

ULTRASTRUCTURE OF MITOSIS IN THE COWPEA RUST FUNGUS *UROMYCES PHASEOLI* VAR. *VIGNAE*

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ABSTRACT

Aspects of the ultrastructure of mitotic nuclei of the fungus *Uromyces phaseoli* var. *vignae* are described from both intercellular hyphae in the cowpea host and infection structures induced to differentiate in vitro. The interphase nucleus-associated organelle (NAO) consists of two trilamellar acircular disks connected by an osmiophilic bar. The intranuclear spindle develops between these disks when they separate. The spindle contains pole to pole, interdigitating, chromosomal, and fragmentary microtubules arranged to form a central bundle along the surface of which lie the metaphase chromosomes. No metaphase plate is found. There are up to three microtubules per kinetochore and approximately 14 chromosomes on the haploid spindle. Telophase elongation appears to involve extension of pole to pole microtubules with no evidence for the remaining presence of interdigitating microtubules. Concomitantly, numerous cytoplasmic microtubules develop from each NAO disk where few or none are present in other phases. Reformation of the interphase NAO involves the formation of a sausage-shaped intermediate at late telophase. The nuclear envelope remains intact and the nucleolus persists throughout division. Various aspects of the spindle and NAOs appear to be evolutionary intermediates between Ascomycetes and higher Basidiomycetes, thus supporting the theory of Basidiomycete evolution from the former group and demonstrating an encouraging correlation between mitotic characteristics and other phylogenetic markers.

Increasingly sophisticated analyses of the mitotic apparatus of numerous evolutionarily primitive organisms have begun to explain more clearly how these organisms achieve equitable chromosome segregation. Several workers have suggested that the observed variations may indicate steps in the evolution of the mitotic system (35, 20). This concept, plus the fact that many of the systems in these organisms are physically small and thus more amenable to detailed ultrastructural analysis, suggests that continued investigations may help to explain the fundamental mechanisms of all

mitotic systems, an approach discussed more fully elsewhere (16). An additional benefit from such work is that in organisms with a very limited fossil history and simple morphology, for example, the fungi, details of the mitotic system may be useful as phylogenetic markers, thus helping to clarify the interrelationships of the various groups. The value of such characters for the aquatic fungi has been discussed by Heath (17), and McCully and Robinow (24) have noted that among the yeasts there is a correlation between details of the mitotic apparatus and other taxonomic characters.

From recent reviews of fungal mitosis (13, 20, 10) it is apparent that the rust fungi have been neglected, yet they represent a group of organisms whose phylogenetic relationships are obscure and somewhat controversial (e.g. 37, 4) and which are undoubtedly somewhat evolutionarily primitive and thus might be predicted to have a "primitive" mitotic apparatus. This paper fills part of the information gap by describing mitosis in a typical rust fungus. The results have been presented in preliminary form at the American Institute of Biological Sciences Meetings in Corvallis, August, 1975, and the Canadian Federation Meetings in Winnipeg, June 1975 (17).

MATERIALS AND METHODS

Leaves of cowpea (*Vigna sinensis* [Torner] Savi) infected with cowpea rust (*Uromyces phaseoli* [Pers.] Wint. var. *vignae* [Barch.] Arth.) were grown and prepared for electron microscopy as described earlier (18) but without sucrose in the glutaraldehyde. In this tissue mitosis occurs in the intercellular hyphae, and micrographs are of mitotic nuclei encountered by chance when searching for other information. Germ tube differentiation on collodion membranes was used to select material containing mitotic nuclei in more predictable division phases, by a system based on that reported by Maheshwari et al. (23). Collodion solution (Fisher Scientific Co., Pittsburgh, Pa.) was mixed with ether and ethyl alcohol (1:3:1 vol/vol) and to each 20 ml of this solution were added seven drops of light mineral oil (Fisher Scientific Co.). The mixture was poured into an acid-washed glass dish to leave a thin film covering its bottom. After drying, the film was floated off with distilled water which was replaced after 10 min with fresh water. The second water wash was left for 5 h, to absorb spore germination inhibitors present in the new membrane, then discarded. Uredospores were then distributed evenly onto the membrane in an air stream, the membrane surface was moistened with a fine spray of double-distilled water, and the spores were allowed to germinate in the dark at 22°C for approximately 8 h, depending on the developmental stage required. The stages in development and the correlated nuclear division times are shown in Figs. 1-5.

The differentiating germ tubes were fixed by flooding the membrane with 2.5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.0) for 5 min. Squares of membrane were then transferred to fresh glutaraldehyde for a further 2 h. After four rinses in the above buffer (10 min each) and 2 h in similarly buffered 1% osmium tetroxide, the membrane fragments were supported on a Millipore filter (Millipore Corp., Bedford, Mass.), coated with space water agar, dehydrated in ethanol, and flat embedded in Epon 812 resin. Germings showing the desired stage of differentiation were selected with the aid

of Nomarski interference contrast microscopy and sectioned, one per block face, in a controlled orientation. All material was examined from serial gray-silver sections mounted on single-hole grids. Sections were stained with uranyl acetate and lead citrate and micrographs were recorded on 35-mm film.

The three-dimensional reconstruction shown in Fig. 29 was made in isometric projection by a computer-driven "Calcomp 925" plotter using dimensions derived from section numbers and an arbitrary two-dimensional grid placed in constant location over each micrograph. The limits of each mass of chromatin were determined subjectively relative to these coordinates.

RESULTS

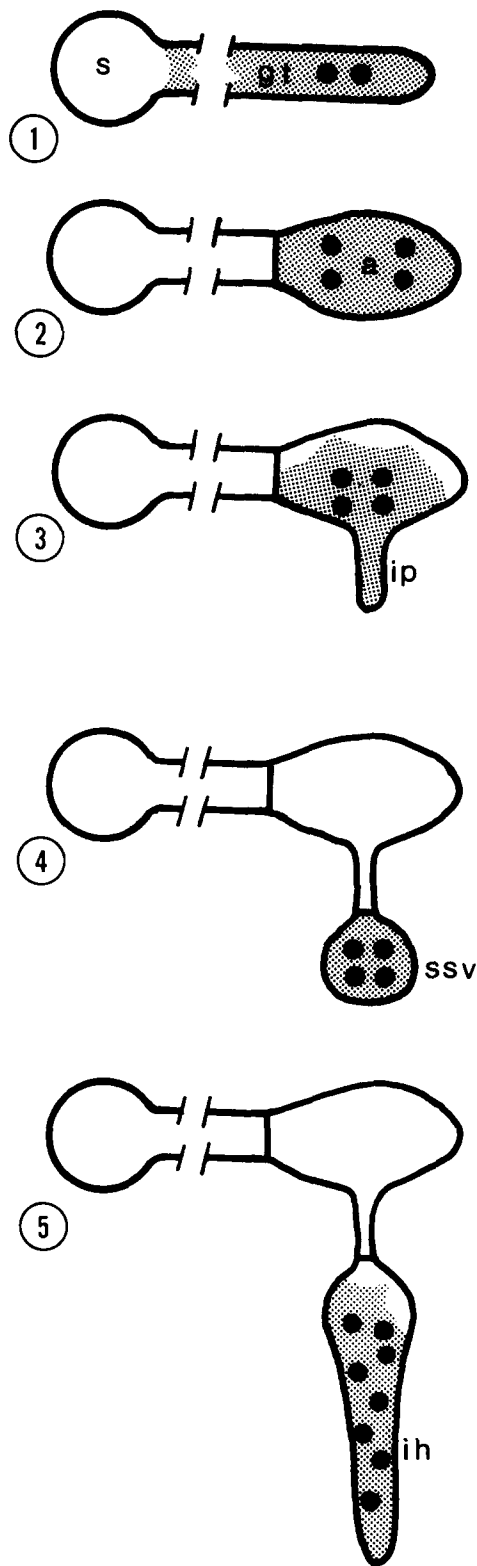
Interphase nuclei are each accompanied by a nucleus-associated organelle (NAO),¹ details of which are shown in Figs. 6-19. The organelle consists of two somewhat acircular three-layered disks connected by an osmophilic bar. The whole structure is closely appressed to the outer membrane of the nuclear envelope which forms a variously deep depression in the region of apposition. Cytoplasmic microtubules do not radiate from the interphase NAOs, even in those nuclei apparently undergoing nuclear migration,² but there are frequently cisternae of endoplasmic reticulum and small vesicle-filled vesicles or multivesicular bodies (*mvb*) lying close to the NAO.

