Untangling Topo II’s function at mitotic centromeres

Two studies reveal a conserved, noncatalytic role for Topo II’s C-terminal domain in recruiting the checkpoint kinase Aurora B.

Sister chromatids get tangled up during DNA replication and so, before they can be segregated during mitosis, they must first be untangled by the enzyme topoisomerase II (Topo II). Surprisingly, even though it isn’t required for the enzyme’s localization or catalytic activity, Topo II’s C-terminal domain (CTD) is also required for accurate chromosome segregation. Two groups now reveal that this may be because Topo II’s C terminus helps recruit the checkpoint kinase Aurora B to mitotic centromeres (1, 2).

As part of the chromosomal passenger complex (CPC), Aurora B localizes to mitotic centromeres, where it helps to activate the spindle assembly checkpoint if spindle microtubules are improperly attached to kinetochores and tension levels are abnormally low. Topo II accumulates at centromeres during mitosis, and studies have suggested that it can regulate Aurora B activity under certain conditions (3, 4). Yoshiaki Azuma’s lab at the University of Kansas previously found that Topo II’s CTD is SUMOylated during mitosis (5), allowing it to recruit a protein called Claspin to mitotic centromeres (6).

Claspin, in turn, binds to an activator of Aurora B called Chk1, and, accordingly, Aurora B’s activity was reduced when Azuma and colleagues, led by graduate student Makoto Yoshida, inhibited the mitotic SUMOylation of Topo II’s CTD in Xenopus egg extract. But Aurora B’s centromeric localization was also reduced, suggesting that Topo II’s CTD might bind to additional proteins involved in Aurora B recruitment (1).

Yoshida et al. performed a series of pulldown experiments and found that, when SUMOylated during mitosis, Topo II’s CTD can bind to Haspin, a kinase that phosphorylates histone H3 to create a binding site for the CPC on centromeric chromatin. This interaction depended on two SUMO-interacting motifs in Haspin’s N-terminal domain, as well as the mitosis-specific phosphorylation of a nearby threonine residue. “So the interaction is cell cycle dependent because two modifications—SUMOylation of Topo II and phosphorylation of Haspin—are required to form a stable association between the two proteins,” Azuma explains.

Blocking this association impaired Haspin’s recruitment to mitotic centromeres in Xenopus egg extract, with a corresponding decrease in H3 phosphorylation that would explain why Aurora B’s centromeric localization is reduced in the absence of Topo II SUMOylation.

Meanwhile, at the University of Minnesota, Duncan Clarke and colleagues found that Topo II’s CTD, and its SUMOylation during mitosis, was also required to recruit Aurora B to mitotic centromeres in budding yeast (2).

After speaking with Azuma, Clarke and colleagues, led by postdoc Heather Edgerton, wanted to test whether this was also due to an interaction between Topo II and Haspin, but, unlike in vertebrates, Haspin and H3 phosphorylation are not thought to be involved in recruiting the CPC in S. cerevisiae. (A second recruitment pathway, involving the phosphorylation of histone H2A by Bub1 kinase, appears to operate in all eukaryotes).

Nevertheless, Edgerton et al. examined yeast lacking both Haspin orthologues, Alk1 and Alk2, and found that Aurora B was no longer efficiently recruited to mitotic centromeres. The researchers also determined that, as in vertebrates, yeast histone H3 is phosphorylated on threonine 3 during mitosis, and that mutating this residue to nonphosphorylatable alanine also disrupted Aurora B localization. H3 phosphorylation was reduced in the absence of Topo II’s CTD or the Haspin orthologues, but expressing a phosphomimetic version of this histone restored Aurora B’s accumulation at mitotic centromeres.

Thus, the mechanism of Aurora B’s recruitment to mitotic centromeres is conserved in Xenopus and S. cerevisiae. “That’s a little surprising, because, unlike other eukaryotes, budding yeast have point centromeres consisting of a single nucleosome,” says Clarke, who now wants to determine whether defects in Aurora B recruitment, and thus in checkpoint activation, are the reason why cells segregate their chromosomes inaccurately in the absence of Topo II’s CTD. Azuma, meanwhile, wants to investigate whether Topo II’s CTD can couple checkpoint activation to the enzyme’s function in untangling sister chromatids.

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