Mem. Fac. Educ., Shimane Univ. (Nat. Sci.) Vol. 13. pp. 17–23. December 1979.

Breakdown of Nuclear Envelope Observed in Cultured Newt Cells with Phase-Contrast Microscopy

Takeshi SETO*

Abstract : With a technique of phase-contrast, time-lapse cinematography, the breakdown of nuclear envelope in cultured newt lung cells was analysed from the selected film sequences. Prophase chromosomes showed a centrifugal movement in the nuclear cavity during about 20 to 30 minutes prior to the nuclear envelope breakdown. The first visible alteration indicating the breakdown at the end of prophase was a conspicuous dent of the membrane. A small break was seen in this area where the centrioles were supposed to be oriented. Then the envelope disrupted into larger segments after the invaginated portion of the membrane ruptured. The disruption extended over other segments of the envelope gradually, which proceeded for about 6 to 12 minutes from the initiation of breakdown. The result indicated that the initial point always oriented perpendicularly to the equatorial plate of the metaphase.

Introduction

The presence of a membraneous envelope enclosing the nuclear material is one of the essential features of eukaryote cells. The nuclear envelope has been revealed by the electron microscope as a complex double membraned structure. Its structural complexity remained many unsolved questions on the structure and function (Feldherr 1972, Franke and Scheer 1974). Behavior of the nuclear envelope in mitosis is especially one of the most misterious phenomena. In the course of cell division, the nuclear envelope undergoes a cyclic breakdown and reformation. A programed breakdown takes place in mitotic prometaphase and meiotic diakinesis in many, though not all, organisms.

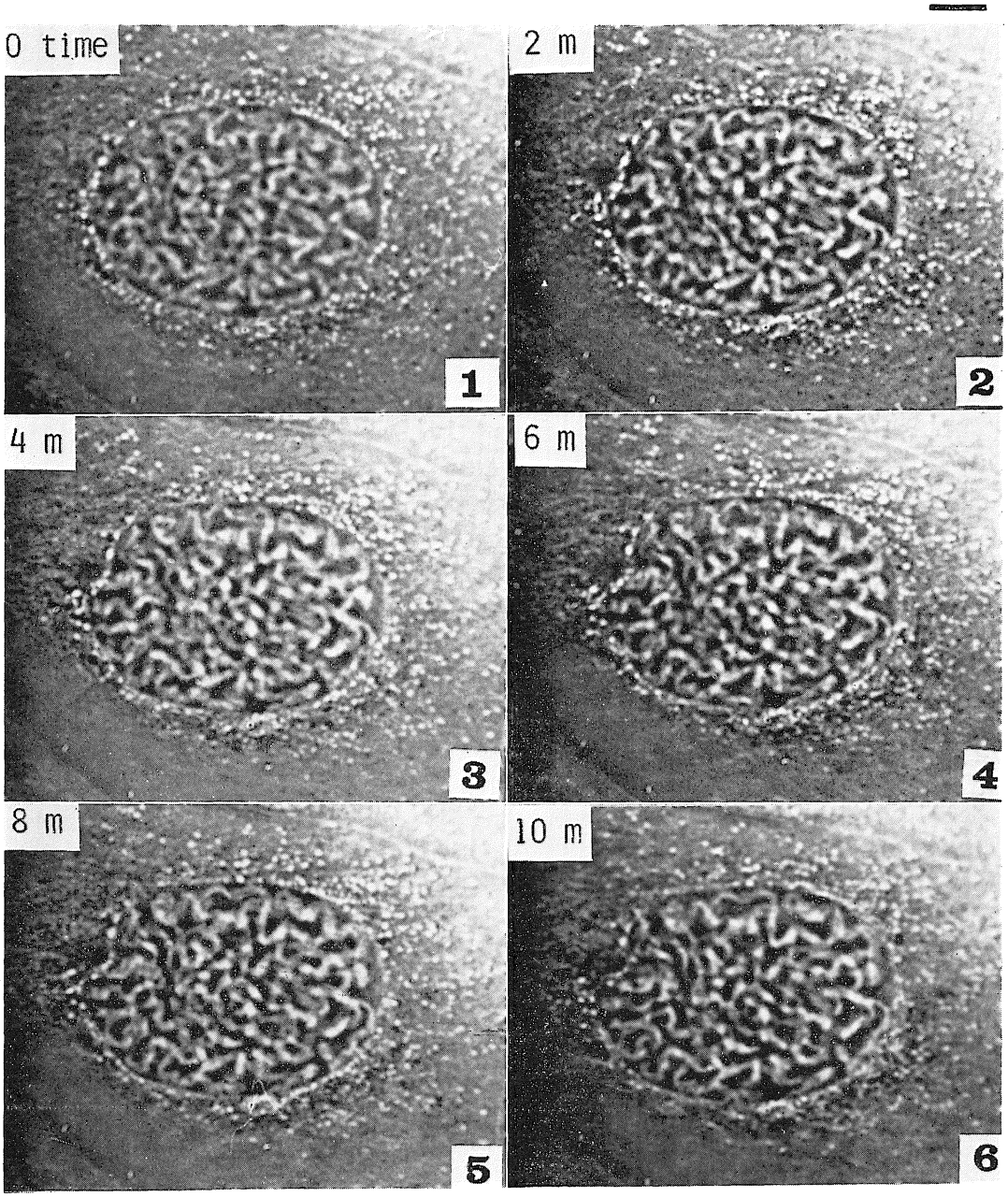
Several reports, as viewed in the electron microscope, illustrated the disappearance of nuclear envelope during mitosis (Robins and Gonatas 1964, Murray *et al.* 1965, Chang and Gibley 1968, Roos 1973). However, the process was scarecely observed in living cells. Izutsu (1968) reported detailed observation of prophase cell alterations in living spermatocytes of grasshopper. With somatic cells in culture the present study was made focusing on the manner of the breakdown of nuclear envelope in the living cells.

