

REVIEW

Biochemistry and genetics of eukaryotic mismatch repair

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The process of mismatch repair was first postulated to explain the results of experiments on genetic recombination and bacterial mutagenesis. Mismatch repair has long been known to play a major role in two cellular processes: (1) the repair of errors made during DNA replication or as the result of some types of chemical damage to DNA and DNA precursors; and (2) the processing of recombination intermediates to yield new configurations of genetic markers. More recent studies have suggested that mismatch repair may also be crucial for (1) the regulation of recombination events between divergent DNA sequences that could result in different types of genetic instability (Rayssiguier et al. 1989; Selva et al. 1995; Datta et al. 1996), (2) some types of nucleotide excision repair responsible for repair of physical/chemical damage to DNA (Karran and Marinus 1982; Fram et al. 1985; Feng et al. 1991; Mellon and Champe 1996), and (3) participating in a cell-cycle checkpoint control system by recognizing certain types of DNA damage and triggering cell-cycle arrest or other responses to DNA damage (Hawn et al. 1995; Anthony et al. 1996).

The most extensively characterized general mismatch repair system is the *Escherichia coli* MutHLS system, which repairs a broad spectrum of mispaired bases and has been reconstituted with purified enzymes. Eukaryotes are known to contain a mismatch repair system that has at least some components that are highly related to key components of the bacterial MutHLS mismatch repair system. The observation that defects in mismatch repair genes are linked to both inherited cancer susceptibility and some sporadic cancers has generated considerable interest in the gene products that function in eukaryotic mismatch repair. The goal of this review is to discuss recent studies on the mechanisms of MutHLS-like mismatch repair in the yeast *Saccharomyces cerevisiae* and in humans and to relate insights derived from these studies to human cancer genetics. Given space constraints, it is difficult to cover everything known about mismatch repair or to reference all of the relevant work that has been done in this area. However, a brief overview of the *E. coli* MutHLS pathway is presented below to allow comparison of the *E. coli* and eukaryotic mismatch repair pathways and proteins. For more detailed information, particularly related to bacterial mis-

match repair, base-specific mismatch repair systems, and cancer genetics, see other recent reviews (Modrich 1991; Eshleman and Markowitz 1995; Fishel and Kolodner 1995; Friedberg et al. 1995; Kolodner 1995; Marra and Boland 1995; Modrich and Lahue 1996).

The *E. coli* MutHLS mismatch repair pathway

The *E. coli* MutHLS mismatch repair pathway is a general DNA repair pathway that recognizes and repairs all single-base mispairs except C-C (Modrich 1991; Fishel and Kolodner 1995; Friedberg et al. 1995; Kolodner 1995; Modrich and Lahue 1996). It also repairs small insertion/deletion mispairs, although it may not efficiently recognize most insertion/deletion mispairs that have more than 4 unpaired bases. The basic repair reaction catalyzed by this pathway is understood in considerable detail because it has been reconstituted in vitro with DNA substrates containing mispaired bases, MutH, MutL, MutS, and UvrD (helicase II) proteins, DNA polymerase III holoenzyme, DNA ligase, single-strand DNA-binding protein (SSB), and any one of the single-stranded DNA exonucleases—Exo I, Exo VII, and RecJ protein (Fig. 1; Lahue et al. 1989; Modrich 1991; Grilley et al. 1993; Fishel and Kolodner 1995; Friedberg et al. 1995; Kolodner 1995; Modrich and Lahue 1996). The reaction involves mismatch-dependent nicking of the unmethylated strand at a hemimethylated GATC site and degradation from the nick past the mismatch followed by resynthesis. The roles of many of the proteins that function in this reaction have been elucidated. The MutS protein binds to DNA at the site of a mispaired base and is responsible for mismatch recognition. No activity has been assigned to MutL, although it interacts with MutS bound to a mispaired base and is required for activation of MutH. MutH is an endonuclease that nicks hemimethylated DNA on the unmethylated strand when activated by MutS and MutL in the presence of a mismatch. The requirement for MutH in the reaction can be replaced by a pre-existing nick in the DNA. The repair reaction can utilize hemimethylated sites that are either 5' or 3' to the mispair. Excision requires UvrD (helicase II) and one of the single-stranded DNA exonucleases—Exo I (3' exonuclease), Exo VII (3' and 5' exonuclease),

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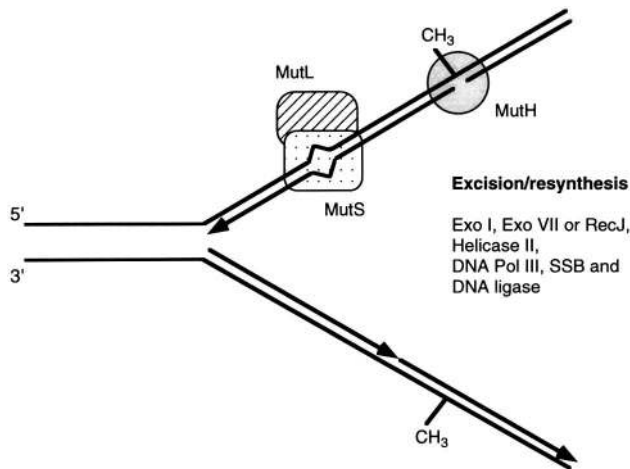


