Electron microscopical observation of the contact between ribosomes and detergent-resistant cytofilaments

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Summary. The contact of cytofilaments with ribosome-like particles in Triton X-100-treated cells of Zajdela ascites hepatoma was revealed. It is suggested that cytofilaments are engaged in the transport of ribosomes from the nuclear surface into definite areas of cytoplasm.

The filaments, 40–120 Å in diameter, have been found in cells of many normal and malignant tissues[1,2], and fulfil structural[3] and contractile[4] functions. The possible role of cytosceleton ultrastructures in intracellular transport[1] and in distribution of so-called free polysomes[5] has been suggested. In this connection, observations of the contact of cytofilaments (CF) with the cell nucleus[1,3,6,7] are of interest. In the present work, ultrastructural localization of CF in the cells of Zajdela ascites hepatoma of rats was studied. For the best observation of CF, the treatment with nonionic detergent polyoxyethylenglycol (9–10) p-t-octylphenol-Triton X-100 (Schuchardt München), which does not influence their distribution in the cytoplasm[5], was applied.

Materials and methods. Ascitic fluid was diluted 1:1 by isotonic saline, pH 7.2, +4 °C, left for 5 min and then treated with 0.05% TX-100 (diluted in saline) at room temperature for 5 min; then cells were fixed in 2 portions of 2.5% glutaraldehyde (phosphate-buffered, pH 7.2) with postfixation in 2% OsO_4 (phosphate-buffered, pH 7.2, with 0.2 M sucrose), 1 h in every fixative. For the control undiluted ascitic fluid was fixed. The pellet of fixed cells was included into agar-agar, dehydrated and embedded into epon-812. Ultrathin sections were contrasted by uranyl acetate and lead citrate according to Reynolds[8], and observed in electron microscope EMV-100L.

Results and discussion. Short treatment of hepatoma cells with 0.05% TX-100 causes partial destruction of cytoplasmic membranes and considerable enlightment of the cytoplasmic ground. Ribosomes bound to membranes and integrity of nuclear envelope are mostly presearved (figure 1). CF, 30–120 Å in diameter, can be seen in the control as well as detergent-treated cells, but in the latter they are more distinct[7]. Large amounts of CF with the diameter 70–90 Å are seen in the vicinity of the nucleus parallelly to its surface. Some of them have a contact with electron-dense particles measuring 170–320 Å, deposited on the outer nuclear membrane and with the annuli of nuclear pores

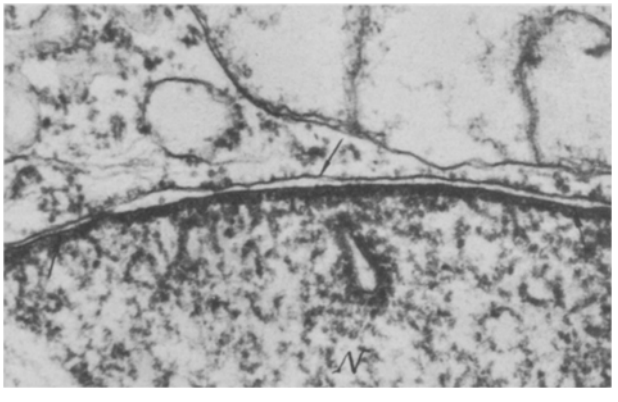
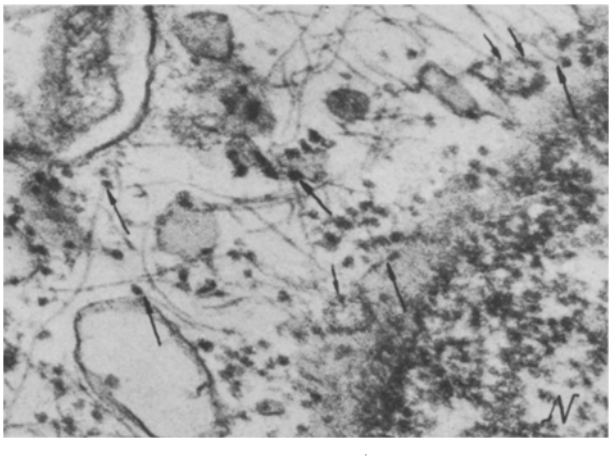
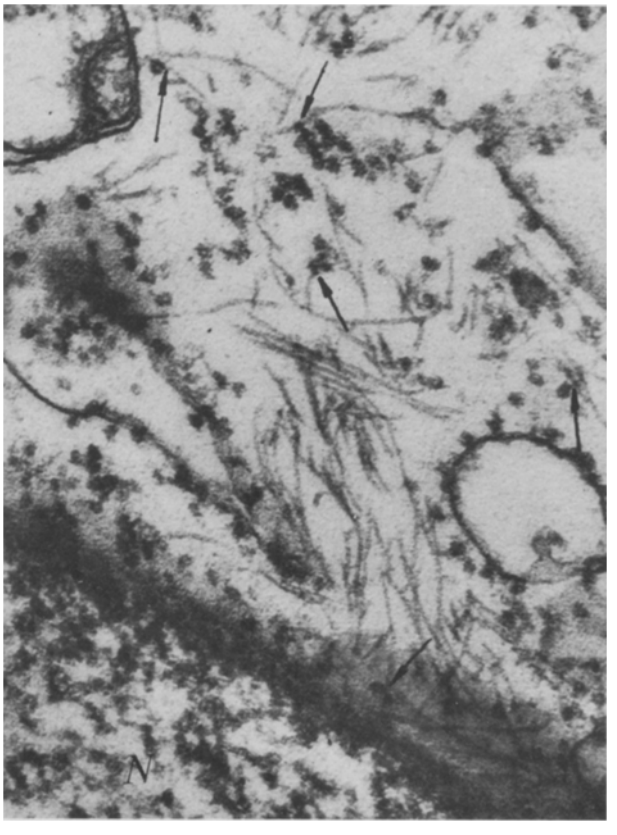


Fig. 1. The fragment of Zajdela ascites hepatoma cell treated with Triton X-100. Cross-section of the nuclear envelope. The integrity of external and internal membranes is preserved (arrows); N, nucleus. × 50,000.





