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Mismatch Repair, Genetic Stability and Tumour Avoidance

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Mismatch repair, genetic stability and tumour avoidance

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SUMMARY

Escherischia coli methyl-directed mismatch repair eliminates premutagenic lesions that arise via DNA biosynthetic errors; components of the repair system also block ectopic recombination between diverged DNA sequences. A mismatch-dependent, methyl-directed excision reaction that accounts for function of the system in replication fidelity has been reconstituted in a purified system dependent on ten activities. The reaction displays a broad specificity for mismatched base pairs and is characterized by an unusual bidirectional excision capability. Human cell nuclear extracts support strand-specific mismatch correction in a reaction that is similar to bacterial repair, with respect to both mismatch specificity and unusual features of mechanism. Like the bacterial system, the human pathway also functions in mutation avoidance because several classes of mutator human cells are deficient in the reaction. These include an alkylation-tolerance cell line that is resistant to the cytotoxic action of *N*-methyl-*N'*-nitro-nitrosoguanidine, as well as hypermutable RER^+ tumour cells such as those associated with hereditary non-polyposis colon cancer. *In vitro* experiments indicate that the human repair reaction is dependent on at least six activities, excluding DNA ligase, and that distinct defects in the system can lead to the RER^+ phenotype.

1. INTRODUCTION

Current appreciation of the mechanisms of mismatch repair and the significance of this process in mutation avoidance has resulted largely from work in bacterial systems (Claverys & Lacks 1986; Meselson 1988; Modrich 1991). The genetic stabilization provided by the prototypic *Escherichia coli* methyl-directed mismatch repair pathway has been attributed to its roles in ensuring the fidelity of both chromosome replication and homologous genetic recombination (Meselson 1988; Radman 1988; Modrich 1991). The discussion that follows will highlight the molecular mechanisms that underly the fidelity functions of the *E. coli* pathway and will summarize evidence indicating that human cells possess a functional homologue of the bacterial system. As in the case of the methyl-directed pathway, genetic inactivation of the human system confers a large increase in spontaneous mutability, as well as resistance to the cytotoxic action of simple DNA alkylators. Moreover, defects in the human system are also associated with a strong predisposition to tumour development in certain tissues.

2. MECHANISM OF METHYL-DIRECTED MISMATCH EXCISION REPAIR

Because mismatches are comprised of normal Watson–Crick bases, postreplication correction of DNA biosynthetic errors relies on secondary signals within the helix that are used to identify the newly synthesized strand. As anticipated by Wagner & Meselson (1976), the requisite strand-specificity for mismatch repair in *E. coli* is based on patterns of adenine methylation at d(GATC) sites (Meselson 1988; Modrich 1991). Owing to the postsynthetic nature of this modification, GATC sequences in newly synthesized DNA are unmethylated and it is this transient existence in unmodified form that permits the repair system to identify the daughter DNA strand.

A mismatch-provoked, methyl-directed excision reaction, which can account for function of mismatch repair in *E. coli* replication fidelity, has been reconstituted in a purified system that depends on ten activities (Lahue *et al.* 1989; Cooper *et al.* 1993). Availability of this system has led to identification of key intermediates in the reaction. As illustrated in figure 1, repair is initiated by the mismatch-provoked incision of the unmodified strand at a hemimethylated GATC site. This reaction, which depends on the products of the *mutH*, *mutL*, and *mutS* mutator genes as well as ATP hydrolysis, displays an exquisite sensitivity for mismatched base pairs (Au *et al.* 1992; Nelson *et al.* 1993). Analysis of interaction of the three Mut proteins with heteroduplex DNA has indicated that initiation involves binding of MutS to a mismatch (Su & Modrich 1986; Su *et al.* 1988) followed by ATP-dependent addition of MutL (Grilley *et al.* 1989). Assembly of this complex leads to activation of a latent, MutH-associated GATC endonuclease in a reaction that depends on ATP hydrolysis by MutS (Au *et al.* 1992).

