Communication

Requirement of the Yeast MSH3 and MSH6 Genes for MSH2-dependent Genomic Stability*

(Received for publication, January 17, 1996)

Robert E. J. Johnson, Gopala K. Kovvali, Louise Prakash, and Satya Prakash
From the Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, Texas 77555-1061

Defects in DNA mismatch repair result in instability of simple repetitive DNA sequences and elevated levels of spontaneous mutability. The human G/T mismatch binding protein, GTBP/p160, has been suggested to have a role in the repair of base-base and single nucleotide insertion-deletion mismatches. Here we examine the role of the yeast GTBP homolog, MSH6, in mismatch repair. We show that both MSH6 and MSH3 genes are essential for normal genomic stability. Interestingly, although mutations in either MSH3 or MSH6 do not cause the extreme microsatellite instability and spontaneous mutability observed in the msh2 mutant, yeast cells harboring null mutations in both the MSH3 and MSH6 genes exhibit microsatellite instability and mutability similar to that in the msh2 mutant. Results from epistasis analyses indicate that MSH2 functions in mismatch repair in conjunction with MSH3 or MSH6 and that MSH3 and MSH6 constitute alternate pathways of MSH2-dependent mismatch repair.

Mutations in the four human mismatch repair genes hMSH2, hMLH1, hPMS1, and hPMS2 are associated with hereditary nonpolyposis colorectal cancer (HNPPCC) as well as other cancers (1–8). Cell lines derived from these tumors are defective in DNA mismatch repair and exhibit increased levels of spontaneous mutations and frequent alterations of microsatellite repeat sequences (1–11). An MSH2 homolog, GTBP/P160, has recently been identified in human. GTBP/P160 exists as a heterodimer with the hMSH2 protein, and this complex binds to a G/T mismatch or to heteroduplexes containing a (dG)2 or (dT)2 insertion on one DNA strand (12, 13). Cell lines that are defective in GTBP exhibit elevated spontaneous mutability and are defective in the repair of base-base and single nucleotide insertion-deletion mismatches (12, 14, 15). However, the contribution of mutations in GTBP to hypermutability and cancer predisposition is not entirely clear at present, because two of the colorectal cancer cell lines, DLD-1 and HCT-15 that are mutated in GTBP, also harbor mutations in the 3′–5′-exonuclease “proofreading” domain of DNA polymerase δ (16). Thus, the observed phenotypes in these cell lines could arise from the mutations in GTBP or those in polδ, or they could be due to the combined effects of the GTBP and polδ mutations.

In Saccharomyces cerevisiae, mutations in the mismatch repair genes MSH2, PMS1, or MLH1 result in marked increases in spontaneous mutation rates and microsatellite instability (17–24). Purified MSH2 protein binds DNA containing mismatched base pairs and insertions (21, 22), and PMS1 and MLH1 form a complex, which then binds the MSH2-DNA complex containing a mismatch (23). Mutations in MSH3, another MSH2 homolog, cause a moderate increase in microsatellite instability (24) and have little effect on spontaneous forward mutation rates (25). The yeast homolog of human GTBP has been identified in the yeast genome sequencing project; we refer to it as MSH6.

The manner by which MSH2, MSH3, and MSH6 proteins effect mismatch repair has not yet been determined. Studies with the human MSH2 and GTBP proteins could be interpreted to mean that while MSH2 can recognize loops larger than one nucleotide, MSH2-GTBP heterodimer is more efficient in the recognition of single base mispairs and one-base loops (12–14). By contrast, recent studies with the yeast MSH3 gene have been interpreted to suggest that MSH2 by itself could recognize single base mispairs (24). Overall, previously published results are in accord with the idea that MSH2 can function in mismatch repair independently of the other MSH proteins. To clarify the roles of the MSH2, MSH3, and MSH6 genes in mismatch repair and to identify the possible functional interaction among them, we have examined the effects of null mutations in these genes on microsatellite instability and spontaneous mutability and have carried out epistasis analyses. Our studies indicate that for its function in mismatch repair, MSH2 requires the MSH3 or the MSH6 gene and that MSH3 and MSH6 constitute alternate MSH2-dependent mismatch repair pathways.

