Communication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 271, No. 45, Issue of November 8, pp. 27987–27990, 1996

Evidence for Involvement of Yeast Proliferating Cell Nuclear Antigen in DNA Mismatch Repair*

(Received for publication, August 12, 1996, and in revised form, September 13, 1996)

Robert E. Johnson‡, Gopala K. Kovvali‡, Sami N. Guzder‡, Neelam S. Amin§, Connie Holm§, Yvette Habraken‡, Patrick Sung‡, Louise Prakash‡, and Satya Prakash‡¶

From the ‡Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, Texas 77555-1061 and the §Department of Pharmacology, Division of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, California 92093-0651

DNA mismatch repair plays a key role in the maintenance of genetic fidelity. Mutations in the human mismatch repair genes *hMSH2*, *hMLH1*, *hPMS1*, and *hPMS2* are associated with hereditary nonpolyposis colorectal cancer. The proliferating cell nuclear antigen (PCNA) is essential for DNA replication, where it acts as a processivity factor. Here, we identify a point mutation, *pol30–104,* in the *Saccharomyces cerevisiae POL30* gene encoding PCNA that increases the rate of instability of simple repetitive DNA sequences and raises the rate of spontaneous forward mutation. Epistasis analyses with mutations in mismatch repair genes *MSH2*, *MLH1*, and *PMS1* suggest that the *pol30–104* mutation impairs *MSH2/MLH1/PMS1*-dependent mismatch repair, consistent with the hypothesis that PCNA functions in mismatch repair. MSH2 functions in mismatch repair with either MSH3 or MSH6, and the MSH2-MSH3 and MSH2-MSH6 heterodimers have a role in the recognition of DNA mismatches. Consistent with the genetic data, we find specific interaction of PCNA with the MSH2-MSH3 heterodimer.

In both prokaryotes and eukaryotes, defects in DNA mismatch repair cause elevated spontaneous mutation rates and increased instability of simple repeat DNA sequences. Mutations in any of the human mismatch repair genes *hMSH2*, *hMLH1*, *hPMS1*, and *hPMS2* are associated with hereditary nonpolyposis colorectal cancer. Cell lines from these cancers are defective in DNA mismatch repair and display increased levels of spontaneous mutations and frequent alterations of microsatellite repeat sequences (1, 2).

Epistasis analyses in yeast have suggested that MSH2 protein functions in conjunction with MSH3 or MSH6 protein in mismatch recognition. Genetic and biochemical studies in both yeast and humans have further indicated that the MSH2-MSH3 and MSH2-MSH6 complexes differ in substrate specificities. In yeast, mutations in *MSH3* cause an increase in instability of microsatellite tracts but have little effect on single-base mispairs, whereas mutations in *MSH6* have a more prominent effect on the incidence of single-base mispairs than on microsatellite tract instability (3–5). From these and other genetic observations, it has been inferred that MSH2-MSH3 complex is more proficient in the removal of insertion-deletion mismatches of two or more nucleotides (4), whereas MSH2-MSH6 is better at removing single nucleotide mismatches (4, 5). Human cell lines defective in the MSH6 component of the MSH2-MSH6 heterodimer hMutSα exhibit a selective loss in the repair of base-base and single-nucleotide insertion-deletion mismatches; the repair of two-, three-, and four-nucleotide insertion-deletion mismatches is reduced 2–4-fold in these cell lines (6, 7). Consistent with genetic observations, hMutSα binds a G/T mismatch or a one nucleotide insertion-deletion mismatch with high efficiency (6). By contrast, the yeast MSH2-MSH3 heterodimer exhibits little affinity for a G/T mismatch but binds insertion-deletion mismatches with high specificity (8). The manner by which PMS1 and MLH1 function in mismatch repair remains to be determined.

