Cell. Mol. Life Sci. 66 (2009) 1021 – 1038
1420-682X/09/061021-18
DOI 10.1007/s00018-009-8739-9
© Birkhäuser Verlag, Basel, 2009

Cellular and Molecular Life Sciences

Mismatched repair: variations on a theme

C. Kunz*, **Y. Saito and P. Schär**

Institute of Biochemistry and Genetics, Department of Biomedicine, University of Basel, Mattenstrasse 28, 4058 Basel (Switzerland), Fax: +41 (0)61 267 3566, e-mail: christophe.kunz@unibas.ch

Online First 21 January 2009

Abstract. Complementary base pairing underlies the genetic template function of the DNA double helix. Therefore, to assure faithful DNA transactions, cells must adhere to a strict application of the Watson-Crick base pairing principle. Yet, mispairing does arise in DNA, most frequently as a result of DNA polymerase errors or base damage. These mismatches need be rectified to avoid mutation. Sometimes, however, mispairing is actively induced to trigger mutagenesis. This happens in activated B-lymphocytes, where the targeted generation and processing of G·U mismatches contributes to somatic hypermutation and antibody diversification. Non-mutagenic mismatches arise in heteroduplex intermediates of homologous recombination, and their processing helps restrict homeologous recombination. Depending on the type of mismatch and the biological context of its occurrence, cells must apply appropriate strategies of repair to properly control mutagenesis. This review will illustrate conceptual and functional challenges of cellular mismatch correction on typical examples of mutagenic base-base mismatches. (Part of a Multi-author Review)

Keywords. DNA mismatches, DNA damage, mutagenesis, mismatch repair, base excision repair, somatic hypermutation.

Matches and mismatches in DNA

Base pairing is an inherent structural and functional feature of deoxyribonucleic acid (DNA). The prevalent structure of DNA is a right-handed double helix, consisting of two antiparallel single strands, each of which represents a linear sequence of adenine (A), cytosine (C), guanine (G) and thymine (T) nucleotides. The bases moieties of these nucleotides point towards the center of the double helix, where they come to lie within hydrogen-bonding distance of each other. Canonical base pairing, also referred to as Watson-Crick pairing, follows a strict rule by which A pairs with T and G pairs with C, establishing two and three hydrogen bonds, respectively (Fig. 1). Due to the complementary nature of these interactions, both DNA strands contain the same sequence information but as mirror images of each other. This ingenious structural template concept provides for endless and error-free duplication and maintenance of genetic information [1]. Be it DNA replication, repair, recombination or gene expression, all genetic transactions make use of the template function of DNA, which in turn is based on a strict application of the Watson-Crick base pairing principle.

Although Watson-Crick base pairing represents the most common base-base interaction in DNA, alternative hydrogen-bonding configurations do occasionally arise. These include so-called reverse Watson-Crick, Hoogsteen or reverse Hoogsteen base pairs [2], some of which may have a biological function [3], such as the stabilization of guanine quadruplex DNA at telomeres [4]. In general, however, bases lacking an appropriate Watson-Crick hydrogen-bonding partner represent erroneous occurrences that threaten the integrity of the DNA structure and the genetic code. Such DNA mismatches can be of different nature. Base-base mismatches consist of non-complementary juxtaposed bases including all possible combinations of non-Watson-Crick pairs, i. e. G·T, A·C, A·A, G·G, A·G, C·T, C·C and T·T. The hydrogen-bonding

* Corresponding author.

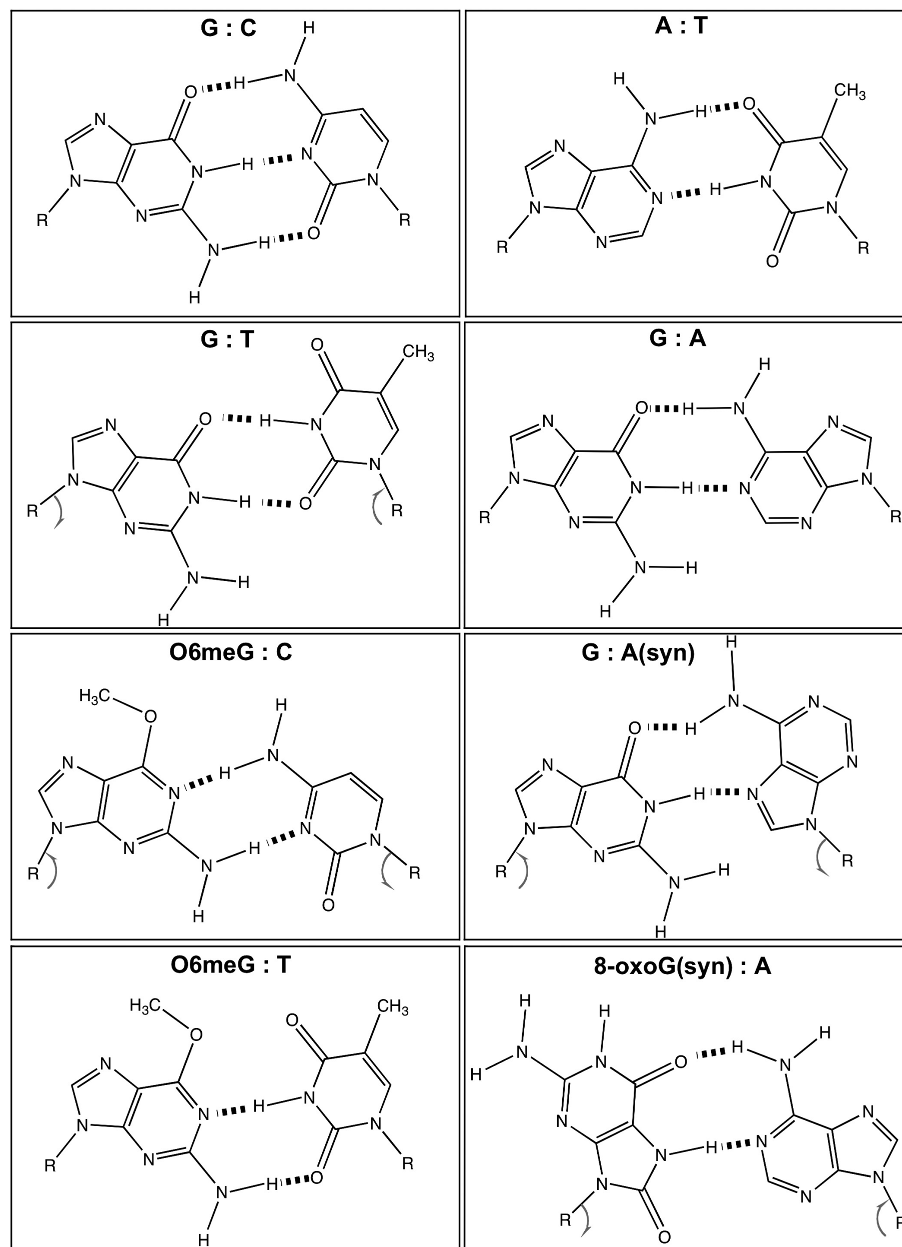

Figure 1. Hydrogen-bonding properties of Watson-Crick base pairs and base pair mismatches as determined by X-Ray and NMR structural analyses. Unless indicated otherwise, bases are shown in the *anti* conformation. Significant deviations from Watson-Crick glycosidic bond angles relative to the C1-C1 vector are indicated by arrows in grey.

potential of these mismatches varies considerably [5–8]. Consequently, individual base-base mismatches affect the helical structure of the DNA to different extents. The G·T mismatch, for instance, adopts a relatively stable and well-fitting wobble configuration, supporting intrahelical stacking, whereas the non-pairing C·C assumes poorly defined extrahelical arrangements [9]. Another type of DNA mismatch arises when, due to the presence of extra nucleotides in one DNA strand relative to the other, one or a few DNA bases remain unpaired and form so-called small nucleotide insertion/deletions loops (IDLs). Finally, mismatches may occur between DNA bases and uracil (U), most frequently in the form of G·U, or when chemical modification of DNA bases alters their hydrogen-bonding potential. Methylation of the O6-position of G, for instance, alters the Watson-Crick surface of the base so that pairing with C gets distorted and pairing with T becomes favorable in the context of the DNA duplex. Similarly, oxidation of G to 8-oxo-7,8-dihydroguanine (8-oxo-G) enables hydrogen bonding with A upon rotation of the damaged base around the N-glycosidic bond into a *syn* conformation (Fig. 2).

DNA mismatches can arise in different contexts of cellular DNA metabolism, and although they usually

consist of chemically regular DNA bases, they must be considered pro-mutagenic DNA aberrations. A notable exception is mismatches that appear in heteroduplex DNA following strand transfer between parental DNA sequences in the process of homologous recombination. In all other cases, failure of mismatch correction by cellular repair systems or misdirected repair to the strand containing the original and correct DNA sequence will inevitably give rise to genetic mutations. Purine-pyrimidine mismatches will give rise to transition mutations in 50 % of the progeny DNA; purine-purine and pyrimidine-pyrimidine mispairs to transversion mutations. Therefore, to maintain a stable genome, it is essential for cells to monitor the state of base pairing in their genomes and to correct mismatches that will occasionally occur. To achieve this, cells are equipped with multiple mismatch correction systems. These differ in reaction mechanisms but share common substrates, so that, depending on the context of mismatch occurrence, they can optimally fulfill the task of mutation avoidance. Two main pathways are to be distinguished: postreplicative mismatch repair (MMR) and base excision repair (BER) (Fig. 3). In MMR, homologs of the bacterial MutS (e.g. human MSH2, MSH3, MSH6) and MutL proteins (e.g. human MLH1, PMS2) constitute functional complexes for mismatch recognition and coordination of nucleolytic excision. The system is capable of discriminating newly synthesized forms of parental DNA strands and is therefore suited to correct DNA polymerase errors. In BER, mismatch-specific DNA glycosylases (e.g. human TDG, MBD4, MYH) recognize and directly hydrolyze mispairing bases. Excision is directed to the base representing a particular form of DNA damage. BER is therefore designed to process mismatches in the context of DNA damage. Mechanistic features of the two repair pathways are discussed below. Generally, defects in mismatch correction give rise to increased mutation rates, which, in humans, are associated with an increased risk of cancer [10, 11].

In the following, we will discuss conceptual aspects of mismatch processing from the perspective of the biological context of mismatch occurrence and the mechanistic requirements for repair. To simplify the illustration of relevant functional networks, we will focus on the formation and repair of typical base-base mismatches.

Origin and genetic consequences of DNA mismatches

The first evidence for the existence of non-Watson-Crick base-pairing in cells came from studies of meiotic recombination in ascomycetous fungi. At a very low frequency, haploid spores produced through meiosis were found to segregate both parental alleles in the first mitotic division after meiosis, a phenomenon known as postmeiotic segregation, PMS. This particular type of non-Mendelian segregation was proposed to reflect the generation and failure of repair of DNA mismatches in intermediates of homologous recombination [12]. At about the same time, mismatch generation and repair was also postulated to account for the occurrence of 5-bromouracil-induced lactose-negative mutants of *Escherichia coli.* This was explained by misincorporation of 5-bromouracil into DNA opposite G, followed by misdirected repair of the G in an attempt to restore Watson-Crick base-pairing [13]. Hence, these very early observations already indicated that DNA mismatches do occur and are processed in different contexts of DNA metabolism. Extensive research into the subject over the subsequent decades provided a comprehensive understanding of the molecular events underlying the generation and repair of DNA mismatches.

Mismatches generated by DNA polymerases

During the biosynthesis of DNA, DNA polymerases use a template DNA strand to select nucleotides for incorporation into the nascent strand. This applies to both semi-conservative DNA replication and synthesis associated with DNA repair, and the precision of the polymerases involved directly affects the base pairing configuration of the resulting double helix. So even if high-fidelity enzymes are at work, the millions of nucleotides that need to be assembled make DNA polymerase errors a major source of DNA mismatches. The overall accuracy and error spectrum of a DNA polymerase is determined mainly by three parameters; the nucleotide selectivity of its active site, its mismatch extension capacity, and its proofreading ability [14, 15]. In eukaryotes, the bulk of nuclear DNA is replicated by two members of the B family of DNA polymerases, Polδ and ε, whereas a third member, Polα, contributes by RNA-DNA primer synthesis [16]. The average fidelity of these enzymes is in the order of one error in 10^5 nucleotides synthesized, which, in the case of Polδ and Polε, is further improved to 1 in 10^7 by their inherent proofreading ability [17]. By structural considerations, mispairings that cause little distortion to the canonical Watson-Crick geometry are more likely to be tolerated by the polymerase active site and, therefore, to escape proofreading. This applies to wobble base pairs such as the G·T, to mismatches composed of rare base tautomers, and to the perfectly-matched nascent base pair in IDLs that arise by dissociation and reassociation of the polymerase, hence allowing template and primer strands to melt and reanneal out of register in

repetitive sequences [9, 18, 19]. Considering the size of the human genome (~3×10^9 nucleotide pairs); however, an error rate of 1 in 10^7 would still generate hundreds of mispairs during DNA replication, mostly of the purine-pyrimidine and IDL types. Thus, to achieve a mutation rate of 1 in $10^9 - 10^{11}$ as estimated for human cells [20, 21], mismatch repair activities must act downstream of the replication fork and reduce the error rate by an additional 100-fold.

