DNA double-strand break repair by homologous recombination is initiated by the Ctp1 protein together with the Mre11–Rad50–Nbs1 nuclease complex in Schizosaccharomyces pombe, but the mechanism by which Ctp1 promotes this process has remained unknown. Andres et al. now use atomic force microscopy to image Ctp1–DNA complexes, demonstrating a striking capacity of Ctp1 filaments to bridge DNA molecules. This unanticipated role of Ctp1 might help explain how the processing of DNA ends is coordinated to facilitate DNA break repair.

DNA double-strand breaks (DSBs) can arise accidentally from exposure to radiation, chemicals, or errors of DNA metabolism. Homologous recombination is a key DSB repair pathway, which functions in a largely error-free manner. In this process, the recombination machinery uses an intact DNA template—most often the sister chromatid in growing cells—to provide instructions for how to rejoin the two DNA pieces of a broken chromosome (Fig. 1A). On the molecular level, recombination is initiated by DNA end resection, which involves controlled degradation of the 5′-terminated DNA strands at the DNA break sites, leading to 3′ single-strand DNA overhangs (1). These structures then help locate the matching DNA template. Subsequently, the 3′-ends prime DNA synthesis to recover any missing DNA sequence (Fig. 1A). Although these steps have been studied extensively, the role of one key human homologous recombination factor, CtIP, has been elusive. A new study from Andres et al. (2) on Ctp1, the equivalent of CtIP in the fission yeast Schizosaccharomyces pombe, provides evidence for a dual DNA binding/DNA bridging function that depends on protein oligomerization. These data help explain how Ctp1 promotes resection and may provide insights into the basis of human diseases, such as Seckel and Jawad syndromes, which are caused by mutations in Ctp1 (3).

What were the initial clues to Ctp1 function? Ctp1 and its orthologues, including human CtIP and Sae2 from the budding yeast Saccharomyces cerevisiae, are known to form tetramers that bind DNA (4, 5). One function of these evolutionarily conserved factors is to promote the initial step of the DNA end resection pathway by stimulating the Mre11 nuclease, which is an integral component of the Mre11–Rad50–Nbs1 (MRN) complex (6, 7). The Mre11 nuclease then endonucleolytically cleaves the 5′-terminated DNA strand at a site internal to the DSB. However, the underlying molecular mechanism of this reaction remains unclear. What is the stoichiometry and three-dimensional organization of the Ctp1–MRN ensemble bound to DNA? How is the DNA break identified? Is the resection of the two broken DNA ends coordinated?

Here, Andres et al. (2) attempt to answer some of these questions using atomic force microscopy. Without double-stranded DNA (dsDNA), Ctp1 forms monodisperse peaks consistent with tetrameric complexes. With dsDNA, two important observations were made. First, filamentous complexes of Ctp1 on dsDNA were seen. Volumetric analysis revealed that these structures likely result from tandem Ctp1 tetramers bound to dsDNA. Furthermore, the authors obtained clear evidence of Ctp1 filaments bridging two dsDNA molecules. These bridging events resulted in the pairing of two dsDNA chains, either intramolecularly or in trans (2). The images revealed a repeating pattern, consistent with adjacent tetrameric Ctp1 units mediating the dsDNA zipping. Importantly, the zipping function of Ctp1 was readily observed with circular dsDNA molecules without ends (2), which is in marked contrast to the previously established capacity of MRN to bridge DNA termini (8).

To determine the role of Ctp1 self-interaction in this process, Andres et al. (2) utilized mutants deficient in oligomerization or containing a disulfide bond to lock oligomers in place. Analysis of these Ctp1 variants by atomic force microscopy and other assays indicated that the DNA bridging function of Ctp1 was severely affected by mutations that impair self-interaction. Furthermore, DNA bridging was impaired by mutations that disrupt DNA binding of Ctp1 (2).

These seminal results collectively demonstrate the DNA binding and bridging activity of Ctp1, which is likely conserved in evolution. Previously, the DNA end binding and bridging by Mre11–Rad50 was hypothesized to coordinate the resection of both ends of the broken DNA molecule (8). As the bridging function of Ctp1 does not require a DNA end, an attractive possibility is that MRN mediates bridging of the DNA ends, while Ctp1 and orthologues promote the pairing of DNA internal to the end (Fig. 1B). This may protect the end from nonspecific degradation and guarantee scheduled resection by dedicated nuclease complexes. Additionally, this arrangement may facilitate simultaneous resection of both DNA ends, which...
could improve the efficacy of DSB repair. Another attractive possibility is that the Ctp1 filament may help direct the DNA cleavage to the 5′-terminated strand by the Mre11 nuclease away from the DNA break. The authors notice a similarity between the filaments of Ctp1 and those of the DNA mismatch repair nuclease complex Mlh1–Pms1 in yeast (MLH1–PMS2 in humans) (9). A cardinal feature of DNA mismatch repair reactions is that a strand discontinuity in the newly synthesized strand helps direct DNA cleavage of the same DNA strand often hundreds of nucleotides away. How this function is coordinated remains to be determined, but filament formation of Mlh1–Pms1 might play an important role. Analogously, a Ctp1/Sae2/CtIP filament might help ensure that Mre11-catalyzed DNA cleavage will occur specifically in the strand carrying the 5′ terminus, even though it might be some distance away.

Beyond these new mechanistic possibilities, another challenge remains in establishing the physiological relevance of DNA bridging by Ctp1. Andres et al. (2) do demonstrate that the mutations that impair DNA binding of Ctp1 result in cellular sensitivity to DNA damaging agents. Likewise, previous work has linked mutations that affect DNA binding of CtIP with Seckel syndrome (3). These results certainly suggest the relevance of the DNA binding and bridging functions, but are not fully unambiguous because Ctp1 (and Sae2/CtIP) also interacts with and stimulates the nuclease activity of Mre11. The domains, which are necessary for DNA binding by Ctp1 and its orthologues, are also likely important to promote the nuclease function of Mre11 (3, 5, 6, 10). The isolation of separation of function mutants that are defective in one but proficient in the other functional attributes of Ctp1 would help resolve this issue. Therefore, more structural and biochemical insights are needed to fully understand how the DNA binding and bridging activities of Ctp1 and Mre11 nuclease enhancement promote DNA repair. The elegant images by Williams and colleagues will certainly inspire further research in this field.

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