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Review Article

Structural view of the yeast Dam1 complex, a ring-shaped molecular coupler for the dynamic microtubule end

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In a dividing eukaryotic cell, proper chromosome segregation requires the dynamic yet persistent attachment of kinetochores to spindle microtubules. In the budding yeast *Saccharomyces cerevisiae*, this function is especially crucial because each kinetochore is attached to a single microtubule; consequently, loss of attachment could lead to unrecoverable chromosome loss. The highly specialized heterodecameric Dam1 protein complex achieves this coupling by assembling into a microtubule-encircling ring that glides near the end of the dynamic microtubule to mediate chromosome motion. In recent years, we have learned a great deal about the structural properties of the Dam1 heterodecamer, its mechanism of self-assembly into rings, and its tethering to the kinetochore by the elongated Ndc80 complex. The most remarkable progress has resulted from defining the fine structures of helical bundles within Dam1 heterodecamer. In this review, we critically analyze structural observations collected by diverse approaches with the goal of obtaining a unified view of Dam1 ring architecture. A considerable consistency between different studies supports a coherent model of the circular core of the Dam1 ring. However, there are persistent uncertainties about the composition of ring protrusions and flexible extensions, as well as their roles in mediating ring core assembly and interactions with the Ndc80 complex and microtubule.

Accurate segregation of duplicated chromosomes is an important step in cell division that depends critically on the kinetochore, a large macromolecular complex that assembles at the centromere and attaches to the plus ends of spindle microtubules [1–3]. Among species, the best-characterized kinetochore is that of budding yeast, which binds to a single microtubule [4] (Figure 1) and exhibits several fascinating features [5,6]. In particular, it hosts the multisubunit Dam1 complex, which is essential for chromosome segregation [7–15]. Different components of this complex were initially identified through genetic screens targeting molecular components that contribute to chromosome segregation [9–12,14,16]. For example, mutations or overproduction of Dam1 polypeptide (Dam1p), Duo1p, and Dad1p increase chromosome missegregation and lead to mitotic defects, such as short, bent or broken spindles [12,14]. Components of this complex are also regulated by Aurora B and Mps1 kinases [17,18]. According to a widely accepted model, the Dam1 complex contributes to chromosome segregation by forming a microtubule-encircling ring, which is fastened to the kinetochore by an elongated Ndc80 protein complex (Figure 1B). Together, these proteins mediate coupling between the kinetochore and dynamic microtubule end. Notably, analogous function is carried out in other organisms by the multimolecular ensemble containing Ndc80 and SKA complexes [15].

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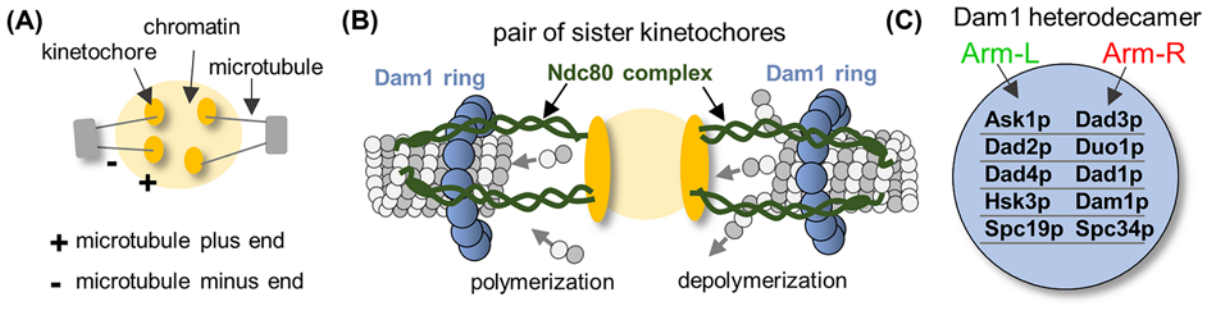

(D)

motility and force / size of assemblies	MT wall diffusion	tracking of depolymerizing end	tracking of polymerizing end	force transduction
small oligomers	10^{-1} - 10^{-3} $\mu m^2/s$ (1-4 subunits)	normal MT depolymerization rate	yes	reduced
ring	estimated 10^{-6} – 10^{-8} $\mu m^2/s$	reduced MT depolymerization rate	not yet examined	strong and enhanced by long tethers
spiral	complete MT wall decoration with no diffusion or tip-tracking. MT depolymerization is impeded.			

Figure 1. Illustration of Dam1 complex function and interactions with microtubules *in vitro*

(**A**) Schematic of kinetochore-microtubule configuration in a dividing yeast cell. (**B**) Proposed configuration of kinetochore-microtubule attachment, in which microtubule-encircling Dam1 rings are tethered to sister kinetochores via Ndc80 complexes [6]. (**C**) Molecular composition of the Dam1 heterodecameric complex, which contains five pairs of homologous polypeptides. (**D**) Summary of structurally diverse budding yeast Dam1 complexes exhibiting distinct behaviors on the microtubule lattice and ends, see text for details.

Structural plasticity of the Dam1 heterodecamers and their interactions with microtubules *in vitro*

The Dam1 complex is a 210-kDa heterodecamer consisting of ten polypeptides, including the Dam1p itself, Duo1p, Ask1p, Spc34p, and other proteins (Figure 1C) [10,14,16]. *In vitro* studies of this complex have provided a great deal of information about its ability to form various oligomeric structures that exhibit substantially different interactions with microtubules. This progress has become possible when the Drubin, Barnes and Harrison laboratories purified recombinant full *Saccharomyces cerevisiae* Dam1 complexes (Sc–Dam1 complex) expressed in bacteria [19,20]. The purified soluble complex exists primarily as monomers (i.e., single Dam1 heterodecamers), along with some dimers, although an appreciable fraction of larger, curved oligomers form at higher Dam1 concentrations [8,19,20]. Subsequent work revealed that Dam1 heterodecamers can assemble a variety of structures on the microtubule surface.

