Structure-function insights into the yeast Dam1 kinetochore complex

Eva Nogales[1,2,*] and Vincent H. Ramey[2,3]

[1]Department of Molecular and Cell Biology, UC Berkeley/Howard Hughes Medical Institute, [2]Life Science Division, Lawrence Berkeley National Laboratory, and [3]Biophysics Graduate Program, UC Berkeley, Berkeley, CA 94720-3220, USA

*Author for correspondence (enogales@lbl.gov)

Journal of Cell Science 122, 3831-3836 Published by The Company of Biologists 2009
doi:10.1242/jcs.004689

Summary

Faithful segregation of genetic material during cell division requires the dynamic but robust attachment of chromosomes to spindle microtubules during all stages of mitosis. This regulated attachment occurs at kinetochores, which are complex protein organelles that are essential for cell survival and genome integrity. In budding yeast, in which a single microtubule attaches per kinetochore, a heterodecamer known as the Dam1 complex (or DASH complex) is required for proper chromosome segregation. Recent years have seen a burst of structural and biophysical data concerning this interesting complex, which has caught the attention of the mitosis research field. In vitro, the Dam1 complex interacts directly with tubulin and self-assembles into ring structures around the microtubule surface. The ring is capable of tracking with depolymerizing ends, and a model has been proposed whereby the circular geometry of the oligomeric Dam1 complex allows it to couple the depolymerization of microtubules to processive chromosome movement in the absence of any additional energy source. Although it is attractive and simple, several important aspects of this model remain controversial. Additionally, the generality of the Dam1 mechanism has been questioned owing to the fact that there are no obvious Dam1 homologs beyond fungi. In this Commentary, we discuss recent structure-function studies of this intriguing complex.

Key words: Mitosis, Microtubules, Spindle checkpoint

Introduction

The accurate segregation of genetic material between daughter cells is an essential step in cell division. Errors in this process lead to aneuploidy and can result in cell transformation (King, 2008). The kinetochore is a network of protein complexes that assembles on centromeric chromatin. It acts as the connection point between chromatids and the microtubules that segregate them into daughter cells (Cheeseman and Desai, 2008; Westermann et al., 2007). Kinetochores can sense that sister chromatids are attached to microtubules from the same spindle pole and can correct such attachments through a mechanism that has been proposed to be mediated by physical tension, in which pulling forces of the spindle put strain on the centromeric region of chromosomes (Li and Nicklas, 1995; Maresca and Salmon, 2009; Uchida et al., 2009). The stable engagement of a kinetochore is regulated by the Aurora-B kinase, the activity of which has been shown to reduce the affinity of several kinetochore proteins for microtubules (Cheeseman et al., 2002; Ruchaud et al., 2007). A single unattached or incorrectly attached kinetochore is sufficient to trigger the spindle-assembly checkpoint and halt progression into anaphase, thereby preventing cell division.

An essential function of the kinetochore is to couple chromosome movement to microtubule depolymerization. Once all of the chromosomes in a cell that is preparing to divide are correctly bi-oriented, they must be pulled apart into daughter cells. After the spindle-assembly checkpoint has been satisfied, the anaphase-promoting complex triggers the destruction of the cohesin complexes that tether sister chromatids together (Cheeseman and Desai, 2008; Westermann et al., 2007). Kinetochores are then able to track microtubule ends as they depolymerize and, in a process that does not require motor activity, harness the energy released during microtubule depolymerization to move chromosomes to the two opposite spindle poles (Koshland et al., 1988).

The molecular mechanisms by which chromosomes attach to kinetochores have remained a great mystery since the mitotic process was first visualized using live movies. In budding yeast, in which there is a single microtubule attachment per kinetochore, a complete component list of kinetochore proteins has recently been determined. The first report on Dam1-complex subunits and their requirement for a functional mitotic spindle was published over a decade ago. In this report, Drubin, Barnes and co-workers identified Duo1p as a protein that contributed to aspects of spindle function sensed by the spindle-assembly checkpoint (Hofmann et al., 1998). Furthermore, a yeast two-hybrid approach was used in this study to identify Dam1p as a Duo1p-interacting protein that was shown to bind directly to microtubules. Soon after these discoveries, Winey and colleagues (Jones et al., 1999) identified the *Dam1* gene in a genetic screen and showed that the protein it encoded was involved in spindle integrity, and that it localized to spindle microtubules and probably to the kinetochore. Major breakthroughs in the next few years included the characterization of the remaining components of the Dam1 complex (Cheeseman et al., 2001a; Cheeseman et al., 2001b; Enquist-Newman et al., 2001; Janke et al., 2002), and the identification of the functional interaction between the Dam1 complex and the checkpoint kinase Ipl1p (Cheeseman et al., 2002; Jung-seog Kang, 2001; Shang et al., 2003).

