THE DISTAL KINETOCHORE IS NOT RESPONSIBLE FOR THE OSCILLATION OF MONOOriented CHROMOSOMES IN NEWT LUNG CELLS

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INTRODUCTION

Monooriented (i.e. centrophilic) chromosomes in bipolar spindles are common during astral prometaphase of vertebrate cells in monolayer tissue cultures. A characteristic ultrastructural feature of these chromosomes is the presence of a single kinetochore fiber (K-fiber) on the pole-facing (proximal) kinetochore. The sister kinetochore, which faces in the opposite direction, is invariably free of microtubules (see refs. in 1). Cine analyses of monooriented newt lung cell (NLC) chromosomes (2; see also 3) has revealed that they undergo oscillatory movements, i.e. they move toward and away from the pole with which they are associated.

The mechanism responsible for the oscillatory movements of monooriented NLC prometaphase chromosomes is unknown. It has been suggested (4) that these movements result from antagonistic forces generated at or by both kinetochores, i.e. that the proximal kinetochore of a monooriented chromosome is responsible for the poleward movement, while the distal kinetochore is responsible for movement away from that pole. Observations on persistently monooriented NLC chromosomes which enter anaphase before becoming amphioriented are cited in support of this hypothesis. Apparently, in these cases, the proximal chromatid moves into the polar area, while the distal one moves radially away from that pole into the cytoplasm. The movement of the distal chromatid into the cytoplasm is envisioned to occur by its kinetochore interacting with and moving radially along astral microtubules (MTs). This hypothesis is consistent with the common belief that the congression of prometaphase chromosomes to the middle of the spindle arises from antagonistic forces generated, at each sister kinetochore, by K-fibers oriented toward opposite poles.

Time-lapse cine observations of living NLCs, however, prompted Bajer (2) to ascribe the oscillatory movements of monooriented chromosomes to a push/pull
generated by the single pole-facing K-fiber. This hypothesis draws some support from the observation that MT elongation, induced by taxol, can exert a pushing force on chromosomes (5). A major implication of this model is that a monopolar (or half) spindle can generate the forces for chromosome transport, not only toward the single pole, but also away from that pole. This suggests that the movement of a prometaphase NLC monooriented chromosome to the forming metaphase plate is produced, in part, from the activity of a single K-fiber (6), i.e., it's congression is not solely dependent on antagonistic pulling forces acting on opposite sister kinetochores (see 7).

We have investigated the mechanism of prometaphase chromosome oscillation in the Northwestern rough skinned newt (Taricha granulosa) using correlated light and high-voltage electron microscopy (HVEM) of normal and laser-irradiated monooriented chromosomes. Our results indicate that the oscillatory movement is not due to the activity of the distal kinetochore. Additional observations of untreated monopolar spindles and of monopolar spindles treated with MT-disrupting agents support the suggestion that monooriented chromosome oscillations—end the positioning of these chromosomes up to 15 μm away from the centrosome—arise from a pull generated at the single active (proximal) kinetochore, balanced by a push generated on the chromosome from astral MTs.

MATERIALS AND METHODS

Tissue Culture and Light Microscopy

Primary NLC cultures were prepared as previously described (8,9). Selected mitotic cells were followed in vivo with a Nikon Diaphot microscope equipped with phase-contrast optics and a UVX automatic 35-mm exposure system.

Electron Microscopy

Untreated or experimentally treated cells were followed in vivo until the desired stage for fixation. They were then fixed by perfusion with 3% glutaraldehyde in 0.1 M PO4 buffer (pH 6.9) for 30 min, osmicated in 1% OsO4 in buffer (10 min, 4°C), stained en bloc with 1% uranyl acetate (3-12 h), dehydrated in ethanol, and embedded in Epon-Araldite (see 10). Cells of interest were then serially thin or semithick (0.25 μm) sectioned and the sections stained as previously described (10). Thin sections were examined in a Philips 300 electron microscope with a 70-μm objective aperture. Semithick sections were examined in the Wadsworth Center's HVEM operated at 800 kV with an objective aperture of 30-μm.

