PREPROPHASE MICROTUBULE BANDS IN SOME ABNORMAL MITOTIC CELLS OF WHEAT

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SUMMARY

Caffeine treatment of growing wheat tissues was used to form binucleate or polyplidal cells; preprophase microtubules in subsequent division cycles in these and some other abnormal cells were then examined. In root tips, binucleate cells or those with greatly enlarged nuclei usually contained one transverse preprophase band of microtubules; sometimes this was slightly asymmetrical or skew, and less commonly two bands were seen. In coleoptile vascular bundles, there were generally two or more bands in the greatly elongated cells, these sometimes appearing in different planes. During formation of the stomatal complexes, preprophase microtubules were almost invariably found where expected, preceding abnormal development both in untreated and also in caffeine-treated material, regardless of the number, disposition or size of nuclei. This occurred even when wall stumps, formed during a previous abortive division, indicated that that previous division was also asymmetrical. It is concluded that the position(s) of preprophase band(s) of microtubules is not particularly influenced by the nucleus or nuclei, being more susceptible to external morphogenetic influences which can persist for some considerable time. Particularly in the case of stomatal complexes, a cell wall seems necessary to seal off or otherwise fulfil the tendency towards asymmetrical division.

INTRODUCTION

A very considerable amount of recent work has been published concerning the ultrastructure of the mitotic spindle in a variety of cells. Spindle microtubules are found to be ubiquitous components following careful fixation, and these structures are almost always correlated with birefringent spindle fibres observed in vivo (e.g. Rebhun & Sander (1967); Behnke & Forer (1966), however, did not find a spatial correspondence of the two in insect spermatocytes). In plant cells, Ledbetter & Porter (1963) demonstrated that glutaraldehyde fixation preserved spindle and other microtubules. Their results have been widely confirmed and extended.

Pickett-Heaps & Northcote (1966a, b) later found another unexpected involvement of microtubules in spindle structure of various wheat cells; microtubules were found concentrated near the cell wall at preprophase in a band whose position predicted with considerable accuracy the plane of future division; this was most strikingly shown in the highly asymmetrical mitoses involved in the formation of the stomatal complex (Pickett-Heaps & Northcote, 1966b; c.f. Stebbins & Jain, 1960; Stebbins & Shah, 1960). Burgess & Northcote (1967) described preprophase microtubules in Phleum, but found that even with a symmetrical disposition of the microtubule band, subsequent division could often be asymmetrical.
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During further investigations into this puzzling phenomenon and its significance, the author undertook a series of experiments in which caffeine treatment of dividing cells was used to inhibit or prevent cell-plate formation. The experimental tissues were subsequently allowed to divide further, and observations were made on preprophase microtubules present in such artificially created multinucleate or polyploid cells (the latter formed due to nuclear fusion in multinucleate cells).

Caffeine has long been known as an inhibitor of cytokinesis, earlier stages of division being apparently unaffected; furthermore, the toxic effect of the drug is low (e.g. see Kihlman, 1966, p. 114). Several papers have described the ultrastructure of such caffeine-treated cells (Detsson & Benbadis, 1966; López-Sáez, Riusueño & Giménez-Martín, 1966); this treatment can also be used to label cells (Giménez-Martín, González-Fernández & López-Sáez, 1965).

A definition of 'preprophase'

Since the word 'preprophase' is used a lot in this paper it is important to define the term. Preprophase is used to describe nuclei which it is believed are or were imminently about to proceed into prophase. In wheat cells, three phenomena generally associated with this condition allow it to be recognized:

(i) Preliminary condensation of chromatin; this does not seem a sufficient criterion in itself, since considerable variation exists in the appearance of chromatin in interphase nuclei. In some epidermal cells, preprophase microtubules are present near nuclei almost indistinguishable from interphase nuclei.

(ii) The very characteristic appearance of the nucleolus (see Pickett-Heaps & Northcote, 1966a, b; Figs. 11, 13-16).

