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Chromatid Behavior in Late Mitosis: A Scanning Electron Microscopy Analysis of Mammalian Cell Lines with Various Chromosome Numbers

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CHROMATID BEHAVIOR IN LATE MITOSIS: A SCANNING ELECTRON MICROSCOPY
ANALYSIS OF MAMMALIAN CELL LINES WITH VARIOUS CHROMOSOME NUMBERS

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Abstract

Chromatid activity during the process of nuclear reformation following metaphase is a period of mitosis where little precise information is available. Nuclear reformation requires that chromosomes, at metaphase and chromatids during anaphase and telophase align, position and associate in a clearly defined sequence to insure the specific design of each nucleus. Four cell lines with chromosome numbers ranging from seven to almost seventy were chosen to determine whether the process of nuclear assembly is the same throughout. Chromosomal alignment at metaphase is found to be radial in all four cell lines. Chromosome positioning is essentially the same in all four, where the smaller chromosomes are located centrally and longer ones are positioned peripherally in a radial alignment. Chromosomal association is directly related to chromosome number. The more chromosomes in a one dimensional plane occupying a given area, the closer the association. In comparing the HeLaS3 and muntjac chromatids, the former has the closer association at metaphase. Since association is the most important aspect of chromatid behavior in nuclear reformation, chromatid positioning becomes a vital process during anaphase movement. Chromatid positions established during anaphase determines later positioning in the interphase nucleus because of the subsequent interconnection of adjacent chromatids by the formation of a fibrous meshwork. This fibrous meshwork, formed in anaphase and early telophase, functions to stabilize chromatids following their positioning and it also serves as a substrate or matrix for the assembly of nuclear envelope.

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Introduction

Welter and Hodge (1985) developed a technique which permits the three-dimensional visualization of mitotic chromatid configurations with scanning electron microscopy (SEM). An analysis of chromatid configurations in HeLaS3 cells using SEM indicates that the process of nuclear assembly following metaphase proceeds in a continuation of distinct steps which involves chromatid positioning, alignment and association (Welter, et al., 1985). Of the three, association is the most important to insure proper nuclear assembly. Late mitotic chromatids must become closely associated with each other so that their surfaces become part of the outer surface of the progeny nucleus. Chromatid alignment is not a late mitotic event. However alignment is an extremely important metaphase event which permits the formation of a hollow sphere by late telophase. It is essential that the longer chromosomes position themselves on the periphery of the metaphase plate so that the length of the chromatid is available for the formation of the non-polar region of the walls of the hollow sphere of late telophase. Positioning, like alignment, is a metaphase and early anaphase event. The positioning of the shorter chromatids is not as essential as the positioning of the longer chromatids. The alignment of the smaller chromatids is not as critical as the alignment of the longer chromatids since they act as filler at the antipolar end of the developing hollow sphere with no apparent pattern in their alignment. The chromatids of the late metaphase/early anaphase configuration is radial with the centromeres near the cluster of the smaller chromatids and the telomeres near the periphery of the circular configuration. It is essential that these long chromatids are parallel to each other in their alignment.

HeLaS3 mitotic cells were used in the development of the isolation procedure because large numbers of figures from a single collection were easily obtained. We were surprised to find that chromatid orientation in anaphase did not conform to that established in the literature. The HeLaS3 anaphase orientation of chromosomes is in contrast to the proposed Rabl (1885) orientation in which the centromeres form a leading pole toward the centriole, and the telomeres trail. Rabl orientation of mammalian

nuclei has been established based on studies using premature chromosome condensation (Cremer et al., 1982; Sperling and Ludtke, 1981), laser-UV-microirradiation (Cremer et al., 1983) and C-banded techniques (Hsu et al., 1971).

The anaphase orientation of chromatids observed in HeLaS3 is not observed in Indian muntjac, CHOK1 and LN anaphases, which suggests that the anaphase orientation is not due to the effects of the acid isolation procedure of Welter and Hodge (1985). In addition, other isolation procedures yield configurations with the same anaphase chromatid orientations as observed with the acid isolation procedure (Welter and Hodge 1985). If the anaphase and telophase orientation of chromosomes affects the internal organization of the interphase nucleus (Avivi and Feldman 1980), then perhaps HeLaS3, or human cells in general, have evolved an alternative arrangement of the chromatin. Another possibility is that the orientation of chromatids in HeLaS3 is due to the excessive number of chromosomes in the complement. In this study, we analyze the orientation of mitotic chromatids in a human diploid cell line (designated LN) and compare these findings to those observed in HeLaS3, Indian muntjac, and Chinese hamster (CHO-K1). These four cell lines were used to determine the influence of chromosome number on anaphase chromatid orientation. The male Indian muntjac has seven, CHOK1 has twenty, LN cell has forty-six and HeLaS3 has 65 + 3 chromosomes. HeLaS3 is a near triploid cell line while the others are diploid.

