Kinetochore Structure: Pulling Answers from Yeast

Despite the identification of multiple kinetochore proteins, their structure and organization has remained unclear. New work uses electron microscopy to visualize isolated budding yeast kinetochore particles and reveal the kinetochore structure on microtubules.

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The kinetochore is a complex macromolecular structure that mediates chromosome–microtubule interactions to direct the dynamic process of chromosome segregation. Despite the discovery of a large number of kinetochore components, the basic structure of this complex remains unknown [1,2]. In this respect, the kinetochore structure has lagged significantly behind other large complexes, such as the ribosome [3] or the nuclear pore [4]. Indeed, the kinetochore is often depicted as a set of interconnected shapes that lack molecular or structural detail. A major limitation in studying kinetochore structure has been that, at least until recently, it was not possible to isolate kinetochore assemblies from cells. Defining the structure and organization of the kinetochore represents a major goal for understanding the mechanisms that ensure the faithful distribution of the genetic material. Now, new work by Gonen *et al.* [5] helps to bridge the gap between the structure of individual kinetochore components and a model for the entire kinetochore complex by directly visualizing isolated kinetochore particles.

The kinetochore connects the centromere of a chromosome to microtubules from the mitotic spindle. Early structural data from electron microscopy of vertebrate cells established a trilaminar model for the kinetochore [6]. An electron-dense region of the kinetochore near the chromosomal DNA was termed the 'inner kinetochore' and a second electron-dense region near the microtubule was termed the 'outer kinetochore'. These images have remained useful structural references while researchers elucidated the molecular composition of the kinetochore. However, the vertebrate kinetochore is an extremely large structure that connects to multiple different microtubule polymers, suggesting that this trilaminar arrangement is likely formed by multiple interlinked repeats of smaller underlying kinetochore units. In contrast, the budding yeast kinetochore is much smaller and thus simpler, connecting to just a single microtubule [7]. Visualizing this minimal structure would be an ideal way to define the organization of the kinetochore proteins that form the microtubule attachment. Unfortunately, due in part to its small size, it has not been possible to observe the kinetochore in intact yeast cells by electron microscopy [8].

Despite the differences in size between the vertebrate and budding yeast kinetochores, there are significant similarities in their molecular composition. The inner kinetochore is composed of several DNA-binding proteins, including the conserved histone variant CENP-A (Cse4 in budding yeast) and a set of sub-complexes termed the centromere-associated network (CCAN) [9]. These proteins provide a platform for the outer kinetochore, which contains the conserved microtubule-binding Ndc80 complex and additional associated proteins, including KNL1 and the Mis12 complex, which together form the KMN network. In yeast, the Dam1 complex contributes an additional microtubule-binding element. High-resolution fluorescence imaging has established the general distribution of these many proteins within the kinetochore [10,11], as well as provided details on their relative stoichiometries [12]. These studies have indicated that, although kinetochore size varies, the number of protein molecules per bound microtubule remains conserved between yeast and vertebrates.

To achieve chromosome segregation, the kinetochore must couple the force of microtubule polymerization and depolymerization to chromosome movement. How this is achieved is a major question that is closely linked to the mysteries of kinetochore structure. There are two major, non-exclusive models for how a kinetochore remains attached to a microtubule during mitosis [13]. The first model, termed biased diffusion, suggests that multiple weak attachments from the kinetochore form a moving interface with a depolymerizing microtubule. This model necessitates a multivalent attachment, a role potentially performed by the Ndc80 complex. The second model, termed 'forced walk', suggests that the curling protofilaments resulting from microtubule depolymerization displace a more tightly associated microtubule-binding protein down the microtubule lattice, allowing the kinetochore to capture the force from microtubule depolymerization. A ring-like structure, such as has been identified *in vitro* for the budding yeast Dam1 complex [14], could act in this way to remain attached even as a microtubule shrinks. The budding yeast kinetochore is unique in that it binds to only one microtubule per centromere, whereas human kinetochores bind to ~15–20 microtubules [15]. This places a unique challenge on the budding yeast kinetochore because it cannot disassociate from this lone microtubule without chromosome loss. The strict requirement for Dam1 in budding yeast and its lack of conservation in higher eukaryotes may reflect this important functional difference [16].

