GENOMICS 36, 288–295 (1996)
ARTICLE NO. 0464

cDNA Sequence, Map, and Expression of the Murine Homolog of *GTBP,* a DNA Mismatch Repair Gene

A. CORRADI, L. CROCI, C. L. STAYTON, M. GULISANO, E. BONCINELLI, AND G. G. CONSALEZ[1]

Department of Biological and Technological Research (DIBIT), San Raffaele Scientific Institute, via Olgettina 58, 20132 Milan, Italy

Received February 14, 1996; accepted May 9, 1996

DNA mismatch repair genes (MMR) have been actively studied in humans for their role in cancer susceptibility. Recently, *GTBP,* a new member of the MMR gene family, has been cloned and found to encode a 160-kDa protein that forms a heterodimer with hMSH2. Our group has isolated and characterized the mouse homolog of *GTBP.* The transcript, termed *Gtmbp* (G/T mismatch-binding protein), encodes a protein highly similar to its human counterpart. The gene is markedly conserved across species and maps to the distal portion of mouse chromosome 17, in a region homologous to human 2p. One *Gtmbp*-related sequence maps to proximal mouse chromosome 4. Studies of the expression of *Gtmbp* suggest that its regulated transcription is tightly linked to the level of DNA replication, consistent with the protein's DNA proofreading function.

INTRODUCTION

DNA mismatch repair (MMR) activity is essential for maintaining the integrity of the cell's genetic material. The MMR pathway is a complex proofreading system, capable of correcting double helix mispairings occurring in the course of DNA replication (Jirichny, 1994; Modrich, 1994). In addition to their primary function in DNA repair, some MMR proteins are known to play a role in suppressing homologous recombination between diverged sequences (Rayssiguier *et al.,* 1989). This functional pathway has been described and studied mostly in prokaryotes. In *Escherichia coli,* initiation of the mismatch repair mechanism requires the recruitment of MutS, which binds DNA and subsequently interacts with a cofactor protein termed MutL. Formation of this complex activates an elaborate excision/repair cascade involving a number of functionally related enzymes (Grilley *et al.,* 1993).

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession No. U42190.

[1] To whom correspondence should be addressed. Fax: +39-2-26434767. E-mail: consaleg@dibit.hsr.it.

Recently, MMR genes have been identified in eukaryotes, and their role has been elucidated mainly by genetic analysis of germline mutations in cancer patients. Specifically, susceptibility to one type of hereditary or sporadic human cancer (human nonpolyposis colon cancer, HNPCC) has been found associated with molecular anomalies within genes encoding MMR factors (Fishel *et al.,* 1993; Leach *et al.,* 1993; Papadopoulos *et al.,* 1994; Nicolaides *et al.,* 1994; Bronner *et al.,* 1994). In most cases, individuals inherit a germline mutation in one copy of a DNA mismatch repair gene (*hMSH2, hMLH1, PMS1,* or *PMS2*). Whenever a somatic mutation of the other allele occurs, this leads to an uncontrolled load of gene mutations and, eventually, to neoplastic transformation. An example of this chain of events was provided by Markowitz *et al.* (1995), who described mutations of the TGFβ receptor II gene, a key factor in the control of cell growth, in MMR-deficient colon cancer cell lines. Although most MMR gene anomalies described so far behave as recessive mutations, some have been found to exert a dominant negative effect, leading to a significant reduction in DNA repair and to cancer susceptibility in the absence of a superimposed somatic mutation (Parsons *et al.,* 1995).

Recently, two groups (Palombo *et al.,* 1995; Drummond *et al.,* 1995) have identified in HeLa cells an MMR complex that preferentially binds heteroduplexes containing G/T mismatches. This dimer contains a 100-kDa protein, which they identified as hMSH2 (a previously isolated MutS homolog), and a novel 160-kDa protein (GT-binding protein, GTBP), which, reportedly, is primarily involved in binding G/T mispairs and repairing base–base and single-nucleotide insertion–deletion mismatches (Drummond *et al.,* 1995). Specifically, GTBP-deficient cell lines show alterations in mononucleotide repeat stretches (Papadopoulos *et al.,* 1995). *GTBP* was physically mapped to chromosome 2p16. In the present paper, we report the isolation and genetic characterization of the mouse ortholog of *GTBP* (Palombo *et al.,* 1995). This gene has been named *Gtmbp* (G/T mismatch binding protein) to avoid confu-

0888-7543/96 $18.00

sion with a previous entry (Le Roy *et al.*, 1992) applying the name *Gtbp* to an unrelated transcript.

MATERIALS AND METHODS

General procedures. Nucleic acid purification, restriction analysis, gel electrophoresis, DNA ligation, cloning, subcloning, dideoxy-sequencing, probe radiolabeling, Northern and Southern analyses, and library screening were performed according to established protocols (Sambrook *et al.*, 1989). Due to the occurrence of multiple compressions in the sequence of the GC-rich 5′ region of the *Gtmbp* transcript, the sequence was double-checked using deaza-dNTP analogs (Pharmacia). Northern blot analysis was conducted on positively charged Biodyne-B nylon filters (Pall) containing 20 or 30 μg total RNA per lane from CD1 mouse embryonic brain at various prenatal stages and various postnatal mouse organs. Hybridizations of Northern, Southern, and zoo blot filters were performed at 65°C in 125 m*M* sodium phosphate (pH 7.2), 250 m*M* NaCl, 7% SDS, 10% PEG. Filters were washed at 65°C to final stringencies of 0.2× SSC for 20–30 min. Phage plaque hybridizations and subsequent washes were carried out under similar stringency conditions.