When Dam1 complexes are added at subnanomolar concentrations to stabilized microtubules *in vitro*, they can be detected via total internal reflection fluorescence microscopy as quickly diffusing single molecules and small oligomeric patches whose diffusion coefficient decreases exponentially as the number of subunits increases [21–24] (Figure 1D). Small Dam1 complexes readily track microtubule depolymerizing ends without significantly changing their shortening rates [23]. These tip-tracking motions are likely to be mediated by diffusion of Dam1 oligomers, biased by the shortening microtubule end (reviewed in [25]). Small Dam1 oligomers can also track the ends of polymerizing microtubules with an apparently normal rate of tubulin assembly [26]. However, small diffusing complexes

are poor transducers of the microtubule depolymerization force, as revealed by mutants with reduced oligomerization potential [22,27].

At higher concentration of soluble Dam1, small oligomers start to assemble spontaneously into single or stacked rings around microtubules [8,19]. Live observations via fluorescence microscopy revealed that Dam1 complexes large enough to form full rings diffuse very poorly [21–23], ruling out a biased-diffusion mechanism for their tracking of the depolymerizing microtubule tip. These non-diffusing complexes can be transported, albeit slowly, by the force developed by depolymerizing microtubules, while carrying loads of up to 30 pN [21,23]. Electron microscopy (EM) studies revealed that the dense circular core of the ring is separated from the microtubule wall by ∼6 nm gap [19,20]. This feature has been suggested to optimize force transduction by the ring, enabling it to capture the power strokes of bending protofilaments [28,29]. One Dam1 ring consists of 16–18 individual subunits [8,30–32], which is surprising because such rings encircle microtubules containing 13–14 tubulin protofilaments. The mismatch between the numbers of ring and tubulin subunits led to the proposal that Dam1 heterodecamers binds to microtubules via flexible regions and with no specific 'footprint' [8,31,33], potentially contributing to Dam1's ability to form assemblies with varying topologies.

Indeed, yet another Dam1 structure can be seen at saturating Dam1 concentration, when microtubules become completely covered with Dam1 complexes. Under these conditions, Dam1 heterodecamers assemble into double spirals. These left-handed antiparallel spirals wind together around the microtubule wall [8,20]. The spirals exhibit no tip-tracking, but instead exert stabilizing effects on microtubules, inhibiting their normal depolymerization. Furthermore, various higher order Dam1 structures are observed even in the absence of microtubules. For example, in the presence of lipid monolayers or at high Dam1 concentrations, large oligomers and 15-subunit rings can assemble on their own [19,32]. Also, Dam1 complexes from *Chaetomium thermophilum* (Ct–ΔTails–Dam1 complex) mutated to remove all flexible extensions can oligomerize in a high concentration of monovalent salts [34]. In the absence of microtubules, Ct–ΔTails–Dam1 complexes form 17-subunit rings stacked into tubes. Thus, as with tubulin dimers, which can polymerize into different structures depending on experimental conditions, Dam1 complexes have the ability to form multisubunit assemblies with various geometries. These findings illustrate the considerable structural plasticity of the Dam1 heterodecamer. This feature represents a profound challenge for *in vitro* analyses of the kinetics of oligomerization of Dam1 heterodecamers, as well as their dynamic interactions with microtubules and force-transducing characteristics. Linking these behaviours with specific Dam1 structural assemblies is an important goal for future research.

The Dam1 coupler in mitotic cells

The structure of Dam1 oligomers within cells is not known with certainty. Diffusing Dam1 spots observed on cytoplasmic and spindle microtubules are likely formed by small Dam1 oligomers [35,36]. Fluorescence correlation spectroscopy and quantitative fluorescence microscopy have revealed that the copy number of Dam1 complex per kinetochore is 16–20 in metaphase and 12 in anaphase [37,38]. These quantitative observations suggest that kinetochore-associated Dam1 can assemble one full ring, although the reduction in the number of Dam1 subunits in anaphase remains poorly understood. Others have proposed that the physiological kinetochore coupler is based on two rings bridged by the Ndc80 complexes [39]. This proposal, however, is contradicted by the estimated number of kinetochore-bound Dam1 subunits, and also by the results of *in vitro* studies suggesting that a single depolymerizing microtubule is not powerful enough to simultaneously pull four Dam1 rings (two per sister kinetochore) [23,29]. Electron tomography of high-pressure frozen budding yeast cells was used to directly visualize the budding yeast kinetochore, revealing partial ring structures at the ends of some spindle microtubules [40]. Recently, a cryo-EM study reported microtubule encircling rings with 17-fold symmetry, presumably formed by Dam1 complexes, but only two such complete structures were observed [41,42]. The remaining structures revealed by this method were partial rings, and some were located too far from the microtubule plus-ends to serve as kinetochore couplers. Future advances in imaging technology, in combination with Dam1 labeling, should provide greater insight into the physiological form of the Dam1 kinetochore coupler.

Together, studies in cells and *in vitro* biophysical assays inspired theoretical analyses of the underlying coupling mechanisms (reviewed in [25,29]). This line of inquiry recently received a significant boost from structural and biochemical studies of the Dam1 complex that greatly advanced our understanding of its molecular organization. The following sections review these advances and discuss remaining and emerging questions to be addressed in future research.

