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Mini-Review

Keeping kinetochores on track☆

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ABSTRACT

The multiple functions of kinetochores are reflected in their complex composition, with over a hundred different proteins, which self-associate in several functional subcomplexes. Most of these kinetochore proteins were identified over the last 10–12 years using a combination of genetic, cell biological, biochemical, and bioinformatic approaches in various model organisms. The key challenge since then has been to determine the structural architecture of kinetochores, define the functions of its different subcomponents, and understand its regulation, both in response to the rapid changes in microtubule dynamics or to sense erroneous attachments for spindle checkpoint signalling. Here, we present some of the key advances obtained in the last six years on the biology of kinetochores, both through our work and through the work of many other groups studying this exciting structure.

Introduction

In every cell cycle the genome must be duplicated and equally distributed into two future daughter cells. Each chromatid is replicated during the S-phase and the duplicated sister chromatids are segregated during mitosis. Chromosome segregation is controlled in eukaryotes by the microtubule-based mitotic spindle and kinetochores, which are microtubule binding sites built on specialised chromosomal regions called centromeres (Santaguida and Musacchio, 2009; Walczak and Heald, 2008). The mitotic spindle has a bipolar structure, as microtubules are anchored with their minus ends at both spindle poles, and bind the kinetochores via their plus ends. Faithful chromosome segregation requires that each sister kinetochore pair binds microtubules emanating from opposite spindle poles (amphitelic or bipolar attachment). In case of errors, or absence of attachments, kinetochores engage the spindle checkpoint, leading to a delay in anaphase onset (Khodjakov and Pines, 2010). This safeguard mechanism guarantees that anaphase only occurs once all kinetochore pairs are attached in a bipolar manner. This set of events is essential to prevent genetic instability at the chromosomal level and the development of chromosomal imbalances (aneuploidy), a phenomenon that is present in about 85% of solid human tumours (Weaver and Cleveland, 2006).

Kinetochores are not only the centromeric microtubule attachment sites and key sensors for the spindle checkpoint, they also form the machinery that is responsible for mitotic chromosome movements, in particular congression to the metaphase plate and separation of the sister chromatids at anaphase onset (Kops et al., 2010). Kinetochores regulate chromosome movements both through microtubule motor proteins (kinesins and dynein) and by regulating the dynamics of the microtubule plus ends they are bound to. Kinetochores also stabilise the microtubules they are bound to, ensuring that chromosomes remain attached to the spindle during all phases of chromosome segregation. The importance of kinetochores is illustrated by the fact that in unicellular organisms, such as the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*, or in multi-cellular organisms such as the nematode *Caenorhabditis elegans* or the fruit fly *Drosophila melanogaster*, most kinetochore proteins are essential. This is also true in vertebrates, where many kinetochores are essential, even at the cellular level.

Kinetochores, a highly conserved structure only at second look

The study of kinetochore proteins has been difficult for a long time for two main reasons: firstly the proteins are only present in low abundance, and until very recently the purification of entire kinetochores was elusive (Akiyoshi et al., 2010). This delayed the functional study of kinetochore proteins, as only a few of them were known. The second difficulty is that the protein sequences of most kinetochore subunits are evolutionarily highly divergent. For a long time, the sequences of fungal kinetochore proteins were not sufficient to identify metazoan kinetochore proteins and vice versa in a BLAST search, a fact that considerably delayed comparative functional studies. As an example, in 2005 only two structural proteins were known in *D. melanogaster*, compared to over 60 in *S. cerevisiae* (Heeger et al., 2005). To make things more

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complicated, it was also known that the structure and size of kinetochores vary strongly from organism to organism (Przewloka and Glover, 2009). The kinetochores of most eukaryotes are built on so-called regional centromeres, long stretches of a repetitive AT-rich sequence that lack any sequence-specificity. Such kinetochores can be built on centromeres that are as small as a few kilobases and only bind three microtubules (*S. pombe*), or sit on centromeres of several megabases and bind up to 25 microtubules (mammalian cells). The range is even wider if one considers the kinetochores of certain budding yeasts (including *S. cerevisiae*), which are built on short (125 bp) and sequence-specific point centromeres and only bind one microtubule, or to the other extreme, certain nematodes (including *C. elegans*), which possess holocentric centromeres that span the entire length of the chromosomes and are bound by a multitude of microtubules. Given the high diversity in structure and sequence, it was unclear to what extent the composition of kinetochores in eukaryotes would be conserved.

