ARCHITECTURE OF THE MICROTUBULE
COMPONENT OF MITOTIC SPINDLES FROM
DICTYOSTELIUM DISCOIDEUM

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SUMMARY

Ten mitotic spindles from Dictyostelium discoideum have been studied by electron microscopy of
serial sections. We have used computer graphics to track individual microtubules (MTs) in three
dimensions and to compare seven spindles at different stages of anaphase and telophase. The central
spindle of early anaphase is formed by the interdigitation of two sets of pole-associated MTs. The
distribution of MT lengths at this stage is hetero-disperse. During anaphase total MT length
decreases by a factor of about 2 as a result of two opposing changes in MT length: the longer MTs
that interdigitate become even longer, while the short MTs, including those attached to kineto-
chores, become shorter still and decrease in number. The extent of MT interdigitation is less in
longer spindles than in short ones. In metaphase and early anaphase, the MTs are not in an ordered
arrangement as seen in spindle cross-sections, but as anaphase proceeds the MTs cluster into a
square-packed, paracrystalline bundle in which most of the nearest neighbours come from opposite
poles. This arrangement and the condensation-like increase in order suggest the existence of specific
interactions between antiparallel MTs. A quantitative analysis of MT positions supports this inter-
pretation, but direct evidence for convincing bridges between MTs is lacking. The pole-distal ends
of the MTs that interdigitate show an irregular termination (C-shaped ends in transverse view), as is
characteristic of MTs that are either adding or losing subunits. Since it is these interdigitating MTs
that elongate, and since the shortening MTs show the customary blunt endings, we conclude that
subunits add to the interdigitating MTs at their pole-distal ends. This inference, combined with
other structural data, suggests that the interdigitating MTs of Dictyostelium are sliding over one
another as they polymerize in anaphase. It also suggests a simple model for why the spindle becomes
thinner as it elongates. We propose that MT interdigitation defines a region where MTs bind a factor
that will associate only with antiparallel MTs. This factor biases the MT assembly equilibrium
toward polymer. As the shorter MTs slide out of this region, they lose their polymerization
advantage and depolymerize, releasing subunits to contribute to the further elongation of the already
longer MTs. The properties of the Dictyostelium spindle are compared with those of both higher and
lower eukaryotes.

INTRODUCTION

Structural analyses of biological machines have often provided important insights
into the mechanisms by which they work. The mitotic spindle is no exception, but this

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apparatus has presented a significant challenge to recent microscopists because spindles are large relative to the thickness of a customary section for electron microscopy, and most spindles lack the order found in vertebrate striated muscle, where the analysis of a small domain permits an understanding of a large structure, thanks to its symmetry. Reconstruction by serial section analysis has therefore become an important route to the elucidation of spindle geometry (Manton, Kawallik & von Stosch, 1969; McIntosh & Landis, 1971; reviewed by Fuge, 1977). For such a study one wants small spindles to facilitate a detailed fine-structure analysis. Dictyostelium is an appropriate organism for such a study because the metaphase spindle is only 3 μm long and contains few microtubules (MTs) compared to the spindles of higher eukaryotes (Moens, 1976; Roos & Camenzind, 1981). Mitosis takes place essentially within the nuclear envelope; the mitotic centrosomes are 'spindle pole bodies', which initiate MTs that grow right into the nuclear space. The chromosomes have visible kinetochores, each of which attaches to a few MTs. Both chromosometo-pole motion and spindle elongation occur during anaphase, so in Dictyostelium we find the features of a classic mitotic spindle, and an important ground-work has been laid by work already published.

In this paper we present the results of an analysis of the MTs from 10 mitotic cells, five of which have been reconstructed in their entirety, the rest studied to varying extents. Two limitations of such an analysis are immediately evident: (1) conclusions are necessarily based on a small sample size; and (2) inferences about time-dependent events must be drawn from comparisons of different cells at different stages of the mitotic cycle. Further, there is the omnipresent problem of preparative artifacts associated with the study of fixed cells. Nonetheless, several features of the behaviour of mitotic microtubules emerge from our study that lead us to believe that the results are descriptive of mitosis in living Dictyostelium. In addition, the results show that at metaphase the microtubules in this spindle form a structure that is very much like a small version of the spindles found in cells of vertebrates, while the well-ordered central spindle that forms in Dictyostelium during anaphase strongly resembles that found from prometaphase through telophase in diatoms. The morphology of the Dictyostelium spindle therefore provides an element of continuity between the almost crystalline spindles of some unicellular organisms and the less-ordered spindles of the higher eukaryotes. The detailed information that we have gathered from Dictyostelium allows us to suggest how certain simple rules for MT assembly and sliding might give rise to some of the structural transformations of the spindle during mitosis.

MATERIALS AND METHODS

Stock cultures of Dictyostelium discoideum Raper, strain NC-4, were maintained as previously described (Roos & Camenzind, 1981). For electron microscopy, cells from log-phase cultures were plated on square coverslips, fixed, and flat-embedded as described. Individual cells at particular stages of mitosis were selected and photographed in plastic under immersion oil, scribed with a diamond-tipped marker, and excised for serial sectioning. Sections were picked up on Formvar-coated slot grids and stained with uranyl acetate and lead citrate. Microscopy was performed either on a Philips 300 electron microscope equipped with a goniometer stage or on a Hitachi HU-11E.
Essentially complete sets of serial sections through the spindle were obtained from three metaphase, four anaphase and three telophase cells. Microtubule (MT) tracking was undertaken with the help of an interactive computer graphics system based on a CDC-6400 as previously described (McIntosh, McDonald, Edwards & Ross, 1979). Complete reconstruction was achieved for three anaphase and two telophase spindles. One additional anaphase (Ana 4) and one telophase (Telo 1) were reconstructed only in the region near the plane equidistant between the poles; these truncations were due to technical problems, such as missing sections or poor staining in some areas. The complexity of the MT structure in the metaphase spindles prevented unambiguous tracking of all the MTs pole-to-pole in any of these cells.

The names assigned to the cells (Ana 2, etc.) reflect the order in which the cells were processed. The cells' relative times in mitosis were deduced from their morphology in both the light and electron microscopes. The principal criterion used was the length of the spindle, which increases monotonically throughout anaphase (Roos & Camenzind, 1981).

Measurements within the plane of section were calibrated with a replica grating ruled at 54,000 lines per inch. Distances perpendicular to the plane of section were estimated by comparing them to the thickness of a single section. In three cells the relative thickness of each section was measured by densitometry. A Joyce-Loebl microdensitometer was used to determine the optical density of the images of the sections on low-magnification electron micrographs taken in the linear region of the film's grey scale. Over many densitometric scans the optical density contributed by the sections varied by less than 10%, so in general we assumed that all sections of a given set were of the same thickness. This thickness was estimated by measuring the pole-to-pole separation on a light micrograph of the fixed cell taken before sectioning and then dividing this distance by the number of sections in the spindle.

