A mad partner for Shugoshin in meiosis

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The centromeric cohesin protector Shugoshin (Sgo) and the spindle checkpoint protein Mad2 are known to act in distinct pathways for ensuring accurate chromosome segregation during eukaryotic cell division. In this issue of *The EMBO Journal*, Orth *et al* (2011) describe an unexpected physical interaction between vertebrate Sgo and Mad2, as well as indications of their potential mutual regulation in meiosis.

Cancer and Down syndrome are two outcomes of chromosome missegregation in mitosis and meiosis, respectively. During the somatic cell cycle, accurate segregation depends on proper regulation of cohesin complexes that hold replicated sister chromatids together (Peters *et al*, 2008). In early mitosis, cohesin at chromosome arms is removed while centromeric cohesin remains protected by Shugoshin bound to PP2A, thus enabling bi-orientation of sister centromeres by kinetochore attachment to microtubules emanating from opposite spindle poles. Anaphase activation of the protease separase, via the APC/C^{Cdc20} ubiquitin ligase (Yu, 2007), then results in cleavage of centromeric cohesin, and thus sister-chromatid separation.

In zygotic divisions, replicated maternal and paternal chromosomes pair during meiosis I to form ‘bivalents’ (Figure 1). Chiasmata formed by crossover homologous recombination allow sister-chromatid arm cohesion to contribute to the tethering of bivalents. In this case, sister kinetochores mono-orient by attaching to microtubules originating from the same spindle pole, and separase cleavage of arm cohesin segregates homologues into the two daughter cells. Here, Shugoshin–PP2A protects centromeric sister-chromatid cohesion from separase during anaphase I (Kitajima *et al*, 2006), until sister-chromatid bi-orientation during meiosis II, when separase also cleaves centromeric cohesin and triggers separation similar to the situation in mitosis.

Spindle microtubule attachment of sister chromatids in mitosis, and of bivalents in meiosis, occurs asynchronously. In each case, a surveillance system called the spindle checkpoint prevents their premature separation, by blocking APC/C^{Cdc20} until all chromosomes are properly attached to the spindle (Musacchio and Salmon, 2007). Central to spindle checkpoint signalling is conformational activation of the two-state protein Mad2 (Luo and Yu, 2008; Figure 1). Mad2 has two natively folded conformers, ‘open’ Mad2 (O-Mad2) and

‘closed’ Mad2 (C-Mad2). Upon checkpoint activation, the Mad1–C-Mad2 core complex at unattached kinetochores recruits cytosolic O-Mad2 through O–C-Mad2 dimerization and converts it to ‘intermediate’ Mad2 (I-Mad2). I-Mad2 can dissociate from the core complex via formation of a tight Cdc20–C-Mad2 complex, which results in the inhibition of APC/C^{Cdc20}, and thus of separase activation.

Despite each being essential for accurate chromosome segregation in mitosis and meiosis, the pathways in which Sgo and Mad2 act have been considered largely distinct. However, the study from the Stemmann laboratory in this issue of *The EMBO Journal* (Orth *et al*, 2011) now changes the picture. Orth *et al* tested whether mammalian Sgo proteins (Sgo1 and Sgo2) interacted with spindle checkpoint proteins, and found that human Sgo2, but not Sgo1, bound to Mad2. Binding of endogenous Mad2 to *Xenopus* Sgo (the sole Shugoshin protein in this organism) in frog oocytes confirmed that this interaction also occurs in meiotic cells. Through a comprehensive set of biochemical and biophysical experiments, the authors showed convincingly that human Sgo2 and *Xenopus* Sgo contain a canonical Mad2-interacting motif (MIM) resembling the MIMs in Mad1 and Cdc20 (Yu, 2007), and formed a similarly tight and specific Sgo–C-Mad2 complex, characterized by its ability to further interact with O-Mad2 or the Mad2 inhibitor p31^{comet} (Luo and Yu, 2008; Figure 1). Because the Sgo2 MIM is located C-terminal to its coiled-coil domain responsible for PP2A binding, binding of Mad2 does not affect Sgo2 binding to PP2A, and *vice versa*. Strikingly, mutation of a single residue in the Sgo2 MIM (R153A) abolished the Sgo2–Mad2 interaction. These results firmly establish a conserved *bona fide* interaction between vertebrate Sgo and Mad2.

