MITOSIS IN THE FUNGUS THRAUSTOTHECA CLAVATA

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ABSTRACT

The ultrastructure of mitosis is described in Thraustotheca clavata, an oomycete fungus. An intranuclear spindle develops between differentiated regions of the nuclear envelope which move apart, each associated with 180° oriented centriole pairs. The spindle contains low numbers of continuous and interdigitating microtubules in addition to chromosomal microtubules. Each kinetochore is attached to only one microtubule. Serial section analysis shows that at meiosis there are probably 12 chromosomes in the diploid nucleus, yet at mitosis the methods utilized in the present study suggest that there may be less than 12 kinetochores connected to each pole. At mitosis many of the kinetochores within a given spindle are not arranged in opposite pairs. The behavior of the spindle microtubules during mitosis is comparable to that of higher organisms but the rarity of short intertubular distances appears to preclude significant force generation by means of intertubular bridge mechanisms. Evidence is presented for a nuclear envelope-microtubule interaction which is capable of generating shear forces during both mitosis and interphase nuclear movements.

INTRODUCTION

Although a considerable amount of information has been obtained, the mechanics of the mitotic process are still far from clear (Forer, 1969; Nicklas, 1971). A common approach to many biological problems is to examine the process under investigation in a variety of organisms which are likely to be evolutionarily primitive so that a possible evolutionary sequence can be deduced. Information on the way a process has developed can help explain the highly evolved, usually more complex process. This approach to understanding mitosis has been taken by Pickett-Heaps (summarized in 1969, 1972) in a series of papers on the ultrastructure of mitosis and cytokinesis in various algae, a group which shows relatively clear evolutionary lines. The fungi also present a group of organisms in which it is generally agreed that there are clear evolutionary lines, but to date there are few detailed ultrastructural papers which describe mitosis in these organisms. The least studied group of fungi with respect to mitosis are the aquatic "phycomycetes," yet this assemblage of organisms is generally considered to include some of the least highly evolved fungi. A detailed ultrastructural analysis of Saprolegnia ferax (Heath and Greenwood, 1968, 1970), an oomycete, clarified a confusing accumulation of light microscopical information by showing clearly the presence of a small, microtubule-containing, intranuclear spindle. However, details of the way in which these microtubules interacted with the chromatin were unclear although simple kinetochores were demonstrated for the first time in a "phycomycetous" fungus. In addition to a description of the spindle, morphological evidence for a microtubule-membrane interaction was presented and the suggestion made that this interaction was partially responsible for force generation in mitosis. In order to establish the evolutionary significance of this unusual mitotic system a survey of mitosis...
in allied genera is underway. The results reported here extend the original observations to another member of the Oomycetes and expand our comprehension of the morphological aspects of this presumably primitive mitotic system. Many of the present observations place restrictions on theories which have been invoked to universally explain mitosis and other microtubule involving processes.

MATERIALS AND METHODS

*Thraustotheca clavata* (de Bary) Humphrey (culture number 371a) was generously supplied by Dr. M. W. Dick from the Aquatic Phycomycete Culture Collection of Reading University. Vegetative hyphae were grown aseptically at 20°C or 25°C in plastic Petri dishes in a medium containing 1% glucose, 0.1% peptone, 0.01% yeast extract, 0.1% KH₂PO₄, 0.03% MgSO₄ · 7H₂O with 1.5% agar if appropriate. Fixation, embedding, and sectioning procedures were essentially those described by Heath and Greenwood (1971). Silver-gray serial sections were collected on Formvar-coated single-hole grids and stained with uranyl acetate and lead citrate. Antheridia and oogonia were produced by growing hyphae from infected hemp (*Cannabis sativa* L.) seeds into “dilute salts” solution (Machlis, 1953) at 20°C for 3 days. The dynamic sequence of events described here are reconstructed from electron micrographs of selected hyphae. The preferable technique of making ultrastructural observations on nuclei fixed after in vivo observation was not attempted because most of the interesting details discussed are below the limit of detectability in living hyphae.

