NUCLEAR AND CYTOPLASMIC TUBULES IN CORTICAL CELLS OF LEAF BELTIAN BODIES

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Beltian bodies are small appendages found at the tips of the leaf rachis and pinnules of certain tropical species of *Acacia*. Ants living on the tree harvest these structures which, along with a sugar solution from petiole glands, furnish the ant's food supply (3). During a histochemical study of these structures, inclusions were noted in the nucleoplasm and cytoplasm that reacted positively to protein stains. With the electron microscope, clusters of tubules were found in these two regions of the cortical cells.

Nuclear crystals have been known since the 1800's, and recent ultrastructural studies have shown a protein-crystalline arrangement for most of these inclusions (8, 9). Schnepf (7) presented electron micrographs of a loosely arranged, small cluster of lamellae (his term) found in the nucleus of *Pinguicula* gland cells. His paper seems to be the only account of fibrillar-like material in plant cell nuclei. Unfortunately, he presents no micrographs of transversely sectioned lamellae.

The clusters of cytoplasmic tubules are apparently new to plant cells. Their small size and massive distribution distinguish them from other known tubules such as microtubules (4), spindle apparatus (2), and the tubular system found in myxomycete plasmodia (5).

MATERIALS AND METHODS

Seeds of *Acacia cornigera* from Costa Rica were supplied by Dr. Daniel Janzen, Department of Entomology, University of Kansas, and were planted in greenhouses in Madison. Seedling and young plant growth was vigorous, and Beltian bodies were produced in large numbers. Janzen (3) states that, in nature, ants harvest the Beltian bodies within 5 days after they become visible. In Madison, they were collected 3 days after their appearance in order to simulate the timing in nature.

Each Beltian body was sliced in half and fixed at 4°C for 6 hr in 6% glutaraldehyde in 0.02 M cacodylate buffer at pH 6.9. The tissue was washed for 1 hr in four changes of buffer and postfixed in 2% osmium tetroxide in distilled water at 4°C for 4 hr. The Beltian bodies were then dehydrated with an acetone series and embedded in Epon 812. Sections were further stained with 2% uranyl acetate in 50% ethyl alcohol followed by lead citrate (6) diluted 1:5 with 0.01 N

