ULTRASTRUCTURE AND TIME COURSE OF MITOSIS IN THE FUNGUS *FUSARIUM OXYSPORUM*

JAMES R. AIST and P. H. WILLIAMS

From the Department of Plant Pathology, The University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT

Mitosis in *Fusarium oxysporum* Schlect. was studied by light and electron microscopy. The average times required for the stages of mitosis, as determined from measurements made on living nuclei, were as follows: prophase, 70 sec; metaphase, 120 sec; anaphase, 13 sec; and telophase, 125 sec, for a total of 5.5 min. New postfixation procedures were developed specifically to preserve the fine-structure of the mitotic apparatus. Electron microscopy of mitotic nuclei revealed a fibrillo-granular, extranuclear Spindle Pole Body (SPB) at each pole of the intranuclear, microtubular spindles. Metaphase chromosomes were attached to spindle microtubules via kinetochores, which were found near the spindle poles at telophase. The still-intact, original nuclear envelope constricted around the incipient daughter nuclei during telophase.

INTRODUCTION

Around the turn of this century, Harper clearly illustrated, in several fungi, ". . . a conspicuous, well-differentiated, disk-shaped granule . . . lying close on the surface of the nuclear membrane and generally occupying a slight depression in it" (24). This "central body", as Harper called it (24), was shown to set up the meiotic spindles in Ascomycetes and Basidiomycetes (21–24, 30). Because the central bodies were observed in all stages of the life cycles of some of the fungi studied, Harper considered these structures to be separate and independent organelles. He concluded that "The position of the central body on the nuclear membrane is characteristic of the fungi . . ." (24). Since Harper's early light microscope studies, others have reported the participation of structures similar to the central body, or Spindle Pole Body (SPB)[1], in spindle development during somatic nuclear division in several fungi (1, 19, 33, 43, 46, 56, 57, 74, 75).

Although some information is available concerning chromosome behavior during fungal, mitosis, the mechanism by which the chromosomes move from their metaphase position to the spindle poles at anaphase is still a matter of considerable controversy. The question of whether kinetochores

[1] The term "central body" fell into general disuse some years ago (32), and it is still considered inappropriate for the structures Harper described (43, 45). The other terms which have been used (43) all suggest a particular function or morphology, which limits their usage to only one or a few examples because of the absence of well-documented information concerning their function and because of the diverse morphology of these structures. In this paper we use the term "Spindle Pole Body" (SPB), which is an English translation of "Spindelpolkörperchen", used by Harper in 1895 (21). In a discussion session on terminology organized by Dr. C. F. Robinow during the First International Mycological Congress held in Exeter, England, September, 1971, a measure of agreement was reached that "Spindle Pole Body", proposed by J. R. Aist, is a more acceptable term, because, among other reasons, it is neutral with respect to morphology and function.

are present in dividing fungal nuclei is, therefore, a crucial one. Kinetochores or kinetochore-like structures have only recently been reported in fungi (9, 26, 27, 39, 61, 68). In none of these studies, however, has the existence of fungal kinetochores been conclusively demonstrated, either because the chromosomes were not visible (26, 27), or because it was not shown in *serial sections* that spindle microtubules actually terminated in the structures described (9, 39, 61, 68). Kinetochores have not previously been reported to occur during mitosis in higher true fungi.

The previous light microscope study of mitosis in *Fusarium oxysporum* Schlect. (1) revealed that three individual chromosomes were attached to the spindle at different points along its longitudinal axis at metaphase. The anaphase migration of chromosomes from this scattered metaphase arrangement usually resulted in a mitotic anaphase configuration which is typical of many fungi, and is often referred to as the "double-bar" (57) or "two-track" stage (14). Telophase involved a rapid nuclear and spindle elongation during which the original nuclear envelope apparently "pinched off" the incipient daughter nuclei just before disappearance of the spindle. The nature of the attachment of chromosomes to the spindle was not determined.

In the present paper we provide evidence for the existence of kinetochores in *F. oxysporum* and details of their structure and behavior during mitosis. The ultrastructure and behavior of chromosomes, SPB's, and spindles, and the time course of mitosis are also described.

MATERIALS AND METHODS

The *Fusarium oxysporum* Schlect. (Indiana isolate) used in this study was obtained from Dr. Charles Bracker, Purdue University, Lafayette, Indiana, and was maintained on carboxymethylcellulose (CMC) agar (42).

Light Microscopy

Living hyphae, growing on CMC agar, were viewed with phase-contrast optics (1). The time course of mitosis was determined at 24–26°C. Only hyphal tip nuclei which were located closer to the septum than to the hyphal apex and which had been under a cover slip less than 1 hr were used. Twenty such nuclei, in which every division stage was clearly seen, were observed continuously during mitosis. The time required for each stage of mitosis was recorded on a Heathkit Chart Recorder (Heath Co., Benton Harbor, Mich.) operated at a chart speed of 15 sec/in. The mean and standard deviation for each time interval were calculated.

Electron Microscopy

Mycelium used for electron microscopy was obtained as follows: CMC agar-coated (one side only) microscope slides were placed on solidified CMC agar in Petri dishes. Then, strips of agar, bearing hyphal tips, were placed beside the slides. When sufficient mycelial growth had occurred over the slides (1–2 days), the hyphae were fixed. Fixation procedures were first evaluated by observing the nuclei, with phase-contrast optics, at the various steps in the fixation-dehydration schedule. Only procedures which preserved the chromosomes and spindle through dehydration were subsequently used for electron microscopy.

In all cases, hyphae were first fixed by flooding the growing cultures, in the Petri dishes, with 3% glutaraldehyde in 0.1 M cacodylate, pH 7.0. The slides were then transferred to fresh fixative in McJunkin Staining Dishes (Arthur H. Thomas Co., Philadelphia, Pa.) for 1 hr at room temperature. Subsequent steps, through embedding, were made in these dishes (61). After glutaraldehyde fixation, the mycelia were washed in buffer and postfixed according to one of three schedules: (*a*) 1% OsO_4 in H_2O (5 min), followed by 0.5% uranyl acetate in H_2O (2 hr); (*b*) 1% OsO_4 plus 0.5% uranyl acetate in H_2O (1 hr), followed by 0.5% uranyl acetate in H_2O (2⅓ hr); (*c*) 1% OsO_4 plus 0.5% uranyl acetate in H_2O (5 min), followed by 0.5% uranyl acetate in H_2O (2⅓ hr). Dehydration in a graded acetone series was followed by a slow embedment in Epon-Araldite diluted with acetone: 5%, 10%, and 15% plastic, 1 hr each; 25% plastic, 1.5 hr; 40%, 55%, 70%, and 85% plastic, 2 hr each; and, finally, 100% plastic, 2 changes, for a total of 24 hr.

The slides, with embedded agar film and mycelia, were then removed from the dishes and placed at an angle of about 40° from horizontal to allow most of the plastic to drain off. Next, the bottoms of the slides were wiped clean of plastic, and the slides were polymerized in horizontal position at 70°C for 36 hr.

The technique used to select hyphal tip cells is a modification of the ones used by Girbardt (18), Robinow (57, and personal communication), Tanaka (61), and Aist and Williams (2) for electron microscopy of selected cells. The embedded slides were scanned under a 100 × oil immersion phase-contrast objective (no cover slip), and hyphal tip cells were selected in which the morphology of the nuclei was similar to that of living dividing nuclei (1). A rough sketch of the desired hyphal tip cell and adjacent hyphae was made at low magnification to

aid in locating the cell at later stages (61, and personal communication). The slide was then removed from the microscope stage, wiped free of oil, and then placed back on the stage in exactly its former position. Next, the microscope stage was lowered and a small bent needle was used to make a square etching in the surface of the plastic (around the circle of light coming through the condenser). This square of plastic contained the desired hypha plus several adjacent ones. The slide was placed onto a hot plate at about 60°C, where a razor blade was used to cut the plastic along the etched lines. This flat plastic square was then easily peeled from the slide by carefully forcing a razor blade between the plastic and the glass at a very low angle. Care was taken to peel the plastic square off from a direction lateral to the desired hypha so as not to bend the hypha. The plastic square was gently flattened between two warm slides. After the plastic square was glued onto a sectioning block, the desired hyphal tip cell was relocated, under a dissecting microscope, by use of the rough sketch. The techniques of trimming and of collecting serial sections were as previously described (2, 42, 70). Sections were poststained in lead citrate and viewed in a JEM-7 electron microscope.

Nuclei were assumed to be in interphase when none of the nuclei in the cell had a spindle. Mitotic nuclei were assigned to the various stages of mitosis on the basis of the presence and positioning of chromosomes, spindle morphology, and the shape of the nuclear envelope (1). The number of nuclei studied in each stage of division was as follows: 15 in interphase, three in the predivision phase, 11 in prophase, 15 in metaphase, two in anaphase, and five in telophase.

Sizes given for SPB's and kinetochores were derived from a total of five single measurements on appropriately oriented sections through five different nuclei. For microtubule width, a total of 10 measurements was made on five sections, each through a different metaphase nucleus. The magnification of the microscope was calibrated using a diffraction grating replica.