As long as the cellular dNTP supply is sufficient and balanced, nucleotide misincorporation by replicative DNA polymerases is a relatively rare and stochastic event. If the dNTP pool gets out of balance and the ratio of correct to incorrect dNTP decreases, base substitution by DNA polymerases increases [22]. Moreover, dNTP perturbations can cause template-primer misalignments that lead to IDLs [23], and an excess of dNTPs may affect proofreading efficiency by driving DNA chain elongation past a mismatch forming at the nascent base pair binding site of the polymerases [24]. All of this is evident from the mutagenic effects of drugs affecting nucleotide metabolism [25, 26]. Indeed, the nucleotide pool is often a target of clinical chemotherapies. The underlying strategy is to perturb DNA replication, and hence cell proliferation, by extensive misincorporation of nucleotides. 5-Fluorouracil (5-FU), for instance, inhibits thymidylate synthase (TS), the enzyme responsible for the reductive methylation of dUMP in the final step of dTMP biosynthesis. Inhibition of TS thus leads to an accumulation of dUMP at the expense of dTMP, which, upon phosphorylation to dUTP, gets incorporated into DNA [27, 28] [C. Kunz, unpublished data]. Mostly, this will happen opposite a template A and generate canonical Watson-Crick base pairs. Occasionally, however, dUMP will get incorporated opposite G, generating U·G mismatches [29]. Through metabolic interconversions and allosteric regulatory mechanisms, the 5-FU-induced depletion of dTTP triggers further alterations in nucleotide levels, giving rise to a general imbalance in the nucleotide pool (reviewed in [30]). Similar scenarios apply to other drugs affecting nucleotide pools, including antifolates such as methotrexate or ribonucleotide reductase inhibitors like hydroxyurea.

Mismatches in DNA can also arise when chemically damaged nucleotides contaminate the dNTP pool. Base modifications in dNTPs, mostly oxidation or alkylation, will exhibit ambiguous base pairing properties and thereby fool the DNA polymerases in the process of chain elongation. The oxidation of dGTP serves as an illustrative example here. Through reactive oxygen species generated by cellular metabolism, dGTP or its precursors oxidize to 8-oxo-dGTP, the most common form of oxidative DNA base damage. 8-oxo-GTP will compete with dTTP for incorporation opposite A during DNA synthesis, generating 8-oxoG·A mispairs (Fig. 1), which will give rise to T·A→G·C transversion mutations if not repaired appropriately [31–34]. Similarly, nucleotide pool alkylation may generate base derivatives, including the highly mutagenic O6-methylguanine (^{Me}G), which, upon conversion to ^{Me}GTP, may get incorporated into DNA opposite a template T. If not repaired properly, the resulting ^{Me}G·T mismatch will give rise to A·T→G·C transitions [35].

Finally, eukaryotic cells possess a number of specialized DNA polymerases that exhibit low template fidelity and are therefore error prone. These include members of the family X enzymes (e. g. Polβ and Polλ) that are responsible for short-patch repair synthesis, as well as several Y-family polymerases (e. g. Pol η, Polι, Polκ) that are able to replicate damaged DNA templates (reviewed in [17]). Besides lacking proofreading activity, the latter also have poorly discriminating active site architectures and therefore accommodate non-fitting nucleotides rather easily (reviewed in [36, 37]). Hence, DNA synthesis by these polymerases leads to frequent generation of mismatches, with frequencies ranging from about 10^{-4} for the X-family enzymes up to about 10^{-1} for the Y-type enzymes [38–41]. However, since these enzymes are designed to synthesize only very short stretches of DNA, mostly in the context of bypassing non-instructive DNA lesions, their overall contribution to mismatch generation in unperturbed cells may be rather low.

Mismatches at sites of DNA damage

DNA damage that may occur spontaneously or be induced by chemical or physical mutagens can alter the hydrogen-bonding potential of bases. In some cases, such base modification will generate a promutagenic mismatch directly; in others, it will arise only upon synthesis across the lesions.

A first mutagenic event to be considered is the hydrolytic deamination of DNA bases. Four out of the five bases present in DNA, namely A, C, 5-methlycytosine (5-meC) and G, possess exocyclic amino groups that contribute to the Watson-Crick surface of the base (Fig. 1). These amino groups are susceptible to spontaneous hydrolytic deamination at physiologically significant rates [42], which, in consequence, alters the hydrogen-bonding potential of the respective base. In the case of C and 5-meC, for instance, deamination will produce U and T, respectively, both mismatched with G in the DNA duplex (Figs. 1, 2). Unlike DNA polymerase errors that occur mainly in the nascent strand during DNA replication, deamination can affect bases in either DNA strand

anytime in the cell cycle, generating an appreciable number of G·U and G·T mismatches. If not restored to the original G·C base pair, the DNA strand containing the deaminated base will give rise to a C·G→T·A transition mutation in the subsequent round of DNA replication. Similarly, A and G are prone to deaminate spontaneously to hypoxanthine (Hx) and xanthine (X), respectively, generating poorly matched Hx·T and X·C base pairs. Hx will pair with C during DNA synthesis, generating a non-Watson-Crick Hx·C base pair that will ultimately give rise to A·T→G·C transitions, unless repaired [43, 44]. The situation is less clear for the deamination product of G. Although incorporation of all four naturally occurring nucleotides opposite X was shown *in vitro*, base pairing with C and T seems unstable due to electrostatic repulsion (reviewed in [45]). Thus, replication across this base may be impaired and result in fork arrest rather than generation of mismatches.

Similarly, the oxidation of G to 8-oxoG in DNA generates a potentially miscoding template base, facilitating the incorporation of dAMP instead of dCMP opposite the lesion during DNA synthesis (Figs. 1, 2). Unless corrected, the resulting 8-oxo-G·A mismatches will give rise to G·C→T·A transversion mutations upon another round of DNA replication [46, 47].

Alkylating agents of the unimolecular nucleophilic substitution (SN1) type (e.g. N-methyl-N′-nitro-N-nitrosoguanidine) generate a range of DNA lesions, including the cytotoxic and highly mutagenic O6-methylguanine (^{Me}G) [48, 49]. Methylation of the O6 position of G alters its hydrogen-bonding potential in such way that it will pair equally well with C or T during DNA replication. Both ^{Me}G·C and ^{Me}G·T then activate mismatch repair processes. If repair fails, the ^{Me}G·T mismatch will give rise to G·C→A·T transition mutations through another replication cycle.

In contrast to conventional base-base mispairing, a special type of mismatch forms at sites of base loss. Base loss is a hydrolytic process generating apyrimidinic or apurinic sites (AP site) in one DNA strand. Purines are more frequently affected than pyrimidines, with experimental evidence suggesting that numbers of events amount up to 10 000 depurinations/cell/day [44]. Base loss thus generates a large number of unpaired bases, mostly pyrimidines, in genomic DNA. Unlike most base-base mismatches, however, which contain coding DNA sequences in both strands, AP sites represent non-instructive lesions that prevent DNA polymerases from properly selecting and fitting incoming dNTPs for a successful nucleotidyl transfer [37]. Consequently, AP sites will obstruct DNA synthesis by replicative DNA polymerases and force the incorporation of random nucleotides. Generally, however, AP sites, like other replication-blocking DNA lesions [50], will trigger the engagement of specialized translesion synthesis (TLS) polymerases (reviewed in [17]). Mutagenesis data suggest that these appear to incorporate preferentially A opposite AP sites, a concept that has been referred to as the A-rule (reviewed in [51]). Thus, TLS polymerases allow DNA synthesis across difficult-to-replicate lesions, thereby tolerating mispairing but avoiding replication fork collapse [50].

Mismatches induced on purpose

A most fascinating recent discovery is that, under specific circumstances, DNA mismatches may be actively generated to facilitate localized mutagenesis or recombination. Somatic hypermutation (SHM) in antigen-stimulated B-cells is a prominent example, where activation induced deaminase (AID), a member of the APOBEC protein family, deaminates DNA cytosines to generate G·U mismatches in the immunoglobulin variable-gene region. The purpose is to induce a mutagenic process that results in an affinity maturation of the antibody expressed (reviewed in [52]). These G·U mismatches are subject to repair, but the process evidently allows for mutations to occur and is therefore error prone. Cytosine deamination by AID also induces class-switch recombination (CSR) at the immunoglobulin locus, presumably by providing excision repair mediated single-stranded DNA breaks that trigger initiation of recombination (reviewed in [53]).

Active deamination may also occur in the context of gene promoter activation. In mammals, cytosine in CpG dinucleotides is frequently methylated at the carbon-5 position (5-meC), a modification that affects chromatin structure and gene expression (reviewed in [54]). Recent evidence suggests that activation of gene transcription is accompanied by cyclic de- and remethylation of specific CpGs in gene promoters [55, 56]. The mechanisms involved in the active demethylation step are not clear at present. Although direct excision and replacement of 5-meC with C by DNA repair-type mechanisms has been observed [57–59], there is also evidence for an indirect process by which 5-meC is first deaminated to T, thereby generating a G·T mismatch, which is then repaired to give rise to an un-methylated G·C pair [56].

Generally, the presence of C and 5-meC deamination activities in cells bears the potential of erroneous formation of mutagenic G·U and G·T mispairs in the genome. Hence, these activities require stringent control, both at the level of targeting and activation, but also by systems that correct mistargeted deamination events [60].

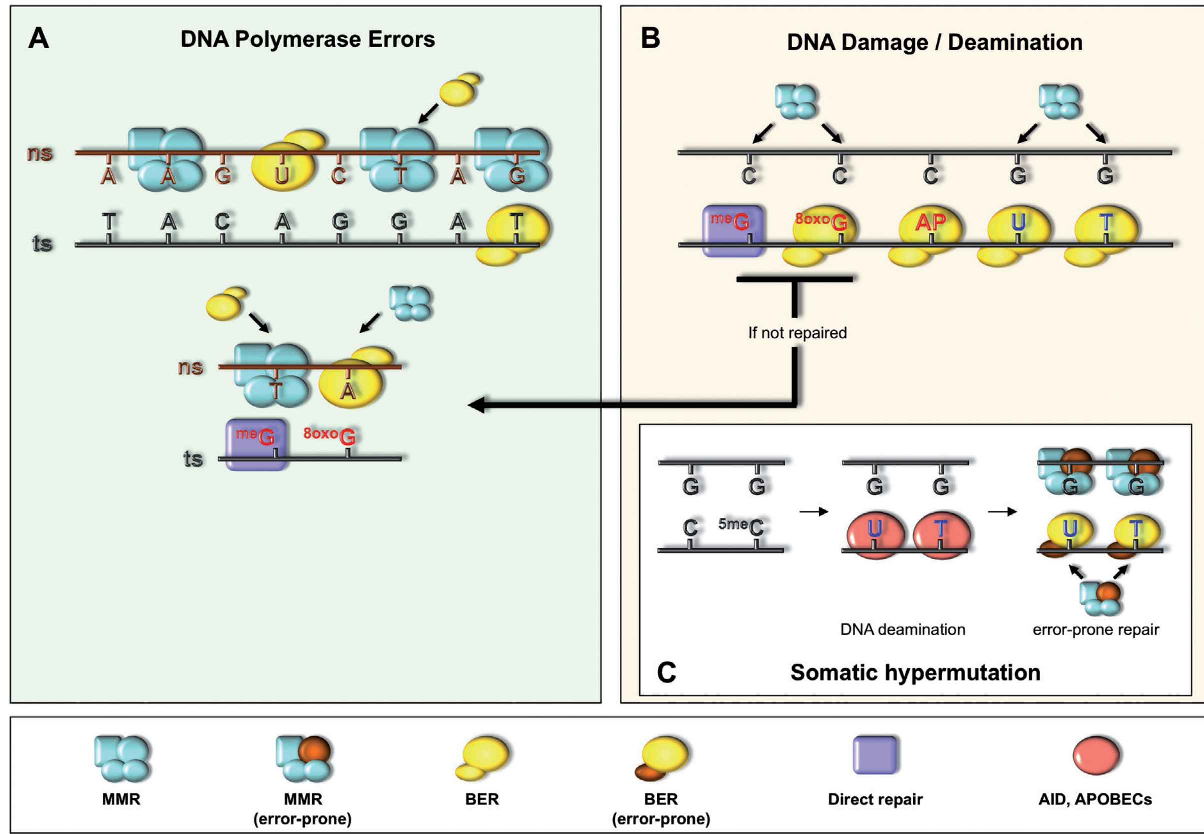

Figure 2. Repair of single base mismatches arising under various circumstances. The panels schematically illustrate the contribution of different pathways contributing to the repair of mismatches emerging from DNA polymerase errors (*A*), by DNA damage (*B*) or induced during somatic hypermutation (*C*). (*A*) Repair of mismatches arising from nucleotide misincorporation during DNA synthesis requires pathways able to rectify the erroneous base in the nascent strand. Owing to its ability to discriminate nascent (ns) from template (ts) strands, postreplicative MMR is the most appropriate system for correction of mispairs between canonical DNA bases, while misincorporated uracil is primarily removed through BER initiated by UDGs. G·T mismatches may also be addressed by TDG/MBD4-mediated BER. Due to the specific removal of T, however, this pathway may generate mutations if G is misincorporated opposite T. (*B*) DNA damage occurring either spontaneously or induced by chemical or physical mutagens can alter the hydrogen-bonding potential of bases. In the case of spontaneous hydrolytic deamination of C or 5meC, this directly generates promutagenic G·U or G·T mispairs, respectively. Due to altered base pairing properties, 8-oxo-G and O6-methylguanine (^{me}G) may lead to mismatches if not repaired prior to replication. As base damage may occur in any DNA strand, repair of damage-induced mismatches requires systems capable of eliminating the damaged and potentially mutagenic base, irrespective of the DNA strand it is located in. As BER is damage directed, most of these lesions are normally fixed by this pathway. In addition, ^{me}G can be repaired by direct damage reversal. MMR may also recognize and process such mispairs, but this would randomly generate mutations if the damage occurred in non-replicating DNA or if the miscoding base was present in the parental DNA strand during DNA replication. (*C*) During somatic hypermutation, mismatches are introduced through active enzymatic deamination of C and possibly also 5me-C. In this context, mismatches are formed and processed for the purpose of introducing local mutations. Consequently, repair initiated by BER and MMR enzymes needs to follow an error-prone strategy.

