DNA-repair enzymes

Dmitry G Vassylyev* and Kosuke Morikawa†

Recent crystallographic studies of DNA-repair enzymes have provided the structural basis for the recognition of damaged DNA. The results imply that flipping out of the base is a common and crucial event in DNA repair. Two classes of repair enzymes that recognize distinct types of damage may exist. DNA-repair enzymes that share similar folds and DNA binding motifs have been proposed to belong to a superfamily.

Addresses
*International Institute for Advanced Research, Central Research Laboratories, Matsushita Electric Industrial, 3-4 Hikari-dai, Seika, Kyoto 619-02, Japan; e-mail: dmitry@crl.mei.co.jp
†Biomolecular Engineering Research Institute 6-2-3, Furuedai, Suita, Osaka 565, Japan; e-mail: morikawa@beri.co.jp

Abbreviations

3D	three-dimensional
AP	apurinic–apyrimidinic
BER	base excision repair
CMP	cytidine monophosphate
DR	direct reversal
ds	double-stranded
endo	endonuclease
FCL	[4Fe–4S] cluster loop
HhH	helix-hairpin-helix
HSV	herpes simplex virus
hUDG	human UDG
MMR	mismatch repair
NER	nucleotide excision repair
PD	pyrimidine dimer
PHL	DNA photolyase
ss	single-stranded
TBP	TATA box binding protein
UDG	uracil-DNA glycosylase
UGI	uracil glycosylase inhibitor

Introduction

Genetic information preserved in DNA is used by all living organisms to ensure their normal function and development. In contrast with globular proteins, DNA molecules are extremely thin and long, so that even the bases are exposed to the solvent. DNA, therefore, is easily modified and damaged by various chemical agents and different types of radiation to yield mutagenic lesions [1]. Mutagenesis of DNA also results in the spontaneous formation of mismatched base pairs in the genome during replication [1]. If not repaired, these DNA lesions disturb the genomic integrity and could consequently become lethal to living cells. All organisms, therefore, contain various repair mechanisms that remove the improper nucleotides from DNA and replace them with the correct ones.

DNA-repair enzymes recognize and excise the damaged portions of the DNA strand. This major step is carried out by a large variety of enzymes or multienzyme complexes, which can be classified according to the different lesions and the mechanisms of repair. In the base excision repair (BER) pathway [1], DNA glycosylases directly excise the modified-base moieties from the DNA to produce apurinic–apyrimidinic (AP) sites, and the phosphate backbone at the abasic site is subsequently cleaved by the actions of AP-endonucleases. Enzymes involved in the direct reversal (DR) pathway [1] catalyze the reverse reaction to the one that originally caused the base modification; thus, excision is not required. Nucleotide excision repair (NER) is the most important and universal DNA-repair mechanism in living organisms [1,2], and it allows the excision of almost all kinds of DNA lesions relevant to chemical modification. NER removes a segment of the DNA strand including the damaged base and several nucleotides on either side of the lesion. Enzymes involved in the mismatch repair (MMR) system have a similar mechanism of action to that of the BER and NER systems [3,4]. The major distinction of MMR from the other repair systems is that it can discriminate an improper base between two normal nucleotides forming a mismatched base pair.

DNA-repair systems are highly conserved among different species, ranging from prokaryotes to eukaryotes. Malfunctional DNA-repair mechanisms often cause cancer and, in most cases, are lethal to organisms [2,3]. Thus, the structural study of DNA repair is one of the most important areas, aiding cell biology and the medical sciences. Successful crystallographic studies of several DNA-repair enzymes in the past two years have greatly contributed to the understanding of how damaged DNA is recognized and excised at the molecular level. It would be impossible to describe all of the aspects of DNA repair available from recent structural studies; therefore, this review focuses on the mechanism by which repair enzymes bind specifically to damaged DNA and comments on the general view of damage recognition.

