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Review

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

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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.

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 β 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

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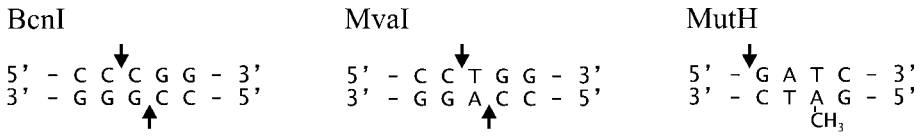

Figure 1. Specificities and activities. The cleavage sites are marked by arrows. MutH cleaves only the unmethylated strand in hemimethylated DNA.

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, '/' 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 unmodified 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.

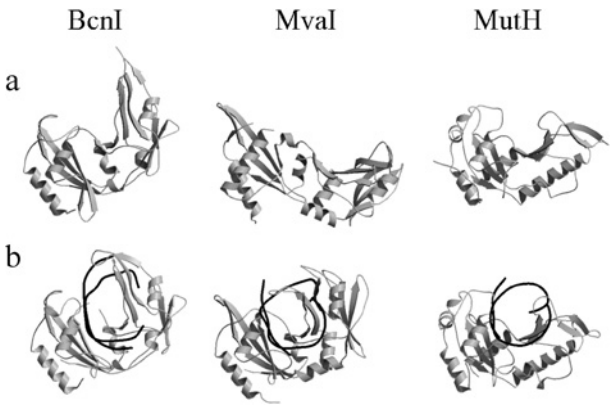

Figure 2. BcnI, MvaI and MutH are two-lobed structures with a flexible hinge**.** The top and bottom rows show crystal structures in the absence (*a*) and presence (*b*) of DNA. The MutH structure without DNA has been determined for the *E. coli* enzyme, but the structure with DNA has been solved for the (very similar) *H. influenzae* enzyme. Structures with DNA were oriented by superimposing nucleic acid residues around the scissile phosphoester bond. Structures without DNA were then positioned by superimposing the catalytic lobes with their counterparts in the structures with DNA. In the orientation presented here, the catalytic lobes are on the left and the recognition lobes are on the right. Coordinates were taken from PDB files with the following accession codes: 2ODH (apo-BcnI), 2OA9 (apo-MvaI), 2AZO (apo-MutH), 2ODI (BcnI-DNA complex), 2OAA (MvaI-DNA complex), 2AOR (MutH-DNA complex).

Catalytic lobes

BcnI and MvaI are structurally very similar throughout, but the similarity to MutH is mostly concentrated in the catalytic lobe, which can be described as the core nuclease fold with a few extra decorations (Fig. 3). Structural superposition of the DNA-bound forms of the catalytic lobes shows that the most conserved regions anchor the active-site residues (Fig. 3). Interestingly, the locations of the active-site aspartates (BcnI Asp55, MvaI Asp50 and MutH Asp70) superimpose only in the DNA-bound conformations, but vary drastically in the apo-structures (Fig. 4). At least in the cases of BcnI and MvaI, it is not clear whether the unproductive active-site loop conformations are due to a lack of divalent metal ions or to a lack of DNA, but either way they suggest that there is substantial flexibility in the active-site.

In their productive conformations, the active-site loops of BcnI, MvaI and MutH bind two divalent metal ions. Protein-DNA cocrystals were grown in the presence of Ca^{2+} ions, which do not support catalysis, and thus the structurally characterized complexes resemble enzyme-substrate complexes [3, 6, 7]. In all