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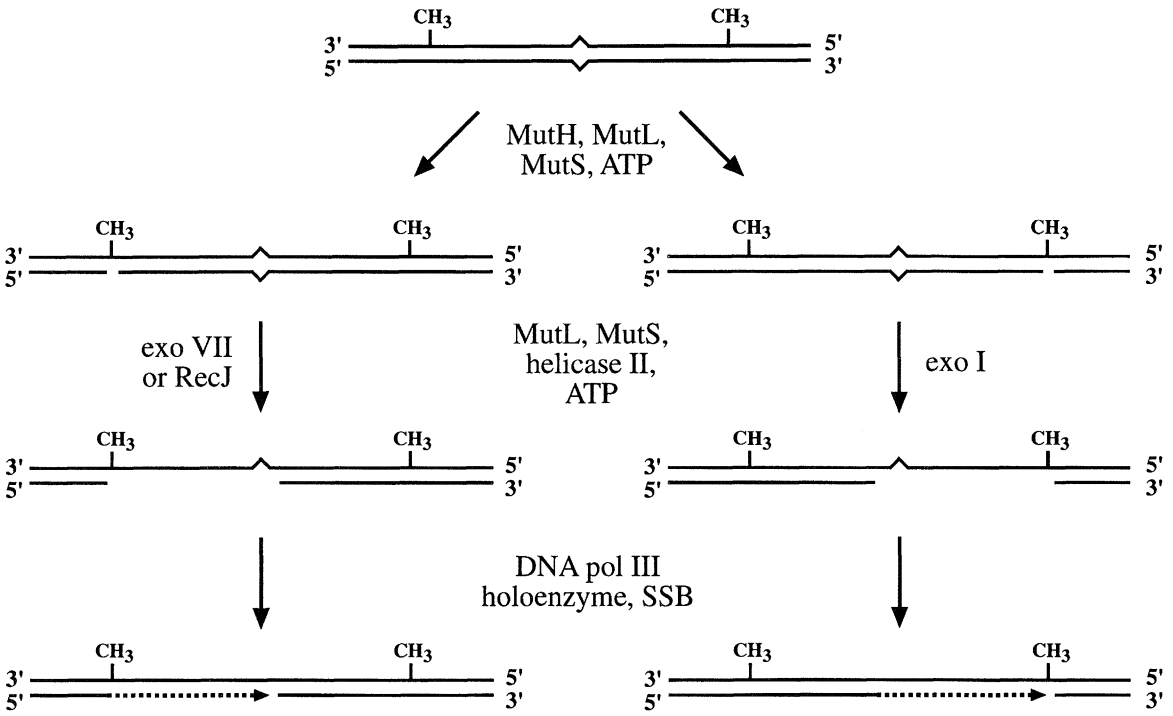

Figure 1. A mismatch-provoked, methyl-directed excision reaction. Repair is initiated by the mismatch-provoked incision of the unmodified strand at a hemimethylated GATC site. Reproduced with permission from Grilley *et al.* (1993).

Interaction of the mismatch and the GATC sequence that determines strand-specificity is thought to involve formation of a DNA loop (Grilley 1992).

Inasmuch as a pre-existing strand-break bypasses the requirement for both MutH and a hemimethylated GATC site (Längle-Rouault *et al.* 1987; Lahue *et al.* 1989), it is clear that the function of these two elements in methyl-directed repair is provision of a single-strand break which serves as the primary signal for targeting correction to the unmodified DNA strand. Surprisingly, GATC incision by MutH during the initiation of repair can occur either 3′ or 5′ to the mismatch on the unmodified strand (Au *et al.* 1992), an observation that reflects the novel bidirectional capability of the methyl-directed system (see figure 1). The excision stage of repair depends on MutS, MutL, DNA helicase II (the *mutU* gene product) and one of several exonucleases (Lahue *et al.* 1989; Cooper *et al.* 1993). This step is strictly exonucleolytic in nature, with hydrolysis initiating at the incised GATC site and proceeding toward the mismatch to terminate a set of discrete sites within a 100 nucleotide region beyond the mispair (Grilley *et al.* 1993). When the incised GATC sequence resides 3′ to the mismatch, excision requires exonuclease I, a 3′→5′ hydrolytic activity, while excision from a 5′ GATC site depends on the 5′→3′ hydrolytic activity of RecJ or exonuclease VII (Cooper *et al.* 1993). Each of these three exonucleases is specific for single-stranded DNA (Lehman & Nussbaum 1964; Chase & Richardson 1974; Lovett & Kolodner 1989). Excision from either side of the mismatch depends on the cooperative action of DNA helicase II and an appropriate hydrolytic activity (Grilley *et al.* 1993). It has, therefore, been inferred that helicase II displacement renders the incised strand sensitive to the appropriate single-strand exonuclease. The gap produced by this complex excision reaction is repaired by DNA polymerase III holoenzyme in the presence of single-strand binding protein SSB, with DNA ligase restoring covalent integrity to the helix (Lahue *et al.* 1989).