Endonuclease V

Endonuclease (endo) V from bacteriophage T4 has high affinity for both cyclobutane-type pyrimidine dimers (PDs) and AP sites within DNA and possesses two distinct catalytic activities, PD N-glycosylase and AP-lyase [5]. The crystal structure of this enzyme (from the inactive mutant, Glu23→Gln) in complex with a DNA duplex containing a PD reveals a strikingly deformed DNA conformation [6••], although the protein conformation remains unchanged (Fig. 1) [7]. The DNA in the complex kinks sharply at the position of the PD. The adenine base complementary to the 5' moiety of the PD is completely

'flipped out' from the DNA; it is accommodated into a cavity on the surface of the enzyme by weak and nonspecific van der Waals interactions. The flipping out of the base opens up free space inside the DNA for the catalytic residues of the enzyme to access their target. Thus, this extrahelical base seems to be required for facilitation of the enzymatic reaction, rather than for specific damage recognition. The other surprising feature of the structure is that specific binding to the PD occurs through an 'indirect readout' mechanism. Endo V makes no direct interactions with the modified moiety of the PD bases. Instead, the deformed DNA backbone in the close vicinity of the lesion is recognized using a number of basic protein residues.

Figure 1

Ribbon model of T4 endo V in complex with a 13-mer oligonucleotide duplex containing a PD (α helices and loop regions are shown in red and green, respectively). The trace of the phosphate backbone is shown in cyan, the DNA bases are shown in yellow, and the thymine dimer and the flipped-out base are highlighted in white. Figure generated using the program O [48].

We believe that the unusual DNA conformation observed in the endo V–DNA complex is induced by the enzyme upon binding, as the enzyme-free DNA duplexes containing a PD do not have such striking deformations of the DNA [8–11].

Endonuclease III

Endo III from *Escherichia coli* has a very broad substrate specificity for various oxidized pyrimidine derivatives within the DNA [12–17]. It possesses N-glycosylase and AP-lyase activities similar to those of endo V [18]. In combination with mutational analyses, the 1.8 Å resolution structure of this enzyme [19••] revealed not only the catalytic site and the DNA-binding interface, but also two novel DNA-binding motifs (Fig. 2). The protruding [4Fe–4S] cluster loop (FCL) and the helix-hairpin-helix

(HhH) motif, previously identified as the binding site of thymine glycol [20], were shown to participate in DNA binding. The HhH motif contains a putative catalytic residue, Lys120, which lies near the deep cleft formed between two domains. Another catalytic residue, Asp138, is completely buried within this cleft. Thus, the global view of the binding cavity suggests that the damaged base should be extrahelical upon the binding of endo III to the damaged DNA duplexes.

Figure 2
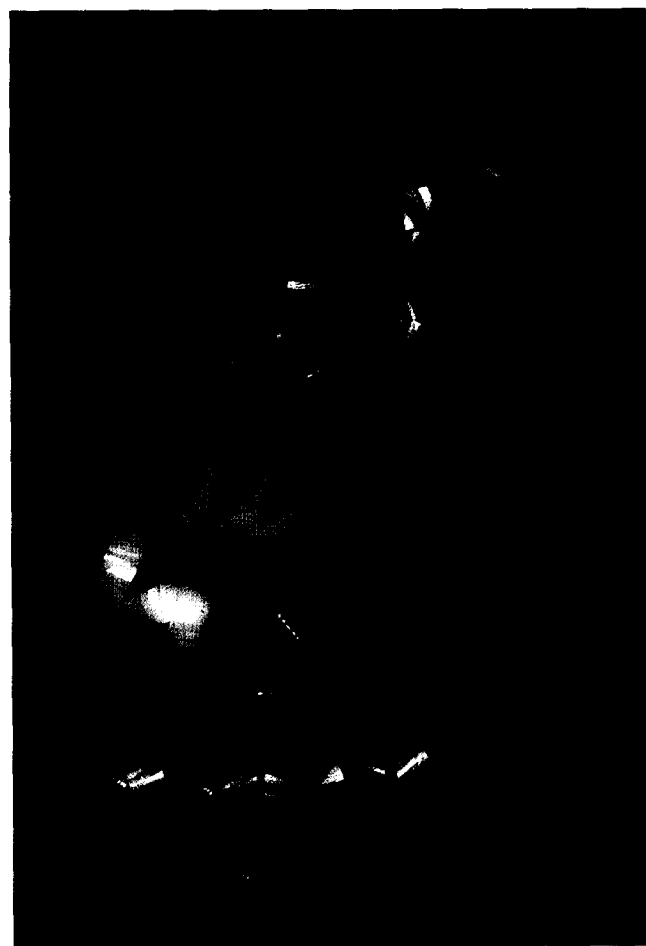
Overview of the endo III structure. The two major domains of the enzyme are shown in green and red, the HhH motif is shown in yellow, and the FCL loop and [4Fe–4S] cluster are shown in magenta and cyan, respectively. Figure generated using the program O [48].

