INTRANUCLEAR AND CYTOPLASMIC ANNULATE LAMELLAE IN PLANT CELLS

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INTRODUCTION

Annulate lamellae (AL) have hitherto been described for animal cells (in particular, for germ cells), for embryonic systems, and for rapidly growing cells like tumor and cancer cells (for review, see references 10, 16) Such cisternae, which are either single or arranged in stacks of parallel lamellae, are characterized by the presence of pore complexes, i.e., pores of a relatively uniform size distribution which are associated with distinct granulo-fibrillar structures and are arranged in a symmetrical subarchitecture identical to that of nuclear pore complexes (e g., 10, 14). No micrograph has so far been published which documents the presence of such structures in plant cells. Kessel (10), though, in an addendum to his review, mentioned nonpublished observations of Skvarla who claimed the existence of "extensive profiles of annulate lamellae in pollen of *Canna* during pollen wall formation" We have been able to confirm this remark and, additionally, have found that stacks of AL occur also in earlier stages of microsporogenesis (e g , pollen mother cells) of *Cannaceae* and *Zingiberaceae* [1] Sen (15) has demonstrated membranous stacks of *Lilium* microsporocyte meiotic prophase and discussed a relationship to annulate lamellar formations, but these lamellae do not show any pore complexes and thus are to be regarded as stacked endoplasmic reticulum (ER)[2] rather than AL

The question is whether AL in plant cells are confined to pollen development of a few species or are of more general occurrence Since in animal cells AL have been described mostly from cell types with a high RNA synthesis including, e g. diverse

[1] Scheer, U., and W. W. Franke. Manuscript in preparation.

[2] Such stacks of ER are commonly very elaborate in various microsporocytes and can form the so-called "nuclear cap" (e.g., reference 11).

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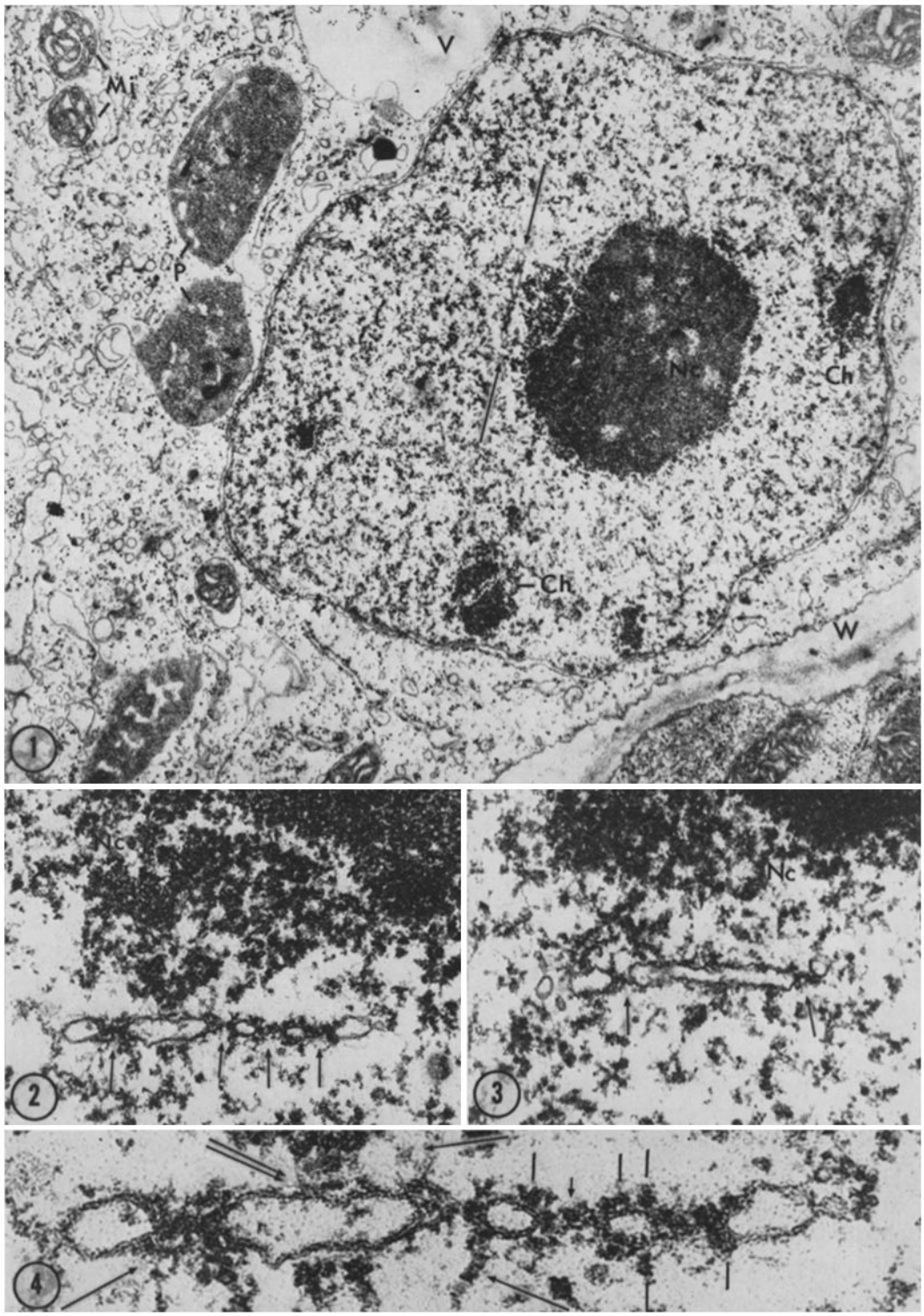

