THE ULTRASTRUCTURE OF THE NUCLEAR ENVELOPE OF THE OOCYTE OF URECHIS UNICINCTUS

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THE ULTRASTRUCTURE OF THE NUCLEAR ENVELOPE OF THE OOCYTE OF *URECHIS UNICINCTUS*

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It is widely recognized that the nuclear envelope of the various kinds of animal cells is composed of two membranous components with pore structure (WATSON, 1955, 1959, GALL, 1956, etc.). From the embryological viewpoint the ultrastructure of the envelope of the large nucleus (germinal vesicle) in the oocyte is of great interest. The first study in this field was made by CALLAN and TOMLIN (1950), who worked with isolated nuclei from amphibian oocytes. In 1955, AFZELIUS, using the ultra-thin sectioning technique, demonstrated the presence of annulated pore structure in the nuclear envelope of the sea urchin oocyte and reported on the dimension of the structure. The structure of the nuclear envelope in *Chaetopterus* eggs was also studied by MERRIAM (1961), who worked especially on the changes during cell division with cytochemical methods and the electron microscope.

The egg of *Urechis* has often been employed as a good material for the study of fertilization and cleavage. The present study was undertaken to enlarge the knowledge of the ultrastructure of the nuclear envelope to the *Urechis* egg.

MATERIAL AND METHODS

The echiuroid, *Urechis unicinctus*, used in this study was collected on the coast of Kanazawa near Yokohama. Eggs and sperm were obtained by dissecting the animals.

The unfertilized egg generally has a large indentation, and a large round germinal vesicle, which remains intact until the egg is stimulated by fertilization or by artifical activation to start the maturation devision. When the egg is fertilized, the nuclear envelope begins to break down in about 15 minutes, and seems to be dissolved completely within 20 minutes when observed with a light microscope.

Unfertilized eggs were fixed in a 1 per cent solution of osmium tetraoxide in filtered sea water. Fixation was for one hour at 0°C. Similarly, after fertilization, aliquots were fixed at five minutes' intervals for 30 minutes. These samples were then dehydrated in alcohols and embedded in an 8 to 2 mixture of butyl- and

methyl-methacrylates. Thin sections were observed under a JEM-T6 electron microscope manufactured by the Japan Electron Optics Co.

RESULTS AND DISCUSSION

The nuclear envelope in the unfertilized egg (*oocyte*). In the unfertilized egg, the nuclear envelope sometimes shows more or less complicated invaginations into the nucleoplasm, giving the nuclear surface a wrinkled appearance (Figs. 1 and 3). The invagination engulfs the cytoplasmic materials. Some of the wrinkles might be due to the artifact produced during fixation and embedding, but the fold as shown in Fig. 1 is so complicated that it cannot be considered to be an artifact. AFZELIUS (1955) suggested that the wrinkled appearance of the nucleus in the sea urchin egg

