JCB Report

Human chromokinesin KIF4A functions in chromosome condensation and segregation

Manjari Mazumdar,[1] Suma Sundareshan,[2] and Tom Misteli[1]

[1]National Cancer Institute, National Institutes of Health, Bethesda, MD 20892
[2]Bangalore Genei Pvt. Ltd, BDA Industrial Suburb, Peenya, Bangalore 560058 India

Accurate chromosome alignment at metaphase and subsequent segregation of condensed chromosomes is a complex process involving elaborate and only partially characterized molecular machinery. Although several spindle associated molecular motors have been shown to be essential for mitotic function, only a few chromosome arm–associated motors have been described. Here, we show that human chromokinesin human HKIF4A (HKIF4A) is an essential chromosome-associated molecular motor involved in faithful chromosome segregation. HKIF4A localizes in the nucleoplasm during interphase and on condensed chromosome arms during mitosis. It accumulates in the midzone from late anaphase and localizes to the cytokinetic ring during cytokinesis. RNA interference–mediated depletion of HKIF4A in human cells results in defective prometaphase organization, chromosome mis-alignment at metaphase, spindle defects, and chromosome mis-segregation. HKIF4A interacts with the condensin I and II complexes and HKIF4A depletion results in chromosome hypercondensation, suggesting that HKIF4A is required for maintaining normal chromosome architecture. Our results provide functional evidence that human KIF4A is a novel component of the chromosome condensation and segregation machinery functioning in multiple steps of mitotic division.

Introduction

Faithful segregation of the genome involves an elaborate macromolecular machine in which the mitotic spindle plays a central role. Defects in components that control spindle organization and function often lead to chromosome mis-segregation, aneuploidy, and cellular abnormalities (Pihan and Doxsey, 1999; Jallepalli and Lengauer, 2001). The dynamic nature of the spindle apparatus is believed to be maintained both by the dynamic instability of microtubules (MT) as well as several force producing MT motors (Scholey et al., 2003). Poleward and away from the pole forces balance each other during metaphase congression and are responsible for chromosome motility toward the poles (Marshall, 2002). Polar ejection forces may be generated either by dynamic MTs or by plus-end–directed motor including the chromokinesins, which associate with chromosome arms (McIntosh et al., 2002). Chromokinesins represent a family of chromosome arm-binding kinesins consisting of two distinct types of members: chromokinesins/KIF4 and the Kid homologues (Sekine et al., 1994; Vernos et al., 1995; Wang and Adler, 1995; Williams et al., 1995; Tokai et al., 1996; Yan and Wang, 1997; Antonio et al., 2000; Funabiki and Murray, 2000). Both types of chromokinesins are nuclear during interphase and localize on condensed chromosome arms during mitosis. In humans, two KIF4 members exist: HKIF4A and HKIF4B (Ha et al., 2000). Human KIF4A (HKIF4A) is a 140-kD protein that contains several conserved structural motifs including a kinesin-like motor domain, a long coiled-coil region, a nuclear localization signal, a DNA-binding motif and a cysteine-rich Zn fingerlike motif. The protein has been shown to interact with BRCA2-associated factor 35 and the DNA methyltransferase DNMT3B (Lee and Kim, 2003; Geiman et al., 2004). Although HKIF4A associates with chromosomes during mitosis, no information as to the function of the protein is available (Lee et al., 2001). Here, we show by RNA interference (RNAi) that HKIF4A is a novel multifunctional component of the chromosome condensation and segregation machinery.

The online version of this article contains supplemental material.

Address correspondence to Manjari Mazumdar, National Cancer Institute, National Institutes of Health, Bldg. 41, Rm. B 507, 41 Library Dr., Bethesda, MD 20892. Tel.: (301) 435-2672. Fax: (301) 496-4951. email: mazumdam@mail.nih.gov

Key words: chromokinesin; spindle; chromosome condensation; molecular motor

Abbreviations used in this paper: HKIF4A, human KIF4A; MT, microtubule; RNAi, RNA interference.

