CYTOPLASMIC MICROTUBULES IN THE LEAF GLANDS OF PHASEOLUS VULGARIS

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SUMMARY
Preliminary observations on the fine structure of the club-shaped glands on Phaseolus vulgaris leaves are reported. The perinuclear cytoplasm of the apical cells of these glands contains an abundance of microtubules. These occur either as aggregates of 2-8 or more tubules, or they may be organized around a central core of material to form a fibre-like structure. The cells also contain cortical microtubules and are rich in rough endoplasmic reticulum and dictyosomes. The nuclei of these cells also contain a proteinaceous fibre, visible in the light microscope. The possible significance of these structures is discussed in relation to cytoplasmic streaming, maintenance of cellular asymmetry, and reaction to virus infection.

INTRODUCTION
In the course of recent investigations into the fine structure of greening plastids in the primary leaves of Phaseolus vulgaris, observations were made occasionally on the glands of these leaves. The glands are multicellular and club-shaped. They can be seen in living material with the highest power of the dissecting microscope and may be distinguished readily from the hooked trichomes which also invest the foliar parts of the bean. They occur upon both the upper and lower epidermis of the leaves and, more sparsely, upon the stem. On young leaves they appear to be most abundant on that part of the lower epidermis which overlies the minor venation.

The cytoplasm of these gland cells contains a remarkable array of microtubules. Since cytoplasmic microtubules currently command such widespread interest, it is felt worthwhile to publish these preliminary observations in order to bring the fine structure of these cells to the attention of other investigators.

MATERIAL AND METHODS
For light microscopy primary leaves from 9 to 10-day-old dark-grown seedlings of P. vulgaris (L), var. dwarf shell red kidney, were fixed in 10% acrolein in tap water (Feder, 1960), dehydrated in methyl cellosolve and embedded in glycol methacrylate (Ashley & Feder, 1966; O'Brien & Thimann, 1965). Sections were cut at 1-4 μ with dry glass knives, stained and photographed as described in the figure legends. For electron microscopy, the tissues were fixed in 3% glutaraldehyde (diluted from 50% Biological Grade, Fisher Scientific Co.) in 0.025 M sodium phosphate buffer, pH 6.8.

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at room temperature for 16 h. The fixed tissues were rinsed in the same buffer (4 changes for 1 h each at 0 °C), treated with 2% phosphate-buffered osmium tetroxide for 12 h, dehydrated in an ice-cold series of graded acetone solutions, and embedded in Araldite (for details, see O'Brien, 1967). Sections showing silver-grey interference colours were doubly stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963), and examined in a Siemens Elmiskop 1 at 80 kV.

RESULTS

Fig. 1 shows a section of part of two cells of a gland. Part of the nucleus, with a prominent nucleolus, can be seen in each cell. The vacuolar contents of the cell on the left are intensely acidophilic, and the reactions of this material to a variety of stains (acrolein/Schiff, eosin, periodic acid/Schiff, toluidine blue o) are consistent with the suggestion that it is rich in protein. The perinuclear cytoplasm is also intensely acidophilic and one can just discern that there are areas within it which stain even more strongly than the rest.

Fig. 2 shows a tangential section of the nucleus, part of a protein-rich vacuole, and the perinuclear cytoplasm. In addition to the normal population of organelles, it is apparent that the region of the cytoplasm which lies between the nucleus and the protein-rich vacuole is traversed by a profusion of microtubules. Part of the same section is shown in more detail in Fig. 3. Individual microtubules, and small groups of them (2–8 or more) can be seen in various planes of section. In addition, other microtubules appear to be aligned quite precisely around a central core of material, the whole constituting a fibre-like structure. Two of these structures, one sectioned transversely and the other somewhat obliquely, occur close to one another in the middle of the field. The one sectioned transversely is shown in more detail in Fig. 3 (inset). There appear to be connexions between the walls of many of the microtubules and the material of the central core, and in two places, small ‘bridges’ appear to connect adjacent microtubules. Many of the microtubules show evidence of substructure within their walls. The microtubules in the perinuclear cytoplasm, ranging from 270 to 320 Å in diameter, are somewhat wider than the cortical ones, which are 190–250 Å in diameter.

Fig. 4 is a light micrograph of a section of a gland cell, stained briefly with iodine/potassium iodide solution and photographed between crossed polaroids. It is clear that the perinuclear cytoplasm contains material which is quite strongly birefringent under these conditions.

