The prometaphase configuration and chromosome order in early mitosis

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Summary

We have examined early mitotic stages in HeLa cells, mouse 3T3 fibroblasts and mitogen-activated mouse lymphocytes by immunofluorescence labelling with anti-tubulin and anti-centromere. Chromatin organization was monitored with the DNA-specific fluorochrome Hoechst 33258. This approach has led us to identify a modified Rabl array of chromosomes and spindle microtubules early in mitosis that is distinct from that at metaphase, and which we have called 'the prometaphase configuration'. In the configuration, chromosomes are oriented so that telomeres are clustered at the outer surface, whereas centromeres are clustered inside the configuration, at the surface of a hollow spindle. Observations on cells earlier in mitosis indicate that the configuration is presaged by the spatial relationship between chromosomes and cytoplasmic microtubules in prophase and early prometaphase. We propose a model in which the prometaphase configuration represents an important step linking prophase and metaphase, serving to translate interphase spatial and intragenomic order into order at the metaphase plate.

Key words: prometaphase, mitosis, microtubules, centromeres, nuclear order, chromosome order.

Introduction

The hypothesis that chromosomes are disposed in an ordered fashion during interphase and mitosis is conceptually attractive. Although yet to be verified unequivocally, it is strongly supported by data from many sources and, as evidenced by the number of recent reviews (Church, 1981; Comings, 1968, 1980; Fussell, 1984, 1987; Heslop-Harrison & Bennett, 1984; Hubert & Bourgeois, 1986) is currently a concept of great interest.

One feature of interphase chromosome order, a relic telophase arrangement (Fussell, 1984), has emerged as apparently universal in higher organisms. Rabl (1885) was first to conclude, from observations on salamander cells, that the arrangement of chromosomes at anaphase–telophase is retained throughout interphase and prophase. Now variously referred to as the Rabl orientation (Fussell, 1987) or model (Cremer *et al.* 1982), the telophase configuration (Foe & Alberts, 1985) or polarized chromosome order (Comings, 1980), this chromosome orientation is characterized by the following: (1) telomeres are clustered towards one side of the nucleus; (2) centromeres are clustered at the opposite side of the nucleus, furthest from the telomeres; (3) the microtubule-organizing centre (MTOC) lies near the centromeres.

Studies using C-banding (Coates & Wilson, 1985), autoradiographic detection of late-replicating chromatin (Fussell, 1987), serial sectioning (Church, 1981) or DNA-specific fluorescent staining (Foe & Alberts, 1985; Gruenbaum *et al.* 1984) have clearly demonstrated the Rabl orientation in many tissues. Furthermore, the elegant laser–u.v.–microbeam irradiation experiments of Cremer *et al.* (1982) have supported the tested predictions of the Rabl model. No exceptions for clustering of telomeres have been reported to date, but centromere clustering at the nuclear periphery appears somewhat less general (Fussell, 1987; Hubert & Bourgeois, 1986). Although present in many cell types, centromere clustering is absent in others, varying according to species and tissue. However, reports indicating that individual chromosomes occupy separate intranuclear domains (Gruenbaum *et al.* 1984; Manuelidis, 1985; Schardin *et al.* 1985) and that chromosome arms move little after early G_1 phase (Fussell, 1987; Hens *et al.* 1983, and references therein; Schardin *et al.* 1985) further support Rabl's conclusions.

Other investigations strongly suggest that a second type of order, a non-random arrangement of homologous and non-homologous chromosomes, overlies the Rabl orientation in interphase nuclei (Comings, 1980; Hubert & Bourgeois, 1986; Schardin *et al.* 1985). This type of order, i.e. intragenomic order, has also been convincingly demonstrated within the metaphase plate (Heslop-Harri-

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son & Bennett, 1984). The relationship between interphase and metaphase intragenomic order is unknown but, by implication, both the Rabl orientation and intragenomic order at interphase contribute in some fashion to the formation of an ordered metaphase plate.

It is clear that chromosomes are physically displaced from their interphase positions during metaphase plate formation, i.e. during prometaphase. Although beginning, at prophase, and ending, at metaphase, with ordered chromosome configurations, prometaphase itself is widely considered a dynamic and somewhat chaotic step. It is generally depicted as a jumble of chromosomes which have been released from the constraints of a nuclear envelope but are not yet securely anchored by kinetochore microtubule (MT) bundles to the spindle poles. There is no hypothesis to explain how interphase order, of either type, might survive the apparently cataclysmic events of prometaphase to be translated into a metaphase order.

In the course of investigations on cycling mammalian cells, we had frequently observed a mitotic configuration that did not conform to any of the well-characterized stages of mitosis. In this report we examine this configuration using immunofluorescence labelling with antibodies to tubulin and to centromeres. The results show that the configuration is a modified Rabl orientation and that it is assumed by chromosomes between nuclear envelope breakdown and metaphase, i.e. during prometaphase. We propose that the prometaphase configuration is a staging step between prophase and metaphase, and that it functions in the translation of interphase nuclear organization into an ordered metaphase plate.

Materials and methods

Cell culture

HeLa cells and mouse 3T3 fibroblasts were cultured at 37°C and 5% CO_2 in Dulbecco's modified Eagle's medium, with 10% foetal calf serum and 100 units ml^{-1} penicillin, 100 $\mu g\,ml^{-1}$ streptomycin. Mouse splenocytes were isolated, cultured and stimulated for 48 h with concanavalin A, as described (Chaly *et al.* 1983).

Double indirect immunofluorescence staining

For immunostaining, HeLa and 3T3 cells were grown on coverslips, and lymphocytes were attached to coverslips with poly-L-lysine. Cells were processed by a modified protocol for immunostaining of cytoskeletal antigens (Rogers *et al.* 1981) that permitted preservation and simultaneous detection of both nuclear and cytoplasmic antigens. Briefly, the method is as follows: 3% paraformaldehyde in phosphate-buffered saline (PBS), 30 s; 1% Triton X-100 in microtubule stabilization buffer (SB), 1 h; 0·2% glutaraldehyde in SB, 4 min; sodium borohydride (1 mg ml^{-1} in PBS, 3×4 min). Antibodies were then applied sequentially: first primary, first secondary; second primary, second secondary, for 45 min each. Non-specific staining was blocked by washes with 0·15% gelatin in PBS before each primary antibody. All samples were counterstained with Hoechst 33258 (1 $\mu g\,ml^{-1}$ in PBS) and mounted in 50% glycerol–PBS with 0·1% *p*-phenylene diamine. Non-specific staining was monitored by preparing coverslips in which one or both primary antibodies were replaced by PBS.