RESULTS

Interphase vegetative nuclei are each accompanied by a pair of centrioles which are aligned at 180° to each other (Fig. 1). These centriole pairs are always associated with a differentiated region of the nuclear envelope termed a “pocket” by Heath and Greenwood (1970). Inside the nucleus a few

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1 The term “pocket” is retained as an accurate descriptive term; a causal role for this differentiated region in microtubule formation is not proven, thus “MTOC” (Pickett-Heaps, 1969) is inappropriate. Kinetochores comparable to traditionally defined kinetochores of other spindles are present, thus “KCE” (Girbardt, 1971) is not applicable and other morphological terms such as “plaque” (Robinow and Marak, 1966) and “spindle pole body” (Aist and Williams, 1972) are less descriptively accurate.
Figure 4  A stage in centriole (c) migration slightly later than that shown in Fig. 3. Two intercentriolar microtubules are present (arrows) and the cytoplasmic microtubules which run from the region of the centrioles along the surface of the nucleus (N) and which are associated with the horns can be clearly seen. X 57,300.

Figures 5 and 6  Part of a series of sections of an early stage in spindle development. Two closely associated intercentriolar tubules are present (arrows, Fig. 5) and inside the nucleus (N) the spindle shows both continuous microtubules (arrow, Fig. 6) running between the pocket (p) regions and probable kinetochores (arrow, Fig. 5). Fig. 5, X 88,100; Fig. 6, X 77,100.

(<10) microtubules may radiate from the pocket region into the nucleus, each microtubule extending about 10 nm. Some of these tubules appear to terminate in kinetochores, but such tubules and kinetochores are not always present. Impending mitosis is indicated by centriole replication, a process which involves the separation of parent centrioles and synthesis of daughter centrioles on the proximal ends of the parentals (Fig. 2). This process appears to be identical to

Figures 7–10  Section numbers 6, 9, 10, and 11, respectively, from a series through an early “metaphase” spindle. The polar pairs of centrioles (c) are seen in Fig. 7 and the obliquely sectioned pocket regions (p) of the nuclear envelope in Figs. 8 and 9. The positions of the kinetochores are marked by arrows. Note the osmiophilic presumed chromatin associated with each kinetochore and the predominantly unpaired and irregular arrangement of the kinetochores. All, X 77,300.
that described in *Saprolegnia* (Heath and Greenwood, 1970). At the end of centriole replication there are two pairs of closely spaced centrioles, each pair associated with a pocket. Mitosis then continues with increasing separation of the centriole pairs and their associated pockets. Subsequent stages of mitosis are most easily considered in two parts, intranuclear and extranuclear events.

**Intranuclear Events**

Initially intranuclear microtubules are few and are similar to those present during interphase but they do radiate from both pockets. As centriole separation continues, these presumptive spindle tubules elongate and increase in number but not until the pockets are approximately 1 µm apart (center to center) are there any spindle tubules which run from pocket to pocket (Figs. 5, 6). When the pockets are approximately 2 µm apart the spindle appears to contain its maximum number of microtubules. Typical spindle structure at this stage is shown in Figs. 7-12 and Table I.

The spindle of *T. clavata* contains four types of microtubules, all of which have at least one end located adjacent to a pocket and all with apparently similar substructures. *Continuous tubules* run continuously from one pocket to the other (Figs. 6, 10, 15). Frequently, even in serial sections, continuity is difficult to prove but there appears to be no more than four or five such tubules per spindle. Continuous tubules have been detected in spindles ranging from 1 to 4 µm in length, the latter length corresponding to an anaphase-telophase stage.

*Interdigitating tubules* extend for most of the length of the spindle but are only associated with one pocket. Where these tubules terminate in the nucleoplasm there are no detectable differentiated structures (Figs. 7-10, 12, 13, 17-19). As with the

![Figure 11](image-url) Selected areas of section numbers 21 (= a) to 14 (= h) from the transverse series used to compile Fig. 12. Kinetochores are marked by arrows and a possible kinetochore by a ?. Each micrograph has been trimmed to show a comparable area of the spindle. Although divergence of the spindle microtubules makes exact comparison difficult, by selecting suitable “marker” microtubules the termination of the kinetochore tubules can be verified. All, × 85,800.
**Table I**

