The Mammalian β-Tubulin Repertoire: Hematopoietic Expression of a Novel, Heterologous β-Tubulin Isotype

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Abstract. We describe the structure of a novel and unusually heterologous β-tubulin isotype (Mβ1) isolated from a mouse bone marrow cDNA library, and a second isotype (Mβ3) isolated from a mouse testis cDNA library. Comparison of Mβ1 and Mβ3 with the completed (Mβ4, Mβ5) or extended (Mβ2) sequence of three previously described β-tubulin isotypes shows that each includes a distinctive carboxy-terminal region, in addition to multiple amino acid substitutions throughout the polypeptide chain. In every case where a mammalian interspecies comparison can be made, both the carboxy-terminal and internal amino acid substitutions that distinguish one isotype from another are absolutely conserved. We conclude that these characteristic differences are important in determining functional distinctions between different kinds of microtubule.

The amino acid homologies between Mβ2, Mβ3, Mβ4, and Mβ5 are in the range of 95–97%; however the homology between Mβ1 and all the other isotypes is very much less (78%). The dramatic divergence in Mβ1 is due to multiple changes that occur throughout the polypeptide chain. The overall level of expression of Mβ1 is low, and is restricted to those tissues (bone marrow, spleen, developing liver and lung) that are active in hematopoiesis in the mouse. We predict that the Mβ1 isotype is functionally specialized for assembly into the mammalian marginal band.

Microtubules are involved in a remarkable variety of cellular processes, including mitosis, morphogenesis, and the motion of cilia and flagella. With the discovery that vertebrate tubulins are encoded by multiple genes, the question arose as to the contribution of different tubulin gene products to the diversity of microtubule function. One hypothesis is that the microtubules involved in each cellular function are composed of special α- and β-tubulins (7, 27). A modification of this view is that some, but not all, α- and β-tubulin isotypes contribute to the functional diversity of microtubules either through their differential polymerization, or by virtue of unique interaction with distinct microtubule-associated proteins. At the other extreme, one could imagine that all α- and β-tubulins function identically, and that the various genes have evolved for the purpose of delivering the different amounts of α- and β-tubulin protein needed in different cells. Some support for this idea comes from genetic evidence in *Drosophila* (11, 12) and *Aspergillus* (20) where it has been shown that a mutation in a single tubulin gene affects many different microtubule functions. In addition, the expression of a chicken/yeast chimeric tubulin in mammalian cells results in its incorporation into both cytoskeletal and spindle microtubules without disruption of their function (3).

With such questions in mind, we have been investigating the mammalian tubulin repertoire by exhaustive screening of cDNA libraries representing several different tissues. We recently reported the structure and patterns of expression of five α-tubulin (17, 31) and three β-tubulin isotypes (17). Here we present the complete sequence of two novel mouse β-tubulin isotypes, and compare them with the extended sequences of the three previously described β-tubulins. Subcloned probes were used to study the expression of these isotypes during development. One is expressed ubiquitously at low levels and in mature testis at very high levels, where it is the dominant β-tubulin. The second is remarkable in that it has only 78% amino acid homology with the other β-tubulin proteins; RNA blot transfer experiments show that the expression of this isotype is restricted to tissues that are active in hematopoiesis. The structure, interspecies conservation, and expression patterns of these proteins seem to imply that the various α- and β-tubulin isotypes are indeed important determinants of functional differences among microtubules.

Materials and Methods

cDNA Cloning and Sequencing

PolyA$^+$ RNA was prepared from the testis and bone marrow of adult Swiss Webster mice for the construction of cDNA libraries in λgtll (33) as described (15). The libraries were screened (1) with ^{32}P nick-translated, excised insert from the chicken β-tubulin clone pT2 (4), and duplicate filters were screened with the ^{32}P-labeled 3′ untranslated region fragments from Mβ2 and Mβ5 (17). Plaques that hybridized to the former probe, but not the latter were picked, purified, and subcloned into bacteriophage M13 for