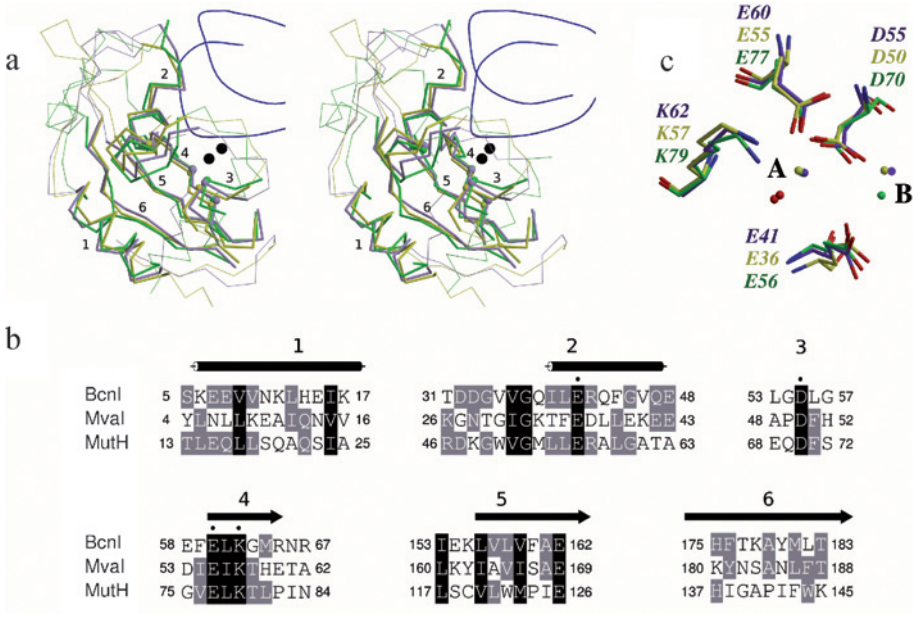

Figure 3. Superposition of BcnI (purple), MvaI (yellow) and MutH (green). (*a*) Superposition of the catalytic lobes. Equivalent regions were determined with the program STRUPRO [27] (equivalence distance to 3.5 Å, extension distance to 4.5 Å, minimal fragment length 5 residues) and are numbered. Purple dots mark the locations of BcnI active-site residues and black balls stand for metal ions. (*b*) Alignment of the structurally most conserved regions. The numbers above the secondary structure elements refer to panel *A*. Dots mark active-site residues. (*c*) Superposition of active-site residues in the DNA-bound conformation. Metal ions are represented by balls in the color of the enzymes. Nucleophilic water molecules are shown as red balls.

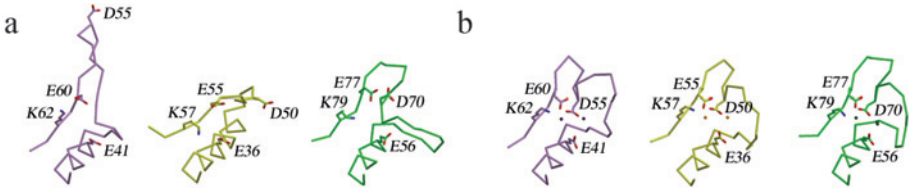

Figure 4. Conformational change in the active-site regions. (*a*) Conformations of BcnI (purple), MvaI (yellow) and MutH (green) in the absence of DNA. (*b*) Conformations of BcnI (purple), MvaI (yellow) and MutH (green) in the presence of their target DNAs.

structures, one metal ion (A) is much better ordered than the other metal ion (B) (Fig. 3c). The better-ordered metal ion A is hexa-coordinated in all structures and is located at the center of an almost perfect octahedral coordination sphere. In all cases, ligands for the metal ion A are a phosphate oxygen atom of the scissile phosphate, a carboxylate oxygen atom of the conserved aspartate, a carboxylate oxygen atom of the glutamate residue from the EXK sequence motif, a main-chain carbonyl oxygen atom and two water molecules. In all enzyme-substrate structures, one of these water molecules is ideally positioned for in-line nucleophilic attack on the scissile phosphate, but is slightly too far away from the phosphorus atom for the attack to actually proceed in the crystal.