These initial genetic and biochemical studies showed that the ten-subunit Dam1 complex (Fig. 1) was essential for regulated microtubule-kinetochore attachment. However, only in the last 4 years has a structural and biophysical description of the interaction between the Dam1 complex and microtubules become available. The expression of the ten Dam1-complex subunits in bacteria by the Harrison laboratory (Miranda et al., 2005) made it possible to

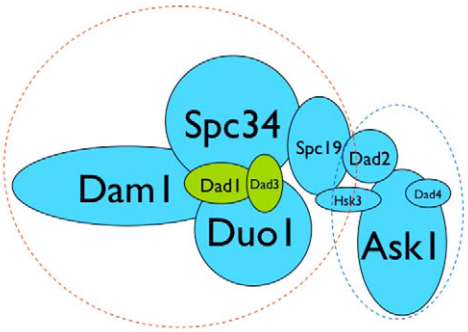

Fig. 1. The hypothetical architecture of the Dam1 complex. This model is based on the Uniprot interaction database (Ito et al., 2001) and on work by Harrison and co-workers (Miranda et al., 2007). The two dashed circles represent the subcomplexes described by Miranda et al., who also observed that Dad1p and Dad3p form a stable subcomplex (green). On the basis of the literature, Dad3p seems to be connected only to Dad1p. The interaction partner of Dad4p is unclear, although it is known that this protein is within a ternary subcomplex with Ask1p and Dad2p.

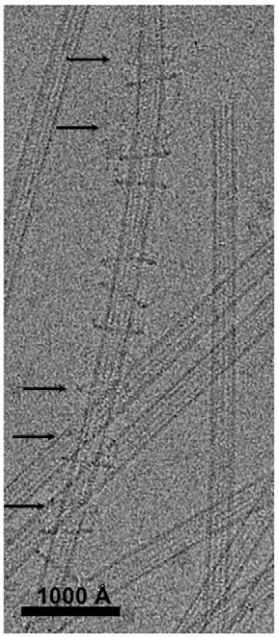

Fig. 2. Cryo-EM showing Dam1-complex rings around microtubules. Rings that are tilted out of the plane of the image, which are harder to see, are indicated with arrows.

purify a functional, recombinant full complex and therefore to explore the structural bases for the interaction of the complex with microtubules. The first electron microscopy (EM) studies of the complex proved to be illuminating and exciting, as they showed the microtubule-induced assembly of Dam1 complexes into rings and spirals (Miranda et al., 2005; Westermann et al., 2005). A ring was thought to be an ideal structure to allow coupling of the energy released during microtubule depolymerization, when GDP-tubulin relaxes into its low-energy state by protofilament peeling.

Although these data and the concepts they inspired have captivated the minds of both structural and cell biologists (Salmon, 2005), the molecular mechanisms that underlie Dam1-complex function remain highly controversial. The character of the interaction between the Dam1 complex and tubulin is under discussion, as is whether ring formation is required for the tracking and functionality of the complex. Finally, although rings have yet to be visualized in vivo, and the Dam1 complex initially seemed to be unique to fungi, a potential alternative complex with similar properties has recently been proposed for metazoans. In this Commentary, we review the highlights of several recent structure-function studies that have helped to increase our understanding of the Dam1 complex and discuss the current controversies that surround this topic.

