THE NUCLEUS OF *NOCTILUCA SCINTILLANS*

Aspects of Nucleocytoplasmic Exchanges and the Formation of Nuclear Membrane

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ABSTRACT

The unicellular organism, *Noctiluca*, has been examined with the electron microscope. The nucleus is small compared to the very large size of the cell, but the nuclear border has an organization which indicates an active nucleocytoplasmic exchange. Whereas annuli are missing over most parts of the nuclear membrane proper, there are "annulated vesicles" in a layer inside the nuclear membrane. The hypothesis is put forth that nuclear substances move through the annuli into these vesicles, and that the annulated vesicles themselves are transported through the nuclear membrane. The various forms of the annulated vesicles are consistent with this hypothesis. An implication of this postulate is the synthesis of annulated membranes inside a closed nucleus which are physically separate from the endoplasmic reticulum. The chromosomes are in a state resembling prophase chromosomes and are surrounded by granular masses. Only a small portion of the entire nuclear volume is occupied by the chromosomes. There are many nucleolus-like bodies.

Following the description of the morphology of the nuclear membrane (1, 8, 42) a number of theories have been proposed which attempt to explain the exchange of substances through the nuclear membrane at a fine structural level. The notion that the annuli of the nuclear membrane contain ribosome-like granules (1) has been taken as evidence for transport of particles through the annuli (*e.g.* reference 41). It has also been proposed that substances may diffuse from the nucleus to the perinuclear space (*i.e.*, the cleft between the two electron-opaque layers of the nuclear membrane) and reach the cavities of the endoplasmic reticulum by means of several open communications between the two structures (17, 42). A third concept is the "membrane flow hypothesis" of Bennett (5), implying a transport of particles from nucleus to cytoplasm by means of a "flowing" of the membranes with attached particles through the pore and in some way around the annulus. In a fourth hypothesis it is assumed that relatively large "emission bodies" may pass the nuclear membrane by tearing a hole in it and thus leaving an open nucleo-cytoplasmic connection (26). Finally, it has been suggested that there might be a passage of large emission bodies, but that the nuclear membrane is closed by a regenerating membrane under the body before its actual emission (reference 2, and, similarly, reference 12). This mechanism presupposes formation of nuclear membrane inside a closed nucleus. The only mechanism of nucleocytoplasmic transport which has received experimental support is the route through the annuli, as demonstrated in amebas by injection of colloidal gold (11).

In a study of the cytology of the luminescent flagellate, *Noctiluca scintillans*, observations have been made on the nuclear membrane which seem to be of interest in this connection. *Noctiluca* belongs to the order Dinoflagellata, a group which is characterized by a single nucleus containing evenly scattered moniliform chromatin threads (27). The chromatin is in a state resembling permanent prophase chromosomes, and the separate chromatin threads do not lose their individuality from one mitosis to another. Several electron microscopic studies have been reported on the peculiar hydrated and spiralized micelles forming the chromatin threads or chromosomes (13, 15, 16, 20, 25, 37) of dinoflagellates.

By light microscopy it has been noted that some of the largest members of the dinoflagellates (*Gymnodinium rubrum*, *Gyrodinium virgatum*, and *Gyrodinium corallinum*) have a peculiar structure at the level of the nuclear membrane. It is an alveolar layer of ellipsoidal vacuoles sometimes seen to have a pink content (27). These species have not been subjected to electron microscopy, nor has the giant of the order, *Noctiluca*. The present paper deals with the fine structure of the nucleus of *Noctiluca scintillans*.

MATERIAL AND METHODS

Noctiluca scintillans (Macartney) (*N. miliaris* S.) organisms were collected in the plankton at Misaki Marine Biological Station, Japan, in October 1958, and at Kristineberg Zoological Station, Sweden, in July 1961 and 1962. They were fixed in 1 per cent osmium tetroxide dissolved in sea water, dehydrated in graded alcohols, and embedded in methacrylate or Epon 812. Sectioning was performed on an LKB "Ultrotome" ultramicrotome and microscopy on a Siemens Elmiskop I electron microscope.