Recognition lobes

As expected from the sequence similarity, the recognition lobes of BcnI and MvaI are very similar and can be approximately described as two β sheets that are stacked against each other to 'almost' form a barrel. The MutH recognition lobe is far more irregular and quite different, despite the presence of some common elements, especially in the region that contacts DNA. In all structures, the recognition lobes are built from two continuous stretches of the linear amino acid sequence, but the length of the two regions varies widely between the REases and MutH. In MutH, the first part is short and comprises only ~28 residues. The corresponding region is expanded by a helix and a β meander to ~85 and ~97 residues in BcnI and MvaI, respectively. In contrast, the second part of the recognition lobe is shorter in the REases than in MutH, in part because the very C-terminus of the protein belongs to the recognition lobe only in MutH (Fig. 5).

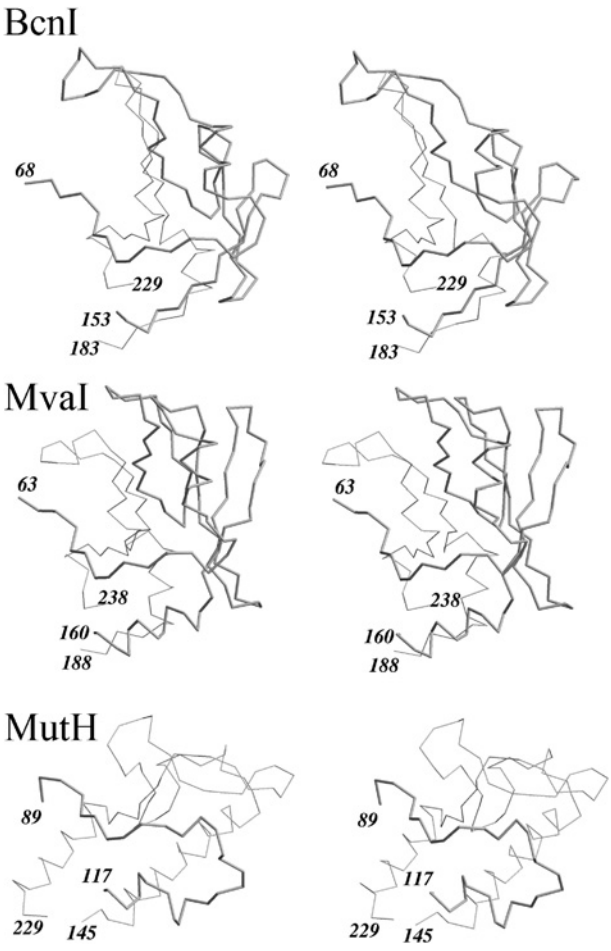

Figure 5. Recognition lobes. The recognition lobes of BcnI, MvaI and MutH are built from two regions of the linear sequence. The Cα traces of the N-terminal and C-terminal parts of the recognition lobes are represented by thick and thin lines, respectively.

DNA binding

The DNA binds analogously to the BcnI, MvaI and MutH monomers. In all cases, the catalytic lobes approach the DNA from the minor groove sides, whereas the recognition lobes interact with the DNA from the major groove sides. This arrangement makes sense, because an unambiguous discrimination of all possible base pairs based on the hydrogen-bonding pattern alone is only possible from the major groove side [19]. Only one DNA strand comes close to the single active-site in the monomer. In line with the nomenclature of a recent bioinformatics paper [18], we refer to this strand as the 'proximal' strand and to the complementary strand as the 'distal' strand. As BcnI catalyzes double strand breaks, the binding modes with central 'C' in the proximal position and with the central 'G' in the proximal position must both occur in solution. Similarly, MvaI must be able to accommodate either the strand with central 'T' (T strand) or the strand with the central 'A' (A strand) proximally. Surprisingly, only the binding modes with proximal pyrimidines have been observed in the cocrystals of BcnI and MvaI with cognate oligoduplexes.