Inside the nucleus, exclusively adjacent to the NAOs, there is always a hemisphere of granular material distinctly differing in appearance from the other components of the nucleoplasm (Figs. 14-19). The above NAO configuration was found on all interphase nuclei examined in detail except the recently post telophase ones referred to below.

Figs. 7, 20, and 21 show what is interpreted as an early stage in mitosis. This interpretation is made because the NAO is on one of the two nuclei present in a developing appressorium (which were thus premitotic but soon destined to undergo mitosis) and because the diameters of the disks are approximately double that of all other interphase

¹ We use the term NAO introduced by Girbardt and Hädrich (12) because "centrosome" has become closely identified with the diglobular structure of the higher Basidiomycetes, and because spindle pole body (SPB, 1) has always seemed inappropriate for a structure which spends most of its time unassociated with a spindle. NAO is functionally neutral and locationally more correct than SPB.

² Heath, I. B., and M. C. Heath. Manuscript in preparation.

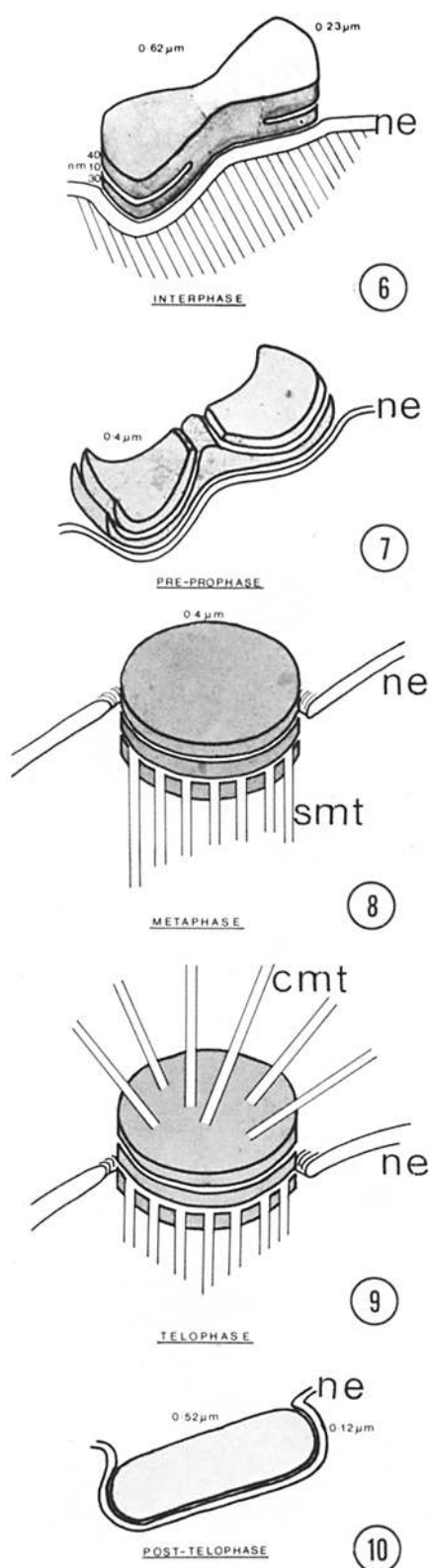


disks and close to the metaphase disk diameter. The disks are partially separated but no intranuclear microtubules are present. Unfortunately, we have no stages between this and the metaphase spindles described below.

Details of the metaphase spindle are known from analysis of three serially sectioned spindles with pole to pole lengths of approximately 2.5, 2.9, and 3.0 μm , and numerous partial series of other spindles of uncertain length. These spindles were considered to be at metaphase either because they had chromosomes with microtubule connections to both poles at various points along their length or because they were similar in length to spindles showing the latter feature.

The poles of the metaphase spindle are occupied by disks resembling the enlarged NAO disks shown in Fig. 21 (Figs. 22, 28). Presumably, continued migration from a stage similar to that shown in Fig. 20 produces the metaphase locations. The metaphase disk lies *within* the nuclear envelope in a "pore," the margins of which closely abut the disk so that there is no apparent cytoplasm-nucleoplasm continuity (Figs. 8, 22). This arrangement should be contrasted with the clear continuity of the nuclear envelope under the NAO during interphase (Figs. 14–19). All of the spindle microtubules which terminate at each pole do so in a distinct additional layer of osmiophilic material internal to the inner layer of the disk (Fig. 22). During metaphase, there may be a few (ca. <5) cytoplasmic microtubules radiating in diverse directions from the vicinity of the disks, but there is

FIGURES 1–5 Diagrammatic representation of the stages of germ tube differentiation in vitro. The spore (s) produces a variously long germ tube (gt) into which the cytoplasm and both nuclei (black circles) (present in the mature and dormant uredospore) move (Fig. 1). The tip of the germ tube enlarges into an appressorium into which the cytoplasm flows. The appressorium is then cut off from the germ tube by a cross wall and, during appressorium formation, the first round of mitosis occurs (Fig. 2). The appressorium produces an infection peg (ip) (Fig. 3) through which the cytoplasm flows into the subsequently developed substomatal vesicle (ssv) (Fig. 4). The second round of mitosis may occur in the developing substomatal vesicle but usually occurs after this structure is cut off from the infection peg (by another cross wall) in the infection hypha (ih) which develops next (Fig. 5). The infection hypha thus contains eight nuclei. The tip of the infection hypha will develop into the haustorium mother cell which will receive two nuclei. The other nuclei will pass into secondary hyphae formed later as branches from the infection hypha (not shown).



invariably a group of *mvbs* clustered about each pole (Figs. 22 and 28). These *mvbs* appear to be more numerous relative to interphase, but detailed quantitative analysis was not performed.