RESULTS

General, and Techniques

Under phase-contrast optics, mitosis in living cells of the Indiana isolate appeared identical to that described for an Arkansas isolate (1). The Indiana isolate was used in the present study because its chromosomes appeared to be slightly more condensed at metaphase than the chromosomes of the Arkansas isolate.

Phase-contrast observation of nuclei fixed for electron microscopy revealed that, although the chromosomes were well-preserved after glutaraldehyde fixation, they were no longer visible after conventional OsO_4 postfixation. The reasoning behind the development of the procedures used was, first, a very short postosmication of the narrow hyphal cell might be enough time for the OsO_4 to preserve the fine structure without providing it time to disperse the condensed chromatin, and, second, uranyl acetate, by virtue of its capacity to bind and precipitate nucleic acids, might counteract the dispersive effects of OsO_4 on the chromatin. Fixation schedule A gave the best preservation of both cytoplasmic and nuclear fine structure.

Shrinkage and wrinkling of the hyphae during embedding was difficult to avoid. The embedding procedure outlined was fairly successful and thin sections were made of only those hyphae which showed little or no wrinkling when viewed, after polymerization, with phase-contrast optics.

Time Course of Mitosis

Designation of mitotic stages was the same as in the previous study (1). The time course data are summarized in Fig. 1. The first visible sign of mitosis is the beginning of nucleolar dispersion (Fig. 1, time "0"). Within about 20 sec the chromosomes are first clearly visible, and 49 sec later they are quite condensed. At this stage, the chromosomes are seen to move in conjunction with (i.e., they are stationary with respect to) a bipolar spindle which traverses the chromosome cluster (Fig. 1, beginning of metaphase). The chromosomes remain in this metaphase configuration for approximately 2 min. Anaphase follows in which the chromatids migrate to the spindle poles in about 13 sec. During early telophase the spindle length and the distance between the incipient daughter nuclei both increase greatly, For this first 65 sec of telophase, the incipient daughter nuclei are apparently connected, i.e., the spindle is visible between them or the distance between them remains constant as they migrate to and fro within the hypha. The daughter nuclei then become disconnected, and about 1 min later new nucleoli are clearly visible within them. The total time required for mitosis is about 5.5 min.

Electron Microscopy

The interphase nuclei in hyphal tip cells of *F. oxysporum* are often oblong and contain a single nucleolus and a rather homogenous dispersion

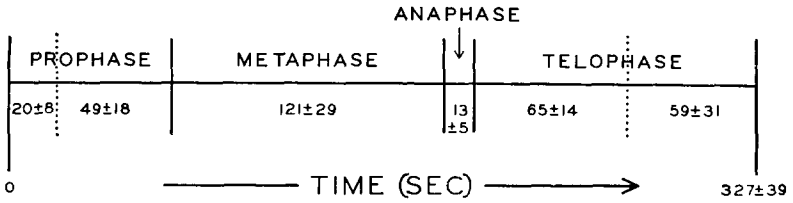


FIGURE 1 Time course of mitosis of living nuclei of *F. oxysporum*. Numbers indicate the time in seconds (mean ± standard deviation) required for each stage, and were derived from data taken on 20 complete mitoses. Mitosis begins (time "0") with the initiation of nucleolar breakdown. About 20 sec later, the chromosomes are first visible, and in another 49 sec they are clearly attached to (i.e., moving in conjunction with) a bipolar spindle. A 2 min metaphase is followed by a rapid (ca. 13 sec) anaphase, during which sister chromosomes migrate to opposite poles of the spindle. The first part of telophase (ca. 65 sec) involves a rapid elongation of the nucleus and spindle, and constriction of the original nuclear envelope around the incipient daughter nuclei. During the last part of telophase (ca. 59 sec), the unattached daughter nuclei enlarge and new nucleoli appear within them. The entire process requires about 5.5 min.

of fine granules and fibrils (Fig. 2), at least part of which is undoubtedly the chromatin. The SPB is 0.49–0.58 μm wide at interphase, and is located within a small inpocketing of the nuclear envelope to which it is attached. At the inner surface of the nuclear envelope, adjacent to the SPB, is found an amorphous granular region(s) which stains more densely than the rest of the nonnucleolar nuclear contents (Figs. 2, 3). This material was often difficult to distinguish on the basis of texture and staining density from the material in the SPB and in the chromosomes at metaphase. The SPB's of interphase nuclei in subapical cells were often seen to have cytoplasmic microtubules radiating from them as shown in Fig. 3. This nucleus is one of two such nuclei seen in which the SPB was at one end and the nucleolus at the opposite end. A similar morphology was seen in living migrating fungal nuclei (71), in which the SPB preceded the bulk of the nucleus during nuclear migration.

Details of the replication of the SPB were not obtained in this study. However, an interphase nucleus with two adjacent SPB's is shown in Fig. 4, a–c. Both of these SPB's were smaller than the SPB's which occurred singly at interphase (Figs. 2, 3).

Hyphae of *F. oxysporum* are multinucleate, and mitoses occur in a wave which in a matter of 5–10 min travels the entire length of a hyphal tip cell (1). A hyphal tip nucleus just about to begin mitosis (i.e., at the wave front) is shown in Fig. 5. Even though the adjacent nucleus (not shown) was well into mitosis, this predivision nucleus has an intact nucleolus and no spindle. Since a similar situation was found in other hyphae, the spindle must be produced very rapidly in the early stages of mitosis (perhaps in 1–2 min; see Fig. 1).

In an early prophase nucleus, two SPB's were found in nuclear inpocketings (Fig. 6, a–c). From the inpocketings numerous microtubules, representing the half-spindles, radiated broadly into the nucleus. At this stage, many of the microtubules were partially covered with a densely-staining finely granular material which gave them a fuzzy appearance. The chromosomes were not sufficiently condensed to be visible in thin sections at early prophase. Later, in prophase, the chromosomes were easily distinguished and the spindle microtubules were less divergent (Fig. 7). The amorphous granular material, typically associated with the nuclear envelope beneath the SPB at interphase, was absent from this region during prophase and metaphase.

By metaphase, the spindle had narrowed down to a rather compact bundle of microtubules (Figs. 8–10, 12), with the chromosomes situated around the periphery (Fig. 12). In cross-sections of the spindle, such as Fig. 12, 35–50 well-defined microtubules could be counted. The microtubules ranged in diameter from 16.0–22.2 nm, with an average diameter of 18.8 nm. Kinetochore microtubules are shown in Figs. 8, 10, 14, 15 b, and 16, and in Fig. 9, a possible continuous microtubule can be seen. Some of the spindle microtubules may not converge toward one of the spindle poles (Fig. 9).

Spindle poles are shown at high magnification in Figs. 11, 13, and 14. The SPB's are 0.51–0.84 μm wide at metaphase. On their nuclear side the SPB's are contiguous with the nuclear envelope. They are composed of moderately dense-staining fibrillo-granular material. The cyto-

plasmic side of the SPB is typically bordered by a light-staining ribosome-free zone. This zone is often traversed by short, broad fibers from the SPB (Fig. 11). Relatively few, usually short, cytoplasmic microtubules were seen associated with SPB's at this stage. The spindle microtubules usually terminated near the inner surface of the nuclear envelope. Nuclear pores were small and few in number in most regions of the nuclear envelope during all stages of mitosis, but at metaphase many, sometimes large (Fig. 13), holes occurred beneath the SPB's. In some micrographs the perinuclear space beneath the SPB's was stained more densely than was the rest of the perinuclear space.

Although plastic-embedded chromosomes were well contrasted when viewed with phase-contrast optics before sectioning, they had medium to low contrast when viewed in thin sections in the electron microscope (Figs. 10, 12, 14, 15 a, 16). The chromosomes were finely granular at high magnification (Fig. 14) and some of the small granules were aggregated into short, thick fibers. Relatively large, dense-staining nuclear particles (probably ribonucleoprotein (RNP) particles from the dispersed nucleolus) were excluded from the chromosomes (Figs. 14, 15 a).

