Genesis of concentric laminated inclusions in the nucleus

F.N. Ghadially and J-M.A. Lalonde

Department of Pathology, University Hospital, University of Saskatchewan, Saskatoon (Saskatchewan, Canada S7N OWO),
20 April 1979

Summary. Single-membrane-bound inclusions containing zymogen granules were found in pancreatic acinar cells. Images were seen suggesting that the concentric laminated inclusion is derived by hydration and fusion of such granules within the inclusion.

The concentric laminated inclusion is a rare variety of intranuclear inclusion which is said[1] to be 'unique among mammals'. It is composed of alternating shells of electron-dense and electron-lucent material. A single smooth-surfaced, loose fitting, membrane demarcates this type of inclusion from the nuclear matrix. The composition and mode of formation of these inclusions is not too clear.

These inclusions have been sighted in only a few instances. These include: a) the oviduct epithelium of hens; b) mucus-secreting cells of human labial salivary glands; c) acinic cell carcinoma of parotid gland; d) murine pulmonary adenomas and adenocarcinomas; and e) a synovial intimal cell from an apparently healthy rabbit (for references see Ghadially[2]).

During the course of examination of pancreatic tissue removed from a case of insulinoma we found numerous intranuclear inclusions in the pancreatic acinar cells, which were unremarkable except for the presence of these inclusions. Such inclusions were not found in islet cells or any other variety of cell in the pancreatic tissue. The purpose of this brief report is to illustrate the morphology of these inclusions and their probable mode of formation.

Within the nuclei of some acinic cells we found single membrane-bound inclusions containing 1 or 2 granules virtually identical, in size and morphology, to the zymogen granules in the cell cytoplasm (figure 1). This we believe is probably the first stage in the formation of the laminated inclusion. In another group of inclusions the granule in the inclusion was no longer uniformly electron-dense but had a mottled appearance, (figure 2) and such granules appeared to be somewhat larger than the zymogen granules in the cytoplasm. Mottled granules were not found in the cytoplasm. It would therefore appear that this is not a processing artefact and that the larger size and lucent zones indicate an influx of fluid into the granule. Appearances seen in figures 3 and 4 may be looked upon as a continuation of this process which lead to the formation of larger inclusions with an electron-dense rim (figure 3) or laminae (figure 4). However, increasing hydration does not appear to be the only mechanism whereby these inclusions grow in size for it would appear (figure 5) that an increase in size may occur by the fusion of 2 such inclusions within a single-membrane-bound space or by the addition of fresh granules to a well formed laminated inclusion (figure 6).

Little is known about the composition of laminated inclusions, but the images seen by us suggest that these inclusions derive from the secretory granules of the cell. In support of this one may point out that these laminated inclusions have been seen in cells that produce a mucous or serous (i.e. zymogenic) secretion. Therefore one may surmise that laminated inclusions probably contain glycoproteins or mucoproteins.

Yet another intriguing feature of these inclusions is that they are bounded by a single membrane, in contrast to the common pseudoinclusion containing cytoplasmic material which is bounded by 2 membranes derived from the double-membraned nuclear envelope.

Zymogen granules are at times found in the cisternae of the rough endoplasmic reticulum[3]. Since this cisternal system is continuous with the perinuclear cistern one may envisage that a zymogen granule entering the nucleus by this route may carry before it the inner membrane of the nuclear