Fig. 5 shows an area of perinuclear cytoplasm in a different cell in which large numbers of microtubules, singly or in groups, penetrate among the cisternae of the endoplasmic reticulum (ER), while a different part of the same cell contains numerous fibre-like structures (Fig. 6).

In addition to the remarkable content of fibrous material in the cytoplasm, the nuclei also contain fibres. These were seen originally in various planes of section in electron micrographs (Fig. 8), but they are large enough to be visible in the light microscope. Fig. 7 shows one stretching the full diameter of the nucleus (part of a
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similar fibre can just be distinguished in the nucleus of the right-hand cell in Fig. 1.

Sections of these cells judged to show the cytoplasm at some distance from the nucleus do not contain the profusion of cytoplasmic microtubules seen in the perinuclear region. Rather, one sees occasional sections of the fibre-like structures and here and there, sections of individual microtubules. On several occasions proplastids and mitochondria have been seen near these isolated cytoplasmic microtubules, apparently lined-up as in Fig. 9. The proplastid on the left in Fig. 9 shows yet another feature of interest, for the stroma appears to contain very fine fibrous elements aligned parallel to the long axis of the proplastid.

The cytoplasm contains abundant rough endoplasmic reticulum (ER) (Figs. 1, 10 and 11) and the cisternae always appear to contain material of low electron density. The ER cisternae are often closely associated with dictyosomes which are also quite abundant. Intercisternal elements (Mollenhauser, 1965) can be resolved between the cisternae of the dictyosome shown in Fig. 11. The 'hairy vesicles', now widely recognized as a cytoplasmic element associated with the cell surface and the dictyosome in a variety of cell-types (see, for example, Bowers, 1964; Manton, 1964; Roth & Porter, 1964; Bonnett & Newcomb, 1966), are also evident in Figs. 10, 11.

Finally, the cortex of these gland cells also contains microtubules, which appear to be oriented in more than one direction (Fig. 10, inset).

DISCUSSION

Haberlandt (1914) regarded these glands as hydathodes and claimed that they had abundant protoplasmic contents but 'showed no trace of oily, resinous or gummy secretion.' Butterfass (1956) has studied the penetration of fluorescent dyes into the glands, and Nestler (1899) has shown that they can exude a fluid rich in potassium. Indeed, Uphof & Hummel (1962) suggest that they might be regarded as 'potassium glands'.

Until more is known of the developmental sequences through which these gland cells pass, it is difficult to interpret their fine structure in a meaningful way. The presence of protein-rich vacuoles, the abundance of rough ER and dictyosomes could mean that the cells are producing a reserve protein. Furthermore, one could postulate that the microtubules and fibre-like structures are engaged in transporting protein to the vacuole. Several investigators have suggested that either microtubules (Porter, Ledbetter & Badenhausen, 1964; Rudzinska, 1965; Tilney & Porter, 1965; Bikle, Tilney & Porter, 1966), or fibres (Nagai & Rebhun, 1966; O'Brien & Thimann, 1966) are involved in cytoplasmic streaming. The central core of the fibre-like structures in these cells bears some resemblance to the structure seen in cross-sectional views of the fibres described in Nitella by Nagai & Rebhun (1966). O'Brien & Thimann (1966) speculated that a similar protein might be involved both in the formation of microtubules and of fibres in the coleoptile. The presence of a sheath of microtubules surrounding the central core in these gland cells of the bean leaf, and the apparent connexions between the walls of the microtubules and the core, could be taken as
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evidence in support of a relationship between these two structures. Unfortunately, there is a serious objection to the suggestion that either the fibre-like structures or the perinuclear microtubules are involved in streaming. To date, all attempts to observe streaming in these gland cells in fresh material have been unsuccessful, even though streaming was obvious in nearby cells of the epidermis. Of course, it is always possible that streaming does occur in these cells and that it is very sensitive to the manipulations involved in examining the glands by phase contrast.

An impressive body of evidence also supports the suggestion that microtubules are involved in the maintenance of cell shape and in the control of cellular asymmetry (see, for example, Ledbetter & Porter, 1963; Byers & Porter, 1964; Pickett-Heaps & Northcote, 1966a, b). It may be suggested that these cytoplasmic microtubules are performing a similar function here. First, it is clear from Figs. 1, 4 and 7 that the cytoplasm is distributed asymmetrically within the cell. While we are not yet in a position to understand why an asymmetric distribution of the organelles is a feature of so many highly differentiated cell types, it is patently clear that it is important to their functions (see Bünning, 1957). The number of cytoplasmic microtubules is so great in the perinuclear cytoplasm of these cells that it is easy to imagine that, in vivo, the organelles in this region of the cell are embedded in a viscous 'gel' (note especially the degree to which the vesicular and cisternal elements shown in Fig. 5 are encased in microtubules).