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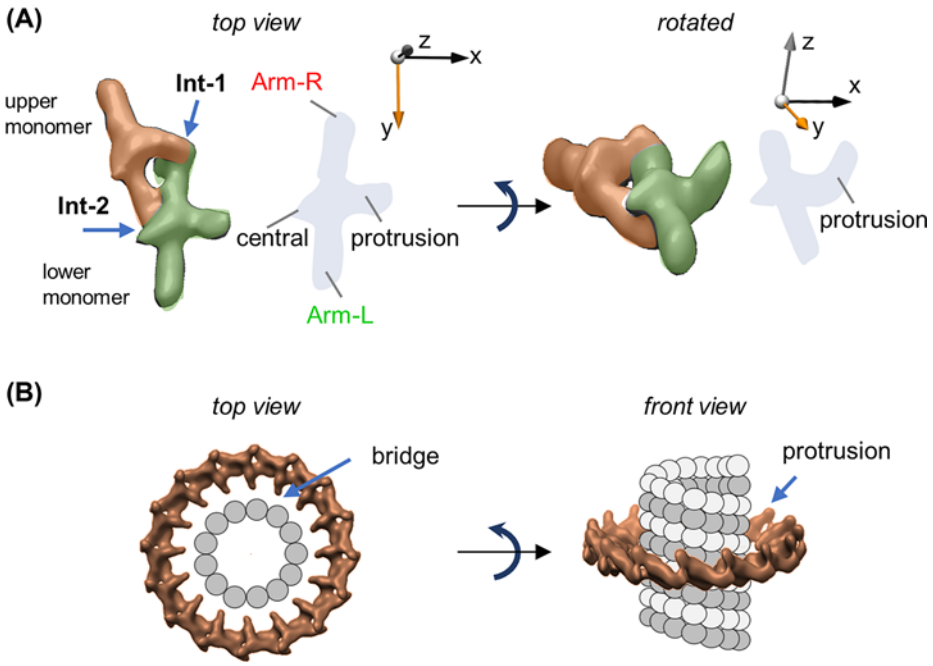

Figure 2. Low-resolution structures of Dam1 dimer, and the microtubule encircling Dam1 ring

(**A**) Three-dimensional reconstruction of Sc–Dam1 dimer (EMDB-ID:1372; [8]) with two monomers colored orange and green. Left cartoon (in gray) shows an approximate two-dimensional projection (2-D outline) of the green envelope in the reconstructed dimer. Cartoon on the right is an analogous 2-D outline generated from the dimer structure that was rotated at ∼70° around x-axis to visualize the protrusion domain. (**B**) Two views of the reconstructed Sc–Dam1 ring encircling a microtubule (EMDB-ID: 5254, [31]). Microtubules are represented by the cartoons of approximate scale. Two domains extend from the circular core of the ring: protrusions that are parallel to the tubulin protofilaments, and bridges that lie approximately in the plane of the ring and point at the microtubule wall.

Structural basis for Dam1 dimerization and ring formation

A series of negative-stain and low-resolution cryo-EM studies from the Nogales and Harrison labs laid the groundwork for an overall structural view of the individual Dam1 subunits and the ring [8,19,20,31,32]. Single-particle analysis of soluble Dam1 dimers, comprising two heterodecamers, revealed a complex branched configuration formed by two T-shaped monomers (Figure 2A). Each monomer is ∼160 Å in length, with two elongated bars merging in the middle. A protruding domain extends perpendicularly from the middle area of the monomer and bends at the distal end. Thus, in a three-dimensional XYZ coordinate system with the elongated bars lying in the xy plane, the distal part of the 'protrusion' domain aligns with the z-axis, as illustrated using projections of Dam1 monomer in two different views (Figure 2A). These elongated bars are called Arm I and Arm II [34]. Here, we will refer to them as Arm-L and Arm-R, respectively, to unambiguously define their orientation relative to the protrusion and to simplify analysis of Dam1 configurations in different ring structures in the following review sections. The short extension from the middle of the two bars on the other side of the protrusion is referred to as the central domain.

In the soluble Dam1 dimer, two monomers lay across each other such that the resultant structure has no symmetry (Figure 2A). The contacts between the monomers are formed by two interfaces, Int-1 and Int-2 [8]. Int-1 involves the base of the protrusion domain of the upper monomer, lying on top of Arm-R of the lower monomer. At the Int-2 interface, the tip of Arm-L of the upper monomer contacts the bottom of the central domain of the lower monomer. In a reconstruction of the Dam1 ring encircling the microtubule (Figure 2B), all dimers are arranged similarly: the protrusions of each monomer are parallel to the microtubule protofilaments and point in one direction [31,32], giving the ring a corona-like appearance. Because Dam1 monomers in the assembled Dam1 ring have the same interaction interfaces as in soluble dimers [32], the Dam1 dimers are unlikely to experience any major conformational change upon ring assembly and microtubule binding, although more subtle changes may be revealed by future high-resolution studies. However, the Dam1 ring assembled around the microtubule exhibits prominent 'bridges', which are absent from soluble dimers and appear to be enlargements of the central domains pointing toward the microtubule wall

