THE FORMATION OF MULTIVESICULAR BODIES FROM THE NUCLEAR ENVELOPE

WINCENTY KILARSKI and ANDRZEJ JASIŃSKI

From the Department of Comparative Anatomy, Jagellonian University, Krakow, Krupnicza 50, Poland

ABSTRACT

Cells of the gas gland of the perch _Perca fluviatilis_ L., stimulated to increased generation of gas by the repeated emptying of the swim-bladder, were examined in the electron microscope. Intense activity of the nuclear envelope was demonstrated. Simple vesicles originating from the external nuclear membrane and the so-called multivesicular bodies derived from the outpocketings of both membranes of the nuclear envelope were observed. The multivesicular bodies were filled with numerous fine vesiculae arising from the active proliferation of their internal membrane. The authors offer two alternative mechanisms of formation of fine vesiculae inside the multivesicular bodies and the mechanism of the tearing away of these bodies from the nuclear envelope.

INTRODUCTION

Watson was the first to suggest in his study on mammalian hepatocytes that the external nuclear membrane may be a continuation of the endoplasmic reticulum (ER) (1). Hence the concept arose that the nuclear envelope belongs to the ER system. Further observations revealed that in skeletal muscles this structure may be a part of the sarcoplasmic reticulum (2, 3).

Several electron microscopists have reported on nuclear "blebbing," the extrusion of nuclear material into the cytoplasm. These extrusions are surrounded by a single or double membrane that originally was an integral part of the nuclear envelope. Blebbing phenomena involving the outer nuclear membrane have been observed mainly in oocytes of different animals. Hsu (4) found several blebs of the outer nuclear membrane in oocytes of the tunicate _Boltenia_. Wischnitzer (5) also noted a blebbing of the outer nuclear membrane in oocytes of _Rana_. However, none of the cells has demonstrated the frequency of blebbing that occurs in oocytes of _Necturus_ (6). Blebbing has also been observed in oocytes of various other species (7–11). In a very recent electron microscopic study of oocytes from _Proopterus_, Scharrer and Wurzelmann (12) described the blebbing of the nuclear envelope during extrusion of nucleolar material into the cytoplasm.

There are only a few electron microscope studies that have described the blebbing phenomena for both nuclear membranes. The blebbing of both nuclear membranes of salivary gland cells in _Drosophila_ was reported by Gay (13, 14). Bell and Mühlethaler (15) observed blebbing of the nuclear envelope in the archegonia of _ferns_. Szollosi (11) described nucleolar extrusion in his electron microscopic observations of rat eggs.

The preceding examples indicate that the nuclear envelope is not a static structure serving only as a material partition between nucleoplasm and cytoplasm, but that it is actively involved in the process of the formation of some cellular organelles, especially under conditions of increased physiological activity of the cell. The purpose of the present
paper is to describe similar phenomena that occur in the nuclei of the somatic cells of the gas gland in the fishes.

MATERIALS AND METHODS

Perch fish (*Perca fluviatilis* L.) ranging from 100 to 150 g in weight were used. Cells of the gas gland, which were stimulated to their maximum activity by the removal of gas from the swim-bladder with a syringe, were used for study. This procedure made the cells of the gland produce gas steadily and intensively, which was manifested by a remarkable decrease in glycogen, clearly visible in histological sections. After 72 hr of stimulation, during which about 30 cc of gas was taken three times at intervals of 24 hr, the fish were killed. The gas glands were excised, cut into small pieces, and fixed in glutaraldehyde and then in osmium tetroxide (16). After fixation, the tissue blocks were dehydrated in ethyl alcohols of increasing concentration and embedded in Epon 812 (17). The polymerized blocks were sectioned with a Porter-Blum ultramicrotome (Ivan Sorvall, Norwalk, Conn.) equipped with a diamond knife. Ultrathin sections were mounted on copper grids and stained with uranium acetate (18) and lead citrate (19). Observations were made with a Japanese JEM-5Y electron microscope.

OBSERVATIONS

Rapid changes were observed in the nuclear envelope at the time of intense activity of the gas gland. They involved either the external membrane or both membranes of the nuclear envelope. The common occurrence of this phenomenon, observed in nearly each cell, was striking. Many nuclei showed marked undulations and outpocketings of the external nuclear membrane. Numerous vesicles which were in close apposition to the nuclear envelope were also observed. These were connected with the nuclear membrane by short "necks." A large number of vesicles which were

![Figure 1](image-url)
detached from the membrane but situated close to the nucleus had probably arisen from the above-mentioned outpocketings, as a result of their tearing away from the membrane (Fig. 1).

The outpocketings of both membranes of the nuclear envelope were observed more frequently. Large vesicles, which were surrounded by a double membrane and filled with numerous, mostly fine vesiculae or, perhaps, tubules, were very often observed to be attached to the nuclear envelope. A single section of the nucleus usually revealed several (3–5) such vesicles (Fig. 2). Their relation to the nuclear membrane has been found to be variable. Sections of these vesicles show them to be most commonly semicircular and firmly seated on the surface of the nucleus (Figs. 3 and 4). They also occur as elongated oval profiles touching the nuclear envelope (Fig. 5) or as more or less round profiles detached and somewhat removed from the surface of the nucleus (Fig. 6).

