Occurrence of Microfilaments in the *Tubifex* Egg Undergoing the Deformation Movement

By

Takashi Shimizu

Zoological Institute, Hokkaido University

(With 3 Text-figures and 3 Plates)

Since Penners discovered the interesting role of pole plasm in the morphogenesis of the *Tubifex* egg in 1922, investigators working on the embryonic development of this worm have made efforts to understand the mechanism underlying pole plasm formation (Lehmann, 1956, for review). It is known that the *Tubifex* eggs show conspicuous activity causing deformation of the egg shape prior to the formation of pole plasm (Woker, 1944; Huber, 1946; Henzen, 1966; Matsumoto and Kusa, 1966). According to Penners (1922), the deformation movement (Protuberanzen) results in the formation of pole plasm. Therefore it seems to be worth performing a causal analytic study of the deformation movement. On this problem, an interesting experiment was carried out by Rötheli (1949, 1950), who revealed that the deformation movement of the *Tubifex* egg is inhibited by the antimitotics naphthoquinon and phenanthrenquinon. Although several authors have performed electron microscopical studies on the *Tubifex* egg, no cytoplasmic components, which probably participate in the deformation movement, have been reported (see Henzen, 1966).

In the present paper, the author describes the ultrastructure of the cortical layer of the eggs of *Tubifex* undergoing the deformation movement.

Materials and Methods

The freshwater oligochaete, *Tubifex hattai*, was collected from the stream running through the campus of Hokkaido University. To obtain newly laid cocoons, the worms were reared by the method described by Hirao (1964, 1965). The deposited cocoons were collected in a Petri-dish containing the culture solution[1] for the *Tubifex* embryo developed by Inase (1960). After careful removal of the elastic cocoon membrane with a pair of watchmaker's forceps, the healthy eggs thus separated were allowed to stand in the same culture solution (18°–20°C) until they attained the desired stages of embryonic development.

1) Culture solution for *Tubifex* embryo: M/50 NaCl 100 parts+M/50 KCl 25.2 parts +M/75 $CaCl_2$ 251.3 parts+M/50 $MgSO_4$ 41.8 parts, (pH 7.1).

The eggs were fixed at 0 (just laid), 1.5, 3, and 4.5 hours after oviposition (Fig. 1). As will be described later, the developing *Tubifex* eggs show characteristic changes in their shape.

For the electron microscopical observations, the developing eggs in the culture solution were fixed in 5% glutaraldehyde for 6 hours. They were rinsed overnight in 6 changes of 0.2 M sucrose and postfixed in 1% osmium tetroxide for 2 hours. These solutions were all made with 0.1M sodium cacodylate-HCl buffer solution, pH 7.4 and the immersions were performed at 0°– 4°C. After dehydration through ethanol, the materials were cleared in propylene oxide and embedded in Epon 812 (Luft, 1961). Silver or gold thin sections were cut on a Poter-Blum ultramicrotome and stained in half-saturated aqueous solution of uranyl acetate and in lead citrate solution (Reynolds, 1963). They were observed in a Hitachi HS–7 electron microscope.

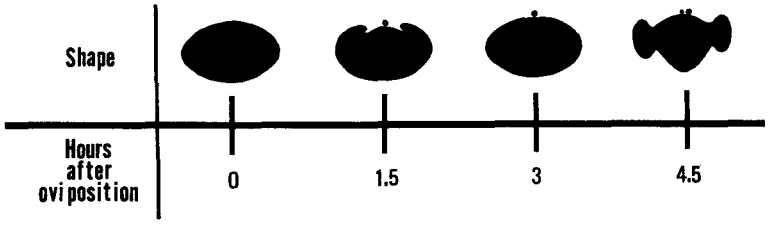


Fig. 1. Schematic illustration of the egg shape at the time of fixation.