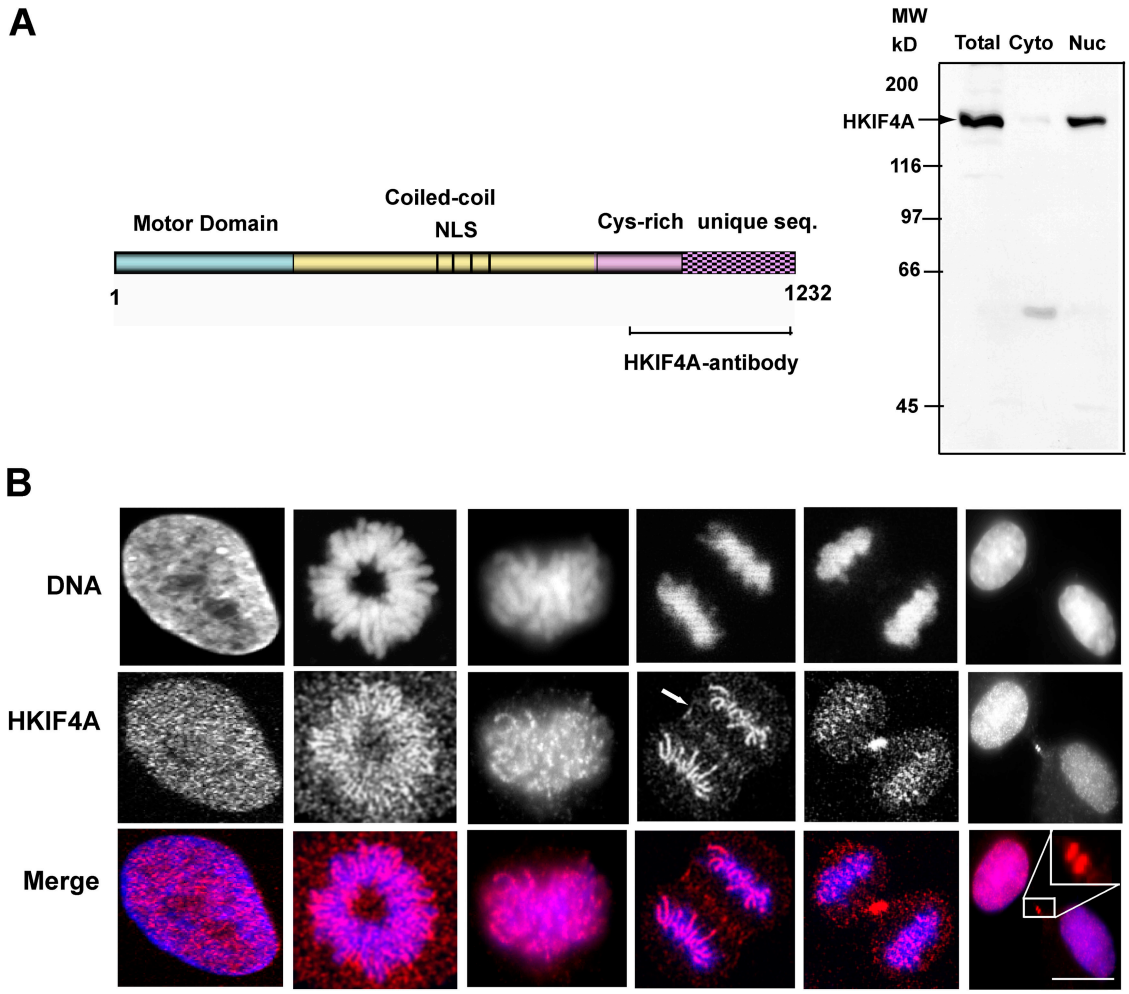

Figure 1. **Dynamic localization of HKIF4A during the cell cycle.** (A) Schematic representation of the protein domain used for generating an mAb against HKIF4A. Subcellular fractionation of nonsynchronized MRC-5 cells showed that the majority of the endogenous protein is present in the nucleus. Total, total cell extract; Cyto, cytoplasmic extract; Nuc, nuclear extract. Same amounts of protein were loaded in each lane. (B) Non-synchronized MRC-5 cells were fixed and double stained with HKIF4A (red) and DAPI (blue) at different phases of the cell cycle. During interphase the protein was prominently nuclear but from prophase to telophase HKIF4A was present on chromosome arms. In addition, the protein accumulated in the mid-zone (arrow) and formed the cytokinetic ring until cytokinesis. The inset shows an amplified image of the mid-body that appears as two rings. Bar, 5 μm.

Results and discussion

To gain insight into HKIF4A function, we raised a mouse mAb specific against the extreme COOH-terminal domain of human chromokinesin HKIF4A (Fig. 1 A; see Materials and methods). In Western blots of MRC-5 cell extracts, the HKIF4A antibody detected a single band of 140 kD (Fig. 1 A). In subcellular fractionation of nonsynchronized cells, the protein was highly enriched in the nuclear extract and only trace amounts were detected in the cytoplasmic fraction (Fig. 1 A). During mitosis HKIF4A associates along the entire arms of condensed chromosomes (Fig. 1 B). In addition to the chromosomal localization, the protein accumulates in the mid-zone from anaphase A to cytokinesis (Fig. 1 B). From anaphase B to cytokinesis HKIF4A is present in the mid-body as two distinct rings connecting the MTs from the two half spindles (Fig. 1 B, inset). In late cytokinesis, until the two daughter cells pinch off, the protein persists in the center of the mid-body (Fig. 1 B). Costaining of HKIF4A with tubulin shows partial colocalization at the spindle poles and at the central spindle (Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200401142/DC1).

HKIF4A is essential for prometaphase organization and metaphase alignment

To determine the in vivo function of HKIF4A, we depleted the protein from MRC-5 cells by RNA interference. The cellular level of HKIF4A decreased by almost 90% of its initial amount after two consecutive transfections 24 h apart (Fig. 2 A). HKIF4A RNAi did not affect cellular tubulin or lamin A/C levels (Fig. 2 A) and RNAi against lamin A/C did not affect HKIF4A levels (not depicted). HKIF4A depletion resulted in an accumulation of mitotic cells. Although the mitotic index was 0.11 ± 0.06 in mock-transfected control cells, it was 0.2 ± 0.01 in HKIF4A-depleted cells 48 h after transfection ($P < 0.05$). HKIF4A-depleted mitotic cells showed pronounced defects in various stages of mitosis (Fig.