The *POL30* gene of *Saccharomyces cerevisiae* encodes PCNA,[1] an essential component of the DNA replication machinery (9, 10). PCNA forms a homotrimer that acts as a sliding clamp around the DNA duplex and increases the processivity of DNA polymerases δ and ϵ (Ref. 11 and references therein). Here, we identify a mutation, *pol30–104*, that causes a dramatic increase in the rate of microsatellite instability and spontaneous mutability. To examine if hypermutability in *pol30–104* arises from a defect in mismatch repair, we compared tract instability and mutability of double mutants carrying *pol30–104* in combination with mismatch repair mutations with single mutants of mismatch repair genes. From these and other studies, we suggest that hypermutability in *pol30–104* derives from a defect in mismatch repair. We have purified the MSH2-MSH3 complex and the MSH2 protein to near homogeneity from yeast (8) and show that PCNA interacts strongly with the MSH2-MSH3 heterodimer but not with MSH2. This observation is highly significant because genetic and biochemical studies have indicated that MSH2-MSH3 heterodimer and MSH2-MSH6 heterodimer but not MSH2 are the biologically relevant species in mismatch recognition.

MATERIALS AND METHODS

Generation of the pol30–104 Mutation—To generate new mutations of yeast PCNA, PCR mutagenesis of the *POL30* gene and plasmid shuffle techniques were used. The *POL30* gene in plasmid pCH1565 was mutagenized by PCR amplification using pCH1565-specific primers and the Perkin-Elmer *Taq* polymerase in the presence of 1 mM $MgCl_2$ and 1 μM $MnCl_2$ under standard conditions (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 200 μm of dNTP). Mutagenized *POL30* and an appropriate pCH1565 fragment were then introduced into yeast strain CH2134 (*pol30Δ::LEU2 pCH1511 [POL30 URA3]*) to allow for recombination of mutagenized *POL30* into vector pCH1565. Transformants

* This work was supported by Grant CA41261 from the NCI, Grants GM19261 and GM36510 from the National Institutes of Health, and Grant DE-FG03-93ER61706 from the Department of Energy. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed: Sealy Center for Molecular Science, University of Texas Medical Branch, 6.104 Medical Research Bldg., 11th and Mechanic St., Galveston, TX 77555-1061. Tel.: 409-747-8602; Fax: 409-747-8608; E-mail: sprakash@scms.utmb.edu.

[1] The abbreviations used are: PCNA, proliferating cell nuclear antigen; PCR, polymerase chain reaction; 5-FOA, 5-fluoro-orotic acid; MMS, methylmethane sulfonate; BSA, bovine serum albumin; MOPS, 3-(*N*-morpholino)propanesulfonic acid; bp, base pair(s).

TABLE I
Effect of the pol30–104 mutation on the stability of poly(GT) tracts
Tract length alterations were monitored in plasmid pSH91. All the strains are isogenic and differ only by the mutations indicated.

Strain	Genotype	Rate of tract instability (± S.D.)	Rate relative to wild type
MS71	Wild type	$6.0 (\pm 0.7) \times 10^{-6}$	1
YPCNA1.14	*pol30–104*	$4.6 (\pm 0.1) \times 10^{-4}$	80
YRP85	*msh2*Δ	$1.4 (\pm 0.2) \times 10^{-3}$	230
YRP23	*mlh1*Δ	$1.9 (\pm 0.4) \times 10^{-3}$	320
AMY101	*pms1*Δ	$2.2 (\pm 0.5) \times 10^{-3}$	370
YPCNA1.19	*pol30–104 msh2*Δ	$2.2 (\pm 0.2) \times 10^{-3}$	370
YPCNA32	*pol30–104 mlh1*Δ	$1.3 (\pm 0.2) \times 10^{-3}$	220
YPCNA31	*pol30–104 pms1*Δ	$1.5 (\pm 0.3) \times 10^{-3}$	250

TABLE II
Types of poly(GT) tract alterations generated in pol30–104 and other mismatch repair defective strains

Strain	Genotype	Number of tracts sequenced	Number of tracts with base pair deletions (−) or additions (+)				
			−4	−2	+2	+4	Others
MS71	Wild type	33	0	2	26	0	5[d]
YPCNA1.14	*pol30–104*	58	1	24	30	1	2[e]
YRP85	*msh2*Δ	28	0	21	7[b]	0	0
MS128	*msh2*Δ	(77)[a]	(0)	(52)	(22)	(3)	(0)
YRP23	*mlh1*Δ	46	1	28	17[c]	0	0
YPCNA1.19	*pol30–104 msh2*Δ	50	2	32	16[b]	0	0
YPCNA32	*pol30–104 mlh1*Δ	36	0	22	14[c]	0	0

