REVIEW ARTICLE

Recognition of DNA alterations by the mismatch repair system

Giancarlo MARRA and Primo SCHÄR[1]

Institute for Medical Radiobiology, August Forel-Strasse 7, CH-8029 Zürich, Switzerland

Misincorporation of non-complementary bases by DNA polymerases is a major source of the occurrence of promutagenic base-pairing errors during DNA replication or repair. Base–base mismatches or loops of extra bases can arise which, if left unrepaired, will generate point or frameshift mutations respectively. To counteract this mutagenic potential, organisms have developed a number of elaborate surveillance and repair strategies which co-operate to maintain the integrity of their genomes. An important replication-associated correction function is provided by the post-replicative mismatch repair system. This system is highly conserved among species and appears to be the major pathway for strand-specific elimination of base–base mispairs and short insertion/deletion loops (IDLs), not only during DNA replication, but also in intermediates of homologous recombination. The efficiency of repair of different base-pairing errors in the DNA varies, and appears to depend on multiple factors, such as the physical structure of the mismatch and sequence context effects. These structural aspects of mismatch repair are poorly understood. In contrast, remarkable progress in understanding the biochemical role of error-recognition proteins has been made in the recent past. In eukaryotes, two heterodimers consisting of MutS-homologous proteins have been shown to share the function of mismatch recognition *in vivo* and *in vitro*. A first MutS homologue, MSH2, is present in both heterodimers, and the specificity for mismatch recognition is dictated by its association with either of two other MutS homologues: MSH6 for recognition of base–base mismatches and small IDLs, or MSH3 for recognition of IDLs only. Mismatch repair deficiency in cells can arise through mutation, transcriptional silencing or as a result of imbalanced expression of these genes.

Key words: base–base mismatches, DNA replication, insertion/deletion loops, mutagenesis.

INTRODUCTION

Irregular base pairing in prokaryotic and eukaryotic DNA is recognized by a group of highly conserved polypeptides that are homologues of the bacterial MutS protein. Together with another group of conserved proteins, the MutL homologues, they are key components of post-replicative mismatch repair systems. The primary role of MutS proteins is the detection of mispaired and misaligned bases in DNA and the initiation of mismatch correction, but they may also be involved in the recognition of a wider spectrum of DNA alterations that cause structural distortions mimicking those of mispaired Watson–Crick bases.

The first biological evidence for the occurrence and the correction of irregular base pairing in genomic DNA was obtained in two separate lines of investigation. In the early 1960s, studies of meiotic homologous recombination in ascomycetous fungi led Holliday [1] to postulate differential correction of mismatches arising in meiotic recombination intermediates to explain various patterns of non-Mendelian segregation of genetic markers. Simultaneously, studies of induced mutagenesis in bacteria led Witkin [2] to postulate mismatch repair to account for the 5-bromouracil-induced generation of lactose-negative *Escherichia coli* clones in lactose-positive colonies. This mutagenic effect was postulated to reflect misincorporation of 5-bromouracil opposite guanine, followed by misrepair of the guanine to restore normal base pairing between 5-bromouracil and adenine.

During the past three decades, the key factors involved in prokaryotic and eukaryotic post-replicative mismatch correction have been identified. Extensive genetic and biochemical investigation into their physiological function and enzymic properties yielded a comprehensive understanding of the molecular mechanisms involved, with the successful *in vitro* reconstitution of the prokaryotic mismatch repair reaction in 1989 representing a significant milestone of achievement [3]. More recently, it has been firmly established that in multicellular organisms the mutability resulting from a failure of the mismatch repair system can generate cells with tumorigenic potential, and these studies have culminated in the remarkable discovery of a causal relationship between deficiencies in post-replicative mismatch repair and a familiar form of human colorectal cancer, called hereditary non-polyposis colorectal cancer (reviewed in [4]).

The objective of the present review is to summarize the general mechanistic aspects of substrate recognition by prokaryotic and eukaryotic post-replicative mismatch repair systems.

DEFINITION, ORIGIN AND CONSEQUENCES OF BASE-PAIRING ERRORS

In principle, two types of erroneous base pairing can arise in DNA. The first consists of non-complementary juxtaposed bases and is generally referred to as base–base mispairing or mismatching. Base–base mispairing can occur in duplex DNA in the form of purine–purine (G·G, A·A, G·A), purine–pyrimidine (G·T, T·G, A·C) or pyrimidine–pyrimidine (C·C, T·T, T·C) mismatches which, with the exception of C·C, are all subject to correction by the mismatch repair system, albeit with variable efficiency. A second type of pairing error arises as a consequence of misalignment of the two complementary single strands in

Abbreviations used: *cis*-Platinum, *cis*-diamminedichloroplatinum; *DHFR*, dihydrofolate reductase gene; HMG, high-mobility group; IDL, insertion/deletion loop; MGMT, O^6-methylguanine methyltransferase; MLH, MutL homologue; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; MSH, MutS homologue; NER, nucleotide-excision repair; O^6meG, O^6-methylguanine.

[1] To whom correspondence should be addressed (e-mail schaer@imr.unizh.ch).

© 1999 Biochemical Society

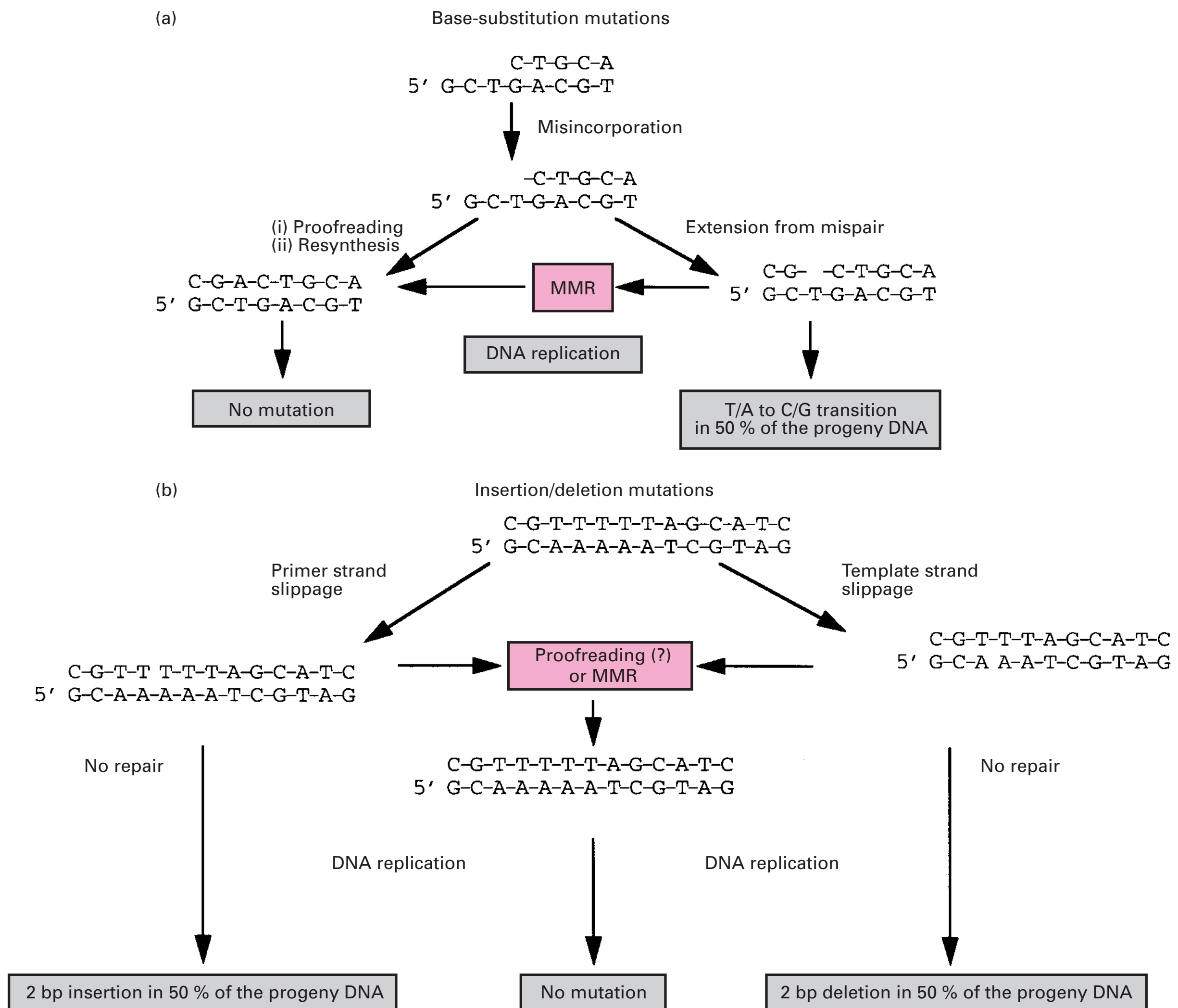

Figure 1 Mutation avoidance by post-replicative mismatch repair

Formation of a mismatch (**a**) or a misalignment (**b**) occurs during a first round of DNA replication. In the absence of repair a point mutation (**a**) or a frameshift mutation (**b**) will be fixed in the subsequent round of DNA replication. Abbreviation: MMR, mismatch repair.

double-stranded DNA. Depending on the extent of misalignment, this results in the formation of single-stranded loops of one or more unpaired bases in the DNA duplex. Such mispairings, termed insertion/deletion loops (IDLs), are also efficiently detected and corrected by the mismatch repair system.

Base–base mismatches and IDLs can arise by several processes, the most important of which in vegetatively growing cells appears to be DNA replication (Figure 1). During DNA synthesis, base–base mismatches are generated by misincorporation of a non-complementary base in the newly replicated DNA strand, whereas IDLs arise from slippage of one DNA strand along the other. If these errors escape correction by the proofreading function of the DNA polymerase, they will be subject to repair by the mismatch repair system. In any case, correction must be directed to the newly synthesized strand in order to ensure fidelity of inheritance. In the absence of repair prior to the successive round of DNA replication, or in the absence of strand discrimination by mismatch repair, mutations will be assimilated in 50% of progeny. Misincorporations generating purine–pyrimidine mispairs will give rise to transition mutations, whereas those generating either pyrimidine–pyrimidine or purine–purine mispairs will lead to transversion. IDLs due to misalignment will produce frameshift mutations; depending on whether a single-stranded loop forms in the template or in the newly synthesized DNA strand, the resulting mutational event will be a deletion or an insertion respectively (Figure 1).

Erroneous base pairing can also occur in heteroduplex DNA intermediates of homologous recombination events between two quasi-homologous DNA sequences. When hybrid DNA is formed as part of the recombinational process, mispaired bases are generated at sites of sequence divergence between two homologous chromosomes. Unlike most of the mismatches produced during DNA replication, mispairings arising in recombinational intermediates are not promutagenic, but are nevertheless substrates for the mismatch repair system. Processing of recombinational heteroduplexes by the mismatch repair system has been shown to contribute to the pattern of non-Mendelian segregation (post-meiotic segregation, gene conversion), to the total frequencies of reciprocal exchange (crossover) and to the regulation of recombination between similar but non-identical

© 1999 Biochemical Society

('homeologous') DNA sequences. The latter function of mismatch repair provides an efficient homology-sensing tool that assists in the maintenance of a genetic barrier between closely related species by actively preventing genetic recombination between related DNA sequences. Failure of mismatch repair in recombination does not appear to be life-threatening, but may favour the interaction between homeologous sequences and thus accelerate the appearance of novel recombinants, which may be disastrous for populations in the long term. In the case of meiotic homologous recombination, which is fundamental for proper chromosome segregation, a possible negative consequence of tolerated homeologous interaction is increased inaccurate chromosome disjunction (reviewed in [5,6]).