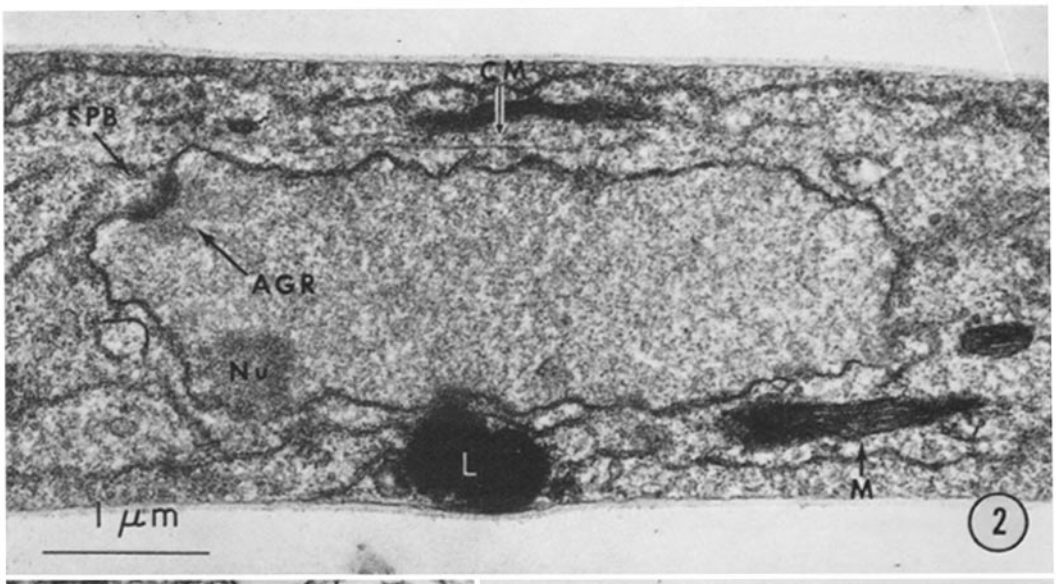
Kinetochores were spherical to pyriform in shape with a diameter of 80–160 nm. They were surrounded by a discontinuous, narrow, light-staining zone (Figs. 14, 15 b, 16, 18, 19). Kinetochores were further differentiated from the chromatin by their finer texture and greater staining density. Several kinetochores were studied in adjacent serial sections, and an example is reproduced in Fig. 15, a–c. In all such cases, one microtubule terminated at the kinetochore. In addition, complete serial sections through two chromosomes (one at metaphase, one at anaphase) revealed the presence of only one kinetochore per chromatid. In incomplete series of sections through several other metaphase nuclei the numbers of single kinetochores connected to the same spindle pole were as follows: four in two nuclei, three in two nuclei, and two in one nucleus. A telophase nucleus had three single kinetochores in an incomplete series of sections (Figs. 18, 19). The kinetochore microtubule in Fig. 14 traverses the entire distance from kinetochore to spindle pole, thus clearly showing the relationship of the chromosomes to the achromatic apparatus.

Sister kinetochores were rarely seen in the same section. However, careful study of incomplete series of sections of three metaphase nuclei confirmed that the kinetochroes of each chromosome occurred in pairs (Figs. 8, 10) and were separated from each other by a distance of 0.5–1.0 μm: four kinetochore pairs were seen in one nucleus and one in each of the other two. The above data indicate a chromosome number of at least four for the Indiana isolate of *F. oxysporum* used in

Key to abbreviations used in Figs. 2–19

AGR, amorphous granular region
CH, chromosome
Ch, chromatid
CM, cytoplasmic microtubule
DN, daughter nucleus
ER, endoplasmic reticulum
K, kinetochore
L, lipid body
M, mitochondrion
N, nucleus
NE, nuclear envelope
Nu, nucleolus
SM, spindle microtubule
SPB, Spindle Pole Body
V, vacuole

FIGURES 2–4 Electron micrographs of interphase nuclei of *F. oxysporum*. Fig. 2, a general view of an interphase nucleus showing nuclear envelope, nucleolus, SPB, diffuse chromatin, and an amorphous, more densely-stained, granular region at the inner surface of the nuclear envelope adjacent to the SPB. Cytoplasmic microtubules, mitochondria, and lipid bodies are also shown. Glutaraldehyde, postfixation schedule A. × 22,500. Fig. 3, this nucleus, with the nucleolus at one end and the SPB at the other, has the same general morphology as living, migrating fungal nuclei (71). Note the microtubules radiating out into the cytoplasm from the left edge of the SPB. Endoplasmic reticulum and a vacuole are also shown. Glutaraldehyde, postfixation schedule C. × 25,800. Figs. 4 a–4 c, three adjacent serial sections through an SPB at interphase. Arrows indicate corresponding regions on each micrograph. This SPB is apparently double, with each half being smaller than the SPB's in Figs. 2 and 3. Note the cytoplasmic microtubules associated with the upper half of the SPB. Glutaraldehyde, postfixation schedule C. × 34,400.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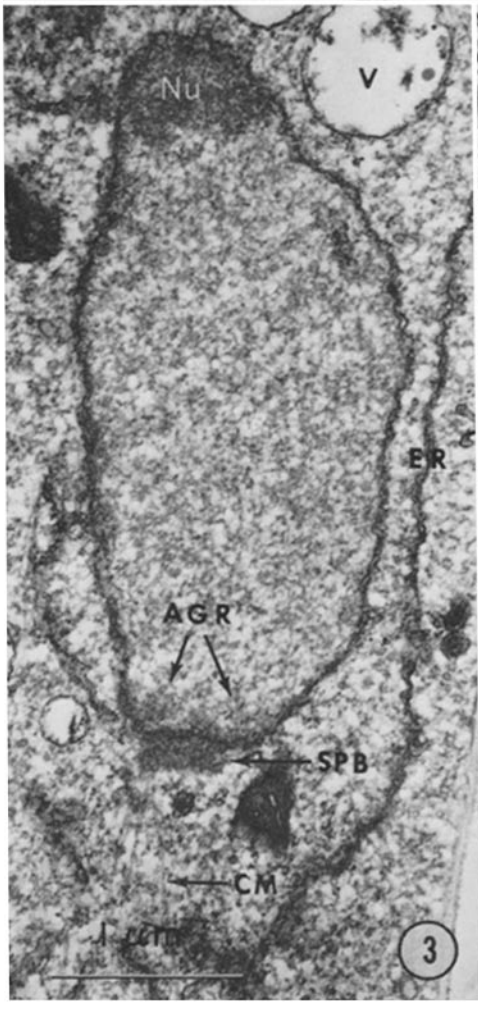
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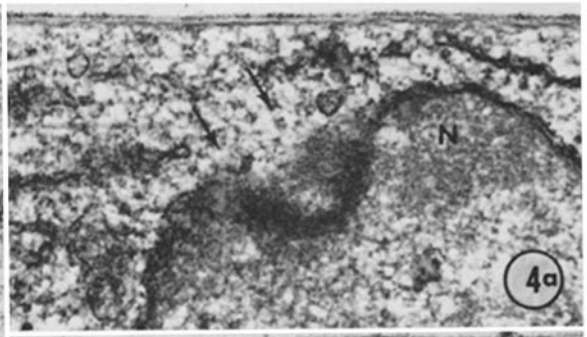
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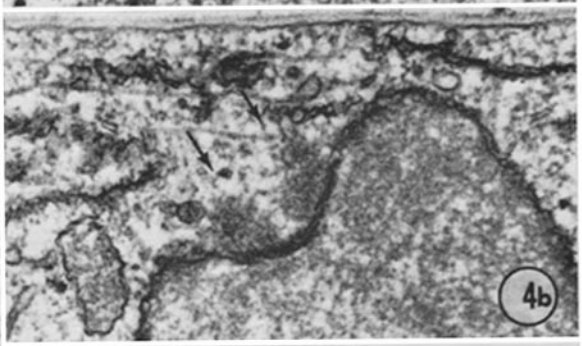
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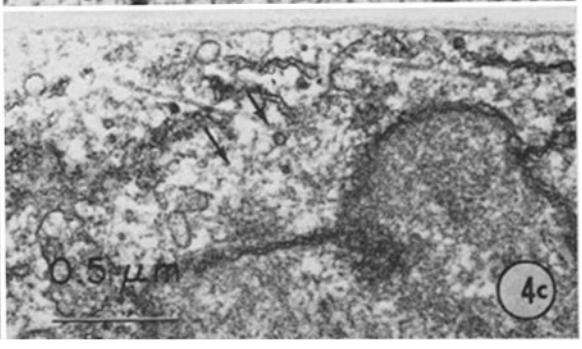
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4c

this study. The pairs of sister kinetochores were not grouped in a metaphase plate region, but instead they were scattered along the longitudinal axis of the spindle (Figs. 8, 10). It was common for the kinetochore region to appear stretched toward one spindle pole at metaphase (Figs. 8, 10, 15 b).

A nucleus at late anaphase is shown in Fig. 16. Most of the chromosomes have reached the spindle poles and numerous continuous spindle microtubules can be seen. Near the center of the figure, at the left edge of the nucleus, is a V-shaped lagging chromosome. The kinetochore of this chromosome, with its attached microtubule, was apparently leading the chromosome in its poleward migration when the cell was fixed.

At telophase the spindle was highly elongated (1) and, in serial sections, one of which is reproduced in Fig. 17, it was seen to be still intact. The nuclear envelope constricted around the spindle behind the incipient daughter nuclei (Fig. 17). Structures identifiable as kinetochores on the basis of their morphology, texture, and staining density were found only near the spindle poles at telophase (Figs. 18, 19). During anaphase and telophase, numerous cytoplasmic microtubules were associated with the SPB's (Figs. 16, 17).

A summary diagram of mitosis in *F. oxysporum*, derived from the results of both the present and the previous (1) investigations, is presented in Fig. 20. The main features which distinguish this form of mitosis from more common forms are (*a*) a possible connection between the chromosomes and an SPB at interphase, (*b*) the scattered arrangement of the chromosomes along the spindle at metaphase, (*c*) an asynchronous chromatid disjunction during anaphase, and (*d*) the persistence of the nuclear envelope throughout mitosis.

DISCUSSION

The present results correspond well with the previous description of mitosis in living hyphae of *F. oxysporum* (1). The breakdown of the nucleolus, the persistence of the nuclear envelope, the behavior of chromosomes, SPB's and spindles, and the attachment of chromosomes to the spindle were all confirmed by the present study. "Pinching-off" of incipient daughter nuclei at telophase (1) was shown here to involve constriction of the original nuclear envelope around the incipient daughter nuclei. Thus, the envelopes of the young daughter nuclei are probably derived from the end portions of the original nuclear envelope.