RNA fingerprinting. DD151.10 was derived through a modification (Consalez *et al.*, in preparation) of the RAP-PCR protocol (Welsh *et al.*, 1992) comparing mRNAs of embryonic mouse telencephalon E10.5 and postnatal mouse brain. RNA fingerprinting was conducted as follows: a reverse transcription reaction was carried out using a $(dT)_{16}$ primer on total RNA extracted by the cesium chloride method (Sambrook *et al.*, 1989). Radioactive PCRs, in duplicate, were performed from 2 μl of each RT reaction in a 50-μl final volume with arbitrary 12-mers DR1 (5′-GGGTCGCGAACA) and DR2 (5′-TCTGGGAACCGG) (final concn 1 μM). PCR conditions were 3 min at 94°C, 2 min at 80°C at which *Taq* polymerase was added (hot start), followed by 35 cycles of 40 s at 94°C, 1 min at 50°C, 1 min at 72°C, with a final elongation step of 5 min at 72°C. [α-^{32}P]dCTP (0.2 μl) was added to each reaction. Amplified products were separated on a 5% denaturing acrylamide gel, visualized by autoradiography. Differentially displayed bands were cut from the gel and electroeluted. The bands were reamplified using the same 12-mer primers and blunt-end cloned into pBluescript II SK+ (Stratagene) as described (Consalez *et al.*, 1996).

Sequence analysis. Data bank searches (GenBank, GenEmbl, SwissProt, and PIR) were run through the BlastN and BlastX network servers (Altschul *et al.*, 1990). Additional sequence analysis and contig assembly was performed using the GCG package (Devereux *et al.*, 1984). The nucleotide sequence of the gene was deposited into the GenBank database under Accession No. U42190.

Probes. Three probes were used in the hybridization experiments described below. cDNA 151.10 spans 570 nt starting at nt 904 of our cDNA sequence (GenBank Accession No. U42190). cDNA p11R1 spans 2.0 kb of coding region from nt 1102 to nt 3120. In addition, a cDNA probe for *Msh2* (Msh2-3′) that spans nt 2665 through 3026 of the published cDNA sequence (Varlet *et al.*, 1994) was generated by PCR.

Genetic mapping. First, an intragenic *Msp*I polymorphism was revealed by hybridization with cDNA probe p11R1 of a panel containing 6 μg/lane C57BL/6j (B6) and *Mus spretus* (*spr*) DNA, cut with five different restriction enzymes. A panel from the (B6 × *spr*)F1 × *spr* interspecific backcross (The Jackson Laboratory, Bar Harbor, ME), containing *Msp*I-cut DNA from the two parentals and 94 N_2 progeny was hybridized with the same probe. Twenty-four progeny (A10-H12) were also typed by SSCP mapping (Beier *et al.*, 1992, 1993) using primers 5′ GGA GTT GGG GAC AGC GAT A and 5′ CGC TCA AGG TAC TCT TGG TTT. The ensuing strain distribution patterns (SDP) were analyzed with Map Manager 2.6.5 (Manly and Elliott, 1991), leading to map localization and linkage analysis with respect to markers independently mapped by others. Probe Msh2-3′ also detected an *Msp*I polymorphism and was used for linkage analysis on the BSS panel.

In situ hybridization of mouse embryonic tissue sections. Radioactive *in situ* hybridization (ISH) (Wilkinson, 1992) was carried out as follows: 7-mm paraffin serial sections from a single embryo were displaced in four to six adjacent series; two alternative series were used for each probe. Four embryos from at least two litters were studied at each developmental stage. Slides were deparaffinated in xylene, hydrated through an alcohol series, treated with paraformaldehyde and proteinase K, acetylated, and dehydrated through an ethanol series. Forty microliters (1×10^6 cpm) of a *Gtmbp* riboprobe (sense or antisense) in the hybridization mix was added to each slide. Hybridization was carried out overnight at 55°C. Slides were washed under stringent conditions (65°C, 2× SSC, 50% formamide) and treated with RNase A. Autoradiography was performed with Kodak NT/B2 emulsion. Exposure times were between 5 and 12 days. After development, sections were stained in Nissl stain and mounted in DPX. Sections were examined and photographed on dark fields using a Zeiss SV11 microscope.

RESULTS

Our group is isolating and characterizing developmentally regulated genes in the embryonic mouse brain, utilizing a modified PCR-based differential screening protocol commonly referred to as RAP-PCR or RNA fingerprinting (Welsh *et al.*, 1992). In this project, in comparing RNA from actively proliferating embryonic neuroepithelium with RNA from postnatal and adult brain tissues, containing mostly postmitotic neurons, we have identified several genes, including *Gtmbp,* that display differential expression.