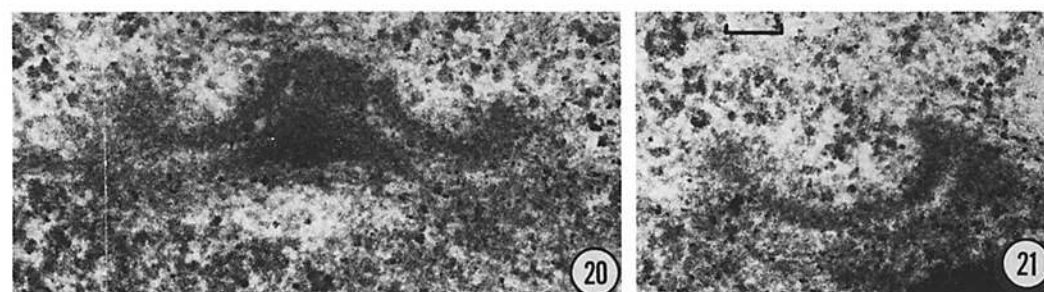
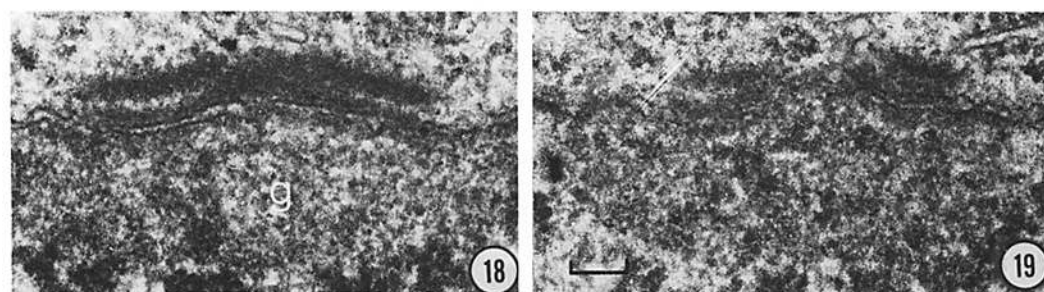
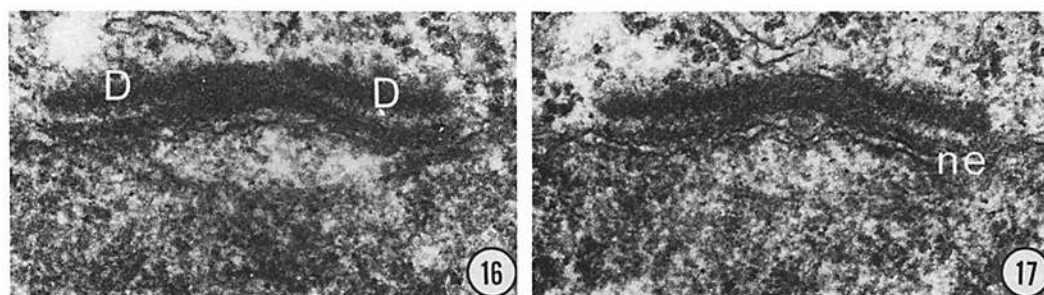
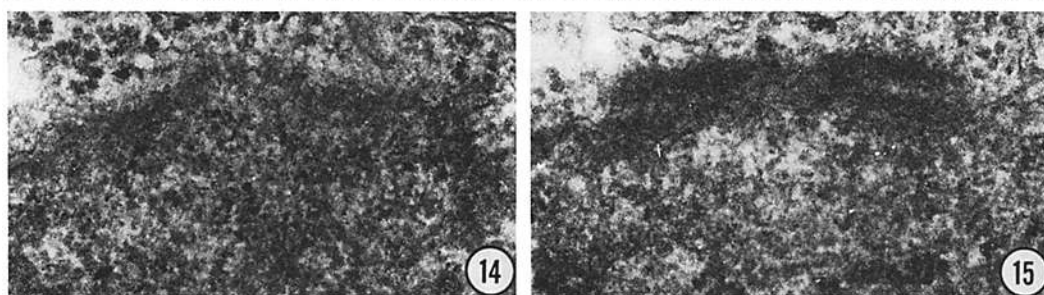
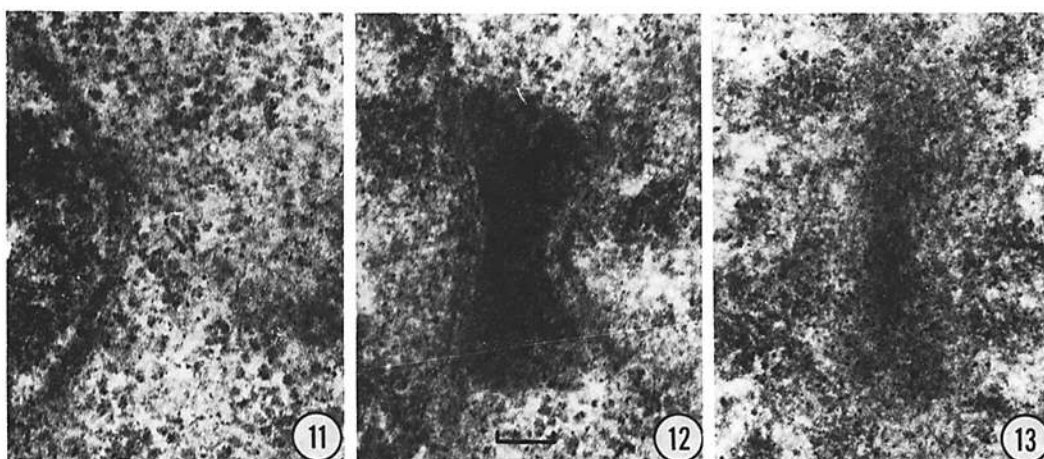
The structure of the spindle itself is illustrated in Figs. 22–26. The essential features of this spindle, as far as can be deduced at present, are as follows.

(a) The chromosomes are not aligned in any form of metaphase plate. They can be found along almost the entire length of the spindle with microtubule connections to both poles irrespective of their position (Figs. 22 and 27).

(b) It has not been possible to obtain unequivocal information on all chromosomes, but there are clear cases showing groups of three microtubules terminating on one side of a chromosome (Fig. 24), thus showing that there can be up to three microtubules per kinetochore. The possibility of numbers per kinetochore above three can be fairly certainly eliminated, but because the kinetochore terminations are not clearly differentiated, lower figures are possible. This number of microtubules per kinetochore compares with the two or more reported in another Basidiomycete genus *Coprinus* (22, 39), but is high relative to the one found in most fungi (see review of Heath, reference 13).

(c) There are 14 individual clusters of chromatin on a metaphase spindle (Fig. 27). Because many of these clusters had connections to both poles of the spindle it seems that anaphase separation had not begun, thus suggesting that 14 is the haploid number in this dikaryotic organism. This figure is higher than that based on light microscopy for other rusts where a remarkably constant $n = 6$ for various species has been reported (e.g., reference 40). Apart from the possible differences between the species, the present figure could be high due to asynchronous onset of anaphase. Thus, some chromatids may be counted as chromosomes. With the present quality of fixation and the closely packed microtubules, it is hard to rule out this possibility, but it certainly does not apply

FIGURES 6–10 Diagrammatic representation of the changes in morphology of the NAO through the mitotic cycle (phases as marked). Approximate dimensions are shown, and the nuclear envelope (*ne*), cytoplasmic microtubules (*cmt*), and spindle microtubules (*smt*) are marked. The cross hatching in Fig. 6 indicates the differentiated nucleoplasm characteristically associated with the interphase NAOs. It is also present in the stages depicted in Figs. 7 and 10.



on a scale sufficient to bring the number down to six. Because the chromosomes are small, the light microscope-based figures could be low due to problems of resolution of imperfectly squashed material. Since no genetic analysis is available, a more accurate figure must await synaptonemal complex analysis of the type performed by Moens and Perkins (30).

(d) Apart from kinetochore or chromosomal microtubules, the spindle also contains both pole to pole and interdigitating (14) microtubules. The partial series of transverse sections summarized diagrammatically in Fig. 25 shows that 64 microtubules run from pole to pole, but this is an overestimate since undoubtedly some of them would have terminated in the missing sections. The longitudinal series shown in part in Fig. 22 was complete, but accurate microtubule counts were not possible because of the problem of superimposition of closely packed microtubules in relatively thick sections. The current best estimate of pole to pole numbers is approximately 50.

Given the poorly differentiated nature of the kinetochore microtubule terminations, it could be argued that the claimed interdigitating microtubules are kinetochore microtubules. Two points refute this argument. (a) In Fig. 26 it can be seen that some of the non-pole to pole microtubules are mixed among the pole to pole ones. Because the chromosomes and thus kinetochores lie on the periphery of this central group of tubules, these internally located microtubules cannot be kinetochore linked. They are thus termed interdigitating. (b) In Fig. 25, there are clearly more non-pole to pole microtubules on the most complete half-spindle (left) than are needed to account for all the likely kinetochore-linked microtubules (i.e. $14 \times 3 = 42$). As seen in Fig. 26, there is no ordered

arrangement detected among the interdigitating microtubules from opposite poles.

(e) The spindle contains a number of "fragmentary" or "free" (26) microtubules which run for various lengths but are connected to neither pole (Fig. 25).

In all metaphase nuclei the spindle occupies a small portion of the entire nucleus. The rest of the nucleus contains the nucleolus and is permeated by osmiophilic material which shows no obvious connection with either the chromatin or the nucleolus (Fig. 23). The nature of this material is unknown. It could be chromatin which is less condensed than that connected to the spindle, thus suggesting poorly differentiated and hard to count chromosomes, or it could be some other unidentified material, in which case interpretation of interphase nuclei becomes difficult; apparently, not all osmiophilic material can be equated with chromosomes or nucleoli as is often thought. Because we have not observed any anaphase stages, we conclude that this phase of division occurs very rapidly.