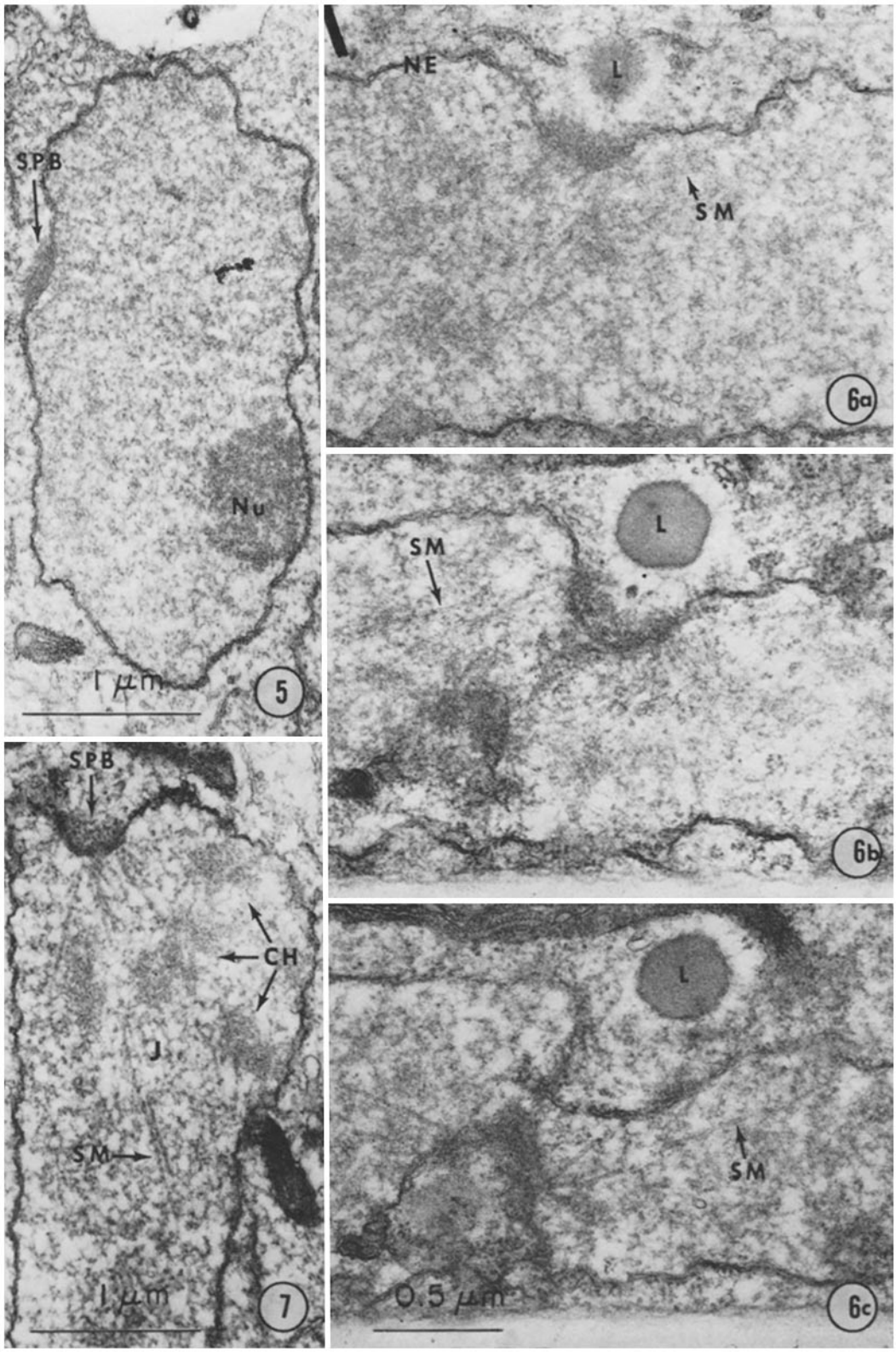
Time Course of Mitosis

Although the somatic nuclei of some fungi require about 20 min to complete division (54, 56, 58), most of those for which data are available, including *F. oxysporum*, require 10 min or less (1, 7, 8, 17, 52, 57, 75). The time required for mitosis in the fungi is at least an order of magnitude lower than for mitosis in higher plants and animals (17, 32). The percentage of the nuclear division time which is occupied by metaphase in *F. oxysporum* (ca. 36%), and in *Basidiobolus* (54, 61) and *Polystictus* (Teilungsphase III) (17) (both ca. 25%), is quite high in comparison to this percentage in many other kinds of organisms (17, 32).

Spindle Pole Bodies (SPB's)

The cross-sectional outline of fungal SPB's is quite variable. Many Ascomycetes have barlike or layered SPB's (1, 33, 58, 60, 68, 69, 74, 75), whereas the SPB's of many Basidiomycetes are

FIGURES 5–7 Nuclei of *F. oxysporum* in predivision (Fig. 5) and in prophase (Figs. 6 and 7) stages. Fig 5, this predivision nucleus is located at the front of the wave of mitoses which was passing through this hypha at the moment of fixation. At this stage, which is before nucleolar breakdown, there is no evidence of intranuclear microtubules associated with the SPB. Glutaraldehyde, postfixation schedule B. × 27,500. Figs. 6 a–6 c, serial sections through a very young spindle. One section is missing between Fig. 6 a and Fig. 6 b. The two SPB's are located within inpocketings of the nuclear envelope. Numerous, widely-diverging, intranuclear microtubules, representing the half-spindles, can be seen radiating from each of the inpocketings. The chromosomes are not well-differentiated at this stage. A lipid body is also shown. Glutaraldehyde, postfixation schedule A. × 40,000. Fig. 7, a prophase nucleus in which the chromosomes have become clearly discernible. The spindle microtubules are less divergent than those in the earlier stage shown in Fig. 6. Glutaraldehyde, postfixation schedule C. × 27,500.


SPB
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spherical (20, 37, 39, 44, 46). Within the Ascomycetes both spherical and barlike forms are present (20), and in the Basidiomycete *Schizophyllum* the SPB's are semicircular (51), a shape we often saw in *F. oxysporum*. The cross-sectional outline of *F. oxysporum* SPB's frequently resembled those of SPB's in parasitic protozoa (28, 63) and a higher green plant (29). Thus, it seems unwise, at present, to attach taxonomic significance to the morphology of fungal SPB's.

It has been suggested that a variety of structures located at or near the ends of microtubules be termed "microtubule-organizing centers" (48). In our opinion, it is too early to assign such a function to most of these structures, since in the majority of cases it has not been conclusively demonstrated that they organize anything. It is possible, of course, that SPB's orient or polymerize microtubules and, indeed, the circumstantial evidence presented in this paper, and in many of the papers cited above, would support such a conclusion. It is also possible, however, that, as postulated for *Plasmodium fallax* (28), the SPB's are pools of microtubule subunits which become organized into microtubules by another structure, such as the nuclear envelope. Thus, the nuclear pores which develop beneath the SPB's during nuclear division may function in allowing microtubule subunits to pass into the nucleus.

Girbardt has recently coined the term "kinetochore equivalent (KCE)" for the structures which occur at the spindle poles in fungi (19, 20). Use of this term may be premature becuase of evidence that fungi possess true kinetochores which are similar in morphology, location, and behavior to kinetochores of some higher organisms (see next section and Introduction). The term Spindle Pole Body seems more appropriate at present since it can be unambiguously applied in fungi such as *F. oxysporum* in which both kinetochores *and* spindle pole structures have been shown to occur during mitosis.