There is, however, another possibility. Murayama (1966) has shown that haemoglobin S, isolated from red cells of patients with sickle-cell anaemia, also forms microtubules. It has been known for some time (Schramm & Zillig, 1955) that the isolated nucleic-acid free protein of tobacco mosaic virus will also form microtubules under appropriate conditions. The wealth of cytoplasmic microtubules present in these cells could be, in a sense, an artifact. It is possible that the cells synthesize a large quantity of some as yet unidentified protein which simply forms microtubules by aggregation. While this is not regarded as a very likely possibility, it cannot be dismissed, for there is a considerable literature on proteinaceous inclusions in epidermal cells and trichomes (see Uphof & Hummel, 1962; Thaler, 1966). As long ago as 1867, Weiss described 'protein spindles' in the glandular hairs of Origanum. The fact that these cells contain an intranuclear fibre is another reason for giving this last possibility serious thought.

Tischler (1922) and Thaler (1966) both present evidence that intranuclear inclusions of protein are often, but not always, indications of virus infection. Similarly, virus-infected cells often show an abundance of spindle-like or fibrous protein accumulations in the cytoplasm. One must face the possibility, therefore, that the intranuclear fibre and the perinuclear microtubules of these cells are the products of a virus infection. Indeed, van Iterson, Hoeniger & van Zanten (1967) point out that is is possible that the microtubules which they have identified in the bacterium Proteus are smooth forms of polysheaths, aberrant assemblies of the tail material of phages.

It remains to discuss the possible significance of the fine filaments in the proplastid of Fig. 9. It is well known that plastids carry out amoeboid movement but it has never been settled whether these movements arise from activity within the plastid or from an interaction of the plastid with the cytoplasm. If the movements do originate within
the plastid, one might expect to find evidence for a fibrous element. Although it is no more than a speculation, the fine filaments evident in the proplastid of Fig. 9 could be such a component.

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REFERENCES


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Fig. 1. Light micrograph showing part of the apical cells of the gland, stained with acid fuchsin (1:10000 in 1% acetic acid (Robinow, 1963)); photographed by phase contrast. The nuclei have prominent nucleoli and the perinuclear cytoplasm is strongly acidophilic. Within this cytoplasm one may just distinguish regions which stain even more strongly (arrow). Note the acidophilia of the vacuole contents in the left-hand cell. × 1200

Fig. 2. Part of the perinuclear cytoplasm and protein-rich vacuole (p) in a region similar to that illustrated in the left-hand cell of Fig. 1. n, nucleus. × 18500.
Fig. 3. Detail of part of Fig. 2, showing two fibre-like structures (f) and numerous cytoplasmic microtubules. The inset shows the fibre-like structure in transverse section in greater detail. Connexions between the walls of the microtubules and the material of the core are apparent at the small arrows, while the large arrows show 'bridges' between microtubules. Fig. 3 is $\times 38000$; inset, $\times 102000$.

Fig. 4. Light micrograph of a gland cell, stained briefly with iodine/potassium iodide solution and examined between crossed polaroids. Note the strongly birefringent regions (arrows) of the perinuclear cytoplasm. $\times 1200$.

Fig. 5. Part of the perinuclear cytoplasm in a different gland, illustrating abundant microtubules penetrating among the cisternae of ER. $\times 50000$.

Fig. 6. The same cell as in Fig. 5, showing numerous fibre-like structures in a different region of the cell. $\times 24000$. 
Fig. 7. An intranuclear fibre. Acid fuchsin/phase contrast. × 1200.

Fig. 8. Part of a similar fibre, seen in oblique transverse section. × 46000.

Fig. 9. Proplastids (pl) apparently aligned near an isolated microtubule (mt) at some distance from the nucleus. Note the fine filaments (arrows) in the proplastid on the left. × 70000.

Fig. 10. Part of the peripheral cytoplasm showing the abundance of rough ER and dictyosomes; × 23000. The inset shows the irregular arrangement of the cortical microtubules (mt); × 73000.

Fig. 11. Part of the peripheral cytoplasm in which intercisternal elements can be seen between the cisternae of the dictyosome. × 42000.