However, a parallel steady increase in fully sequenced genomes and in the number of annotated kinetochore proteins over time allowed a more precise bioinformatic analysis, which revealed that most kinetochore proteins are conserved amongst eukaryotes. In particular, our group developed an algorithm to identify novel kinetochore proteins both in fungi, plants and metazoans using known *S. cerevisiae* or *S. pombe* kinetochore proteins as a starting point (Meraldi et al., 2006). Since the sequences of kinetochore proteins are often only conserved in very short stretches, we generated multiple-sequence alignments of fungal kinetochore proteins to identify these short stretches and use them as search patterns against metazoan or plant databases. This approach identified seven novel orthologous kinetochore proteins in mammals, three novel proteins in *D. melanogaster* and two novel proteins in plants. These results implied that most kinetochore proteins are conserved in eukaryotes, a finding that was confirmed by biochemical and genetic studies identifying those same proteins as *bona fide* kinetochore proteins (Cheeseman et al., 2004; Foltz et al., 2006; Obuse et al., 2004; Okada et al., 2006; Schittenhelm et al., 2007). The overall conclusion of these studies was that the core of eukaryotic kinetochores is composed of two large conserved protein networks: on one side the KMN network, which consists of Knl-1, the hetero-tetrameric MIND/Mis12 subcomplex, and the hetero-tetrameric-NDC80 subcomplex, and on the other side the CCAN network (Constitutive Centromere Associated Network, which is also called CENP-A NAC/CAD or CENP-H/I complex), which consists of 15 subunits. Soon after the NDC80 subcomplex was recognised as the key microtubule attachment site at kinetochores, which is essential for the binding to spindle microtubules in all eukaryotes (Cheeseman et al., 2006; DeLuca et al., 2006).

Kinetochores, key drivers of chromosome movements

Once kinetochores have established a stable microtubule-attachment through the NDC80 complex, one of their essential functions is to drive the chromosomes to the metaphase plate, a process that is less well understood. In mammalian cells, one can distinguish two phases in this process: first kinetochores establish lateral attachments to microtubules (in an NDC80 independent manner), allowing them to congress to the centre of the spindle to form stable bipolar, end-on attachments via the NDC80 complex (Cai et al., 2009). Microtubule motors, in particular the kinesin CENP-E and dynein, control the movements in this initial phase (Kapoor et al., 2006; Vorozhko et al., 2008). In a second step, once kinetochore pairs are attached in a bipolar manner, chromosome movements are driven by changes in the dynamics of kinetochore-microtubules (Jaqaman et al., 2010; Tirnauer et al., 2002). However, the mechanisms by which kinetochores control plus-end microtubule dynamics have only started to emerge recently. One difficulty is that these dynamics cannot be easily measured since kinetochores are not bound to a single microtubule, but rather are attached to 25 microtubules, which are bundled into a kinetochore-fibre, all of which have different inherent dynamics (VandenBeldt et al., 2006). Therefore, one key step in the study of kinetochore movements was the joint development (in collaboration with the Danuser, McAinsh and Swedlow groups) of a kinetochore-tracking assay in human cells (Jaqaman et al., 2010). This assay, which was based on the computational analysis of rapid and high-resolution recordings of GFP-labelled kinetochores in dividing human cells, allowed for the first time to quantify and characterise the exact movements of human sister-kinetochores in 4-dimensions, and to screen for perturbations that affect these movements. It revealed that metaphase sister-kinetochore pairs undergo semiregular oscillations along the spindle axis, a phenomenon that had been previously described in a qualitative manner (Skibbens et al., 1993). Our automated assay further showed that the speed of kinetochore movements is controlled antagonistically by two microtubule-depolymerases located at kinetochores, the kinesins MCAK and Kif18a, consistent with previous studies on Kif18a (Jaqaman et al., 2010; Stumpff et al., 2008). However, the depletion of either depolymerase did not affect the semi-regularity of sister-kinetochore oscillations, indicating that other components must be controlling the regularity of chromosome movements. Soon after, using the same assay we could show that the CCAN network is essential for sister-kinetochore oscillations and that it plays an essential role in the control of microtubule dynamics (Amaro et al., 2010). Indeed, CENP-H depletion, which disrupts the entire CCAN complex, abolishes regular sister-kinetochore oscillations, leading to rapid and erratic kinetochore movements and a disorganised metaphase plate (Fig. 1). This phenotype is due to the ability of the CCAN network to control the turnover of kinetochore-microtubules. While free mitotic microtubules have a half-life of 10–15 s, kinetochore-microtubules have a much slower turnover of 4–6 min (Zhai et al., 1995). However, in the absence of CENP-H, kinetochore-microtubule plus-ends show a turnover of 10–15 s, indicating that kinetochores have lost the ability to suppress the rapid turnover of mitotic microtubules (Amaro et al., 2010). Importantly, the CCAN network is most likely directly controlling microtubule dynamics: at least one CCAN subunit, CENP-Q, can efficiently bind microtubules *in vitro* and our live cell imaging of GFP-CENP-I, another CCAN subunit, indicates that it preferentially accumulates on the sister-kinetochore bound to growing microtubules, a behaviour that is only known for a few microtubule-binding proteins (Amaro et al., 2010). Interestingly, the CCAN network directly binds to the centromeric CENP-A nucleosomes and contributes to the assembly of the centromeric nucleosomes, suggesting that it acts as a link between centromeric DNA and the microtubule plus-ends (Carroll et al., 2009; Foltz et al., 2006; Okada et al., 2006). One critical challenge for the future will be to determine the molecular mechanisms by which the CCAN network controls the turnover rate of kinetochore-microtubules, and the precise function of the individual components of this large protein network in this process.