The small size of metaphase and anaphase spindles in D. discoideum sometimes prevented us from orienting the cells correctly for sectioning, so a goniometer stage was an essential tool for cross-section microscopy. Oblique sections present a difficulty, however, which is not corrected by reorientation of the sections in the microscope. When sectioning obliquely into a spindle pole, one encounters microtubules on one edge of the pole before reaching the other edge (Fig. 1). This situation is evident in the early anaphase cell Ana 1 (Fig. 10), where four sections were necessary to get from one edge of the pole to the other. Since the spindle pole body of some cells is oblique to the spindle axis, the stereo projections of the spindle are presented without a correction for the sectioning distortion (Fig. 1f), but in our studies of the positions of microtubule ends along the spindle axis, we have brought the polar ends of all the MTs to the same polar section, and then extended them by their natural length along the spindle axis (Fig. 1e). There is a small, natural variability in the position of the polar end of spindle tubules even in cells sectioned perpendicular to the spindle axis (±1 section), so some error in the position of the pole-distal end results from our method of justifying the position of all polar ends to a single section. This error is small, however, compared to the errors that would have resulted from leaving obliquely sectioned cells uncorrected. Further, during anaphase and telophase, many of the pole-distal MT ends near the plane equidistant from the two spindle poles (the spindle mid-plane) are 'C-MTs', i.e., MTs with jagged ends in which some protofilaments extend further than others. The paraxial location of the pole-distal MT end is therefore impossible to define with precision. While tracking, we followed the convention that a MT was scored so long as it was represented by enough wall to make more than 180° of arc at a normal MT diameter. The total length of the non-tubular portion of the MTs was usually about 0.15 μm and rarely exceeded 0.35 μm. MT lengths described here are therefore accurate to only about ±2 sections (about 0.15 μm).

The interactive computer system was used to prepare informative reconstructions of the spindles for view and analysis. We fitted fourth-order polynomials to the points along each MT and then generated stereo projections of these families of curves (e.g. see Figs 8, 9), as previously described (McIntosh et al. 1979). When the MTs are present in only a few transverse sections, however, there are insufficient data to generate a smooth curve, and the MTs appear as zigzags made from line segments one section long. In simple stereo projections, the clustering of the MTs at the poles and the equator usually prevented a meaningful viewing of their interrelationships. To improve image clarity, we therefore modified all the stereo projections in the same, systematic way: the spindles were broadened in the plane perpendicular to the spindle axis by a factor of 2, increasing the spacing between the lines representing the MTs without lengthening the pole-to-pole distance. This transformation has the advantage that one can then follow the trajectories of individual MTs in three
Schematic of Microscopy and Reconstruction for a spindle

cut in Oblique Section

Fig. 1
**Microtubules of Dictyostelium spindles**

dimensions with a stereo viewer. It has the disadvantage that all the spindles are made to appear fatter and more curved than they really are.

**RESULTS**

**Longitudinal views of the spindle**

*Electron micrographs.* At metaphase (Fig. 2) MTs run through the centre of the nucleus, converging at either end on the spindle pole bodies (SPBs). Only one SPB is shown in this micrograph. Size, shape and orientation of each SPB relative to the spindle axis can vary considerably. Dense material fills the space between the MTs near the SPB. Two chromosomes are visible in this micrograph; the section passed peripherally through the kinetochores of the chromosome on the left (CH1) and medially through the kinetochores of the chromosome on the right (CH2). The chromosomal ends of the kinetochore MTs (kMTs) are in an amorphous, electron-opaque layer at some distance from the curved plaque that lies close to the chromatin. The polar ends of the kMTs insert into the SPB, as do the polar ends of many other spindle MTs. The MTs marked by arrows provide evidence that one kMT can connect a chromosome directly to the SPB at metaphase. The granular material of the nucleoplasm is largely the dispersed nucleolus that fills the bulges of the nucleus.

In anaphase nuclei, most kMTs are shorter than at metaphase and some are oblique to the interpolar axis (Fig. 3). Some of the MTs that are not attached to chromosomes cluster in a bundle that runs between the poles of the elongated spindle. Such an
interpolar MT bundle is often called a 'central spindle'. In the region approximately midway between the poles the MTs are evenly spaced (insert in Fig. 3). Many MTs appear to end in this region. In some cases the normal MT image terminates as a single line that can be up to 0.5 μm long. We interpret these images as longitudinal or oblique profiles of jagged MT ends in which some protofilaments extend further than others (often called C-MTs because of their images in cross-section).

In Dictyostelium chromatin condensation is not always visible in thin section, but we can recognize telophase by the fact that the nucleus becomes shaped like a dumbbell (Fig. 4). The nuclear envelope wraps each nascent daughter nucleus, but at some stage during the initiation of telophase it breaks, leaving the middle portion of the central spindle bare. Kinetochores with short kMTs are clustered near the spindle poles (Fig. 5). The MTs of the central spindle form an apparently continuous shaft, but they are more tightly bundled near the poles than midway between the nuclei (Figs 4–6). An amorphous, dark-staining material lies between the MTs near the middle of the interzone, and many jagged MT ends are apparent (Fig. 6).

Axial distributions of microtubules. With the data available from serial sections one can represent spindle structure at different mitotic stages as distributions of MT number along the spindle axis (Fig. 7). The curves from all stages display peaks near the spindle poles, but the trough in the mid-region of the metaphase and early anaphase spindles (Meta and Ana 1 in Fig. 7) is replaced by a hump in the mid-region of the later anaphases and telophases (Ana 4, 3, and Telo 2, 1). Taken together, the six curves show that the number of MTs per cross-section decreases markedly with chromosome segregation and spindle elongation. From metaphase to telophase this decrease is by a factor of about 3 in the spindle mid-region, and by 4–6 halfway between the spindle mid-plane and the poles (Fig. 7).

Stereo projections of spindle structure and the distributions of MT length and position along the spindle axis. Three-dimensional reconstructions of the MT component in spindles at different stages of mitosis give a more graphic view of the time-dependent changes in spindle architecture (Figs 8, 9, 11–14). Owing to the transformation explained in Materials and Methods the spindles shown here appear thicker and more bowed than they really are, but because all the spindles shown have been transformed equivalently, comparisons between them should be informative.

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**Fig. 2.** Part of a longitudinally sectioned metaphase nucleus of *D. discoideum*. The material of the dispersed nucleus (nu) fills most of the nucleus. A spindle pole body (spb) lies in a hole in the nuclear envelope (ne). The other spb was in a different section. Two chromosomes (ch1 and ch2) with pairs of sister kinetochores are visible. The MTs marked by open arrows run without interruption from kinetochore to pole. The obliquely sectioned non-kinetochore MTs constitute the central spindle of metaphase. A piece of double membrane, similar to the ne, lies inside the nucleus (filled arrow). ×61 500.