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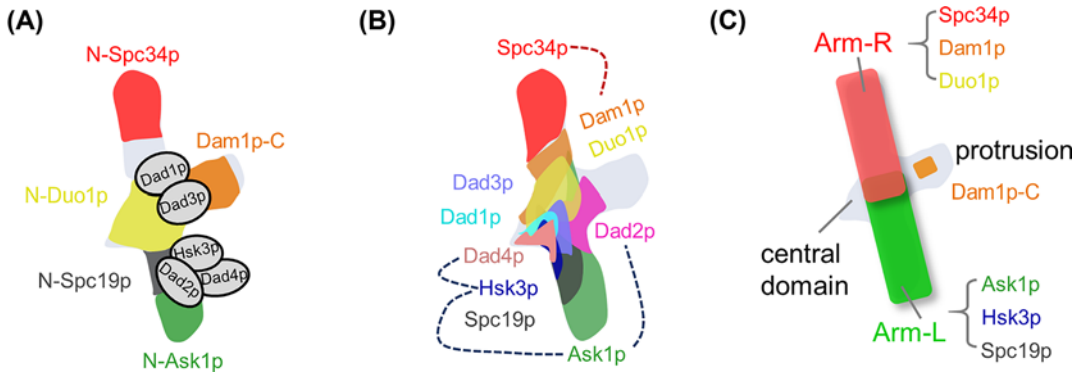

Figure 3. Locations of polypeptides, as determined by negative-stain EM and cross-linking studies using various Dam1 assemblies

(**A**) Schematic of proposed polypeptides locations within the complex, based on studies that used structural (colored subunits) and biochemical (gray ovals) approaches, see text for details. (**B**) Illustration of subunit organization based on the cross-linking model in [44]. Polypeptide interactions identified in [45] are shown as blue and red dotted lines. (**C**) Consensus model derived from approaches summarized in panels (A,B).

[8,32]. Rings formed by Dam1 complexes in which the 138 amino acids (AAs) of the C-terminal extension of Dam1p were deleted (from Q205 to R343), referred to as Dam1ΔC complex, exhibit reduced density in the bridge, suggesting that the bridge is formed in part by the Dam1p tail [8]. Consistent with this, biochemical and cross-linking studies revealed that the unstructured C-terminal tails of Dam1p and Duo1p polypeptides make a major contribution to microtubule binding [19,43–45]. Thus, binding to microtubule may trigger structural transitions within these tails, leading to formation of 'bridge' structures extending from the circular core of the ring to the microtubule wall. The mechanical properties of these bridges have been proposed to play important roles in ring's wobbling and force transduction at the shortening microtubule end [23,28]. Determining the molecular basis of microtubule binding by the ring, and the ring's ability to translocate on microtubules under force, represent important challenges for future research.

Fine structure of the core of Dam1 heterodecamer

Ramey et al. first probed subunit distribution within the Sc–Dam1 complex using maltose-binding protein fusions to N-termini of various polypeptides, which were subsequently visualized by negative-stain EM [32]. This led to the mapping of Spc34p, Duo1p, Spc19p, and Ask1p to the Arm domains, as shown in Figure 3A. Because the Dam1ΔC complex lacks some density in the protrusion domain, the Dam1p C-terminus was mapped to the protrusion [8,32]. The positions of other remaining polypeptides have been proposed based on known biochemical interactions (Figure 3A) [18,43,46]. Building on these results, Zelter et al. used cross-linking mass spectrometry to propose the locations of all ten polypeptides [44]. By taking into account the reported chemical cross-links, Dam1p, Duo1p, and Scp34p were mapped to Arm-R, and Ask1p, Spc19p, and Hsk3p to Arm-L; the central domain was crowded with multiple proteins (Figure 3B). Another study confirmed cross-linking between Spc34p and Dam1p, as well as within the protein network formed by Dad4p, Hsk3, Ask1p, and Dad2p [45]. Although there are considerable differences in the specific AA contacts identified by these cross-linking studies, the overall subunit distributions within the Dam1 complex were similar; together with the EM analyses these studies led to the consensus low-resolution model shown in Figure 3C.

Structural studies of the Dam1 complex entered the 'atomic age' when the cryo-EM structure of the Ct–ΔTails–Dam1 complex was determined with 4.5-Å resolution [34] (Figure 4A). Wild type Ct–Dam1 complex was truncated to remove all flexible extensions and assembled into tubes, which were subsequently locked with bifunctional cross-linkers in high-salt buffer. Using AA sequences of *S. cerevisiae* Dam1 components and the Swiss-Model software [47], we built a homology model of the Sc–ΔTails–Dam1 heterodecamer to facilitate comparison with the results of previous studies of budding yeast Dam1 complex (Figure 4B). This fully automated protein structure homology-modelling program used as a template the cryo-EM structure of the Ct–ΔTails–Dam1 heterodecamer (PDB: 6cfz, atom coordinate file) to generate a structural model for the Sc–ΔTails–Dam1 complex, which closely resembles the original cryo-EM structure.