The first cDNA probe for *Gtmbp* (151.10) was identified by RNA fingerprinting analyzing total RNA from E10.5 embryonic mouse CNS and P2 postnatal mouse brain. A 600-nt band that was strikingly more abundant at the former stage was excised from polyacrylamide, reamplified, and cloned as described (Consalez *et al.*, 1996). Sequence analysis through the BLASTX program (Altschul *et al.*, 1990) revealed homology to a yeast MutS homolog (PIR entry S51246). cDNA 151.10 was used as a probe to screen an E11.5 whole embryo library. Six hundred thousand plaque-forming units were plated and transferred to nylon filters. The membranes were hybridized and washed under high stringency conditions. Three phage clones hybridized by 151.10 were subcloned into plasmid and manually sequenced. Independently, the human homolog of the same gene (GenBank Accession No. U28946) was published by others and its function extensively characterized (Palombo *et al.*, 1995; Drummond *et al.*, 1995; Papadopoulos *et al.*, 1995). The full coding sequence of *Gtmbp* has been deposited with GenBank under Accession No. U42190. Here, we report the protein sequence, evolutionary conservation, map location, and expression of the mouse *Gtmbp* gene.

cDNA and deduced peptide sequence. Figure 1 shows the sequence of the mouse GTBP protein aligned with the published human protein sequence. The deduced amino acid sequence of mouse GTBP spans the distance between the most N-terminal and C-terminal peptides (shaded areas in Fig. 1) isolated and se-

FIG. 1. Amino acid sequence deduced by analysis of the *Gtmbp* gene. Peptides isolated and sequenced by Palombo *et al.* (1995) from the N-terminal and C-terminal regions of the human protein (p160/GTBP) are shaded. Regions of identity between human and mouse GTBP are boxed. At the N-terminal and C-terminal ends, exact coincidence is observed between the human and mouse proteins.

quenced by Palombo *et al.* (1995) from the corresponding human protein. No in-frame stop codons are encountered in our cDNA contig upstream of the putative initiation codon, thus the location of our start codon remains conjectural. However, our deduced peptide sequence matches the human protein sequence, as updated in Nicolaides *et al.* (1996), both at the N-terminal and at the C-terminal end. The two proteins show 86.1% identity and 92.1% similarity. At the nucleotide level, the two sequences are 84.6% identical, strongly suggesting orthology between them.

Evolutionary conservation. A 2.0-kb cDNA from the coding region of the *Gtmbp* transcript (p11R1) was used as a probe to hybridize a zoo blot containing DNA from various species. The cDNA probe contains no *Eco*RI sites. This analysis, performed at high stringency, revealed clear specific signals (Fig. 2) in lower primate, bovine, hamster, mouse, and *Xenopus laevis* DNA, probably reflecting the existence of orthologs of *Gtmbp* in each of those species. In mouse, the probe lights up two *Eco*RI bands of 7.2 and 3.5 kb, whereas only one 6.8-kb band is hybridized in human.

Map assignment. The human *GTBP* gene has been mapped by others to human chromosome 2p16 (Papadopoulos *et al.*, 1995), in the physical proximity of its close cognate *hMSH2*, suggesting the possible duplication of a primordial *MutS* gene. We mapped *Gtmbp* in mouse by linkage analysis of the BSS backcross panel

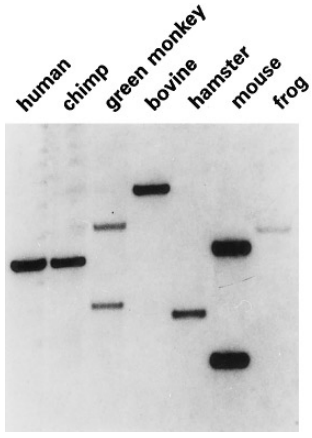

FIG. 2. Zoo blot analysis of 8 μg DNA from different species, cut with *Eco*RI. The blot was hybridized with a cDNA encoding an N-terminal domain of GTBP (p11R1). Bands are 7.2 and 3.5 kb in mouse DNA. The experiment used high-stringency hybridization and washing conditions (see Materials and Methods).