There is strong evidence suggesting that interphase chromosome arrangements in both plants and animal cells is nonrandom (Ashley and Pocock 1981; Avivi and Feldman 1980; Comings, 1980). Fussell (1975, 1983) demonstrated that the telophase orientation of telomeres is maintained throughout interphase, suggesting that nuclear contouring is a function of the chromatid arm. Mitotic alignment (John, 1976), synthesis and assembly of ribosomes (Steffensen et al., 1974) and gene regulation (Steffensen, 1977) are functions thought to be related to nonrandom interphase chromosomal arrangements. The alignment and subsequent stabilization of the chromatids following meshwork formation supports the metaphase/interphase relationship of chromosome positioning. This study contributes to the understanding of the behavior of chromatids throughout mitosis.

Materials and Methods

Cell culture and synchronization.

Human primary cells (designated LN), CHOK1 and Indian muntjac cells were grown in Ham's F-12 media supplemented with 20% fetal calf serum. Mitotic cells were obtained by selective detachment from cells in logarithmic growth. HeLaS3 cells were maintained in suspension culture at 37°C. Mitotic cells were obtained by selective detachment following a double thymidine block (Simmons et al., 1973).

Preparation of mitotic cells for scanning electron microscopy (SEM).

Mitotic configurations were isolated as previously described (Welter et al., 1985). Briefly, mitotic cells were washed in 75 mM KCl and fixed in 3:1 methanol:acetic acid. A drop of the cell suspension was placed on a #2-22 diameter coverglass and immersed for 2 seconds in 50% glacial acetic acid at 102°C, then placed in 50% ethanol. This treatment freed mitotic chromatid configurations from cytoplasmic and spindle elements. The mitotic configurations were critical point dried and sputter coated with gold-palladium (5-6 nm thickness). An AMR-1000A scanning electron microscope was used for the three-dimensional observation of mitotic configurations. Selected configurations were photographed on Polaroid type 55 P/N film.

Morphometric determinations

Morphometric data were obtained using the Steriometric Measurement and Analysis Program (Scientific Microprograms, Raleigh, NC) on an Apple computer. Area, perimeter, maximum diameter and form factor were determined for the different stages of mitosis.

Results

SEM Analysis of Late Mitotic Chromatid Configurations

Metaphase: Metaphase, anaphase and telophase configurations were analyzed for similarities in alignment, positioning and association of chromatids throughout the late stages of mitosis. Prophase was not analyzed because of the difficulty in obtaining sufficient figures in this stage of mitosis.

At metaphase alignment of longer chromatids is consistent in the four cell lines studied. The longer chromatids are arranged radially with the centromeres nearer the center of the configuration and with the telomeres away from the center at the periphery (Figure 1). Long metacentric chromatids are V-shaped and long submetacentric chromatids are J-shaped with only a few long chromosomes not flexed (Figure 1). The shorter chromatids which generally occupy the center of the configuration are not flexed at the centromere.

Light microscopic analysis of non-colcemid collected metaphases, that are slightly hypotonically shocked and air dried, demonstrates a circular arrangement of the centromeres of the peripherally located longer chromatids in all cell lines (Figure 1). However the HeLaS3, with its approximately one-third more (triploid) chromatids, demonstrates two concentric rings of centromeres whereas the other three cell lines demonstrate only one ring.

SEM analysis of metaphase plates demonstrate that positioning of chromosomes is generally related to size. Rarely are the large chromosomes found within the center of the plate and equally so rarely are the short chromosomes found at the periphery. It is not clear what role the small chromosomes play in the formation of the outer surface of the spherical progeny nucleus since they appear to be recessed beneath the telomeres at the antipolar end of the configuration.

Chromosome association at metaphase is directly related to chromosome number and size. Association is the closeness of one chromosome to another. Table 1 demonstrates that the greatest diameter of the metaphase configuration is found in Indian muntjac, which has the fewest number of