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                                                                                            1                                        10                                       20
Ms5  CCAAAAACCTTAATTTTCTTTCTTGTTCGGTACCTACATTGGAACCACCAAAAACAATTATTTCAGTAAACCGTAGCC ATG AGG GAA ATC GTG CAC ATC CAG GCC GGA CAG TGT GGC AAC CAG ATC GGT GCT AAG TTC
Ms4              GCTAGAGCCACAGCGCCGGATCGGCACCCGTCCATCAGACGCCACCAGCGCCACCACC     C                       C G   A        T    A   C   T                G   C
Ms3                                     GCTCCCTTCTACAGCTGTTCCGCATGTCGCCGCCGCC              G               C G               G       C                    T   C   C
Ms1                                                          TCTTGGTTCAGGCTAGG       A   G                          ATT   C                                  A   C

                                30                                      40                                       50                                       60
Ms5  TGG GAG GTC ATA AGC GAT GAA CAT GGC ATC GAC CCC ACC GGT ACC TAC CAC GGT GAC AGC GAC CTG CAG CTG GAC CGA ATC TCT GTG TAC TAT AAT GAA GCC ACA GGT GGC AAG TAT GTC
Ms4              C   T       G   C                   T   G       T   T   G       T       C           G A G   AA    C       C       G               A   C       T
Ms3          A           C   G           T    T        T  C   T          A   T           C           G   C   AAC           C   C           C                   G
Ms1          G   C G G   A           G   T       TG  G T   G  G      TGT    G ACG TCT   CT   C           G A G   AGC    T       C   C           TAC     AAG           G

                                70                                      80                                       90                                      100
Ms5  CCT CGA GCT ATC TTG GTG GAT CTA GAA CCT GGG ACT ATG GAC TCC GTT CGC TCA GGT CCT TTT GGC CAG ATC TTC AGA CCA GAC AAC TTC GTT TTC GGT CAG TCT GGG GCA GGC AAC AAC
Ms4  C A     C G G C       C   C   G       C   C   C               T   C       C   C               T C G               T   A   T       A   C   A
Ms3  C   C   C G G C C       C T G   G   C   C   C               G   G   T       G   C   C   G           T   T           C   T       AG         T   G
Ms1  G       C G   C T       C   G       C       A           AG  A C   A    C A C AGA    G   A GTC CTC    T CAG  T       GT   T    CAT    A C        T   T   T

                                110                                     120                                      130                                     140
Ms5  TGG GCT AAA GGC CAC TAC ACA GAG GGA GCT GAG TTG GTT GAC TCT GTC TTG GAT GTG GTG CGG AAG GAG GCG GAG AGC TGT GAT TGC CTG CAA GGC TTT CAG CTG ACC CAC TCA CTG GGT
Ms4      C   G   T           C       C   C       A   G   T G C    C       C           C   A           A       C   C   T   C   G       C       C           G   C   A
Ms3      C   G   G   T           A   T   A       C           G   G       C   T       A A       A   T           C           G       C                   C
Ms2                                                                                                   |               T   C   G   T   C                               G
Ms1      G   G                           G       C   A C   A AA       A           A A     G       AGC          C   C       A   G   T   C       A C GTG   T   T   G

                                150                                     160                                      170                                     180
Ms5  GGA GGC ACT GGC TCT GGC ATG GGC ACC CTG CTC ATC AGC AAG ATC CGG GAA GAA TAT CCT GAC CGT ATC ATG AAT ACC TTC AGT GTG GTG CCC TCG CCC AAA GTC TCT GAT ACC GTG GTC
Ms4  T       C       A       G   T                           A   G   G   T   A       A G               G       C           A   A       G   G       C   G       G
Ms3  G   G       G       G       T       C   T               G   G   C   A       A A           C               A       T   C       G   G   G   C   A       T
Ms2                  A       G                           A A   G   G   C   A       C           C           C A           A       G               T       G
Ms1          G       A                       G A           A       G   G   C   G   T   G       T   C T       T   C A           A   A       G   A   A   C   G       A