Figure 1. Illustration of the action of the *E. coli* MutHLS mismatch repair system on a mismatch at a replication fork. Repair is initiated by binding of MutS protein to a mismatch. The subsequent binding of MutL to MutS is required to activate MutH, which then nicks the unmethylated strand of DNA at hemimethylated GATC sites. Nicking of the unmethylated strand is then followed by the excision from the nick to the mismatch and resynthesis to fill in the resulting gap. These interactions result in the coupling of mismatch repair to DNA replication, so that mismatches formed during DNA replication are repaired using the methylated parental strand as template, resulting in a reduction of misincorporation errors. (Adapted from Kolodner 1995.)

and RecJ protein (5' exonuclease)—depending on whether the nicked, unmethylated site is 5' or 3' to the mismatch. Once excision has occurred, resynthesis is mediated by DNA polymerase III holoenzyme, SSB, and DNA ligase.

A crucial feature of the MutHLS mismatch repair pathway is its ability to preferentially repair the daughter DNA strand after DNA synthesis, thus increasing the fidelity of DNA replication. Normally, DNA in *E. coli* is methylated at GATC sites by the Dam methylase. This is a postreplication modification, however, and the daughter DNA strand is transiently unmethylated after DNA replication. The MutHLS system utilizes this modification asymmetry to direct repair to the unmethylated daughter strand via the activity of MutH, which nicks the unmethylated DNA strand at hemimethylated GATC sites. It is important to point out that the requirement for MutH and the use of Dam methylation to distinguish between newly replicated and parental DNA appear to be unique to certain bacteria. Other bacteria like *Streptococcus pneumoniae* have a mismatch repair system that is closely related to the MutHLS system but the *Streptococcus* system, referred to as the Hex system, is not presently known to utilize a MutH homolog or DNA methylation as a mechanism for strand discrimination in the repair process (Modrich 1991; Fishel and Kolodner 1995; Friedberg et al. 1995; Kolodner 1995; Modrich and Lahue 1996). DNA methylation does not occur in *S. cerevisiae* (Proffitt et al. 1984), and no con-

vincing evidence has implicated DNA methylation in strand discrimination during mismatch repair in other eukaryotes (Modrich 1991; Fishel and Kolodner 1995; Friedberg et al. 1995; Kolodner 1995; Modrich and Lahue 1996). Thus, it seems possible that the mechanism of strand discrimination in eukaryotes will prove to be quite different from that observed in *E. coli*.

MutHLS-like mismatch repair in eukaryotes

Two lines of experimentation have indicated that eukaryotes have a broad-spectrum mismatch repair system related to the bacterial MutHLS system. First, a series of genetic studies led to the identification of *S. cerevisiae* homologs of the bacterial *mutL* and *mutS* gene products and the demonstration that these gene products were required for mismatch repair (Williamson et al. 1985; Bishop et al. 1987; W. Kramer et al. 1989; Reenan and Kolodner 1992a; Reenan and Kolodner 1992b; Kolodner 1995). Second, biochemical studies of higher eukaryotic cells have demonstrated in vitro nick-directed repair of a variety of different mispaired bases similar to the repair reactions catalyzed by the *E. coli* MutHLS system (Fang and Modrich 1993; Modrich and Lahue 1996). More recently, biochemical studies have demonstrated that these mismatch repair reactions are dependent on eukaryotic homologs of the bacterial *mutL* and *mutS* gene products (Umar et al. 1994; Boyer et al. 1995; Drummond et al. 1995; Li and Modrich 1995). A considerable body of evidence indicates that this pathway plays an important role in maintaining replication fidelity, processing recombination intermediates, and regulating recombination in response to sequence divergence. These subjects, however, are beyond the scope of this review.

Homologs of the bacterial MutS proteins

A series of studies has demonstrated that there are at least six *S. cerevisiae* proteins, MSH1–MSH6, which show a high degree of amino acid similarity with the Bacterial MutS proteins (Fig. 1; Table 1). Homologs of many of these proteins have been identified in other organisms and are the subject of intense study. As will be discussed below, there is evidence that three of these proteins, MSH2, MSH3, and MSH6, function in a eukaryotic MutHLS-like mismatch repair pathway. The involvement of three MutS homologs in mismatch repair provides evidence for the relative complexity of eukaryotic mismatch repair compared to the bacterial paradigm.

