THE ULTRASTRUCTURE OF THE NUCLEAR ENVELOPE OF AMPHIBIAN OOCYTES: A REINVESTIGATION

III. Actinomycin-Induced Decrease in Central Granules within the Pores

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INTRODUCTION

Since its first mention by Pollister et al. (28), the central granule within the nuclear pore complex ("central dot," "pore plug") has been repeatedly described in plant and animal cells from sections as well as from metal-shadowed or negatively stained, isolated nuclear membranes (e.g. 1, 7, 8, 11, 42). Moreover, such granules with diameters varying in the range of 50 to 300 A are known to exist not only in nuclear pores but also in pores of the cytoplasmic annulate lamellae (19, 32, 39). While Kessel (20) ascribes to such internal pore granules a gatekeeper's role, i.e. closing and opening the pore channel for cytoplasmic exchange, observations by various other authors rather suggest that they represent ribonucleoprotein (RNP) material in a transitional binding to the constituent pore material. There are some indications which particularly support this view. (*a*) The relative amount of pore complexes showing a central granule varies according to the different stages during amphibian oogenesis (10, 26). The central granule frequency here is markedly higher in younger stages of oogenesis, especially in the lampbrush stage which is well known for an extreme RNA-synthesis. (*b*) In synchronized cultures of the ciliate *Tetrahymena pyriformis* a correlation exists between physiological states of high RNA-synthesis and the frequency of granules in the macronuclear pores (42). (*c*) Cross-sections through the nuclear envelopes of cells highly active in RNA-synthesis often show dense dumbbell-shaped clumps of material, presumably of RNP nature, lying on either side of the pore complex and connected by an about 100–150 A broad rod (2, 10, 21, 31, 36). Such configurations are widely interpreted as material in transit through the "central channel" of the pore and obviously can correspond to the appearance of the central granules in the tangential sections and in the negatively stained preparations of isolated nuclear envelopes.

The present study was undertaken further to elucidate whether a correlation exists between nuclear RNA-synthesis and the frequency of central granules within the pore complexes. When isolated amphibian oocytes are incubated in a medium containing actinomycin D in concentrations above 10 μg/ml, chromosomal RNA-synthesis a well as nucleolar RNA-synthesis is completely blocked (17, 23, 33). According to the hypothesis outlined above, a decrease in nucleocytoplasmic migration of RNP material, then, should follow the actinomycin-induced inhibition of nuclear RNA-synthesis.

MATERIALS AND METHODS

The newts, *Triturus alpestris* Laur., were collected in June and July in Black Forest ponds near Freiburg i.Br. The ovaries from decapitated animals were placed in a watch glass containing sterile commercial tissue culture medium TC 199, supplemented with 100 μg/ml penicillin. Only late lampbrush stage oocytes (diameter 800–900 μ, cf. 10) were used for the present study.

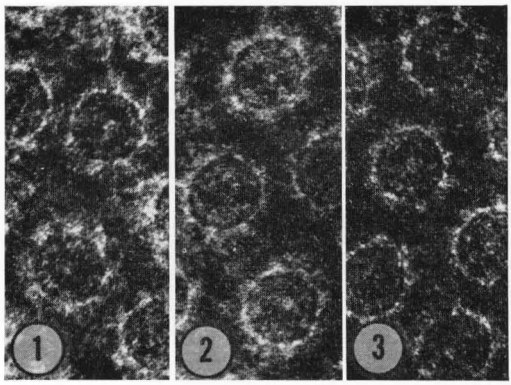
40 lampbrush oocytes were incubated in TC 199 with penicillin and 20 μg/ml actinomycin D. 40 oocytes of the very same stage were kept as controls in the penicillin-supplemented TC 199 without actinomycin. The experiments were run at 21°C. After different times, three oocytes each of the control and the experiment were transferred into watch glasses containing a solution of 0.1 M KCl and 0.1 M NaCl in a ratio of 5:1 (4, 12). Nuclei were isolated and prepared as described previously (9). For negative staining a 2% phosphotungstic acid (PTA) solution, adjusted with NaOH to pH 7.0, was used. Electron micrographs were made with a Siemens Elmiskop IA.

Particular care was taken that the time of preparation, i.e. the interval from tearing the oocytes to the addition of the fixative, was the same in all experiments. In experiments concerned with the 0 to 30 min range of actinomycin effects, precise stoppage was achieved by adding ice-cold TC 199. All values de-

scribed below are mean values of three different experiments.

