# Review

# DNA repair nucleases

**T. M. Marti[a] and O. Fleck[b],***

Institute of Cell Biology, University of Bern, Baltzerstrasse 4, 3012 Bern (Switzerland), Fax: +41 31 631 4684,
e-mail: fleck@izb.unibe.ch
[a] Present address: UCSF Comprehensive Cancer Center, University of California, 2340 Sutter Street, Box 0808,
San Francisco, California 94143 (USA)
[b] Present address: Department of Genetics, Institute of Molecular Biology, University of Copenhagen,
Øster Farimagsgade 2 A, 1353 Copenhagen K (Denmark), Fax: +45 35 32 2113, e-mail: fleck@my.molbio.ku.dk

**Abstract.** Stability of DNA largely depends on accuracy of repair mechanisms, which remove structural anomalies induced by exogenous and endogenous agents or introduced by DNA metabolism, such as replication. Most repair mechanisms include nucleolytic processing of DNA, where nucleases cleave a phosphodiester bond between a deoxyribose and a phosphate residue, thereby producing 5′-terminal phosphate and 3′-terminal hydroxyl groups. Exonucleases hydrolyse nucleotides from either the 5′ or 3′ end of DNA, while endonucleases incise internal sites of DNA. Flap endonucleases cleave DNA flap structures at or near the junction between single-stranded and double-stranded regions. DNA nucleases play a crucial role in mismatch repair, nucleotide excision repair, base excision repair and double-strand break repair. In addition, nucleolytic repair functions are required during replication to remove misincorporated nucleotides, Okazaki fragments and 3′ tails that may be formed after repair of stalled replication forks.

## Introduction

DNA, the carrier of genetic information of every living organism, is composed of a sugar-phosphate backbone and four organic bases. DNA is subject to cellular metabolic processes such as replication, transcription and repair. Several functions, especially during repair, require controlled cleavage of DNA. In 1903, Araki first reported the enzymatic breakdown of nucleic acids, and in the same year Iwanoff introduced the expression 'nucleases' for such enzymes [1, 2]. Today the nucleases are classified as sugar-specific nucleases, i.e. DNA nucleases and RNA nucleases, and sugar nonspecific nucleases [3]. Nucleases can be further divided into exonucleases, which hydrolyse either from the 5′ or the 3′ end of nucleic acids, and en-

donucleases, which hydrolyse internal phosphodiester bonds without the requirement of a free DNA end (fig. 1). DNA nucleases cleave a phosphodiester bond between a deoxyribose and a phosphate group. One cleavage product contains a 5′-terminal phosphate, and the second product contains a 3′-terminal hydroxyl group (fig. 1). In contrast to DNA nucleases, AP lyase activities process DNA either by a β-elimination reaction producing a 3′-terminal phosphoglyceraldehyde residue (fig. 1) or, like *Escherichia coli* MutM, by β-δ-elimination, which results in a 3′-phosphate end [4–7]. Similarly, the dRPase activity of DNA polymerase β (Pol β), removes an abasic sugar-phosphate molecule by β-elimination [6]. Special types of nucleases are AP endonucleases, which cleave 5′ to apurinic/apyrimidinic (AP) sites (fig. 1).
This review focuses on DNA nucleases with a function in repair. The various repair processes will be briefly de-
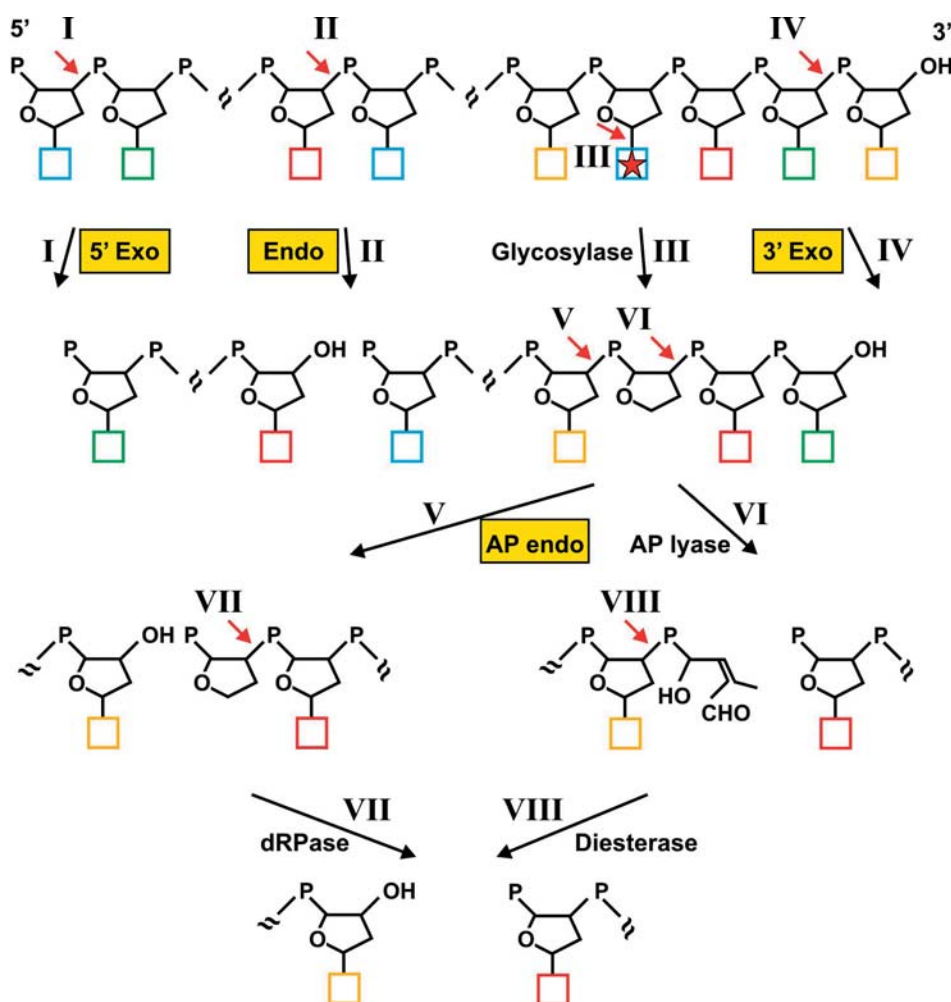
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Figure 1. Enzymatic cleavage of DNA. Enzymatic incisions are indicated by red arrows and roman numbers, DNA nucleases are highlighted in yellow boxes. Phosphate groups are drawn as P's, deoxyriboses as pentagons and bases as coloured squares. The asterisk indicates a damaged or mismatched base, which is substrate of a base excision repair glycosylase. Only the products remaining in the DNA chain are shown. DNA nucleases cleave a phosphodiester bond between a deoxyribose and a phosphate residue in a way that one of the products contains a 5′-terminal phosphate group and the second a 3′-terminal hydroxyl group. For excision DNA exonucleases need a free 5′ DNA end (5′ Exo) or a free 3′ end (3′ Exo). Most exonucleases excise a single nucleotide from the 5′ or 3′ end of DNA (I and IV). The DNA strand can be degraded by consecutive nucleotide excisions. Some exonucleases, however, hydrolyse in an internal DNA region distant from the free DNA end (not shown). DNA endonucleases (Endo) do not require a free DNA end and cleave DNA at an internal phosphodiester bond, thereby creating a single-strand break (II). A special type of endonucleases are AP endonucleases (AP endo), which hydrolyse 5′ of an AP site (V). An AP site can be produced spontaneously or enzymatically by a DNA glycosylase, which cleaves the N-glycosylic bond between base and sugar (III). AP sites can exist with a cyclic sugar moiety (shown) or as open chain configurations (not shown) [5, 6]. An AP site can be also processed by the AP lyase activity of bifunctional DNA glycosylases, which in contrast to AP endonucleases cleave 3′ of the AP site by a β-elimination reaction, producing a fragmented sugar with a double bond (VI). Subsequently, a single nucleotide gap between termini with a 5′ phosphate and a 3′ hydroxyl group is produced by phosphodiesterase activity (VIII). The same products are formed when the 5′-terminal AP site created by an AP endonuclease (V) is further processed by a dRPase activity (VII).

scribed, followed by a more detailed description of the role and substrate specificity of DNA nucleases. Repair nucleases of eukaryotes are listed in table 1. The substrates and products of representative DNA repair nucleases are schematically shown in figure 2. In general, DNA products that result from nucleolytic cleavage are further processed by factors acting downstream in a pathway. This is, as a minimum, DNA synthesis and ligation, but can also be a cascade of events, as e.g. the whole process of

homologous recombinational (HR) repair of double-strand breaks (DSBs), which is initiated by resection of 5′ DNA ends. It should also be pointed out that many repair processes include redundant nuclease functions, and that a given nuclease can be involved in more than one process. For example, degradation of mismatched DNA in *E. coli* can be redundantly carried out by one out of four exonucleases (fig. 2A), and to different degrees each of them is involved in other processes. Repair mechanisms

