Planta (1986) 168:11–23

Planta

Pollen wall formation in *Lilium*: The effect of chaotropic agents, and the organisation of the microtubular cytoskeleton during pattern development

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Abstract. Using a combination of electron-microscopic and immunocytochemical techniques the behaviour of the microtubular cytoskeleton has been followed throughout microsporogenesis in *Lilium henryi* Thunb. Cells treated with colchicine at specific stages and then permitted to develop to near maturity were used to investigate any participation by microtubules in the regulation of pollen wall patterning. The microtubular cytoskeleton assumes four principal forms during the meiotic process; in pre-meiosis it resembles that characteristic of meristematic somatic cells, during meiotic prophase it becomes associated with a nuclear envelope and, perhaps, with the chromosomes and, as the nuclear and cell divisions commence, it takes the form of a normal spindle apparatus. In the young microspores, microtubules assume a radial organisation extending from sites at the nuclear envelope to the inner face of the plasma membrane. No firm evidence was found linking any one of these forms of cytoskeleton with the generation of patterning on the cell surface. Experiments with colchicine revealed that the drug would readily dislocate the colpus, but did not affect the general reticulate patterning. The radial cytoskeleton was present during the deposition of the early primexine, but evidence from these and other studies (J.M. Sheldon and H.G. Dickinson 1983, J. Cell. Sci. **63**, 191–208; H.G. Dickinson and J.M. Sheldon, 1984, Planta **161**, 86–90) indicates patterning to be imprinted upon the plasma membrane prior to the appearance of this type of cytoskeleton. These results are discussed in terms of a recent model proposed to explain pattern generation on the surface of *Lilium* pollen grains, based on the "self-assembly" of patterning determinants within the plasma membrane.

Abbreviation: MTOC = microtubule-organising centre

Key words: Cytoskeleton – *Lilium* – Microtubule – Pollen wall (pattern generation, self-assembly).

Introduction

One of the first events in the angiosperm archesporial tissue is an abandonment of the organized 'somatic' fibrillar cellulosic wall for a homogeneous matrix of the β-1,3-linked glucan, callose. This new wall isolates the developing meiocytes physically from the surrounding sporophytic tissues and is held to be impermeable to many large molecules (Heslop-Harrison and Mackenzie 1967). Callosic cross-walls are formed following the cell divisions of meiosis and, after this, development of the young pollen wall commences around each member of the tetrad (Dickinson 1970; Heslop-Harrison 1971a; Sheldon and Dickinson 1983). The first stages of this process include the positioning of the non-patterned apertural areas and formation of the primexine, a structure later elaborated by materials derived from the tapetum (Dickinson and Bell 1976). Little is known of the factors which determine the basic patterning of this wall, save that they are of sporophytic origin (Heslop-Harrison 1971a). Since the microtubular cytoskeleton plays an important part in the organisation of cell walls in somatic tissues, it is clearly of interest to investigate whether it is similarly active in the organisation of the pollen wall.

It is now firmly established that the microfibrillar helices of somatic cell walls are organised by microtubules which direct the course of the cellulosic synthetase units over the surface of the plasma membrane (Heath 1974; Heath and Seagull 1982). Callosic cell walls are not, however, synthesised in the same fashion; instead they are produced by the apparent discharge of cisternae de-

rived from smooth endoplasmic reticulum (Dickinson and Lewis 1973, 1975). Evidence is also available that, in *Lilium*, the deposition of the second wall layer, the patterned "pollen wall", is phasic, having its origins early in prophase with the synthesis of a 'pre-pattern determinant'. This determinant, produced in the sporophytic pollen mother cell appears to be inserted into the plasma membrane throughout the duration of meiosis (Sheldon and Dickinson 1983). In the tetrad stage this imprinting of the plasma membrane is followed by the establishment of the various components of the pollen wall, the first indication of which is the apposition of sheets of endoplasmic reticulum to the plasma membrane, shielding the colpal region from further wall-forming activities. Wall construction then follows with the deposition of a shallow layer of cellulosic fibrils between the plasma membrane and the inner face of the callose wall in all but the areas protected by the membranous colpal 'shield' (Heslop-Harrison 1968). The establishment of the sexine begins during the deposition of this layer with the production of lamellate structures, apparently associated with vesiculation of the plasma membrane (Dickinson 1970) which then develop into probacula and muri. While still retained within the tetrad these initials are thickened with protosporopollenin and, on release from the tetrad, tapetally derived sporopollenin. The formation of the two final layers of the wall, the lamellate nexine and the cellulosic intine, then takes place.

Microtubules have been implicated in the establishment of aperture position (Heslop-Harrison 1971 b, c), but the function of radial arrays of microtubules which emanate from microtubule-organising centres (MTOCs) at the nuclear envelope early in the tetrad stage (Dickinson and Sheldon 1984) is unclear. It is possible that they represent evidence of re-establishment of the cytoskeleton following the meiotic divisions, but they may also be important in the transport of precursors of a second wall layer which confers height to the sporopollenin wall (Dickinson 1970; Dickinson and Sheldon 1984). There is little doubt that, later, helically-orientated microtubules influence the orientation of the intine fibrils (Dickinson and Heslop-Harrison 1971; Mattsson 1976).

We remain in ignorance, however, of any part played by microtubules in the insertion of patterning determinants into the plasma membrane. Certainly these organelles must be involved in the construction of the meiotic spindle, for treatment with chaotropic agents results in no, or incomplete cell division taking place.

Material and methods

Protoplasts for the immuno-cytochemical study were released from young microspores of *Lilium henryi* Thunb. by treatment as described by Dickinson and Sheldon (1984). The cells obtained were washed in protoplast incubation medium (PIM) and fixed for 15 min in an 8% solution of paraformaldehyde in PIM. After washing with water they were affixed to poly-L-lysine (MW 150000–300000) (Sigma, Poole, Dorset, UK)-coated coverslips (Mazia et al. 1975). The coverslips were washed with phosphate-buffered saline (PBS) and treated for 1 h with 1:1000 dilution of antitubulin (YL1/2) (kindly supplied by J. Kilmartin, University Medical School, Cambridge, UK; Kilmartin et al. 1982). After washing with PBS, the cells were stained with 1:50 dilution of fluoroscein-conjugated species-specific antibody (Sigma) for 1 h in the dark, washed again and mounted in glycerol containing 'Citifluor' (kindly supplied by D. Goodwin, The City University, London, UK; Davidson and Goodwin 1983). The material was examined in a Leitz (Wetzlar, FRG) Dialux microscope fitted with epifluorescence optics. Control preparations (containing no antisera to tubulin) gave virtually no fluorescence.

In the study of wall modification, staged buds were treated with colchicine as described by Dickinson and Sheldon (1984). After staining in a 0.1% aqueous solution of Auramine O (Sigma), young pollen was observed under incident UV irradiation.

Microspores of *Lilium* were prepared for electron microscopy as described in Dickinson and Lewis 1973. Ultrathin sections of this material were conventionally stained with lead citrate and uranyl acetate, and examined in a Hitachi (Nissei Sangyo Ltd., Reading, UK) H-800 transmission electron microscope, operating at 75 kV.

