C-MICROTUBULES IN ISOLATED MITOTIC SPINDLES

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SUMMARY

Microtubules with incomplete cylindrical structure are present in isolated mitotic spindles of the sea urchin, Arbacia punctulata. In cross-section they appear C-shaped, and are thus similar to the 'C-microtubules' or 'C-filaments' observed previously in other systems. The C-microtubules are not uniformly distributed within isolated spindles, but are typically numerous in the interzonal region of anaphase spindles and in the metaphase chromosome 'plate'. In chromosome-to-pole regions they are seen much less frequently, and microtubules with the usual O-configuration predominate. Counts of C- and O-microtubules in anaphase spindle cross-sections of known location show an inverse relationship between the number of C-microtubules present and the total number of microtubules present. The observations suggest that the C-microtubules are not simple artifacts of fixation or isolation, but rather may represent a stage of microtubule disassembly which occurs in the interzone during isolation or during anaphase in vivo. The alternate possibility of assembly is not excluded, however. The significance of C-microtubules is further discussed with respect to their occurrence in other systems, and to potential differences between mitotic microtubules.

INTRODUCTION

Structures appearing to be microtubules with an incomplete or open cylindrical configuration have been observed previously in several systems (Tucker, 1967; Behnke, 1967; Roth & Shigenaka, 1970). They are sometimes referred to as 'C-microtubules' or 'C-filaments' because they resemble the letter C in cross-section rather than the O which is usually encountered. While their significance is as yet undetermined, these C-microtubules may be microtubules fixed during formation or disassembly in vivo.

Evidence of naturally occurring structural alteration in mitotic microtubules during the course of mitosis has been rather limited, and the precise manner in which microtubules of mitotic systems are assembled or broken down remains unknown. In his structural study of binary fission in the ciliate Nassula, Tucker (1967) observed C-microtubules in both metaphase micronuclei and anaphase separation spindles of the macronucleus and micronuclei (that is, in the region between separating sets of chromosomes). The dimensions of these 'C-filaments' were compatible with the hypothesis that they were essentially microtubules with a longitudinal separation between 2 of their component protofilaments. Tucker reported that the macronuclear spindle contained about 3 C-filaments per 10 complete microtubules, and the micronuclear spindles 1 C-filament per 10 microtubules. Thus they were present in considerable numbers.
We have found C-microtubules in the isolated mitotic apparatus of sea-urchin eggs, and have studied their number and distribution in cross-sections through known positions in isolated spindles.

MATERIALS AND METHODS

Mitotic apparatuses and spindles devoid of asters were isolated from eggs of the sea urchin *Arbacia punctulata* prior to first cleavage, according to the method of Kane (1962, 1965). Gametes were obtained by the electrical method (Harvey, 1956), and fertilization membranes were removed using thioethylglucosamide (Mazia, Mitchison, Medina & Harris, 1961), as described previously (Cohen & Rebhun, 1970). Mitotic apparatuses were stabilized by exposure to Mg$^{++}$ immediately after isolation (Goldman & Rebhun, 1969). Variations in details of isolation procedure used for different preparations of mitotic apparatus are as follows:

(a) The isolation medium was 12% hexylene glycol, 0.01 M maleate, pH 6.2. Eggs were lysed in the medium by brief swirling on a vortex mixer. The preparation was placed at 0 °C, and one-tenth volume of 0.01 M MgCl$_2$ in isolation medium added, giving a final concentration of 0.001 M MgCl$_2$. The mitotic apparatuses were washed 5 times in isolation medium containing 0.001 M MgCl$_2$, at 0-4 °C, and resuspended in the same medium.

(b) The isolation medium was 12% hexylene glycol, 0.01 M phosphate, pH 6.3. Procedure was the same as in (a) except that MgCl$_2$ was made to 0.003 M by adding to the preparation of isolates an equal volume of isolation medium containing 0.006 M MgCl$_2$. Some of the spindles lost their asters during the 5 washes in isolation medium containing 0.003 M MgCl$_2$, at 0-4 °C. Final resuspension was in the same medium.