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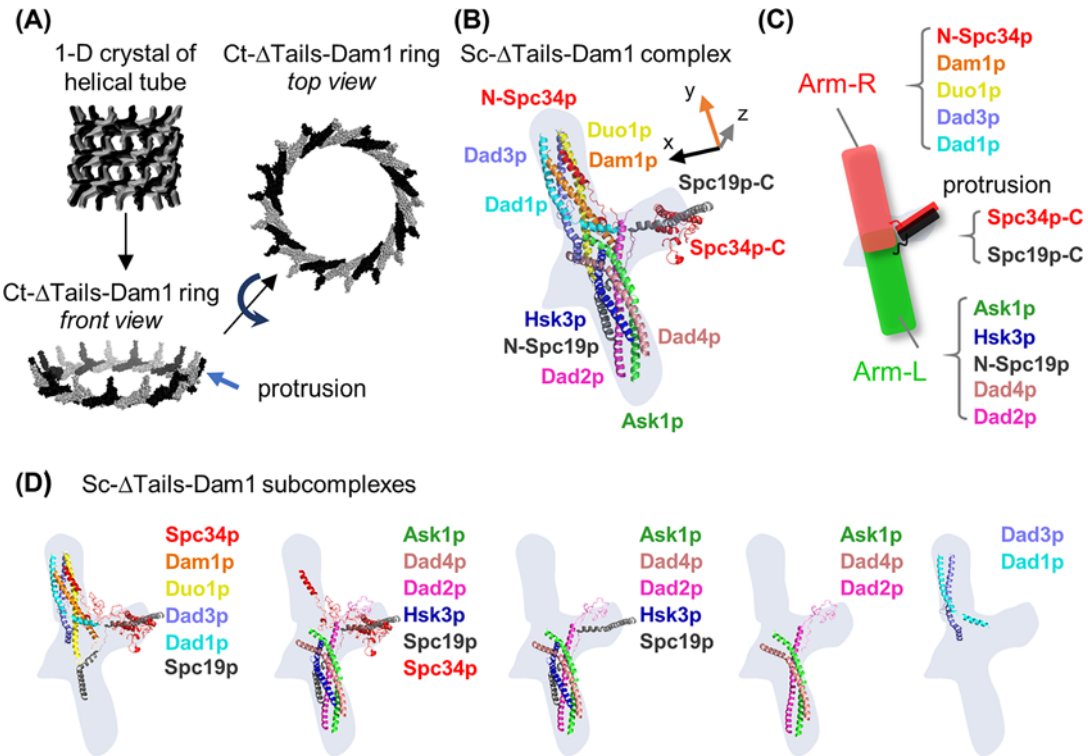

Figure 4. Cryo-EM structure of Dam1 complex

(**A**) Cartoon of the 1-D crystal formed by Ct–ΔTails–Dam1 complex lacking all flexible extensions (drawing is based on [34]), and two views of the Ct–ΔTails–Dam1 ring composed of 17 heterodecamers (PDB-ID: 6cfz). (**B**) Atomic homology model of the Sc–ΔTails–Dam1 ring complex lacking all flexible extensions, based on the corresponding structure of the Ct–ΔTails–Dam1 complex, see text for details. Cartoon in gray is the 2-D outline of the low-resolution reconstruction of Sc–Dam1 monomer, see Figure 2A. (**C**) Simplified schematic of protein components in different domains, as identified by high-resolution cryo-EM. (**D**) Polypeptides within the Sc–ΔTails–Dam1 atomic model that have been biochemically purified as stable subcomplexes and their approximate position relative to the 2-D monomer's outline.

The computer-generated structure matches the 2-D outline of the Sc–Dam1 monomer derived from earlier low-resolution studies (Figure 4B). The two arms of the main core of the atomic model are formed by parallel five-helix bundles, with the N-termini of the subunits at the distal ends of the arms. Within this intricate web, some helices span different parts of the T-shaped outline, preventing their assignment to only one domain. Nonetheless, the locations of subunits within the Arm domains, as identified by this approach, are highly consistent with previous assignments (compare Figures 4C and 3C). The α-helices of Ask1p, Dad2p, Dad4p, Hsk3p, and the N-terminus of Spc19p form one bundle that matches the composition of Arm-L, whereas the α-helices of Dam1p, Duo1p, Dad1p, Dad3p, and the N-terminus of Spc34p form the bundle of Arm-R. Interestingly, Dam1 complex has approximate internal two-fold symmetry: polypeptides forming Arm-L and Arm-R can be assigned to five pairs (Figure 1C), with some pairs representing structural paralogs with homologous sequences [15,34]. The two five-helix bundles cross each other in the middle of the heterodecamer but lack a clear central domain, presumably because this domain is formed by flexible regions that are absent from the Ct–ΔTails–Dam1 complex. The extensive contacts between helices belonging to specific protein subunits are consistent with the ability of these polypeptides to assemble stable subcomplexes, which were identified in prior biochemical studies [19,43] (Figure 4D).

Although the contacts between the Dam1 monomers at the interface Int-1 in 1-D crystals could not be resolved, our understanding of the atomic composition of Int-2 has improved dramatically. This interface is formed by a tight cluster of charged residues in Ask1p and Dad4p of the upper face of Arm-L and residues of Dad3p in the lower central domain [34]. Additionally, a hydrophobic patch of Dad4p in upper Arm-L contacts Dad3p in the lower central domain. Targeting these highly conserved residues will provide a powerful experimental tool for future studies of the mechanism of Dam1 ring assembly. Future work should also investigate the contributions of unstructured tails to Dam1 oligomerization, and how it is regulated by phosphorylation.