We refer to these vesicular outpocketings of the nuclear envelope as “multivesicular bodies” because they are filled with a large number of fine vesiculae or tubules. The multivesicular bodies described here, unlike those studied by Bruni and Porter (20), are enclosed in two membranes, an external one and an internal one, which are derived, probably, from the corresponding nuclear membranes. The numerous fine vesiculae which fill such a body have wavy or oval outlines and originate from the infoldings of its internal membrane.

Two types of these fine vesiculae were observed: smaller ones (51–72 μ in diameter) containing relatively dense, granulofibrous material, and larger ones (168–290 μ) filled with homogeneous

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Figure 2  Section through a cell of gas gland of a perch stimulated to increased “secretion” of gas into the swim-bladder. In addition to the folded external nuclear membrane studded thinly with ribosomes, there are numerous multivesicular bodies (arrows) and the presumed remnants of the bodies, after disintegration, in the form of a very small vesiculae (short arrows). × 5250.

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FIGURE 3  A fragment of the nucleus of a gas gland cell showing multivesicular bodies in various phases of development. The connections between the fine vesiculae and the internal membrane of the multivesicular body (arrows) are visible. × 34,650.

material of slight density. There is, of course, the possibility that the differences in size and density observed between the vesiculae are, to some degree, due to the plane of section. If the vesiculae were sectioned obliquely, i.e. tangential section through the vesiculae, they would have a greater density and a smaller size than vesiculae bisected in their median plane. The first type of vesiculae is observed in vesicles which are still connected with the surface of the nucleus (Figs. 3 and 4). Vesicles which are detached from the surface of the nucleus (Fig. 6) or, as may be supposed, are just at the phase of separating from the nuclear envelope are filled with both small dark and large light vesiculae. The external nuclear membrane also has the potential to form vesiculae, but it is more limited. Fine vesiculae budding to the outside from the membrane surrounding a multivesicular body were seen occasionally (Fig. 7).

Vesicles which have the form of cups seated on the surface of the nucleus are not completely separated from the interior of the nucleus by the membrane at this phase. However, elements of the karyoplasm never were observed in the lumen of such vesicles. The differences in density between the contents of the nucleus and those of the vesicles must have prevented the vesicles from being filled with karyoplasmic elements. The internal nuclear membrane, which, at this phase of formation of the vesicle, exhibits a relatively large opening (Fig. 4), grows and cuts off the lumen of the vesicle from the content of the nucleus. Originally, however, the vesicle, which has not been separated completely from the nuclear envelope, has its external membrane in common with the nuclear envelope (Fig. 7). After the thorough restoration of the internal membrane of the vesicle, the vesicle tears away from the external nuclear membrane. A phase of this process is the formation of a connecting “neck,” which tears away from the external nuclear membrane, thus setting the vesicle free from this membrane, and which, in turn, also tears away from the external vesicular membrane, to remain as an intermediate vesicle between the nuclear envelope and the multivesicular body (Fig. 6).

DISCUSSION

The term “multivesicular body” as used in this paper is employed only to convey a graphic description of the structure of these formations; the formations themselves cannot be identified with the multivesicular bodies often distinguished in hepatic cells (20, 21). The “multivesicular bodies” described in this paper seem to derive from the nuclear envelope, whereas the multivesicular bodies described by Bruni and Porter (20) take origin from the Golgi apparatus. Rosenbluth and Wissig (21) also described multivesicular bodies which arise from the aggregates of micropinocytotic vesicles which separate from the cell membrane. In both these cases the multivesicular bodies are enclosed in one membrane, and their origin is not related to an extremely increased physiological activity of the cells.

The formation of multivesicular bodies from the nuclear envelope of cells of the gas gland in fishes is distinctly associated with the increased physiological activity of these cells, since these bodies
FIGURE 4 A multivesicular body, showing the continuity between its wall and the membranes of the nuclear envelope (arrows). In this phase the multivesicular body still opens into the interior of the nucleus. X 34,650.

FIGURE 5 A multivesicular body at the time of separation from the nuclear envelope. Note the presence of large, light vesiculae within it in this phase. X 15,400.

FIGURE 6 A multivesicular body after tearing away from the nuclear envelope. Between the body and the nuclear envelope is the so-called intermediate vesicle formed from the neck connecting the multivesicular body with the external membrane of the nuclear envelope immediately before their separation. IV-intermediate vesicle. X 34,650.

FIGURE 7 This image of a multivesicular body suggests that the fine vesiculae with which it is filled may in fact be sections of one or more tubules formed from its internal membrane. Also shown is a vesicle which is developing from the external membrane of the multivesicular body. The few ribosomes that are visible on the outer membrane of the multivesicular body suggest that this membrane originates from the external nuclear membrane. X 35,000.

may be induced in an artificial manner by emptying the swim-bladder and thus provoking the intense secretion of gas by the glandular cells (22, 23).