General concepts of mismatch correction

As we have seen, DNA mismatches arise under different circumstances in cells. Most of them represent promutagenic structures, as one of the two DNA stands contains a sequence alteration. Such mismatches generally need to be rectified in a conservative manner so that mutation is avoided. Somatic hypermutation represents a notable exception. Here, mismatches are generated and processed for the purpose of introducing mutations. Evidently, the different contexts of mismatch generation require different concepts of mismatch correction, and cells must be able to apply and coordinate their repair activities accordingly (Fig. 2).

Correction of DNA polymerase errors

Because nucleotide misincorporation generates genetic rather than physical damage to DNA, the resulting mismatches usually lack distinctive chemical alterations that help target DNA repair activities. However, mismatches establish unusual base-base pairing interactions that can be detected by specialized mismatch binding factors. Proteins with such function include members of the highly conserved MutS protein family (reviewed in [61]) as well as

mismatch-specific DNA glycosylases including TDG and MYH, the mammalian orthologs of bacterial Mug and MutY, respectively. MutS proteins act as dimeric complexes (e. g. MutSα and MutSβ in eukaryotes) to recognize mismatches and initiate repair by the postreplicative MMR system. By contrast, mismatch-specific DNA glycosylases bind and excise mismatched bases, thereby triggering a DNA BER process [62, 63] (Fig. 3).

In the context of DNA polymerase errors, it is imperative that nucleotide excision be directed to the nascent DNA strand because this is where the error occurred. Thus, besides the ability to recognize DNA mismatches, a repair system dealing with such errors must provide strand discrimination competence to avoid mutation by accidental excision of the correct template nucleotide. Ideally, such a system should be coupled to DNA replication as a postreplicative process, whereas other mismatch processing activities without strand discrimination function should be prevented from repairing DNA polymerase errors (Fig. 2).

The MMR system controlled by members of the MutS and MutL protein families is not only the most intensely studied but also the most suitable for the correction replication errors. This system functions by degradation and resynthesis of the nascent DNA strand including the mismatched nucleotide (Fig. 3). In eukaryotes, the nucleolytic degradation appears to be directed to the DNA stand containing discontinuities in the proximity of the mismatch. This suffices to discriminate the nascent DNA strands in the replication process by the presence of DNA primer ends and/or strand-interruptions between unprocessed Okazaki fragments. The MMR system is able to process most base-base mismatches and small IDLs, albeit with different efficiencies: G·T, G·G, A·A and A·C mismatches and IDLs with less than four unpaired bases are repaired with high, G·A, T·T and C·T mismatches with intermediate, and the C·C mismatch with exceptionally poor efficiency [64–67]. In view of MMR acting as a backup of polymerase proofreading, it makes perfect sense that this system acts most efficiently on mispairs that are likely to escape polymerase proofreading, i. e. the G·T wobble base pair and IDLs occurring at microsatellite sequences away from the nascent base pair binding site of the polymerase.

Although, owing to its ability to direct repair to the newly synthesized DNA strand, the MMR system is best suited for the correction of DNA polymerase errors; it may face competition from other repair activities. In mammals, DNA glycosylases like TDG and MBD4 may compete for G·T and MYH for G·A or C·A mismatch binding and processing (Fig. 2) [62, 68, 69]. However, DNA glycosylases are designed to eliminate damaged DNA bases, irrespective of whether they are located in the template or nascent strand during DNA synthesis. In the case of the G·T mismatch, this will be the T because it represents a deaminated 5-meC substrate for TDG or MBD4; in the case of the G·A mismatch, this will be the A because it mimics the 8-oxoG·A substrate for MYH. This damage directedness will compromise strand discrimination in the context of DNA replication, particularly if G happens to be misincorporated in the nascent strand opposite a template T or a template A. Hence, if the glycosylases gained access to the mismatch, BER would correct the template strand, thereby generating a mutation.

Coordination between different mismatch repair activities is therefore required to facilitate productive repair and avoid unfavorable mutagenic interferences (Fig. 2). This can be achieved by spatiotemporal regulation of mismatch recognition, be it at the levels of supply and/or activity of mismatch binding proteins and/or by targeting such factors to specific sites where their action might be needed.

For MMR, little is known about regulation at expression and activity levels, but a large body of evidence suggests a direct physical coupling of key components of the system to the DNA replication apparatus. The eukaryotic mismatch recognition complexes MutSα and MutSβ as well as MLH1 all interact physically and functionally with PCNA (proliferating cell nuclear antigen), the trimeric sliding clamp facilitating processive DNA synthesis (Fig. 3) [70–74].

MYH expression is increased in replicating cells and an interaction with PCNA was also reported. Thus, this DNA glycosylase is targeted to the replication fork where it may compete with the MMR system for the processing of G·A (or C·A) mismatches. Notably, the interaction with PCNA was proposed to orient MYH to the newly synthesized DNA strand [75, 76]. Such strand discrimination functionality in the context of DNA replication would allow MYH to excise A that gets misincorporated opposite a template G (or C), while preventing it from mutagenic processing of a parental A following misincorporation of G.

G·T processing in the context of DNA replication appears to be coordinated at protein availability and activity levels. TDG, the predominant G·T glycosylase in mammalian cells, is eliminated from cells in S-phase of the cell cycle by the ubiquitin-proteasome system, whereas MBD4, a minor G·T glycosylase, is present throughout S-phase but, apparently, in an inactive form [77]. Hence, BER is unlikely to disturb G·T processing by MMR in the context of DNA replication.

Repair of DNA damage associated mismatches

Unlike DNA polymerase errors, mismatches that occur as a consequence of DNA base modification require repair systems capable of excising the irregular and potentially mutagenic base, irrespective of the DNA strand it is located in. The postreplicative MMR system seems of little use here. Even if such mismatches arise in replicating DNA, repair directed to the newly synthesized strand would generate mutations whenever the miscoding base lesion occurred in the parental DNA strand. On the other hand, MMR in non-replicating DNA would randomly fix mutations because of the inability of this system to identify the mutagenic base.

Considering cytosine deamination, repair of the resulting G·U mismatch appears a straightforward task. Because the uracil itself represents a distinctive foreign base in DNA, a specific recognition of the non-Watson-Crick configuration of the G·U mispair is not necessarily involved here. Therefore, any DNA glycosylase with an ability to recognize and excise U from DNA will suffice to initiate a BER process that will restore the original G·C base pair [68, 78–80]. The situation is entirely different for G·T mismatches that arise from deamination of 5-meC. Here, the mutagenic T is not different from any other T in the DNA except that it happens to be mispaired with G. Repair in this case therefore requires an activity that is capable of recognizing and processing a mismatched T without attacking normal A·T base pairs. Such an activity is provided by TDG and MBD4, the two mismatch-specific thymine DNA glycosylases [68, 81]. Both are capable of excising T from the G·T mismatch, thereby initiating BER to restore the G·C base pair. Although a G·T mismatch arising from 5-meC deamination represents a perfect substrate for the MMR system, an engagement of this pathway seems useless in this context; its inability to recognize T as the mutagenic base would lead to frequent mutagenic repair.

A similar conflict arises upon generation of 8-oxo-G. If this lesion is not eliminated by Ogg1-dependent BER [82], it will give rise to 8-oxo-G·A mispairs upon DNA replication (Figs. 1, 2). 8-oxo-G mispaired with A is no longer a substrate of Ogg1, which seems reasonable because 8-oxo-G excision in this context would generate G·C to T·A mutations. The MMR system might get involved here but is not preferable because it would target the A in the nascent DNA strand, leaving 8-oxo-G in the parental DNA strand untouched. Attempts of repair would lead to reiterated excision and reincorporation of A and eventually be fatal. To overcome this dilemma, human cells employ MYH, an ortholog of the *E. coli* MutY protein that excises A from 8-oxo-G·A mispairs [83]. In doing so, MYH provides an opportunity to restore the initial 8-oxo-G·C base-pair, which can then be further processed by Ogg1 to yield the original G·C base pair. Yet, this requires C be preferentially incorporated opposite 8-oxo-G in MYH-dependent BER, a functionality which may be provided by DNA Polλ. Polλ, more than the canonical BER polymerase Polβ, is capable of inserting C opposite 8-oxo-G with a high preference (1200-fold), provided it is assisted by the auxiliary proteins PCNA and RPA [84].

The highly mutagenic and cytotoxic ^{Me}G favors misincorporation of T but also pairs with C. Both ^{Me}G·C and ^{Me}G·T represent non-Watson-Crick base pairs (Fig. 1) and hence are substrates for mismatch-processing activities [85]. However, an efficient first-line defense against ^{Me}G is achieved by direct repair involving the O^6 methylguanine-DNA-methyltransferase (MGMT). MGMT transfers the methyl group of ^{Me}G to a cysteine residue in its active site, thereby directly restoring G while irreversibly inactivating its repair activity (reviewed in [48]). This wasteful but straightforward and non-invasive repair process avoids base misincorporation opposite ^{Me}G, and it prevents futile excision repair. If MGMT is inactive or saturated, however, ^{Me}G·T and ^{Me}G·C base pairs will arise during DNA synthesis and MMR engages in an attempt to correct these mispairs. This, however, will not be productive because nascent strand-directed repair will fail to remove the template ^{Me}G and therefore continuously regenerate ^{Me}G·T (or C) mismatches [86, 87]. This way, on treatment of cells with S_N1-alkylating agents, the MMR system will generate persistent DNA damage that triggers DNA damage signaling and eventually induces cellular apoptosis [49, 88]. However, BER may come into play as well. Both, TDG and MBD4/MED1 were shown to process T opposite ^{Me}G *in vitro* [89, 90]. Like MMR, though, BER is likely to be non-productive and cytotoxic in this context, mainly because the associated Polβ will synthesize across ^{Me}G only inefficiently and preferentially reinserts a T [91]. Hence, direct repair rather than MMR or BER is to be engaged to prevent futile processing of alkylation induced ^{Me}G·T and ^{Me}G·C mismatches.

Repair of enzymatically induced mismatches

It is our general understanding that mismatch repair systems act to avoid mutation, although the considerations above illustrate how their misengagment can in fact generate mutations. It seems counterintuitive, though, that under certain circumstances these very systems are used to actively induce mutation. So it happens during somatic hypermutation (SHM) in activated B-lymphocytes, where AID triggers mutation by deaminating C to generate G·U mispairs [52].