Interaction between the Dam1 complex and microtubules

In vitro reconstitution of the ten-protein Dam1 complex made it possible to carry out EM studies of the complex bound to microtubules, which revealed an assembly of rings and spirals interacting in a novel manner with the underlying tubulin (Fig. 2) (Miranda et al., 2005; Westermann et al., 2005). Fourier analysis of images of microtubules covered with double spirals showed that the axial repeats of Dam1-complex subunits and tubulin are different from one another (Westermann et al., 2005), and end-on views suggested that 13-protofilament microtubules are surrounded by 16 repeats of the Dam1 complex oligomerized into a ring (Westermann et al., 2006). Furthermore, initial analysis of frozen hydrated samples by cryo-EM showed that the mass of the Dam1 complex is positioned approximately 20 Å away from the ordered microtubule lattice that underlies it (Westermann et al., 2005). This hinted at the possibility that the ring-microtubule interaction is mediated by the C-terminal tails of α- and/or β-tubulin – these unstructured and highly extended segments of about 15 amino acids are also known as E-hooks owing to an abundance of glutamic acids within them. This C-terminal region of each tubulin molecule can be selectively cleaved by the serine protease subtilisin. In the original experiments by Westermann et al., subtilisin cleavage of tubulin abrogated the binding of the Dam1 complex, supporting the idea that the C-terminal regions of tubulin molecules were involved in the ring-microtubule interaction (Westermann et al., 2005). By contrast, however, a later study by Harrison and co-workers reported a negligible effect of subtilisin cleavage on binding of the Dam1 complex to microtubules or on ring formation (Miranda et al., 2007). The disparity of results concerning the effect of subtilisin might have been caused by the different experimental conditions – in particular, the relative ratios of Dam1 complex to tubulin. At concentrations of Dam1 complex that saturate the lattice of native microtubules, little or no Dam1 is observed to bind to subtilisin-cleaved microtubules. Only when the Dam1 complex is present in excess, at levels that cause it to accumulate in solution, do the subtilisin-cleaved microtubules become significantly bound by Dam1-complex rings (Hong-Wei Wang, V.R., E.N., Julie Welburn and Yuko Nakajima, unpublished observations). This result suggests that the C-terminal tails of tubulin are relevant for its interaction with the Dam1 complex under physiological conditions.

Structural analysis of the yeast Dam1 complex containing a truncated form of Dam1p lacking its C-terminus revealed that the this region is located in a strategic location in the complex that potentially affects oligomerization and interactions with the microtubule (Fig. 3C) (Wang et al., 2007). Dam1p is a major target of the spindle-checkpoint kinase Ipl1p (Cheeseman et al., 2002). Interestingly, a phosphomimetic mutant of the Ipl1p-phosphorylation sites in Dam1p (three out of four of which are at

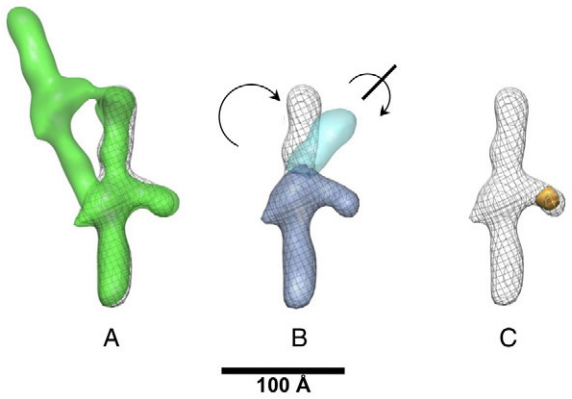

Fig. 3. EM reconstruction of the negatively stained, unassembled Dam1 complex. (A) Reconstruction of the Dam1 dimer (green) and the average monomer derived from it (mesh). (B) Illustration of the conformational change that is proposed to occur upon binding of the Dam1 complex to microtubules and its oligomerization into a ring. (C) The wild-type Dam1 complex is shown as a mesh together with the difference map that is derived when the yeast Dam1p-ΔC mutant reconstruction is subtracted from it. The C-terminus of Dam1p (shown in yellow) is located near the site of microtubule interaction and oligomerization of the Dam1 complex.

the Dam1p C-terminus) shows little effect on the binding of the Dam1 complex to the microtubule but causes a reduction in ring assembly (Westermann et al., 2005). Therefore, we proposed that one role of Ipl1p might be to fine-tune the coupling of the microtubule interaction with the conformational change in the Dam1 complex required for its oligomerization (Fig. 3B), and that Ipl1p-mediated phosphorylation of Dam1p results in ring breakdown (Wang et al., 2007).