3. MutS AND MutL MODULATE RECOMBINATION BETWEEN DIVERGED DNAs

In addition to their fidelity role in chromosome replication, components of the methyl-directed system also act to reduce the yield of recombinants when crossovers are selected in regions of imperfect homology. Analysis of recombination in *E. coli* and *Salmonella typhimurium* has demonstrated that the frequency of crossovers is reduced by one to several orders of magnitude when homology in the region of interest differs by 1% to 20% at the nucleotide level (Rayssiguier *et al.* 1989; Shen & Huang 1989; Petit *et al.* 1991). This effect is clearly dependent on MutS and MutL proteins because recombinant yield in crosses involving such quasi-homologous (i.e. homeologous) sequences is increased dramatically in *mutS* and *mutL* mutants (Rayssiguier *et al.* 1989; Petit *et al.* 1991). Radman and colleagues (Rayssiguier *et al.* 1989) proposed that the antirecombination activity of MutS and MutL is indicative of their function as a barrier to illegitimate exchanges between divergent sequences. This idea was confirmed by the demonstration that *mutS* and *mutL* products control the frequency of *E. coli* chromosomal rearrangements that result from recombination between divergent *rhs* repeats (Petit *et al.* 1991).

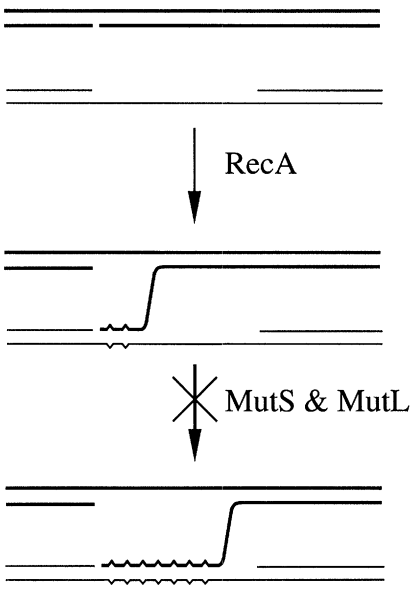

Figure 2. Analysis of homeologous strand-transfer intermediates that accumulate in the presence of MutS and MutL demonstrated that the Mut proteins block the branch migration step of the reaction. This is, presumably, in response to occurrence of mismatches within the recombination heteroduplex.

In contrast to the large increase in homeologous exchange observed in *mutS* and *mutL* mutants, *mutH* and helicase II mutations have little effect on recombinant yield in crosses involving sequences that are divergent by 1% or more (Rayssiguier *et al.* 1989; Petit *et al.* 1991). This suggests that the antirecombination activity of MutS and MutL may involve features of mechanism that are either distinct from, or in addition to, that of the mismatch-provoked excision reaction outlined above. Whereas the molecular basis of this phenomenon remains unclear, a dramatic effect of MutS and MutL on RecA-catalysed strand transfer between divergent sequences has been demonstrated *in vitro*. Worth *et al.* (1994) have demonstrated that although MutS and MutL are without effect on RecA-mediated M13-M13 or fd-fd strand transfer, these proteins abolish heteroduplex formation between M13 and fd DNAs – which are 3% divergent at the nucleotide level. Inhibition of strand transfer between these homeologous DNAs can be observed in the presence of MutS alone but this effect is dramatically potentiated by MutL, an observation consistent with the known order of addition of the two proteins to a mispair (Grilley *et al.* 1989). Analysis of homeologous strand-transfer intermediates that accumulate in the presence of MutS and MutL demonstrated that the Mut proteins block the branch migration step of the reaction, presumably in response to occurrence of mismatches within the recombination heteroduplex (see figure 2). Although MutS and MutL block branch migration through regions of diverged sequence, the proteins do not destabilize previously formed homeologous heteroduplex (Worth *et al.* 1994). Thus it is postulated that branch migration intermediates trapped by the proteins are subject to disassembly by an, as yet, unidentified mechanism.

