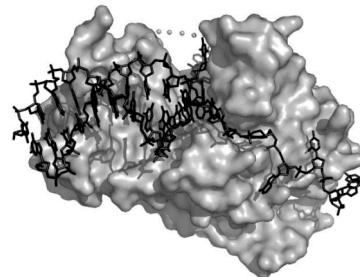


W0256

**Co-crystal of Bacteriophage T4 RNase H with a Fork DNA Substrate: Insight into Lagging Strand RNA Primer Removal from Okazaki Fragments.** J.M. Devos<sup>1</sup>, C.E. Jones<sup>2</sup>, N.G. Nossal<sup>2</sup>, T.C. Mueser<sup>1</sup>, <sup>1</sup>Dept. of Chemistry, The Univ. of Toledo, Toledo, OH. <sup>2</sup>National Institutes of Health, Bethesda, MD.

Bacteriophage T4 RNase H is a 5' exonuclease responsible for removal of RNA primers from lagging strand fragments during DNA replication. T4 RNase H, a member of the flap endonuclease (Fen-1) family of enzymes, acts as a 5' exonuclease on duplex substrates and as a flap endonuclease on branched substrates. We have previously reported the structure of native T4 RNase H (pdb 1TFR). Here we present the crystal structure of the D132N active site mutant of T4 RNase H with a fork DNA substrate. The complex reveals the molecular interactions for substrate recognition and provides structural insight into the catalytic mechanism. The duplex interacts with an extended loop of the HhH motif. The 5' overhang crosses over the active site through the central cleft of the protein. The 3' overhang reaches close to binding sites for the T4 32 protein and the T4 45 clamp, at the C- and N-termini respectively. The preferred cut site for T4 RNase H on this fork DNA is between the first two duplex nucleotides next to the 5' overhang.



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