A new piece in the kinetochore jigsaw puzzle

Kevin D. Corbett[1,2] and Arshad Desai[1,2]

[1]Ludwig Institute for Cancer Research and [2]Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093

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.

Kinetochores 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, kinetochores 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, 2014; Przewloka et al., 2011; Screpanti 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 (Spc105 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 alphabet soup 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 Okp1/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

Correspondence to Arshad Desai: abdesai@ucsd.edu

The Rockefeller University Press
J. Cell Biol. Vol. 206 No. 4   457–459
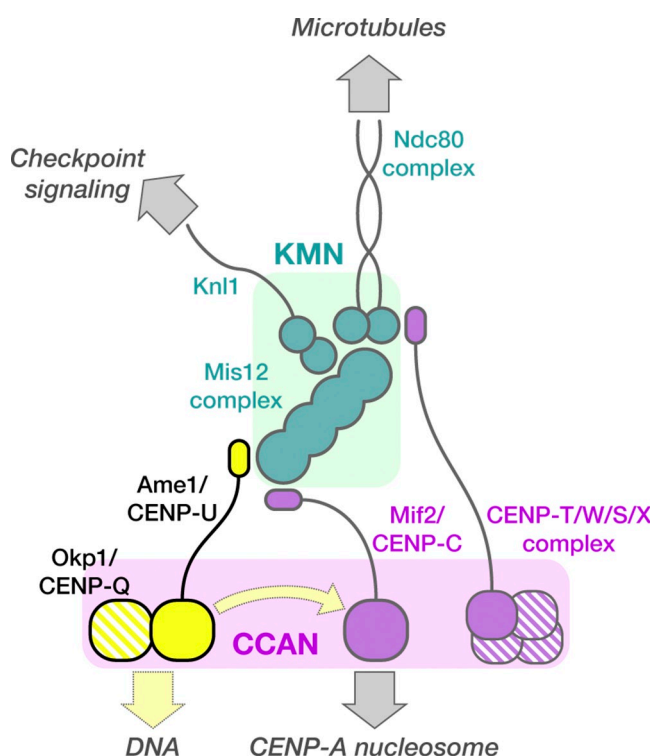www.jcb.org/cgi/doi/10.1083/jcb.201407048

JCB   457

Figure 1. **Contacts between inner and outer kinetochore protein complexes.** The architecture of the outer kinetochore KMN complex (green box) is broadly conserved, with Knl1 and the Ndc80 complex binding at one end of the rod-like Mis12 complex. Within the constitutive centromere-associated network (CCAN; pink box), there are now two Mis12 complex binding activities: one in the N terminus of the dimeric CENP-C/ Mif2 (magenta; for simplicity only one monomer is drawn), and the second in Ame1/CENP-U (yellow; for simplicity other CCAN subunits known to associate with Ame1 and Okp1 are not depicted). The Ame1/CENP-U–Mis12 complex interaction, mediated by an N-terminal motif in Ame1, is described in this issue by Hornung et al. (2014) as being essential for outer kinetochore assembly in budding yeast. They also demonstrate DNA binding activity and direct Mif2/CENP-C–binding activity for the Ame1–Okp1 complex (yellow arrows). In addition to CENP-C and Ame1/CENP-U, the CENP-T subunit of a different CCAN complex (T/W/S/X) binds directly to the Ndc80 complex.

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.

embryonic stem cells, though curiously not for the viability of mouse embryonic fibroblasts (Kagawa et al., 2014).

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,

References

Basilico, F., S. Maffini, J.R. Weir, D. Prumbaum, A.M. Rojas, T. Zimniak, A. De Antoni, S. Jeganathan, B. Voss, S. van Gerwen, et al. 2014. The pseudo GTPase CENP-M drives human kinetochore assembly. *Elife (Cambridge)*. 3:e02978. http://dx.doi.org/10.7554/eLife.02978

Bock, L.J., C. Pagliuca, N. Kobayashi, R.A. Grove, Y. Oku, K. Shrestha, C. Alfieri, C. Golfieri, A. Oldani, M. Dal Maschio, et al. 2012. Cnn1 inhibits the interactions between the KMN complexes of the yeast kinetochore. *Nat. Cell Biol.* 14:614–624. http://dx.doi.org/10.1038/ncb2495

Carroll, C.W., K.J. Milks, and A.F. Straight. 2010. Dual recognition of CENP-A nucleosomes is required for centromere assembly. *J. Cell Biol.* 189:1143–1155. http://dx.doi.org/10.1083/jcb.201001013

Gascoigne, K.E., K. Takeuchi, A. Suzuki, T. Hori, T. Fukagawa, and I.M. Cheeseman. 2011. Induced ectopic kinetochore assembly bypasses the requirement for CENP-A nucleosomes. *Cell.* 145:410–422. http://dx.doi.org/10.1016/j.cell.2011.03.031

Haruki, H., J. Nishikawa, and U.K. Laemmli. 2008. The anchor-away technique: rapid, conditional establishment of yeast mutant phenotypes. *Mol. Cell.* 31:925–932. http://dx.doi.org/10.1016/j.molcel.2008.07.020