These will attract primarily uracil DNA glycosylases (UDGs) to activate BER, but occasionally also the MMR system (Fig. 2). Paradoxically however, the purpose of U excision in this context is to initiate mutagenic repair to broaden the spectrum of possible mutations following cytosine deamination, i.e. to allow mutations other than C→T transitions to occur around the deaminated G·C base pair [92].
Different scenarios of G·U-dependent mutation have been proposed on the basis of experimental evidence and theoretical considerations. C→T mutations at the deaminated G·C itself may occur by replication across the G·U mismatch, thus generating C·G and T·A duplexes, or by MMR directed to the G strand, generating a A·U base pair. For this to happen, however, UDGs would have to be prevented from excising the U prior to replication or MMR.
Removal of U by a UDG may explain the occurrence of non-C→T mutations at the deaminated G·C [93, 94]. UDGs would thus generate an AP site (Fig. 3) that will trigger non-instructed base incorporation during DNA replication, presumably involving translesion synthesis by a lesion-tolerant DNA polymerase [17, 37, 95]. The nucleotide preferentially incorporated opposite the AP site will depend on the properties of the polymerase in action, as well as on the DNA sequence context. This scenario, however, strictly requires an uncoupling of U excision from the downstream steps of BER, so that the unprocessed AP site can serve as a template for DNA synthesis during subsequent DNA replication. Exactly how AP-site protection is achieved remains unclear, although the engagement of a UDG with a very slow AP-site dissociation rate like TDG might provide an answer here [62].
Another way to explain non C→T mutations at G·U base pairs is mutagenic BER. This scenario would require the excision of U be followed by error-prone repair synthesis across the undamaged template G, presumably involving a DNA polymerase other than the relatively accurate Polβ, which is normally associated with BER. Although such a process is hypothetical, Polβ indeed appears to be downregulated in B-lymphocytes undergoing SHM [96] and substituted for by DNA polymerases with poor template base selectivity such as Polθ, Polη and Polζ, providing sufficiently high misincorporation rates [97].
Mutations at A·T base pairs in the vicinity of AID-generated G·U mispairs must be explained by long-patch excision repair events that must be associated with MMR rather than BER. Indeed, inactivation of the MMR pathway was shown to significantly reduce A·T site mutations [92, 98–100], and further experimental evidence suggests that these MMR-dependent mutations arise by coupling G·U recognition by MUTSα with long-patch nucleolytic excision by Exo I and repair synthesis by an error-prone DNA polymerase, possibly Polη [101–105]. This suggests that mismatch recognition by MUTSα can be variably associated with error-free or error-prone downstream processing for two diametrically opposed purposes; mutation avoidance or mutagenesis. The regulatory mechanisms assuring the correct engagement of these MMR subpathways remain to be established.
In the context of hormone-induced gene activation, DNA methyltransferases (DNMT3a/b) were reported to deaminate 5-meC at methylated CpG dinucleotides to generate G·T mismatches as intermediates of excision repair mediated local DNA demethylation [55, 56]. Hence, unlike in SHM, the repair of these G·T mismatches must be accurate and restore G·C base pairs in all cases. BER appears to be the pathway primarily dealing with these mispairs and TDG was reported to be responsible for T excision. This goes in line with evidence showing that TDG is targeted to gene promoters through interactions with transcription factor complexes, including nuclear and hormone receptors [106–108], but also DNA methyltransferases [56, 109, 110]. Whether or not MDB4/MED1, the second G·T-processing activity in mammalian cells, contributes to the repair of such mismatches is currently unclear [68]. Because of the potential of mutagenic misrepair in the absence of a strand-discriminating signal (DNA strand interruption), MUTSα-dependent MMR must be prevented from processing these gene activation-induced G·T mismatches. Also, long-patch DNA excision associated with MMR would be unfavorable because methylated CpGs not destined for demethylation might be co-repaired and hence demethylated. The mechanisms assuring the preferential engagement of BER and preventing misengagement of MMR are not clear, but the direct physical association of TDG with transcription factors at gene promoters is likely to play a critical role [106–110].

Basic mechanisms of mismatch repair

Research over the last two decades has generated fairly detailed knowledge about the molecular transactions involved in MutS and DNA glycosylase-dependent mismatch repair. These pathways have been summarized in numerous excellent reviews throughout the years [61, 111–113], and there is little we could add to this. Since this review is about general concepts of cellular mismatch processing, including the biological complexity of coordinating repair activities to properly control mutagenesis, the following section is meant to provide just a quick overview

on relevant mechanistic features of the repair systems involved, mainly at the level of mismatch recognition and strand discrimination (Fig. 3).

The postreplicative mismatch repair system

Much of our mechanistic understanding of MMR comes from studies of the *E. coli* MutHLS system, which comprises all requirements for successful mismatch excision. Mismatch recognition by the MutHLS system is accomplished by a homodimer of MutS proteins, which, upon binding, recruits and forms a ternary complex with a homodimeric complex of the matchmaker protein MutL. Both MutS and MutL possess ATPase activities [114–116] that are required for mismatch repair [117,118]. Complex formation then leads to MutL-driven activation of the latent MutH endonuclease [119], which binds to Dam methylation sequences (GATC) that remain transiently hemi-methylated in the newly synthesized DNA. MutH then incises the unmethylated DNA strand, thereby directing repair to the newly synthesized strand. This provides an entry point for single-strand binding protein (SSB) and DNA helicase II (UvrD). Unwinding of the DNA duplex by the UvrD then facilitates exonucleolytic degradation of the nascent strand from the nick to and slightly past the mismatch. Depending on whether nicking occurred 5′ or 3′ relative to the mismatch, excision of the strand is carried out by exonucleases with either 5′–3′ (RecJ or ExoVII) or 3′–5′ (ExoI or ExoX) polarity, respectively. Resynthesis by Polymerase III is then followed by sealing of the resulting nick by DNA ligase.

The engagement of this system in mismatch repair entirely depends on the recognition of a mismatch by the MutS complex, a process that is understood at the three-dimensional structural level. High-resolution structures of MutS bound to heteroduplex DNA show that both MutS subunits together form an oval disk with two composite channels of 30 and 40 Å diameter. The heteroduplex DNA, passing through the larger channel, is kinked by 60°, which leads to widening of the minor and narrowing of the major groove [120, 121]. Remarkably, the two MutS subunits contribute asymmetrically to mismatch binding and act as functional heterodimers, whereby only one subunit establishes mismatch-specific DNA interactions, involving the highly conserved G-X-FYE motif. It was proposed that mismatch recognition by MutS depends on increased DNA pliability caused by the helix destabilizing effect of the mispair (reviewed in [122]). However, while this may explain the preference of MutS for heteroduplex over homoduplex DNA, it does not account for the lack of a correlation between the MutS binding efficiency and the extent of helix destabilization caused by different mismatches. The G·T mismatch, for instance, is bound by MutS most efficiently but represents a comparably stable wobble base pair, affecting the DNA helical structure only marginally. By contrast, the C·C mismatch assumes unsteady pairing configurations and affects helix stability more dramatically, but is hardly processed by the MutS system [8, 123, 124]. Interestingly, atomic force microscopy revealed binding of MutS to both homo- and heteroduplex DNA. In the presence of heteroduplex DNA, however, two types of MutS-DNA complexes were found, one with the DNA in a bent state and one with the DNA in straight arrangement [124]. Together with crystallographic evidence [120, 121], this has been interpreted as MutS first binding to DNA unspecifically and bending it while searching for a mismatch. Upon encountering a mismatch, MutS would then undergo sequential conformational changes to form the initial recognition complex (IRC) containing kinked DNA and, upon mismatch verification, the ultimate recognition complex (URC), in which the DNA adopts an unbent state (Fig. 3). Hence, the relative stabilities of these two complexes would determine the rate of transition from the IRC to the URC and, thus, the efficiency of recognition and repair of a mismatch. According to this model, the more a mismatch destabilizes the stiffness of the DNA double helix (i. e. C·C), the more it would favor the bent IRC conformation and therefore be refractory to repair.

As in bacteria, mismatch recognition in eukaryotes is provided by dimeric complexes of MutS proteins. These, however, are heterodimeric rather than homodimeric, consisting of pairs of MutS homologs, the so-called MSH proteins. MSH2 dimerizes with MSH6 or MSH3 to form the two complexes MutSα and MutSβ, respectively [125, 126]. The two have complementary mismatch recognition functions, MutSα binding preferentially to base-base mismatches and small IDLs, MutSβ preferring larger IDLs [127–129]. In the case of MutSα, the heterodimeric functionality is illustrated by the fact that only the MSH6 subunit makes contact with the mismatched base pair [130]. Accordingly, the critical phenylalanine and glutamate residues of the G-X-FYE motif have been conserved in MSH6 homologues, but not in MSH2 or MSH3 [131, 132], and mutation of either of these residues reduces mismatch repair activity of MutSα *in vitro* and *in vivo* [131–133]. Although the architectures of human and prokaryotic MutS complexes are similar, they differ in some details. The MSH6 subunit in the MutSα structure shows an additional ordered element at its N-terminus, forming a positively charged coiled region. Nonspecific interactions of this region with DNA may help stabilize the interaction with suboptimal substrates such as C·C [130]. Remarkably, co-

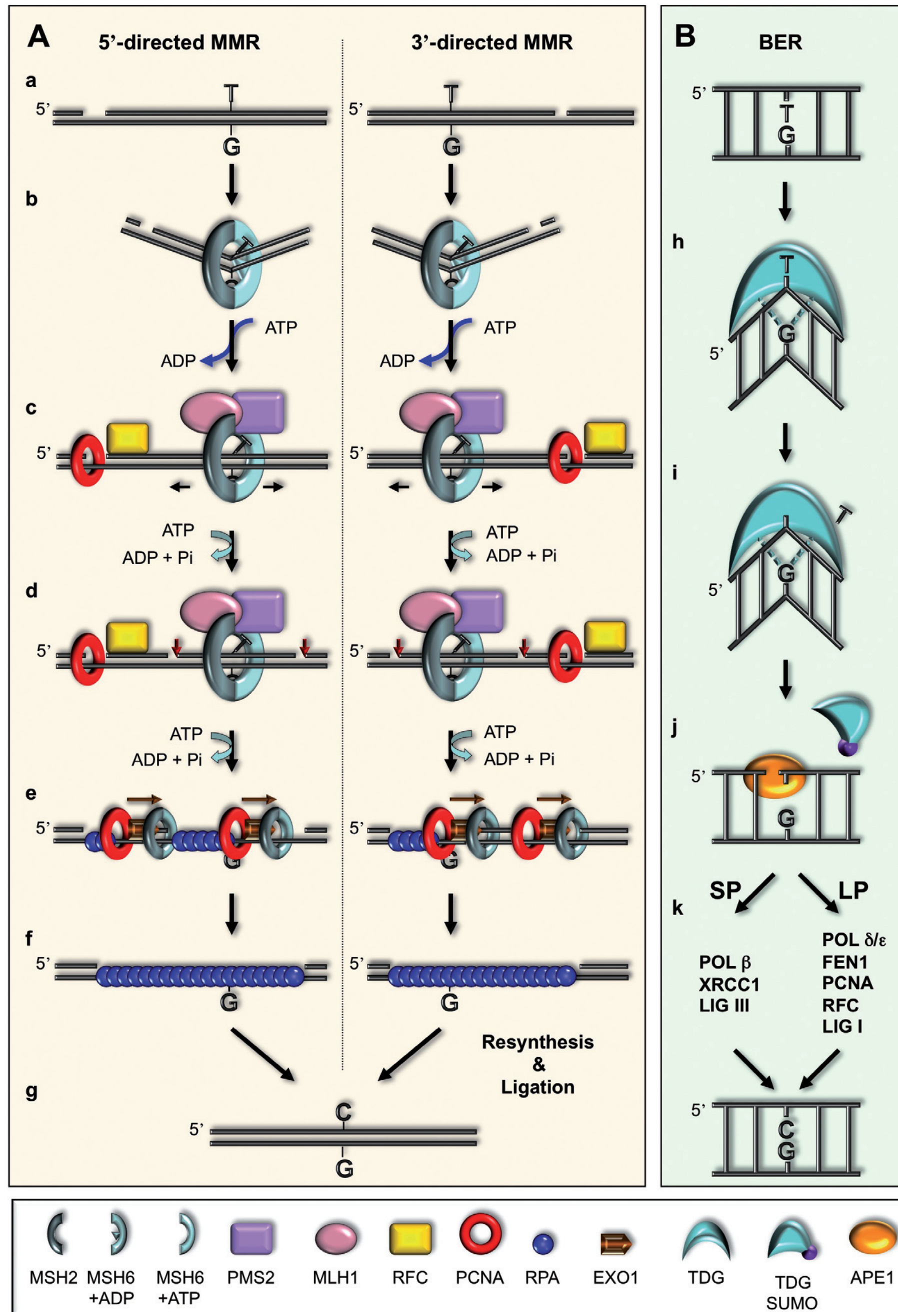

Figure 3. Simplified overview of mismatch repair by eukaryotic MMR (*A*) or BER (*B*). Bidirectional MMR requires strand discontinuities located either 5′ or 3′ to the mismatch (a). MutSα, a heterodimer composed of MSH2 and MSH6, binds the mismatch (b), recruits the MLH1-PMS2 heterodimer (MutLα) and undergoes a conformational switch upon exchange of ADP with ATP, allowing sliding away from the mismatch (c). A latent endonuclease activity in the PMS2 subunit of MutLα is activated in a MutSα-, RFC-, PCNA- and ATP-dependent manner and introduces nicks in the discontinuous strand (red arrowhead) (d). This generates 5′ entry points for the 5′ to 3′ nuclease EXO1, independently of whether the initial strand discontinuity was located 5′ or 3′ to the mismatch. MutSα activated EXO1 subsequently degrades the nicked strand (e), generating single stranded gaps which are protected by RPA (f). POLδ then loads at the 3′ terminus and fills in the gap with help of its cofactors PCNA and RFC. Finally, DNA Ligase I completes repair by sealing the remaining nick (g). (*B*) Recognition of a G·T mismatch by TDG involves flipping of the substrate T into the active site pocket but also the establishment of contacts to the opposite G, allowing excision of T from G·T but not from A·T pairs (h). Flipping of the substrate base allows hydrolysis of the glycosydic bond, thus releasing the T and generating an AP-site in the DNA strand (i). SUMOylation of TDG induces a conformational change, reducing its DNA binding affinity and facilitating dissociation of the glycosylase from the AP-site, which is then cleaved by AP-endonuclease 1 (APE1). Further processing of the single strand break may proceed by short (SP) or long patch (LP) repair (k). SP repair involves the action of Polβ, which inserts one nucleotide and removes the dRP residue, followed by sealing of the DNA strand by the LIG III-XRCC1 complex. In LP repair, Polδ/ε assisted by the cofactors PCNA and RFC synthesizes a more extensive stretch of DNA, resulting in displacement of the parental DNA strand. The resulting flap structure is cleaved by FEN1 and the nick sealed by LIG I.

crystal structures of MutSα with mismatched DNA substrates revealed identical modes of binding to G·T, G·U and O6^{me}G·T mismatches. Considering that G·T and O6^{me}G·T are structurally quite different (Fig. 1), this supports the view that the interaction with a mismatch is determined by helix stability rather than by particular features of non-Watson-Crick base pairing configurations.

