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Some structural differentiations in the Hela cell: heavy bodies, annulate lamellae, and cotte de maillet endoplasmic reticulum

Strukturelle Differenzierungen in der Hela-Zelle

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Abstract

A small fraction of HeLa cells within an exponentially growing culture showed cisternal differentiations, such as cytoplasmic as well as intranuclear annulate lamellae and special smooth surfaced endoplasmic reticulum aggregates with a typical "Cotte de maillet" appearance. Additionally, clusters of dense granules were observed in the cytoplasm which were often associated with polysomes and strongly resembled the so-called "heavy bodies" known in particular in diverse oocytes. The functional meaning of these structures is discussed. Moreover, it is deduced from the ultrastructural identity of the pore complexes in the nuclear envelope and the cytoplasmic and intranuclear annulate lamellae that the pore complex material with its highly ordered arrangement is not a structure characteristic for nucleocytoplasmically migrating material, but rather is a general structural expression of a tight binding of ribonucleoprotein (RNP) to cisternal membranes. The pore complexes are thought of as representing sites of a RNP-storage. A similar functioning is hypothesized for the "heavy body"-like aggregates. To the current hypotheses on the formation of annulate lamellae and the nuclear envelope, which are based on the concept of membrane continuities and constancies, the alternative view of a self assembly mechanism of membrane constituents on nucleoprotein structures is added.

Introduction

Among the mammalian cells grown in vitro the HeLa cell line is one of the best studied ones, at least concerning the biochemical cytology. Thus, any structural information on this cell type is especially needed for understanding the correlation of structures and function in this system. Although the population of an exponentially growing HeLa

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cell culture appears widely homogeneous at the ultrastructural level, single cells can sometimes be encountered which are distinguished by conspicuous structural features of the cytoplasm as well as within the nucleus. So, e. g., an abundance of perinuclear microtubules and filaments has recently been described to occur in a small fraction of cultivated HeLa cells [30]. In addition, the present study deals with special cisternal differentiations and with the occurence of particulate aggregates, which are usually associated with polysomes and which suggest a close relationship to the "heavy bodies" known from other cell types.

Materials and methods

HeLa cells were grown and processed for thin section electron microscopy as described elsewhere [12, 29, 30]. The degree of contamination with Mycoplasmataceae was routinely checked by quantitative evalutions of electron micrographs, and only those cultures in which less than one PPLO was found among one hundred HeLa cells were used throughout the present study.

Results

Throughout the present study, it was repeatedly observed that a small fraction of HeLa cells within the cultures contained cytoplasmic annulate lamellae (AL) as being either single cisternae (e. g. Fig. 2) or arranged into more or less comprehensive stacks (Fig. 1). The existence of such cytoplasmic AL (for recent reviews see, e. g. [52, 80, 89]) has been documented for HeLa cells by EPSTEIN [20] and ERLANDSON and DE HARVEN [21]. In the HeLa material of this study, the pore frequency of the AL was usually higher than that of the corresponding nuclear envelope in the same cell by a factor of two to three, a difference which is characteristic for a great many of the AL-possessing cells (c. f., e. g., [42, 70, 80]. The cytoplasmic AL generally seemed to be more frequent in the immediate vicinity of the nucleus (e. g. Fig. 1 and 2; c. f. also [21]). As has already been noted by the above mentioned investigators, the cytoplasmic annulate cisternae are in manifold luminal continuity with normal rough endoplasmic reticulum (ER) cisternae (e. g. Fig. 1). No differences were recognized in the subarchitecture of the pore complexes of the AL and those of the nuclear envelope, which has also been reported from various other cell systems (e. g., [52, 80, 89]). In histochemical examinations using the procedure of BERNHARD [7] the non-membraneous pore complex material of the AL behave as positively as did the nuclear pore complexes [33], thus suggesting a ribonucleoprotein character. ERLANDSON and DE HARVEN [21] reported fluctuations in AL frequency in synchronized HeLa cell cultures during the course of the cell cycle. With the non-synchronized cells of our study we were not able to precisely determine the interphase stage of the AL-containing cells.