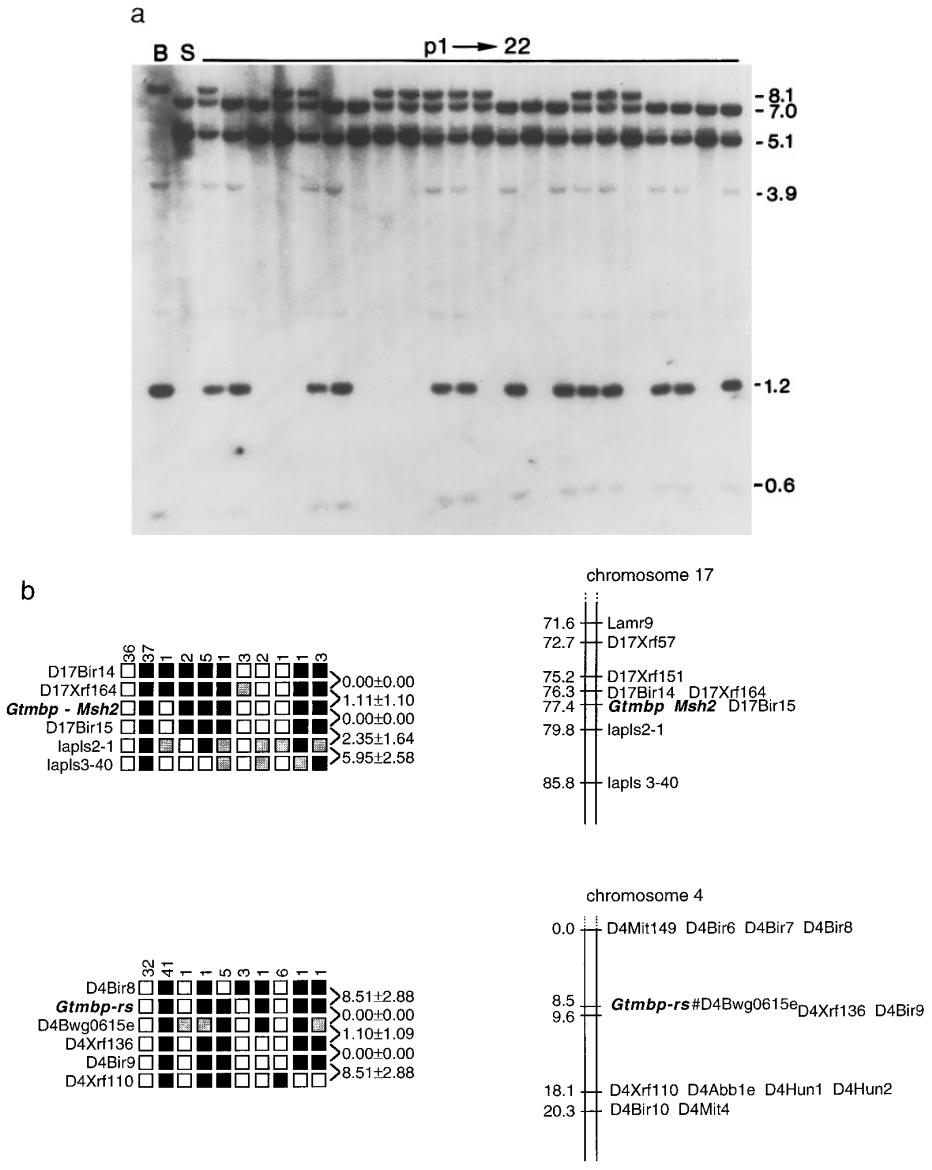

FIG. 3. (a) Representative polymorphic banding pattern obtained by hybridizing *Msp*I-cut DNA from the BSS backcross with cDNA probe p11R1. Two independently segregating polymorphisms are detectable. B, B6 parental; S, *Mus spretus* parental; p, progeny C1 → H3. Molecular weights (kb) are listed on the right. In addition, a faint 2.4-kb band is visible. (b) Genetic mapping of *Gtmbp* and *Gtmbp-rs* on mouse chromosomes 17 and 4, respectively. Left: Rows represent strain distribution patterns, i.e., alleles observed at each locus across the 94 individual N_2 progeny of the BSS cross on the chromosome inherited from the F_1 parent. Empty squares indicate the *M. spretus* allele; solid squares indicate the B6 allele; stippled squares: genotype not determined. Numbers between rows indicate recombination fractions ± standard error. Columns represent different haplotypes observed on chromosomes 17 and 4. Numbers above columns define the number of individuals sharing each haplotype. Right: Positions of *Gtmbp* and *Gtmbp-rs* on chromosomes 17 and 4 with respect to nearby markers independently mapped by others on the BSS backcross. Numbers on the left represent approximate genetic distances from the most centromeric chromosome 17 and 4 markers in this cross.

distributed by The Jackson Laboratory (Rowe *et al.*, 1994) (Fig. 3). Figure 3a shows a representative *Msp*I banding pattern obtained by hybridizing a Southern blot of the BSS backcross with probe p11R1. The analysis of this pattern led us to the map localization of two *Gtmbp*-related loci in the mouse genome. One polymorphism allowed us to assign *Gtmbp* to chromosome 17, in the known region of synteny with human chromosome 2p16. This polymorphism consists of a composite B6-specific banding pattern, containing 3.9-, 1.2-, and 0.6-kb *Msp*I fragments. After a longer exposure, one additional B6-specific *Msp*I band of 2.4 kb appears. cDNA p11RI contains two *Msp*I sites; the fact that this probe lights up four chromosome 17-specific bands in *Msp*I-digested B6 DNA suggests that it spans a genomic region containing introns and exons. Segregation of an *Msp*I polymorphism at the *Msh2* locus was analyzed on the same cross in 94 meioses, showing no recombinations with *Gtmbp* (LOD = 28.3). In the BSS cross (Fig. 3b, top), *Gtmbp* cosegregates with D17Bir15 (no recom-

