Rif2-mediated regulation of MRX activity at DNA double-strand breaks

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DNA double-strand breaks (DSBs) are among the most cytotoxic DNA lesions, because failure to repair them can lead to genome instability. DSBs can be repaired by either nonhomologous end joining (NHEJ), which directly ligates the two broken DNA ends, or homologous recombination (HR), which uses an intact homologous DNA sequence as a template for repair. The key process in determining which pathway is used to repair DSBs is the initial processing of the DSB ends. While NHEJ requires little or no DNA end processing, HR is initiated by nucleolytic degradation of the 5′ terminated strands at both DNA ends by a concerted action of nucleases in a process termed DNA end resection. The Mre11-Rad50-Xrs2/NBS1 complex (MRX in *Saccharomyces cerevisiae*, MRN in humans) has structural and enzymatic activities to initiate DSB resection and to maintain the DSB ends tethered to each other for their repair. Several studies have shown that ATP binding and hydrolysis activities of the Rad50 subunit are crucial to regulate DNA binding, tethering and nuclease functions of the MRX complex.

In *S. cerevisiae*, MRX is known to interact with Rif2, which is recruited to telomeric DNA ends and negatively regulates telomerase-mediated telomere elongation. We have previously shown that Rif2, which is recruited to DSBs in a manner partially dependent on MRX, enhances the ATP hydrolysis activity of Rad50 and attenuates MRX function in end-tethering. This observation, together with the finding that the lack of Rif2 by itself increases both end-tethering and NHEJ, suggests that Rif2 can regulate MRX activity at DSBs by modulating ATP-dependent conformational changes of Rad50. To better understand the crosstalk between Rad50 and Rif2, we have searched for *rad50* mutants that phenocopy *RIF2* deletion and therefore that increase both NHEJ and end-tethering. We identified a mutation in Rad50 that is located on the surface of the protein, suggesting that it can affect a possible Rif2-Rad50 interaction. We will present data regarding the structural and functional characterization of this Rad50 mutant variant.