Another cisternal system which could be seen in continuity with the rough ER cisternae are the stacks of smooth surfaced cisternae (Fig. 1) which are arranged in a characteristic meshwork-like pattern resembling the typical "cotte de maillet" aspect known in other plant and animal cell types (for references see [6, 22, 67]). Such a cisternal organization has hitherto not been reported from HeLa cells. Another sort of stack-aggregation of tightly associated smooth cisternae has, though, been noted by EPSTEIN [20]: These are "paired smooth cisternae" similar to those known from diverse other cells, especially from tumorous material (for review see [34, 39]). In contrast to what EPSTEIN has reported for such "paired cisternae", the "cotte de maillet"

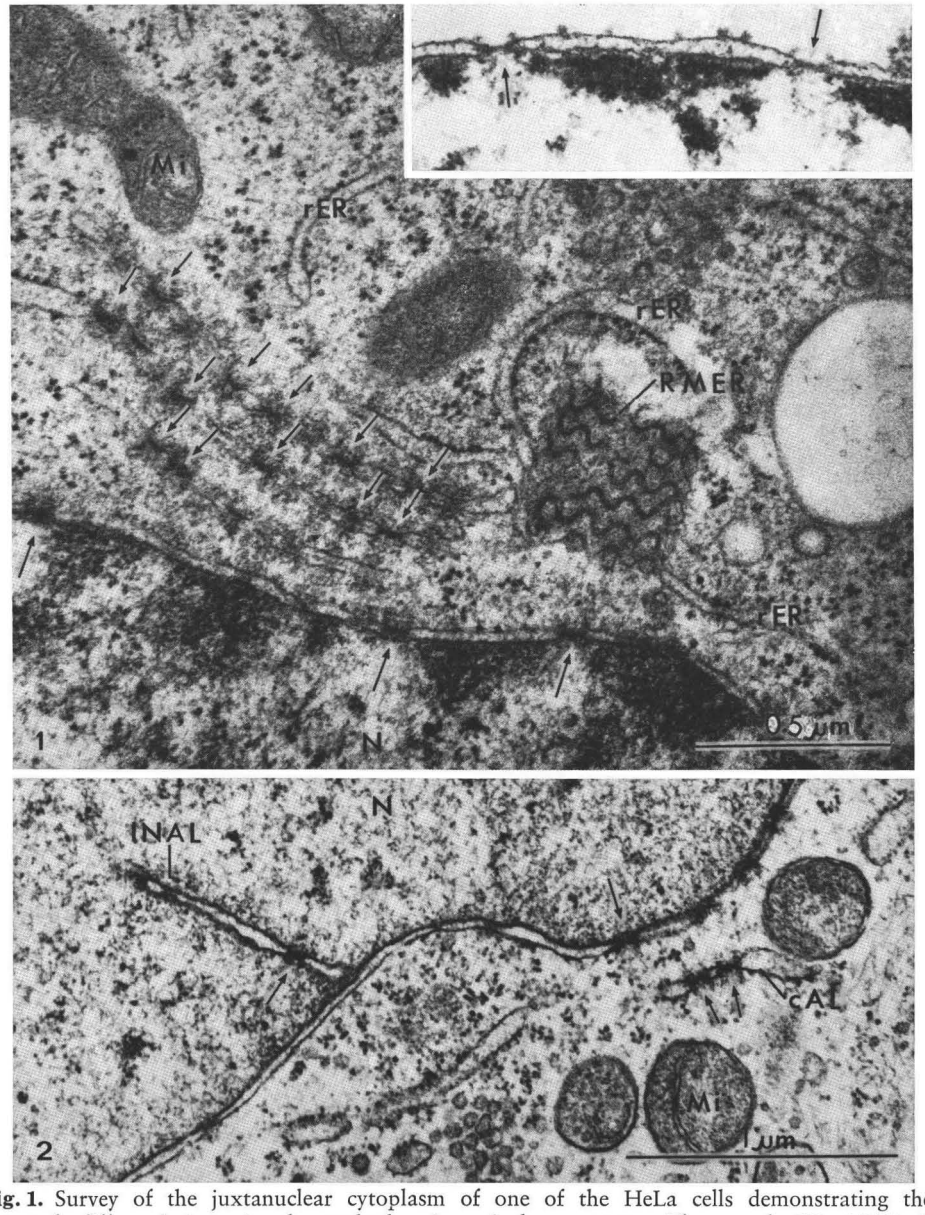

Fig. 1. Survey of the juxtanuclear cytoplasm of one of the HeLa cells demonstrating the cisternal differentiations in the endoplasmic reticulum system: The rough ER (rER) is continuous with stacked annulate lamellae (pore complexes denoted by the short arrows) and with the smooth surfaced cisternal aggregates of the "robe de maillet" appearance (RMER). – The lower series of long arrows denote nuclear pore complexes which are recognized in more detail with isolated HeLa nuclei as shown in the inset. – N nucleus. – Mi mitochondrion. – 52 000 ×. – Inset 62 000 ×.

Fig. 2. HeLa cell showing an intranuclear annulate cisterna (INAL), the nuclear envelope, and a cytoplasmic annulate cisterna (cAL) lying nearby in the juxtanuclear zone. The arrows denote the pore complexes. – 36 000 ×.