RESULTS

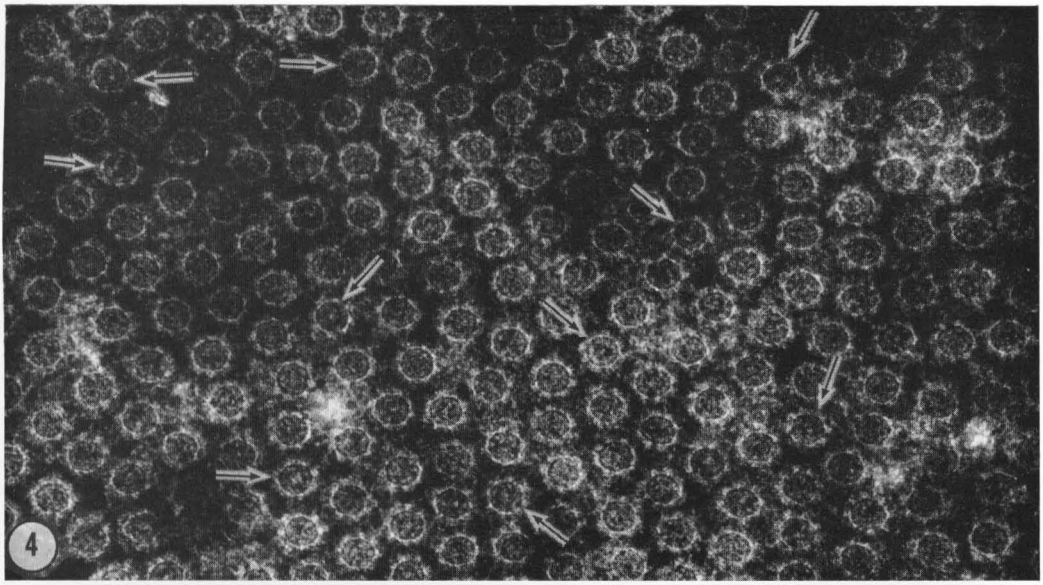
Since the aim of the present investigation was a quantitative comparison of central granule frequencies, the delimiting definition of this pore complex structure was particularly critical. The substructural details of the nuclear pores of the Alpine newt oocytes have been communicated in previous articles (9, 10). In these studies it was also demonstrated that diverse kinds of inner pore material such as dumbbell-shaped large masses, rods, granular aggregates, or single granules can contribute to the image of a central dot in tangential sections and negatively stained preparations of isolated envelope fragments. Although the central pore granule typically appears as a homogeneous spherical or cylindrical body, it can be discerned in many instances as being composed of smaller globules or finely filamentous material (Figs. 1–3; cf. also 9, 20, 43, and P. Comes, H. Kleinig, and W. W. Franke, *Z. Zellforsch. Mikroskop. Anat.*, in press). No considerable loss of central granule material occurs during the aforementioned isolation and staining procedures, as is indicated by the fact that herewith envelope fragments could be obtained from certain immature stages of *Xenopus laevis* oogenesis which exhibited more than 90% of central granule-containing pores (unpublished results).