1A **B**

C **D**
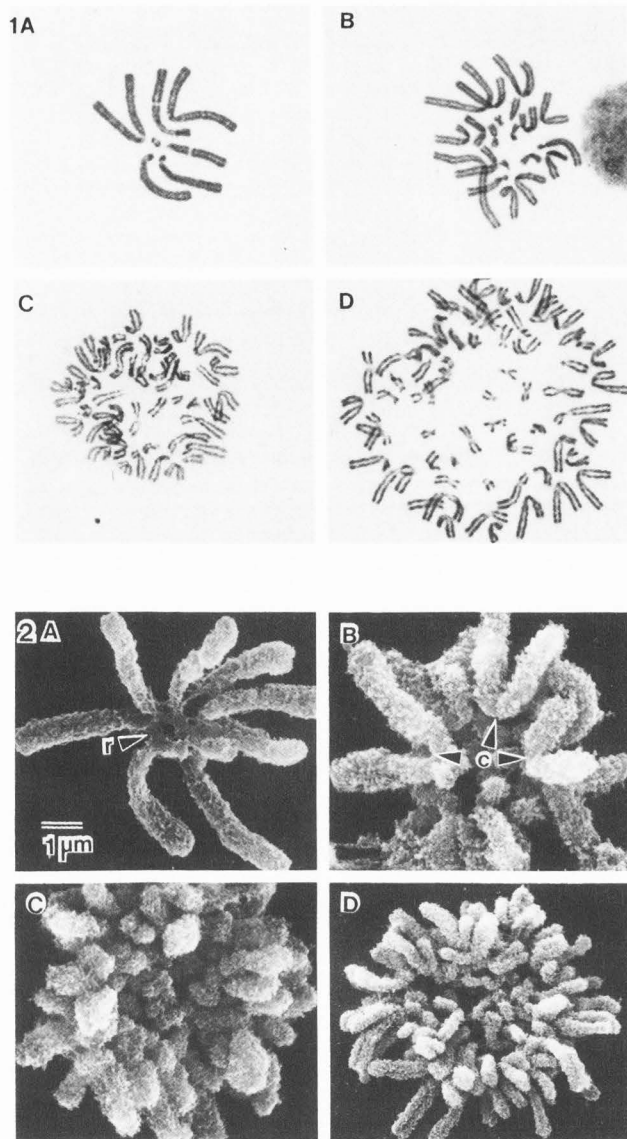

2A **B**

C **D**

Figure 1. Metaphase chromosomes present a radial array at the periphery.
The six long chromosomes of Indian muntjac (A) are arranged such that there is a great distance between adjacent chromosomes. The small Y2 chromosome is found at the center of the configuration adjacent to each of the centromeres. CHOK1 (B) with its 20 chromosomes have the same peripheral appearance as muntjac, however there are six small chromosomes occupying the center. As in muntjac the centromeres occupy a circular position intermediate between the peripheral telomeres and the centrally located small chromosomes. Although there is some displacement of the chromosomes from the methanol:acetic acid fixative and the air drying of the metaphases LN (C) presents a configuration with radially aligned chromosomes that are crowded at the periphery. The centromeres of the peripheral chromosomes occupy a circular position between the telomeres and the smaller chromosomes occupying the center. The chromosomes of HeLaS3 (D) appears to be less crowded than LN. This spreading is the result of the hypotonic shock that is required to spread the chromosomes so that they can be analyzed. Again the larger chromosomes occupy the periphery, however, the centromeres are arranged in two concentric rings at the periphery.

Figure 2. Polar view of metaphase plates.
The radial arrangement of the peripheral chromosomes is apparent in all four cell lines. Indian muntjac (A) is the only line that demonstrates clearly a continuous structure that interconnects adjacent centromeres. This ring (r) cannot be seen in CHOK1 (B), LN (C) or HeLaS3 (D) because of the close association of the chromosomes in this region. However, digestion of the chromosomes with DNase or hypotonic spreading reveals that there are interconnecting fibers found between adjacent chromosomes at the centromeres (c). The centromeres of the longer chromosomes are found within the flexed region.

TABLE 1. Maximum Diameter (μm) of Mitotic Configurations

Mitotic	HeLaS3	LN	Cell Line	Indian Muntjac	CHO-K1
Metaphase	11.4 ± 0.8	7.8 ± 0.9		12.1 + 1.0	8.3 ± 0.7
Anaphase	7.3 ± 0.6	5.3 ± 0.7		5.5 ± 1.3	5.8 ± 0.9
Anaphase-Telophase Transition	7.2 ± 0.4	5.7 ± 0.4		5.5 ± 0.6	5.6 ± 0.7
Late Telophase	6.8 ± 0.5	6.3 ± 0.7		5.9 ± 0.6	5.8 ± 0.5
G_1 (Progeny)	6.8 ± 0.4	6.5 ± 0.6		6.9 ± 0.5	6.2 ± 0.4

Measurements were obtained using the Steriometric Measurement and Analysis Program (Scientific Microprograms, Raleigh, NC) with an Apple II Computer. At least 20 measurements were obtained for each mitotic stage.