OBSERVATIONS

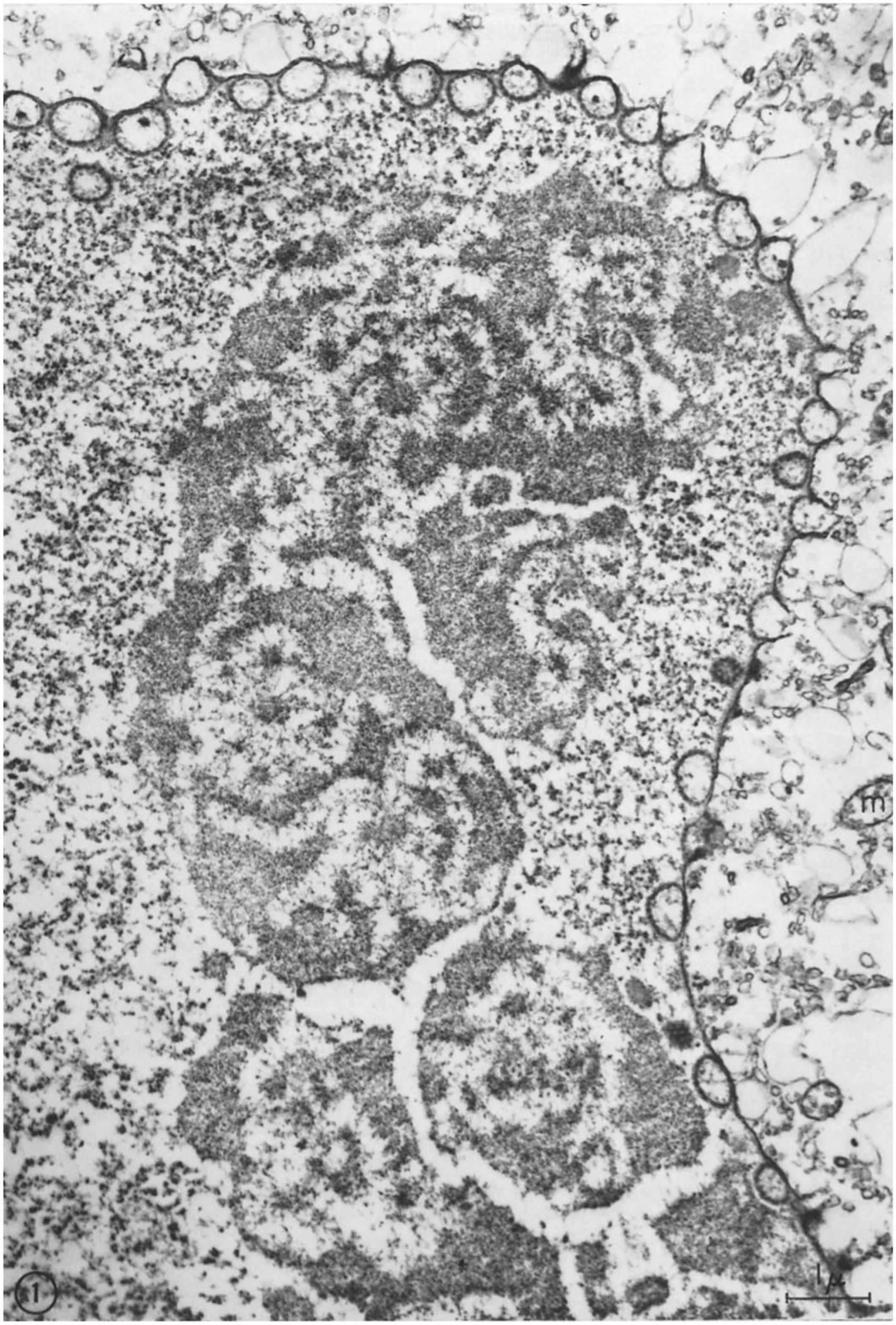
The *Noctiluca* organism is a spherical cell 0.4 to 1.2 mm in size. Its nucleus is round or oval and about 30 μ in diameter. When seen in the light microscope the outline of the nucleus is fairly even and, in contrast to most other large nuclei, it is devoid of fissures and outpocketings. Observations on the living nucleus have not been rewarding, partly because of the large size of the cell.

