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Q.S. Xu, R.B. Kucera, R.J. Roberts, and H.-C. Guo, "An Asymmetric Complex of Restriction Endonuclease MspI on its Palindromic DNA Recognition Site," *Structure,* **12**, 1741-1747 (2004).

FUNDING
National Institutes of Health

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An Asymmetric Complex of Restriction Endonuclease MspI on its Palindromic DNA Recognition Site

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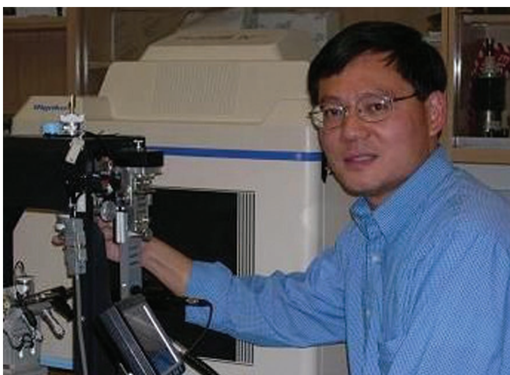
Most well-known restriction endonucleases recognize palindromic DNA sequences and are classified as Type IIP. Due to the recognition and cleavage symmetry, Type IIP enzymes are usually found to act as homodimers in forming two-fold symmetric enzyme-DNA complexes. Now we find an asymmetric complex of the Type IIP restriction enzyme MspI in complex with its cognate recognition sequence. Unlike any other type IIP enzyme reported to date, an MspI monomer and not a dimer binds to a palindromic DNA sequence. The enzyme makes specific contacts with all four base pairs in the recognition sequence. This MspI-DNA structure represents the first example of asymmetric recognition of a palindromic DNA sequence by two different structural motifs in one polypeptide.

Restriction endonucleases (REases) are enzymes that recognize specific DNA sequences and cleave at the sugar-phosphate backbone of DNA. They are components of restriction-modification systems that protect bacteria from invasive foreign DNA. However, REases are best known for their roles in recombinant DNA technology and genetic manipulation. More than 3,570 REases with 240 unique specificities have been biochemically characterized. Of the 588 commercially available REases, a great majority recognize double-strand DNA sequences of 4-8 base-pairs with a dyad axis of symmetry, called palindromes. In the presence of Mg^{2+}, these REases cleave both strands of the DNA at fixed symmetrical locations to generate cohesive or blunt ends suitable for molecular cloning. These symmetrically cleaving enzymes are grouped into a subtype, called Type IIP. Biochemical and structural evidence for several Type IIP prototype enzymes indicate that they form symmetric dimers or tetramers to recognize their palindromic recognition sequences. One of these Type IIP Reases, known as MspI, produces a different cleavage pattern from those of known structures. It recognizes the palindromic tetranucleotide sequence 5′-CCGG and cleaves between the first and second nucleotides, leaving two-base cohesive 5′ overhangs.



Authors (top): Dr. Qian Steven Xu and (bottom) Prof. Hwai-Chen Guo

We recently reported the crystal structure of an MspI-DNA complex at 1.95 Å resolution, using a combination of multiple isomorphous replacement with anomalous scattering (MIRAS) and multiwavelength anomalous diffraction (MAD) methods. This represents the first crystal structure of a restriction enzyme that recognizes a tetranucleotide sequence. Unexpectedly, MspI interacts with the palindromic recognition sequence as a monomer (**Figure 1**). As a result, two symmetric half-sites of DNA are recognized asymmetrically by the MspI monomer (**Figure 2**). Together, there are six direct and five water-mediated hydrogen bonds from the MspI monomer to the four base-pair recognition sequence, which nearly saturates the hydrogen-bonding potential in the major grove of the recognition site. In addition, the two dyad-related half-sites on DNA make asymmetric van der Waals contacts with different motifs in one MspI polypeptide. Although homodimers had been previously reported to bind pseudo-palindromic DNA sequences asymmetrically, the MspI-DNA complex represents the first structure of a palindromic DNA

sequence bound by an asymmetric protein monomer.