(iii) The appearance of preprophase microtubule bands.

That these criteria have some validity is shown by the observations on asymmetrical divisions in epidermal cells since they were associated with nuclei which are known to be about to divide; they are also applicable to other wheat cells examined. The characteristic dispersal of nucleoli at preprophase is also seen for example in *Chara* (Pickett-Heaps, 1967a) and *Spirogyra* (Godward, 1966), where adherence of some nucleolar component to the chromosomes throughout division is clearly apparent; this is also seen in some animal cells (see Hsu, Arrighi, Klevecz & Brinkley, 1965). Significantly, telophase and preprophase nucleoli in wheat often appear very similar, suggesting that dispersal of nucleoli may be the reverse sequence of events that occur during reformation. Such results would appear to conflict somewhat with the very detailed work of Lafontaine & Chouinard (1963); their 'preprophase' nucleus (Fig. 4) is not like preprophase nuclei in wheat and they state: 'No evidence has been obtained that the structural components of the nucleolus become associated as such with the chromosomes to form an external or internal matrix.' However, Lafontaine & Lord (1966) find a coating of material around telophase chromosomes that appears to condense, forming the nucleolus, and more recently Subramanyam & Royan-Subramaniam (1967) have other evidence suggesting an association of some nucleolar material with chromosomes in *Allium.*
MATERIALS AND METHODS

General considerations

Conditions for the caffeine treatment were derived empirically, after considerable experimentation. It was necessary that: (i) cytokinesis in a reasonably large number of cells was inhibited or prevented; (ii) the nuclei of these cells should then proceed into division, with as few side effects being exhibited as possible.

However, the following experimental observations needed to be taken into account: (i) excision, etc., of the coleoptile has a marked inhibitory effect on cell division, therefore the caffeine must enter through the roots or coleoptile epidermis; (ii) total immersion of the coleoptile was also found to inhibit mitosis severely; (iii) concentrations of caffeine higher than about 0.1 % also have an increasingly inhibitory effect on division (Kihlman & Levan, 1949).

Compromise conditions, with uptake of caffeine by the roots only, gave the best results, although the degree of inhibition of cytokinesis was somewhat variable, since many of the cells of interest were not in direct contact with the drug. It was found that about 24 h of treatment resulted in a comparatively large proportion of affected cells; removal of the tissue into distilled water for a further variable period helped to stimulate further division in these cells.

Procedure in detail

Previously sterilized germinating wheat seedlings (Triticum vulgare) were placed intact on filter paper saturated with 0.01-0.2 % solutions of caffeine in Petri dishes at 21 °C; the coleoptiles were between 0.2 and 0.5 cm long. The periods of exposure were arbitrary, ranging from 3 h to 2 days; over longer periods, the seedlings though apparently healthy, exhibited a decrease in growth rate when compared with controls. Many seedlings were then removed from the caffeine solution and placed in water for periods of recovery up to 24 h.

The effect of caffeine was often somewhat variable, some seedlings being apparently unaffected, while at the other extreme some failed to grow. Seedlings were chosen for microscopy which exhibited the retarded growth on caffeine treatment, followed by an increased growth rate when incubated subsequently in water. Such seedlings were found to contain many binucleate and polyploid cells.

For fixation, root tips were excised and coleoptiles were cut into 1-1 mm segments; these were placed immediately into 6 % glutaraldehyde, buffered to pH 7 with phosphate (0.025 M) containing 0.002 M calcium chloride. After 1-2 h at room temperature, the specimens were well washed with buffer, postfixed in 1 % osmium tetroxide, buffered with veronal acetate for 1 h and dehydrated as usual. Araldite-embedded specimens were sectioned on glass knives; sections were stained with uranyl acetate and lead tartrate, and were examined using a Philips EM200 electron microscope.