THE NUCLEAR BORDER: Electron micrographs of the nuclear border demonstrate a layer of vesicles inside the nuclear membrane (Figs. 1 to 6). For descriptive purposes these vesicles will be called annulated vesicles. The nuclear membrane itself is relatively thin, around 150 A, and is a layer that covers most of the surface of the nucleus. It consists of two parallel electron-opaque lamellae and is, over most of its extension, devoid of annuli.

Whereas annuli are missing over most of the nuclear membrane, the presence of annuli is a distinctive characteristic of the annulated vesicles. The membranes of these vesicles contain tightly packed annuli, evidenced as rings in grazing sections of the membranes (Fig. 4) and as interruptions of the two electron-opaque strata in cross-cut membranes (Figs. 3 to 6). The annuli are arranged in a hexagonal packing, and many of them have a central granule in the grazing sections. The dimensions of the annuli are comparable to those measured in the nuclear membrane of the sea urchin oocyte (1) or to those seen in the cytoplasmic annulated lamella (1, 2, 36, 40).

The connection between the annulated vesicles, the nuclear membrane, and vesicles outside the nuclear membrane is of particular interest here. The majority of the annulated vesicles occupy a zone immediately below the nuclear membrane and in contact with it. Only a minority of the annulated vesicles appear to be free in the nucleoplasm, and none has been found separated from the nuclear membrane for a distance of more than 1 μ. The annulated vesicles that are close to the nuclear membrane and those that appear to be free resemble each other in size and in the complexity of their limiting membrane, but they differ in shape. The "free" vesicles are spherical; the

FIGURE 1 Survey electron micrograph of the nucleus of *Noctiluca scintillans*. The nuclear border runs along the upper and right margins of the figure. The nuclear membrane is cross-cut along the entire distance shown. Under the nuclear membrane or in association with it are "annulated vesicles." In the upper left corner, a round annulated vesicle is seen to be removed some distance from the nuclear membrane. Note the various shapes exhibited by the annulated vesicles at various levels in relation to the nuclear border. The dark patches with inner tortuous threads represent the chromosomes. The remainder of the nucleoplasm is occupied by a finely granular substance. *m*, mitochondrion. × 13,000.


m
1
1 μ

"attached" ones are flattened towards the nuclear membrane or else have a shape that appears to be a function of their distance away from the center of the nucleus. They are plane-convex when flattened towards a straight nuclear membrane (Figs. 1 and 5), biconvex where the nuclear membrane protrudes outward slightly (Fig. 3), or pear-shaped with a narrow end protruding, together with the nuclear membrane, towards the cytoplasm (Figs. 1 and 5). The membrane of the protruding annulated vesicle is usually thinned distally and about 170 A as compared to 300 A in deeper regions. The nuclear membrane also is sometimes missing over the top of such a protruding vesicle, and the vesicle itself is open towards the cytoplasm. There is, however, even here, no break in the continuity of a nuclear covering, as the sides of the open annulated vesicles seem to be fused with the nuclear membrane proper, the bottom of the annulated vesicles thus being integrated as a part of the nuclear membrane (Figs. 4 and 5). The various shapes exhibited by the annulated vesicles are thus suggestive of a sequence of transformations in which the nuclear membrane is also involved (see Discussion). Whereas the membrane of the annulated vesicles is provided with annuli at places bordering on the nucleoplasm, there are no annuli in sections facing the nuclear membrane or protruding free into the cytoplasm (Figs. 3 and 5). Extending from the nuclear membrane and attached to it with a rather broad base are simple-walled cytoplasmic vesicles with an appearance similar to the protruding annulated vesicles. These vesicles are, as a rule, slightly larger than the annulated vesicles and have a more irregular outline, but in their frequency and shape they are quite reminiscent of the protruding annulated vesicles (Figs. 1 and 5). Simple-walled vesicles also appear free in the cytoplasm, together with mitochondria, dictyosomes, lipid inclusions, and other cytoplasmic components.

The interior of the annulated vesicles appears as a fluffy substance resembling the nucleoplasmic matrix. There is also, in a fair number of the annulated vesicles, an electron-opaque inclusion, visible in Figs. 1, 2, and 6.

