NUCLEAR P PROTEIN IN SIEVE ELEMENTS OF *TILIA AMERICANA*

RAY F. EVERT and BHARATI P. DESHPANDE. From the Department of Botany, University of Wisconsin, Madison, Wisconsin 53706

Tubular and fibrillar forms of P protein (slime) have been reported in sieve elements of a wide range of higher vascular plants. However, all P protein components encountered in previous investigations have been extranuclear in their occurrence. At this time we are reporting on the presence of tubular and fibrillar forms of P protein in both nucleus and cytoplasm of differentiating sieve elements of *Tilia americana*.

MATERIALS AND METHODS

The materials used in this investigation were obtained from the trunks of apparently healthy *Tilia americana* trees growing in the Eagle Heights area of the University of Wisconsin campus at Madison. The method of obtaining tissues was similar to that of a previous study of *Ulmus americana* phloem (3). Some tissues were fixed in 6% glutaraldehyde and postfixed in 2% osmium tetroxide; others were fixed in glutaraldehyde-formaldehyde and postfixed in 2% osmium tetroxide (4). Similar results were obtained with both fixation procedures. All sections were dehydrated in acetone and embedded in Araldite-Epon. Sections were cut on a Sorvall MT-2 ultramicrotome (Ivan Sorvall Inc., Norwalk, Conn.) with diamond knives, stained with uranyl acetate and lead citrate, and viewed and photographed with a Hitachi HU-11C microscope.

RESULTS AND DISCUSSION

The tubular P protein components in the nuclei and cytoplasm of differentiating sieve elements of *Tilia americana* are similar in appearance and occur in groups or clusters in both places (Figs. 1 and 3). (Such clusters of P protein components commonly are called P protein bodies or slime bodies.) As seen in transection, each tubule consists of an electron-transparent lumen and an electron-opaque wall, which shows some evidence of substructure (Fig. 4). At the time of tubule synthesis the sieve-element nucleus is normal in appearance (Fig. 1).

As for some other species of dicotyledons, in *T. americana* formation of the tubular form of P protein precedes that of the fibrillar form (2, 3, 6, 10, 13). Fibrillar components of similar appearance arise more or less simultaneously in nucleus and cytoplasm of *T. americana* sieve elements. At that time both nucleoplasm and hyaloplasm are less dense than earlier. The fibrils occur in aggregates (Figs. 2, 5, and 6) and are narrower than the tubules of younger sieve elements. The tubules average about 180 A in diameter (the electron-transparent lumen about 60 A in diameter), the fibrils about 130 A in both nucleus and cytoplasm,