Antibodies

Primary antibodies. The production and characterization of monoclonal PI1 (ascites fluid, 1:500) has been described (Chaly *et al.* 1984). Microtubules were detected using a monoclonal anti-tubulin (ascites fluid, 1:1000) produced in mouse against *Polytomella agilis* flagellar axoneme tubulin (Aitchison & Brown, 1986). The anti-centromere (Brenner *et al.* 1981; Chaly *et al.* 1983; Moroi *et al.* 1980, 1981) was serum (1:40) from a patient with the CREST syndrome of scleroderma (gift from Dr M. J. Fritzler, University of Calgary, Calgary, Alberta).

Secondary antibodies. Fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG(H+L) (Cappel Laboratories) (1:75); FITC-conjugated goat anti-mouse Igm (μ-chain specific) (Kierkegaard & Perry) (1:80); tetramethyl rhodamine isothiocyanate (TRITC)-conjugated goat anti-mouse IgG(H+L) (Zymed) (1:100); TRITC-conjugated goat anti-mouse IgG (γ-chain specific) (Zymed) (1:100); FITC-conjugated rabbit anti-human Ig (Zymed) (1:60).

Microscopy

Samples were observed with a Zeiss Universal microscope or a Zeiss Photomicroscope III equipped with ×63 (n.a. 0·8–1·25) Plan-Neofluar objectives, a 50 W mercury burner and epifluorescent illumination. Photographs were recorded on Ilford XP1-400 film.

Results

Results with HeLa and 3T3 cells, and lymphocytes were similar and the micrographs selected apply equally to the three cell types.

The prometaphase configuration

The configuration is depicted in Figs 1–3. The images in Figs 1B–B″ and 3 are interpreted as representing polar views of the equational orientation of the configuration in Figs 1A–A″ and 2.

Equatorial view. The ovoid spindle lay at the centre of the configuration and was coated with chromosomes (Figs 1A,A″, 2). As well as the spindle poles, it comprised aster MTs, MTs apparently extending between the poles, and MT bundles radiating between the chromosomes (Figs 1A,A″, 2D–E, D′–E′). MT density appeared greater at the margins of the spindle than in the centre (Figs 1A″, 2D′–E′).

The chromosomes were arranged radially, positioned so that the centromeres outlined the spindle in curvilinear arrays (Figs 1A′, 2B–G) and the telomeres were at the outer surface of the configuration (Figs 1A, 2). The centromere/chromosome array was continuous about one spindle pole, but was in many instances interrupted at the other (Figs 1A–A″, 2B–G, B′–G′). Some chromosomes were generally visible midway between the spindle poles, out of the plane of focus (Figs 1A, 2C–G).

The configuration was asymmetrical, in that centromeres were not distributed uniformly over the entire surface of the spindle. It appeared that the spindle retained a 'cytoplasmic' and a 'nuclear' face, as present in prophase. Chromosomes were preferentially associated with the nuclear face (Fig. 2).

Polar view. The chromosomes were arranged radially

around the spindle with centromeres towards the centre and telomeres at the surface of the configuration (Figs 1B–B″, 3). In each focal plane, the centromeres formed a circle around the edge of the spindle (Fig. 3). In many instances, the circle was interrupted at one spot (Fig. 1B′).

The spindles were bipolar (Fig. 3C,C″) and hollow (Figs 1B″, 3C′). They comprised primarily kinetochore MT bundles, arranged in circles apparently coincident with the centromeres (Fig. 1B–B″). MT bundles inside the circles were not prominent.

Frequency of the configuration. The frequency of the configuration was determined in unsynchronized populations of HeLa and 3T3 cells, and of mouse lymphocytes stimulated with concanavalin A for 48 h. Cells scored as early prometaphase were characterized by a chromosome mass resembling that in prophase nuclei but showing signs of no longer being restrained by a nuclear envelope (Figs 4B–B′, 5). Only those mitotic figures clearly identifiable as forming the configuration were scored as

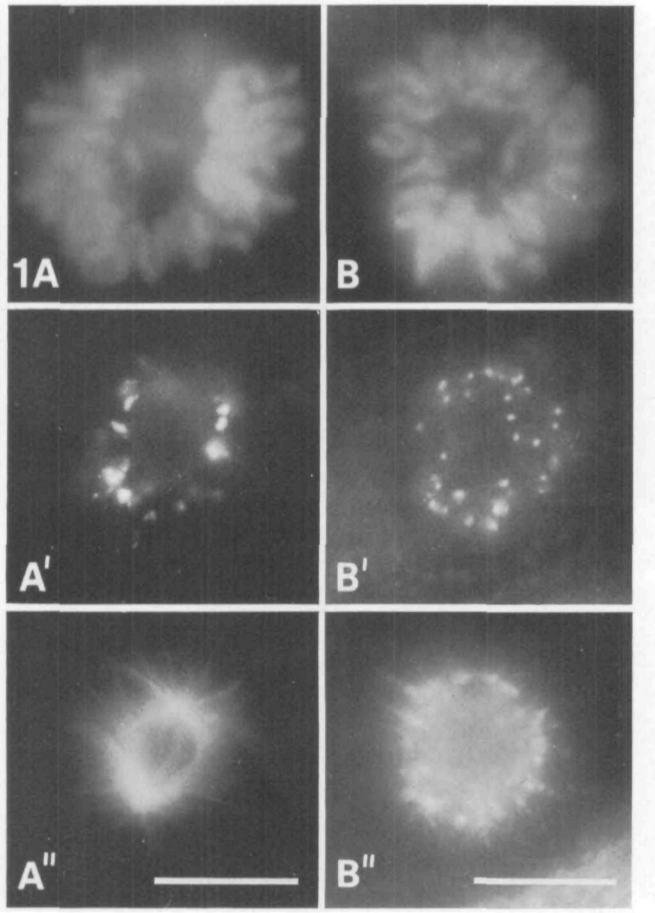

Fig. 1. Equatorial (A–A″) and polar (B–B″) views of the prometaphase configuration in HeLa cells, stained with Hoechst (A, B) and double-labelled with anti-centromere (A′,B′) and anti-tubulin (A″,B″). ×1900. Bars on all micrographs, 10 μm.