The frequency of the annulated vesicles has been estimated, from sections which include large areas of the nuclear membrane, to be approximately $2.6/\mu^2$. The total number of annulated vesicles in a *Noctiluca* nucleus is hence of the order of 8,000. A calculation of the number of annuli in one annulated vesicle has given a value of approximately 80. (The total surface area of an average-sized annulated vesicle is 1 μ^2, the frequency of annuli is close to $100/\mu^2$, and approximately 80 per cent of its membrane is annulated.)

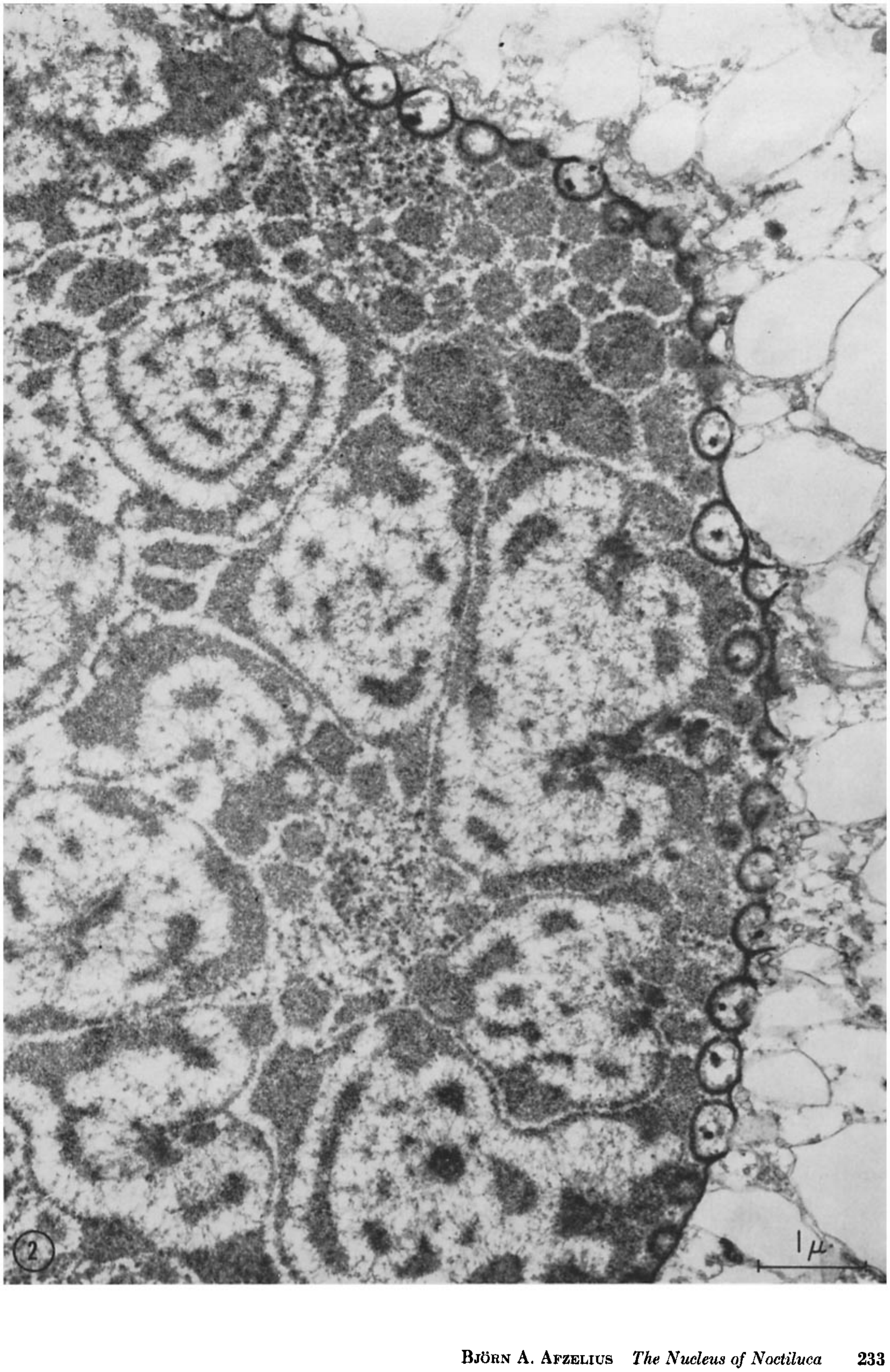
The entire nuclear membrane, or most of it, is connected to an underlying fibrous layer. This consists of a web of fine fibers which are oriented parallel to the nuclear membrane (Fig. 6). The thickness of the fibrous layer is 0.25 μ or less, and of the individual fibers approximately 70 A.