3-Methyladenine DNA glycosylase II (AlkA)

3-Methyladenine DNA glycosylase II (AlkA) is an *E. coli* monofunctional enzyme, that exhibits only N-glycosylase activity and broad substrate specificity for alkylated DNA [21–26]. Crystallographic studies of this enzyme [27••,28••] show that it folds into a compact globular structure, consisting of three domains that have approximately equal sizes (Fig. 3). Domain I is topologically identical to

one half of the DNA-binding domain of the TATA box binding protein (TBP) [29]. The folds and the arrangements of domains II and III have high structural similarity to endo III, although no significant homology in the sequence is present. The endo-III-like domains of AlkA are directly involved in DNA binding and catalysis, whereas the biological function of the TBP-like domain is not clear. AlkA lacks the [4Fe–4S] cluster and therefore the FCL DNA-binding motif that was found in endo III. The HhH motif, however, is highly conserved both in conformation and in sequence across the two proteins. There is no lysine residue which is crucial for the AP-lyase activity in endo III inside the HhH motif of AlkA, which implies that the HhH motif is more important for the recognition and binding of damaged DNA than for catalysis. In AlkA, a catalytic residue, Asp238, projects into the cleft between the two endo-III-type domains, as Asp138 in endo III does. This also suggests that the damaged base would be flipped out of the DNA duplex upon binding to the protein.

Endonuclease III superfamily

The structure of the 26 kDa catalytic domain of the MutY protein, the DNA-glycosylase which possesses a BER mechanism against adenine bases in A–G mispairs (MMR) [30,31], has recently been solved (John Tainer, personal communication) and was found to be very similar to that of endo III, including the FCL and HhH motifs. A high level of structural similarity is observed between endo III and AlkA, whereas the sequence homology is limited to only the HhH motif and the adjacent active-site loop. Thus, the presence of this structural homology in a number of other BER enzymes (e.g. yeast 8-oxoguanine DNA glycosylase [Ogg1], PD specific endonuclease from *Micrococcus luteus*) provides evidence for the existence of an endo III superfamily, which adopts a common fold but which has extremely broad substrate specificity [32,33•]. Notably, the HhH motif was also observed in the NER enzymes [34], which highlights its general significance for recognition of damaged DNA.

Uracil-DNA glycosylase

Uracil-DNA glycosylase (UDG) has a N-glycosylase activity that is highly specific to the uracil base [35] and its analogous derivative, hydroxy-2'-deoxyuridine [17] within the DNA. Two crystal structures of UDG, herpes simplex virus type-1 (HSV-1) and human (h), have recently been reported with respect to both the free-enzyme form and the enzyme in complex with either a trinucleotide and uracil (for HSV-1UDG) or 6-aminouracil (for hUDG) [36•,37•]. In agreement with the 39% sequence similarity between the two enzymes, both structures have a similar fold and have a remarkably conserved 3D arrangement of the uracil-binding pocket. As the uracil base is completely buried inside the pocket, it must flip out from DNA duplexes during complex formation. The specific hydrogen bond network between the protein and the

Figure 3

Ribbon diagram of the AlkA DNA glycosylase structure, shown at the same orientation as endo III in Figure 2 after superposition of the two proteins by the conserved HhH motifs (shown in yellow). The TBP-like domain I is shown in cyan, the two endo III-type domains (II and III) are shown in green and red, respectively, to emphasize their similarity to the endo III fold (Fig. 2). Figure generated using the program O [48].

uracil allows UDG to discriminate uracil from structurally similar cytosine, while the steric collision with protein residues prevents binding of the thymine to UDG.

The crystallographic studies of UDG were expanded to include structural determinations of HSV-1UDG and hUDG in complex with a protein inhibitor, UGI [38•,39•]. These structures revealed that the negatively charged residues of UGI bind to a positively charged DNA-binding groove in UDG and mimic the interactions between UDG and the phosphate backbone of the DNA. Leu272, in a protruding loop of hUDG, is packed into the hydrophobic pocket at the center of the negatively charged surface of UGI. Leu272 was proposed to penetrate into the dsDNA at the position of the uracil base, thereby facilitating the flipping-out of the base.

Figure 4

Overview of hUDG–DNA complex shown along the helical axis of dsDNA. The protein is represented by a ribbon model (α helices and β strands are shown in blue and orange, respectively). The DNA is shown as a ball-and-stick model. The uracil base (shown in white) in the complex is extrahelical, and is cleaved from its sugar moiety (also shown in white). The sidechains of Arg272, His268, Asn204, and Asn145 (shown as yellow ball-and-stick models) surround the flipped-out nucleotide. Reproduced with permission from [40**].