4. STRAND-SPECIFIC MISMATCH REPAIR IN HUMAN CELLS

Study of strand-specific mismatch repair in eukaryotic organisms has been hampered by the lack of definitive information on the nature of strand signals that might operate in these organisms. For this reason we (Holmes *et al.* 1990), and Kunkel and colleagues (Thomas *et al.* 1991) have used heteroduplexes containing a site-specific, strand-specific incision to explore the potential existence of such reactions in higher cells. The rationale for use of such substates was based on previous observations in bacterial systems. As mentioned above, a preexisting strand break bypasses the requirements for MutH and a hemimethylated GATC site in the *E. coli* methyl-directed reaction (Längle-Rouault *et al.* 1987; Lahue *et al.* 1989); it has been postulated that DNA termini are the natural signal governing mismatch repair in *Streptococcus pneumoniae* (Claverys & Lacks 1986). Use of such substrates has led to identification of mismatch-provoked, nick-directed excision repair systems in extracts derived from both *Drosophila melanogaster* (Holmes *et al.* 1990) and human (Holmes *et al.* 1990; Thomas *et al.* 1991) cells.

Analysis of the human nick-directed reaction has revealed striking similarities to the bacterial methyl-directed pathway with respect to both specificity and mechanism. Both systems are characterized by a similar broad specificity for base–base (Holmes *et al.* 1990; Modrich 1991; Thomas *et al.* 1991; Fang *et al.* 1993; Fang & Modrich 1993) and insertion–deletion mispairs (Thomas *et al.* 1991; Parker & Marinus 1992; Parsons *et al.* 1993). Furthermore, repair efficiencies for different mispairs are similar in the two systems with C–C being the weakest substrate in both cases (Holmes *et al.* 1990; Modrich 1991; Thomas *et al.* 1991). In addition to differential rates of correction of different heteroduplexes, which is indicative of mismatch recognition, nick-directed repair events observed in human cell-free extracts are mismatch-provoked as judged by two additional criteria. Repair, which is inhibited by aphidicolin, is accompanied by mismatch-dependent, aphidicolin-sensitive DNA synthesis that is localized to the region between the mispair and the nick that directs the reaction (Holmes *et al.* 1990; Thomas *et al.* 1991). Furthermore, inhibition of extract DNA synthesis by aphidicolin or by omission of exogenous dNTPs leads to the mismatch-dependent formation of single-strand gaps that span the mispair and the single-strand break that directs the reaction (Fang *et al.* 1993; Fang & Modrich 1993). With circular heteroduplexes, in which the nick is asymmetrically disposed relative to the mismatch, these excision tracts are localized to the shorter path joining the two sites – irrespective of whether the strand break is located 3′ or 5′ to the mispair as viewed along this path (see figure 3). With either kind of heteroduplex, single strand gaps were found to extend from the site of the strand break to terminate at a number of discrete sites in the region 90 to 170 nucleotides beyond the location of the mismatch. This is reminiscent of excision tracts observed in the bacterial pathway (Grilley *et al.* 1993) and it, therefore, appears the human nick-

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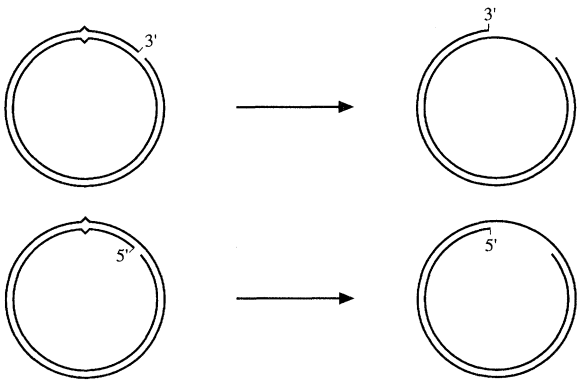

Figure 3. Circular heteroduplexes. The nick is asymmetrically disposed relative to the mismatch; these excision tracts are localized to the shorter path joining the two sites irrespective of whether the strand break is located 3′ or 5′ to the mispair, as viewed along this path.

directed reaction displays a bidirectional excision capability similar to that documented for *E. coli* methyl-directed repair.

