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EXTRUSION OF NUCLEAR MATERIALS INTO CYTOPLASM IN THE POSTERIOR SILK GLAND CELLS OF SILKWORM, *BOMBYX MORI*

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INTRODUCTION

The transfer of microscopically visible material from the nucleus into the cytoplasm has been described from time to time for many years (1). Application of electron microscopic techniques to the study of nucleocytoplasmic interactions has made it clear that several methods may exist by which materials or substances are transported between the nucleus and cytoplasm (2). The pores or annuli of the nuclear envelope appear to represent, in most cases, main sites of nucleocytoplasmic exchange (3–5). Further, a variety of blebs and outpocketings of the nuclear envelope have been suggested to represent a morphological stage in the transfer of nuclear material to cytoplasm (6, 7, 2).

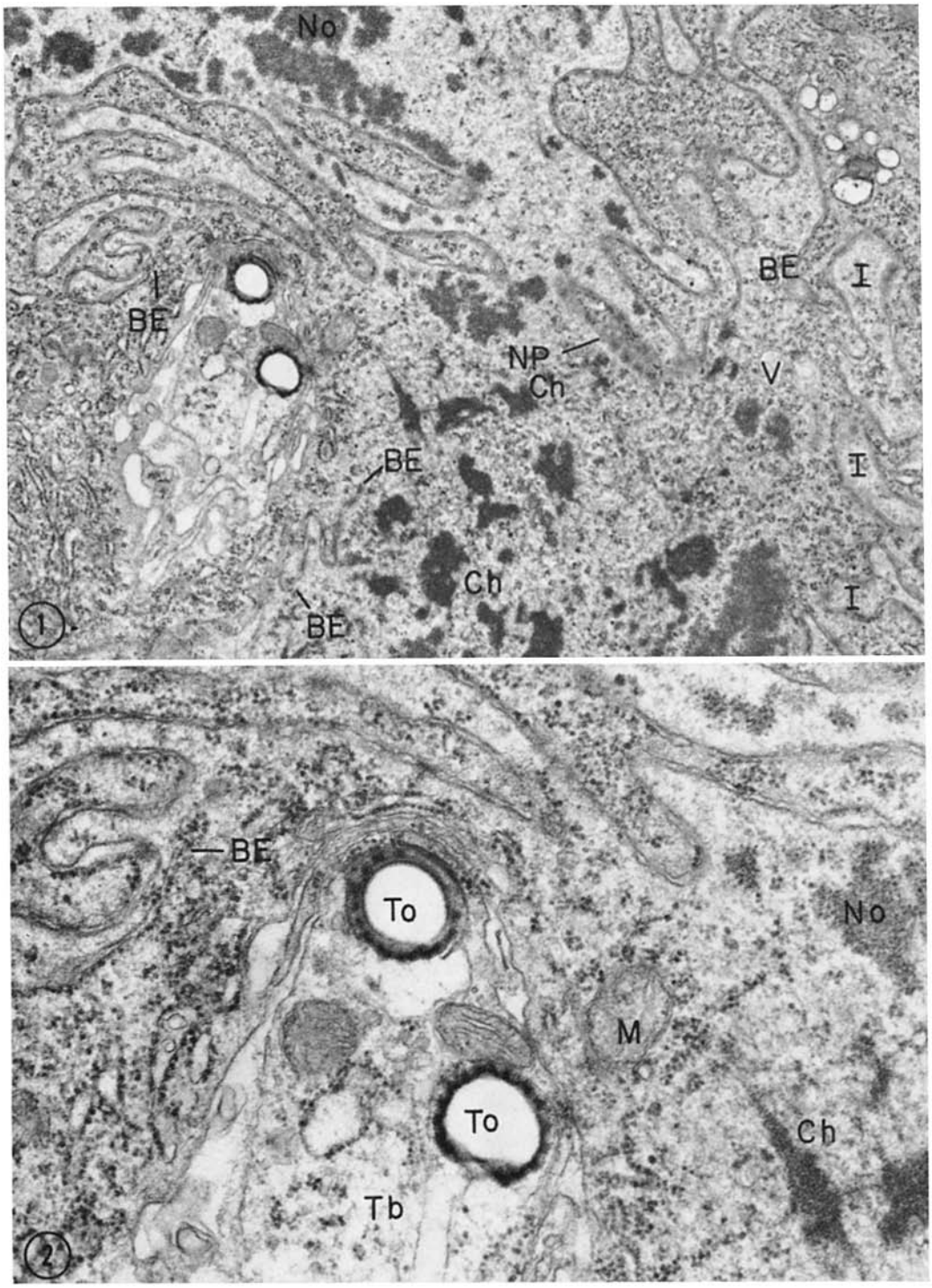
While studying the ultrastructure of the cells of posterior silk gland during the fourth and fifth larval instars, we have had a chance to observe the partial disappearance of the nuclear envelope and the subsequent extrusion of the nuclear materials directly into cytoplasm.