Kinetochore function has a global effect on the dynamics of the mitotic spindle

Our investigation of CCAN kinetochore proteins suggested early on that kinetochores are not just controlling chromosome movements, but that in addition they globally affect spindle morphology and dynamics. Indeed, when we depleted the CCAN proteins CENP-O or CENP-L, we found that those depletions led to an accumulation

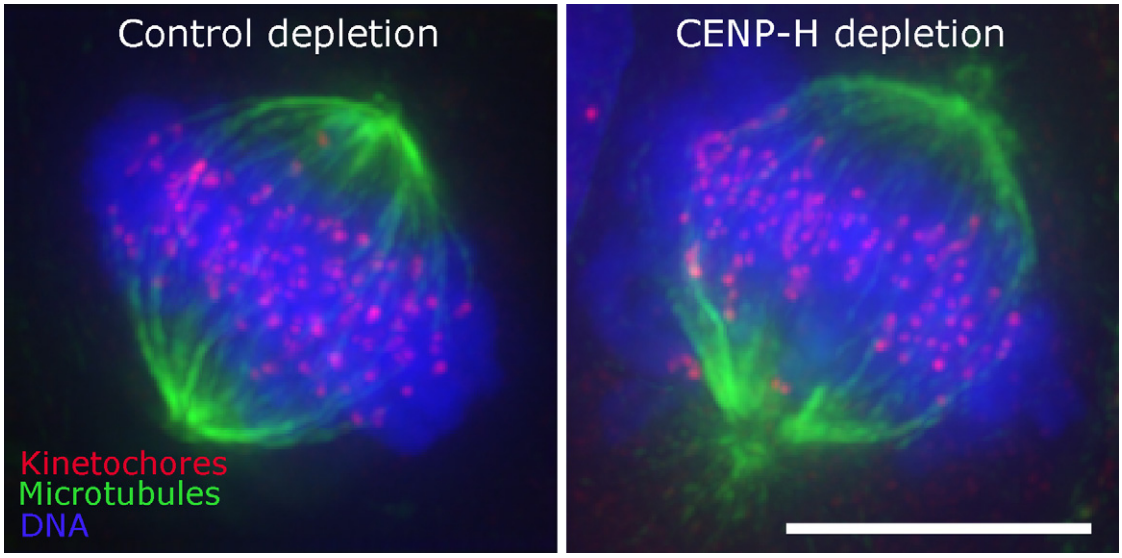

Fig. 1. Disruption of the CCAN network impairs correct chromosome movements. Shown are representative immunofluorescence pictures of CENP-H- or control-depleted HeLa metaphase cells stained with antibodies against CREST (red; kinetochores), antibodies against alpha-tubulin (green; microtubules) and 4,6-diamidino-2-phenylindole (DAPI for DNA; blue; courtesy of A.C. Amaro). Note the tight metaphase plate in control-depleted cells, and the multiple unaligned chromosomes in cells lacking the CCAN network (CENP-H depletion; white arrows). Scale bar = 10 μm.