This high degree of conservation with respect to specificity and mechanism, led to the suggestion that the human strand-specific pathway is a functional homologue of the bacterial methyl-directed system (Holmes *et al.* 1990; Thomas *et al.* 1991; Fang & Modrich 1993). This idea has been confirmed dramatically during the past year, with the demonstration that strand-specific mismatch repair plays a critical role in maintenance of genetic stability in human cells.

5. A METHYLATION-TOLERANT CELL LINE IS DEFECTIVE IN MISMATCH REPAIR

The initial demonstration of a human mismatch-repair deficiency was in the MT1 cell line. This cell line was derived from TK6 lymphoblastoid cells after frameshift mutagenesis and selection for mutants resistant to the cytotoxic effects of *N*-methyl-*N*′-nitro-nitrosoguanidine (MNNG) (Goldmacher *et al.* 1986). Although several hundred times more resistant to killing by MNNG than the TK6 parental line, MT1 cells do not display an elevated capacity for repair of alkylated DNA. Rather, alkylated bases simply persist in chromosomes of MT1 cells and, in fact, this line is somewhat more sensitive to mutagenesis by MNNG than the parental line is (Goldmacher *et al.* 1986). Therefore, the MT1 line tolerates DNA adducts that lead to death in wild-type cells.

In addition to this alkylation-tolerant phenotype, MT1 cells are also hypermutable in the *absence* of alkylating agent (Goldmacher *et al.* 1986). The rate of generation of spontaneous *HPRT* mutations is elevated about 60-fold, with exon mutations including transitions, transversions and single nucleotide insertions (Kat *et al.* 1993). MT1 and TK6 cells are also distinguished by poor clone-forming ability at low cell densities, in the case of the former line, and by differences in cell-cycle progression phenotype after exposure to MNNG doses that lead to 90% killing (Goldmacher *et al.* 1986). Because the mutagenesis procedure used to isolate MT1 yields a very small *HPRT* mutant fraction, the several phenotypes of MT1 cells have been attributed to a single genetic alteration (Goldmacher *et al.* 1986).

Our interest in the MT1 line was prompted by its hypermutable phenotype and by the observation of Karran and Marinus (1982) that *E. coli* mismatch repair mutants are more resistant than wild-type cells to killing by MNNG. Indeed, these observations led Thilly and coworkers (Goldmacher *et al.* 1986) and Karran & Bignami (1992) to propose that tolerance of mammalian cells to the cytotoxic action of DNA methylating agents might be associated with a mismatch-repair deficiency. This proposal was confirmed with the demonstration that MT1 cells are defective in strand-specific mismatch correction (Kat *et al.* 1993). As judged by *in vitro* assay, the mutator line is defective in repair of each of the eight base–base mismatches, with repair blocked prior to the excision stage of the reaction. In addition to demonstrating that mismatch repair contributes to genetic stability of human cells, these findings also imply that the human repair system recognizes mispairs involving certain kinds of damaged bases. In particular, they indicate that recognition and processing of such lesions by the mismatch-repair system is involved in targeting for death those cells that have suffered unacceptable levels of alkylation damage. Although the affected gene responsible for the MT1 phenotype has not been identified, the nature of the MT1 mutation provides further support for the contention that the human strand-specific mismatch-repair pathway is a functional homologue of the bacterial methyl-directed system.