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chromosomes (seven). This diameter reflects the length of the chromosomes of Indian muntjac. With our isolation procedure the large metacentric chromosomes are approximately 10-12 µm in length. With a radial alignment of six of the seven chromosomes in muntjac, there is a large distance between adjacent chromosomes, thereby little close association (Figure 2). The only region of the muntjac where close association occurs is at the centromeric ring. We have demonstrated an interconnection of the centromeres of all of the muntjac chromosomes, except the Y2, at metaphase and have called this interconnecting structure the centromeric ring (Welter et al., 1984).

The association of adjacent chromosomes in the metaphase plates of the CHO-K1, LN and HeLaS3 is apparent from the diameters of these configurations given on Table 1. HeLaS3 with 65 or more, the chromosomes are crowded into a configuration which has a smaller diameter than the muntjac. However you must remember that the HeLaS3 and LN have the shortest of the long chromosomes of these cell lines. In our preparations the longest chromosomes in HeLaS3 and LN are about 6 µm while the longest in CHOK1 is about 8-9 µm. You will note from Table 1 that the diameter of the LN metaphase is the smallest even though this cell line has 24 more chromosomes than the CHOK1 line. In ranking these cell lines for closeness in association the chromosomes of HeLaS3 and LN have about the same distance separating them with CHOK1 next and muntjac chromosomes having the greatest distance between adjacent chromosomes.

In a lateral prospective of all metaphase plates the centromeres occupy the same plane. This was established by C-banding air dried metaphase preparations and analysed with light microscopy. However the telomeric arrangement at the periphery of the plates varied considerably. Muntjac always demonstrated a plate of the same thickness from center to periphery. However the periphery of the metaphase plate of HeLaS3 was much thicker than the central region. Telomeres of the peripheral chromosomes were directed toward each pole giving the lateral view of the HeLaS3 metaphase a bi-convexed appearance. There was a similar appearance to the CHOK1 and LN metaphases but not the degree found in HeLaS3. This peripheral orientation is a result of crowding of a large number of long chromosomes into the outer ring.

Because of the close association of the chromosomes in the HeLaS3, LN, and CHOK1 metaphase plate it is difficult to demonstrate a distinct centromeric ring in these lines. Excessive hypotonic shock prior to fixation will spread the chromosome sufficiently to demonstrate chromatid interconnections but a clearly defined centrometic ring, as in muntjac, is not evident.

Anaphase: Early anaphase does not differ significantly from metaphase with respect to alignment, positioning and association. The diameter of the configurations is not reduced until chromatid positioning is altered. Initial separation of homologous chromatids begins at the centromeres of the larger peripherally placed chromatids. The homologous chromatids of the centrally located small chromosomes remain together, briefly creating a central depression on each side of the plate. The telomeres of the peripherally located chromatids remain associated as the anaphase structures separate thereby producing a trailing effect of the peripheral chromatids (Figure 3). All four lines position the chromatids, as described above, in early anaphase configurations. However because of the excessive number of chromatids crowded into the two concentric rings of chromatids found in HeLaS3 a large number of telomeres of the inner ring do not trail in the anaphase separation, but lead toward the centriole.

As anaphase progresses a central chamber is formed which is composed of a floor at the antipolar end composed of the smaller chromatids, and walls which are composed of the centromeres and the proximal regions of the chromatids. C-banding studies of air dried anaphase structures, when analyzed from a lateral perspective, indicate that the centromeres are recessed within the lateral wall of the chamber below the rim of the chamber (Figure 4). The recession of the centromeres and early tethering of the adjacent chromatids makes it impossible to identify the centromeric ring found in muntjac metaphase preparations. The chromatids extend a short distance toward the polar end of the configuration before flexing at almost 180° to form the lateral wall of this hollow-half spherical structure (Figure 3). The greater the number of chromatids in the periphery, the greater the depth of the chamber. Indian muntjac has only the Y2 chromatid forming the floor of the chamber and six chromatids forming the inner wall of the chamber and sides of the sphere and has a very shallow chamber. In CHOK1 the chamber is slightly deeper and LN even deeper. HeLaS3 has a very deep chamber by mid-anaphase because of the polar orientation of the telomeres of the inner ring of chromatids. At this stage the chamber depth is approximately half the diameter of the configuration. These inner chromatids of HeLaS3 flex at the rim of the chamber at approximately 90° to overlap the chromatids of the outer ring which trailed in the anaphase separation forming the lateral surface (Figure 5). The flexion of these chromatids forming the inner wall of the chamber was in the mid to distal region of the chromatid and left the centromere deeply recessed within the chamber.