*Distribution of microtubules and kinetochores in three serially sectioned mitotic spindles*

| Phase | Metaphase | Metaphase | Anaphase |
|-------|-----------|-----------|----------|
| Interpolar distance, µm | 1.35 | 2.0 | 4.0 |
| Section thickness, nm | 70 | 70 | 140 |
| Plane of sections cut | Longitudinal | Transverse | Longitudinal |
| Section number | A | B | C | D | E | A | B | C | D | E | A | B | C | D | E |
| 1 | 0 | 1 | | | | | | | | | | | | | |
| 2 | 0 | 7 | | | | | | | | | | | | | |
| 3 | 2 | 21 | | | | | | | | | | | | | |
| 4 | 13 | 35 | | | | | | | | | | | | | |
| 5 | 38 | 34 | | | | | | | | | | | | | |
| 6 | 33 | 32 | | | | | | | | | | | | | |
| 7 | 41 | 34 | | | | | | | | | | | | | |
| 8 | 45 | 32 | | | | | | | | | | | | | |
| 9 | 43 | 31 | | | | | | | | | | | | | |
| 10 | 34 | 24 | 1 | 1 | | | | | | | | | | | |
| 11 | 27 | 27 | | | | | | | | | | | | | |
| 12 | 24 | 22 | 1 | | | | | | | | | | | | |
| 13 | 28 | 3 | 1 | 2 | | | | | | | | | | | |
| 14 | 20 | 2 | | | | | | | | | | | | | |
| 15 | 17 | 1 | 1 | 1 | | | | | | | | | | | |
| 16 | 17 | 2 | | | | | | | | | | | | | |
| 17 | 22 | | | | | | | | | | | | | |
| 18 | 24 | | | | | | | | | | | | | |
| 19 | 24 | | | | | | | | | | | | | |
| 20 | 30 | | | | | | | | | | | | | |
| 21 | 36 | | | | | | | | | | | | | |
| 22 | 41 | | | | | | | | | | | | | |
| 23 | 35 | | | | | | | | | | | | | |
| 24 | 42 | | | | | | | | | | | | | |
| 25 | 32 | | | | | | | | | | | | | |
| 26 | 12 | | | | | | | | | | | | | |
| 27 | 4 | | | | | | | | | | | | | |
| 28 | 0 | | | | | | | | | | | | | |
| 29 | 0 | | | | | | | | | | | | | |
| 30 | 0 | | | | | | | | | | | | | |
| 31 | | | | | | | | | | | | | |
| 32 | | | | | | | | | | | | | |
| 33 | | | | | | | | | | | | | |
| 34 | | | | | | | | | | | | | |
| 35 | | | | | | | | | | | | | |

The microtubule counts from the transverse sections are direct counts from actual sections of assumed 70 nm thickness. The length of the spindle was calculated on this assumption. The counts from the longitudinally sectioned spindle were determined by constructing a grid representing transverse sections of the indicated thickness. This grid was located in precisely the same position on each serial section and counts were arrived at by adding the number of microtubule profiles appearing in each section in each grid space. Column A = number of microtubules per section, B = number of definite kinetochores attached to the upper half spindle, C = number of uncertain but possible kinetochores attached to the upper half spindle, D and E = numbers of definite (D) and possible (E) kinetochores attached to the lower half spindle. Thus "3" in column B, section 13 of the longitudinally sectioned metaphase spindle indicates that three kinetochores attached to the upper half spindle occurred in that region of the spindle.

I. BRENT HEATH *Mitosis in the Fungus Thraustotheca clavata* 209
Reconstruction of an anaphase spindle prepared in a similar manner to that shown in Fig. 12 but from serial longitudinal sections. In order to trace the tubules through adjacent sections their positions were recorded in a superimposed grid corresponding to transverse 140 nm thick "sections." Because of the obliquity of the pockets and the divergence of the spindle tubules the polar terminations of the tubules (i.e., below approximately section 8 and above approximately section 27) may not be accurate representations and for similar reasons the polar tubules have been omitted completely. The short interzonal tubules are clearly seen. Their terminations were unambiguous because of the wide spacing of the tubules in this region.

FIGURE 13

Diagram illustrating microtubular composition of a metaphase spindle. This diagram was reconstructed from a series of serial transverse sections part of which is shown in Fig. 11. The numbers indicate the section number and pocket denotes the position of the obliquely sectioned pocket areas of the nuclear envelope. A section thickness of 70 nm was assumed. Solid circles denote microtubule terminations which were judged to be clear kinetochores and the open circles denote terminations which were possibly kinetochores but could not be positively identified as such. The identification of kinetochores was a subjective assessment by the author, Figs. 7–11 and 15 and 16 give an indication of the structures conforming to the definition of a kinetochore used in this assessment. The 7 denotes a section in which a clear decision on the presence or absence of the microtubule could not be made due to the general background granularity of the nucleoplasm and the poorly contrasted nature of the tubule. Lines not terminating in circles indicate microtubules which terminated with no detectable differentiation although at the poles these terminations were adjacent to the pocket region of the nuclear envelope. Not all the polar microtubules are included in the diagram. Because of the difficulties of achieving exact correspondence of sections over a considerable distance of the spindle the horizontal location of the lines is arbitrary and should not be taken to indicate pairing of kinetochores.