Nomenclature for monomeric enzyme-DNA interactions

For most comparisons of restriction enzyme specificities, the natural reference point is the center of symmetry of the palindromic or pseudopalindromic recognition sequences. Here, this choice is unsuitable,

because the center of (approximate) symmetry is at a different position with respect to the cleavage site in the BcnI/MvaI REases and in the MutH nickase (Fig. 1). In order to name base pairs in equivalent locations consistently, we suggest a nomenclature that is modeled on the Schechter and Berger nomenclature [20] for peptidase substrates. We designate nucleotides in the proximal strand by 'P' and count them from the cleavage site, using primed symbols when proceeding in a 5′-3′ direction (P1′, P2′, P3′, etc) and non-primed symbols when proceeding in the 3′-5′ direction (P1, P2, P3, etc). Nucleotides of the distal strand are designated by the letter 'D' and numbered according to the nucleotides which they interact with (e.g. P1-D1, P1′-D1′ are base paired) (Fig. 6).

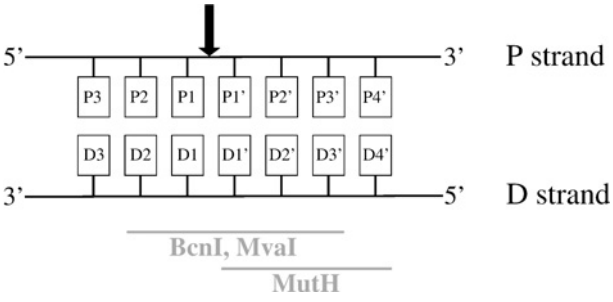

Figure 6. Nomenclature for the DNA substrates of monomeric restriction endonucleases and nicking enzymes. The abbreviations 'P' and 'D' stand for 'proximal' and 'distal' and distinguish the DNA strands by their distance from the active-site. Nucleotides of the P strand are counted starting at the cleavage site, using primed symbols when proceeding in a 5′-3′ direction (P1′, P2′, P3′, etc.) and non-primed symbols when proceeding in the 3′-5′ direction (P1, P2, P3, etc.). Nucleotides of the D strand are named according to the P strand nucleotides which they interact with (e.g. P1-D1, P1′-D1′ are base paired).

Detailed protein-DNA interactions

Hydrogen-bonding interactions of BcnI, MvaI and MutH are mostly limited to the specifically recognized bases. In the case of BcnI and MvaI, these are the P2-D2 to P3′-D3′ base pairs. MutH recognizes the P1′-D1′ to P4′-D4′ base pairs (Fig. 7).

As BcnI (CC/SGG) and MvaI (CC/WGG) are similar and recognize related sequences, one might have expected that they recognize equivalent bases analogously, and indeed, there are many interactions that are conserved. However, there is also substantial 'drift', which might be neutral, but might also indirectly affect the specificity for the central base pair. On the major groove side, only the P2-D2 makes exactly equivalent interactions with BcnI and MvaI. In the P1-D1 position, Arg216 in BcnI plays the role of His225 in MvaI, and the Nε atom of its guanidino group plays the role of the Nδ atom of His225 in the