Sgo2-deficient mice are viable but infertile, and Sgo2-deficient mouse embryonic fibroblasts undergo normal mitotic divisions, indicating that murine Sgo2 is essential for meiosis but not for mitosis (Llano *et al*, 2008). However, earlier studies on human Sgo2 function produced conflicting results. Watanabe and coworkers showed that Sgo2 depletion by RNAi caused premature sister-chromatid separation (PSCS) in HeLa cells (Kitajima *et al*, 2006; Tanno *et al*, 2010). By contrast, Yen and coworkers found that Sgo2 depletion with a different siRNA did not produce PSCS, but perturbed chromosome alignment and the kinetochore binding of MCAK, a microtubule depolymerizing motor

Figure 1 Function and regulation of Mad2 and Sgo during mitosis and meiosis. Mad2 has two conformers, open-Mad2 (O-Mad2) and closed-Mad2 (C-Mad2). Ribbon diagrams of O/C-Mad2 are shown with the conformationally malleable region coloured yellow. In mitosis, Sgo1-PP2A protects centromeric cohesin, which enables sister-chromatid bi-orientation. In response to lack of tension or microtubule attachment at kinetochores, O-Mad2 is recruited and activated by Mad1-C-Mad2 to become I-Mad2, which then forms Cdc20-C-Mad2 and inhibits APC/C. Similar conformational activation of Mad2 likely occurs during meiosis, and may require Sgo2 during meiosis II. Mad2 may also regulate Sgo2's function in centromeric cohesin protection during meiosis I.

(Huang *et al*, 2007). To further explore the significance of the Sgo2-Mad2 interaction, Orth *et al* revisited the mitotic function of Sgo2 in human cells. They showed that both of the previously employed siRNAs depleted Sgo2 equally efficiently, and could confirm that the former siRNA (Kitajima *et al*, 2006; Tanno *et al*, 2010) produced PSCs, mitotic arrest, and cell death. On the other hand, siRNA used previously by Huang *et al* diminished MCAK kinetochore localization, but failed to produce any discernable mitotic or spindle checkpoint defects. These results are, therefore, consistent with the results on mouse Sgo2 (Llano *et al*, 2008), and suggest that Sgo2 may be dispensable for sister-chromatid cohesion and mitotic progression also in human cells (where Sgo1 is the likely centromeric cohesin protector during mitosis). The data further suggest that the mitotic defects upon Sgo2 seen by Watanabe and colleagues may be due to off-targets effects; while other technical differences might underlie the chromosome alignment defects upon Sgo2 RNAi observed previously by Huang *et al*.

What then is the function of the Sgo2-Mad2 interaction? Because Sgo2 does not appear to have non-redundant functions in mitosis, a definitive answer to this question awaits generation of knock-in mice harbouring the Mad2 binding-deficient mutant Sgo2-R153A and characterization of their meiotic phenotypes. Orth *et al* did, however, provide a tantalizing clue to a potential role of Mad2 in regulating Sgo2. They showed that the centromeric localization of Sgo2 at metaphase became less focused in cells treated with Mad2 RNAi. Consistently, Sgo2-R153A exhibited less focused centromeric localization. Thus, Mad2 may conceivably regulate Sgo2 localization and sister-chromatid cohesion in meiosis I. In this scenario, Sgo2 would be a downstream effector of Mad2, similar to Cdc20 (Figure 1).

Because budding yeast Sgo1 has been implicated in tension sensing in the spindle checkpoint (Indjeian *et al*, 2005), an alternative hypothesis is that mammalian Sgo2 is also a Mad1-like upstream regulator of Mad2 during meiosis (Figure 1). Bivalents segregate normally during male and female meiosis in Sgo2-null mice (Llano *et al*, 2008), arguing

against a role of Sgo2 in regulating Mad2 during meiosis I. By contrast, although many Sgo2-null spermatocytes undergo metaphase II arrest and apoptosis, some escape from mitosis and form aneuploid spermatids. Thus, it remains possible that the Sgo2–Mad2 interaction contributes to spindle checkpoint signalling during meiosis II. Future studies on the function of the Sgo2–Mad2 interaction may unveil intricate mutual regulation between these two critical regulators of chromosome segregation in meiosis.

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Conflict of interest

The author declares that he has no conflict of interest.