THE NUCLEAR CONTENT: The chromosomes are easily recognized in the present material, although they have not been well fixed (*cf.* reference 25). They appear as coiled threads embedded in a granular matrix of high electron opacity (Figs. 1 and 2). The threads have a thickness of 0.3 μ and an indeterminate length. The cross-sectional area of a single chromosomal body equals that of a total nucleus in mammalian tissue cells.

Besides containing the prominent chromosomal bodies, the diffusely mottled nuclear sap contains many electron-opaque, subspherical bodies with the morphological characteristics of a nucleolus. Several bodies of this type are shown in Fig. 2 and one is shown in Fig. 5.

The nucleoplasm, as well as the nuclear membrane, is modified in huge cell nuclei. In small or moderate-size nuclei the hydrated chromosomes fill the entire space within the membrane; in giant nuclei the chromosomes occupy but a small portion of the entire volume (6, 10, 12). The reminder of the nucleus consists of another phase, a "nucleogel," which has tinctorial properties and ultrastructural characteristics deviating from those

FIGURE 2 Electron micrograph of an area of the nucleus occupied by chromosomes and nucleolus-like bodies. The borders between the separate chromosomes appear distinct. In the upper right portion of the nucleus are nucleolus-like bodies which are composed of small granules, similar to those outlining the chromosomes. Several of the annulated vesicles, in contact with the nuclear membrane, contain electron-opaque inclusions. In the cytoplasm surrounding the nucleus there are many prominent, simple-walled vesicles. × 17,000.


2
1 μ

of common nuclei. Unfortunately, either a lack of a well defined structure of this nucleogel or a faulty preservation of it prevents one from extending the electron microscopical study any further.

The preceding description of nuclei of *Noctiluca* is based on observations on both Japanese and Swedish forms. Identical results were obtained in both cases.

DISCUSSION

This investigation is part of a more extensive study of large cells (1–3). The emphasis has been placed on the relationships between the nucleus and the cytoplasm. With an increasing cell volume, the ratio of nuclear surface area to cell volume decreases. In many types of giant cells the nucleus presents an extensive branching, which partly compensates this feature (28, 39, 43). In *Noctiluca*, which is one of the giants among the protozoans, the nucleus appears spherical and is rather small compared to the cell as a whole. Some approximate calculations may be given of ratios between nuclear surface area and cell volume: rat liver cell or full grown sea urchin oocyte, 1:50 μ^{-1}; the resting sea urchin egg after meiosis, 1:1000 μ^{-1}; a 1 mm *Noctiluca* organism 1:200,000 μ^{-1}.

These figures suggest an increased rate of nucleocytoplasmic exchanges in large cells when measured per unit area of the nuclear membrane. Another feature of *Noctiluca* gives further support for the presumption of an unusually high rate of transport over the nuclear border: the mitotic divisions are performed, and the chromosomes separate, apparently within a closed nucleus (7, 14, 22, 34, 35). No mixing of nucleoplasm and cytoplasm seems to take place during division, as in most other cell types.