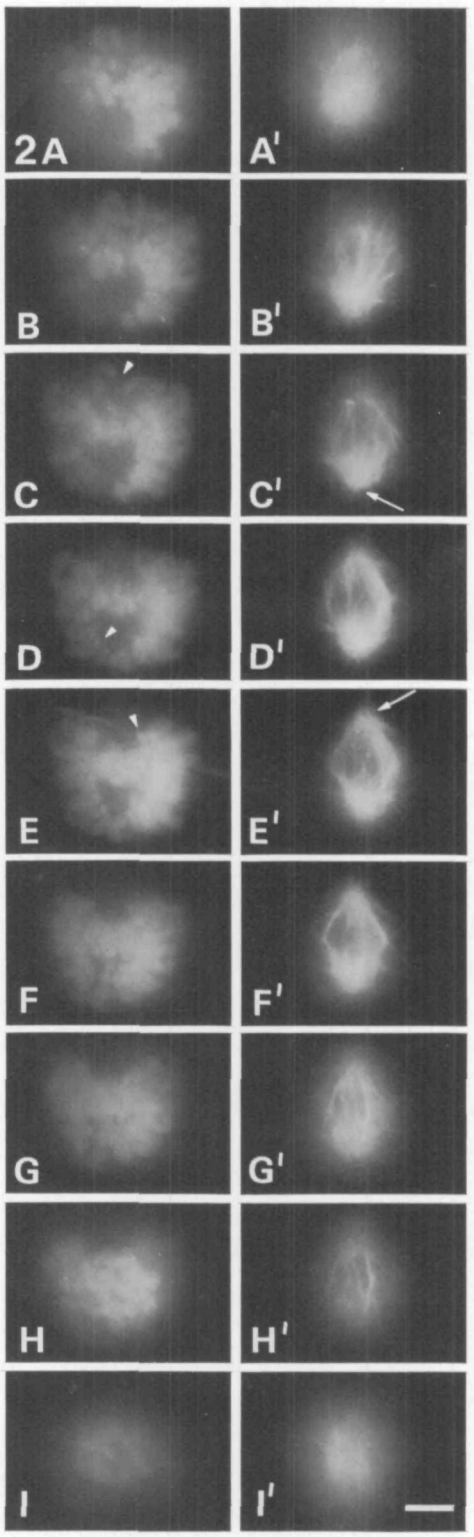

Fig. 2. Nine consecutive focal planes (A–A′, B–B′, C–C′, D–D′, E–E′, F–F′, G–G′, H–H′, I–I′) of an equatorial prometaphase configuration of 3T3 cells, stained with Hoechst (A–I) and labelled with anti-tubulin (A′–I′). The configuration is slightly tilted so that one MTOC (arrow) is visible in plane 3 (C–C′) and the other in plane 5 (E,E′). Mouse centromeres, located at A+T-rich pericentromeric heterochromatin, are visualized by Hoechst due to preferential binding of the dye to A+T rich DNA (e.g. arrowheads in C–E). Centromeres can be identified in planes 2–6 (B–G); no centromeres are evident in the last two planes. ×850.

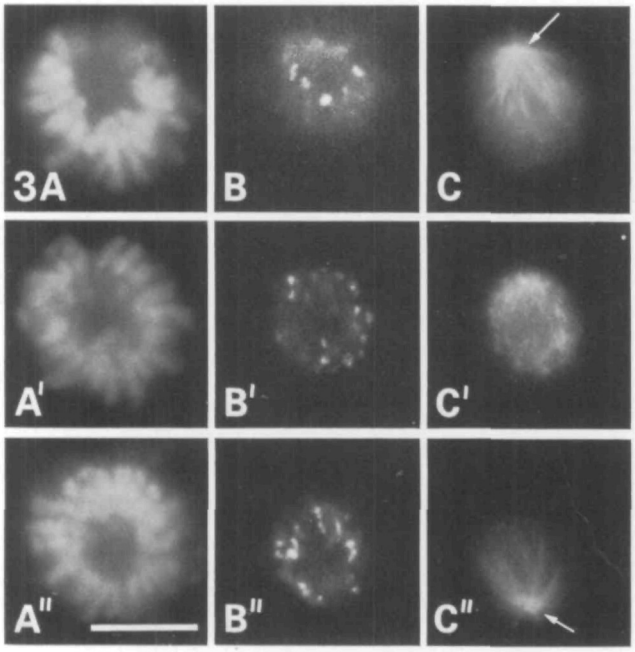

Fig. 3. Three sequential focal planes (A–A″, B–B″, C–C″) of a polar prometaphase configuration in HeLa cells, stained with Hoechst (A–C) and double-labelled with anti-centromere (A′–C′) and anti-tubulin (A″–C″). One spindle pole is visualized in each end plane (C,C″) (arrows). ×1400.

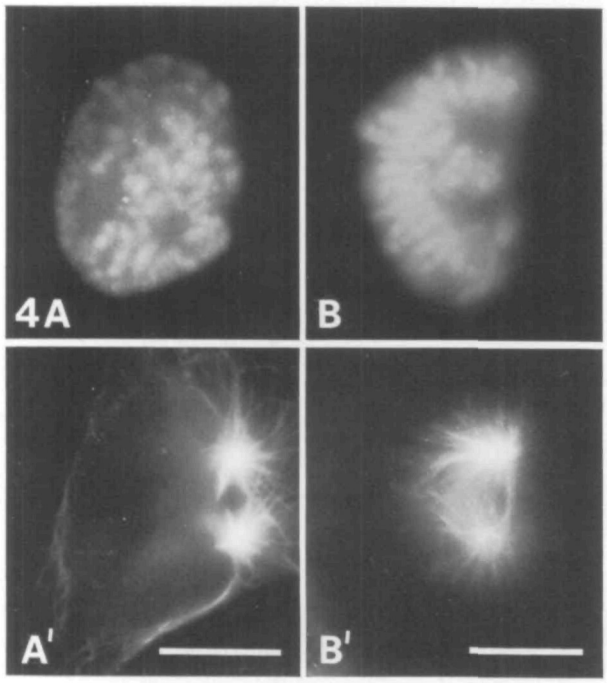

Fig. 4. HeLa cells stained with Hoechst (A–B) and labelled with anti-tubulin (A′–B′). In late prophase (A–A′), the cytoplasmic MT network is partly disaggregated and two asters are evident (A′) near the nucleus and apparently associated with indentations of the nuclear surface. In early prometaphase (B–B′), the MTOCs (B′) appear embedded in the chromosome mass. A. ×1600; B, ×1400.