Materials and Methods

Selected cells in mitosis were obtained from cultured lung tissue of newts, *Taricha granulosa* and *Cynops pyrrhogaster*. The *in vitro* culture technique and nutrient medium employed were the same as that described by Seto and Rounds (1968). Continuous observation with phase-contrast microscopy of living cells was made use of the Rose multipurpose culture chamber (Rose *et al.* 1958). The cells grown in the chamber with the necessary optical properties facilitated a long-term phase cinematographic record.

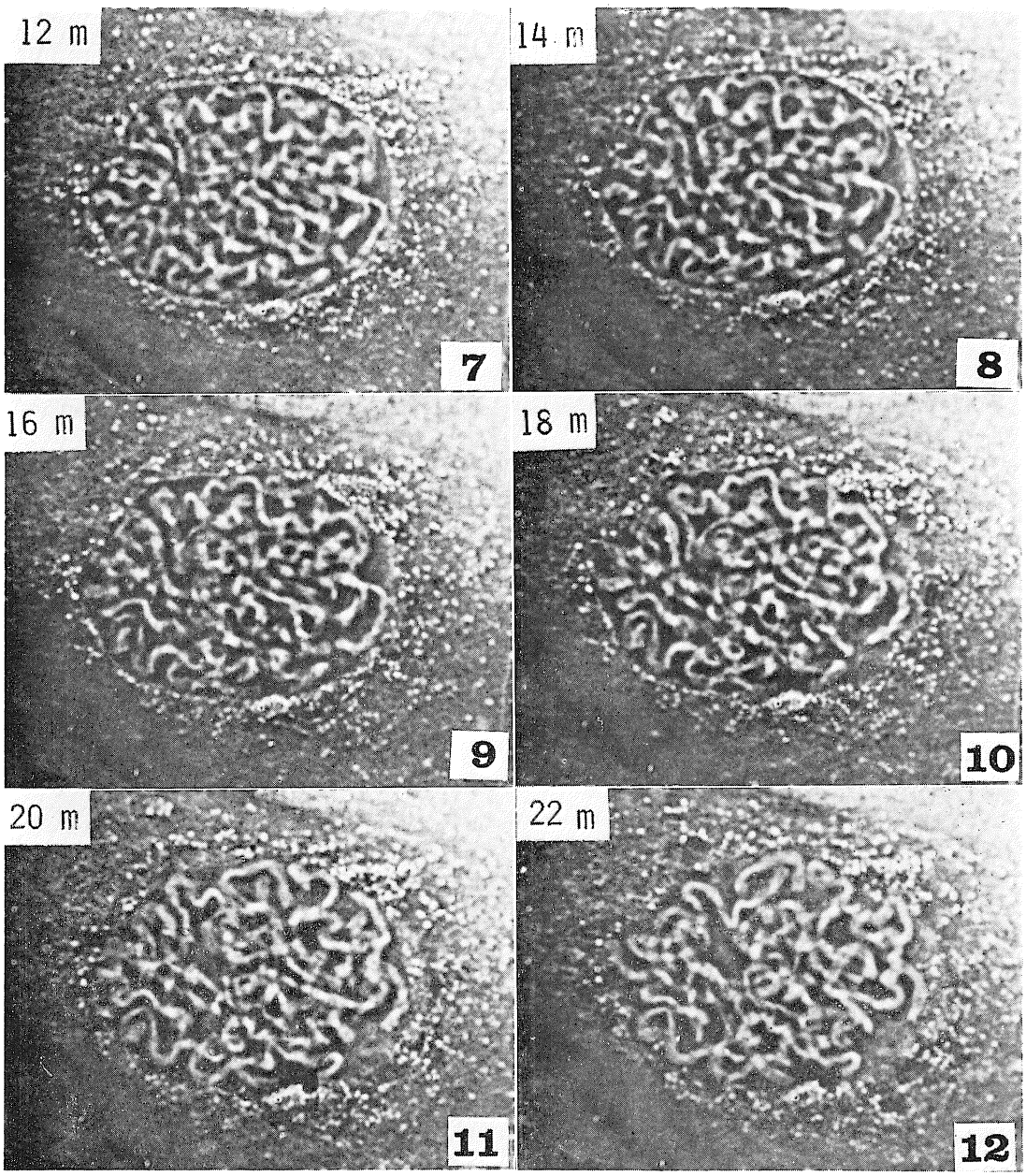
Motion picture records of selected cells with 16 mm monochrome film (Kodak : R 66) were made at 8 frames per minute with the aid of Cine Kodak Camera. Sequences of