Results

The results are presented in two sections, firstly, those relating to the nature of the cytoskeleton in sporogenous tissue obtained from the examinations by immunocytochemistry and transmission electron microscopy and, secondly, those gained by disruption of this cytoskeleton during microsporogenesis using colchicine. In both these studies the cytoskeleton was examined during all stages of microsporogenesis, namely in the archesporial tissue, the meiocytes, post-meiotic cells, and in young spores.

1) The nature of the cytoskeleton in sporogenous cells

The archesporial tissue. Treatment with fluorescent-labelled antitubulin (see Fig. 1) shows the cells of the archesporial tissue to possess a cytoskeleton largely composed of orbiting microtubules running at different depths between the surface of the protoplast membrane and the nucleus (Fig. 1). Microtubules with this configuration are also seen with the electron microscope (Fig. 2).

The meiocytes. In early-prophase cells the microtubular cytoskeleton extends from centres of organ-

isation situated at the nuclear surface (Figs. 3–5). These 'centres', of which there appear to be a small number, are composed of a tubulin-free region from which the microtubules spread through the cytoplasm (Fig. 4). The situation of the microtubules with regard to the nuclear surface is well illustrated by electron microscopy (Fig. 6), and it is interesting to note the close association between the microtubules external to the nuclear envelope and the chromatin within it (Figs. 6, 7).

As prophase proceeds, the microtubules gradually disperse from these centres of organisation, but nevertheless remain in some form of association with the nuclear envelope (Fig. 8). At even later prophase stages a number of cells are commonly observed with a strikingly disorganized microtubular system (Fig. 9). The electron microscope also shows microtubules of this type of cytoskeleton to run through the cytoplasm (Fig. 10).

During metaphase I the cytoskeleton becomes rearranged into the meiotic spindle (Fig. 11). This is composed of thick fascicles each containing 100–130 microtubules, which are seen to nucleate at sites on the condensed chromosomes (Fig. 12). The electron microscope reveals the area of cytoplasm contained within the spindle to be devoid of membranous organelles (Fig. 13). The microtubular bundles of the spindle become more loosely packed towards the end of anaphase I and, at telophase, the phragmoplast is formed (Figs. 14, 15).

The cytoskeleton of cells at the diad stage resembles that of the late-prophase cells to which reference was made earlier, possessing a randomly orientated structure, with microtubules fairly evenly distributed through the cytoplasm (Figs. 16, 17). In some cells the cytoskeleton has a striate appearance, which could indicate the reinitiation of spindle formation. At the second meiotic division, spindle development takes place as for the first (Fig. 18). However, as the second phragmoplast forms, microtubules become rearranged into radiating arrays extending from the nuclear envelope (Fig. 19).

Post meiotic cells. The microtubular cytoskeleton in tetrad cells is unusual, in that it is composed of a system of radial arrays of microtubules extending out from the nuclear envelope to the surface of the plasma membrane (Fig. 20). These microtubules, or groups of microtubules, extend from sites apparently evenly distributed over the whole of the nuclear surface. Electron microscopy confirms that they radiate out from centres of organisation at the nuclear envelope (Fig. 21), and about directly onto the plasma membrane (Fig. 22). At early tetrad stages, vesicles are observed to be associated with this microtubular cytoskeleton (Fig. 23).

Young spores. The radial disposition of the cytoskeleton of post-meiotic cells is initially retained by the young, mononucleate, spores (Fig. 24). This configuration is later lost and cortical microtubules are seen again to run in large numbers under the surface of the plasma membrane (Fig. 25).

2) The effect of colchicine treatment

The meiocytes. Throughout the major part of meiosis, from early prophase to diad, the primary effect of treatment with colchicine is the disruption of the meiotic divisions. When stained with Auramine-O, treated cells which have been cultured to maturity reveal that in addition to those which have divided normally, there are grains with fully formed pollen-walls that have remained undivided (Figs. 26, 27), others that have undergone the first meiotic division (Figs. 27, 28), and yet others which have divided but not separated, sharing a common sporopollenin wall (Fig. 29). The number of affected grains varied, depending upon the meiotic stage at which they were originally treated (Fig. 30).

The colpus is affected in a variety of ways, depending upon the level of division attained. Those cells remained undivided either have no aperture or a varying number of irregular colpi (Figs. 26, 27). Although a few irregular colpi are seen where one division has occurred, more often these cells possess a very regular 'banded' aperture (Figs. 27, 28). All fully divided pollen grains possess a normal colpus.

At no stage in meiosis did treatment with colchicine affect the nature of the reticulate pattern later assumed by the exine (Figs. 26–29).

Post-meiotic cells. Colchicine treatment of tetrad cells disrupts neither the reticulate patterning nor the positioning of the colpus. When cells are treated at early tetrad stages, it does, however, affect the height of the exine giving the muri of the wall a squat and thickened appearance (Fig. 31).

Colchicine and microtubules. While most of the information from the colchicine experiments was obtained through examination of mature spores, this approach did not reveal whether the drug disrupted microtubules over the period of its application. For this reason, colchicine-treated material

Figs. 1, 3–5, 8, 9, 11, 14, 16, 18–20, 24. Protoplasts of *Lilium* microspores stained with fluorescent-labelled antitubulin

Figs. 2, 6, 7, 10, 12, 13, 15, 17, 21–23, 25. Transmission electron micrographs of *Lilium* microspores

Fig. 1. The orbiting cytoskeleton of a cell from the archesporial tissue. ×3400; bar = 5 μm

Fig. 2. Cortical microtubules (*M*) at the plasma membrane (*PM*) of archesporial cells. The cell walls (*W*) have not yet been thickened with callose. ×21000; bar = 0.5 μm

Fig. 3. The cytoskeleton of an early prophase meiocyte showing two MTOCs (*arrowed*) at the surface of the nucleus (*N*). ×3100; bar = 5 μm

Fig. 4. The same cell as depicted in Fig. 3 but showing another MTOC. Note the unstained centre and the radiating microtubules. ×3100; bar = 5 μm

Fig. 5. Cross section of a MTOC (*arrowed*) at the nucleus (*N*) of an early-prophase meiocyte. ×3100; bar = 5 μm

Fig. 6. Microtubules (*M*) close to the nuclear envelope (*NE*) of an early-prophase meiocyte. ×56000; bar = 0.2 μm

Fig. 7. Microtubules (*M*) at the nuclear envelope (*NE*) of an early-prophase cell. Note the chromatin (*C*) associated with the microtubules. ×59000; bar = 0.2 μm

Fig. 8. The cytoskeleton of a late-prophase meiocyte. The microtubules have now dispersed from the MTOCs depicted in Figs. 3–5, but still remain close to the nuclear surface (*NS*). ×3100; bar = 5 μm

Fig. 9. Cytoskeleton of a random composition in a late-prophase cell. ×3100; bar = 5 μm

Fig. 10. Microtubules (*M*) running through the cytoplasm of a late-prophase cell of the type depicted in Fig. 8. Note that the nuclear envelope (*NE*) possesses a number of nuclear pores (*NP*). ×23000; bar = 0.5 μm

Fig. 11. The spindle of a cell during the first meiotic division. ×3100; bar = 5 μm

Fig. 12. The kinetochore (*K*) of a chromosome (*C*) observed during division I. Note the large numbers of microtubules (*M*). ×21000; bar = 0.5 μm

Fig. 13. The spindle region (*S*) during division I appears devoid of membranous organelles between the chromosomes (*C*). ×5400; bar = 2 μm

