Tubifex*, ooplasmic segregation is accompanied by noticeable egg deformation. During the second meiotic division, the yolk-free cytoplasm comes first to the egg periphery and then is concen-

trated in the animal and vegetal areas (Shimizu, 1982, 1996, 1999). Similar aggregates of the animal and vegetal teloplasm and the perinuclear cytoplasm are formed in the leech egg before the beginning of cleavage (Fernández *et al.*, 1998). In the course of cleavage, the cytoplasm of these domains lands in five pairs of teloblasts that give rise to the ectoderm and mesoderm (Weisblat and Shankland, 1985).

Although ooplasmic segregation has been studied for a long time and has been described in many animals (see, for example, Dohmen and Verdonk, 1979; Jeffery, 1988; Jeffery and Bates, 1989; Fernandez *et al.*, 1998); studies of its mechanisms are still in the early stages. At present, it seems that the cytoskeletal elements are essential for ooplasmic segregation. Note that in some cases, the cell cortex, where molecules or some organelles are "anchored," plays an important role in the provision of segregation processes and maintenance of the already existing cytoplasmic domains (Dohmen and Verdonk, 1979; Jeffery, 1988).

Specific mechanisms providing for these processes are very diverse in representatives of different groups of animals (Zalokar, 1974; Sawada and Osanai, 1981; Hill and Strome, 1988; Astrow *et al.*, 1989; Kluge, 1990; Dorresteyn and Kluge, 1990; Shimizu, 1996, 1999; Fernández *et al.*, 1998).

In the polychaete *P. dumerilii*, microtubules appear to play the key role in the realization of the first phase of ooplasmic segregation, which is blocked by nocodazole and taxol but is not sensitive to cytochalasin B. The second phase is less sensitive to the inhibitors of microtubules, which only delay, but do not block it. The main role is ascribed to actin filaments, since cytochalasin B fully blocks this phase (Dorresteyn and Kluge, 1990; Kluge, 1990).

In leeches, the mechanism of ooplasmic segregation is rather complicated. According to Fernández *et al.* (1998), ooplasmic segregation in the eggs of these animals includes at least six successive stages. The beginning of this process is markedly an altered orientation of the meiotic spindle, whose axis turns perpendicularly to the zygote surface. A so-called grey spot is formed in the area of contact of the peripheral centromere of this spindle with the cell surface. This process is blocked by colchicine; i.e., its realization requires the presence of microtubules.

The second stage is related to the formation of a discoid animal domain of clear cytoplasm, which appears as a result of the spreading of a contractile ring arising at the egg equator to the animal pole. The second stage is blocked by cytochalasin B; i.e., it is mediated by actin filaments.

The third stage is characterized by the formation of a domain of perinuclear cytoplasm located in the center of the zygote around the sperm centrosome. The formation of this aggregate of clear cytoplasm is sensitive to colchicine, thus suggesting the involvement of microtubules in this process.

The fourth stage also requires the presence of microtubules of the spermaster: it is characterized by the movement of the animal cytoplasmic domain, together with female karyomeres that remain after meiotic division, along the aster microtubules to the center of the zygote.

The fifth stage consists of a thickening of the ectoplasm layer, which is determined by a centrifugal transport of the organelles along the microtubules.

Finally, the sixth stage is characterized by the appearance of two contractile rings at both egg poles, which shift the ectoplasm to the animal and vegetal poles. As a result of this process, whose realization requires both actin and microtubules, aggregates of teloplasm are formed on both poles of the egg, which land in the course of subsequent cleavage divisions in blastomere *CD* and, then, in teloblasts.

Fernandez *et al.* (1998) noted many similar features in the mechanisms of ooplasmic segregation in the leeches and ascidians. In ascidians, the cortex of the unfertilized egg includes a peripheral submembrane cytoskeleton formed predominantly by F-actin and a deeper cortical cytoskeleton whose elements are structurally similar to the intermediate filaments. The first phase of ooplasmic segregation related to the movement of myoplasm to the vegetal area is blocked by cytochalasin B (Zalokar, 1974; Sawada and Osanai, 1981), thus suggesting that the driving force of the first phase is produced by the actin filaments of the submembrane cytoskeleton (Jeffery and Swalla, 1990; Jeffery, 1992). Since the second phase, when the myoplasm is shifted to the subequatorial area, is blocked by colchicine and is sensitive to a low temperature (Jeffery and Bates, 1989), it has been proposed that its realization is mediated by microtubules, possibly those of the spermaster (Zalokar, 1974; Sawada and Shatten, 1988).