* Department of Biology, Faculty of Education, Shimane University, Matsue, Japan 690.



Figures 1 to 12. Process of the nuclear envelope breakdown at prophase in a living newt cell in culture. Each photomicrograph was taken at intervals of two minutes. These were enlarged with selected film frames of 16 mm cinerecord. Scales on upper right of both plates indicate 10 micra. 10X ocular, 40X objective, phase-contrast optics (Zeiss).

Fig. 1, a nucleus at middle prophase, the membrane can be clearly traced along its whole



surface. Figs. 4–6, a conspicuous dent of the nuclear envelope is seen on upper left of the nucleus, where centrioles located. Figs. 8–10, the envelope disrupts into several larger fragments after the invaginated portion disappeared. Figs 11 & 12, disappearance of the membrane expands over the whole surface of the nucleus and chromosomes rapidly move toward the equatorial plate.

nuclear envelope alteration at prophase were projected from film frames onto tracing paper, utilizing the cinefilm perforations as reference points for alignment. Selected frames taken every two minutes were printed on photographic paper for figure plates.

Observations

A common type of cell originating in cultures of newt lung was the epithelial-like cell. Under the dialysis membrane in the Rose chamber, the cells in the outgrowth became flattened to form a monolayer of epithelium on the coverglass, and mitotic figures were found among outgrowing cells within 6 or 7 days of the cultivation at 26°C. The newt cell was a particularly favorable material for study of cell division, since the beginning of mitotic prophase was more distinguishable among interphase cells than was found in mammalian mitotic cells. Nearly every mitotic figures were observable with good resolution and structural detail with the phase-contrast microscope due to the cellophane technique employed.

The beginning of prophase was recognized by the appearance of chromosomes as thin threads inside the nucleus. As the prophase progresses, the chromosomal condensation was observed, which makes clear visualization of chromosome set distributed in the nuclear cavity (Figs. 1–3). During the nuclear envelope persisted in recognizable form, whole chromosomes showed a centrifugal movement in the nuclear cavity. This movement lasted for about 20 to 40 minutes prior to the envelope disruption.

There was a remarkably different view between inside and outside of the nuclear envelope before its breakdown. In contrast to abundant granules on the periphery of the membrane, nuclear cavity beside chromosomes appeared a more homogeneous structure without any granular materials. Accordingly the occurrence of the membrane disruption can be detected by the rapid alteration of this contrast even under lower magnification.