Base-pairing abnormalities can also occur in the absence of ongoing DNA replication or recombination as a result of spontaneous or induced base damage. A classical example of spontaneous base modification is hydrolytic deamination of cytosine or 5-methylcytosine. These deamination events are estimated to occur at appreciable rates in the genomes of living cells, generating uracil or thymine respectively mispaired with guanine. Thus G·T mismatches can arise from at least three different cellular processes: (i) DNA synthesis, (ii) DNA recombination, and (iii) deamination of 5-methylcytosine. Unlike during DNA replication, where correction of G·T mismatches can be to either G·C or A·T base pairs, depending on whether T or G respectively was misincorporated into the newly synthesized strand, the very same mismatches arising after deamination of 5-methylcytosine to thymine need to be restored to G·C base pairs in order to avoid mutation. In human cells this appears to be achieved by a separate, DNA-glycosylase-mediated G·T mismatch repair pathway that has been reviewed elsewhere [6,7]. In contrast, mispairing that is induced as a consequence of chemical base modification in DNA, such as the O^6meG·C base pairs (where O^6meG is O^6-methylguanine) generated after treatment of cells with alkylating agents, has been reported to provoke correction by the mismatch repair system. Similarly, it has been found that mismatch repair factors play a role in the processing of more complex chemically induced DNA adducts, such as the diguanyl intrastrand cross-links arising after treatment with some platinum-based anti-cancer drugs, including cisplatin. In the following sections we will focus our review on the molecular mechanism of base–base mismatch and IDL recognition by the post-replicative mismatch repair system, and discuss its role in the detection and processing of some chemically induced DNA adducts relevant for the treatment of human cancers.

MECHANISM OF POST-REPLICATIVE MISMATCH CORRECTION

Although the most extensively studied mismatch repair systems to date are the MutHLS-dependent pathways of *E. coli* and *Salmonella typhimurium* (reviewed in [8]), early evidence for mismatch correction in prokaryotes was obtained in transformation experiments with *Streptococcus pneumoniae*. Whereas *Strep. pneumoniae* wild-type cells show donor-marker-specific transformation efficiencies, a series of *hex*$^-$ mutants were isolated which behaved as high-efficiency recipients in transformation with any DNA donor marker ('hex' stands for 'high-efficiency transformation with all markers'). These *hex*$^-$ strains were found to be deficient in mismatch correction, and it has become evident that marker discrimination in *Strep. pneumoniae* reflects donor-strand-directed mismatch correction in heteroduplex recombination intermediates formed after transformation [9]. Thus strand directionality in this system was proposed to be signalled by the presence of DNA ends in the donor DNA fragments [10]. The mutator phenotype associated with *hex*$^-$ mutants nourished the hypothesis that mismatch correction might not only be active in recombination intermediates, but also function in the avoidance of spontaneous mutation [11], and it is believed that during DNA replication interruptions present on a nascent DNA strand are signals for the Hex mismatch repair system to target removal of misincorporated bases in the newly synthesized strand.

A different mode of strand discrimination, characterized and understood in more detail, is employed by the *E. coli* MutHSL system. In 1975, Marinus and Morris [12] found a spontaneous mutator phenotype to be associated with inactivating mutations in the DNA adenine methylase (*dam*) gene of *E. coli*. The *dam* gene encodes a DNA methyltransferase which methylates the 6-position of adenine in GATC sequences. Wagner and Meselson [13] then suggested that transiently unmethylated GATC sites in the nascent DNA strand present immediately after DNA synthesis could serve as a signal for the post-replicative mismatch repair apparatus to discriminate between template and newly synthesized DNA strands. A number of observations corroborate this hypothesis: a genome-wide alteration of GATC methylation results in increased spontaneous mutation rates in *E. coli*; the presence of hemi-methylated GATC sites in phage heteroduplexes was found to be essential for the proper function of the mismatch repair system; and *in vitro* experiments have established that repair is strongly biased towards the unmethylated strand even in the presence of only a few GATC sites, with the nearest one being 1000 bp away from the mismatch [14–18].

The discovery of the protein factors involved in the recognition and processing of mismatched bases in *E. coli* was facilitated by detailed genetic characterization of the pathway. Mutations inactivating four different *E. coli* genes, *mutH*, *mutS*, *mutL* [19,20] and *uvrD* (*mutU*) [21], were found to give rise to high spontaneous mutability in an epistatic manner, and this phenotype was correlated with a deficiency in methyl-directed mismatch repair. Later, an *in vitro* mismatch repair assay developed by Modrich and co-workers [16] allowed the biochemical dissection of the pathway and of the protein functions involved. In this assay, which was based on the detection of strand-specific restoration of a mismatch-containing recognition site for a restriction endonuclease within bacteriophage f1 DNA, extracts derived from *mutH*, *mutS*, *mutL* and *uvrD* mutants were correction-deficient. Using such extracts from *E. coli* mismatch repair mutants in this or similar types of assays in an *in vitro* complementation-based biochemical approach, Modrich and colleagues managed to identify, isolate and study individual core components of the methyl-directed mismatch repair system and, ultimately, to reconstitute the entire mismatch repair reaction with purified proteins [3,22]. This appreciable biochemical achievement has provided an invaluable model for investigation of the molecular mechanisms involved in a generic mismatch repair reaction.

A current model for the mechanism of methyl-directed mismatch repair in *E. coli* is illustrated in Figure 2. The pathway is dependent on the functions encoded by the *mutS*, *mutL* and *mutH* genes. Base–base mispairs and IDLs of up to four extrahelical nucleotides are recognized efficiently and bound specifically by a homodimer of the MutS protein [23,24], an interaction that leads to a DNaseI-digestion-resistant footprint of about 20 bp around the mismatched site in the substrate. Although a weak ATPase activity of MutS was identified, and an ATP-binding site was found to be conserved in MutS proteins encoded by the *Salmonella typhimurium mutS* and the *Streptococcus pneumoniae hexA* genes, ATP hydrolysis is not required for MutS to bind to DNA [25–27]. It has been proposed, however, that ATP is important in steps downstream of mismatch recognition and binding by MutS. Experimental evidence

© 1999 Biochemical Society

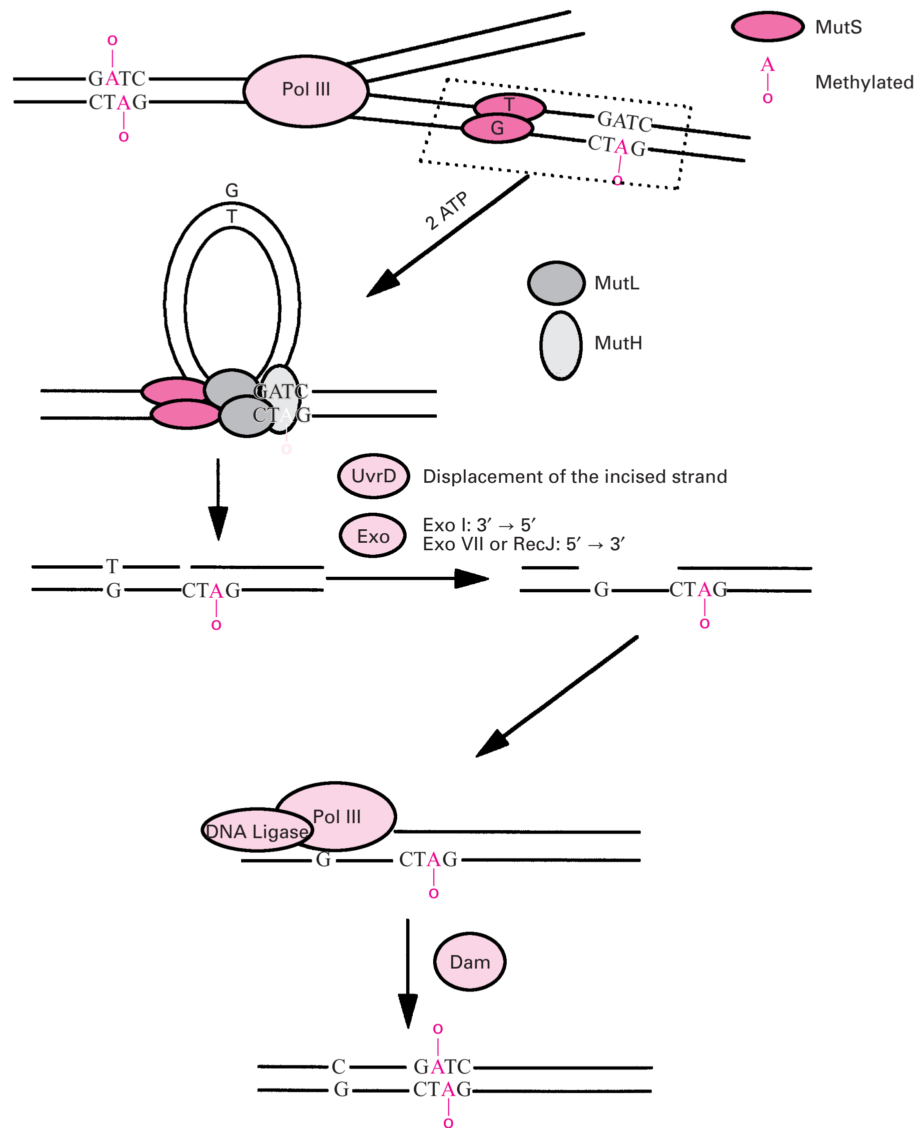

Figure 2 Model for the methyl-directed DNA mismatch repair pathway in *E. coli*

A detailed description of the model is provided in the text. Abbreviations: Exo, exonuclease; Pol III, DNA polymerase III holoenzyme.

suggests a scenario in which ATP binding and hydrolysis is required for the induction of conformational changes in the MutS–DNA complex that lead to an association with a homodimer of the MutL protein and the translocation of DNA through or along the MutS–MutL recognition complex [28–30]. This translocation has been visualized by electron microscopy as the formation of protein-stabilized α-shaped double-stranded DNA loop structures in which MutS protein co-localizes with MutL at the base of the nascent α-loop [30]. Consistent with this, in the presence of ATP, the forming MutS–MutL complex protects a region of about 100 bp from digestion by DNase I, which is considerably larger than the footprint observed with MutS homodimer alone [28]. The ATP-dependent translocation mechanism might allow co-ordinated interaction between the mismatch recognition complex consisting of MutS and MutL proteins in a complex with heteroduplex DNA and the nearest hemi-methylated GATC site. This may result in association with and activation of the latent MutH endonuclease, which will incise at unmethylated GATC sites and thus initiate the excision process [31]. A significant observation regarding strand discrimination by the MutHLS system is that the requirement for MutH endonuclease can be obviated *in vitro* by the introduction of a strand-specific nick located up to 1000 bp from the mismatched site [3,32].