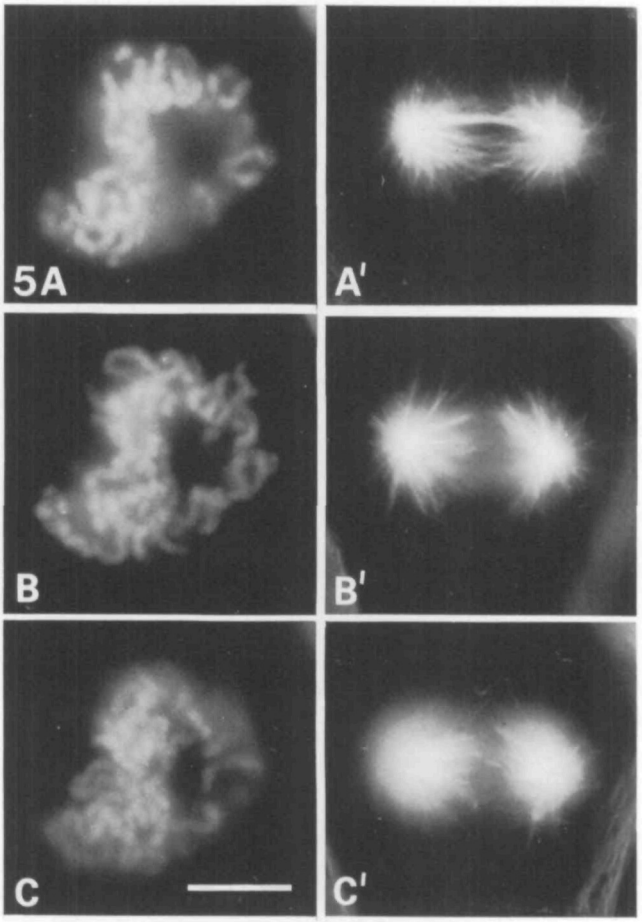

Fig. 5. Three focal planes (A–A′,B–B′, C–C′) of a HeLa cell in early prometaphase, stained with Hoechst (A–C) and labelled with anti-tubulin (A′–C′). A′–C′ have been printed to optimize visualization of MTs. In consequence, the two MTOCs in A′ are not clearly visible. ×1400.

prometaphase. Figures apparently in transition to metaphase (as in Fig. 9) were scored as metaphase.

Despite these rigorous criteria, it is clear from Table 1 that the configuration represented a significant proportion (10%) of the mitotic cells in all three cell types. In the mouse cells, it was 1½–2 times as abundant as metaphase or anaphase.

The prometaphase configuration exhibited features that could not readily be explained from the current understanding of mitosis. With some exceptions (Aubin *et al.* 1980; McIntosh *et al.* 1975; Roos, 1973*a*), in descriptions of MTOC separation during prophase, the MTOCs are depicted as moving to diametrically opposite sides of the nucleus. This implies that in prometaphase, after nuclear envelope breakdown, the chromosome mass would lie entirely *between* the MTOCs, as it does at metaphase. Images of the prometaphase configuration (Figs 1–3), however, clearly demonstrated that the chromosomes *surround* the spindle. In an attempt to explain these observations, we carried out observations on cells in prophase and early prometaphase.

Prophase–early prometaphase

Specifically, we examined the relative position of the

Table 1. *Frequency of mitotic stages*

Cell type	% Mitotic cells*†						
	Prophase	Early prometaphase	Prometaphase	Metaphase	Anaphase	Telophase	Early G_1
3T3	35·8	4·7	12·5	6·8	5·2	11·0	23·8
HeLa	20·8	6·8	9·1	21·6	9·5	10·6	21·6
Mouse splenocyte‡	56·4	9·5	9·5	6·4	6·9	6·2	5·1

*The mitotic index was 3·3 % for 3T3, 4·4 % for HeLa cells and 2·5 % for splenocytes.
† % are based on 400 mitotic figures in 3T3 cells and splenocytes, and on 250 figures in HeLa cells.
‡ Splenocyte populations were examined at 48 h after addition of mitogen.

MTOCs with respect to one another, with respect to the nuclear periphery during late prophase and early prometaphase, and with respect to the nuclear surface at these stages.

The MTOCs lay immediately adjacent to the prophase nucleus in all 3T3 cells and lymphocytes observed, as well as in most HeLa cells. In less than 1 % of the HeLa cells, one or both MTOC(s) lay in the cytoplasm at some distance from the nucleus. Variability in position of MTOCs has been reported in PtK cells (Aubin *et al.* 1980; Roos, 1973*a*) as well as in other cell types (Bajer & Molè-Bajer, 1981).

Contrary to expectations, however, initial observations indicated that, not only did the MTOCs not move to opposite sides of the ovoid nucleus during late prophase (Fig. 4A–A′), but that even in early prometaphase they were separated only by a partial arc of the nuclear surface (Figs 4B–B′, 5). Aubin *et al.* (1980) have reported similar observations for 3T3 cells.

The subjective evaluation was tested by scoring the position of MTOCs with respect to one another and to the nuclear periphery in late prophase in 3T3 and HeLa cells (Table 2). The results support the initial observation. In over 80 % of late prophase cells, the MTOCs were separated by only a partial arc of the nuclear surface.

These data suggested that after nuclear envelope breakdown the chromosome mass would lie to one side of the immature spindle rather than being sandwiched between the two spindle poles. The observation that the spindle in the prometaphase configuration showed differentiated cytoplasmic and nuclear faces was consistent with this.

The results also implied, however, that the spindle and its poles would lie in the cytoplasm 'above' the chromosomes at prometaphase, i.e. at a different focal plane from them. Extensive through-focusing observation demonstrated, on the other hand, that the spindle/MTOCs were somewhat embedded in the chromosome mass (Fig. 2) and that both components were readily visualized in the same focal plane (Figs 1B,B″, 2C–E, C′–E′).

We therefore examined the position of the MTOCs

Table 2. *Position of MTOCs relative to the nuclear periphery in late prophase*

Cell line	% Cells with depicted MTOC/nucleus relationship*†						
					‡	§	§
3T3	33	27	15	8·7	10	3	3·3
HeLa	35	29	14	5	15	1	1

*% are based on 300 3T3 and 100 HeLa in late prophase.
† Relative MTOC positions considered equivalent are grouped in one column.
‡ Late prophase cells in which one MTOC was on the substratum side of the nucleus and the other on the side facing the medium.
§ Late prophase cells in which MTOC were on diametrically opposite sides of the nucleus.

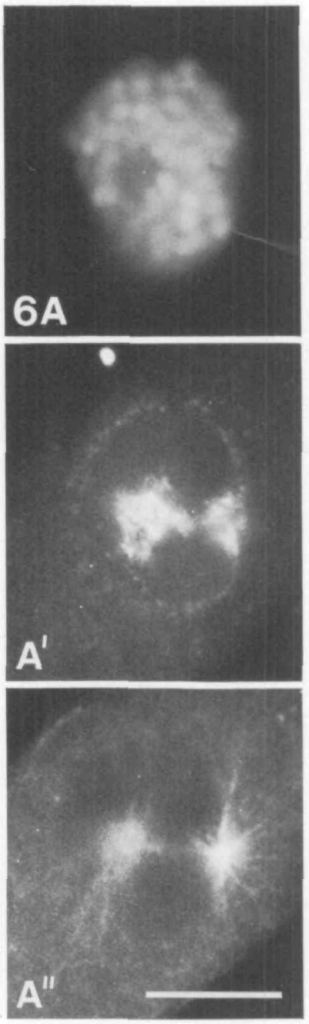

Fig. 6. 3T3 cell in late prophase, stained with Hoechst (A) and double-labelled with antibody P11 (A′) and anti-tubulin (A″). ×1800.

with respect to the surface of the nucleus/chromosome mass during late prophase and early prometaphase.