MATERIALS AND METHODS

A hybrid of strain Shingyoku x Gunko of the silkworm, *Bombyx mori*, was exclusively used, and the season of rearing was spring. The larvae were fed mulberry leaves and cultivated at 25° ± 1°C. Events in the larval instar have been precisely timed in hours from the moment of the preceding eclosion from exuviae. Under the present experimental conditions, the duration of the fourth and the fifth larval instars was 120 ± 6 and 192 ± 9 hr, respectively. The specimens for electron microscopy were prepared successively from the beginning of the fourth instar at intervals of 6 ~ 24 hr. Posterior silk glands, dissected out of the larvae, were immediately fixed for several hours in ice-cold 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 (8). After having been washed several times with the same buffer, the silk glands were postfixed for another several hours with 1% OsO_4 in the same buffer. Block staining with uranyl acetate was frequently used to increase contrast (9). Specimens were dehydrated with alcohol and embedded in Epon 812 according to Luft's method (10). Sections were cut on a Porter-Blum MT-2 ultramicrotome, doubly stained with uranyl acetate (11) and lead citrate (12), and examined with a JEM-6C electron microscope.

OBSERVATIONS

Fig. 1. shows a posterior silk gland cell 12 hr after the beginning of the fourth larval instar, and Fig. 2 is the higher manification of a part of Fig. 1. As has been reported by various authors (13–15), a silk gland cell has a large, complicatedly ramified nucleus loaded with large numbers of nucleoli and chromatin blocks. These two nuclear bodies are



FIGURES 1–3 Figs. 1 and 3 are electron micrographs of the posterior silk gland cells 12 hr after the beginning of fourth instar. Fig. 2 is a higher magnification of a part of Fig. 1. In both cases, the nuclear envelope is partially lost, the nucleoli (*No*) and chromatin blocks (*Ch*) being in direct contact with cytoplasm. In Figs. 1 and 2, blind ends of the nuclear envelope (*BE*) are observable. *To*, tracheolus; *Tb*, tracheoblast; *NP*, nuclear pore; *M*, mitochondria; *V*, cytoplasmic vesicle; *I*, islet of nucleus. Fig. 1, × 15,000; Fig. 2, × 40,000; Fig. 3, × 26,000.