Proteolysis experiments carried out by Miranda et al. showed that treatment of the Dam1 complex with elastase, which causes cleavage of the complex components Ask1p, Dam1p and Duo1p, abrogates the capacity of the complex to bind to microtubules (Miranda et al., 2007). Interestingly, the outcome of elastase treatment is the same when the Dam1 complex is in the assembled ring form around microtubules during treatment. The fact that the cleavage sites are accessible in this assembled form supports the notion that there is a loose interaction between the oligomeric ring and the microtubule. The alternative possibility is that individual Dam1 complexes have a short dwell time within a dynamic ring assembly; however, this idea conflicts with experimental results showing that there is no turnover once the ring is formed (Westermann et al., 2006).

Today, it is generally accepted that the Dam1 complex is a unique microtubule-binding factor that self-assembles to form a ring structure around the microtubule lattice. Importantly, there is a mismatch between the arrangement of the subunits in the microtubule and in the ring: this is made possible by interactions between tubulin and Dam1-complex subunits that involve flexible protein regions, probably in both interaction partners.

Structure and organization of the Dam1 complex

Defining the architecture of the Dam1 complex and its microtubule-driven self-assembly into a ring structure is essential for understanding the mechanisms by which rings might contribute to the end-on attachment of spindle microtubules to chromosomes demonstrated for the Dam1 complex (Shimogawa et al., 2006; Tanaka et al., 2007) and how the complex couples microtubule disassembly to processive chromosome movement. It is also crucial for determining how the assembly of the ring could be regulated and how the ring could attach to other components of the kinetochore. EM-based single-particle and helical analyses have been used to obtain initial structures of the Dam1 complex before and after its oligomerization around microtubules. This work defined the architecture of the Dam1 complex as well as its mechanism of self-assembly (Wang et al., 2007). Individual Dam1 complexes are elongated structures of about 150 Å with a central bulge from which a protrusion extends (Fig. 3A). Structural comparison of unassembled complexes with the repeating unit of the assembled helical oligomeric complex (Fig. 4) strongly suggests that, once within the ring, the structure of each individual complex changes. We propose that a conformational change that would twist the complex near the central protrusion would be sufficient to accommodate 16 copies of the structure around a single turn of the spiral density, without physical overlapping of individual Dam1 complexes or unfilled density regions (Fig. 3B). Therefore, ring oligomerization seems to be facilitated by a conformational change upon binding to microtubules, which might explain the coupling between interaction with the microtubule and ring assembly. This molecular mechanism, which requires a microtubule for ring assembly to occur, overcomes the hypothetical issue of kinetochore microtubules having to thread through a pre-formed circular structure barely larger that their outer diameter. In this context it is worth mentioning that Tanaka and co-workers showed that the Dam1 complex accumulates on the kinetochore secondarily to the NDC80 complex, and provided experimental evidence that the kinetochore-microtubule interaction does not involve a threading process (Tanaka et al., 2005).

Deletion of specific subunits from coexpression vectors results in the formation of a number of subcomplexes that have shed light on how the complex is organized (Miranda et al., 2007) (Fig. 1). Removal of Hsk3p results in two subcomplexes – Ask1p-Dad2p-Dad4p (which does not bind to microtubules) and Dam1p-Duo1p-Spc34p-Spc19p-Dad1p-Dad3p (which binds to microtubules but does not form rings). In the absence of Dam1p, the complex that forms is also missing Duo1p and the Dad1p-Dad3p dimer (which can be expressed and purified independently and is a stable structural module). Thus, Dam1p and Duo1p have been proposed to form a structural unit. The localization of the C-terminus of

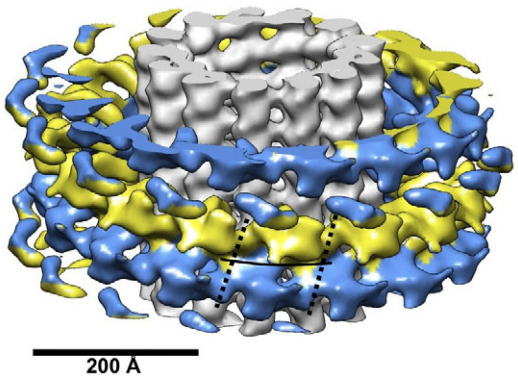

Fig. 4. Cryo-EM reconstruction of Dam1-complex double spirals (blue and yellow) around a microtubule (white) (EMDB-ID:1371). Each rotation of the spiral, which corresponds to a skewed single ring, is shown in a different color. Dashed lines separate adjacent Dam1-complex subunits along the spiral, and the solid black line denotes the two-fold axis of symmetry in the double spiral.

