A new piece in the kinetochore jigsaw puzzle

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In eukaryotic cell division, the kinetochore mediates chromosome attachment to spindle microtubules and acts as a scaffold for signaling pathways, ensuring the accuracy of chromosome segregation. The architecture of the kinetochore underlies its function in mitosis. In this issue, Hornung et al. (2014. J. Cell Biol. http://dx.doi.org/201403081) identify an unexpected linkage between the inner and outer regions of the kinetochore in budding yeast that suggests a new model for the construction of this interface.

Kinetochore are enormous multiprotein complexes built on centromeric chromatin that link chromosomes to spindle microtubules and serve as hubs for signaling pathways that prevent incorrect chromosome segregation. Based on early electron microscopy, the kinetochore has been partitioned into the inner kinetochore, which includes chromatin-proximal components, and the outer kinetochore, which harbors microtubule-binding and signaling activities. As proteomic and functional screens have by now identified most, if not all, kinetochore components, the next major challenge is to solve the puzzle of the kinetochore’s higher-order architecture and connect this understanding to its essential mechanical and checkpoint signaling functions. An important step toward this long-term goal involves the reconstitution of kinetochore subcomplexes, the definition of their interactions with one another, and the identification of specific activities that reside within them. There has been much recent progress on this front, but many important questions remain and new mysteries continue to emerge. In this issue, Hornung et al. use a reconstitution approach with the budding yeast kinetochore to reveal an unexpected link between the chromatin-proximal inner kinetochore and the microtubule-binding outer kinetochore that stimulates a rethinking of current views of this interface.

In the majority of eukaryotes, kinetochore are specified by and built on a foundation of specialized nucleosomes in which histone H3 is replaced by a variant called CENP-A (for centromere protein A). Recent biochemical and structural studies have elucidated a conserved mechanism for CENP-A nucleosome recognition by the inner kinetochore protein CENP-C (known as Mif2 in budding yeast; Carroll et al., 2010; Kato et al., 2013). CENP-C, in turn, binds to the Mis12 complex (Mtw1 complex in budding yeast), which acts as a hub for interactions linking the inner and outer kinetochore (Maskell et al., 2010; Petrovic et al., 2010; Przewloka et al., 2011; Screpani et al., 2011). Specifically, the Mis12 complex associates with the microtubule-binding Ndc80 complex, now accepted to be the key component of the dynamic microtubule-coupling interface, as well as with Knl1 (Spcl105 in budding yeast), which recruits signaling molecules that ensure accuracy of segregation (Fig. 1).

Although significant strides have been made toward understanding the architecture of the outer kinetochore Knl1–Mis12–Ndc80 (KMN) complex, there have remained considerable questions regarding the functions and importance of the alpha-synthetase辅酶 complex of inner kinetochore components, referred to as the constitutive centromere-associated network (CCAN; CENP-C, -H, -I, -K, -L, -M, -N, -O, -P, -Q, -R, -S, -T, -U, -V, -W, and -X; for a detailed review see Perpelescu and Fukagawa, 2011). Among the CCAN proteins, CENP-C is the most widely conserved and best understood. Of the others, CENP-T (along with its binding partners CENP-W, -S, and -X) has been shown to bind directly to both DNA (Nishino et al., 2012) and to the outer kinetochore Ndc80 complex (Malvezzi et al., 2013; Nishino et al., 2013), leading to a model in which the CENP-T and CENP-C–Mis12 complexes provide two distinct “hands” for holding Ndc80 complexes at the kinetochore (Gascoigne et al., 2011; Hori et al., 2013; for an alternative view challenging this “two hands” model, see Carroll et al., 2010; Basilico et al., 2014). However, although CENP-T is important for kinetochore assembly in vertebrate cells, its budding yeast orthologue Cnn1 is not essential and its deletion does not affect the amount of Ndc80 recruited to the kinetochore (Bock et al., 2012).