It was originally thought that the eukaryotic MSH2 protein, a homolog of the bacterial MutS protein, was the mismatch repair protein that recognizes mispaired bases in DNA (Reenan and Kolodner 1992a,b). Purified MSH2 from both *S. cerevisiae* and human cells recognizes both single-base mispairs and, with a higher affinity, multiple base insertion/deletion mispairs (Fishel et al. 1994; Alani et al. 1995). More recently, it has been appreciated that human MSH2 copurifies with a second MutS homolog, human GTBP/p160 (Drummond et al. 1995; Pal-

Table 1. Names and alternate names for mismatch repair genes and proteins discussed

<i>E. coli</i> protein	<i>S. cerevisiae</i> protein	Human protein
MutS	MSH2	MSH2
	MSH3	MSH3, DUG1, MRP1
	MSH6	GTBP, p160
MutL	PMS1	PMS2
	MLH1	MLH1
	MLH2	PMS1

General nomenclature: Gene names, uppercase italics; mutation names, lowercase italics; protein names, uppercase except for *E. coli* proteins, where only the first letter of each word is capitalized.

Note on MutL-related proteins: Human PMS2 is more closely related to *S. cerevisiae* PMS1 than human PMS1 is related to *S. cerevisiae* PMS1. Human PMS1 is more closely related to *S. cerevisiae* MLH2 (see Fig. 4).

References: *E. coli* MutS-related proteins: (Reenan and Kolodner 1992a, Fishel et al. 1993; Leach et al. 1993; New et al. 1993; Drummond et al. 1995; Palombo et al. 1995; Marsischky et al. 1996; Watanabe et al. 1996). *E. coli* MutL-related proteins: (Bronner et al. 1994; Nicolaidis et al. 1994; Papadopoulos et al. 1994; Prolla 1994a,b).

ombo et al. 1995), which appears to be the homolog of *S. cerevisiae* MSH6 (Figs. 2 and 3, and below). The observation that the heterodimer of human MSH2 and GTBP (MSH6) recognizes mispaired bases initially suggested that the entire complex could function as the MutS equivalent in eukaryotic mismatch repair (Drummond et al. 1995; Palombo et al. 1995). The available data on mismatch repair-defective tumor cell lines, however, suggest that the role of a MSH2–GTBP (MSH6) complex in mismatch recognition may not be simple. In vivo, tumor cell lines lacking MSH2 appear to have a high degree of microsatellite instability at both dinucleotide and mononucleotide repeat loci (Bhattacharyya et al. 1994; Shibata et al. 1994; Umar et al. 1994b; Boyer et al. 1995; Papadopoulos et al. 1995). In contrast, tumor cell lines lacking GTBP (MSH6) appear to have less pronounced microsatellite instability: Dinucleotide repeat instability is difficult to detect in such cell lines, and mononucleotide repeat instability is less pronounced compared to *msh2* mutant lines (Bhattacharyya et al. 1994; Shibata et al. 1994; Papadopoulos et al. 1995). Biochemical analysis also suggests that mutations in *MSH2* and *GTBP* (*MSH6*) have somewhat different effects on mismatch repair in *in vitro* mismatch repair assays (Drummond et al. 1995). Finally, mutations in *MSH2* are common in hereditary nonpolyposis colorectal cancer (HNPCC) families, whereas mutations in *GTBP* (*MSH6*) have not been found (Papadopoulos et al. 1995; Liu et al. 1996). These data suggest that *MSH2* and *GTBP* (*MSH6*) are not genetically equivalent.

Genetic and biochemical analyses of mismatch repair in *S. cerevisiae* have suggested that mismatch recognition involves three MutS homologs: MSH2, MSH3, and MSH6 (the homolog of human GTBP/p160), which form two different heterodimeric complexes (Marsischky et

al. 1996). Mutations in *MSH2* were found to cause a strong general mutator phenotype resulting in the accumulation of both frameshift and single-base substitution mutations, high rates of frameshift mutation reversion, and dinucleotide repeat instability, consistent with a general role in mismatch repair (Reenan and Kolodner 1992a; Strand et al. 1993; Marsischky et al. 1996). In contrast, mutations in *MSH6* caused only a modest mutator phenotype that was confined primarily to the accumulation of single-base substitution mutations, suggesting that *MSH6* might be required primarily for recognition of single-base substitution mispairs and to a lesser extent single base insertion/deletion mispairs (Marsischky et al. 1996). Consistent with this latter view, *pms3* mutants, which had been shown previously in transformation assays that detect mismatch repair directly to cause a defect in the repair of single-base substitution mispairs but not in the repair of single-base insertion/deletion mispairs, are caused by mutations in *MSH6* (B. Kramer et al. 1989; Marsischky et al. 1996). Also consistent with this view is the observation that *msh6* mutations cause only a small increase in dinucleotide repeat instability (Johnson et al. 1996).