Table 1. Eukaryotic DNA repair nucleases.

| Nuclease[a] | Activity | Pathway[b] | References |
|---|---|---|---|
| EXO1 | 5′→3′ exo/ endo | MMR, rec.[c], DSB repair | 26–30, 35 |
| XPG/scRAD2 | endo | NER | 184–187 |
| XPF-(ERCC1)/scRAD1-(RAD10) | endo | NER, SSA | 83, 186–189 |
| hAPE1 | AP endo / 3′→5′ exo | BER | 101, 103, 190 |
| hAPE2/scAPN2 | AP endo / 3′→5′ exo | BER | 106, 108, 109 |
| scAPN1 | AP endo / endo | BER, NIR | 107, 115 |
| FEN1 | endo / 5′→3′ exo | repl.[d] (Okazaki), BER, UVER, NIR | 63, 110–115 |
| ENDO V | endo | AER | 116, 117, 191 |
| MRE11-(RAD50-NBS1) | 3′→5′ exo | DSB repair, repl. (restart), rec. (?) | 118 |
| UVDE | endo | UVER | 112, 127, 130 |
| Pol δ | 3′→5′ exo | repl. (proofreading), MMR (?) | 37, 132, 192 |
| Pol ε | 3′→5′ exo | repl. (proofreading), MMR (?) | 37, 132, 193 |
| Pol γ | 3′→5′ exo | repl. of mtDNA[e] (proofreading) | 132, 194 |
| TREX1 | 3′→5′ exo | unknown (proofreading?) | 151 |
| TREX2 | 3′→5′ exo | unknown (proofreading?) | 151 |
| ExoN | 3′→5′ exo | unknown (proofreading?) | 154, 155 |
| Dna2 | endo | repl. (Okazaki) | 110, 158, 195 |
| MUS81-(EME1) | endo | repl. (restart) | 162, 166, 168 |
| WRN | 3′→5′ exo | Unknown | 171 |
| p53 | 3′→5′ exo | Unknown | 179, 196 |
| hRAD9 | 3′→5′ exo | unknown (checkpoint activation?) | 183 |

[a] Although different nomenclatures have been established for different species, for simplification we used uppercase letters for eukaryotic proteins in this review. A few exceptions have been made to avoid confusion, e.g. we further used ExoN instead of EXON. Prefixes h and sc indicate proteins from human and *S. cerevisiae*, respectively.
[b] Mechanisms for that nuclease function is required.
[c] Recombination.
[d] Replication.
[e] Mitochondrial DNA.

without the need of a nuclease also exist, such as damage repair by O[6]-methylguanine-DNA methyltransferase, which transfers the methyl group of the damaged base to one of its own cysteine residues in a suicide reaction, and by photolyases, which split covalent bonds of ultraviolet (UV) light-induced pyrimidine dimers [8].

With respect to their substrate preference, DNA nucleases can be structure, damage or sequence specific. Structure-specific nucleases recognise intermediates of DNA repair. For example, during nucleotide excision repair (NER), enzymatic unwinding of DNA around a lesion results in a bubblelike structure which is incised by the endonucleases XPF-ERCC1 and XPG at or near the junctions between double-stranded (ds) and single-stranded (ss) regions (fig. 2C). Damage-specific DNA repair nucleases usually have a specific- and a nonspecific binding mode. These nucleases recognise DNA in a nonspecific manner and scan along DNA to find damage. Once detected, the damage is bound in a specific manner, allowing the nuclease to dock at the damage with the active-site residues. Sequence-specific nucleases are rather rarely used in repair. One example is MutH of *E. coli*, which introduces a nick in GATC sequences when the adenine is not methylated [9].

The primary sequences of nucleases do not show high similarity except for some conserved residues in their catalytic sites [10]. Therefore, it is often not possible to conclude from the primary sequence what the specific function of a nuclease could be. Based on the three-dimensional structure that has been solved for many DNA repair nucleases, they can be classified in the following folding families: RNase H-like, resolvase-like, restriction endonuclease-like, RecJ-like, metallo-dependent phosphatase, DNase I-like, TIM α/β barrel and His-Me finger endonuclease [10].

**Long-patch mismatch repair in *E. coli***

DNA mismatches can arise during replication by strand slippage or false integration of nucleotides, by spontaneous or induced base alterations, and during recombination. The major defence against manifestation of premutagenic mismatches that arise during replication is the long-patch mismatch repair (MMR) pathway. The basic principle of MMR appears to be quite similar between prokaryotic and eukaryotic organisms, with some striking differences [11].

In *E. coli*, MutS binds to a mismatch, while MutH binds a hemimethylated *dam* (GATC) site located either 5′ or 3′ to the mismatch [11, 12]. MutS can exist as a homodimer and a homotetramer, the latter being likely the native state on mismatched DNA [13, 14]. MutL mediates complex formation of MutS and MutH and thereby enables activa-
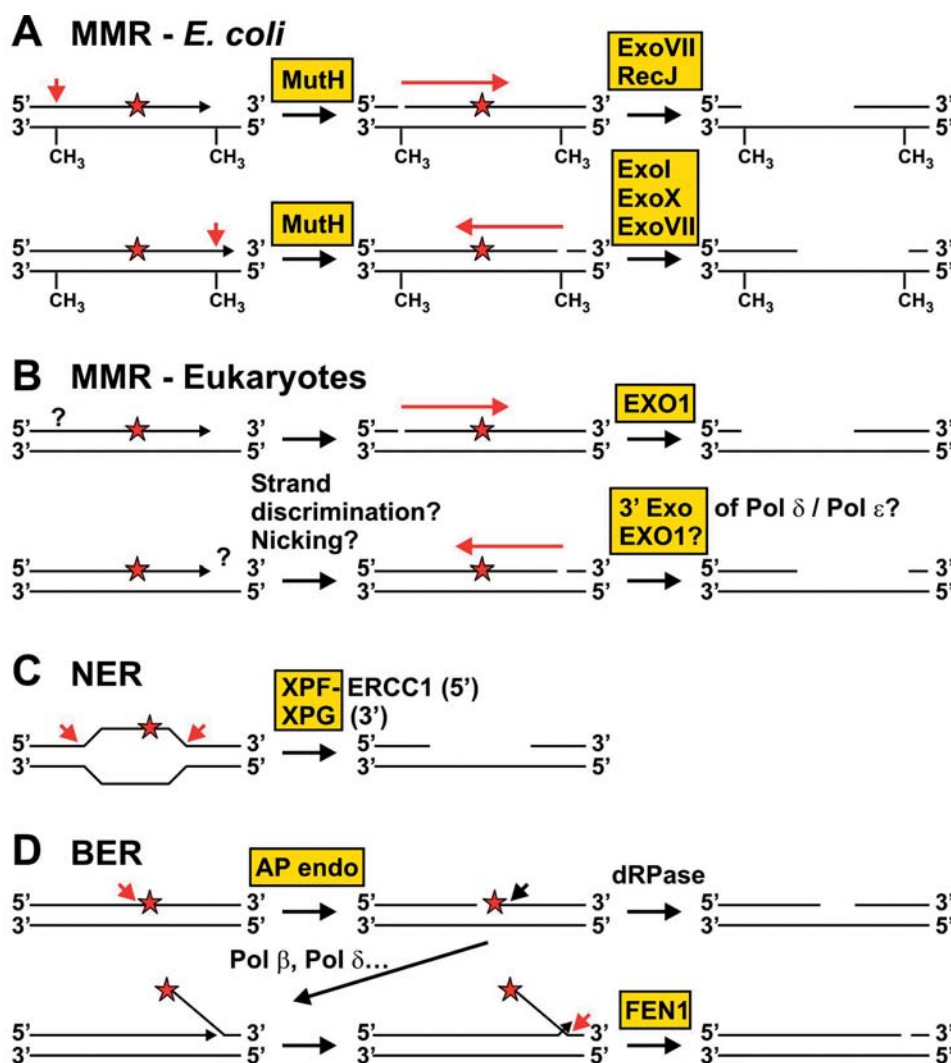
Figure 2. Substrates and products of DNA nucleases in repair and replication processes. Nucleolytic activities are shown as red arrows; nucleases are highlighted in yellow boxes. DNA damage is indicated as red asterisks. (*A*) MMR in *E. coli*. MutH incises the newly synthesised strand at a hemimethylated *dam* site when a mismatch is produced during replication and not removed by the proofreading activity of the polymerase (see *H*). The nick can be either 5′ or 3′ to the mismatch. Exonucleolytic degradation towards and beyond the mismatch can occur by ExoVII or RecJ from the 5′ side (top line) and by ExoI, ExoX or ExoVII from the 3′ side (bottom line). (*B*) MMR in eukaryotes. It is not yet known how eukaryotic MMR can discriminate between the new and the template strand and whether enzymatic nicking is required. Exo1 is involved in eukaryotic MMR for excision from the 5′ side (top line) and likely also has a function for excision from the 3′ side (bottom line). In addition, the 3′ exonucleolytic activities of Pol δ and ε may be involved in 3′ excision. (*C*) NER. After unwinding of the DNA around the damage, dual incision by XPF-ERCC1 (5′ to the damage) and XPG (3′ to the damage) occurs. (*D*) BER. AP sites that are produced spontaneously or by monofunctional DNA glycosylases (compare with fig. 1) are incised on the 5′ side by an AP endonuclease. The resulting 5′ abasic terminus can be processed by the dRPase activity of Pol β (short-patch BER, top line). After strand displacement, a flap structure is formed that can be cleaved by FEN1 (long-patch BER, bottom line).