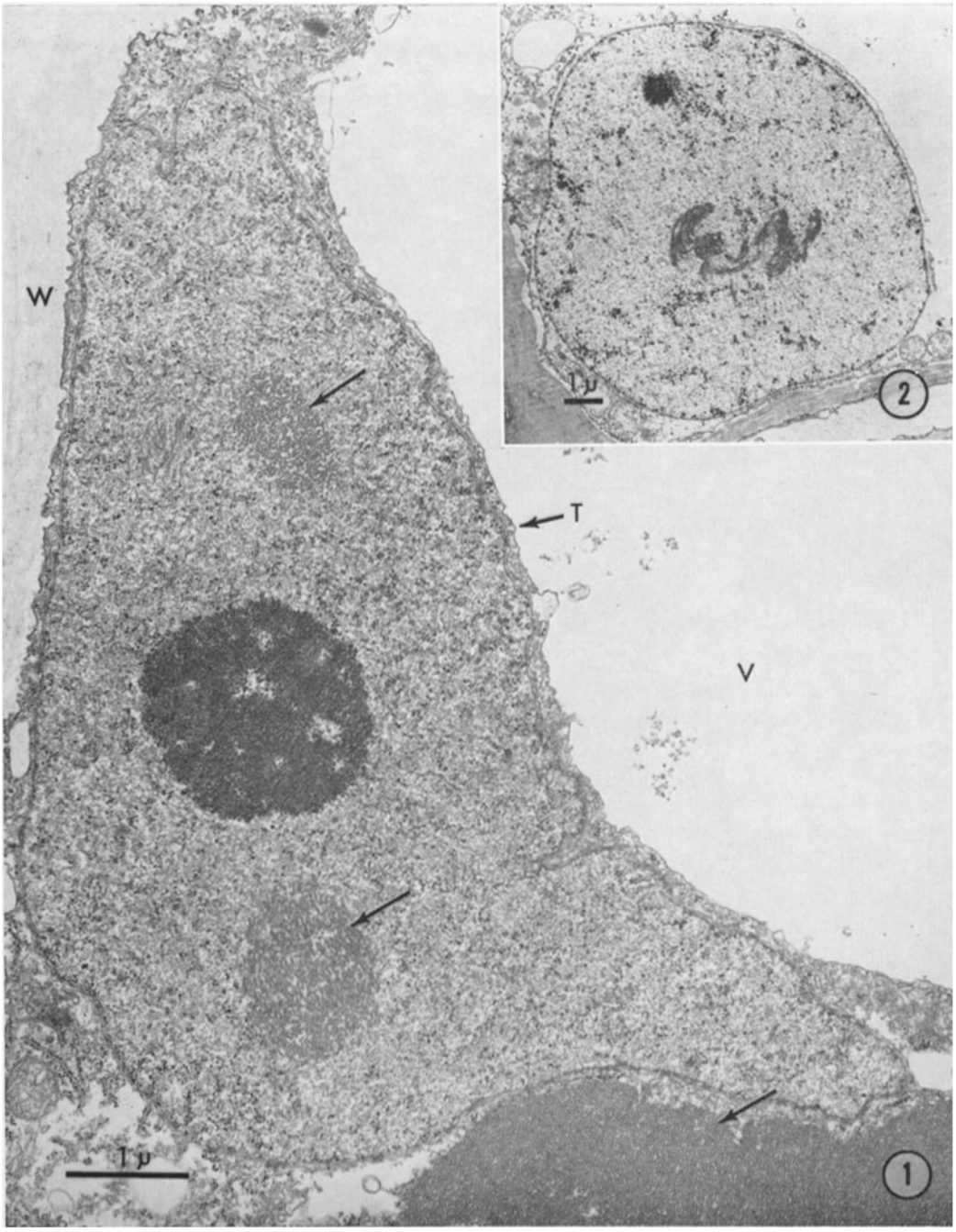

FIGURE 1 Transection of a young sieve element showing clusters of P protein tubules (unlabeled arrows) in nucleus and cytoplasm. *T*, tonoplast; *V*, vacuole; *W*, wall. × 18,000.

FIGURE 2 Transection of sieve element at late stage of development showing aggregates of fibrillar P protein components in nucleus. × 5500.