MATERIALS AND METHODS

Construction of Null Mutations of the MSH2, MSH3, and MSH6 Genes—Null mutations of MSH2, MSH3, and MSH6 were generated by transformation of yeast strain S71 [MATα, ade5–1 his7–2 trp1–289 ura3–52 CAN11] or its msh2 derivatives with the plasmids pPM600, pEN33, or pBJ271, respectively. All deletion-generating plasmids utilize the URA3 “geneblaster” fragment, which carries the URA3 gene flanked by two identical 1.15-kilobase Salmonella typhimurium HisG sequences. pPM600 contains the MSH2 gene in which nucleotides (nt) +11 to +2279 of the 2898-nt open reading frame (ORF) have been replaced with the URA3 geneblaster fragment. The msh2– generating DNA fragment was obtained by digestion with EagI and XbaI. pEN33 contains the MSH3 gene in which nucleotides +638 to +3135 of the 3141-nt MSH3 ORF have been replaced with the URA3 geneblaster fragment. The linear msh3–generating DNA fragment was obtained by digestion with EcoRI. pBJ271 contains PCR fragments of 692 and 751 nt corresponding to the 5′- and 3′-regions of the MSH6 gene, respectively, separated by the URA3 geneblaster fragment. This plasmid deleted nucleotides +114 to +3637 of the 3726-nt MSH6 ORF. The linear msh6–generating DNA fragment was obtained by digestion with EcoRI. Genomic mshA mutations were generated by the gene replacement method by transformation of yeast to Ura− with the appropriate linear DNA fragments. Deletion mutations were confirmed by Southern (DNA) blot analyses. Loss of the URA3 gene following recom
Role of MSH3 and MSH6 in Mismatch Repair

**Fig. 1.** MSH6 is the yeast homolog of human GTBP/p160. A, homology between the GTBP and MSH6 proteins is shown schematically. Shaded areas represent identical or highly conserved regions of the proteins. Spaces indicate gaps for optimal alignment. The position of the Walker type A nucleotide binding sequence (GKS) is indicated. aa, amino acids. B, alignment of C-terminal regions of the E. coli MutS, S. cerevisiae MSH2, MSH3, and MSH6, and human GTBP proteins. This highly conserved region contains the Walker type A nucleotide binding motif (GKS), indicated by asterisks. Identical residues are boxed, and conserved residues are shaded. Amino acid positions are in parentheses. For optimal alignment a deletion of amino acids NGKAYCV was introduced in the GTBP sequence at the position indicated by □.

### Table 1

| Strain | Genotype | Tract instability detected by | Rate of tract instability (± S.D.) | Rate relative to wild type |
|--------|----------|-----------------------------|------------------------------------|--------------------------|
| MS71   | Wild type| FOAr                        | 6.0 (±0.7) × 10^{-6}               | 1                        |
| YRP85  | mshΔ     | FOAr                        | 1.4 (±0.2) × 10^{-3}               | 233                      |
| YRP29  | mshΔ     | FOAr                        | 1.9 (±0.3) × 10^{-4}               | 32                       |
| YRP74  | mshΔ     | FOAr                        | 4.0 (±0.7) × 10^{-5}               | 7                        |
| YRP90  | mshΔ     | FOAr                        | 1.2 (±0.2) × 10^{-3}               | 200                      |
| YRP56  | mshΔ     | FOAr                        | 1.1 (±0.1) × 10^{-3}               | 183                      |
| YRP77  | mshΔ     | FOAr                        | 1.3 (±0.2) × 10^{-3}               | 217                      |
| YRP91  | mshΔ     | FOAr                        | 1.5 (±0.2) × 10^{-3}               | 250                      |
| MS71   | Wild type| β-Gal                       | 2.9 (±0.2) × 10^{-6}               | 1                        |
| YRP85  | mshΔ     | β-Gal                       | 2.6 (±1.0) × 10^{-3}               | 897                      |
| YRP29  | mshΔ     | β-Gal                       | 3.0 (±0.8) × 10^{-4}               | 103                      |
| YRP74  | mshΔ     | β-Gal                       | 1.7 (±0.2) × 10^{-5}               | 6                        |
| YRP90  | mshΔ     | β-Gal                       | 2.6 (±0.6) × 10^{-3}               | 897                      |
| YRP56  | mshΔ     | β-Gal                       | 2.3 (±0.6) × 10^{-3}               | 793                      |
| YRP77  | mshΔ     | β-Gal                       | 1.8 (±0.6) × 10^{-3}               | 621                      |
| YRP91  | mshΔ     | β-Gal                       | 2.0 (±0.6) × 10^{-3}               | 690                      |