Aside from CENP-C and the CENP-T complex, relatively few CCAN components have been characterized to date in depth. In budding yeast, Ame1/CENP-U and its binding partner Otk1/CENP-Q have long been known to be essential for viability (Ortiz et al., 1999), in contrast to many other CCAN components, including Cnn1/CENP-T. Early work on the Ame1 orthologue CENP-U and its associated CENP-O/P/Q/R complex in chicken DT40 cells indicated that this protein set was dispensable for kinetochore assembly and viability in vertebrate cells (Hori et al., 2008). However, recent work has shown that CENP-U is required for the viability of mouse embryos and...
In this issue, Hornung et al. (2014) build on a prior observation (Hornung et al., 2011) to provide compelling evidence in budding yeast for a new inner–outer kinetochore linkage involving Ame1/CENP-U (Fig. 1). Starting with mass spectrometry–based evidence that Ame1 associates closely with other CCAN proteins as well as members of the KMN complex, they show that a reconstituted Ame1–Okp1 complex binds directly to the reconstituted yeast Mis12 complex in vitro. Moreover, this binding is mediated by a short motif in the N terminus of Ame1, which by itself is sufficient for the interaction (Fig. 1). Importantly, mutations in the Ame1 motif that eliminate the interaction with the Mis12 complex in vitro are lethal in vivo. Using functional assays in conjunction with the “anchor-away” method that enables rapidly inducible inactivation of essential nuclear proteins by driving their nuclear export (Haruki et al., 2008), the authors show that this lethality is caused by chromosome missegregation. This result, combined with the observation of a severe outer kinetochore assembly defect, led to the conclusion that the Ame1–Mis12 complex interaction is critical for inner–outer kinetochore linkage in budding yeast. Intriguingly, Ame1–Okp1 not only provides a direct attachment site for the Mis12 complex but also associates directly with Mif2/CENP-C, as well as with DNA, in vitro (Fig. 1, arrows with broken lines). However, these other properties have not yet been analyzed to the same depth as the Mis12 complex interaction, leaving open the question of their functional contributions.

Although Hornung et al. (2014) provide compelling biochemical evidence for a new linkage between the inner and outer kinetochore mediated by Ame1/CENP-U, their in vivo analysis suggests additional and as yet unexplained complexity. For example, deletion of the N-terminal motif of Ame1 required for interaction with the Mis12 complex not only reduces Mis12 localization at centromeres but also reduces Ame1 localization equivalently. This observation suggests interdependence in the assembly pathway, with Ame1 recruiting the Mis12 complex and the Mis12 complex in turn stabilizing Ame1. It is also possible that the motif deletion has a second effect, such as alteration of Ame1–Okp1 DNA binding activity. Although these issues will likely be addressed in future work, the most pressing question emerging from the current study is whether the new connection defined in budding yeast is relevant in other species. Primary sequence conservation of the Ame1 motif that binds to the Mis12 complex is restricted to fungi. However, Hornung et al. (2014) note that the N termini of vertebrate CENP-U orthologues contain similar predicted secondary structure to the fungal proteins. This, together with the recent finding that CENP-U is essential in mouse embryonic stem cells and in embryonic development (Kagawa et al., 2014), suggests that discounting a role for CENP-U in vertebrate kinetochore assembly may have been premature. As human kinetochore complexes continue to be reconstituted (e.g., Nishino et al., 2012; Basilico et al., 2014; Petrovic et al., 2014), a straightforward approach will be to follow the lead of Hornung et al. (2014) and analyze potential interactions with purified components. In addition, the availability of a conditional knockout in mice should enable analysis of outer kinetochore assembly in the absence of CENP-U, either on its own or in combination with removal of other CCAN components. The knockout will also facilitate characterization of the fascinating context dependence of the essentiality of CENP-U in vertebrates (Kagawa et al., 2014).

In the ongoing quest to complete the kinetochore jigsaw puzzle, Hornung et al. (2014) have placed a new and unanticipated piece. Their work highlights the continued importance of reconstitution approaches as a complement to genetic and cell biological analysis of chromosome segregation.

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