                                190                                     200                                      210                                     220
Ms5  GAG CCC TAC AAT GCC ACC CTG TCT GTC CAT CAG TTG GTT GAG AAC ACG GAT GAG ACC TAC TGC ATC GAC AAC GAG GCC CTC TAC GAC ATC TGC TTC CGT ACC CTC AAG CTC ACC ACG CCA
Ms4                      A                   C   G       T                                               G                       G               G           C
Ms3                          T   A       C   C       A   A                   T   T   T   T   T   A   A   T   T                   A A     A       G           A   C
Ms2          T               C   A   G   C   C   A       A       A           T   C   T       T       T   G   T               T       C       G       G           A
Ms1                      GTG   A   C A     C   C   A A     A       CC TG   T                   A   G       T           T   T       C       G   G   G   G   A   C

                                230                                     240                                      250                                     260
Ms5  ACC TAC GGA GAC CTG AAC CAT CTC GTC TCG GCC ACC ATG AGC GGC GTC ACC ACC TGC CTC CGT TTC CCG GGC CAG CTT AAT GCT GAC CTT CGA AAG CTG GCT GTC AAC ATG GTG CCA TTC
Ms4  G       G       C       C       G   A               T   A                   A                   G       C       A   T   A   C                   G
Ms3  T       T               A   G   C               T   G   A       T           G   A       T               A       C           G   G   A           A   T           C
Ms2      T   C   T   C       C   G   G   A               T   A   G               G   C       A               G   C   A           G   C               C   G
Ms1          G   T   C       C   G   G   C TTG               A A     G   T CA    G   C   T   C   T           C       A           G   C               C   G   T           C   T

                                270                                     280                                      290                                     300
Ms5  CCA CGT CTC CAC TTC TTC ATG CCT GGC TTT GCC CCT CTC ACC AGC CGT GGA AGC CAG CAG TAC CGG GCC CTC ACT GTG CCT GAA CTT ACC CAG CAG GTC TTC GAT GCC AAG AAC ATG ATG
Ms4  C                               A   A   C   A   C T G       A G   C                       C   T       G   G       A       A G           T
Ms3  T   C   G                                       C T G       G   C               T       G   A   T       G   C           A G   T
Ms2      C   G                       A           A       G       G   C               A       G   G       C   G   G           A G       C T
Ms1  T   T   T                           C   T   A       A GC   AG   T               A       T A   A G G   G   C       A       A G           CGC   T   C

                                310                                     320                                      330                                     340
Ms5  GCC GCC TGC GAC CCG CGC CAC GGC CGG TAC CTC ACA GTT GCC GCC GTC TTC CGT GGA CGG ATG TCC ATG AAG GAG GTG GAT GAG CAG ATG CTC AAC GTG CAG AAC AAG AAT AGC AGC TAC
Ms4  T   G   T           A A       T   C       G   T   G   T   T       G                               A   C           T A   GT          G       C           T
Ms3  T       T   T   A A A   T   G   C       TG   T   G   T       A G   C   C       T                       C   A           T   T   C   A       C
Ms2  T           T       T       C       G   C   G       A T       C   C                                                                       C
Ms1  T       T       T       G       C       G   T   G   T TGT A A       G   T AA       CC       A       C C A       C       G TC   A T       CA   G   C           A   G

                                350                                     360                                      370                                     380
Ms5  TTC GTG GAA TGG ATC CCC AAC AAT GTC AAG ACA GCT GTC TGT GAC ATC CCA CCG CGT GGC CTC AAG ATG GCA GTC ACC TTC ATT GGA AAC AGC ACA GCC ATC CAG GAG CTG TTC AAG CGC
Ms4      T   G                           G       C   A               G   C   C   G                   C               C   C           T
Ms3  T   T   G                           G                       T       T   G       G   A       T G   C               C           C                   A
Ms2          G                       C           G   C   G           T   T                       T   C               C           T
Ms1  T                   T                   GT       G               G   C       G   G   C           CT           C G   C       AT                   A       C       CC A G