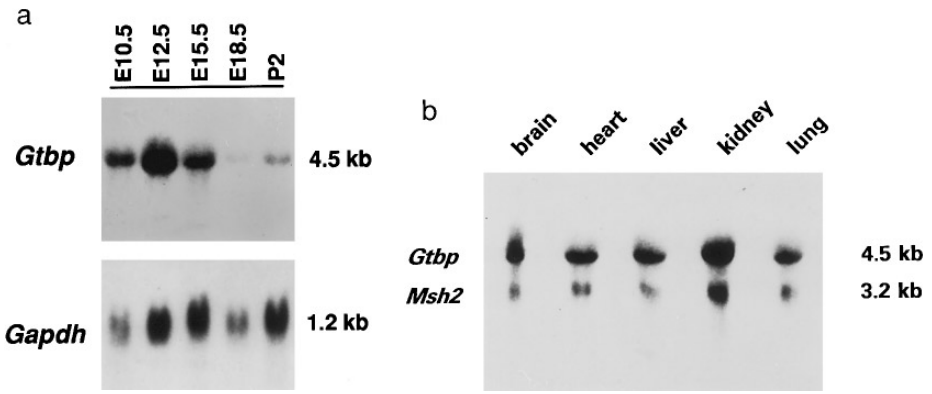

FIG. 4. (a) Northern blot analysis of 20 μg total RNA/lane extracted from mouse embryonic brain at different stages of gestation (10.5, 12.5, 15.5, and 18.5 days postcoitum and 2 days postnatal) hybridized with a *Gtmbp* cDNA probe (p11R1) (indicated here as *Gtbp*). Normalization was performed with a *Gapdh* probe. (b) Northern blot analysis of 30 μg total RNA/lane extracted from different newborn mouse tissues (brain, heart, liver, kidney, and lung) hybridized with p11R1 (indicated here as *Gtbp*) and an *Msh2* cDNA (Msh2-3′).

binations in 94 meioses; LOD = 28.3) and with D17Xrf164 (one recombinant in 90 meioses; LOD = 24.7). D17Xrf164, which maps centromeric to *Gtmbp*, was mapped by others using a human expressed sequence tag (EST) (GenBank Accession No. T03795) as a probe (Spencer *et al.*, in preparation). On the BSS map, two recombinations separate *Gtmbp* from D17Xrf151, a mouse locus mapped by others with a human EST probe (GenBank Accession No. R17485) localized on human chromosome 2 (Spencer *et al.*, in preparation).

In addition to the primary map localization, segregation of a single B6-specific 8.1-kb band in the backcross led us to the unequivocal assignment of a *Gtmbp*-related sequence to the centromeric portion of mouse chromosome 4, a region not reported to be syntenic with human chromosome 2p. Map assignment of this chromosome 4-linked locus was confirmed by SSCP mapping (Beier *et al.*, 1992) of 24 progeny of the same cross (not shown). *Gtmbp-rs* (Fig. 3b, bottom) is closely linked to D4Bwg0615e (Brady, Her, and Beier, unpublished results) (no recombination in 91 meioses; LOD = 27.4), and cosegregates with D4Bir9 (Rowe *et al.*, 1994) and D4Xrf136 (Spencer *et al.*, in preparation) (one recombination out of 94 meioses tested; LOD = 25.9). The latter corresponds to human EST 163190, mapped in human through a somatic cell hybrid line containing human chromosomes 3 and 8.

Expression. We have analyzed the expression of the gene by Northern analysis and by *in situ* hybridization of pre- and postnatal tissue sections. *Gtmbp* is expressed at high levels in the embryo starting at least as early as Embryonic Day 7 (not shown). Northern blot analysis of total RNA extracted from mouse brain at five different stages of development (10.5–18.5 days postcoitum and 2 days postnatal) reveals higher levels of a single *Gtmbp* mRNA species at E10.5 and E12.5, coincident with the peak of neuroepithelial proliferation (Fig. 4a). Northern analysis of the distribution of *Gtmbp* in various mouse tissues at Postnatal Day 1 reveals widespread transcription of the gene, at levels apparently higher (about three times) than those of the *Msh2* transcript (Fig. 4b).

mRNA *in situ* hybridization of tissue sections (Fig. 5) shows that *Gtmbp* expression is widespread and related to sites of active cell proliferation and DNA replication. We have analyzed sections from Embryonic Day 10.5 to Postnatal Day 1. At E10.5 (Fig. 5a) almost all embryonic tissues are proliferating. A strong hybridization signal proportional to cell density within the tissue is present throughout the embryo. During late gestation, neural cells start differentiating. At this stage, hybridization becomes restricted to proliferative zones within the CNS, as the ventricular zone (arrowheads in Figs. 5b, 5c, and 5d), or the external germinal layer (EGL) (Hatten and Heintz, 1995) in the developing cerebellum (E18.0; arrow in Fig. 5d). In the neonatal CNS (Postnatal Day 2; Fig. 5e), *Gtmbp* is expressed in areas where proliferation is still present, e.g., the cerebellar EGL (arrow) and hippocampus (arrowhead).

DISCUSSION

In the present paper, we report the cloning and genetic characterization of the mouse *Gtmbp* gene, which encodes a DNA mismatch repair enzyme. This protein participates in a complex involved in the early stages of mismatch recognition.

At the protein level, GTBP is related to MutS homologs in various species. Evidence of cross-specific conservation of *Gtmbp* comes primarily from significant homology at the protein level to a putative yeast MMR factor (PIR Entry S51246). In addition, high stringency zoo blot analysis points to the existence of orthologs of *Gtmbp* in primates, rodents, bovines, and amphibians at the nucleotide level. Striking conservation of this gene in various vertebrates suggests that GTBP, which forms a heterodimer with hMSH2, is an essential factor for MMR activity in this class of organisms, although its exact role is still a matter of active investigation.

