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The Human PMS2L Proteins Do Not Interact with hMLH1, a Major DNA Mismatch Repair Protein[1]

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The human *PMS2* gene encodes one of the bacterial *mutL* homologs that is associated with hereditary nonpolyposis colorectal cancer (HNPCC). One of the interesting features of the *hPMS2* gene is that it is part of a multiple gene family which is localized on chromosome bands 7p22, 7p12-p13, 7q11, and 7q22. Here we report four newly identified *hPMS2*-like (*PMS2L*) genes. All four novel members of the *PMS2L* gene family encode relatively short polypeptides composed of the amino-terminal portion of hPMS2 and are expressed ubiquitously except in the heart. To clarify whether the PMS2L polypeptides contribute to the DNA mismatch repair (MMR) pathway through an interaction with hMLH1, we have performed a yeast two-hybrid assay and an immunoprecipitation study using an *hPMS2* mutant cell line, HEC-1-A. Our results clearly indicate that hMLH1 does not interact with two representative PMS2Ls, whereas the carboxyl-terminal portion of hPMS2, not the amino-terminal portion, does interact with hMLH1. Thus, PMS2Ls are not likely to participate in the MMR pathway through association with hMLH1; they must play some other roles in the living cells.

Key words: immunoprecipitation study, mismatch repair, PMS2L, RT-PCR, yeast two-hybrid assay.

The DNA mismatch repair (MMR) system plays an essential role in the correction of replication errors that have escaped DNA polymerase proofreading mechanisms, mismatches that have arisen from spontaneous chemical base changes such as deamination of 5-methylcytosine, and mispairs that form during genetic recombination of homologous but nonidentical sequences (*1*). Several human mismatch repair proteins have been recently discovered; among these, hMSH2, hMSH3, hMSH6, hMLH1, and hPMS2 are the major players in human MMR system. Biochemical studies have clarified the roles of hMSH2, hMSH3, and hMSH6 in the mismatch recognition reaction. hMSH2 forms heterodimers with either hMSH6 or hMSH3, and the resulting hMSH2-hMSH6 (hMutSα) and hMSH2-hMSH3 (hMutSβ) protein complexes carry different mismatch binding specificities (*2*). hMutSα binds to the G/T mismatch and small extrahelical DNA loops preferentially, whereas hMutSβ shows higher binding affinity to larger extrahelical DNA loops. By analogy to the bacterial MutL homodimer, a heterodimer of hMLH1 and hPMS2 (hMutLα) may interact with either hMutSα or hMutSβ and act as a part of the multiprotein complex (*3*).

We and others previously cloned several genes homologous to *hPMS2*, termed *PMS2L* (*PMS2*-like) genes (*4, 5*). These are small genes with very high similarity to the 5′ portion of *hPMS2*. Most of them contain several conserved motifs among various MutL homologues, such as KELVEN (codons 40 through 45 in *hPMS2*) and GFRGEAL (codons 105 through 111 in *hPMS2*) (*6*). Moreover, the high-level expression of the truncated polypeptide encoded by the

TABLE I. **Nucleotide sequences of the oligomers used for PCR amplification and/or hybridization.**

Primer	Gene/ location	Nucleotide sequence
43-5[a]	PMS2L13	5′-CTGCCGTGCATGTTGGGG-3′
43-21[b]	PMS2L13	5′-TGGTCCAGGCTCTTTGACG-3′
31-M[a]	PMS2L14	5′-CGATGGGAGAATCATCCAGA-3′
31-3[b]	PMS2L14	5′-TGCCGTGATTGTCAGTTTCTT-3′
B-13[a]	PMS2L15	5′-TCGAGGTGAGCTGGGCTC-3′
B-1[b*]	PMS2L15	5′-CCGGAATTCTCAAAGAGGGCGAGCG-3′
71-1[a]	PMS2L16	5′-TCTGGAACTCCGGAGCTCA-3′
71-12[b]	PMS2L16	5′-ACACATCCTAATTTGCGTAGC-3′
PMS2R12[a*]	hPMS2/ exon 2	5′-ATGGCCTTAGCAGGTTCTGT-3′

[a]Oligomers used to amplify each PMS2L cDNA. [b]Internal oligomers used for hybridization with each RT-PCR product. *Oligomers corresponding to the antisense strands. Note that all four PMS2Ls analyzed in this study contained the nucleotide sequence corresponding to PMS2R12. Therefore, PMS2R12 was used in all PCR reactions as the antisense primer.

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Abbreviations: HNPCC, hereditary nonpolyposis colorectal cancer; MLH, MutL homology; MMR, mismatch repair; MSI, microsatellite instability; ONPG, *o*-nitrophenyl-1-thio-β-D-galactopyranoside; RT-PCR, reverse transcriptase-polymerase chain reaction.

hPMS2-134 (an HNPCC-causative germline truncating mutation at codon 134 of the *hPMS2* gene) in an MMR-proficient cell line caused a fatal defect in MMR pathway (7). The hPMS2-134 polypeptide contains the highly conserved amino-terminal domain of hPMS2 and structurally resembles PMS2Ls. These findings suggest a functional role for PMS2L proteins in the MMR system. Furthermore, the germline mutations in the existing MMR genes in about one-third of HNPCC kindreds have not yet been identified. The possibility remains that other genes may be involved in this disease (8), and *PMS2L* genes are among the candidates. To understand the possible role of the PMS2L proteins in human MMR system, we have examined the interaction of the PMS2L proteins with hMLH1 by two different approaches: yeast two-hybrid assay and immunoprecipitation study. Herein we report that hMLH1 does not interact with PMS2Ls but does interact with the carboxyl-terminal portion of hPMS2.