The bacterial as well as the eukaryotic MutS complexes possess composite ATPase motifs and ATPase activity [120, 121, 130]. ATP binding, hydrolysis and ADP-ATP exchange by MutS coordinate a sequence of molecular transactions that couple mismatch recognition with initiation of nucleolytic processing [134, 135]. In an ADP bound form, MutS forms a stable complex with mismatched DNA [120, 134]. For MutSα an exchange of ADP with ATP induces a conformational change that allows it to slide away from the mismatch along the DNA duplex [136, 137]. It is not clear whether MutSα moves along the DNA by diffusion in the form of a sliding clamp or by translocation through repeated cycles of ATP hydrolysis and reloading [138–140] (discussed in [141]). Irrespective of the mechanism, however, sliding of MutSα provides a plausible mechanistic model for how mismatch recognition links to the search for DNA strand interruptions at a distance that will serve as strand-discrimination signals for repair. Corroborating the functional asymmetry of the MutS dimer, the consumption of ATP by the two subunits contributes differently to the mismatch repair function of the complex, both in bacterial MutS and in human MutSα (reviewed in [142]).

The initial steps of MMR are similar in prokaryotes and eukaryotes, but processes downstream may differ. For instance, neither a MutH-type function nor a DNA helicase has been identified or implicated in eukaryotic MMR. Eukaryotic homologs of the bacterial MutL, however, do exist [143–146], and they form structural and functional heterodimers. In the human system, hMLH1 heterodimerizes with hPMS2, hPMS1 or hMLH3 to form the MutLα, MutLβ and MutLγ complexes, respectively. Whereas MutLα contributes to general MutSα/β-dependent mismatch repair, MutLγ appears to participate in IDL repair and in processes associated with meiotic recombination, i. e. in the context of more complex DNA structures [144, 147–152]. Little is known about the biological function of the MutLβ complex. Besides the MutS and MutL complexes, eukaryotic mismatch repair depends on additional factors, most of which are components of the replication machinery. Prominent examples are PCNA and its DNA loading factor RFC (replication factor C) (reviewed in [153]). Interestingly PCNA, known for its processivity stimulating effect on replicative DNA polymerases, was shown to function both at the mismatch recognition as well as in DNA synthesis steps of MMR [74, 154, 155]. Consistent with this, PCNA was reported to interact with MutSα and MutSβ [70–72] to form a ternary complex that binds to DNA, but strikingly, only in the absence of a mismatch. In the presence of a G·T mismatch, PCNA seemed to be excluded from the mismatch-bound MutSα complex. This biochemical evidence indicates that MutSα is targeted to the replication fork by binding to PCNA, loaded onto newly replicated DNA, and is then transferred from PCNA to the DNA upon encountering a mismatch [156].

Whereas transiently hemi-methylated GATC sites serve as a signal to discriminate the newly synthesized DNA strand for MMR in *E. coli*, it is not clear how eukaryotes assure nascent DNA strand-directed repair. However, because single-strand nicks and gaps can direct strand-specific mismatch repair *in vitro* [157–160], it was postulated that replication-associated strand discontinuities may serve this purpose *in vivo* [74, 161, 162].

Given that strand discontinuities may be located on either side of the mismatch, it might be expected that mismatch-driven degradation would involve exonucleases with 5′–3′ or 3′–5′ polarity. While this is so in *E. coli*, it may not be true for eukaryotes. Although eukaryotes express a substantial number of exonucleolytic activities, the 5′–3′ nuclease EXO1 is the only one that has been directly implicated in mismatch repair [163–168]. Not only was EXO1 found to interact with eukaryotic MutS and MutL proteins [169–171], MutSα also converts its distributive mode of DNA hydrolysis into a processive mode, facilitating degradation of up to 2000 nucleotides before dissociation *in vitro* [155]. Moreover, the rate of DNA resection by the MutSα-EXO1 complex is controlled by the replication protein A (RPA), which binds and protects the single-stranded DNA strand as it is being generated by the nuclease. The effect of RPA appears to be twofold: it reduces the processivity of the MutSα-EXO1 complex to about 250 nucleotides, and its binding to the nascent single-stranded DNA gaps affects the reloading of the nuclease [155]. Degradation of the DNA strand is therefore thought to include multiple cycles of mismatch-dependent reloading of MutSα and EXO1 and 5′–3′ degradation until there is no more mismatch present [172]. Although MutLα is not required for activation of EXO1, it has been implicated at the excision step as well. It appears to enhance mismatch dependency of the nucleolytic process by suppressing hydrolysis of homoduplex DNA, thereby possibly participating in termination of the excision step [155, 172, 173]. Regarding the 5′–3′ polarity of EXO1, its requirement for processing of

DNA strand interruptions located on the 3′ side of the mismatch seems counterintuitive [174]. However, this mechanistic dilemma could be resolved by a striking discovery, namely that MutLα possesses a latent endonuclease activity that is activated by MutSα, PCNA and RFC in an ATP-dependent manner [175]. This activity, located in the PMS2 subunit of the complex, introduces DNA single-strand breaks in the discontinuous strand of heteroduplex DNA substrates, irrespective of the relative position of the discontinuity. Thereby, MutLα possesses the ability to generate an entry point for 5′ to 3′ hydrolysis by MutSα-activated EXO1, even if the strand-discriminating discontinuity is located on the 3′ side of the mismatch (Fig. 3). Following nucleolytic excision of the mismatch, repair synthesis is accomplished by DNA Polymerase δ together with PCNA [153, 154, 176]. Synthesis may be promoted by phosphorylation of DNA-bound RPA, facilitating the release of the protecting protein filament from the template DNA strand [177]. The final step of the mismatch repair process involves sealing of the nick by DNA ligase I.

DNA base excision repair

The BER system is designed to repair modifications or damage to DNA bases (Fig. 3), as described in detail in the accompanying review by Robertson et al. [this issue]. Here, we will summarize specific mechanistic features of mismatch-directed BER, focusing on the recognition and processing of mismatched bases by DNA glycosylases. DNA glycosylases catalyze the hydrolysis of the N-glycosidic bond of a damaged deoxynucleoside, generating an AP site in DNA, which is then subject to excision repair by the general BER system.

As most typical BER substrates cause only minor perturbations of the DNA double helix, they represent a challenge for recognition by DNA glycosylases as most base-base mismatches do for recognition by MutS. In contrast to MMR, however, the coupling of damage recognition with base excision in BER requires discrimination of the damaged versus undamaged base at the first step of repair. Thus, in the case of mismatch processing by BER, the DNA glycosylases involved not only need functionalities to recognize the mismatch but also to ensure that the right base be excised.

How DNA glycosylases achieve substrate specificity and selectivity first became evident with the crystal structure of substrate-bound human UDG [178]. The structure revealed that, when bound to UDG, the substrate U is rotated by 180° out of the base stack and accommodated in a tightly fitting active site pocket of the DNA glycosylase. The rotation of the base with its sugar moiety is associated with substantial kinking of the DNA and positions the C1′ of the deoxyribose for the nucleophilic attack. Thus, an initial step in damage recognition appears to exploit the deformability of the DNA helix at the site of a base lesion. The principle of base flipping and fitting into an active site pocket is common to all DNA glycosylases for which structural analyses have been performed (reviewed in [179]). DNA glycosylases are necessarily damage-specific. So, only bases that can be readily accommodated in the restrictive substrate binding pocket of a glycosylase will be processed. Thereby, the geometry of the binding pocket and the hydrogen-bonding potential of the damaged base play critical roles [180].

Considering mismatch recognition by DNA glycosylases, the fitting of a base into the active site pocket is not sufficient as a criterion for damage recognition, as paired and mispaired bases do not necessarily differ in chemistry. Thus, mismatch-directed glycosylases like TDG, MBD4 and MYH need strategies to discriminate normal bases in paired versus mispaired configurations. Crystal structures of the TDG catalytic domain in complex with substrate DNA revealed that, besides forming contacts allowing the extrusion and excision of the mismatched T, this glycosylase additionally forms a wedge invading the complementary strand at the opposite G [181, 182]. The G remains in the double helix and stacks with the 3′-located base. The 5′ neighboring base, however, is displaced by the wedge, which establishes a cleft surrounding the G at its 5′ face, its Watson-Crick and minor groove edges. Two residues of the insertion loop (A274, P280) establish specific contacts with the Watson-Crick surface of the G which are not compatible with A. Thus, in the case of TDG, recognition of the substrate T involves specific interactions with the opposite base, thereby allowing discrimination between G·T and A·T base pairs.

A similar mechanism applies to the discrimination of 8-oxo-G·A from T·A base pairs by MutY. Also in this case, the substrate A is rotated out of the DNA helix, whereas the 8-oxo-G opposite remains intrahelical. Remarkably, the 8-oxo-G appears trapped between the N- and C-terminal domains of MutY, which establish extensive hydrogen-bonding contacts with the surface of the oxidized G [183]. In addition, the MutY-bound 8-oxoG nucleoside is swiveled about its glycosidic bond to adopt an *anti* conformation, while its conformation is *syn* when paired with adenine (Fig. 1). Thus, MutY establishes a chemically complementary geometry that is highly specific to the surface of the 8-oxo-G nucleoside, which is inappropriate for recognition of A base paired with T.

Concluding remark

Mismatches arise in DNA under different circumstances through diverse DNA metabolic processes, and most often they represent a threat to genetic information. In order to appropriately control mutagenesis, cells must be able to apply suitable strategies of repair, depending not only on the type of a mismatch but also on the particular context of its occurrence. Simply the fact that individual mismatches can be processed by different repair systems implies a fair degree of higher-level regulation, into which we have very little insight at present. Yet, if our aim is to fully understand the biology of mismatch generation and repair, including the consequences of defective repair or misrepair, these questions will have to be addressed.