tion of the endonuclease activity of MutH, which incises the transiently nonmethylated strand. Since this strand is the newly synthesised strand, incision by MutH allows strand discrimination and, in a subsequent step, removal of the falsely incorporated nucleotide. Helicase II unwinds the DNA, and the nicked ssDNA is exonucleolytically degraded, resulting in excision tracts of up to 1 kb [12]. Depending on the position of the nicked *dam* site, either a 5′→3′ or a 3′→5′ exonuclease degrades the nicked strand towards and beyond the mismatch (fig. 2A). Finally, the resulting gap is filled in by DNA Pol III and ligated by DNA ligase I.

Mutational analysis revealed that Tyr$^{212}$ of MutH is important, if not the only amino acid residue that is responsible for verification of the DNA methylation status at *dam* sites [15]. Methylation of both adenine residues in a
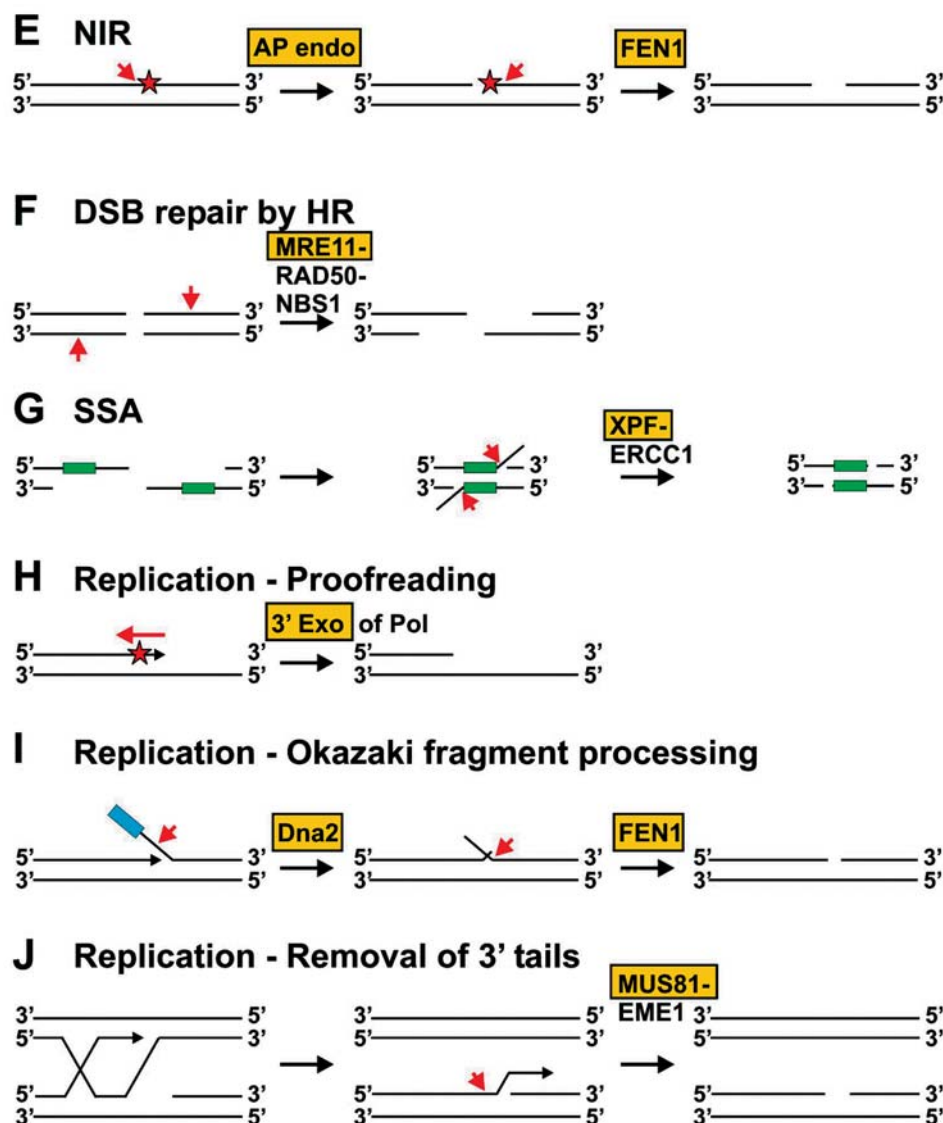
Figure 2 (continued). (*E*) NIR. Alternatively to BER, oxidative damage can be directly incised by an AP endonuclease. The 5′-terminal damage can be released by FEN1. (*F*) DSB repair by HR. A DSB produced by chemical agents or radiation can be repaired by HR. In the first step of HR, the 5′ ends may be endonucleolytically cleaved by MRE11-RAD50-NBS1. Note that the polarity is different to the polarity found for the MRE11 complex in vitro (see fig. 3D). (*G*) SSA. SSA represents a specific mode of HR in which repeats (green boxes) flank the DSB. After resection of the 5′ ends (see *F*), a joint molecule can be formed by pairing of the repeats. The NER endonuclease XPF-ERCC1 (RAD1-RAD10 in *S. cerevisiae*) can remove the nonhomologous ends by incision. (*H*) Proofreading during replication. Mismatches produced during replication can be removed by the 3′ exonuclease activity of replicative polymerases or by associated 3′→5′ exonucleases. (*I*) Processing of Okazaki fragments. Okazaki fragments are displaced by DNA synthesis (the RNA part is indicated as blue box). Dna2 cleaves inside the 5′ flap, thereby removing the RNA and most of the ssDNA tail. After branch migration, a double-flap structure with a one-nucleotide tail on the displacing strand can be produced and cleaved by FEN1. (*J*) Removal of 3′ tails that can be formed after repair of stalled replication forks. MUS81-EME1 (-MMS4) incises dsDNA 5′ to the 3′ tail.

*dam* site drastically reduces the endonuclease activity of MutH. Similar to restriction endonucleases, structural adaptations of MutH may prevent cleavage at fully methylated *dam* sites. The Tyr[212] forms a hydrogen bond with the unmethylated $N^6$ atom of the adenine in the nascent strand and simultaneously makes van der Waals binding with the methyl group of the adenine of the opposite DNA strand. Binding is verified when all contacts of the bases of the recognition sequence are properly formed. This leads to activation of the catalytic centre and subsequent cleavage of the nonmethylated nascent strand [15].

After DNA unwinding by helicase II and protection of the template strand by single-strand binding (SSB) protein, the nicked strand is degraded towards and beyond the mismatch by one out of four exonucleases [16]. Degradation occurs by the 5′→3′ exonuclease activity of either ExoVII

or RecJ, when the nick was introduced 5′ to the mismatch, or by the 3′→5′ exonuclease activity of ExoI, ExoX or ExoVII when the nick was introduced 3′ to the mismatch (fig. 2A). In contrast to the other exonucleases, ExoVII possesses both 5′→3′ and 3′→5′ exonuclease activities [17]. ExoI, ExoVII and RecJ are ssDNA-specific exonucleases, while ExoX can also degrade dsDNA, although with lower efficiency [11].

In mutants defective in three of the four exonucleases, ExoI, ExoVII, ExoX and RecJ, repair initiated at a nick located on one site of a mismatch is strongly reduced, while repair initiated from a nick at the opposite site remains considerably efficient [17]. One exception is the *exoI exoX recJ* triple mutant, which shows significant repair from both sites, due to the presence of 5′→3′ and 3′→5′ exonuclease functions of ExoVII. Mutation rates of the various triple mutants are not increased when compared with wild type [17]. Thus, the function of one remaining exonuclease is sufficient for MMR. The *exoI exoVII exoX recJ* quadruple mutant shows a moderately increased mutation rate when compared with wild type, which is clearly not as strong as in a *mutS* mutant. However, since the mutation rate of the quadruple mutant is epistatic to *mutS*, the mutator phenotype of the *exoI exoVII exoX recJ* mutant is likely due to a defect in MMR [17]. It has been proposed that the weak mutator phenotype of the quadruple mutant is caused by frequent chromosome loss, which may result in underrecovery of mutation events [16].