[a] These data are taken from Strand *et al.* (3).
[b] The frequencies of the −2- and +2-bp alterations were similar in the *msh2*Δ and *pol30–104 msh2*Δ strains (χ^2 for 1 degree of freedom = 0.6; $p > 0.25$).
[c] The frequencies of the −2- and +2-bp alterations were similar in the *mlh1*Δ and *pol30–104 mlh1*Δ strains (χ^2 for 1 degree of freedom = 0.008; $p > 0.90$).
[d] Other alterations observed in the wild type strain were −16, −14, −10, +14, and +14.
[e] Other alterations observed in the *pol30–104* strain were −10 and −10.

were replica plated onto 5-FOA containing medium to select for loss of wild type PCNA plasmid pCH1511[*POL30 URA3*] and screened for cold sensitivity (14 °C) and sensitivity to the alkylating agent methylmethane sulfonate (MMS).

Construction of Mutant Strains—Isogenic mutant strains were generated by transformation of the respective wild type or mismatch repair defective strains with the appropriate plasmid. The *pol30–104* mutation was introduced into the yeast strain MS71 (*MATα ade5–1 his7–2 trp1–289 ura3–52 CAN1*^{s}) or its leu$^-$ (*leu2–3,-112*) isogenic parental strain AMY125 by the gene replacement method. Plasmids pCH1577 (*pol30–104:LEU2*) or pBJ300 (*pol30–104:hisG::URA3::hisG*) were digested with *Sac*I and introduced into yeast. Genomic deletion mutations of *MSH2* (4), *MLH1*, and *PMS1* (12) were generated by the gene replacement method. All strains used in this study are isogenic and derived from AMY125. Integration of mutations were confirmed by Southern (DNA) blot analyses. Loss of the *URA3* gene by recombination of the *HisG* sequences was selected for by plating on medium containing 5-FOA.

Rates of Microsatellite Instability—Wild type and isogenic mutant strains were transformed with plasmid pSH91. To monitor alterations in the pSH91 repeat tract, for each strain 19 independent 100-μl cultures, each starting from ~10 5-FOA-sensitive cells, were grown at 30 °C in yeast extract-peptone-dextrose (YPD) medium to ~10^5–10^6 cells before being plated onto medium containing 5-FOA. The rates of tract alterations were determined by the method of the median (13).

DNA Sequencing—Alterations in the pSH91 repeat tract were determined by PCR analysis. Using plasmid DNA isolated from 5-FOA-resistant cells, a single-stranded ~130-nucleotide region encompassing the repeat tract was amplified by assymetric PCR using ^{35}S-dATP (Amersham Corp.) and the primers 5′-CCATTCTAATGTCTGCCCC-3′ and 5′-GTTTTCCCAGTCACGAC-3′. To determine the size of the alterations, the products were compared with labeled PCR products of predetermined length on 6% polyacrylamide denaturing gels.

Rates of Spontaneous Forward Mutation—For each strain, 19 independent cultures were grown at 30 °C in 100 or 500 μl of YPD, each starting from ~10 canavanine-sensitive cells. Cells were then plated onto arginine-deficient medium containing canavanine. Rates of spontaneous forward mutation at the *CAN1*^{s} locus were determined from the number of canavanine-resistant colonies by the method of the median (13).

PCNA Affi-Gel 15 Beads—Yeast PCNA was purified to homogeneity from *Escherichia coli* strain B834 containing the plasmid pBL228 as described (10). PCNA (2 mg) and BSA (5 mg) were coupled to 1 ml of Affi-Gel 15 in 0.1 M potassium MOPS, pH 7.5, following the instructions of the manufacturer (Bio-Rad). The coupling efficiency was greater than 95% for both PCNA and BSA, as determined by analyzing a sample before and after the coupling reaction by SDS-polyacrylamide gel electrophoresis.

