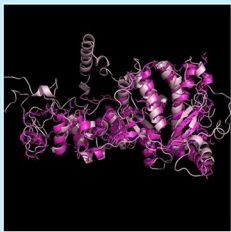




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Protein Overlay of CtGEN1 (fuchsia)
with DmGEN (light pink)

Altering Substrate Specificity of the Nuclease GEN

DNA damage occurs in many settings, including normal replication or exposure to radiation. Branched DNA structures such as Holliday junctions (HJs) and 5' flaps are important intermediates formed in the process of DNA repair, and *H. sapiens* GEN1 is a nuclease that can repair these intermediates. *In vitro*, human GEN1 and the structurally similar *Drosophila melanogaster* Gen (DmGen) cleave 5' flaps more efficiently than HJs. Substrate specificity is thought to be determined by a helical arc region, adjacent to the active site, through which ssDNA may be threaded for cleavage. Homologs of GEN have variable arc regions, and thus different substrate specificities— while DmGen has specificity for HJs, 5' flaps, and replication forks, its ortholog from *C. thermophilum* (CtGEN1) lacks the arc region, and thus is limited to HJ activity. Conversely, its homolog found in *H. sapiens* (HsFEN1) exhibits specificity for 5' flaps due to a different arc region. To manipulate the substrate specificity of DmGen, I replaced the arc region with the corresponding region from both CtGEN1 and HsFEN1 to yield two chimeric proteins termed GEN^[CtGEN1] and GEN^[HsFEN1]. I hypothesized that GEN^[CtGEN1] would have specificity to only HJs and GEN^[HsFEN1] would have specificity only to 5' flaps. Nuclease assays revealed that both GEN^[CtGEN1] and GEN^[HsFEN1] are unable to cleave HJs, suggesting that the swapped region plays a role in substrate binding or cleavage. Further research, including 5' flap nuclease assays and *in vivo* experiments, is needed to better understand the consequences of only cleaving 5' flaps or HJs in DNA repair. Ultimately, this work will offer novel insights into the role of the DmGen nuclease including its substrate specificity in isolation and in the greater context of DNA damage repair pathways in *Drosophila* and humans alike.