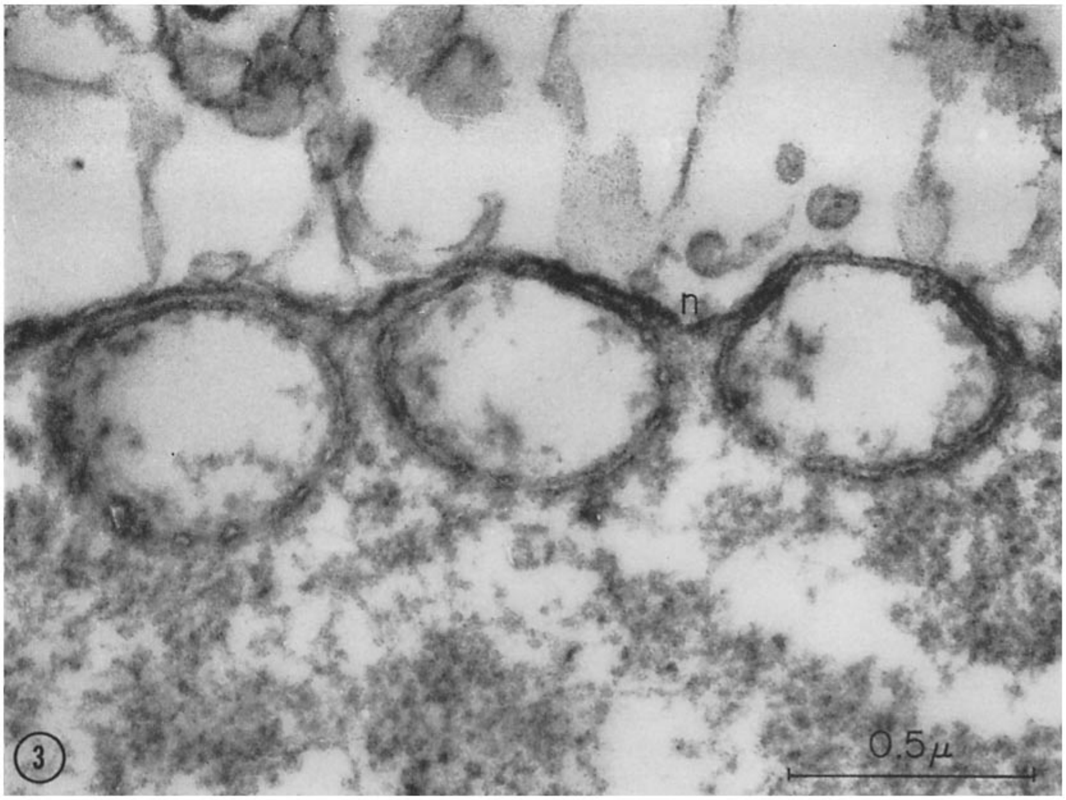
The appearance of the nuclear border of *Noctiluca* will be discussed in the light of these considerations. Whereas cell types with intensive metabolic activities are characterized by nuclear membranes with densely packed annuli, the *Noctiluca* cell has a nuclear membrane proper which is largely devoid of them. It has, on the other hand, a number of annulated vesicles under or at the nuclear membrane, making the total sum of annuli in a *Noctiluca* nucleus about half a million. This value is of the same order as the calculated value of annuli in the nuclear membrane of the sea urchin oocyte (from data in reference 1). If the annuli are engaged in nucleocytoplasmic exchanges in *Noctiluca*, it is necessary to assume that the annulated vesicles themselves are transported through the nuclear membrane or open at it. There is nothing in the present electron micrographs to gainsay such an interpretation. The various forms exhibited by the annulated vesicles are actually very suggestive of such a passage over the nuclear border. Specifically, it appears that the direction of the transport is one from the nucleus to the cytoplasm, as judged from the direction of the bulges of the nuclear membrane and the shapes of the annulated vesicles.

The close association between the nuclear membrane and the annulated vesicles at some stage of their existence seems to constitute a mechanism for securing the release of nuclear substances in the vesicles without making holes in the nuclear membrane. The unique cytoplasmic and nucleoplasmic milieux are thus retained, whereas a free mixing of the two fluids *via* an open hole would require much energy to restore the equilibrium inside and outside the membrane. The similarity between this release mechanism and that of the "heavy bodies" in sea urchin oocytes should be noted (2).

The formation of annulated vesicles within the

FIGURE 3 Higher magnification electron micrograph of three cross-sectioned annulated vesicles. Note that most of the membrane of the vesicles is provided with numerous interruptions corresponding to the annuli, but that the part of the membrane which borders on the nuclear membrane is simpler and thinner. The vesicles appear to be flattened towards the nuclear membrane, or protrude slightly, together with the membrane, towards the cytoplasm. There are only wisps of material inside the annulated vesicles. *n*, nuclear membrane. × 64,000.

FIGURE 4. Electron micrograph of the nuclear border showing parts of six annulated vesicles. The left one is open towards the cytoplasm and is in close contact with the nuclear membrane. The annulated vesicle marked with an arrow is cut in a grazing section and shows some of the cross-cut annuli. × 47,000.


