Proper chromosome segregation in mitosis requires tethering of spindle microtubules to the kinetochore. Using electron tomography of mammalian cells, McIntosh et al. (2008) now report the presence of fibrils that connect the inner kinetochore to the curved protofilaments at microtubule ends, suggesting a new model for force generation in chromosome movement.

Chromosome segregation is one of the more aesthetically pleasing processes in cell biology. Dynamic microtubules collide with chromosomes until chance encounters at the centromere initiate a cascade of reactions that lead to robust attachment of microtubule plus ends to the kinetochore. How this assembly harnesses energy from microtubule dynamics into directed chromosome motion is an area of active investigation. Chromosome segregation presents the following problem: How does one microtubule—a hollow protein tube 25 nm in diameter and several microns long—attach to the chromosome, a DNA structure that is 2 nm in diameter but nearly 1 m in length in humans, to exert force and ensure segregation fidelity during cell division? McIntosh et al. (2008) now present images obtained by electron tomography revealing the presence of fibrils connecting the curved protofilaments at microtubule ends to the inner kinetochore. These findings suggest a new model for the attachment of microtubules to kinetochores and for the mechanism of force generation in chromosome movement.

Influential insights into the attachment of microtubules to kinetochores came from Terrell Hill, who proposed that the microtubule is inserted into a sleeve or channel within the kinetochore (Hill, 1985). In this model, the end of the microtubule is free to gain and lose subunits given its accessibility to the solvent phase in the sleeve. Since then the field has been in search of sleeves or rings that fit this proposed structure. To the field’s great satisfaction, the Dam/Dash complex of yeast was found to form rings in vitro (Westermann et al., 2006; Miranda et al., 2005) (Figure 1). Yet, it has been surprisingly difficult to demonstrate whether the Dam/Dash complex forms rings in vivo. In addition, in several other organisms, this complex has either not been found or is not abundant enough for ring formation (Joglekar et al., 2008).

The work of McIntosh et al. (2008) allows us to peer deeper into the structures of flanking pericentric chromatin and the microtubule plus end, revealing several surprises. The first is the structure of the microtubule plus end in mitosis. McIntosh et al. examine kinetochore-microtubule attachments in PtK1 cells (derived from the Kangaroo rat) by electron tomography and show that the plus end is not a linear assembly of 13 protofilaments, as once thought. Instead, the protofilaments are curved at the growing and shortening plus ends. This curvature expands the diameter of the plus end (from 25 to ~35 nm), thus increasing the surface area available for interactions with kinetochore proteins. Indeed, McIntosh et al. find 2–4 nm filaments that connect to the bent tips of these curved microtubules. They propose that these end-on attachments can do mechanical work. From this emerges a new model that couples
energy from the shortening of microtubules to chromosome movement. This model also suggests that attachment to the kinetochore is not mediated by a sleeve around the microtubule but rather through fibrils connected to the inside of the microtubule.

How does the existence of 2–4 nm filaments fit within the known structural information of the kinetochore and its interaction with chromatin? The kinetochore is comprised of 65–70 different proteins whose stoichiometries within the complex are known for the budding yeast (Joglekar et al., 2006). This information provides important geometric constraints to help us understand the in vivo structure of the attachment of microtubules to the kinetochore. The kinetochore is composed of a series of complexes consisting of eight NDC80 complexes, five to six globular complexes (MIND), two members of the COMA complex (containing Ctf19, Okp1, Mcm21, Ame1), and one centromere-specific nucleosome. In eukaryotes, the centromere-specific nucleosome is characterized by the replacement of histone H3 with a highly conserved histone H3 variant, CENP-A. The centromere DNA locus is bent in such a way that the flanking pericentric chromatin is paired via intra-molecular interactions (Yeh et al., 2008). This intramolecular configuration has key structural implications, including the notion that the eukaryotic kinetochore physically links two dynamic polymers, the microtubule and centromere DNA C loops. Both of these polymers are dynamically unstable; microtubules grow and shorten from their plus ends, whereas centromere DNA loops extend or contract depending on the degree of intra-or intermolecular pairing of DNA. The flanking DNA loops might fluctuate in chromatin between a 30 nm fiber and an extended 2 nm double helix. Alternatively, stiff linkers such as NDC80 might bind laterally or to microtubule plus ends depending on the state of tension. In either scenario, the kinetochore harnesses energy by linking these two dynamic polymers via multiple weak interactions.