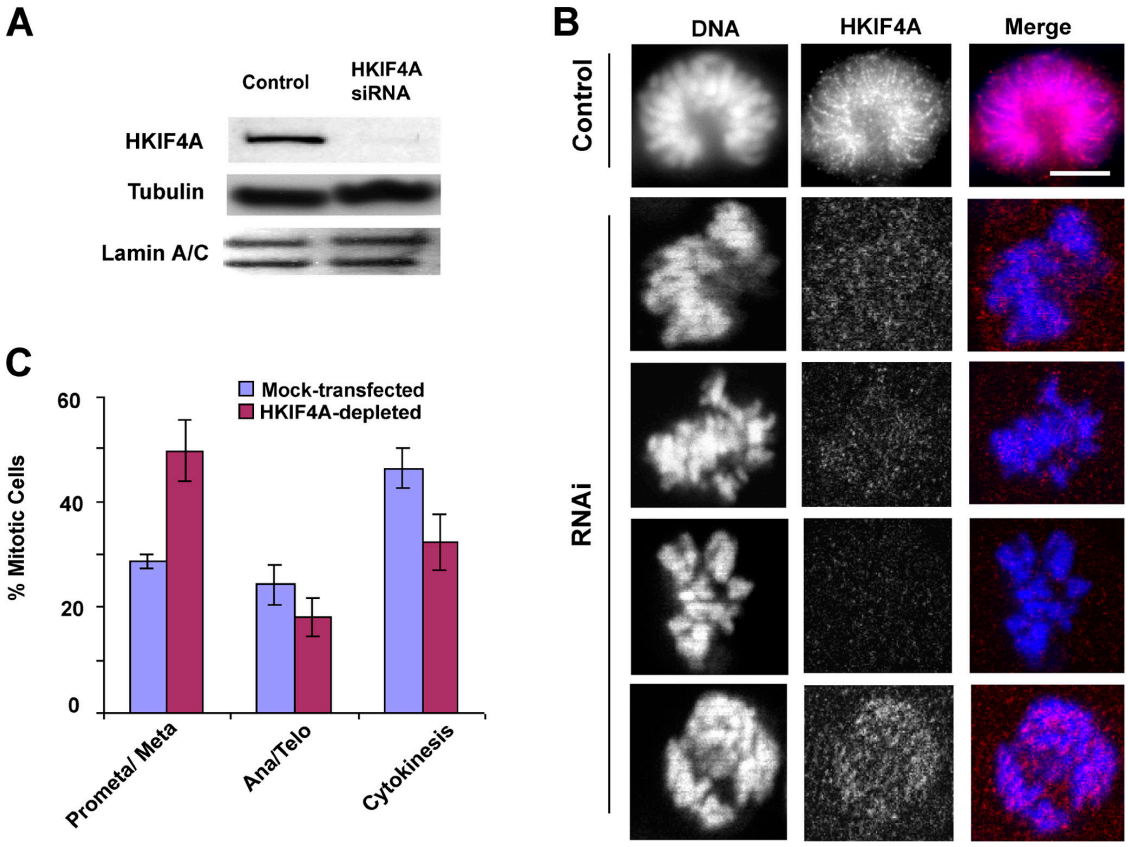

Figure 2. **Depletion of HKIF4A reduces sister chromatid resolution and causes chromosome misalignment.** (A) Western blot of HKIF4A in total extracts of MRC-5 cells after 48 h of mock (control) or HKIF4A RNAi transfection. (B) MRC-5 cells were fixed 48 h after transfection with RNAi and stained with DAPI (blue) and anti-HKIF4A (red). The chromosomal signal of HKIF4A was lost due to the RNAi effect. Bar, 5 μm. (C) Quantitation of distribution of mitotic phases in mock- and HKIF4A RNAi-transfected cells 48 h after transfection. Values represent averages from three experiments ± SD.

2 B). 65% of prometaphase cells lacked the typical doughnut shape arrangement of chromosomes, chromosomes were frequently misaligned, and anaphase separation was often incomplete (Fig. 2 B). Similar observations were made upon microinjection of anti-HKIF4A antibody into prometaphase cells (Fig. S2, available at http://www.jcb.org/cgi/content/full/jcb.200401142.DC1). Quantitation of the distinct mitotic stages of RNAi-transfected cells (Fig. 2 C) indicated that 48 h after transfection the fraction of prometaphase and metaphase cells was dramatically higher (50 ± 5.7%) than in mock-treated cells (29 ± 1.3%). The severity of the observed mitotic defects appeared roughly correlated with the level of HKIF4A depletion (not depicted).

Depletion of chromokinesin HKIF4A causes mitotic spindle defects, anaphase bridges, and aneuploidy

To determine the role of HKIF4A in mitotic spindle function, we analyzed MTs and chromosomes from mock-transfected and HKIF4A-depleted MRC-5 cells 48 h after transfection with RNAi (Fig. 3). Compared with mock-transfected cells, immunofluorescence microscopy showed dramatic mitotic spindle defects (Fig. 3 A). MT organization of both prometaphase and metaphase spindles was abnormal and was accompanied by chromosome alignment defects (Fig. 3 A, arrow). In a number of cases spindle poles appeared less focused, and in extreme cases chromosomes scattered out of the spindle axis and the spindle completely lost its integrity (Fig. 3 A, solid arrowheads). In addition to prometaphase and metaphase defects, HKIF4A depletion also caused defective cytokinesis (Fig. 3 A). Cells lacking HKIF4A frequently exhibited lagging chromosomes in anaphase and after anaphase, and although the cells started to constrict, the cleavage furrow did not ingress completely (Fig. 3 A, forked arrow). Almost 50% of anaphase cells exhibited lagging chromosomes or chromatin bridges. Some anaphase bridges were observed to persist into telophase, resulting in formation of a large nucleus, binucleate cells, and multiple micronuclei (not depicted).

Amongst all prometaphase cells, 66% of RNAi-treated cells showed disorganized prometaphase figures compared with 11% of control cells (Fig. 3 B). Similarly, 63% of RNAi-treated metaphase cells showed mis-aligned metaphases compared with 14% control cells. In depleted cells, defective spindles were observed in 78% of cells ($n = 220$; Fig. 3 C). 40 ± 4.2% of spindles were disorganized, 27 ± 5.3% of spindles were defocused, and 11 ± 3.8% of spindles were multipolar (Fig. 3 C).

To determine whether these mis-segregation and cytokinesis defects resulted in aneuploidy of daughter cells, metaphase chromosome spreads from mock or HKIF4A RNAi-depleted