Once initiated by the MutS, MutL and MutH proteins, the repair reaction proceeds by exonucleolytic degradation of the nicked DNA strand from the incised GATC site towards and past the mismatch, followed by DNA resynthesis and ligation. *In vitro* reconstitution experiments have shown that the excision and resynthesis steps can be bi-directional and require the functions of UvrD (MutU) helicase II, exonuclease I, exonuclease VII or RecJ, DNA polymerase III holoenzyme, single-stranded DNA-binding protein and DNA ligase [22,29,33].

The identification of MutS and MutL homologues in eukaryotes from unicellular yeast to multicellular organisms, including mammals, suggests that the key components of the bacterial mismatch correction system have been conserved throughout evolution, and it is thought that the mechanistic

© 1999 Biochemical Society

principles of eukaryotic post-replicative mismatch repair mirror those of the bacterial prototype reaction illustrated in Figure 2. Apparently, the major differences lie in the multiplicity and heterodimeric organization of the MutS and MutL factors, indicating a higher complexity of the eukaryotic system. Mismatch recognition in eukaryotes is mediated by either of two heterodimers of MutS homologues: MutSα, a heterodimer of MSH2 and MSH6 (where MSH stands for MutS homologue), or MutSβ, a heterodimer of MSH2 and MSH3. The MutS proteins MSH1, MSH4 and MSH5 do not contribute to base–base mismatch or IDL correction in nuclear DNA. The role of ATP in the mismatch recognition step resembles observations made with the bacterial pathway, indicating that a similar strategy of initial mismatch processing, including the formation of an α-loop structure, may be employed by the eukaryotic system. In this scenario, the α-loop would be stabilized at its base by MutSα or MutSβ in a complex with MutLα, which is a heterodimer of two MutL homologues [MLH1 and PMS2 (human) or PMS1 (yeast)]. The lack of evidence for eukaryotic MutH homologues or analogues and the absence of DNA methylation in yeast and *Drosophila melanogaster*, coupled with the irregular distribution of cytosine methylation in higher eukaryotes, suggests a strand discrimination mechanism distinct from the methylation-directed mechanism described for *E. coli*. As in *Strep. pneumoniae*, directionality appears to be imparted by the presence of DNA-strand-specific nicks ensuring that, during DNA replication, mismatch correction would be directed by DNA ends in leading-strand synthesis or nicks between Okazaki fragments in lagging-strand DNA synthesis [34–36]. Exonucleolytic degradation of the incised strand can be bi-directional, and thus involves either 5′–3′ or 3′–5′ exonucleases [22,36]. One 5′–3′ exonuclease, the product of the *exo1* gene in *Schizosaccharomyces pombe* and its homologue in *Saccharomyces cerevisiae*, could be genetically and physically associated with the mismatch repair process [37,38]. The gap-filling reaction is most probably carried out by DNA polymerase δ [39], and the nick is probably sealed by DNA ligase I. In addition, proliferating cell nuclear antigen has been shown to be involved in steps preceding DNA synthesis in mismatch correction, which may indicate an association of mismatch repair components with the replication apparatus [40], although the nature of this is unclear. For more detailed information on the enzymology of the eukaryotic mismatch correction reaction, the reader is referred to recent reviews [6,41,42]

In the following section we will focus our discussion on the recognition step of the mismatch repair process. In particular, we will discuss the role of eukaryotic MutS homologues in mismatch recognition and binding, and compare their properties with those of their prokaryotic counterparts.

SPECIFICITY, FACTORS AND MECHANISM OF MISMATCH RECOGNITION

A common parameter for mismatch recognition by the mismatch repair systems is the nature of the DNA structural alterations imposed by mispairing bases. It is therefore important to start our considerations of the specificity of mismatch recognition with a general evaluation of the relationship between mismatch structure and repair efficiency. In *E. coli* the methylation-directed mismatch repair system was demonstrated to correct G·T, A·C, G·G and A·A mismatches and IDLs consisting of up to four unpaired bases with high efficiency, and T·T, C·T and G·A mismatches with intermediate and variable efficiencies, whereas C·C mispairs and larger IDLs appeared to be very poor substrates for mismatch repair [17,24,43,44]. Thus, allowing for some sequence-context-dependent variability in the middle range of this spectrum, purine–pyrimidine and purine–purine mismatches and small IDLs are more effective substrates for mismatch repair than pyrimidine–pyrimidine mismatches and larger IDLs. This observation appears to be universal, as a similar general trend for mismatch correction efficiencies was reported for the Hex-dependent pathway of *Strep. pneumoniae* [9,45], for a eukaryotic mismatch repair system active in vegetatively growing and meiotically differentiating *Sacch. cerevisiae* and *Schiz. pombe* cells [46–49], and for an activity present in mammalian cell extracts [34–36,50].

With the caveat that mismatch repair efficiencies measured *in vivo* and *in vitro* truly reflect the mismatch recognition capacities of the MutS factors involved [51,52], it could be anticipated that differences in repair efficiencies would be correlated with differences in the structural properties of individual mismatches. However, examination of structural parameters such as heteroduplex stabilities or helix dynamics of mismatched oligonucleotide duplexes has revealed surprisingly little correlation with the correction efficiencies observed: G·T and G·A are among the most stable mismatches, whereas A·C and T·C are among the least stable mispairs, but G·T and A·C are corrected more efficiently than G·A and T·C [53,54]. In contrast, estimates of enthalpies of mismatch stack melting obtained on the basis of the thermodynamic properties of octadecameric heteroduplexes were correlated to some extent with correction efficiencies, suggesting that the stack melting behaviour of mismatches might be a structural feature that affects correction efficiency [54]. In this study, base–base mismatches with stack melting enthalpies similar to those of Watson–Crick pairs were defined as wobble pairs (T·G, G·G, C·A, A·A and A·G), those with enthalpies about half that of A·T or G·C were classified as weak pairs (G·T, A·C and G·A), and those with enthalpies near to zero were inferred to be unstacked or extrahelical (T·T, C·T, T·C and C·C). Thus, according to this classification, the most efficiently repaired mismatches *in vivo* are all of the wobble type, whereas the most poorly repaired substrates fall into the group of open or unstacked mismatches, which consists of pyrimidine–pyrimidine mispairs only. It was suggested that wobble mispairs may cause a rigid deformation of the helix, which is stabilized by co-operative hydrogen-bonding and intrahelical stacking interactions. This structure, rather than local instabilities in the double strand, may be recognized by mismatch repair enzymes. On the other hand, mismatches consisting of two small pyrimidines adopt a less defined and less solid structure due to their higher intrinsic flexibility, and can escape recognition by swinging in and out of the helix [54,55]. Since (with the possible exception of extra pyrimidine bases and C·C mispairs) most mismatches studied can adopt an intrahelical configuration [55–59], it is an attractive possibility that mispair-specific features of base functional groups in the major and minor grooves of the helix are determinants for mismatch recognition.

If the primary role of post-replicative mismatch repair is the elimination of promutagenic base-pairing errors following DNA synthesis, a reasonable biological concept would be that mismatches that are more likely to arise as DNA polymerase errors are better substrates for correction than others that occur only rarely. Indeed, it has been found for prokaryotes that patterns of mismatch-specific repair efficiencies are correlated with the spectrum of mismatches generated by DNA polymerase errors during DNA replication: (i) the mutational spectra displayed by *E. coli mutH*, *mutS* and *mutL* mutants are similar to those derived from errors of the DNA polymerase III holoenzyme [60], and (ii) the mispairs that are most frequently generated by DNA polymerase III show the highest repair efficiency [17]. It can be anticipated that similar correlations exist between mismatch

© 1999 Biochemical Society

repair affinities and the spectrum of DNA polymerase errors in eukaryotic systems.

The multiplicity of MutS-related proteins in eukaryotic organisms suggests that, during evolution, the mismatch recognition function has been refined to accommodate the demands of increasingly complex genomes. Whereas in *E. coli* mismatch affinity is defined by the structure and sequence context of a particular DNA base-pairing error and a single MutS protein, in yeast and mammalian cells it additionally depends on the functional properties of different heterodimeric combinations of various MutS proteins. Of the six known eukaryotic MutS homologues, three have been shown to engage in pairwise interactions that are relevant for mismatch correction in both yeast and mammalian cells.

In yeast, a mismatch-binding activity was found to be associated with a 110 kDa protein [52], which was later identified as the product of the *MSH2* gene [61]. *Sacch. cerevisiae msh2* mutants display increased post-meiotic segregation and elevated rates of spontaneous mitotic mutation [62]. Recombinant MSH2 protein could be shown to bind selectively to duplex oligonucleotide substrates containing a G·T mismatch, 1–14-nucleotide and palindromic (PAL) insertion mispairs with an affinity decreasing along the sequence $+14$ PAL, $+12$ PAL $> +14 > +8 >$ G·T, $+6$, $+4$, $+2$, $+1$ [61]. These mismatch-binding properties of MSH2 clearly differ from those of bacterial MutS, which does not appear to bind IDLs larger than four nucleotides [24], and also from the above-mentioned *MSH2*-dependent activity observed in *Sacch. cerevisiae* nuclear extracts, which binds base–base mismatches with high specificity [52]. This discrepancy may be explained by the fact that mismatch recognition under physiological conditions is primarily accomplished by a heterodimer of two MutS homologues. There is genetic and biochemical evidence for an interaction between MSH2 and MSH3 or MSH6 in *Sacch cerevisiae*. The most compelling sets of data show a partially redundant involvement of *MSH3* and *MSH6* in *MSH2*-dependent mismatch repair, whereby the substrate specificity of the repair process may be dictated by interaction of MSH2 with either MSH3 or MSH6. The mutational spectra observed in *msh3* and *msh6* mutants are consistent with the MSH2–MSH3 heterodimer preferentially recognizing IDLs and the MSH2–MSH6 heterodimer recognizing both IDLs and base–base mispairs [63–65]. Corroborating these observations, Iaccarino and colleagues [66] purified a G·T-mismatch-binding factor from yeast nuclear extracts and identified MSH2 and MSH6 as its protein components, and Alani [67] reported on the selective binding of a recombinant MSH2–MSH6 heterodimer to oligonucleotide substrates containing a G·T mismatch or a $+1$ base IDL. Interestingly, in the latter study both G·T and $+1$ IDL binding were abolished by the addition of ATP to the reaction, which led the author to conclude that ATP hydrolysis by the MSH2–MSH6 heterodimer is required to activate events downstream of mismatch recognition in the base–base mismatch repair reaction [67].