Results

External observation of deformation movement:

The *Tubifex* egg immediately after oviposition is spheroidal in shape and is 0.1×0.3 mm on its short and long axes. The animal pole of the egg is discernible by the presence of a clear spot in the egg surface. According to Hirao (1968), this spot is a yolk-free area having the metaphase spindle of the first meiotic division. About 60 minutes after oviposition, the egg surface of the animal hemisphere becomes wrinkled (Fig. 2A). This has been called the first deformation movement by earlier investigators. Through the movement, many protuberances are formed on the egg surface of the animal hemisphere. At the same time, the first meiotic division proceeds and the first polar body is extruded from the egg, and this is observable in the fresh material. The first movement lasts for about 40 minutes. The wrinkles of the egg surface completely disappear and the egg returns to the original smooth-surfaced spheroidal shape. After a lapse of 1.5 to 2 hours, the deformation movement again occurs in the egg (Fig. 2B). This has been known as the second deformation movement (about 4 hours after oviposition). The extrusion of the second polar body occurs during this movement. Many protuberances appear on the equatorial surface of the egg. The second deformation movement lasts for about 80 minutes and the egg returns to the original smooth-surfaced spheroidal shape. At about 10 hours after oviposition, the egg divides into 2 cells.

The first and second deformation movements are clearly distinguished by the

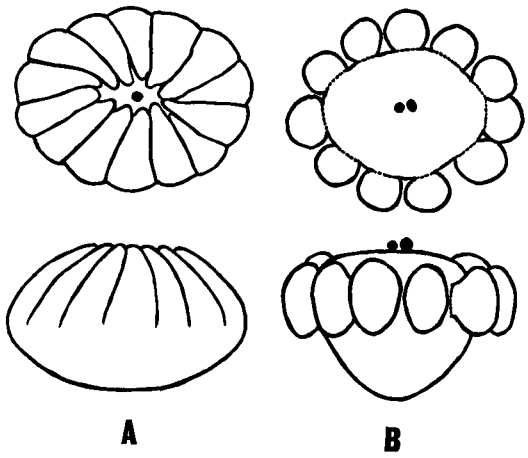


Fig. 2. Diagrammatic representation of the egg undergoing the first (A) and the second (B) deformation movements. Polar bodies extruded during the movements are indicated by black dots. Upper, viewed from the animal pole; lower, side view.

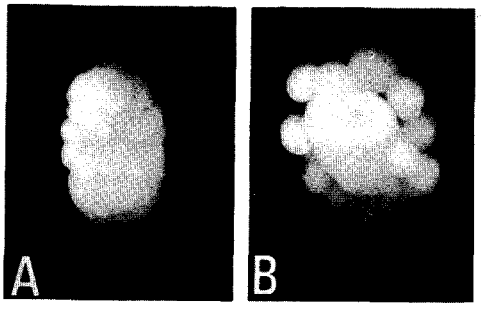


Fig. 3. *Tubifex* egg undergoing the first (A) and the second (B) deformation movement. Photographed from the animal pole of the fresh eggs. Note the difference in the distribution of protuberances. ×35.

distribution of protuberances (Figs. 2 and 3): In the first movement it is restricted to the surface of the animal hemisphere. Therefore the egg surface is very wrinkled in the animal hemisphere but is smooth in the vegetal one. On the other hand, the protuberances are formed around the equatorial circumference during the second movement. Invariably, in this case, the entire surface of the egg participates in the formation of protuberances.

Electron microscopical observation of the egg surface:

When the cytoplasmic layer of the egg surface was observed in the electron microscope, cytoplasmic organelles and inclusions such as mitochondria, endoplasmic reticulum, poorly developed Golgi complex, ribosomes and various-sized vesicles were found in all eggs examined. In addition to these organelles, bundles of microfilaments were detected in the most peripheral cytoplasm just

beneath the plasma surface. So far as the present study goes, the existence of microfilaments is, however, limited to the particular developmental stages examined, namely those of the first and the second deformation movements. The diameter of individual microfilaments is in the range of 5–7 nm. The distribution of the microfilamentous bundles in the egg surface shows a characteristic pattern. The filaments are found only in the surface area of the animal hemisphere but cannot be detected in the vegetal surface of the egg during the first deformation movement. On the other hand, they are distributed in the peripheral cytoplasm of both the animal and the vegetal hemispheres of the egg at the second deformation movement. The details of the distribution pattern and the profile of microfilamentous bundles in the eggs undergoing the first and second deformation movements will be described in the following pages.

The first deformation movement: The egg surface is covered with a thin cytoplasmic layer which is characteristically devoid of ribosomes and membraneous organelles. This layer is about 0.5 μm thick on average and is called the cortical layer in the present paper. The cortical cytoplasmic layer contains bundles of microfilaments in the region covering the entire surface of the animal hemisphere. The cortical layer with the bundles is also observed in the floor of grooves appearing as a result of the formation of protuberances.