**Fig. 3.** Nucleus in mid- to late anaphase. The elongated central spindle is a bundle of MTs that interconnects the poles. Its MTs are regularly spaced in the mid-region of the interzone (see also the insert). Some MTs end in the interzone as a single line (insert, arrow). The kinetochores (k) are single, and some are close to the spindle poles. Note that the MT of the kinetochore nearest the left pole slants across the central spindle. ×34 500; insert, ×73 900.
Fig. 4. Nucleus in telophase. The MTs of the central spindle form an interpolar shaft (the SPB of the left pole was in a serial section). They are more closely packed near the poles than in the cytoplasm between daughter nuclei (n1, n2). ×20 400.

Fig. 5. Serial section to Fig. 4. The MTs of the central spindle penetrate deep into the nucleus and are tightly bundled in parallel alignment, except for a single one (open arrow). Kinetochore MTs (solid arrow) are more or less oblique to the central spindle. ×41 500.

Fig. 6. Detail of the extranuclear portion of the central spindle (from the same cell as Figs 4, 5). The lines marked by open arrows are the jagged, non-tubular ends of MTs. There is much dense material between the MTs. ×79 300.
Microtubules of Dictyostelium spindles

The early anaphase spindle (Figs 8, 9) is just longer than that of metaphase (cf. Fig. 7). The longitudinal view shown in Fig. 8 is familiar, but the transverse view of Fig. 9 may need explanation. The entire spindle is seen along the pole-to-pole axis, so stereo viewing reveals the convergence of the MTs on both spindle poles and the expansion of the spindle equator. Unfortunately, kinetochores were not sufficiently distinct in transverse sections to let us identify them unambiguously. Consequently, we were unable to distinguish kMTs from non-kinetochore MTs (nkMTs) in all cases and could not define exactly the separation of the daughter chromosomes. Nonetheless, we

![Graph showing distributions of MT number along the spindle axis.](image)

**Fig. 7.** Distributions of MT number along the spindle axis, as explained for Fig. 1E. The median plane between the poles was in each case taken as the plane of spindle symmetry and distance along the axis is expressed in μm from that plane. The differences in the lengths of the spindles fixed at metaphase, anaphase and telophase, as well as the differences in MT number at each transverse section are evident. In metaphase and early anaphase (Meta, Ana 1) the profile shows a trough at the equator and a peak near each spindle pole. The profiles of the later anaphases (Ana 4, Ana 3) and of the telophase (Telo 2) have a hump in the interzone and peaks near the spindle poles. The arrows at the ends of some of the lines imply that data collection stopped at that point.
have determined that most spindle MTs are nkMTs. Taking account of the seven chromosomes of *D. discoideum* (e.g., see Robson & Williams, 1977) and two to three kMTs per chromosome (Moens, 1976; and our own unpublished observations), the predicted 14–21 kMTs are only a fraction of the 140–160 MTs seen in these half-spindles. The nkMTs are of diverse lengths: many are short and end between pole and equator, whereas a few extend all the way from one pole to the immediate vicinity of the other. It is difficult to see this because there are so many MTs superimposed in the stereo views.

Figs 8, 9. Stereo views of an early anaphase spindle (Ana 1) in paraxial projection (Fig. 8) and in polar projection (Fig. 9). The orientation of the sections was oblique with respect to the spindle axis, therefore the artifact illustrated in Fig. 1 is evident. Some MTs run long distances, whereas others are only a few sections long. Most of these short MTs are associated with the poles (pMTs), but a few exist as fragments scattered through the body of the spindle. ×17 000 parallel to the axis; ×34 000 perpendicular to it.
Fig. 10. Distribution of MT lengths and positions along the axis of the early anaphase spindle shown in Figs 8, 9. The MTs are arranged as explained in Fig. 1g, with unbroken and broken lines enveloping the populations of MTs associated with the two poles. The abscissa represents position in μm from the spindle equator; the ordinate is used to lay out individual MTs side-by-side on top of one another. The polar ends have all been justified to a single section (see Materials and Methods). Microtubules not associated with a pole (fragments) are displayed as horizontal lines at the top of the graph. The cross-hatch portion of the figure represents the length of MTs that extends beyond the spindle mid-plane. The bracket on the abscissa at the mid-plane marks the region analysed for positional specificity of near-neighbour MTs (see below).
It is easier to study the distribution of MT lengths and the axial positions of the MTs in representations of spindle structure like Fig. 1G. We call these graphs MT axial position distributions. From the axial position distribution of Ana 1 (Fig. 10), it is evident that there is a broad spread of MT lengths ranging from approximately 0.25 \( \mu m \) to just over 3 \( \mu m \), essentially the pole-to-pole length of the spindle. Another feature apparent in this diagram is that the spindle is constructed from two families of MTs, one emanating from each pole. The cross-hatched area that is below both lines on the graph represents the fraction of the total MT contour length, which is long enough to extend into the opposite half-spindle.

We tried to reconstruct metaphase spindles for comparison with this and other anaphases. The greater number of MTs in the metaphase spindle, the obliqueness of the MTs as they converge towards the poles, and perhaps bad luck, defeated five attempts. The MT distributions of the three metaphase cells from which essentially complete sets of serial sections were obtained all looked similar to the one metaphase shown in Fig. 7, with varying degrees of asymmetry about the spindle equator. We are convinced from the tracking we have done, however, that there is one difference between the metaphase and anaphase spindles of Dictyostelium: considerably more MTs extend from one pole to the other at metaphase. In our two best metaphase trackings we counted 11 and 15 MTs, respectively, that we could confidently call continuous from one polar region to the other. By early anaphase, however, these continuous MTs have largely gone (Fig. 10). Except for this change and the slightly increased pole-to-pole distance, we could find no difference between the MT arrangements of metaphase and early anaphase.

Figs 11—13 are stereo drawings of two other spindles in anaphase and one in telophase. The mid- and late anaphase spindles (Figs 11, 12) resemble Fig. 3. Spindle elongation was well under way at the time of fixation, and the bunching and parallel alignment of the MTs in the central spindle are evident, even though spindle breadth is exaggerated in our stereo projections. Overall, there are visibly fewer MTs than in the early anaphase spindle (cf. Figs 7, 8). MT density is greatest near the poles and near the spindle mid-plane. Furthermore, by following individual MTs one can recognize that many nkMTs interdigitate in a region approximately 2 \( \mu m \) wide near the spindle mid-plane.