Fig. 14. The phragmoplast (*P*) after division I. Note the terminal position of the nuclei (*N*). ×3100; bar = 5 μm

Fig. 15. Microtubules (*M*) directing vesicles (*V*) to the phragmoplast (*P*) during cytokinesis I. Note the lack of mitochondria and plastids in the vicinity. ×21000; bar = 0.5 μm

Fig. 16. Member of a diad at telophase I showing its randomly orientated cytoskeleton. ×3100; bar = 5 μm

Fig. 17. Microtubules (*M*) in the cytoplasm of a diad cell during telophase I. ×13000; bar = 1 μm

Fig. 18. A spindle of the second meiotic division. ×3100; bar = 5 μm

Fig. 19. A diad cell immediately prior to the second cytokinesis. Note how the microtubules form radiating arrays from around each nucleus (*N*). ×3100; bar = 5 μm

Fig. 20. A post meiotic cell showing microtubules radiating from the nucleus (*N*) to the protoplast surface (*P*). ×3100; bar = 5 μm

Fig. 21. Microtubules (*M*) radiating out from a MTOC (*arrowed*) at the nuclear envelope (*NE*) of a tetrad cell. ×45000; bar = 0.2 μm

Fig. 22. Microtubules (*M*) abutting onto the plasma membrane (*PM*) of a tetrad cell. Note the callose wall (*C*). ×80000; bar = 0.2 μm

Fig. 23. Vesicles (*V*) close to microtubules (*M*) in the cytoplasm of an early tetrad cell. ×35000; bar = 0.5 μm

Fig. 24. Cross section of a young mononucleate pollen grain showing the radiating cytoskeleton. ×3100; bar = 5 μm

Fig. 25. Cortical microtubules (*M*) running under the surface of the plasma membrane of a mature pollen grain. Note the intine (*I*) and the electron-opaque sexine (*S*). ×15000; bar = 1 μm

Figs. 26–29, 31. *Lilium* microspores stained with Auramine O and observed under UV irradiation

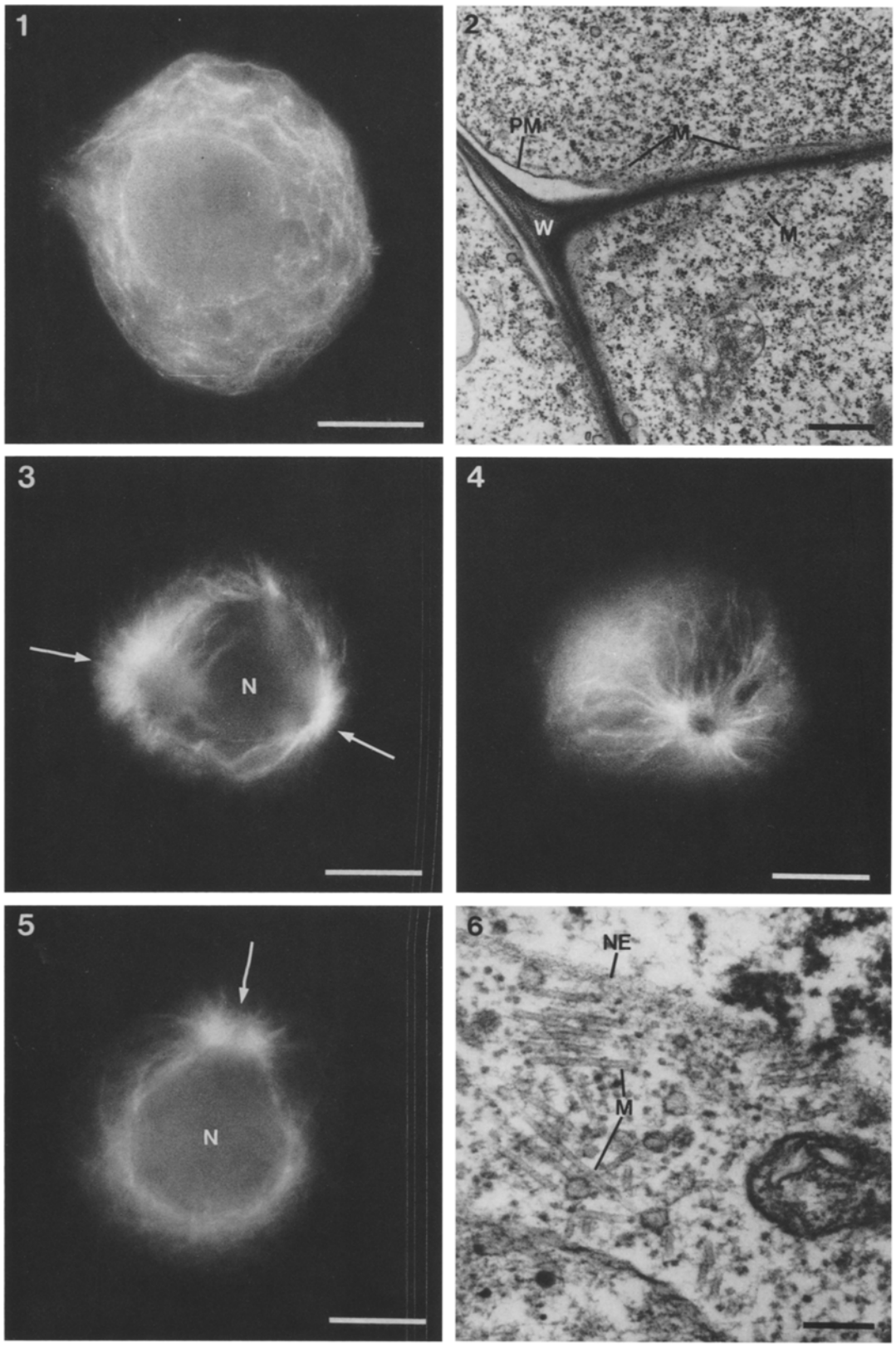
Fig. 26. Young "pollen grain" exposed to colchicine at the late prophase stage of microsporogenesis. It has remained undivided. Note the normal reticulate pattern and the irregular colpus (*arrowed*). ×2500; bar = 5 μm

Fig. 27. Two grains formed after colchicine treatment during pachytene. Both grains have a normal reticulate pattern although one grain has remained undivided (*A*) with an irregular colpus, while the other has undergone one division (*B*) and has a "banded" aperture. ×2300; bar = 5 μm

Fig. 28. Pollen grain formed after treatment with colchicine at the diad stage, having undergone only the first meiotic division. Note the normal reticulate pattern and the "banded" aperture (*arrowed*). ×2700; bar = 5 μm

Fig. 29. Four pollen grains which have divided normally after exposure to colchicine during pachytene, but which share common dividing walls. ×2500; bar = 5 μm