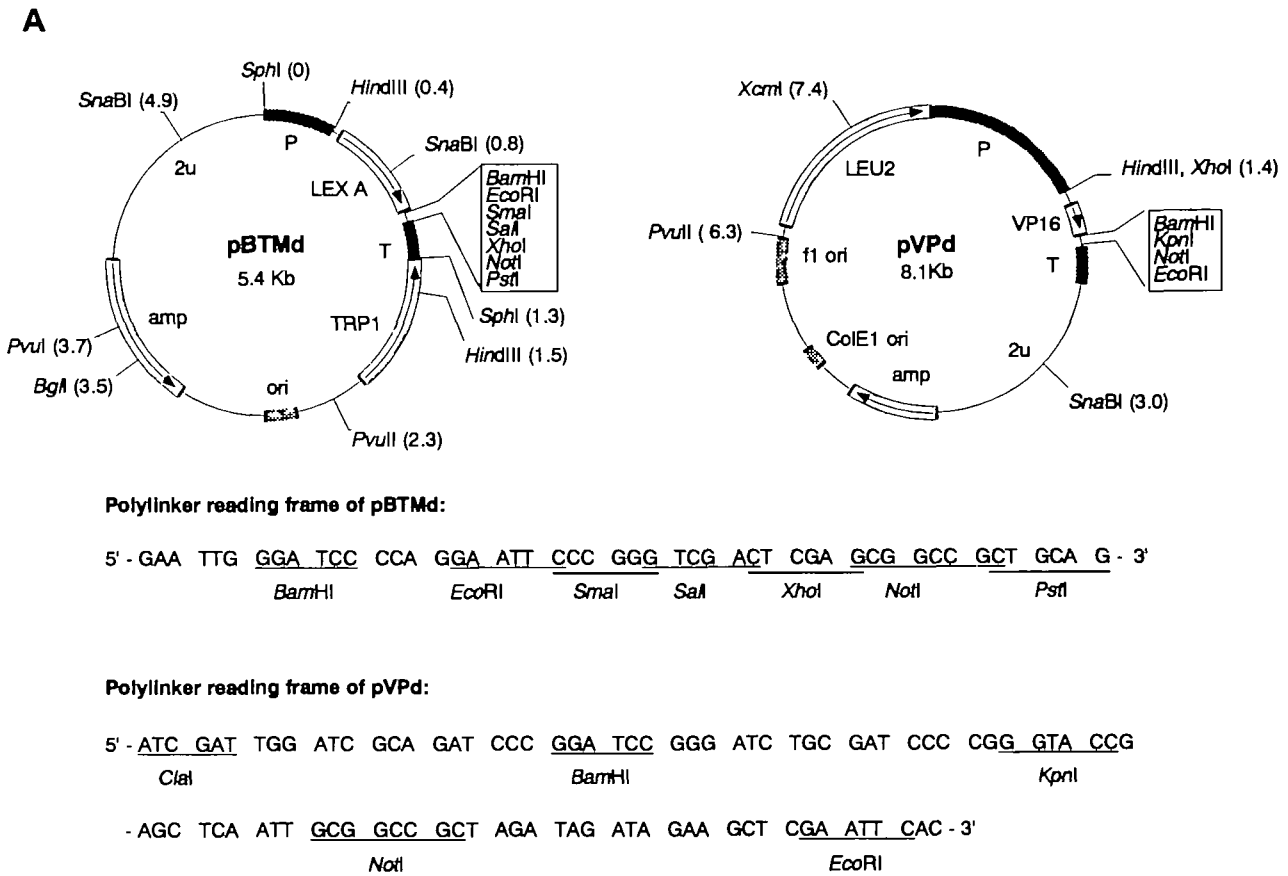

Fig. 1. **Schematic diagrams of yeast two-hybrid vectors and of the various DNA fragments analyzed in the present study.** (A) The lexA fusion vector pBTMd and the activation domain vector pVPd are shown. pBTMd is a derivative of pBTM116 carrying the alcohol dehydrogenase (ADH) promoter (P) to express bait proteins as fusions to the DNA-binding protein lexA. Several restriction sites immediately upstream of the ADH terminator (T) are available for insertion of coding sequences. pVPd is a derivative of pVP16 containing a nuclear localization signal (NLS)-VP16-linker unit driven by the ADH promoter (P). Numbers indicate relative map positions. Reading frames of the polylinker sites for pBTMd and pVPd are shown at the bottom. (B) Schema of the six DNA fragments cloned into pBTMd and pVPd vectors are shown. Each DNA fragment was inserted into the polylinker site of one of the two vectors and was fused in-frame with the lexA DNA-binding domain or the VP16 activation domain. For *PMS2L13* and *PMS2L16*, regions homologous to *hPMS2* are indicated by filled boxes.

A

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PMS2L13                    AAPAVH VGEPVHAGGL HTERGADPVI GLYLVHRGGA   36
PMS2L8                       MAQPKQE RVARARHQRS ETARHQRSET   27
PMS2L9     MCPTRPRLGR RCMVSPREAD LGPQKDTRLD LPRSPARAPR EQNSLGEVDR   50
PMS2L11                        MEKLS AASGYSDVTD SKAMGPLAVG   25

PMS2L13    CQTPTVGNRQ TPTLGI---- ----HARPRR RATTSLLTLL LAFGKNAVRC   78
PMS2L8     AKTPTLGNRQ TPTLGNRQTP RLGIHARPRR RATTSLLTLL LAFGKNAVRC   77
PMS2L9     RGPREQTRAP ATAAPPRPLG SRGAEAAEPQ EGLSATVSAC FQEQQEMNTL  100
PMS2L11    CLTKCSHAFH LLCLLAMYCN GNKGPEHPNP GKPFTARGFP ASATFQTTPG   75

PMS2L14      LDTCLITM GESSRKPPTP TPEGPTVSVK QLFSTLPVRH KEFQRNIKKK   48
PMS2L13    ALIGPGSLTS RTRPLTEPLG EKERREVFFP PRPERVEHNV ESSRWEPRRR  128
PMS2L8     ALIGPGSLTS RTRPLTEPLG EKERREVFFP PRPERVEHNV ESSRWEPRRR  127
PMS2L15                  ELRA RGELGSRSPL SLALFENHGF RPPTKWGEHG   34
PMS2L9     QGPVSFKDVA VDFTQEEWRQ LDPDEKIAYG DVMLENYSHL VSVGYDYHQA  150
PMS2L11    PQASRGFQNP ETLADIPASP QLLTDGHYMT LPVSPDQLPC DDPMAGSGGA  125