Laser Ablation

The methods used to ablate NLC kinetochores by laser microbeam irradiation were essentially those detailed by McNeil and Berns (11). A 0.2 μm diameter microbeam (λ=532 nm, energy/pulse = 130 nJ, 10 pulses/sec) was used to cut chromosomes by
irradiation over 0.5-2 min. Cells were observed on a Zeiss AxioMat using a Neofluor 100x/1.33 objective and images were recorded using a time-lapse VCR. The experimental cells were processed for electron microscopy as outlined above.

RESULTS

Monooriented chromosomes on bipolar NLC spindles usually became bipolar oriented and congressed to the metaphase plate before the initiation of anaphase. However, 10% of NLCs entered anaphase before one or more centrophilic chromosomes achieved a metaphase position. When this happened, one of two outcomes was observed. In most (90%) cases the proximal anaphase chromatid moved into the polar region, while the distal chromatid remained motionless (Fig. 1). However, in 10% of the cases the proximal chromatid moved into the pole, while the distal chromatid moved radially away from that pole, the kinetochore leading the way, 15-μm or more into the cytoplasm (Fig. 2). Ultrastructural analysis of the distal chromatid in the former cases revealed a kinetochore free of MTs (Fig. 3). In the latter cases the kinetochore possessed a well-developed K-fiber, which was attached to an ectopic and acentriolar spindle pole adjacent to the plasma membrane (Fig. 4). Similar results were observed even when a centrophilic chromosome was positioned between the pole and the metaphase plate as anaphase was initiated: the proximal chromatid moved into the polar area, while the distal chromatid remained motionless (not shown).

When the spindle poles failed to separate during prophase, all of the prometaphase chromosomes initially monooriented toward the single polar area to form a monopolar spindle. Subsequently one of two outcomes was observed: (a) the single pole finally split and separated into two poles, which led to the formation of a normal bipolar spindle, or (b) the chromosomes remained monooriented at 10-15 μm away from the single pole, which never split. In these latter cases one of two outcomes was observed. In most cells the chromosomes moved progressively closer to the pole, over a period of hours, until they were tightly grouped around it (not shown; see 2). They remained associated with this pole until a restitution nucleus was formed. Alternatively all of the mono-oriented chromosomes established a stable position 10-15 μm distal to the single polar area. As a result a half-spindle was formed (Fig. 5). When these half-spindles entered anaphase, the proximal chromatids moved into the polar area, while the distal chromatids remained stationary at the periphery of the spindle.

To determine whether the oscillatory movements of monooriented chromosomes were the result of transient distal kinetochore function (by lateral association with astral MTs or by linkage to free MTs), we used a laser to create monooriented chromosomes by cutting one kinetochore off a bipolar-oriented chromosome. Our correlative light and electron-microscopic results (Fig. 6, 7)
reveal that chromosomes with one kinetochore removed immediately begin moving to
the pole which the nonirradiated kinetochore was facing. In many cases these
chromosomes swing laterally out of the spindle as they approach the pole. These
artificially created mono-oriented chromosomes show continual oscillations as they
move, in average, closer to the pole. The oscillatory movements did not appear to
depend on the size of the chromosome containing the single functional
kinetochore.

In all cases acentric fragments, generated by cutting chromosomes with the
laser, were transported at a constant velocity (1-2 μm/min) radially away from
the closest pole into the cytoplasm (not shown).

Finally, to determine how disassembly of non-kinetochore MTs effects the
position of mono-oriented chromosomes relative to the spindle pole, we treated
newly formed monopolar spindles with cold (6°C), colcemid (0.1 μg/ml), or
nocodazol (10 μM). In every case these MT-disrupting agents quickly (within 2-5
min) inhibited the oscillations and induced the chromosomes to move into and pack
tightly around the single polar area (Fig. 8). A similar natural packing of
chromosomes around the single polar area occurred in untreated monopolar spindles
(see above) but this process occurred over a period of hours as the spindle aged.

DISCUSSION

Chromosome congression to the equator of a bipolar spindle has been explained
solely by the action of antagonistic pulling forces applied at the opposing
kinetochores by K-fibers oriented toward opposite poles (see 7,12). Since the
poleward force on a kinetochore appears to be a linear function of K-fiber
length, a chromosome congresses until the antagonistic forces are balanced (7).
The studies described in this report were designed to determine whether the
movement of a mono-oriented chromosome away from its associated pole is a result
of forces acting on the distal kinetochore. Our observations of persistently
mono-oriented chromosomes in anaphase and of prometaphase chromosomes containing a

Fig. 1. Anaphase in a NLC which contains a mono-oriented chromosome. The prox-
imal chromatid moves into the pole, while the distal chromatid (arrowhead) re-
mains motionless. Elapsed time (in min) is shown in lower corner of each frame.

