Chromosome Segregation: Keeping Kinetochores in the Loop

The Ndc80 complex is a key component of the kinetochore–microtubule interface. Two studies now demonstrate that a conserved loop region within the extended coiled-coil of Ndc80 plays an unexpected role in recruiting proteins to the kinetochore.

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Chromosome segregation in eukaryotes requires that the macromolecular kinetochore complex, built on a foundation of centromeric chromatin, interacts with the microtubules from the mitotic spindle [1]. The four protein Ndc80 complex is a component of the conserved microtubule-binding KNL-1/Mis12 complex/Ndc80 complex (KMN) network, which is a key player in the kinetochore–microtubule interface. The Ndc80 complex is essential for the formation of kinetochore–microtubule interactions by binding to microtubules directly [2]. The Ndc80 complex also plays other important roles in kinetochore function in addition to binding to microtubules, including the recruitment of components of the spindle assembly checkpoint [3].

Previous structural analyses have revealed that the Ndc80 complex is a rod-shaped molecule with an extended coiled-coil domain spanning ~55 nm with globular domains at either end of the coiled coil [2,4,5]. The binding of Ndc80 to microtubules requires its calponin homology (CH) domain and its positively charged amino-terminal tail, which directly contact microtubules and contain key residues involved in the correction of erroneous kinetochore–microtubule interactions [2,6,7].

Previous structure–function analyses have focused primarily on the role of these two regions and have assumed that the extended coiled-coil region functions exclusively as a linker between the inner and outer kinetochore.

Interestingly, the Ndc80 coiled-coil is not uniform but instead contains a ‘kink’ in its structure whose location coincides with a conserved ‘loop’ inserted into the coiled-coil domain [5,8] (Figure 1A). Despite this conserved structural feature within the coiled-coil, no specific function had been associated with the loop region of Ndc80. Now, two papers analyzing the fungal Ndc80 complex in this issue of Current Biology [9,10] have defined protein–protein interactions involving this loop that are critical for proper kinetochore–microtubule attachments.

To generate an interface with microtubules, Ndc80 does not function in isolation. Recent studies have demonstrated that the budding yeast Dam1 complex, which also binds to microtubules, associates with the microtubule-bound Ndc80 complex to generate a synergistic microtubule-binding and plus-end tracking unit [11,12]. However, how two distinct microtubule-binding activities could be coordinated in the context of microtubules was unclear. Importantly, although Ndc80 is present in all eukaryotes, Dam1 homologues have thus far only been identified in fungi.

Since the presence of the Ndc80 loop region is also conserved, its function has likely diverged in higher eukaryotes. Work from the Tanaka and Toda labs [9,10] now demonstrates that the loop region of Ndc80 is required to recruit the Dam1 complex in Saccharomyces cerevisiae and the XMAP215/Stu2 homologue Dis1 in Schizosaccharomyces pombe, respectively.

To analyze the role of the Ndc80 loop, Maure and colleagues [9] took a targeted approach to generate mutations that specifically disrupt the loop region of Ndc80 in budding yeast. These mutants retain kinetochore targeting of the Ndc80 complex, indicating that the Ndc80 complex remains intact, but are temperature sensitive for growth. Phenotypic analysis of these mutants revealed that chromosomes fail to achieve bi-orientation and only infrequently form end-on kinetochore–microtubule interactions in a chromosome recapture assay, phenotypes reminiscent of defects in the Dam1 complex [13]. Based on the phenotypic resemblance between the ndc80 loop mutants and loss of function mutations in the Dam1 complex, Maure et al. [9] tested whether there is a loop-dependent interaction between Ndc80 and the Dam1 complex. Indeed, the Ndc80–Dam1 interaction is eliminated in the loop mutants based on yeast two-hybrid analysis, and Dam1 less efficiently associated with kinetochores in cells expressing the Ndc80 loop mutants based on localization and chromatin immunoprecipitation (ChIP) experiments. However, the capacity of the Dam1 complex to recruit the Ndc80 complex to microtubules in vitro is unchanged in the ndc80 loop mutants, suggesting that the interaction between the Ndc80 loop-region and the Dam1 complex in the context of microtubules may involve more than the tested loop region. In total, Maure et al. [9] conclude that the loop region of Ndc80 is required for the formation of stable end-on kinetochore–microtubule interactions via the recruitment of the Dam1 complex to kinetochores.