hPMS2                          MERAESSST EPAKAIKPID RKSVHQICSG   29
PMS2L3                           SST EPAKAIKPID RKSVHQICSG   23
PMS2L14    RACFPFAFCR DCQFLEGSPA MLPVQPAKLT EPAKAIKPID RKSVHQICSG   98
PMS2L13    GACGSRGGNF PSPRGGSGVA SLERAESSST EPAKAIKPID RKSVHQICSG  178
PMS2L8     GACGSRGGNF PSPRGGSGVA SLERAENSST EPAKAIKPID RKSVHQICSG  177
PMS2L1         RGGNF PSPRGGSGVA SLERAESSST EPAKAIKPID RKSVHQICSG   45
PMS2L2             F SSSRGGSGVA SLERAESVST EPAKAIKPID RKSVHQICSG   41
PMS2L16                        MHFYLLGT EPAKAIKPID RKSVHQICSG   28
PMS2L15    RGAVEAASVR PRSGILSAPA IRCPLDFFST EPAKAIKPID RKSVHQICSG   84
PMS2L9     KHHHGVEVKE VEQGEEEPTM EGEFPCQHSP EPAKAIKPID RKSVHQICSG  200
PMS2L6                    ILA LFLVTDFFST EPAKAIKPID RKSVHQICSG   33
PMS2L5                                  AKAIKPID RKSVHQICSG   18
PMS2L4                       RVLS PMSELRARGT EPAKAIKPID RKSVHQICSG   34
PMS2L11    PVLRVGHDHG CHQQPRICNA PLPGPGPYRT EPAKAIKPID RKSVHQICSG  175

hPMS2      QVVLSL-STA VKELVENSLD AGATNIDLKL KDYGVDLIEV SDNGCGVEEE   78
PMS2L3     PVVLSL-STA VKELVENSLD AGATNIDLKL KDYGVDLIEV SDNGCGVEEE   72
PMS2L14    PVVLSL-STA VKKIVGNSLD AGATNIDLKL KDYGMDLIEV SGNGCGVEEE  147
PMS2L13    PVVPSL-STA VKELVENSLD AGATNIDLKL KDYGVDLIEV SGNGCGVEEE  227
PMS2L8     PVVPSLRPNA VKELVENSLD AGATNVDLKL KDYGVDLIEV SGNGCGVEEE  227
PMS2L1     PVVPSL-STA VKELVENSLD AGATNIDLKL KDYGVDLIEV SGNGCGVEEE   94
PMS2L2     PVVLSL STA VKKIVENSLD AGATNIDLKL KDYGVDLIEV SGNGCGVEEE   90
PMS2L16    PVVLSL STA VKKIVGNSLD AGATNIDLKL KDYGMDLIEV SGNGCGVEEE   77
PMS2L15    PVVLSL-STA VKKIVGNSLD AGATNIDLKL KDYGMDLIEV SGNGCGVEEE  133
PMS2L9     PVVLSL-STA VKELVENSLD AGATNIDLKL KDYGVDLIEV SDNGCGVEEE  249
PMS2L6     PVVPSL-STA VKELVENSLD AGATNIDLKL KDYGVDLIEV SGNGCGVEEE   82
PMS2L5     PVVLSL STA VKKMVENSLD AGATNIDLKL KDYGMDLIEV SGNGCGVEEE   67
PMS2L4     PVVLSL STA VKELVENSLD AGATNIDLKL KDYGVDLIEV SDNGCGVEEE   83
PMS2L11    PVVLSL STA VKELVENSLD AGATNIDLKL KDYGMDLIEV SGNGCGVEEE  224

hPMS2      NFEGLT-- L KHHTSKIQEF ADLTQVETFG FRGEALSSLC ALSDVTISTC  125
PMS2L3     NFEGLTLSAL KHHTCKIQEF ADLTEVETFG FQGEALSSLC ALSDVTISTC  122
PMS2L14    NFEGLSLSAL KHHTSKIREF ADLTRVETFG FQGKALSSLC ALSDVTISTC  197
PMS2L13    NFEGLT---L KHHTSKIQEF ADLPQVETFG FRGEALSSLC ALSDVTISTC  274
PMS2L8     NFEGFT---L KHHTCKIQEF ADLTQVETFG FRGEALSSLC ALSDVTISTC  274
PMS2L1     NFEGLT---L KHHTSKIQEF ADLPQVETFG FRGEALSSLC ALSDVTISTC  141
PMS2L2     NFEGLT---L KHHTSKIQEF ADLPQVETFG FRGEALSSLC ALSDVTISTC  137
PMS2L16    NFEGLISFSS ETSHI*                                        92
PMS2L15    NFEGLISFSS ETSHI*                                       148
PMS2L9     NFEGLISFSS ETSHM*                                       264
PMS2L6     NFEGLSKLTF SNPIIK*                                       98
PMS2L5     NFEGLMMSPF LPATYRRRLG LDTCLITMGK SSRKPPTPTP EDHSQREAVI  117
PMS2L4     NFEGLTMSPF LPATHRRRLG LDTCLITMGK SSRKPPTPTP EDHSQREAVI  133
PMS2L11    NFEGLMMSPF LPATSRRRLG LDTCLITMGK SSRRPPTPTP EG-PQSA*    270

hPMS2      HASAKVGTRL MFDHNGKIIQ KTPYPRPRGT TVSVQQLFST LPVRHKEFQR  175
PMS2L3     HASVKVGTRL VFDHDGKIIQ ETPYPPPQRT TVSVKQLFST LPVRHKEFQR  172
PMS2L14    HVSAKVGTRL VFDHDGKIIK KTPYPHPRGT TVSVKQLFST LPVRHKEFQR  247
PMS2L13    HVSAKVGTRL VFDHYGKIIQ KTPYPHPRGM TVSVKQLFST LPVHHKEFQR  324
PMS2L8     RVSAKVGTRL VFDHYGKIIQ KTPYPRPRGM TVSVKQLFST LPVHHKEFQR  324
PMS2L1     HVSAKVGTRL VFDHYGKIIQ KTPYPHPRGM TVSVKQLFST LPVHHKEFQR  191
PMS2L2     HVSAKVGTRL VFDHDGKIIQ KTPYPHPRGT TVSVKQLFST LPVHHKEFQR  187
PMS2L5     --- ----- ---------- ----- --- ----- ST YCAHK-EFQR  128
PMS2L4           --  -  -  --       --- ST LPVHHKEFQR  145
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hPMS2      NIKKEYAKMV QVLHAYCIIS AGIRVSCTNQ LGQGKRQPVV CTGGSPSIKE  225
PMS2L3     NIKKTCLLPL RLLP*                                        186
PMS2L14    NIKKKRACFP FAFCRDCQFL EGSPAMLPVQ PAKLTVTGEL RACRSMKTRE  297
PMS2L13    NIKKKRACFP FAFCRDCQFP EASPAMLPVQ PAELTPRSTP PHPCSLEDNV  374
PMS2L8     NIKKKRACFP FAFCRDCQFP EASPAMLPVQ PVELTPRSTP PHPCSLEDNV  374
PMS2L1     NIKKKRACFP FAFCRDCQFP EASPAMLPVQ PAELTPRSTP PHPCSLEDNV  241
PMS2L2     NIKKKRACFP FAFCRDCQFL EGSPAMLPVQ PAKLTPRSTP PHPCSLEDNV  237
PMS2L5     NIKKKRACFP FAFCRIVSFL RAPQPCFLYS LQN*                   161
PMS2L4     NIKKTCLFPL RLLP*                                        159