Fig. 31. Grain exposed to colchicine early in the post-meiotic stage. Note that the normal reticulate pattern is present but expressed in squat and thickened muri. ×3300; bar = 5 μm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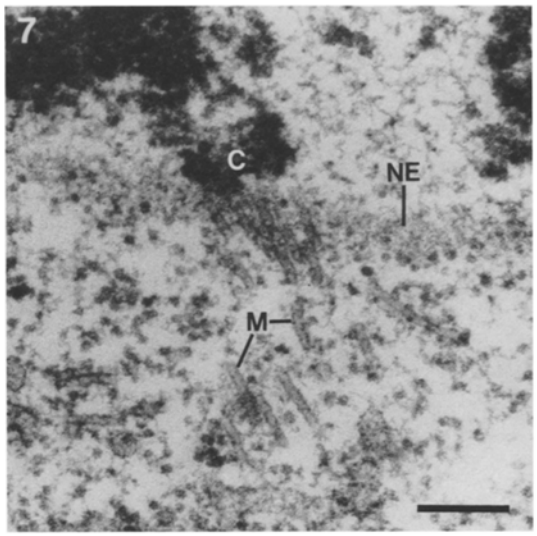
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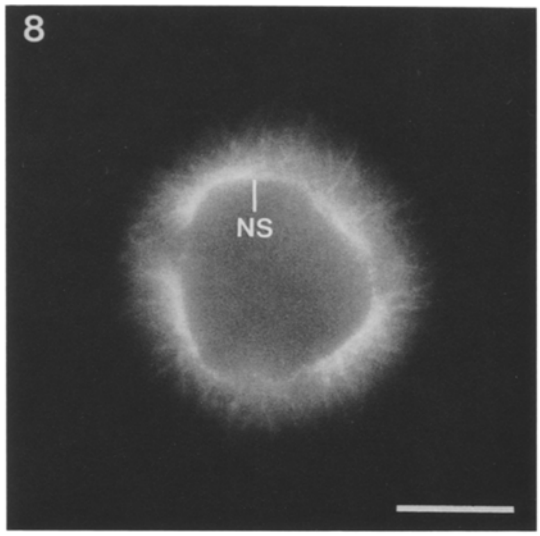
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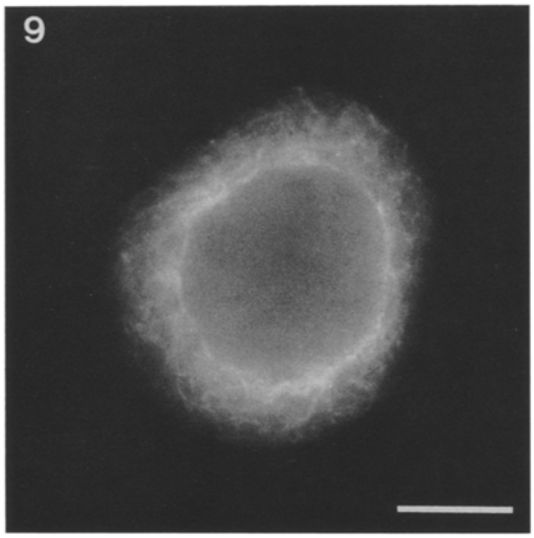
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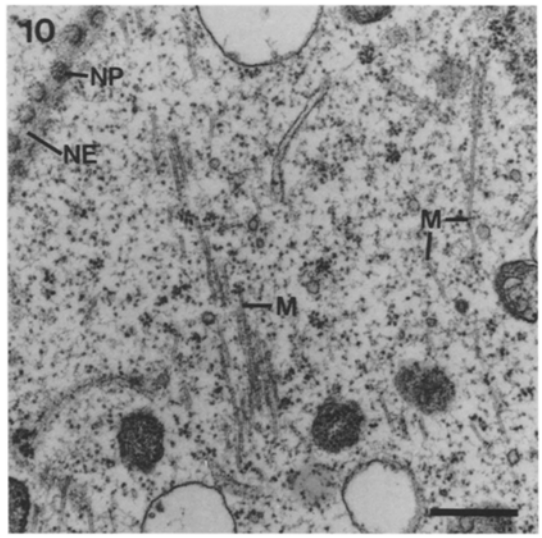
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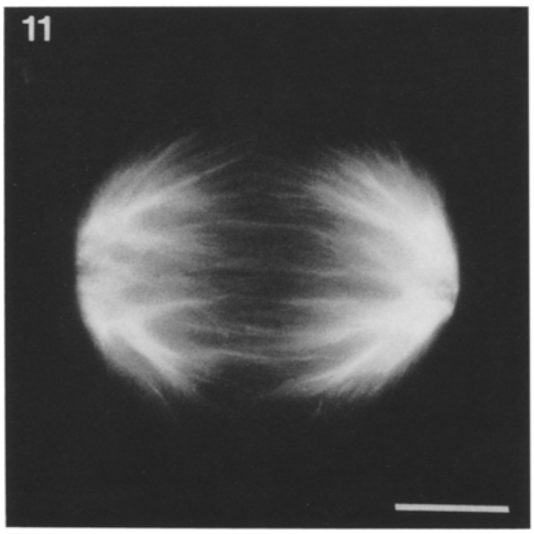
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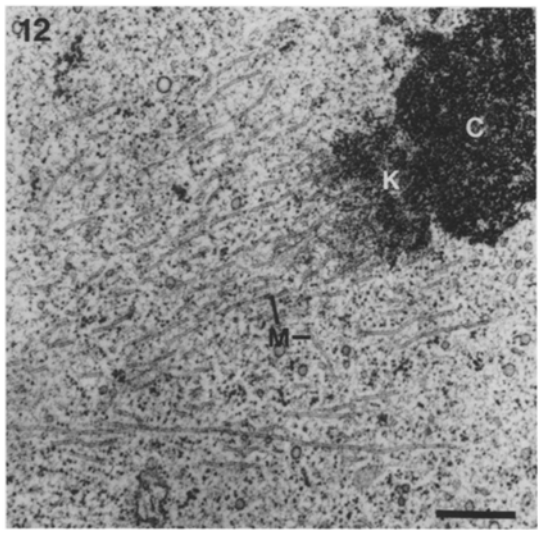
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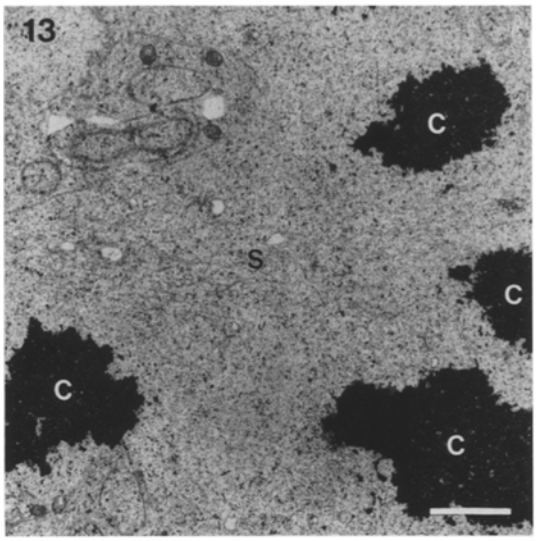