FIGURES 1–4. Intranuclear annulate lamellae in a culture cell of *H. gracilis*. Fig. 1 gives a nuclear survey Arrows indicate an intranuclear annulate cisterna which lies at the nucleolar chromatin. Figs. 2 and 3 are serial sections of this situation (small arrows indicate pore complexes). Details of the pore complex substructure are recognized at the higher magnification of Fig. 4. Annular granules (denoted by the bars) are associated with the pore margins; the arrowhead points to a central granule-like density. The long arrows indicate fibrils terminating at the annular regions. The double arrow points to a site of chromatin attachment at the cisternal surface. *Nc*, nucleolus; *Ch*, dense chromatin; *W*, cell wall; *V*, vacuole; *Mi*, mitochondria; *P*, plastids. Fig. 1, × 21,000; Fig. 2. × 67,500; Fig. 3, × 54,000; Fig. 4, X 150,000.

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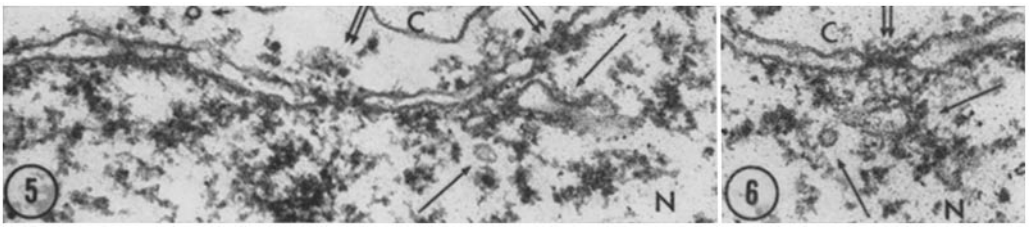

FIGURES 5 and 6. Membrane profiles in the periphery of *H. gracilis* nuclei are indicated by the arrows. Double arrows denote nuclear pore complexes. Arrowhead points to a juxtanuclear microtubule. Note strand-like connections between such intranuclear membrane profiles and the pore complex (Fig. 6). *N*, nucleoplasm; *C*, cytoplasm. Fig. 5, × 70,000. Fig. 6, × 88,000.

cultivated tumorous cells, we have focused our attention on plant material which has a high RNA turnover and is in exponential growth conditions.

MATERIALS AND METHODS

Cell suspension cultures from the stalk of *Haplopappus gracilis* were cultivated as described by Fritsch et al. (5). Cells of an exponentially growing culture were fixed for 30 min at 25°C in 2% glutaraldehyde (buffered with 0.05 M sodium cacodylate to pH 7.2 and containing 0.1 M KCl and 2 mM $CaCl_2$). The cells were then washed thoroughly in the buffer and postfixed in 2% OsO_4 (pH 7.2) for 2 hr in the cold. Dehydration was carried out through graded ethanol steps in the cold. The material was embedded in Epon 812 and sectioned on a Reichert ultramicrotome OmU2 (C. Reichert, Buffalo, N. Y.). Micrographs were made with a Siemens Elmiskop IA.