Binding of MSH2 and MSH2-MSH3 Complex to PCNA Affinity Matrix—MSH2 and MSH2-MSH3 heterodimer were purified to near homogeneity from a yeast strain carrying either the plasmid for overproducing MSH2 or the plasmids for overproducing both MSH2 and MSH3 (8). Purified MSH2 protein (500 ng) and MSH2-MSH3 complex (1 μg) were mixed with 10 μl of the PCNA or BSA containing Affi-Gel beads in 140 μl of buffer A (20 mM Tris acetate, pH 7.0, 20% glycerol, 0.01% Nonidet P-40, 100 μg/ml BSA, 1 mM dithiothreitol) containing 0.1 M KCl at 4 °C for 1 h. The unbound proteins were removed by centrifugation, and the affinity matrix was washed with 0.2 ml of buffer A containing 1 M KCl at 4 °C. Proteins were then eluted from the Affi-Gel beads by incubating in 25 μl of 2% SDS at 37 °C for 10 min. The supernatant (2 μl) containing unbound proteins and the SDS eluates (0.5 μl) were subjected to immunoblot analysis to determine their content of MSH2 protein and the MSH2-MSH3 heterodimer. Immunoblot analysis of the 1 M KCl washes from all the binding reactions revealed that they did not contain any MSH2 protein or MSH2-MSH3 complex.

RESULTS

Mutations of the *POL30* gene were obtained using PCR mutagenesis and plasmid shuffle. A total of eight different *pol30* mutations were identified by their sensitivity to MMS and by their inability to grow at the restrictive temperature of 14 °C. We screened these mutations for their effects on spontaneous mutability at the *CAN1*^{s} locus and found that one of these, *pol30–104*, caused a dramatic increase in mutability at the permissive temperature (30 °C), whereas the others had little or no effect. UV sensitivity is not affected by *pol30–104*. The sequence of the entire coding region of the *pol30–104* allele was determined. The *pol30–104* mutation is a C to T transition at nucleotide 752 of the coding sequence, which results in an alanine to valine change at residue 251 of the PCNA protein.

TABLE III

Effect of the pol30–104 mutation on the rate of spontaneous forward mutation to canavanine resistance (can1^r)

Strains were identical to those in Table I, except that they did not carry plasmid pSH91.

Strain	Genotype	Rate of forward mutation to *can1*^{r} (± *S.D.*)	Rate relative to wild type
MS71	Wild type	$3.8 (\pm 1.0) \times 10^{-7}$	1
YPCNA1.14	pol30–104	$1.2 (\pm 0.2) \times 10^{-5}$	32
YRP23	*mlh1*Δ	$9.6 (\pm 3.3) \times 10^{-6}$	25
YPCNA32	*pol30–104 mlh1*Δ	$1.5 (\pm 0.5) \times 10^{-5}$	39

FIG. 1. **MSH2-MSH3 heterodimer but not MSH2 interacts with PCNA.** *A*, purified MSH2 protein (*lane 1*) was mixed with BSA Affi-Gel 15 and PCNA Affi-Gel 15 beads, which were treated with 2% SDS to elute bound MSH2 protein. The supernatants containing unbound MSH2 protein from the BSA Affi-Gel (*lane 2*) and the PCNA Affi-Gel (*lane 3*) and the SDS eluates containing MSH2 protein bound to the BSA Affi-Gel (*lane 4*) and the PCNA Affi-Gel (*lane 5*) were subjected to immunoblot analysis with affinity purified anti-MSH2 antibodies (8). *B*, purified MSH2-MSH3 complex (*lane 1*) was mixed with BSA Affi-Gel 15 and PCNA Affi-Gel 15 beads, which were treated with 2% SDS to elute bound MSH2-MSH3 complex. The supernatants containing unbound MSH2-MSH3 complex from the BSA Affi-Gel (*lane 2*) and the PCNA Affi-Gel (*lane 3*) and the SDS eluates containing MSH2-MSH3 complex bound to the BSA Affi-Gel (*lane 4*) and the PCNA Affi-Gel (*lane 5*) were subjected to immunoblot analysis with affinity purified anti-MSH2 and anti-MSH3 antibodies (8). MSH3 protein migrates in SDS gels as a triplet.

This residue is in a stretch of four amino acids that are conserved in *S. cerevisiae*, *Schizosaccharomyces pombe*, *Drosophila*, mouse, and human PCNA. Because the *pol30–104* mutation differs from the other cold-sensitive and MMS-sensitive *pol30* mutations by increasing spontaneous mutability dramatically, we examined the possibility that *pol30–104* may confer a defect in mismatch repair.