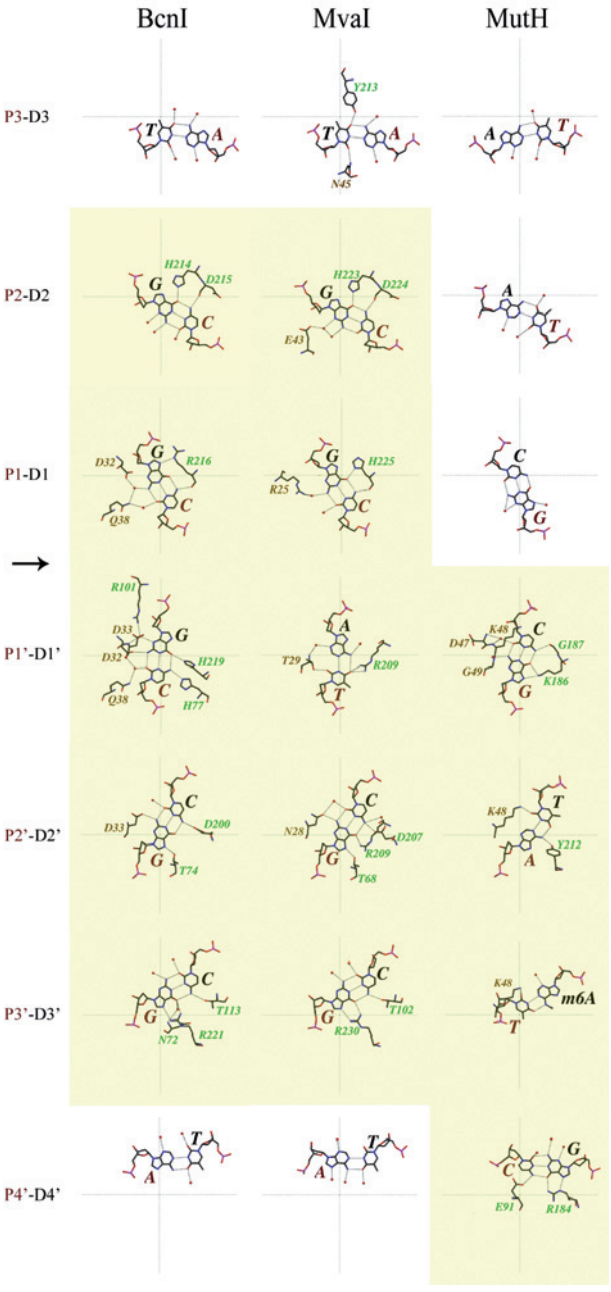

Figure 7. Specific interactions with the bases. Hydrogen bond interactions in the planes of the bases are indicated by dotted lines. P strand bases are labeled in brown and D strand bases are labeled in black (all in single-letter code). Amino acids of the recognition lobe are shown in green, and amino acids of the catalytic lobe are drawn in brown. The faint yellow background marks base pairs that are part of the recognition sequence. Note that van der Waals interactions and indirect hydrogen-bonding interactions via more than one water molecule are not shown in the figure.

MvaI-DNA complex. In the P1′-D1′ and P2′-D2′ positions, BcnI misses an equivalent for Arg209 in MvaI. BcnI and MvaI have different specificities for the central base pair (C-G in the case of BcnI, T-A in the case of MvaI). Therefore, it does not come as a

surprise that interactions with this base pair (P1′-D1′) are not conserved. Note that BcnI and MvaI must also be able to accommodate the alternative binding mode, which switches the roles of purine and pyrimidine. On the minor groove side, most residues form indirect, water-mediated hydrogen bonds with the bases that are not conserved between BcnI and MvaI. Ordered water molecules are often found in equivalent places, but are anchored by different residues in the BcnI- and MvaI-DNA complexes. Such changes are found in the P2-D2, P1-D1, P1′-D1′ and P2′-D2′ positions of the DNA. Moreover, MvaI is engaged in two direct hydrogen bonds with the P3-D3 base pair outside the recognition site, while BcnI does not form any confidently detected direct hydrogen-bonding interactions with DNA outside its target sequence.

The specificities of the BcnI/MvaI REases and of MutH are unrelated. Therefore and because of the substantial differences in the architecture of the recognition lobes of BcnI, MvaI and MutH, these enzymes use entirely different machineries for sequence discrimination, even for the positions P1′-D1′, P2′-D2′ and P3′-D3′, which make specific contacts with the BcnI/MvaI REases and with MutH (Fig. 7).

Differences between BcnI/MvaI and MutH

Detailed comparison of BcnI, MvaI and MutH highlights the similarities between these enzymes. In all cases, approximately symmetric target sequences are recognized asymmetrically. Whether the enzymes nick a single strand or cleave both strands depends on their sensitivities to the slight asymmetries in their substrates.