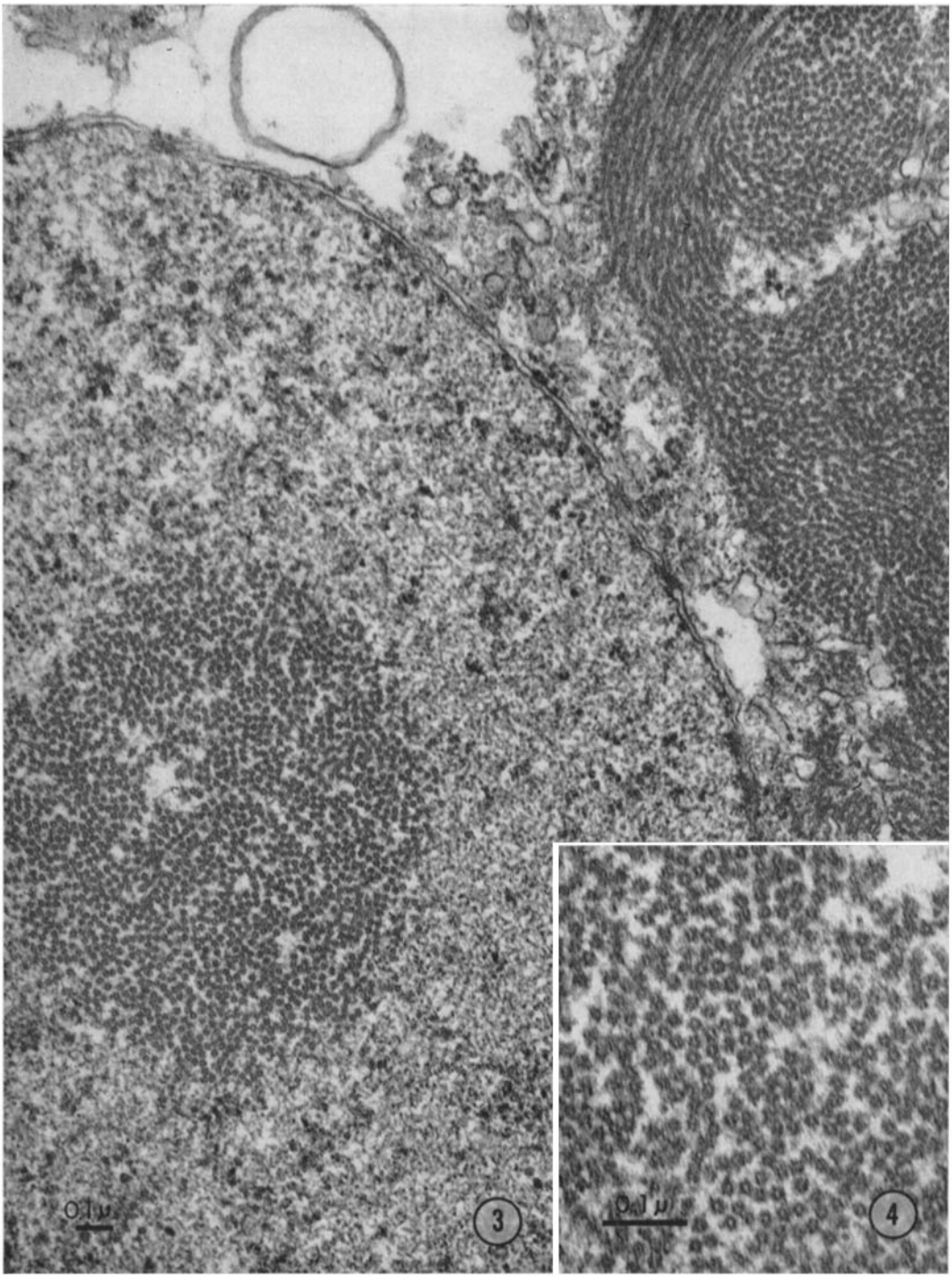

FIGURE 3 Transection of young sieve element showing clusters of P protein tubules in nucleus and cytoplasm. × 55,000.

FIGURE 4 View at higher magnification of portion of cluster of tubules from nucleus of Fig. 3. The tubular nature of these P protein components is very apparent at this magnification. × 126,500.