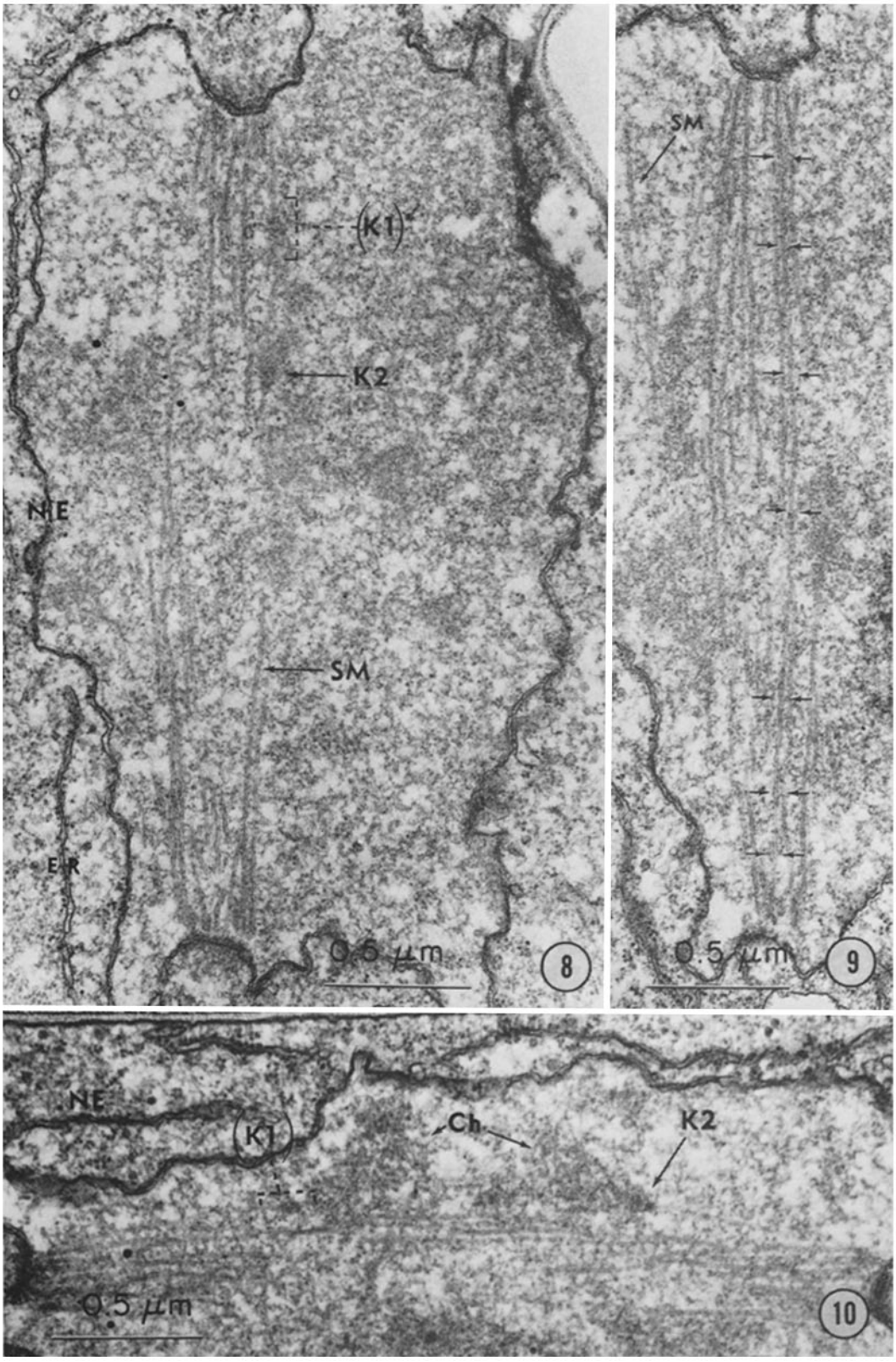
Kinetochores

Structures are designated as kinetochores in this paper because they are " . . . the differentiated chromosomal structures to which spindle fibers attach during mitosis . . . " (41). This designation is further supported by the observations that (*a*) these structures occur in pairs at metaphase, (*b*) they appear to lead the chromosomes to the spindle poles at anaphase, and (*c*) they are found near the spindle poles at telophase. These results also confirm the conclusion that " . . . the chromosomes usually attach to the spindle at different points . . . " along the axis of the spindle (1).

F. oxysporum kinetochores are reminiscent of the "ball-in-cup" type of kinetochores found in higher plant cells (5, 73) and recently reported to occur during meiosis in the fungus *Xylosphaera polymorpha* (9). They also resemble the regions of "chromosome spindle element attachments" illustrated in mitotic nuclei of the fungus *Armillaria mellea* by Motta (46; Fig. 6). Furthermore, the attachment points of individual microtubules to the kinetochores of the worm *Urechis* (40), of the sedge *Cyperus* (11), and of the fungus *Coprinus lagopus* (39) bear some resemblance to the kinetochores of *F. oxysporum*. The significance of these structural similarities is unknown.

Little can be said concerning the function(s) of *F. oxysporum* kinetochores, other than that they serve as organelles of attachment. Since they appear to lead the chromosomes in their migration to the spindle poles at anaphase, they probably transmit a force from the kinetochore microtubules to the chromosomes.

