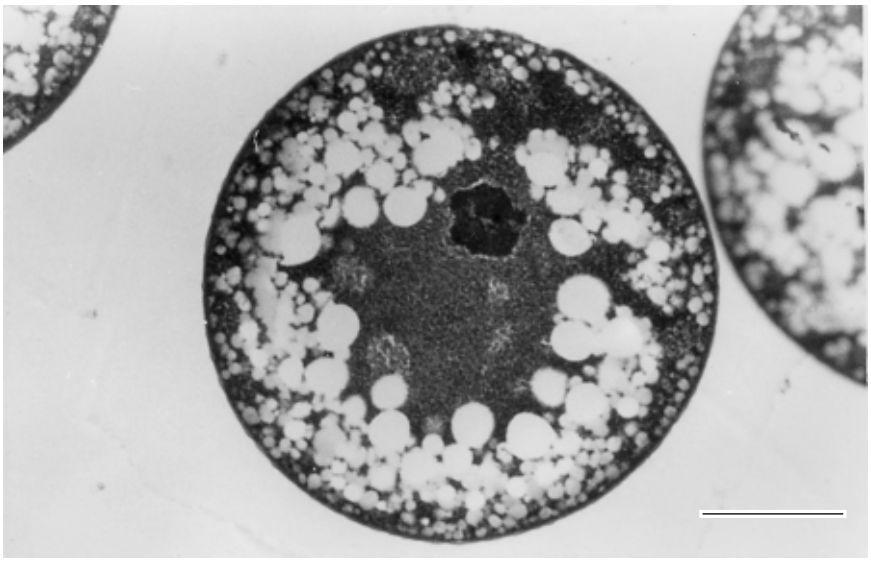

Fig. 8. Continuous action of colchicine (25 μg/ml) on the *N. virens* embryo for 4 h starting from 25 min after fertilization; block of the I meiotic division, preservation of the main mass of the domain of clear cytoplasm in the egg center. Semithin section, staining by toluidine blue.

disintegration. The central zone was surrounded by a sphere rich in large lipid inclusions, a sphere containing predominantly yolk granules was closer to the periphery, and finally, the outer sphere with cortical alveoles was limited by the egg plasma membrane (Figs. 1c and 9). In the oocytes fixed within 3 h after fertilization, a characteristic polarization of the central cytoplasmic domain, which was elongated towards the periphery with special reference to the first meiotic spindle formation, was sometimes observed. In the experiments that started within 70 min after fertilization, this asymmetric position of clear cytoplasm was already noted, like in the normal oocytes, within 2 h after fertilization. It was preserved for a long time and was noted even within 8 h after fertilization (7 h of keeping in cytochalasin B). In the experiments where the inhibitor was added within 20 min after fertilization, the interphase nucleus was usually formed within 6 h of development, but the normal stratification of the cytoplasm was absent. The normal animal cap of clear cytoplasm was not formed in the presence of cytochalasin B.

In one experiment, the eggs were placed in a mixture of colchicine (25 μg/ml) and cytochalasin B (5 μg/ml) within 70 min after fertilization. During the entire period of observations (8 h), the oocytes preserved their initial structure. The nuclear material represented by a homogeneous substance and dark-stained chromosomes was located in the center of the clear cytoplasm. The cytoplasm with large lipid drops and the cytoplasm with yolk granules were located closer to the periphery (Figs. 1c and 10). Ooplasmic segregation proved to be fully inhibited in the presence of both microtubule and microfilament inhibitors.