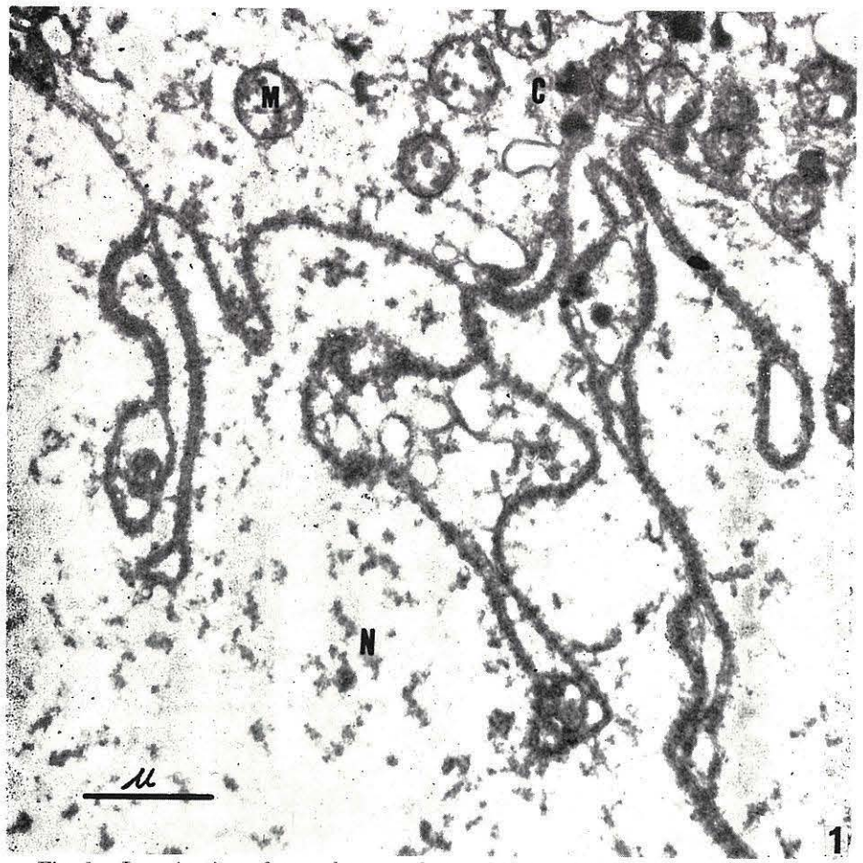

Fig. 1. Invagination of a nuclear envelope. *M*, mitochondria, *N*, nucleus, *C*, cytoplasm (The same abbreviation applies to the following figures). Magnification ×17,000.

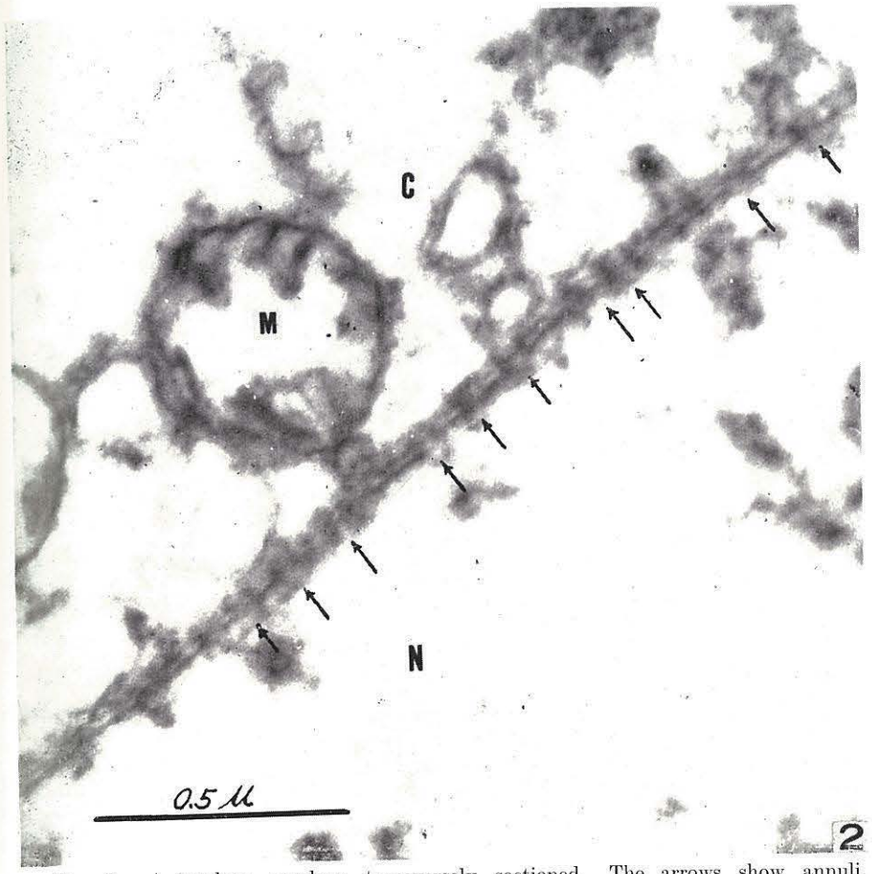

Fig. 2. A nuclear envelope transversely sectioned. The arrows show annuli. Mangification ×72,000.

may explain the well-known ability of the nucleus to swell. A possiblity has also been suggested by several workers that the interaction between the nucleus and cytoplasm may be enlarged by wrinkling of the nuclear envelope. In the grown-up oocyte of *Urechis*, the nuclear envelope is broken soon after fertilization. It may be therefore considered that the fold or invagination may have some meaning, if any, during the growth period of the oocyte.