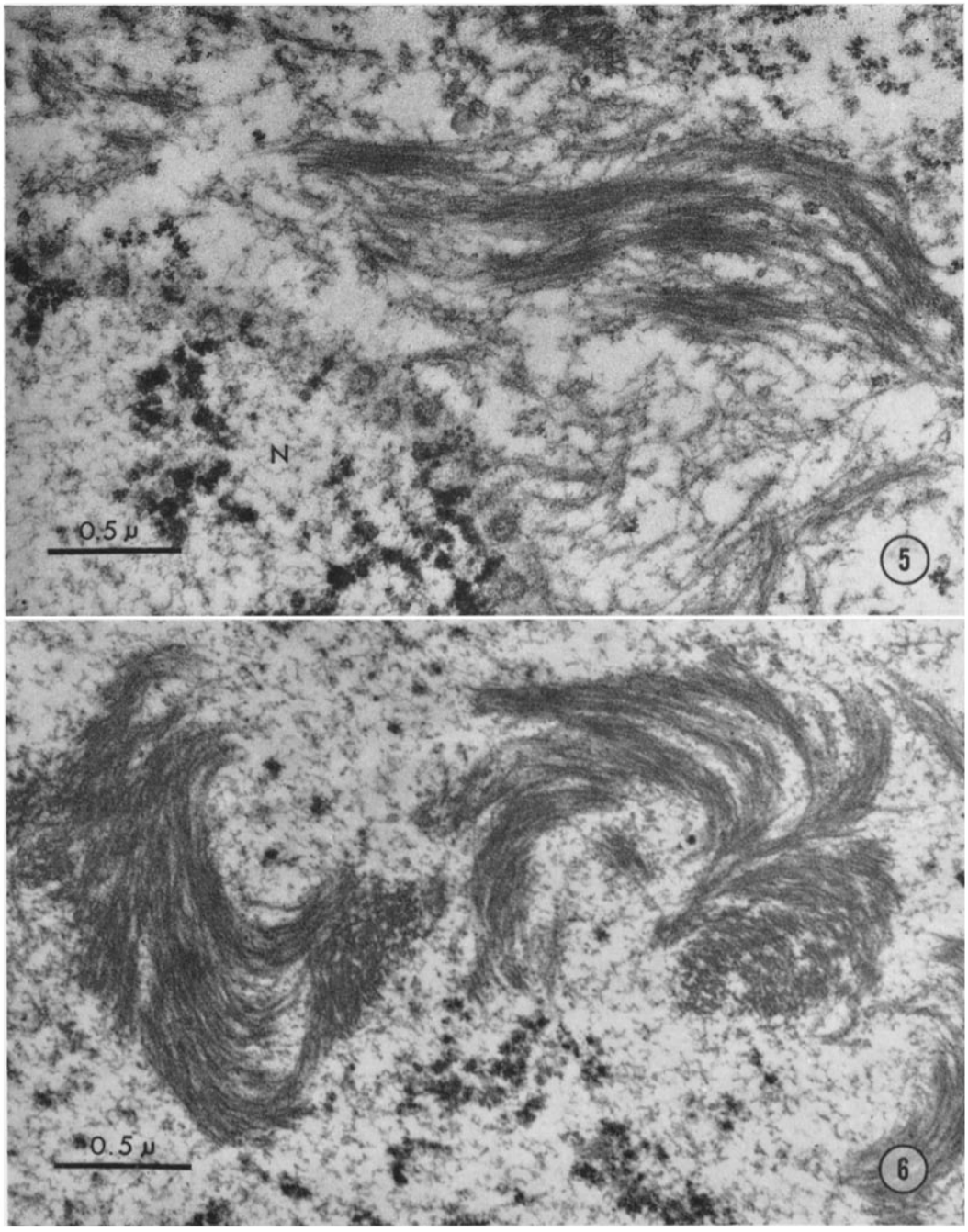

FIGURE 5 Transection of sieve element at late stage of development showing aggregates of fibrillar P protein components in cytoplasm. Part of nucleus (*N*) is shown at lower left. × 38,500.

FIGURE 6 View at higher magnification of aggregates of fibrillar P protein components from nucleus of Fig. 2. × 39,500.

at the stages of development studied thus far. About half of the nuclei observed in differentiating sieve elements contained P protein components of either tubular or fibrillar form. Whether the remainder contained P protein could not be determined, for complete series of sections of those nuclei were not obtained.

Some investigators (2, 6, 10, 13) have suggested that in the species they have studied the tubular form of P protein gives rise to the fibrillar form. Whether a similar relationship exists between the tubular and fibrillar forms of P protein in *T. americana* remains to be determined. Older sieve-element nuclei apparently contain only fibrillar P protein components. This suggests that such a relationship might exist between the two forms of P protein in *T. americana*.

Because of their close spatial relation to groups of P protein tubules during early stages of P protein body development, various cytoplasmic components have been implicated with tubule synthesis in other species. Especially notable among such cytoplasmic components are the endoplasmic reticulum and "spiny" vesicles (1, 3, 5, 10). The presence of P protein tubules within nuclei of sieve elements of *T. americana* seemingly precludes any direct relationship between such cytoplasmic components and tubule synthesis in that species.

This is not the first report of the occurrence of proteinaceous inclusions in plant cell nuclei. Nuclear protein crystals have long been known to occur in plant cells, although such inclusions have only recently been demonstrated with the electron microscope (8, 9, 11, 12). Some of these nuclear inclusions are viral inclusions (9), while others apparently are not (11, 12). The only previous report of nuclear tubules is that of Rickson (7). He found clusters of 76 A tubules in both nucleus and cytoplasm of cortical cells of leaf Beltian bodies in *Acacia cornigera*.

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