DISCUSSION

Cortical reaction. Just after the contact of the spermatozoon with the egg, which in *N. virens* is at the stage of prophase I, activation of the oocyte begins and meiotic divisions are resumed. The fertilization cone is

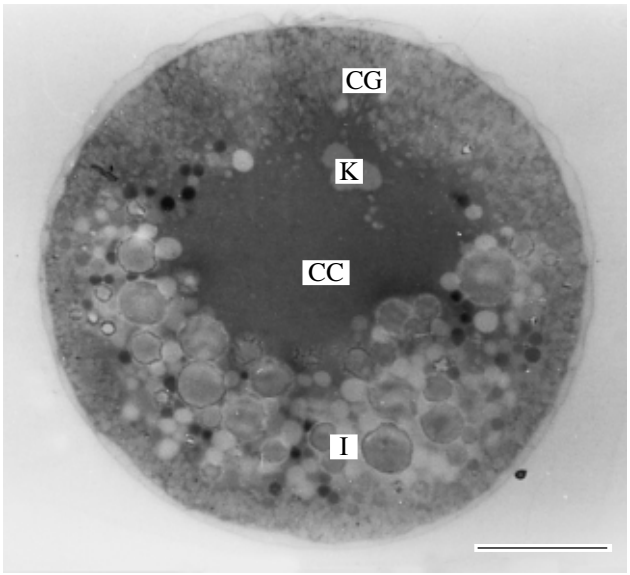

Fig. 9. Effect of cytochalasin B (0.2 μg/ml) on the cortical reaction in the *N. virens* egg, semithin section, staining by methylene blue. Designations: K, karyomeres; for other designations see Fig. 2.

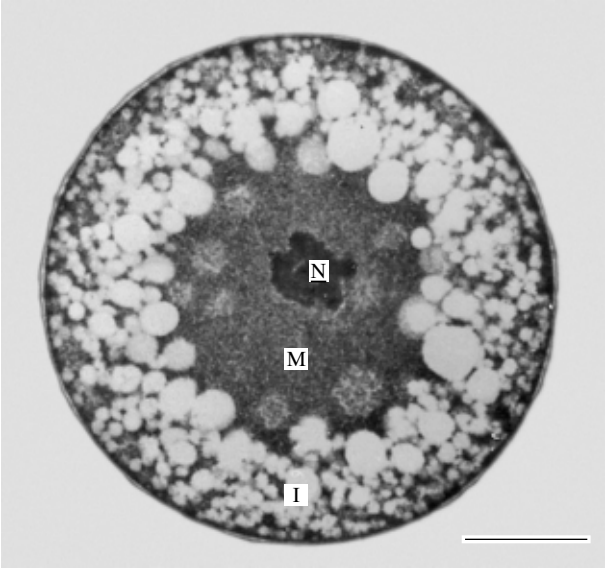

Fig. 10. Combined effects of colchicine (25 μg/ml) and cytoshalasin B (5 μg/ml) on the *N. virens* embryo for 8 h. Complete block of ooplasmic segregation. Semithin section, staining by methylene blue. Designations: M, myxoplasm; N, nuclear material and chromosomes; for other designations, see Fig. 2.

formed at the site of contact (Sato and Osanai, 1983; Anderson and Eckberg, 1983; Kluge, 1990). The spermatozoon does not fuse with the egg until the end of the first meiotic division, and this coincides with the observation of Lillie (1911) on another *Nereis* species, *N. limbata*. In the case of polyspermy, which is readily induced in *N. virens* by an excess of spermatozoa in the medium, several (up to several tens) fertilization cones are formed. These data suggest that, firstly, the fertilization cone is formed under the influence of the spermatozoon and, secondly, the site of penetration of the spermatozoon into the egg is not restricted to a special area of the oocyte's surface. Note, however, that after the animal-vegetal axis is formed, a definite position of the fertilization cone relative to this axis becomes evident. According to our unpublished data, in most cases the fertilization cone is located in the equatorial zone of the egg, with a distinct animal-vegetal axis. It remains uncertain whether this phenomenon is due to the organizing role of the spermatozoon or to the predetermination of the animal-vegetal axis and the presence of a very wide, but limited, zone of the highest probability of contact of the spermatozoon with the surface.