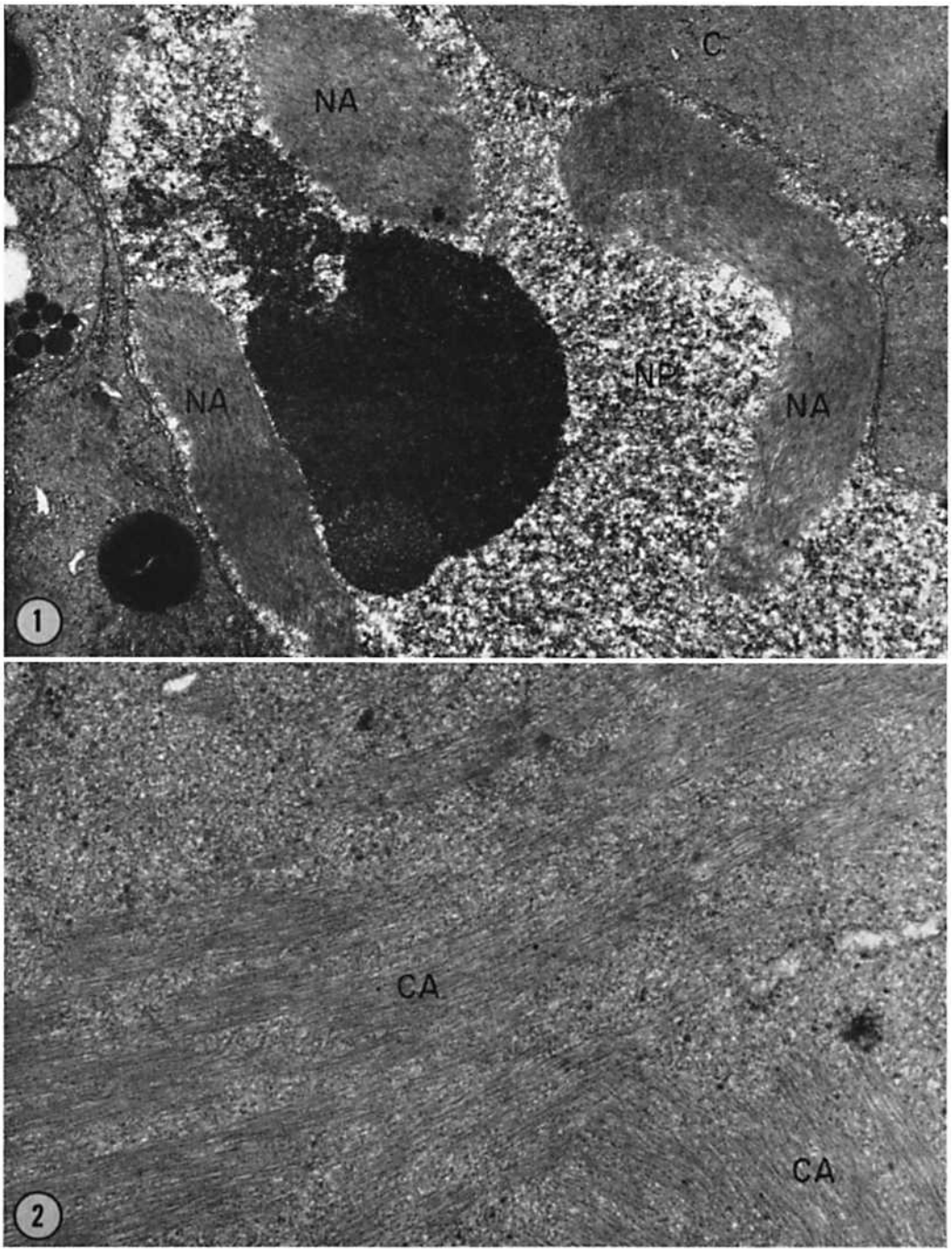

FIGURE 1 Section of a cortical cell nucleus showing three nuclear aggregates (*NA*) in the nucleoplasm (*NP*). Tubules can also be seen in the cytoplasm (*C*). × 18,000.

FIGURE 2 Portion of the cytoplasm with two cytoplasmic aggregates (*CA*) present. × 54,000.