Unlike either *msh2* or *msh6* mutants, *msh3* mutants have little if any mutator phenotype in forward mutation and frameshift reversion assays and show increased dinucleotide repeat instability, albeit at lower levels, than that seen in *msh2* mutants (New et al. 1993; Alani et al. 1994; Strand et al. 1995; Marsischky et al. 1996). Strikingly, when double mutant strains were analyzed, *msh3* and *msh6* mutations showed a large synergistic effect on the rate of accumulation of mutations in frameshift reversion assays that measure the formation and repair of single-base insertion/deletion mispairs (Marsischky et al. 1996). A similar synergistic effect was seen in forward

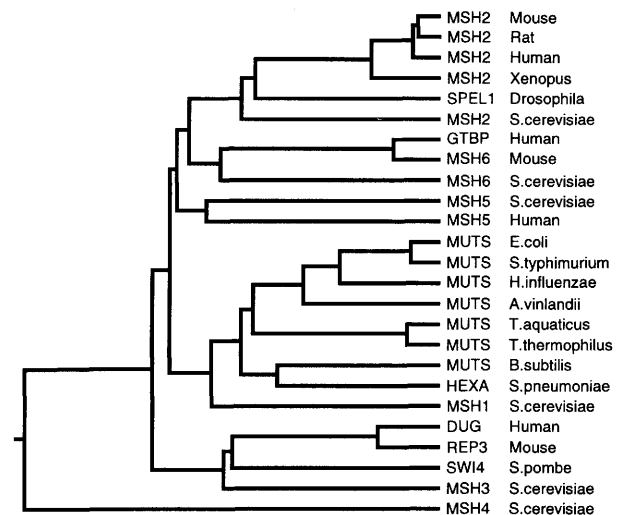


Figure 2. Family tree of known MutS homolog proteins. All of the sequences used for this construction were either retrieved from GenBank or are from unpublished studies; human MSH5, N. Winand and R. Kolodner (unpubl.); mouse MSH6, G. Crouse and R. Kolodner (unpubl.).

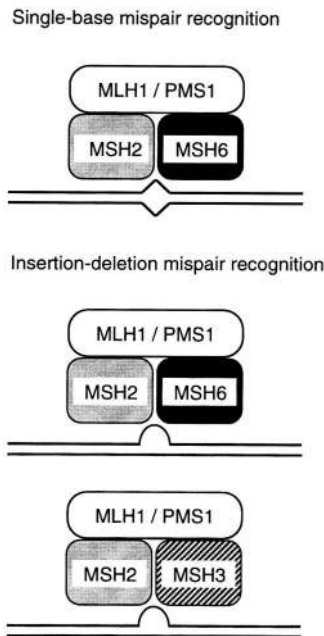


Figure 3. Model for mismatch recognition in *S. cerevisiae*. The various postulated complexes between MSH2 and either MSH3 or MSH6 are illustrated interacting with either a single-base substitution mispair or an insertion/deletion mispair; exactly which of the proteins in these complexes—MSH2, MSH3, or MSH6—actually interacts with the mispaired base is not known. Also indicated is the previously described MLH1–PMS1 complex that interacts with the mispair recognition complex. The *S. cerevisiae* protein names are given as primary names; the human protein names are the same except for PMS1, which is called PMS2 in humans, and MSH6, which has been called GTBP or p160 in humans. (Reprinted, with permission, from Marsischky et al. 1996).

mutation assays that detect a broader variety of mutation events, and in these experiments, the mutation rate and mutation spectrum in the *msh3 msh6* double mutant were essentially the same as that seen in the *msh2* single mutant (Marsischky et al. 1996). These and other genetic data indicate that MSH3 and MSH6 encode redundant activities that act in MSH2-dependent mismatch repair (Johnson et al. 1996; Marsischky et al. 1996). Consistent with this hypothesis, MSH2 forms heterodimeric complexes with both MSH3 and MSH6 proteins (Marsischky et al. 1996).

The analysis of *S. cerevisiae* MSH2, MSH3, and MSH6 has led to the proposal of a model in which there are two different pathways of MSH2-dependent mismatch repair: repair that is primarily specific for single-base substitution mispairs and requires a MSH2–MSH6 complex, and repair that is primarily specific for insertion/deletion mispairs and requires either a MSH2–MSH3 complex or a MSH2–MSH6 complex (Fig. 3; Marsischky et al. 1996). The sum of the mutagenesis data reported suggests that repair of single-base substitution mispairs requires the MSH2–MSH6 complex, that repair of single-base insertion/deletion mispairs can utilize the MSH2–MSH6 or

MSH2–MSH3 complexes about equally, and that repair of larger insertion/deletion mispairs likely utilizes the MSH2–MSH3 complex 5–10 times as frequently as the MSH2–MSH6 complex (Johnson et al. 1996; Marsischky et al. 1996). A crucial test of this model will require the purification of these two complexes and the demonstration that they have the required mispair recognition properties.