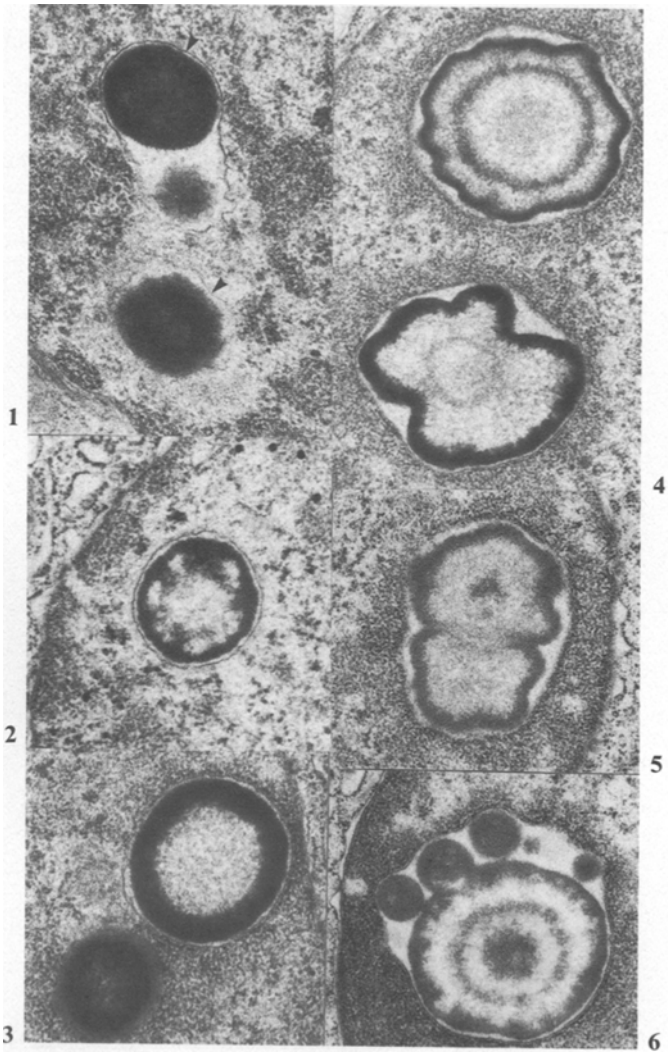

Fig. 1. 2 single-membrane-bound (arrowheads) inclusions are seen in this nucleus. One contains a single zymogen granule the other contains 2 granules. ×28,000. Fig. 2. A single-membrane-bound inclusion containing a mottled granule with a clear center. ×21,000. Fig. 3. 2 inclusions are seen in this nucleus. The one in the bottom left corner is tangentially cut and difficult to evaluate. The other inclusion bounded by a single membrane presents an electron dense shell and a lucent center containing flocculent material. ×20,500. Fig. 4. Laminated inclusions with a crenulated border are seen lying within single-membrane-bound spaces in the nucleus. ×17,000. Fig. 5. Appearances seen here suggest that 2 inclusions are fusing within a single-membrane-bound space in the nucleus. ×16,500. Fig. 6. A laminated inclusion and some zymogen granules are seen in a single-membrane-bound space. 1 of the granules appears to have fused with the laminated inclusion. ×16,500.

envelope and thus form a single-membrane-bound inclusion.

However the usual variety of zymogen granule seen in the cell cytoplasm is bounded by a membrane derived from the Golgi complex. For such a granule to form a single-membrane-bound inclusion one would have to speculate that probably a fusion of the membrane bounding the granule and the outer membrane of the nuclear envelope occurs and that dissolution of the membranes in the zone of fusion liberates the granule into the perinuclear cistern from whence it proceeds into the nucleus as a single-membrane-bound inclusion derived from the inner membrane of the nuclear envelope.

A similar hypothesis has been proposed previously[4] for intranuclear inclusions containing Russell bodies which are also bounded by a single membrane derived presumably from the inner membrane of the nuclear envelope.