Figure 5

Overall structure of exo III represented by a ribbon model. The α helices, β strands and loops are shown in blue, orange and magenta, respectively. The magnesium ion is represented by a green ball. Figure kindly provided by Cliff Mol and John Tainer.

Very recently, John Tainer and coworkers [40**] have succeeded in solving the structure of a hUDG–dsDNA com-plex using a double mutant, Leu272→Arg/Asp145→Asn, of hUDG. The DNA in this complex essentially adopts the normal B-form (15°bend) with the local strand separation on the 5'-side to the uracil and the uracil base flipped-out (Fig. 4). The DNA conformation is very similar to that observed in the HhaI methyltransferase–DNA complex [41]. The sidechain of Arg272 is inserted into the abasic site in place of uracil, which is in good agreement with the role of Leu272 predicted for the wild-type enzyme. The nonspecific interactions between the protein and the DNA phosphate backbone are found within a very limited region in one strand only around the lesion. This observation suggests how UDG may bind to both dsDNA and ssDNA in a similar manner.

DNA photolyase

DNA photolyase (PHL) is a unique DNA-repair enzyme with a narrow spectrum of specificity for the cyclobutane-type PD. In contrast to endo V, which repairs the PD using the BER mechanism, PHL uses the DR pathway, reverting the PD to normal pyrimidines by splitting the cyclobutane ring using photoreactivation [42].

The molecular structure of PHL from *E. coli* in complex with two organic cofactors has been solved [43**]. PHL consists of two major (N- and C-terminal) domains. The PD-binding site is located in a hole, which lies at the center of the C-terminal domain in close proximity to the catalytic cofactor. The proposed mode of PD binding, in which the lesion is accommodated deeply within this hole,

provides evidence that the PD must be flipped out of the dsDNA.

Exonuclease III

Exo III is a major AP endonuclease in *E. coli,* and it possesses a number of additional catalytic activities against undamaged DNA and RNA substrates [44]. In terms of DNA repair, however, exo III is only highly specific to AP sites in the DNA. The enzymatic activities of exo III require a single divalent-metal ion. It was not surprising, therefore, that the 3D structure of this enzyme [45••] revealed a fold similar to that found in a number of other metal-dependent DNA-processing enzymes (e.g *E. coli* RuvC resolvase, RNase H, HIV-1, integrase) [46]. The crystal structure of exo III in complex with Mn^{2+} and dCMP determined the active site of the enzyme (Fig. 5). The arrangement of the residues in the vicinity of the active site raises the possibility that the DNA base opposite the AP site may be flipped out of the double helix and bound to the enzyme, in a manner similar to that observed in the endo V–DNA complex.

Conclusions

The crystal structures of endo V in complex with a dsDNA substrate and the UDG–dsDNA complex revealed three possible steps of damage recognition: first, protein-induced DNA kinking around a lesion; second, indirect readout of the target damage via the deformed phosphate backbone; and third, flipping out the base from the DNA. The first two steps are inherent only in endo V and seem to be most important for the enzyme to distinguish damaged DNA from normal DNA. The third step is important in the mechanisms of both enzymes. The flipping out of the base and its subsequent accommodation into a highly specific cavity on the protein surface seem to be the major factors used by UDG to discriminate damage. Endo V probably uses the flipping of the base to achieve effective catalysis, rather than to enhance specific recognition.

Why do endo V and UDG, both DNA N-glycosylases, recognize their substrates buried within the DNA in such different manners? One reason may be the difference in the specificities of these enzymes. Endo V recognizes both PD and AP sites with almost the same affinity, although the two types of damage are very different in terms of their 3D architectures. Moreover, the different violations of the intact DNA double helix associated with these lesions are likely to provide two distinct local conformations of DNA in the absence of the enzymes. Thus, in principle, endo V should have two different modes for DNA recognition and binding for the modified bases. For endo V, the induced-fit conformational change of the DNA, once bound to the enzyme, and the indirect readout of the lesion form a very elegant solution to this problem. In contrast, UDG has a very specific affinity for uracil bases only, and, like many other sequence-specific DNA-binding proteins, this enzyme essentially recognizes B-form DNA. The outward resemblance of uracil to other bases, however, requires it to be flipped out to enable accurate identification by the enzyme.