Continuous tubules, the interdigitating tubules also elongate with the overall elongation of the spindle, being present from the onset of spindle formation through to late telophase. There are typically approximately five such tubules extending for a variable length (in excess of the half spindle length) from each pole.

Polar tubules radiate for typically less than 0.5 µm from each pocket region (Figs. 7–10, 12, 13, 15, and Table 1). They terminate with no detectable differentiation in the nucleoplasm. Approximately 20 such tubules are present at each pole, each maintaining a fairly constant length throughout mitosis.

Chromosomal tubules are defined here as spindle microtubules terminating in identifiable kinetochores. Kinetochores are morphologically defined as structures comparable to those shown in Figs. 7–11, 15, 16, 19, in which (by means of serial sectioning) a single microtubule can be shown to terminate. Such morphologically simple kinetochores are not always easily identified but have been detected in interphase nuclei (as defined by the presence of only one pair of centrioles) and throughout mitosis.

In all observed spindles with a pole-to-pole length of less than 3.7 µm, kinetochores are located at approximately the equator of the spindle (e.g., Figs. 7–10). However, interdigitation of chromosomal tubules from opposite poles does occur (Figs. 7–10), thus a conventional "flat" metaphase plate is lacking. It has not been possible to determine whether this interdigitation is due to extensive oscillations of chromosomes or is a static configuration. Preanaphase kinetochores can occur in pairs (Fig. 18) but this configuration is the exception rather than the rule in T. clavata.

In spindles over 4.0 µm from pole to pole, kinetochores are no longer located at the equator but are found in two groups close to each pole
(e.g. Figs. 15, 16, 19). Typically in these spindles the chromosomal tubules are less than 0.8 µm in length. These changes in kinetochore location clearly suggest that anaphase movement occurs when the spindle is approximately 3.8 µm long, although this figure could be variable due to the small number of spindles examined. The number of kinetochores detected in three serially sectioned spindles is shown in Table I.

At all stages in mitosis all categories of microtubules diverge from each other towards the equator of the spindle and are typically widely spaced (before anaphase > 30 nm tubule to tubule and during anaphase-telphase far more, as seen in Figs. 17-19) with no suggestion of aggregation into bundles. Rarely two tubules can be closely (approximately 10 nm tubule to tubule) spaced (Figs. 11, 15), but this configuration is not a consistent feature of the many spindles examined. Furthermore, if two tubules do lie close together they normally run from the same pole. It is very rare to find closely spaced microtubules which originate from opposite poles. If close lateral association of spindle tubules does occur routinely in significant numbers, it must be for a very short period in the total mitotic process.

After anaphase separation of the chromosomes, spindle elongation continues. During telophase the nucleus and persistent nucleolus both become dumbbell shaped with approximately 10 spindle microtubules in any one cross-sectional area of the interzone. Telophase nuclei with a pole-to-pole distance of 7.4 µm occur. At some length greater than this, mitosis is presumed to be completed by median constriction with appropriate membrane fusions.

In all serially sectioned mitotic spindles there are in addition to the above described microtubules a number of short microtubules which terminate at both ends in undifferentiated regions of the nucleoplasm. The position of these tubules is shown in Figs. 12, 13, 17-19.

**Extranuclear Events**

During interphase (defined by a serial sectioning demonstration of only one pair of centrioles per nucleus) there are approximately 20 microtubules which radiate predominantly parallel to the long axis of the hyphae. These microtubules radiate for an undetermined distance from the region of the centriole pairs (Figs. 4, 14, 20, 24) but they do not directly connect to the centrioles. Typically half of the tubules run towards the apex and half run posteriorly. The nuclear envelope is associated with some of these tubules (Figs. 21, 23) such that the nucleus forms one or two long pointed horn-like projections. At the onset of centriole migration both opposite ends of the nucleus form one or more tubule-associated "horns" (e.g. Figs. 14, 15, 20, 21). This arrangement of horns projecting beyond the poles of the spindle is found consistently at all stages of mitosis in all mitotic nuclei examined. All horns examined by serial sectioning were associated with one or two microtubules.

