Structural dissection of the Holliday junction resolvase GEN1

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DNA repair and maintenance pathways depend on the correct and efficient processing of DNA by structure-specific nucleases. Holliday junction resolvase GEN1 is a versatile enzyme with the ability to cleave various DNA substrates such as DNA flaps, gaps, replication fork intermediates and four-way junctions. Therefore, it is considered to be a nuclease of last resort for the processing of replication and recombination intermediates.
To understand the mechanism of GEN1's versatility, we recently solved a crystal structure of human GEN1 in complex with Holliday junction DNA. The overall architecture of GEN1 is similar to other Rad2/XPG nucleases; however, it revealed an unexpected appearance of a chromodomain, which are commonly found in histone readers for chromatin recruitment. So far, GEN1 is the only example of a nuclease extended by a chromodomain, which may imply further roles in the interaction with chromatin or other factors. We found that the GEN1 chromodomain plays a crucial role in efficient DNA recognition and cleavage.
Using structure-guided mutagenesis, we characterized different DNA-binding modes of GEN1 biochemically. We found that GEN1 distinguishes different DNA substrates through its helical arch, aided by a robust recognition of DNA by a cluster of positive amino acids shadowing the chromodomain.
Stabilizing GEN1 by protein engineering facilitated a direct reconstitution of GEN1 with 5' flap DNA in a monomeric and with Holliday junction in a dimeric form. Dimer formation of GEN1 is a unique feature in the Rad2/XPG nuclease family and it is the key to ensure the resolution of DNA junctions in a strictly symmetric manner. We demonstrate a remarkable dimerization mechanism and we propose that the arch domain works as a switch to change GEN1's DNA recognition mode, making GEN1 a versatile tool for DNA processing and for maintaining genome integrity.