Fixation was carried out immediately after preparation. Final resuspension in isolation medium containing Mg$^{++}$ was followed by addition of an equal volume of the same medium containing 5% glutaraldehyde, at the same pH. Preparations were fixed in glutaraldehyde at 0 °C for 60 min, sedimented, washed once with water (for procedure a, above) or with isolation medium containing Mg$^{++}$ (for procedure b, above), and post-fixed in 1% OsO$_4$ in the same wash medium for 45-60 min. After passage through an ethanol dehydration series and propylene oxide, mitotic apparatuses and free spindles were embedded in Beem capsules or in LKB flat-embedding moulds in Epon 812 (Luft, 1961).

In the case of flat embedments, embedded mitotic apparatuses and spindles were examined under phase contrast, and selected ones of known mitotic stage were oriented for cross-sectioning with the long axis of the spindle approximately perpendicular to the face of the block. Sections were cut with diamond knives on a Sorvall MT-2 ultramicrotome, mounted on Formvar-coated mesh grids (for non-serial sections) or slot grids (serial sections), stained with uranyl acetate followed by lead citrate (Stempak & Ward, 1964; Reynolds, 1963; Venable & Coggeshull, 1965), and examined in a Hitachi HS-7S or HS-8 electron microscope. When serial sectioning was performed, each section was ‘numbered’ in sequence, so that precise location of any section within the spindle could be ascertained. In some cases, non-serial sections representing a certain length of spindle were mounted on a numbered grid series, so that approximate location of a section (within about 5 μm) was known.

In certain cross-sections through known spindle locations, detailed observations and counts of microtubules were made for areas approximately 1/4 of the entire cross-section. Measurements of area counted and of entire cross-sectional area were made by means of tracing enlargements of sections and portions of sections on to paper of known weight per unit area. Areas were then determined from the weights of the tracings. The generally uniform distribution of microtubules in entire cross-sections permitted results for large areas examined in detail to be extrapolated to the entire cross-section.
RESULTS

Initial observations

Cross-sections through spindles of whole isolated mitotic apparatuses representing several different preparations (that is, different batches of eggs on different dates) were sometimes found to contain numerous C-microtubules (Fig. 3). These were present in significant numbers in only some of the sections, however; in most sections, the classical O of the complete cylindrical structure predominated, and C-microtubules were rare. In those sections in which C-microtubules were abundant, there were also numerous normal-looking microtubules in close proximity, as shown in Fig. 3, arrow a. The C-microtubules were not identical in appearance; rather, they ranged from slightly 'open' C-profiles (Fig. 3, arrow b) through intermediate ones (arrow c), to nearly straight profiles (arrow d).

The abundance of C-microtubules in some sections but not others could be explained most directly by either of the following suppositions: (1) in each preparation of isolated mitotic apparatus, the C-microtubules are present in only a percentage of the total number of spindles isolated, or (2) in each isolated spindle, the C-microtubules are present in only certain regions. While cross-sectioning of large numbers of spindles to assess possibility (1) would have been a prodigious task, cross-sectioning of relatively few spindles through known positions along the spindle axis was feasible in assessing possibility (2).

Location of C-microtubules in isolated anaphase spindles

Examination of cross-sections through known spindle positions in intact isolated anaphase mitotic apparatuses and in an anaphase spindle devoid of asters substantiated possibility (2) above rather strikingly: the C-microtubules are characteristic of the interzonal region of isolated anaphase spindles. In the chromosome-to-pole regions, C-microtubules are relatively rare. Figs. 4 and 5 provide a comparison of the chromosome-to-pole and interzonal regions of the same isolated anaphase spindle, seen in cross-section.

Numbers of C-microtubules in chromosome-to-pole and interzonal regions

A more quantitative approach to the distribution of C-microtubules was taken by making counts of the numbers of C-microtubules and normal O-microtubules in selected sections along the axis of a single isolated anaphase spindle (devoid of asters). The positions of the cross-sections, known through use of serial sectioning (see Materials and Methods), are shown in Fig. 1. The data obtained from counts of microtubules in these sections are presented in Table 1. The results show clearly the relative abundance of C-microtubules in the interzone, not far from the chromosome set (Fig. 1, Table 1, section II). Closer to the mid-plane of the interzone the C-microtubules are even more abundant (section III); however, on the poleward side of the chromosome set (section I), the number of C-microtubules drops off markedly. Observations made on additional cross-sections show that the distribution of
C-microtubules is similar in each half-spindle, as expected; relatively few of them appear in either chromosome-to-pole region of the spindle.