Mutational analyses revealed that inactivation of the ATP-binding domain of MSH2 does not affect the mismatch-binding specificity of the MSH2–MSH6 heterodimer *in vitro*, but brings about a dominant-negative phenotype if the mutant gene is overexpressed in a wild-type strain. The dominant-negative effect may reflect the activity of the mutant MSH2–MSH6 complexes that can still bind base–base mismatches but are inactive in the subsequent steps of the repair reaction. Also, mutation of the helix–turn–helix domain of MSH2, a degenerate peptide motif common to all MutS homologues, resulted in mutant proteins that displayed properties similar to those observed for the proteins defective in ATP binding. Therefore the helix–turn–helix domains were proposed to function in modulating mismatch recognition by mediating conformational changes in the MSH2–MSH6 complex stimulated by ATP hydrolysis [68]. The physical interaction of MSH2 with MSH3 is less well understood. Habraken and colleagues [69] reported purification of a yeast MSH2–MSH3 complex to near homogeneity from extracts of MSH2- and MSH3-overexpressing yeast cells. The purified heterodimer was shown to bind IDLs with high specificity, and to have only a low affinity for G·T mispairs [69]. In a later contribution, the same group showed that IDL binding by the MSH2–MSH3 complex is stimulated in the presence of purified MLH1–PMS1 heterodimer [70].

Evidence for a MutHLS-related, nick-directed mismatch-correction activity in multicellular eukaryotes has been obtained in transfection experiments with mammalian cell lines [71,72] and by biochemical examination of *in vitro* repair capacities of protein extracts derived from mammalian cells, *Xenopus laevis* eggs and *Drosophila melanogaster* cells [22,34–36,50,73,74]. The existence of a factor in HeLa cell extracts with the capacity to specifically bind different mismatched oligonucleotide substrates was first reported in 1988 [75,76]. This factor could be purified to near homogeneity and was shown to consist of two polypeptides of molecular masses 160 and 100 kDa [76]. Sequencing of tryptic peptides generated from these proteins [77] revealed the 100 kDa species to be identical with the product of the *hMSH2* gene, the first human *MutS* homologue that had been identified some months previously [78,79]. This gene encodes a protein of 934 amino acids which shows a high degree of identity with *Sacch. cerevisiae* MSH2, and so the protein was designated hMSH2. The second protein with a molecular mass around 160 kDa was designated GTBP/p160, and only after cloning of its cDNA did it become evident that it was a second human MutS homologue [80,81]. On the basis of its close relationship with *Sacch. cerevisiae* MSH6, the human homologue was later renamed hMSH6. hMSH3, a protein closely related to *Sacch. cerevisiae* MSH3 and *Schiz. pombe* Swi4, was the first human MutS homologue to be genetically characterized, in 1989 [82]. At that time, the gene was named *DUC-1* (for *d*ivergent *u*pstream *c*lone), since it was shown to be divergently transcribed from the dihydrofolate reductase (*DHFR*) gene in both rodents [83,84] and humans [82].

Human cells with defects in hMSH2, hMSH3 or hMSH6 display different mutator phenotypes reminiscent of those observed with the corresponding yeast mutants. Some are predominantly affected in the recognition and repair of base–base mispairs, others in the processing of IDLs, and again others in the repair of both. This is reflected also in the pattern of microsatellite DNA instability, which is a prominent marker of mismatch repair deficiency; some mismatch-repair-deficient human cells show instability of mono-, di- and tri-nucleotide repeats, while others show instability only in poly(A) repeats [85]. It is now well established that both the extent and type of genomic instability are related to the MutS homologue mutated, and thus reflect the differential contributions of hMSH2, hMSH3 and hMSH6 to the recognition and repair of specific base-pairing errors. Although hMSH2 has been shown to bind to G·T and IDL heteroduplex substrates containing 8–14-nucleotide heterologies [86,87], it appears that mismatch recognition in human cells is carried out primarily by heterodimeric MSH complexes, consisting of either hMSH2 and hMSH6 (hMutSα) or hMSH2 and hMSH3 (hMutSβ). The existence of a functional hMSH2–hMSH6 complex was demonstrated in two ways. Isolation of hMSH2 from HeLa cells by *in vitro* complementation of a mismatch repair deficiency in extracts of an MSH2-deficient cell line yielded equimolar amounts of the two proteins, which were shown to form a stable heterodimer [81]. Secondly, production of

© 1999 Biochemical Society

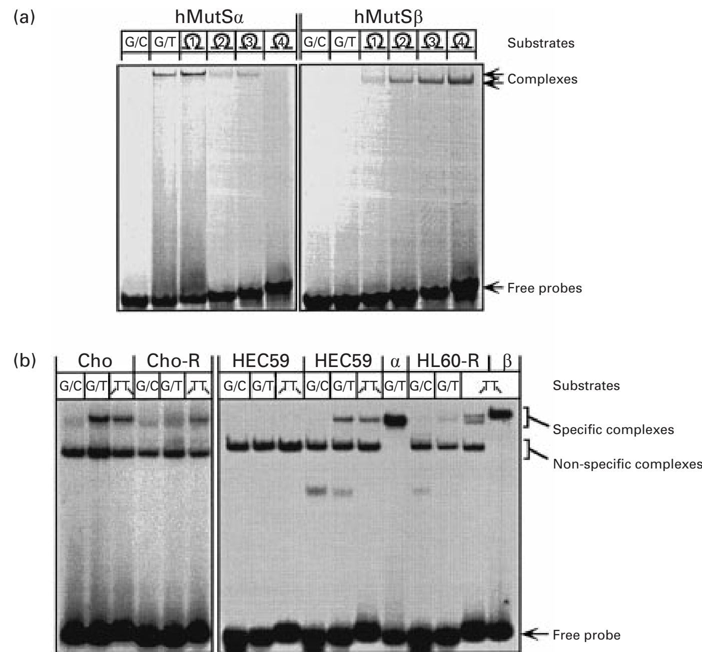

Figure 3 Base–base mismatch and IDL recognition patterns of recombinant hMutSα and hMutSβ complexes (a), and of activities in extracts of CHO, CHO-R, HEC59, HL60, and HL60-R cells (b)

The recombinant proteins or cell extracts were incubated with radioactively labelled oligonucleotide duplexes that either were perfectly complementary (G/C) or contained a single mispair (G/T) or an IDL with two extrahelical thymines (-T-T-) [89]. Non-denaturing polyacrylamide gels were used to analyse the electrophoretic mobility shifts of the protein–DNA complexes. (**a**) The specificity of hMutSα and hMutSβ for a G/T mismatch or IDLs is shown (see the text). (**b**) The electrophoretic mobilities of the complexes between oligonucleotide substrates and proteins extracted from the cell lines specified were compared with those of complexes formed upon incubation of the DNA with hMutSα (lane α) or hMutSβ (lane β). Note that, unlike the recombinant hMSH2–hMSH3 heterodimer, the hMutSβ detectable in extracts of human cells overproducing hMSH3 gives rise to two distinct protein–DNA complexes (lane HL60-R TT). Neither of these complexes co-migrates with hMutSα-bound substrates (e.g. lane HL60-R G/T). CHO, Chinese Hamster ovary cells; CHO-R, CHO cells overproducing MSH3 protein; HEC59, MSH2-deficient endometrial human cancer cell line; HL60; human leukaemia cell line; HL60-R, HL60 cells overproducing MSH3 protein.

hMSH2 and hMSH6 by *in vitro* translation [80,88] or in the baculovirus system [89,90] has demonstrated that both proteins are required for efficient binding to a G·T heteroduplex. Similarly, the existence of a functional hMSH2–hMSH3 complex was demonstrated by co-immunoprecipitation and co-purification of proteins produced in insect cells infected with both hMSH2- and hMSH3-encoding baculovirus vectors. The purified hMSH2–hMSH3 heterodimer was shown to bind IDL heteroduplex substrates, but not G·T mispairs [88,89].

As in yeast, interaction of hMSH2 with hMSH3 or hMSH6 yields mismatch recognition complexes with distinct, but partially overlapping, substrate recognition patterns. The *in vitro* binding specificity of these complexes was demonstrated in mobility-shift DNA-binding assays, as shown in Figure 3(a), and these data are integrated into the model illustrated in Figure 4. MutSα recognizes substrates with base–base mismatches and small IDL heterologies, and is able to restore repair of these mismatches in extracts of MSH2-deficient cell lines, whereas MutSβ binds preferentially IDL heteroduplexes and restores repair of only IDLs, but not of base–base mismatched substrates, in hMSH2-deficient cell extracts [89,91,92]. These data explain why a mutation in hMSH2 causes a more severe mutator phenotype than mutations in either hMSH6 or hMSH3; cells mutated in hMSH6 (MT1 or HCT15) show a mild mutator phenotype, with instability of only mononucleotide repeats, whereas hMSH2-mutated cells (LoVo, HEC59, etc.) show a strong mutator phenotype [81,85]. However, as is well established for the yeast system [65], human hMSH3/hMSH6 double-mutant cells would be expected to have synergistically enhanced effects and give rise to a mutator phenotype similar to that of hMSH2 single mutants.

The functional separation of the two MutS heterodimers has been further documented by two independent observations reported recently by different groups [91,92]. It was shown that hMutSα was able to complement *in vitro* a base–base mismatch repair defect in human and rodent cell lines, which had been acquired by treatment with methotrexate. In these cells, methotrexate-induced amplification of the *DHFR* locus, which spans the *MSH3* gene, leads to overexpression of MSH3 and thus to a dramatic change in the relative amounts of MutSα and MutSβ. Under such conditions, it is believed that the over-abundant MSH3 protein sequesters the available MSH2 protein into MutSβ complexes, leading to destabilization of the partnerless MSH6 protein and, ultimately, to MutSα depletion. Thus these cell lines lack the base–base mispair recognition factor, while loop repair by MutSβ is maintained or even improved. The mismatch binding data that led to this interesting conclusion are shown in Figure 3(b).

The molecular mechanism of base–base mismatch binding by hMutSα and the role of ATP in the recognition step of the human mismatch repair reaction has been addressed in recent studies by two laboratories. Gradia et al. [90] found that an ADP-bound form of hMutSα was active for mismatch binding, while the ATP-bound form was not. The authors stated that ATP binding by MutSα interferes negatively with its capacity for mismatch binding, whereas hydrolysis of ATP by the complex results in the recovery of binding activity, and they concluded

© 1999 Biochemical Society

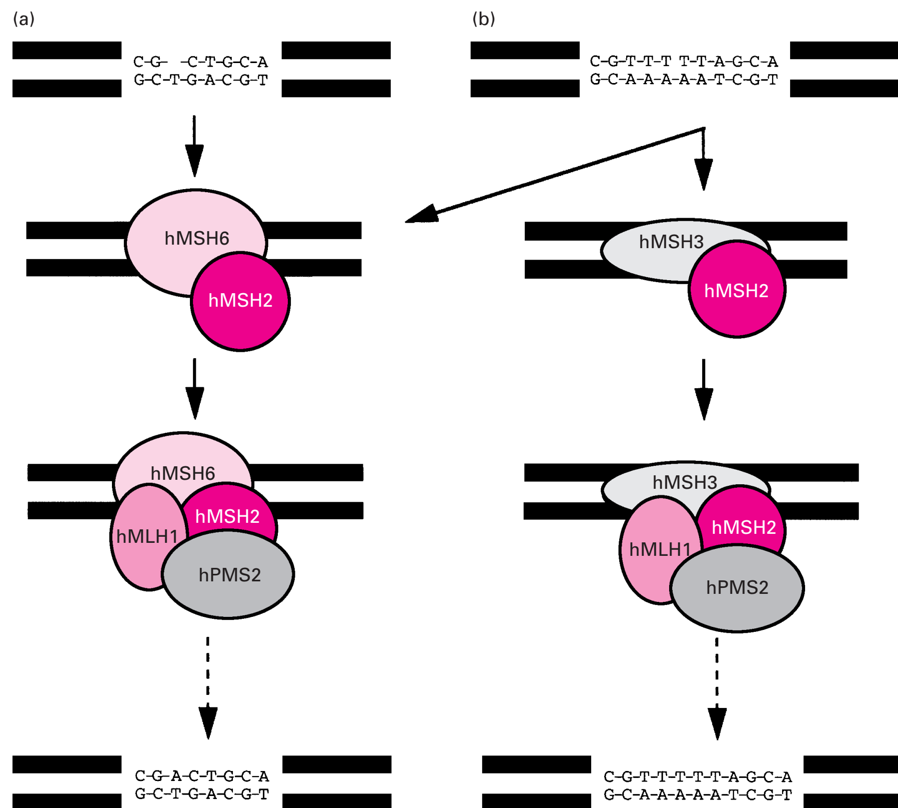

Figure 4 Schematic representation of mismatch (a) and IDL (b) recognition in human cells