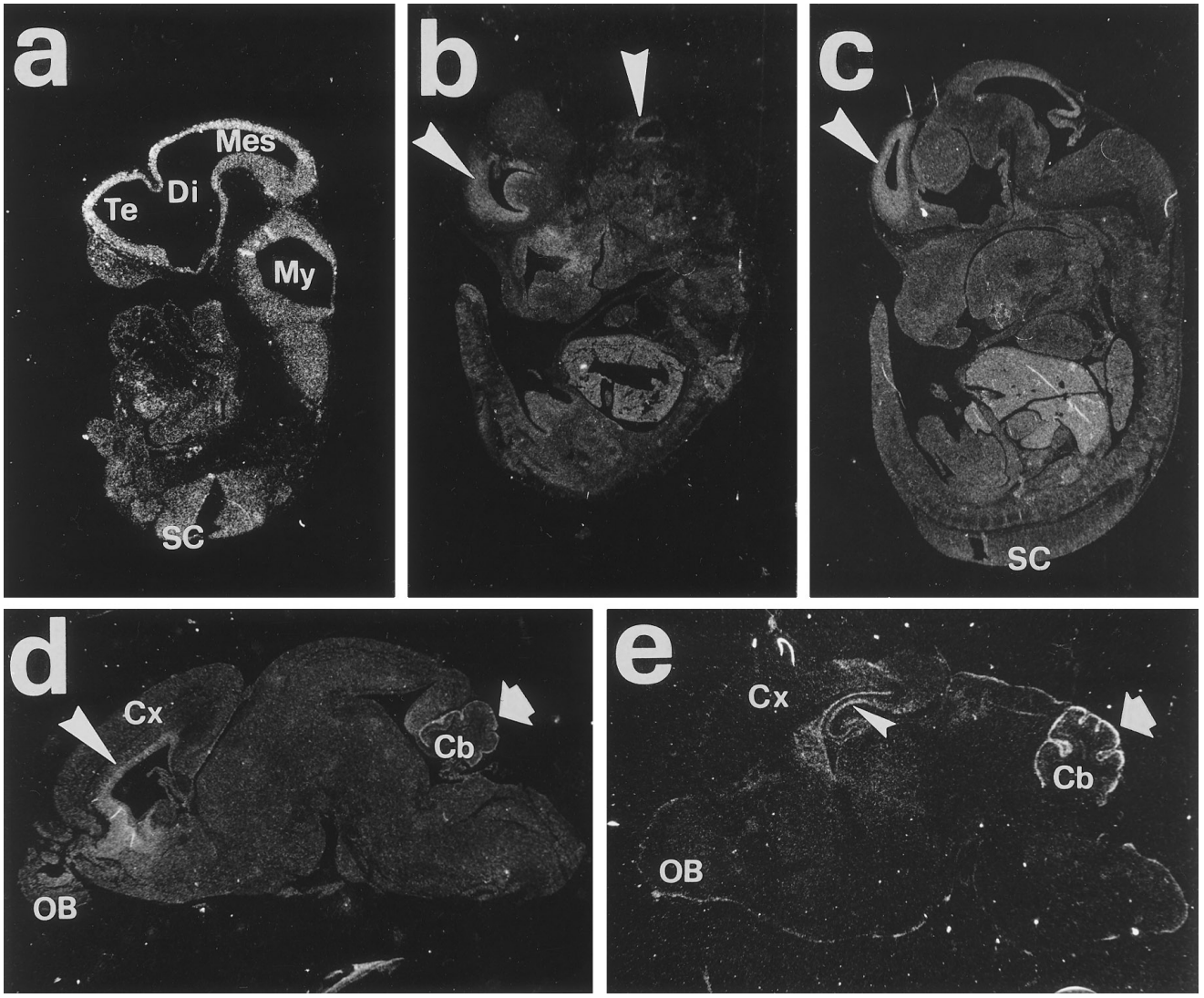

FIG. 5. *Gtmbp* gene expression during mouse embryonic development, assessed by mRNA *in situ* hybridization of embryonic sections. Sagittal sections; anterior is to the left. (a) E10.5; (b) E12.5; (c) E14.5; (d) E18.0; (e) P2. At all stages analyzed, a clear hybridization signal is evident in proliferating tissues and areas. Strong signal is clearly visible in the ventricular zone throughout the CNS, a layer devoted exclusively to neuroepithelial proliferation (arrowhead in b, c, and d). When proliferation starts during cerebellar development hybridization signal is confined to a highly proliferating layer (external germinal layer; arrow in d and e) In P2 brain sections (e) hybridization signal is present in the hippocampus (small arrowhead), a proliferative brain area at this stage. Cb, cerebellum; Cx, cerebral cortex; Di, diencephalon; Mes, mesencephalon; My, myelencephalon; OB, olfactory bulb; SC, spinal cord; Te, telencephalon.