Except for a few divergent MTs, the spindle of the telophase cell is more tightly bundled than those of anaphase (compare Fig. 13 with Figs 11, 12). Near the poles

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Fig. 11. Stereo view of a spindle in mid-anaphase (Ana 2) in paraxial projection. This spindle is longer than that of Ana 1 (Fig. 8) and the MTs of the central spindle are more highly ordered. Many quite short MTs are associated with the poles. \( \times 17000 \) parallel to the spindle axis; 34,000 perpendicular to it.

Fig. 12. Stereo view of a late anaphase spindle (Ana 3). Further spindle elongation and MT clustering in the interpolar bundle are evident. The dissimilarity of the MT packing at the two spindle ends is associated with a difference in the shape of the spindle pole bodies (data not shown). The MT fragments at the upper right were highly oblique to the spindle axis and resemble some MTs near the left pole of Fig. 3. \( \times 17000 \) parallel to the spindle axis; \( \times 34000 \) perpendicular to it.
Microtubules of Dictyostelium spindles

Figs 11, 12
Figs 13, 14
Microtubules of Dictyostelium spindles

there are mostly short MTs. The longer MTs of the central spindle clearly interdigitate in the mid-zone, where the spindle diameter is greater and where MTs are more widely spaced than closer to the poles. Because the ends of the interdigitating MTs are not in register, a 'zone of overlap' is not clearly set off from the rest of the spindle, but scrutiny of the MT ends near the spindle mid-plane shows that the extent of interdigitation in this cell is less than that in the anaphase cells. Even in late telophase (Fig. 15) the interpolar spindle retains its design as a fibre formed from two interdigitating sets of MTs, with a relatively short segment of overlap.

Comparisons of the MT axial position distributions from different stages of anaphase and telophase (Figs 10, 15–19) reveal two aspects of the structural differences between the MT components of spindles fixed at different times in mitosis. (1) Short MTs that are prevalent in Fig. 10 and in the partial reconstructions we have obtained from metaphase cells are fewer in number in the longer spindles of late anaphase and telophase. While we may assume from longitudinal images (e.g. Figs 2, 3, 5) that kMTs shorten as the spindle elongates, the distributions show that short nkMTs also are fewer and shorter in cells fixed after spindle elongation has begun. (2) The long MTs that remain in elongated spindles are longer than the longest MTs of earlier anaphase. Clearly there is a complex shift in the factors affecting MT assembly to permit this bidirectional change in length distributions during Dictyostelium anaphase.

The comparison of spindles at different mitotic stages is facilitated by examining these raw data in different quantitative ways. The areas under the broken and the unbroken lines of Figs 10 and 15–19 correspond to the total amount of polymer associated with each spindle pole. The area beneath both curves (e.g. the cross-hatched region of Fig. 10) represents the total length of those portions of MTs that are long enough to extend into the opposite half-spindle where they might interact with MTs from the opposite pole. These different areas are plotted in Fig. 20 as a function of spindle length, interpreted as time in mitosis. The increase in MT length as the spindle elongates does not quite compensate for the MT shortening and the decrease in MT number that occur in anaphase, so the total length of polymer decreases between early anaphase and telophase. The area that is simultaneously beneath both curves also decreases, suggesting that the extent of interdigitation of the two half-spindles lessens as spindle length increases.

The frequency distributions of MT lengths in three anaphase spindles and one telophase are depicted in Fig. 21. In early anaphase (Ana 1) there are two shoulders on

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Fig. 13. Stereo view of an early telophase spindle (Telo 2), showing an elongated central spindle and some short MTs clustered near the poles. The endings of the MTs from each pole can be seen near the spindle mid-plane. Although they are not in register they lie closer to the mid-plane than do the endings of MTs in earlier mitosis (compare with the spindles of early, mid and late-anaphase; Figs 8, 11, 12). ×17,000 parallel to the spindle axis; 34,000 perpendicular to it.

Fig. 14. The equatorial part of a later telophase cell (Telo 1). Because some sections were lost, the reconstruction could not be carried from pole to pole. The region of MT interdigitation is, however, drawn in its entirety. 17,000 parallel to the spindle axis; 34,000 perpendicular to it.
Figs 15, 16
Figs 15–19. MT axial position distributions as in Fig. 16. The graphs are arranged in order of increasing spindle length (the spindle of Telo 1 measured 8.5 μm from pole to pole in the light micrograph taken before sectioning). The brackets on the abscissas are as in Fig. 10. See text for a detailed explanation.
a steeply decreasing curve. The distribution from the mid-anaphase spindle (Ana 2) is similar, but the number of short MTs has decreased and the common lengths of those remaining have decreased, while the MTs that define a distribution peak at greater lengths (2–3 µm) have increased slightly in length. The curves for anaphase and telophase are again similar, but here the distribution peaks representing long MTs are broadened and shifted to the right, an indication of uneven MT elongation. The area under each of these curves and bounded by any two vertical lines that represent the extreme values of MT lengths under consideration is equal to the number of MTs in a given spindle whose lengths are between these limits. If one determines the total area under all the anaphase and telophase curves, it is evident, as expected from Fig. 7, that the total number of MTs decreases with time in mitosis. If, however, one considers only MTs longer than 1.8 µm – that is, just longer than one-half the length of the early anaphase spindle – the total number of MTs in this category remains approximately constant during mitosis. The number of MTs shorter than 1.8 µm decreases dramatically.

Transverse structure of the spindle

Electron micrographs. The transverse image of the Dictyostelium spindle varies with position along the spindle axis. Further, the different cross-sectional morphologies show different changes with time in mitosis. Right at the pole, the MTs are almost close-packed throughout mitosis (Figs 22A, 23A, 24A,B, 25A). The predominant packing is hexagonal, with many MTs so close to their neighbours that their
walls practically touch. At the spindle mid-plane, the spacing between MTs is generally larger and more varied; at metaphase and early anaphase (Figs 22c, d, 23c) there is no obvious order to the cross-sectional arrangement of the MTs, apart from their tendency to lie within the boundary of the spindle. By mid-anaphase, however, the arrangement at the mid-plane is obviously ordered, showing domains of semi-crystalline arrangement with a predominance of square packing (Fig. 24d). Through telophase, the mid-plane retains a similar appearance (Figs 25d, 26b). The region of the spindle between the pole and the mid-plane shows more variation in structure. In metaphase, anaphase and early telophase it appears to be the least-ordered portion of the spindle (Figs 22c, 23b, 24c, 25c). By late telophase, however, the MTs in this area

![Diagram](image_url)

**Fig. 21.** Four curves showing the distributions of MT lengths in cells ranging from early anaphase (Ana 1) to mid-telophase (Telo 2). These are presented as running averages of three adjacent bins to provide a limited degree of smoothing to the data. Note that area on this graph does not correspond to total MT length, because unit area on the right side implies more polymer than unit area on the left.
have become as tightly packed and ordered as the region adjacent to the poles (Fig. 26A). Here again, hexagonal packing predominates, and the appearance of the MT bundle is distinct from that seen near the spindle mid-plane where MTs from the two half-spindles interdigitate.

