REV7/MAD2L2: the multitasking maestro emerges as a barrier to recombination

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REV7/MAD2L2 plays important roles in translesion DNA synthesis and mitotic control. Two new papers extend its gamut by revealing its unexpected participation in pathway choice during DNA double-strand break repair. By inhibiting 5' DNA end resection downstream of 53BP1 and RIF1, REV7/MAD2L2 promotes non-homologous end joining at the expense of homologous recombination. Importantly, loss of REV7/MAD2L2 renders PARP inhibitors ineffective in BRCA1-deficient tumours, suggesting another possible mechanism for the acquisition of resistance to this important new class of drug.

See also: **G Xu *et al*** (May 2015) and
V Boersma *et al* (May 2015)

REV7/MAD2L2, a protein of just 211 amino acids, has emerged over the years as a potentially important component of multiple cellular pathways. REV7 was first identified in a screen for yeast mutants that exhibited reduced reversion of a reporter allele after exposure to UV light (Lawrence *et al*, 1985). The hypomutability of *rev7* mutants is explained by its role as a subunit of DNA polymerase ζ , a key enzyme involved in replicating damaged DNA by translesion synthesis. REV7 acts as an adaptor between REV1 and REV3, the catalytic subunit of Pol ζ , and helps coordinate the sequential insertion and extension steps of lesion replication (Fig 1A). REV7 was rediscovered as MAD2L2 (or MAD2B) by its homology with yeast MAD2 (Cahill *et al*, 1999), one of the *mitotic arrest-deficient* genes that are components of the spindle assembly checkpoint (SAC). Although MAD2L2 is not a SAC component, it nonetheless helps prevent premature

activation of the anaphase promoting complex/cyclosome (APC/C) by the activator protein CDH1 (Listovsky & Sale, 2013) (Fig 1B).

Enter the two new papers from the Rottenberg (Xu *et al*, 2015) and Jacobs groups (Boersma *et al*, 2015), which, somewhat confusingly, maintain the distinct names of REV7 and MAD2L2 in their respective titles. Hereon, I will use MAD2L2. The two papers adopt quite distinct approaches to search for factors that modify the choice cells make between homologous recombination (HR) and non-homologous end joining (NHEJ) when faced with DNA ends.

Xu *et al* asked how cells lacking the tumour suppressor BRCA1 become resistant to inhibitors of poly-ADP ribose polymerase (PARP), which block the repair of single strand nicks in DNA. Normal cells cope with this quite well, as the nicks are transmitted into S phase and are repaired by HR. BRCA1 is essential for efficient recombination and cells lacking BRCA1 are exquisitely sensitive to PARP inhibition, a feature that is now exploited in treatment of HR-deficient tumours. However, emergence of resistance is a common problem and, somewhat surprisingly, is associated with reactivation of HR, even in the continued absence of BRCA1. A breakthrough in understanding how this happens came with the observation that the HR defect of BRCA1-deficient cells could largely be reversed by deletion of 53BP1 (Bunting *et al*, 2010). 53BP1 is recruited to double-strand breaks and plays an important role in promoting non-homologous end joining by inhibiting the resection of the DNA ends through recruitment of RIF1 (Fig 1C) (reviewed in Zimmermann & de Lange, 2014). This effect can be countered during S/G2 by the action of

BRCA1 and phosphorylated CtIP, which prevent RIF1 recruitment allowing initiation of 5' end resection. Thus, in BRCA1-deficient cells, the 53BP1-dependent recruitment of RIF1 can be relieved by deletion of 53BP1, HR restored and sensitivity to PARP inhibition lost. Xu *et al* therefore employed an RNAi screen to search for additional proteins that promote loss of sensitivity to PARP inhibition in BRCA1-deficient cells, identifying MAD2L2 as a high-confidence hit.

In contrast, Boersma *et al* were interested in identifying factors that influence DNA repair at telomeres. Loss of Trf2, a component of the telomeric cap that protects the ends of chromosomes and prevents them being recognised as a double-strand break, results in loss of telomere end protection, activation of the DNA damage response and cell death caused by telomere-telomere chromosome fusions. Therefore, using a conditional depletion of Trf2, Boersma *et al* screened for genes whose knockdown promoted survival in the absence of telomere end protection. The most prominent hit in their screen was also MAD2L2, alongside expected DNA damage response factors such as 53BP1.

From this point, both teams converged on similar overall conclusions. MAD2L2 is recruited to DSB sites but is not needed to initiate damage signalling or to bring in 53BP1 or RIF1. Similar to 53BP1 and RIF1, MAD2L2 is needed to inhibit DNA 5' end resection, likely downstream of RIF1 (Fig 1C). Consistent with this model, MAD2L2 is required for telomere fusion when capping is defective and for effective immunoglobulin class switch recombination. Its knockdown also reverses the loss of RAD51 focus formation and sensitivity to PARP inhibition of BRCA1-deficient

Figure 1. The cellular roles of MAD2L2.

(A) MAD2L2 helps coordinate nucleotide insertion opposite a lesion by Y-family polymerases with mismatch extension by REV3/Polζ during translesion synthesis. (B) MAD2L2 helps to prevent premature activation of the APC/C by sequestering CDH1. (C) The newly uncovered role for MAD2L2 in pathway choice during double-strand break repair. A highly stripped-down scheme of the DNA damage response showing the role of 53BP1 in limiting resection through recruitment of RIF1. Genetically, MAD2L2 appears to act downstream of RIF1. BRCA1 and phosphorylated CtIP prevent RIF1 recruitment, allowing displacement of 53BP1, resection and recombination. Thus, in the absence of BRCA1, 53BP1 and MAD2L2 block resection. Removing either of these factors in BRCA1-deficient cells alleviates this block and can allow resection to proceed.

tumours. However, restoration of fully functional HR in a gene conversion reporter assay is much less impressive. This is an interesting point as several lines of evidence suggest that Polζ is required for effective homologous recombination, possibly during the DNA synthesis steps of the reaction. Thus, while MAD2L2 appears to be required for promoting NHEJ, at the same time it may be needed to facilitate completion of HR as part of Polζ.

So, while this new genetic evidence places MAD2L2 in the hot seat for promoting pathway choice through inhibition of DNA end resection, its mechanism and the contexts in which it operates remain completely unclear. Interaction studies do not place it in a complex with 53BP1 or RIF1 directly (Xu *et al*, 2015). However, a previously identified MAD2L2 complex hints at a particular role in heterochromatin. In a screen for readers of lysine methylation in human cells, MAD2L2 was found in a complex with isoforms of the heterochromatin-binding protein HP1, and two zinc finger proteins POGZ and ZNF828 (Vermeulen

et al, 2010). It will thus be interesting to examine whether these interactions are relevant for MAD2L2's role in double-strand breaks processing.

Finally, it is worth considering these observations in the context of a whole mouse, given the multiple roles MAD2L2 seems to have acquired. Unexpectedly, MAD2L2-deficient mice can reach term and even adulthood (Pirouz *et al*, 2013; Watanabe *et al*, 2013). Curiously, however, their most prominent phenotype is complete failure of primordial germ cell development, which does not appear to be explained by DNA damage accumulation but rather by an unanticipated failure of epigenetic reprogramming. Thus, together with the new observations from Xu *et al* and Boersma *et al*, it seems likely that MAD2L2 is going to keep several fields busy for some time to come.

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