### RESULTS

Identification of the Yeast GTBP Homolog—We have identified a yeast homolog (GenBank accession number Z47746) of human GTBP and refer to this gene as MSH6. The two proteins share 27% identical and 50% conserved residues (data not shown). As shown schematically in Fig. 1A, the two protein sequences are colinear without major gaps and the homology extends throughout the entire lengths of MSH6 and GTBP. As shown in Fig. 1B, the homology among MSH2, MSH3, and MSH6 and their E. coli counterpart MutS is particularly striking in the C-terminal portion of these proteins, which also contains the highly conserved Walker type A nucleotide-binding motif. In this region of 107 residues, the MSH proteins share a very high degree of identity among themselves and with the GTBP and MutS proteins.

Tract Instability in mshΔ Strains—We used two plasmid-based assays (17, 27) to examine the effect of mutations in MSH genes on the stability of simple repetitive DNA sequences. Centromeric plasmid pSH91 contains an in-frame 33-base pair (bp) insertion of poly(GT){sub}16 in the coding sequence of a hybrid gene containing the yeast URA3 gene. The
Role of MSH3 and MSH6 in Mismatch Repair

TABLE II

Types of poly(GT) tract alterations generated in mismatch repair defective strains

| Strain     | Genotype         | Number of tracts sequenced | Number of tracts with base pair deletions (−) or additions (+) |
|------------|------------------|----------------------------|---------------------------------------------------------------|
|            |                  |                            | −2     | +2     | +4     | Others |
| MS71       | Wild type        | 33                         | 0      | 2      | 26     | 0      | 5²    |
| YRP85      | msh2Δ           | 28                         | 0      | 2      | 3      | 0      | 0     |
| YRP29      | msh3Δ           | 28                         | 0      | 5      | 32     | 2      | 2²    |
| YRP74      | msh6Δ           | 41                         | 1      | 16     | 20     | 1      | 0     |
| YRP90      | msh3Δ msh6Δ     | 58                         | 2      | 3      | 6      | 1      | 0     |
| YRP56      | msh2Δ msh3Δ     | 30                         | 2      | 9      | 2      | 0      | 0     |
| YRP77      | msh2Δ msh6Δ     | 33                         | 0      | 35     | 11     | 1      | 0     |
| YRP91      | msh2Δ msh3Δ msh6Δ | 47                      |        |        |        |        |        |

² The five other alterations observed in the wild type strain were −16, −14, −10, +14, and +14.
² The two other alterations observed in the msh6 strain were −8 and +20.

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 are resistant to 5-FOA. Centromeric plasmid pSH31 contains an out-of-frame 29-bp poly(GT)₄G insertion in the coding sequence of the E. coli β-galactosidase gene. Yeast cells carrying pSH31 do not produce β-galactosidase and form white colonies on medium containing X-Gal. Alterations in tract length that restore the correct reading frame result in blue colonies on X-Gal medium.