Despite their involvement in MMR, ExoI, ExoVII, ExoX and RecJ also have a function in repair of UV-induced damage and in suppression of homologous recombination [18, 19]. The 3′→5′ exonucleases ExoI and ExoX appear to be less important for UV damage repair than the 5′→3′ exonuclease activities of RecJ and ExoVII.

## Long-patch MMR repair in eukaryotes

Eukaryotic cells have multiple MutS and MutL homologues but obviously no MutH homologue [11]. It is currently not known how the template and the nascent strand are distinguished during eukaryotic MMR. One possibility is that the asymmetric sliding clamp, proliferating cellular nuclear antigen (PCNA), which has a function in DNA replication, is responsible for strand discrimination [20, 21]. On the other hand, nicks or free 3′ ends that are present in the nascent strand during replication may be sufficient for strand discrimination. Indeed, in vitro experiments showed that preferentially the strand with a nick or, even more efficiently, a short gap is degraded by eukaryotic MMR [22]. Since the nick or gap can be located either 5′ or 3′ to the mismatch, eukaryotic MMR appears to be bidirectional, like MMR of *E. coli* [22, 23]. Eukaryotic MMR is initiated by binding of a MutS-related heterodimer, which is either MutSα (MSH2-MSH6) or

MutSβ (MSH2-MSH3). MutSα and MutSβ differ in their ability to recognise base-base mismatches and small loops [11]. Subsequently, a MutL-related heterodimer stabilises binding of MutSα and MutSβ to the mismatched substrate. The major MutL-related dimer is MutLα (MLH1-PMS2 in humans). In some but not all eukaryotes, additional MutL-related heterodimers exist, which play a minor role in MutSβ-dependent repair of loops [11]. Binding of MutLα induces a conformational change, which may allow interaction with downstream factors. One such factor is likely the 5′→3′ exonuclease EXO1 (fig. 2B) [24–26]. EXO1 is member of the FEN1 family of structure-specific flap endonucleases, which is defined by two conserved motifs. Eukaryotic EXO1, however, is not homologous to ExoI of *E. coli*. Despite its role in MMR, EXO1 also has a function in recombination and DSB repair [27–30].

The *Saccharomyces cerevisiae EXO1* gene was identified in a two-hybrid screen with *MSH2* [25]. Compared with MMR mutants defective in MSH2 or MLH1, mutation frequencies are only slightly increased in the *exo1* mutant, which, similar to the situation in *E. coli*, may be due to functional redundancy with other exonucleases. Mutation rates of the *msh2 exo1* double mutant are in the same range as those of the *msh2* single mutant, indicating that EXO1 is involved in the MMR pathway. However, since the mutator effect of *exo1* is rather small, a possible additive effect in the *msh2 exo1* double mutant may not be detectable. Since MSH2 and EXO1 also have a function in recombination [27, 31], it is possible that physical interaction between MSH2 and EXO1 reflects a role during a recombinational process and that the mutator of *exo1* cells is due to a defect in an MMR-independent mechanism.

Besides their dsDNA- and ssDNA-specific 5′→3′ exonuclease activities, human and *S. cerevisiae* EXO1 also possesses a 5′ flap-endonuclease activity, similar to the other members of the FEN1 family (fig. 3) [32, 33]. In *S. cerevisiae*, site-specific mutation analysis revealed that the dsDNA 5′→3′ exonuclease and flap-endonuclease activities largely depend on the Asp$^{173}$ residue, which, however, is not required for the ssDNA 5′→3′ exonuclease activity [32]. The flap-endonuclease activity of scEXO1 does not have a preference for a specific flap structure, in contrast to RAD27 (*S. cerevisiae* homologue of FEN1), which preferentially cleaves a double flap with an additional one-nucleotide 3′ flap (fig. 3B) [32, 34].

Besides its 5′→3′ exonuclease activity human EXO1 also has a function in the excision step of MMR when the strand break is located 3′ to the mismatch. This function is either of a structural nature or due to a cryptic 3′→5′ exonuclease activity of EXO1 [35]. In crude protein extracts, excision tracts of human MMR span several hundred nucleotides from the initial strand break, reaching 90–170 bp beyond the mismatch [23]. In contrast, with purified human MutSα, MutLα and EXO1, the excision
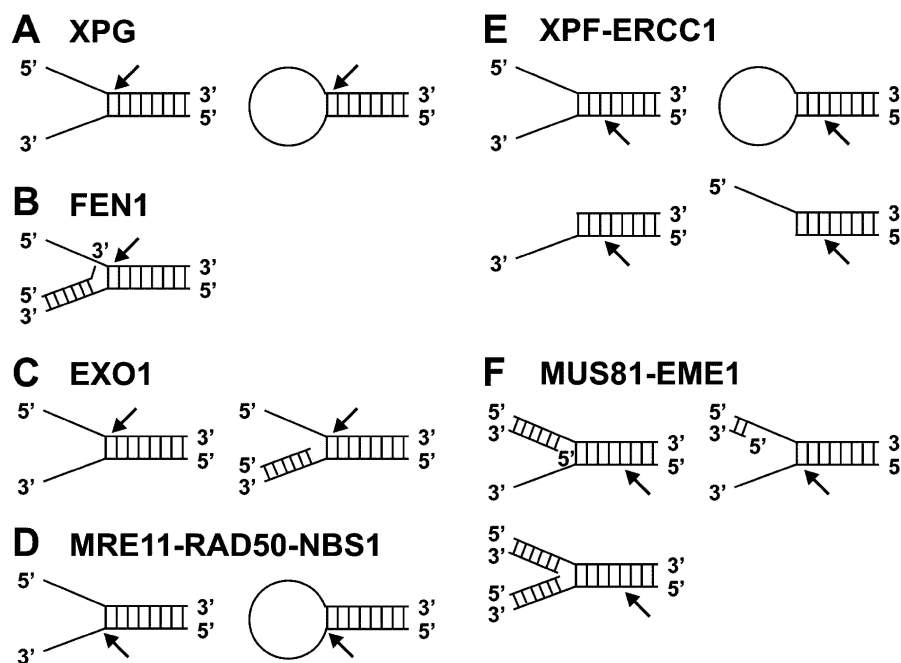
Figure 3. DNA structures preferentially cleaved by flap endonucleases. Members of the FEN1 family incise at ssDNA/dsDNA junctions (*A–C*). Preferred substrate of FEN1 is a double-flap structure in which the 3′ dsDNA arm contains a one-nucleotide flap (*B*). The MRE11 complex preferentially cleaves 3′ ssDNA at the junction to dsDNA (*D*). XPF-ERCC1 incises in the dsDNA region two to eight nucleotides from the junction (*E*). MUS81-EME1 (-MMS4) preferentially incises Y structures with 5′ dsDNA tails (*F*). Cleavage occurs five nucleotides from the 5′ end of the 5′ dsDNA arm. The NER endonucleases XPG (*A*) and XPF-ERCC1 (*E*) and the MRE11 complex (*D*) can incise stem-loop structures, which are not cleaved by FEN1, EXO1 and MUS81-EME1.

patches extend over several thousand nucleotides from the strand break [35]. This difference may be due to control of EXO1-dependent excision by as yet unidentified MMR components.

Mouse EXO1 has a function in the repair of base mismatches and loops with one unpaired nucleotide in both 5′ and 3′ nick-directed repair [36]. In addition, EXO1-deficient mouse cells exhibit instability of a mononucleotide repeat. EXO1 knockout mice show reduced survival, frequently develop lymphoma and are sterile due to a defect in meiosis [36].

Genetic studies with *S. cerevisiae* indicate that the 3′→5′ exonuclease activities of the DNA polymerases δ and ε participate in MMR (fig. 2B), besides their function in MMR-independent mutation avoidance mechanisms, e.g. proofreading during replication [37].

**Very short patch repair in *E. coli***

In *E. coli* a very short patch (VSP) repair pathway exists, which recognises T/G mismatches in *dcm* ($CC^A/_TGG$) methylation sites and related sequences and restores them exclusively to C:G pairs [38–40]. The Dcm methyltransferase methylates the internal cytosines of both strands of *dcm* sites. The resulting 5-methylcytosine can be deaminated to thymine, which if not repaired pairs with an ade-

nine during the next replication. The VSP repair system counteracts the formation of such C:G to T:A transitions. The sequence-specific Vsr endonuclease is the initial factor of VSP repair and incises DNA immediately 5′ to the mismatched thymine. DNA Pol I performs nick translation, creating a repair patch size of less than 10 nucleotides. Finally, DNA ligase I seals the nick.

VSP is stimulated by the MMR factors MutS and MutL, but not by MutH [38, 41]. Independent of VSP, T/G mismatches in *dcm* sites can also be repaired by MMR. However, this error-prone repair is largely avoided by cell cycle regulation, since MMR is induced during exponential growth, whereas VSP repair is most efficient during stationary phase [42].