In addition to the above cytoplasmic microtubules, a further group of two or three tubules develops between the replicated centriole pairs at the onset of their separation and before the formation of the first continuous spindle microtubules (Figs. 3-5). These cytoplasmic intercentriolar microtubules elongate throughout mitosis as centriole separation continues. At later stages in mitosis (e.g., after the spindle is approximately 2 µm long) some of the intercentriolar tubules elongate on beyond the opposite centriole pair and thus run on for an undetermined distance into the cytoplasm.

The mitotic process is shown diagrammatically in Fig. 25.

**Chromosome Number in T. clavata**

Chromosome counts derived from light microscopy of members of the Oomycetes are notoriously unreliable due to the small size of both nuclei and chromosomes. In order to obtain an accurate chromosome count for *T. clavata* an analysis of meiotic prophase I was attempted. An extensive series of antheridia were examined but at present the information is far from complete. However, a series of sections through one prophase nucleus has revealed what appear to be developing pre-synapsis axial cores (Fig. 22). Such cores clearly attach to the nuclear envelope but they cannot be traced far into the nucleus. They appear to merge with the "nucleoplasm" at their unattached ends. By analogy with the work of Moens (1969) in *Locusta migratoria*, this nucleus is interpreted as one in which the axial cores have begun to develop from opposite ends of each chromosome and are thus not yet fully formed. If this interpretation is correct, one would predict that there should be twice as many attachment points as the diploid number of chromosomes. The series of sections at hand covered almost the entire nucleus and re-
FIGURES 17–19 Serial sections of the interzone region of the anaphase spindle shown in Figs. 13 and 15. The micrographs show comparable areas of the spindle and illustrate the way in which it is possible to trace tubules $a$, $b$, and $d$ through serial sections, thus supporting the reality of the terminations of tubules $e$ and $f$ in this region. The functional significance of tubules $d$, $e$, and $g$ which clearly are not parallel to the long axis of the spindle is obscure. However, they are difficult to reconcile with any intertubular bridge force-generating system. Note kinetochore (arrow) in Fig. 19. All $\times$ 43,000.

FIGURE 14 A “late metaphase” nucleus showing the spindle extending between the pocket regions ($p$) of the nuclear envelope and the microtubule associated (e.g., large arrow) hornlike projections ($h$) of the nucleus. Serial sections showed that each horn was associated with only two or three microtubules. This spindle is identified as preanaphase by the equatorial location of the presumed chromatin (small arrows) and equatorial kinetochores identified in other sections. Scale = $1.0 \mu m. \times 43,900$.

FIGURE 15 An anaphase nucleus again showing microtubule associated horns ($h$) and kinetochores (arrows) located near the poles. The arrowed pair of microtubules in the interzone are the only pair in the spindle which were as closely spaced. $p$, pocket regions. Other views of this spindle are shown in Figs. 17–19 and diagrammatically in Fig. 13. Scale = $1.0 \mu m. \times 47,400$.

FIGURE 16 An enlarged view of kinetochores 1, 2, and 3 shown in Fig. 15. $\times$ 70,700.
FIGURE 95 Diagrammatic summary of mitosis in *T. clavata* showing the behavior of the centriole pairs (c), persistent nucleolus (Nu), cytoplasmic microtubules, kinetochores (denoted by cross bars on some spindle tubules), and nuclear envelope. The inter-pocket distance at metaphase (B) may vary from approximately 1.0 to 3.7 μm and at “anaphase-telophase” (C and D) from 4.0 to in excess of 7.4 μm. ?denotes uncertainty in the extent of the microtubules. In D the dashed line indicates the probable existence of pole-to-pole tubules. Certainly there are tubules in the interzone at this time but it has not been possible to demonstrate continuity.

FIGURE 90 An early metaphase spindle showing the horns (h) of the nucleus. The position of the centriole pairs at the poles of the spindle (X) was determined from serial section. A clear microtubule is associated with the upper horn (arrow) and a comparable configuration was evident in serial sections of the lower horn. Scale = 1.0 μm. × 92,300.

FIGURE 91 A detail of Fig. 90 showing the acuminate nature of the nuclear envelope and its very close association with the microtubule (arrows). × 80,500.