The msh6Δ mutation resulted in a 7-fold increase in tract instability in pSH91 and a 6-fold increase in pSH31 (Table I). The msh3Δ mutation conferred a 32-fold elevation in tract instability in pSH91 and a 103-fold increase in pSH31, an effect similar to that reported recently (24). By contrast, the msh2Δ mutation increases tract instability 233-fold in pSH91 and 897-fold in pSH31 (Table I) (17, 20). To determine the functional relationship between MSH3 and MSH6, we examined tract instability in strains carrying null mutations in both these genes. Interestingly, in both plasmids pSH91 and pSH31, the rate of tract instability in the msh3Δ msh6Δ double mutant was nearly identical to the rate observed in the msh2Δ strain (Table I). The synergistic increase in the rate of tract destabilization in the msh3Δ msh6Δ double mutant over that in the msh3Δ or the msh6Δ single mutant indicates that MSH3 and MSH6 provide for alternate pathways for maintaining tract stability. To determine the manner of interaction of MSH3 and MSH6 with MSH2, we examined tract instability in the msh2Δ msh3Δ, msh2Δ msh6Δ, and msh2Δ msh3Δ msh6Δ mutant strains. For both plasmid systems, we found the rate of tract instability in these mutants to be the same as that in the msh2Δ single mutant (Table I). These data indicate that the effect of the msh2Δ mutation is epistatic to that of the msh3Δ and msh6Δ single mutations, as well as to that of the msh3Δ msh6Δ double mutant.

Types of Tract Alterations Generated in mshΔ Strains—To determine the types of repeat alterations generated in various mshΔ strains, we sequenced the tracts in plasmid pSH91 that had undergone alterations. As has been shown previously, in wild type cells there is a bias for additions of 2 bp with a ~15% occurrence of large (>8 bp) alterations, whereas in the msh2Δ strain, there is a bias toward accumulation of 2-bp deletions (Table II) (17, 20). The msh3Δ mutant exhibits an even higher bias for 2-bp deletions than does the msh2Δ mutant (χ² for 1 degree of freedom = 6.5; p < 0.01). The msh6Δ mutation does not appear to alter the pattern of insertions and deletions in comparison with wild type. The msh3Δ msh6Δ double mutant strain, however, exhibits a pattern of repeat length alterations that resembles the msh2Δ pattern (Table II). The msh2Δ msh3Δ msh6Δ and msh2Δ msh3Δ msh6Δ mutant strains exhibit a pattern of repeat length alterations similar to that observed in the msh2Δ strain (Table II), providing further evidence for an epistatic effect of the msh2Δ mutation on the msh3Δ and msh6Δ mutations.

Spontaneous Mutation Rates in mshΔ Strains—To determine the role of MSH genes in spontaneous mutability, we examined the rates of spontaneous forward mutations at the CAN1 locus in the various mshΔ strains. In contrast to its modest effect on microsatellite instability, the msh6Δ mutation caused a 12-fold increase in the rate of spontaneous forward mutation to canavanine resistance (can1¹) (Table III). The msh3Δ mutation, however, resulted in only a 3-fold increase in the spontaneous forward mutation rate. The rate of can1¹ mutation in the msh3Δ msh6Δ double mutant strain increased to a level similar to that in the msh2Δ strain (Table III), indicating that, as for microsatellite stability, both genes have a role in maintaining normal levels of spontaneous mutability. However, while MSH3 appears to have a more predominant role than MSH6 in microsatellite stability, MSH6 contributes more significantly to normal spontaneous mutability. The rate of formation of can1¹ mutations in the msh2Δ msh3Δ, msh2Δ msh6Δ, and msh2Δ msh3Δ msh6Δ mutant strains was the same as that in the msh2Δ mutant alone (Table III), indicating an epistatic effect of the msh2Δ mutation on the msh3Δ and the msh6Δ mutations.