FIGURES 8–10 Skipped serial sections of the same metaphase nucleus of *F. oxysporum*. In Fig. 8, an SPB can be seen at each spindle pole, and a narrow bundle of microtubules runs from one SPB to the other. Most of the microtubules do not contact the nuclear envelope beneath the SPB's. The nuclear envelope is continuous, with an occasional nuclear pore. A kinetochore (*K2*) can be seen in the upper half of the figure, and above it is shown part of its sister kinetochore (*K1*), the remainder of which was in an adjacent section. In Fig. 9, a possibly continuous microtubule can be seen (between the paired arrows) running almost the entire distance from pole to pole. Discontinuities in the microtubule make it impossible to completely trace one microtubule with certainty. In the upper left of the figure are a few microtubules which do not converge toward the upper spindle pole. A pair of sister kinetochores is shown in Fig. 10. The kinetochore at the left (*K1*) is only partially contained in the section. Note that the sister chromatids are already separated at the kinetochore region. Glutaraldehyde, postfixation schedule A. × 55,000.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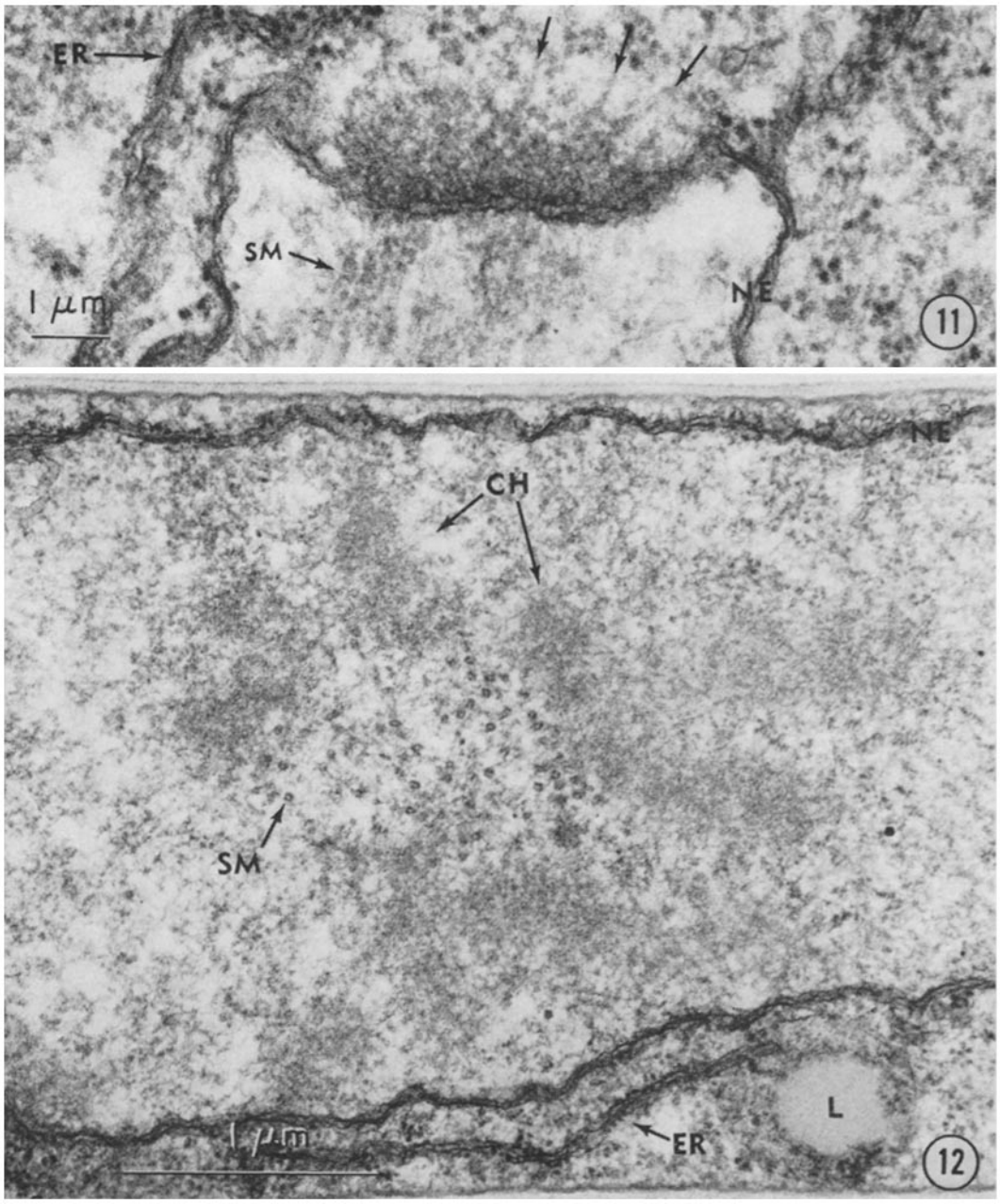
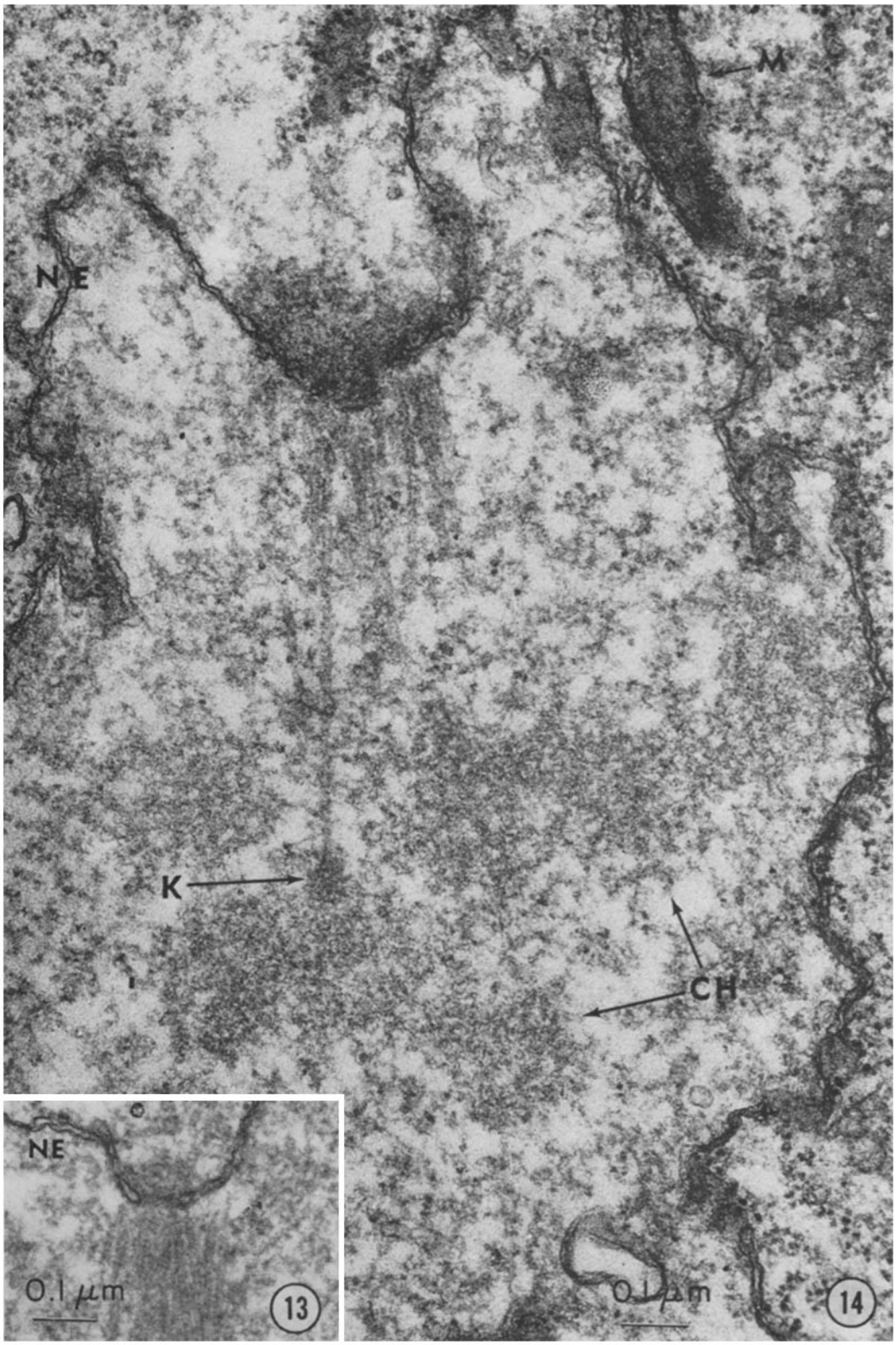
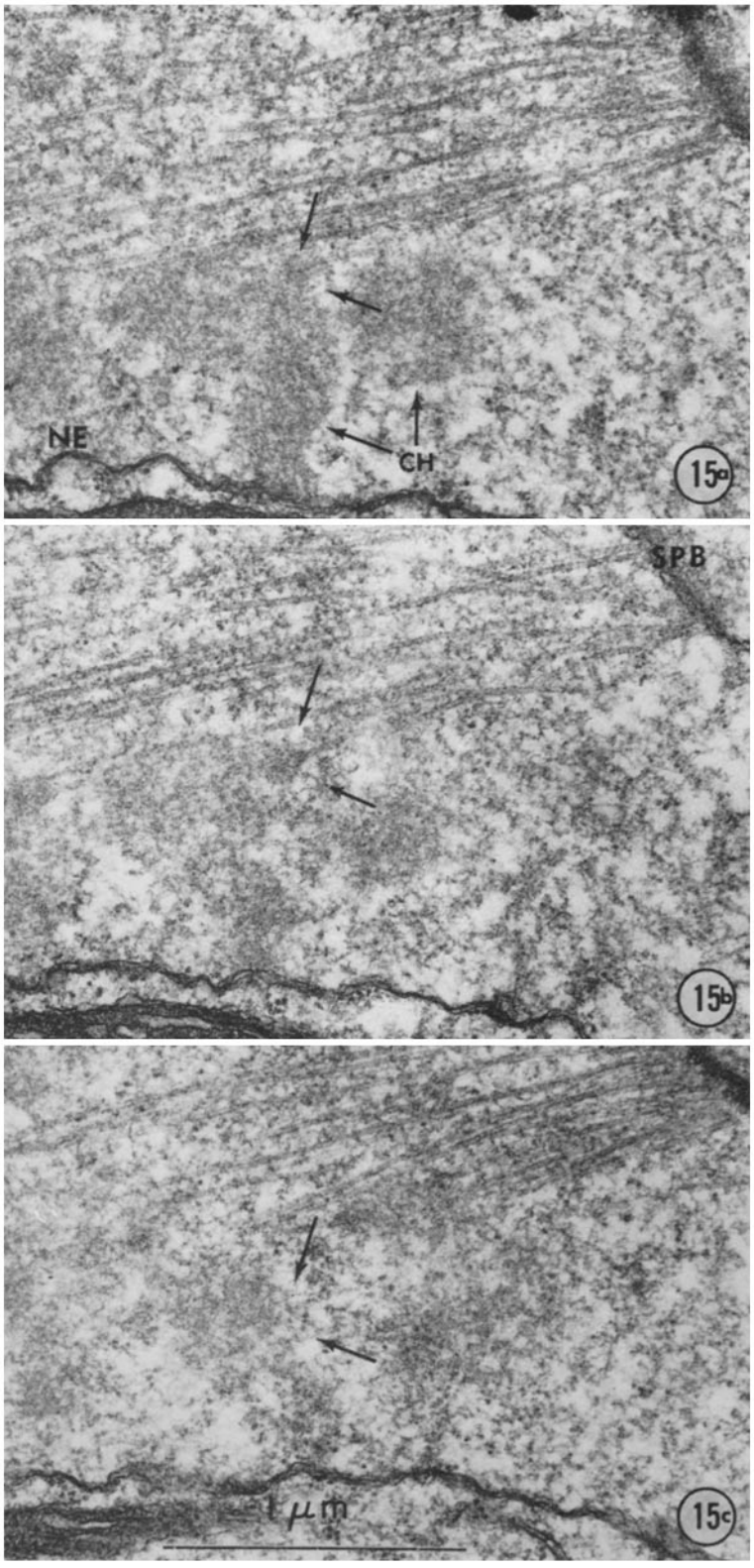


FIGURES 11 and 12 Metaphase nuclei of *F. oxysporum*. Fig. 11, a spindle pole region at high magnification. The SPB is a hemispherical fibrillo-granular structure closely appressed to the nuclear envelope. On the cytoplasmic side of the SPB is a light-staining, ribosome-free region which is traversed by thick fibers (arrows). These fibers appear to be a continuation of the material in the SPB. Intranuclear spindle microtubules can be seen below the SPB. Glutaraldehyde, postfixation schedule A. × 110,000. Fig. 12, the spindle in cross-section. Chromosomes appear as accumulations of fibrillo-granular material around the periphery of the microtubular spindle. Glutaraldehyde, postfixation schedule A. × 50,000.



FIGURES 13 and 14 Metaphase nuclei of *F. oxysporum*. Fig. 13, a spindle pole region showing a large hole in the inpocketing of the nuclear envelope. Such holes allow direct contact of the SPB with the spindle microtubules. Glutaraldehyde, postfixation schedule A. × 99,000. Fig. 14, a general view which shows one of the spindle poles sectioned obliquely. A single microtubule can be seen connecting the kinetochore to spindle pole. The chromosomes appear as regions of primarily finely granular material from which RNP particles are excluded. Glutaraldehyde, postfixation schedule A. × 110,000



FIGURES 15 a–15 c A series of three adjacent serial sections which demonstrate the termination of a microtubule at a kinetochore. The unlabeled arrows indicate a corresponding region in each section. In Fig. 15 a, only chromosomal material can be seen in this region. In Fig. 15 b, the kinetochore with attached microtubule can be seen, and in Fig. 15 c only a slice of the side-wall of the end of the kinetochore microtubule is present in this region. Glutaraldehyde, postfixation schedule A. × 40,000.

Mechanism of Chromosome Movement

Chromosome movement during mitosis in *F. oxysporum* involves at least two phases: first, the migration of sister chromosomes to the spindle poles (anaphase) and, second, the separation of incipient daughter nuclei (early telophase) (1). Anaphase movement is primarily a result of shortening of the kinetochore microtubules (as evidenced by the polar location of kinetochores at telophase), since the spindle elongates only slightly during this stage (1). There is no evidence that in *F. oxysporum* the metaphase or anaphase chromosomes are attached to the nuclear envelope, so it is highly unlikely that the nuclear envelope plays a direct role in chromosome movement at anaphase. Thus, anaphase movement of chromosomes in *F. oxysporum* may have a similar structural basis as that described for certain algae (49, 50), higher plants (6), and animals (41).