Dam1p to part of the protrusion (as shown by EM; Fig. 3C) (Wang et al., 2007) suggests that this domain, and probably the central bulge, are formed by Dam1p and Duo1p, which is in agreement with their involvement in microtubule binding.

In summary, we now have a low-resolution architecture of the Dam1 complex (both unassembled and in its oligomeric form around microtubules), and a significant amount of information about the protein-protein interactions of the complex and the subcomplexes that have been defined within it. However, these two types of information have not yet been put together to generate a map of the complex that identifies the position of each protein, the details of its self-assembly, or its interaction with microtubules. This task will be a major aim for the near future.

Dynamic properties of microtubule attachment

The flexible electrostatic interaction between the mismatched symmetries of the Dam1-complex ring and the microtubule suggested that the Dam1-complex ring would be able to diffuse along the microtubule lattice by switching stochastically from one E-hook to another. The implications of this idea for kinetochore function were considerable and led to experiments showing that rings were capable not only of one-dimensional diffusion on the microtubule lattice but also of tracking depolymerizing microtubule ends, with high processivity and without energy consumption of their own (Westermann et al., 2006). In the model put forward, it was proposed that Dam1-complex ring structures interact with the microtubule via flexible elements and lack a 'footprint' on the microtubule lattice, which would allow for diffusion that becomes biased and unidirectional when the microtubule depolymerizes (Westermann et al., 2006; Nogales and Wang, 2006).

A number of microtubule-binding proteins, including the Dam1 complex, have been reported to engage in one-dimensional diffusion on the microtubule lattice (Gestaut et al., 2008; Grishchuk et al., 2008b; Powers et al., 2009; Westermann et al., 2006). The fact that these proteins share this common property attests to both the minimal physical requirements for microtubule-based gliding and its potential usefulness. To diffuse on the microtubule lattice, a protein must bind to assembled tubulin with an affinity that allows robust attachment but that is not so tightly bound that the energetic barrier for jumping between adjacent binding sites becomes prohibitive. Processivity of diffusion can be enhanced by the cooperation of multiple copies of the binding protein, as was demonstrated recently for NDC80 (Powers et al., 2009), either through oligomerization, as for the Dam1-complex ring, or by being associated with a scaffold, or both. Regulation of the affinity of a microtubule binder for the microtubule, or its assembly state, could therefore produce a wide range of effects in vivo. Consequently, many recent biophysical studies of the Dam1 complex have been and are still being aimed at determining the effects of phosphorylation, mutation and oligomerization on these binding properties.

Robust connection of the depolymerizing spindle microtubule to the kinetochore requires a highly processive and mobile attachment. There is currently disagreement about whether this is accomplished by the Dam1 complex through a biased diffusion or a 'forced-walk' mechanism (Grishchuk et al., 2008b; Westermann et al., 2006). The Dam1-complex ring has been proposed to harness an impressive amount of force (Efremov et al., 2007) – up to the 70 pN generated at depolymerizing microtubule ends, as calculated from single-molecule studies (Grishchuk et al., 2005). Conversely, experiments using laser-trapped beads have reported that the force harnessed by the Dam1 complex is a more modest 3 pN (Asbury et al., 2006). However, forces in this range have been interpreted by McIntosh and co-workers as an underestimation caused by the geometry of the experimental setup used. These authors proposed a geometrical correction for the lateral attachment of the bead to the ring, which estimates that the force that the Dam1 ring experiences from bending protofilaments is an average of ~30 pN (Grishchuk et al., 2008a). This discrepancy, together with the fact that measurements of the binding affinity of Dam1 to microtubules have ranged over several orders of magnitude (see below), make it difficult to determine unambiguously which of these two distinct models – biased diffusion versus force-walk – is more appropriate. Thus, the motility mechanism of the Dam1 complex on the microtubule remains an area of heated controversy (Gestaut et al., 2008; Grishchuk et al., 2008a; Liu and Onuchic, 2006; Westermann et al., 2005).