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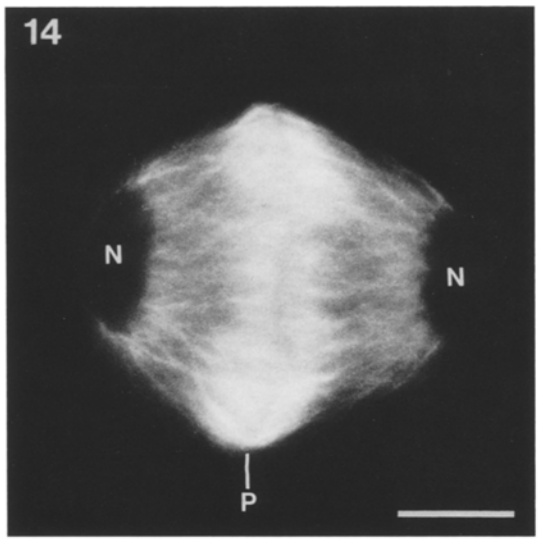
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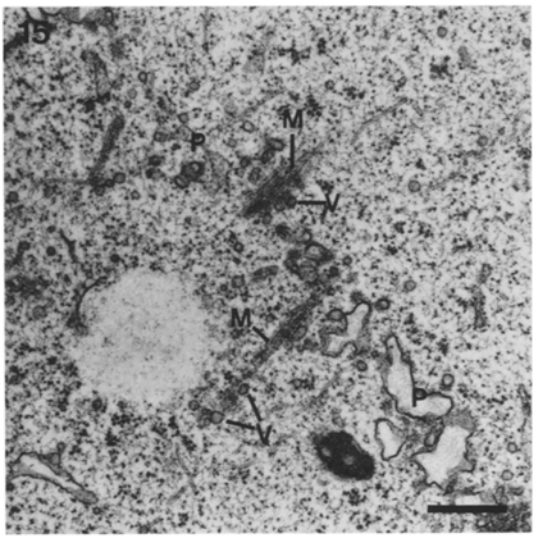
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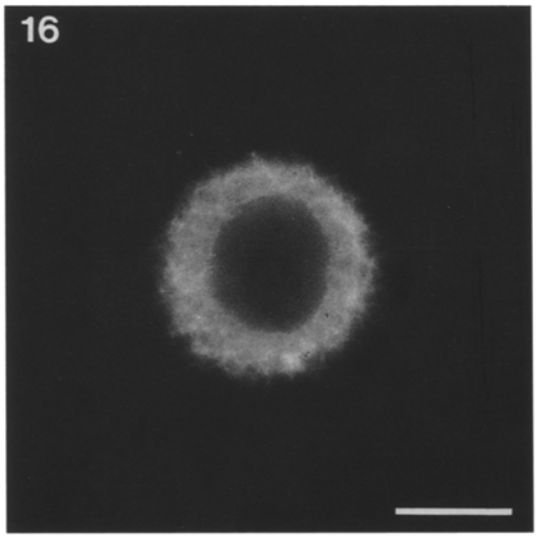
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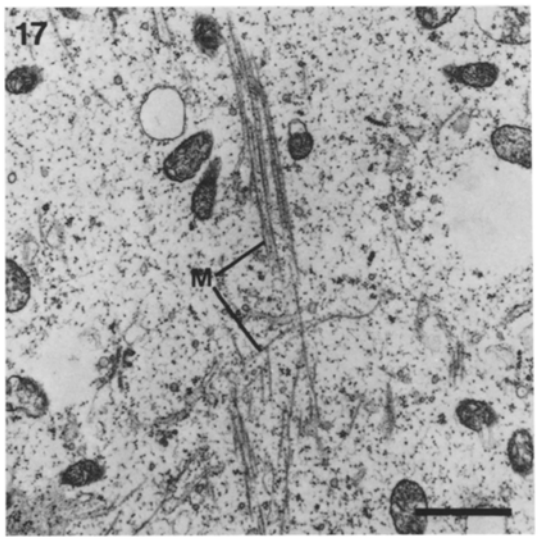
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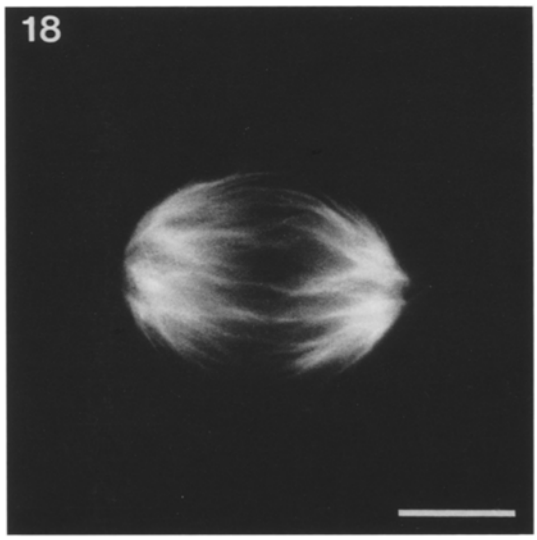
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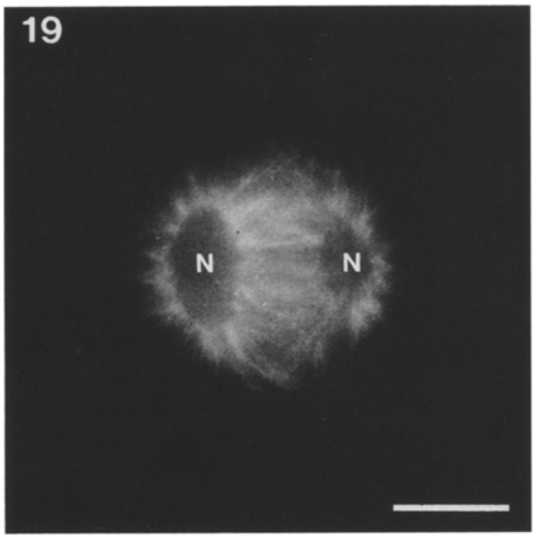
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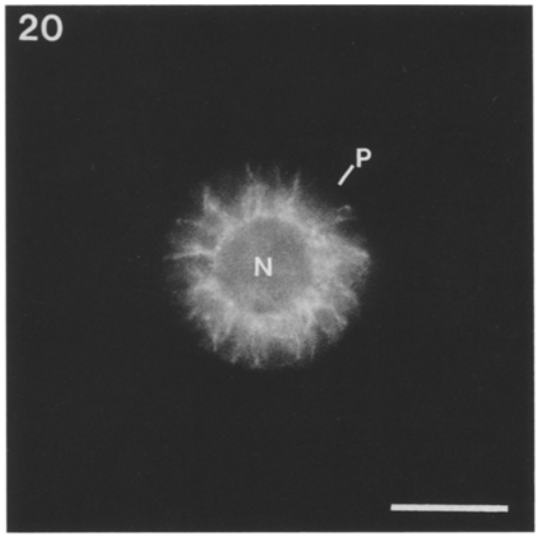
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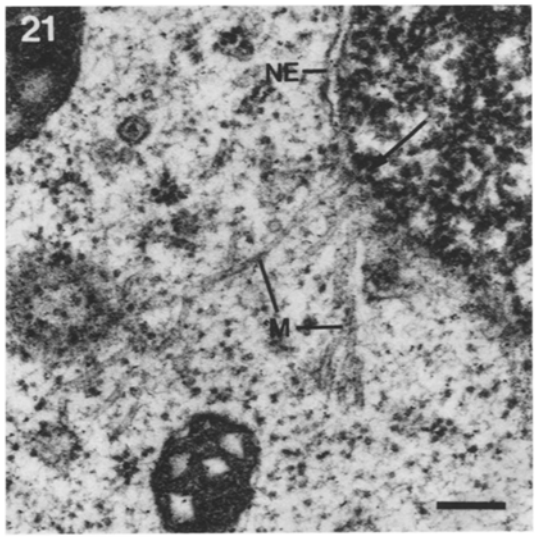
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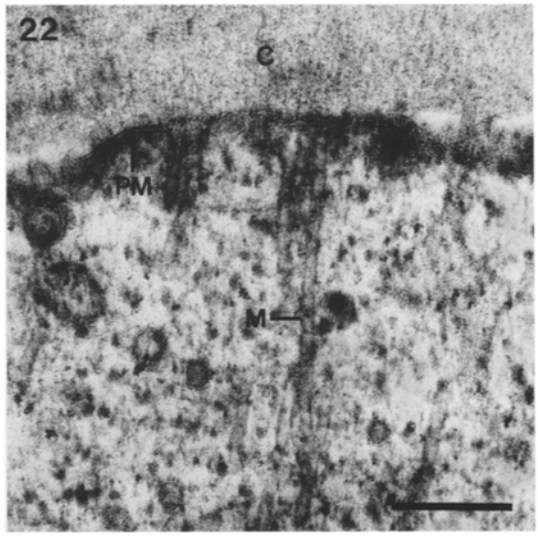