FIGURE 92 One of a series of sections of a nucleus in an antheridium showing what are interpreted as developing presynapsis axial cores each attached at one end to the nuclear envelope (arrow heads). Scale = 1.0 μm. × 43,800.

FIGURE 93 Typical transverse section of a pair of microtubules (arrows) associated with the nuclear envelope. Note the conspicuous clear zone around each tubule, an arrangement typical of these microtubules. × 128,400.

FIGURE 94 Transverse section of a centriole located adjacent to a pocket region (p) of the nuclear envelope. The single microtubules between the pocket and the centriole run out into the cytoplasm from this region and are those with which some of the horns of the nucleus will be associated. × 128,400.
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216

DISCUSSION

Mitosis in *T. clavata* is similar to that process previously described in *S. ferax* (Heath and Greenwood, 1968, 1970) and may thus prove to be widespread in the Oomycetes. In both species the centrioles replicate by an unusual end-to-end process which has been discussed more fully elsewhere (Heath and Greenwood, 1970). However, subsequent centriole migration and spindle development can now be described more accurately. Because the centrioles are always associated with the pockets of the nuclear envelope it will be assumed that some undetected structure holds them together. Thus in the following discussion they will be considered as one entity, referred to only as the centriole. In order to separate the centrioles some force-generating system must operate. The only detected structures suitably located at the onset of centriole migration are the nuclear envelope and the intercentriolar cytoplasmic microtubules. McCully and Robinow (1971) suggested that in *Schizosaccharomyces pombe* migration of the equivalent structures could be caused by appropriately localized membrane growth. If the nucleus behaved as a turgid balloon such a system could work, but nuclear morphology suggests that this is not so. Alternatively, the fluid mosaic membrane model (Singer and Nicolson, 1972) does not lend itself to the possibility of the interpocket region of the nuclear envelope acting as a pushing structure. In *T. clavata* the development of the intercentriolar cytoplasmic microtubules must have some functional significance. It is suggested that the elongation of these tubules is responsible for initial centriole migration, although since there are only two or three present it is difficult to differentiate between elongation per se and intertubule sliding with concomitant elongation as the probable means of force production. At present it is difficult to ascertain how widespread this postulated system may be because careful serial sectioning of the appropriate stages of migration has not been reported for other species. However, the concept of microtubule-dependent centriole migration is supported by the work of Brinkley et al. (1967) on hamster cells. As soon as the intranuclear spindle is located between the migrating pockets of the *T. clavata* nucleus it is no longer possible to differentiate the cause and effect relationships of the intra- and extranuclear microtubules since both sets elongate synchronously. Because more nuclear envelope is clearly needed to surround the elongating nucleus it is probable that membrane synthesis occurs at some stage during migration, but that this synthesis is the cause of elongation seems dubious.

A number of recent reviews (Forer, 1969; Luykx, 1970; Nicklas, 1971; Heath, 1973) have adequately discussed the mechanics and possible evolution of chromosome movement systems. Thus, only the specialized aspects of the *T. clavata* system need be discussed. Fundamental to this discussion is the assumption that the kinetochores are correctly named. This assumption is supported by three main points. (a) They are identifiable, differentiated, terminations of spindle microtubules. The only differentiated microtubule terminations reported in mitotic spindles are kinetochores (excluding midbodies which are clearly absent in the *T. clavata* spindles). (b) The terminations are associated with material which is slightly more osmiophilic than the general nucleoplasm. Increased osmiophilia is a common property of chromosomes. The relatively low osmiophilia of oomycete chromosomes is not enhanced by the technique of Aist and Williams (1972) and remains unexplained. (c) The sequential location of the terminations during mitosis is closely comparable to that shown by kinetochores in many organisms. Thus, kinetochore appears to be a valid term if used exclusive of its genetic implications.