A detailed description of the model is provided in the text. Dashed arrows indicate that several other factors are involved in steps following mismatch or loop recognition, such as strand displacement, excision, resynthesis and DNA ligation.

that the process of ATP binding and hydrolysis by the mismatch recognition complex might fulfil the role of a 'molecular switch' for mismatch binding and regulation of subsequent steps of the repair reaction. Iaccarino et al. [93] reported that recombinant hMutSα bound a G·T-mismatch-containing oligonucleotide with a >20–fold higher affinity than a corresponding homoduplex substrate, and their data from cross-linking experiments suggested that mismatch binding is accomplished through a physical contact between the mismatched DNA substrate and the hMSH6 subunit of the MutSα complex. In the presence of ATP, hMutSα was shown to dissociate from the mismatched oligonucleotide, and this reaction was attenuated when the ATP-binding domains of hMSH2, hMSH6 or both were mutated. ATP binding, and not its hydrolysis by hMutSα, was required for dissociation from the mismatch. However, ATP hydrolysis appears to be essential for the subsequent steps of the mismatch repair reaction, as indicated by the fact that the double-mutant complex defective in the ATP-binding domains of both hMSH2 and hMSH6 was not able to complement mismatch-repair-deficient human cell extracts *in vitro*. In agreement with corresponding observations on bacterial MutS and yeast MutSα, this supports the idea that ATP binding and possibly hydrolysis by hMutSα is required for the induction of conformational changes in the MutSα–heteroduplex-DNA complex after mismatch recognition. This is believed to activate translocation of DNA through the mismatch recognition factor in search of a strand discrimination signal, leading to the displacement of MutSα from the mismatch and, ultimately, to initiation of the exonucleolytic excision process. On a comparably small linear heteroduplex substrate, such as is normally used in mismatch-binding assays, this process would result in dissociation of the mismatch recognition complex from the substrate, which is consistent with the experimental observations. A central role for ATP hydrolysis in the formation of the mismatch repair initiation complex, and in particular in the establishment of a physical association of hMLH1 and hPMS2 with hMSH2 and proliferating cell nuclear antigen, has been further corroborated recently by the work Gu et al. [94].

In addition to recognition and repair by the MutS-dependent post-replicative mismatch repair system, a subset of specific base–base mispairs appear to be substrates for other mismatch processing pathways *in vivo*. These systems, which are beyond the scope of this review, display much more confined mismatch recognition spectra and appear to involve reaction mechanisms resembling those of base-excision repair, e.g. DNA-glycosylase-mediated excision of erroneous bases. Well documented examples are the *E. coli* MutY-dependent mismatch repair pathway, which specifically repairs A·G or A·8-oxo-G to C·G and A·C to G·C [16,95], and the very short patch repair pathway, which repairs G·T, derived from deamination of 5-methylcytosine, to G·C [96]. We have already mentioned the mammalian G·T-specific mismatch repair pathway, which is thought to counteract the mutagenic consequences of the deamination of methylated cytosines [71,97], but other mismatch-specific activities have been described in human cell extracts, e.g. an A·G-specific nicking activity [98] and an A·C-mismatch-binding factor [99,100]. In *Schiz. pombe*, genetic evidence was reported for a short patch

© 1999 Biochemical Society

pathway correcting C·C mismatches occurring in meiotic recombination intermediates [49,101]. More recently this C·C mismatch correction pathway was shown to be independent of *msh2* and *pms1* functions, but to involve the *Schiz. pombe* homologues of human XPA, ERCC1 and ERCC4, which constitute key components of the nucleotide excision repair pathway ([102]; P. Schär, unpublished work; O. Fleck, unpublished work). In *Sacch. cerevisiae* cell extracts, another MSH2-independent activity that recognizes 4–9-base IDLs has been described, but not studied further [103].

CHEMICALLY INDUCED DNA ADDUCTS AND MISMATCH REPAIR

It is becoming evident that the mismatch correction system addresses also chemically induced DNA adducts or lesions that mimic the structure of mispaired Waston–Crick bases. A strong stimulus for investigating the impact of cellular DNA repair activities on various types of chemically induced DNA adducts has come from a clinical interest in the development and application of genotoxic agents for cancer chemotherapy, as well as from an increased public alertness to environmental mutagens and carcinogens. The therapeutic use of agents that induce the formation of DNA adducts is based on the idea that this type of damage can interfere with DNA synthesis and thus affect rapidly dividing tumour cells in a more dramatic way than cells from normal tissue. Consequently, during the last decade, a wealth of studies addressing the role of DNA repair in the processing of DNA adducts has appeared in the literature.

Regarding the role of the mismatch repair system in this context, the most attractive observation appears to be a positive correlation between mismatch repair deficiency and tolerance to several DNA-damaging agents. The first evidence for this phenomenon was reported in 1986 by Goldmacher et al. [104]. They demonstrated that a human cell line, TK6, can be induced to alkylation tolerance by treatment with an acridine. They speculated that this acquisition of alkylation tolerance could be related to a deficiency in mismatch repair. This hypothesis was verified later and, furthermore, it was established that tolerance not only to alkylating agents, but also to a wider spectrum of DNA-damaging agents, seems to be a basic characteristic of mismatch-repair-deficient mammalian cells. In this section, we limit our discussion to two types of chemicals: alkylating agents and *cis*-Platinum (*cis*-diamminedichloroplatinum).

Alkylating agents

A wide range of carcinogenic chemicals, including intermediates of normal cellular metabolism, can react with DNA to produce alkylated bases. These agents can have either one (monofunctional) or two (bifunctional) reactive groups which tend to interact covalently with nucleophilic centres in DNA. Such reactive sites are present in all four bases, and they are attacked with different affinities and specificities by different alkylating agents. Most reactive are the ring nitrogen atoms of the bases, in particular N^7 of guanine and N^3 of adenine, but methylation of less nucleophilic oxygens, such as the O^6 position of guanine, appears to have more severe biological consequences, as the resulting adducts are mispairing and thus mutagenic (reviewed in [105]).

Generally, treatment of cells with simple alkylating agents is mutagenic and cytotoxic. For the frequently used agents *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG) and *N*-methyl-*N*-nitrosourea, both effects can be attributed to the formation of O^6meG in DNA. G·C to A·T transitions represent the predominant type of mutation induced by these alkylating agents, and they are thought to arise as a consequence of frequent misincorporation of thymine opposite O^6meG during DNA replication. Structural studies on a set of oligonucleotide duplexes in which O^6meG was juxtaposed with each of the four DNA bases revealed that the most stable base pair is formed between O^6meG and cytosine rather than between O^6meG and thymine (reviewed in [106]). As illustrated in Figure 5(a), O^6meG pairs with cytosine through two hydrogen bonds in a wobble alignment. Hydrogen bonding between O^6meG and thymine is much weaker and allows for accommodation of a geometry consistent with unperturbed Watson–Crick base pairing, which causes little distortion of the phosphodiester backbone. These structural features may explain why the DNA polymerase favours incorporation of thymine opposite O^6meG in the process of DNA replication. However, the mutagenicity of treatment with alkylating agents may also depend on DNA sequence context, as suggested by non-random distribution of alkylation-induced mutations in the H-*ras* or *hprt* genes (reviewed in [106]), and the presence of mutational ‘hot spots’ could indeed reflect the compound effects of sequence context on base alkylation, DNA synthesis and repair.

The cytotoxicity of O^6meG is strongly enhanced in cells lacking the demethylating O^6-methylguanine methyltransferase (MGMT) activity ($MGMT^-$, Mex^- or Mer^- cells). This $MGMT^-$ phenotype is commonly found among tumour cell lines and is thought to arise mainly by transcriptional silencing of the *MGMT* gene. The MGMT protein is the most efficient repair factor for the selective removal of methyl groups from the O^6 position of guanine. It acts by transferring methyl groups from O^6meG to its cysteine residues and is thereby consumed. If O^6meG bases escape restoration to guanine due to an absence or insufficiency of MGMT activity, DNA replication will result in the above-mentioned preponderance of O^6meG·T mispairs over O^6meG·C base pairs. Both of these pairs have been shown to be recognized by human MutSα and are therefore assumed to provoke mismatch repair [107]. Attempts at mismatch correction at DNA modifications have been proposed to be the underlying reason for the cytotoxic effects of the modifying agents, the idea being that post-replicative mismatch correction directed to the newly synthesized strand would be ineffectual, leaving the methylated base in the parental strand and thus leading to a reiterative cycle of DNA synthesis, O^6meG·T mispair formation and mismatch repair. Such abortive repair events are presumed to result in cell-cycle arrest and lethality, but the underlying biological processes are poorly understood [104,108,109]. Other experimental evidence implicates mismatch repair also in alkylation-associated chromosomal instability. While the presence of high concentrations of O^6meG residues in the genomic DNA of $MGMT^-$ cells is highly recombinogenic [110], methylation-provoked chromosomal aberrations are reduced in mismatch-repair-deficient human and hamster cells [111,112]. An attractive explanation of this phenomenon is that mismatch correction at sites of O^6meG bases is associated with the generation of recombinogenic DNA intermediates. Taken together, these observations predict that inactivation of the mismatch repair system would result in decreased sensitivity to the killing effects of DNA alkylating agents accompanied by cellular hypermutability, a phenomenon referred to as methylation tolerance.