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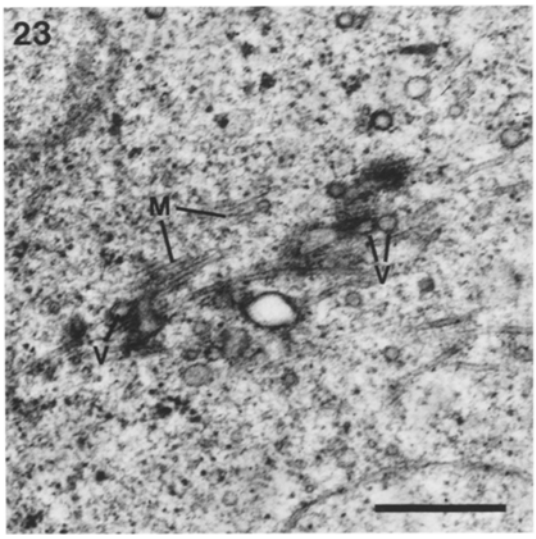
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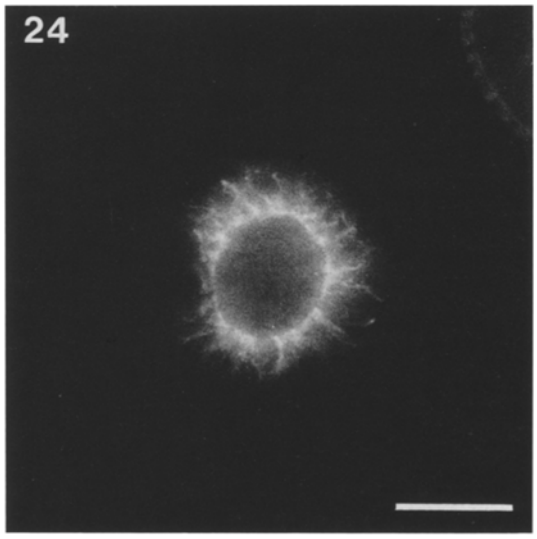
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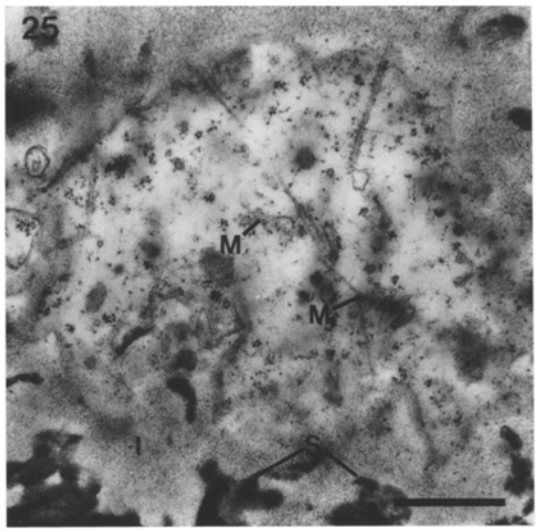
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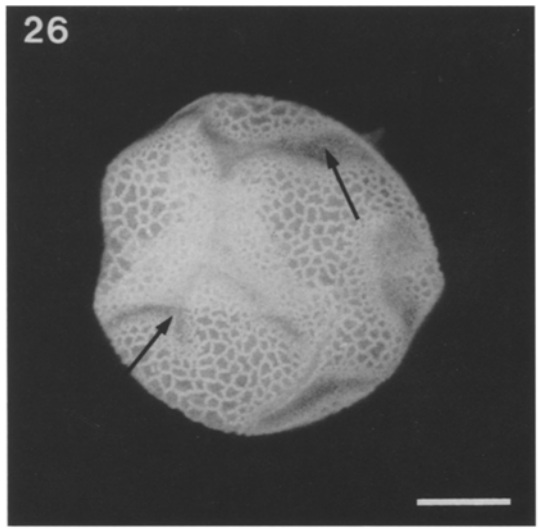
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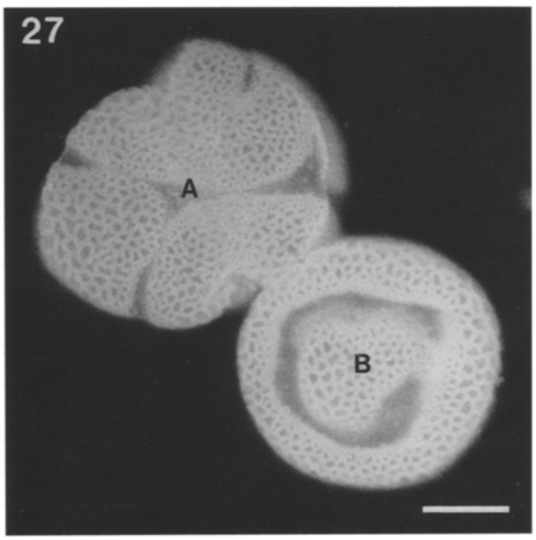
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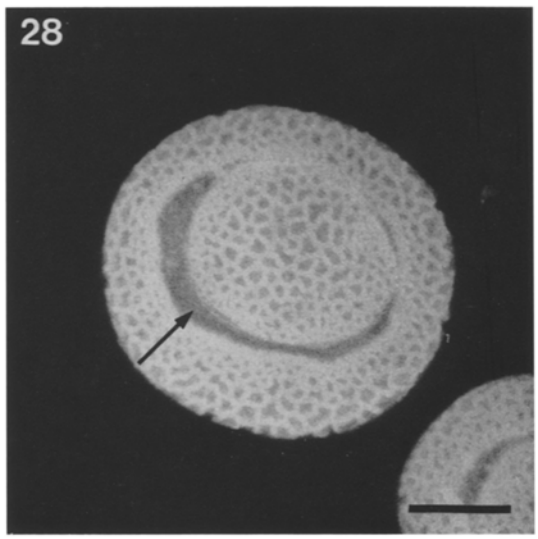
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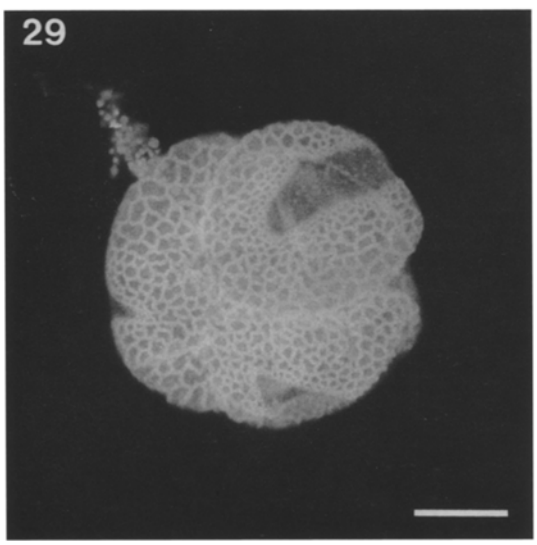
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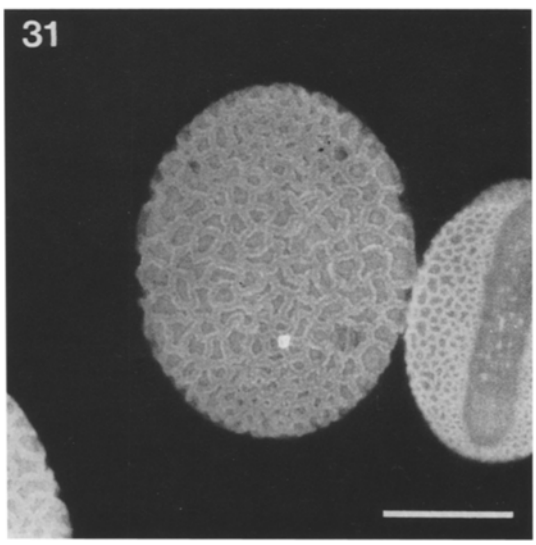
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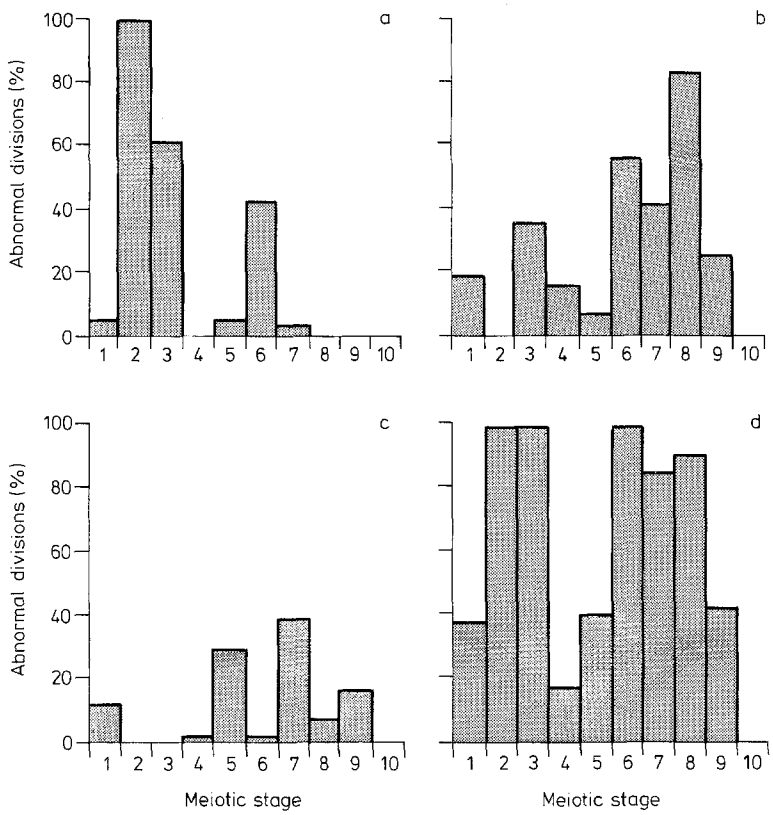