How is the model proposed by McIntosh et al. different from other models of chromosome segregation? Some provocative work from bacterial systems further expands possible strategies for chromosome segregation. Polymer extension, in which an actin-like polymer segregates replicated strands of DNA by extension between the replicated mini-chromosomes, is one such example (Garner et al., 2007). This mechanism is quite different from two different polymers held together via a kinetochore linker; in bacteria the “linkage” is dynamic, whereas in eukaryotes the attached polymers are dynamic. Interestingly, when one considers the polymer from a purely theoretical perspective, it is evident that the tendency for a polymer to adopt a form with the highest degree of freedom (entropy) will also have the consequence of segregating replicated strands that are spatially confined (Jun and Mulder, 2006).

The common feature of all of these mechanisms (ring, fibrils, polymer extension, entropic recoil) is the requirement for force generation. How much force is needed to segregate a chromosome, and is it realistic to consider a mechanism based on entropy? Although a number of biophysical experiments have been performed with purified components in vitro, these hypotheses ultimately need to be tested in vivo. The mitotic spindle is an extremely weak machine, in fact, one of the weakest machines for its size (Nicklas, 1988). Chromosome segregation is about accuracy and not speed. Indeed, problems may arise if the speed of segregation was increased. Speed would reduce time available for error correction. It would also increase the chances of chromosome breakage, as chromosomes are soft materials and susceptible to shear force. A physically accurate way to think about chromosome segregation is that a very small force is imposed at the centromere, enough to overcome random fluctuations from thermal motion. Following centromere segregation, residual mechanical linkages between chromosome arms are destroyed (such as cohesin degradation), allowing entropic recoil to drive chromosome arm segregation.

As we start to dissect mechanisms of force generation, knowing the number, position, and physical properties of individual kinetochore components, including those of microtubules and...
chromatin, becomes the next challenge. α-helical coiled proteins are stiff over short length scales, comparable to microtubules and naked DNA, and are significantly stiffer than the average chromosome. The kinetochore is likely to be comprised of a series of stiff mechanical linkages. These linkages may assemble on the microtubule lattice, either displaced from the plus end (for instance, a Hill sleeve) or along the inward surface of a curved protofilament (as suggested by McIntosh et al.). The structures and hypotheses that emerge from such studies will help us understand how cells ensure that every last chromosome is faithfully segregated to generate a thriving organism.

References
Figure 1. Kinetochore-Microtubule Attachment

(A) Geometric configuration of conserved kinetochore components in budding yeast. The kinetochore provides the physical linkage between the microtubule plus end (green) and the centromeric DNA (wrapped around the centromere nucleosome, Cse4). The drawing reflects the number of individual complexes based on quantitative fluorescence microscopy, the structure of complexes from sedimentation velocity or electron microscopy, and the assumption that there is three-dimensional symmetry around the microtubule lattice.

(B) The microtubule (green, right) is a 25 nm diameter filament. The ring (red) depicts the notion that an element in the kinetochore encircles the growing or shortening plus end of the microtubule. The centromere-specific histone (orange circle) is at the apex of a loop of...
intramolecularly paired pericentric chromatin. In contrast, the findings of McIntosh et al. (2008) suggest that 2–4 nm fibrils bind the inner surface of the curved protofilaments. These fibrils may be proteinaceous (black coiled-coil α-helical protein) or DNA (stretched pericentric chromatin).