To examine the effect of the *pol30–104* mutation on the stability of simple DNA repeats, we used the centromeric plasmid pSH91 that contains an in-frame 33-bp insertion of poly$(GT)_{16}$G in the coding sequence of a hybrid gene containing the yeast *URA3* gene (12). The pSH91 repeat tract is in-frame with the *URA3* gene, resulting in Ura$^+$ cells. Alterations of the tract that produce an out-of-frame mutation give rise to Ura$^-$ cells that become resistant to 5-FOA.

Compared with wild type, the *pol30–104* mutation caused an 80-fold increase in the rate of tract instability (Table I). Mutations in the yeast mismatch repair genes *MSH2*, *MLH1*, and *PMS1* result in elevated rates of tract instability (12). To determine whether the increased tract instability in the *pol30–104* mutant could be due to a defect in mismatch repair, we examined tract instability in the *pol30–104 msh2*Δ, *pol30–104 mlh1*Δ, and *pol30–104 pms1*Δ double mutant strains. If tract instability in the *pol30–104* mutant had resulted from an increase in slippage events during DNA replication rather than from a defect in mismatch repair, then the rate of tract instability in these double mutant strains would have increased in a multiplicative fashion. In fact, a *pms1* mutation, in combination with the *pol3–01* mutation, which inactivates the 3′ to 5′ proof reading exonuclease function of DNA polymerase δ, results in mutation rates that are the product of the relative rates observed in the *pms1* and *pol3–01* single mutants (14). For example, compared with wild type, although forward mutation rates of *URA3* are elevated 40- and 130-fold in the *pms1* and *pol3–01* single mutants, respectively, the mutation rate in the *pms1 pol3–01* double mutant increases 19,000-fold (14). On the other hand, if tract instability in the *pol30–104* mutant had resulted from a defect in mismatch repair, then double mutant strains carrying the *pol30–104* mutation in combination with the mismatch repair mutations will exhibit the same level of tract instability as the *msh2*, *mlh1*, and *pms1* mutants. The results in Table I show that tract instability in the *pol30–104 msh2*Δ, *pol30–104 mlh1*Δ, and *pol30–104 pms1*Δ double mutant strains was about the same as in the *msh2*Δ, *mlh1*Δ, and *pms1*Δ strains. These data are consistent with epistasis of mutations in mismatch repair genes with *pol30–104*. Although the possibility of a role of PCNA in a parallel and independent pathway cannot be excluded, these results suggest an involvement of PCNA in MSH2/MLH1/PMS1-dependent mismatch repair.

We determined the nature of tract alterations in the *pol30–104* mutant by sequencing the tracts that had undergone changes in plasmid pSH91 (Table II). Additions or deletions of two base pairs represent the most common alterations in all the strains tested. In the wild type strain, most of the tract alterations are additions of 2 bp. In the *msh2*Δ and *mlh1*Δ mutants, there is about a 2-fold bias in favor of 2-bp deletions over 2-bp additions, whereas in the *pol30–104* mutant, 2-bp additions and deletions occur about equally frequently (Table II). Thus, the pattern of tract alterations in *pol30–104* resembles more closely the pattern in the mismatch repair mutants than that in the wild type. Because *pol30–104* is a missense mutation, it is not surprising that it does not cause the same degree of tract destabilization and bias as do null mutations in mismatch repair genes. To verify that tract destabilization in the *pol30–104* mutant arose from inactivation of mismatch repair, we compared the pattern of tract alterations in the *pol30–104 msh2*Δ and *pol30–104 mlh1*Δ double mutant strains with that in the *msh2*Δ and *mlh1*Δ single mutant strains. When our data for the *msh2*Δ mutant are combined with those of Strand *et al.* (3) (Table II), then the numbers of −2 and +2 tracts in this mutant are 73 and 29, respectively, compared with 32 and 16, respectively, in the *pol30–104 msh2*Δ mutant (χ^2 for 1 degree of freedom = 0.70; $p > 0.25$). Thus, the pattern of tract alterations in these mutant strains is almost identical. The incidence of −2 and +2 tracts was also the same in the *pol30–104 mlh1*Δ and *mlh1*Δ mutants (Table II).