The first visible alteration indicating the breakdown was a conspicuous tortuorsity or dent of the membrane, which indicated partial penetration into tne nucleus (Figs. 4 & 5). A small break was seen initially in the penetrated area where was the centriole region and the aster was supposed to be oriented (Fig. 6). While these processes were taking place in the nucleus, the two pairs of centrioles, each one surrounded by the so-called aster, migrated toward the poles along with the asters. The phenomenon was not conspicuously observed by the phase-contrast microscopy, but polarized optics well visualized the migration of centrioles in the same cells (Sato, personal communication).

The nuclear envelope initially disruption into larger fragments after the invaginated membrane disappeared and gaps between these fragments were quite small in the beginning (Figs. 7 & 8). Then larger fragments somewhat undulated before the complete breakdown occurred. The disruption extended gradually over other segments of nuclear envelope, which proceeded for about 6 to 12 minutes from the initial feature of breakdown as measured with cinematographic records (Figs. 9 to 11). The initial point of breakdown would be related to the spindle axis, which oriented perpendicularly to the equatorial plate of the metaphase.

Discussion

The brief description on the process of the nuclear envelope breakdown in the somatic cells of newts was ascribed to inadequate number of instances. However, it is of significance to provide manifold informations on the breakdown comparing living aspects with the ultrastructures. The present observation revealed the breakdown initiated at the centriolar region which was indicated by the partial penetration of membrane into the nuclear cavity. Also a manner of membrane disruption was mentioned.

Ultrastructural studies of dividing animal cells have been published by many workers (Robbins and Gonatas 1964, Krishan and Buck 1965, Murray *et al.* 1965, Brinkley and Stubblefield 1970, McIntosh and Landis 1971, and others). They dealt mainly with general features and specialized structures of mitotic apparatus, and there is virtually no information on the breakdown of nuclear envelope. Exceptionally, Roos (1973) reported more comprehensive ultrastructural study of mammalian cells in mitosis and dealt with alterations of the structure of nuclear envelope at prophase. He stated that regardless of the position of the asters relative to the nucleus, fragmentation of the envelope always began in their vicinity. Accordingly the membrane was fragmented at one or two sites possibly the site of parent centriole position.

A rare example on the study of the nuclear envelope breakdown was presented by Izutsu (1958). With living spermatocytes of grasshoppers he observed the aspects of the disruption at diakinesis of the first division and demonstrated the relations between centriolar region and initial point of the breakdown. He noticed that the nuclear membrane protruded towards the centriole only at the portion touched with the astral rays, and then the membrane began disappearance at its protruding portion at prometaphase. The possibility of surface-active substances involved has long been suggested for the mechanism of the breakdown (Anderson 1953). Recent works extended a considerable interpretation that the invagination of the nuclear envelope was attributed to forces applied by asters or microtubules (Bajer and Molé-Bajer 1969, 1972). They presented evidences from the events in living *Haemanthus* endosperm cells, that microtubules connected to the envelope caused rupturing by pulling and pushing. Our film sequences of similar records suggested the above interpretation that the presence of a certain cite to initiate the disruption, although phase-contrast optics did not show distinct association between microtubules and nuclear envelope. Similarly Sato (1975) stated that nuclear membrane breakdown may be caused by the rapid invasion of spindle microtubules previously located outside of the envelope. He further estimated the speed of invasion at 10−12 μm/min in the endosperm cell division of *Haemanthus katherinae*.

In addition to this mechanical pressure exerted by the microtubules, the substances such as enzymes released from nucleoplasm or perinuclear space were supposed to act on the nuclear envelope breakdown. This mechanism could work either independently or in conjunction with enzymatic processes (Feldherr 1972). However, it is not exactly known what induces the membrane disintegration. Neither the molecular mechanism nor the biological function of the nuclear envelope breakdown is understood (Franke and Scheer 1974).

We saw large fragments of intact portions of the nuclear envelope remained in the both side of spindle axis for two thirds of the duration needed for whole membrane

disintegration, although electron micrograph of mouse oocyte nucleus represented that membrane broken into several large segments (Szollosi *et al.* 1972). We measured approximately 6 to 12 minutes required for completion of breakdown in the somatic newt cell. According to the measurements by Szollosi *et al.* (1972) the time for the mouse follicular oocyte maturing *in vitro* took about three and one-half hours. This is only an example available for comparing the time required, which indicated a great difference between somatic cell and gamete cell nuclei.