Light microscopy. Thick ('blue-green') Araldite sections adjacent to ultra-thin sections, were mounted on clean glass slides and stained for a few seconds with hot 1 % toluidine blue in 1 % borax solution. The sections were washed, dried, mounted and examined in a light microscope, being photographed as required.
OBSERVATIONS

Inhibition of cytokinesis by caffeine led to the formation of binucleate cells, increasing in number as the treatment was prolonged. The nuclei of these cells could divide further, not necessarily synchronously which is somewhat surprising (Fig. 1). In many cases, large or very large nuclei were present, apparently resulting from nuclear fusion. These nuclei were generally lobed and often contained ‘nuclear vesicles’ (see below—Figs. 7, 14 and 17); they will be termed ‘polyploid’ nuclei.

Effect of caffeine on ultrastructure generally

Cytoplasmic organelles of caffeine-treated cells were similar in appearance to those of untreated cells. Long exposures to caffeine did however often alter the appearance of nuclei. Large, lobed nuclei were frequent (e.g. Fig. 4) and such nuclei generally contained the large nuclear vesicles, whose contents were dissimilar to the cytoplasm surrounding the nucleus and hence probably not continuous with it (Fig. 7). The large nuclear vesicles contained a conspicuous number of much smaller vesicles (Fig. 7); these latter appear to be unfused cell-plate vesicles which had become trapped and isolated within nuclei following inhibition of cytokinesis. Other less frequent abnormalities included ‘sticky’ chromosomes at anaphase and telophase (Kihlman & Levan, 1949). In the highly elongated cells of the coleoptile vascular bundle, nuclei were found which were markedly inhomogeneous in texture; chromatin in some regions of such nuclei was highly condensed, but in others it was often dispersed and intermingled with spindle elements (microtubules and cell-plate vesicles). Telophase in caffeine-treated cells appeared normal; in particular, vesicles collected amongst the typical phragmoplast microtubules. No particular reason for inhibition or prevention of plate formation was apparent following cursory inspection of micrographs (see Whaley, Dauwalder & Kephart, 1966); this aspect is beyond the scope of this paper. Fusion of vesicles in small regions often resulted in partial formation of cell plates. One most useful result of this was the frequent appearance of a stump or incomplete wall on one or both older side walls (Figs. 2, 3, 11 et al.); such stumps were used as markers to correlate the cell-plate position and plane of a previous attempted division with that of the preprophase band(s) of subsequent mitosis in binucleate or polyploid cells.

This paper is particularly concerned with the preprophase microtubules in the artificially created, multinucleate or polyploid cells.

Untreated mononucleate cells

Preprophase nuclei in normal mononucleate cells have been described previously (Pickett-Heaps & Northcote, 1966a). The appearance of the nucleolus was highly characteristic (as in Figs. 11, 13–16 and 17) and the preprophase band of microtubules was almost invariably very conspicuous. In no case is there any evidence that the plane of subsequent division is different from that of the plane of preprophase microtubules (cf. Burgess & Northcote, 1967). In root tip meristems there are always a few files of cells that have divided longitudinally. The microtubule band in the few preprophase
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cells found at the head of such files, was also always oriented longitudinally. Otherwise,
preprophase bands and planes of cell division were always transverse. In coleoptile
vascular bundles, the course of events is far more complex. Cells divide transversely,
longitudinally and even apparently diagonally. Correspondingly, the preprophase band
was seen in similar equivalent situations but it is as yet impossible to prove in these cells
that the plane of division would have followed the plane of preprophase microtubules.

An interesting, but uncommon, abnormality in stomatal development can sometimes
be found when two subsidiary cells (sc) are formed on one side of the guard mother
cell (gmc) (Fig. 6). This occurs if the gmc wall is shared by two epidermal cells (see
Stebbins & Jain, 1960). Two equivalent preprophase epidermal cells have been found;
as expected, the preprophase band in each predicted the position of the cell plate
(Fig. 5).