The great diversity of the mechanisms underlying ooplasmic segregation in the Spiralia having a similar type of development needs to be interpreted. In this respect, a comparative-embryological analysis of ooplasmic segregation seems essential. The chosen object, *Nereis virens* (Polychaeta), is not only interesting as a representative of a primitive group of animals but also very convenient for experimental studies, since the period of ooplasmic segregation lasts several hours.

MATERIALS AND METHODS

The materials were collected at the Marine Biological Station, St. Petersburg State University, in the Chupa Bay of the White Sea. Sexually mature individuals were caught on the water surface by a hand net during the period of spawning, in the second half of June–beginning of July. The females and males were kept separately. The eggs obtained from mature females were kept in two changes of pure seawater, taken outside the spawning area, for 1.5 h. Artificial insemination and further cultivation of the embryos were carried out in

thermostated rooms at $11 \pm 0.5^\circ\text{C}$ (Dondua, 1975). Within 10 min after the addition of sperm preliminarily diluted with water, the eggs were washed in three changes of sea water and cultivated in 4-liter glass vessels, with constant mixing of the water with stirrers.

The effects of cytochalasin B, as well as colchicine and other mitostatics, were studied by placing the fertilized eggs in solutions of the corresponding substances in pasteurized sea water (80°C for 20 min) filtered through a millipore filter (Synpor 8, VUFS, Chemapol, Czechoslovakia) with a pore diameter of $0.23 \mu\text{m}$.

Several variants of experiments, which differed in the time of the beginning of the action of inhibitors and in the concentration of inhibitors, were carried out. When the incubation was begun within 10–15 min after insemination, the oocytes were at the stage of cortical reaction. When the experiment was started within 70 min and 3 h, the oocytes were at the stages of cortical reaction completion and first polar body extrusion, respectively. Colchicine (Ferak Berlin, Germany) was used at concentrations of 25, 100, and $200 \mu\text{g/ml}$; cytochalasin (Sigma, USA), at concentrations 0.2, 5, and $10 \mu\text{g/ml}$; and nocodazole (Sigma), at a concentration of $5 \mu\text{g/ml}$. The inhibitors were also used in com-

binations: colchicine ($5 \times 10^{-4} \text{ M}$) + cytochalasin B ($5 \mu\text{g/ml}$).

The embryos were fixed every 0.5–1 h after fertilization by Bouin fluid until the two-cell stage. Histological sections were stained by Mayer hematoxylin and poststained by eosin or by Hansen hematoxylin.

In order to obtain serial semithin sections, the materials fixed by Zenker fluid were stained by gallocyenin (Fluka, Switzerland), pH 0.85, and the plates were then dehydrated in an increasing series of alcohols and embedded in araldite. Sections were made on an LKB-Nova ultratome (Sweden) and poststained by methylene blue.

For total preparation to be made, the materials were fixed by 3.7% formaldehyde on phosphate buffer or on artificial sea water free of Ca and Mg ions and complemented with Tris and EDTA. The materials were then analyzed using differential interference contrast (DIC or Nomarski optics) under an Axioplan microscope (Carl Zeiss, Germany).

Using an Olympus camera, Semithin sections were photographed with Agfa Plan 100 ASA negative film,