An encircling ring is clearly an attractive model for processivity and has been an evolutionary solution for tracking another linear substrate, DNA, by processive clamps such as those of proliferating cell nuclear antigen (PCNA). However, some of the required properties of the Dam1 complex at the kinetochore have been recapitulated in vitro by non-encircling oligomers or single complexes, suggesting that a ring might not be necessary for function (Gestaut et al., 2008; Grishchuk et al., 2008b). Although fluorescence data have put the number of Dam1 complexes at the budding yeast kinetochore at 16-20 during metaphase (Joglekar et al., 2006), which is in agreement with the number seen in a single ring, no direct evidence exists for the oligomerization state of the complex in vivo (McIntosh, 2005), in part owing to the difficulty in visualizing kinetochores in this organism.

Using Dam1-complex-coated beads, Asbury et al. showed that the complex could follow both growing and shrinking microtubule ends (Asbury et al., 2006). The attachments made between the beads and the microtubule were load-bearing, and sliding experiments suggested that Dam1 complexes were forming a ring that sensed growing and shrinking ends as barriers. Interestingly, the presence of the bead at microtubule ends had no apparent effect on microtubule dynamics. However, Asbury and co-workers went on to show that applying tension to the bead against the direction of microtubule depolymerization decreased the frequency of catastrophe (the switch from growth to shrinkage), slowed microtubule shortening and increased the frequency of rescue (the switch from shrinkage to growth) frequency (Franck et al., 2007). The authors noted the compatibility of a ring model with the force-dependent changes in dynamic parameters that they observed: the ring would transmit tension to the peels at the end of depolymerizing microtubules and tend to straighten them, resulting in a microtubule-stabilizing effect. The dependency of microtubule stabilization on tension also agrees well with the idea of multiple weak binding sites for the ring: weak attachments would allow for diffusion and by themselves have little effect on disassembly parameters, although the ring would be able to affect the stability of the microtubule when force was exerted through it against the depolymerizing direction. However, at the time that these concepts were proposed, they had already been put into question. Davis and co-workers developed a high-sensitivity fluorescence-based assay to measure the affinity of the Dam1 complex for microtubules at extremely low concentrations of tubulin (Gestaut et al., 2008). They reported values in the nanomolar range that were 30-fold greater than previously described. Furthermore, single-molecule experiments showed that, at low concentrations of the Dam1 complex, when only short oligomers of one to four copies in size are formed, this oligomeric complex can follow disassembling microtubule ends, although with reduced processivity.

The issue of the oligomerization state of Dam1 and its functional relevance has been further analyzed by McIntosh and co-workers (Grishchuk et al., 2008b). On the basis of fluorescence assays, they proposed that ring oligomers of the Dam1 complex are unable to diffuse but able to track depolymerizing ends. Smaller oligomers diffuse with rates that are inversely proportional to their sizes. Interestingly, they also found that single rings can track microtubule ends, but larger assemblies cannot. The presence of one tracking ring reduces the depolymerization rate, whereas a second ring results in halting of the depolymerization process until one of the rings disassembles, most probably the one that is in contact with the microtubule edge. These results resemble the effect of force on depolymerization speed and rescue rates in the bead experiments of Asbury and co-workers. Experiments by McIntosh and co-workers using Dam1-complex-coated beads showed two different behaviors depending on whether soluble Dam1 complexes were also available in solution. Without soluble Dam1 complexes, the beads bound only to the GMPCPP section of microtubules (a region that would mimic the stabilizing GTP cap at growing microtubule ends). The beads increased the depolymerization rate as the amount of Dam1 complex on the beads increased. The authors also proposed that these beads tracked depolymerizing ends by rolling over the microtubule, rather than sliding, on the basis of experiments with unevenly fluorescent beads. The authors interpreted these results as a lack of ring formation, and thus postulated that there are two distinct mechanisms for end tracking by rings and small oligomers (Grishchuk et al., 2008b).

The recent burst of biophysical data concerning the interaction between the Dam1 complex and microtubules highlights the interest of the scientific community in understanding the detailed molecular mechanisms that underlie microtubule-spindle interactions. Although these findings are exciting, some reported results remain controversial and will require further studies designed specifically to resolve crucial aspects of Dam1 mechanism, such as whether Dam1 tracks depolymerizing microtubule ends through a biased diffusion or a forced-walk mechanism, as well as the biological relevance of the ring structure.