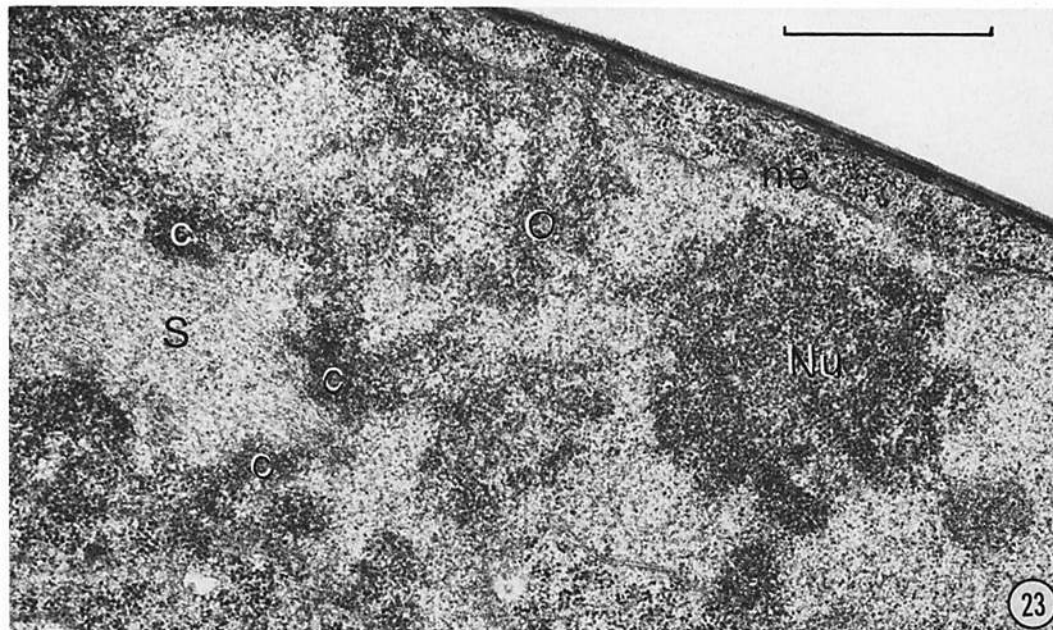
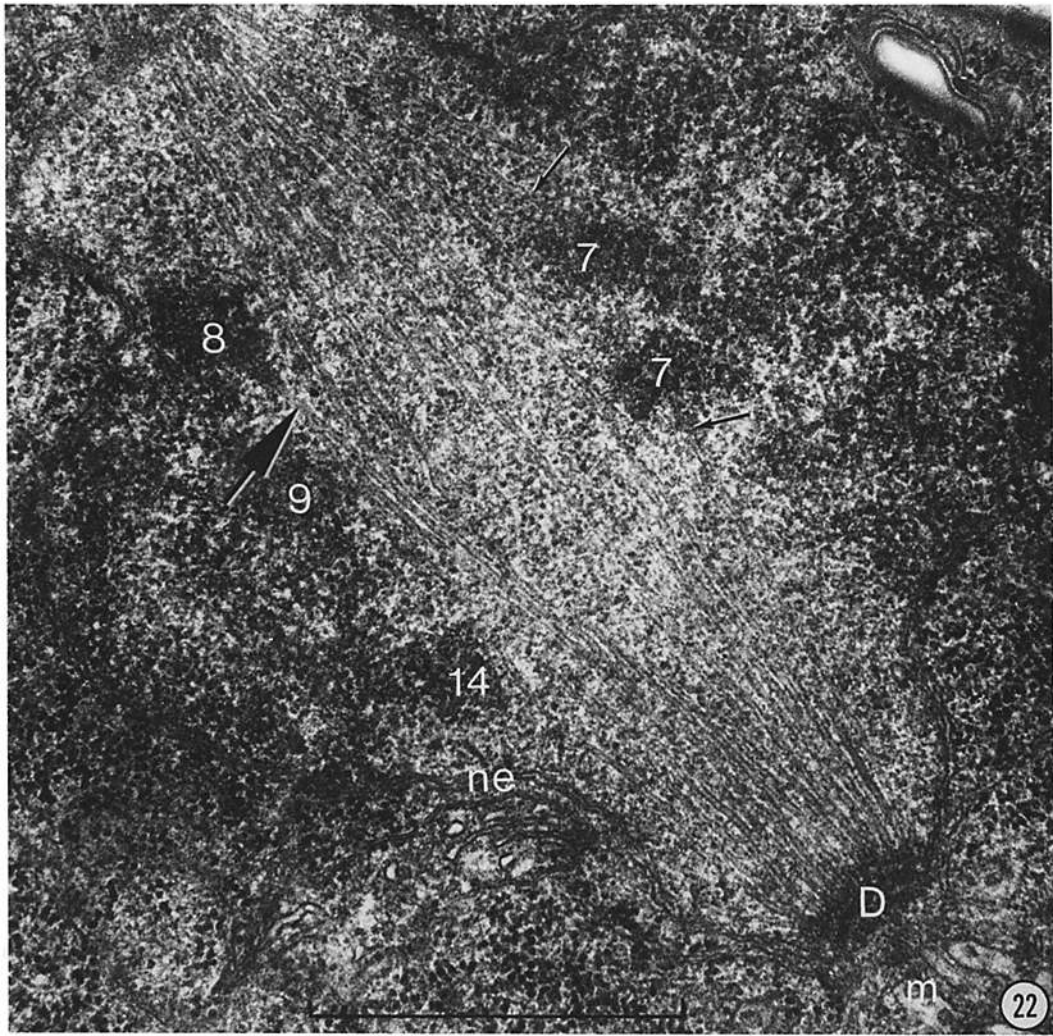
Telophase is described from incomplete series of sections of three nuclei with pole to pole distances of approximately 7.3, 11.2, and 12.3 μm . The polar NAO disks remain essentially unchanged from their metaphase configuration, but the number of cytoplasmic microtubules radiating from them increases dramatically to approximately 60 (Figs. 29 and 30). These microtubules run predominantly away from the nucleus, but a few also pass back along the nuclear envelope. The polar disks define the approximate ends of the elongated nuclei. There are no hornlike extensions of the nucleus as shown in some other fungi (14).

The telophase spindle appears to consist solely

FIGURES 11-13 Serial sections cut through an interphase NAO parallel to its face. The general surface morphology is clearly seen in Fig. 12, while the other figures indicate its thinness. $\times 76,000$. Scale = 0.1 μm .

FIGURES 14-19 Serial sections cut through an interphase NAO parallel to its long axis and at right angles to its surface. The dark-light-dark, three-layered nature of the two disks (D) is evident as is the nonlayered connecting component. Note the close apposition of the NAO to the nuclear envelope (ne) and the hemisphere of granular material (g) adjacent to the NAO inside the nucleus. $\times 75,500$. Scale = 0.1 μm .

FIGURES 20-21 Sections 3 (Fig. 20) and 5 (Fig. 21) of a series through a preprophase NAO showing the apparent separation (Fig. 20) and increased diameter of the disks (Fig. 21) relative to the interphase condition (e.g., Fig. 16). $\times 77,300$. Scale = 0.1 μm .



of a closely packed bundle of approximately 40 pole to pole microtubules (Fig. 31). Clearly, the unambiguous demonstration of pole to pole continuity in such long spindles is hard, but there is no equatorial zone of increased microtubule number as would be expected if extensive interdigitation of microtubules occurred and as has been shown in *Dictyostelium* (29). The chromatin is no longer differentiated from the general osmiophilic material permeating the nucleus at this and earlier stages (Figs. 31–33). The nuclear envelope remains intact but the nucleoplasm is drawn predominantly into two masses at either end of the spindle. The nucleolus persists at telophase and lies close to the equatorial region of the spindle where it is located in a projecting lobe of nucleoplasm (Fig. 33). We cannot prove the point, but we have strong circumstantial evidence to suggest that at late telophase the nucleolus becomes expelled from the nucleus and lies free in the cytoplasm (Fig. 32).

In one developing infection hypha (Fig. 34) which contained eight nuclei, and thus had recently completed the final round of mitosis before haustorial mother cell formation, each thus-defined post telophase nucleus examined contained no spindle remains but possessed an unusual NAO. The NAOs lay in an unusually deep invagination of the nuclear envelope and had the form of an elongated sausage as shown in Fig. 35. Because of the above-mentioned stage of differentiation, we interpret these NAOs to be undergoing reversion from the mitotic disk to the interphase double-disk state. This sausage-shaped, reversion-associated structure is very similar to that shown in *Polystictus* by Girbardt and Hädrich (12).