**DISCUSSION**

Our results indicate that MSH3 and MSH6 are both required for maintaining wild type levels of tract stability and spontaneous mutability in yeast cells; MSH3, however, has a more
prominent role in maintaining tract stability, and MSH6 plays a more active role in forward spontaneous mutability, indicative of single bp alterations. Mutations in human MSH3 and MSH6 exhibit epistasis to MSH2, and the simultaneous absence of MSH3 and MSH6 results in the same high levels of tract instability and spontaneous mutability as in the msh2Δ strain. Our observations are consistent with the following suggestions: (i) for its action in mismatch repair, MSH2 functions either with MSH3 or MSH6, and MSH2 is non-functional when both MSH3 and MSH6 are absent, (ii) in wild type yeast cells, MSH2 and MSH6 function together in the preferential repair of 2–4 bp insertions and deletions, whereas MSH2 and MSH6 together have a preference for removing single base mismatches, and (iii) the absence of either MSH3 or MSH6 can be compensated to varying extents by the other gene, depending upon the type of mismatch, accounting for the synergistic effects observed in the msh3a msh6Δ double mutant. Based on our results, we propose the following model. The MSH2 protein combines physically with either the MSH3 or the MSH6 protein, and the MSH2-MSH3 and MSH2-MSH6 complexes have different substrate specificities (Fig. 2). The MSH2-MSH3 complex is more efficient at removing 2–4 bp insertions and deletions, while the MSH2-MSH6 complex is more efficient at removing single bp mismatches. Thus, even though purified MSH2 protein from both humans and yeast has been shown to bind DNA containing a G/T mismatch or insertion-deletion loop-type mismatches of up to 14 nucleotides (21, 22), our results predict that complex formation with MSH3 or MSH6 is obligatory for the action of MSH2 in mismatch repair.

In Mutations in human MSH2, MLH1, PMS1, and PMS2 account for the majority of cancers in HNPCC kindreds. However, a large proportion of sporadic colon cancers and other types of cancer do not have mutations in these genes, and no germline GTBP mutations have been identified in HNPCC kindreds that harbored no mutations in these four mismatch repair genes (14). Our results with the yeast MSH3 and MSH6 genes would suggest that mutations in the human MSH3 or the GTBP gene are unlikely to cause as severe a defect in mismatch repair as do mutations in MSH2. Mutational inactivation of both hMSH3 and GTBP, however, should result in increased microsatellite instability, hypermutability, and cancer predisposition characteristic of mutations in the hMSH2 gene.

Acknowledgment—We thank P. Sung for discussions.