Microtubule attachments in the absence of Dam1

The fact that the Dam1 complex is unique to fungi contrasts with the more conserved nature of other outer-kinetochore components. The Dam1-complex ring could be the perfect coupler in budding yeast cells, in which the attachment of a single microtubule per kinetochore requires constant molecular engagement at the microtubule end to avoid kinetochore release. In systems in which more than one microtubule engages each chromatid, one or more microtubules might disengage without release, and the requirements for each microtubule-kinetochore interaction might be less stringent. In budding yeast, the number of Dam1 complexes per kinetochore is compatible with the size of the ring observed in vitro, but fission yeast seems to express the Dam1 complex at a lower copy number per kinetochore, which is insufficient for ring closure. Interestingly, the Dam1 complex is not essential in fission yeast (Sanchez-Perez et al., 2005).

Conversely, the NDC80 complex is another crucial site of kinetochore-microtubule attachment in yeast that seems to be conserved and is essential in all eukaryotes. This four-protein complex has a long coiled-coil domain with globular domains at each end for microtubule and kinetochore binding. Importantly, it has recently been shown that NDC80 is a member of a conserved network of protein complexes known as the KMN network (Cheeseman et al., 2006). This network also includes the KNL-1 protein, which is reported to have microtubule-binding activity of its own, and the MIS12 complex, which seems to act as a coordination hub that tethers the network to the inner kinetochore. Recently, an electron tomography study of PtK1 cells suggested that there are fibrils associated with the inside surface of microtubule ends at the site of kinetochore attachment (McIntosh et al., 2008). The authors suggested that these filamentous proteins could couple shortening microtubules to cargo movement and, furthermore, that they could be NDC80 complexes. In support of this idea, in vitro studies have shown that this complex can track microtubule ends (McIntosh et al., 2008; Powers et al., 2009). However, there is no direct evidence for whether these filaments form part of the KMN complex or whether they correspond to a yet-unidentified component of the kinetochore.

A newly identified molecular player is the Ska1 complex (Gaitanos et al., 2009; Hanisch et al., 2006). This three-protein kinetochore complex is present in all eukaryotes except yeast, and therefore in all species that lack the Dam1 complex. Interestingly, the Ska1 complex seems to share a number of properties with the Dam1 complex, such as its direct binding to microtubules via the Ska1 protein and its microtubule-induced oligomerization (Welburn et al., 2009). It is currently difficult to judge whether the mechanistic details of the attachment of the Ska1 and Dam1 complexes to spindle microtubules will define them as functional homologs.

Conclusions and perspectives

The last 4 years of structure-function studies of the microtubule-kinetochore interaction mechanism have been extremely exciting. The discovery that the yeast Dam1 complex self-assembles into rings in a microtubule-dependent manner, and that it can follow shortening microtubule ends, provided a simple and elegant explanation for how kinetochores can not only remain attached to the depolymerizing ends of microtubules but also harness the energy of microtubule disassembly for processive movement. The role of each of the ten components of the Dam1 complex – in terms of microtubule interaction, self-assembly and attachment to other kinetochore components – is only now starting to be explored. The physical bases for the interaction of the complex with microtubules and for its tracking properties remain controversial, but published studies have now laid the foundation for further experiments that will more directly test the existing models. There are still several outstanding issues that have yet to be explored, including how the Dam1 complex interacts with other kinetochore components such as the KMN network. The possible relationship between the Dam1 complex and other complexes that lack obvious sequence conservation but share certain essential properties, such as the Ska1 complex, is also an exciting area of investigation. There is no doubt that, in the next few years, structural and biophysical characterization of these kinetochore complexes (individually or in combination) will provide us with a better mechanistic understanding of a process that is at the heart of a cell's most essential property: to reproduce by faithfully passing on its genetic program to its offspring.

We thank Greg Alushin for critical reading of the manuscript. This work was supported by training grants from the US National Institutes of Health, by a grant from the National Institute of General Medical Sciences of the US National Institutes of Health and by a Biomedicine chair from the BBVA Foundation to E.N. E.N. is a Howard Hughes Medical Institute Investigator. Deposited in PMC for release after 6 months.

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