Several lines of investigation corroborate a relationship between methylation-induced killing and mismatch correction (reviewed in [113]). Selection for resistance to MNNG or *N*-methyl-*N*-nitrosourea has led to the identification of several methylation-tolerant mammalian cell lines which typically have acquired a spontaneous mutator phenotype associated with a deficiency in mismatch repair [104,114–117]. On the other hand,

© 1999 Biochemical Society

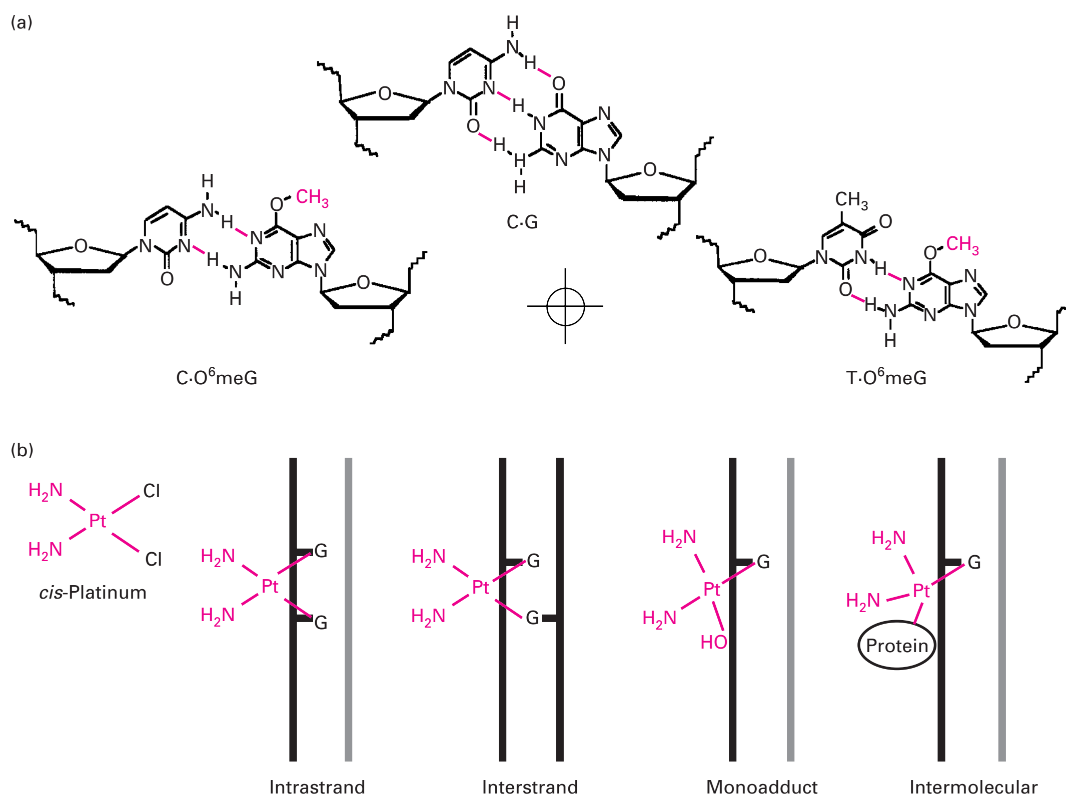

Figure 5 DNA adducts caused by alkylating agents (a) or *cis*-Platinum (b)

(**a**) O^{6}meG is one of the most frequent DNA adducts induced by alkylating agents, and it can pair with thymine or cytosine. (**b**) In the cells, *cis*-Platinum is converted into a charged electrophilic drug that reacts with DNA to form intrastrand and interstrand cross-links, monoadducts and protein–DNA adducts.

tumour cell lines with known defects in mismatch repair activities have been reported to exhibit alkylation tolerance. For instance, HCT116 tumour cells, which are defective in both alleles of *hMLH1*, are hyper-resistant to killing by MNNG and fail to activate a G2 cell-cycle checkpoint after MNNG treatment. Consistent with this, the sensitivity to MNNG killing and the ability to activate DNA-damage-induced checkpoint arrest in HCT116 cells can be restored by expression of a functional *hMLH1* gene in chromosome transfer experiments [118,119]. However, the situation may be more complex, as other DNA repair systems are likely to contribute to the repair of alkylation damage *in vivo*. A glycosylase activity specifically removing thymine in O^{6}meG·T mispairs has been described in human cells [120], and it has been reported that the nucleotide-excision repair (NER) system can recognize distortions produced by O^{6}meG (reviewed in [106]). Another significant factor determining cellular responses to alkylation damage is the timing of adduct formation during the cell cycle. For instance, it is likely that O^{6}meG·C pairs can be efficiently restored by MGMT throughout the entire cell cycle. Likewise, base- and nucleotide-excision repair pathways may also act independently of the cell cycle, whereas the mismatch repair system would process damage mainly during post-replicative phase, when the highest concentration of O^{6}meG·T mispairs is expected to be present in the newly replicated DNA.

A remarkable contribution to the investigation of the above-described phenomena was made by Wei and co-workers [121,122]. In an attempt to study DNA methylation tolerance in yeast, they tested the MNNG sensitivity of *msh2*, *msh3*, *msh5*, *msh6*, *pms1* and *mlh1* null mutants in the background of MGMT deficiency (*mgt1*) and found no significant differences compared with corresponding mismatch-repair-proficient wild-type strains. However, a specific allele of the *MSH5* gene, *msh5-14*, was shown to confer methylation tolerance to cells in a co-dominant manner [121,122]. These data suggest that allele-specific defects, rather than null mutations, in MutS homologues may cause alkylation tolerance through some form of dominant-negative interference with the processing of methylation damage by different DNA repair pathways.

cis-Platinum

This agent is of particular interest as it is in widespread use in chemotherapy, especially of ovarian and testicular tumours [123]. Figure 5(b) shows the chemical structure of *cis*-Platinum and its possible interactions with DNA. The two chloride ligands shown in a *cis* geometry are lost under physiological conditions where the chloride concentration is low, and the drug is converted into a charged electrophilic agent [124,125]. As depicted in Figure 5(b), the converted *cis*-Platinum then reacts with nucleophilic sites in the DNA, resulting in the formation of mainly 1,2-intrastrand cross-links between adjacent purines, i.e. 1,2-d(GpG) or 1,2-d(ApG), but also of 1,3-d(GpNpG) dipurinyl intrastrand cross-links, dipurinyl interstrand cross-links, monoadducts with purines, and DNA–protein cross-links [124,126].

The cytotoxic effect of *cis*-Platinum is thought to be a consequence of the formation of DNA adducts which are poorly repaired and which block DNA replication and/or transcription and, by an as yet unknown mechanism, trigger G2 cell-cycle arrest and eventually apoptosis (reviewed in [127,128]). It is not

© 1999 Biochemical Society

presently clear which of the known *cis*-Platinum DNA adducts is primarily responsible for these effects, but the fact that *cis*-isomer-induced 1,2-d(GpG) cross-links are the most abundant lesions [129,130] which, in addition, are poorly repaired [131], may suggest that these intrastrand adducts contribute significantly to the cytotoxicity of *cis*-Platinum. Consistent with this view is the observation that the related agent *trans*-Platinum which generates adducts in DNA that are repaired more efficiently, has failed in anti-cancer therapy. Consequently, to better understand the chemotherapeutic effect of *cis*-Platinum, biochemical studies on the role of individual DNA repair pathways in the processing of platinum-induced DNA damage have focused on the use of platinum-modified DNA probes carrying intrastrand adducts.

Treatment of tumours with cisplatin leads to cellular resistance, a possible cause of which has been established by the finding that ovarian carcinoma cell lines selected *in vitro* for cisplatin resistance are defective in mismatch repair, with the same phenotypic consequences as described above for cells tolerant to methylating agents [132–134]. The degree of acquired tolerance caused by inactivation of the mismatch repair system appears to be clinically relevant, as $MSH2^{-/-}$ human xenografts were shown to be significantly less responsive to cisplatin treatment than $MSH2^{+/+}$ tumours [135]. These findings correlate with biochemical observations indicating that human mismatch recognition proteins, the purified native heterodimer hMutSα [107] and the overexpressed hMSH2 subunit alone [136], can specifically interact with DNA that contains cisplatin 1,2-d(GpG) cross-links. Interestingly, Mello and co-workers [136] also observed overexpression of *hMSH2* in testicular and ovarian tissue, the tumours of which are most responsive to cisplatin treatment. If indeed hMSH2 levels are likely to reflect the cell's capacity to interact with cisplatin adducts and thus to interfere with their repair, these data may support the idea that cisplatin is killing cells by provoking abortive mismatch repair attempts.

Cisplatin adducts are repaired mainly by the NER system, with efficiencies varying in a lesion-specific manner. In particular, the abundant 1,2-adducts appear to be less efficiently removed by NER than the less frequent 1,3-diguanyl cross-links [130, 137–142]. This may be due to the fact that the latter substrate, which causes bulkier helix distortions, is more efficiently recognized by the NER system. Indeed, Moggs et al. [142] demonstrated that increasing the structural alteration of an intrastrand cross-link by opposing a thymine to one or both of the platinated guanines stimulated NER efficiency. Interestingly, this mismatch-dependent stimulation of NER repair efficiency was also observed with hMutSα-deficient cell extracts, arguing against a direct involvement of the mismatch repair system in the processing of such compound lesions. These observations were corroborated indirectly by another study in which hMutSα was found to have reduced the affinity for a G·T mismatch in the context of a cisplatin di-adduct [143]. This is in apparent contrast to reports regarding the binding of hMutSα to matched platinated DNA [107,136,144] and would suggest that, although mismatch recognition factors may interact with cisplatin 1,2-d(GpG) adducts, they seem to be unimportant for processing of the lesions by NER *in vitro*.

Nevertheless, mismatch correction deficiency is correlated with the cisplatin tolerance of cells, and it appears that the most likely form of interference *in vivo* is replication-associated physical competition between mismatch recognition factors and other lesion-recognizing factors, including proteins containing HMG (high-mobility-group)-box motifs [145–148]. It is not clear, however, how mismatch repair proteins, HMG-box proteins or other cellular factors can modulate NER, but it is possible that they either facilitate lesion processing by attracting repair factors or inhibit correction by shielding the adducts. As has been shown for the rat HMG1 protein [149], the mismatch-binding complex might interact physically with cisplatin di-adducts during the process of DNA replication and thus block translesion synthesis by directly or indirectly stalling the DNA polymerase. As replication bypass at sites of cisplatin–DNA lesions has been described [149–152], one scenario could be that, in proliferating cells, mismatch repair recognition factors enter the scene after a DNA polymerase bypasses a cisplatin adduct and incorporates a mispairing base opposite the lesion. In doing so they would exclude the NER system from becoming active by simple physical competition and thus would prevent repair of the lesion. This could trigger futile repair processes in a way similar to that described above for O^6meG·T, and could eventually result in cell death. One prediction by this model is that inactivation of factors required for the formation of a functional mismatch recognition complex should allow for both more frequent translesion synthesis and more efficient repair of the damage by the NER system, resulting in increased cellular tolerance to cisplatin associated with a spontaneous and cisplatin-induced hypermutability phenotype. This hypothesis is supported by the evidence that human cell lines, especially ovarian cancer cells, acquire resistance to *cis*-Platinum concomitantly with the appearance of spontaneous or selected mismatch repair deficiency [132–134]. Future studies will have to address this question in more detail.