hPMS2      NIGSVFGQKQ LQSLIPFVQL PPSDSVCEEY GLSCSDALHN LFYISGFISQ  275
PMS2L14    GITEAVG*                                                304
PMS2L13    ITVFSSVKNG PGSSR*                                       389
PMS2L8     ITVFSSVKNG PGSSR*                                       389
PMS2L1     ITVFSSVKNG PGSSR*                                       258
PMS2L2     ITVFSSVKNG PGSSR*                                       252

hPMS2      CTHGVGRSST DRQFFFINRR PCDPAKVCRL VNEVYHMYNR HQYPFVVLNI  325

hPMS2      SVDSECVDIN VTPDKRQILL QEEKLLLAVL KTSLIGMFDS DVNKLNVSQQ  375

hPMS2      PLLDVEGNLI KMHAADLEKP MVEKQDQSPS LRTGEEKKDV SISRLREAFS  425

hPMS2      LRHTTENKPH SPKTPEPRRS PLGQKRGMLS SSTSGAISDK GVLRPQKEAV  475

hPMS2      SSSHGPSDPT DRAEVEKDSG HGSTSVDSEG FSIPDTGSHC SSEYAASSPG  525

hPMS2      DRGSQEHVDS QEKAPETDDS FSDVDCHSNQ EDTGCKFRVL PQPTNLATPN  575

hPMS2      TKRFKKEEIL SSSDICQKLV NTQDMSASQV DVAVKINKKV VPLDFSMSSL  625

hPMS2      AKRIKQLHHE AQQSEGEQNY RKFRAKICPG ENQAAEDELR KEISKTMFAE  675

hPMS2      MEIIGQFNLG FIITKLNEDI FIVDQHATDE KYNFEMLQQH TVLQGQRLIA  725

hPMS2      PQTLNLTAVN EAVLIENLEI FRKNGFDFVI DENAPVTERA KLISLPTSKN  775

hPMS2      VTFGPQDVDE LIFMLSDSPG VMCRPSRVKQ MFASRACRKS VKIGTALNTS  825

hPMS2      EMKKLITHMG EMDHPWNCPH GRPTMRHIAN LGVISQN*               862
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Fig. 2. **Characterization of *PMS2L* cDNA clones.** (A) Alignment of the deduced amino acid sequences of *hPMS2* and *PMS2L* genes. Underlines indicate conserved amino acid sequences common to all PMS2L polypeptides. Asterisks show termination codons. (B) Schematic diagram of the encoded polypeptides of PMS2Ls compared to hPMS2. The shaded areas indicate regions of hPMS2 similarity among various PMS2Ls. The numbered sections within hPMS2 indicate its exons. Based on the deduced amino acid sequences, the *PMS2L* genes were divided into two groups, A and B. Group A contains exons 1 through 5 (residues 1 through 179) of the *hPMS2* gene (*PMS2L1, 2, 3, 8, 13*, and *14*), and group B contains exons 2 and 3 (residues 9 through 83) of the *hPMS2* gene (*PMS2L4, 5, 6, 9, 11, 15*, and *16*). Group B was further divided into subtypes B-1 (*PMS2L6, 9, 15*, and *16*) and B-2 (*PMS2L4, 5*, and *11*) according to the homology of the encoding amino acid sequences of the carboxyl-termini.