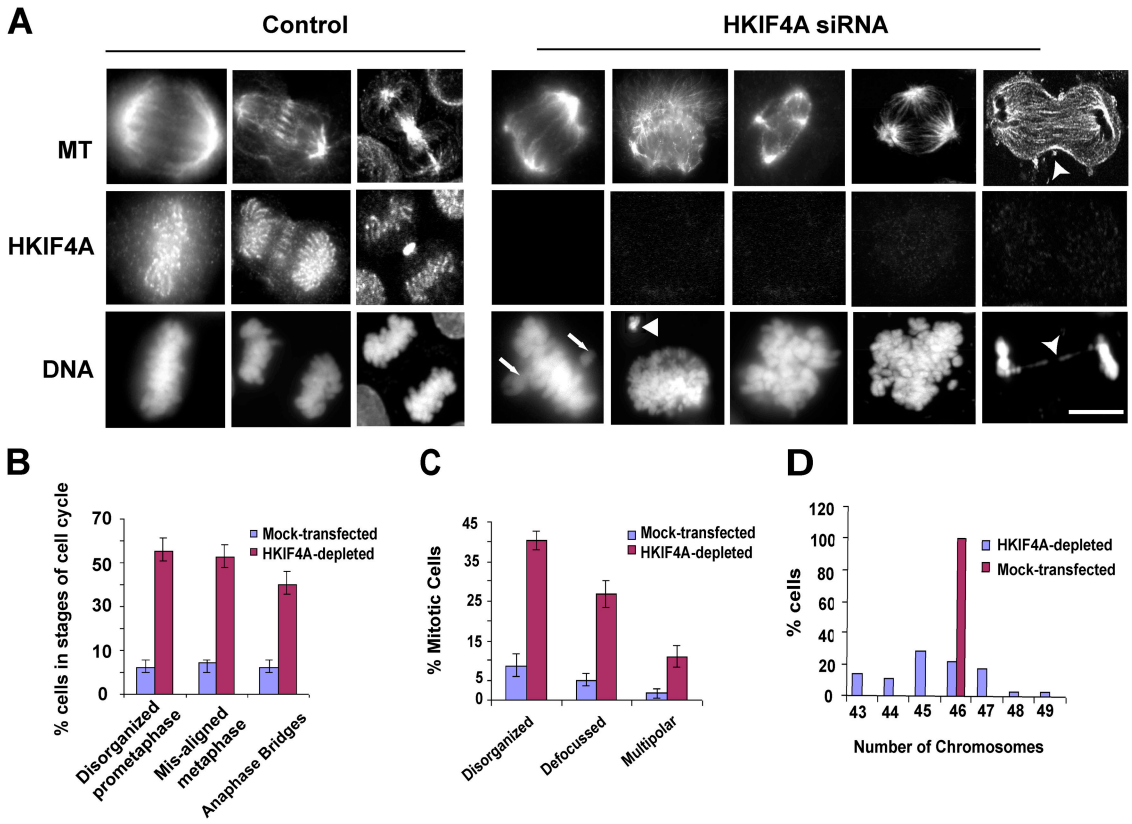

Figure 3. **HKIF4A is required for maintenance of mitotic spindle integrity and cellular ploidy.** (A) Mitotic MRC-5 cells were stained with anti-tubulin, anti-HKIF4A antibodies, and DAPI to reveal the spindle and the chromosomal states in mock-transfected (control) or RNAi-transfected MRC-5 cells 48 h after transfection. The HKIF4A-depleted cells showed dramatic defects in spindle structure accompanied by chromosome mis-segregation. Anaphase and late telophase cells showed lagging chromosomes or lagging chromosomes forming a bridge (forked arrowheads). Bar, 5 μm. (B) Mitotic phenotypes 48 h after transfection with HKIF4A RNAi were scored against the total number of mitotic cells in a particular phase of the cell cycle. The frequency of disorganized prometaphase, mis-aligned metaphase chromosomes, and anaphase bridges was increased compared with the control cells. Values represent averages from 220 cells ± SD from three experiments. (C) Quantitation of different spindle phenotypes obtained 48 h after RNAi treatment of cells. The numbers of disorganized and defocused spindles in the mitotic figures of HKIF4-depleted cells is increased compared with mock-transfected cells. Values represent averages from three experiments ± SD. (D) Quantitation of aneuploidy caused by chromosome mis-segregation in HKIF4A-depleted MRC-5 cells. The percentage of chromosome spreads containing the indicated number of chromosomes was determined. Values represent data from four independent experiments ($n = 30$).

cells were prepared. 80% of spreads of HKIF4A-depleted cells were aneuploid. 53% of HKIF4A-depleted cells had lost one or more chromosomes and 25% of cells had gained one or more chromosomes (Fig. 3 D). Less than 1% of aneuploid spreads were found in control cells.

HKIF4A is required for maintaining normal metaphase chromosome morphology

Because HKIF4A is localized all along the condensed chromosome arms, we examined the consequences of HKIF4A depletion on the structural integrity of mitotic chromosomes. Metaphase chromosome spreads from mock-transfected or HKIF4A RNAi-transfected cells were prepared after 2 h of colcemid block and stained with DAPI (Fig. 4). RNAi-mediated depletion of HKIF4A induced significant hypercondensation and chromosomes from HKIF4A-depleted cells were dramatically shorter than chromosomes from mock-transfected cells (Fig. 4 A). The average length of control chromosomes was 4.88 μm with a range between 1.5 and 10 μm, reflecting the variable sizes of human chromosomes. The width of the chromosomes was on average 0.68 μm with a range of 0.5–0.8 μm (Fig. 4 B). In contrast, chromosomes from depleted cells were on average 3-μm long and 1.2-μm wide, with a range of 1.1–5.5 μm in length and 0.8–1.5 μm in width (Fig. 5 B). These differences were statistically significant at the $P < 0.001$ level. To rule out that the observed hypercondensation of chromosomes was caused by artifacts of chromosome spread preparation, and more importantly, to exclude the possibility that hypercondensation was caused by prolonged presence of chromosomes in mitosis, we analyzed chromosomes in intact cells. We followed progression of mitosis from nuclear envelope breakdown to telophase in single living HeLa cells stably expressing histone H2B-GFP that are either mock-transfected or RNAi-transfected (Fig. 4 C). In the majority of cells in the RNAi-treated population, chromosomes were more condensed compared with mock-transfected cells even before prometaphase (Fig. 4 C). As expected, cells containing hypercondensed chromosomes experienced a delay in mitotic progression (Fig. 4 C). Furthermore, chromosomes were already significantly more condensed in HKIF4A-depleted cells compared with control cells even before breakdown of the nuclear envelope in early prophase