Structural analysis and biochemical data indicate that Vsr has a function similar to MutH and that both have a structure similar to type II restriction endonucleases [43, 44]. Binding of Vsr to a T/G mismatch leads to insertion of amino acid side chains into the minor and major grooves of DNA, which causes bending and widening of both grooves of the DNA. Insertion of three aromatic side chains into the major groove leads to separation of the T/G mismatch from the adjacent A:T base pair [43].

High cellular levels of Vsr are mutagenic, which may be due to interaction of Vsr with MutL, which inhibits dimerization of MutL [45, 46]. Vsr interacts with MutL through five C-terminal residues, which are similar to the MutL-

interacting residues of MutH. Although MutS has some function in VSP, it cannot simultaneously bind with Vsr to a mismatch. MutS and MutL may deform the DNA around a mismatch, thereby enhancing recognition and binding of Vsr [43].

### NER in *E. coli*

DNA photoproducts induced by UV radiation and other bulky DNA lesions, such as psoralen monoadducts and intrastrand crosslinks, can be repaired by NER. In addition, methylated bases and AP sites, usually repaired by base excision repair (BER), and to some extent even mismatches are 'repaired' by NER [47].

In *E. coli*, a protein complex composed of $UvrA_2$ and UvrB binds to undamaged DNA, with gene promoters being the preferred docking sites for initial binding [48]. The protein complex slides along the DNA in an ATP hydrolysis-dependent manner. When a damaged base is encountered, the molecular matchmaker $UvrA_2$ is released, resulting in a UvrB-DNA complex and bending of the DNA. UvrC recognises UvrB-DNA with high specificity, forming a stable UvrB-UvrC-DNA complex. Dual incision occurs four to five nucleotides 3′ and eight nucleotides 5′ to the damage [49]. The catalytic sites for both incision activities are located in UvrC [50]. After incision, DNA helicase II is required for release of UvrC and for excision of the damage in a 12–13-nucleotide-long oligonucleotide. NER is completed by DNA Pol I-dependent repair synthesis and ligation [51]. UvrC contains a UvrBC domain, which is responsible for interaction with UvrB, and two domains that are required for damage incision [52, 53]. 3′ incision is catalysed by the N-terminal Uri domain and 5′ incision by the endoV domain, which contains conserved aspartate residues [53]. The C-terminal helix-hairpin-helix $(HhH)_2$ domain with two HhH motifs is required for 5′ incision and, depending on the sequence context of a lesion, can be also involved in 3′ incision [54]. The $(HhH)_2$ domain specifically binds to ssDNA/dsDNA junctions with a preference for bubblelike DNA structures containing at least six unpaired bases. It has been proposed that two interacting UvrC molecules bind via their $(HhH)_2$ domains to the two ssDNA/dsDNA junctions of a bubble and that the $(HhH)_2$ domains stabilise such DNA structures for dual incision [54, 55].

Cho (Uvr<u>C</u> <u>ho</u>mologue), an *E. coli* protein with homology to the N-terminal part of UvrC, can perform 3′ incision during NER [56]. Compared with UvrC, incision by Cho occurs four nucleotides further downstream of a lesion, which could be due to a different interaction with UvrB. It has been speculated that Cho could be required for 3′ incision of DNA adducts that are poor substrates of UvrC [57]. Since Cho lacks 5′ incision activity, 5′ incision has still to be done by UvrC, also when Cho has made the 3′

incision. In the absence of UvrC, 3′ incision by Cho may be followed by DNA excision carried out by a 3′→5′ exonuclease.

### NER in eukaryotes

The process of human NER has been uncovered by analysis of NER-defective individuals with the inherited disorder xeroderma pigmentosum (XP) [58]. Individuals afflicted by XP show an extreme photosensitivity and exhibit a high incidence of skin cancer. During NER, a preincision complex is formed by transcription factor IIH (TFIIH), XPA, replication protein A (RPA) and the two structure-specific endonucleases XPF-ERCC1 and XPG. Two subunits of TFIIH, the ATP-dependent helicases XPB (3′→5′) and XPD (5′→3′) are responsible for opening the DNA double helix around the lesion [59]. After dual incision by XPG and XPF-ERCC1, the damage is released in a 24–32-nucleotide-long oligonucleotide (fig. 2C) [60]. XPG cleaves predominantly 4-6 nucleotides 3′ to the lesion, and XPF-ERCC1 cleaves predominantly 20–24 nucleotides 5′ to the lesion [60]. The resulting gap is filled in by Pol δ or ε, and the remaining nick is sealed by ligase I.

NER consists of two subpathways, global genome repair (GGR) and transcription-coupled repair (TCR) [61]. A specific defect in TCR causes Cockayne syndrome (CS). Most CS patients have a mutation in CSA, significantly fewer in CSB and rarely in XPB, XPD or XPG. Patients suffering from CS are UV sensitive, but they do not show an enhanced incidence of skin cancer, which distinguishes CS from XP. XPC in complex with hHR23B and UV-DDB have a specific function in the damage recognition step of GGR, while TCR is thought to be initiated by RNA polymerase II stalled at a lesion [62].

Like EXO1 and FEN1, XPG belongs to the FEN1 family of structure-specific endonucleases. Members of the family contain an N-terminal and an internal domain with highly conserved acidic amino acid residues that are essential for nuclease activity [63]. All FEN1 family members cleave ssDNA/dsDNA junctions in the double-stranded part adjacent to the 5′ ssDNA arm. The endonucleases have overlapping but different substrate specificity, which may reflect adaptation of their function to individual repair processes. For example, XPG can efficiently incise bubble or stem-loop substrates, whereas FEN1 and EXO1 only process substrates with free 5′ ssDNA ends (fig. 3) [33, 59, 64, 65].

In addition to 3′ incision during NER, XPG has a structural function during NER and in the not well characterised transcription-dependent BER pathway [66–69]. XPG helps to open the DNA around a lesion and to stabilise the damage recognition complex [66]. In addition, a role for the *S. cerevisiae* homologue RAD2 in RNA Pol II-dependent transcription has been reported [70].

XPG is able to cleave several ssDNA/dsDNA structures with the same polarity that is required for 3′ incision during NER. Such structures are bubbles, splayed arms, stem loops and flaps (fig. 3 A) [59, 65]. Although not cleaved, XPG has a higher binding affinity for three-way junctions than for splayed arms and flap structures [65]. The 3′ and not the 5′ ssDNA arm adjacent to the dsDNA region of an ssDNA/dsDNA junction is important for binding and incision by XPG. The presence of at least two nucleotides of the 3′ arm is sufficient for substantial binding and cleavage by XPG, while complete lack of the 5′ arm reduces incision activity only by 50%. Although the 5′ arm is not required for binding and incision, the nuclease activity of XPG, but not binding to DNA, is inhibited when the 5′ arm is double stranded in the junction, indicating that accessibility or flexibility of the junction is important for incision but not for binding of XPG [65]. Recognition and incision by XPG might have different claims on a substrate, which may reflect the different functions of XPG in different repair systems.

Mutated XPG can cause XP, CS or a combined XP/CS phenotype, depending on the type and position of the mutation [71]. Mutations affecting the nuclease activity of XPG generally cause a XP phenotype, while mutations that result in truncation of the protein lead to a phenotype with symptoms from both XP and CS [71]. It is assumed that a deficiency of the NER function of XPG causes XP, whereas a deficiency of the function of XPG in transcription and in transcription-coupled BER is responsible for the characteristics of CS [69, 70, 72].

XPF and homologues in other eukaryotes (RAD1 in *S. cerevisiae*) belong to a family of structure-specific endonucleases which includes MUS81 and the recently identified archaeal Hef endonuclease [73, 74]. XPF and MUS81 have catalytic activities but perform their function as heterodimers with ERCC1 (RAD10 in *S. cerevisiae*) and EME1 (MMS4 in *S. cerevisiae*), respectively, while Hef forms a homodimer [73–78]. XPF-ERCC1 acts as a structure-specific endonuclease which incises the DNA 5′ to the damage in a bubble structure (fig. 3E) [58, 60]. XPF-ERCC1 and RAD1-RAD10 also cleave nonhomologous 3′ flap structures and have an NER-independent role in homologous recombination, during single-strand annealing (SSA) (fig. 2G) and in repair of interstrand crosslinks [79–83]. In addition, XPF-ERCC1, but not other NER factors, has a role in the formation of UV-induced chromosome exchanges, which indicates that XPF-ERCC1 functions in bypass or repair of damage during replication [84].

de Laat and co-workers extensively analysed the structure specificity of XPF-ERCC1 endonuclease activity [79]. As assumed from its function in 5′ incision during NER, XPF-ERCC1 is able to cleave stem-loop structures 5′ to the loop in dsDNA two to eight nucleotides away from the junction (fig. 3E). XPF-ERCC1 also cleaves 3′ ssDNA arms from splayed arms and flap structures. The presence of either a 3′ or 5′ ssDNA arm is sufficient for cleavage by XPF-ERCC1. This is in contrast to XPG, which requires an at least two-nucleotide-long 3′ arm for efficient cleavage [65].