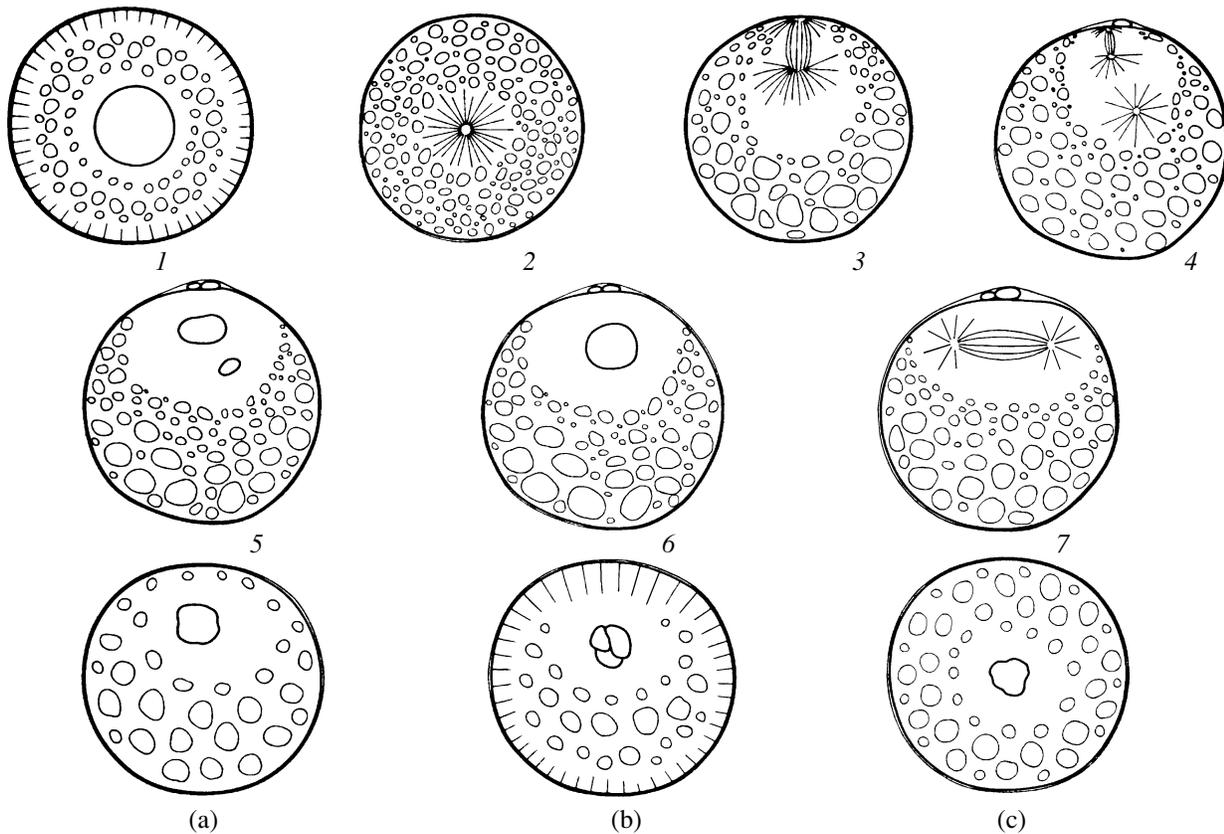


Fig. 1. A scheme of ooplasmic segregation under normal and experimental conditions. 1–7, Normal development: 1, oocyte before fertilization; 2, I meiotic division, view from the animal pole; 3, I meiotic division; 4, II meiotic division; 5, approach of male and female pronuclei; 6, zygote; 7, 1st cleavage spindle. (a–c) Development in the presence of inhibitors: (a) colchicine, (b) cytochalasin B, and (c) colchicine and cytochalasin B.