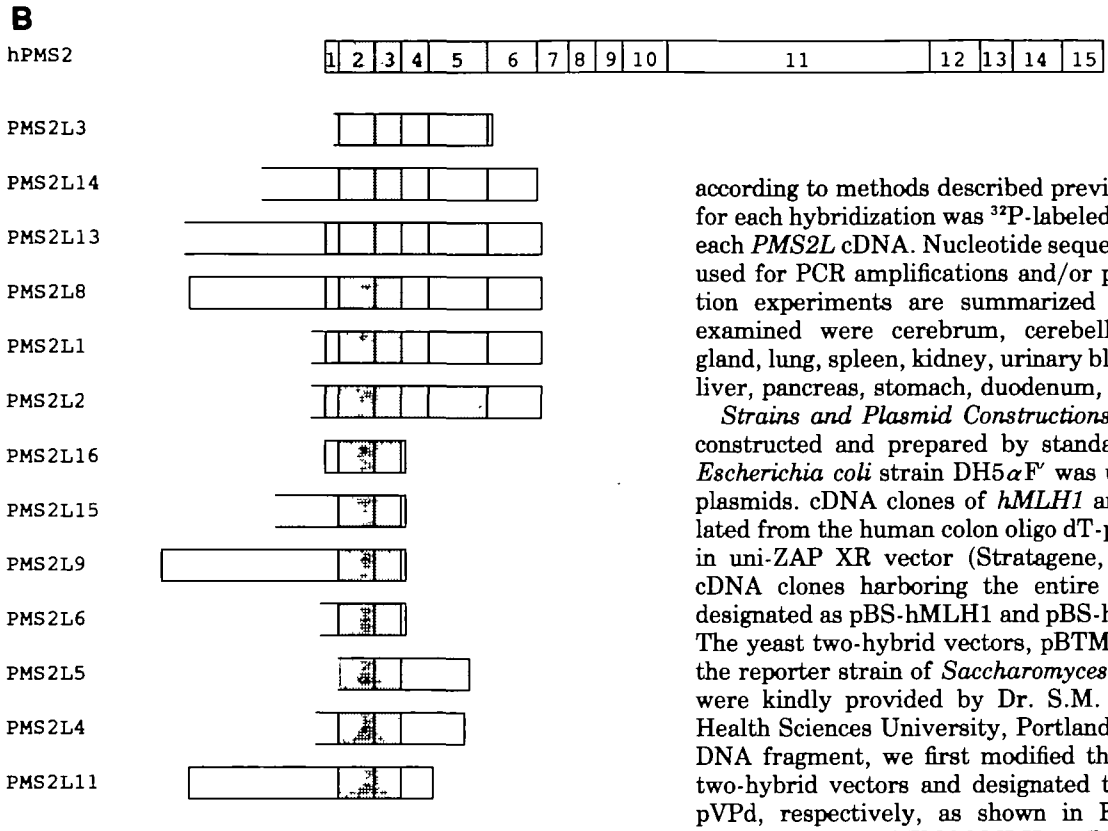

Fig. 2B

MATERIALS AND METHODS

Isolation of PMS2L cDNAs—A total of 1×10^6 clones from a human fetal brain oligo dT- and random-primed cDNA library in λgt11 (Clontech, Palo Alto, CA) was screened with an α-^{32}P-labeled cDNA fragment cut out from the *PMS2L1* gene that corresponded to exons 2 and 3 of the *hPMS2* gene. Filters were hybridized at 65°C overnight, washed twice in 2×SSC at 65°C for 15 min, and autoradiographed overnight at −80°C with intensifying screens. Following plaque purification, insert DNAs from positive clones were excised by digestion with *Eco*RI, subcloned into pBluescript II SK(+), and subjected to DNA sequencing by the dideoxy chain termination method with T3, T7, or synthetic gene specific oligonucleotide primers. The DNA sequence of each *PMS2L* cDNA clone was analyzed using a Thermo Sequenase core sequencing kit with 7-deaza-GTP (Amersham, Little Chalfont, UK) and a Hitachi SQ-5500 DNA sequencer (Hitachi Electronics Engineering, Tokyo) according to suppliers' recommendations.

Analysis of the Expression of PMS2Ls by RT-PCR—RNA was purified from various tissues using a guanidine isothiocyanate-based method. Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed according to methods described previously (*9*). DNase I pretreatment (0.24 U/μl for 15 min at rt) was also performed. PCR amplification was performed at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s for 40 cycles. Reaction products were separated by electrophoresis in 2% agarose gels and transferred to nylon membranes for Southern hybridization according to methods described previously (*9*). The probe for each hybridization was ^{32}P-labeled oligomer specific for each *PMS2L* cDNA. Nucleotide sequences of the oligomers used for PCR amplifications and/or probes for hybridization experiments are summarized in Table I. Organs examined were cerebrum, cerebellum, heart, adrenal gland, lung, spleen, kidney, urinary bladder, ovary, uterus, liver, pancreas, stomach, duodenum, and colon.

Strains and Plasmid Constructions—All plasmids were constructed and prepared by standard techniques (*10*). *Escherichia coli* strain DH5αF′ was used to propagate all plasmids. cDNA clones of *hMLH1* and *hPMS2* were isolated from the human colon oligo dT-primed cDNA library in uni-ZAP XR vector (Stratagene, La Jolla, CA), and cDNA clones harboring the entire coding region were designated as pBS-hMLH1 and pBS-hPMS2, respectively. The yeast two-hybrid vectors, pBTM116 and pVP16, and the reporter strain of *Saccharomyces cerevisiae*, L40 (*11*) were kindly provided by Dr. S.M. Hollenberg (Oregon Health Sciences University, Portland, OR). To clone each DNA fragment, we first modified the polylinker sites of two-hybrid vectors and designated them as pBTMd and pVPd, respectively, as shown in Fig. 1A. Altogether, plasmid clones pBTMd-hMLH1, pBTMd-hPMS2, pVPd-hMLH1, and pVPd-hPMS2 were constructed. Derivatives containing some portions of the cDNAs (see Fig. 1B) were also constructed.

Yeast Transformation and β-Galactosidase Assays—Yeast transformation was performed by the polyethylene glycol-lithium acetate method (*12*). For the color filter assay, colonies with pairing hybrids were lifted on nylon membranes, frozen in liquid nitrogen, and incubated at 30°C on Whatman 3MM paper soaked with Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, 50 mM 2-mercaptoethanol, pH 7.0). To measure β-galactosidase activity, 0.1 ml of the cell mixture was added to 0.9 ml of Z buffer, permeabilized with two drops of chloroform and one drop of 0.1% SDS, and combined with 0.2 ml of 4 mg/ml *o*-nitrophenyl-1-thio-β-D-galactopyranoside (ONPG) substrate (*13*). Reactions were stopped with Na_2CO_3 when an appropriate level of color had developed. β-Galactosidase activity was calculated by the following formula: $U=1{,}000\times[(OD_{420})-(1.75\times OD_{550})]/(t)\times(v)\times(OD_{600})$. The protein concentration was measured by the Bradford protein assay (Bio-Rad, Hercules, CA) with bovine serum albumin as the standard.

Immunoprecipitation Study—A unique *Sma*I site of a mammalian expression vector pFLAG-CMV-2 (Eastman Kodak, Scientific Imaging Systems, New Haven, CT) was replaced by a *Xho*I site by linker insertion, and the resulting vector was designated as pFLAG-CMV-2d. hPMS2, hPMS2(1-261), hPMS2(203-862), PMS2L13(1-179), and PMS2L16(9-83) (see Fig. 1B) were subcloned into pFLAG-CMV-2d and used to transfect a *hPMS2* mutant endometrial adenocarcinoma cell line HEC-1-A (ATCC, Rockville, MD) (*14*). Cells were maintained in McCoy's 5a medium supplemented with 10% fetal bovine