## BER

BER is responsible for repair of DNA damages that are produced by oxidation, alkylation, deamination or hydroxylation of DNA bases. BER also processes mismatched and fragmented bases, exocyclic adducts and cyclobutane pyrimidine dimers (CPDs) [85]. BER is initiated by a damage-specific DNA glycosylase which cleaves the N-glycosylic bond between base and sugar, producing an AP site (fig. 1) [6, 86]. AP sites can also occur spontaneously by depurination and depyrimidination events [87]. AP sites can be further processed by BER in several ways (figs 1, 2D). An AP endonuclease can hydrolyse the DNA sugar-phosphate backbone 5′ of an AP site, producing a 3′-terminal hydroxyl group and a 5′-dRP moiety (fig. 1) [88]. The 5′-dRP moiety can be processed during short-patch BER by the dRPase activity of Pol β, leading to a one-nucleotide gap between nucleotides with a 3′-hydroxyl and a 5′-phosphate terminus. Subsequently, a single nucleotide is inserted by Pol β, and the remaining nick is sealed either by DNA ligase I or by a complex of DNA ligase III with XRCC1 [86, 88]. Depending on the nature of the 5′-dRP moiety and the DNA substrate, the 5′-dRP moiety can be also removed by long-patch BER, which further processes the nick. Strand displacement during long-patch BER by Pol β, Pol δ and perhaps Pol ε results in incorporation of two to eight nucleotides and formation of a flap structure (fig. 2D) [86]. The flap structure is cleaved by FEN1 in a PCNA-dependent reaction. As in short-patch BER, the remaining nick is either sealed by DNA ligase I or by DNA ligase III-XRCC1 [6, 86]. AP sites can also be processed by the AP lyase activity of bifunctional DNA glycosylases (fig. 1). The resulting fragmented sugar residue is cleaved by a diesterase activity (likely contributed by an AP endonuclease), which produces a one-nucleotide gap between nucleotides with 3′-hydroxyl and 5′-phosphate termini [6, 86]. Thus, sequential cleavage by AP lyase and diesterase activities leads to the same DNA products as sequential cleavage by an AP endonuclease and a dRPase activity (fig. 1). Alternatively to the mechanisms described above, the bifunctional DNA glycosylases MutM (Fpg) and Nei of *E. coli* and the mammalian Nei-like enzymes NEIL1 and NEIL2 incise AP sites by β-δ-elimination, resulting in a one-nucleotide gap between nucleotides with 3′ and 5′-phosphate ends [89–94]. Before repair can be completed by gap filling and ligation, the 3′-phosphate group has to be removed. A

third mammalian Nei-like glycosylase, NEIL3, has not yet been characterised [94, 95].

Several BER subpathways have been reconstituted in vitro with purified proteins. Short-patch BER of U/G mismatches is achieved by UDG glycosylase, hAPE1, Pol β and DNA ligase III [96]. XRCC1 is not absolutely required but enhances efficient ligation. Short-patch BER of 8-oxoG is sufficient with the bifunctional glycosylase OGG1, hAPE1, Pol β and ligase I [97]. Long-patch BER of a reduced AP site requires FEN1, which is stimulated by proliferating cell nuclear antigen (PCNA), and additionally the proteins involved in short-patch BER [98]. Pol β, δ and ε can perform the DNA synthesis step [98–100]. Human APE1 (hAPE1) has a strong AP endonuclease activity and less efficient 3′→5′ exonuclease, 3′ phosphodiesterase and 3′ phosphatase activities [101]. The exonuclease activity of hAPE1 is influenced by reaction conditions and by the structure of the 3′-terminal nucleotide [102, 103]. The exonuclease activity of hAPE1 preferentially removes nucleoside analogues, 3′ mismatched nucleotides and the natural dinucleotide compound Gp$_4$G [102–105]. The exonuclease activity seems to have an important function as a proofreading activity for Pol β. A second ExoIII-like protein, called hAPE2, has been identified in human cells [106]. Although the critical catalytic residues are conserved between hAPE1 and hAPE2, hAPE2 shows only weak AP endonuclease activity.

With APN1 and APN2, two AP endonucleases have been identified in *S. cerevisiae* [107, 108]. APN1 belongs to the EndoIV family and APN2 to the ExoIII family. Like EndoIV, besides an AP endonuclease function, APN1 also possesses 3′-phosphatase and 3′-phosphodiesterase activities, which can remove 3′-modified termini [88]. APN1 is responsible for almost all of the cellular AP endonuclease activity. APN2 possess weak AP endonuclease activity, but has strong 3′→5′ exonuclease and 3′-phosphodiesterase activities [109]. *S. cerevisiae* cells defective in APN1 are moderately sensitive to the alkylating agent methyl methanesulphonate (MMS), which primarily introduces AP sites, and sensitivity is further increased when APN2 is additionally defective [108]. Cells defective in APN1 and APN2, but not in only one of the AP endonucleases are sensitive to the oxidising agent H$_2$O$_2$. Thus, APN1 and APN2 play redundant roles in repair of oxidative damage and AP sites through alternate pathways, and APN1 is more important for repair of AP sites [108, 109].

During long-patch BER, strand displacement produces a flap structure that is processed by FEN1 (fig. 2D) [86]. FEN1 has also a function in other DNA metabolism such as in Okazaki fragment maturation during DNA replication (fig. 2I), in nonhomologous end joining (NHEJ), and in some organisms in an NER-independent UV-damage excision repair (UVER) pathway [63, 110–112]. FEN1 is

a structure-specific 5′ flap endonuclease, which cuts the ssDNA arm at the junction of a branched DNA structure (fig. 3B) [113]. It is thought that FEN1 binds to a nicked end and then translocates along the ssDNA to the branch point before it performs incision [64]. Recent studies with the *S. cerevisiae* homologue RAD27 showed that the preferred in vitro substrate is a double-flap structure that possess an one-nucleotide 3′ flap (fig. 3B) [34, 114]. In addition to its endonuclease activity, FEN1 also possesses a 5′→3′ exonuclease activity [63].

## Nucleotide incision repair

A repair system has been identified in *E. coli*, *S. cerevisiae* and human cells that, alternatively to BER, repairs various types of oxidative damage [7, 115]. In this pathway, the AP endonucleases Nfo (EndoIV) of *E. coli* and APN1 of *S. cerevisiae* incise DNA 5′ to the damage, thereby producing a 3′-hydroxyl end and a 5′-phosphate end that includes the damaged nucleotide (fig. 2E). Damage-specific 5′ incision occurs also in human cell extracts by a not yet identified endonuclease. The 5′-terminal damage can be released as a mononucleotide by *E. coli* DNA Pol I and in the presence of PCNA by human FEN1 (fig. 2E) [115]. The biological advantage of nucleotide incision repair (NIR) is that it can process oxidative damage without producing AP sites and blocking 3′-ends, genotoxic intermediates that are produced by BER. The existence of NIR may explain why DNA glycosylase-deficient *E. coli* and mice cells are not sensitive to reactive oxygen species [7].

## Damage-specific 3′ incision by endonuclease V

Endonuclease V of *E. coli* recognizes a wide spectrum of DNA damage, including AP sites, deaminated bases and mismatches [116]. Damage repair initiated by EndoV represents an alternative to BER initiated by DNA glycosylases, and has been termed alternative excision repair (AER) pathway [116]. EndoV hydrolyses the second phosphodiester bond 3′ to a lesion, resulting in a 3′-terminal hydroxyl group and a 5′-terminal phosphate group adjacent to the damaged nucleotide. It has been suggested that subsequent removal of the damage is carried out by a not yet identified 3′→5′ exonuclease in a short repair patch of a few nucleotides [116]. EndoV shares significant similarities with motifs of UvrC required for catalytic activity. Sequence homologues of EndoV are present in eukaryotes, suggesting that AER represents a conserved back-up system for BER [116]. In fact, it has recently been shown that the mouse homologue mENDO V incises DNA containing a hypoxanthine residue in the same way as the bacterial enzyme, but that it has a more limited sub-

strate spectrum [117]. Like NIR, AER avoids the forma-
tion of genotoxic repair intermediates which are formed
during BER.

