MITOSIS

BRCA1 — keeping excessive activities in check

> …provides evidence for a distinct function of BRCA1–BARD1 in mitosis…

Loss of BRCA1 function causes a predisposition to breast and ovarian cancer by incompletely defined mechanisms. Joukov *et al.* now uncover a new function of BRCA1 in the assembly of the mitotic spindle, which might be relevant to BRCA1-mediated tumour suppression.

In vivo, BRCA1 and its structural relative, BARD1, form a heterodimeric complex that has E3 ubiquitin ligase activity. Both proteins also contain BRCA1 C-terminal (BRCT) motifs that function as specific phosphopeptide-binding domains. Although it has been previously proposed that BRCA1–BARD1 might participate in mitosis, it is difficult to assign specific mitotic roles to proteins that are also important during interphase.

Joukov *et al.* used *Xenopus laevis* egg extracts and mammalian cells to examine the mitotic function of the BRCA1–BARD1 heterodimer and showed that this complex is localized in the nucleus and binds to chromatin during interphase. The complex is released from chromatin in mitosis. Depletion of BRCA1–BARD1 from extracts using specific antibodies showed that the heterodimer is dispensable for S-phase progression, but it is required for proper nuclear assembly upon mitotic exit.

The involvement of BRCA1–BARD1 in post-mitotic nuclear formation led the authors to investigate the requirements of this heterodimer for the execution of mitosis and spindle assembly. Compared to control extracts, which had bipolar spindles with focused spindle poles and congressed chromosomes, spindles in BRCA1–BARD1-depleted extracts had unfocused poles and chromosome-congression defects. Joukov *et al.* recapitulated these findings in mammalian cells: small-interfering-RNA-mediated depletion of BRCA1–BRAD1 resulted in increased numbers of disorganized bipolar spindles and defects in chrom osome alignment.

These defects were reminiscent of those induced by perturbations of the Ran GTPase pathway, which prompted the authors to examine the effects of BRCA1–BARD1 depletion on the formation of microtubule (MT) asters that form after the addition of exogenous RanGTP to meiotic egg extracts. Asters that assembled in BRCA1–BARD1-depleted extracts were larger and had perturbed poles with poorly focused MTs, indicating that BRCA1–BARD1 regulates microtubule organization downstream of RanGTP. Experiments using the wild-type and mutant BRCA1–BARD1 showed that this function requires the E3 ubiquitin ligase activity of the heterodimer.

How does BRCA1–BARD1 function? The authors investigated the localization of four proteins that are known to be involved in spindle-pole formation — NuMA, γ-tubulin, TPX2 and XRHAMM. All these proteins bind to MTs and concentrate on aster poles in *X. laevis* egg extracts. However, in BRCA1–BARD1-depleted extracts, γ-tubulin and XRHAMM localization was more diffuse, and TPX2 was localized along the length of microtubules instead of the poles. Polar localization of TPX2 was restored by the addition of recombinant wild-type BRCA1–BARD1, but not by the addition of its mutant counterpart that is deficient in ubiquitin transfer.

Joukov *et al.* then showed that BRCA1–BARD1 interacts with XRHAMM, NuMA and TPX2 and attenuates the activity of XRHAMM. In the absence of BRCA1–BARD1, XRHAMM is hyperactive and it is this hyperactivity that causes the mislocalization of TPX2 and aberrant spindle assembly.

This study provides evidence for a distinct function of BRCA1–BARD1 in mitosis that might contribute to its role in the maintenance of genome integrity and tumour suppression.

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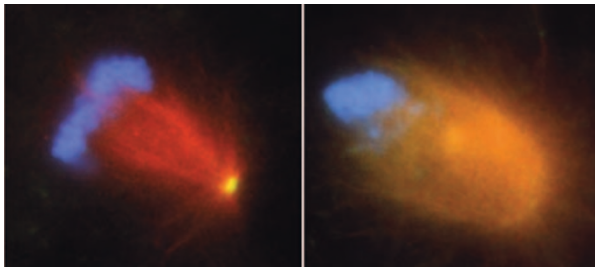

Chromatin-induced asters assembled in mock-treated (left) and BRCA1–BARD1-treated (right) extracts. Blue, DAPI; red, rhodamine-labelled tubulin; green, TPX2. Image courtesy of V. Joukov, Dana-Farber Cancer Institute, Boston, USA.

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