RESULTS AND DISCUSSION

Fig. 1 presents a survey micrograph of a *Haplopappus* cell nucleus to demonstrate the position of the intranuclear annulate lamellae (IAL). Such an annulate cisternal sheet is shown in detail in two different section planes in Figs. 2 and 3 (from a section series) to give an impression of the three-dimensional extension of the cisterna. A coating of the IAL membranes with condensed chromatin as was observed in rat placental giant cells (9, 13), in the rabbit zygote (6), in human melanoma cells (12), in HeLa cells (3), and in insect spermatogonia (4) is not so apparent here. However, nucleolus-associated heterochromatin[3] and the IAL often display remarkably close proximity (e.g., Figs. 2–4). Structural details of the pore complexes of the IAL are presented in Fig. 4. Granular annular subunits lying upon the pore margin on both sides of the cisterna are clearly visible, as well as the relatively compact, electron-opaque inner pore material, which is typical for pore complexes in general (2). Fibrils extending from the annulus region and central granules are also sometimes recognized (Fig. 4). Besides the IAL, some smaller intranuclear membranous cisternae or vesicles were found in the peripheral part of the nuclei (Figs. 5, 6, 8, 10). These resemble to a certain degree the intranuclear membranes described in spider crab oocytes by Hinsch (7) who further hypothesized that they may play a role in nucleocytoplasmic exchange. Such membrane vesicles can reveal conspicuous associations with the inner nuclear membrane (Fig. 5), or with the fibrils extending from the nuclear pore complexes (Fig. 6). It is interesting to mention in this connection that typical IAL were recognized almost exclusively in posttelophase cells in which the cell plate formation was still not finished. This observation fits into the concept of Maul (12) who visualized the IAL as being residues of the former interphase nuclear envelope which are entrapped in the course of anaphase nuclear envelope restoration (for more detailed account, see reference 3). It is also conceivable, however, that such IAL represent nuclear membrane assemblies at chromatin structures which is erratic in the sense that they are not integrated into the new nuclear envelope.

In particular, cytoplasmic annulate lamellae (CAL) were encountered in the juxtanuclear region (Figs. 7–9). They are not extensively developed and usually consist of single cisternae with relatively few pore complexes (e.g., Fig. 10). The CAL in a *Haplopappus* cell may be characterized as cisternae of rough ER with occasional pore complexes. Such cisternae either are isolated from the nuclear envelope (Figs. 7 and 8) or are in direct

[3] Identifiable as deoxyribonucleoprotein with the selective staining procedure of Bernhard (1) in parallel preparations fixed with aldehyde alone.