A key challenge in the kinetochore field has been to analyze kinetochore function biochemically. One approach for such studies has been a 'bottom up' approach to reconstitute individual kinetochore proteins and complexes to analyze their associations and activities. In an elegant alternative approach to isolate intact kinetochore particles, the Biggins lab previously developed one-step purifications to pull out the structure from budding yeast cells. For these studies, they utilized FLAG-tagged Dsn1, a member of the Mis12 complex that bridges the inner and outer kinetochore, as a 'handle' [17]. When Dsn1 is affinity purified under low stringency conditions, the majority

of the defined components of the budding yeast kinetochore co-purify. The choice of lower salt conditions was extremely important, as higher stringency purifications have been shown to isolate individual sub-complexes rather than the entire kinetochore.

These isolated kinetochore particles have provided a powerful tool for a range of studies on kinetochore function. In addition to defining kinetochore components [17], the Biggins lab has previously collaborated with the Asbury lab to analyze the microtubule-binding behavior of these kinetochore particles [18]. They found that the particles displayed many properties of native kinetochores, including microtubule-binding activity and the ability to track with depolymerizing microtubule ends. The particles also exhibited an intriguing catch-bond property in the presence of force, in which the connection with microtubules was enhanced with increased force.

Now, the Biggins group has collaborated with the Gonen lab to directly visualize the kinetochore particles by electron microscopy [5]. This work revealed that these particles are 126 nm in diameter with a dense central domain and globular outer domains. In the presence of increased salt, these particles appeared more extended, which could indicate conformational flexibility of the proteins. The number of globular outer domains present in each particle ranged from five to seven. The authors hypothesized that each of these globular domains represents a KMN network unit. This result suggests that a component of the particle's central hub dictates the stoichiometry and organization of the particle. This organizational player could be an oligomeric protein or a DNA-based structure.

When the authors mixed the isolated kinetochore particles with stabilized microtubules, they observed structures decorating the microtubules. As predicted by the earlier biophysical work, the particles were capable of associating with microtubules in both side-on and end-on configurations. When they observed side-on attachments, they were able to visualize a structure that appeared to be an extended Ndc80 complex gripping the microtubule. Most strikingly, when they observed end-on
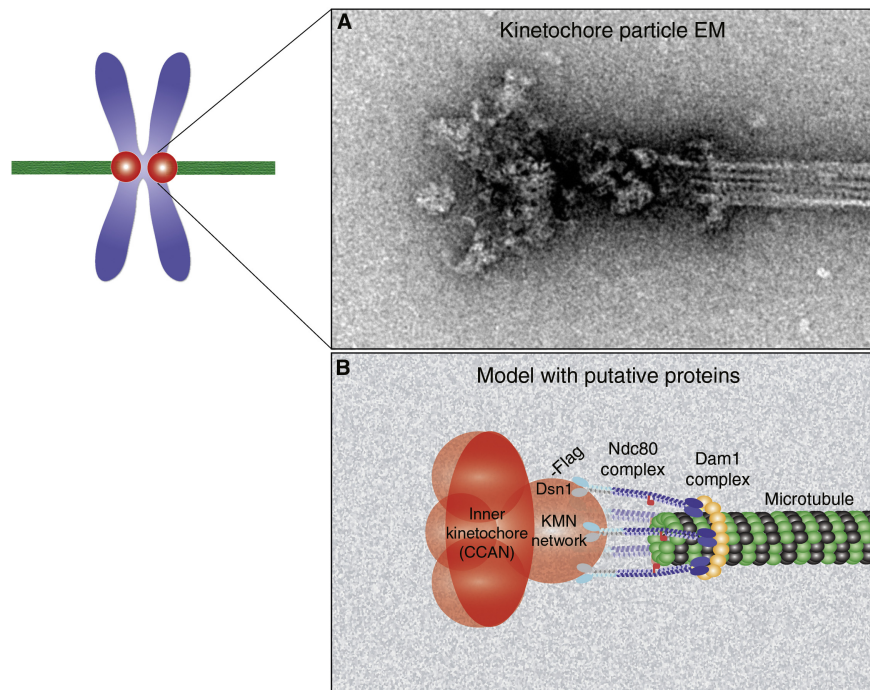
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Figure 1. Electron microscopy of isolated kinetochore particles.