We also examined the effect of the *pol30–104* mutation on spontaneous forward mutations of the *CAN1* locus (Table III). The *pol30–104* mutation caused ~30-fold increase in the rate of spontaneous mutability, which is similar to that observed in the *mlh1*Δ strain and the *pol30–104 mlh1*Δ strains (Table III). The results for *can1*^{r} are consistent with the epistasis observed for microsatellite instability.

To examine whether PCNA interacts physically with mismatch repair proteins, we purified PCNA from *E. coli* cells expressing the protein and covalently coupled it to Affi-Gel 15 beads for use as affinity matrix. The PCNA beads and control

beads containing BSA were mixed with MSH2 protein and MSH2-MSH3 heterodimer that we had purified to near homogeneity from *S. cerevisiae* (8). After washing with a large volume of 1 M KCl, the beads were incubated with 2% SDS to elute bound proteins followed by immunoblot analysis of the SDS eluates to determine the amount of MSH2 protein or of MSH2-MSH3 heterodimer that was retained on the Affi-Gel beads in each case. Interestingly, while MSH2 protein alone did not show any affinity for the PCNA beads (Fig. 1*A*), the majority (~80%) of the input MSH2-MSH3 heterodimer was retained on the PCNA beads (Fig. 1*B*). This interaction between PCNA and the MSH2-MSH3 complex is highly specific because (i) we observed no binding of MSH2-MSH3 heterodimer to the control BSA Affi-Gel 15 beads (Fig. 1*B*) and (ii) the association of MSH2-MSH3 with the PCNA beads was stable to washing with 1 M KCl, as indicated by the absence of the heterodimer in the 1 M KCl wash (data not shown).

DISCUSSION

We show here that the *pol30–104* mutation in PCNA causes an increase in the rate of instability of $(GT)_n$ repeat sequences and in the rate of forward mutations at the *CAN1*^{s} locus. The effect of *pol30–104* on tract alterations, however, is not quite as severe as that of null mutations in mismatch repair genes. This is likely to be due to the fact that *pol30–104* is a missense mutation and not a null mutation. Null mutations in *POL30* are lethal. The strong mutator phenotype of *pol30–104*, which is observed at the permissive temperature, distinguishes this mutation from the other cold-sensitive and MMS-sensitive *pol30* mutations that we have identified. This suggested to us that hypermutability in *pol30–104* may arise from a defect in mismatch repair rather than from a defect in DNA replication. To test the validity of this hypothesis, we carried out epistasis analyses of *pol30–104* with null mutations in mismatch repair genes *MSH2*, *MLH1*, and *PMS1*. We found that the rates of tract instability, the pattern of tract alterations, and spontaneous *can1*^{r} mutability were the same in double mutants of *pol30–104* with null mutations in mismatch repair genes and in single mismatch repair mutants. From these genetic observations, we infer that hypermutability in *pol30–104* results from a defect in mismatch repair.

Our recent biochemical studies have indicated that while the MSH2-MSH3 heterodimer binds insertion/deletion mismatches with high affinity but does not bind a G/T mismatch, MSH2 by itself does not recognize any of these mismatches (8). We now show that PCNA interacts with the MSH2-MSH3 complex but not with MSH2 protein, and the interaction between PCNA and MSH2-MSH3 is strong, being stable to challenge with 1 M KCl, further emphasizing the significance of the MSH2-MSH3 heterodimer in mismatch repair. Besides binding the MSH2-MSH3 complex, PCNA may also interact with additional components of the mismatch repair machinery, such as the MSH2-MSH6 heterodimer, PMS1, and MLH1.

Our findings have implications regarding which DNA polymerase may function in mismatch repair. The aphidicolin sensitivity of mismatch repair-associated DNA synthesis (15, 16) had suggested that any of the three DNA polymerases, α, δ, or ϵ, might act in this process, as they are all aphidicolin-sensitive. The evidence implicating PCNA in mismatch repair would suggest that Polδ may be the polymerase involved in mismatch repair, because PCNA is an essential subunit of this DNA polymerase. Polϵ, however, could also be involved, because PCNA also stimulates this DNA polymerase. One way by which PCNA might function in mismatch repair could be as follows. The mismatch repair proteins including MSH2-MSH3 or the MSH2-MSH6 heterodimer and PMS1 and MLH1 could be targeted to the mismatch via their affinity for the mismatch and for one another, and PCNA, along with the DNA polymerase, could be loaded onto the nick remaining at the 5′ side of the mismatch prior to the removal of RNA primers and joining of nascent DNA fragments. Via DNA looping, perhaps catalyzed by the MSH proteins in a manner analogous to the MutS catalyzed α-shaped loop structures in *E. coli* (17), the mismatch bound proteins may become associated with the PCNA-DNA polymerase complex, and these interactions could be important for the subsequent excision and repair synthesis reactions. The *pol30–104* mutation might impair any of these interactions.