DISCUSSION

The mitotic system described here does not help differentiate between the major theories postu-

lated to explain mitosis. It is essentially compatible with all of them. The intertubular distance is close enough to permit cross-bridge mechanisms (27, 33) and, whilst the arrangement of interdigitating microtubules is not so ordered as that elegantly shown in *Diatoma* (25), this is no serious impediment to the intertubule cross-bridge hypothesis for mitotic force generation. Superficially, telophase elongation would appear to be most likely to occur by microtubule polymerization (19), but as argued elsewhere (16), this could also be generated by intertubule sliding with concomitant synchronous polymerization even in the absence of morphologically interdigitating microtubules. The lack of success in finding actin filaments is hardly compelling evidence against Forer's (8) hypothesis, and the close spacing of the microtubules makes unambiguous rejection of the "zipper hypothesis" (3) impossible. Likewise, the present observations can easily be accommodated in the model proposed by Heath (16). However, there are two significant contributions which should be emphasized. First, the fragmentary or free (26) microtubules of the spindle are demonstrated in a spindle phylogenetically far removed from previous reports (i.e. oomycete—reference 14; mammal—reference 26; insect—reference 9) suggesting that they may be of fundamental importance in spindle mechanics. The only current model which specifically predicts the existence of such fragments is that of Dietz (6). Second, we again have a spindle lacking a metaphase plate, a common occurrence in the fungi (13). If one assumes that during the metaphase stages described here the chromosomes are not moving, as seems likely because they are the most abundant and thus presumably the longest lasting stage, then theories relying on equally opposed forces to explain metaphase are inadequate. The hypothesized use of individually controlled microtubule depolymerization to control or pre-

FIGURE 22 One of the series of sections of a metaphase spindle from which Fig. 27 was prepared. Portions of chromosomes 7 (in fact one as judged from serial sections), 8, 9, and 14 (numbers refer to Fig. 27) are shown. Note the microtubular connections of chromosome 7 to both poles (arrows) and one of the connections of chromosome 8 to the farther pole (large arrow). The other connection of chromosome 8 was in an adjacent section. Note the three osmiophilic layers of the polar disk (*D*) and compare with Fig. 8. The inner layer is less well defined than the outer two. The nuclear envelope (*ne*) is intact, and while *mvbs* (*m*) are present at the poles cytoplasmic microtubules are not. $\times 49,700$. Scale = $1.0 \mu\text{m}$.

FIGURE 23 Metaphase nucleus showing small, microtubule-containing spindle (*S*) with chromosomes (*c*) and the nucleolus (*Nu*) inside the persistent nuclear envelope (*ne*). Note the osmiophilic material (*O*) which differs in appearance from both the nucleolus and the chromosomes. $\times 27,600$. Scale = $1.0 \mu\text{m}$.

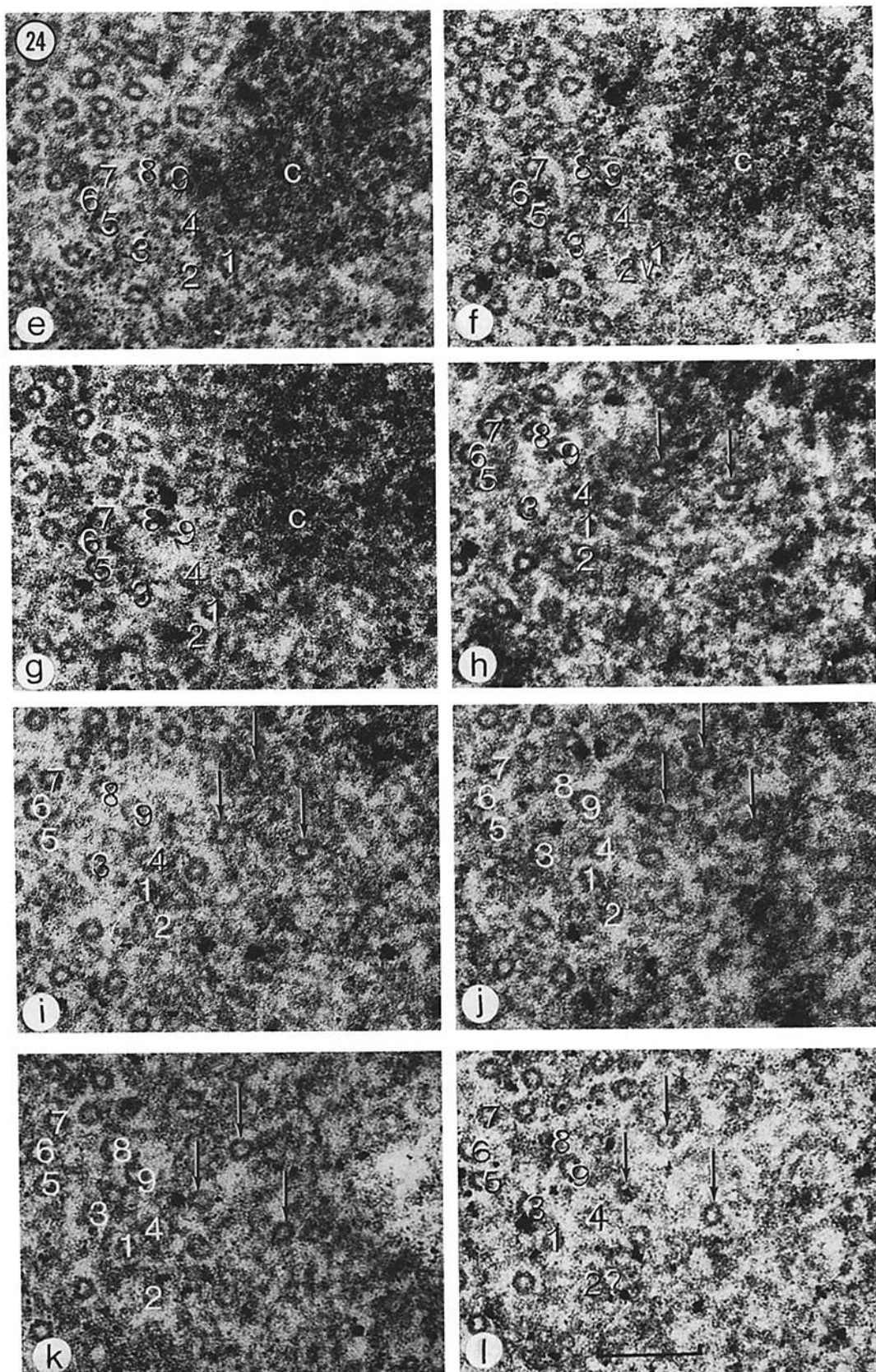


FIGURE 24 Portions of sections *e-l* from the series used to construct Fig. 25. The three microtubules arrowed terminate in a chromosome (*c*). Marker microtubules are numbered for easy reference. There is a small shift of field between *g* and *h* in order to show a larger area of the chromosome. $\times 166,800$. Scale = $0.1 \mu\text{m}$.