The genetics of *S. cerevisiae* MSH2, MSH3, and MSH6, where comparable data exist, are consistent with the results of the analysis of human tumor cell lines. Like *S. cerevisiae* *msh2* mutants, human *msh2* mutant cell lines (e.g., LoVo, HEC59, 2774) have a strong general mutator phenotype and strong microsatellite instability including instability at dinucleotide and mononucleotide repeat loci (Bhattacharyya et al. 1994; Shibata et al. 1994; Boyer et al. 1995; Liu et al. 1995). Similar to the mutator phenotype of *S. cerevisiae* *msh6* mutants, *gtbp* (*msh6*) mutant tumor lines (DLD1/HCT15, VACO543) have a strong mutator phenotype when the accumulation of mutations at *HPRT* (forward mutation assay) is measured, but their microsatellite instability phenotype is reduced compared to *msh2* mutant tumor lines (Bhattacharyya et al. 1994; Shibata et al. 1994; Eshleman et al. 1995; Papadopoulos et al. 1995). Little is known about the possible presence of *msh3* mutations in either tumor cell lines or HNPCC families other than the fact that like *GTBP* (*MSH6*) mutations they must be rare because most HNPCC families studied have either *msh2* or *mlh1* mutations (Papadopoulos et al. 1995; Liu et al. 1996).

The genetic properties of MSH2, MSH3, and MSH6 in *S. cerevisiae* have important implications for the analysis of mismatch repair-defective mutations and their association with cancer susceptibility. First, the redundancy of MSH3 and MSH6 compared to the apparently universal requirement for MSH2 in mismatch repair provides an explanation for the high prevalence of *msh2* mutations in HNPCC families compared to mutations in either *GTBP* (*MSH6*) or *MSH3* (Papadopoulos et al. 1995; Liu et al. 1996). This is because independent mutations in both MSH3 and *GTBP* (*MSH6*) would be required to produce the same mismatch repair defect as that caused by mutations in MSH2. Second, selection for tumors and syndromes (HNPCC) associated with high degrees of microsatellite instability may preclude cancers and syndromes caused by *msh6* or *msh3* mutations. In contrast, one might expect to find *msh3* or *gtbp* (*msh6*) mutations associated with cancers and cancer susceptibility syndromes with mutator phenotypes that more closely resemble the mutator phenotypes caused by *msh3* and *msh6* mutations in *S. cerevisiae*. Third, it is possible that the mutator phenotype caused by *msh3* or *gtbp* (*msh6*) mutations compared to *msh2* mutations is insufficient to cause cancer initiation or progression or that such mutations have lower penetrance than *msh2* mutations. Fourth, it is possible that the lack of another entirely MSH2-dependent process, rather than the loss of mismatch repair, is the underlying cause of cancer susceptibility. Fifth, it is possible that there are tumors and tumor cell lines showing microsatellite instability that

are *msh3*, *gtbp* (*msh6*) double mutants. A particularly intriguing possibility is that mutations in *MSH3* and *GTBP* (*MSH6*) segregate in the human population and are relatively silent until *msh3* *gtbp* (*msh6*) double mutant individuals with increased cancer susceptibility are generated. It would require two independent events to inactivate the wild-type *MSH3* and *GTBP* (*MSH6*) alleles as expected in tumor cells from HNPCC individuals (Leach et al. 1993; Hemminki et al. 1994; Borresen et al. 1995) and the occurrence of two such events might be infrequent. However, the loss of the second allele of one of the genes, *MSH3* or *GTBP* (*MSH6*), would cause a weak mutator phenotype that could speed inactivation of the remaining functional gene. It is also possible that *MSH3/msh3*, *GTBP* (*MSH6*)/*gtbp* (*msh6*) cells might have an increased mutation rate resulting from unlinked partial noncomplementation, which would increase the loss of the second alleles of these genes.

Homologs of the bacterial MutL proteins

A variety of studies have documented a large number of eukaryotic genes encoding proteins that show a high degree of amino acid similarity with the bacterial MutL proteins (Figs. 3,4). It should be noted that the complete gene sequence is not known in all cases. Historically, these proteins have been designated as either PMS or MLH proteins because the first eukaryotic gene encoding a MutL homolog was named *PMS1* before it had been cloned. These eukaryotic proteins, however, are all related to the bacterial MutL proteins. The available evidence suggests that at least two of these proteins, *MLH1* and *PMS1* (*PMS2* in humans), are involved in mismatch repair. The involvement of two such proteins in mismatch repair, as compared to the involvement of a single MutL protein in bacteria, parallels the complexity of eu-

karyotic mismatch repair seen in regard to the MSH proteins.

In both *S. cerevisiae* and human cells, there appear to be at least two MutL homologs that are required for mismatch repair: *MLH1* and *PMS1* in *S. cerevisiae*, and *MLH1* and *PMS2* (the closest homolog of *S. cerevisiae* *PMS1*) in human cells (Prolla et al. 1994; Kolodner 1995; Li and Modrich 1995; Modrich and Lahue 1996). These proteins appear to exist in a 1:1 complex (Prolla et al. 1994b; Li and Modrich 1995). The purified human *MLH1*–*PMS2* complex complements the in vitro mismatch repair defect caused by *mlh1* mutations found in human tumor cell lines, indicating that it is functional in mismatch repair (Li and Modrich 1995). In *S. cerevisiae*, this *MLH1*–*PMS1* complex interacts with *MSH2* bound to a mispaired base, consistent with the idea that the *MLH1*–*PMS1* (human *PMS2*) complex plays the same role in mismatch repair in eukaryotes that MutL plays in bacteria. The available biochemical and genetic data (discussed in more detail below) and the various protein sequence homology relationships support the idea that the *S. cerevisiae* *MLH1*–*PMS1* and human *MLH1*–*PMS2* complexes play equivalent roles in mismatch repair.