Monoclonal antibody PI1 was applied as a tag for the nuclear surface. This antibody detects peripheral and internal components of the nucleus in interphase (Chaly *et al.* 1984). The peripheral component becomes more prominent as the internal labelling disperses during prophase. Furthermore, two involutions of the nuclear periphery, separated by a partial arc of the nuclear surface, are always identified by PI1 in late prophase (Chaly *et al.* 1984). Double immunofluorescence labelling with PI1 (Fig. 6A′) and anti-tubulin (Fig. 6A″) showed that in late prophase (Fig. 6A) the MTOCs were located in the cytoplasm adjacent to and perhaps lying within the nuclear involutions. Centriole-containing nuclear involutions late in prophase have been described in a number of cell types, including HeLa (Paweletz, 1974; Robbins & Gonatos, 1964) and PtK1 (Roos, 1973*a*).

At nuclear envelope breakdown, PI1-staining disperses (Chaly *et al.* 1984). However, observation of early prometaphase spindle/chromosome complexes indicated that the MTOC/nucleus relationship in late prophase was carried through to early prometaphase. As depicted in Fig. 5, the spindle poles were apparently embedded in chromosome-free 'holes' surrounded by the chromosome mass. This through-focus series also demonstrates the asymmetrical position of the immature spindle with respect to the chromosomes at this stage of mitosis. Whereas MT bundles apparently connecting the poles are clearly visible in focal plane no. 1 ('cytoplasmic face') (Fig. 5A,A′), aster and kinetochore MT bundles and their cross-sections predominate in the other two planes (Fig. 5B–C, B′–C′). Analogous focal planes in another cell at a similar mitotic stage can be identified in Fig. 4B′.

Prometaphase to metaphase

Metaphase spindle/chromosome arrays (Figs 7–8) were readily distinguishable from those of the prometaphase configuration by several characteristics. (1) In the configuration, chromosomes were arranged around a chromosome-free space, whether in equatorial (Fig. 1A) or in polar (Fig. 1B) views. There was no chromosome- or centromere-free space in either edge-on (Figs 7A–A′, B–B′, C–C′) or cross-section (Fig. 8B) views of the metaphase plate. (2) The prometaphase spindle lay within the chromosome array (Fig. 1A,A″,B,B″), whereas the half-spindles lay on either side of the chromosome plate at metaphase (Fig. 7A,A″,B,B″,C,C″). (3) In the prometaphase configuration, MT bundles were more prominent at the edges of the spindle than in the centre (Fig. 1A″,B″), presumably because of a concentration of kinetochore MTs. On the other hand, MT bundles were relatively uniformly distributed from

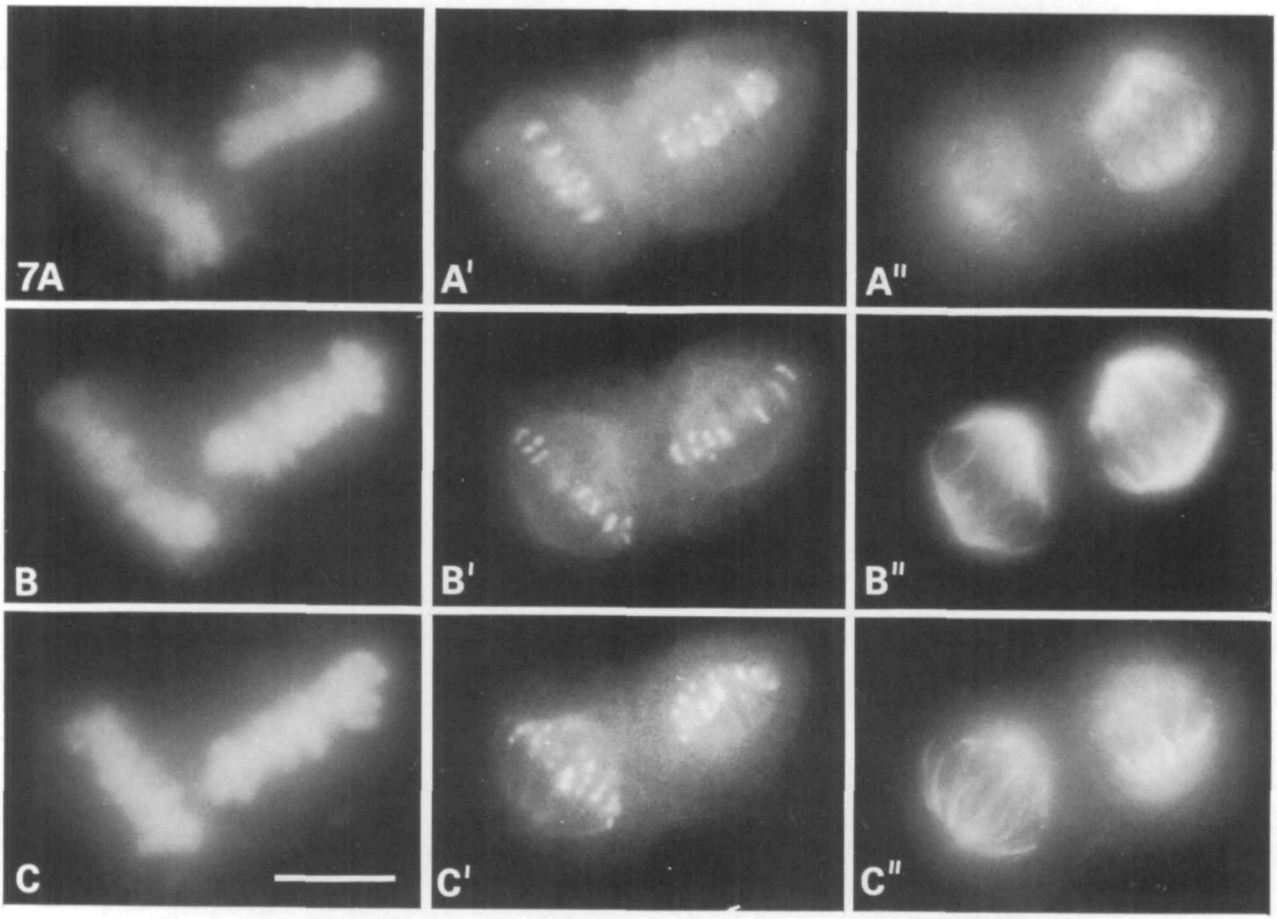

Fig. 7. Three sequential focal planes (A–A″, B–B″, C–C″) of two HeLa cells in metaphase, viewed edge-on. The cells were stained with Hoechst (A–C) and double-labelled with anti-centromere (A′–C′) and with anti-tubulin (A″–C″). ×1600.