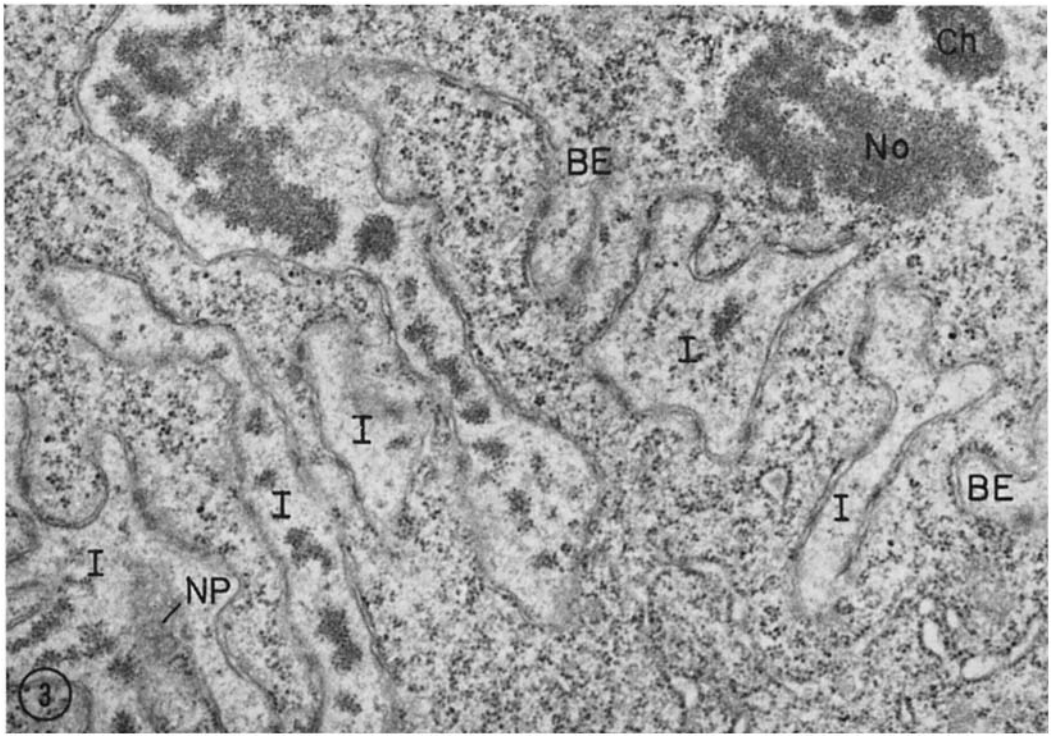
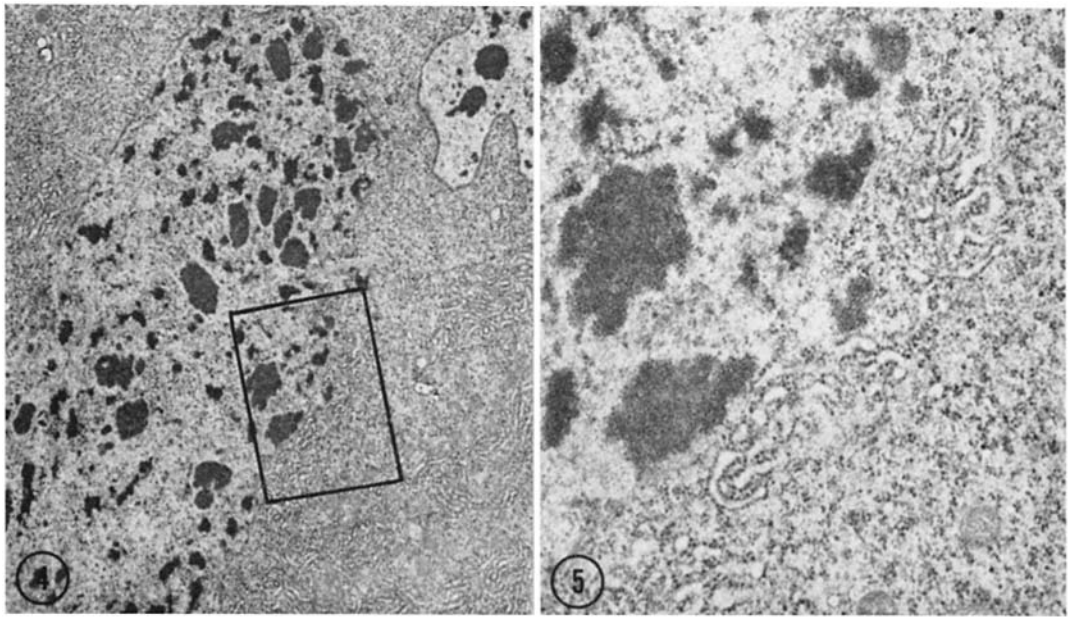


FIGURE 3 See legend under Figs. 1 and 2.

sometimes difficult to distinguish from one another. The nucleoli are, however, usually associated with ribosome-like particles and are slightly less dense than the chromatin blocks. The nucleus is usually surrounded completely by a nuclear envelope, on which a number of usual nuclear pores are found. In the middle part of Fig. 1, however, a large opening in the nuclear envelope is clearly shown. In the lower part of this micrograph a mitochondrion, chromatin blocks, and nucleoli exist in the same compartment. Blind ends (*BE*) of the perinuclear cisterna can be identified in both figures. Fig. 3 shows another cell of the posterior silk gland. The upper right part of this micrograph appears to be a nuclear region, as judged from the existence of nucleoli and chromatin blocks, while the lower part is recognizable as a cytoplasmic region, on account of its free ribosomes and rough endoplasmic reticulum (ER). What is peculiar is that the nuclear and cytoplasmic regions are in direct continuity with each other, without being separated by a nuclear envelope. Between these two regions, there are two isolated profiles of nuclear lobes, each completely surrounded by its own envelope. Blind ends of the perinuclear cisterna are observed on the both sides of these "islets." Three similar nuclear islets can be seen on the lower left side of this micrograph. They could be true islets of nucleoplasm or could be just peninsulas of nucleoplasm still in continuity with the nuclear main body in another plane than that of the section.

These electron micrographs suggest that the extrusion of nuclear materials into the cytoplasm is preceded by extensive, irregular infoldings of the nuclear envelope followed, in some cases, by fusion of adjacent folds which results in the formation of a nuclear islet and in the opening of large gaps in the nuclear envelope through which nucleoplasm and cytoplasm come into direct contact. This interpretation is supported by the fact that the direct communication of nucleus with cytoplasm is frequently observed in such irregularly infolded regions of the nucleus.