In human cells there is a third MutL homolog, *PMS1*, that has been implicated in mismatch repair by virtue of the observation of a germ-line mutation in *PMS1* in a single patient with a family history of colon cancer (Nicolaidis et al. 1994). The tumor from this patient was not examined for either loss of the second *PMS1* allele or for microsatellite instability. Therefore, it is not clear whether *PMS1* behaves like other mismatch repair and tumor suppressor genes during tumor development or whether *pms1* mutations cause a mismatch repair defect in tumors. At present, little is known about the biochemical role of h*PMS1* in mismatch repair. Additional studies of human *PMS1* are clearly needed.

The genetics of *S. cerevisiae* *PMS1* and *MLH1* and human *MLH1* and *PMS2* parallel each other to a great extent. The available data indicate that mutations in *S. cerevisiae* *MLH1* and *PMS1* cause a similar, strong general mutator phenotype that is virtually the same as that caused by mutations in *MSH2*; no differences in the mutator phenotypes caused by *pms1* and *mlh1* mutations have yet been published (Williamson et al. 1985; Bishop et al. 1987; B. Kramer et al. 1989; Strand et al. 1993; Prolla et al. 1994a; Jeyaprakash et al. 1996). Interestingly, in a recent study on the effects of *msh2*, *msh3*, and *pms1* (human *pms2*) mutations on crossing over between divergent DNA sequences, *PMS1* (human *PMS2*) appeared to play significantly less of a role in suppressing homologous recombination than *MSH2* (Datta et al. 1996; Hunter et al. 1996). This suggests that defects in *PMS1* (human *PMS2*) might not be equivalent to defects in other mismatch repair genes (unfortunately *MLH1* was not evaluated in these studies; Datta et al. 1996; Hunter et al. 1996). Analysis of human tumor cell lines with mutations in either *MLH1* or *PMS2* support this view: Such cell lines have a strong, global microsatellite instability phenotype, and extracts prepared from such cell

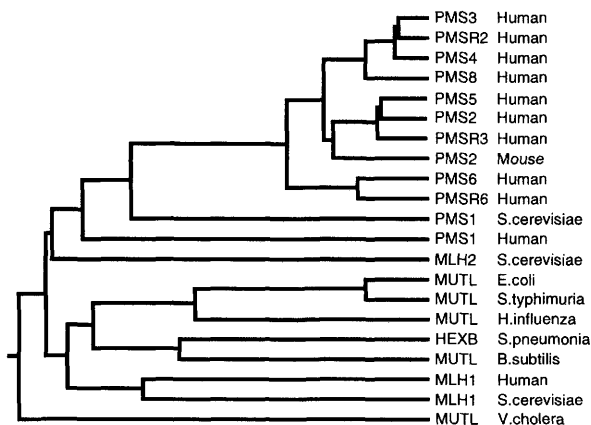


Figure 4. Family tree of known MutL homolog proteins. All of the sequences used for this construction were retrieved from GenBank except *S. cerevisiae* *MLH2*, which was provided by Tom Prolla and R. Michael Liskay (Prolla 1994). Some of the sequences used (h*PMS5*, h*PMS6*, h*PMSR6*, h*PMS8*) are not complete coding sequences.

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lines are mismatch repair defective (Parsons et al. 1993; Bhattacharyya et al. 1994; Shibata et al. 1994; Boyer et al. 1995; Li and Modrich 1995; Risinger et al. 1995). Furthermore, the mismatch repair defect caused by *mlh1* mutations can be complemented in vitro by the addition of the MLH1-PMS2 complex (Li and Modrich 1995). In addition, cells derived from homozygous *pms2* mutant mice show microsatellite instability, consistent with a mismatch repair defect (Baker et al. 1995). One striking feature about the genetics of the human *MLH1* and *PMS2* genes is that there is a strikingly lower frequency of germ-line *pms2* mutations compared to germ-line *mlh1* mutations in HNPCC families, especially given that mutations in both genes appear to cause the same type of mismatch repair defect (Boyer et al. 1995; Risinger et al. 1995; Liu et al. 1996). These data may reflect trivial possibilities such as founder effects (Nystrom-Lahti et al. 1995), or DNA sequence contexts may make *MLH1* more mutable than *PMS2*. Alternately, these data raise the intriguing possibilities that *MLH1* and *PMS2* do not play equivalent roles in the cell even though they are subunits of the same complex (see above discussion of Datta et al. 1996; Hunter et al. 1996), that *pms2* mutations confer a selective disadvantage to the population compared to *mlh1* mutations (Baker et al. 1995), or that like *S. cerevisiae* *MSH3* and *MSH6*, there is a function that is redundant with *PMS2*. The resolution of this question could provide important information about how mismatch repair defects give rise to cancer susceptibility.