1 Watson, J. D. and Crick, F. H. (1953) Genetical implications of the structure of deoxyribonucleic acid. Nature 171, 964–967.
2 Ghosal, G. and Muniyappa, K. (2006) Hoogsteen base-pairing revisited: resolving a role in normal biological processes and human diseases. Biochem Biophys. Res. Commun. 343, 1–7.
3 Sühnel, J. (2001) Beyond nucleic acid base pairs: from triads to heptads. Biopolymers 61, 32–51.
4 Patel, D. J., Phan, A. T. and Kuryavyi, V. (2007) Human telomere, oncogenic promoter and 5′-UTR G-quadruplexes: diverse higher order DNA and RNA targets for cancer therapeutics. Nucleic Res. 35, 7429–7455.
5 Allawi, H. T. and SantaLucia, J., Jr. (1997) Thermodynamics and NMR of internal G.T mismatches in DNA. Biochemistry 36, 10581–10594.
6 Boulard, Y., Cognet, J. A. H. and Fazakerley, G. V. (1997) Solution structure as a function of pH of two central mismatches, C·T and C·C, in the 29 to 39 K-ras gene sequence, by nuclear magnetic resonance and molecular dynamics. J. Mol. Biol. 268, 331–347.
7 Tikhomirova, A., Beletskaya, I. V. and Chalikian, T. V. (2006) Stability of DNA duplexes containing GG, CC, AA, and TT mismatches. Biochemistry 45, 10563–10571.
8 Peyret, N., Seneviratne, P. A., Allawi, H. T. and SantaLucia, J., Jr. (1999) Nearest-neighbor thermodynamics and NMR of DNA sequences with internal A.A, C.C, G.G, and T.T mismatches. Biochemistry 38, 3468–3477.
9 Johnson, S. J. and Beese, L. S. (2004) Structures of mismatch replication errors observed in a DNA polymerase. Cell 116, 803–816.
10 Galiatsatos, P. and Foulkes, W. D. (2006) Familial adenomatous polyposis. Am. J. Gastroenterol. 101, 385–398.
11 Rustgi, A. K. (2007) The genetics of hereditary colon cancer. Genes Dev. 21, 2525–2538.
12 Holliday, R. (1964) A mechanism for gene conversion in fungi. Genet. Res. 5, 282–304.
13 Witkin, E. M. and Sicurella, N. A. (1964) Pure clones of lactose-negative mutants obtained in *Escherichia coli* after treatment with 5-bromouracil. J. Mol. Biol. 8, 610–613.
14 Beard, W. A. and Wilson, S. H. (2003) Structural insights into the origins of DNA polymerase fidelity. Structure 11, 489–496.
15 Joyce, C. M. and Benkovic, S. J. (2004) DNA polymerase fidelity: kinetics, structure, and checkpoints. Biochemistry 43, 14317–14324.
16 Hubscher, U., Maga, G. and Spadari, S. (2002) Eukaryotic DNA polymerases. Annu. Rev. Biochem. 71, 133–163.
17 McCulloch, S. D. and Kunkel, T. A. (2008) The fidelity of DNA synthesis by eukaryotic replicative and translesion synthesis polymerases. Cell Res. 18, 148–161.
18 Garcia-Diaz, M. and Kunkel, T. A. (2006) Mechanism of a genetic glissando: structural biology of indel mutations. Trends Biochem. Sci. 31, 206–214.
19 Streisinger, G., Okada, Y., Emrich, J., Newton, J., Tsugita, A., Terzaghi, E. and Inouye, M. (1966) Frameshift mutations and the genetic code. Cold Spring Harb. Symp. Quant. Biol. 31, 77–84.
20 Drake, J. W. (1991) A constant rate of spontaneous mutation in DNA-based microbes. Proc. Natl. Acad. Sci. USA 88, 7160–7164.
21 Loeb, L. A. (2001) A mutator phenotype in cancer. Cancer Res 61, 3230–2329.
22 Roberts, J. D. and Kunkel, T. A. (1988) Fidelity of a human cell DNA replication complex. Proc. Natl. Acad. Sci. USA 85, 7064–7068.
23 Bebenek, K., Roberts, J. D. and Kunkel, T. A. (1992) The effects of dNTP pool imbalances on frameshift fidelity during DNA replication. J. Biol. Chem. 267, 3589–3596.
24 Mathews, C. K. (2006) DNA precursor metabolism and genomic stability. FASEB J. 20, 1300–1314.
25 Bianchi, V. (1982) Nucleotide pool unbalance induced in cultured cells by treatments with different chemicals. Toxicology 25, 13–18.
26 Kunz, B. A., Kohalmi, S. E., Kunkel, T. A., Mathews, C. K., McIntosh, E. M. and Reidy, J. A. (1994) International Commission for Protection Against Environmental Mutagens and Carcinogens. Deoxyribonucleoside triphosphate levels: a critical factor in the maintenance of genetic stability. Mutat. Res. 318, 1–64.
27 An, Q., Robins, P., Lindahl, T. and Barnes, D. E. (2007) 5-Fluorouracil incorporated into DNA is excised by the Smug1 DNA glycosylase to reduce drug cytotoxicity. Cancer Res. 67, 940–945.
28 Longley, D. B., Harkin, D. P. and Johnston, P. G. (2003) 5-fluorouracil: mechanisms of action and clinical strategies. Nat. Rev. Cancer 3, 330–338.
29 Sowers, L. C., Eritja, R., Kaplan, B., Goodman, M. F. and Fazakerly, G. V. (1988) Equilibrium between a wobble and ionized base pair formed between fluorouracil and guanine in DNA as studied by proton and fluorine NMR. J. Biol. Chem. 263, 14794–14801.
30 Meyers, M., Hwang, A., Wagner, M. W., Bruening, A. J., Veigl, M. L., Sedwick, W. D. and Boothman, D. A. (2003) A role for DNA mismatch repair in sensing and responding to fluoropyrimidine damage. Oncogene 22, 7376–7388.
31 Lipscomb, L. A., Peek, M. E., Morningstar, M. L., Verghis, S. M., Miller, E. M., Rich, A., Essigmann, J. M. and Williams, L. D. (1995) X-ray structure of a DNA decamer containing 7,8-dihydro-8-oxoguanine. Proc. Natl. Acad. Sci. USA 92, 719–723.
32 Kouchakdjian, M., Bodepudi, V., Shibutani, S., Eisenberg, M., Johnson, F., Grollman, A. P. and Patel, D. J. (1991) NMR structural studies of the ionizing radiation adduct 7-hydro-8-oxodeoxyguanosine (8-oxo-7H-dG) opposite deoxyadenosine in a DNA duplex. 8-Oxo-7H-dG(syn)cntdot.dA(anti) alignment at lesion site. Biochemistry 30, 1403–1412.
33 Maki, H. and Sekiguchi, M. (1992) MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. Nature 355, 273–275.
34 Sekiguchi, M. (1996) MutT-related error avoidance mechanism for DNA synthesis. Genes Cells 1, 139–145.
35 Eadie, J. S., Conrad, M., Toorchen, D. and Topal, M. D. (1984) Mechanism of mutagenesis by O6-methylguanine. Nature 308, 201–203.
36 Prakash, S., Johnson, R. E. and Prakash, L. (2005) Eukaryotic translesion synthesis DNA polymerases: specificity of structure and function. Annu. Rev. Biochem. 74, 317–353.
37 Fleck, O. and Schar, P. (2004) Translesion DNA synthesis: little fingers teach tolerance. Curr. Biol. 14, R389–391.

38 Bebenek, K., Garcia-Diaz, M., Blanco, L. and Kunkel, T. A. (2003) The frameshift infidelity of human DNA polymerase lambda. Implications for function. J. Biol. Chem. 278, 34685–34690.
39 Bebenek, K., Matsuda, T., Masutani, C., Hanaoka, F. and Kunkel, T. A. (2001) Proofreading of DNA polymerase eta-dependent replication errors. J. Biol. Chem. 276, 2317–2320.
40 Matsuda, T., Bebenek, K., Masutani, C., Hanaoka, F. and Kunkel, T. A. (2000) Low fidelity DNA synthesis by human DNA polymerase-eta. Nature 404, 1011–1013.
41 Ohashi, E., Bebenek, K., Matsuda, T., Feaver, W. J., Gerlach, V. L., Friedberg, E. C., Ohmori, H. and Kunkel, T. A. (2000) Fidelity and processivity of DNA synthesis by DNA polymerase kappa, the product of the human DINB1 gene. J. Biol. Chem. 275, 39678–39684.
42 Ehrlich, M., Norris, K. F., Wang, R. Y., Kuo, K. C. and Gehrke, C. W. (1986) DNA cytosine methylation and heat-induced deamination. Biosci. Rep. 6, 387–393.
43 Hill-Perkins, M., Jones, M. D. and Karran, P. (1986) Site-specific mutagenesis in vivo by single methylated or deaminated purine bases. Mutat. Res. 162, 153–163.
44 Lindahl, T. (1993) Instability and decay of the primary structure of DNA. Nature 362, 709–715.
45 Kulikowska, E., Kierdaszuk, B. and Shugar, D. (2004) Xanthine, xanthosine and its nucleotides: solution structures of neutral and ionic forms, and relevance to substrate properties in various enzyme systems and metabolic pathways. Acta Biochim. Pol. 51, 493–531.
46 Wang, D., Kreutzer, D. A. and Essigmann, J. M. (1998) Mutagenicity and repair of oxidative DNA damage: insights from studies using defined lesions. Mutat. Res. 400, 99–115.
47 Pavlov, Y. I., Minnick, D. T., Izuta, S. and Kunkel, T. A. (1994) DNA replication fidelity with 8-oxodeoxyguanosine triphosphate. Biochemistry 33, 4695–4701.
48 Kaina, B., Christmann, M., Naumann, S. and Roos, W. P. (2007) MGMT: key node in the battle against genotoxicity, carcinogenicity and apoptosis induced by alkylating agents. DNA Repair (Amst) 6, 1079–1099.
49 Stojic, L., Brun, R. and Jiricny, J. (2004) Mismatch repair and DNA damage signalling. DNA Repair (Amst) 3, 1091–1101.
50 Cox, M. M., Goodman, M. F., Kreuzer, K. N., Sherratt, D. J., Sandler, S. J. and Marians, K. J. (2000) The importance of repairing stalled replication forks. Nature 404, 37–41.
51 Strauss, B. S. (2002) The 'A' rule revisited: polymerases as determinants of mutational specificity. DNA Repair (Amst) 1, 125–135.
52 Neuberger, M. S. (2008) Antibody diversification by somatic mutation: from Burnet onwards. Immunol. Cell Biol. 86, 124–132.
53 Stavnezer, J., Guikema, J. E. and Schrader, C. E. (2008) Mechanism and regulation of class switch recombination. Annu. Rev. Immunol. 26, 261–292.
54 Miranda, T. B. and Jones, P. A. (2007) DNA methylation: the nuts and bolts of repression. J. Cell. Physiol. 213, 384–390.
55 Kangaspeska, S., Stride, B., Metivier, R., Polycarpou-Schwarz, M., Ibberson, D., Carmouche, R. P., Benes, V., Gannon, F. and Reid, G. (2008) Transient cyclical methylation of promoter DNA. Nature 452, 112–115.
56 Metivier, R., Gallais, R., Tiffoche, C., Le Peron, C., Jurkowska, R. Z., Carmouche, R. P., Ibberson, D., Barath, P., Demay, F., Reid, G. et al. (2008) Cyclical DNA methylation of a transcriptionally active promoter. Nature 452, 45–50.
57 Barreto, G., Schafer, A., Marhold, J., Stach, D., Swaminathan, S. K., Handa, V., Doderlein, G., Maltry, N., Wu, W., Lyko, F. et al. (2007) Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. Nature 445, 671–675.
58 Gehring, M., Huh, J. H., Hsieh, T. F., Penterman, J., Choi, Y., Harada, J. J., Goldberg, R. B. and Fischer, R. L. (2006) DEMETER DNA glycosylase establishes MEDEA polycomb gene self-imprinting by allele-specific demethylation. Cell 124, 495–506.
59 Jost, J. P., Siegmann, M., Sun, L. and Leung, R. (1995) Mechanisms of DNA demethylation in chicken embryos. Purification and properties of a 5-methylcytosine-DNA glycosylase. J. Biol. Chem. 270, 9734–9739.
60 Liu, M., Duke, J. L., Richter, D. J., Vinuesa, C. G., Goodnow, C. C., Kleinstein, S. H. and Schatz, D. G. (2008) Two levels of protection for the B cell genome during somatic hypermutation. Nature 451, 841–845.
61 Li, G. M. (2008) Mechanisms and functions of DNA mismatch repair. Cell Res. 18, 85–98.
62 Cortazar, D., Kunz, C., Saito, Y., Steinacher, R. and Schar, P. (2007) The enigmatic thymine DNA glycosylase. DNA Repair (Amst) 6, 489–504.
63 David, S. S., O'Shea, V.L. and Kundu, S. (2007) Base-excision repair of oxidative DNA damage. Nature 447, 941–950.
64 Kramer, B., Kramer, W. and Fritz, H. J. (1984) Different base/base mismatches are corrected with different efficiencies by the methyl-directed DNA mismatch-repair system of *E. coli*. Cell 38, 879–887.
65 Modrich, P. and Lahue, R. (1996) Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu. Rev. Biochem. 65, 101–133.
66 Parker, B. O. and Marinus, M. G. (1992) Repair of DNA heteroduplexes containing small heterologous sequences in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 89, 1730–1734.
67 Dohet, C., Wagner, R. and Radman, M. (1985) Repair of defined single base-pair mismatches in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 82, 503–505.
68 Hendrich, B., Hardeland, U., Ng, H. H., Jiricny, J. and Bird, A. (1999) The thymine glycosylase MBD4 can bind to the product of deamination at methylated CpG sites. Nature 401, 301–304.
69 Yang, H., Clendenin, W. M., Wong, D., Demple, B., Slupska, M. M., Chiang, J.-H. and Miller, J. H. (2001) Enhanced activity of adenine-DNA glycosylase (Myh) by apurinic/apyrimidinic endonuclease (Ape1) in mammalian base excision repair of an A/GO mismatch. Nucleic Acids Res. 29, 743–752.
70 Clark, A. B., Valle, F., Drotschmann, K., Gary, R.K. and Kunkel, T. A. (2000) Functional interaction of proliferating cell nuclear antigen with MSH2-MSH6 and MSH2-MSH3 complexes. J. Biol. Chem. 275, 36498–36501.
71 Flores-Rozas, H., Clark, D. and Kolodner, R. D. (2000) Proliferating cell nuclear antigen and Msh2p-Msh6p interact to form an active mispair recognition complex. Nat. Genet. 26, 375–378.
72 Kleczkowska, H. E., Marra, G., Lettieri, T. and Jiricny, J. (2001) hMSH3 and hMSH6 interact with PCNA and colocalize with it to replication foci. Genes Dev. 15, 724–736.
73 Johnson, R. E., Kovvali, G. K., Guzder, S. N., Amin, N. S., Holm, C., Habraken, Y., Sung, P., Prakash, L. and Prakash, S. (1996) Evidence for involvement of yeast proliferating cell nuclear antigen in DNA mismatch repair. J. Biol. Chem. 271, 27987–27990.
74 Umar, A., Buermeyer, A. B., Simon, J.A., Thomas, D. C., Clark, A. B., Liskay, R. M. and Kunkel, T. A. (1996) Requirement for PCNA in DNA mismatch repair at a step preceding DNA resynthesis. Cell 87, 65–73.
75 Boldogh, I., Milligan, D., Lee, M. S., Bassett, H., Lloyd, R. S. and McCullough, A. K. (2001) hMYH cell cycle-dependent expression, subcellular localization and association with replication foci: evidence suggesting replication-coupled repair of adenine:8-oxoguanine mispairs. Nucleic Acids Res. 29, 2802–2809.
76 Parker, A., Gu, Y., Mahoney, W., Lee, S. H., Singh, K. K. and Lu, A. L. (2001) Human homolog of the MutY repair protein (hMYH) physically interacts with proteins involved in long patch DNA base excision repair. J. Biol. Chem. 276, 5547–5555.
77 Hardeland, U., Kunz, C., Focke, F., Szadkowski, M. and Schar, P. (2007) Cell cycle regulation as a mechanism for functional separation of the apparently redundant uracil