3
n
0.5 μ


4
0.5 μ

nucleus thus appears to be the most likely hypothesis. A corollary of this hypothesis is the synthesis of annulated membranes inside a closed nucleus which is physically separated from the endoplasmic reticulum. Earlier conclusions concerning the precursors of the nuclear membrane have emphasized the role of the endoplasmic reticulum (4, 19, 23, 24, 30–33, 38, 44) which is supposed to wrap the chromosomes during telophase to form chromosomal vesicles. The present interpretation is at variance with these conclusions, and gives another route of formation of annulated membranes in *Noctiluca* cells and in sea urchin oocytes (2).

The continuity between the endoplasmic reticulum and the nuclear membrane, in some cell types, has been used as a frequently repeated argument for the fundamental similarity of the two types of membranes. The different morphology of the two types seems to speak against this viewpoint, however, as does the present conclusion that the annulated membranes are synthesized, or put together, in the nucleus. There are several other distinct differences between the nuclear membrane and other cytoplasmic membranes examined, which make the grouping together of the two membranes into one membrane type appear oversimplified. The electrical properties of the nuclear membrane are strongly different from those of the cell membrane (29), the nuclear membrane cannot regenerate after puncture (9), and the chemical properties deviate (21).

No attempts have been made to analyse the chemical nature of the material within the annulated vesicles. The cytoplasm around the *Noctiluca* nucleus has been reported to have a reddish color (18, 34), giving dense populations of *Noctiluca* a pink hue (the "red tides"). In the related dinoflagellate, *Gyrodinium corallinum*, the nuclear membrane has been described as containing a layer of pink vacuoles (27).

The fibrous layer under the nuclear membrane remains to be discussed. In the giant ameba, *Pelomyxa carolinensis*, there is a similar fibrous layer in the prophase stage (38). The nuclei of this ameba are of moderate size, are numerous in each cell, and undergo acentric divisions with a breakdown of the nuclear membrane in metaphase. It cannot be judged whether or not the fibrous layer has a function in the mitotic divisions. It is possible that the fibrous layer in *Noctiluca* may have a role different from that of the fibrous layer in *Pelomyxa*, such as perhaps mediating the transport of the annulated vesicles through the nuclear membranes.