Caffeine-treated root-tip cells

Typical preprophase microtubules were found in both binucleate and polyploid
cells. In both cases, usually only one, often very large (Fig. 8), band of symmetrically
situated microtubules was present. In some binucleate cells (and in one trinucleate
cell), the band was displaced towards one end of the cell; in others, it was slightly
skew. In a few cases, two separate preprophase bands were present. In preprophase
root-cap initials, however, the situation in several observed cases was much more
complex. A band or bands of microtubules in different parts of the cell were sectioned
in several planes. Attempts at serial sectioning of such cells did not clarify the dis-
position of the bands.

Caffeine-treated cells of the coleoptile vascular-bundle

Cells of the vascular-bundle are much more elongated than those of root tips. The
plane of cell division is also much more variable (see above). Preprophase micro-
tubules were again present in treated cells, but the organization of the bands was more
complex and variable than that found in the root. Two or even three preprophase
bands were often seen oriented transversely across the cell. These were generally
symmetrical, i.e. if one was present, it was centrally placed; if two, their planes divided
the cell roughly into three equal portions. Sometimes two or more such preprophase
bands appeared to be present in different planes (Figs. 9 and 10 show the bands along
two adjacent parts of the same wall).

Caffeine-treated mesophyll cells of the young leaf

Internal cells of the very young leaf (treated or untreated) undergo active division;
in the future mesophyll cells, this division is almost always transverse at first (Figs. 2,
11), leading to a simple cubical structure composed of very young cells. Preprophase
microtubule bands were oriented transversely in all of the few treated cells examined.
The number of bands present was normally two, symmetrically situated in the cell
(Figs. 11, 11 A-D); this number was not related to the number of nuclei (Fig. 2).
Caffeine-treated leaf epidermal cells

These cells were of special interest to the author, since highly asymmetrical, but very precise and predictable cell divisions lead to the formation of the stomatal complex. For a definition of the terms used, see Pickett-Heaps & Northcote (1966b).

First asymmetrical division. Caffeine treatment of cells which were to provide gmc’s, normally produced rows of small compact binucleate cells (Fig. 4). Wall stumps in some of these indicated that previous inhibition of cytokinesis had usually occurred at the first asymmetrical division (Figs. 12, 12A). When the nucleus or nuclei of these cells were at preprophase, the single band of microtubules in all cases examined was displaced in the same direction for a given row regardless of the number, size or distribution of the nuclei (Figs. 4, 12–14). The band also coincided with the position of wall stumps which indicated a previous attempt at asymmetrical division (Figs. 12, 12A). In one particular cell, wall stumps were centrally situated about a single large nucleus (Fig. 14); two separate preprophase bands were present in this cell, each being displaced in the same direction, with one of the bands apparently located as if the wall stumps represented a completed wall (Figs. 14, 14A). In another cell, two adjacent separate stumps on one side wall suggested that perhaps two previous attempts at asymmetrical division had occurred; preprophase microtubules were again present near these stumps.

Second asymmetrical division. In a few preprophase epidermal cells, the microtubule band was oriented transversely and symmetrically; in such cases the adjacent gmc was invariably small and formation of sc’s in this region had not been initiated. If the gmc’s were larger however, and some sc’s had been formed in the area, then the microtubule band was almost always (cf. Fig. 18) in the expected position in the preprophase cells (i.e. adjacent to the gmc, as shown previously (Pickett-Heaps & Northcote, 1966b)). This occurred with either binucleate (Figs. 16, 16A, B) or polyploid cells (Figs. 15, 17 and 17A, B).

In many cases, large or small wall stumps showed without doubt that the previous abortive attempt had been at the second asymmetrical division (Figs. 15, 17). At subsequent preprophase in these cells, the microtubule band was always still in the position predicting another second asymmetrical division. In one instance where the portion of sc wall was quite long, the band was found near the end of this stump (Figs. 17, 17B).