A centrifugal movement of chromosomes, originally noticed by Shimakura (1957), was evidently seen in the somatic cell shortly before the nuclear envelope breakdown. The identical movement has been widely observed by other investigators in meiotic prophase in the first division of grasshopper spermatocytes (Shimakura 1957, Izutsu 1958). However, no interpretation on the source of the movement has been presented yet.

Acknowledgments : I wish to thank the late Dr. Charles M. Pomerat and Dr. James Kezer for their valuable advice through this study. My appreciation extended to Mr. George Lefeber for many helpful suggestions, and to Mr. Etsuo Watanabe who provided the equipment for film analysis.

References

Anderson, N. G. 1953. On the nuclear envelope. Science **117**, 517–521.

Bajer, A. and Molé-Bajer J. 1969. Formation of spindle fibers, kinetochore orientation, and behavior of the nuclear envelope during mitosis in endosperm. Chromosoma **27**, 448–484.

Bajer, A. and Molé-Bajer, J. 1971. Architecture and function of the mitotic spindle. In : Advances in Cell and Molecular Biology (E. J. DuPraw, ed.), Academic Press, New York, Vol. 1, pp. 213–266.

Brinkley, B. R. and Stubblefield, E. 1970. Ultrastructure and interaction of the kinetochore and centriole in mitosis and meiosis. In : Advances in Cell Biology (D. M. Prescott, L. Goldstein and E. McConkey, eds.) Appleton-Century-Crofts, New York. Vol. 1, pp. 119–185.

Chang, J. P. and Gibley, C. W., Jr. 1968. Ultrastructure of tumor cells during mitosis. Cancer Res. **28**, 521–534.

Feldherr, C. M. 1972. Structure and function of the nuclear envelope. In : Advances in Cell and Molecular Biology. (E. J. DuPraw, ed.), Academic Press, New York, Vol. 2, pp. 273–307.

Franke, W. W. and Scheer, U. 1974. Structure and functions of the nuclear envelope. In : The Cell Nucleus (H. Busch, ed.), Academic Press, New York, Vol. 1, pp. 220–345.

Izutsu, K. 1958. Phase-contrast cinematographic studies on meiosis in Orthopteran spermatocytes I. Development of the chromosomal spindle fibers and the kinetic behavior of the chromosomes before metaphase. Cytologia **23**, 485–495.

Krishan, A. and Buck, R. C. 1965. Structure of the mitotic spindle in L strain fibroblasts. J. Cell Biol. **24**, 433–414.

McIntosh, J. R. and Landis, S. C. 1971. The distribution of spindle microtubules during mitosis in cultured human cells. J. Cell Biol. **49**, 468–497.

Murray, R. G., Murray, A. S. and Pizzo, A. 1965. The fine structure of mitosis in rat thymic lymphocytes. J. Cell Biol. **26**, 601–619.

Robbins, E. and Gonatas, N. K. 1964. The ultrastructure of a mammalian cell during the mitotic cycle. J. Cell Biol. **21**, 429–463.

Roos, U. P. 1973. Light and electron microscopy of rat kangaroo cells in mitosis I. Formation and breakdown of the mitotic apparatus. Chromosoma **40**, 43–82.

Rose, G. G., Pomerat, C. M., Shindler, T. O. and Trunnell, J. B. 1958. A cellophane-strip technique for culturing tissue in multipurpose culture chambers. J. Biophysic. Biochem. Cytol. 4, 761–764.

Sato, H. 1975. The mitotic spindle. In : Aging Gametes. Internationl Symposium, Seattle 1973, Karger, Basel, pp. 19–49.

Seto, T. and Rounds, D. E. 1968. Cultivation of tissues and leukocytes from amphibians. In : Methods in Cell Physiology (D. M. Prescott, ed.), Academic Press, New York, Vol. 3, pp. 75–94.

Shimakura, K. 1957. Some observational facts and their interpretations concerning the mechanism of prometaphase movement in mitosis (in Japanese). Symposia Soc. Cell. Chem. 5, 201–212.

Szollosi, D., Calarco, P. G. and Donahue, R. P. 1972. The nuclear envelope : Its breakdown and fate in mammalian oogonia and oocytes. Anat. Rec. 174, 325–340.