The orientation of microfilaments in the cortical layer is not constant. Most of the filaments run parallel to the egg surface (Fig. 4) but in some portions their orientation is perpendicular or oblique to the latter (Fig. 5). Careful examination reveals that the microfilaments are arranged perpendicularly to the egg surface in the cortical layer covering the distal surface of the protruding portion (Fig. 6) and the floor of grooves (Fig. 7). Furthermore it appears that the outer end of the perpendicularly arranged filaments has some association with the outermost limiting membrane of the egg cytoplasm, plasma membrane. The other end seems to anchor on the surface of such organelles as mitochondria, endoplasmic reticula or in the cortical cytoplasmic matrix.

The second deformation movement: Electron micrographs of the egg undergoing the second deformation movement also reveal the presence of microfilamentous bundles in the cortical cytoplasm. In contrast with the case of the first deformation movement, however, the bundles can be detected at this stage not only in the animal surface layer but also in the vegetal cortical cytoplasm of the egg.

Figure 8 shows the section of a groove cut parallel or somewhat obliquely to the egg axis. The cortical layer is evidently thickest in the floor of the groove and is 0.8–0.9 μm thick. No significant differences in the distribution and orientation of the microfilamentous bundles are found between the animal and the vegetal hemispheres. It should be noted that microfilaments are not found in the cortical cytoplasm covering the protuberances formed by the deformation movement (Figs. 8a and 11a).

The microfilamentous bundles show a perpendicular or oblique arrangment to the plasma surface in the floor of grooves (Fig. 8b). The outermost limiting

membrane of the egg cytoplasm, plasma membrane, seems to be connected with mitochondria, endoplasmic reticula and ribosomes by these microfilaments. Sometimes a direct association of obliquely arranged microfilaments with the plasma membrane is distinctly observed: At the site of association, two electron-dense layers of the plasma membrane slightly approach each other, thus the width of the electron-transparent layer of the membrane appears to decrease (Fig. 9). Since the bundles of microfilaments running parallel to the egg surface exist somewhat below the surface of the cortical layer, a narrow space of the cortical cytoplasmic matrix can be seen between the bundles and the plasma membrane (Fig. 10).

Tangential sections of the groove disclose that the floor is slightly undulated (Fig. 11a). In these sections many punctuate components are found in the cortical cytoplasmic layer (Fig. 11b). These may represent the sagittal sections of microfilaments, since their diameter is apparently smaller than that of ribosomes.

Discussion

It has long been known that the developing *Tubifex* egg undergoes the deformation movement prior to cleavage. In the early ultrastructural studies of *Tubifex* eggs, several authors failed to demonstrate any cytoplasmic structure which seemed likely to participate in the deformation movement (Hess, 1959; Lehmann and Henzen, 1963; Henzen, 1966). The present study revealed the presence of microfilaments in the cortical cytoplasmic layer of the *Tubifex* egg. The morphological properties of these microfilaments are similar to those reported in many invertebrate and vertebrate eggs (Goodenough *et al.*, 1968; Szollosi, 1970; Selman and Perry, 1970; Conrad *et al.*, 1973, *etc.*).

As for the physiological role of microfilaments, Cloney (1966) demonstrated the contractile role of caudal epitheliar microfilaments during tail retraction in ascidian larvae. Since that, their roles in cellular locomotion have been reported in many non-muscle cells of various organisms (Cloney, 1969; Schroeder, 1970; Wrenn and Wessels, 1970, *etc.*). These may also suggest the participation of microfilaments in the deformation movement of the developing *Tubifex* eggs.

Raff (1972) reported that the polar lobe formation in the *Ilyanassa* eggs is prevented by cytochalasin B which is known to exert specific effects on microfilaments (Wessels *et al.*, 1971; Schroeder, 1972; Spudich, 1972). Electron microscopical observations by Conrad and Williams (1974) revealed that microfilaments associated with the cleavage furrow and the polar lobe constriction in the *Ilyanassa* egg disappear after treatment with cytochalasin B. Together with these findings, the following evidence suggests that microfilaments detected in the cortical cytoplasmic layer of the *Tubifex* egg play an important role in the deformation movement through their contractile property: (1) They can only be detected in the cortical layer during the deformation movement. (2) Their occurrence is restricted to those areas of the egg closely associated with the

deformation movement. No microfilaments were found in the cortical cytoplasm of the vegetal hemisphere during the first deformation movement.