serum. Transfection of DNA into cells grown in 10-cm dishes using Lipofectamine Reagent (Life Technologies, Gaithersburg, MD) was performed according to the supplier's recommendation. At 48 h post-transfection, the cells were washed with 8 ml of ice-cold PBS(−) and recovered by scraping. The cells were collected by centrifugation at 3,000 rpm for 5 min at 4°C and lysed in 200 μl of NP-40 lysis buffer (1% NP-40, 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 1 mM PMSF, 1% aprotinin). The cell extracts were divided into two aliquots: 10 μl for Western blotting and 190 μl for immunoprecipitation. For Western blotting, the sonicated cell extracts were boiled for 5 min, electrophoresed on 6 or 15% SDS-polyacrylamide gels, and blotted onto a Clear blot membrane-P (Atto, Tokyo). The blots were then probed with a 1:50 diluted anti-hPMS2 monoclonal antibody PMS2(Ab-1) (Oncogene Science, Cambridge, MA) or with a 1:300 diluted anti-FLAG monoclonal antibody M2 (Eastman Kodak). The sheep anti-mouse horseradish peroxidase-conjugated immunoglobulin G antibody (Amersham International PLC, Buckinghamshire, UK) was used as secondary antibody with a 1:1,000 dilution. The blot was visualized with enhanced chemiluminescence detection (Amersham International PLC). For immunoprecipitation, the cells were kept on ice for 20 min and pelleted at 12,000 rpm for 5 min at 4°C. The supernatant was carefully removed and incubated with 3.5 μl of 2.8 μg/μl anti-FLAG M2 monoclonal antibody at 4°C overnight. The immunoprecipitates were then incubated with 20 μl of protein G-Sepharose (Pharmacia-LKB Biotechnologies, Uppsala, Sweden) for 3 h at 4°C and pelleted at 3,000 rpm for 5 min at 4°C. The immunoprecipitates were washed three times with NP-40 lysis buffer, suspended in 20 μl of sample buffer (125 mM Tris-HCl, pH 6.8, 10% 2-mercaptoethanol, 20% glycerol, 4.6% SDS, 0.0025% BPB), and denatured by boiling for 5 min. Denatured samples were resolved by SDS-PAGE (6 or 15% acrylamide) and electroblotted onto a Clear blot membrane-P. The membranes were probed with a 1:500 diluted anti-hMLH1 monoclonal antibody G168-728 (Pharmingen, San Diego, CA) and immune complexes were visualized using enhanced chemiluminescence detection.

RESULTS

We and others previously isolated and characterized *PMS2L* (*PMS2*-like) genes (*4, 5*). However, entire coding sequences have been reported for only a small number of genes. Moreover, the total number of *PMS2L* genes in the human genome is unclear, as are the functions of their protein products. To isolate and characterize the *PMS2L* genes further, a human fetal brain cDNA library was screened with a DNA fragment from the *PMS2L* conserved region corresponding to exons 2 and 3 of the *hPMS2* gene as the probe. Thirty-two cDNA clones were obtained: among these, four similar but distinct cDNA clones were selected because these four harbored an ORF or a strong candidate for an ORF. Nucleotide sequences of these four clones differed from those of *hPMS2* and *PMS2L1* through *PMS2L12*, so we designated these as *PMS2L13* through *PMS2L16*. The deduced amino acid sequences and polypeptide structures of 13 *PMS2L* genes are shown in Fig. 2. Three *PMS2Ls*, *PMS2L7*, *PMS2L10*, and *PMS2L12*, are excluded from the figure because their sequences are not available from the database. Sequence analyses of newly isolated cDNA clones suggested that only *PMS2L13* or *PMS2L16* harbored a poly(A) tract or an in-frame methionine preceded by an in-frame stop codon, respectively. As shown in Fig. 2, all 13 *PMS2L* genes encode relatively small polypeptides composed of amino-terminal regions of hPMS2 and share at least 96% identity in nucleotides and 91% identity in amino acids with *hPMS2* at codons 9 through 83 (encoded by exons 2 and 3). Based on the deduced amino acid sequences, these genes were roughly divided into two groups, A and B: group A consists of *PMS2L1, 2, 3, 8, 13*, and *14*, and group B, *PMS2L4, 5, 6,*

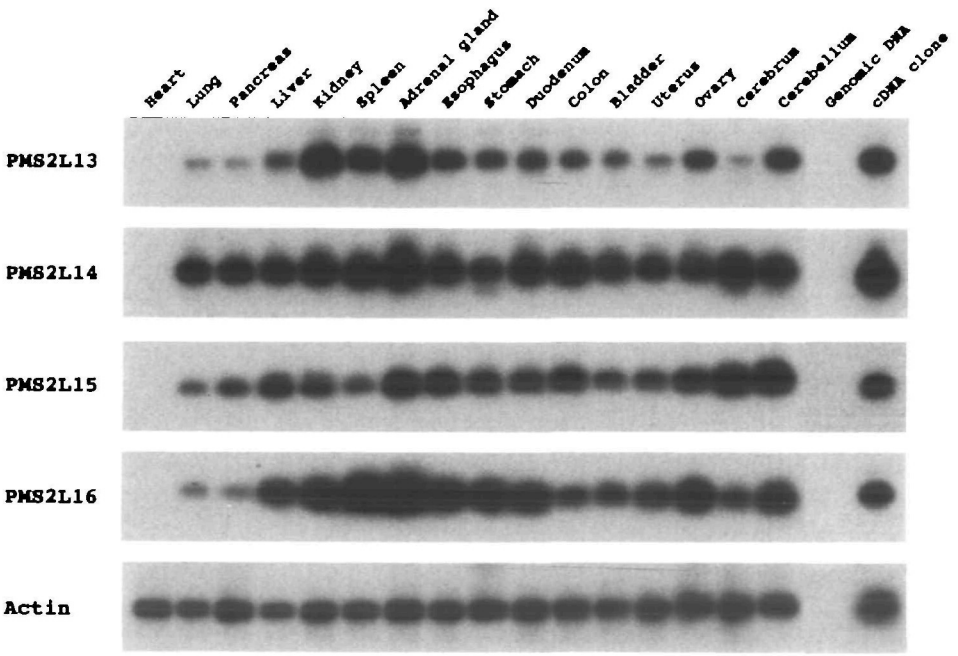

Fig. 3. **Expression profile of the four *PMS2L* genes by RT-PCR in normal human tissues.** All tissues except heart expressed every *PMS2L* transcript. Probes used for detection of *PMS2L13, PMS2L14, PMS2L15*, and *PMS2L16* were 43-21, 31-3, B-1, and 71-12, respectively (see Table I). Expression of the actin gene was monitored as a control. Autoradiography was performed for 15, 90, 240, 240, and 15 min with intensifying screens for *PMS2L13, PMS2L14, PMS2L15, PMS2L16*, and *actin*, respectively.

9, 11, 15, and *16* (see Fig. 2).