A few cell complexes were found in which the situation was a great deal more complex. For example, two preprophase epidermal cells of great size were seen on each side of two gmc’s (Fig. 18); one contained a very large preprophase nucleus, and the other two smaller nuclei. The preprophase bands in each epidermal cell were sectioned at several regions of the wall, some of these related to the position of the two gmc’s, and others suggesting a transverse orientation of the bands. The exact interrelationship of such bands could not however be worked out in detail. It is suspected that in this region of epidermis, the young gmc’s were not exerting their full ‘influence’ on the epidermal cells, since no sc’s had been formed in this particular region.
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‘Abnormalities’ in preprophase microtubule distribution

In the results on treated tissues described above, the plane and position of the preprophase microtubules for given cells was predictable (particularly for those of the developing stomatal complex) and such predictions were generally correct. Apart from isolated, inexplicable deviations from the patterns of behaviour described above, two interesting ‘abnormal’ distributions of preprophase microtubules were encountered. In each case, the abnormalities were in fact consistent. In one set of five adjacent, preprophase epidermal cells, preprophase microtubules were oriented transversely even though these epidermal cells were alongside partially formed stomatal complexes (i.e. when one might have expected an asymmetrical distribution of the bands). In each of another set of three gmc’s, again all in the same row, the preprophase microtubule band was oriented transversely instead of longitudinally as would be expected. In both cases, it is probably significant that the abnormalities were consistent and confined to the given row.

DISCUSSION

It may be valuable at this point to re-emphasize a difference in the cytological effects of caffeine and colchicine. Caffeine specifically inhibits cytokinesis, the process whereby the plant cell forms a new wall between daughter cells. If the concentration of caffeine is not high, the course of mitosis, and the ultrastructural appearance of the spindle, seem relatively unaffected; there is no reason to believe that caffeine affects microtubules. The phragmoplast initially appears normal, but the coalescence of vesicles, normally giving the new cell wall, is apparently inhibited or prevented.

Colchicine, however, has a profound and very well-known effect on the mitotic spindle, causing a disruption and dissolution of the birefringent fibre apparatus. This coincides with a disappearance of microtubules (Inoué & Sato, 1967; Pickett-Heaps, 1967b), and more recently, evidence has been obtained suggesting that colchicine binds directly on to a subunit of microtubules (Borisy & Taylor, 1967a, b; Shelanski & Taylor, 1967).

There has been considerable speculation and much diversity of opinion regarding the function of microtubules (see review by Porter, 1966) and appropriate evidence is generally circumstantial. However, a growing body of evidence suggests that these organelles may be involved in morphogenesis in various cells as well as in other phenomena such as the maintenance of structural rigidity and intracellular movement.

In plant cells, evidence has been presented suggesting an involvement of microtubules in control over xylem wall formation (Pickett-Heaps, 1967b, c); it may perhaps follow that ‘wall’ microtubules exercise a general control over cell-wall synthesis which ultimately determines cell shape. Furthermore, while describing the presence of spindle microtubules in plant cells, Pickett-Heaps & Northcote (1966a, b) also showed that the organization of cytoplasmic microtubules at preprophase could be very significantly correlated with the future plane of cell division in Triticum. Burgess & Northcote (1967) confirmed that a similar phenomenon occurs in Phleum (and in pea...
roots) but the author has been unable to demonstrate it in a complex alga, Chara (Pickett-Heaps, 1967a, 1968a). However, during spermatogenesis in Chara, another manifestation of morphogenetic activity associated with microtubules has been described (Pickett-Heaps, 1968b), since elongation of the organism, and highly specific movements and spatial reorganization of cell organelles (nucleus, mitochondria, basal bodies, plastids and lipid bodies) were seen to be directly related to the growth and organization of the manchette microtubules. Thus, microtubules are seen as significant morphogenetic agents in the plant cell. (It is not of course, implied that this is their only role.)

In view of such varied evidence, it is of some importance to examine in more detail the preprophase band discovered previously. With this end in view, the use of drugs whose effects are often largely unknown is considered a valid and useful tool of the cytologist, provided care is exercised in interpreting the results.