stacks are continuous with both types of ER system, namely the rough cisternae and the AL (Fig. 1). A general hypertrophy of ER-elements, including the smooth ones, has been ascribed to a treatment of HeLa cells with metaphase-arresting agents by ERLANDSON and DE HARVEN [21].

Besides the cytoplasmic AL, a small number of cells also showed intranuclear AL (Figs. 2 to 4). These occured generally as single cisternal sheets and were never found to accumulate in stacks. Intranuclear AL have so far been documented only for special cellular situations including, e. g., diverse germ cells, embryonic cells, rat placenta trophoblasts (for literature see, e. g., [15, 16, 23, 24, 28, 44, 45, 47, 49, 50, 52, 66, 73]). Since MAUL [66] describes them in melanoma cells, their occurence in HeLa – as another tumorous material – is of particular interest. Fig. 3 presents a survey of a HeLa nucleus in order to demonstrate the low frequency and the mode of distribution of such intranuclear AL. The profiles of intranuclear AL, encountered in the course of this study were of a maximal length of ca. 6 μm. Linear forms were observed besides curved and branched ones (e. g. Fig. 3). Since both forms of AL, nucleoplasmic and cytoplasmic, are relatively rare in HeLa cells it might be worth mentioning that cells which had AL often showed them in both compartments (e. g. Fig. 2). Details of the