The detail of the nuclear envelope sectioned transversely is shown in Fig. 2. The envelope is composed of two membranes. Generally speaking, the inner membrane appears denser and thicker than the outer one, though in some parts of the envelope no differences in density and thickness are found between the two membranes. In Table 1, some data on the thickness of the membrane are shown.

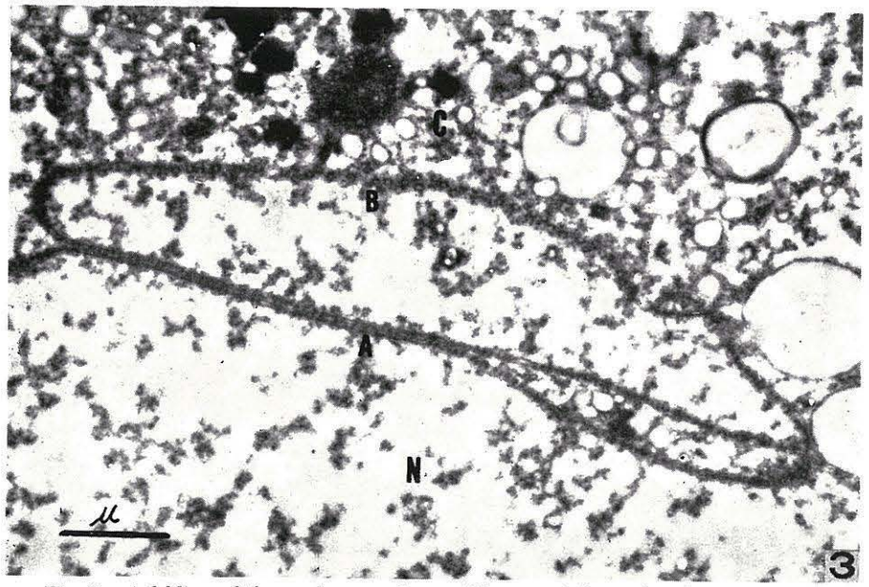

Fig. 3. A folding of the nuclear envelope. The part *A* is sectioned tangentially and the part *B* transversely. Magnification ×15,000.

Table 1

Dimension of the nuclear envelope

Each mean value was calculated from five measurements in the same nucleus. Values are in Å unit.

	Total thickness of the envelope	Thickness of the inner membrane	Thickness of the outer membrane	Distance between the two membranes
Part A	280	119	84	77
Part B	286	137	94	57
Part C	270	124	83	63
Part D	276	134	87	55
Part E	257	113	68	76

There are found discontinuities in the sectioned envelope, as shown by the arrows in Fig. 2. The discontinuous part is considered to be a pore. A single membrane covering the pore is sometimes observed, but is not always observable, possibly because of its extreme thinness. An electron dense region is found on both sides of the pore in the section. This region protrudes both into the cytoplasm and into the nucleoplasm. When the nuclear envelope is cut tangentially, it can be seen that this electron dense region is of a cylindrical shape, and that it corresponds to the annulus reported by several investigators in the amphibian and sea urchin oocytes. Fig. 3 shows a folded part of the envelope. A part (A)

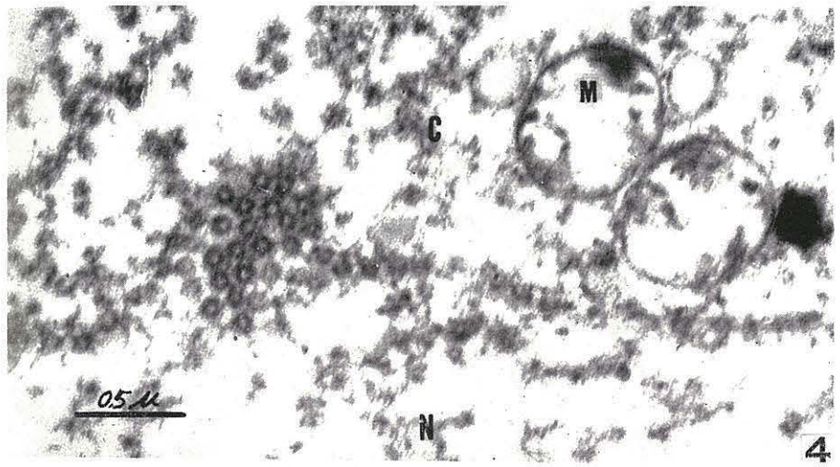

Fig. 4. A wrinkled part of the nuclear envelope tangentially sectioned. Magnification ×30,000.