of transient monopolar spindles in about 30–40% of the cells (McAinsh et al., 2006; McClelland et al., 2007; Mchedlishvili et al., in press; Toso et al., 2009). This suggested that kinetochores are not only driving chromosome movements at the local level, but have a more global effect on spindle architecture. Our live-cell imaging analysis of CENP-O or CENP-L-depleted cells revealed that this effect was linked to the respective history of centrosome separation at the single cell level. If cells had been able to separate their centrosomes before nuclear envelope breakdown, CENP-O or CENP-L depletion did not affect spindle formation, chromosome alignment or chromosome segregation (Mchedlishvili et al., in press; Toso et al., 2009). In contrast, if cells had not yet separated their centrosomes at nuclear envelope breakdown – a phenomenon that has been observed in many cancerous or primary cell lines – CENP-O or CENP-L depletion delay centrosome separation by 6–9 min, leading to a prolonged monopolar spindle conformation (Fig. 2; Mchedlishvili et al., in press; Toso et al., 2009). Our recent data show that even such a small delay will result in a very severe delay of chromosome alignment and a 2.5-fold increase in the error rate of chromosome segregation, indicating that the rapid formation of a bipolar spindle with the help of a kinetochore-based pushing force plays a crucial role in faithful transmission of chromosomes (Mchedlishvili et al., in press). At the more mechanistic level, our investigations further revealed that this kinetochore-pushing force in early prometaphase depends on the ability of kinetochores to incorporate tubulin subunits at the microtubule plus end,

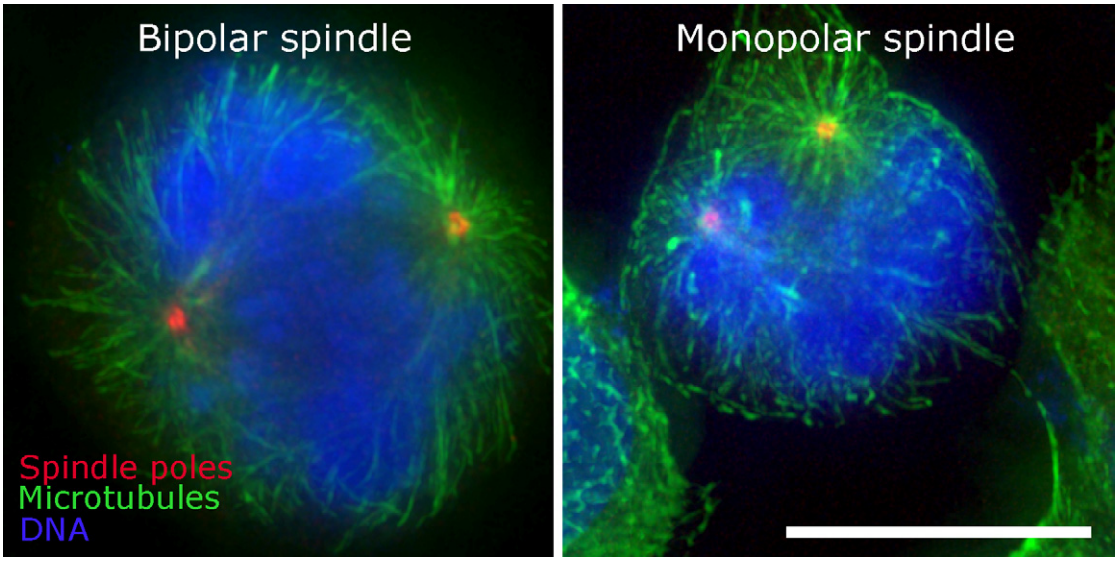

Fig. 2. Depletion of the CCAN kinetochore protein CENP-L leads to the formation of transient monopolar spindles in 30% of the cells. Shown are representative immunofluorescence pictures of CENP-L depleted HeLa cells in prometaphase stained with antibodies against gamma-tubulin (red; spindle poles), antibodies against alpha-tubulin (green; microtubules) and 4,6-diamidino-2-phenylindole (DAPI for DNA; blue; courtesy of N. Mchedlishvili). While the majority of CENP-L depleted cells display a normal bipolar spindle (left panel), about 30% of cells lacking CENP-L show a monopolar configuration. This population of cells with monopolar spindles reflects the inability of CENP-L depleted cells to rapidly separate the two spindle poles during prometaphase. This shows how a local dysfunction at kinetochores can affect the global architecture of the mitotic spindle. Scale bar = 10 μm.