In some fungi the separation of sister genomes is accompanied by spindle and nuclear envelope elongation (1, 26, 34, 43, 57, 58). It has beea suggested that in certain of these fungi nuclear elongation is caused by membrane growth, whereas the elongating central spindle plays no active role in force production in this phenomenon (43, 58). This proposal is based largely on the observation that in certain fungi the ends of elongating, dividing nuclei are not pointed, as might be expected if the spindle was providing the force. Such a mechanism seems unlikely to occur in fungi for three reasons: first, it is difficult to imagine how membranes alone could exert a sufficient force parallel to their plane of growth, whereas microtubules are known to be able to exert considerable force in their direction of growth (6, 62); second, mitotic nuclei of *F. oxysporum* and *Ceratocystis fagacearum* are typically pointed immediately after anaphase, although during telophase the ends of the nucleus become rounded (1); and, third, the rounded appearance of the ends of telophase nuclei can easily be accounted for by visualizing the chromosomes of the incipient daughter nuclei as being tightly clustered around a nearly central point, the SPB. Such a cluster would be expected to approach a spherical shape if the chromosomes were spherical and unhindered. We envision the incipient daughter nuclei and the regions of the nuclear envelope in contact with them as exerting opposing forces on each other. This interaction could result in either (*a*) blunt or rounded ends of the nuclear envelope, or (*b*) the location of part of the chromatin and nuclear envelope forward of the SPB. Which of these two conditions [(*a*) or (*b*)] is present at a particular stage would depend on the magnitude of the forces exerted by the chromosomes and by the nuclear envelope at the moment of fixation. Such a model could explain the nuclear shapes observed in yeasts if the daughter genomes are already at the spindle poles before the spindle length is as great as the over-all nuclear diameter. This is apparently the case in *Schizosaccharomyces pombe* (43). We believe that the elongating central spindle, not the nuclear envelope, is the primary source of forces which cause nuclear elongation and separation of incipient daughter nuclei during telophase.

The "Double Strand" Theory of Somatic Fungal Nuclear Division

A number of papers have been published in which fungal chromosomes are described as being connected to each other to form a linear or ring-shaped chain of chromosomes (3, 12, 13, 25, 36, 47, 64, 65, 66, 67, 71). The chain of chromosomes would duplicate during interphase to produce a two-chain or double-strand configuration. During nuclear division, each chromosome chain would function more or less as a unit and would eventually form one of the daughter nuclei. An evaluation of the evidence for this theory is meaningful only when it is compared to evidence from studies which contradict it. It has been possible for several years now to study a fungal nucleus in the living state, with light microscopy, and to subsequently study the *same* nucleus, after fixation and embedding, with electron microscopy (1, 18, 56, 57). A similar continuity was achieved in the present study, and in a recent study of *Basidiobolus* mitosis (61), by studying nuclei whose morphology after fixation corresponded to the morphology of living mitotic nuclei. In addition, the technique of sequentially staining the spindle, and then the chromosomes, of the *same* nucleus (58) has provided insight into the relation between chromosome behavior and spindle behavior (1, 57, 58). Results obtained using these conbined techniques, as well as results of correlated light and electron microscope studies of *different* somatic fungal nuclei (43, 69, 75), demonstrate many similarities between fungal nuclei and the nuclei of most other eucaryotes. A microtubular spindle can now be considered to be a marker for nuclear division in many fungi. These studies have further enabled us to envision the space limitations within which the

chromosomes must sort themselves out into daughter nuclei. Such space limitations are not compatible with some descriptions of double-strand nuclear division (66, 67). Furthermore, the evidence is now fairly conclusive that the chromosomes of *F. oxysporum* are separate structures which migrate independently to the spindle poles during mitosis. Separate, well-defined somatic chromosomes have also been demonstrated to occur in other fungi (1, 35, 53).

Evidence comparable to that described above has not been presented in support of the double-strand theory. The observations on living cells reported by Laane (36) and by Weijer et al. (64) have not been supported by fine-structural observations on the same structures which they believed to be chromosomes and nuclei. Fine-structural observations by Namboodiri and Lowry (47) were made on randomly selected hyphae which had been subjected to extremely rough treatment before fixation. Their material was then fixed in $KMnO_4$, which is known to destroy microtubules. Such techniques would not be expected to reveal the nature of dividing nuclei. Here, too, the *same* nuclei were not studied both in the living condition and after fixation and embedding.

Although many double-stranded nuclei may be artifactual (4, 35), some are apparently not. The anaphase stage of fungal mitosis appears double-stranded in many fungi, particularly in stained preparations (1, 14, 57). However, the evidence from *F. oxysporum* clearly shows that this stage is extremely short-lived, and that the chromosomes do not migrate as if they were attached to each other. Furthermore, Robinow and Caten (57) and Robinow (55) have shown that the double-strand appearance of anaphase in *Aspergillus* is often an illusion due to the plane of focus.

In our opinion, most of the support for the double-strand theory of nuclear division has come from observations on nondividing nuclei. This probably explains why Heale et al. (25) found a large discrepancy between the percentage of "dividing" nuclei in material stained to show chromatin, and the percentage of nuclei with spindles in different material stained to show spindles. Until concrete evidence, such as that obtainable by combined light and electron microscope studies of the *same* nuclei, is provided in favor of the double-strand theory of fungal nuclear division, this theory will remain unsubstantiated.

Relation of Chromosomes to the SPB

Harper's meticulous drawings of fungal nuclei clearly showed that in his material there was always a chromatinic connection between SPB's and interphase chromosomes. This direct connection was, however, lost momentarily during meiosis. Harper found a measure of agreement between the number of countable chromosomes at meiosis and the number of chromatinic strands connected to the SPB's at interphase. He, therefore, suggested (24) that fungal chromosomes are individually and permanently attached to SPB's, either by direct connections at interphase or via spindle fibers during meiosis. Considerable support for this idea has now accumulated: (*a*) several workers have published light micrographs of stained interphase fungal nuclei which show the main mass of chromatin to be connected to a nearby granule (possibly the SPB) by a chromatinic thread or two (1,

FIGURES 16 and 17 Nuclei of *F. oxysporum* in late divisional stages. Fig. 16, a late anaphase nucleus in an unusually narrow hypha. Most of the chromosomes have reached the spindle poles, and numerous continuous microtubules can be seen between them. A lagging chromosome can be seen at the left side of the nucleus, near the center of the figure. Note that the kinetochore, with its attached microtubule, appears to be leading this V-shaped chromosome in its migration toward the upper spindle pole. The nuclear envelope is still intact. Complete serial sections were obtained through both SPB's which were found to have a large number of cytoplasmic microtubules associated with them. A few of these microtubules are visible in the upper left corner of the figure. Glutaraldehyde, postfixation schedule B. × 24,400. Fig. 17, a nucleus at mid-telophase in an unusually narrow hypha. The spindle is quite long and narrow, and it extends from one SPB to the other. The intact nuclear envelope has constricted around the upper daughter nucleus, and it was apparently in the process of constricting around the lower incipient daughter nucleus when the hypha was fixed. In a complete series of serial sections through this spindle, numerous cytoplasmic microtubules were seen radiating from the SPB's. Some of these microtubules can be seen in the upper right corner of the figure. Glutaraldehyde, postfixation schedule B. × 21,650.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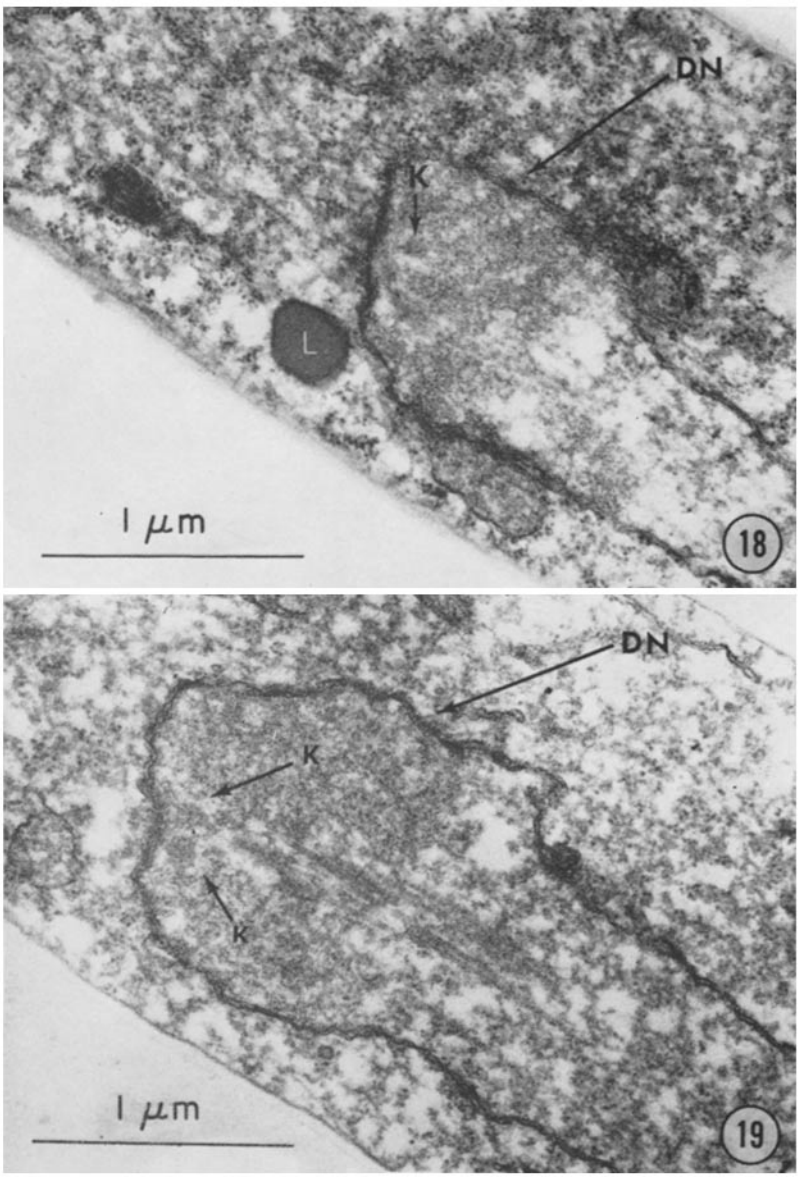