Fig. 30A–D. Percentage of abnormal divisions observed after treatment of developing *Lilium* meiocytes with colchicine for 12 h at various meiotic stages. **A** Cells undergoing no divisions; **B** cells undergoing the first meiotic division; **C** cells sharing a common dividing wall; **D** total abnormal divisions. Meiotic stages: *1*) leptotene-zygotene, *2*) zygotene, *3*) pachytene, *4*) pachytene-diplotene, *5*) diplotene-metaphase I, *6*) diakinesis-metaphase I, *7*) anaphase I, *8*) diad, *9*) anaphase II – early tetrad, *10*) tetrad

was examined with the electron microscope immediately following the period of application. At no time were any microtubules observed. The general aspect of the cytoplasm did, however, differ considerably depending upon whether the cells were treated in prophase or as young spores. In prophase meiocytes the cytoplasm appeared very vesicular, containing numerous small vacuoles. The spore cytoplasm, on the other hand, resembled "normal" tissue very closely, except in that massive swarms of coated vesicles were distributed at random throughout the cell. A careful search was made of the regions of each type of cell normally occupied by large numbers of microtubules; none were found either in the central or perinuclear regions of prophase meiocytes, or radiating from the nuclear envelope of young spores in the tetrad.

Discussion

The meiotic divisions. Although Lloyd et al. (1980) have proposed that colchicine has a reduced effect on plant microtubules as compared with those of animal cells, it is clear from the results obtained in this study that the drug not only affects these organelles, but also the meiotic divisions of pollen mother cells. The disruption caused to the divisions by colchicine varies with the stage of microsporogenesis at which treatment takes place. With time, cells recover from the effects of colchicine treatment and it is interesting to note that meiotic divisions are not only disrupted when material is treated at stages where microtubules are present as, or actively reorganising into, the spindle, but also at earlier stages in meiotic prophase (leptotene to early pachytene). Very early treatment with colchicine may thus affect the first stages in spindle formation. The organisation of tubulin monomers differs between cytoplasmic and spindle microtubules (Forer et al. 1980; Forer 1982) and, since spindle microtubules must clearly be composed of tubulin derived from the depolymerisation of cytoplasmic tubules, the first stages of spindle construction must include the generation of this pool of protein. If this takes place in early prophase, treatment with colchicine at this point will not only serve to depolymerise any cytoplasmic microtubules present, but also render the pool of free tubulin no longer available for use in spindle formation.

Alternatively colchicine has also been reported to affect the pairing of meiotic chromosomes when applied during leptotene and zygotene (Shephard et al. 1974), and it may be for this reason that division of the meiocytes is prevented in our experi-

ments, rather than through the inhibition of spindle formation. Such an inference is supported by the presence of the complex arrays of microtubules that radiate from tubulin-free centres on the outer surface of the nucleus at this time. The nature of this nuclear "exoskeleton" presumes some form of interaction with the nuclear envelope, and the association between microtubules on the outer surface of the envelope and chromatin on the inner indicates that it plays some part in the regulation of pairing. The precise nature and function of MTOCs at the nuclear surface are thus far from clear, as indeed is the eventual fate of this type of cytoskeletal organisation. Far more detailed studies of these prophase stages are required before any conclusion may be drawn as to whether these nuclear-associated MTOCs go on to organise the spindle, or simply disassemble. Nevertheless, these observations add to a fast-accumulating body of evidence indicating that the nuclear envelope plays a pivotal role in the regulation of meiotic prophase. Further structural evidence of this activity is provided by the formation of the striking nuclear vacuoles characteristic of the zygotene nuclear envelope of all plants so far studied (Sheffield et al. 1979).