The cortical reaction, i.e., exocytosis of the granular-fibrillar contents of large membranous vacuoles located in the peripheral layer of the oocyte and designated as cortical granules in nereids (Costello, 1948), is the first morphological expression of egg activation. A very large amount of densely packed cortical granules, arranged in several rows, are organized in a common cortical layer in *N. virens*. Such a structure of the cortical layer was also described for many other polychaetes (Fallon and Austin, 1967; Dhainaut, 1969, 1970; Takashima and Tominaga, 1978; Kluge *et al*., 1995). The cortical granules of nereid oocytes contain polysaccharides, including acid mucopolysaccharides (Dhainaut, 1970; Takashima and Tominaga, 1978). This material may rapidly swell in water (Lillie, 1911; Fallon and Austin, 1967; Dhainaut, 1969; Takashima and Tominaga, 1978).

In the most extensively studied examples of cortical reaction in sea urchins and lower vertebrates (Jaffe, 1983; Longo, 1988; Chandler, 1991), exocytosis of cortical granules rapidly spreads over the egg surface from the site of contact of the spermatozoon with the plasmalemma and is completed within 10 s. *N. virens*, like other nereids (Lillie, 1911; Kluge *et al*., 1995), are characterized by slow changes in the cortical layer: the first large-scale processes were noted only within 10 min and the end of the cortical reaction approximately within 1 h after insemination. It is known that the cortical reaction in many animals is a calcium-dependent process; this appears to be true for *Nereis* as well. There are published data that the cortical granules in some polychaetes serve as a depot for Ca ions (Emanuelson and Odselius, 1985), but such studies have not been carried out in nereids.

Exocytosis of the cortical granules in *N. virens*, like in other nereids (Kluge, 1990; Kluge *et al*., 1995), embraces initially the peripherally located cortical granules, while the cortical granules of deeper cortical layers excrete their contents later as they approach the plasma membrane.

The results of experiments with colchicine, nocodazole, and cytochalasin B suggest that the cortical reaction in *N. virens* depends on the actin rather than tubulin structures of the cytoskeleton (Dondua and Sidorova, 1986).

Cytochalasin B not only blocks the cortical reaction but also prevents elongation of the microvilli and even induces the resorption of the latter. Taking into account a high concentration of F-actin in the microvilli, as visualized by rhodamine-phalloidin, it may be concluded that actin plays the leading role in the formation of microvilli. Kluge *et al*. (1995) came to a similar conclusion with respect to *Platynereis dumerilii*.

Ooplasmic segregation in *Nereis virens*. In the *N. virens* egg prepared for fertilization, the animal-vegetal axis is not morphologically shaped. Unlike many other polychaetes (for review see Dorresteijn and Fischer, 1988), the unfertilized *N. virens* egg is spherical and consists as if of several spheres surrounding the nucleus: a sphere of cytoplasm free of yolk and rich in mitochondria and ribosomes, a sphere of yolk and lipid inclusions, and, finally, cortical layers that occupy the egg periphery.

Ooplasmic segregation in *N. virens* oocytes leads to the transformation of this polyaxial radial-symmetrical structure into a polarized stratified structure, where dif-

ferent kinds of cytoplasm are located in a certain succession along the animal-vegetal axis. Our data suggest that the formation of this axis is based on two main events related to the activities of cytoskeletal elements: microtubules and actin filaments.

At the first stage of ooplasmic segregation, the central cytoplasmic domain is polarized. The aggregate of clear cytoplasm loses its spherical shape and, while spreading along the meiotic spindle axis, reaches the egg surface. This site becomes the animal pole of the egg, and here, soon after fertilization, the first polar body is extruded. The next stage is realized mostly after the second polar body extrusion and is characterized by the displacement of the cytoplasm with yolk granules and lipid vacuoles to the vegetal hemisphere and of all cytoplasm free of inclusions to the animal hemisphere.

The first stage is sensitive to the inhibitors of microtubules, colchicine and nocodazole, thus suggesting the leading role of microtubules in the initial polarization of the *N. virens* oocyte, which appears to be a common property of polychaetes (Dorresteijn and Kluge, 1990; Shimizu, 1999). The dependence of the shape of the central cytoplasmic domain on the presence of spindle microtubules stresses the conditionality of the widely cited "Hertwig rule" (see, for example, Tokin, 1987), according to which the division spindle is located in the direction of the longest axis of yolk-free cytoplasm. In our case, the active organizing role of microtubules of the division spindle is revealed in the modification of the cytoplasmic domain shape: the suppression of the spindle assembly by colchicine or nocodazole prevents the formation of an animal domain of clear cytoplasm.