With the exception of *PMS2L14*, the group A genes had significant homology with *hPMS2* at codons 1 through 179 (exons 1 through 5). *PMS2L8* and *PMS2L13* had significant homology in the entire region (*PMS2L8* codons 50 through 389), with 99% identity in nucleotides and 97% identity in amino acids. Four *PMS2Ls* of this group, *PMS2L1, 2, 8*, and *13*, carry the same carboxyl termini. Group B had a significant homology with *hPMS2* at codons 9 through 83 (encoded by exons 2 and 3) and was further divided into two subtypes, B-1 and B-2, according to the homology of the amino acid sequences of the carboxyl termini. In group B-1, the deduced polypeptides terminate at 10 or 11 amino acids after the homologous region to *hPMS2* in exon 3. Two *PMS2Ls* of group B-1, *PMS2L15* and *16*, carry the same carboxyl-terminal domain: the last five amino acids were ETSHI. Group B-2 is composed of three *PMS2Ls, PMS2L4, 5*, and *11*, and the carboxyl-terminal domain is longer than in group B-1. Although belonging to different groups, *PMS2L3* and *PMS2L4* share the same carboxyl terminus. To guess the possible function of PMS2Ls, we have also searched the database for portions of PMS2L outside of the homologous region to hPMS2, but no significant homology with known proteins was found.

Expression of *PMS2L13* through *PMS2L16* was monitored using mRNA samples prepared from a variety of human tissues. Figure 3 shows the results of RT-PCR: *PMS2L13* through *PMS2L16* genes were expressed in all tissues except heart.

To examine the possible role of PMS2Ls in the MMR

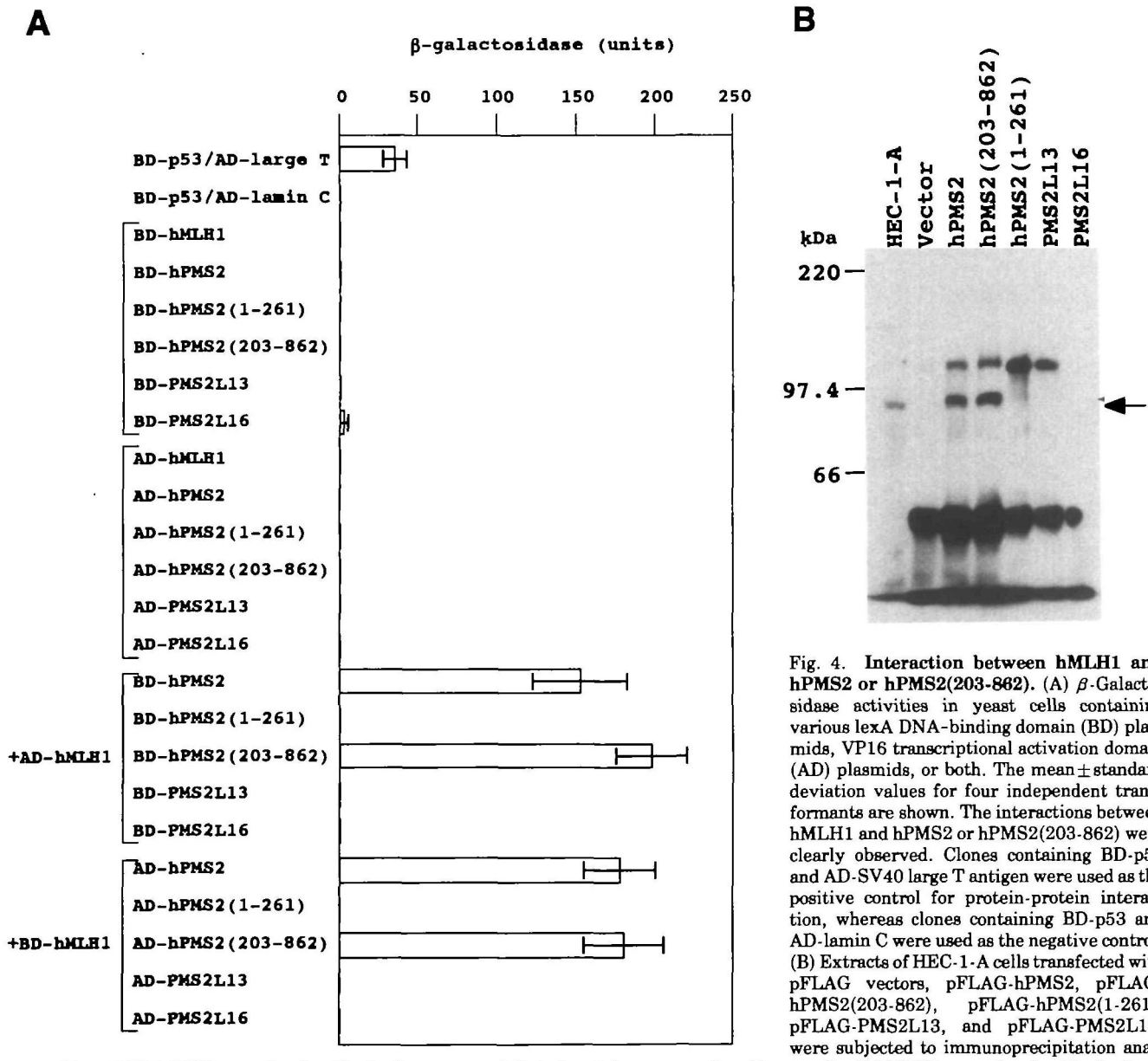

Fig. 4. **Interaction between hMLH1 and hPMS2 or hPMS2(203-862).** (A) β-Galactosidase activities in yeast cells containing various lexA DNA-binding domain (BD) plasmids, VP16 transcriptional activation domain (AD) plasmids, or both. The mean ± standard deviation values for four independent transformants are shown. The interactions between hMLH1 and hPMS2 or hPMS2(203-862) were clearly observed. Clones containing BD-p53 and AD-SV40 large T antigen were used as the positive control for protein-protein interaction, whereas clones containing BD-p53 and AD-lamin C were used as the negative control. (B) Extracts of HEC-1-A cells transfected with pFLAG vectors, pFLAG-hPMS2, pFLAG-hPMS2(203-862), pFLAG-hPMS2(1-261), pFLAG-PMS2L13, and pFLAG-PMS2L16, were subjected to immunoprecipitation analyses with anti-FLAG M2 monoclonal antibody. Immunoprecipitated proteins were analyzed by use of anti-hMLH1 monoclonal antibody. Lane HEC-1-A represents 10 μg of the cell extract that was subjected to immunoblotting without prior immunoprecipitation as the control. The arrow indicates the position of the hMLH1 protein.