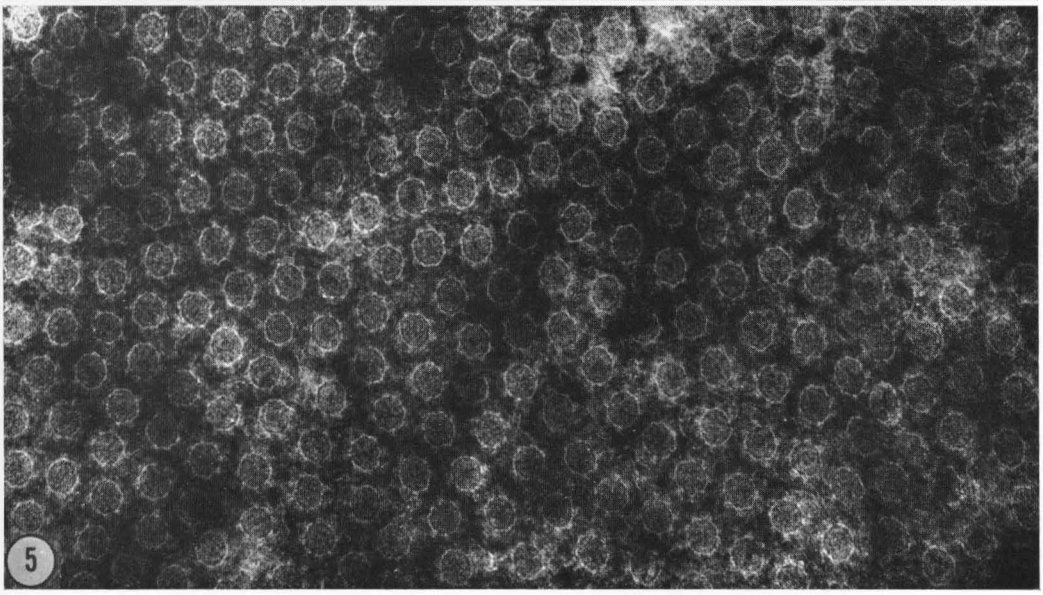


FIGURES 1–3 Structural details of central granules in the pores of manually isolated newt oocyte nuclear envelopes as revealed after negative staining with PTA (pH 7.0). A compound structure of the central granule and its association with the fibrillar material of the pore complex, especially with that of the so-called "inner ring" (9, 43), is suggested in Fig. 1. Typical "compact" central granules are seen in Fig. 3 in which the uppermost pore shows a more hollow interior of the central granule. Variations in central granule dimensions are presented in Fig. 2. Structures below 40 A as detectable, for example, in the middle pore of Fig. 2, were not taken into account as central granules in the quantitative evaluations of the present study. × 125,000.

In the present study, only pores revealing distinct particles with diameters above 40 A in their lumina were considered as "central granule containing." Any fibrillar structures were not regarded. Some of the modifications in the central granule appearance in the newt oocyte nuclear pores can be seen from Figs. 1–3. Only moderately stained envelopes areas as shown in Figs. 4 and 5 were evaluated.

While other structural data of the pore complex as, for example, the pore frequency (61 ± 4 pores per square micron) and the mean pore diameter (730 ± 15 A) did not alter during the experiments described, either in the controls or in the actinomycin-treated preparations, the percentage of central granule-containing pores decreased significantly (Figs. 4–6). The central granule frequency in the nontreated, directly prepared nuclear envelopes was at about 46%. (This value and that of the mean pore diameter differ somewhat from those found in slightly smaller but still comparable lampbrush eggs, 750 μ egg diameter, which exhibited a mean pore diameter of 630 A and a central granule frequency of 55%; [cf. 10]. This might be due to the later stage in oogenesis and/or reflect seasonal changes.) In the control experiments a slight decrease in central granule frequency from 46 to *circa* 30% is observed, caused either by passive wash-off or by something like an active transport into the surrounding medium. In both actinomycin-treated preparations and controls, a slight "loosening" of the central granules to a somewhat fainter appearance in the negatively stained preparations was recognized (compare with the directly stained preparations presented in references 9 and 10). It is interesting to note, in this connection, that the central granule is much more resistant to washes in bivalent cation-free media than, for instance, the granular subunits of the annulus. It was recently found in our laboratory that central granules in nuclear membranes from mammalian liver can withstand even a 6 hr extraction with high salt (1.5 M KCl) media (W. W.





FIGURES 4 and 5 Typical nuclear envelopes manually isolated from late lampbrush oocytes of *Triturus alpestris* and negatively stained with neutralized PTA. Note the apparent higher frequency of central granules (arrows) in Fig. 4 (50 min control) in comparison with the frequency in Fig. 5 (after 50 min treatment with actinomycin D). × 56,000.

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Treatment with actinomycin D, however, brings about a relatively rapid, exponential decrease in the percentage of pores containing a central granule which exceeds that of the control by far. After 50 min treatment with the antibiotic the central granule frequency is constantly below 10%.

DISCUSSION

Actinomycin D is known to inhibit the DNA-dependent RNA-synthesis by preferential binding to the guanine-containing sites of the DNA (e.g.