is sectioned obliquely, showing a row of the annuli, while the other part (B) is sectioned transversely. Fig. 4 shows a tangential section of a winkled part of the envelope.

The dimension of the annulus is shown in Fig. 5. The annulus is about 850Å in diameter, its wall being 170Å in thickness. The total height of the annulus is about 660Å the part protruding on the cytoplasmic side being 250Å and the part on the nuclear side 140Å. The distance between two annuli is 430–870Å. It may be said that the annulus structure of the *Urechis* oocyte resembles that of the sea urchin oocyte in shape and size.

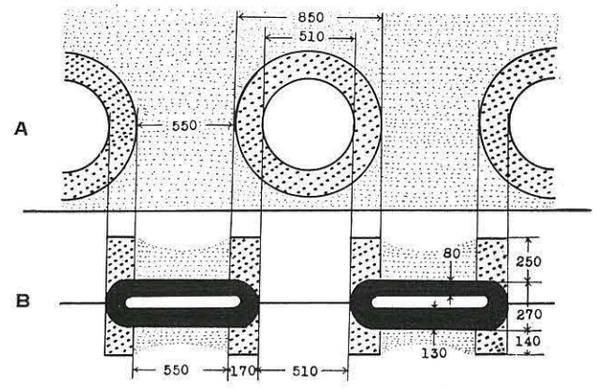

Fig. 5. Dimensions of the annulus structure. *A*, tangential section, *B*, transverse section. Values are in Å unit.

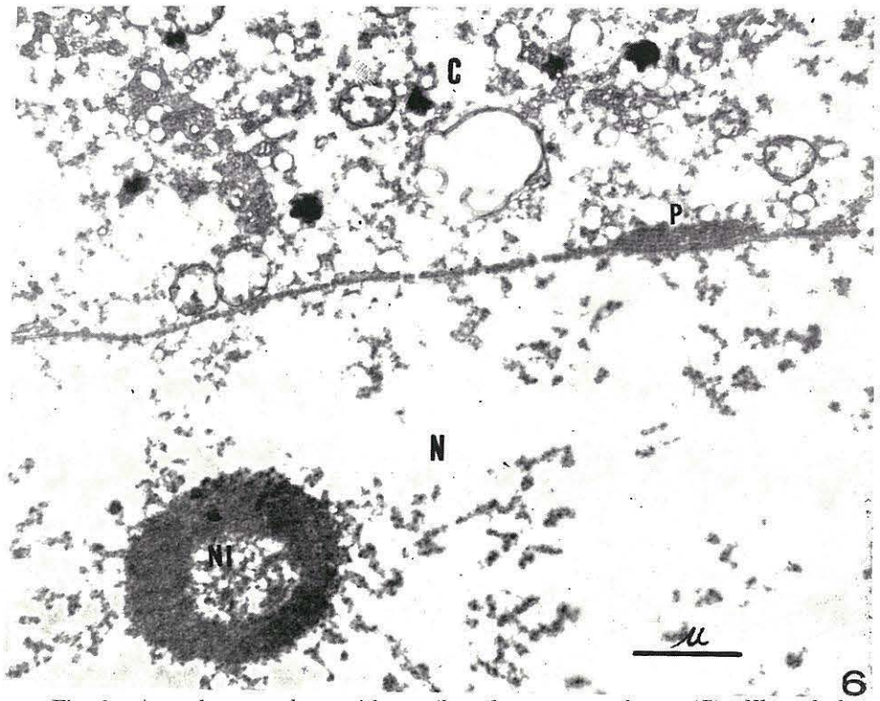

Fig. 6. A nuclear envelope with a pile of excess membrane (*P*). *Nl*, nucleolus. Magnification 15,000×.