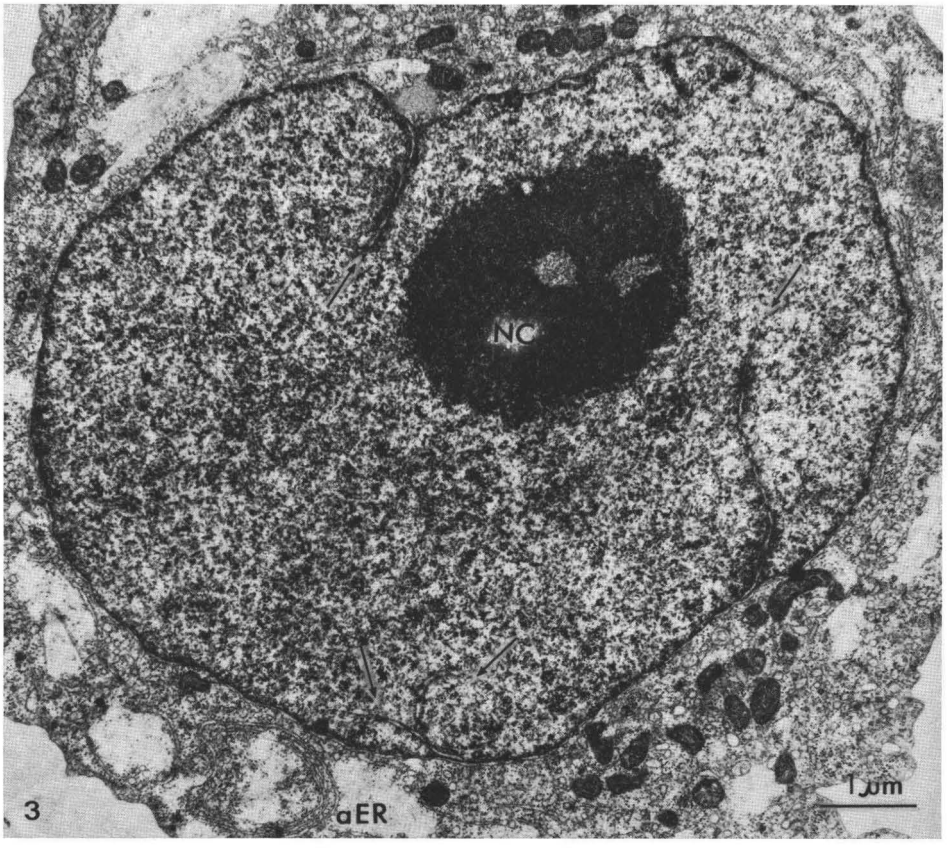

Fig. 3. Survey of a HeLa nucleus showing the distribution of the intranuclear annulate lamellar sheets (arrows). Nc, nucleolus; aER, aggregations of rER cisternae. – 13 000 ×.

intranuclear AL are presented in Fig. 4. A continuity of the AL membrane with the inner nuclear membrane such as, e. g., EVERINGHAM [23] has convincingly demonstrated for the ascidian oocytes was never clearly identifiable in this study. Very frequently, though, the intranuclear AL cisternae were recognized as closely abutting the inner aspect of the nuclear envelope under a relatively low angle and then running parallel to it for a certain distance (e. g. Fig. 4). In all such associations, however, both membranes were separated by a ca. 25 nm wide interspace which was usually filled with condensed chromatin (Fig. 4). A coating of the intranuclear AL with condensed chromatin patches was also detectable in deeper areas of the nucleoplasm where