In experiments based on a similar premise, Karran and colleagues (Branch *et al.* 1993) have found that extracts of alkylation-resistant variants of CHO and RajiF12 Burkitt's lymphoma cells are deficient in a G–T binding activity. However, in contrast to MT1, these variant cell lines are only slightly hypermutable. This difference may, at least, partly reflect the distinct methods used to isolate the two classes of alkylation-resistant cell lines. While MT1 was isolated after frameshift mutagenesis and single-step selection for high level MNNG resistance (Goldmacher *et al.* 1986), the CHO and RajiF12 variants were isolated after multistep selection for cells able to survive exposure to continuously increasing doses of alkylating agent (Aquilina *et al.* 1988; Branch *et al.* 1993). It is evident that such differing selection protocols may yield distinct genetic outcomes. It is also evident that rationalization of results obtained with the two kinds of alkylation-resistant cell lines must await identification of the genetic defect in MT1 cells and clarification of the DNA metabolic role of the G–T binding protein that is defective in the CHO and RajiF12 variant lines.

6. MISMATCH REPAIR DEFICIENCY AND CANCER PREDISPOSITION

Nowell (1976) suggested a model for cancer development based on genetic destabilization (giving rise to mitotic errors and simpler mutations), progressive genetic change and clonal selection. The demon-

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stration that progressive genetic alterations can be associated with development of several human cancers (Weinberg 1989; Fearon & Vogelstein 1990; Stanbridge 1990) confirmed a key element of this proposal but the origins of the mutations involved in tumourigenesis have been uncertain. For example, Loeb (1991) has argued that although a one or two mutation mechanism for tumour development can be reconciled with the mutation rate of a healthy human cell (estimated to be approximately 10^{-10} per nucleotide per generation), it is difficult to rationalize cancer incidence with normal spontaneous mutability if three or more mutations are necessary for disease development. Although such arguments neglect the impact of exposure to exogenous mutagens, the finding that cancer cells can harbour a substantial number of mutations (Callahan & Campbell 1989; Fearon & Vogelstein 1990) has resulted in reiteration of the argument that acquisition of a genetically unstable phenotype may play a role in tumourigenesis (Harwood *et al.* 1991; Loeb 1991).

Dramatic evidence indicative of a role for hypermutability in development of certain cancers has appeared during the past year with the demonstration that certain sporadic tumours and most tumours developing in patients afflicted with hereditary nonpolyposis colorectal cancer (HNPCC) harbour frequent mutations within $(CA)_n$ and other simple microsatellite repeat sequences (Aaltonen *et al.* 1993; Han *et al.* 1993; Ionov *et al.* 1993; Peltomäki *et al.* 1993*b*; Risinger *et al.* 1993; Thibodeau *et al.* 1993; Merlo *et al.* 1994; Mironov *et al.* 1994; Shridhar *et al.* 1994). HNPCC is one of the most common familial cancer syndromes, and in addition to colorectal carcinoma, a subset of affected individuals is also predisposed to endometrial, hematologic, gastric, pancreatic, ovarian, skin, and urinary tract cancer (Lynch *et al.* 1993). With the apparent exception of some small cell and non-small cell lung cancers (Merlo *et al.* 1994; Shridhar *et al.* 1994), the tissue distribution of sporadic cancers in which microsatellite variability has been observed is similar to that observed in HNPCC patients (Han *et al.* 1993; Peltomäki *et al.* 1993*b*).

$(CA)_n$ and other simple repeat sequences are prone to slipped-strand mispairing and are thought to be particularly susceptible to insertion–deletion mutagenesis during replication (Levinson & Gutman 1987*b*; Kunkel 1993). Thus tumours displaying microsatellite mutations have been designated RER^+ (as opposed to RER^-) based on the supposition that observed sequence variations result from Replication ERrors (Aaltonen *et al.* 1993; Ionov *et al.* 1993). That hypermutability does indeed contribute to incidence of $(CA)_n$ mutations in RER^+ tumour cells has been confirmed by clonal analysis of $(CA)_n$ repeats in colorectal tumour cells (Parsons *et al.* 1993). In contrast to stability of the microsatellite sequence in RER^- colorectal tumour cells, cell lines derived from two independent RER^+ tumours displayed a 100-fold elevation in mutability of $(CA)_n$ repeats (Parsons *et al.* 1993).