                                390                                     400                                      410                                     420
Ms5  ATC TCT GAG CAG TTT ACG GCT ATG TTC CGC CGG AAG GCT TTC CTC CAC TGG TAC ACG GGT GAG GGC ATG GAC GAG ATG GAG TTC ACC GAG GCT GAG AGC AAC ATG AAC GAC CTG GTG TCT
Ms4      G           C   C   C           A A   C       C   G                   C   A                       T   G   A   A                   T
Ms3      A           C   A   C           A   C           A                       A       T                   T                                               C   C
Ms2      G           C   T   C           G   C           G                                               G                               T
Ms1  G           C   C T A               A A A   GA       T G G           C A       A   G       ATA   GT   A   T GGG   A           T G T   C C T

                                430                                     440
Ms5  GAG TAC CAG CAG TAC CAG GAT GCC ACC GCG GAA GAG GAA GAG GAT TTC GGA GAG GAG *** GCA GAA GAG GAG GCC TAA CGGCAGAGAGCCCTGCATCAGCTCAGGCTGCTTAGATCCCTCAGCCTTTCTCCAA
Ms4                                  T   T   ***   G   GC   G       A           GCT   A   G       T   T TAA GTCTCCTGCCATCACTCTGTCCCTGGGGCCCACCAGCAAAGCTTTGACCCTAAGC
Ms3                          C   T   A   T   G   A   G   GA   G   T   AG       GCT   AG           T   T TAG AGCTGTCTTAGTCACTAAAGCATGGGAGCAGTGTGAACTCTTTATTCATTCACAG
Ms2                                  G   T   T       C G   GC   G       AG       GAG   GT       T       T TGA GAACTTCTCAGATACAGTGTGCACCCTTAGTGAACTTCTGTTGTCCTCCAGCATT
Ms1          A   A T   A       T   GA   A   GG CTA   G   C AGT GAA   AG   T C   GAG   AG C       T   AG GCA GAA GAC AAA GAT CAT TAG CTAGGAGAGAAGCTATAGGGCAGCCGATGCT

Ms5  CTGCCCTTTGTCCTCCAGTTTCTTTCTGCTGCCTCTGTCTTGTATTTGTTTTGCTTCTGTTTTCTCATTGGGGGTAAATGGTGCCTGGCACATGGCAGGCACTCAATAAATATTTGTTTGTGG(A)n
Ms4  ATCACACCCCTGCACCTAGTTGCCTCATTCCCTAGGACCCCATGAGCATCTTCACCATGAGGCCAAGCCCAGGTTGCTTCTATTTGCTTCACCTTTAACTCCTAAACCCCACTGTCTCTCCAACCTGCCAGGGAAGGGCTCTTCTAGTTCCCATGAGCG
Ms3  CCTGTCTGCTAGCCATGTCCACTGTGCATTAGCTGTCCTGTGTCCTGACATCACTTGTACAGATACCACCATTAAAGCAATTCATAGTGTG(A)n
Ms2  GGTCTTTCTATTTGTAAATTATGGTGCTCAGTTTGCCTCTGTCAGAAATTCACTGTTGATGTAATAGTGTGAACCTCTTTCAAGATCACAGTATTGTCTCAGAAATCTATATGAATAAAAAAGCATGTGG
Ms1  TGCTTTTCCTTCTTCCTTTTTAAATAGTGAGTGAGTTTACTGTAGTGAGCCCTATTTATTTTTTATTAGTTGTAGGATAGACATGCAGACAAAGTGTGCAAGTTTGCCAGGAGCCGTTTAGCAGTGTTGCTGGGTACC
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dideoxy sequencing (24). Approximately 30 β-tubulin cDNA clones from each library were sequenced. The sequences of selected clones were completed by subcloning *Bal*31 exonuclease-treated fragments into M13 (16), and a 3′ untranslated region probe for Mβ3 was also constructed by this method. A 3′ untranslated region probe for Mβ1 was constructed by subcloning into pUC a 176-bp *Sau*3A to *Kpn*I fragment from this region. In the initial screening of 2×10^5 recombinant phage only one cDNA representing Mβ1 was obtained. Two antisense oligodeoxyribonucleotide probes corresponding to heterologous regions of this isotype were therefore synthesized (see Fig. 1), ^{32}P-end-labeled, and used to screen 2×10^5 further cDNA clones to obtain six overlapping cDNAs encoding Mβ1, all of which were sequenced as described above.