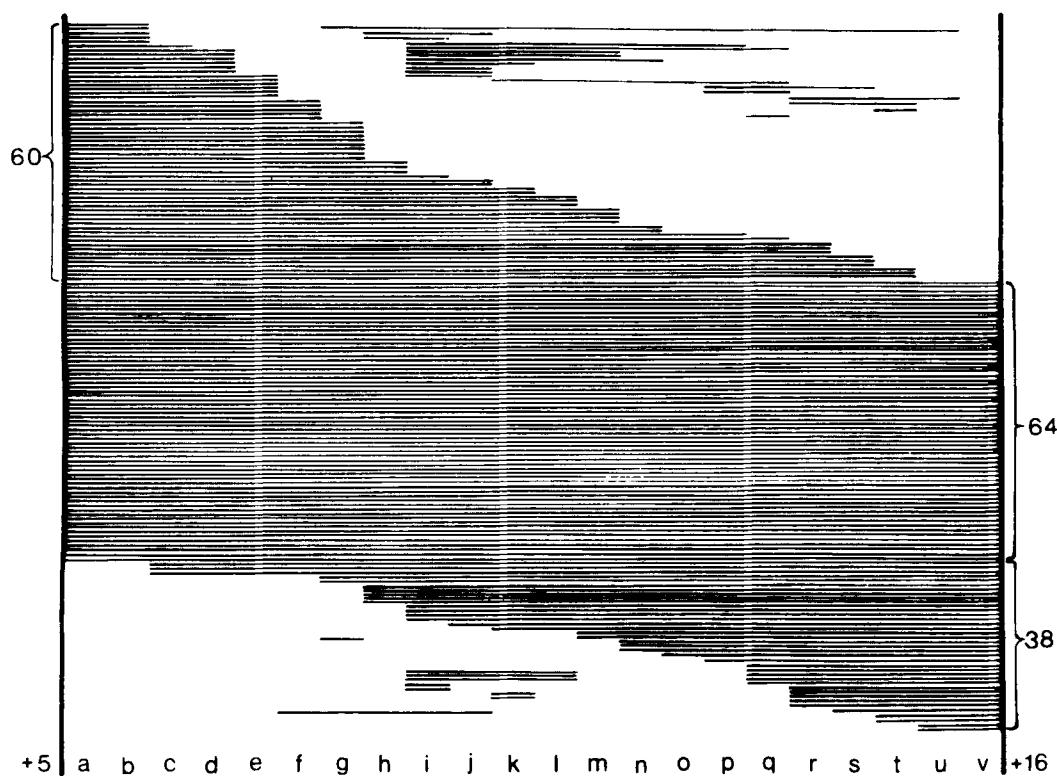


FIGURE 25 Diagram of the distribution of microtubules through a series of 22 transverse sections of a metaphase spindle. The series did not go uninterrupted from pole to pole; the figures +5 and +16 indicate the approximate number of sections not analyzed between this series and the poles of the spindle. Each line represents a microtubule traced through serial sections. The letters of the X axis designate each section for cross reference to Figs. 24 and 26. The vertical order has no significance.

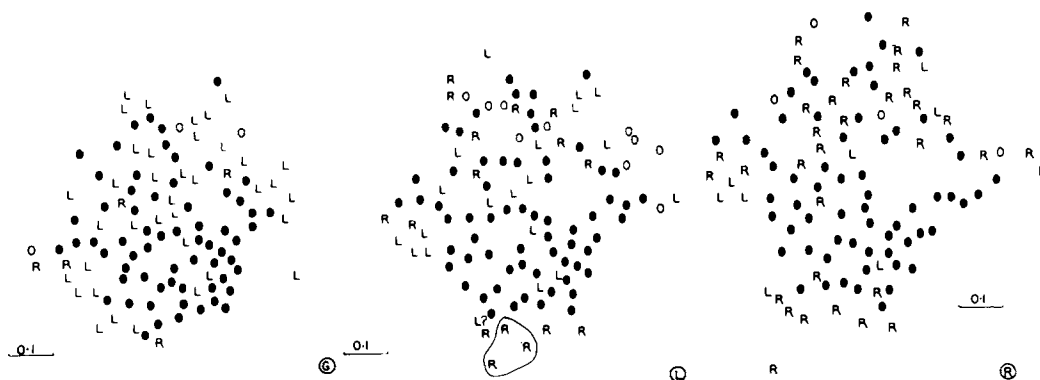


FIGURE 26 The total microtubule distribution of sections g, l, and r from the series shown in Fig. 25. Solid circles indicate microtubules which ran throughout the series. Open circles are fragmentary microtubules (i.e. ones which did not connect to either pole). "L" designates microtubules connected only to the left-hand (as in Fig. 25) side of the series, and "R" designates microtubules connected only to the right-hand side of the series. The three microtubules enclosed by the line in l are those arrowed in Fig. 24. To facilitate comparison with Fig. 24, rotate these figures approximately 90° counterclockwise.

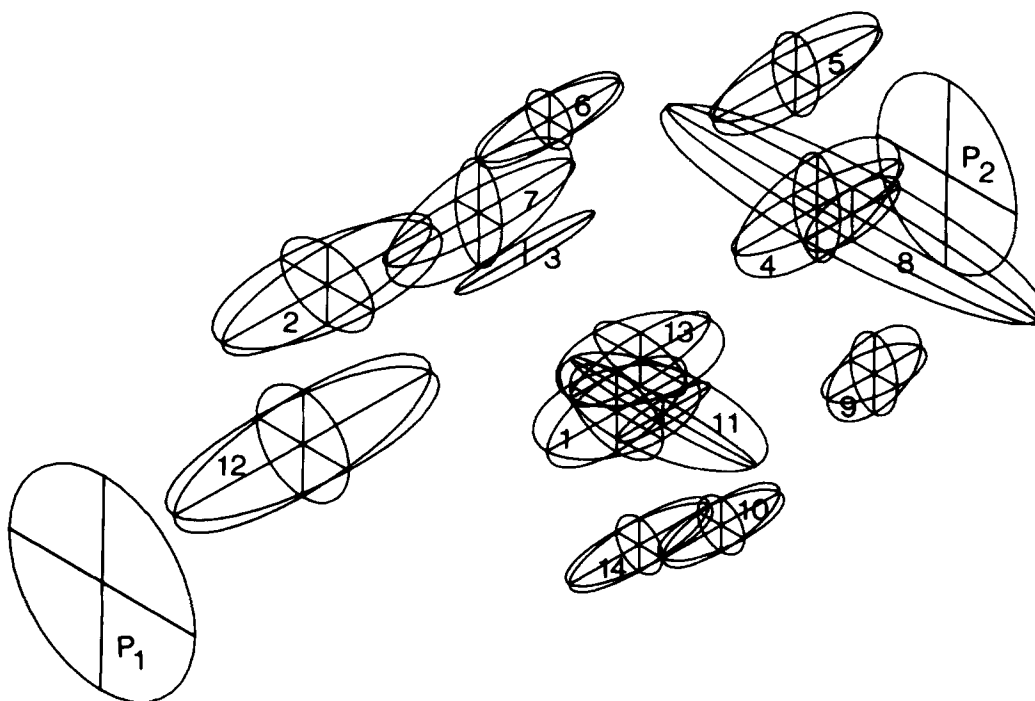


FIGURE 27 Isometric projection of the distribution of chromosomes from a serially sectioned metaphase spindle, part of which was shown in Fig. 22. The polar disks of the spindle are designated P_1 and P_2 .