Other components required for mismatch repair

It seems clear that HNPCC families, sporadic tumors, and tumor cell lines that are associated with mismatch repair defects or that exhibit microsatellite instability exist where it has not yet been possible to demonstrate defects in known mismatch repair genes (da Costa et al. 1995; Katabuchi et al. 1995; Liu et al. 1995, 1996; Papadopoulos et al. 1995; Wijnen et al. 1995, 1996). This observation has provided additional interest in the identification of other components of eukaryotic mismatch repair. At present, there is little definitive additional information about the other proteins that function in eukaryotic MutHLS-like mismatch repair pathways. On the basis of our current understanding of the enzymes that promote MutHLS mismatch repair in *E. coli*, obvious candidates for proteins required for eukaryotic mismatch repair are exonucleases, helicases, and enzymes required for DNA synthesis (Modrich 1991; Kolodner 1995; Modrich and Lahue 1996). A particularly interesting unresolved issue is the identification of the proteins and signals that function in the recognition of the newly replicated DNA strand during mismatch repair; this is because DNA methylation and MutH homologs seem unlikely to play this role as they do in *E. coli* (Modrich 1991; Kolodner 1995; Modrich and Lahue 1996). Whereas most of the progress reported in eukaryotic mismatch repair enzymology has been in the area of the MSH and MLH proteins, there has been some recent

progress in the identification of exonucleases that might play a role in eukaryotic mismatch repair.

A 5' → 3' endo/exonuclease, *S. cerevisiae* RTH1 (RAD27, YKL510), has been proposed to function in mismatch repair (Johnson et al. 1995). Mutations in *RTH1* (*RAD27*) cause a mutator phenotype (Johnson et al. 1995; Reagan et al. 1995; Vallen and Cross 1995) that in one study was shown to be as strong as that caused by mutations in *MSH2*, *MLH1*, and *PMS1* (Johnson et al. 1995). This result seems at odds with mechanistic studies of mismatch repair promoted by human cell extracts indicating that, like the *E. coli* reaction, eukaryotic mismatch repair is likely to involve redundant 5' → 3' and 3' → 5' exonucleases (Modrich 1991; Fang and Modrich 1993; Modrich and Lahue 1996). Double mutant combinations of *rth1* and either *msh2*, *mlh1*, or *pms1* showed a three- to fivefold synergistic effect (Johnson et al. 1995). One possible interpretation of this result is that *RTH1* functions in an entirely different pathway from *MSH2*, *MLH1*, and *PMS1*. Additional genetic analysis of *RTH1* (*RAD27*) has indicated that *RTH1* (*RAD27*) plays a role in repair of other types of DNA damage, that *RTH1* (*RAD27*) is a member of the *RAD6* epistasis group that functions in DNA damage tolerance, and that *rth1* (*rad27*) mutants also have a cell cycle defect (Reagan et al. 1995; Vallen and Cross 1995). Studies of the *S. cerevisiae* (called RTH1, RAD27, YKL510) and human (called FEN-1, MF-1, exonuclease IV) protein have suggested that RTH1 functions in the processing of 5' ends of Okazaki fragments and processing branched DNA structures formed by different DNA repair pathways (Ishimi et al. 1988; Harrington and Lieber 1994; Waga and Stillman 1994; Waga et al. 1994; Hiraoka et al. 1995; Sommers et al. 1995). There are several possible interpretations of the data on *RTH1* with regard to a possible role in mismatch repair. (1) RTH1 functions as an exonuclease during mismatch repair; however, the strong mutator phenotype of *rth1* mutants seems inconsistent with the mechanistic studies implicating bidirectional excision during mismatch repair. This suggests that if RTH1 functions in mismatch repair, it likely functions in other repair reactions that act to reduce mutation rates. (2) RTH1 might not function as an exonuclease but rather as an endonuclease that cleaves branched structures that must be processed during different DNA repair pathways, possibly including mismatch repair. (3) The mutator phenotype seen in *rth1* mutants is not attributable to a mismatch repair defect but is rather the result of defects in the fidelity of DNA synthesis, or other repair defects. This latter possibility could account for the modest synergistic effect of *rth1* mutations in combination with *msh2*, *mlh1*, and *pms1* mutations. Clearly caution must be taken in the interpretation of the meaning of mutator phenotypes.