From the values given in Table 1 it is apparent that the majority of C-microtubules in the interzone do not extend all the way to the poles of the spindle in the C-configuration, for there are simply not enough of them between chromosomes and poles. Thus, either most of the C-microtubules may end before the chromosome sets and different, O-microtubules start and continue poleward from there, or else individual microtubules may be in the C-configuration in the interzone and then transform into closed O-microtubules between chromosomes and poles.

**Fig. 1.** Positions of cross-sections through an isolated anaphase spindle. These sections are the same ones listed in Table 1, in which counts were made of numbers of C- and O-microtubules. ch, chromosome sets.

*C-Microtubules in longitudinal section*

What would a single microtubule look like in thin longitudinal section if one segment of it in the C-configuration were continuous with an adjacent segment in the O-configuration? Suggested possibilities are illustrated in Fig. 2. Fig. 2A depicts a 'closed' O-segment as it would appear in both cross- and longitudinal section. The 2 parallel electron-dense lines normally seen in longitudinal section are observed; each of these lines presumably represents a certain depth of electron-dense material being viewed, and this depth must be greater than just one or two protofilaments, for otherwise the lumen of the microtubule would also appear as electron-dense. In Fig. 2 B, an 'open' C-segment is caught in the section in such a manner as also to give rise to 2 parallel lines in longitudinal section. These would not be distinguishable from those of A, except, perhaps, for a somewhat greater separation between the lines (giving a false impression of a normal O-microtubule of increased diameter). In c, however, the plane of the section is such that a C-microtubule gives rise to only a single electron-dense line in longitudinal section. Therefore, if there were a transition from an O to a C configuration within a single microtubule, a fortuitous longitudinal section might show this as in D or E.

With these possibilities in mind, we examined longitudinal sections of isolated anaphase spindles, and found in some of them the images of apparent O-to-C transition suggested in Fig. 2. This is illustrated in Fig. 6, showing the region of one of the anaphase chromosome sets. Longitudinal sections of apparently normal-looking O-microtubules (arrow a) are accompanied by single electron-dense lines, at least one
Table 1. Numbers of C-microtubules and O-microtubules observed in selected cross-sections of an isolated anaphase spindle

<table>
<thead>
<tr>
<th>Section no. †</th>
<th>Region</th>
<th>Total no. ‡ microtubules studied</th>
<th>No. of C-microtubules</th>
<th>No. of O-microtubules</th>
<th>Ratio of no. of C's per 10 O's</th>
<th>Total no. microtubules</th>
<th>No. of C-microtubules</th>
<th>No. of O-microtubules</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Chrom.-to-pole</td>
<td>985</td>
<td>42</td>
<td>943</td>
<td>0.4/10</td>
<td>3349</td>
<td>143</td>
<td>3206</td>
</tr>
<tr>
<td>II</td>
<td>Interzone</td>
<td>1030</td>
<td>312</td>
<td>718</td>
<td>4.3/10</td>
<td>2235</td>
<td>677</td>
<td>1558</td>
</tr>
<tr>
<td>III</td>
<td>Interzone</td>
<td>622</td>
<td>326</td>
<td>296</td>
<td>11.0/10</td>
<td>1611</td>
<td>848</td>
<td>763</td>
</tr>
</tbody>
</table>

* Isolation procedure (da) as described in Materials and Methods.
† The section numbers correspond to the positions of cross-sections shown in Fig. 1.
‡ Total number refers to C-microtubules + O-microtubules.
of which appears to be continuous with an O-segment (arrow b) as illustrated in Fig. 2 E. In another example (Fig. 6, arrow c), a transition appears as illustrated in Fig. 2 D. Other microtubule segments in Fig. 6 seem intermediate between these 2 cases: one of the two lines is more electron-dense than the other, and the diameter of the microtubule is greater than normal. These are probably C-microtubule segments (arrows d).

Fig. 2. Proposed appearance of C-microtubules as seen in longitudinal section.
A, Typical complete O-microtubule; 2 parallel lines in longitudinal section (heavy lines).
B, C-microtubule; 2 parallel lines in longitudinal section.
C, C-microtubule; only 1 line in longitudinal section.
D, Transition from O- to wide C-configuration, one view; both lines diverge and fade away.
E, Transition from O- to wide C-configuration, another view; one line stops while the other continues.