causing bipolarly attached kinetochores to accelerate the separation of the two spindle poles (Toso et al., 2009). Both CENP-O and CENP-L depletions reduce this force, by weakening kinetochore-microtubules, reflecting the influence of the CCAN complex on kinetochore-fibre dynamics. These studies therefore indicate that kinetochores are not just local players that control the movements of individual chromosomes, but that they play a crucial global role in the dynamics of the entire mitotic spindle. These studies also open up a new exciting field for the future where we will not only have to understand the function and composition of a single kinetochore, but we will increasingly have to dissect how local microtubule dynamics at kinetochores at one end, and spindle poles at the other end are coordinated during cell division, to ensure the correct systemic function of the mitotic spindle.

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References

Akiyoshi, B., Sarangapani, K.K., Powers, A.F., Nelson, C.R., Reichow, S.L., Arellano-Santoyo, H., Gonen, T., Ranish, J.A., Asbury, C.L., Biggins, S., 2010. Tension directly stabilizes reconstituted kinetochore-microtubule attachments. Nature 468, 576–579.

Amaro, A.C., Samora, C.P., Holtackers, R., Wang, E., Kingston, I.J., Alonso, M., Lampson, M., McAinsh, A.D., Meraldi, P., 2010. Molecular control of kinetochore-microtubule dynamics and chromosome oscillations. Nat. Cell Biol. 12, 319–329.

Cai, S., O'Connell, C.B., Khodjakov, A., Walczak, C.E., 2009. Chromosome congression in the absence of kinetochore fibres. Nat. Cell Biol. 11, 832–838.

Carroll, C.W., Silva, M.C., Godek, K.M., Jansen, L.E., Straight, A.F., 2009. Centromere assembly requires the direct recognition of CENP-A nucleosomes by CENP-N. Nat. Cell Biol. 11, 896–902.

Cheeseman, I.M., Chappie, J.S., Wilson-Kubalek, E.M., Desai, A., 2006. The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. Cell 127, 983–997.

Cheeseman, I.M., Niessen, S., Anderson, S., Hyndman, F., Yates 3rd., J.R., Oegema, K., Desai, A., 2004. A conserved protein network controls assembly of the outer kinetochore and its ability to sustain tension. Genes Dev. 18, 2255–2268.

DeLuca, J.G., Gall, W.E., Ciferri, C., Cimini, D., Musacchio, A., Salmon, E.D., 2006. Kinetochore microtubule dynamics and attachment stability are regulated by Hec1. Cell 127, 969–982.

Foltz, D.R., Jansen, L.E., Black, B.E., Bailey, A.O., Yates, J.R., Cleveland, D.W., 2006. The human CENP-A centromeric nucleosome-associated complex. Nat. Cell Biol. 8, 446–457.

Heeger, S., Leismann, O., Schittenhelm, R., Schraidt, O., Heidmann, S., Lehner, C.F., 2005. Genetic interactions of separase regulatory subunits reveal the diverged Drosophila Cenp-C homolog. Genes Dev. 19, 2041–2053.

Jaqaman, K., King, E.M., Amaro, A.C., Winter, J.R., Dorn, J.F., Elliott, H.L., McHedlishvili, N., McClelland, S.E., Porter, I.M., Posch, M., Toso, A., Danuser, G., McAinsh, A.D., Meraldi, P., Swedlow, J.R., 2010. Kinetochore alignment within the metaphase plate is regulated by centromere stiffness and microtubule depolymerases. J. Cell Biol. 188, 665–679.

Kapoor, T.M., Lampson, M.A., Hergert, P., Cameron, L., Cimini, D., Salmon, E.D., McEwen, B.F., Khodjakov, A., 2006. Chromosomes can congress to the metaphase plate before biorientation. Science 311, 388–391.

Khodjakov, A., Pines, J., 2010. Centromere tension: a divisive issue. Nat. Cell Biol. 12, 919–923.