(A) Electron microscopy image of an isolated kinetochore particle with an end-on attachment to a microtubule. The particle is composed of a central density with surrounding globular structures. A clear ring around the microtubule is connected to the kinetochore particle through elongated structures. (Image provided by Tamir Gonen and reproduced with permission from [5].) (B) A model of the predicted proteins that correspond to the visualized EM density. Gonen *et al*. [5] propose that the globular domains represent KMN network components (red spheres). The central density (red oval) likely contains inner kinetochore components. The extended molecules that contact the microtubule are proposed to be the Ndc80 complex. Finally, the ring is represented as an oligomer of the Dam1 complex.

attachments, they also observed a ring around the microtubule that likely corresponds to the Dam1 complex (Figure 1). This ring was attached to the main particle by elongated, Ndc80-like structures. Importantly, the ring was observed even though the Dam1 complex was not present at stoichiometric concentrations in the kinetochore affinity purifications. This result supports the model that Dam1 ring formation is a cooperative process aided by both microtubules and the Ndc80 complex [19,20]. Gonen *et al.* [5] also made tomographic reconstructions that show that the isolated kinetochore assembly encircles the microtubule and that the ring is contacted at regular intervals by protrusions from the kinetochore particle. Finally, deletion of key subunits from the Dam1 and Ndc80 complex almost completely abolished rings and attachment, respectively. Although this is not a definitive

identification of the complexes, these results are consistent with current models.

The work from Gonen *et al.* [5] presents thought-provoking images and enhances our knowledge of kinetochore structure. The observations of an oligomeric structure, the multivalent attachment site with microtubules, and the formation of a ring for end-on attachments all support models of kinetochore function. Specific identification of the proteins within this structure is an important next step for this work. Protein labels, specific truncations, and mutations will be interesting additions to the experiment. This work opens the question of whether the kinetochore in higher eukaryotes will emerge as a tessellation of the yeast kinetochore, or whether the structure will be fundamentally different. Collectively, these experiments provide a face for

the yeast kinetochore and a foundation for future studies.

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Programmed Cell Death: A New Way Worms Get Rid of Unwanted Cells

The genetics and predictable cell death lineages in *Caenorhabditis elegans* have been critical for identifying a conserved apoptosis pathway. Yet, cells still die in mutants that disrupt this pathway. A recent study shows that this death occurs by cell shedding.

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The simplified genetics of *Caenorhabditis elegans* was critical for elucidating the programmed cell death pathway conserved throughout most species [1–3]. Because these worms have a streamlined version of the conserved genetic pathway required for apoptosis, it had confused researchers for some time that, in certain instances, the apoptotic pathway is redundant [1]. A recent paper from Denning, Hatch, and Horvitz [4] demonstrates that cell shedding can compensate for the death pathway in a number of cells targeted to die by developmental programmed cell death. A number of *C. elegans* mutants in which apoptosis is inhibited at different stages of the pathway shed cells that then eventually die by a caspase-independent type of apoptosis.

One feature of *C. elegans* development that is critical for defining the apoptotic pathway is the ability to precisely predict which cells die, making it easy to identify those that do not. To investigate what controls cell shedding, the authors screened various mutants engineered to express GFP in cells that consistently shed when cell death is inhibited (i.e. in worms lacking the caspase CED-3). In cases where these GFP-positive cells do not shed, they instead divide, producing two GFP-positive cells. Thus, by screening for mutations that produced two GFP-positive cells, they found that cell shedding requires the genes PIG-1, a serine–threonine kinase related to AMP-activated kinase, and a complex that phosphorylates PIG-1, composed of LKB1, STADα and MO25α. By finding genes required for shedding, they discovered that the apoptotic and shedding pathways act redundantly. GFP-labeled cells would still die by programmed cell death in single *ced-3* or *pig-1* mutants, but in a double mutant lacking both the apoptotic and shedding pathways these cells instead divide and produce two cells of the same fate — in the example they studied, an excretory cell (Figure 1).

Although these findings suggest that shedding can compensate to promote cell death in cases where the apoptotic pathway is blocked, another possibility is that normally these cells can both die and be shed. Because *C. elegans* has highly efficient phagocytosis mechanisms, shed cells that are also targeted for cell death may be engulfed so rapidly that they are not apparent. Indeed, mutations in engulfment genes also produce 'floaters', suggesting that typically cells are shed but become engulfed so rapidly that they are not noticeable. Blocking the apoptotic pathway reveals shed cells because