FIGURES 18 and 19 Skipped serial sections through one end of a middle telophase nucleus. Structures identifiable as kinetochores on the basis of their morphology, texture, and staining density are embedded among the closely-packed chromosomes of the daughter nucleus. The kinetochore in Fig. 18 is shown in side view, whereas those in Fig. 19 are seen in end view. Some of the spindle microtubules can be seen in Fig. 19. Glutaraldehyde, postfixation schedule B. × 34,400.

8, 10, 35, 71, 72); (*b*) the stretching of the nucleolus in predivision migrating nuclei of *Polystictus* may be due to a connection between the nucleolus-organizing region of a chromosome and the SPB (=KCE), as suggested by Girbardt (20); (*c*) in electron micrographs of somatic interphase nuclei of many fungi, including *F. oxysporum*, an amorphous granular region, possibly heterochromatin, is seen near the inner surface of the nuclear envelope adjacent to the SPB (16, 20, 44); and (*d*) the study of Rosenberger and Kessel (59) showed that chromosomes of *Aspergillus* which were newly syn-

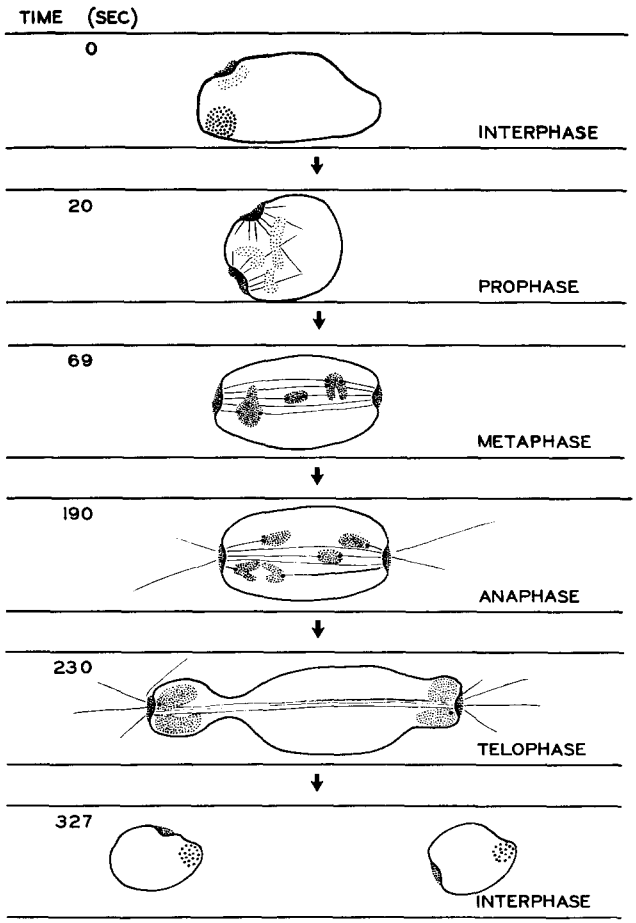

FIGURE 20 Semidiagrammatic summary of mitosis in *F. oxysporum* based on the present study, and on the previous light microscope study (1). The numbers given at the left represent the approximate time (in seconds) after the beginning of mitosis that the nuclear configuration shown would occur. At time "0" the nucleolus is essentially intact and a spindle has not yet appeared. After about 20 sec, the nucleolus has partially or completely broken down and chromosomes are visible. A half-spindle has developed from each of two nuclear envelope inpocketings which are occupied by the SPB's. The amorphous granular region is no longer associated with the nuclear envelope beneath the SPB. Metaphase begins after about 69 sec, and it is characterized by the appearance of separate chromosomes attached at different points along the longitudinal axis of the spindle. Sister kinetochores are located on opposite sides of the chromosomes and are, therefore, already separated. Kinetochore, and possibly also continuous, microtubules can be distinguished at this stage. The total number of kinetochores per nucleus has not been demonstrated. After about 190 sec, asynchronous disjunction of sister chromatids, and the migration of chromosomes toward the spindle poles from spatially separated spindle attachment sites, produces an anaphase configuration typical of many fungi. This stage is sometimes referred to as the double-track (57) or two-track (14) stage. The kinetochores appear to lead the chromosomes in their migration to the spindle poles. Elongation of the entire nucleus follows, and near midtelophase (ca. 230 sec), the still-intact nuclear envelope constricts around the incipient daughter nuclei. At this stage, kinetochores can be seen among the chromosomes near the spindle poles. During anaphase and telophase, numerous cytoplasmic microtubules are associated with the SPB's. About 5.5 min from the beginning of mitosis, new nucleoli reappear in the enlarging daughter nuclei thus completing the mitotic process. This diagram has many similarities to one prepared to summarize mitosis in *Aspergillus nidulans* (15), which was based on the work of Robinow and Caten (57).

thesized during interphase segregated as a unit to the same pole during mitosis. As was suggested by Day (15), this could be achieved by the permanent attachment of all newly-synthesized chromatids to a structure, such as the SPB, which is also replicated at interphase (20, 45). Luykx (41), however, has pointed out that this nonrandom segregation of chromosomes during mitosis can be explained on the basis of preservation of kinetochore and spindle orientation from one mitosis to the next. Much of the evidence for a permanent connection between chromosomes and the nuclear envelope in higher organisms, and in fungi, has been recently summarized and discussed by Girbardt (20).

Observations on nuclear behavior in *F. oxysporum* (1, 71) support Harper's model of permanent connections between fungal chromosomes and SPB's. In *F. oxysporum* it is likely to be the kinetochore regions of the chromosomes which are connected to the SPB (or to the nuclear envelope adjacent to the SPB). The amorphous granular region associated with the inner surface of the nuclear envelope adjacent to the SPB at interphase would be interpreted as heterochromatin. Such an interpretation is consistent with the observation that kinetochore DNA is often associated with blocks of heterochromatin (41). As the kinetochore microtubules grow during prophase, the specific connections between the chromatids and the SPB's (which would have been established during chromatid and SPB replication) would be maintained via the kinetochore microtubules. Migration of the kinetochores to the spindle poles at anaphase would reestablish the original connection between the chromosomes and the SPB's. This course of events has been shown to occur during the mitotic cycle in *Barbulanympha* (31), where densely-staining structures located on the nuclear envelope are directly connected to the chromosomes at interphase, but are only indirectly attached (via spindle fibers) to them during nuclear division. Mitosis in *S. pombe*, where kinetochore microtubules may not develop (43), may represent a variation on this same theme. In this case, the chromosomes would be directly connected to the SPB at all stages of the mitotic cycle. Harper's model must, of course, be considered tentative until proof of interphase connections between the SPB and the chromosomes of fungal nuclei is available.

Microtubules, SPB's, and Nuclear Migration

The SPB was perhaps first suggested to have an active role in migration of fungal nuclei by Levine in 1913 (38). Girbardt (17), Wilson and Aist (71), and Aist (1) confirmed Levine's observations by direct phase-contrast observations on living, migrating fungal nuclei. Recent observations on fixed and stained fungal nuclei studied by light microscopy have also implicated the SPB in nuclear migration (1, 10, 71, 72).

A correlation between nuclear motility and the presence of cytoplasmic microtubules attached to the SPB has been demonstrated in *P. versicolor* by Girbardt (19). Such a correlation also occurs in the data on *F. oxysporum:* first, cytoplasmic microtubules were seen radiating from the SPB's of certain interphase nuclei which appeared to have been in the process of migrating when they were fixed, and, second, the marked increase, during anaphase and telophase, in the number of cytoplasmic microtubules radiating from the SPB's is correlated with a migration of the dividing nucleus through the hypha (1). The occasional migration of a metaphase nucleus and the rotation of metaphase nuclei (1) could be due to a transitory association of cytoplasmic microtubules with the SPB's. Thus, there is growing evidence which suggests that certain motility of fungal nuclei may require the presence of cytoplasmic microtubules attached to the SPB's. The questions of what forces are involved and how they are deployed are yet unsolved (19).

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