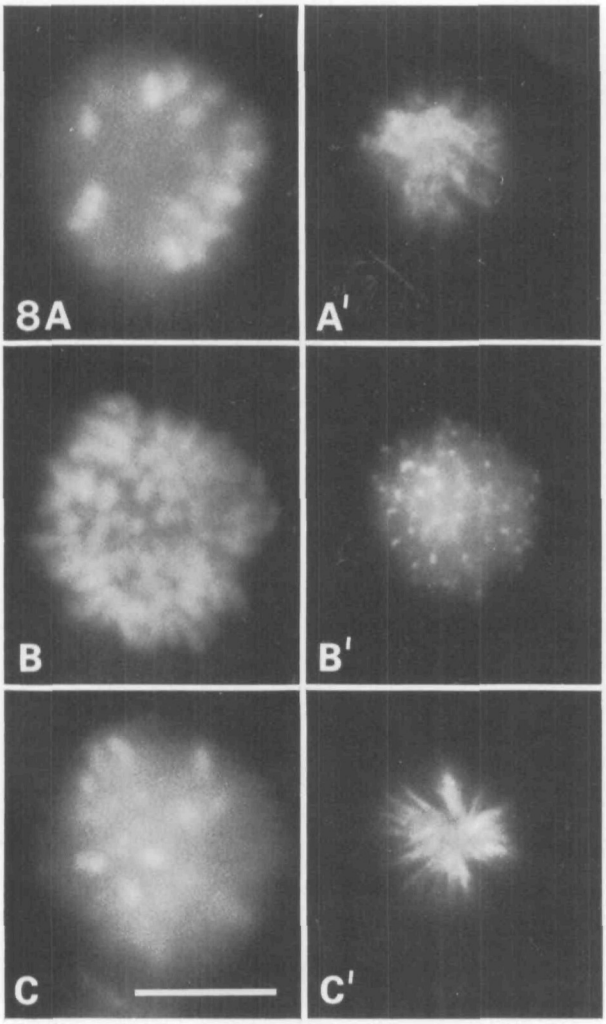

Fig. 8. Three sequential focal planes (A–A′, B–B′, C–C′) of a polar view of a HeLa cell in metaphase. The cell was stained with Hoechst (A–C) and labelled with anti-tubulin (A′–C′). A spindle pole is seen in the first (A′) and third (C′) planes. ×1900.

one edge of the metaphase plate to the other in all views (Figs 7A″–C″, 8A′–C′). (4) The configuration was roughly circular, both in equatorial (Fig. 1A–A″) and in polar (Fig. 1B–B″) views. The metaphase chromosome plate is distinctive in this regard, being roughly rectangular when viewed edge-on (Fig. 7A–C). Although circular in cross-section views (Fig. 8B), the absence of a chromosome-free space (see (1) above) distinguished it from the prometaphase configuration.

As well as the readily identifiable prometaphase configuration and metaphase plates, numerous mitotic figures were observed with spindle/chromosome arrays apparently intermediate between the configuration and the mature metaphase figure (Fig. 9). Most chromosomes in such views were arrayed in a plate-like group midway between the spindle poles, but were less tightly packed than at metaphase (Fig. 9A; cf. Fig. 7A–C). Centromeres were visualized as paired dots (Fig. 9A′), indicating that chromatid separation had not yet occurred (Brenner *et al.* 1981). Some characteristics of the con-

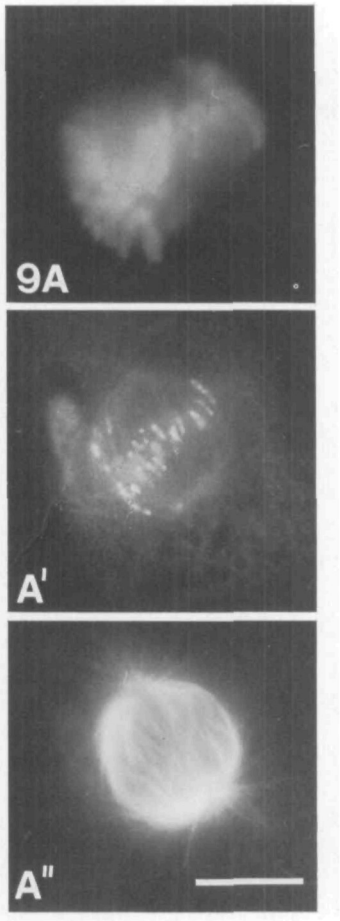

Fig. 9. HeLa cell in late prometaphase stained with Hoechst (A) and double-labelled with anti-centromere (A′) and anti-tubulin (A″). ×1400.

figuration were identifiable in short curvilinear arrays of centromeres at the margins of the broad chromosome plate in these mitotic figures (Fig. 9A′). The spindle closely resembled that at metaphase, although each half-spindle was somewhat ragged at the centromere end (Fig. 9A″; cf. Fig. 7A″–C″).

Discussion

As discussed in the Introduction, order with respect to the nucleus/chromosomes can be of at least two types: spatial (e.g. Rabl orientation) and intragenomic. At metaphase, spatial order is evidenced by alignment of centromeres at the centre of the plate with telomeres at the external surfaces. The plate might be described as two populations of chromosomes, each in the Rabl orientation, in contact *via* the centromere clusters. The orderly arrangement of homologues and non-homologues within the plate appears to be determined by the relative lengths of the arms on adjacent chromosomes (Fussell, 1984; Heslop-Harrison & Bennett, 1984). In the case of the interphase nucleus, neither type of order has been proven definitively, although considerable evidence points to their existence (Manuelidis, 1985; Schardin *et al.* 1985). The non-random localization of readily identifiable portions of the genome, such as rDNA (i.e. nucleoli), chromatin bodies, centromeres and telomeres (reviewed by Church, 1981; Comings, 1968, 1980; Fus-

sell, 1987; Hubert & Bourgeois, 1986), as well as extensive evidence in favour of the Rabl orientation (see Introduction) all point to spatial ordering of interphase chromosomes. Direct evidence for intragenomic order is still somewhat contradictory (Gruenbaum *et al.* 1984), but genetic data (Comings, 1968; Hubert & Bourgeois, 1986), the non-random localization of chromosomes in interspecies hybrids (Manuelidis, 1985; Schardin *et al.* 1985) and a variety of other microscopic observations (see Schardin *et al.* 1985, for review) strongly suggest that the positioning of homologues and non-homologues within the interphase nucleus also is non-random. The relationship between interphase order, of either type, and metaphase order has not been established.