These two distinct recognition mechanisms may be used to classify other DNA-repair enzymes with high and broad substrate specificities into two groups (Table 1). It is worthwhile mentioning that all of the enzymes containing the HhH motif belong to the endo V class (Table 1), and hence damage recognition should be associated with enzyme-induced DNA deformations. Thus, the HhH motif may have a role as a universal 'DNA deformation' motif for DNA-repair enzymes that have broad specificity.

As DNA-repair enzymes may induce substantial deformations of the cognate DNA, structural studies of the protein–DNA complexes will be crucial for a full understanding of DNA repair mechanisms. The design of substrate analogs for DNA repair [47] should facilitate successful crystallization of the enzyme–DNA complexes.

Table 1

Recognition of damaged DNA by DNA-repair enzymes.

Enzyme	Mechanism	Target DNA	Specificity	HhH motif	Possible recognition event			Classification
					Induced-fit of DNA	Indirect readout	Flipped-out base	
Endo V	BER	ds	Broad	No	Yes	Yes	Yes	Endo V
Endo III	BER	ds	Broad	Yes	Yes	Yes	Yes	Endo V
AlkA	BER	ds	Broad	Yes	Yes	Yes	Yes	Endo V
MutY	BER/MMR	ds	Broad	Yes	Yes	Yes	Yes	Endo V
UvrABC	NER	ds	Broad	Yes	Yes	Yes	?	Endo V
ERCC1	NER	ds	Broad	Yes	Yes	Yes	?	Endo V
MutHLS	MMR	ds	Broad	No	Yes	Yes	?	Endo V
UDG	BER	ds/ss	High	No	No	No	Yes	UDG
PHL	DR	ds/ss	High	No	?	No	Yes	UDG
ADA*	DR	ds	High	No	?	No	Yes	UDG
Exo III	BER	ds	High	No	?	No	Yes	UDG

*O^6-methylguanine-DNA methyltranserase [49].

Acknowledgements

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- •   of special interest
- ••   of outstanding interest

1.   Friedberg EC, Walker GC, Siede W: **Spontaneous alterations of and damage to DNA**. In *DNA Repair and Mutagenesis*. Washington DC: ASM Press; 1995:2–19.

2.   Lehman AR: **Nucleotide excision repair and the link with transcription**. *Trends Biochem Sci* 1995, 20:402–405.

3.   Kolodner RD: **Mismatch repair: mechanisms and relationship to cancer susceptibility**. *Trends Biochem Sci* 1995, 20:397–401.

4.   Friedberg EC, Walker GC, Siede W: **Mismatch repair**. In *DNA Repair and Mutagenesis*. Washington: ASM Press; 1995:367–405.

5.   Latham KA, Lloyd RS: **T4 endonuclease V: perspectives on catalysis**. In *DNA Damage*. Edited by Wallace S, Houten BV, Kow YW. New York: New York Academy of Sciences; 1994:181–197.

6.   Vassylyev DG, Kashiwagi T, Mikami Y, Ariyoshi M, Iwai S,
••   Ohtsuka E, Morikawa K: **Atomic model of a pyrimidine dimer excision repair enzyme complexed with a DNA substrate: structural basis for damaged DNA recognition**. *Cell* 1995, 83:773–782.
The crystal structure of an inactive mutant of T4 endo V in complex with a cognate DNA substrate refined to 2.75 Å resolution is reported. Implications for the mechanisms of damage recognition and catalytic reaction at the molecular level are discussed.

7.   Morikawa K, Ariyoshi M, Vassylyev DG, Matsumoto O, Katayanagi K, Ohtsuka E: **Crystal structure of a pyrimidine dimer-specific excision enzyme from bacteriophage T4: refinement at 1.45 Å resolution and X-ray analysis of the three active site mutants**. *J Mol Biol* 1995, 249:360–375.

8.   Kemmink J, Boelens R, Koning TMG, Kaptein R, Van der Marel GA, Van Boom JH: **Conformational changes in the oligonucleotide duplex d(GCGTTGCG)d(CGCAACGC) induced by formation of cis-syn thymine dimer**. *Eur J Biochem* 1987, 162:37–43.

9.   Kemmink J, Boelens R, Koning TMG, Van der Marel GA, Van Boom JH, Kaptein R: **¹H NMR study of the exchangeable protons of the duplex d(GCGTTGCG)·d(CGCAACGC) containing a thymine dimer**. *Nucleic Acids Res* 1987, 15:4645–4653.