Analytical study of the deformation movement in the *Tubifex* egg using cytochalasin B is in progress.

Summary

Electron microscopical observation was performed on the cortical cytoplasmic layer of the eggs of a freshwater oligochaete, *Tubifex hattai*, undergoing the deformation movement. Besides the cytoplasmic organelles and inclusions such as mitochondria, endoplasmic reticulum, Golgi complex, ribosomes and various-sized vesicles, bundles of microfilaments were found in specific regions of the egg. Their occurrence was limited to particular developmental stages, *i.e.* the stages of the first and second deformation movements. No microfilaments were detected in that part of the cortical layer of the egg having the smooth cytoplasmic surface. The bundles of microfilaments were found only in the animal hemisphere during the first deformation movement but were detected in both the animal and the vegetal hemispheres, except on the surface of protuberances, during the second deformation movement. These distributions suggest the participation of microfilaments in the deformation movement of *Tubifex* egg.

The author wishes to express his sincere appreciation to Asst. Professor T.S. Yamamoto for his kind guidance throughout the course of this study and reading through the manuscript. Techinical advice was given by Mr. Y. Takakuwa to whom the author is much grateful.

References

Cloney, R.A. 1966. Cytoplasmic filaments and cell movements: epidermal cells during ascidian metamorphosis. J. Ultrastruct. Res. **14**: 300–328.

———— 1969. Cytoplasmic filaments and morphogenesis: the role of the notochord in ascidian metamorphosis. Z. Zellforsch. **100**: 31–53.

Conrad, G.W. and D.C. Williams 1974. Polar lobe formation and cytokinesis in fertilized eggs of *Ilyanassa obsoleta*. I. Ultrastructure and effects of cytochalasin B and colchicine. Develop. Biol. **36**: 363–378.

Conrad, G.W., D.C. Williams, F.R. Turner, K.M. Newrock and R.A. Raff 1973. Microfilaments in the polar lobe constriction of fertilized eggs of *Ilyanassa obsoleta*. J. Cell Biol. **59**: 228–233.

Goodenough, D.A., S. Ito and J.P. Revel 1968. Electron microscopy of early cleavage stages in *Arbacia punctulata*. Biol. Bull. **135**: 420.

Henzen, M. 1966. Cytologische und mikroskopische Studien über die ooplasmatische Segregation während der Meiose des *Tubifex*-Eies. Z. Zellforsch. **71**: 415–440.

Hess, O. von 1959. Phasenspezifische Änderung im Gehalt an ungesättigten Fettsäuren beim Ei von *Tubifex* während der Meiosis und der ersten Furchung. Z. Naturforsch. **14b**: 342–345.

Hirao, Y. 1964. Reproductive system and oogenesis in the fresh-water oligochaete, *Tubifex hattai*. J. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. **15**: 439–448.

——— 1965. A method for the observation of oviposition in a fresh-water oligochaete, *Tubifex hattai*. Zool. Mag. **74**: 283–285. (In Japanese with English abstract).

——— 1968. Cytological study of fertilization in *Tubifex* egg. *Ibid.* **77**: 340–346. (In Japanese with English abstract).

Huber, W. 1946. Der normale Formwechsel des Mitoseapparates und der Zellrinde beim Ei von *Tubifex*. Rev. suisse Zool. **53**: 468–474.

Inase, M. 1960. The culture solution of the eggs of *Tubifex*. Sci. Rep. Tôhoku Univ. Ser. IV. (Biol.). **26**: 65–67.

Lehmann, F.E. 1956. Plasmatische Eiorganisation und Entwicklungsleistung beim Keim von *Tubifex* (Spiralia). Naturiwss. **43**: 289–296.

——— und M. Henzen 1963. Zur Mikrocytologie der Meiose und Mitose von *Tubifex*. Rev. suisse Zool. **70**: 298–304.

Luft, J.H. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. **9**: 409–414.

Matsumoto, M. and M. Kusa 1966. Time-lapse cinemicrographic recording of *Tubiex*-eggs during maturation and early cleavage. Zool. Mag. **75**: 270–275. (In Japanese with English abstract).

Penners, A. 1922. Die Furchung von *Tubifex*. Zool. Jb. Abt. Anat. Ontog. **43**: 323–367.

Raff, R.A. 1972. Polar lobe formation by embryos of *Ilyanassa obsoleta*. Effects of inhibitors of microtubule and microfilament function. Exptl. Cell Res. **71**: 455–459.

Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**: 208–211.

Rötheli, A. 1949. Auflössung und Neubildung der Meiosespindel von *Tubifex* nach chemischer Behandlung. Rev. suisse Zool. **56**: 322–326.

——— 1950. Chemische Beeinflussung plasmatischer Vorgänge bei der Meiose des *Tubifex*-Eies. Z. Zellforsch. **35**: 69–109.

Schroeder, T.E. 1970. Neurulation in *Xenopus laevis*. An analysis and model based upon light and electronmicroscopy. J. Embryol. exp. Morph. **23**: 427–462.

——— 1972. The contractile ring. II. Determining its brief existence, volumetric changes, and vital role in cleaving *Arbacia* eggs. J. Cell Biol. **53**: 419–434.

Selman, G.G. and M.M. Perry 1970. Ultrastructural changes in the surface layers of the newt's egg in relation to the mechanism of its cleavage. J. Cell Sci. **6**: 207–228.

Spudich, J.A. 1972. Effects of cytochalasin B on actin-filaments. Cold Spring Harbr. Sym. Quant. Biol. **37**: 587–593.

Szollosi, D. 1970. Cortical cytoplasmic filaments in cleaving eggs: a structural element corresponding to the contractile ring. J. Cell Biol. **44**: 192–209.

Wessels, N.K., B.S. Spooner, J.F. Ash, M.O. Bradley, M.A. Luduna, E.L. Taylor, J.T. Wrenn and K.M. Yamada 1971. Microfilaments in cellular and developmental processes. Science **171**: 135–143.

Woker, H. 1944. Die Wirkung des Colchicins auf Furchungsmitosen und Entwicklungsleistungen des *Tubifex* Eies. Rev. suisse Zool. **51**: 109–170.

Wrenn, J. and N.K. Wessels 1970. An ultrastructural study of lens invagination in the mouse. J. exp. Zool. **171**: 359–367.

Explanation of Plates

Figs. 4 and 5. Electron micrographs showing the cortical layer of the animal hemisphere of the egg during the first deformation movement. Microfilaments arranged parallel (arrows in Fig. 4) or obliquely (arrows in Fig. 5) to the egg surface are seen. Note the compact cytoplasmic matrix and the absence of cellular organelles in the cortical layer. Mt, mitochondria.

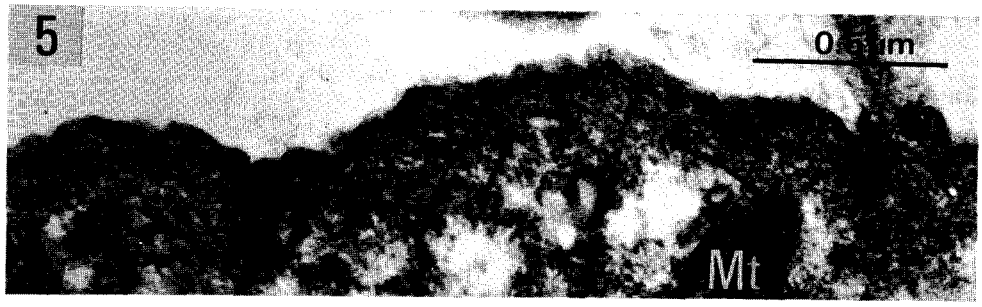
Figs. 6 and 7. The cortical layer covering the animal pole (Fig. 6) or constituting the floor of the groove (Fig. 7) appeared in the animal hemisphere of the egg undergoing the first deformation movement. Note a clear difference in the orientations of microfilaments (emphasized by black lines). ER, endoplasmic reticulum; Mf, microfilament; Mt, mitochondria.

Fig. 8. Electron micrograph showing the section of groove formed by the second deformation movement. Note that the cortical layer of protuberance (Prt) contains no microfilaments. Fig. 8b is a high magnification of the area outlined in Fig. 8a. ER, endoplasmic reticulum; Mf, microfilament; Mt, mitochondria.