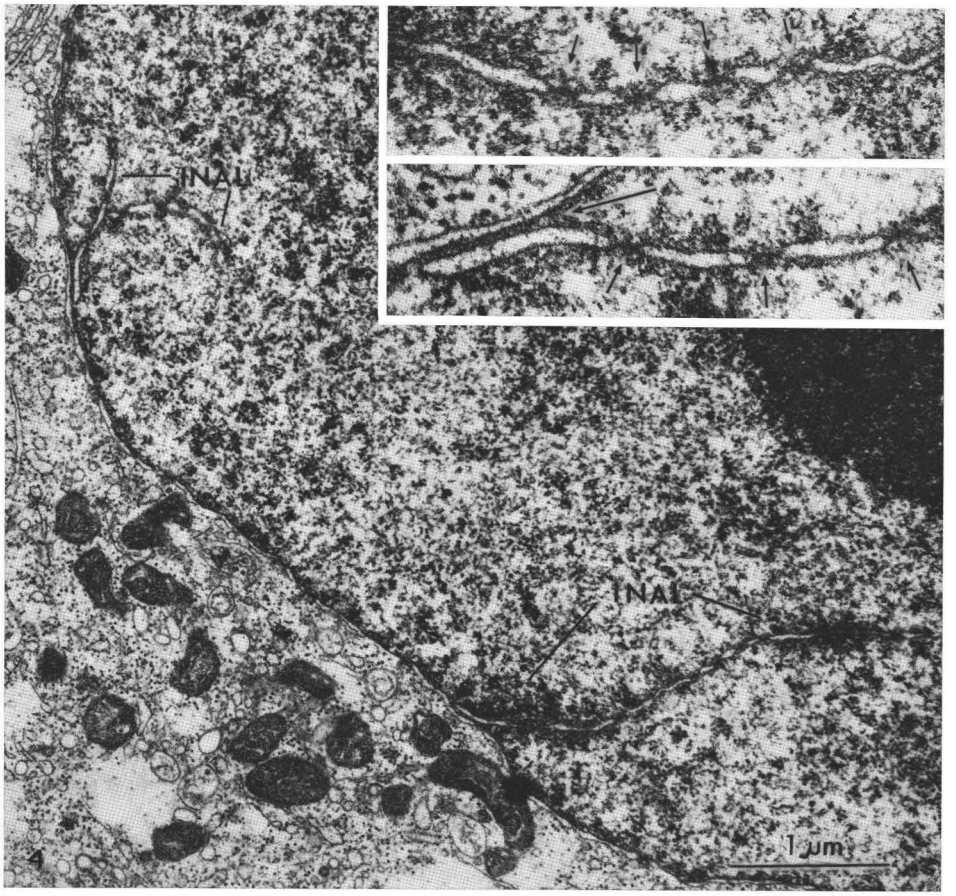

Fig. 4. Details of Fig. 3. The INAL are often intimately associated with clumps of condensed chromatin. They tend to abut the inner aspect of the nuclear envelope under a low angle and can run parallel to it for a certain distance. However, in such parallel associations they are always separated by a ca. 25 nm interspace which usually appears to be filled with condensed chromatin (long arrow in the lower inset) in a similar pattern as is characteristic for the inner nuclear membrane. The pore complexes of the INAL (arrows in the inset) show all basic structural features known from the cytoplasmic AL and the nuclear envelope pores such as, e. g., the annulus material and particles associated with the pore center. – 21 000 ×. – Insets 51 000 ×.

condensed chromatin normally does not occur (e. g. Fig. 3). In this respect the situation in HeLa bears some resemblance to the chromatin-AL association in the melanoma [66], the rat placental trophoblasts [47, 73] and the spermatogonia of the insect. *Philaenus spumarius* [28]. A membrane-coating with similarly electron dense fuzzes is also noted in the cisternal aggregates associated with the nucleolar periphery in diverse oocytes (e. g. [15, 16, 57, 58]) and tumor cells [4].

The pores appeared to be less densely packed in the intranuclear AL than within the nuclear envelope and cytoplasmic AL in the same cell (see, e. g. [73]). The pore complexes of the intranuclear AL revealed all structural details which are known for the pore complexes of the nuclear envelope and the cytoplasmic AL. In particular, the annulus material at the pore margins was identified as well as the intraporous matter and the particles and filaments, which are can be associated with the pore center (Fig. 4). The similarities of the structural organization of the pore complexes in all these three types of porous cisternae is also emphasized in the findings of other authors in different cell systems (e. g. [16, 23, 73]).

The appearance of AL as well as of the tangles of perinuclear microtubules and filaments [30] in certain HeLa cells was often accompanied by the occurence of non-