RNA Blot Transfer Experiments

RNA was prepared (2) from 10 different tissues dissected from Swiss Webster mice of various ages (see legend to Fig. 1). RNA concentrations were determined by absorbance at 260 nm, and 10- or 20-μg aliquots were electrophoresed on 1% agarose gels containing 2.2 M formaldehyde. The gel contents were transferred to nitrocellulose (26) and the blots hybridized with gene specific probes for Mβ1 or Mβ3. Oligonucleotides were ^{32}P-labeled with polynucleotide kinase, and excised fragments were ^{32}P-labeled by nick-translation (23). Hybridization and wash conditions are given in the figure legends.

Results

Isolation of Two Novel Mouse β-Tubulin Isotypes

Accumulating evidence on the tissue-restricted expression of several tubulin isotypes and the interspecies conservation of isotype-specific amino acid sequences suggests a role for the primary structure of these isotypes in defining microtubule function (5, 17, 28, 31). The expression of unique tubulin isotypes might therefore be expected in tissues and/or cell types that contain specialized kinds of microtubules, such as platelets (which contain the marginal band [30]) or spermatozoa (which contain a flagellum and the manchette). We therefore performed exhaustive screening experiments on cDNA libraries constructed using polyA$^+$ mRNA from mouse bone marrow and testis. To facilitate the isolation of novel β-tubulin cDNAs, each library was simultaneously screened with two probes: a chicken β-tubulin coding region cDNA (4) that would indiscriminately identify all β-tubulin coding sequences, and a mixed probe consisting of the subcloned 3′ untranslated regions of two previously described mouse β-tubulin isotypes, Mβ2 and Mβ5, that are expressed in most (if not all) tissues, though at varying levels. This approach served to eliminate from study many of those clones encoding β-tubulin isotypes we had characterized previously (17).

These experiments resulted in the identification of two novel β-tubulin cDNAs, one (Mβ3) isolated from the testis cDNA and bone marrow cDNA libraries, the other (Mβ1) only from the bone marrow cDNA library. The complete sequence of each isotype was determined from a set of extensively overlapping clones, each bearing sequence identity within the region of overlap. The compiled sequence data from these clones is shown in Fig. 1, together with the extended sequences of cDNAs encoding three previously described mouse β-tubulin isotypes, Mβ2, Mβ4, and Mβ5. Each cDNA possesses both unique untranslated regions and multiple substitutions throughout the coding regions, and each therefore represents a cloned copy of a distinct gene transcript. The β-tubulin isotypes encoded by each cDNA are compared in Fig. 2. The 15 carboxy-terminal amino acids of each isotype are distinct, and there is significantly less homology between isotypes in this region than in any other portion of the polypeptide chain. Multiple amino acid substitutions also exist throughout the polypeptide, particularly in Mβ1, which is exceptionally divergent from all other mammalian β-tubulin isotypes described hitherto, and, in addition, encodes a slightly larger polypeptide chain containing 451 amino acids. While the great majority of amino acid differences among Mβ2, Mβ3, Mβ4, and Mβ5 are the result of conservative substitutions, a significant proportion of the divergent amino acids in Mβ1 are nonconservative (Fig. 2), resulting in a polypeptide that is two charges less acidic than that encoded by, for example, Mβ5.

Patterns of Expression of Mβ3 and Mβ1 in the Adult Mouse

To determine the overall pattern of expression of the isotypes encoded by Mβ1 and Mβ3, non-crosshybridizing (i.e., isotype-specific) probes were used in blot transfer experiments using total RNA from adult mouse brain, heart, kidney, liver, lung, skeletal muscle, spleen, stomach, and testis. The data show abundant expression of Mβ3 in testis, with a much lower (10–20-fold) and essentially invariant level of expression in all other tissues examined except brain, where it is lower still (Fig. 3). On the other hand, in the tissues examined, Mβ1 is expressed most strongly in spleen, and (at a much lower level) in lung. The relative exposure times of the RNA blots shown in Fig. 3 suggest that the level of expression of Mβ1 is much lower in these tissues than that of any other co-expressed tubulin isotype. No expression of Mβ1 was detectable in adult brain, heart, kidney, liver, skeletal muscle, stomach, or testis.