Kops, G.J., Saurin, A.T., Meraldi, P., 2010. Finding the middle ground: how kinetochores power chromosome congression. Cell. Mol. Life Sci. 67, 2145–2161.

McAinsh, A.D., Meraldi, P., Draviam, V.M., Toso, A., Sorger, P.K., 2006. The human kinetochore proteins Nnf1R and Mcm21R are required for accurate chromosome segregation. EMBO J. 25, 4033–4049.

McClelland, S.E., Borusu, S., Amaro, A.C., Winter, J.R., Belwal, M., McAinsh, A.D., Meraldi, P., 2007. The CENPA NAC/CAD kinetochore complex controls chromosome congression and spindle bipolarity. EMBO J. 26, 5033–5047.

Mchedlishvili, N., Wieser, S., Holtackers, R., Mouysset, J., Belwal, M., Amaro, A.C., Meraldi, P. Kinetochores accelerate centrosome separation to ensure faithful chromosome segregation. J. Cell Sci., doi:10.1242/jcs.091967, in press.

Meraldi, P., McAinsh, A.D., Rheinbay, E., Sorger, P.K., 2006. Phylogenetic and structural analysis of centromeric DNA and kinetochore proteins. Genome Biol. 7, R23.

Obuse, C., Iwasaki, O., Kiyomitsu, T., Goshima, G., Toyoda, Y., Yanagida, M., 2004. A conserved Mis12 centromere complex is linked to heterochromatic HP1 and outer kinetochore protein Zwint-1. Nat. Cell Biol. 6, 1135–1141.

Okada, M., Cheeseman, I.M., Hori, T., Okawa, K., Mc-Leod, I.X., Yates, J.R., Desai, A., Fukagawa, T., 2006. The CENP-H-I complex is required for the efficient incorporation of newly synthesized CENP-A into centromeres. Nat. Cell Biol. 8, 458–469.

Przewloka, M.R., Glover, D.M., 2009. The kinetochore and the centromere: a working long distance relationship. Annu. Rev. Genet. 43, 439–465.

Santaguida, S., Musacchio, A., 2009. The life and miracles of kinetochores. EMBO J. 28, 2511–2531.

Schittenhelm, R.B., Heeger, S., Althoff, F., Walter, A., Heidmann, S., Mechtler, K., Lehner, C.F., 2007. Spatial organization of a ubiquitous eukaryotic kinetochore protein network in Drosophila chromosomes. Chromosoma 116, 385–402.

Skibbens, R.V., Skeen, V.P., Salmon, E.D., 1993. Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: a push–pull mechanism. J. Cell Biol. 122, 859–875.

Stumpff, J., von Dassow, G., Wagenbach, M., Asbury, C., Wordeman, L., 2008. The kinesin-8 motor Kif18A suppresses kinetochore movements to control mitotic chromosome alignment. Dev. Cell 14, 252–262.

Tirnauer, J.S., Canman, J.C., Salmon, E.D., Mitchison, T.J., 2002. EB1 targets to kinetochores with attached, polymerizing microtubules. Mol. Biol. Cell 13, 4308–4316.

Toso, A., Winter, J.R., Garrod, A.J., Amaro, A.C., Meraldi, P., McAinsh, A.D., 2009. Kinetochore-generated pushing forces separate centrosomes during bipolar spindle assembly. J. Cell Biol. 184, 365–372.

VandenBeldt, K.J., Barnard, R.M., Hergert, P.J., Meng, X., Maiato, H., McEwen, B.F., 2006. Kinetochores use a novel mechanism for coordinating the dynamics of individual microtubules. Curr. Biol. 16, 1217–1223.

Vorozhko, V.V., Emanuele, M.J., Kallio, M.J., Stukenberg, P.T., Gorbsky, G.J., 2008. Multiple mechanisms of chromosome movement in vertebrate cells mediated through the Ndc80 complex and dynein/dynactin. Chromosoma 117, 169–179.

Walczak, C.E., Heald, R., 2008. Mechanisms of mitotic spindle assembly and function. Int. Rev. Cytol. 265, 111–158.

Weaver, B.A., Cleveland, D.W., 2006. Does aneuploidy cause cancer? Curr. Opin. Cell Biol. 18, 658–667.

Zhai, Y., Kronebusch, P.J., Borisy, G.G., 1995. Kinetochore microtubule dynamics and the metaphase-anaphase transition. J. Cell Biol. 131, 721–734.