Positioning of the colpus. It has been proposed by Dover (1972) that the MTOCs may be responsible for aperture positioning. The work with colchicine has revealed that this may be so for *Lilium,* although there does not seem to be a direct relationship between the number of MTOCs and the number of apertures, as predicted for *Triticum* (Dover 1972). Colpal position corresponds with the location of the MTOC at the second meiotic division, but it is interesting to note that if these bodies are directly responsible for colpal positioning they produce the opposite effect in grains resulting from only one meiotic division. Here, MTOC position coincides with the patterned centre of the circular colpus (Fig. 32A) rather than the unpatterned colpal region, as seen in normally divided grains (Fig. 32B).

The possibility thus exists that normal colpal positioning is determined after the first meiotic division, as a band-shaped region of the plasma membrane of each member of the diad. We have obtained no clear indication as to the mechanism by which this band could be determined, but a clue may lie in the cytoplasmic reorganisation that accompanies the cell divisions (Hawes et al. 1981; Ryan 1984). During this process, organelles are first confined to a region investing the spindle, and later displaced from its poles by movement of the

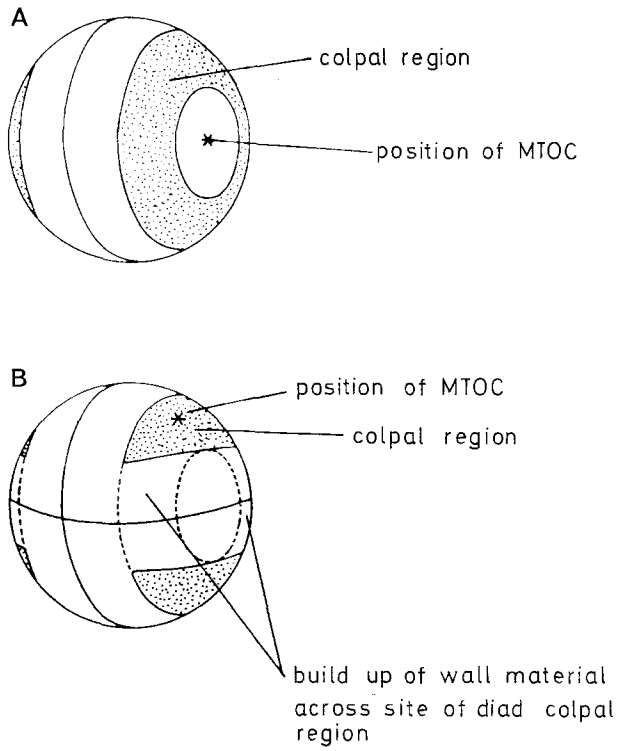

Fig. 32A, B. A possible mechanism for colpal formation in *Lilium* pollen grains. **A** Colpus position in relation to MTOC after only one meiotic cell division. **B** Colpus position in relation to MTOC and diad colpus after the two normal meiotic divisions

chromosomes and the formation of the new nuclei close to the plasma membrane. The breakdown of the spindle and the subsequent formation of the phragmoplast induces movement of the organelles back into the body of the cytoplasm, thus generating a narrow layer of inclusions locked between the phragmoplast and the nucleus. This layer of organelles, which extends out to the plasma membrane, survives through to the second meiotic division, perhaps being fixed in this position by the MTOC of the second meiotic spindle. It is at this stage that 'stabilisation' of the plasma membrane would most probably occur.

Formation of the second dividing wall then results in phragmoplast material being deposited across the diameter of the predetermined "colpal band", thus causing in its bisection and, subsequently, the form of the colpus with which we are familiar (Fig. 32).

The expression of pattern. In previous work (Sheldon and Dickinson 1983) evidence was presented indicating that pattern is established as a result of the inclusion of a substance or substances into

the plasma membrane. This material is proposed to be conveyed to the surface via coated vesicles, since the patterning may be affected by preventing their movement by centrifugation. Further, it appears that any pattern-inducing material is transferred to the cell surface either over some considerable period or in two phases, for treatment at different times may selectively affect the generation of patterning on first the 'old' meiocyte walls, and then the 'new' cross-walls formed between the meiotic products.

There are two possibilities by which a cytoplasmically synthesised pattern determinant might act to generate pattern within the plasma membrane; either a cytoplasmic structure may act as a stencil and control the insertion of material into the membrane, or this material could be included into the plasma membrane at random and reorganise into the reticulate pattern in situ. As microtubules are often associated with the directional movement of vesicles (Palevitz 1982; Gunning 1982), the microtubular cytoskeleton is an obvious contender for involvement in the former alternative.

Examination of the cytoskeleton using immunofluorescent techniques has not, however, revealed a microtubular configuration which can clearly be held responsible for the determination of the reticulate pre-pattern. The cytoskeleton at the monad and diad meiotic stages, when the first phase of deposition of the pattern determinant within the plasma membrane has been proposed to occur, shows no obvious relationship with the plasma membrane.

Although at the later tetrad stage of microsporogenesis the cytoskeleton is radially organised and forms associations with the plasma membrane as well as with the nuclear envelope, a configuration previously seen in plants by De May et al. (1982) in the endosperm of *Haemanthus,* it cannot be involved in the deposition of the pattern determinant for, by this time, the majority of the patterning is already established within the plasma membrane. Further, the microtubules are found not only to radiate beneath the colpal shield, where they would not be required for pre-pattern deposition, but also they do not increase in frequency at the colpal periphery, where the "patterning units" become much reduced in size.

When considering the three-dimensional expression of the hexagonal arrays of the final wall pattern, however, the radial cytoskeleton of the tetrads is of interest. While this apparent radiation of microtubules from the nucleus may certainly be evidence of re-establishment of the cytoskeleton following the meiotic divisions (Dickinson and Sheldon 1984; Dr. L. Clayton, John Innes Institute, Norwich, UK, personal communication), such an organisation must also be of importance in the transport of the precursors of the several cellulosic fibrillar layers of the wall. This inference is supported by the observations that treatment with colchicine at these stages results in grains with squat walls and also that, in normal cells, numerous vesicles are seen associated with the radially orientated microtubules.

The colchicine experiments also strongly indicate that the cytoskeleton is not responsible for the determination of the hexagonal pre-pattern, in that application of the drug at all stages of meiosis does not affect the generation of the pattern, although pairing, division and colpal formation are all disrupted. As far as it has been possible to determine from electron-microscopic studies, no other cytoplasmic structures are evidently responsible for the imprinting of pattern onto the plasma membrane.

These data thus reinforce the view (Sheldon and Dickinson 1983), that the reticulate pattern is established not by imposition from some cytoplasmic structure, but by biophysical reorganisation of material inserted into the bimolecular leaflet over the course of meiosis.

The authors' thanks are due to Dr. J.V. Kilmartin for the provision of antibody and Dr. D. Goodwin for the provision of 'Citifluor'. J.S. has a CASE studentship funded by the Science and Engineering Research Council and sponsored by the Royal Botanic Gardens, Kew.

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Received 22 June; accepted 6 December 1985