The prometaphase configuration is an ordered, asymmetrical mitotic figure that exhibits the essential features of the Rabl orientation: clustering of centromeres and telomeres at opposite faces of the configuration, and positioning of the centromeres at the face near the MTOC(s). It appears to be a widespread phenomenon and was observed in all mammalian primary and continuous cultures we examined. As well as 3T3, HeLa and mouse splenocytes, these include Chinese hamster ovary cells, human and bovine mitogen-activated lymphocytes, and mouse L929, embryonal carcinoma P19 and El4 cells (data not shown). Similar chromosome/spindle arrays have also been reported in studies of PtK1 (Roos, 1976), PtK2 (Peterson & Berns, 1980), human laryngeal carcinoma HEp-2 (Moroi *et al.* 1981), HeLa (Robbins & Gonatos, 1964), mouse splenocytes and *Triton* fibroblasts (Hughes, 1952), mouse spermatogonia (Fogg & Cowing, 1953) and the grasshopper *Caledia captiva* (Coates & Wilson, 1985).

We propose that the configuration functions in maintaining intragenomic order throughout the cell cycle by translating the order of the interphase nucleus into order at the metaphase plate. A model for the interphase to metaphase transition, an extension of a model recently proposed by Fussell (1984), is described below (Fig. 10). Our model is consistent with observations *in vivo* of dividing HeLa S3 (Robbins & Gonatos, 1964), PtK1 (Roos, 1973*a*) and new lung cells (Bajer & Molè-Bajer, 1981), as well as with descriptions of early mitosis in *C. captiva* (Coates & Wilson, 1985).

During interphase

Centrioles have been shown to duplicate during interphase in several cell types (Aubin *et al.* 1980; Kuriyama & Borisy, 1981). By the end of G_2 (Fig. 10A), two unseparated MTOCs are usually evident near the nucleus.

Data indicate that chromatin is anchored in large loops at the nuclear periphery during interphase, and that the anchoring may be mediated by components of the nuclear matrix such as the lamins (Hubert & Bourgeois, 1986; Schardin *et al.* 1985). Thus, each interphase chromosome appears to be tethered to the nuclear periphery at several points, probably including the telomeres (Fig. 10A). From the evidence (Fussell, 1987), centromeres are either distributed throughout the nuclear interior and are not tethered (Fig. 10A), or may be tethered if clustered opposite the telomeres.

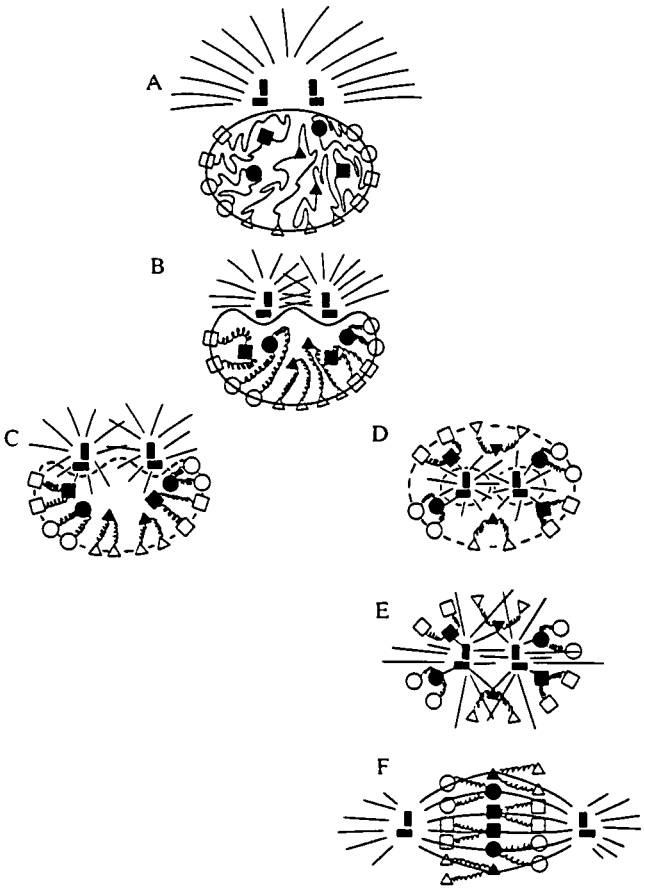

Fig. 10. Proposed model for the interphase to metaphase transition: interphase (A), prophase (B), early prometaphase (side view) (C), early prometaphase (equatorial view) (D), equatorial prometaphase configuration (E), edge-on metaphase (F). The nucleus is represented as containing $2n = 6$ metacentric chromosomes. The order in which the chromosomes have been affixed to the envelope was chosen at random. (□, △, ○) telomeres; (■, ▲, ●) centromeres; (▙) centriole pair (MTOC).

During prophase

As chromatin condenses during prophase, chromatin loops are believed to detach from the nuclear periphery until chromosomes are anchored at the envelope only by the telomeres (Fig. 10B) (Fussell, 1984; McKeon *et al.* 1983). If the telomeres lie to one side of the nucleus (Fig. 10B), Rabl spatial order and intragenomic order would be maintained.

Although the model has been developed specifically for mitosis, it allows for the ordered chromosome movements reported during meiotic prophase (Church, 1981). Telomeres can be displaced by sliding along the nuclear envelope in a manner analogous to that of bacterial chromosomes, and movement is similarly possible for centromeres, whether free (Fig. 10B) or tethered. However, the extent to which subsequent steps in the model might apply to the meiotic prophase–metaphase transition is unclear. Accompanying meiotic prophase

chromosome movement is a 180° rotation of the relative positions of centromeres and MTOCs. At nuclear envelope dissolution, therefore, the Rabl orientation has become transformed into the 'bouquet', in which it is the telomeres that are juxtaposed with the MTOCs (Church, 1981; Fussell, 1987).

The duplicated MTOCs, lying at the opposite side of the nucleus from the telomeres, move apart during prophase (Aubin *et al.* 1980; Paweletz, 1974; Roos, 1973*a*). In late prophase, they sink (or are pulled down) into the nucleus at the involutions (Fig. 10B) (Paweletz, 1974). Though still separated from the chromosomes by the nuclear envelope, the immature spindle is then in effect positioned with respect to the chromosomes as it will be in the prometaphase configuration, i.e. chromosomes beneath it on the nuclear face, to either side of it and with a few chromosomes between the poles, but with essentially no chromosomes above it on the cytoplasmic face.