The antipolar end of the late anaphase configuration in the muntjac and CHOK1 cells is entirely composed of telomeres of the peripheral chromatids. However it is not possible to determine whether telomeres of the smaller centrally located chromatids in LN and HeLaS3 form the majority of the antipolar end of the sphere. Because of the short length of the peripheral chromatids in these two lines and the fact that lateral length of the anaphase configuration is greater than this length, the central region of the antipolar end is probably composed of the smaller chromatids.

Anaphase positioning of chromatids is extremely important in contouring the final shape of the nucleus. The positioning is also important in bringing the chromatids in close
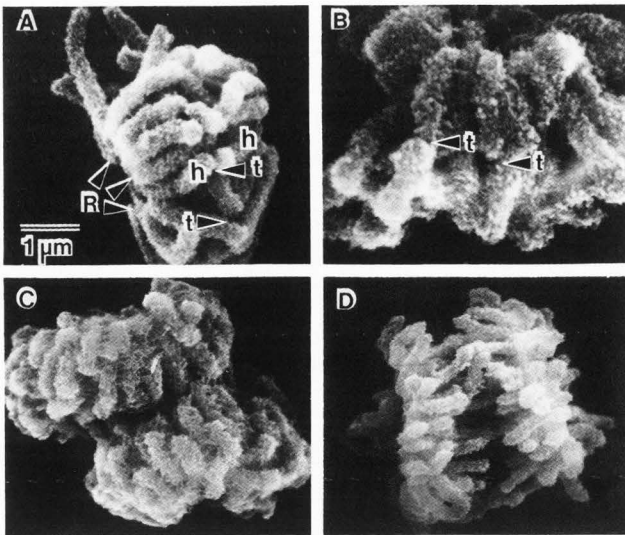
Figure 3. Early Anaphase.
Indian muntjac (A) and CHOK1 (B) clearly
demonstrate the telomeric (t) association as
homologous chromatids (h) separate in early
anaphase. LN (C) have separated and been
slightly displaced so that the telomeric
association of homologous chromatids can not be
demonstrated. It is this delayed separation
which pulls the telomeres of the chromatids from
their radial alignment in the parallel alignment
along the lateral walls of anaphase cylinder seen
in Figure 5. In HeLaS3 (D) the more centrally
located group of remain attached telomere to
telomere, however those of the peripheral group
have separated and assume a radial and/or polar
alignment. Note that the chromatids near the
chamber rim (R) in muntjac (A) and CHOK1 (B) are
flexed at greater than 90° with the centromeres
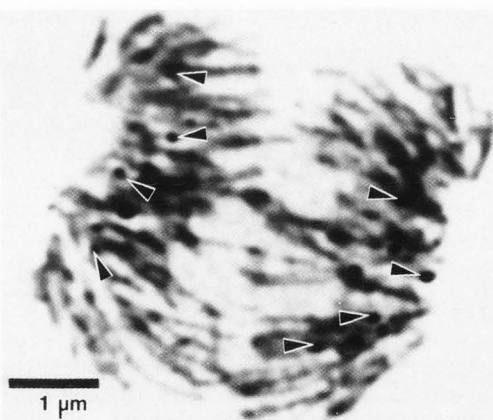of these chromatids found within the chamber.

Figure 4. C-Banded HeLaS3 anaphase.
This air dried C-banded HeLaS3 anaphases
demonstrates that centromeres (arrows) are found
extending from the polar margin to 2/3 the
distance into the anaphase cylinder with the
majority near the mid-region. This position of
centromeres demonstrates a non-Rabl orientation
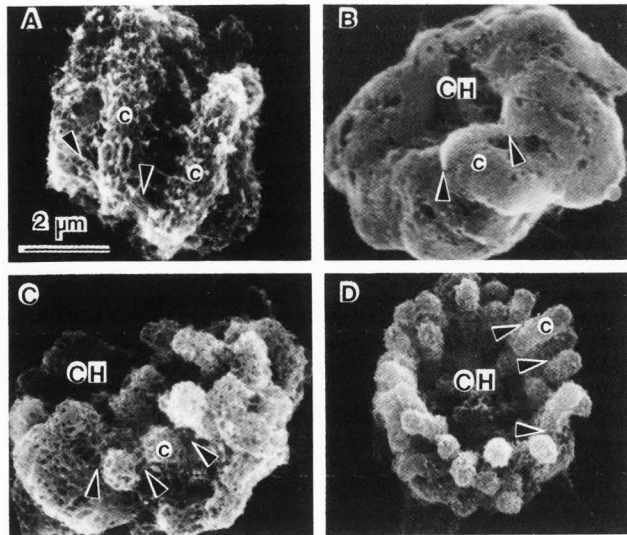of some of the chromatids.