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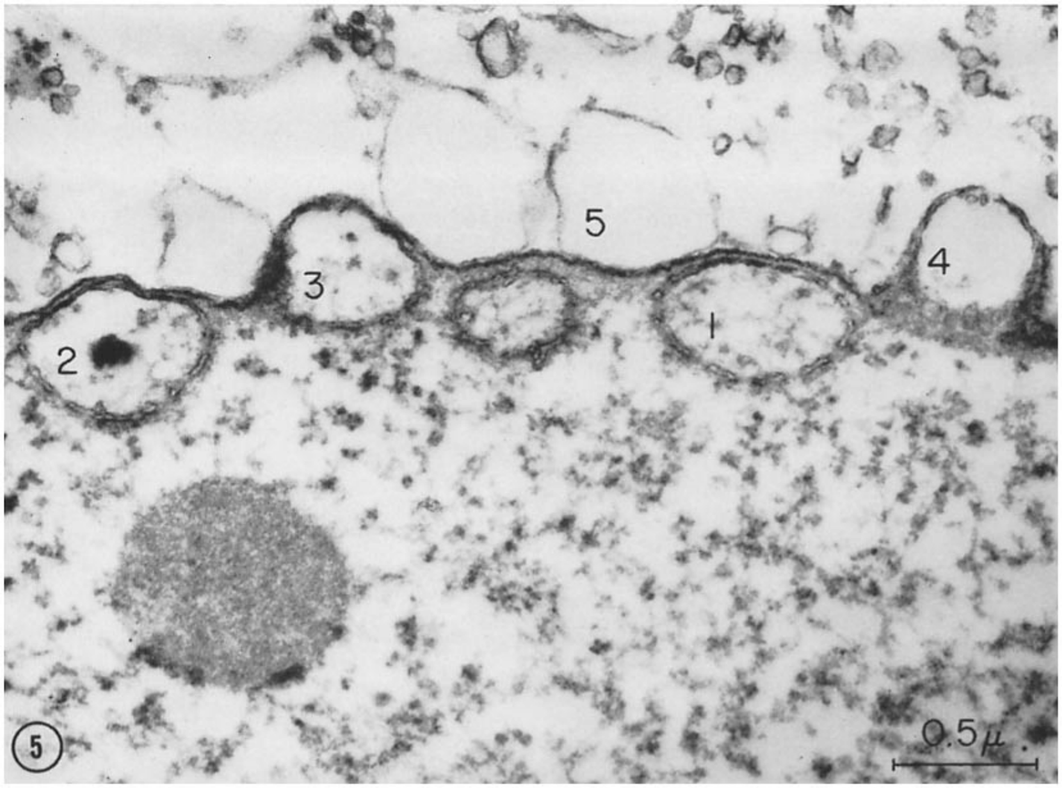
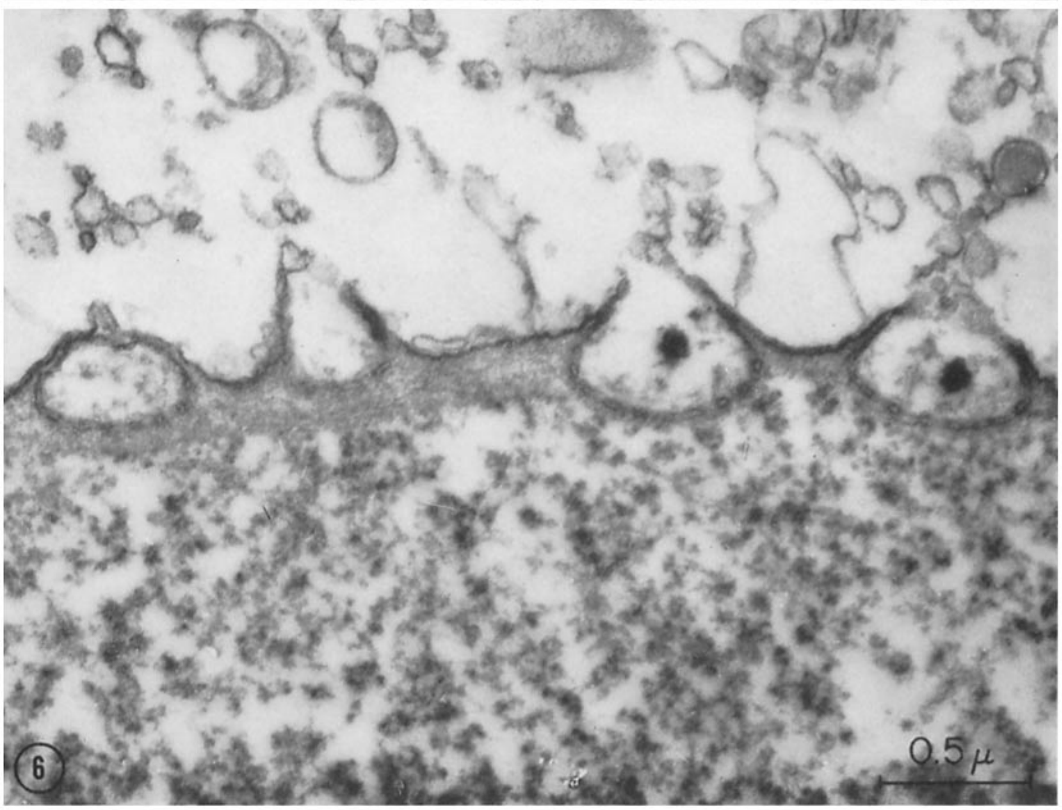
Figure 5 A portion of the nuclear membrane with five adjacent annulated vesicles and three cytoplasmic vesicles. Note the similarity in shape between the protruding annulated vesicle on the right and the three cytoplasmic vesicles. The numbers *1* to *5* represent suggested steps in a sequence of transformations from annulated vesicles to cytoplasmic vesicles. Annuli in a cross-section are indicated at the basal part of the annulated vesicle marked *4*. A nucleolus-like body is at the lower left. × 37,000.

Figure 6 A portion of the nuclear border where the fibrous layer is particularly prominent. This fibrous layer is quite distinct from the granulated nucleoplasm and consists of finer filaments. × 40,000.


5
2
3
1
4
5
0.5 μ


6
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