The results reported above do not suggest any specific function of the microtubule band other than has been suggested previously. During preprophase of the second asymmetrical division, the band could obviously function in positioning the nucleus (or nuclei) and in all wheat cells examined it predicts the position of the cell plate (Pickett-Heaps & Northcote, 1967a, b). Burgess & Northcote (1967) have good evidence to suggest that in Phleum root tips the band is 'associated with the prior positioning of the nucleus rather, than with the future development of the mitotic spindle', although a few lines later, they also say 'the preprophase band of microtubules which we think has this influence on the orientation of the mitotic structure at this time... ' (Burgess & Northcote, 1967, p. 325). Perhaps, mitosis and cytokinesis should be considered more as two separately controlled processes that normally closely follow one another. For example during spermatogenesis in Chara, diagonal spindle structures can form transverse cell plates (Pickett-Heaps, 1968b), but in differentiation of oogonia and vegetative Chara cells, a relatively symmetrical mitosis can be followed by the formation of a very highly curved cell plate (Pickett-Heaps, 1967a, d). It must not be forgotten, also, that the preprophase grouping could well be a result of polarization, and not a cause. Other experiments at present being undertaken seem to indicate that the microtubule band is not even directly related to preprophase positioning of the nucleus, so (perhaps like the relationship between microtubules and wall microfibrils) the association of these two organelles might be quite indirect and of subtle significance.

The experiments do however confirm the remarkably consistent association of the microtubule band with the preprophase condition in wheat cells, to some extent regardless of the number and size of the nuclei. The results suggest quite strongly that:

First, microtubules are grouped together as a result of a stimulus either emanating from the nucleus as it goes into preprophase or, more likely, associated with the (external) factors inducing the prophase condition in a cell; in some epidermal cells under the influence of the gm, preprophase bands are detectable before the nucleus shows much sign of incipient division.

Secondly, the positioning of the band is highly susceptible to external morphogenetic influences, being not noticeably affected in these experiments by the size,
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shape, position or number of the nuclei in the cell. The localized effect of the gmc can be observed even during abnormal stomatal development (Figs. 5, 6).

Thirdly, the morphogenetic field(s) responsible for this positioning can last a relatively long time (much longer than the duration of cell division).

Fourthly, unless the morphogenetic influence is satisfied, perhaps geometrically by the formation of a cell wall, interposition of a nucleus in a certain fixed region, etc., the cell will evidently continue for some time to try to satisfy the demands of a morphogenetically determined geometric pattern. This is most clearly seen in the results shown on asymmetrical divisions which form the stomatal complex. Some abnormal or unexpected orientations of the preprophase bands appeared to fall into a pattern even if this pattern was unusual. For example, transverse (and not longitudinal) orientation of preprophase microtubules was encountered in three sequential gmc's (about to undergo the third symmetrical division) in one row of cells. Similarly, several adjacent preprophase epidermal cells (which were expected to show the asymmetrical preprophase band) all had a single, transverse band of tubules (see Results).

In cases where two or more preprophase bands were present (generally in long cells) it follows that these were probably the result of morphogenetic influences that would normally act on smaller, divided cells (see particularly Fig. 14). Again the size, shape, number, etc., of nuclei could not in these experiments, be related definitively to the position and number of preprophase bands.

The nature of the processes which lead to stomatal differentiation is still very obscure. The gmc could obviously produce some compound(s) which, diffusing only laterally, induces competent epidermal cells to divide (Stebbins & Jain, 1960; Stebbins & Shah, 1960), the plane of the division (and of the preprophase microtubules) being influenced by the geometry of the system. It is difficult to see how such an explanation could apply to the first asymmetrical division, which however appears less tightly controlled. For example, one often finds series of cells of only slightly different sizes in a very young, stomatal row, and it may not be obvious which are destined to become gmc's (except that, in the author’s experience, any small cell containing a vacuole can never induce sc formation). Likewise, the most striking grouping of microtubules in a small region occurs in the second asymmetrical division. Perhaps, therefore, the first asymmetrical division occurs when competent cells respond to generalized physiological gradients associated with nearby meristematic regions, and the second asymmetrical division is similar except that a single cell (the gmc) acts as the small but potent ‘meristem’. Both these divisions might occur at right angles to the field or gradient as is seen generally in the rest of the tissue, but with the first and second asymmetrical divisions giving curved cell plates (Pickett-Heaps & Northcote, 1966) in a ‘curved’ field; the third symmetrical division also follows this pattern, being at right angles to the field created by the young complex.