## Repair of DSBs

DNA DSBs can be induced by ionising radiation, chemi-
cal agents, during replication and enzymatically during re-
combination [83]. DSBs can be repaired either by HR
(fig. 2F) or NHEJ and in some special cases by SSA
(fig. 2G). Repair of DSBs requires the MRE11 complex,
which is composed of MRE11, RAD50 and NBS1
(XRS2 in *S. cerevisiae*). Inactivation of the MRE11 com-
plex causes pleiotrophic defects in DSB repair, replica-
tion, checkpoint signalling, early meiosis, V(D)J recom-
bination and telomere-length maintenance [118]. In
addition, the MRE11 complex may play a role in homol-
ogous recombination, although an increasing amount of
data argues against this [118]. In humans, mutations in
MRE11 and NBS1 cause AT-like syndrome (ATLS) and
Nijmegen breakage syndrome (NBS), respectively [119,
120]. The AT-like syndrome is related to ataxia telangiec-
tasia, which is caused by a defect in the checkpoint pro-
tein ATM [121].
MRE11 exhibits DNA nuclease, strand-dissociation and
strand-annealing activities [118]. MRE11 contains an N-
terminal nuclease domain with four conserved phospho-
esterase motifs. The MRE11 nuclease domain has a ds-
DNA-specific $3'{\rightarrow}5'$ exonuclease activity and a $3'$ flap
endonuclease activity, which are structure specific [118].
The DNA-processing activity of MRE11 has a wide sub-
strate spectrum which partially depends on the interaction
with the other proteins of the MRE11 complex and on the
accessibility of ATP [118]. The exonuclease is active on
dsDNA substrates with blunt ends and with $3'$ recessed
ends, but not on dsDNA with $3'$ overhangs. The endonu-
clease cleaves $3'$ ssDNA flaps in splayed-arm structures
and at ssDNA/dsDNA junctions of stem-loop structures
and hairpins (fig. 3D) [118]. Remarkably, the polarity of
the nuclease is not compatible with the mechanistic re-
quirement for $3'$ overhangs during homologous recombi-
national (HR) (compare fig. 2F and fig. 3D). An interest-
ing model for how $3'$ ssDNA tails could be created by the
MRE11 complex has been suggested by Trujillo and Sung
[122]. After unwinding the dsDNA by a helicase, cleavage
of secondary structures, such as stem loops and hairpins
in the $5'$ ssDNA arm, would lead to $3'$ ssDNA tails. This
model, however, predicts that the $3'$ ssDNA arm has to be
selectively protected against incision.
In *S. cerevisiae*, the MRE11 complex seems to be in-
volved in NHEJ [123]. This is in contrast to the situation
in *Schizosaccharomyces pombe* and chicken cells, where
the MRE11 complex plays a minor role in NHEJ, if that
[124–126].

## UVDE-dependent repair

UVDE (also termed UVE1) is an ATP-independent en-
donuclease that has been found in *S. pombe*, *Bacillus sub-
tilis* and *Neurospora crassa*, but not in other species so far
studied [127–129]. UVDE is the key factor of the NER-
independent UVER pathway and initiates UVER by inci-
sion of a phosphodiester bond either immediately or one
or two nucleotides $5'$ to damage. UVDE incises at a vari-
ety of lesions, including the two major photoproducts,
CPDs and 6-4 photoproducts (6-4PPs), AP sites, oxidative
damage, base-base mismatches and small loops, indicat-
ing a more general role of UVDE in DNA repair [112].
RAD2 (the *S. pombe* FEN1 homologue) further processes
DNA incised by UVDE through its $5'{\rightarrow}3'$ exonuclease
activity [130]. The resulting gap can be filled in by Pol δ.
In the absence of RAD2, DNA repair intermediates
produced by UVDE are channelled into an error-prone
or sometimes lethal process [112]. Factors involved
in RAD2-independent UVER are EXO1, RHP18 and
RHP51, which have a function in recombinational
processes [112].

## Repair processes during replication

Before mitosis and cell division can take place, a cell has
to duplicate its genetic information with high accuracy.
DNA replication is performed by a multiprotein complex
called the replisome, which itself is located in so-called
nuclear replication factories [131]. Key factors of the
replisome are DNA polymerases, which replicate dsDNA
in a semiconservative manner.
Replication is initiated at origins of replication by syn-
thesis of an RNA primer by the primase activity of the Pol
α-primase complex. Subsequently, the RNA primer is ex-
tended to Okazaki fragments by Pol α [132]. For replica-
tion elongation, Pol α is replaced by Pol δ or Pol ε, which
are able to synthesise long stretches of DNA [132, 133].
Besides replication, DNA polymerases are also involved
in DNA repair, recombination and translesion synthesis
[133]. Some DNA polymerases have $3'{\rightarrow}5'$ exonuclease
activity, and DNA Pol I of *E. coli* additionally has a $5'{\rightarrow}3'$
exonuclease function. DNA polymerases that lack an in-
trinsic $3'{\rightarrow}5'$ exonuclease can in principle be associated
with factors exhibiting such activity. During replication,
nuclease functions are required for proofreading (fig. 2H),
for removal of Okazaki fragments (fig. 2I) and for cleav-
age of DNA intermediates that result from recombina-
tional repair of stalled or collapsed replication forks
(fig. 2J).

## Proofreading by 3′→5′ exonucleases

DNA Pol I of *E. coli* is involved in replication but shows low processivity [8]. Pol I has 3′→5′ and 5′→3′ exonuclease activity. The 5′→3′ exonuclease activity is structure specific and cuts at ssDNA/dsDNA junctions [134]. The cut is made between the first two nucleotides of dsDNA [135]. The 5′→3′ exonuclease activity of Pol I has structural homology to members of the FEN1 family [136, 137]. Pol I without 5′→3′ exonuclease activity is called a Klenow fragment.

DNA Pol III is the primary replication enzyme of *E. coli* and consists of 10 different subunits. The 3′→5′ exonuclease activity is located in the ε subunit and the polymerase activity in the α subunit [132]. The rate-limiting step in the proofreading reaction of Pol III is melting of DNA in which the 3′ terminus undergoes a conformational change before the DNA becomes a substrate of the 3′→5′ exonuclease [138].

DNA Pol II has a function in error-free and error-prone translesion synthesis and exhibits 3′→5′ exonuclease activity [132, 139, 140]. After blocking of replication by DNA damage, Pol II plays a central role in restarting replication by a damage bypass mechanism.

In eukaryotic cells, DNA Pol α, Pol δ and Pol ε are essential for replication of nuclear DNA and Pol γ for replication of mitochondrial DNA. Pol δ, Pol ε and Pol γ, but not Pol α, have a 3′→5′ exonuclease activity for proofreading of misincorporated nucleotides [132].

Pol δ has a function in DNA synthesis during BER, NER, MMR and recombination. Mice with a homozygous deficiency in Pol δ proofreading activity develop cancer, with skin squamous cell carcinoma being the most common form [141]. PCNA stimulates the processivity of Pol δ, and concomitantly reduces its fidelity by promoting misincorporation of nucleotides and extension of synthesis at mismatched substrates [142]. Surprisingly, in vitro replication fidelity of *S. pombe* Pol δ is one to two orders of magnitude lower than the fidelity of *E. coli* Pol III [143]. While the misinsertion rate of the two enzymes is nearly the same, the proofreading activity of *S. pombe* Pol δ appears to be very inefficient. The authors assumed that additional cofactors may be required for efficient proofreading by *S. pombe* Pol δ [143].

Similar to Pol δ, Pol ε is likely involved in DNA synthesis during BER, NER, MMR and recombination. In contrast to Pol δ, processivity of Pol ε is not stimulated by PCNA [133]. Pol ε of *S. cerevisiae* consists of four subunits with a proposed 1:1:1:1 stoichiometry [144]. The proofreading activity of Pol ε is located in the N-terminal region of the catalytic subunit POL2 [132]. The proofreading activity of Pol ε efficiently removes mismatched nucleotides during DNA replication, but is slowed down when dsDNA with matched nucleotides is encountered [145, 146].

The mitochondrial Pol γ is a member of the nuclease family A, in contrast to Pol α, β and ε, which belong to the nuclease family B [132]. In *S. cerevisiae*, mutation of one of the conserved residues of the exonuclease domain of Pol γ leads to a drastic decrease in 3′→5′ exonuclease activity and to an increased mutation frequency in mitochondrial DNA [147, 148]. However, the residual activity is sufficient to maintain enough mitochondrial DNA molecules for correct segregation into the daughter cells [147]. Pol α, Pol β and the translesion polymerases lack intrinsic proofreading activity [149]. Several 3′→5′ exonucleases have been identified which may substitute for the missing proofreading function of some of the polymerases. TREX1, exhibiting the major 3′→5′ exonuclease activity in human cells, processes ssDNA and dsDNA [150]. TREX2 is a TREX1 homologue with an overall amino acid identity of 44%, and an 80% identity in the Exo motifs (I, II, III_ε), which are conserved in exonucleases from phages to humans [151]. In an in vitro reconstituted DNA repair system, containing Pol β, DNA ligase III and XRCC1, the TREX1 proofreading activity is required for rejoining of an ssDNA break containing a mismatch at the 3′ end [152]. These data indicate that TREX1 can substitute for the missing proofreading activity of Pol β. TREX1 and TREX2 excise 3′ unpaired nucleotides from dsDNA more efficiently than nucleotides from dsDNA with blunt ends and from ssDNA [153].

Besides TREX1 and TREX2, 3′→5′ exonuclease activities have been also discovered for WRN, p53, hRAD9 and MRE11 [149]. However, the 3′→5′ exonuclease of TREX1 and TREX2 appears to be about 1000-fold more active than that of the other proteins [153].