While stressing the leading role of microtubules in the initiation of ooplasmic segregation in the *N. virens* oocytes, the data obtained also reveal a complex pattern of its mechanisms. Indeed, under the conditions when the microtubules are blocked by colchicine or nocodazole, the central cytoplasmic domain establishes a connection with the peripheral zone of the egg (although with a significant delay). Colchicine and nocodazole are known to enhance the polymerization of microtubules (De Brabander *et al*., 1986), while translocation of the spindle asters is provided by rearrangement of these organelles, i.e., assembly at the plus-end and disassembly at the minus-end. As shown by Kuriyama *et al*. (1986), the translocation of the first cleavage spindle in the *Spisula* zygote is stopped in the presence of taxol, a drug that stabilizes microtubules. These data suggest that depolymerization of the microtubules is a necessary condition for the translocation of the division spindle. Kluge (1990) came to a similar condition while studying the formation and translocation of the division spindle in *Platynereis* in the presence of cytoskeleton inhibitors.

The results of studies with the use of immunocytochemistry and confocal laser microscopy on the mollusc *Dreissena* have shown that the spindle asters of cells kept in a solution of nocodazole were reduced in size. The contacts of asters with the cortex were also reduced at metaphase. However, even at high nocodazole concentrations, the spindle system was still capable of directed movement, but much more slowly (Luetjens, 1997). Thus, under the conditions when the assembly of microtubules was blocked by nocodazole, the continuing depolymerization of tubulin was capable of providing for translocation of the division spindle. The formation of a cytoplasmic outgrowth from the center to the periphery under the conditions of the blocked assembly of microtubules may be partially explained by the displacement of the cortical cytoplasm rich in inclusions to the sides from a certain point on the egg surface.

The results presented suggest that the driving forces of this translocation are provided by the actin microfilaments of the egg cortex. Indeed, when the microtubules and microfilaments were simultaneously inhibited by a mixture of colchicine and cytochalasin B, the central cytoplasmic domain remained without visible changes even within 8 h after fertilization, when the egg cleavage took place in the control. The suggestion about an important role of microfilaments in ooplasmic segregation is confirmed by the results of studies suggesting the role of the egg cortex as a whole, and microfilaments specifically, as a peculiar anchor and driving force of the organelles and diverse morphogenetically active molecules (Jeffery, 1984; Yisraeli *et al*., 1989; Kluge, 1990; Fernández *et al*., 1998). The cytoskeleton is a dynamic continuously rearranging structure, and related cellular components undergo regular positional changes. The simultaneous loss of both the major part of the motor function of the microtubules and the effect of fixation of the already translocated substances by microfilaments leads to a practically complete arrest of segregation.

Microfilaments play the key role at the second stage of ooplasmic segregation in *N. virens*, since under the conditions of cytochalasin block, the egg stratification is markedly slowed down and modified. A similar cytochalasin B-induced prevention of the second phase of segregation, including the translocation of yolk granules and lipid inclusions in the vegetal hemisphere, was also described for *Platynereis dumerilii* (Kluge, 1990).

Thus, the data obtained suggest that ooplasmic segregation in *N. virens* takes place due to active interaction between microtubules and microfilaments. The blocking of even one of these systems suffices to disturb the process as a whole. Their simultaneous switching off arrests ooplasmic segregation completely. The integration of the function of microtubules and microfilaments is also characteristic for ooplasmic segregation in leeches (Fernández *et al*., 1994, 1998).

Note in conclusion that the type of ooplasmic segregation observed in the *N. virens* oocytes has primitive features, as expressed in both a relatively late development of the animal-vegetal axis and in the fact that initiation of the rearrangement of the cytoplasm is coupled

with the processes providing for meiotic divisions. In our opinion, the mechanisms underlying this relationship could serve as a basis for the formation of different, more specialized types of ooplasmic segregation in the course of evolution.

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