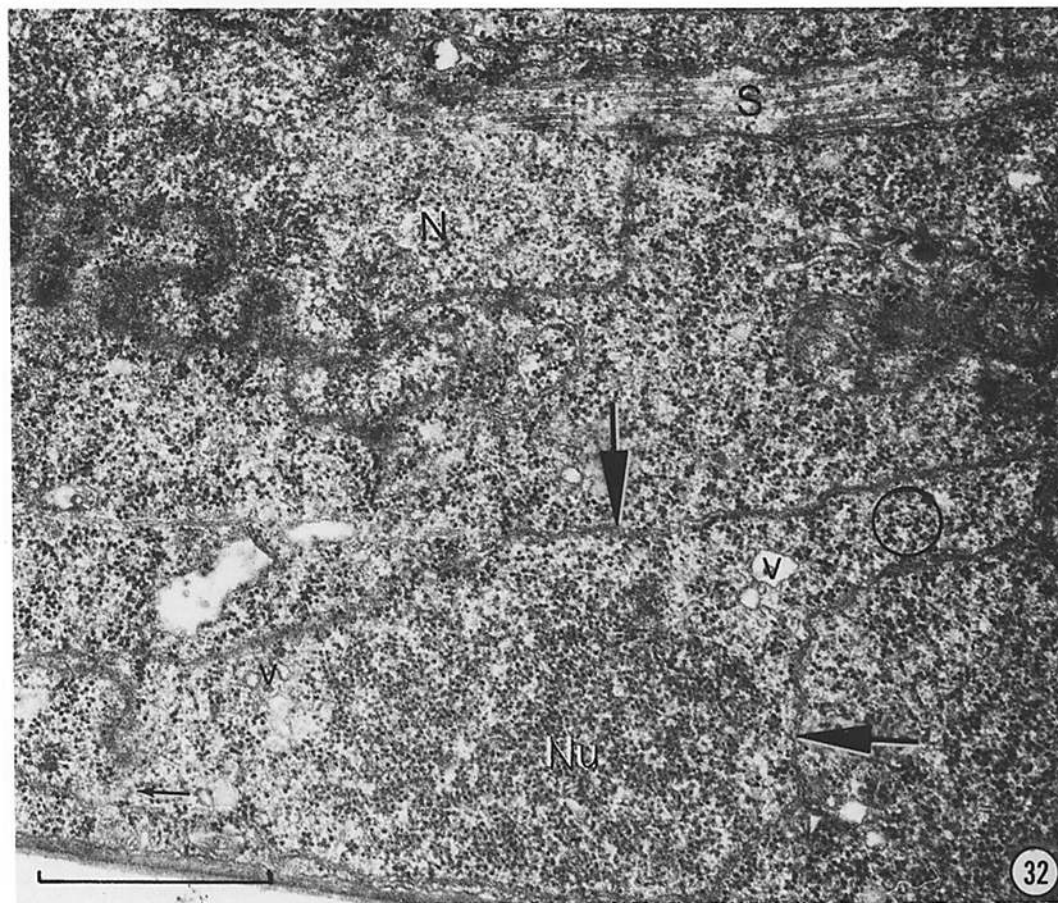
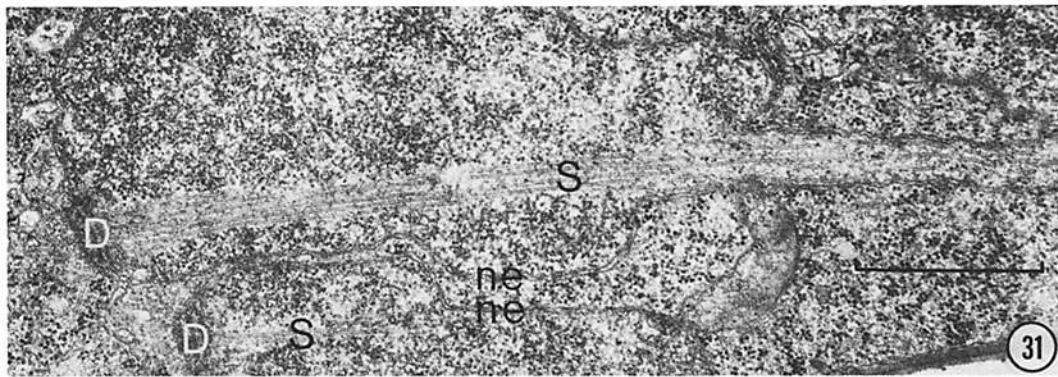
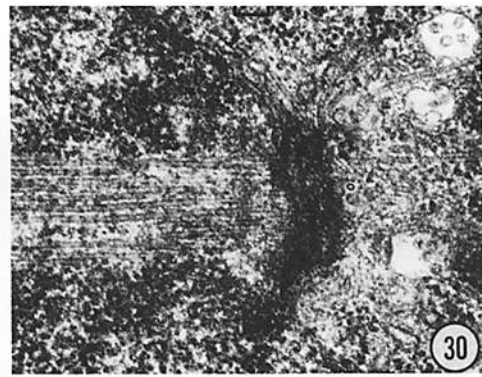
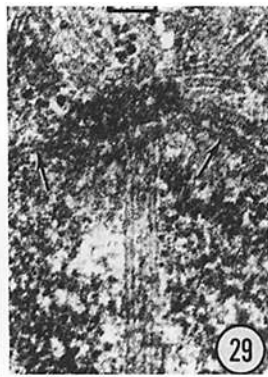
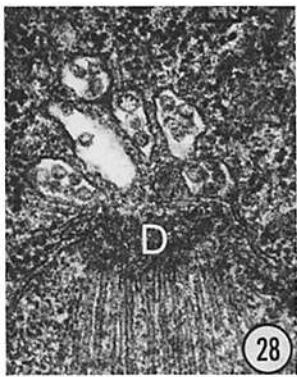
FIGURE 28 Detail of the pole of a longitudinally sectioned metaphase spindle showing numerous *mvbs*, no cytoplasmic microtubules, and the NAO disk (D). $\times 48,600$. Scale = $0.1 \mu\text{m}$.

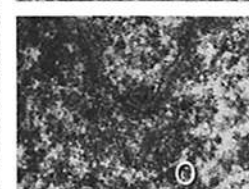
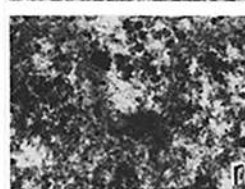
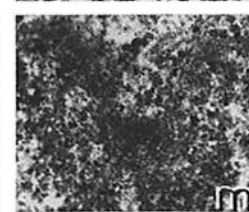
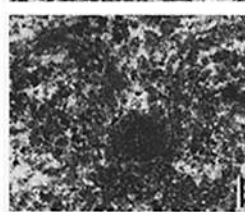
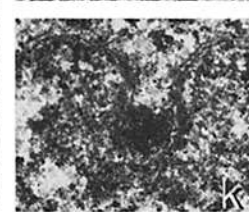
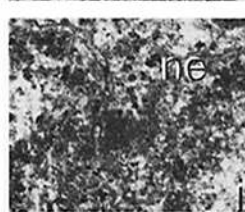
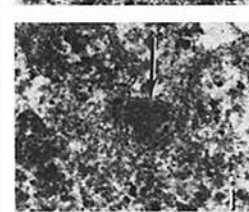
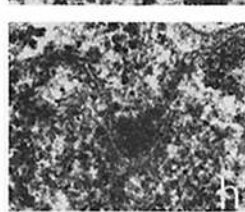
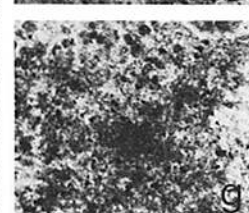
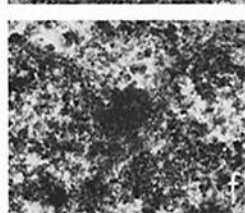
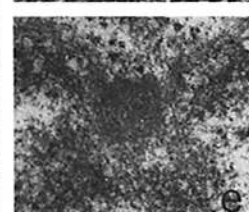
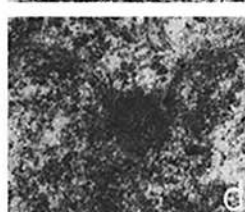
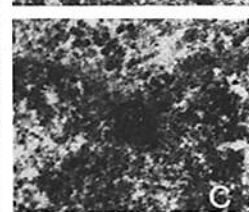
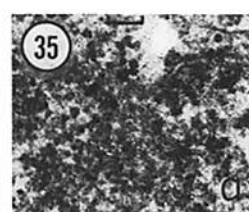
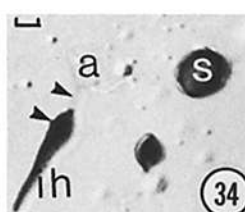
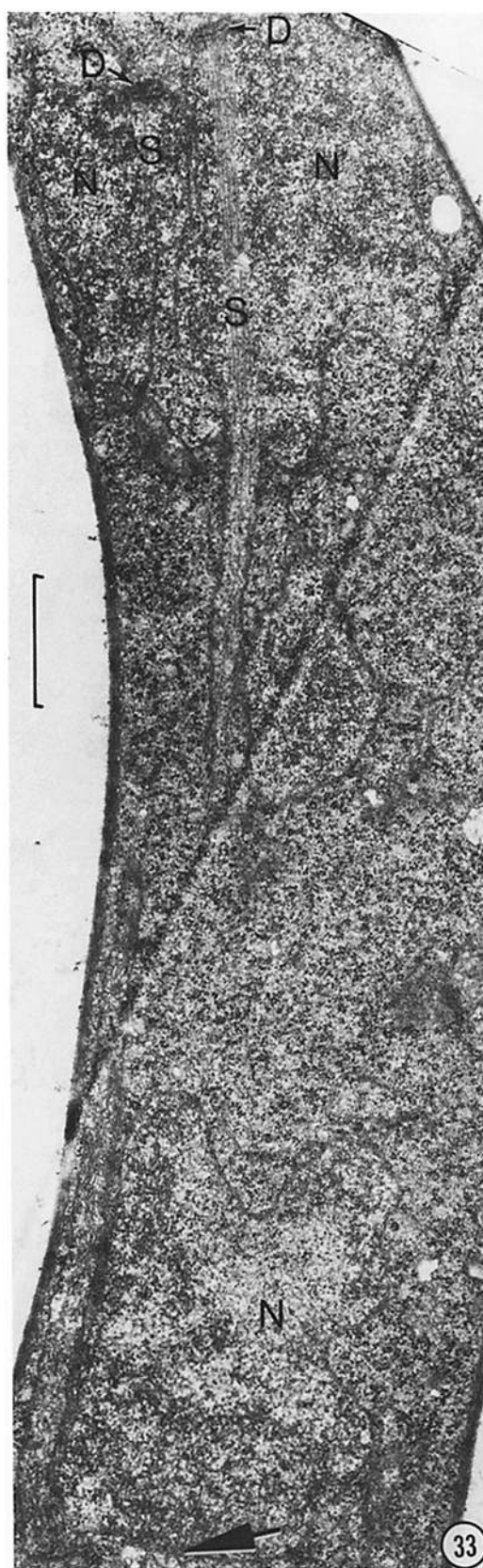
FIGURE 29 Detail of the pole of a longitudinally sectioned telophase spindle. Note the way in which the nuclear envelope (arrows) appears to be aligned more with the innermost layer of osmiophilic material rather than the intermediate layers as found at metaphase. Compare with Figs. 22 and 28. Numerous cytoplasmic microtubules are evident. $\times 61,000$. Scale = $0.1 \mu\text{m}$.