Human ExoN possesses 3′→5′ exonuclease activity which excises nucleotides from ssDNA and dsDNA in a nonprocessive way [154]. Fidelity and elongation from mismatched base pairs by Pol α are increased in the presence of ExoN [155].

## Processing Okazaki fragments

Synthesis of Okazaki fragments is initiated by the primase activity of the Pol α-primase complex, which synthesises an RNA primer of 8–12 nucleotides in length [156]. The RNA primer is further extended by 20–30 DNA nucleotides by Pol α. In a reaction requiring replication factor C (RFC) and PCNA, Pol α is replaced by Pol δ. When Pol δ encounters the next downstream Okazaki fragment, strand displacement synthesis occurs. PCNA stabilises the strand displacement reaction in a way that Pol δ does not dissociate from DNA at RNA-DNA sites [157]. On the other hand, RPA ensures that strand displacement is limited to about 30 nucleotides and regulates the sequential function of Dna2 and FEN1 in removing the whole Okazaki fragment [157, 158]. Binding of RPA to the 5′

ssDNA flap protects the structure from FEN1 binding and cleavage [158]. Dna2 cuts the flap just 3′ to the end of the RNA-DNA primer (fig. 2I) synthesised by Pol α and thereby releases Dna2 and RPA [156, 158]. The remaining 5–7-nucleotide flap, which is too short for RPA binding, is subsequently cleaved by FEN1 (fig. 2I) or alternatively by other nucleases, such as EXO1 or RNase H [158]. The cleavage reaction results in a nicked duplex which can be sealed by ligase I [156].

## Repair of DNA structures formed after replication fork arrest

Various causes lead to DNA replication fork arrest. For example, inhibition of ribonucleotide reductase leads to depletion of the dNTP pool and thereby pauses replication [159]. In addition, DNA damage, such as chemical DNA crosslinks and strand breaks as well as DNA-protein complexes formed during cellular metabolism, can induce replication arrest [160]. Stalling of replication forks can lead to formation of abnormal DNA structures. The abnormal DNA structures induce a checkpoint response, and subsequent cell cycle arrest allows time for repair before replication is reinitiated [161]. In a recent study in yeast, it has been suggested that the abnormal DNA structure formed during replication is ssDNA and not a DSB [162]. This ssDNA can be repaired either by recombination or nonrecombinational gap filling. In contrast to the situation in yeast, stalling of replication in human cells leads to the formation of DSBs [163].

MUS81 was first identified in *S. cerevisiae* as a protein which interacts with the recombination protein RAD54 [164]. Shortly afterwards, the MUS81 homologue of *S. pombe* was identified [165]. spMUS81 interacts with the checkpoint kinase CDS1, which has a function in survival of cells confronted with replicational stress. MUS81 from *S. cerevisiae*, *S. pombe* and humans has endonuclease activity [75, 166, 167]. Endonuclease activity of *S. pombe* and *S. cerevisiae* MUS81 is performed in complex with EME1 and MMS4, respectively, which show limited homology to each other [77]. Human MUS81 interacts with hEME1, which is more homologous to EME1 of *S. pombe* than to MMS4 of *S. cerevisiae*. A second protein, hEME2, exhibits some homology to hEME1, which is mainly restricted to the C-terminal part of hEME1 [77].

Initial studies indicated that the MUS81 complex plays a role in cleavage of stalled replication forks and in resolution of Holliday junctions [75, 166, 167]. However, more recent studies gave new insights into the function of the MUS81 complex. Splayed-arm structures, which are efficiently cleaved by RAD1-RAD10 and XPF-ERCC1 (fig. 3E), are poorly processed by scMUS81-MMS4 and hMUS81-hEME1 [77, 168]. Notably, MUS81 complexes

poorly incise Holliday junctions [77, 166, 169]. 3′ flap structures are efficiently cleaved by scMUS81-MMS4. Importantly, incision occurs five nucleotides upstream of a 5′ end and thus requires a 5′ terminus near the junction (fig. 3F) [168]. It was further shown that hMUS81-hEME1 cleaves replication fork structures with two dsDNA arms [77].

A model for cleavage by the MUS81 complex has been proposed in which a stalled or collapsed replication fork is processed by synthesis-dependent strand annealing (SDSA) (fig. 2J), requiring the functions of recombinational repair proteins of the RAD52 epistasis group [162, 168]. After displacement and reannealing of the invading strand, a 3′ flap structure is produced that can be cleaved by the MUS81 complex (fig. 2J). Alternatively, the TOP3-SGS1 helicase can complete reannealing of the 3′ tail, thereby producing a 5′ flap structure, which may be processed by FEN1. That cleavage by the MUS81 complex occurs downstream and not upstream of recombinational repair is supported by the finding that synthetic lethality of a *sgs1 mus81* double mutant of *S. cerevisiae* is suppressed by additional mutation of *rad54* [162]. Inactivation of both pathways that deal with 3′ tails leads to accumulation of lethal HR intermediates, which in the *sgs1 mus81 rad54* triple mutant can be channelled into the nonrecombinational gap-filling process. In addition, it is unlikely that the MUS81 complex directly cleaves stalled replication forks since the presence of extensive stretches of ssDNA can be assumed. Such structures are likely not processed by the MUS81 complex, since cleavage requires a 5′ end near the junction (fig. 3F) [168].

## 3′→5′ exonuclease activity of WRN, p53 and hRAD9

The Werner syndrome is a disease of premature ageing and is caused by mutations in WRN [170]. The WRN protein belongs to the family of the RecQ-like helicases, which includes *E. coli* RecQ, *S. cerevisiae* SGS1 and human RECQL and BLM [170]. In contrast to the other helicases, WRN also has 3′→5′ exonuclease activity which is able to degrade dsDNA [171]. The 3′→5′ exonuclease activity of WRN is more efficient on dsDNA with a 5′ overhang than on dsDNA with a 3′ overhang or blunt-ended dsDNA [172]. WRN removes a terminal mismatched nucleotide just as well as terminal matched nucleotides. WRN is able to initiate degradation from gapped or nicked dsDNA, a substrate preference similar to that of *E. coli* ExoIII [172].

WRN interacts with FEN1 and EXO1 and increases their nuclease activities [170, 173, 174]. WRN also interacts with the Ku70/80 heterodimer, which is required for repair of DSBs by NHEJ [175, 176]. In complex with Ku70/80, WRN efficiently digests dsDNA with either a 3′ or 5′ overhang and blunt-ended dsDNA.

The tumour suppressor protein p53 functions to maintain the genomic integrity of mammalian cells by playing a role in cell-cycle control, apoptosis and DNA repair [177]. p53 has sequence-specific DNA binding activity, which is required for transactivation functions, and 3′→5′ exonuclease activity, which may be involved in various aspects of DNA repair.

More than 50% of all human cancers have a mutation in p53, frequently in the conserved central part, which is responsible for DNA binding and 3′→5′ exonuclease activity [178]. The major substrate of the 3′→5′ exonuclease activity of p53 is ssDNA and dsDNA with mismatched nucleotides, while matched nucleotides effectively retard excision [179]. p53 enhances the replication fidelity of Pol α, but not of Pol ε or *E. coli* Pol I [179]. In addition, p53 interacts with hAPE1 and Pol β, stabilises the interaction of Pol β with AP sites and stimulates the BER of uracil in DNA [180].

In *S. pombe*, six so-called checkpoint Rad proteins were identified which have a function in the early DNA damage checkpoint response [181]. It was shown by immunoprecipitation that a highly modified form of human Rad9 (hRad9) forms a complex with hRad1 and hHus1 (9-1-1 complex) [182]. The three proteins form a heterotrimeric checkpoint clamp complex (CCC), which is likely loaded by a checkpoint-specific clamp loading factor onto damaged sites of DNA [161]. CCC is phosphorylated in a damage-dependent manner, but it is not clear whether this is necessary for downstream signalling. Surprisingly, hRad9 has 3′→5′ exonuclease activity, which is located at its N-terminal part [183]. It has been suggested that hRad9 exonuclease activity has a function in primary DNA damage processing which may be important for the DNA damage checkpoint response [183].

## Conclusions

With a few exceptions, DNA repair mechanisms require the function of one or more nucleases to ensure removal of lesions from DNA and thus integrity of the genetic information. Several nucleases have endonuclease and exonuclease activity and are implicated in multiple pathways. A defect in a repair pathway can result in accumulation of mutations, cancer, inherited diseases and cell death. However, only a few human disorders are correlated with a defect in a DNA repair nuclease. This may be in part due to functional redundancy of DNA nucleases within a given pathway and to repair of the same type of lesion by multiple repair mechanisms. In recent years, new DNA nuclease activities and DNA repair pathways have been discovered which provide further evidence for such back-up functions. Clearly, more studies are necessary to further understand the functions of classical and novel repair nucleases and pathways and their interconnections.

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