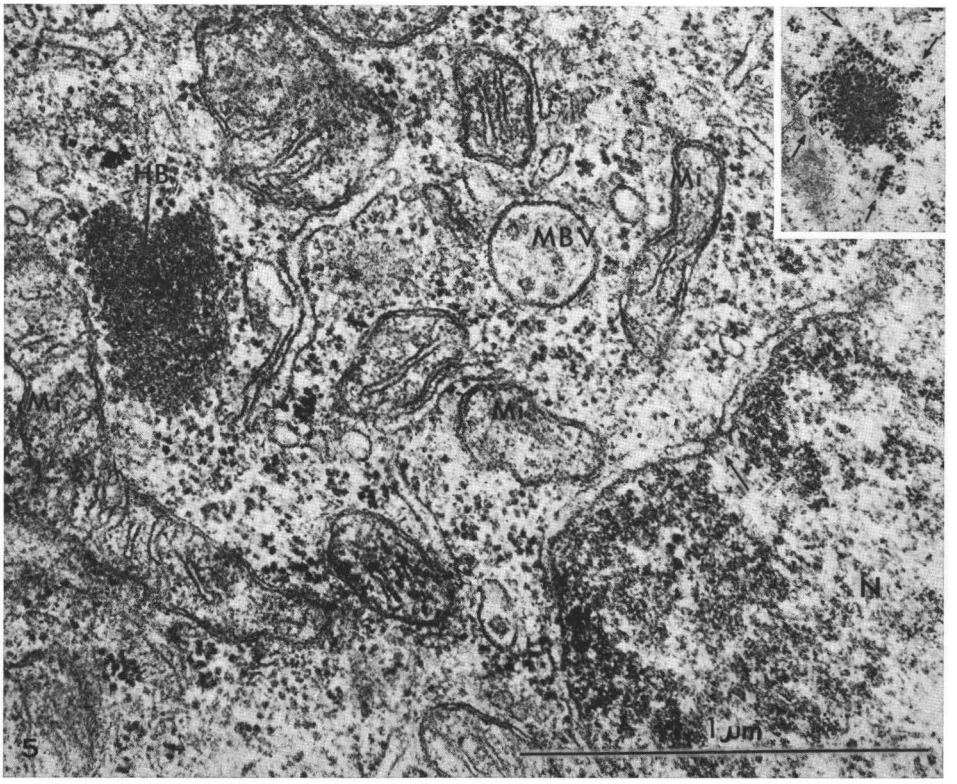

Fig. 5. Dense clusters, in which granular components can often be identified, lie in the HeLa cytoplasm and are interpreted as being related to "heavy bodies" (HB). Frequently, these aggregates are conspicuously associated with polysomes (inset). N, nucleus; arrow denotes a nuclear pore; Mi, mitochondria; MVB, multivesicular body. 54 000 ×. – Inset 27 000 ×.

membraneous, almost spherical dense aggregates measuring up to 0.6 μm in diameter. These were relatively evenly distributed over the cytoplasm and sometimes suggested a composition of tightly packed 120 to 180 Å large granules (e. g. Fig. 5). Their periphery was often characterized by a conspicuously close association with polysomes, especially with the helical, non-membrane-bound ones (Fig. 5 inset.). Such spherical aggregates of dense granules strongly resemble the class of "heavy bodies" [2]) known in other cell types, so in particular in germ cells of various species (for literature see the recent article by [19]; further [1, 2, 3, 11, 14, 17, 25, 27, 38, 40, 41, 51, 53, 54, 56, 60, 64, 65, 69, 71, 81, 84, 87, 91, 92]), but also in the nurse cells of *Dytiscus marginalis* [26], in differentiated and embryonic cells as well as neoblasts in *Turbellaria* [35, 61, 62, 72, 77] and in the siphonous green alga *Acetabularia mediterranea* [8, 85, 88].

Discussion

In view of the rarity of the aforedescribed structures in a HeLa cell population it might be of interest to state that all such differentiations, including also the "perinuclear beards" of microtubules and filaments [30], can occur coincidently within the very same cell.

The aggregations of the "cotte de maillet" type of organization are likely to belong to a class of paired or stacked formations of smooth ER cisternae which are known in diverse plant and animal cells and which apparently are especially frequent in tumorous material and in the developing sieve elements of higher plants (for detailed discussions see, e. g. [6, 22, 34, 39]). Although such cisternae appear in frequent luminal continuity with normal rough ER, we see no point contradictory to the currently dominating interpretation (c. f. also [90]) that such aggregates represent cisternae of formerly rough ER which tend to closely associate after becoming detached from the ribosomes. Such a behaviour might represent some sort of a membrane-to-membrane self assembly process of "inactivated" cisternae in general (see also [34]).

Some important conclusions can be simply drawn from the structural identity of the pore complexes of the cytoplasmic and intranuclear annulate lamellae with those of the nuclear envelope:

1. The symmetrically ordered components of nuclear envelope pore complexes cannot merely represent the structural "snapshot" image of material during a nucleocytoplasmic translocation, as such is often discussed in the literature since the findings of Stevens and Swift [82]: the annular components as well as the intraporous material, including the diverse categories of filaments and the central granule, are recognized with both forms of AL as well.

2. Cytoplasmic annulate lamellae are most likely to contain ribonucleoproteins (see, e. g., [36, 52, 75, 89]; this study). Cytochemical and autoradiographic attemps to detect DNA in association with this type of structure have hitherto been negative (these authors; see also [37]). Thus, this might support the general concept that the nuclear pore complexes do not contain considerable amounts of DNA, as has been recently

[2]) Some probable synonyms for closely related structures of this category: dense bodies, dense granular masses, granular cytoplasmic bodies, granular-fibrillar bodies, dense lumps, »amas denses", "agrégates cytoplasmiques", "extrusion nucléaire", perinuclear "nuages", nuclear emissions, polar granules, intermitochondrial cement, nucleolus-like bodies, "pseudo-nucléoles", "endoplasmatische Einschlüsse".

suggested through the work of COMINGS and associates (e. g. [13]), but are made up to a high degree of ribonucleoproteins (e. g., [30, 33, 68, 78, 79]; for contradictory literature speaking for a purely proteinaceous character compare, e. g., [18, 59, 70]).