Hori, T., M. Okada, K. Maenaka, and T. Fukagawa. 2008. CENP-O class proteins form a stable complex and are required for proper kinetochore function. *Mol. Biol. Cell.* 19:843–854. http://dx.doi.org/10.1091/mbc.E07-06-0556

Hori, T., W.-H. Shang, K. Takeuchi, and T. Fukagawa. 2013. The CCAN recruits CENP-A to the centromere and forms the structural core for kinetochore assembly. *J. Cell Biol.* 200:45–60. http://dx.doi.org/10.1083/jcb.201210106

Hornung, P., M. Maier, G.M. Alushin, G.C. Lander, E. Nogales, and S. Westermann. 2011. Molecular architecture and connectivity of the budding yeast Mtw1 kinetochore complex. *J. Mol. Biol.* 405:548–559. http://dx.doi.org/10.1016/j.jmb.2010.11.012

Hornung, P., P. Troc, F. Malvezzi, M. Maier, Z. Demianova, T. Zimniak, G. Litos, F. Lampert, A. Schleiffer, M. Brunner, et al. 2014. A cooperative mechanism drives budding yeast kinetochore assembly downstream of CENP-A. *J. Cell Biol.* In press.

Kagawa, N., T. Hori, Y. Hoki, O. Hosoya, K. Tsutsui, Y. Saga, T. Sado, and T. Fukagawa. 2014. The CENP-O complex requirement varies among different cell types. *Chromosome Res.* http://dx.doi.org/10.1007/s10577-014-9404-1

Kato, H., J. Jiang, B.-R. Zhou, M. Rozendaal, H. Feng, R. Ghirlando, T.S. Xiao, A.F. Straight, and Y. Bai. 2013. A conserved mechanism for centromeric nucleosome recognition by centromere protein CENP-C. *Science.* 340:1110–1113. http://dx.doi.org/10.1126/science.1235532

Malvezzi, F., G. Litos, A. Schleiffer, A. Heuck, K. Mechtler, T. Clausen, and S. Westermann. 2013. A structural basis for kinetochore recruitment of the Ndc80 complex via two distinct centromere receptors. *EMBO J.* 32:409–423. http://dx.doi.org/10.1038/emboj.2012.356

Maskell, D.P., X.-W. Hu, and M.R. Singleton. 2010. Molecular architecture and assembly of the yeast kinetochore MIND complex. *J. Cell Biol.* 190:823–834. http://dx.doi.org/10.1083/jcb.201002059

Nishino, T., K. Takeuchi, K.E. Gascoigne, A. Suzuki, T. Hori, T. Oyama, K. Morikawa, I.M. Cheeseman, and T. Fukagawa. 2012. CENP-T-W-S-X forms a unique centromeric chromatin structure with a histone-like fold. *Cell.* 148:487–501. http://dx.doi.org/10.1016/j.cell.2011.11.061

Nishino, T., F. Rago, T. Hori, K. Tomii, I.M. Cheeseman, and T. Fukagawa. 2013. CENP-T provides a structural platform for outer kinetochore assembly. *EMBO J.* 32:424–436. http://dx.doi.org/10.1038/emboj.2012.348

Ortiz, J., O. Stemmann, S. Rank, and J. Lechner. 1999. A putative protein complex consisting of Ctf19, Mcm21, and Okp1 represents a missing link in the budding yeast kinetochore. *Genes Dev.* 13:1140–1155. http://dx.doi.org/10.1101/gad.13.9.1140

Perpelescu, M., and T. Fukagawa. 2011. The ABCs of CENPs. *Chromosoma.* 120:425–446. http://dx.doi.org/10.1007/s00412-011-0330-0

Petrovic, A., S. Pasqualato, P. Dube, V. Krenn, S. Santaguida, D. Cittaro, S. Monzani, L. Massimiliano, J. Keller, A. Tarricone, et al. 2010. The MIS12 complex is a protein interaction hub for outer kinetochore assembly. *J. Cell Biol.* 190:835–852. http://dx.doi.org/10.1083/jcb.201002070

Petrovic, A., S. Mosalaganti, J. Keller, M. Mattiuzzo, K. Overlack, V. Krenn, A. De Antoni, S. Wohlgemuth, V. Cecatiello, S. Pasqualato, et al. 2014. Modular assembly of RWD domains on the Mis12 complex underlies outer kinetochore organization. *Mol. Cell.* 53:591–605. http://dx.doi.org/10.1016/j.molcel.2014.01.019

Przewloka, M.R., Z. Venkei, V.M. Bolanos-Garcia, J. Debski, M. Dadlez, and D.M. Glover. 2011. CENP-C is a structural platform for kinetochore assembly. *Curr. Biol.* 21:399–405. http://dx.doi.org/10.1016/j.cub.2011.02.005

Screpanti, E., A. De Antoni, G.M. Alushin, A. Petrovic, T. Melis, E. Nogales, and A. Musacchio. 2011. Direct binding of Cenp-C to the Mis12 complex joins the inner and outer kinetochore. *Curr. Biol.* 21:391–398. http://dx.doi.org/10.1016/j.cub.2010.12.039