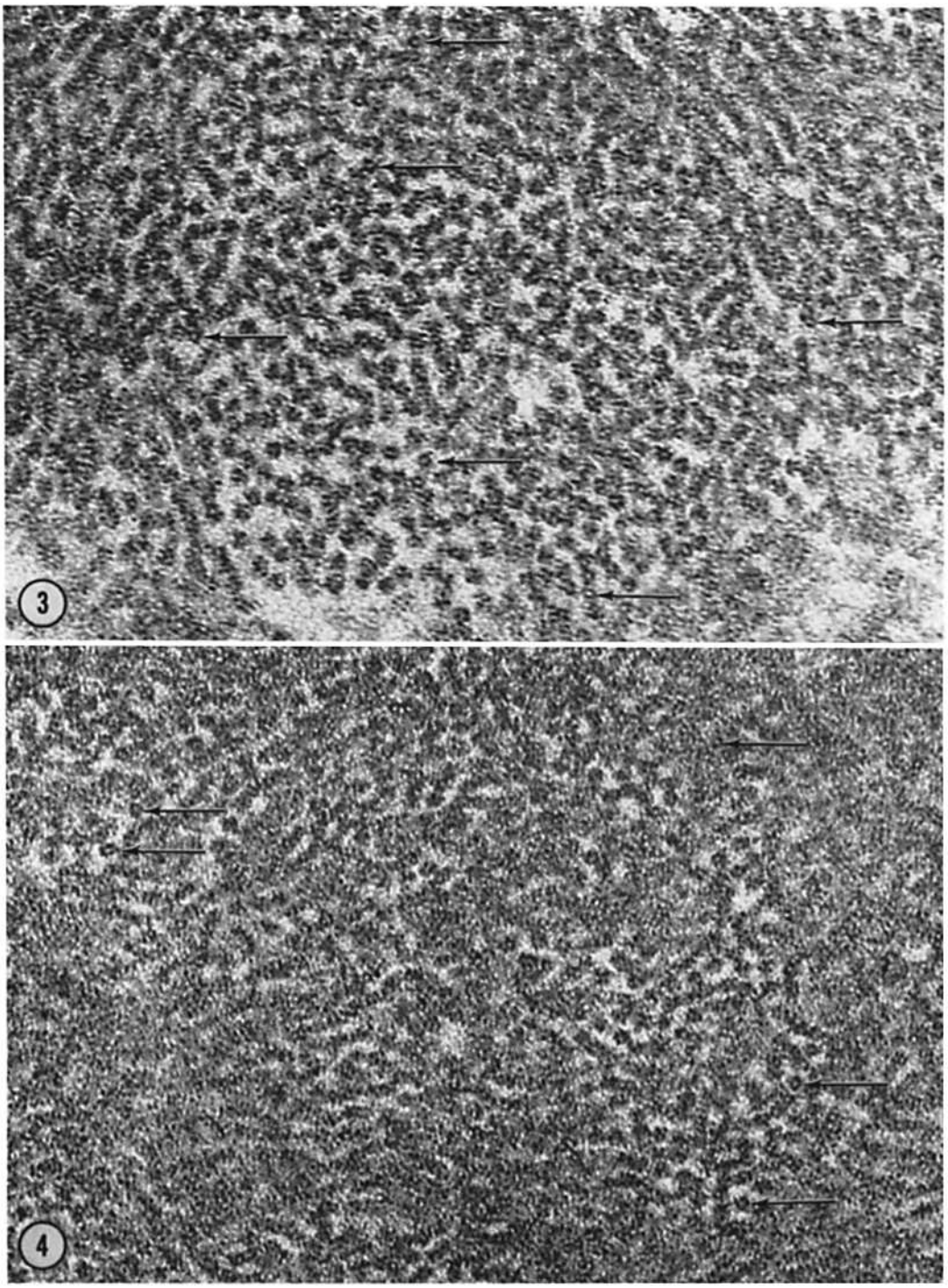

FIGURE 3 Transverse section through part of a nuclear aggregate. Tubules oriented perpendicular to the plane of section are noted by arrows. × 258,000.

FIGURE 4 Transverse section through part of a cytoplasmic aggregate. Tubules oriented perpendicular to the plane of section are noted by arrows. × 258,000.

(CO_2-free) NaOH. Micrographs were made with a Hitachi 11C electron microscope.

RESULTS AND COMMENTS

Both nuclear and cytoplasmic tubules are organized into clusters or aggregates (Figs. 1, 2). The aggregates are randomly distributed in the nucleoplasm and cytoplasm. They are clearly evident in the nucleoplasm, but, owing to background density, it is more difficult to delineate them in the cytoplasm. While the cytoplasm has a large population of ribosomes, these particles were never observed within an aggregate. Thus, on viewing micrographs of the cytoplasm, one finds large areas free of ribosomes; close examination reveals that the area devoid of ribosomes is occupied by an aggregate. This might imply that, although composed of individual tubules, the aggregate functions as a unit. The clusters maintain their integrity even when two or more touch one another.

Owing to the random bending of the clusters, it was difficult to measure the over-all length of an individual aggregate. However, if the bending was not particularly excessive, single cytoplasmic clusters could be traced in serial sections. Two such aggregates measured 8 and 10 μ in length. No particular association between the clusters and any membrane or organelle was found.

Within a cluster, the tubules are oriented more or less parallel to one another. The individual tubules do not appear to be as rigid as microtubules or spindle fibers, but are wavy in outline. Occasionally long, straight profiles of individual tubules are found in the cytoplasmic aggregates.

The tubular nature of each individual in a cluster can be seen in Figs. 3 and 4. The random appearance of transversely sectioned tubules is a result of the wavy nature of each individual member within a cluster. The outer tubule diameter, in both the nucleus and cytoplasm, is approximately 76A. When this value is compared to 65 A diameter for the *Pinguicula* nuclear lamellae, 200–300 A diameter for plant microtubules, 140–220 A diameter for tubular slime in various sieve elements, and 150 A diameter for the tubules of myxomycete plasmodia, it seems most likely that the elements described here are similar to the *Pinguicula* lamellae of Schnepf. However, these elements are more highly concentrated and organized in the Beltian body nuclei, and are clearly tubular. The tubule walls and intertubule areas appear to have a substructure of electron-opaque dots. However, more research is needed before anything positive can be said about these regions.

The similarity in structure, morphology, position, and concentration between these tubular elements and the *Pinguicula* nuclear lamellae makes it tempting to homologize them, and to assign them a function. However, at this stage of the investigation, it is perhaps best simply to summarize the data for the cells in which these tubular components are found. Salient points include: (*1*) the tubules are found in cells having a high protein content (Rickson, unpublished); (*2*) the Beltian bodies appear to be evolutionarily derived from glandular secretory tissue (*1*); and (*3*) similar-appearing nuclear fibrils have been found in enzymatic protein-secreting cells of *Pinguicula*. These data would indicate a possible correlation between protein secretion and the tubules. Further investigation of the ontogeny of these tubules and on Beltian body metabolism of labeled amino acids is now underway.

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