Extra-nuclear membranes have been reported by several workers (Afzelius 1955, Dalton and Felix 1954). In *Urechis* oocytes, excess membranes are often found in the cytoplasm close to the nuclear envelope, forming a pile of many layers (Figs. 6 and 7). Each layer seems to have a structure the same as that of the nuclear envelope. The significance of such figures are still obscure.

Breakdown of the nuclear envelope after fertilization. When eggs are fertilized, the nuclear envelope begins to break down after about 15 minutes at 18°C. The envelope is first broken to fragments or pieces at several parts, and the nuclear content begins to mix with the cytoplasm. The pieces or fragments of the broken envelope are found both in the nuclear side and in the cytoplasmic side, forming vesicles of various size (Figs. 8 and 9).

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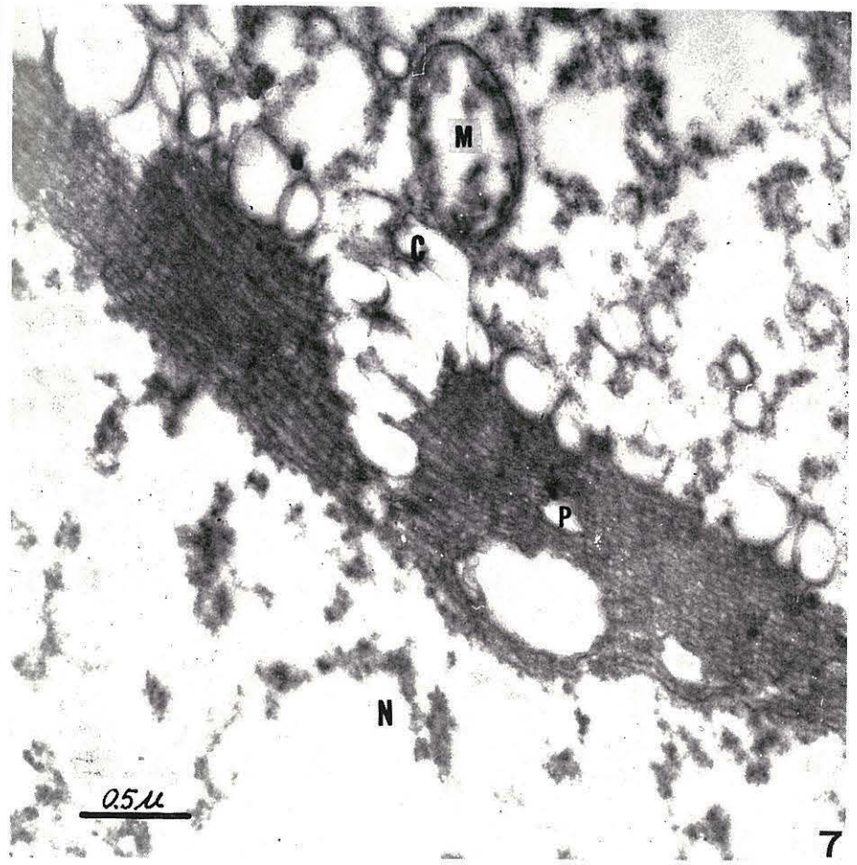

Fig. 7. A plie of excess membranes. Magnification 29,000×.

SUMMARY

The nuclear envelope of the oocyte of *Urechis unicinctus* was studied with the electron microscope. The nuclear envelope sometimes shows more or less complicated invaginations into the nucleoplasm, engulfing the cytoplasmic materials.

The nuclear envelope is composed of two membranes with the annulated pores which resemble those found in the sea urchin eggs. The annulus is 850Å in diameter, and its wall is 170Å in thickness. The annulus protrudes 250 Å on the cytoplasmic side and 140Å on the nuclear side, the total height being about 660Å. The distance between two annuli is 430–870Å. The total thickness of the envelope is 270 Å, the inner single membrane measuring 110–130Å and the outer 70–90Å.

Piles of pieces of the double membranes, the structure of which is the same as the nuclear envelope, are often found in the cytoplasm close to the nucleus.