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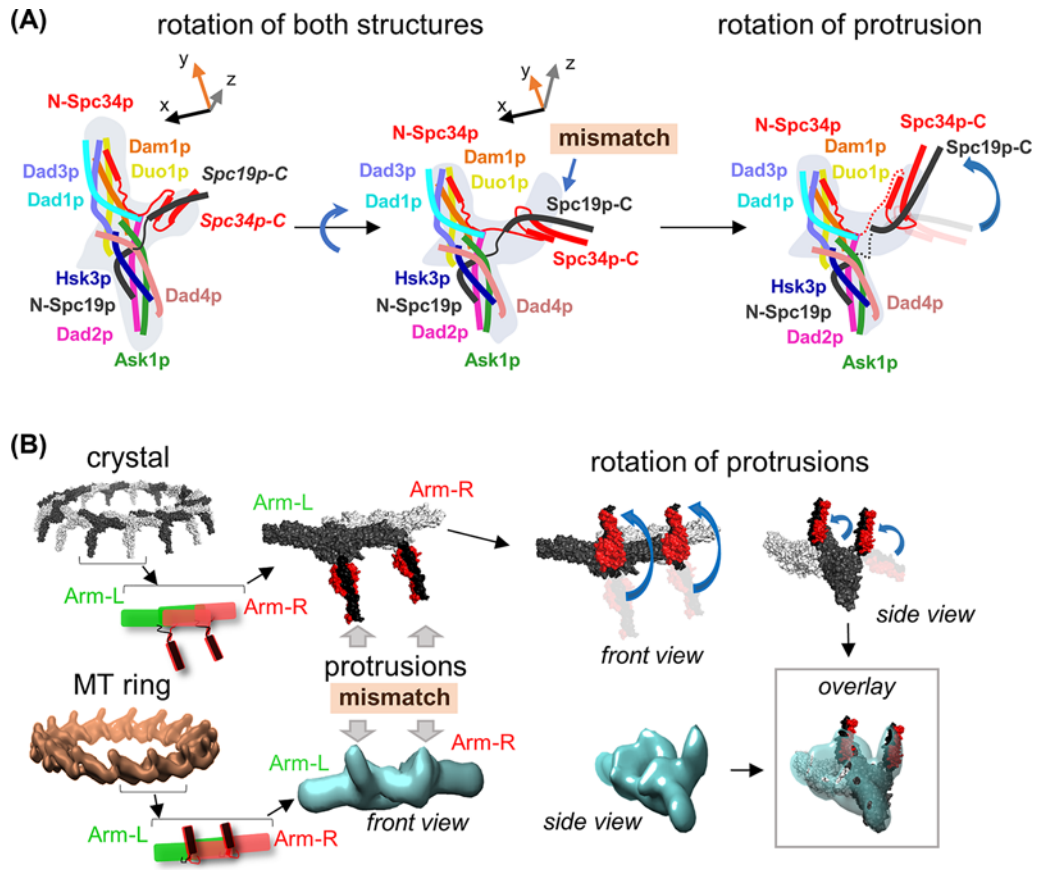

Figure 5. Comparison of the Dam1 heterodecamer and ring structures derived from different studies

This figure compares three-dimensional structures from the studies summarized in Figures 2 and 3 against structures based on a recent cryo-EM study by Jenni and Harrison (summarized in Figure 4). (**A**) Overlay of the atomic Sc–ΔTails–Dam1 model (colored components) and the 2-D outline (in gray) of a soluble Dam1 heterodecamer structure from [8]. Two views are shown to illustrate that the protrusion in the atomic structure does not fit within the 2-D outline. Blue arrows show rotation of these protrusions to improve the match. (**B**) Dam1 rings—from the 1-D crystal (upper, gray scale) and from the assembly around the microtubule (lower, orange)—oriented to match the polarity of the arms forming the circular core of these rings. Enlargements show dimers from the 1-D crystals (top row) and dimers reconstructed from the negatively stained soluble Dam1 complexes (lower row), which match closely the overall shape of dimers in the microtubule-encircling ring. The 2-D overlay of dimer models illustrates that the protrusion domains in rings recreated in these different studies are pointing in opposite directions.

Opposite orientation of the protrusion domains in different Dam1 structures

Although the Arm domains of the cryo-EM-based Sc–ΔTails–Dam1 model match well the elongated part of the soluble Dam1 complexes, these structures differ strikingly in the orientation of the protrusion domain (Figure 5A). Indeed, if the Arms of these two models are aligned to match the locations of individual polypeptides, the protrusion domain of the cryo-EM structure will point in the opposite direction relative to the earlier low-resolution model of the soluble Dam1 dimer. It is not possible to simultaneously match the relative position of the polypeptides within the Arms and the recently resolved protrusion domain within the 2-D outline by simply rotating the dimer's envelope or the atomic model. However, flipping the protrusion of the Sc–ΔTails–Dam1 model complex allows it to fit within the T-shaped 2-D outline while preserving the location of polypeptides within the Arms (Figure 5A).

An analogous rotational transformation is needed to reconcile the ring structures derived from these different studies. Indeed, both circular assemblies—the microtubule-encircling Dam1 ring from [31] and the ring found within the 1-D crystal [34]—have characteristic corona-like appearance. However, the orientation of the protrusions relative

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to the circular core and the oligomerization interfaces differ between these structures. This discrepancy is readily resolved by rotating the protrusion domains of the cryo-EM structure (Figure 5B).

The difference in the direction of the protrusions in Dam1 assemblies from different studies [8,31,32,34] could be explained in several ways. First, these Dam1 assemblies correspond to Dam1 protein complexes from different fungal species, so the differences may be species-specific. Second, the orientation of the protrusion in the Ct–ΔTails–Dam1 heterodecamer could be affected by the absence of all flexible extensions from this complex. Third, the inappropriate orientation of the protrusion in the Ct–ΔTails–Dam1 structure may have been imposed during the crystal formation or chemical fixation. Fourth, orientation of the protrusion may be affected by microtubule binding. The latter possibility seems less likely because protrusions in the Sc–Dam1 rings formed in the absence of microtubules retain the same orientation as seen in the rings formed around the microtubules [32]. It is worth noting that deletion of flexible extensions is likely to preclude Dam1 binding to microtubule [8,19,43–45], so it might be difficult to examine orientation of the protrusions in the Ct–ΔTails–Dam1 rings in the presence of microtubules.

Although it is currently not possible to discriminate between these possibilities, this intriguing topological difference highlights the deficits in our knowledge about the mechanical properties of the protrusion and the polypeptides that link it to the core. Cryo-EM reconstruction reveals that the protrusion domain, consisting of two winding helices of the C-terminal halves of Spc19p and Spc34p, is linked to the Arm domains by short disordered regions [34]. These links, which form the base of the protrusion, appear to be flexible enough to allow the protrusions to adopt opposite orientations in different Dam1 assemblies. The flexibility of the protrusions, which participate in forming the interaction interface Int-1 between Dam1 monomers, may explain the structural plasticity of Dam1 monomers and may contribute to their ability to adopt various angles within Dam1 rings with different numbers of subunits. On the other hand, all protrusions within the ring maintain same polarity, indicating that they are not entirely floppy but have some inherent rigidity. Future work should seek to characterize the mechanical properties of the protrusion domains and elucidate their functions in Dam1 complex assembly and translocation on microtubule walls.