The observation of a monomeric MspI-DNA complex in crystals (where MspI is in 18 mM concentration) suggests that such a unique DNA recognition by MspI is likely to occur in an optimal reaction solution where MspI concentrations are much lower (in the sub-μM range). Since there is only one catalytic site in an MspI monomer, some kind of enzyme dimerization or flipping is necessary to cleave both strands of DNA at the recognition site. It is thus plausible that MspI binds to its recognition sequence through the monomer-DNA intermediate captured in the crystals, and then proceeds to a final homodimer-DNA complex (**Figure 3**) responsible for symmetrical cleavage of both strands of DNA. Alternatively, MspI may achieve double-strand DNA cleavage by bringing together two monomers bound on two separate recognition sites, similar to the model proposed for the Type IIS REase FokI that recognizes a specific asymmetric DNA sequence. A third possibility is that MspI undergoes some conformational rearrangements to use a single active site to sequentially cut both DNA strands, similar to that proposed for BfiI, another Type IIS REase.

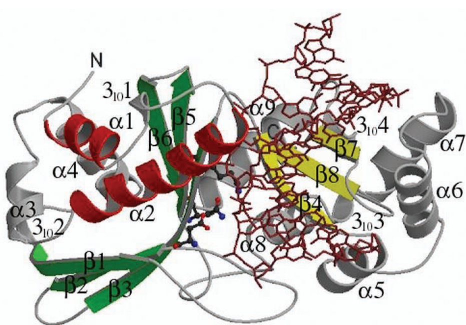


Figure 1. *The tertiary structure of the MspI-DNA complex. The enzyme is represented as ribbons and the DNA as a brown stick model. Secondary structure elements of MspI are labelled and the conserved structural core are colored in red for N-terminal helices, green for the five-stranded central β-sheet, and yellow for the β-sheet involved in DNA recognition. Side chains of the catalytic site residues (Asp99, Asn117, and Lys119) are shown as ball-and-stick representations with carbons in black, nitrogens in blue, and oxygens in red.*

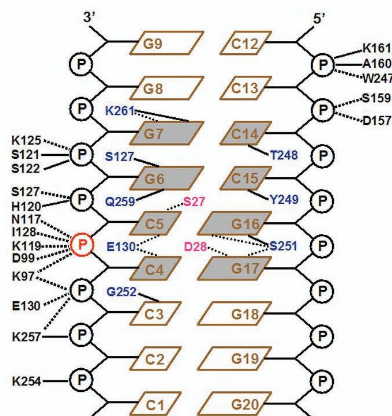


Figure 2. *Schematic diagram of hydrogen bonding between MspI and DNA. The DNA recognition sequence is shaded in gray, with the scissile phosphate (C4-C5) circled in red. One DNA base pair (G10:C11) is omitted in the final model. Blue and pink represent amino acids that bind to DNA bases in the major and minor groove, respectively. Amino acids that bind to the phosphate backbone of DNA are colored in black. Solid and dotted lines represent direct and water-mediated hydrogen bonds, respectively.*

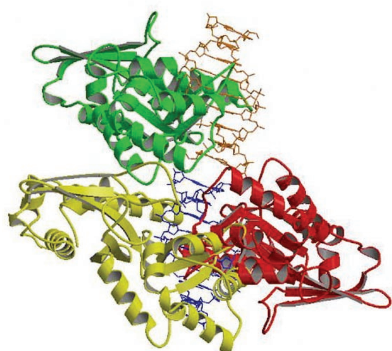


Figure 3. *The relationship between a putative MspI dimer and the crystallographic dimer in the MspI-DNA cocrystal. The enzymes are represented as ribbons and the DNAs as stick models. In the crystal, two MspI monomers (green and red) bind to two separate recognition sequences (brown and blue). To generate a putative dimer (gold and red), the red monomer was rotated 180º along the dyad axis (perpendicular to the paper plane) of the palindromic DNA (blue).*