This attempt to simplify the problem and so explain it in terms of a general mechanism applicable to other cells of the meristem may prove useful. It is compatible with the results described above and can be made to fit the most interesting results recently described by Stebbins, Shah, Jamin & Jura (1967), where some delay of division by
their chemical treatments might allow the weakening transverse gradients of the ageing gmc complex to be overcome by the general physiological gradients of the young leaf.

REFERENCES


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(Received 24 May 1968)
ABBREVIATIONS ON PLATES

A, B, C, D Areas indicated by black bars and letters are shown in detail in the adjacent pictures.
Black bars These indicate the position of the preprophase band(s) of microtubules, being placed on the opposite side of the wall to them.

e epidermal cell

n nucleus

g guard mother cell (on figures)

p preprophase nucleolus

gc guard cell (formed following division of the gmc)

s subsidiary cell (on figures)
gmc guard mother cell (in caption)

sc subsidiary cell (in caption)

ls longitudinal section

v nuclear vesicle

Fig. 1. Light micrograph; Longitudinal section of a very young leaf mesophyll cell. Tissue exposed to 0.1% caffeine (24 h), then water (6 h). Nuclei in trinucleate cell are at different phases in division, one clearly being early prophase. Adjacent is a binucleate interphase cell. x 1600 approx.

Fig. 2. As for Fig. 1 except that the second incubation in water lasted 18 h. Two early prophase nuclei are seen, and wall stumps (arrows) indicate plane of previous division. Two preprophase bands were found (bars), very similar in position to those shown in Fig. 1, (an equivalent, but mononucleate cell). x 2000 approx.

Fig. 3. Light micrograph; Longitudinal section of leaf epidermis. Tissue treated with 0.1% caffeine for 24 h, then with water for 12 h. Both nuclei in the epidermal cell are at meta- or early anaphase; they are separated by relatively large wall stumps (arrows) which is probably why they are both undergoing the second asymmetrical division towards separate gmc’s. x 960 approx.

Fig. 4. Low-power, light micrograph of leaf epidermis in longitudinal section. Tissue treated with 0.1% caffeine for 24 h, then water for 18 h. Abortive telophases are indicated (double arrows) and many binucleate or polyploid cells are present. In two rows, asymmetrical division has been attempted (to form gmc’s, or possibly, epidermal hairs). In all cases where such cells were proceeding to preprophase again (small arrows), the preprophase microtubules were asymmetrically situated. For example, one cell (large arrow) is seen again in more detail in Figs. 13, 13 A, B. x 360.
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Fig. 5. Abnormal stomatal development (untreated leaf). The end wall between two epidermal cells coincides with the position of a gmc (note that it is already flanked by an sc). The nucleus in one of these epidermal cells is at preprophase, and the preprophase microtubules are appropriately located (black bars, c.f. Fig. 6). \( \times 2700 \) approx.

Fig. 6. The end result of the situation seen in Fig. 5, with a stomatal complex containing two sc's on one side. The gmc has divided to form the future guard cells (gc). (This light micrograph was taken from another block of tissue.) \( \times 1100 \) approx.

Fig. 7. 'Nuclear vesicle', from coleoptile cambial tissue treated with 0.1% caffeine for 24 h, and then water for 18 h. These inclusions in large, polyploid nuclei from treated tissue, are not uncommon (c.f. Figs 14, 17). The author suspects the large vesicle (v) to contain the trapped components of the phragmoplast following abortive telophase, including many cell plate vesicles. \( \times 40,500 \).