DNA glycosylases TDG and UNG2. Nucleic Acids Res. 35, 3859–3867.
78 Kavli, B., Sundheim, O., Akbari, M., Otterlei, M., Nilsen, H., Skorpen, F., Aas, P. A., Hagen, L., Krokan, H. E. and Slupphaug, G. (2002) hUNG2 Is the major repair enzyme for removal of uracil from U:A matches, U:G mismatches, and U in single-stranded DNA, with hSMUG1 as a broad specificity backup. J. Biol. Chem. 277, 39926–39936.
79 Haushalter, K. A., Todd Stukenberg, P., Kirschner, M. W. and Verdine, G. L. (1999) Identification of a new uracil-DNA glycosylase family by expression cloning using synthetic inhibitors. Curr. Biol. 9, 174–185.
80 Neddermann, P. and Jiricny, J. (1994) Efficient removal of uracil from G.U mispairs by the mismatch-specific thymine DNA glycosylase from HeLa cells. Proc. Natl. Acad. Sci. USA 91, 1642–1646.
81 Neddermann, P. and Jiricny, J. (1993) The purification of a mismatch-specific thymine-DNA glycosylase from HeLa cells. J. Biol. Chem. 268, 21218–21224.
82 Bjoras, M., Luna, L., Johnsen, B., Hoff, E., Haug, T., Rognes, T. and Seeberg, E. (1997) Opposite base-dependent reactions of a human base excision repair enzyme on DNA containing 7,8-dihydro-8-oxoguanine and abasic sites. EMBO J. 16, 6314–6322.
83 Slupska, M. M., Luther, W. M., Chiang, J. H., Yang, H. and Miller, J. H. (1999) Functional expression of hMYH, a human homolog of the *Escherichia coli* MutY protein. J. Bacteriol. 181, 6210–6213.
84 Maga, G., Villani, G., Crespan, E., Wimmer, U., Ferrari, E., Bertocci, B. and Hubscher, U. (2007) 8-oxo-guanine bypass by human DNA polymerases in the presence of auxiliary proteins. Nature 447, 606–608.
85 Duckett, D. R., Drummond, J. T., Murchie, A. I., Reardon, J. T., Sancar, A., Lilley, D. M. and Modrich, P. (1996) Human MutSalpha recognizes damaged DNA base pairs containing O6-methylguanine, O4-methylthymine, or the cisplatin-d(GpG) adduct. Proc. Natl. Acad. Sci. USA 93, 6443–6447.
86 Cejka, P. and Jiricny, J. (2008) Interplay of DNA repair pathways controls methylation damage toxicity in *Saccharomyces cerevisiae.* Genetics 179, 1835–1844.
87 Cejka, P., Mojas, N., Gillet, L., Schar, P. and Jiricny, J. (2005) Homologous recombination rescues mismatch-repair-dependent cytotoxicity of S(N)1-type methylating agents in *S. cerevisiae*. Curr. Biol. 15, 1395–1400.
88 Stojic, L., Mojas, N., Cejka, P., Di Pietro, M., Ferrari, S., Marra, G. and Jiricny, J. (2004) Mismatch repair-dependent G2 checkpoint induced by low doses of SN1 type methylating agents requires the ATR kinase. Genes Dev. 18, 1331–1344.
89 Sibghat, U., Gallinari, P., Xu, Y. Z., Goodman, M. F., Bloom, L. B., Jiricny, J. and Day, R. S., 3rd. (1996) Base analog and neighboring base effects on substrate specificity of recombinant human G:T mismatch-specific thymine DNA-glycosylase. Biochemistry 35, 12926–12932.
90 Cortellino, S., Turner, D., Masciullo, V., Schepis, F., Albino, D., Daniel, R., Skalka, A. M., Meropol, N. J., Alberti, C., Larue, L. et al. (2003) The base excision repair enzyme MED1 mediates DNA damage response to antitumor drugs and is associated with mismatch repair system integrity. Proc. Natl. Acad. Sci. USA 100, 15071–15076.
91 Singh, J., Su, L. and Snow, E. T. (1996) Replication across O6-methylguanine by human DNA polymerase beta *in vitro*. Insights into the futile cytotoxic repair and mutagenesis of O6-methylguanine. J. Biol. Chem. 271, 28391–28398.
92 Rada, C., Di Noia, J.M. and Neuberger, M. S. (2004) Mismatch recognition and uracil excision provide complementary paths to both Ig switching and the A/T-focused phase of somatic mutation. Mol. Cell 16, 163–171.
93 Di Noia, J. and Neuberger, M. S. (2002) Altering the pathway of immunoglobulin hypermutation by inhibiting uracil-DNA glycosylase. Nature 419, 43–48.
94 Rada, C., Williams, G. T., Nilsen, H., Barnes, D. E., Lindahl, T. and Neuberger, M. S. (2002) Immunoglobulin isotype switching is inhibited and somatic hypermutation perturbed in UNG-deficient mice. Curr. Biol. 12, 1748–1755.
95 Ling, H., Boudsocq, F., Woodgate, R. and Yang, W. (2004) Snapshots of replication through an abasic lesion; structural basis for base substitutions and frameshifts. Mol. Cell 13, 751–762.
96 Poltoratsky, V., Prasad, R., Horton, J. K. and Wilson, S. H. (2007) Down-regulation of DNA polymerase beta accompanies somatic hypermutation in human BL2 cell lines. DNA Repair (Amst) 6, 244–253.
97 Casali, P., Pal, Z., Xu, Z. and Zan, H. (2006) DNA repair in antibody somatic hypermutation. Trends Immunol. 27, 313–321.
98 Rada, C., Ehrenstein, M. R., Neuberger, M. S. and Milstein, C. (1998) Hot spot focusing of somatic hypermutation in MSH2-deficient mice suggests two stages of mutational targeting. Immunity 9, 135–141.
99 Wiesendanger, M., Kneitz, B., Edelmann, W. and Scharff, M. D. (2000) Somatic hypermutation in MutS homologue (MSH)3-, MSH6-, and MSH3/MSH6-deficient mice reveals a role for the MSH2-MSH6 heterodimer in modulating the base substitution pattern. J. Exp. Med. 191, 579–584.
100 Martomo, S. A., Yang, W. W. and Gearhart, P. J. (2004) A role for Msh6 but not Msh3 in somatic hypermutation and class switch recombination. J. Exp. Med. 200, 61–68.
101 Bardwell, P. D., Woo, C. J., Wei, K., Li, Z., Martin, A., Sack, S. Z., Parris, T., Edelmann, W. and Scharff, M. D. (2004) Altered somatic hypermutation and reduced class-switch recombination in exonuclease 1-mutant mice. Nat. Immunol. 5, 224–229.
102 Delbos, F., De Smet, A., Faili, A., Aoufouchi, S., Weill, J. C. and Reynaud, C. A. (2005) Contribution of DNA polymerase eta to immunoglobulin gene hypermutation in the mouse. J. Exp. Med. 201, 1191–1196.
103 Wilson, T. M., Vaisman, A., Martomo, S. A., Sullivan, P., Lan, L., Hanaoka, F., Yasui, A., Woodgate, R. and Gearhart, P. J. (2005) MSH2-MSH6 stimulates DNA polymerase eta, suggesting a role for A:T mutations in antibody genes. J. Exp. Med. 201, 637–645.
104 Martomo, S. A., Yang, W. W., Wersto, R. P., Ohkumo, T., Kondo, Y., Yokoi, M., Masutani, C., Hanaoka, F. and Gearhart, P. J. (2005) Different mutation signatures in DNA polymerase eta- and MSH6-deficient mice suggest separate roles in antibody diversification. Proc. Natl. Acad. Sci. USA 102, 8656–8661.
105 Zeng, X., Winter, D. B., Kasmer, C., Kraemer, K. H., Lehmann, A. R. and Gearhart, P. J. (2001) DNA polymerase eta is an A-T mutator in somatic hypermutation of immunoglobulin variable genes. Nat. Immunol. 2, 537–541.
106 Um, S., Harbers, M., Benecke, A., Pierrat, B., Losson, R. and Chambon, P. (1998) Retinoic acid receptors interact physically and functionally with the T:G mismatch-specific thymine-DNA glycosylase. J. Biol. Chem. 273, 20728–20736.
107 Chen, D., Lucey, M. J., Phoenix, F., Lopez-Garcia, J., Hart, S. M., Losson, R., Buluwela, L., Coombes, R. C., Chambon, P., Schar, P. and Ali, S. (2003) T:G mismatch-specific thymine-DNA glycosylase potentiates transcription of estrogen-regulated genes through direct interaction with estrogen receptor {alpha}. J. Biol. Chem. 278, 38586–38592.
108 Lucey, M. J., Chen, D., Lopez-Garcia, J., Hart, S. M., Phoenix, F., Al-Jehani, R., Alao, J. P., White, R., Kindle, K. B., Losson, R. et al. (2005) T:G mismatch-specific thymine-DNA glycosylase (TDG) as a coregulator of transcription interacts with SRC1 family members through a novel tyrosine repeat motif. Nucleic Acids Res. 33, 6393–6404.
109 Li, Y.-Q., Zhou, P.-Z., Zheng, X.-D., Walsh, C. P. and Xu, G.-L. (2007) Association of Dnmt3a and thymine DNA glycosylase links DNA methylation with base-excision repair. Nucleic Acids Res. 35, 390–400.
110 Gallais, R., Demay, F., Barath, P., Finot, L., Jurkowska, R., Le Guevel, R., Gay, F., Jeltsch, A., Metivier, R. and Salbert, G. (2007) Deoxyribonucleic acid methyl transferases 3a and 3b