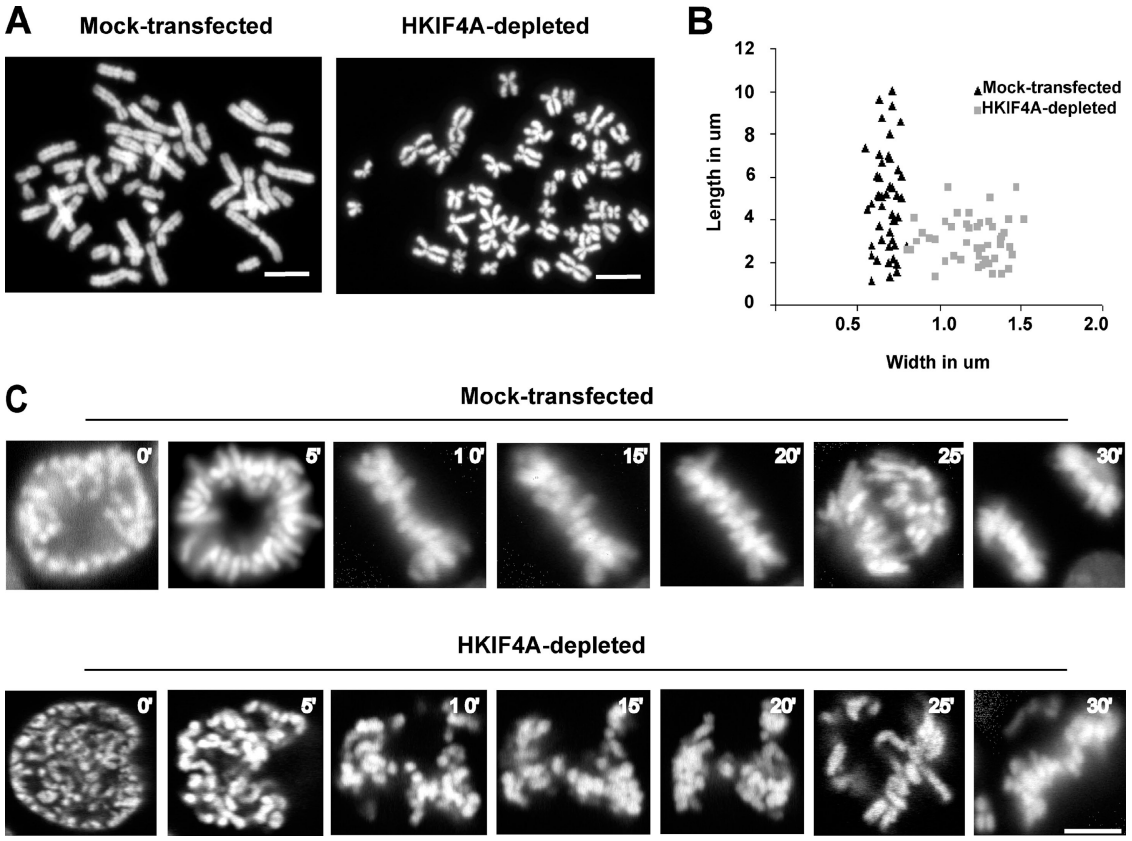

Figure 4. **HKIF4A is required for maintenance of normal metaphase chromosome morphology.** (A) Chromosome spreads from control and HKIF4A-depleted MRC-5 cells were stained with DAPI. Metaphase chromosomes from RNAi-treated cells show longitudinal shortening and widening compared with mock-transfected control cells. Bars, 5 μm. (B) Projected images of chromosome spreads were analyzed and the width (x axis) and length (y axis) of all chromosomes in a spread measured. Note that the large variance in length in the control sample is due to the differences in size amongst human chromosomes. Values represent typical spreads as in A. (C) Time-lapse imaging of HeLa cells stably expressing GFP-histone 2B. Cells were either mock transfected or transfected with HKIF4A RNAi for 48 h and were imaged every 5 min by acquiring a complete z-stack of 10 sections each 1 μm apart. All images were acquired and processed under identical conditions. Bar, 5 μm.

(Fig. S3, available at http://www.jcb.org/cgi/content/full/jcb.200401142/DC1).

HKIF4A interacts with condensin

To ask whether HKIF4A depletion leads to hypercondensation of chromosomes via the condensation machinery, we tested whether HKIF4A physically interacts in vivo with condensin. Two distinct condensin complexes, condensin I and II, which share the SMC subunits hCAP-C and -E, but differ in their non-SMC components, hCAP-D, -G, and -H, have been described previously (Ono et al., 2003). Immunoprecipitation with anti-HKIF4A antibody from nuclear extract of nonsynchronized MRC-5 or HeLa cells or from mitotic HeLa extracts specifically pulled down hCAP-E, -G, and -G2 (Fig. 5 A; see Fig. S3 for controls). The physical association of HKIF4A with both condensin complexes I and II was corroborated by immunofluorescence microscopy of chromosomes in intact mitotic cells (Fig. 5, B–D). HKIF4A partially colocalized with hCAP-E, -G, and -G2 along the length of the chromosomes in what appeared as overlapping punctate regions, possibly indicating that only a subpopulation of HKIF4A interacts with condensin subunits (Fig. 5, B–D).

If HKIF4A indeed functionally interacts with the condensin complex, one might predict that loss of HKIF4A affects condensin distribution. To test this prediction, we localized hCAP-E in HKIF4A-depleted MRC-5 cells. RNAi targeted against HKIF4A did not affect the overall protein level of condensin hCAP-E in extracts of nonsynchronized or mitotic cells (Fig. 5 E). The distribution of hCAP-E, -G, and -G2 on chromosomes was altered in HKIF4A-depleted intact dividing cells (Fig. 5 F). HKIF4A-depleted chromosomes lacked the axial localization of hCAP-E, -G, and-G2, partially relocalized and appeared diffusely distributed over the condensed mitotic chromatin mass. These data support an interaction between HKIF4A and the condensin complex.

Although HKIF4 has previously been localized to mitotic chromosomes (Lee et al., 2001) our results extend these observations by demonstrating a functional role for HKIF4A in chromosome segregation, cytokinesis, and structural integrity of chromosomes.

A role of HKIF4A as a molecular motor is suggested by its close homology with the other KIF4 kinesin family members mouse KIF4 (Sekine et al., 1994), *Xenopus* Xklp1 (Vernos et al., 1995), and *Drosophila* KLP3A (Williams et al., 1995). In this function, it may contribute to generating an away from the pole force and cooperate with other plus- and minus-end–directed motors to create the force balance required for spindle bipolarity and chromosome alignment at