The involvement of PCNA in mismatch repair suggests the possibility that mutations in human PCNA that inactivate only its mismatch repair function may contribute to sporadic colorectal cancers (18) and to other cancers, including those of the stomach, lung, breast, and pancreas, that are associated with microsatellite instability (19–22).

Acknowledgment—We thank P. Burgers for plasmid pBL228.

REFERENCES

1. Modrich, P., and Lahue, R. (1996) *Annu. Rev. Biochem.* **65,** 101–133
2. Umar, A., and Kunkel, T. A. (1996) *Eur. J. Biochem.* **238,** 297–307
3. Strand, M., Earley, M. C., Crouse, G. F., and Petes, T. D. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 10418–10421
4. Johnson, R. E., Kovvali, G. K., Prakash, L., and Prakash, S. (1996) *J. Biol. Chem.* **271,** 7285–7288
5. Marsischky, G. T., Filosi, N., Kane, M. F., and Kolodner, R. (1996) *Genes & Dev.* **10,** 407–420
6. Drummond, J. T., Li, G.-M., Longley, M. J., and Modrich, P. (1995) *Science* **268,** 1909–1912
7. Papadopoulos, N., Nicolaides, N. C., Liu, B., Parson, 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
8. Habraken, Y., Sung, P., Prakash, L., and Prakash, S. (1996) *Curr. Biol.* **6,** 1185–1187
9. Bauer, G. A., and Burgers, P. M. J. (1990) *Nucleic Acids Res.* **18,** 261–265
10. Ayyagari, R., Impellizzeri, K. J., Yoder, B. L., Gary, S. L., and Burgers, P. M. J. (1995) *Mol. Cell. Biol.* **15,** 4420–4429
11. Krishna, T. S. R., Kong, X.-P., Gary, S., Burgers, P. M., and Kuriyan, J. (1994) *Cell* **79,** 1233–1243
12. Strand, M., Prolla, T. A., Liskay, R. M., and Petes, T. D. (1993) *Nature* **365,** 274–276
13. Lea, D. E., and Coulson, C. A. (1949) *J. Genet.* **49,** 264–285
14. Morrison, A., Johnson, A. L., Johnston, L. H., and Sugino, A. (1993) *EMBO J.* **12,** 1467–1473
15. Holmes, J. J., Clark, S., and Modrich, P. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 5837–5841
16. Thomas, D. C., Roberts, J. D., and Kunkel, T. A. (1991) *J. Biol. Chem.* **266,** 3744–3751
17. Modrich, P. (1991) *Annu. Rev. Genet.* **25,** 229–253
18. Liu, B., Nicolaides, N. C., Markowitz, S., Willson, J. K. V., Parsons, R. E., Jen, J., Papadopoulos, N., Peltomaki, P., de la Chapelle, A., Hamilton, S. R., Kinzler, K. W., and Vogelstein, B. (1995) *Nat. Genet.* **9,** 48–55
19. Han, H. J., Yanagisawa, A., Kato, Y., Park, J. G., and Nakamura, Y. (1993) *Cancer Res.* **53,** 5087–5089
20. Glebov, O. K., McKenzie, K. E., White, C. A., and Sukumar, S. (1994) *Cancer Res.* **54,** 3703–3709
21. Mironov, N. M., Aguelon, M. A., Potapova, G. I., Omori, Y., Gorbunov, O. V., Klimenkov, A. A., and Yamasaki, H. (1994) *Cancer Res.* **54,** 41–44
22. Merlo, A., Mabry, M., Gabrielson, E., Vollmer, R., Baylin, S. B., and Sidransky, D. (1994) *Cancer Res.* **54,** 2098–2101