Figures 2 and 3. Tangential sections of the nuclear envelope of Zajdela ascites hepatoma cells, treated with TX-100. Cytofilaments are in a contact with electron-dense particles of the outer nuclear membrane and of cytoplasm (arrows), with the annuli of nuclear pores (short arrows); N, nucleus. Fig. 2, × 60,000. Fig. 3, × 90,000.

(figure 2 and 3). In the cytoplasm of detergent-treated and control cells, similar particles can also be seen in the contact with the ends or middle portions of CF, which are 30-90 Å in diameter. The morphology and contrasting capacities allow us to identify these particles as ribosomes. One can suggest that CF are engaged in the transport of so-called free ribosomes from the nuclear surface into definite areas of cytoplasm, where they function as the membrane-free ribosomes. The failure to confirm the contact of CF with ribosomes in the similar experiments[5,9], is probably due to the destructive action of a relatively high concentrations of TX-100 applied in this work. The data on quite certain distribution of CF in the cytoplasm[10], as well as the fact on the abundance of CF and free ribosomes in the malignant cells[11], correlate with the notion on transport function of these ultrastructures.

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Evidence of relationship between the dopaminergic neurotransmitters and the nuclei of the retinal cells

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Summary. Biochemical and quantitative autoradiographic methods indicated the accumulation of labelled dopamine inside the nuclei of retinal cells, and this decreased after treatment with 6 OH dopamine.

In previous papers[1,2] we reported that, as a result of blocking the dopaminergic transmission in the retina, the neurotrophic false neurotransmitter 6 OH dopamine inhibited the generation of outer segments and the turnover of rhodopsin. Subsequent studies[3] showed that the drug also affected the quantity of RNA in the retina. This led us to postulate that the dopaminergic neurotransmitter might be tied to the nucleic acid in some unknown manner. To pursue this interesting and important problem one step further, the answers to the following questions have to be sought. Firstly, is it possible that the dopaminergic transmitters enter into the nuclei of retinal cells? If so, will this entry be affected by such agents as 6 OH dopamine? Furthermore, since the retina consists of a heterogenous population of neuronal cells, will the different nuclei of different cell types display variations in quantities of entry of dopaminergic transmitters?

Materials and methods. 40 neonatal albino rats (1-week-old, strain Simonsen) were used, of which two-thirds were for biochemical studies. The animals were divided into controls and experimentals. The latter were injected with 100 mg/kg 6 OH dopamine i.p. After 3 days, both the controls and experimentals in this group were sacrificed and the retinas dissected out and incubated with 0.005 mCi tritiated dopamine (New England Nuclear) in oxygenated TC199 medium at 37 °C for 45 min to 1 h. The retinas were then homogenized, and using a sucrose gradient method[4], the nuclei were separated from the cytoplasm. The activities of both nuclei and cytoplasmic fractions were counted with a Liquid Scintillation Counter.

The remaining one-third of the rats were used in autoradiography. The retinas were again isolated and incubated with labelled dopamine. The retinas were then embedded in paraffin and sectioned. The sections were coated with Ilford emulsion and stored in the dark (4 °C) for 1.5 months. The emulsion was then developed and the sections stained with hematoxylin. The grains on the nuclei of the different layers of the retinas were counted.

Results. In the normal retina, the activity in the nuclei portion, as determined by the Liquid Scintillation Counter, was 551.8±38.78 cpm/0.1 g tissue wet weight (x±SE, n=6). The activity in the cytoplasmic portion was 15462.6±1639.8 cpm/0.1 g tissue wet weight (n=6). In the nuclei portion of 6 OH dopamine-treated retinas, the activity was only 405.6±26.72 cpm/0.1 g tissue net weight (n=6). The difference between this, and that of the controls, was statistically significant ($p < 0.05$).

Autoradiographic studies indicated that the number of grains on the nuclei of cells of the outer nuclear layer of the retina was 1.68±0.43 (n=30), of the nuclei of the cells of the inner nuclear layer it was 1.20±0.08 (n=30) and of the nuclei of the ganglion cell layer it was 4.32±0.65 (n=30).

Discussion. Our results indicate firstly that labelled dopamine, a precursor of dopaminergic transmitters, enters the nuclei of cells of the retina. This strengthens our view that perhaps the dopaminergic transmitters do enter the nuclei. Secondly, the application of 6 OH dopamine will decrease the labelled dopamine entry into the nucleus. Furthermore, the entry of labelled dopamine into the different nuclei of the different cell types varies in quantity. The nuclei of the ganglion cells have the highest amount of labelled dopamine entry.

If our hypothesis on the entry of dopaminergic neurotransmitters into the nuclei is correct, then neurotransmitters which enter can either directly or indirectly moderate the DNA activities and cause changes in the RNA patterns, resulting in the manipulation of development and various metabolic activities.

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