Review

Restriction endonucleases that resemble a component of the bacterial DNA repair machinery

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\textbf{Abstract.} It has long been known that most Type II restriction endonucleases share a conserved core fold and similar active sites. The same core folding motif is also present in the MutH protein, a component of the bacterial DNA mismatch repair machinery. In contrast to most Type II restriction endonucleases, which assemble into functional dimers and catalyze double-strand breaks, MutH is a monomer and nicks hemimethylated DNA. Recent biochemical and crystallographic studies demonstrate that the restriction enzymes BcnI and MvaI share many additional features with MutH-like proteins, but not with most other restriction endonucleases. The structurally similar monomers all recognize approximately symmetric target sequences asymmetrically. Differential sensitivities to slight substrate asymmetries, which could be altered by protein engineering, determine whether the enzymes catalyze only single-strand nicks or double-strand breaks.

\textbf{Keywords.} Restriction endonuclease, DNA repair, crystal structure.

The superfamily of restriction endonuclease-like proteins is defined by a common core fold that includes a mixed \(\beta\) sheet and two helices on either side [1]. The superfamily is extremely diverse and includes various restriction endonuclease (REase) families, very short patch repair (Vsr) endonuclease, TnsA endonuclease, endonuclease I, archaeal Holliday junction resolvase and the DNA repair protein MutH [1]. The presence of the nuclease core fold in many Type II REases and MutH-type proteins has long been known [2–4]. Recent work has shown that a group of REases shares many common features with MutH-like proteins, but not with other REases [5–7]. MutH is part of a DNA mismatch repair protein complex and shows no catalytic activity alone, at least under physiological conditions [4]. In complex with its partner proteins, MutH is a nickase that cleaves only the unmethylated strand in hemimethylated DNA, its physiological substrate [4]. Biochemical and crystallographic studies indicate that MutH interacts with its target DNA as a monomer. Although the MutH target sequence GATC is palindromic, N6-methylation of the adenine in one strand breaks this symmetry (Fig. 1), so that MutH binds DNA only in one orientation and cleaves only one strand [3, 4].

In contrast to MutH, most Type II REases do not require the assistance of other proteins and act on
unmethylated DNA under physiological conditions [8]. By definition, Type II REases catalyze double-strand breaks. In many cases, the target sequences of Type II REases are symmetric (palindromic) or nearly symmetric (pseudopalindromic), and in almost all cases, the enzymes match the two-fold symmetry of their target sequences, either as dimers or as higher-order assemblies of dimers [8]. The presence of two active-sites makes the dimer a minimal functional unit which can cleave both strands in target DNA [9].

The neat distinction between monomeric MutH-type DNA repair enzymes that nick DNA and dimeric REases that catalyze a double-strand cut broke down when it was discovered that the restriction enzymes MspI [10] and HinP1I [11, 12] are monomers in solution. Various models have been considered to explain how these enzymes can nonetheless catalyze double-strand breaks: they might dimerize transiently or, alternatively, they might cleave the two DNA strands sequentially [10–12]. MspI and HinP1I are unusual REases in other ways as well: they are the first crystallographically characterized palindrome cutters that generate 2 nt 5\'-overhangs.

The distinction between the MutH-like nickases and REases has been blurred further by the recent biochemical and crystallographic characterization of the related REases BcnI (CC/SGG, S stands for C or G, \(\sim\) designates the cleavage position) and MvaI (CC/WGG, W stands for A or T) [6, 7]. These two endonucleases recognize similar 5 nt pseudopalindromic sequences and cleave them to generate single nt 5\'-overhangs [13]. MvaI cleaves the two strands of unmethylated DNA at different rates [14] and is exceedingly tolerant to DNA modifications, which often affect cleavage of the two DNA strands differentially [15–17]. These unusual results have been explained by the demonstration that MvaI, like HinP1I and MspI, is a monomer that recognizes its target sequence asymmetrically [6]. For BcnI, no detailed kinetic data have been published, but it was found that the enzyme is active as a monomer [7].

Remarkably, the similarity between the REases BcnI and MvaI on one side and the DNA repair protein MutH on the other side goes even further: crystallographic studies have confirmed the sequence based prediction that BcnI and MvaI are more similar to MutH than to any other crystallographically characterized REase [6, 7]. Moreover, the three enzymes share many additional features, including a two-lobed architecture, mobility of the hinge region that connects the lobes, active-sites that take their productive conformations only in the presence of metal and DNA, and very similar DNA-binding modes, despite unrelated target sequences.

Crystal structures of Escherichia coli MutH in the absence of DNA [2] and of Haemophilus influenzae MutH in the presence of unmethylated and hemimethylated DNA [3] have been known for several years. Crystal structures of BcnI and MvaI in the absence and presence of DNA have been determined very recently [6, 7]. In addition, an independent prediction of the structure of MvaI has appeared while the first version of this manuscript was under review [18]. Here, we take advantage of the new structural information to compare BcnI [7] and MvaI [6] with the DNA repair protein MutH [2, 3].

BcnI, MvaI and MutH are two-lobed monomers with a flexible hinge

BcnI, MvaI and MutH are all monomeric and share a characteristic two-lobed structure (Fig. 2). In the case of MutH, the two lobes have been termed the ‘N-arm’ and the ‘C-arm’ of the protein [2]. This terminology is unfortunate, because the BcnI and MvaI counterparts of the MutH N-arm contain structural elements from both the N- and C-termini of the proteins. Therefore, we prefer the alternative terms ‘catalytic lobe’ and ‘recognition lobe’, which reflect the functional role of the lobes (see below) and are applicable in all cases. For all three enzymes, there is strong evidence that the hinge is highly mobile (Fig. 2). In the absence of DNA, the angles between the catalytic and the recognition lobes are very different for the different enzymes. The most extreme case is the very ‘open’ MvaI conformation that was trapped in crystals without DNA. Although hard evidence is lacking for BcnI and MvaI, it is probable that the detailed conformations are dictated by crystal contacts, so that the apo-structures alone tell only that the hinge regions are flexible. In the presence of DNA, all enzymes take very similar conformations, which appear to be dictated by the interactions with DNA. It is therefore tempting to speculate that all three enzymes can act as ‘clamps’ for DNA.