Taking a parallel approach in fission yeast, Hsu and Toda [10] identified mutations in the loop of Ndc80 by random mutagenesis followed by a screen for defects in spindle assembly. Mutations in the S. pombe Ndc80 loop lead to an unstable spindle phenotype, in which kinetochore microtubules are formed inefficiently and hence stable kinetochore–microtubule interactions are never established. The authors demonstrate that this phenotype can be suppressed by overexpression of Dis1, the S. pombe homologue of the microtubule polymerase XMAP215/Stu2. Dis1 localizes to kinetochores and is required for proper
microtubule dynamics but has not been implicated in mediating kinetochore microtubule attachments directly [14,15]. Consistent with a loop-dependent recruitment of Dis1, the localization of Dis1 to kinetochores is lost when the Ndc80 loop is mutated. In addition, based on several distinct approaches, Hsu et al. [10] demonstrate Ndc80 and Dis1 interact directly in a manner that is dependent on the loop region of Ndc80. Interestingly, expression of an Ndc80 mutant lacking the amino-terminal 95 amino acids, which are crucial for the Ndc80–microtubule interaction, was able to partially rescue the loop mutant phenotype, demonstrating a separation of function between the loop region and the microtubule-binding activity of Ndc80. Strikingly, artificially localizing Dis1 to kinetochores by tethering it to the Ndc80 complex component Nuf2 was able to partially suppress the phenotypes associated with the loss of Ndc80 loop function, indicating that recruitment of Dis1 to the kinetochore is an important function of the Ndc80 loop in fission yeast. In total, Hsu et al. [10] propose a model in which Ndc80 recruits Dis1 to kinetochores via its loop region to facilitate proper spindle formation.

Based on these two studies, it is now clear that the conserved loop region of Ndc80 plays a key role in controlling spindle dynamics in fission yeast and the formation of proper end-on kinetochore–microtubule interactions in budding yeast. Due to its elongated shape, the Ndc80 complex can associate with microtubules laterally, while spanning the dynamic end of the microtubule. A protein–protein interaction domain located within the coiled-coil stalk would enable Ndc80 to specifically recruit other proteins into close proximity with the microtubule plus-end (Figure 1B). Although the presence of this loop appears to be conserved throughout eukaryotes, the protein activity recruited by Ndc80 may depend on the specific requirements posed by the kinetochore architecture of each species. It is striking that the conserved loop region of Ndc80 has such different apparent functions in S. cerevisiae and S. pombe, raising important questions about their differential requirements for kinetochore–microtubule interactions. In addition, while homologues of the Dam1 complex have not been found in metazoans, the loop region of Ndc80 is conserved throughout eukaryotes. Since the Dam1 complex is present, but non-essential in fission yeast [16], it is tempting to speculate that fission yeast might be an evolutionary intermediate and the divergent function of the Ndc80 loop in this species reflects an adaptation to the requirements of multiple microtubule-binding sites at a single kinetochore (budding yeast kinetochores, in contrast, interact with a single microtubule). Future work to define the contribution of the Ndc80

![Figure 1. The conserved loop in the Ndc80 complex coiled-coil provides a platform for protein interactions.](image-url)
loop region in other species will be important to understand how kinetochore architecture is tailored to the functional requirements of kinetochore–microtubule attachment in different organisms. In total, these studies provide significant insight into how the multiple microtubule-binding activities at a kinetochore are integrated to facilitate kinetochore–microtubule interactions.

References

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Cell Evolution: Gene Transfer Agents and the Origin of Mitochondria

Recently, α-proteobacteria have been shown to possess virus-like gene transfer agents that facilitate high frequency gene transfer in natural environments between distantly related lineages. This system could have driven the genomic integration of the mitochondrial progenitor and its proto-eukaryote host and contributed to the evolutionary mosaic of genes seen in modern-day prokaryotic and eukaryotic genomes.

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Understanding how eukaryotes and their mitochondria evolved is an important unsolved problem in evolutionary biology. If, as textbooks now tell us, mitochondria evolved from bacterial endosymbionts belonging to the α-proteobacteria, how is it that present-day nuclear genomes have come to possess genes from seemingly every corner of the bacterial world [1–3]? A recent paper by McDaniel et al. [4] published in Science has provided a potentially important piece of the puzzle. The authors show that α-proteobacterial ‘gene transfer agents’ drive an extremely high rate of genetic exchange in nature. This discovery has implications for understanding the ancestry of α-proteobacterial, mitochondrial, and nuclear genomes.

Debate continues over the extent to which horizontal gene transfer (HGT) plays a role in the evolution of microbes and their genomes. One view is that HGT is so pervasive, especially among prokaryotes, as to render too few evolutionary characters to accurately classify life into one bifurcating phylogenetic tree and a unified taxonomic hierarchy [3,5,6]. In short, there is no tree of life, just a web of gene ancestries. In contrast, others have argued that careful targeting of specific gene markers combined with sophisticated phylogenetic methods can identify a skeleton tree of life, upon which hangs an extensive web of gene transfers [7,8].

An exciting development has been the discovery that α-proteobacteria possess a virus-like gene transfer agent (GTA) that produces small tailed phages [9] and packages and transfers ~4.5 kilobase fragments of genomic DNA [10]. The GTA system was first characterized in the α-proteobacterium Rhodobacter capsulatus [11] and different GTA systems have since been identified in diverse prokaryotes, including the δ-proteobacterium Desulfovibrio desulfuricans, the spirochete Brachyspira hydysenteriae, and the archaeon Methanococcus voltae [10]. However, little is known about the function and taxonomic distribution of these different systems or their prevalence in natural environments [10]. The α-proteobacterial R. capsulatus-type GTA system differs from other viral systems in two significant ways: firstly, it seems to function only in genomic DNA transfer and appears incapable of transferring enough genetic material to encode its own protein components