Wischnitzer (5) observed a similar behavior of the nuclear envelope in maturing oocytes of amphibians. However, the formations which he dealt with had a simpler structure; they were formed only from the external membrane of the nuclear envelope and were not filled with fine vesiculae. Kessel (6) observed the formation of many thousands of vesicles from the external nuclear membrane of the oocytes in Necturus. He suggested that these vesicles are needed as raw material for the formation of the annulate lamellae. In his description of the mechanism of synthesis of yolk in crustacean oocytes, Kessel (24) recently has emphasized the part played by the external membrane of the nuclear envelope in the process.
of the formation of new vesicular cisternae of the ER needed for the yolk synthesis. The constant demand for new ER vesicles somehow stimulates the nuclear envelope to produce them.

Our observations agree to some extent with those discussed above. The external nuclear membrane is stretched to a great degree. In many places it forms vesicles which exhibit the ability to become detached; large numbers of such vesicles are seen near the nucleus (Fig. 1). It is difficult to determine whether they participate in the formation of new ER, however, since scattered vesicular profiles of smooth and rough ER, which are visible in the perinuclear cytoplasm, seem to have a close morphological relationship to the outer layer of the nuclear envelope and seem to be derived, at least in part, from the outpocketing of the outer membrane of the nuclear envelope (Fig. 1).

Scharrer and Wurzelmann (12) have postulated that the ER cisternae and the Golgi complex are formed from the outer nuclear membrane. Those authors seem to return to the older idea that mitochondria also may be derived from the nuclear envelope: according to them (12), “It appears that these cytoplasmic organelles derive from the nuclear envelope not only their membranous component but some of the intramitochondrial contents as well.” However, their micrograph (Fig. 10 in ref. 12) does not show a continuity between these organelles and the nuclear envelope.

In 1964 Bell and Mühlethaler (15) described evaginations of the nuclear membrane in the fern archegonia. These evaginations were said to have arisen from large vesicles limited by two membranes, of which the internal one developed evident infoldings which were considered by those authors to be cristae mitochondriales. Thus they postulate another conception of formation of new mitochondria from the nuclear envelope.

Although we do not regard the multivesicular bodies observed by us as being mitochondria, nevertheless the process which Bell and Mühlethaler described resembles very closely the process that occurs in the cells of the fish gas gland. In both cases both membranes of the nuclear envelope are involved in the formation of a new organelle.

![Figure 8](image)

**Figure 8** Diagrams illustrating: A-C1—successive phases of development of the multivesicular body from the nuclear envelope and the formation of fine vesiculae from single infoldings of the internal membrane of this body. A-C2—another possible mechanism of formation of the multivesicular body with its internal membrane producing one or more long tubules of an intricate course; both types of bodies (C1 and C2) may present similar pictures in sections.

![Figure 9](image)

**Figure 9** A possible process of separation of the multivesicular body from the nuclear envelope, accompanied by the transitory appearance of an elongated “neck,” which then turns into an intermediate vesicle.
The multivesicular bodies described here have been recognized only in the close vicinity of the nucleus and never have been observed in any other part of the cell (25). They cannot be traced to the cell periphery, and we assumed, therefore, that they must either disintegrate rapidly or transform immediately into very small vesiculae, as observed in Fig. 2, and in such a form eventually reach their destination, if any, within the cytoplasm. However unknown their function is, they provide a structural component suggesting the omnipotence of the nuclear envelope.

Two possible modes of formation of fine vesiculae or tubes, which fill a multivesicular body are presented in Fig. 8 (ABC, and ABC2). In the first case, the fine vesiculae would arise from numerous small infoldings of the internal membrane of the budding nuclear envelope. In the second case, the internal membrane would produce narrow tubules, one to several in number, which in the process of growing would roll themselves up irregularly inside the vesicle. This manner of formation seems more probable (Figs. 6 and 7). Fig. 9 illustrates the presumable mechanism of separation of a multivesicular body from the nuclear envelope. The process of separation seems to be complicated because of the specific properties of the nuclear envelope. Unlike the cell membrane, the nuclear envelope does not undergo restoration when artificially damaged. This is perhaps owing to the fact that the nuclear envelope is positively charged and therefore cannot bind Ca++ ions needed to synthesize calcium proteinates which would constitute the framework for the newly produced membrane. Thus, the separation of a relatively large structure such as the multivesicular body must proceed in two phases. In the first phase the internal membrane of the nuclear envelope is closed and cuts off the contents of the nucleus from those of the multivesicular body. In the second phase the external membrane is restored. The tubules or vesiculae may partly contribute to the restoration of the internal membrane, as they contain available membranous material of the same quality as that of this membrane from which they have arisen. The restoration of the membrane takes place as the multivesicular body moves away from the nucleus. The external membrane stretches into a long thin neck, which in the final phase tears away from both the nuclear envelope and the multivesicular body and may form an intermediate vesicle (Figs. 6 and 9).

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