Trapping Rap1 at the telomere to prevent chromosome end fusions

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The prevention of nonhomologous end-joining (NHEJ) reactions between chromosome ends is crucial for maintaining genome stability. Although this protective function is known to be fulfilled by a core of conserved telomeric proteins that are collectively known as Shelterin, its mechanistic details remain a mystery. In this issue, Sarthy *et al* (2009) lend fresh insight by developing an ingenious method to dissect the role of hRAP1 in preventing telomeric NHEJ independently of other Shelterin components.

Nearly 70 years ago, McClintock (1941) and Muller (1938) deduced that natural chromosome ends are distinct from the ends created by chromosome breakage in their ability to avoid fusion reactions. The lethal consequences of telomere dysfunction are graphically illustrated by more recent studies in which loss of the human telomere repeat-binding protein TRF2 leads to ‘trains’ of chromosomes hitched together through NHEJ (Celli and de Lange, 2005). Although these studies show the central role of TRF2 in the defining protective function of telomeres, the multitude of molecular interactions in which TRF2 engages has confounded an understanding of precisely how it prevents NHEJ.

Rap1 has been both one of the most well-studied and enlightening of telomeric components and one of the most enigmatic. Budding yeast Rap1 (ScRap1) binds directly to telomeric repeats (as well as numerous promoters) and coordinates several telomeric functions, including silencing and telomerase regulation. Moreover, landmark studies have

shown that ScRap1 prevents NHEJ between chromosome ends (Pardo and Marcand, 2005). Interestingly, although both human and fission yeast Rap1 were identified by their homology to ScRap1, they lack DNA-binding ability and associate with telomeres through interactions with the related telomere-binding proteins TRF2 and Taz1, respectively. Indeed, loss of either Taz1 or Rap1 in fission yeast leads to NHEJ-mediated telomere fusions (Ferreira and Cooper, 2001; Miller *et al*, 2005). Technical challenges to developing a mammalian RAP1 knockout model have hindered our ability to extend this idea to metazoans (Tan *et al*, 2003). However, by combining the insights gained from previous *in vitro* experiments with an innovative approach to constructing informative chimeric molecules, Sarthy *et al* have pinpointed a role of hRAP1 in NHEJ inhibition.

The Baumann lab previously addressed the minimal elements required for protection from chromosomal end-joining in human cell extracts (Bae and Baumann, 2007). They found that as few as eight telomeric (TTAGGG) repeats were sufficient to block the nearby NHEJ. As this tract length is too short to confer t-loops, these observations suggested a direct NHEJ-blocking function that would not rely on telomeric higher-order structure. Immunodepletion and add-back of specific proteins showed that protection depends on both TRF2 and hRAP1. However, as hRAP1 fails to localize to telomeres in the absence of TRF2, the sufficiency of hRAP1 for blocking NHEJ could not be addressed. To circumvent this problem, Sarthy *et al* devised a way to target hRAP1 to

Figure 1 Tethered hRAP1 inhibits telomeric NHEJ. In wild-type cells (left), TRF2 recruits Shelterin components, including hRAP1, to telomeres. TRF2 is known to prevent local activation of the ATM checkpoint pathway, which is upstream of NHEJ; however, the role of hRAP1 has been difficult to address, as its telomere localization relies on interaction with TRF2. In cells with reduced TRF2 function (right), ATM activation triggers telomeric NHEJ. By tethering a truncated version of hRAP1 to telomeres independently of TRF2, Sarthy *et al* have shown that NHEJ prevention is restored, suggesting that hRAP1 blocks NHEJ at a step downstream of ATM activation.

telomeres in the absence of TRF2 by exploiting the specific binding of human telomere repeats by an unrelated fission yeast protein, *Teb1*. The authors constructed a chimeric molecule (hRAP1-*TebDB*) in which the DNA-binding domain of *Teb1* (*TebDB*) is fused with hRAP1. Unlike wild-type hRAP1, hRAP1-*TebDB* binds telomere repeats and inhibits telomeric NHEJ in TRF2-depleted cell extracts.

To investigate a protective role for hRAP1 *in vivo*, a dominant-negative form of TRF2 lacking its basic and Myb domains, 'TRF2 Δ B Δ M', was expressed in HeLa cells. TRF2 Δ B Δ M dimerizes with both endogenous TRF2 and itself, displacing a subset of endogenous TRF2 and all detectable hRAP1 from telomeres (Sarthy *et al*, 2009). The result is a striking telomere deprotection phenotype in which the ATM checkpoint is triggered and end-to-end fusions are rampant (van Steensel *et al*, 1998). To tether hRAP1 to telomeres in a TRF2-independent manner, the authors removed the C-terminal TRF2-interacting region of hRAP1 (creating 'hRAP1 Δ CT'), ensuring that the only mode of telomeric hRAP1 delivery was *via* the chimeric hRAP1 Δ CT-*TebDB*. Strikingly, although neither *TebDB* nor hRAP1 Δ CT confers protection from telomeric NHEJ upon TRF2 Δ B Δ M expression, hRAP1 Δ CT-*TebDB* reduced the incidence of end fusions by 10-fold, showing a potent NHEJ-inhibition ability for tethered hRAP1 Δ CT *in vivo*.

Is hRAP1 the sole mediator of TRF2's role in preventing telomeric NHEJ? The answer to this question awaits tethering of hRAP1 to telomeres in cells that lack TRF2 entirely. This experiment is crucial not only because of the persistence of telomeric TRF2, which may cooperate with the tethered

hRAP1, in TRF2 Δ B Δ M-expressing cells, but also because of a key property of telomere fusions through the cell cycle: The telomere fusions observed in cells lacking TRF2 are formed almost exclusively during the G1 phase, as expected because of the restriction of high levels of NHEJ to the G1 phase of the cell cycle (Ferreira and Cooper, 2004; Konishi and de Lange, 2008). In contrast, TRF2 Δ B Δ M expression leads to NHEJ during the G2 phase (Bailey *et al*, 2001), an event that would be rare under physiological conditions. Hence, it will be crucial to confirm that hRAP1 can prevent telomeric fusions during G1, as molecular events promoting or inhibiting NHEJ (like generation or removal of a 3' overhang) will differ significantly between G1 and G2.

Notably, although hRAP1 Δ CT-*TebDB* alleviates telomeric NHEJ, it does not block the ATM activation triggered by TRF2 Δ B Δ M expression (Sarthy *et al*, 2009). Hence, hRAP1 may harbour the ability to block NHEJ even in the face of ATM signalling, suggesting a model in which hRAP1 acts downstream of checkpoint signalling to impede NHEJ directly, perhaps by physically obstructing access of the NHEJ machinery (Figure 1). The validity and details of such a model will be deciphered by experiments that address whether hRAP1 can inhibit NHEJ at the self-same telomere that is devoid of TRF2 and harbours activated ATM, whether other Shelterin components assist hRAP1 with NHEJ inhibition, and at precisely which step of the pathway from ATM activation to Ligase IV-mediated end-joining hRAP1 acts. The approach presented by Sarthy *et al* will accelerate our understanding of these issues.

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