**Analysis of the spacings between MTs.** We have taken advantage of the numerical representations of MT position characteristic of our computer-facilitated method for spindle reconstruction to analyse the distribution of MTs around one another at

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**Fig. 22.** Transverse sections of a metaphase spindle (A, B, C, D, E are the 1st, 3rd, 6th, 11th, 12th sections inside the SPB, respectively). From the pole towards the equator the MTs diverge rapidly from a tightly packed to a largely disordered array (A–C). Several staining densities that we interpret as chromosomes are labelled with solid arrows in E. The density marked $k$ in E is a chromosome whose kinetochore is visible in D. The small circles of light staining marked with open arrows in D are relative clear examples of the beginnings of kMTs. Note the C-shaped profiles and other incomplete MTs in C, D, E. ×65 000.
different positions along the spindle axis and at successive mitotic stages. Fig. 27 shows a radial distribution analysis of the MTs near one pole of a mid-anaphase spindle (Ana 2 of Fig. 24). Although only a single cell is shown here, the other cells at metaphase, anaphase and early telophase gave essentially the same pattern. The frequency with which MTs lie at a given distance from one another, centre-to-centre, has been calculated as described in the legend to Fig. 27. From Fig. 15 it is clear that the sections represented here are from a region of the spindle containing MTs of only one polarity. Near the pole, the MTs are so tightly bunched that the most common spacing is about 30 nm, only slightly larger than the MT diameter (cf. Fig. 24B). With increasing distance from the pole the MTs are spread farther apart and become more disordered (cf. Fig. 24C), as evidenced by the loss of the peak in the radial frequency distribution.

Near the spindle equator, MTs of two polarities interdigitate, and a new kind of order appears in their arrangement (Fig. 28). There is a marked preference for neighbouring MTs from opposite poles to lie at a specific spacing of about 42 nm (unbroken lines). The most common spacing decreases slightly from early to mid-anaphase (compare Fig. 28A, B, C). MTs from the same pole seem largely to ignore each other and lie at random (broken lines). The only exceptions emerge in the better-ordered zones of interdigitation, which occur in mid-anaphase–telophase, where peaks are also seen in the like-neighbour distributions. In Ana 2 and Telo 3 these

Fig. 23. Three transverse sections (the 1st, 3rd, 13th from the left-hand pole of Fig. 10) from an early anaphase spindle (Ana 1). The oblique orientation of many of the MTs required us to take as many as five pictures of each section at different angles of tilt to identify and locate each MT with confidence. There are several C-profiles and other incomplete ends of MTs in the middle section (C). ×85,000.
Figs 24, 25
Fig. 26. Two transverse sections from a late telophase spindle that was not tracked. A. A section of the central spindle inside one of the daughter nuclei near a spindle pole. Note the close, hexagonal packing of the MTs and the links between many of them. B. A section approximately midway between the pole in the cytoplasmic region of the spindle. The MTs are more widely spaced than in A; there are several C-shaped profiles and other incomplete MTs. Links between the MTs are not obvious, but dense material fills the space between many of them. ×139,000.

Fig. 24. Four transverse sections from a mid-anaphase spindle (Ana 2). They are the sections containing one SPB (A), and then sections 2, 4, 24 (B–D) from the left pole as seen in Fig. 15. The longitudinally and very obliquely cut MTs in A and B are extranuclear 'astral' MTs. The fine granular staining around the spindle MTs in B is the tangentially sectioned nuclear envelope. We interpret the dark grey blobs marked with solid arrows in C as chromosomes, but we were unable to identify kMTs in this cell. D. The middle section of this set. ×90,000.

Fig. 25. Four transverse sections from a telophase cell (Telo 2), 1, 8, 28, 34 sections from the right pole as seen in Fig. 18. The MTs of the central spindle are tightly bundled near the pole (B), but more loosely packed in the mid-zone (D). The single MT in the upper right of C is one of the two MTs deviating from the central spindle in Fig. 13. Hook-shaped appendages decorate two MTs in C. These are jagged ends of MTs that begin as true MTs several sections away. ×90,000.
departures from a uniform distribution of like neighbours can be interpreted as a second-order periodicity at \( \sqrt{2} \) times the lattice constant of a regular square array in which nearest neighbours are all of opposite kind. Indeed, one has the impression of a predominance of square packing in most of the pictures from near the mid-plane of cells in mid-anaphase to telophase (e.g. see Fig. 24d). This impression is substantiated by an analysis of the frequency of angles between adjacent vectors constructed to run from one MT to all its near neighbours, i.e. those separated from it by less than 50 nm, centre-to-centre. For the mid-region of cells Ana 2, 3 and 4, and Telo 3, such histograms show a well-defined peak at 90°, a characteristic of square packing (Fig. 29 for Ana 3, 4). In early anaphase (Ana 1), there is only a tendency toward such a peak, and by late telophase (Telo 1) the peak has broadened and shifted to nearer 60°, suggesting a change to closest packing. An equivalent analysis of the MTs immediately adjacent to the poles shows a favouring of 60° (data not shown). The region midway between the poles and the spindle mid-plane shows no evidence for angular order until late telophase, when the blatantly hexagonal arrangement seen in Fig. 26A appears.

![Fig. 27. Radial distribution of MTs near one pole of a mid-anaphase spindle (Ana 2). Distances between MT centres were determined from the digitized coordinates of the MT centres. All inter MT spacings up to 135 nm were considered. For each micrograph the calculated distances were filed in bins 2.5 nm wide, e.g. all MTs separated from a neighbour by 30–32.5 nm were pooled. The number of MTs in each bin was divided by the average distance represented by that bin to correct for the linearly increasing probability in a uniform distribution of finding MTs separated by greater distances. The unbroken line represents data from the three sections of the spindle nearest to the pole (i.e. the section shown as Fig. 24a and one section on either side of it). The broken line represents data from the next three (Fig. 24c and the next two beyond it); the dotted line data from the three beyond that.](image-url)
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Analysis of changes in MT packing during anaphase. The data describing near-neighbour positions provide evidence for a specific structural inter-relationship between antiparallel MTs in the anaphase–telophase zone of interdigitation. The fact that a particular spacing between MTs from opposite poles predominates even in early anaphase (Fig. 28A), when the eye perceives no marked order in the spindle.