GTBP and *hMSH2* are tightly linked in the human genome: both genes have been mapped to 2p16 through hybridization of YAC DNA and FISH experiments (Papadopoulos *et al.*, 1995). Work by our group (this paper) has assigned *Gtmbp*-related loci to mouse chromosomes 17 and 4, whereas others (Fishel *et al.*, 1993) have mapped *Msh2* to mouse chromosome 17, in a region syntenic with human chromosome 2p. Several indirect lines of evidence support the candidacy of *Gtmbp* (chromosome 17) and not *Gtmbp-rs* (chromosome 4) as a coding locus orthologous to human *GTBP*. First, *Gtmbp* maps within a conserved linkage group including *Msh2*, as demonstrated by complete cosegregation of the two markers in the entire N_2 progeny of the BSS cross (this paper). This is consistent with the tight physical linkage between the two genes in humans. Second, our evidence indicates that a 2.0-kb *Gtmbp* cDNA (p11R1) hybridizes a composite banding pattern, suggesting the existence of introns and exons, as opposed to an intronless retrotransposed pseudogene. Third, only one transcript is detected by Northern blot analysis of several pre- and postnatal brain RNAs and of RNA from various postnatal tissues, suggesting that, most likely, only one of the two *Gtmbp*-related sequences localized by genetic mapping in mouse represents a coding region. Likewise, only one cDNA species was obtained from the high-stringency screening of an E11.5 whole embryo cDNA library. Further work will

be required to determine the nature of the chromosome 4 locus (*Gtmbp-rs*).

The distribution of the *Gtmbp* transcript supports the notion that expression of this gene is strictly dependent on DNA replication and therefore varies greatly during embryogenesis. The apparent imbalance in the levels of the two transcripts at birth is at odds with the fact that the corresponding proteins are known to assemble into a heterodimer, suggesting the possible existence of additional partners for GTBP. It has been speculated that these two distinct gene loci originated from a primordial duplication of a genomic region containing a *MutS* homolog. This and subsequent rearrangements might have affected the ancestral gene's *cis*-acting regulatory elements in such a way that the two descendants utilize independent, although coordinated, transcriptional control mechanisms.

The analysis of mutants generated by gene targeting of *Msh2* (de Wind *et al.*, 1995) and *Pms2* (Baker *et al.*, 1995) has demonstrated the absence of consistent developmental defects in these animals, suggesting that the corresponding proteins may not be quintessential for completion of embryonic development or that residual function may be ensured by as yet unrecognized, redundant members of the MMR family. The generation of knockout mutants for *Gtmbp* will shed further light on the developmental significance (or lack thereof) of this class of genes. More importantly, the analysis of double mutants and of cell lines derived from them will dramatically outline the molecular effects of genomic instability in MMR-deficient tissues and the potential role played by mutation of DNA mismatch repair genes in determining predisposition to various forms of cancer. Molecular and breeding strategies aimed at the production of double homozygous mutants for *Msh2* and *Gtmbp* should keep into account the fact that the two genes are tightly linked in mouse and that *Gtmbp*-related sequences of unknown nature exist in the mouse genome.

ACKNOWLEDGMENTS

We are indebted to Celia Pardini for her mRNA *in situ* hybridization work. Thanks to Lucy Rowe and Mary Barter at The Jackson Laboratory (Bar Harbor, ME) for their very efficient assistance in genetic mapping. This work was funded by the Italian Telethon. Carol L. Stayton was supported by a Telethon fellowship for foreign scientists.

REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215: 403–410.

Baker, S. M., Bronner, C. E., Zhang, L., Plug, A. W., Robatzek, M., Warren, G., Elliott, E. A., Yu, J., Ashley, T., Arnheim, N., *et al.* (1995). Male mice defective in the DNA mismatch repair gene PMS2 exhibit abnormal chromosome synapsis in meiosis. *Cell* 82: 309–319.

Beier, D. R. (1993). Single-strand conformation polymorphism (SSCP) analysis as a tool for genetic mapping. *Mamm. Genome* 4: 627–631.

Beier, D. R., Dushkin, H., and Sussman, D. J. (1992). Mapping genes in the mouse using single-strand conformation polymorphism analysis of recombinant inbred strains and interspecific crosses. *Proc. Natl. Acad. Sci. USA* 89: 9102–9106.

Bronner, C. E., Baker, S. M., Morrison, P. T., Warren, G., Smith, L. G., Lescoe, M. K., Kane, M., Earabino, C., Lipford, J., Lindblom, A., Tannergard, P., Bollag, R. J., Godwin, A. R., Ward, D. C., Nordenskjold, M., Fishel, R., Kolodner, R., and Liskay, R. M. (1994). Mutation in the DNA mismatch repair gene homologue hMLH 1 is associated with hereditary nonpolyposis colon cancer. *Nature* 368: 258–261.

Consalez, G., Corradi, A., Ciarmatori, S., Bossolasco, M., Malgaretti, N., and Stayton, C. (1996). A new method to screen clones from differential display experiments prior to RNA studies. *Trends Genet.*, in press.

de Wind, N., Dekker, M., Berns, A., Radman, M., and te Riele, H. (1995). Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. *Cell* 82: 321–330.

Devereux, J., Haeberli, P., and Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* 12: 387–395.

Drummond, J. T., Li, G.-M., Longley, M. J., and Modrich, P. (1995). Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells. *Science* 268: 1909–1912.