Observations on isolated metaphase spindles

Metaphase spindles do not have an interzonal region, in the usual meaning of the term as it applies to the region of the spindle between separating sets of chromosomes at anaphase. If a metaphase spindle could be said to have an interzone of any sort, however, it would perhaps be the relatively short distance through which a continuous microtubule would pass as it traversed the metaphase 'plate' of chromosomes. Curiously, it is this region of the isolated metaphase spindle in which C-microtubules are relatively abundant. This is illustrated in Figs. 7 and 8, in which the chromosome-to-pole region and metaphase-plate region of the same isolated metaphase spindle are compared.
DISCUSSION

Possibility of artifact

It is unlikely that the C-microtubules observed in isolated spindles represent any simple artifact of either the isolation procedure or of fixation and subsequent steps of preparation for electron microscopy. If all of the microtubules were initially structurally identical and equally labile, and if 'opening' into C-microtubule configuration occurred because of incomplete stabilization in isolation medium or fixatives, one would expect the same percentages of C-microtubules in all regions of the spindle. This is not the case, however, and thus the results suggest some native difference between microtubules as they occur in the interzone of the anaphase spindle and in the chromosome-to-pole region. Conceivably, such a difference could be a secondary result of the absence of some stabilizing factor from the interzone, as opposed to an intrinsic difference in the microtubules themselves. This situation would still, nevertheless, be beyond the realm of simple artifact. In addition, the close proximity of C-microtubules to normal-looking O-microtubules in many sections indicates that stabilization and fixation were adequate for many O-microtubules even in the interzonal region.

The possibility of fixation artifact has also been considered in previously published reports of C-microtubules, involving fixation of microtubules in situ. In the case of micronuclear and macronuclear spindles of Nassula, Tucker (1967) concluded that the C-microtubules should not be dismissed as fixation artifacts without further investigation. Behnke (1967), discussing the C-microtubules which appear in the marginal bundle of human and rat blood platelets during the rewarming period following cooling, argued that they were not fixation artifacts but rather a stage of reassembly of microtubules in which 'curved sheets' of microtubule protein are present. Roth & Shigenaka (1970), observing C-microtubules during the rapid retraction of heliozoan axopods induced by cupric and nickelous ions, described them as part of the disassembly process rather than artifacts. Thus the C-microtubules observed in these systems in situ may well reflect dynamic activity of the in vivo system.

An additional comment concerning the C-microtubules of isolated spindles should perhaps be made: the fact that this is an isolated system does not necessarily imply a greater likelihood of artifact than in an in situ system. Rapid stabilization of microtubules by the isolation medium might equally well improve chances of retaining subtleties of structure which are present in vivo. In the absence of definitive information, at least, one cannot assume that exposure of microtubules to the isolation medium is any more or less effective, in this regard, than exposure to fixative directly. At present, then, there are no grounds for assuming that the C-microtubules of isolated Arbacia spindles must be produced by breakdown of O-microtubules during or after isolation, although this is, of course, a possibility.
Distribution of C-microtubules in isolated spindles

The observations indicate that the C-configuration is characteristic primarily, or perhaps entirely, of the non-chromosomal microtubules of the isolated spindle. The majority of these are probably related in some way to the continuous spindle fibres of light microscopy, but without additional data the C-microtubules are better described as being 'non-chromosomal' or 'interpolar' (Brinkley & Cartwright, 1970) rather than 'continuous' microtubules derivatives. The C-microtubules are found in abundance only in the interzone of isolated anaphase spindles and within the chromosome plate of isolated metaphase spindles. In the anaphase spindle they were observed in greatest numbers towards the middle of the interzone, accounting for approximately 50% of the total of C- and O-microtubules present. They occur in great, though lesser numbers in the interzone nearer the chromosome set, and in relatively low numbers in the chromosome-to-pole region. The comparison is perhaps best illustrated by the ratio of C- to O-microtubules for the 3 sections described in Table 1. It is of interest that the mitotic C-microtubules seen in the ciliate *Nassula* during fission (Tucker, 1967) were also abundant in the region between separating chromosome sets, with the reported ratio of 3 C-microtubules per 10 O-microtubules in the macronucleus being comparable to that of section II of the interzone in the isolated anaphase spindle (Table 1).