In *T. clavata* at least two force-requiring events occur during mitosis, continuous elongation of the spindle and the anaphase separation of the chromosomes. While there appears to be extranuclear mechanisms contributing to the elongation process (see below) it will be assumed that, because the spindle is comparable to other spindles which lack the extranuclear component, the spindle also contributes in part to the elongation forces. The morphology of the spindle enables certain hypotheses for mitotic force production to be ruled out. For example, the intertubule cross-bridge system proposed by McIntosh et al. (1969) seems inappropriate because in *T. clavata* the spindle tubules are typically widely spaced, especially those from opposite poles. The occurrence of close spaced tubules of opposite
polarity as required by McIntosh's model is too rare to merit inclusion as a significant force-generating system. The small number of tubules which run completely from pole to pole are possible candidates for generating spindle-elongating forces by simple elongation due to microtubule polymerization as proposed by Inoué and Sato (1967). The very small number present is not an argument against this proposal if the probable additional extranuclear force-generating system described below is also considered. Furthermore, these continuous tubules exist from the onset of spindle development through until at least anaphase. However, in addition the interdigitating tubules are presumably functionally important and would appear to be associated with spindle elongation since their elongation mirrors that of the whole spindle. Similar interdigitating tubules are reported in Lithodesmium (Manton et al., 1969a, b; 1970a, b) and various mammalian cells (Brinkley and Cartwright, 1971; McIntosh and Landis, 1971). In both these systems intertubular distances and the existence of cross-links support a sliding force-generating system as suggested by McIntosh et al. (1969), but in T. clavata the intertubule spacing is again above that which would favor the cross-bridge hypothesis. If the interdigitating tubules of T. clavata are involved in generating force for elongating the spindle, then the only conceivable mechanisms are elongation and pushing against an undetectable "base" or some form of shear-force producing interaction between the tubules and the spindle matrix as proposed by Subirana (1968). Neither system can be ruled out at present but the recent demonstration of actin-like filaments in certain mitotic apparatuses (Forer and Behnke, 1972) might point towards the tubule-matrix interaction hypothesis, using "matrix" to include actin and other nontubular spindle components. Similarly, while the observation of anaphase chromosomal microtubule shortening is clear, the mechanism of force production is uncertain, although again intertubule interactions seem unlikely for the same space relationship referred to above. Again, a tubule-matrix interaction seems possible, but a depolymerization model is not ruled out. Tubule-matrix interactions imply polarity of the tubules (Subirana, 1968). Because all the spindle microtubules focus on the pockets of the nuclear envelope, it was suggested that in S. ferax (Heath and Greenwood, 1970) these pockets may be analogous to microtubule organizing centers (MTOC's of Pickett-Heaps, 1969). By analogy a similar suggestion is made for T. clavata. Since most of the spindle tubules only focus on one pocket the concept of opposite polarity of tubules from opposite poles is easy to envisage. One further point concerning possible microtubule polarity deserves mention. While tubules from opposite poles need opposite polarity, both interdigitating and chromosomal tubules from a single pole would be expected to have similar polarity because both types of tubule need to generate poleward motion. Only in the few continuous tubules is there the problem of potentially opposite polarity in one tubule.

The absence of a metaphase plate in T. clavata, and other fungi such as Fusarium (Aist and Williams, 1972) would seem to place a severe restriction on balance hypotheses for the metaphase condition (Subirana, 1968; McIntosh et al., 1969) because the varying positions of the chromosomes are unlikely to lead to equal oppositely directed forces. However, prometaphase in higher mitosis is characterized by considerable jiggling of chromosomes. It is possible that in such a rapid mitotic system as in Fusarium (Aist and Williams, 1972) and also in T. clavata the "signal" for anaphase is received before metaphase equilibrium is achieved.

The above components of the spindle of T. clavata are not greatly different from those of the other mitotic systems, but the so-called polar microtubules certainly represent an anomaly. Because they do not change in length or number during mitosis, it is difficult to postulate a function for them. At the present time they are merely recorded as being present. Similarly, the significance of the short fragments of microtubule which lie at various points in the spindle with no kinetochore or pocket association is obscure. There is no evidence to suggest that they are remains of more extensive tubules which existed before fixation. If they are true fragments in vivo their existence raises the possibility of either undetectable organizing centers dispersed throughout the spindle as opposed to the more obvious potential MTOC's, the kinetochores and pockets, or microtubule assembly in the absence of "organizing centers."

In addition to the intranuclear spindle the morphology of the microtubule-associated horn-like projections strongly suggests a force-generating
During interphase the presence of unidirectional horns would suggest that they may be involved in nuclear motility. Movement of fungal nuclei independently of cytoplasmic streaming is a well-known phenomenon, e.g., Wilson and Aist, 1967, and occurs in T. clavata. It is suggested that the cytoplasmic microtubules behave as tracks along which the nuclei are able to move utilizing a nuclear envelope-microtubule force-generating interaction of unknown nature. While only one or two microtubules are associated with each horn there is probably significant additional associated, differentiated cytoplasm not detected with the present techniques, because Hartog (1895) described similar structures in closely allied species.

