Review of: "RIF1-ASF1-mediated high-order chromatin structure safeguards genome integrity"

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Commentary on “RIF1-ASF1-mediated high-order chromatin structure safeguards genome integrity” by Feng*et al.* 2022.

The authors add further complexity to our emerging understanding of pathway choice in the DNA double strand break (DSB) response. Prior work established that 53BP1-dependent recruitment of RIF1 is required to prevent the resection required for homologous recombination (HR), thereby directing DSBs towards non-homologous end joining (NHEJ). Recently, RIF1-dependent recruitment of the Shieldin complex to minimally resected DSBs has been reported to prevent further resection (Findlay et al., 2018; Ghezraoui et al., 2018; Gupta et al., 2018). It does this by not only generating a ‘*shielded*’ chromatin structure flanking DSBs, but also by fill-in DNA synthesis via recruitment of DNA polymerase alpha primase.

Feng and colleagues describe a new role for RIF1-mediated DSB protection. They identify an interaction between RIF1 and ASF1 required for condensing flanking chromatin into a heterochromatin-like state, critical to preventing resection. The RIF1-ASF1 pathway functions independently of the Shieldin complex, providing the initial impediment to resection. However, once limited resection occurs, Shieldin is then recruited to prevent further resection. Mechanistically, the authors establish that ASF1’s histone chaperone activity is required to exchange histone H3-H4 in the chromatin flanking DSBs. Newly formed H3-H4 dimers are cytosolic, and mono-methylated on H3K9 (H3K9me1) by SETDB1, effectively priming H3K9me1 for di- and tri-methylation by SUV39H1 and 2 in the nucleus, essential for formation of heterochromatin (Jasencakova et al., 2010; Rivera et al., 2015), which favours NHEJ repair of DSBs.

Interestingly, Isobe *et al.,* 2021 previously reported another RIF1-dependent method of impeding resection: recruitment of PP1 (protein phosphatase 1) to the vicinity of DSBs. Proteins such as CtIP require phosphorylation for their activity, and PP1-dependent removal of these phosphates prevents the initiation of resection. It is likely that RIF1-dependent recruitment of both ASF1 and PP1 contribute additively to the prevention of initiation of resection, but this remains to be tested. Regardless, once limited resection does occur, Shieldin is recruited to prevent further resection, maintaining the ability to repair by NHEJ.

The study of Feng and colleagues also highlights the interesting alterations that occur to chromatin surrounding a DSB. Recent work suggests that there is an initial rapid and short-lived PARP-dependent expansion of chromatin (that favours fast-kinetic cNHEJ), followed by condensation (which favours slow-kinetic cNHEJ), and subsequent expansion at later

times that favours HR (reviewed in Kieffer and Lowndes, 2022). The 53BP1-RIF1-ASF1-SUV39H-HP1 axis identified is important during the condensation phase to produce a ‘heterochromatin-like’ and slow-kinetic cNHEJ-promoting state.

Finally, a noteworthy technical innovation is their development of a technique to investigate the chromatin state proximal and distal to DSBs. They used a high-energy laser to induce DSBs in a 1-2μm spot, and studied the recruitment of proteins within and flanking this laser-induced DSBs. RPA, RAD51, BRCA1, and MRN are recruited to chromatin proximal to DSBs (within the spot), whereas 53BP1 and RIF1 are recruited to chromatin distal to the DSBs (flanking the spot). The relative simplicity of this technique, which requires further optimisation, may well prove useful to the field, albeit, detecting the recently reported 53BP1 nanodomains via this technique may not be possible (Ochs et al., 2019).

Altogether, this manuscript presents an interesting addition to the role of RIF1 in the regulation of DSB repair pathway choice. Their data suggests that Shieldin provides a secondary backup role to the initial end protection provided by the 53BP1-RIF1-ASF1-SUV39H-HP1 chromatin compaction, as well as the separately reported recruitment of PP1. This work adds significantly to our understanding of DSB repair pathway choice, adding further complexity to our understanding of the time-dependent reorganization of chromatin flanking DSBs.

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