The physiological substrates of BcnI and MvaI are unmethylated DNA duplexes with recognition sequences that are symmetric except at the central base pair [21, 22]. The enzymes are apparently not very sensitive to this asymmetry and can accept either strand in the proximal or the distal position, although not necessarily with equal efficiency [16]. Methylation that introduces extra asymmetry into the substrate can break the degeneracy. In the case of MvaI, it has been shown experimentally that N6-methylation of the central adenine interferes with cleavage of the methylated strand, but not with cleavage of the unmethylated strand [15]. Similar effects have been observed with N4-methylation of the outer cytosine residue, which also protects the methylated strand, but not the unmethylated strand, from cleavage [15]. Hence, MvaI acts as a nickase at least on some substrates. Alterations of the recognition machinery (Arg209) for the central base pair might convert MvaI into a nickase for unmethylated DNA. The wild-type MvaI does not cleave either strand in hemimethylated DNA with a single N4-methylated inner cytosine, apparently because a methyl group in the proximal strand would clash with a carbonyl carbon atom of His225 and a methyl group in the distal strand would bump into the side chain of Asp207. It would be interesting to test whether mutation of Asp207 might convert MvaI into a nickase for hemimethylated DNA with a single N4-methylated inner cytosine.

The MutH target sequence (GATC) is fully palindromic, but hemimethylation introduces a slight asymmetry into the physiological MutH substrate. The enzyme is very sensitive to this slight asymmetry, because it nicks the unmethylated (recently replicated daughter) strand but does not cleave the methylated (original parental) strand [4]. In structural terms, this means that the unmethylated strand always binds proximally, and the methylated strand always binds distally to the MutH active-site [3]. This propensity could indicate that a methyl group is required distally for binding or rejected proximally due to a clash with the enzyme. Both effects probably contribute, but the latter seems to predominate. DNA-binding tests show that MutH does not bind fully methylated DNA, but has almost as high affinity for unmethylated as for hemimethylated DNA [3]. Moreover, cleavage assays indicate that MutH nicks hemimethylated DNA between 5- and 20-fold faster than unmethylated DNA [23] and much faster than fully methylated DNA, which is a very poor substrate [3, 4, 23]. Together, these data suggest that MutH cannot catalyze double-strand breaks, because it cannot accept N6-methyladenine in the proximal strand. If this is the case, MutH should be able to introduce double-strand breaks in unmethylated DNA with the GATC recognition sequence. Although it was concluded from early work that such substrates are usually subject to scission of only one strand [4], later work clearly shows that MutH can also introduce double strand breaks in unmethylated DNA [23]. It remains unclear whether MutH acts processively in such cases, or whether the enzyme cleaves one strand, dissociates, and then later rebinds to cleave the complimentary strand. Cleavage of both strands of hemimethylated DNA or of symmetric, doubly methylated DNA would require a variant of MutH that would tolerate N6-adenine methylation in the proximal strand. Modeling this extra methyl group into the complex of MutH with hemimethylated DNA [3] (without any adjustments to the DNA-binding mode or conformation) reveals a major clash with Tyr212 (and a close contact with a lysine residue). This tyrosine residue has been mutated to serine, resulting in a MutH variant that can cleave fully methylated DNA at a rate comparable to that at which it cleaves

unmethylated DNA [23, 24]. Moreover, experiments show that the mutant enzyme can introduce not only nicks, but also double-strand breaks in the DNA [23, 24].

A more efficient solution to the engineering problem to convert MutH into an REase has already been found by nature. Sau3AI introduces a double-strand break into DNA with the recognition sequence/GATC. The enzyme comprises two MutH-like domains within a single polypeptide chain [25]. Crystallographic information on Sau3AI is not yet available, but the crystallization and preliminary X-ray analysis of the Sau3AI C-terminal fragment have been reported recently [26].

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