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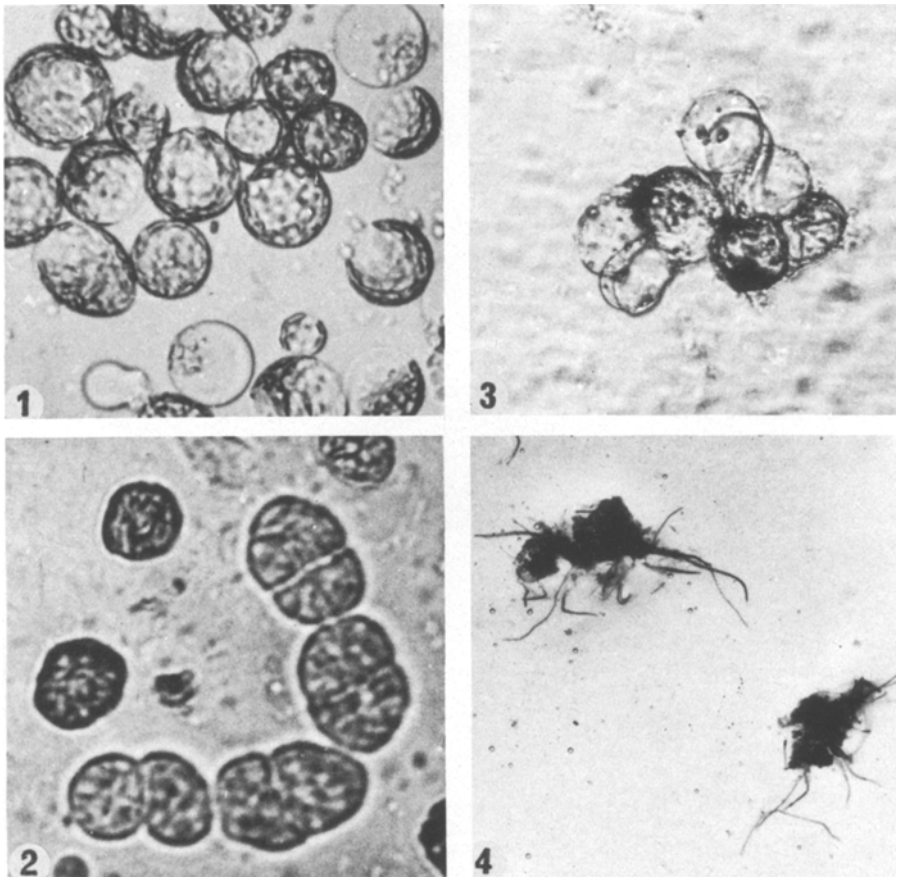
The isolation, culture and organ differentiation from mesophyll protoplasts of *Mollugo nudicaulis* Lam., a C_4 species

K.P. Chellappan, S. Seeni and A. Gnanam

School of Biological Sciences, Madurai Kamaraj University, Madurai 625021 (India), 20 March 1979

Summary. Isolation and culture of mesophyll protoplasts from *Mollugo nudicaulis*, a C_4 species, is reported. Protoplasts developed into callus with root formation. However, no shoot was differentiated. This work was carried out as a part of our overall objective of inducing somatic fusion between the protoplasts of C_3 and C_4 species of *Mollugo*.

The use of the plant protoplast as a potential tool for somatic hybridization, and its significance in crop improvement, has been emphasized[1,2]. Both interspecific and intergeneric fusions of protoplasts and their subsequent divisions are reported in a number of plants. But complete regeneration of somatic hybrids has been achieved only from interspecific somatic fusion of protoplasts[3]. However, no somatic fusion has been reported between the physiologically classified C_3 and C_4 species within a genus. The C_4 species have been demonstrated to be photosynthetically efficient and productive as compared with C_3 species[4]. Of the 180 genera having C_4 species, only 18 genera are known to possess both C_3 and C_4 species. The production of somatic hybrids between C_3 and C_4 plants would help us to



Figures 1–4. Isolation, division and callus formation in mesophyll protoplasts of *Mollugo nudicaulis* Lam.
Fig. 1. Freshly isolated protoplasts at the end of a 4-h digestion in the enzyme mixture. × 450.
Fig. 2. First division of the cultured protoplasts. × 525.
Fig. 3. 8–10 celled colonies on the 10th day. × 750.
Fig. 4. 2-Month-old callus with roots.