Figure 5. Anaphase/Telophase stage.
Chromatids have positioned themselves in a
parallel alignment along the cylinder wall. This
positioning bring the chromatids (c) in close
association with each other which permits
tethering of the adjacent chromatids. This
lateral view of muntjac (A) is at a slightly
earlier stage and the formation of a continuous
meshwork of fibers (arrows) has not been
completed. Note that the chamber (CH) in HeLaS3
(D) is much deeper and has a larger diameter.
The chamber wall is also composed of chromatids
from the outer most chromatids found in the
metaphase radial array. The surface of the
chromatids at this stage is a fibrous meshwork
punctuated with pore-like opening seen best in LN
(C). The meshwork formation between adjacent
chromatids has interconnected the entire length
of the arm in this late CHO-K1 anaphase (B). The
arrows in A, B, C and D demonstrate the
interconnecting fibers between adjacent
chromatids. All four configurations are at same
magnification.

association with each other. The chromatid is
composed of looping fibers which have been
reported to be 20-50 nm in diameter, depending on
the isolation procedure. These looping fibers
make contact with each other at which time, they
become transformed into a fibrous meshwork on the
surface of the reforming nucleus (Welter,
unpublished data). However, if the chromatids
are displaced from alignment and anaphase
position, as can be done with radiation and other
methods, the chromatids will appear as a
projecting appendage or micronucleus (unpublished
observation). By late anaphase the fibrous
meshwork has completely covered all of the
chromatids.

Telophase: The chromatid alignment and
association remain essentially the same as found
in late anaphase because of the tethering of the
chromatids by the fibrous meshwork. The
individual chromatids within the configuration
are recognizable only because some of the
telomeres protrude at this stage and because the
meshwork between adjacent chromatids is not fully

developed or stretched in the isolation process. There is additional chromatid positioning in early telophase. In muntjac the Y2 migrates toward the polar end of the structure to reduce the chamber to a shallow fossa. However the closing of the polar end of the chamber of the CHOK1, LN and HeLaS3 remains obscure. It appears that a portion of the smaller centrally located chromatids migrate from the chamber floor toward the polar region to close this opening. However, if this is true these chromatids would have to leave the plane they occupied in earlier stages. Possibly the chromatid plane becomes warped permitting these smaller chromatids to occupy this polar position. Telophase is completed with the formation of a spherical disc being formed in CHOK1, LN and HeLaS3 (Figure 6). Muntjac by late telophase is an elongated sphere.

The telophase to G1 transition finds all of the structures undergoing internal reorganization of the chromatin and surface contouring to assume the shape of a G1 nucleus.

Discussion

Mosolov and Bondareva (1976) proposed a hypothetical centromeric ring which would join the chromosomal centromeres together, maintaining the strict order of their arrangement into interphase. Indian muntjac, CHOK1, HeLaS3 and LN cells exhibit a radial array of metaphase chromosomes. At anaphase, a putative centromeric ring is formed by centromeric interconnections of the peripheral chromatids. Although a clearly defined ring is only identified in muntjac such a structure cannot be identified in CHOK1, LN and HeLaS3 because of the crowded nature of the centromeric region. There is evidence to support such a structure. Prolonged hypotonic shock will spread the chromosomes enough to reveal fibers that interconnect adjacent chromatids near the centromeres. There has been considerable discussion of the possibility that these interconnections are artifact. However, configurations collected from non-colcemid treated cultures will retain their chromatid alignment and association following considerable mechanical and chemical treatment. This is not true for colcemid treated cultures. Such treatment prevents alignment and association and chromosomes are easily dispersed once the plasma membrane and organelles are removed. This putative ring aligns chromatids in Indian muntjac, CHOK1 and LN anaphases so that a typical Rabl orientation, with centromeres clustered at the centriolar pole (centromeric ring) and the telomeres trailing toward the opposite pole, is exhibited. HeLaS3 is a variant in that some of the peripheral telomeres lead toward the centriolar pole, ahead of the centromeric ring. Also, the centromeres forming the putative centromeric rings are in two layers (D.A. Welter et al., in preparation).

HeLaS3 is a transformed cell line with 65 chromosomes. Preliminary observations in another human transformed cell line (HT1080 with 46 chromosomes) would indicate that the HeLaS3 anaphase configuration is not indicative of transformation (unpublished observation).

Instead, we believe that the variation seen in HeLaS3 is brought about to accommodate the additional chromosomes associated with the centromeric ring. Heteroploid metaphases with large chromosome numbers exhibit this layering effect at the periphery (unpublished observation). With the additional chromosomes packed into the centromeric ring, some of the peripheral chromatids may be unable to flex lateral from the ring. Thus, some of the peripheral telomeres would lead toward the centriolar pole rather than trailing behind the centromeric ring at the mid-anaphase stage.