Fig. 8. Longitudinal section through the single preprophase band of microtubules in a root-tip cell; tissue treated with 0.1% caffeine for 24 h, water for 6\( \frac{1}{2} \) h. The band is very large (in this case around a large polyploid nucleus). \( \times 52,000 \).

Figs. 9, 10. Same cell as that in Fig. 7. Preprophase bands along the same wall, separated by a short distance. They seem to indicate some conflict in the state of polarization of the cell. \( \times 27,000 \).
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Fig. 11. Young mesophyll cell of leaf; tissue treated with 0.1% caffeine for 24h, and then water for 18h. The plane of previous attempted division is indicated by wall stumps (arrows). This cell contained two preprophase bands (A-C, B-D), as did the binucleate cell in Fig. 2 in similar positions. ×3500.

Figs. 11 A-D. Preprophase microtubules from the cell in Fig. 11, shown at higher magnification. ×49000.
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Fig. 12. Leaf epidermis, tissue treated as that in Fig. 11. Previous attempt was at the first asymmetrical division—see wall stump (arrow). At subsequent preprophase, single band of microtubules was still at the same end of the cell (bars). \( \times 3500 \).

Fig. 12A. Detail of Fig. 12, showing the wall stump and preprophase microtubules. \( \times 29000 \).

Fig. 13. Electron micrograph of one of the cells in Fig. 4 (large arrow). Although the disposition of the two nuclei is different from that in Fig. 12, the single band of preprophase microtubules (bars) is similarly highly polarized (in the same direction as that for all equivalent asymmetrically dividing cells—see lower cell, and others in Fig. 4). \( \times 2650 \).

Figs. 13A, B. Preprophase microtubules from positions A, B in Fig. 13. \( \times 40500 \).
Fig. 14. Leaf epidermis, tissue treated with 0.1% caffeine (24 h) and then water (18 h). Typical row of preprophase cells, all with polarized microtubules (bars). The middle cell is interesting in that previous attempted division was symmetrical (wall stumps at arrows); preprophase microtubules are situated as if this were two cells. × 2200 approx.

Figs. 14A, B. Sections of two of the bands shown in Fig. 14 (regions A, B). × 33 500.

Fig. 15. Leaf epidermis; tissue treated with 0.2% caffeine (23 h) and then water (8 h). Previous abortive division was second asymmetrical, next to gmc (g); note wall stumps (arrows). Preprophase microtubules were in appropriate regions (bars) for another attempt at asymmetrical division. × 5000.
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Fig. 16. Leaf epidermis; tissue treated with 0.1% caffeine (24 h) and then water (18 h). Epidermal cell is binucleate (c.f. Figs. 15, 17 and 18), plane of previous division not apparent. Preprophase microtubules are adjacent to gmc (g), which already has one sc (s). Some spindle microtubules were also detected between the nuclei (arrow). × 3000 approx.

Figs. 16a, b. Details of serial section of Fig. 16, showing microtubules in their characteristic position. × 33 500.
Fig. 17. As for Fig. 15; wall of previous asymmetrical division is quite well formed. Preprophase microtubules in this interesting example were found at A and B. × 6300.

Figs. 17A, B. Details of Fig. 17; preprophase microtubules are shown. × 49000.
Fig. 18. Leaf epidermis, tissue treated with 0.1% caffeine (24 h) and then water (18 h). The gmc's in this region were not yet exerting their full effect on adjacent epidermal cells (no sc's were present in this region). Both epidermal cells were in early preprophase (one is binucleate and the other 'polyploid'). The preprophase microtubules were detectable at regions indicated by bars. Their position in each case seems to have been affected partly by the gmc's, while conforming to the more usual transverse orientation. The irregular black lines across the section are due to folds in it. Wall stump indicated by arrow. × 1700 approx.