Because mismatch-repair mutations dramatically destabilize $(CA)_n$ repeats in both *E. coli* and *S. cerevisiae* (Levinson & Gutman 1987*a*; Strand *et al.* 1993), we have begun to screen extracts derived from RER^- and RER^+ tumour cell lines for mismatch-repair defects. Although normal mismatch-correction activity was observed with four RER^- colorectal tumour lines, six independent RER^+ colorectal lines were found to be defective in nick-directed correction of base–base, as well as insertion–deletion mispairs of the 2-, 3-, or 4-nucleotide slipped-strand class (Parsons *et al.* 1993; M. Longley, G.-M. Li, S. Markowitz, B. Vogelstein and P. Modrich, unpublished results). Similar results have been obtained by Kunkel and colleagues with four colorectal and two endometrial RER^+ tumour lines (Umar *et al.* 1994). These repair-defective lines fall into several classes based on *in vitro* complementation data (Parsons *et al.* 1993; Umar *et al.* 1994). Furthermore, although the observed defect in repair of slipped-strand mismatches accounts for microsatellite instability in these RER^+ cell lines, their inability to correct base–base mismatches led to the suggestion that they would also prove to be unstable with respect to base substitution mutagenesis (Parsons *et al.* 1993). The finding that *HPRT* mutability is increased 100-fold in several mismatch repair-deficient RER^+ lines supports this view (J. R. Eshleman, E. Z. Lang, G. K. Bowerfind, R. Parsons, B. Vogelstein, J. K. V. Wilson, M. L. Veigl, W. D. Sedwick and S. D. Markowitz, unpublished results).

HNPCC is inherited in an autosomal dominant fashion and is genetically heterogeneous. The responsible loci map to chromosomes 2p16, 3p21 and elsewhere in the human genome (Leach *et al.* 1993; Lindblom *et al.* 1993; Peltomäki *et al.* 1993*a*). The affected locus in 2p16 kindreds encodes a homologue of bacterial MutS that has been designated hMSH2 (Fishel *et al.* 1993; Leach *et al.* 1993), whereas that at 3p21 specifies the MutL homologue hMLH1 (Bronner *et al.* 1994; Papadopoulos *et al.* 1994). Affected individuals examined to date are heterozygous for germline *hMSH2* (Leach *et al.* 1993) or *hMLH1* mutations (Bronner *et al.* 1994; Papadopoulos *et al.* 1994). In the single instance in which an HNPCC colorectal cancer has been examined in detail, tumour cells retained the germline mutation and acquired a distinct somatic mutation in the other *hMSH2* copy (Leach *et al.* 1993). A similar situation has been observed with RER^+H6 cells that were derived from a sporadic colorectal tumour. This mismatch-repair-deficient line (Parsons *et al.* 1993) harbours defects in both *hMLH1* genes (Papadopoulos *et al.* 1994). Since somatic cells from an HNPCC patient have been found to be proficient in mismatch repair (Parsons *et al.* 1993), these genetic and biochemical findings strongly suggest that the initial event in development of most HNPCC and other RER^+ tumours is genetic destabilization due to loss of function of critical mismatch-repair activities.

7. FRACTIONATION OF THE HUMAN MISMATCH REPAIR SYSTEM

Using traditional biochemical methods and complementation of repair defective extracts derived from alkylation-tolerant and RER^+ tumour cells, we have

identified six activities required for nick-directed mismatch repair in human cells. This set excludes the DNA ligase that we presume to be involved in the last stage of the reaction. One of the six activities, which complements the *hMLH1* mutant H6 line, has been obtained in homogeneous form (Guo-Min Li & Paul Modrich, unpublished results). The characterization of this activity will be described elsewhere, but it is interesting to note that this result implies involvement of the *hMLH1* MutL homologue in mismatch repair. Similarly, Hughes & Jiricny (1992) have isolated several mismatch-binding activities from human cells and peptide analysis of one of these indicates it to be the *hMSH2* product. The evolutionary conservation of general features of specificity and mechanism of mismatch repair outlined above therefore appears to reflect participation of related activities. Further evaluation of the degree to which details of mechanism have been preserved must await isolation and characterization of the other components of the system.

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