CONCLUSIONS

The immense progress made in the understanding of the mechanism of post-replicative mismatch correction during the past few years has been considerably accelerated by the discovery of a direct link between mismatch repair deficiency and the microsatellite DNA instability found in some sporadic and in most inherited colorectal cancers [4,153–156]. The study of mismatch repair has been a good example of how basic research in appropriate model systems can lead to discoveries relevant to human health. Thanks to the invaluable knowledge gained from DNA repair studies with bacteria and yeast, humans carrying defects in individual components of the mismatch repair system can now be identified in the population, their cancer risk can be assessed and appropriate strategies to increase their quality of life can be developed. A future challenge will be to understand the mismatch repair reaction not only by the function of its individual components but also in terms of the entire network of molecular interactions involved in a proper physiological context. Integrative approaches to studying mismatch correction will be necessary in order to unravel the still existing mysteries about this repair pathway, such as the nature and origin of the strand-discriminating signal in eukaryotic pathways and the regulatory mechanisms that ensure accurate co-ordination between mismatch correction and other interfering DNA metabolic processes. These and other questions should stimulate future research on this exciting topic that will lead us towards understanding the complexity of the physiological role of the recognition and correction of erroneous base pairing.

We thank Josef Jiricny for helpful discussion and for critical reading of the manuscript.

REFERENCES

1 Holliday, R. (1964) Genet. Res. **5**, 282–304
2 Witkin, E. M. (1964) J. Mol. Biol. **8**, 610–613
3 Lahue, R. S., Au, K. G. and Modrich, P. (1989) Science **245**, 160–164

© 1999 Biochemical Society

4 Marra, G. and Boland, C. R. (1995) J. Natl. Cancer Inst. **87**, 1114–1125
5 Petes, T. D., Malone, R. E. and Symington, L. S. (1991) in The Molecular and Cellular Biology of the Yeast *Saccharomyces*: Genome Dynamics, Protein Synthesis and Energetics (Broach, J. R., Jones, E. and Pringle, J., eds.), pp. 407–521, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
6 Schär, P. and Jiricny, J. (1998) in Nucleic Acids and Molecular Biology (Eckstein, F. and Lilley, D. M. J., eds.), pp. 199–247, Springer-Verlag, Berlin, Heidelberg
7 Wiebauer, K., Neddermann, P., Hughes, M. and Jiricny, J. (1993) in DNA Methylation: Molecular and Biological Significance (Jost, J. P. and Saluz, H. P., eds.), pp. 510–522, Birkhäuser Verlag, Basel
8 Rasmussen, L. J., Samson, L. and Marinus, M. G. (1998) in DNA Damage and Repair, vol. 1: DNA Repair in Prokaryotes and Lower Eukaryotes (Nickoloff, J. A. and Hoekstra, M. F., eds.), pp. 205–228, Humana Press, Totowa, NJ
9 Claverys, J. P. and Lacks, S. A. (1986) Microbiol. Rev. **50**, 133–165
10 Guild, W. R. and Shoemaker, N. B. (1976) J. Bacteriol. **125**, 125–135
11 Tiraby, J. G. and Fox, M. S. (1973) Proc. Natl. Acad. Sci. U.S.A. **70**, 3541–3545
12 Marinus, M. G. and Morris, N. R. (1975) Mutat. Res. **28**, 15–26
13 Wagner, R. and Meselson, M. (1976) Proc. Natl. Acad. Sci. U.S.A. **73**, 4135–4139
14 Herman, G. E. and Modrich, P. (1981) J. Bacteriol. **145**, 644–646
15 Pukkila, P. J., Peterson, J., Herman, G., Modrich, P. and Meselson, M. (1983) Genetics **104**, 571–582
16 Lu, A. L., Clark, S. and Modrich, P. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 4639–4643
17 Kramer, B., Kramer, W. and Fritz, H. J. (1984) Cell **38**, 879–887
18 Laengle, R. F., Maenhaut, M. G. and Radman, M. (1986) EMBO J. **5**, 2009–2013
19 Cox, E. C. (1976) Annu. Rev. Genet. **10**, 135–156
20 Rydberg, B. (1978) Mutat. Res. **52**, 11–24
21 Nevers, P. and Spatz, H. (1975) Mol. Gen. Genet. **139**, 233–243
22 Cooper, D. L., Lahue, R. S. and Modrich, P. (1993) J. Biol. Chem. **268**, 11823–11829
23 Su, S. S. and Modrich, P. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 5057–5061
24 Parker, B. O. and Marinus, M. G. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 1730–1734
25 Haber, L. T. and Walker, G. C. (1991) EMBO J. **10**, 2707–2715
26 Haber, L. T., Pang, P. P., Sobell, D. I., Mankovich, J. A. and Walker, G. C. (1988) J. Bacteriol. **170**, 197–202
27 Priebe, S. D., Hadi, S. M., Greenberg, B. and Lacks, S. A. (1988) J. Bacteriol. **170**, 190–196
28 Grilley, M., Welsh, K. M., Su, S. S. and Modrich, P. (1989) J. Biol. Chem. **264**, 1000–1004
29 Modrich, P. (1991) Annu. Rev. Genet. **25**, 229–253
30 Allen, D. J., Makhov, A., Grilley, M., Taylor, J., Thresher, R. and Griffith, J. D. (1997) EMBO J. **16**, 4467–4476
31 Welsh, K. M., Lu, A. L., Clark, S. and Modrich, P. (1987) J. Biol. Chem. **262**, 15624–15629
32 Langle, R. F., Maenhaut, M. G. and Radman, M. (1987) EMBO J. **6**, 1121–1127
33 Au, K. G., Welsh, K. and Modrich, P. (1992) J. Biol. Chem. **267**, 12142–12148
34 Holmes, J. J., Clark, S. and Modrich, P. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 5837–5841
35 Thomas, D. C., Roberts, J. D. and Kunkel, T. A. (1991) J. Biol. Chem. **266**, 3744–3751
36 Fang, W. H. and Modrich, P. (1993) J. Biol. Chem. **268**, 11838–11844
37 Szankasi, P. and Smith, G. R. (1995) Science **267**, 1166–1169
38 Tishkoff, D. X., Boerger, A. L., Bertrand, P., Filosi, N., Gaida, G. M., Kane, M. F. and Kolodner, R. D. (1997) Proc. Natl. Acad. Sci. U.S.A. **94**, 7487–7492
39 Longley, M. J., Pierce, A. J. and Modrich, P. (1997) J. Biol. Chem. **272**, 10917–10921
40 Umar, A., Buermeyer, A. B., Simon, J. A., Thomas, D. C., Clark, A. B., Liskay, R. M. and Kunkel, T. A. (1996) Cell **87**, 65–73
41 Kolodner, R. (1996) Genes Dev. **10**, 1433–1442
42 Modrich, P. (1997) J. Biol. Chem. **272**, 24727–24730
43 Dohet, C., Wagner, R. and Radman, M. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 503–505
44 Jones, M., Wagner, R. and Radman, M. (1987) Cell **50**, 621–626
45 Lacks, S. A., Dunn, J. and Greenberg, B. (1992) Cell **31**, 327–336
46 Bishop, D. K., Andersen, J. and Kolodner, R. D. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 3713–3717
47 Kramer, B., Kramer, W., Williamson, M. S. and Fogel, S. (1989) Mol. Cell. Biol. **9**, 4432–4440
48 Detloff, P., Sieber, J. and Petes, T. D. (1991) Mol. Cell. Biol. **11**, 737–745
49 Schär, P., Munz, P. and Kohli, J. (1993) Genetics **133**, 815–824
50 Umar, A., Boyer, J. C. and Kunkel, T. A. (1994) Science **266**, 814–816
51 Jiricny, J., Su, S. S., Wood, S. G. and Modrich, P. (1988) Nucleic Acids Res. **16**, 7843–7853
52 Miret, J., Milla, M. and Lahue, R. (1993) J. Biol. Chem. **268**, 3507–3513
53 Aboul-Ela, F., Koh, D., Tinoco, I. J. and Martin, F. H. (1985) Nucleic Acids Res. **13**, 4811–4824
54 Werntges, H., Steger, G., Riesner, D. and Fritz, H. J. (1986) Nucleic Acids Res. **14**, 3773–3790
55 Hunter, W. N., Brown, T., Anand, N. N. and Kennard, O. (1986) Nature (London) **320**, 552–555
56 Cornelis, A. G., Haasnoot, J. H., den Hartog, J. F., de Rooij, M., van Boom, J. H. and Cornelis, A. (1979) Nature (London) **281**, 235–236
57 Ho, P. S., Frederick, C. A., Quigley, G. J., van der Marel, G. A., van Boom, J. H., Wang, A. H. and Rich, A. (1985) EMBO J. **4**, 3617–3623
58 Brown, T., Hunter, W. N., Kneale, G. and Kennard, O. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 2402–2406
59 Hare, D., Shapiro, L. and Patel, D. J. (1986) Biochemistry **25**, 7456–7464
60 Echols, H. and Goodman, M. F. (1991) Annu. Rev. Biochem. **60**, 477–511
61 Alani, E., Chi, N. W. and Kolodner, R. (1995) Genes Dev. **9**, 234–247
62 Reenan, R. and Kolodner, R. D. (1992) Genetics **132**, 975–985
63 Strand, M., Earley, M. C., Crouse, G. F. and Petes, T. D. (1995) Proc. Natl. Acad. Sci. U.S.A. **92**, 10418–10421
64 Johnson, R. E., Kovvali, G. K., Prakash, L. and Prakash, S. (1996) J. Biol. Chem. **271**, 7285–7288
65 Marsischky, G. T., Filosi, N., Kane, M. F. and Kolodner, R. (1996) Genes Dev. **10**, 407–420
66 Iaccarino, I., Palombo, F., Drummond, J., Totty, N. F., Hsuan, J. J., Modrich, P. and Jiricny, J. (1996) Curr. Biol. **6**, 484–486
67 Alani, E. (1996) Mol. Cell. Biol. **16**, 5604–5615
68 Alani, E., Sokolsky, T., Studamire, B., Miret, J. J. and Lahue, R. S. (1997) Mol. Cell. Biol. **17**, 2436–2447
69 Habraken, Y., Sung, P., Prakash, L. and Prakash, S. (1996) Curr. Biol. **6**, 1185–1187
70 Habraken, Y., Sung, P., Prakash, L. and Prakash, S. (1997) Curr. Biol. **7**, 790–793
71 Brown, T. C. and Jiricny, J. (1987) Cell **50**, 945–950
72 Brown, T. C. and Jiricny, J. (1988) Cell **54**, 705–711
73 Glazer, P. M., Sarkar, S. N., Chisholm, G. E. and Summers, W. C. (1987) Mol. Cell. Biol. **7**, 218–224
74 Brooks, P., Dohet, C., Almouzni, G., Mechali, M. and Radman, M. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 4425–4429
75 Jiricny, J., Hughes, M., Corman, N. and Rudkin, B. B. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 8860–8864
76 Hughes, M. J. and Jiricny, J. (1992) J. Biol. Chem. **267**, 23876–23882
77 Palombo, F., Hughes, M., Jiricny, J., Truong, O. and Hsuan, J. (1994) Nature (London) **367**, 417
78 Fishel, R., Lescoe, M. K., Rao, M. R., Copeland, N. G., Jenkins, N. A., Garber, J., Kane, M. and Kolodner, R. (1993) Cell **75**, 1027–1038
79 Leach, F. S., Nicolaides, N. C., Papadopoulos, N., Liu, B., Jen, J., Parsons, R., Peltomaki, P., Sistonen, P., Aaltonen, L. A., Nystrom, L. M. et al. (1993) Cell **75**, 1215–1225
80 Palombo, F., Gallinari, P., Iaccarino, I., Lettieri, T., Hughes, M., D'Arrigo, A., Truong, O., Hsuan, J. J. and Jiricny, J. (1995) Science **268**, 1912–1914
81 Drummond, J. T., Li, G. M., Longley, M. J. and Modrich, P. (1995) Science **268**, 1909–1912
82 Fujii, H. and Shimada, T. (1989) J. Biol. Chem. **264**, 10057–10064
83 Liu, K., Niu, L., Linton, J. P. and Crouse, G. F. (1994) Gene **147**, 169–177
84 Wells, J., Held, P., Illenye, S. and Heintz, N. H. (1996) Mol. Cell. Biol. **16**, 634–647
85 Perucho, M. (1996) Biol. Chem. Hoppe-Seyler **377**, 675–684
86 Fishel, R., Ewel, A. and Lescoe, M. K. (1994) Cancer Res. **54**, 5539–5542
87 Fishel, R., Ewel, A., Lee, S., Lescoe, M. K. and Griffith, J. (1994) Science **266**, 1403–1405
88 Acharya, S., Wilson, T., Gradia, S., Kane, M. F., Guerrette, S., Marsischki, G. T., Kolodner, R. and Fishel, R. (1996) Proc. Natl. Acad. Sci. U.S.A. **93**, 13629–13634
89 Palombo, F., Iaccarino, I., Nakajima, E., Ikejima, M., Shimada, T. and Jiricny, J. (1996) Curr. Biol. **6**, 1181–1184
90 Gradia, S., Acharya, S. and Fishel, R. (1997) Cell **91**, 995–1005
91 Drummond, J. T., Genschel, J., Wolf, E. and Modrich, P. (1997) Proc. Natl. Acad. Sci. U.S.A. **94**, 10144–10149
92 Marra, G., Iaccarino, I., Lettieri, T., Roscilli, G., Delmastro, P. and Jiricny, J. (1998) Proc. Natl. Acad. Sci. U.S.A. **95**, 8568–8573
93 Iaccarino, I., Marra, G., Palombo, F. and Jiricny, J. (1998) EMBO J. **17**, 2677–2686
94 Gu, L., Hong, Y., McCulloch, S., Watanabe, H. and Li, G. M. (1998) Nucleic Acids Res. **26**, 1173–1178
95 Radicella, J. P., Clark, E. A. and Fox, M. S. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 9674–9678
96 Jones, M., Wagner, R. and Radman, M. (1987) J. Mol. Biol. **194**, 155–159
97 Wiebauer, K. and Jiricny, J. (1989) Nature (London) **339**, 234–236