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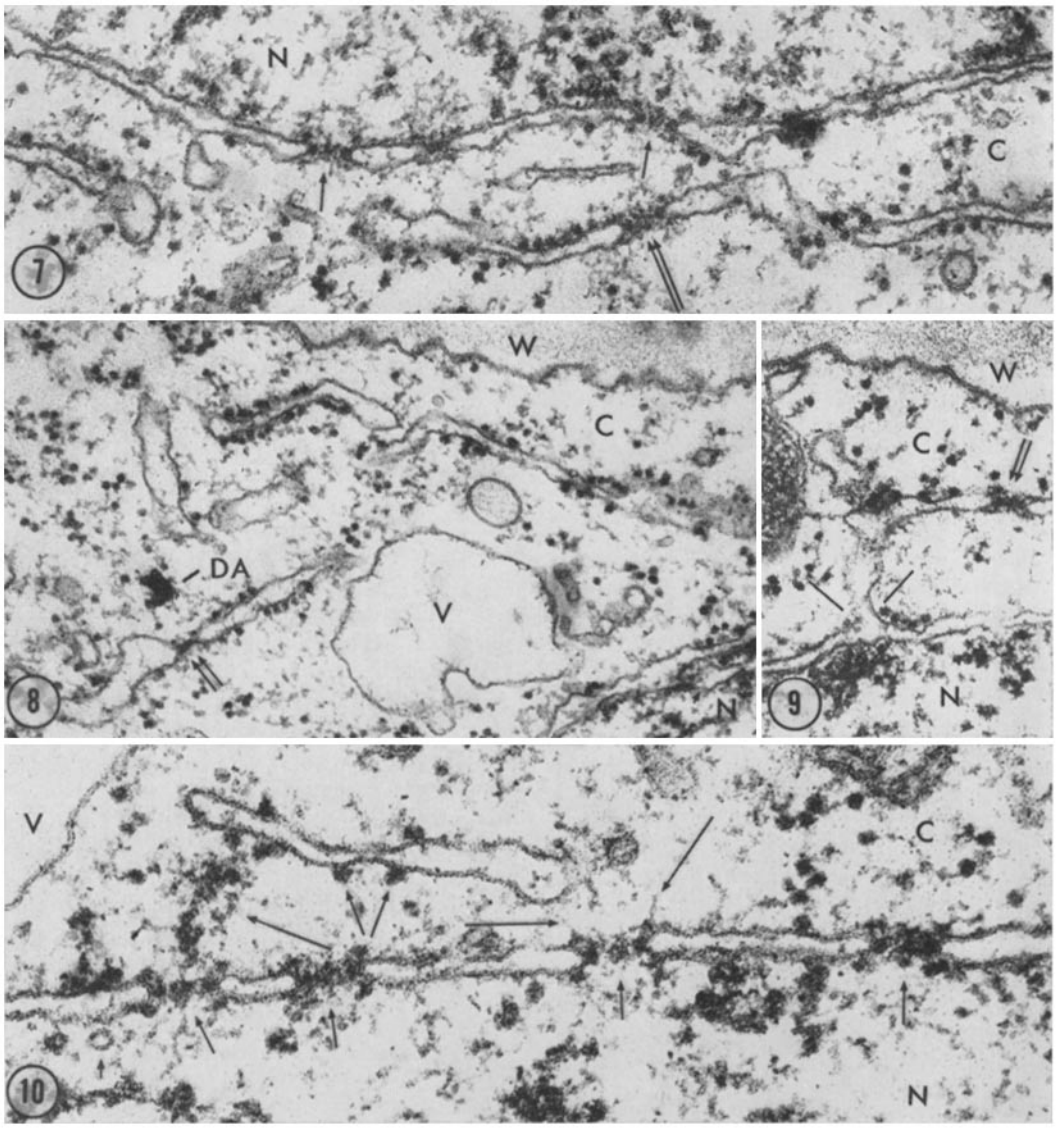

FIGURES 7–9. Pore complexes in cytoplasmic ER cisternae in *H. gracilis* culture cells (double arrows). Nuclear pore complexes are indicated by the small single arrows. In Fig. 8 a "dense aggregate" (*DA*) is seen in the vicinity of an ER pore complex. Fig. 9 shows the presence of such an ER pore complex in an ER cisterna which is continuous with the nuclear envelope (connection indicated by the bars). The arrowhead in the lower right of Fig. 8 points to an intranuclear vesicle. Fig. 7, × 90,000; Fig. 8, × 56,000; Fig. 9, × 70,000.

FIGURE 10. A peculiar ribosomal arrangement which suggests an initial stage of pore complex formation is recognized at the ER cisterna (pair of arrows) Note that fibrillar connections extend from the nuclear pore complexes (small arrows) toward this ER cisterna (connecting strands indicated by the long arrows). The arrowhead in the lower left again points to an intranuclear vesicle. × 110,000.

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continuity with the perinuclear cisterna (Fig. 9, for examples in animal cells, see also references 10, 14) Sometimes a close relationship between AL pore complexes and "dense aggregates" is visible (Fig. 8) Another conspicuous but not infrequent formation is shown in Fig. 10. In between membrane-attached ribosomes, rough ER sections can exhibit indications of local membrane fusion which might result in the production of a "pore complex" It is possible that such membrane fusions are initial stages in the transition of rough ER sections toward an AL-like (i.e., pore complex-bearing) character: ribonucleoproteins, appearing either as ribosome-like particles or as coarse strands which are sometimes connected with nuclear pore complexes (e g , Fig 10), may cause a local breakdown of an ER cisterna followed by subsequent rearrangement, thus producing a hole (= pore) with the ribonucleoprotein material associated This view is in accordance with observations made by Hoage and Kessel (8) on spermatogenesis in drone honeybee in which pore complexes are differentiated in restricted regions of preformed ER membranes. In any case, the AL pore complexes (and also the nuclear pore complexes) appear to represent the general structural expression of a nucleoprotein-lipoprotein interaction rather than being only gates for translocation processes (3) However, one has to admit that clearcut information on the chemical nature and functional meaning of AL is generally still lacking

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