A second candidate for an exonuclease involved in mismatch repair was identified in a two-hybrid screen with *S. cerevisiae* MSH2 as a bait protein (D.X. Tishkoff, A. Beddow, M.F. Kane, and R. Kolodner, in prep.). The *S. cerevisiae* *EXO1* gene was identified as encoding a protein that interacts with both *S. cerevisiae* and human

MSH2, and a *S. cerevisiae* MSH2–EXO1 complex was identified in vivo by coimmunoprecipitation. *S. cerevisiae* EXO1 appears to be a homolog of the *Schizosaccharomyces pombe* 5' → 3' exonuclease EXO1 (Szankasi and Smith 1992, 1995; D.X. Tishkoff, A. Beddow, M.F. Kane, and R. Kolodner, in prep.), and characterization of the overproduced and purified *S. cerevisiae* EXO1 has indicated that it preferentially degrades double-stranded DNA compared with single-stranded DNA. Mutations in *S. cerevisiae* EXO1 cause a weak general mutator phenotype and low levels of microsatellite instability, consistent with the redundant exonuclease proposal. Furthermore, analysis of double mutants was consistent with EXO1 functioning in a MSH2-dependent pathway (D.X. Tishkoff, A. Beddow, M.F. Kane, and R. Kolodner, in prep.). *S. pombe* *exo1* mutants also have a mutator phenotype, and *S. pombe* EXO1 has been proposed to function in either a general MutHLS-like mismatch repair system or a more specific mismatch repair system like the base-specific systems described in bacteria (Szankasi and Smith 1995). At this stage of analysis additional studies are clearly needed to define the potential roles of RTH1 (RAD27) and EXO1 in mismatch repair and to identify additional exonucleases that might be required.

Is there another eukaryotic general mismatch repair pathway?

A number of lines of investigation have suggested that there may be another eukaryotic mismatch repair pathway that has an overlapping specificity with the MutHLS-like pathway. When gene conversion was analyzed in *S. cerevisiae* *pms1*, *msh2*, or *mlh1* mutants by use of tetrad analysis, it was observed that there was a low, residual level of gene conversion independent of the marker analyzed (single-base substitution mutations or small insertion/deletion mutations) (Williamson et al. 1985; Reenan and Kolodner 1992a; Alani et al. 1994; Prolla et al. 1994a). This could occur for two possible reasons: Either a proportion of gene conversion events are caused by gap repair and do not involve the formation of a heteroduplex recombination intermediate (discussed in Alani et al. 1994), or there is an inefficient mismatch repair pathway functional in *pms1*, *msh2*, or *mlh1* null mutants. Similarly, when transformation assays were used to analyze mismatch repair directly, not all apparent mismatch repair was eliminated by mutations in mismatch repair genes (Bishop et al. 1987, 1989; B. Kramer et al. 1989). Furthermore, the frequency of insertion events approximately equaled the frequency of deletion events when insertion/deletion mispairs were repaired after transformation of wild-type *S. cerevisiae* cells, whereas there was a strong disparity in favor of deletion events among the fraction of insertion/deletion mispairs that appeared to be repaired in a *pms1* mutant (Bishop and Kolodner 1986; Bishop et al. 1987). A similar shift toward deletion events in mismatch repair mutants was seen when alterations in GT tract length resulting from replication errors were analyzed (Strand et al. 1993,

1995). One of several possible explanations for these data is that there could be a separate, less-efficient repair pathway that selectively repairs insertion/deletion mispairs by deletion. Consistent with this view, analysis of mismatch repair catalyzed in extracts of human tumor cell lines has suggested there may be an *MLH1*-independent mismatch repair pathway that promotes the repair of some insertion/deletion mispairs >3 nucleotides in length and that repair of some such substrates may also be MSH2-independent (Umar et al. 1994a).

More recently, several workers have detected *S. cerevisiae* and human mispair binding activities present in *S. cerevisiae* *msh2*, *msh3*, and *msh4* mutants and human *msh2* and *gtbp* (*msh6*) mutants (Miret et al. 1996; O'Regan et al. 1996). Other studies have detected activities that nick DNA at the site of a mispair (Chang and Lu 1991; Yao and Kom 1994; Yeh et al. 1994). However, it has not been established whether these activities play a role in a general mismatch repair pathway such as the MutHLS-like pathway or whether these activities act in other pathways such as crossing over during meiotic recombination, which is known to require MSH4 and MSH5 (Ross-Macdonald and Roeder 1994; Hollingsworth et al. 1995), or in base-specific mismatch repair (Modrich 1991; Kolodner 1995; Modrich and Lahue 1996). Whether these types of activities play roles in other mismatch repair pathways and whether alternate mismatch repair pathways that recognize a broad spectrum of mispaired bases including larger insertion/deletion mispairs exist remain important unanswered questions at this time.

Conclusions

A considerable amount of progress has been made in the identification of components of eukaryotic MutHLS-like mismatch repair pathways. These pathways, however, appear more complex than their bacterial counterparts. Furthermore, not all of the proteins that function in these eukaryotic pathways have been identified, nor has it been resolved how many such repair pathways might exist. Particular challenges for the future include the complete identification of the components of these repair pathways, the understanding of how defects in these pathways lead to cancer susceptibility, and the unraveling of the genetics of these pathways in model organisms, which may provide greater insights into the role of inherited and acquired repair defects in cancer susceptibility in the human population.

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