10.   Taylor JS, Garret DS, Brockie IR, Svoboda DL, Tesler J: **¹H NMR assignment and melting temperature study of cis-syn and trans-syn thymine dimer containing duplex d(GCTATTATGC)·d(GCATAATACG)**. *Biochemistry* 1990, 29:8858–8866.

11.   Lee BJ, Sakashita H, Ohkubo T, Ikehara M, Doi T, Morikawa K, Kyogoku Y, Osafune T, Iwai S, Ohtsuka E: **Nuclear magnetic resonance study of the interaction of T4 endonuclease V with DNA**. *Biochemistry* 1994, 33:57–64.

12.   Bremier LH, Lindahl T: **A DNA glycosylase from *Escherichia coli* that releases free urea from polydeoxyribonucleotide containing fragments of base residues**. *Nucleic Acids Res* 1980, 8:6199–6211.

13.   Katcher HL, Wallace SS: **Characterization of the *Escherichia coli* X-ray endonuclease, endonuclease III**. *Biochemistry* 1983, 22:4071–4081.

14.   Bremier LH, Lindahl T: **DNA glycosylase activities for thymine residues damaged by ring saturation, fragmentation, or ring contraction are functions of endonuclease III in *Escherichia coli***. *J Biol Chem* 1984, 259:5543–5548.

15.   Boorstein RJ, Hilbert TP, Cadet J, Cunningham RP, Teebor GW: **UV-induced pyrimidine hydrates in DNA are repaired by bacterial and mammalian DNA glycosylase activities**. *Biochemistry* 1989, 28:6164–6170.

16.   Dizdaroglu M, Laval J, Boiteux S: **Substrate specificity of the *Escherichia coli* endonuclease III: excision of thymine- and cytosine-derived lesions in DNA produced by radiation-generated free radicals**. *Biochemistry* 1993, 32:12105–12111.

17.   Hatahet Z, Kow YW, Purmal AA, Cunningham RP, Wallace SS: **New substrates for old enzymes. 5-hydroxy-2′-deoxycytidine and 5-hydroxy-2′-deoxyuridine are substrates for *Escherichia coli* endonuclease III and formamidopyrimidine DNA N-glycosylase, while 5-hydroxy-2′-deoxyuridine is a substrate for uracil DNA N-glycosylase**. *J Biol Chem* 1994, 269:18814–18820.

18.   Kow YW, Wallace SS: **Mechanism of action of *Escherichia coli* endonuclease III**. *Biochemistry* 1987, 26:8200–8206.

19.   Thayer MM, Ahern H, Xing D, Cunningham RP, Tainer JA: **Novel
••   DNA binding motifs in the DNA repair enzyme endonuclease III crystal structure**. *EMBO J* 1995, 14:4108–4120.
On the basis of the high-resolution crystal structure and a mutational analysis of the enzyme, the authors propose a structural basis for endo III–DNA binding and catalysis, involving two novel HhH and FCL DNA-binding motifs.

20.   Kuo C-F, McRee DE, Fisher CL, O'Hadley SF, Cunningham RP, Tainer JA: **Atomic structure of the DNA repair [4Fe–4S] enzyme endonuclease III**. *Science* 1992, 258:434–440.

21.   Thomas L, Yang C-H, Goldthwait DA: **Two DNA glycosylases in *Escherichia coli* which release primarily 3-methyladenine**. *Biochem J* 1982, 21:1162–1169.

22.   McCarthy TV, Karran P, Lindahl T: **Inducible repair of O-alkylated DNA pyrimidines in *Escherichia coli***. *EMBO J* 1984, 3:545–550.

23.   Matijasevic Z, Sekiguchi M, Ludlum DB: **Release of N²,3-ethenoguanine from chloracetaldehyde-treated DNA by *Escherichia coli* 3-methyladenine DNA glycosylase II**. *Proc Natl Acad Sci USA* 1992, 89:9331–9334.

24.   Bjelland S, Birkeland N-K, Benneche T, Volden G, Seeberg E: **DNA glycosylase activities for thymine residues oxidized in the methyl group are functions of AlkA in *Escherichia coli***. *J Biol Chem* 1994, 269:30489–30495.