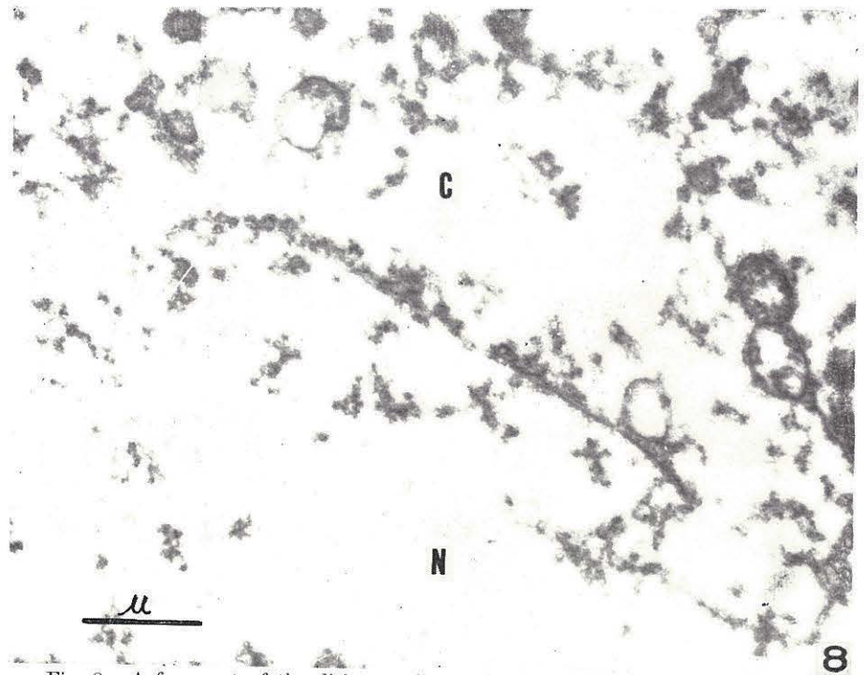

Fig. 8. A fragment of the disintegrating nuclear envelope 20 minutes after fertilization. Magnification 17,000×.

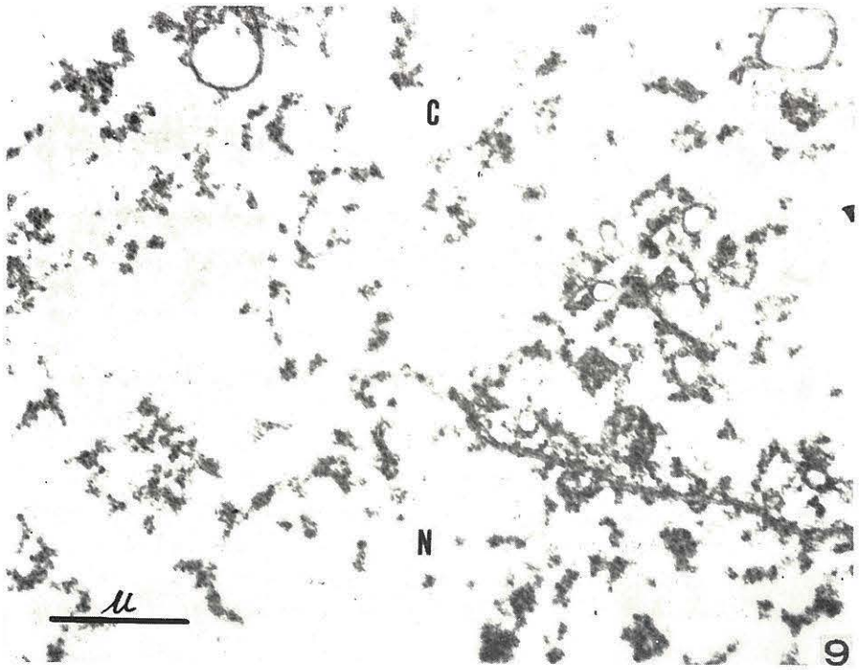

Fig. 9. An end of a fragment of the disintegrating nuclear envelope 20 minutes after fertilization. Magnification 20,000×.

When the eggs are fertilized, the nuclear envelope is broken to pieces or fragments at several places after 15 minutes, followed by the formation of small or large vesicles.

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