These conclusions, taken together with the finding that nuclear membrane fractions contain a distinct portion of RNA tightly bound to the membrane (e. g., [31, 78, 79, 93]), lead us to the concept that the nuclear pore complex structures are the general expression of a type of RNP-binding to cisternal membranes. Pore complexes thus might be structures characteristic for a special type of RNP storage in a membrane-bound state (c. f. also [5, 86]), be it either a transitory dynamic stage or a more static one.

With respect to the general formation of such pore complexes two alternatives are consequently conceivable: namely (i), that the cisterna exists first and the pore complexes with their typical structural organization are produced by interaction with the nucleoprotein component (e. g. [52, 76]); for a detailed discussion on structural concepts of such modes of pore formations in cisternae see also [32], or (ii), that the nucleoprotein matter, be it RNP or DNP, organizes the membrane material in the mode of a true assembly membranogenesis, or a membrane transformation, using either preexisting membrane pieces such as vesicles and ER-like cisternae (c. f., e. g. [48, 63, 66, 74]) or using micellar membraneous subunits. This latter type (ii) would then be somewhat analoguous to the "initiation" of phage particle assembly by nucleoprotein (e. g. [46]).

With the intranuclear AL of HeLa we found no indication for the hypothesis of MAUL [66], who envisaged the intranuclear AL of melanoma cells as generally representing fragments of the nuclear envelope which are entrapped during the mitotic telophase within the reconstituting nuclear membranes. However, such an origin, which is also suggested in the micrographs of LONGO and ANDERSON [63] on the nuclear envelope formation around the dispersed chromatin of the male pronucleus in surf clam fertilization, seems to be excluded for intranuclear AL in oocytes of, e. g., tunicates: Here they are not present in the young stages but appear later in the growing phase of oogenesis ([49]; compare also [44, 45]). Again we would like to add to the current views on the modes of the formation of both forms of AL, as well as of the nuclear envelope and all other types of intranuclear membrane-like structures (see, e. g., also [9, 43]), which are exclusively based on the assumption of membrane coherence and constancy (e. g., [23, 44, 45, 49, 50, 52, 66, 73]), the alternative hypothesis that self-assembly of membranes might be nucleated at nucleoprotein structures such as, e. g., the condensed chromatin (e. g. also telophase chromosomes) or the nucleolus. Both of these concepts obviously do not exclude each other.

The lack of a special distinct structural feature is ostensibly a part of the character of the heavy body-like aggregates and the listing of some of the structures quoted in the previous chapters in this connection under this "family" is still very questionable. Although the heavy body-like aggregates are especially frequent and particularly well studied in germ material, the present study as well as a good number of other reports [8, 35, 61, 62, 72, 77, 85, 88] clearly demonstrate that their occurence is not limited to germ cells alone (e. g. [19]). From some cell systems at least, it appears to be justified to ascribe a RNP-nature to these structures as has been demonstrated by cytochemistry [1, 11, 14, 17, 55, 60, 65, 71, 87], autoradiography [17, 18] and as is also suggested from the conspicuously intimate association with ribosomes and polysomes (see also [65]). On the other hand, such a RNA content is denied in the work of CLEROT [10] and EDDY and ITO [19]. Moreover, in connection with the previous paragraphs, it is

particularly interesting to recall to mind the findings in the sea urchin oocyte which show that such heavy bodies can occur in a close association with AL as well (e. g., [1, 5, 38, 40, 41, 86]). The functional meaning of such aggregates is totally unknown. Some authors speculated a RNA storage function, especially of those RNAs carrying information for differentiating processes (e. g., [41, 64, 83]). As far as the HeLa cell is concerned, however, we can state that this type of aggregate is not a structure obligatory for the average cell in an actively growing population.

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