25.   Saparbaev M, Laval J: **Excision of hypoxanthine from DNA containing dIMP residues by the *Escherichia coli*, yeast, rat and human alkylpurine DNA glycosylases**. *Proc Natl Acad Sci USA* 1994, 91:5873–5877.

26.   Saparbaev M, Kleibl K, Laval J: **Escherichia coli, Saccharomyces cerevisiae, rat, and human 3-methyladenine DNA glycosylases repair 1,N⁶-ethenoadenine when present in DNA**. *Nucleic Acids Res* 1995, 23:3750–3755.

27.   Yamagata Y, Kato M, Odawara K, Tokuno Y, Nakashima Y,
••   Matsushima N, Yasumura K, Tomita K, Ihara K, Fujii Y *et al.*: **Three-dimensional structure of a DNA repair enzyme, 3-methyladenine DNA glycosylase II, from *Escherichia coli***. *Cell* 1996, 86:311–319.
The structural analysis of AlkA at 2.3 Å resolution reveal that the 3D structures of the DNA-binding and catalytic domains are similar to those of two domains of endo III. The major catalytic residue, Asp238, determined by mutational analysis, is located in the cavity on the interface between the two domains. A tentative model of the enzyme–DNA complex is proposed.

28.   Labahn J, Scharer OD, Long A, Ezaz-Nikpay K, Verdine GL,
••   Ellenberger TE: **Structural basis for the excision repair of alkylation-damaged DNA**. *Cell* 1996, 86:321–329.
The crystal structure of the DNA repair enzyme AlkA refined to 1.8 Å resolution is presented. The significance of Asp238 in catalysis was confirmed by mutagenesis studies. The binding site for the alkylated bases is proposed to reside in a large hydrophobic cleft that is mostly occupied by aromatic residues.

29.   Nikolov DB, Hu SH, Lin J, Gasch A, Hoffman A, Horikoshi M, Ohua NH, Roeder RG, Burley SK: **Crystal structure of TFIID TATA box-binding protein**. *Nature* 1992, 360:40–46.

30.   Michaels ML, Tchou J, Grollman AP, Miller JH: **A repair system for 8-oxo-7,8-dihydrodeoxyguanine**. *Biochemistry* 1992, 31:10964–10968.

31.   Tsai-Wu J-J, Liu H-F, Lu A-L: **Escherichia coli MutY protein has both N-glycosylase and apurinic/apyrimidinic endonuclease activities on C and A·G mispairs**. *Proc Natl Acad Sci USA* 1992, 89:8779–8783.

32.   Hilbert P, Boorstein RJ, Kung HC, Bolton PH, Xing D, Cunningham RP, Teebor GW: **Purification of a mammalian homologue of *Escherichia coli* endonuclease III: identification**

of a bovine pyrimidine hydrate-thymine glycol DNA-glycosylase/AP lyase by irreversible cross linking to a thymine glycol-containing oligodeoxynucleotide. *Biochemistry* 1996, 35:2505–2511.

33. Nash HM, Bruner SD, Scharer OD, Kawate T, Addona TA,
• Spooner E, Lane WS, Verdine GL: **Cloning of a yeast 8-oxoguanine DNA glycosylase reveals the existence of a base-excision DNA-repair protein superfamily.** *Curr Biol* 1996, 6:968–980.
The yeast DNA glycosylase (Ogg1) specific to 8-oxoguanine is suggested to be closely related in 3D structure to endo III. The sequence motif shared between Ogg1, endo III, and AlkA was found in a number of other enzymes. This finding led to the proposal of a structural superfamily of DNA repair enzymes.

34. Seeberg E, Eide L, Bjoras M: **The base excision repair pathway.** *Trends Biochem Sci* 1995, 20:391–397.

35. Slupphaug G, Eftedal I, Kavli B, Bharati S, Helle NM, Haug T, Levine DW, Krokan HE: **Properties of recombinant human uracil-DNA glycosylase from the UNG-gene and evidence that UNG-gene encodes the major uracil-DNA glycosylase.** *Biochemistry* 1995, 34:128–138.

36. Savva R, McAuley-Hecht K, Brown T, Pearl L: **The structural basis**
• **of specific base-excision repair by uracil-DNA glycosylase.** *Nature* 1995, 373:487–493.
The high-resolution crystal structures of UDG from herpes simplex virus, both in its free form and in complexes with a trinucleotide and uracil, reveal a new protein fold and allow the authors to propose mechanisms of DNA binding and catalysis.