The centromeric ring appears to be one of the major factors involved in organizing anaphase chromosomes during nuclear reformation. The clustering of centromeres throughout interphase in the Indian muntjac (Korf et al., 1982) suggests that the ring may also play a role in the organization of the interphase nucleus. The fact that the centromeres are not clustered at interphase in cells with a large number of chromosomes, such as HeLaS3, would indicate that other factors may be involved (Hsu et al., 1971; Moroi et al., 1981; Welter et al., 1985).

What is involved in the maintenance of the centromeric ring? One explanation is that the centromeric constitutive heterochromatin contains highly repetitive (satellite) DNA. Mayfield & Ellison (1975) suggest that these regions might have an affinity for one another, leading to their association. Another explanation is that the centromeres are physically interconnected.
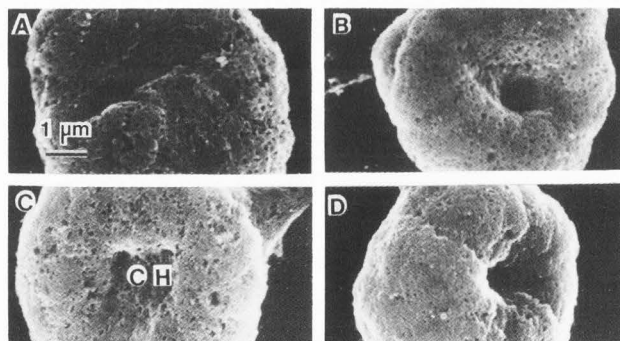

Figure 6. Late telophase stage.
In the transition from late anaphase to this stage there is a surface contouring which obscures the underlying chromatids. This obscuring of the chromatids is accomplished by internal changes of the chromatids up to this stage and the completion of the fibrous meshwork which completely surrounds this telophase configuration. Internal reorganization has also reduced the chamber (CH) to a small depression found in the polar end of the configuration. As this stage progresses into G1 the chamber will be reduced to a shallow fossa or completely eliminated. This Indian muntjac (A) and HeLaS3 (D) telophase demonstrates some chromatid detail near the chamber (LH). CHOK1 (B) and LN (C) have completed meshwork formation and chromatid detail is absent. The configurations in A, B, C and D are at same magnification.

Korf & Diacumakos (1977) reported the presence of centromeric interconnecting fibers in Indian muntjac. These results were confirmed by Welter et al. (1984). While it is possible that the interconnections represent remnants of the mitotic spindle (Korf & Diacumakos, 1977), they could also represent a distinct organizational unit such as nuclear matrix.

The interconnecting fibers seen in early anaphase connecting adjacent chromatids become more numerous at the surface as mitosis proceeds. After the anaphase to telophase transition, the fibers have enclosed the chromatids, not only connecting adjacent chromatids but also covering the surface of the chromatids. This fibrous meshwork thought to be the skeletal framework for the inner nuclear envelope, is interrupted by numerous, small pore-like structures. We would suggest that the fibers of this meshwork represent the deposition of the dense lamina on the reforming nucleus or looping fibers of the chromatid that are transformed following metaphase. Studies are currently underway, using monoclonal antibodies, to determine whether or not these fibers are composed of lamins.

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Discussion with Reviewers

K.W. Adolph: Do the authors believe that identical chromosome associations are found in each mitosis? The authors state, for example, that longer chromosomes are positioned peripherally at metaphase, but are the same long chromosomes adjacent to each other from mitosis to mitosis? Is the resolution of the SEM technique sufficient to answer this experimental question?

Authors: Somatic pairing has been a topic of discussion for seventy five years. Plant and lower animal species have demonstrated this phenomenon but the results obtained from

mammalian cell lines are questionable because of the use of c-mitotic agents and hypotonic salts in the collection of metaphases. In a C-banded analysis of non-colcemid collected metaphases from Indian muntjac we found that the long chromosome did not always associate with each other in their radial arrangement. However, we did find that the Y_2 chromosome always occupied a central position in the metaphase plate. CHO, Human diploid, and HeLaS3 were not analysed for somatic pairing. Based on results obtained from all four mammalian cell lines (manuscript submitted elsewhere), somatic pairing does not occur nor does specific chromosome association at metaphase occur. One would like to speculate that chromosomes do not wander from position to position in the cell during any phase of the cycle and we have evidence that the chromosomes are fixed in place from metaphase to S. However we do not know whether chromosomes retain their relative position from S to metaphase. The resolution of the SEM is sufficient to analyze the chromosomes in their relative position to each other, however the techniques available for isolation of these intact configurations does not permit subsequent banding which is necessary for positive identification of chromatids.