The observed distribution of C-microtubules lends itself to 2 direct interpretations. In the first, the mid-plane of the spindle (metaphase-plate plane, or mid-interzone plane of anaphase) is viewed as the plane in which new material is incorporated into growing or assembling non-chromosomal microtubules. This interpretation assumes the C-microtubules to represent a stage of microtubule formation in this system, as in the platelets studied by Behnke (1967), or the axopods of *Echinopsphaerium nucleofilum* recovering from colchicine treatment as reported by Tilney (1968). Such an interpretation suffers, perhaps, from forcing the extrapolation of the in vitro results to the in vivo system.

A second view assumes the opposite: the C-microtubules are ones caught in an initial stage of disassembly, and either may or may not reflect in vivo activity. The data of Table 1 are in accord with a disassembly process: the total number of microtubules is greatest in the chromosome-to-pole region, lower upon entering the interzone (this is expected here because of ending of chromosomal microtubules at kinetochores), but even lower nearer the mid-interzone. In contrast, the total number of C-microtubules shows the inverse pattern, with the greatest number of C-microtubules near the mid-interzone. Thus it appears as if microtubule disassembly (at least, to the point of non-recognizability in cross-section) occurs by way of C-formation.

The question of whether such disassembly is characteristic only of isolated spindles, or whether it might occur in the living sea-urchin zygote cannot be answered at present. It is known that O-microtubules disappear from isolated spindles under certain storage conditions (Kane & Forer, 1965; Goldman & Rehun, 1969), and therefore the C-microtubules could represent the initial stage of this disappearance. In the work reported here, only Mg\(^{2+}\)-stabilized isolated spindles were studied, so as to avoid
microtubule breakdown after spindle isolation (Goldman & Rebhun, 1969). Conceivably such stabilization might permit partial loss of structure as indicated by the C-configuration.

The C-microtubules of isolated Arbacia spindles may, on the other hand, reflect the initial stage of a disassembly process occurring \textit{in vivo}, as is thought to be the case for the C-microtubules observed in retracting axopods of \textit{Echinospaerium} (Tilney, 1968; Roth & Shigenaka, 1970). In this case, the following dynamic picture would emerge: at metaphase, nearly all of the microtubules would be complete except for some segments of non-chromosomal microtubules in the metaphase plane. As each chromosome set moved poleward during anaphase, the chromosomes would leave in their 'wake' microtubules which began to disassemble. Admittedly this picture is a rather speculative one, but it would account for loss of interzonal microtubules and correspondingly of some of the interzonal birefringence during anaphase. In many species the interzone of anaphase spindles is not very birefringent compared to the chromosome-to-pole region (Inoué & Sato, 1967), and this is true of the isolated Arbacia mitotic spindle as well (Cohen, unpublished observations). Assuming spindle birefringence to be attributable, at least in part, to complete (O-configuration) chromosomal and continuous microtubule segments (Inoué & Sato, 1967; Rebhun & Sander, 1967; Goldman & Rebhun, 1969), the chromosome-to-pole region of the Arbacia spindle would remain birefringent at all stages of anaphase. As chromosomes moved poleward, segments of non-chromosomal microtubules would cease to contribute to spindle birefringence as they were 'passed by', became unstable, and began to disassemble.

**General significance of C-microtubules**

The occurrence of C-microtubules in various systems may shed some light on the general problem of microtubule assembly and disassembly. Using a relatively simple 11-13 protofilament model of microtubule structure, one can begin with the assumption that the stability of the entire structure depends upon the extent of bonding between subunits. That is, the greater the number of neighbours a given subunit has, the greater the number of inter-subunit bonds possible and the greater the probability of the subunit remaining within the structure. On this basis, free ends of microtubules are obvious sites of assembly or disassembly, as the subunits there are not bonded maximally. Relatively slow growth or shortening of microtubules might involve addition or removal of such subunits. In contrast, larger numbers of subunits would become unstable more rapidly if some mechanism existed which permitted formation of the C-configuration along a certain length of microtubule. All of the subunits of the 2 protofilaments at the free edges of the C-microtubule are then left in a relatively unstable condition. C-microtubules may thus be indicative of \textit{rapid} assembly or disassembly in systems in which they occur. For one such system, the retracting \textit{Echinospaerium} axopod (cupric and nickelous ion-induced), Roth & Shigenaka (1970) described the shortening as 'cataclysmic' compared with shortening of flagella of \textit{Chlamydomonas} after one of the two flagella has been amputated (Rosenbaum,
Moulder & Ringo, 1969). In the latter case, shortening is thought to occur by removal of subunits from microtubule tips.