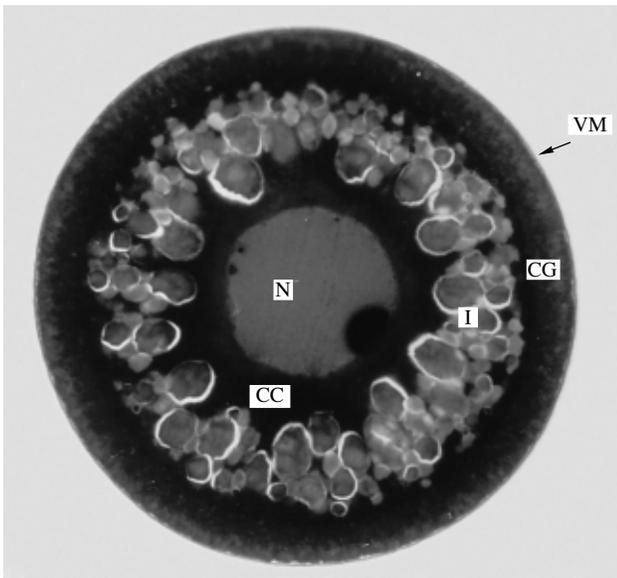


Fig. 2. A fully grown *N. virens* oocyte, semithin section, staining by methylene blue. Designations: CG, a layer of cortical granules; VM, vitelline membrane; I, zone of lipid and yolk inclusions; CC, clear cytoplasm; N, nucleus with nucleolus. Scale: 50 μm (here and in Figs. 3–6 and 8–10).

and total unstained preparations (Nomarski optics), with Copex Rapid 40 ASA film.

RESULTS

Normal development. The unfertilized *N. virens* egg has a spherical shape with a diameter of about

200 μm . Its structure does not show visible signs of polarization and is characterized by radial symmetry (Figs. 1, 1, and 2). The cortical zone with many cortical granules is located under the vitelline membrane and weakly expressed perivitelline space, along the periphery of the oocyte. A zone of lipid drops and yolk inclusions is located closer to the center. The central area is occupied by yolk and lipid-free cytoplasm, rich in mitochondria and ribosomes (so-called clear cytoplasm). A large (about 60 μm in diameter) nucleus is located in the center of the oocyte.

The cortical reaction proceeds just after fertilization, during which the contents of the cortical granules are released in the perivitelline space. A fertilization cone is formed at the site of attachment of the spermatozoon to the plasma membrane (Fig. 3). The spermatozoon remains connected with this cone until the first polar body extrusion.

After the cortical reaction, which is completed within 1 h after fertilization, the surface layer of the oocyte is occupied by the cytoplasm, rich in inclusions (Fig. 1, 2). By this moment, the nuclear envelope has disintegrated and the karyoplasm is mixed with the clear cytoplasm.

Within 1.5 h after fertilization, the first meiotic spindle is formed (Figs. 1, 3, and 4). One of the spindle asters is located in the cortical area. The second aster is located in the depth of the cytoplasm, while the spindle axis is arranged perpendicularly to the cell surface. Within 2.5–3 h after fertilization, the first polar body is formed. As the first meiotic spindle is formed, the initially spherical domain of clear cytoplasm spreads along the spindle axis and comes to the egg surface, thus designating the appearance of the animal pole, the

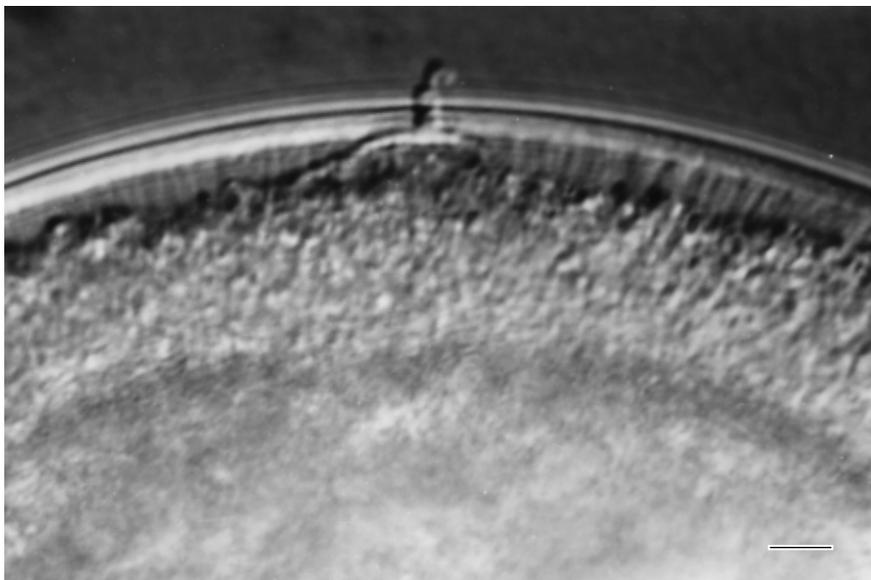


Fig. 3. Fertilization cone of *N. virens* oocyte.