FIGURE 30 Median longitudinal section of the same telophase spindle pole as shown in Fig. 29 (two sections not illustrated between Figs. 29 and 30). The osmiophilic layers of the NAO disk are evident (compare with Fig. 9) as are the microtubules of the spindle. *Mvbs* are interspersed with the cytoplasmic microtubules which radiate both away from the back along the nucleus. $\times 43,100$. Scale = $0.1 \mu\text{m}$.

FIGURE 31 The ends of two telophase spindles, the lower one of which is the opposite end of the same spindle shown in Figs. 29 and 30. The spindle microtubules (S) and NAO disks (D) are evident. Note the intact nuclear envelopes (ne) and the generally dispersed osmiophilic material permeating the nucleoplasm. $\times 24,500$. Scale = $1.0 \mu\text{m}$.

FIGURE 32 Part of one of the series of sections of the telophase spindles from which Figs. 29-31 are taken. This region is near the equator of the spindles, part of which is shown (S). The structure labeled Nu appears to be a nucleolus (cf. Fig. 23) which is partially enveloped by endoplasmic reticulum (large arrows) or possibly nuclear envelope. However, it appears to be open to the cytoplasm (small arrow), a conclusion supported by the presence of vesicles (v) and cytoplasmic ribosomes (compare those near the nucleolus [circled] with those in the cytoplasm and the nucleoplasm [N] adjacent to the nucleolus. $\times 30,600$. Scale = $1.0 \mu\text{m}$.





vent movement (8, 34) seems to offer the best explanation of this type of metaphase configuration.

One of the more puzzling features of this mitotic system is the behavior of the cytoplasmic microtubules. Interphase nuclei migrate through the differentiating germ tube with the apparent aid of a microtubule-nuclear envelope interaction,² but there is a conspicuous absence of NAO involvement of the type predicted from the work of Girbardt (11) on the higher basidiomycete *Polystictus*. A similar absence of cytoplasmic microtubules associated with the interphase NAO is reported in another rust *Melampsora* by Coffey et al. (5), thus suggesting that the rust NAO is not involved in nuclear motility. However, the conspicuous increase in number of NAO-associated cytoplasmic microtubules at telophase indicates some function at that phase of division. The only role conceivable at present is one in aiding telophase nuclear elongation, possibly utilizing microtubule-cytoplasm interactions of the type hypothesized by Heath (15).

The second area to which this study contributes is the field of spindle evolution and the use of the spindle details as phylogenetic markers. While few workers would deny that the heterobasidiomycetous rusts are among the most primitive Basidiomycetes, the question of the ancestry of the Basidiomycetes is more controversial. Most authors now view the rusts as being derived from some ascomycetous ancestor such as a *Taphrina*-like organism (e.g., references 37, 10, 38), but others (e.g., reference 4) suggest that there is no relationship between the Ascomycetes and Basidiomycetes. The main causes of uncertainty in fungal phylogeny are a very imperfect fossil record, relatively simple gross morphology, and essentially no "embryology" to "recapitulate phylogeny," hence

the quest for additional characteristics. The mitotic apparatus reported here supports an Ascomycete-like ancestry for the rusts on three points: (a) the nuclear envelope remains intact throughout mitosis, a feature typical of Ascomycetes as compared with the Basidiomycetes in which the nuclear envelope is more labile (see reviews, references 13, 10). The value of this characteristic seems uncertain because the behavior of the nuclear envelope can obviously vary in different physiological environments as shown by its behavior in *Physarum* (2), but its behavior among the higher fungal taxa does seem to be reasonably consistent; (b) the compact central group of spindle microtubules is apparently more commonly found in the Ascomycetes (e.g., references 31, 36, 32, 1) than in the Basidiomycetes (e.g., references 21, 11), but the value of this characteristic is restricted by relatively few detailed studies and some reported variability. For example, Zickler (41) shows Ascomycete spindles apparently lacking a compact core of microtubules; (c) the appearance of the NAO disks at the poles of the rust spindle is very similar to the typical Ascomycete plaque (see references above and in reviews, references 13, 10, 20) and contrasts with the spherical globular ends of the NAO typically found at the poles of higher Basidiomycete spindles (e.g., references 28, 39 and references in above reviews). Likewise, the interphase double disk NAO morphology, apparently characteristic of the rusts, having also been reported in both *Melampsora* (5) and *Puccinia* (7), indicates a degree of lack of relationship with the homobasidiomycetes which possess the double globular NAO at interphase (e.g., above references to Basidiomycete spindles and NAOs).

Because there has proved to be generally good morphological similarity of NAOs and their equiv-

FIGURE 33 Portions of the two telophase nuclei (*N*) shown in Figs. 29–32. Parts of the spindles (*S*) and NAO disks (*D*) can be seen. The extent of the spindle can be judged by the fact that the position of the NAO disk opposite to that in the upper right was close to the arrow on the lower lobe of the nucleus. $\times 18,400$. Scale = $1.0\ \mu\text{m}$.

FIGURE 34 Nomarski interference contrast micrograph of an embedded infection hypha (*ih*) stage of differentiation. Note spore (*s*), appressorium (*a*), infection peg (upper arrow), and substomatal vesicle (lower arrow). This infection hypha is the one from which the NAO shown in Fig. 35 was taken. $\times 300$. Scale = $10\ \mu\text{m}$.

FIGURE 35 Serial transverse sections through a recently post-telophase NAO (arrowed). Note deep indentation in the nuclear envelope (*ne*). $\times 54,600$. Scale = $0.1\ \mu\text{m}$.

alents within higher fungal taxa and because it is hard to think of a strong selection pressure for any of the morphological variants of a structure of apparent functional constancy (i.e. a role in microtubule metabolism), it seems unlikely that convergent evolution would occur, thus suggesting that NAO morphology is a good phylogenetic marker.

It is not hard to imagine a double disk as an evolutionary intermediate between the *single* Ascomycete plaque and the *double* Basidiomycete NAO.

Thus, the above three points support the hypothesis that the rusts are evolutionary intermediates between Ascomycete-like fungi and the higher Basidiomycetes. It is encouraging to note that details of the mitotic apparatus can correlate with other taxonomically useful characteristics.

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