© 1999 Biochemical Society

98 Yeh, Y. C., Chang, D. Y., Masin, J. and Lu, A. L. (1991) J. Biol. Chem. **266**, 6480–6484
99 Stephenson, C. and Karran, P. (1989) J. Biol. Chem. **264**, 21177–21182
100 O'Regan, N. E., Branch, P., Macpherson, P. and Karran, P. (1996) J. Biol. Chem. **271**, 1789–1796
101 Schär, P. and Kohli, J. (1993) Genetics **133**, 825–835
102 Schär, P., Baur, M., Schneider, C. and Kohli, J. (1997) Genetics **146**, 1275–1286
103 Miret, J. J., Parker, B. O. and Lahue, R. S. (1996) Nucleic Acids Res. **24**, 721–729
104 Goldmacher, V. S., Cuzick, R. A. and Thilly, W. G. (1986) J. Biol. Chem. **261**, 12462–12471
105 Pegg, A. E. (1990) in Chemical Carcinogenesis and Mutagenesis II (Cooper, C. S. and Grover, P. L., eds.), pp. 104–131, Springer Verlag, Berlin
106 Basu, A. K. and Essigmann, J. M. (1990) Mutat. Res. **233**, 189–201
107 Duckett, D. R., Drummond, J. T., Murchie, A. I., Reardon, J. T., Sancar, A., Lilley, D. M. and Modrich, P. (1996) Proc. Natl. Acad. Sci. U.S.A. **93**, 6443–6447
108 Karran, P. and Marinus, M. G. (1982) Nature (London) **296**, 868–869
109 Karran, P. and Bignami, M. (1992) Nucleic Acids Res. **20**, 2933–2940
110 Rasouli, N. A., Sibghat, U., Mirzayans, R., Paterson, M. C. and Day, III, R. S. (1994) Mutat. Res. **314**, 99–113
111 Galloway, S. M., Greenwood, S. K., Hill, R. B., Bradt, C. I. and Bean, C. L. (1995) Mutat. Res. **346**, 231–245
112 Armstrong, M. J. and Galloway, S. M. (1997) Mutat. Res. **373**, 167–178
113 Karran, P. and Bignami, M. (1996) Chem. Biol. **3**, 875–879
114 Branch, P., Aquilina, G., Bignami, M. and Karran, P. (1993) Nature (London) **362**, 652–654
115 Kat, A., Thilly, W. G., Fang, W. H., Longley, M. J., Li, G. M. and Modrich, P. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 6424–6428
116 Branch, P., Hampson, R. and Karran, P. (1995) Cancer Res. **55**, 2304–2309
117 Papadopoulos, N., Nicolaides, N. C., Liu, B., Parsons, R., Lengauer, C., Palombo, F., D'Arrigo, A., Markowitz, S., Willson, J. K. V., Kinzler, K. W., Jiricny, J. and Vogelstein, B. (1995) Science **268**, 1915–1917
118 Koi, M., Umar, A., Chauhan, D. P., Cherian, S. P., Carethers, J. M., Kunkel, T. A. and Boland, C. R. (1994) Cancer Res. **54**, 4308–4312
119 Hawn, M. T., Umar, A., Carethers, J. M., Marra, G., Kunkel, T. A., Boland, C. R. and Koi, M. (1995) Cancer Res. **55**, 3721–3725
120 Sibghat, U. and Day, III, R. S. (1992) Biochemistry **31**, 7998–8008
121 Xiao, W., Rathgeber, L., Fontanie, T. and Bawa, S. (1995) Carcinogenesis **16**, 1933–1939
122 Bawa, S. and Xiao, W. (1997) Cancer Res. **57**, 2715–2720
123 Fram, R. J. (1992) Curr. Opin. Oncol. **4**, 1073–1079
124 Eastman, A. (1987) Pharmacol. Therap. **34**, 155–166
125 Chu, G. (1994) J. Biol. Chem. **269**, 787–790
126 Pinto, A. L. and Lippard, S. J. (1985) Biochim. Biophys. Acta **780**, 167–180
127 Zamble, D. B. and Lippard, S. J. (1995) Trends Biochem. Sci. **20**, 435–439
128 Barry, M. A., Behnke, C. A. and Eastman, A. (1990) Biochem. Pharmacol. **40**, 2353–2362
129 Eastman, A. (1983) Biochemistry **22**, 3927–3933
130 Szymkowski, D. E., Yarema, K., Essigmann, J. M., Lippard, S. J. and Wood, R. D. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 10772–10776
131 Ciccarelli, R. B., Solomon, M. J., Varshavsky, A. and Lippard, S. J. (1985) Biochemistry **24**, 7533–7540
132 Aebi, S., Kurdi, H. B., Gordon, R., Cenni, B., Zheng, H., Fink, D., Christen, R. D., Boland, C. R., Koi, M., Fishel, R. and Howell, S. B. (1996) Cancer Res. **56**, 3087–3090
133 Anthoney, D. A., McIlwrath, A. J., Gallagher, W. M., Edlin, A. R. and Brown, R. (1996) Cancer Res. **56**, 1374–1381
134 Drummond, J. T., Anthoney, A., Brown, R. and Modrich, P. (1996) J. Biol. Chem. **271**, 19645–19648
135 Fink, D., Zheng, H., Nebel, S., Norris, P. S., Aebi, S., Lin, T. P., Nehme, A., Christen, R. D., Haas, M., MacLeod, C. L. and Howell, S. B. (1997) Cancer Res. **57**, 1841–1845
136 Mello, J. A., Acharya, S., Fishel, R. and Essigmann, J. M. (1996) Curr. Biol. **3**, 579–589
137 Bedford, P., Fichtinger-Schepman, A. M. J., Shellard, S. A., Walker, M. C., Masters, J. R. and Hill, B. T. (1988) Cancer Res. **48**, 3019–3024
138 Eastman, A. and Schulte, N. (1988) Biochemistry **27**, 4730–4734
139 Moggs, J. G., Yarema, K. J., Essigmann, J. M. and Wood, R. D. (1996) J. Biol. Chem. **271**, 7177–7186
140 Zamble, D. B., Mu, D., Reardon, J. T., Sancar, A. and Lippard, S. J. (1996) Biochemistry **35**, 10004–10013
141 Calsou, P., Frit, P. and Salles, B. (1992) Nucleic Acids Res. **20**, 6363–6368
142 Moggs, J. G., Szymkowski, D. E., Yamada, M., Karran, P. and Wood, R. D. (1997) Nucleic Acids Res. **25**, 480–490
143 Mu, D., Tursun, M., Duckett, D. R., Drummond, J. T., Modrich, P. and Sancar, A. (1997) Mol. Cell. Biol. **17**, 760–769
144 Yamada, M., O'Reagan, E., Brown, R. and Karran, P. (1997) Nucleic Acids Res. **25**, 491–495
145 Bruhn, S. L., Pil, P. M., Essigmann, J. M., Housman, D. E. and Lippard, S. J. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 2307–2311
146 Pil, P. M. and Lippard, S. J. (1992) Science **256**, 234–237
147 Hughes, E. N., Engelsberg, B. N. and Billings, P. C. (1992) J. Biol. Chem. **267**, 13520–13527
148 Cryer, J. E., Johnson, S. W., Engelsberg, B. N. and Billings, P. C. (1996) Cancer Chemother. Pharmacol. **38**, 163–168
149 Hoffmann, J. S., Locker, D., Villani, G. and Leng, M. (1997) J. Mol. Biol. **270**, 539–543
150 Heiger, B. W., Essigmann, J. M. and Lippard, S. J. (1990) Biochemistry **29**, 8461–8466
151 Mamenta, E. L., Poma, E. E., Kaufmann, W. K., Delmastro, D. A., Grady, H. L. and Chaney, S. G. (1994) Cancer Res. **54**, 3500–3505
152 Hoffmann, J. S., Pillaire, M. J., Maga, G., Podust, V., Hubscher, U. and Villani, G. (1995) Proc. Natl. Acad. Sci. U.S.A. **92**, 5356–5360
153 Jiricny, J. (1996) Cancer Surv. **28**, 47–68
154 Ionov, Y., Peinado, M. A., Malkhosyan, S., Shibata, D. and Perucho, M. (1993) Nature (London) **363**, 558–561
155 Aaltonen, L. A., Peltomaki, P., Leach, F. S., Sistonen, P., Pylkkanen, L., Mecklin, J. P., Jarvinen, H., Powell, S. M., Jen, J., Hamilton, S. R. et al. (1993) Science **260**, 812–816
156 Thibodeau, S. N., Bren, G. and Scheid, D. (1993) Science **260**, 816–819

© 1999 Biochemical Society