associate with the nuclear orphan receptor COUP-TFI during gene activation. Mol. Endocrinol. 21, 2085–2098.
111 Modrich, P. (2006) Mechanisms in eukaryotic mismatch repair. J. Biol. Chem. 281, 30305–30309.
112 Hegde, M. L., Hazra, T. K. and Mitra, S. (2008) Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. Cell Res. 18, 27–47.
113 Zharkov, D. O. (2008) Base excision DNA repair. Cell. Mol. Life Sci. 65, 1544–1565.
114 Wu, T. H. and Marinus, M. G. (1994) Dominant negative mutator mutations in the mutS gene of *Escherichia coli*. J. Bacteriol. 176, 5393–5400.
115 Haber, L. T. and Walker, G. C. (1991) Altering the conserved nucleotide binding motif in the *Salmonella typhimurium* MutS mismatch repair protein affects both its ATPase and mismatch binding activities. EMBO J. 10, 2707–2715.
116 Ban, C. and Yang, W. (1998) Crystal structure and ATPase activity of MutL: implications for DNA repair and mutagenesis. Cell 95, 541–552.
117 Baitinger, C., Burdett, V. and Modrich, P. (2003) Hydrolytically deficient MutS E694A is defective in the MutL-dependent activation of MutH and in the mismatch-dependent assembly of the MutS.MutL.heteroduplex complex. J. Biol. Chem. 278, 49505–49511.
118 Spampinato, C. and Modrich, P. (2000) The MutL ATPase is required for mismatch repair. J. Biol. Chem. 275, 9863–9869.
119 Hall, M. C. and Matson, S. W. (1999) The *Escherichia coli* MutL protein physically interacts with MutH and stimulates the MutH-associated endonuclease activity. J. Biol. Chem. 274, 1306–1312.
120 Lamers, M. H., Perrakis, A., Enzlin, J. H., Winterwerp, H. H., de Wind, N. and Sixma, T. K. (2000) The crystal structure of DNA mismatch repair protein MutS binding to a G x T mismatch. Nature 407, 711–717.
121 Obmolova, G., Ban, C., Hsieh, P. and Yang, W. (2000) Crystal structures of mismatch repair protein MutS and its complex with a substrate DNA. Nature 407, 703–710.
122 Schofield, M. J. and Hsieh, P. (2003) DNA mismatch repair: molecular mechanisms and biological function. Annu. Rev. Microbiol. 57, 579–608.
123 Su, S. S., Lahue, R. S., Au, K. G. and Modrich, P. (1988) Mispair specificity of methyl-directed DNA mismatch correction *in vitro*. J. Biol. Chem. 263, 6829–6835.
124 Wang, H., Yang, Y., Schofield, M. J., Du, C., Fridman, Y., Lee, S. D., Larson, E. D., Drummond, J. T., Alani, E., Hsieh, P. et al. (2003) DNA bending and unbending by MutS govern mismatch recognition and specificity. Proc. Natl. Acad. Sci. USA 100, 14822–14827.
125 Acharya, S., Wilson, T., Gradia, S., Kane, M. F., Guerrette, S., Marsischky, G. T., Kolodner, R. and Fishel, R. (1996) hMSH2 forms specific mispair-binding complexes with hMSH3 and hMSH6. Proc. Natl. Acad. Sci. USA 93, 13629–13634.
126 Drummond, J. T., Li, G. M., Longley, M. J. and Modrich, P. (1995) Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells. Science 268, 1909–1912.
127 Genschel, J., Littman, S. J., Drummond, J. T. and Modrich, P. (1998) Isolation of MutSbeta from human cells and comparison of the mismatch repair specificities of MutSbeta and MutSalpha. J. Biol. Chem. 273, 19895–19901.
128 Marra, G., Iaccarino, I., Lettieri, T., Roscilli, G., Delmastro, P. and Jiricny, J. (1998) Mismatch repair deficiency associated with overexpression of the MSH3 gene. Proc. Natl. Acad. Sci. USA 95, 8568–8573.
129 Umar, A., Risinger, J. I., Glaab, W. E., Tindall, K. R., Barrett, J. C. and Kunkel, T. A. (1998) Functional overlap in mismatch repair by human MSH3 and MSH6. Genetics 148, 1637–1646.
130 Warren, J. J., Pohlhaus, T. J., Changela, A., Iyer, R. R., Modrich, P. L. and Beese, L. S. (2007) Structure of the human MutSalpha DNA lesion recognition complex. Mol. Cell 26, 579–592.
131 Bowers, J., Sokolsky, T., Quach, T. and Alani, E. (1999) A mutation in the MSH6 subunit of the *Saccharomyces cerevisiae* MSH2-MSH6 complex disrupts mismatch recognition. J. Biol. Chem. 274, 16115–16125.
132 Dufner, P., Marra, G., Raschle, M. and Jiricny, J. (2000) Mismatch recognition and DNA-dependent stimulation of the ATPase activity of hMutSalpha is abolished by a single mutation in the hMSH6 subunit. J. Biol. Chem. 275, 36550–36555.
133 Drotschmann, K., Yang, W., Brownewell, F. E., Kool, E. T. and Kunkel, T. A. (2001) Asymmetric recognition of DNA local distortion. Structure-based functional studies of eukaryotic Msh2-Msh6. J. Biol. Chem. 276, 46225–46229.
134 Junop, M. S., Obmolova, G., Rausch, K., Hsieh, P. and Yang, W. (2001) Composite active site of an ABC ATPase: MutS uses ATP to verify mismatch recognition and authorize DNA repair. Mol. Cell 7, 1–12.
135 Lamers, M. H., Winterwerp, H. H. and Sixma, T. K. (2003) The alternating ATPase domains of MutS control DNA mismatch repair. EMBO J. 22, 746–756.
136 Gradia, S., Acharya, S. and Fishel, R. (1997) The human mismatch recognition complex hMSH2-hMSH6 functions as a novel molecular switch. Cell 91, 995–1005.
137 Iaccarino, I., Marra, G., Dufner, P. and Jiricny, J. (2000) Mutation in the magnesium binding site of hMSH6 disables the hMutSalpha sliding clamp from translocating along DNA. J. Biol. Chem. 275, 2080–2086.
138 Blackwell, L. J., Martik, D., Bjornson, K. P., Bjornson, E. S. and Modrich, P. (1998) Nucleotide-promoted release of hMutSalpha from heteroduplex DNA is consistent with an ATP-dependent translocation mechanism. J. Biol. Chem. 273, 32055–32062.
139 Gradia, S., Subramanian, D., Wilson, T., Acharya, S., Makhov, A., Griffith, J. and Fishel, R. (1999) hMSH2-hMSH6 forms a hydrolysis-independent sliding clamp on mismatched DNA. Mol. Cell 3, 255–261.
140 Martik, D., Baitinger, C. and Modrich, P. (2004) Differential specificities and simultaneous occupancy of human MutSalpha nucleotide binding sites. J. Biol. Chem. 279, 28402–28410.
141 Jiricny, J. (2006) The multifaceted mismatch-repair system. Nat. Rev. Mol. Cell. Biol. 7, 335–346.
142 Kunkel, T. A. and Erie, D. A. (2005) DNA mismatch repair. Annu. Rev. Biochem. 74, 681–710.
143 Bronner, C. E., Baker, S. M., Morrison, P. T., Warren, G., Smith, L. G., Lescoe, M. K., Kane, M., Earabino, C., Lipford, J., Lindblom, A. et al. (1994) Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 368, 258–261.
144 Lipkin, S. M., Wang, V., Jacoby, R., Banerjee-Basu, S., Baxevanis, A. D., Lynch, H. T., Elliott, R. M. and Collins, F. S. (2000) MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. Nat. Genet. 24, 27–35.
145 Nicolaides, N. C., Papadopoulos, N., Liu, B., Wei, Y. F., Carter, K. C., Ruben, S. M., Rosen, C. A., Haseltine, W. A., Fleischmann, R. D., Fraser, C. M. et al. (1994) Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 371, 75–80.
146 Papadopoulos, N., Nicolaides, N. C., Wei, Y. F., Ruben, S. M., Carter, K. C., Rosen, C. A., Haseltine, W. A., Fleischmann, R. D., Fraser, C. M., Adams, M. D. et al. (1994) Mutation of a mutL homolog in hereditary colon cancer. Science 263, 1625–1629.
147 Flores-Rozas, H. and Kolodner, R. D. (1998) The S*accharomyces cerevisiae* MLH3 gene functions in MSH3-dependent suppression of frameshift mutations. Proc. Natl. Acad. Sci. USA 95, 12404–12409.
148 Harfe, B. D., Minesinger, B. K. and Jinks-Robertson, S. (2000) Discrete *in vivo* roles for the MutL homologs Mlh2p and Mlh3p in the removal of frameshift intermediates in budding yeast. Curr. Biol. 10, 145–148.
149 Raschle, M., Marra, G., Nystrom-Lahti, M., Schar, P. and Jiricny, J. (1999) Identification of hMutLbeta, a heterodimer of hMLH1 and hPMS1. J. Biol. Chem. 274, 32368–32375.

150 Svetlanov, A. and Cohen, P. E. (2004) Mismatch repair proteins, meiosis, and mice: understanding the complexities of mammalian meiosis. Exp. Cell Res. 296, 71–79.
151 Santucci-Darmanin, S. and Paquis-Flucklinger, V. (2003) Homologs of MutS and MutL during mammalian meiosis. Med. Sci. (Paris) 19, 85–91.
152 Lipkin, S. M., Moens, P. B., Wang, V., Lenzi, M., Shanmugarajah, D., Gilgeous, A., Thomas, J., Cheng, J., Touchman, J. W., Green, E. D. et al. (2002) Meiotic arrest and aneuploidy in MLH3-deficient mice. Nat. Genet. 31, 385–390.
153 Johnson, A. and O'Donnell, M. (2005) Cellular DNA replicases: components and dynamics at the replication fork. Annu. Rev. Biochem. 74, 283–315.
154 Gu, L., Hong, Y., McCulloch, S., Watanabe, H. and Li, G. M. (1998) ATP-dependent interaction of human mismatch repair proteins and dual role of PCNA in mismatch repair. Nucleic Acids Res. 26, 1173–1178.
155 Genschel, J. and Modrich, P. (2003) Mechanism of 5′-directed excision in human mismatch repair. Mol. Cell 12, 1077–1086.
156 Lau, P. J. and Kolodner, R. D. (2003) Transfer of the MSH2.MSH6 complex from proliferating cell nuclear antigen to mispaired bases in DNA. J. Biol. Chem. 278, 14–17.
157 Iams, K., Larson, E. D. and Drummond, J. T. (2002) DNA template requirements for human mismatch repair in vitro. J. Biol. Chem. 277, 30805–30814.
158 Holmes, J., Jr., Clark, S. and Modrich, P. (1990) Strand-specific mismatch correction in nuclear extracts of human and *Drosophila melanogaster* cell lines. Proc. Natl. Acad. Sci. USA 87, 5837–5841.
159 Thomas, D. C., Roberts, J. D. and Kunkel, T. A. (1991) Heteroduplex repair in extracts of human HeLa cells. J. Biol. Chem. 266, 3744–3751.
160 Varlet, I., Canard, B., Brooks, P., Cerovic, G. and Radman, M. (1996) Mismatch repair in *Xenopus* egg extracts: DNA strand breaks act as signals rather than excision points. Proc. Natl. Acad. Sci. USA 93, 10156–10161.
161 Lopez de Saro, F. J. and O'Donnell, M. (2001) Interaction of the beta sliding clamp with MutS, ligase, and DNA polymerase I. Proc. Natl. Acad. Sci. USA 98, 8376–8380.
162 Pavlov, Y. I., Mian, I. M. and Kunkel, T. A. (2003) Evidence for preferential mismatch repair of lagging strand DNA replication errors in yeast. Curr. Biol. 13, 744–748.
163 Genschel, J., Bazemore, L. R. and Modrich, P. (2002) Human exonuclease I is required for 5′ and 3′ mismatch repair. J. Biol. Chem. 277, 13302–13311.
164 Szankasi, P. and Smith, G. R. (1995) A role for exonuclease I from *S. pombe* in mutation avoidance and mismatch correction. Science 267, 1166–1169.
165 Tishkoff, D. X., Boerger, A. L., Bertrand, P., Filosi, N., Gaida, G. M., Kane, M. F. and Kolodner, R. D. (1997) Identification and characterization of *Saccharomyces cerevisiae* EXO1, a gene encoding an exonuclease that interacts with MSH2. Proc. Natl. Acad. Sci. USA 94, 7487–7492.
166 Wei, K., Clark, A. B., Wong, E., Kane, M. F., Mazur, D. J., Parris, T., Kolas, N. K., Russell, R., Hou, H., Jr., Kneitz, B. et al. (2003) Inactivation of Exonuclease 1 in mice results in DNA mismatch repair defects, increased cancer susceptibility, and male and female sterility. Genes Dev. 17, 603–614.
167 Lee, B. I. and Wilson, D. M., 3rd. (1999) The RAD2 domain of human exonuclease 1 exhibits 5′ to 3′ exonuclease and flap structure-specific endonuclease activities. J. Biol. Chem. 274, 37763–37769.
168 Wilson, D. M., 3rd, Carney, J. P., Coleman, M. A., Adamson, A. W., Christensen, M. and Lamerdin, J. E. (1998) Hex1: a new human Rad2 nuclease family member with homology to yeast exonuclease 1. Nucleic Acids Res. 26, 3762–3768.
169 Schmutte, C., Marinescu, R. C., Sadoff, M. M., Guerrette, S., Overhauser, J. and Fishel, R. (1998) Human exonuclease I interacts with the mismatch repair protein hMSH2. Cancer Res. 58, 4537–4542.
170 Schmutte, C., Sadoff, M. M., Shim, K. S., Acharya, S. and Fishel, R. (2001) The interaction of DNA mismatch repair proteins with human exonuclease I. J. Biol. Chem. 276, 33011–33018.
171 Tran, P. T., Simon, J. A. and Liskay, R. M. (2001) Interactions of Exo1p with components of MutLalpha in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 98, 9760–9765.
172 Zhang, Y., Yuan, F., Presnell, S. R., Tian, K., Gao, Y., Tomkinson, A. E., Gu, L. and Li, G. M. (2005) Reconstitution of 5′-directed human mismatch repair in a purified system. Cell 122, 693–705.
173 Nielsen, F. C., Jager, A. C., Lutzen, A., Bundgaard, J. R. and Rasmussen, L. J. (2004) Characterization of human exonuclease 1 in complex with mismatch repair proteins, subcellular localization and association with PCNA. Oncogene 23, 1457–1468.
174 Dzantiev, L., Constantin, N., Genschel, J., Iyer, R. R., Burgers, P. M. and Modrich, P. (2004) A defined human system that supports bidirectional mismatch-provoked excision. Mol. Cell 15, 31–41.
175 Kadyrov, F. A., Dzantiev, L., Constantin, N. and Modrich, P. (2006) Endonucleolytic function of MutLalpha in human mismatch repair. Cell 126, 297–308.
176 Longley, M. J., Pierce, A. J. and Modrich, P. (1997) DNA polymerase delta is required for human mismatch repair *in vitro*. J. Biol. Chem 272, 10917–10921.
177 Guo, S., Zhang, Y., Yuan, F., Gao, Y., Gu, L., Wong, I. and Li, G. M. (2006) Regulation of replication protein A functions in DNA mismatch repair by phosphorylation. J. Biol. Chem. 281, 21607–21616.
178 Slupphaug, G., Mol, C. D., Kavli, B., Arvai, A. S., Krokan, H. E. and Tainer, J. A. (1996) A nucleotide-flipping mechanism from the structure of human uracil-DNA glycosylase bound to DNA. Nature 384, 87–92.
179 Hitomi, K., Iwai, S. and Tainer, J. A. (2007) The intricate structural chemistry of base excision repair machinery: implications for DNA damage recognition, removal, and repair. DNA Repair (Amst) 6, 410–428.
180 Kavli, B., Slupphaug, G., Mol, C. D., Arvai, A. S., Peterson, S. B., Tainer, J. A. and Krokan, H. E. (1996) Excision of cytosine and thymine from DNA by mutants of human uracil-DNA glycosylase. EMBO J. 15, 3442–3447.
181 Maiti, A., Morgan, M. T., Pozharski, E. and Drohat, A. C. (2008) Crystal structure of human thymine DNA glycosylase bound to DNA elucidates sequence-specific mismatch recognition. Proc. Natl. Acad. Sci. USA 105, 8890–8895.
182 Barrett, T. E., Savva, R., Panayotou, G., Barlow, T., Brown, T., Jiricny, J. and Pearl, L. H. (1998) Crystal structure of a G:T/U mismatch-specific DNA glycosylase: mismatch recognition by complementary-strand interactions. Cell 92, 117–129.
183 Fromme, J. C., Banerjee, A., Huang, S. J. and Verdine, G. L. (2004) Structural basis for removal of adenine mispaired with 8-oxoguanine by MutY adenine DNA glycosylase. Nature 427, 652–656.

To access this journal online:
http://www.birkhauser.ch/CMLS