Fig. 28. Radial distributions of MTs at the equator of six mitotic cells arranged on the basis of the length of their spindles (Ana 1 to Telo 1). The distribution from Ana 3 was similar to that of Telo 3 and is not shown. The abscissae are identical, but the scales on the ordinates vary depending on the height of the peak. All data sets are based on the five sections distributed symmetrically around the mid-plane of the spindles (see the brackets on the abscissae of Figs 10, 15–19). The unbroken line shows the relationships between near-neighbour MTs from opposite poles (MTs from the west pole as neighbours of MTs from the east); the broken line illustrates the same for neighbours from like poles (west around west and east around east).
Fig. 29

Frequency

Angle (°)

Ana 1
Ana 4
Ana 3
Telo 1

Fig. 29
cross-section (Fig. 23c), suggests that the formation of defined spacings precedes the formation of ordered packing. That is, local order appears first, followed by the gradual establishment of long-range order. If increased numbers of interactions that define the spacing between MTs are a cause for the order that subsequently appears, one would predict that successive stages in mitosis should show increased order in MT arrangement and that there should be a gradual increase in the numbers of MTs in groups whose members are separated by less than 50 nm centre-to-centre.

Fig. 30. Radial distributions of east MTs around west MTs in the five sections near the equator from three spindles graphed on a single set of axes. The cells were selected to span the early to mid-anaphase period when the central spindle appears to be taking on order (Ana 1, 4, 2). The data were normalized to minimize the impact of different numbers of MTs per spindle cross-section on the comparison between cells. In a random arrangement of two classes of points, one would expect proximity of the two classes in analogy with mass action: the concentration of near neighbours should be proportional to (concentration of class A) times (concentration of class B). The number of MTs per unit area in the equatorial cross-sections of the different cells is approximately constant (±5 %), so the cross-sectioned area is proportional to total number of MTs. The equation can therefore be rewritten: (no. near neighbours)/(NE + NW) = K[NW/(NE + NW)][NW/(NE + NW)], where K is a proportionality constant and NE = no. east MTs and NW = no. west MTs. Edge effects become important with small NE + NW, because MTs on the edge cannot have as many near neighbours as those in the centre of the array. One must therefore correct for the ratio of the perimeter to the area of the bundle. This should be proportional to \(\sqrt{NE + NW}/(NE + NW)\), so an observed 'concentration' of interactions should be multiplied by a number proportional to \(\sqrt{NE + NW}\) to estimate the number that would have been seen under conditions of constant cross-sectioned area. Our normalization for the number of interactions was therefore division by \(NE + NW\) for the data from each section.

Fig. 29. Distributions of the angles between vectors connecting each MT in the five equatorial sections described for Fig. 28 to all its near neighbours, i.e. those lying closer than 50 nm, centre-to-centre. For the early anaphase spindle (Ana 1) there may be a broad peak around 90°. The later anaphases Ana 4 and Ana 3 show sharp peaks at 90°, whereas the peak for the telophase (Telo 1) is broader and ranges to smaller angles.
Fig. 31
We have tested this prediction of increasing order by comparing the peaks in the radial density distributions at different stages of mitosis. Fig. 30 shows the radial distributions of near-neighbour positions for three cells normalized for comparison as described in the figure legend; the increase in order between early and mid-anaphase is evident. The order, measured by either peak height or peak area, increases by a factor of about 3, while the increase in MT packing density is modest (about 5%). Late anaphase and telophase cells resemble mid-anaphase.

The prediction concerning the tendency of MTs to cluster has been tested by constructing a programme to determine the number of MTs in ‘groups’ where each member of a group must be 30–50 nm from at least one other member of the group (Fig. 31). MTs from one pole are called ‘east’ (E) and MTs from the other ‘West’ (W). Each graph in Fig. 31 shows the frequency distribution of MTs in groups as a function of group size (unbroken lines), first for E/W pairs and then for E/E and W/W pairs. The number of MTs in large groups is always greater when E/W pairs are considered (compare Fig. 31A with B, C with D, and E and F). This tendency increases with time in mitosis (compare Fig. 31A with C and E). As a control for a fortuitous packing effect independent of MT polarity, we have added a feature to the programme that analyses the groups. After the real distribution of the groups has been determined, the co-ordinates that represent the MTs from each pole are juggled randomly between E and W, so that polarity assignments become random while MT positions are retained. The analysis of group sizes is then rerun and compared with the analysis using real polarity assignments. The broken lines in the graphs of Fig. 31 display the juggled group distributions. In each case the distribution of E/W group sizes is shifted to the left, indicating a smaller average group size with random polarity assignments (Fig. 31A, C, E), while the distribution of E/E and W/W group sizes is moved to the right (Fig. 31B, D, F). This demonstrates that the groups that form, even early in anaphase, are biased from random, preferring to be made with E and W MTs as near neighbours.

Bridges between microtubules. The morphometric evidence for a specific inter-relationship between MTs from the two poles in the zone of MT interdigitation suggests the existence of specific inter-MT bonds. We have looked for structurally defined bridges between MTs in different regions of the spindle and at different stages of mitosis. Clear bridges are usually rare in our preparations. They are frequent only
in telophase when they are seen outside the zone of MT interdigitation (e.g. see Fig. 26A). The space between the MTs in the zone of interdigitation is filled with poorly structured material. Even in longitudinal section the regions between the interdigitating MTs have failed to reveal order by two objective criteria (translational superposition of transparencies and optical diffraction).

*Jagged microtubule ends.* Another feature of the cross-section image displayed in Figs 22–26 is that the equatorial region of the spindle contains numerous images of incomplete or C-MTs. This morphology is prevalent at the non-polar ends of those nkMTs that interdigitate throughout the period of mitosis in which the spindle is elongating. Those kinetochore MTs that have been identified never show a C-shaped end, and the polar ends of all MTs are abrupt. The non-polar ends of the nkMTs that lie outside the zone of interdigitation are also abrupt, ending as a complete circle in passing from one section to the next.

**DISCUSSION**

*Conclusions and inferences*

The spindle of *D. discoideum* is constructed from two interdigitating sets of MTs, one structurally associated with each pole. We infer a special relationship between each MT and one pole, partly from the structural polarity of MTs in general (reviewed by McIntosh, 1981), partly from the pathway for formation of the *Dictyostelium* spindle (Moens, 1976; Roos, 1980; Roos, de Brabander & de Mey, 1984), and partly from the subsequent behaviour of the spindle in anaphase, during which MTs maintain an association with one pole or the other, but not both. It seems likely that the MTs that run continuously from pole to pole at metaphase also possess a polarity and a special association with a single pole like that seen by negative staining of the yeast SPB (Byers, Schrive & Goetsch, 1978), even though we cannot see anything special about one of their ends in thin sections.

