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Structural Basis for Sequence-Dependent DNA Cleavage by the Non-Specific Endonuclease. W. Yang, Y. Wang, L. G. Doudeva, C.-L. Li, H. S. Yuan, Inst. of Molecular Biology, Academia Sinica, Taipei, Taiwan, ROC.

Non-specific endonucleases hydrolyze DNA without sequence specificity but with sequence preference that they cleave at some sites more efficiently than others. However, the structural basis for sequence-dependent cleavage by the non-specific endonucleases remained elusive. Here we use the non-specific endonucleases ColE7 to dissect this problem. DNA foot printing assays showed that the nuclease domain of ColE7 (N-ColE7) cleaves DNA with a preference for making nicks after (at 3'-O-side) thymine bases. The crystal structure of N-ColE7 (H545Q mutant) in complex with an 18-bp DNA was determined at a resolution of 2.8 Å. In the N-ColE7-DNA structure, a preferred thymine residue is located right before the scissile phosphate and the structure of this "preferred" complex was compared with the previously determined "non-preferred" complexes in which a guanine is located before the scissile phosphate. The structural comparison shows that the phosphate backbone in the "preferred" complex is distorted the most, leading to a shorter distance between the zinc ion and the scissile phosphate. This result suggests a general structural basis for the sequence-dependent DNA cleavage that the enzyme-induced DNA backbone conformational change is the local determinant for non-specific endonucleases to decide whether to cleave or not to cleave a DNA.