C-microtubules and mitotic systems

The observation of C-microtubules in some regions of isolated spindles but not others raises the general possibility that there may be different kinds of mitotic microtubules, with differences in stability under given conditions. Chromosomal microtubules and continuous ones may represent 2 such categories, and some evidence for this in crane-fly spermatocytes is referred to by Forer (1969). In addition, Brinkley & Cartwright (1970) describe experiments with mammalian cells in culture, in which chromosomal microtubules are more stable to cold treatment than interpolar ones in metaphase and early anaphase cells. Differential stability may account for the solubility pattern of isolated spindles in certain solvents (Mazia, 1955; Zimmerman, 1960), in which dissolution begins at the equator and then moves poleward. Certainly, more information concerning the possibility that there are different kinds of mitotic microtubules would be desirable.

A few final comments concerning mitotic C-microtubules may be of some value. Several papers have described cross-bridges between mitotic microtubules, seen in situ (Wilson, 1969; Hepler, McIntosh & Cleland, 1970). In at least one case, involving the interzonal region of anaphase nuclei of the alga Blastophysa (Wilson, 1969), some of the cross-bridges look somewhat like partial C-microtubules in cross-section. In addition, in the interzone of isolated Arbacia spindles, C-microtubules are occasionally found immediately adjacent to O-microtubules, giving a superficial appearance of arms extending from the O-microtubule (Fig. 5, arrow d). We suggest, therefore, that some caution be exercised in interpreting various structures as cross-bridges, at least until more information is available concerning the occurrence of C-microtubules in situ in the spindles of Arbacia and of other species. Certain other structural components, such as the 'wispy filamentous material' observed in continuity with the ends of microtubules in isolated meiotic spindles of Spisula (Rebhun & Sander, 1967), may be portions of C-microtubules in longitudinal section.

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REFERENCES


C-microtubules in isolated spindles


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Fig. 3. C-microtubules in a cross-section through an anaphase mitotic apparatus (isolated according to procedure $a$, Materials and Methods). Microtubules of usual O-configuration (arrow $a$) often appear adjacent to C-microtubules. The C-microtubules range from slightly 'open' profiles ($b$) through intermediate ones ($c$), to wide open profiles ($d$). $\times 80000$. 
Fig. 4. Appearance of microtubules in cross-section through the chromosome-to-pole region of an isolated anaphase spindle (isolation procedure b, Materials and Methods). C-microtubules occur infrequently (arrows). × 52000.
Fig. 5. Appearance of microtubules in cross-section through the interzonal region of the same isolated anaphase spindle as in Fig. 4. C-microtubules are relatively abundant, occurring singly (arrows a) and in groups (b). Many O-microtubules are also present (as at c). Occasionally a C-microtubule lies immediately adjacent to an O-microtubule, giving the latter the appearance of having projecting arms (d). × 66,000.
Fig. 6. Longitudinal section through one chromosome set of the spindle of an isolated anaphase mitotic apparatus (isolation procedure a, Materials and Methods). In addition to longitudinal sections of apparently typical O-microtubules (arrow a), there are single electron-dense lines, one of which (b) appears to be in continuity with an O-microtubule segment. Another O-microtubule segment appears to undergo transition to the C-configuration (c), and there are additional microtubule segments in which one of the two lines is more electron-dense than the other, with the microtubule diameter slightly greater than normal (d); these are believed to be segments of C-microtubules. (An interpretation of the appearance of C-microtubules in longitudinal section is given in Fig. 2.) ch, chromosomes. ×40000.
Fig. 7. Cross-section through the chromosome-to-pole region of an isolated metaphase spindle (isolation procedure b, Materials and Methods). Numerous O-microtubules are present, while C-microtubules are infrequently observed (arrows). × 30,000.

Fig. 8. Cross-section through the metaphase plate region of the same isolated metaphase spindle as in Fig. 7. Numerous C-microtubules are present (arrows). ch, chromosomes. × 30,000.
C-microtubules in isolated spindles