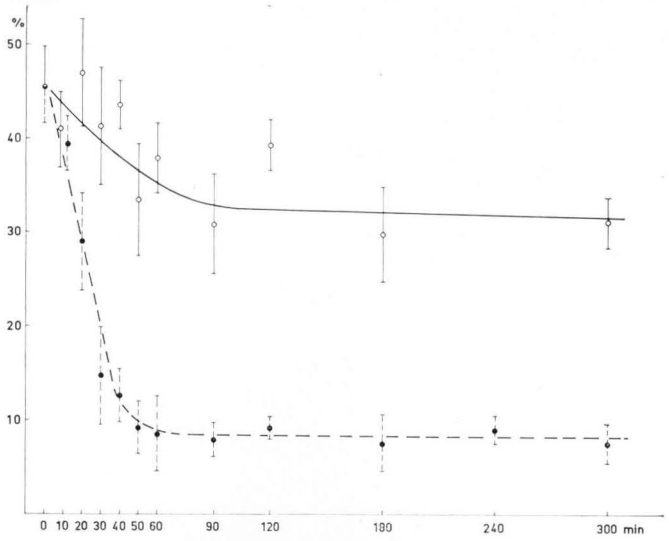

FIGURE 6 Plot of percentage of central granule-containing nuclear pores (ordinate) vs. time after isolation and incubation of the lampbrush oocytes in penicillin-supplemented TC 199 medium. Open circles, control; filled circles, medium made 20 μg/ml with respect to actinomycin D. Bars indicate mean standard deviation. For each value, an average of 1500 pores was evaluated.

15, 29). While in general the synthesis of rRNA is more sensitive to actinomycin than that of the other RNA-species, in amphibian oocytes concentrations as used by Izawa et al. (10 μg/ml [17]) and Lane (50–100 μg/ml [23]) are high enough to prevent the synthesis of all kinds of RNA.

The present finding that application of actinomycin D causes a decrease in the frequency of central granules in the nuclear pore complexes (cf. also 42) could be explained either by an RNP-character of these granules or by a specific or nonspecific inhibition of nucleo-cytoplasmic migration processes by this drug. There is special support for the first line of interpretation in the work of Stevens (35) who reported an actinomycin-induced disappearance of the RNP-helices of *Amoeba proteus* which likewise are also known to represent nucleo-cytoplasmically migrating particles. These helices can be frequently observed during their passage through the innermost part of the nuclear pore lumen and thus are structures comparable to the central granules. An RNP-nature of the central granule is furthermore indicated by the demonstrations of the material derived from Balbiani-rings in *Chironomus* salivary glands (3, 36), from lampbrush loops in amphibian eggs (37) and from the nucleolar periphery in diverse oocytes (2, 10, 21, 22, 31) as migrating through the 100–200 A narrow central channel of the pore. Furthermore, there seems to exist a general correlation between the RNA-synthesis activity of nuclei and the frequency of central granules in their pore complexes (10, 42, P. Comes, H. Kleinig, and W. W. Franke, *Z. Zellforsch. Mikroskop. Anat.*, in press). Mentre (25) reported an RNase digestion of the central granules in the nuclear pores of rat liver cells.

A further step in examining the hypothesis that the central granule is RNA-containing material on its transit from nucleus to cytoplasm, now, would be to make use of the inhibition gap selective for the synthesis of rRNA and that of tRNA and mRNA. Thus it might be possible to clarify whether the central granule is identical to ribosomal or preribosomal RNP-material which is by far predominantly synthesized in the lampbrush stage of amphibian oogenesis (5, 6, 30).

While it is well established that actinomycin blocks the nuclear RNA-synthesis, reports on affecting the migration of RNA-containing material to the cytoplasm are scarce and contradictory. Some authors describe a block or reduction of transfer of labeled RNA in actinomycin-chase experiments (13, 16, 24, 34, 38, 40, W. Eckert and W. W. Franke, In preparation). This disagrees with remarks by Perry (27) and Geuskens (14). Considering a possible reduction of nucleo-cytoplasmic RNA-transfer in the presence of actinomycin, one should keep in mind, however, that other antibiotics which interfere with quite different steps of protein synthesis can also bring about such an RNA-transport reduction (e.g. 18, 41). Therefore, it seems conceivable that reduction of nucleo-cytoplasmic RNA-exchange is a relatively nonspecific concomitant phe-

nomenon of many types of protein synthesis inhibition.

Although one can not decide at this moment whether the observed decrease in central granule frequency after application of actinomycin is due to the drop in nuclear RNA-content or to the reduced rate of nucleo-cytoplasmic transfer, the finding that a substructure of the nuclear pore complex responds to a cell physiological experiment might stimulate further studies on the function of the nuclear pore complex in this direction.

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Note added in proof: Meanwhile similar results were obtained with lampbrush oocytes (diameter 400–520 μ) of *Xenopus laevis*. At 50 min and 2 hr after incubation in TC 199 with actinomycin 17.4 and 12.8%, respectively, of the pores revealed a central granule. Controls maintained a central granule frequency of 50.3 and 52.1%.

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