A complicated infolding of the nuclear envelope, however, does not always seem to be a necessary condition for the direct contact of the nucleoplasm



FIGURES 4 and 5 Fig. 4 shows that loss of the nuclear envelope also has been observed on even parts of the nuclear surface. Fig. 5 is higher magnification of a part of Fig. 4. In this part, the nuclear envelope is not observed. Fig. 4, × 3,800; Fig. 5, × 25,000.

with the cytoplasm, because loss of the nuclear envelope also has been observed on even parts of the nuclear surface (Fig. 4). Fig. 5 is a higher magnification of a part of Fig. 4.

Nothing is known about the fate of these nuclear materials which are extruded from the nucleus. It is suggested that they function for a while in the cytoplasm and then disappear completely. Fig. 6 shows the nuclear materials extruded into the cytoplasm. That the extruded nucleolus is associated with a number of ribosome-like particles suggests that this nucleolus is still actively forming ribosomes. Since the nuclear materials were not found in the cytoplasm from the larvae in the later stage of the fourth instar or in the fifth instar, they are probably degraded rapidly.

The cells of the posterior silk gland examined in this experiment are from larvae at the beginning of the fourth instar to the end of the fifth instar, and, so far as we have observed, this extrusion of nuclear materials has been found only in the posterior silk gland cell from the larvae in the logarithmic growth phase of the fourth instar, that is, from larvae 12, 24, 36, 48, and 60 hr old. The frequency of the nuclear envelope discontinuity observed during this phase was 13 cells per approximately 300 cells. This would be the minimum estimate, because only a small part of the individual cells has been observed. The posterior silk gland cells in these later larval instars are so large (18) and, moreover, the complicatedly ramified nucleus is so extensively distributed within the cell that it is quite difficult to observe thoroughly the entire nuclear surface of even a single cell.

DISCUSSION

The silk gland cell of Lepidoptera has an extremely large nucleus, which is extensively and complicatedly ramified, extending to all parts of the cell. Because of this structure, the function of the nucleus and especially the nucleocytoplasmic interaction in the silk gland cells have attracted the attention of a number of investigators. For example, Maziarski (16) first suggested in 1911 the migration of the nucleoli to the cytoplasm. Nakahara (17) confirmed this finding and further described the partial disappearance of the nuclear membrane when the nucleoli are extruded into cytoplasm. This kind of work has often been treated with a certain amount of skepticism, mainly because of limitation in the resolution of the light microscope. The present electron microscopic observations have clearly shown the partial disappearance of the nuclear envelope and the subsequent extrusion of some nuclear materials