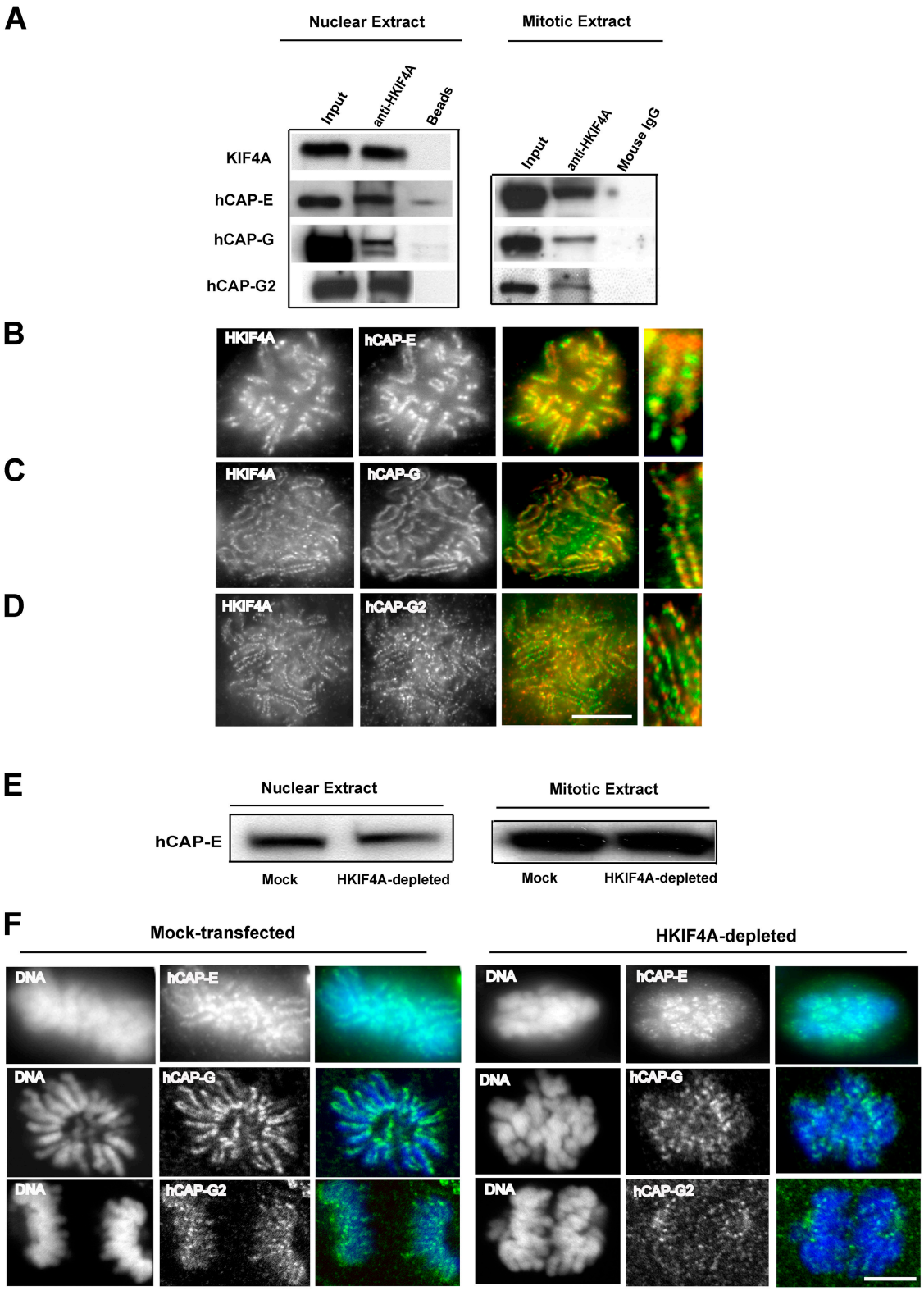

Figure 5. **HKIF4A interacts with components of condensin I and II.** (A) Western blot of anti-HKIF4A pull-down from nuclear or mitotic extract. hCAP-E, -G, and -G2 physically associate with HKIF4A. Protein G beads without antibody or mouse IgG were used as controls. Input loading was one fifth of total. (B–D) Colocalization of HKIF4A with condensin I and II complex. HKIF4A (green) partially colocalized with condensin components hCAP-E, hCAP-G, and hCAP-G2 (red) in punctate regions along the length of the chromosome arms. Far right panels show higher magnifications of individual chromosomes from corresponding merged panels. Bar, 5 μm. (E) Western blot of cell extracts from mock- and HKIF4A RNAi-transfected MRC-5 nonsynchronized and mitotic cells with hCAP-E antibody showed that the condensin level remained unchanged after depletion of HKIF4A. (F) Loss of chromokinesin HKIF4A results in condensin components failing to localize along the chromosome axis in a defined pattern. hCAP-E, G, and G2 (green) were diffusely distributed over the condensed mitotic chromatin mass (DNA, blue). Bar, 5 μm.

metaphase (Goshima and Vale, 2003; Kwon et al., 2004; Bringmann et al., 2004). This interpretation is consistent with our observations because in the majority of HKIF4A-depleted cells, chromosomes were scattered along the length of the spindle, and a large number of aberrant spindle structures were generated. Furthermore, localization of HKIF4A on the cytokinetic mid-body is reminiscent of its *Drosophila* homologue KLP3A in cytokinesis during *Drosophila* male meiosis (Williams et al., 1995, 1997). Our observation that chromosome segregation is not completely blocked but continues at a low level until at least three cycles suggests that HKIF4A is redundant with the other chromokinesin Kid (Levesque and Compton, 2001) and kinetochore-associated plus-end motors (Yen and Schaar, 1996; Kapoor and Compton, 2002). Consistent with redundancy amongst these motors, KLP3A has been shown to be dispensable in *Drosophila* (Goshima and Vale, 2003; Kwon et al., 2004).

Apart from its possible role as a molecular motor, our observations suggest that HKIF4A might also have an additional, and possibly complementary, function as a critical component in chromosome condensation. We find that HKIF4A interacts with both condensin I and II complexes and the depletion of the protein in vivo leads to hypercondensation of chromosomes. Similar to condensin I and II complexes, topoisomerase II and some condensin subunits, HKIF4A localizes in an alternating, punctate pattern along the metaphase chromosome axis (Maeshima and Laemmli, 2003; Ono et al., 2003). Depletion of HKIF4A from chromosomes appeared to partially delocalize condensin subunits from the chromosome axis, which is consistent with their physical interaction. We speculate that HKIF4A might function as a molecular linker and/or spacer between chromosome condensation proteins and DNA to contribute to higher order organization of metaphase chromosomes. Its depletion might thus be expected to result in a collapse of the chromosome fiber, giving rise to the observed hypercondensation phenotype. HKIF4A may, together with condensin and other nonhistone proteins, form the structural framework of the metaphase chromosome (Earnshaw and Laemmli, 1983; Hudson et al., 2003; Swedlow and Hirano, 2003; Gassmann et al., 2004; Strick et al., 2004). Consistent with such a role of HKIF4A, we find multiple defects both in chromosome structure and mitotic spindle organization. Similar phenotypes including formation of anaphase bridges have recently been observed in studies in which the function of components of chromosome condensation machinery have been inhibited (Saka et al., 1994; Steffensen et al., 2001; Kaitna et al., 2002; Lavoie et al., 2002; Chang et al., 2003; Coelho et al., 2003; Hagstrom and Meyer, 2003; Hudson et al., 2003; Somma et al., 2003; Wignall et al., 2003; Ono et al., 2004). The sum of these results suggests a functional link between chromosome condensation and subsequent steps of chromosome segregation.

Materials and methods

Cell lines

MRC-5 human fetal lung fibroblast cells (CCL-171; American Type Culture Collection) were grown in DME (GIBCO BRL) supplemented with 10% FBS, L-glutamate, and penicillin-streptomycin.

Antibodies

The human chromokinesin HKIF4A monoclonal mouse antibody was generated as described previously (Geiman et al., 2004). The culture supernatant was used at 1:50 for Western blots or undiluted for immunoprecipitation reactions. Antibodies for immunofluorescence were goat anti–mouse or anti–rabbit IgG conjugated with Alexa 488 or Alexa 568 (Molecular Probes) and donkey anti–rat IgG conjugated with cy3 (Jackson Labs)

RNAi depletion of KIF4A in MRC-5 cells

Two siRNA duplexes (HKIF4A RNA1, 5′-GCAATTGATTACCCAGTTA-3′; HKIF4A RNA2, 5′-GAAAGATCCTGGCTCAAGA-3′) targeting HKIF4A were obtained from SMARTPOOL (Dharmacon Research) and gave identical results. Cells were transfected with 100 nM RNAi duplexes using Oligofectamine (Invitrogen). Cells were transfected for a second time 24 h after the first transfection (Elbashir et al., 2002). For protein analyses, the transfected cells were washed twice with PBS and extracted with SDS sample buffer. Cells on coverslips were fixed at different time points after transfection up to 55 h.