Fig. 2. Similar to Fig. 1 except that the distal chromatid (arrowhead) moves
radially into the cytoplasm.

Fig. 3 a,b Serial electron micrographs of 0.25-μm sections from the kinetochore
of the chromatid indicated by the arrow in Fig. 1c. Note the absence of MTs.

Fig. 4 a,b Serial electron micrographs of 0.25-μm sections from the chromatid in-
dicated by the arrow in Fig. 2c. The K-fiber MTs are attached to an ectopic
pole.
single kinetochore clearly demonstrate that monooriented chromosomes can move
away from the pole in the absence of a functional distal kinetochore.

This conclusion raises the question as to how monooriented NLC (or other types
of) chromosomes can achieve a position up to 15 \( \mu \text{m} \) away from the single pole
(e.g. Fig. 5; see also 6,13). Since the distal kinetochore of a monooriented
chromosome is non-functional, what force antagonizes the poleward force applied
at the single functional proximal kinetochore so that the chromosome becomes
positioned many micra distal to the pole?

Bajer (2) hypothesized that the movement of a monooriented chromosome away
from the pole was the result of a push generated by the growth of the single
pole-facing K-fiber. However, since the K-fiber always spans the distance
between the kinetochore and the pole, a force applied anywhere along the
chromosome, which tends to push it away from the pole, could be manifested by an
elongation of the K-fiber (see 7,12). Our experiments show that acentric
centromeres, generated by the laser, immediately move out of and away from the
centrosome at a constant velocity of 1-2 \( \mu \text{m} \)/ min without a noticeable deformation
of the chromatid. This behavior is identical to the outward movement of
monooriented chromosomes and appears to arise from well characterized but little
understood elimination forces of the half-spindle or aster (reviewed in 14). Thus
the expulsion force on a monooriented chromosome generated by the aster or
half-spindle could antagonize the poleward pulling force applied at the
kinetochore and the balancing of these forces would in turn regulate the length
of the K-Fiber. In this hypothesis monooriented chromosomes become positioned,
relative to the spindle pole, at that point where the poleward force on the
chromosome applied at the kinetochore is balanced by the outward expulsion force.

Fig. 5a,b Anaphase in a NLC half-spindle. Note the position of the chromosomes
relative to the spindle pole. See text for details.

Fig. 6a) Phase contrast micrograph of a spindle containing a monooriented
chromosome (arrowhead) after fixation. This monooriented chromosome was created
by laser ablation of a single kinetochore on a metaphase chromosome. b) Graph of
the kinetochore to pole distance versus time in the cell pictured in (a). The
chromosome was congressing toward the metaphase plate at 0 time. Between 1-4 min
later the distal kinetochore was cut off of the chromosome. The chromosome then
monooriented, swung laterally out of the spindle, and begun oscillating as it
moved toward the proximal pole.

Fig. 7a,b Electron micrographs of sections 1 and 3, respectively, from a serial
series through the chromosome noted by the arrow in Fig. 6a. One kinetochore
(circle) is oriented toward the polar area. The region of the chromosome
containing the second kinetochore (arrowheads) has been completely removed with
the laser.

Fig. 8a,b Monopolar NLC which was treated at (a) with 10-\( \mu \text{g/mL} \) of nocodazole.
The chromosomes quickly move into, and form a tight group around, the spindle
pole (b).
Dynamic changes in the expulsion force, possibly due to fluctuations in the assembly of non-kinetochore polar MTs (15), will be manifested by oscillations in chromosome position. This hypothesis is consistent with our observation that agents which selectively depolymerize non-kinetochore MTs (e.g. cold, colcemid, nocodazole) immediately inhibit oscillations and induce the chromosomes to move into the centrosome (our Fig. 8). It is also consistent with our observation (see also 2,3) that the kinetochore region of a monooriented chromosome shows pronounced stretching during poleward movement, whereas the chromosome undergoes very little shape change during movement away from the pole, i.e. no deformation of the kinetochore region.

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