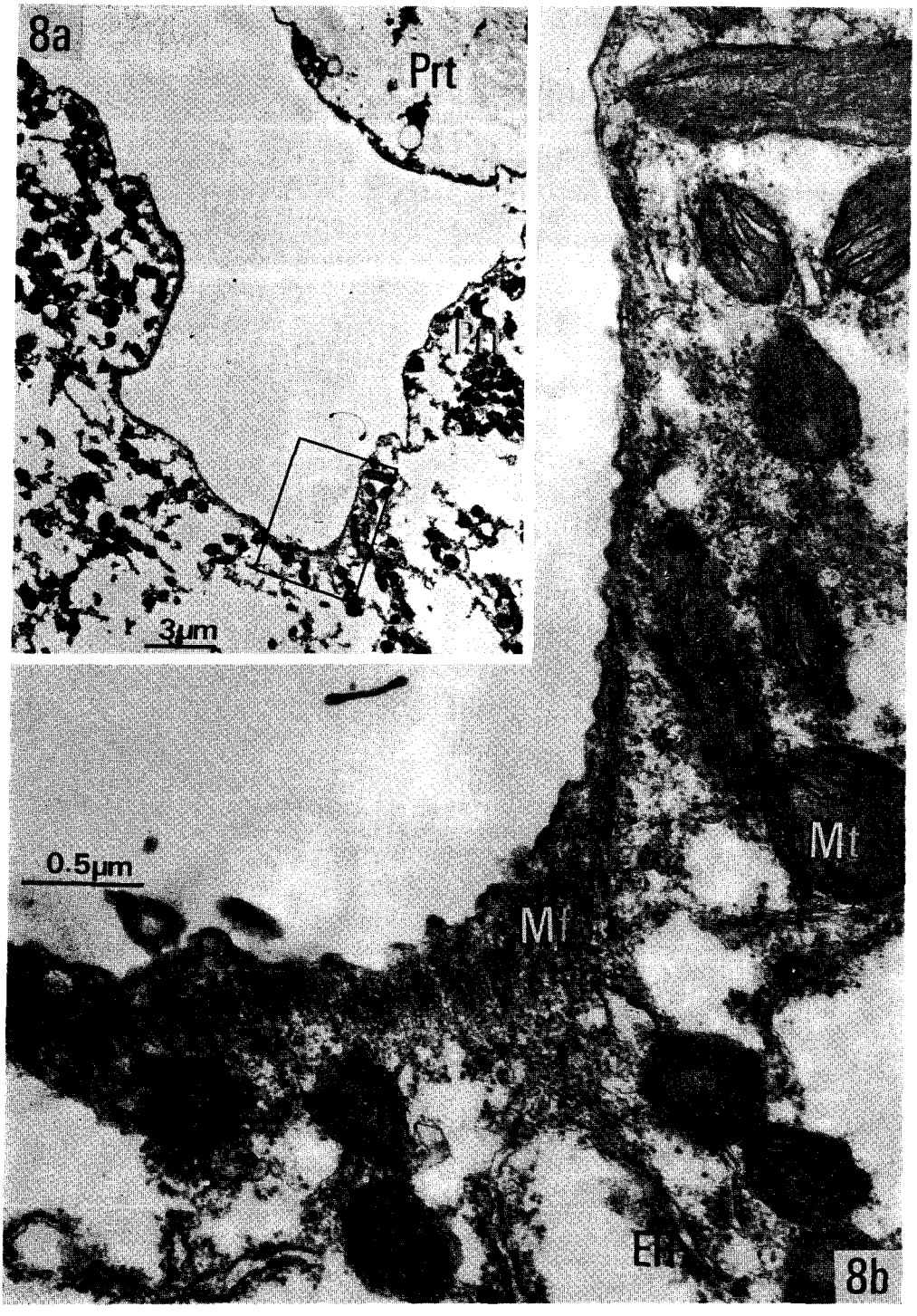
Fig. 9. The cortical layer of the vegetal hemisphere of the egg undergoing the second deformation movement. The orientation of microfilaments is oblique to the egg surface. Fig. 9b is a high magnification of the area outlined in Fig. 9a and shows a direct association of microfilaments with the plasma membrane. ER, endoplasmic reticulum; Mf, microfilament; Mt, mitochondria.

Fig. 10. Electron micrograph showing the cortical layer of the vegetal hemisphere of the egg undergoing the second deformation movement. Note the narrow space of cytoplasmic matrix between the plasma membrane and the bundles of microfilaments (Mf). ER, endoplasmic reticulum; Mt, mitochondria.