Immunofluorescent staining of MRC-5 cells and chromosomes

Immunofluorescence was performed as described previously (Misteli and Spector, 1996). For double staining with tubulin, cells were pre-extracted with 0.5% Triton X-100 before fixation. For spindle staining, anti-HKIF4A was coincubated with rat anti-tubulin antibody (YL/2; Sera Lab) at a dilution of 1:200.

In situ chromosome and metaphase chromosome spreads were prepared and subjected to immunofluorescent staining as described previously (Ono et al., 2003) except that the chromosomes were treated with 0.056 M of hypotonic solution and chromosome spreads were prepared by vertically dropping the cell suspension with a Pasteur pipette onto the slide. Image analysis was performed using either an Eclipse microscope (Nikon) fitted with a cooled CCD camera (Micromax) or a 510 LSM META confocal microscope (Carl Zeiss MicroImaging, Inc.).

Coimmunoprecipitation and Western blotting

Nuclear extracts were prepared and coimmunoprecipitations were performed essentially as described previously (Nielsen et al., 1999). Mitotic extracts from HeLa S3 were prepared as described previously (Gaglio et al., 1995). Precipitated proteins were separated by 7.5% SDS-PAGE and analyzed by Western blotting. Immunoblots were blocked with 5% Carnation nonfat milk in TBST (20 mM Tris, pH 7.5, 137 mM NaCl, 0.1% Tween 20). Primary and secondary antibodies were diluted in 1% blocking solution. Immunoreactive bands of proteins were detected using ECL (Amersham Biosciences).

Online supplemental materials

Fig. S1 A shows the colocalization of HKIF4A with MTs at different phases of the cell cycle. Fig. S1 B shows microinjection of HKIF4A antibody into prometaphase cells causes mis-alignment of chromosomes and mitotic delay. Fig. S2 shows HKIF4A depletion hypercondenses chromosomes even before nuclear envelope breakdown. Fig. S3 shows immunoprecipitation controls. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200401142/DC1.

We thank T. Hirano, K. Yokomori, and M. Bustin for reagents, L. Parada for insightful discussion, T. Cheutin and T. Karpova for help with imaging, and A. Karande for help with raising the mAbs. Imaging was performed at the NCI Fluorescence Imaging Facility.

T. Misteli is a Fellow of the Keith R. Porter Endowment for Cell Biology.

Submitted: 29 January 2004
Accepted: 16 July 2004

References

Antonio, C., I. Ferby, H. Wilhelm, M. Jones, E. Karsenti, A.R. Nebreda, and I. Vernos. 2000. Xkid, a chromokinesin required for chromosome alignment on the metaphase plate. *Cell.* 102:425–435.

Bringmann, H., G. Skiniotis, A. Spilker, S. Kandels-Lewis, I. Vernos, and T. Surrey. 2004. A kinesin-like motor inhibits microtubule dynamic instability. *Science.* 303:1519–1522.

Chang, C.J., S. Goulding, W.C. Earnshaw, and M. Carmena. 2003. RNAi analysis reveals an unexpected role for topoisomerase II in chromosome arm congression to a metaphase plate. *J. Cell Sci.* 116:4715–4726.

Coelho, P.A., J. Queiroz-Machado, and C.E. Sunkel. 2003. Condensin-dependent localization of topoisomerase II to an axial chromosomal structure is required for sister chromatid resolution during mitosis. *J. Cell Sci.* 116:4763–4776.

Earnshaw, W.C., and U.K. Laemmli. 1983. Architecture of metaphase chromosomes and chromosome scaffolds. *J. Cell Biol.* 96:84–93.

Elbashir, S.M., J. Harborth, K. Weber, and T. Tuschl. 2002. Analysis of gene function in somatic mammalian cells using small interfering RNAs. *Methods.* 26: 199–213.

Funabiki, H., and A.W. Murray. 2000. The *Xenopus* chromokinesin Xkid is essential for metaphase chromosome alignment and must be degraded to allow anaphase chromosome movement. *Cell.* 102:411–424.

Gaglio, T., A. Saredi, and D.A. Compton. 1995. NuMA is required for the organization of microtubules into aster-like mitotic arrays. *J. Cell Biol.* 131:693–708.

Gassmann, R., P. Vagnarelli, D. Hudson, and W.C. Earnshaw. 2004. Mitotic chromosome formation and the condensin paradox. *Exp. Cell Res.* 296: 35–42.

Geiman, T.M., U.T. Sankpal, A.K. Robertson, Y. Chen, M. Mazumdar, J.T. Heale, J.A. Schmiesing, W. Kim, K. Yokomori, Y. Zhao, and K.D. Robertson. 2004. Isolation and characterization of a novel DNA methyltransferase complex linking DNMT3B with components of the mitotic chromosome condensation machinery. *Nucleic Acids Res.* 32:2716–2729.

Goshima, G., and R.D. Vale. 2003. The roles of microtubule-based motor proteins in mitosis: comprehensive RNAi analysis in the *Drosophila* S2 cell line. *J. Cell Biol.* 162:1003–1016.

Ha, M.J., J. Yoon, E. Moon, Y.M. Lee, H.J. Kim, and W. Kim. 2000. Assignment of the kinesin family member 4 genes (KIF4A and KIF4B) to human chromosome bands Xq13.1 and 5q33.1 by in situ hybridization. *Cytogenet. Cell Genet.* 88:41–42.

Hagstrom, K.A., and B.J. Meyer. 2003. Condensin and cohesin: more than chromosome compactor and glue. *Nat. Rev. Genet.* 4:520–534.

Hudson, D.F., P. Vagnarelli, R. Gassmann, and W.C. Earnshaw. 2003. Condensin is required for nonhistone protein assembly and structural integrity of vertebrate mitotic chromosomes. *Dev. Cell.* 5:323–336.

Jallepalli, P.V., and C. Lengauer. 2001. Chromosome segregation and cancer: cutting through the mystery. *Nat. Rev. Cancer.* 1:109–117.

Kaitna, S., P. Pasierbek, M. Jantsch, J. Loidl, and M. Glotzer. 2002. The aurora B kinase AIR-2 regulates kinetochores during mitosis and is required for separation of homologous Chromosomes during meiosis. *Curr. Biol.* 12:798–812.