During mitosis the oppositely directed horns are arranged in a way strongly suggesting that the nucleus is being pulled apart. Because the nucleus is relatively large yet has a small spindle whose poles do not coincide with the extremities of the elongating nucleus, it is suggested that the nuclear envelope-microtubule interaction discussed above is also responsible for generating part of the necessary mitotic forces. An association between membranes and microtubules is becoming increasingly frequent in reports of cell ultrastructure (e.g. Franke, 1971a, b; La Fountain, 1972; Järlfors and Smith, 1969), but only in the migrating nuclei of virus-induced syncytia (Holmes and Choppin, 1968), Closterium (Pickett-Heaps and Fowke, 1970), and the mitotic system of Saprolegnia (Heath and Greenwood, 1970) is the evidence for a dynamic shear force-generating nuclear membrane-microtubule interaction really compelling. However, there is increasing evidence for a membrane-microtubule force-generating system in vesicle transport in axons (Järlfors and Smith, 1969; Schmitt, 1968; Ochs, 1972) and possibly also in pollen tubes (Franke et al., 1972) and the melanophores of Fundulus (Bikle et al., 1966), and the changes in nuclear shape found in certain sperm cells seem likely to involve sliding nuclear envelope-microtubule interactions (e.g., Ferraguti and Lanzavecchia, 1971). Thus the force-generating interactions of membranes, including nuclear membranes, and microtubules, may be a widespread phenomenon. In the aquatic fungi this interaction is not restricted to an involvement in vegetative nuclear motility and mitosis but also occurs in the zoospores of many species where it appears to help attach the flagella to the spore (e.g. Fuller and Calhoun, 1968; Heath and Greenwood, 1971; Heath, 1973b). However, in the mitotic system of T. clavata, as in other systems, an important unsolved problem is the mode of anchorage of the microtubules. Because those involved in the nuclear movements have one end located near the centrioles, these could function as anchors, but, as the centrioles themselves move during the mitotic cycle, complex rearrangements are needed.

Regardless of the force-generating systems, the central objective of mitosis is equipartitioning of the genetic material. Analysis of chromosome movements in the Oomycetes is hampered because of the poor staining of chromosomes in ultrastructural investigations and “the extremely small size of the nuclei and of the chromosomes in any of the water molds makes the problem of determining their number with any degree of certainty an extremely difficult one” (Shanor, 1937) in the light microscope. Despite Flanagan’s (1970) claim that “observations by this author . . . do not bear out these statements” he was not able to be more precise than “10 or more” as the diploid number of chromosomes for S. ferax. In T. clavata, Shanor (1937) reported a diploid chromosome number of 10–14. By use of the technique first used by Moens and Perkins (1969), this study has demonstrated a probable diploid number of 12, thus predicting 12 kinetochores in each half spindle. Such numbers were not observed.

Because the kinetochores are such small structures it is conceivable that some were not detected, yet the data from the six half spindles are reasonably consistent and usually less than 12. If this inequality between meiotic chromosome number and kinetochore number can be established with more clearly visualized kinetochores, it will mean that the concepts of chromosome organization in this group need to be reconsidered. It should be noted that, for example, in Lithodesmium (Manton et al., 1970a) the chromatin is not apparently differentiated into chromosomes at metaphase, and in Ascaris (Boveri, 1904) and various insects (Gurdon and Woodland, 1968) there is not always an equivalent number of “chromosomes” at mitosis and meiosis. Thus the common concept of chromosome organization is not necessarily universal. The fact that the kinetochores of T. clavata are not paired is additional evidence for a nonstandard arrangement of chromatin in these nuclei. However, the simplicity of the kinetochores is not unusual, similar ones

218  THE JOURNAL OF CELL BIOLOGY  •  VOLUME 60, 1974
being present in many fungi such as Fusarium (Aist and Williams, 1972), Ustilago (Poon and Day, 1973), and Schizosaccharomyces (detectable in Fig. 33 of McCully and Robinow, 1971). Clearly, further study of these evolutionarily primitive mitotic systems is required before an accurate picture of their evolution and functional characteristics can be deduced.

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