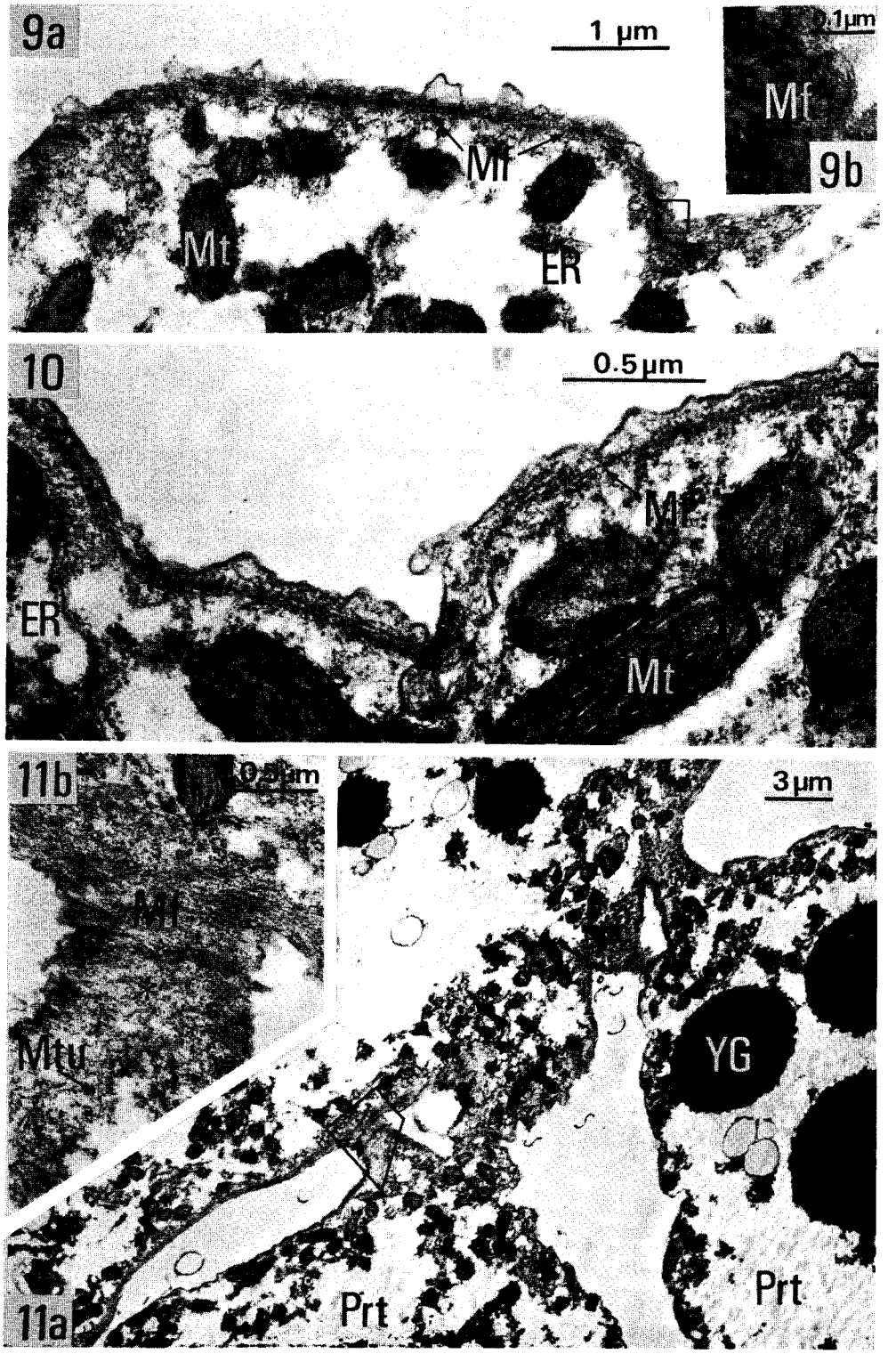
Fig. 11. Tangential section of the floor of groove formed by the second deformation movement. Note the absence of microfilaments in protuberances (Prt). Fig. 11b is a high magnification of the area outlined in Fig. 11a and shows a number of punctuate components (asterisk) representing the sagittal sections of microfilaments. Mf, microfilament; Mt, mitochondria; Mtu, microtubule; YG, yolk granule.



T. Shimizu: Microfilaments in Tubifex *Egg*



T. Shimizu: Microfilaments in Tubifex *Egg*



T. Shimizu: Microfilaments in Tubifex *Egg*