Kapoor, T.M., and D.A. Compton. 2002. Searching for the middle ground: mechanisms of chromosome alignment during mitosis. *J. Cell Biol.* 157:551–556.

Kwon, M., S. Morales-Mulia, I. Brust-Mascher, G.C. Rogers, D.J. Sharp, and J.M. Scholey. 2004. The chromokinesin, KLP3A, drives mitotic spindle pole separation during prometaphase and anaphase, and facilitates chromatid motility. *Mol Biol Cell.* 15:219–233.

Lavoie, B.D., E. Hogan, and D. Koshland. 2002. In vivo dissection of the chromosome condensation machinery: reversibility of condensation distinguishes contributions of condensin and cohesin. *J. Cell Biol.* 156:805–815.

Lee, Y.M., and W. Kim. 2003. Association of human kinesin superfamily protein member 4 with BRCA2-associated factor 35. *Biochem. J.* 374:497–503.

Lee, Y.M., S. Lee, E. Lee, H. Shin, H. Hahn, W. Choi, and W. Kim. 2001. Human kinesin superfamily member 4 is dominantly localized in the nuclear matrix and is associated with chromosomes during mitosis. *Biochem. J.* 360: 549–556.

Levesque, A.A., and D.A. Compton. 2001. The chromokinesin Kid is necessary for chromosome arm orientation and oscillation, but not congression, on mitotic spindles. *J. Cell Biol.* 154:1135–1146.

Maeshima, K., and U.K. Laemmli. 2003. A two-step scaffolding model for mitotic chromosome assembly. *Dev. Cell.* 4:467–480.

Marshall, W.F. 2002. Polar wind left flapping in the breeze? *Trends Cell Biol.* 12:9.

McIntosh, J.R., E.L. Grishchuk, and R.R. West. 2002. Chromosome-microtubule interactions during mitosis. *Annu. Rev. Cell Dev. Biol.* 18:193–219.

Misteli, T., and D.L. Spector. 1996. Serine/threonine phosphatase 1 modulates the subnuclear distribution of pre-mRNA splicing factors. *Mol. Biol. Cell.* 7: 1559–1572.

Nielsen, A.L., J.A. Ortiz, J. You, M. Oulad-Abdelghani, R. Khechumian, A. Gansmuller, P. Chambon, and R. Losson. 1999. Interaction with members of the heterochromatin protein 1 (HP1) family and histone deacetylation are differentially involved in transcriptional silencing by members of the TIF1 family. *EMBO J.* 18:6385–6395.

Ono, T., A. Losada, M. Hirano, M.P. Myers, A.F. Neuwald, and T. Hirano. 2003. Differential contributions of condensin I and condensin II to mitotic chromosome architecture in vertebrate cells. *Cell.* 115:109–121.

Ono, T., Y. Fang, D.L. Spector, and T. Hirano. 2004. Spatial and temporal regulation of condensins I and II in mitotic chromosome assembly in human cells. *Mol Biol Cell.* 15:3296–3308.

Pihan, G.A., and S.J. Doxsey. 1999. The mitotic machinery as a source of genetic instability in cancer. *Semin. Cancer Biol.* 9:289–302.

Saka, Y., T. Sutani, Y. Yamashita, S. Saitoh, M. Takeuchi, Y. Nakaseko, and M. Yanagida. 1994. Fission yeast cut3 and cut14, members of a ubiquitous protein family, are required for chromosome condensation and segregation in mitosis. *EMBO J.* 13:4938–4952.

Scholey, J.M., I. Brust-Mascher, and A. Mogilner. 2003. Cell division. *Nature.* 422:746–752.

Sekine, Y., Y. Okada, Y. Noda, S. Kondo, H. Aizawa, R. Takemura, and N. Hirokawa. 1994. A novel microtubule-based motor protein (KIF4) for organelle transports, whose expression is regulated developmentally. *J. Cell Biol.* 127:187–201.

Somma, M.P., B. Fasulo, G. Siriaco, and G. Cenci. 2003. Chromosome condensation defects in barren RNA-interfered *Drosophila* cells. *Genetics.* 165:1607–1611.

Steffensen, S., P.A. Coelho, N. Cobbe, S. Vass, M. Costa, B. Hassan, S.N. Prokopenko, H. Bellen, M.M. Heck, and C.E. Sunkel. 2001. A role for *Drosophila* SMC4 in the resolution of sister chromatids in mitosis. *Curr. Biol.* 11:295–307.

Strick, T.R., T. Kawaguchi, and T. Hirano. 2004. Real-time detection of single-molecule DNA compaction by condensin I. *Curr. Biol.* 14:874–880.

Swedlow, J.R., and T. Hirano. 2003. The making of the mitotic chromosome: modern insights into classical questions. *Mol. Cell.* 11:557–569.

Tokai, N., A. Fujimoto-Nishiyama, Y. Toyoshima, S. Yonemura, S. Tsukita, J. Inoue, and T. Yamamota. 1996. Kid, a novel kinesin-like DNA binding protein, is localized to chromosomes and the mitotic spindle. *EMBO J.* 15:457–467.

Vernos, I., J. Raats, T. Hirano, J. Heasman, E. Karsenti, and C. Wylie. 1995. Xklp1, a chromosomal *Xenopus* kinesin-like protein essential for spindle organization and chromosome positioning. *Cell.* 81:117–127.

Wang, S.Z., and R. Adler. 1995. Chromokinesin: a DNA-binding, kinesin-like nuclear protein. *J. Cell Biol.* 128:761–768.

Wignall, S.M., R. Deehan, T.J. Maresca, and R. Heald. 2003. The condensin complex is required for proper spindle assembly and chromosome segregation in *Xenopus* egg extracts. *J. Cell Biol.* 161:1041–1051.

Williams, B.C., M.F. Riedy, E.V. Williams, M. Gatti, and M.L. Goldberg. 1995. The *Drosophila* kinesin-like protein KLP3A is a midbody component required for central spindle assembly and initiation of cytokinesis. *J. Cell Biol.* 129:709–723.

Williams, B.C., A.F. Dernburg, J. Puro, S. Nokkala, and M.L. Goldberg. 1997. The *Drosophila* kinesin-like protein KLP3A is required for proper behavior of male and female pronuclei at fertilization. *Development.* 124:2365–2376.

Yan, R.T., and S.Z. Wang. 1997. Increased chromokinesin immunoreactivity in retinoblastoma cells. *Gene.* 189:263–267.

Yen, T.J., and B.T. Schaar. 1996. Kinetochore function: molecular motors, switches and gates. *Curr. Opin. Cell Biol.* 8:381–388.