REFERENCES

1. Fishel, R., Lescoe, M. K., Rao, M. R. S., Copeland, N. G., Jenkins, N. A., Garber, J., Kane, M., and Kolodner, R. (1993) Cell 75, 1027–1038
2. Leach, F. S., Nicolaidis, N. C., Papadopoulos, N., Liu, B. J., et al., Parsons, R., Peltonaki, P., Sistonen, P., Aalto, M., Lappalainen, L., Nyström-Lahti, M., Guan, X.-Y., Zhang, J., Meltzer, P. S., Yu, J.-W., Kao, F.-T., Chen, D. J., Ceresaletti, K. M., Fournier, R. E. K., Tros, S., Lewis, T., Leach, R. J., Naylor, S. L., Weissenbach, J., Medlinski, J.-P., Arvinen, H., Peterson, G. M., Hamilton, S. R., Green, J., Jali, J., Watson, P., Lynch, H. T., Trent, J. M., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. (1993) Cell 75, 1215–1225
3. Parsons, R., Li, G.-M., Longley, M. J., Fang, W.-H., Papadopoulos, N., et al., de la Chapelle, A., Kinzler, K. W., Vogelstein, B., and Modrich, P. (1993) Cell 75, 1227–1236
4. Bronner, C. E., Baker, S. M., Morrison, P. T., Warren, G., Smith, L. G., Lescoe, M. K., Kane, M., Aarabakun, C., Liford, J., Linnblom, A., Tillnergard, P., Blafja, R. J., Godwin, A. R., Ward, D. C., Nordenskjold, M., Fishel, R., Kolodner, R., and Liskay, R. M. (1994) Nature 368, 258–261
5. Papadopoulos, N., Nicolaidis, N. C., Wei, Y. F., Ruben, S. M., Carter, K. C., Rosen, C. A., Hasseltine, W. A., Fleischmann, R. D., Fraser, C. M., Adams, M. D., Venter, J. C., Hamilton, S. R., Peterson, G. M., Watson, P., Lynch, H. T., Peltonaki, P., Meclinski, J.-P., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. (1994) Science 263, 1625–1629
6. Umar, A., Boyer, J. C., Thomas, D. C., Nguyen, D. C., Risiger, J. I., Boyd, J., Ionov, Y., Peruch, M., and Linkel, T. A. A. (1994) J. Biol Chem. 269, 14367–14370
7. Nicolaidis, N. C., Papadopoulos, N., Liu, B., Wei, Y.-F., Carter, K. C., Ruben, S. M., Rosen, C. A., Hasseltine, W. A., Fleischmann, R. D., Fraser, C. M., Adams, M. D., Venter, J. C., Dunlop, M. G., Hamilton, S. R., Peterson, G. M., de la Chapelle, A., Vogelstein, B., and Kinzler, K. W. (1994) Nature 368, 75–80
8. Liu, B., Nicolaidis, N. C., Markowitz, S., Willson, J. K. V., Parsons, R. E., et al., Papadopoulos, N., Pettonaki, P., de la Chapelle, A., Hamilton, S. R., Kinzler, K. W., and Vogelstein, B. (1995) Nat Genet. 9, 48–53
9. Altonen, L. A., Peltonaki, P., Leach, F. S., Sistonen, P., Pykkanen, L., Meclinski, J.-P., Arvinen, H., Powell, S. M., Jen, J., Hamilton, S. R., Peterson, G. M., Kinzler, K. W., Vogelstein, B., and de la Chapelle, A. (1993) Science 260, 812–816
10. Thibodeau, S. N., Bren, G., and Schaid, D. (1993) Science 260, 816–819
11. Ionov, Y., Pelinak, M. A., Makhotyos, S., Shibata, D., and Peruch, M. (1993) Nature 363, 558–561
12. Drummond, J. T., Li, G.-M., Longley, M. J., and Modrich, P. (1995) Science 268, 1909–1912
13. Drummond, J. T., Li, G.-M., Longley, M. J., and Modrich, P. (1995) Science 268, 1915–1917
14. Bhattacharyya, N. P., Skandalis, A., Ganesh, A., Groden, J., and Meuth, M. (1994) Proc Natl Acad Sci USA 91, 6319–6323
15. da Costa, L. T., Liu, B., Wallik, S. E. D., Hamilton, S. R., Kinzler, K. W., Vogelstein, B., Markowitz, S., Willson, K. V., de la Chapelle, A., Downey, K. M., and So, A. G. (1995) Nat Genet. 9, 10–11
16. Strand, M., Prola, T. A., Liskay, R. M., and Peters, T. D. (1993) Nature 365, 274–277
17. Reenan, R. A. G., and Kolodner, R. D. (1992) Genetics 132, 975–985
18. Prola, T. A., Christie, D. M., and Modrich, L. R. (1994) Mol. Cell. Biol. 14, 407–415
19. Johnson, R. E., Koval, G. K., Prakash, L., and Prakash, S. (1995) Science 269, 238–240
20. Fishel, R., Ewel, A., Lee, S., Lescoe, M. K., and Griffith, J. (1994) Science 266, 1403–1405
21. Alani, E., Chi, N. W., and Kolodner, R. D. (1995) Genes & Dev. 9, 234–247
22. Prola, T. A., Pang, Q., Alani, E., Kolodner, R. D., and Liskay, R. M. (1994) Science 265, 1091–1093
23. Strand, M., Earley, M. C., Brou, G. F., and Peters, T. D. (1995) Proc Natl Acad Sci USA 92, 10418–10421
24. New, L., Liu, K., and Brou, G. F. (1993) Mol. Med. Genet. 239, 97–108
25. Lea, D. E., and Coulson, C. A. (1949) J. Genet. 49, 264–285
26. Henderson, S. T., and Peters, T. D. (1992) Mol. Cell. Biol. 12, 2749–2757
Requirement of the Yeast *MSH3* and *MSH6* Genes for *MSH2*-dependent Genomic Stability

Robert E. Johnson, Gopala K. Kovvali, Louise Prakash and Satya Prakash

*J. Biol. Chem.* 1996, 271:7285-7288.
doi: 10.1074/jbc.271.13.7285

Access the most updated version of this article at [http://www.jbc.org/content/271/13/7285](http://www.jbc.org/content/271/13/7285)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 27 references, 16 of which can be accessed free at [http://www.jbc.org/content/271/13/7285.full.html#ref-list-1](http://www.jbc.org/content/271/13/7285.full.html#ref-list-1)