K.W. Adolph: Are interchromosomal connections consisting of DNA possibly significant in determining the arrangement of chromatids? Do chromatin fibers extend between different chromosomes and therefore serve to connect telomeres and fix the arrangement of chromosomes? Mitotic spindle remnants or the nuclear matrix may be components of the centromeric ring, but could non-telomeric DNA connections, at the centromere or at other regions along the chromosome arms, be of importance?
Authors: The interchromosomal connections, whether they are DNA, histone or some non-histone polypeptide are responsible for the positioning the chromatids in their specific alignment on the metaphase plate. It is not known whether the formation of these interconnections are mediated by specific DNA sequences. However the arrangement or alignment of the smaller chromosomes centrally and the longer peripherally each time would suggest that there is some control mechanism. There are interconnecting fibers (composition is unknown) which extend between adjacent chromatids that do indeed serve to interconnect the entire chromatid and fix the arrangement. This process of interconnecting begins at early anaphase near the centromere and is completed at the telomere by telophase. All of the interconnections whether centromeric, telomeric or any point in between along the length of the chromatid are important in forming the fibrous meshwork, on the surface of the newly assembled nucleus, that is found adjacent to the inner nuclear envelope.

K.W. Adolph: What is the evidence that the fibrous meshwork formed in anaphase and early telophase is actually deposited during these periods and is not simply material that is more resistant to extraction by the acid/alcohol preparation procedure at these times?

Authors: There is no morphological evidence to support the deposition of fibrous interconnections in anaphase and telophase. However there are changes in the 30 nm of the chromatid at the onset of anaphase. The free ended 30 nm fibers flex and associate with each other at the periphery of the chromatid. This association results in a tight bonding which is resistant to breakage by the acid/alcohol procedure. These surface changes begin near the centromeric region and progress toward the telomere as the nuclear assembly progresses from anaphase to telophase. This fibrous meshwork formed in late mitosis and found on the interphase nuclear surface can be reversed to free-ended fibers by various protein extraction procedures, with no apparent loss in fiber density on the surface.

T.D. Allen: At the earliest stage of the coalescence of the chromosome surface to form the fibrous meshwork of nuclear surface, is it possible to see if those regions of chromosomes on the inner aspect are decondensing differently?
Authors: It is not possible to analyze these regions with SEM unless there has been an opening torn in this fibrous surface and even then the area is not large enough to fully study the process of decondensation. However, TEM of the acid isolated late mitotic configurations demonstrate that there is little decondensation of the chromatid deep to the fibrous surface as late as telophase.

T.D. Allen: SEM of C banded chromosomes (Jack et al. (1985) Chromosoma 91:363-368) shows apparently 'extra' material over the C banding positive regions. Do you think this material could represent remnants of centromeric ring structure? If so, why might this be restricted to a relatively small proportion of the total human chromosome complement?
Authors: The appearance of the chromatid in those C banded regions reported by Jack et al. is almost identical to what we are seeing along the area of the chromatid that becomes fibrous meshwork of the surface of the assembled nucleus. In the C banded regions there is an obvious morphous "filler" substance which tightly binds the 30 nm fiber and renders them relatively resistant to protein extraction procedures. It is possible that this "filler" substance at the C banded heterochromatic regions of specific chromosomes is the same material that transforms the 30 nm looping chromatin fiber into a heterochromatic fibrous shell located deep to the inner nuclear envelope.

T.D. Allen: Do you feel that this well ordered structure during chromosome separation has evolutionary significance as a method for the exclusion of non-diploid chromosomal material?
Authors: We don't know the answer to this question. However, from experiments where we X-irradiated CHOK1 cell we found that those chromosomes dislodged from the radial arrangement of the metaphase did not separate at anaphase and subsequently become part of the nucleus. These nondisjuncted chromosomes became micronuclei.

Whether some non-diploid chromosomes fail to
align at metaphase and are excluded at anaphase
we can only speculate that this is a possibility.

J.B. Rattner: Movies of mitosis frequently
illustrate that at the completion of anaphase the
chromatids appear to contract somewhat towards
the polar end of the cell. Has this contraction
been noted in preparations such as are present
here and does this contraction play a role in the
formation of the telophase basket-like
structures?
Authors: In our 1985 paper (Welter et al., 1985)
we found that there is a shortening in polar
length of the configuration between late anaphase
and telophase. The reduction in length could be
the result of contraction of the chromatids or a
flexion of the chromatids at the centromeric and
telomeric ends of the configuration. The
formation of the basket-like structure seen in
late anaphase and telophase results from the
warping of the flattened metaphase plate into the
basket-like structure during early anaphase.
Those cell lines which have a true Rabl
orientation during mitosis do not form
basket-like intermediate structures.