Beyond the core structure: insights into the roles of the protrusion and Dam1p C-terminal extension

Although the corona-like appearance of the Dam1 ring was first reported more than a decade ago [8], the exact functions of these conspicuous protruding structures remain enigmatic. Previous structural studies have generated several insights about the ring protrusions.

First, the molecular organization of this domain strongly suggests that it contributes to heterodecamer assembly by what we call the 'shoelacing' mechanism (Figure 6A). Indeed, the protrusion is a coiled-coil of the C-terminal halves of Spc19p and Spc34p, whereas the N-terminal helices of these proteins are tightly interwoven with the four helices that form the robust bundles of Arm-L and -R, respectively (Figure 4B). Such an organization suggests an assembly pathway, in which two Arms, representing pseudo-dimers, become knotted together by the Spc34p and Spc19p 'shoelaces'. The partial interdigitation of the helices from the two Arms reinforces this arrangement, contributing to a strong and rigid core structure. The biochemical isolation of Dam1 subdomains that contain the protrusion and one of the Arms, but lack the second Arm, provide indirect support for this assembly pathway (Figure 4D).

Second, as described earlier in this review, the base of the protrusion domain participates in oligomerization of Dam1 monomers during ring assembly by contributing to interface Int-1 [8] (Figure 6A). Third, the protrusion domain could provide a structural platform for fastening the Dam1 ring to the kinetochore via the elongated Ndc80 complex, as well as acting as the connection site for other kinetochore proteins. The interaction with Ndc80 could be particularly important for inducing specific orientation for Dam1 ring at kinetochores [34]. On microtubules *in vitro*, one study suggested that the ring protrusion points toward microtubule plus-ends [44]. However, direct EM visualization of the orientation of the microtubule-encircling Dam1 rings revealed that ring protrusions are insensitive to microtubule polarity [8,31]. Given the profound structural differences between two faces of the ring [34], the orientation of the ring at the kinetochore is unlikely to be random (Figure 6B).

Several lines of evidence support a model in which Dam1 binding to kinetochore-associated Ndc80 promotes assembly of the Dam1 ring with the protrusion domains pointing toward the microtubule plus-ends (Figure 6B) [34]. First, such ring orientation is consistent with the locations of the proposed contact sites between the Ndc80 complex and Dam1 ring [39,49,50]. Although these complexes exhibit almost no interaction in solution [26,48], their binding is enhanced in the presence of microtubules [26,48]. Furthermore, cross-linking studies suggest interactions between the C-terminal tail of Dam1p and the Ndc80 region proximal to its globular head; between the C-terminal tail of Ask1p and the N-terminal part of the Ndc80 stalk (Figure 6B). Additionally, the C-terminus of Spc34p, which forms the protrusion, cross-links to the C-terminal part of Ndc80 stalk [39]. Second, cryo-EM tomography of kinetochore