37. Mol CD, Arvai AS, Slupphaug G, Kavli B, Alseth I, Krokan HE,
• Tainer JA: **Crystal structure and mutational analysis of human uracil-DNA glycosylase: structural basis for specificity and catalysis.** *Cell* 1995, 80:869–878.
The crystal structures of the free form of glycosylase and its complex with 6-aminouracil, in combination with a mutational analysis, reveal the uracil-binding site, the catalytic residues, and the positively charged groove that is involved in DNA binding. The mechanism of the catalytic reaction is proposed.

38. Mol CD, Arvai AS, Sanderson RJ, Slupphaug G, Kavli B,
• Mosbaugh DW, Krokan HE, Tainer JA: **Crystal structure of human uracil-DNA glycosylase in complex with a protein inhibitor: protein mimicry of DNA.** *Cell* 1995, 82:701–708.
The atomic structure of the complex of hUDG with a protein inhibitor, UGI, has been refined to 1.9 Å resolution. UGI was shown to bind to the DNA-binding loop of the hUDG, and to form no contacts with the uracil-binding pocket. A specific role in DNA binding is proposed for Leu272 of UDG.

39. Savva R, Pearl L: **Nucleotide mimicry in the crystal structure of**
• **the uracil-DNA glycosylase-uracil glycosylase inhibitor protein complex.** *Nat Struct Biol* 1995, 373:752–757.

The crystal structure of HSV-1UDG in complex with a protein inhibitor (UGI) shows that UGI directly mimics the enzyme–DNA interactions observed in the HSV-1UDG–trinucleotide complex. UGI is shown to block access to the active site of the enzyme but to form no contacts within the uracil-binding pocket.

40. Slupphaug G, Mol CD, Kavli B, Arvai AS, Krokan HE, Tainer
•• JA: **Structure of human uracil-DNA glycosylase bound to DNA shows a nucleotide flipping mechanism.** *Nature* 1996, 384:87–92.
The structure of hUDG(Leu272→Arg/Asp145→Asn)–DNA directly reveals the DNA–binding mechanism of hUDG and its cognate DNA substrate. The mutation Leu272→Arg abolishes the release of the product DNA from the protein but does not affect the glycosylase activity of the enzyme, so that the uracil in the complex is cleaved from the sugar moiety.

41. Klimasauskas S, Kumar S, Roberts RJ, Cheng X: **Hha1 methyltransferase flips its target base out of the DNA duplex.** *Cell* 1994, 76:357–369.

42. Sancar A: **Structure and function of DNA photolyase.** *Biochemistry* 1994, 33:2–9.

43. Park H-W, Kim S-T, Sancar A, Deisenhofer J: **Crystal structure**
•• **of DNA photolyase from *Escherichia coli*.** *Science* 1995, 268:1866–1872.
The high resolution (2.3 Å) crystal structure of DNA photolyase complexed with its light-harvesting, MTHF, and catalytic, FAD, cofactors is reported. The binding site for PD is determined and a mechanism of photoreactivation through electron transfer between FAD and PD is proposed.

44. Demple B, Harrison L: **Repair of oxidative damage to DNA: enzymology and biology.** *Annu Rev Biochem* 1994, 63:915–948.

45. Mol CD, Kuo C-F, Thayer MM, Cunningham RP, Tainer JA:
•• **Structure and function of the multifunctional DNA-repair enzyme exonuclease III.** *Nature* 1995, 374:381–386.
The atomic structure of exo III has been refined to 1.7 Å resolution. It exhibits a twofold symmetric fold similar to that of RNase H. The ternary complex of the enzyme with Mn^{2+} and dCMP leads to proposals of enzymatic and DNA-binding mechanisms.

46. Rice P, Craigie R, Davies DR: **Retroviral integrases and their cousins.** *Curr Opin Struct Biol* 1996, 6:76–83.

47. Scharer O, Verdine GL: **A designed inhibitor of base-excision DNA repair.** *J Am Chem Soc* 1995, 117:10781–10782.

48. Jones TA, Zou JY, Cowan SW, Kjeldgaard M: **An improved methods for building protein models in electron density maps and the location of errors in these models.** *Acta Cryst A* 1991, 47:110–119.

49. Moore MH, Gulbis JM, Dodson EJ, Demple B, Moody PCE: **Crystal structure of a suicidal DNA repair protein: the Ada O^6-methylguanine-DNA methyltransferase from *E. coli*.** *EMBO J* 1994, 13:1495–1501.