Two or three MTs are attached to each kinetochore, but the majority of spindle MTs do not associate with chromosomes. There are only a few MTs at any stage of mitosis in *Dictyostelium* that are 'free', i.e. that do not have at least one end associated with a pole. During anaphase spindle elongation a few MTs associated with each pole get longer, while most MTs, including the kMTs, shorten and disappear. There is therefore both polymerization and depolymerization of spindle MTs going on at the same time. Over this time, the total amount of polymer decreases only slightly.

The zone of interdigitation at the spindle equator shortens a little as the spindle elongates, but this change is small and cannot account for the extent of spindle elongation observed (Roos & Camenzind, 1981). Elongation of the interdigitating MTs, on the other hand, seems to be a major factor in the increase of spindle length.

The positional inter-relationships between MTs in the zone of interdigitation during anaphase imply the existence of an interaction between antiparallel MTs. The high incidence of MTs from opposite poles spaced at about 42 nm suggests some sort of bond that depends on MT orientation. The square packing of antiparallel MTs...
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suggests a specific interaction even more than hexagonal packing would, because the denser hexagonal arrangement could result simply from tight bunching of the MTs, while the square arrangement requires some bonding specificity to maintain the inter-MT space and position. Further, the square packing allows each MT to be surrounded entirely by near neighbours of the opposite kind. We infer that the packing is a result of specific bonds between the antiparallel MTs in the zone of interdigitation. These bonds are probably important for the mechanical connection between the two spindle poles, given that no MT runs from pole-to-pole during the later stages of anaphase.

A specific spacing between antiparallel near-neighbour MTs can be detected in early anaphase when there is no obvious ordering of the MT array. The fact that small groups of antiparallel MTs are found in early anaphase, while larger groups have formed by mid-anaphase, suggests that the increase in overall order that occurs at the zone of interdigitation during anaphase results from the interaction between MTs from the two poles. Long-range order seems to form as a result of short-range order, as in condensation. On the other hand, the spacing between the MTs in the zone of interdigitation never becomes as small as it does midway between the spindle mid-plane and the pole. Perhaps the amorphous material between the MTs characteristic of the zone of interdigitation prevents close approach of antiparallel MT surfaces as well as contributing to the attachment between them.

The fact that short MTs disappear from the spindle during anaphase while some other MTs get longer can be understood by the concept of a micro-environment that favours assembly in the zone of MT interdigitation. Since similar behaviour has been reported for the spindles of diatoms (McIntosh et al. 1979; and reviewed by Pickett-Heaps, Tippit & Porter, 1982), a golden-brown alga (Tippitt, Pillus & Pickett-Heaps, 1980) and a fungus (Tippit et al. 1984), the phenomenon may be a common property of spindles. A simple explanation for the hypothetical micro-environment can be found in the fact that this is the only region of the anaphase spindle where antiparallel MTs are neighbours. McDonald, Edwards & McIntosh (1979) have suggested that interactions between antiparallel near-neighbour MTs in the zone of interdigitation might protect these polymers from an otherwise ubiquitous tendency for MTs to depolymerize in anaphase. According to this model, both kMTs and nKMTs that fail to be stabilized by antiparallel near neighbours would shorten (see also Tippit, Pillus & Picket-Heaps, 1983). Those MTs whose non-polar ends are located in the zone of interdigitation could even lengthen as a result of the stabilizing influence of their antiparallel near neighbours because 'stabilizing' may be the result of a shift in the value of the assembly equilibrium constant (Fig. 32). Work by Pickett-Heaps and others supports the idea that interdigitation promotes MT stability by showing that either naturally occurring (Soranno & Pickett-Heaps, 1982) or experimentally induced (Leslie & Pickett-Heaps, 1983) loss of MT overlap leads to MT disassembly in diatoms. It remains to be seen whether interdigitation actually promotes MT assembly.

In the context of a microenvironment that might promote assembly, the distribution of C-shaped MT ends is important. Such a morphology could \textit{a priori} be
Assume $K^+ > K^-$

Assume Mt sliding

$K^+_L$ is the association constant for microtubule subunits with MTs at their plus end in the region of interdigitation where they can be 'linked' by a connection that requires antiparallel near neighbours. $K^+_L$ is the analogous constant for MTs whose ends are free. We assume $K^+_L > K^-$ and show that in the context of heterogeneous MT lengths, the different equilibrium constants explain not only the elongation of the spindle but the fact that its diameter decreases as well (see text for details).

Fig. 32. A diagram representing the changes in MT length and position seen in our electron micrographs. The circles represent the poles, the lines MTs. Each plus sign marks the free MT end that is likely to be the end that exchanges subunits rapidly, by analogy with other cells where MT polarity is known (McIntosh & Euteneuer, 1984). A model is presented based on the assumptions that: (1) subunits add and leave MTs at or near their plus ends; (2) interdigitating MTs slide apart (mechanism of sliding unspecified); (3) interdigitation confers stability on MTs. $K^+_L$ is the association constant for microtubule subunits with MTs at their plus end in the region of interdigitation where they can be 'linked' by a connection that requires antiparallel near neighbours. $K^+_L$ is the analogous constant for MTs whose ends are free. We assume $K^+_L > K^-$ and show that in the context of heterogeneous MT lengths, the different equilibrium constants explain not only the elongation of the spindle but the fact that its diameter decreases as well (see text for details).
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associated with either polymerization or depolymerization of subunits from an MT end (Lambert & Bajer, 1977; Bajer & Mole-Bajer, 1972). However, the MTs that are too short to interdigitate in early anaphase are almost certainly those that shorten further and disappear as anaphase progresses. Neither end of these MTs is jagged. The longer MTs that interdigitate at early anaphase are almost certainly those that elongate further, continuing to compose the interpolar bundle of mid-to-late anaphase. The fact that their number is approximately constant in anaphase and telophase (Fig. 21) supports this assertion. The jagged or C-shaped morphology is therefore found only on the pole-distal end of elongating MTs in the Dictyostelium spindles we have analysed. This observation is consistent with the frequent occurrence of such MT images in prometaphase when the spindle is forming (Roos, 1980). Our observations suggest that the elongating MTs of the interzone bundle add subunits at or near their pole-distal ends. These are probably the ‘plus’ or fast-growing ends of the spindle MTs (McIntosh & Euteneuer, 1984).

If subunits are adding to the non-polar ends of nkMTs in the zone of interdigitation, then one would expect the length of MT overlap at the spindle equator to increase (Fig. 32a). The three-dimensional reconstructions of the spindle equator show, however, that the extent of MT interdigitation actually decreases during spindle elongation. It follows that a process of MT sliding must be going on during MT and spindle elongation to prevent the overlap from increasing (Fig. 32c). A similar mechanism for Dictyostelium spindle elongation based on some combination of MT sliding and polymerization was suggested by Moens (1976). One cannot say, of course, whether this is an ‘active sliding’ as with the MTs of flagella (Summers & Gibbons, 1971). There is certainly a fundamental difference between the two motile systems: the neighbouring spindle MTs in the zone of interdigitation are antiparallel, while adjacent axonemal MTs are parallel. If a dynein-like molecule is involved in spindle MT sliding, it must be a molecule quite different from flagellar dynein.