Fishel, R., Lescoe, M. K., Rao, M. R., Copeland, N. G., Jenkins, N. A., Garber, J., Kane, M., and Kolodner, R. (1993). The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* 75: 1027–1038. [Published erratum appears in *Cell* (1994) 77(1):167.]

Grilley, M., Griffith, J., and Modrich, P. (1993). Bidirectional excision in methyl-directed mismatch repair. *J. Biol. Chem.* 268: 11830–11837.

Hatten, M. E., and Heintz, N. (1995). Mechanisms of neural patterning and specification in the developing cerebellum. *Annu. Rev. Neurosci.* 18: 385–408.

Jiricny, J. (1994). Colon cancer and DNA repair: Have mismatches met their match? *Trends Genet.* 10: 164–168.

Le Roy, H., Simon-Chazottes, D., Montagutelli, X., and Guenet, J. (1992). A set of anonymous DNA clones as markers for mouse gene mapping. *Mamm. Genome* 3: 244–246.

Leach, F. S., Nicolaides, N. C., Papadopoulos, N., Liu, B., Jen, J., Parsons, R., Peltomaki, P., Sistonen, P., Aaltonen, L. A., Nystrom-Lahti, M., Guan, X.-Y., Zhang, J., Meltzer, P. S., Yu, J.-W., Kao, F.-T., Chen, D. J., Cerosaletti, K. M., Fournier, R. E. K., Todd, S., Lewis, T., Leach, R. J., Naylor, S. L., Weissenbach, J., Mecklin, J.-P., Jarvinen, H., Petersen, G. M., Hamilton, S. R., Green, J., Jass, J., Watson, P., Lynch, H. T., Trent, J. M., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. (1993). Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell* 75: 1215–1225.

Manly, K. F., and Elliott, R. W. (1991). RI Manager, a microcomputer program for the analysis of data from recombinant inbred strains. *Mamm. Genome* 1: 123–126.

Markowitz, S., Wang, J., Myeroff, L., Parsons, R., Sun, L., Lutterbaugh, J., Fan, R., Zborowska, E., Kinzler, K., Vogelstein, B., Brattain, M., and Willson, J. (1995). Inactivation of the type II TGFβ receptor in colon cancer cells with microsatellite instability. *Science* 268: 1336–1338.

Modrich, P. (1994). Mismatch repair, genetic stability, and cancer. *Science* 266: 1959–1960.

Nicolaides, N. C., Palombo, F., Kinzler, K. W., Vogelstein, B., and Jiricny, J. (1996). Molecular Cloning of the N-Terminus of GTBP. *Genomics* 31: 395–397.

Nicolaides, N. C., Papadopoulos, N., Liu, B., Wei, Y.-F., Carter, K. C., Ruben, S. M., Rosen, C. A., Haseltine, 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). Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature* 371: 75–80.

Palombo, F., Gallinari, P., Iaccarino, I., Lettieri, T., Hughes, M., D'Arrigo, A., Truong, O., Hsuan, J. J., and Jiricny, J. (1995). GTBP, a 160-kilodalton protein essential for mismatch-binding activity in human cells. *Science* 268: 1912–1914.

Papadopoulos, N., Nicolaides, N. C., Liu, B., Parsons, R., Lengauer, C., Palombo, F., D'Arrigo, A., Markowitz, S., Willson, J. K., Kinzler, K. W., *et al.* (1995). Mutations of GTBP in genetically unstable cells. *Science* 268: 1915–1917.

Papadopoulos, N., Nicolaides, N. C., Wei, Y. F., Ruben, S. M., Carter, K. C., Rosen, C. A., Haseltine, W. A., Fleischmann, R. D., Fraser, C. M., Adams, M. D. *et al.* (1994). Mutation of a mutL homolog in hereditary colon cancer. *Science* 263: 1625–1629. [See comments]

Parsons, R., Li, G. M., Longley, M., Modrich, P., Liu, B., Berk, T., Hamilton, S. R., Kinzler, K. W., and Vogelstein, B. (1995). Mismatch repair deficiency in phenotypically normal human cells. *Science* 268: 738–740.

Rayssiguier, C., Thaler, D. S., and Radman, M. (1989). The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. *Nature* 342: 396–401.

Rowe, L. B., Nadeau, J. H., Turner, R., Frankel, W. N., Letts, V. A., Eppig, J. T., Ko, M. S. H., Thurston, S. J., and Birkenmeier, E. H. (1994). Maps from two interspecific backcross DNA panels available as a community genetic mapping resource. *Mamm. Genome,* 5: 253–274.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Cloning: A Laboratory Manual." Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Varlet, I., Pallard, C., Radman, M., Moreau, J., and de Wind, N. (1994). Cloning and expression of the *Xenopus* and mouse Msh2 DNA mismatch repair genes. *Nucleic Acids Res.* 22: 5723–5728.

Welsh, J., Chada, K., Dalal, S. S., Cheng, R., Ralph, D., and McClelland, M. (1992). Arbitrarily primed PCR fingerprinting of RNA. *Nucleic Acids Res.* 20: 4965–4970.

Wilkinson, D. G. (1992). "In Situ Hybridization—A Practical Approach." Oxford Univ. Press, Oxford.