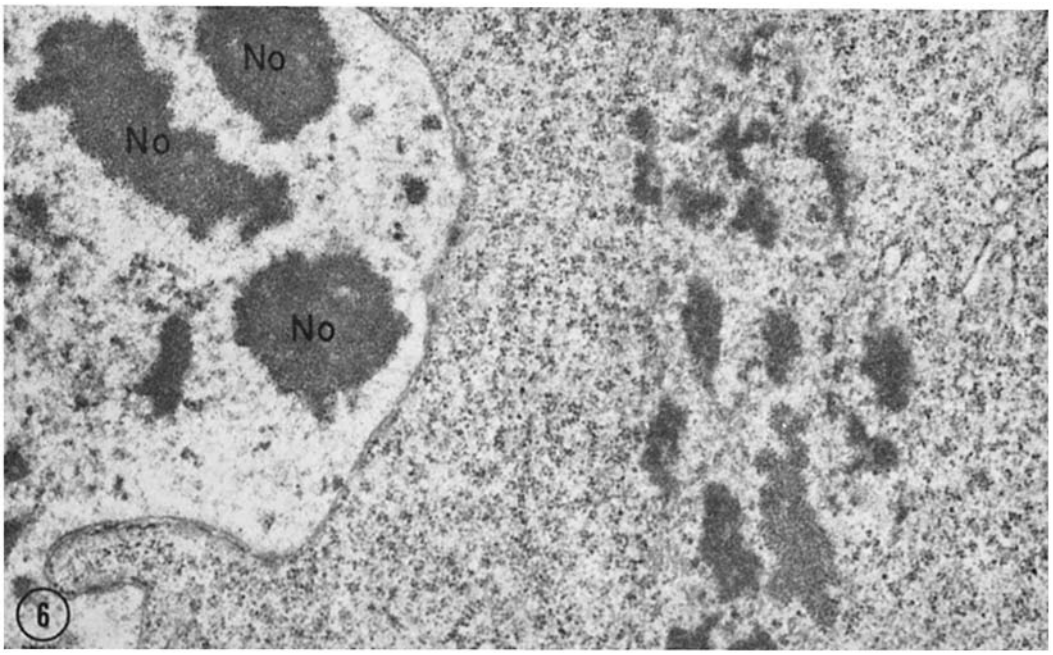


FIGURE 6 Nuclear materials in cytoplasm of the posterior silk gland cell of a fourth instar larva, 12 hr old. Nucleoli (*No*) are associated with ribosome-like particles. Nucleus on the left side is completely covered with a nuclear envelope. × 27,000.

into cytoplasm and have confirmed the suggestions of Maziarski and Nakahara.

Several criticisms, however, should be considered carefully. The first one is that the nuclear envelope is not observed simply because it is tangentially sectioned. It has been repeatedly confirmed that there exist a number of usual nuclear pores on the nuclear envelope in the cells of the posterior silk gland as shown in Figs. 1 and 3. Since no nuclear pore is observable on that part of the nucleus which is supposed to be in direct continuity with cytoplasm, such a possibility could be confidently neglected. The second criticism is that the nuclear envelope is lost because the cell is in division. This also is not probable, because it has been well established that the number of the cells in the posterior silk gland does not increase throughout the larval life, cell division being observed only during the embryonic development (14, 18). It is true that the total amount of DNA in the posterior silk gland of the silkworm increase during the fourth larval instar as has been shown recently,[1] and probably endomitosis is responsible for it. It is not probable that the nuclear envelope is lost during such endomitosis, because, if so, nuclear extrusion also should be observed in the logarithmic growth phase of the fifth instar. The third criticism is that the cells were observed here under pathological conditions. However, all the silkworms, except those which were sacrificed for our experiments, had grown well and had made normal cocoons; moreover, the whole cell or that part of the cell which shows this extrusion phenomenon did not show any sign of degenerative or pathological changes, at least from the morphological point of view. The last possibility is, therefore, not probable, though it is difficult for us to exclude it definitely.

The biological significance of this extrusion phenomenon is not at all clear at the present time. It may play an active role in the biogenesis of ribosomes and other cytoplasmic organelles, or it may be a mechanism for removal of excess nuclear

[1] Morimoto, T., S. Matsuura, S. Nagata, and Y. Tashiro. Manuscript in preparation.

materials, as in the case of the extrusion of small tertiary nucleoli from the pronuclei of rat (19).

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REFERENCES

1. WILSON, E. B. 1928. The Cell in Development and Heredity. The Macmillan Company, New York.
2. WISCHNITZER, S. 1960. *Intern. Rev. Cytol.* **10**:137.
3. ANDERSON, E., and H. W. BEAMS. 1956. *J. Biophys. Biochem. Cytol.* **2**(Suppl.):439.
4. KESSEL, R. G. 1966. *J. Ultrastruct. Res.* **15**:181.
5. STEVENS, B. J., and H. SWIFT. 1966. *J. Cell Biol.* **31**:55.
6. GAY, H. 1956. *J. Biophys. Biochem. Cytol.* **2**(Suppl.): 393.
7. CLARK, W. H. 1960. *J. Biophys. Biochem. Cytol.* **7**:345.
8. SABATINI, D. D., K. BENSCH, and R. J. BARRNETT. 1963. *J. Cell Biol.* **17**:19.
9. FARQUHAR, M. G., and G. E. PALADE. 1965. *J. Cell Biol.* **26**:263.
10. LUFT, J. H. 1961. *J. Biophys. Biochem. Cytol.* **9**: 409.
11. WATSON, M. L. 1958. *J. Biophys. Biochem. Cytol.* **4**:475.
12. REYNOLDS. E. S. 1963. *J. Cell Biol.* **17**:208.
13. HELM, F. E. 1876. *Z. Wiss. Zool. Abt. A.* **26**:434.
14. TANAKA, T. 1928. The Anatomy of the Silkworm. Meibun-Do Co, Tokyo.
15. AKAI, H. 1965. *Bull. Sericult. Exptl. Sta. (Tokyo).* **19**:375.
16. MAZIARSKI, S. 1911. *Arch. Zellforsch.* **6**:397.
17. NAKAHARA, W. 1917. *J. Morphol.* **29**:55.
18. ONO, M. 1951. *Bull. Sericult. Exptl. Sta. (Tokyo).* **13**:247.
19. SZOLLOSI, D. 1965. *J. Cell Biol.* **25**:545.