system, we analyzed the interactions between hMLH1 and the protein product of the newly isolated *PMS2L* genes by two methods: yeast two-hybrid assay and immunoprecipitation study. Two PMS2Ls, PMS2L13 and PMS2L16, were used as representative proteins of the two groups of *PMS2Ls*. We first constructed a series of lexA DNA-binding domain (BD) bait plasmids: pBTMd-hMLH1, pBTMd-hPMS2, pBTMd-hPMS2(1-261), pBTMd-hPMS2(203-862), pBTMd-PMS2L13(1-179), and pBTMd-PMS2L16 (9-83). The same inserts were also subcloned into the VP16 activation domain (AD) plasmid, pVP16d. Transformation of yeast strain L40 with these BD and AD plasmids and filter β-galactosidase assay of the resulting transformants showed that hMLH1 interacted with hPMS2 and hPMS2 (203-862) but not with hPMS2(1-261), PMS2L13(1-179), or PMS2L16(9-83), as summarized in Fig. 4A. These results were confirmed by independent experiments in which each insert in the BD and AD vectors was replaced. We next measured the β-galactosidase activity using *o*-nitrophenyl-1-thio-β-D-galactopyranoside (ONPG) as the substrate. As shown in Fig. 4A, β-galactosidase activity of the combinations of BD-hMLH1 and AD-hPMS2, BD-hMLH1 and AD-hPMS2(203-862), BD-hPMS2 and AD-hMLH1, and BD-hPMS2(203-862) and AD-hMLH1 were 177.6 ± 21.9U, 179.2 ± 25.8U, 155.3 ± 30.7U, and 197.5 ± 21.8U, respectively. Given that the β-galactosidase activity of BD-p53 and AD-large T antigen used as a positive control was 35.7 ± 7.6U, the interaction of hMLH1 and hPMS2 or hPMS2(203-862) was very strong in yeast. To examine whether hMLH1 and hPMS2 or hPMS2 (203-862) are associated in mammalian cells, *hPMS2* mutant HEC-1-A cell lines were transfected with FLAG-tagged hPMS2, hPMS2(1-261), hPMS2(203-862), PMS2L13(1-179), or PMS2L16(9-83) expression vectors. Protein complexes associated with endogenous hMLH1 were first immunoprecipitated with the anti-FLAG monoclonal antibody, and the bound proteins were subsequently analyzed by immunoblotting with an hMLH1-specific antibody. To clarify the position of the hMLH1 protein in SDS-PAGE, we also loaded cell extract of the HEC-1-A on the same gel (see Fig. 4B). As shown in Fig. 4B, this immunoprecipitation study clearly demonstrated that hMLH1 forms protein complexes *in vivo* with hPMS2 and hPMS2(203-862) but shows no interaction with hPMS2(1-261), PMS2L13(1-179), or PMS2L16(9-83).

DISCUSSION

The DNA mismatch repair (MMR) system is essential in living organisms and is evolutionarily highly conserved. In *E. coli*, a complex of three proteins, MutS, MutL, and MutH, plays a major role in the MMR system, while in mammalian cells, many functionally differentiated homologues of the MutS and MutL proteins work cooperatively and form a complex and elaborate MMR system. In the human MMR system, three MutS homologues, hMSH2, hMSH3, and hMSH6, form two different heterodimers, designated hMutSα and hMutSβ, and are involved in the mismatch recognition pathway with different mismatch-binding specificities (*2*). On the other hand, two MutL homologues, hMLH1 and hPMS2, also form a heterodimer, designated hMutLα, which is anticipated to interact with either mismatch-bound hMutSα or hMutSβ (*3*).

The most highly conserved region of the MutL homologues is confined to an 80 amino acid stretch in the amino-terminal region in hPMS2 at residues 40-119, the MutL homology (MLH) domain. Although this region is approximately 50% identical in most MutL homologues, its biological function is not yet known. Among the MutL homologues, the *hPMS2* gene constitutes a huge gene family on human chromosome 7; at least 13 *hPMS2*-like (*PMS2L*) genes have been reported. To clarify the role of PMS2L proteins in normal cells and their involvement in human cancers, we first screened *PMS2L* genes by a hybridization approach using the highly conserved domain as the probe and isolated 4 novel *PMS2L* genes. We then compared their amino acid sequences with those of the previously isolated *PMS2L* genes and divided the *PMS2Ls* into two groups: group A contains exons 1 through 5 (residues 1 through 179) of the *hPMS2* gene, and group B contains exons 2 and 3 of the *hPMS2* gene (residues 9 through 83).

To understand the possible involvement of PMS2Ls in the MMR system, we examined their interactions with hMLH1, the partner of hPMS2 in the human MMR system. However, we could not find any association between these PMS2Ls and hMLH1. Results from the yeast two-hybrid assay and the immunoprecipitation study using the *hPMS2*-deficient cell line HEC-1-A clearly indicated that hMLH1 interacts with the carboxyl-terminal domain of hPMS2. Our results are also consistent with those recently reported in *Saccharomyces cerevisiae* (*15*) and in man (*7*) from immunoprecipitation studies of *in vitro*-translated proteins: the MLH1-interacting domain of PMS2 (PMS1 in the case of yeast) was localized at the carboxyl terminus.

It has recently been reported that a nonsense mutation at codon 134 of the *hPMS2* gene is sufficient to reduce MMR and induce MSI in cells containing a wild-type *hPMS2* allele (*7*). This experimental result indicates that the highly conserved amino-terminal domain of hPMS2 may have an important role in the downstream pathway, but not through interaction with hMLH1, in the MMR system. Since PMS2Ls carry a highly conserved amino-terminal domain, they may also be involved in the downstream pathway of the human MMR system or they may have completely different role(s) in the cell. If the former possibility is true, the abnormal expression of some *PMS2Ls* may give rise to defects in the MMR pathway. Analysis of the proteins interacting with PMS2Ls may lead to the elucidation of the function of PMS2Ls or of the downstream pathway in the MMR system.

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