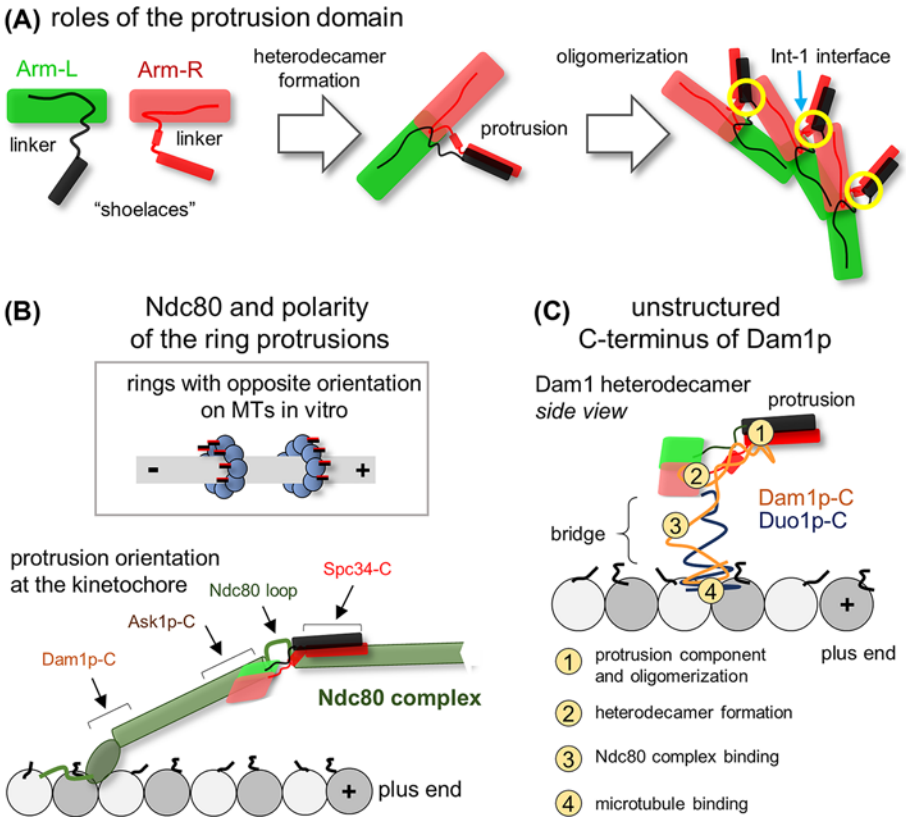

Figure 6. Functions of ring protrusions and Dam1p flexible extension

(**A**) Proposed functions of the protrusion domain in assembly and oligomerization of Dam1 heterodecamer. Elongated arms forming rigid connections are held together by polypeptides of the protrusion domain. During oligomerization, the protrusion of one Dam1 monomer binds to the Arm-R of another monomer (yellow circles). (**B**) Upper cartoon depicts two Dam1 rings with protrusions pointing in different directions [31]. Lower schematics shows a side view of one Dam1 heterodecamer tethered to the kinetochore by a Ndc80 complex. Arrows point to specific segments within the Ndc80 complex that have been implicated in interactions with Dam1 complex. (**C**) Multiple roles of the Dam1p C-terminal tail. Cartoon shows a side view of one Dam1 heterodecamer interacting with a microtubule via the flexible extensions of Dam1p and Duo1p polypeptides.

microtubules in budding yeast cells reported that Dam1 protrusions point toward the microtubule plus-end, although this conclusion is based on images of only two electron-dense ring-like structures [41]. Additionally, this relative orientation of the Dam1 and Ndc80 is supported by the results of fluorescence microscopy studies in yeast cells [51,52], and by the requirement of Ndc80 loop region (490–510) for Dam1–Ndc80 interaction in yeast two-hybrid assay [53]. The finding that the Dam1 and Ndc80 complexes are joined by multiple sites suggests that this macromolecular ensemble can mediate robust coupling between the microtubule end and kinetochore. Elucidating the roles of these interfaces in facilitating specific ring orientation and force transduction in cells and *in vitro* will provide critical insight into the mechanism of microtubule coupling by these macromolecular complexes.

Future work should also seek to characterize the molecular composition of the protrusion domain and determine whether it changes following binding of Dam1 complex to the microtubule. As pointed earlier in this review, the currently available atomic model of the Dam1 heterodecamer lacks flexible extensions for all polypeptides, decreasing the molecular weight of this complex by 50%. These missing unstructured tails may also provide important contributions to protrusion formation or function. Indeed, dimers of the Dam1ΔC heterodecamers lack density specifically in this domain [8], suggesting that a segment of the Dam1p C-tail may represent an integral part of the protrusion domain (Figures 3A and 6C) [32]. This idea is corroborated by the cross-links found between this tail and both Spc34p and Spc19p [44,45]. These results highlight the diverse and still enigmatic functions of the flexible extensions of Dam1p and other polypeptides of this macromolecular complex. In addition to forming part of the protrusion, the C-tail of Dam1p also participates in other processes, including subunit oligomerization. Indeed, Dam1ΔC heterodecamers are

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less able to assemble rings [8], although it is unclear whether the Dam1p C-tail directly contributes to the dimerization interface. The C-tail also makes an essential contribution to heterodecamer formation [19], and Ndc80 binding [39,50]. Finally, this extension is involved in microtubule binding, as confirmed by multiple studies [8,19,43–45].

This picture is further complicated by the reported interactions between flexible extensions from various polypeptides of Dam1 heterodecamer. For example, biochemical studies revealed that Duo1p directly interacts with Dam1p to form a structural unit [14,43]. Flexible C-tail of Duo1p contributes to subunit oligomerization and microtubule binding [43–45], but the molecular organization and the specific roles of the Dam1p and Duo1p C-terminal extensions within the bridge structure are not yet known. If all flexible extensions of the Dam1 heterodecamer have multiple functions, similarly to the Dam1p and Duo1p C-tails, precise elucidation of the structure, assembly, and function of this fascinating complex will pose a major challenge, which we are only beginning to address.

Conclusions

In dividing yeast cells, the heterodecameric Dam1 complex is a key component of the kinetochore-microtubule attachment. In recent years, great strides have been made in uncovering the structure of the Dam1 heterodecamer, advancing our understanding of the molecular underpinnings of Dam1 subunit organization, assembly of the microtubule encircling rings, and the interactions between Dam1 and the Ndc80 complexes. These discoveries led to an atomic-scale model of the circular core of the ring and revealed the important roles of the Dam1 ring protrusions and flexible tails. However, the mechanical properties of these various domains, as well as their connecting joints and extensions, remain unknown. Considerable further effort work is needed to elucidate the mechanism that regulates Ndc80-mediated Dam1 ring assembly and allows this sophisticated molecular ensemble to form a robust coupler that translocates bidirectionally along microtubules under force.

Summary

- Dam1 heterodecamers are capable of forming various oligomeric structures, depending on the concentration of soluble Dam1, the presence of microtubules, and the biochemical milieu.
- A combination of approaches, including high- and low-resolution structural and biochemical investigations, have yielded a cohesive atomic-scale model of polypeptide organization within the core of the Dam1 heterodecamer.
- Recent studies have highlighted the protrusion domain of the Dam1 complex, which exhibits consistent polarity within the circular assemblies but assumes opposite orientations in one-dimensional crystals vs. microtubule-encircling rings.
- The protrusion domain and flexible extensions of Dam1 heterodecamer remain to be fully explored. These structures appear to play crucial roles in heterodecamer formation, ring assembly, and interactions with microtubules and the Ndc80 complex.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

S.W. researched the literature, generated structural models, and prepared the figures. S.W. and E.L.G. critically analyzed structural models and wrote the manuscript.

Abbreviations

AA, amino acid; Ct–ΔTails–Dam1, *Chaetomium thermophilum* Dam1 complex with no flexible extensions; EM, electron microscopy; Sc–Dam1, *Saccharomyces cerevisiae* wild type Dam1 complex.

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