By late prophase, the position of the MTOCs would identify the site and plane of the future metaphase plate. This will be midway between the MTOCs and perpendicular to the long axis of the spindle.

During prometaphase

As the nuclear envelope begins to break down in early prometaphase, remaining chromosome contacts are dissolved, i.e. telomeres are released (Fig. 10C–D). Nuclear envelope breakdown is coincident with depolymerization of the lamins (Krohne & Benavente, 1986). According to our model, the telomeres remain clustered at the far side of the chromosome mass from the spindle, held in place perhaps by non-lamin nuclear proteins such as peripherin (formerly P1) (Chaly *et al.* 1984, 1985) and perichromin (Chaly *et al.* 1985; McKeon *et al.* 1983).

Early prometaphase chromosomes are not fully condensed (Roos, 1973*a*), and undergo further coiling from wavy threads to semi-rigid rods. If telomeres are held in a cluster to one side of the chromosome mass (Fig. 10C), the centromeres perforce will become clustered at the other side, towards the MTOCs, once chromosomes become rigid (Fig. 10C).

The model is consistent with current knowledge about both the timing and the mechanism of kinetochore–MT attachment. There is still some controversy as to the source of kinetochore MTs , but the weight of evidence indicates that, *in vivo*, kinetochores capture MTs that originate at the poles (Kirschner & Mitchison, 1986). Kinetochores in HeLa (Paweletz, 1974) and PtK1 cells (Rieder, 1982; Roos, 1973*b*, 1976) begin to associate with MTs almost as soon as the nuclear envelope begins to fragment. The envelope generally breaks first at the involutions, i.e. near the MTOCs, and MTs appear immediately in the nucleus, apparently penetrating through gaps in the envelope (Rieder, 1982; Roos, 1976). Various studies have demonstrated that chromosomes acquire MTs asynchronously, with those kinetochores closest to the poles becoming attached first (reviewed by Rieder, 1982). They have also demonstrated that such chromosomes are initially oriented only towards one pole – the one they are closer to (Fig. 10C–E, chromosomes (■, ●)). Chromosomes further from the poles have been shown to attach later (Fig. 10C–D, chromosome (▲) and, if near the equator, to have a kinetochore attached to each pole, i.e be amphi-oriented, from the beginning (Fig. 10E, chromosomes (▲)). Mono-oriented chromosomes generally become amphi-oriented before congression (Rieder, 1982; Roos, 1976).

Two chromatids could be resolved in some chromosomes in equatorial views of the prometaphase configuration (Figs 1–2), suggesting that these chromosomes are amphi-oriented. Only one chromatid was detected in other chromosomes (Fig. 1A) and these may be mono-oriented. Paired chromatids were more frequently visualized in mouse cells. This may reflect less difficulty in achieving amphi-orientation of the mouse genome, in which most chromosomes are sub-telocentric. Mouse chromosomes are thus less subject to the formation of V, U and J shapes, in which only kinetochore may be in the direct 'line of sight' of pole-originating MTs (e.g. see Fig. 10C, chromosomes (■)).

With attachment of the centromeres *via* kinetochore MT bundles to the spindle lying near by, the prometaphase configuration is complete (Fig. 10E), still retaining both the spatial and intragenomic order of the interphase nucleus.

During prometaphase to metaphase

Observation of prometaphase–metaphase intermediates, as in Fig. 9, suggests that the metaphase plate is created by a 'compression' of the prometaphase configuration towards the equator. The mechanism of this compression is not clear.

As noted by Roos (1976), those chromosomes between the spindle poles at nuclear envelope breakdown (Fig. 10C–D, chromosomes (▲)) appear to identify the future site of the metaphase plate. In PtK1, some of these chromosomes remain near the equator until metaphase but others oscillate between the equator and the poles (Roos, 1976). The sequence in which chromosomes reach the plate would appear to depend in part on their location at prometaphase. Observations on PtK1 indicate that, for chromosomes lying in approximately the same focal plane, the chromosome nearer the equator congresses before that nearer the pole (Roos, 1976). The transition figures between prometaphase and metaphase (Fig. 9) may thus arise as the chromosomes are progressively reeled in and/or pushed towards the plate (Rieder, 1982).

Chromosome oscillation appears to be a major feature of prometaphase (Rieder, 1982; Roos, 1973*a*, 1976), and has been investigated most particularly in newt lung cells (Bajer & Molè-Bajer, 1981). These cells show great variability in the distance separating the prometaphase MTOCs, ranging from essentially no separation and a 'monopolar' prometaphase to extreme separation and 'anaphase-like' prometaphase. Both extremes may result in completion of a normal mitosis, or may lead to abortion, multipolar division, or some other defective mitosis. In all cases, the MTOC/chromosome arrays are analogous to the prometaphase configuration, comprising MTOC(s) surrounded by a ring of chromosomes in the Rabl orientation. The chromosomes undergo extensive

oscillations before either entering metaphase and completing mitosis, or beginning an abnormal mitotic sequence. Of particular relevance to the present study is the analysis of chromosome movement in a monopolar configuration that resulted in normal mitosis (Bajer & Molè-Bajer, 1981, fig. 6A). The paths of 17 chromosomes were tracked over a period of 10 min, with striking results. Despite the complex movements of the kinetochores, and oscillations of greater than 10 μm in some instances, the relative order of the chromosome centromeres in the ring did not change during that time. Centromeres that were adjacent to one another at time zero were still adjacent after 10 min, with one exception. (A chromosome located somewhat outside the centromere ring shifted by 1–2 centromeres.) These results suggest that there is a mechanism by which chromosome order can be retained within mitotic figures, despite extensive chromosome movement. The observation that adjacent chromosomes tended to oscillate together (Bajer & Molè-Bajer, 1981, Fig. 7) further suggests that the chromosomes may be physically linked, perhaps by some form of interchromosomal 'glue' along the arms, such as peripherin (Chaly *et al.* 1984) or perichromin (McKeon *et al.* 1983), by temporary lateral association of kinetochore MTs (Bajer & Molè-Bajer, 1981), or by both.

The final order of chromosomes at metaphase has been shown to depend on the length of chromosome arms, arranged apparently so that adjacent arms are of most similar length (Fussell, 1984; Heslop-Harrison & Bennett, 1984). Circumstantial evidence indicates that such a relationship also exists in the interphase nucleus (Fussell, 1984). It remains to be determined whether this parameter is itself sufficient to determine intragenomic order.

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