The model drawn in Fig. 32 also suggests an explanation for why the interpolar MT bundle becomes thinner as it elongates. The lengths of nkMTs in Dictyostelium are heterodisperse. Assuming that interdigitation is required to confer MT stability during anaphase, then continued sliding of the interdigitating MTs would pull the free end of one MT after another out of the microenvironment that promotes stability and into the environment that induces disassembly. The subunits released by disassembly could then be recycled by diffusion, permitting elongation of those MTs still long enough to have their free ends in the region of interdigitation.

Comparisons with spindles from other organisms

Mitotic spindles of several organisms have been studied in sufficient detail to permit a comparison with the data presented here. The general form of the spindle in Dictyostelium is strikingly similar to that of many other cells. Kinetochore and non-kinetochore MTs are present at metaphase. The focused pole is distinct from the anastral spindles of higher plants, but is similar to the spindle design seen in some algae, fungi and animal cells (see reviews by Pickett-Heaps et al. 1982; Heath, 1978;
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Fuge, 1977). Quantitative description of mammalian spindles based on MT distributions has yielded data essentially identical to the distributions shown here (Fig. 7) (McIntosh & Landis, 1971; McIntosh, Cande & Snyder, 1975), although these are not stringent criteria for structural identity (Roos, 1981). The distributions all show a general decrease in MT number as the spindle elongates, and all indicate that there is something special about the region around the spindle mid-plane that leads to a smaller decrease in MT number in that part of the spindle during anaphase.

*Dictyostelium* is similar to algae and fungi but different from crane-flies in the paucity of MT fragments in its mitotic spindles. (For diatoms, see Tippit, Schultz & Pickett-Heaps, 1978; McIntosh *et al.* 1979; for a golden-brown alga, see Tippit *et al.* 1980, 1983; for fungi, see Heath, 1978; Tippit *et al.* 1984; for crane-flies, see Fuge, 1977; Steffan & Fuge, 1982. On the other hand, see La Fountain, 1976, for a different view of the crane-fly spindle.) *Dictyostelium* is similar to mammals (PtK1 cells; Rieder, 1981) and yeasts (*Saccharomyces*; Peterson & Ris, 1976) in having kMTs that run all the way from kinetochore to pole. In this respect it differs from two anastral spindles: the micronucleus of *Tetrahymena* (La Fountain & Davidson, 1980) and the mitotic spindle of the green alga *Oedogonium* (Schibler & Pickett-Heaps, 1981). Apparently such continuity is not essential for chromosome-to-pole movement, but in types of cells where poles are well structured, we know of no spindles that lack the connection.

The anaphase–telophase interpolar bundle of MTs in most organisms is composed of two interdigitating families of MTs (mammals, McIntosh & Euteneuer, 1984; algae, reviewed by Pickett-Heaps *et al.* 1982; fungi; Tippit *et al.* 1984). In some fungi, however, the interdigitation extends over the majority of the length of the spindle (reviewed by Heath, 1978; see Peterson & Ris, 1976). The yeast *Saccharomyces cerevisiae* may be an exception on the basis of a recent report that a single MT appears to interconnect the poles late in spindle elongation (King, Hyams & Luba, 1983).

An interaction between neighbouring MTs from opposite poles in the zone of interdigitation has been suggested on the basis of radial distribution analysis in diatoms (McDonald *et al.* 1979), golden-brown algae (Tippit *et al.* 1983) and mammals (McDonald & Euteneuer, 1983). Given an essentially universal tendency of interzonal MTs to form bundles (e.g. see Bajer & Mole-Bajer, 1972 for *Haemanthus*; Fuge, 1977, and La Fountain, 1976, for crane-flies; and Heath, 1978, for fungi), it seems likely that such interactions are universal. The extent to which the zone of interdigitation decreases in length as the spindle elongates is, however, highly variable. In diatoms, the decrease is marked, and only the longest MTs continue to interdigitate at the end of anaphase (Tippit *et al.* 1978; McIntosh *et al.* 1979). Likewise, the zone of overlap decreases in mammals (McIntosh & Landis, 1971; McIntosh *et al.* 1975; McDonald & Euteneuer, 1983). On the other hand, in *Ochromonas* the MTs of the interpolar bundle elongate markedly as the poles move apart, but the zone of overlap remains essentially constant in length (Tippit *et al.* 1980). The spindle of *D. discoideum* is intermediate between these extremes. If, however, our interpretation of the C-MTs as sites of subunit addition should prove to be both correct and
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general, then this variability in behaviour would simply result from the extent to which MTs elongate as they slide during spindle growth. This view is quite different from that expressed by Tippit et al. (1984), who prefer the hypothesis that MTs add subunits at their polar ends.

Our analysis of spindle structure says little about the mechanisms for the movement of chromosomes to the poles. Near-neighbour analysis of kMTs compared to nkMTs in the half-spindle proved to be impossible in Dictyostelium because the kinetochores could not be identified unambiguously in transverse section. Detailed investigation of possible interactions between kMTs and nkMTs must await analysis in a more favourable organism. A superficial study of the Dictyostelium kMTs implies that they are too distant from nkMTs, except right at the pole, to permit force-producing interactions. Our results thus agree with the work on Saccharomyces (Peterson & Ris, 1976), Cryptomonas (Oakley & Heath, 1978) and Ochromonas (Tippit et al. 1983), which suggest that the forces for chromosome-to-pole movement are generated elsewhere.

We are impressed by some similarities between the spindle of D. discoideum and spindles of many other eukaryotes. While diatoms appear in certain ways to be unique, given their organized interpolar spindles and their permitting some kinetochore-to-pole movement before anaphase begins, the mitotic structures described here show a diatom-like interpolar spindle developing during anaphase, probably from the disordered sets of antiparallel nkMTs that are present at metaphase. Similar ordering occurs in mammalian spindles, but the number of MTs is so large that it occurs first in small bunches, the interzone spindle fibres. Each of these shows some similarity to the central spindle of Dictyostelium (McIntosh & Euteneuer, 1984; McDonald & Euteneuer, 1983). The final interzonal MT bundle of the mammalian spindle, the midbody, is architecturally equivalent to the ordered MTs of the central spindles in lower eukaryotes. It would seem that many aspects of structural diversity in spindles can be accounted for by simple variation in the relative timing of essentially similar events, and that the mechanisms of mitosis may be conserved.

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REFERENCES


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