Developmental Regulation of Mβ3 and Mβ1

The preponderance of Mβ3 in adult mouse testis (Fig. 3) suggested that the expression of this isotype might be linked to the process of spermatogenesis. To investigate this possibility, blot transfer experiments were done using RNA from various tissues of the developing mouse. The data (Fig. 4) show that, in testis, the expression of Mβ3 is relatively low until postnatal day 32, when there is a dramatic increase. By contrast, in all somatic tissues examined, a low level of Mβ3 expression is maintained at an essentially constant level throughout development.

The isolation of cDNA clones encoding Mβ1 from a bone marrow cDNA library and its expression in adult spleen raised the possibility that expression of this unusually heterologous isotype might be restricted to tissues involved in hematopoiesis. Because spleen and immature liver are sites of hematopoiesis in the mouse, the expression of Mβ1 was

Figure 1. Nucleotide sequence of five mouse β-tubulin isotypes, Mβ5, Mβ4, Mβ3, Mβ2, and Mβ1, derived from a series of overlapping cDNA clones. The composite data encompass the entirety of the coding region, with the exception of Mβ2, which lacks sequences 5′ to amino acid 125 (indicated by a vertical bar in the figure). Spaces denote sequence identity with respect to Mβ5; asterisks indicate deletions introduced so as to maximize homology. Termination codons and polyadenylation signals are underlined. Heterologous regions of Mβ1 selected for the synthesis of Mβ1-specific antisense oligonucleotides are also underlined.

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            10        20        30        40        50        60        70        80
β5 MREIVHIQAGQCGNQIGAKFWEVISDEHGIDPTGTYHGDSDLQLDRISVYYNEATGGKYVPRAILVDLEPGTMDSVRSGP
β4       L                                     E  N         N     V
β3       L                                     E  N               V
β1         I               GE     CA S C T A   E         Y K_____ V_____      I  SR

            90       100       110       120       130       140       150       160
β5 FGQIFRPDNFVFGQSGAGNNWAKGHYTEGAELVDSVLDVVRKEAESCDCLQGFQLTHSLGGGTGSGMGTLLISKIREEYP
β4                                   A                                           F
β3
β2                                            |
β1 L VL Q  S  H N                  IEN      R S          IV  ________     MN

            170       180       190       200       210       220       230       240
β5 DRIMNTFSVVPSPKVSDTVVEPYNATLSVHQLVENTDETYCIDNEALYDICFRTLKLTTPTYGDLNHLVSATMSGVTTCL
β4
β3
β2          M                              S
β1    L S   M       _____   V  A   I    ACF                              L    I  S_

            250       260       270       280       290       300       310       320
β5 RFPGQLNADLRKLAVNMVPFPRLHFFMPGFAPLTSRGSQQYRALTVPELTQQVFDAKNMMAACDPRHGRYLTVAAVFRGR
β4                                                     M
β3                                                     M
β2                                                     M  S                   I
β1 ____                              AQ        S G     M   R I       R       CI   K

            330       340       350       360       370       380       390       400
β5 MSMKEVDEQMLNVQNKNSSYFVEWIPNNVKTAVCDIPPRGLKMAVTFIGNSTAIQELFKRISEQFTAMFRRKAFLHWYTG
β4            S  S                             A
β3                                            SA
β2                                            SA
β1   T    Q L SI TR  NC          V          N  A  L  N       T V  H S     R  V    S

            410       420       430       440
β5 EGMDEMEFTEAESNMNDLVSEYQQYQDATAEEEEDFGEE*AEEEA
β4                                * GE E  AE  V
β3                                  GE E  *    VA
β2                               D QGE E  EG D
β1     IS  G    DIH        F  VR GL DSEEDAEEA VEAEDKDH
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Figure 2. Amino acid sequences of five distinct β-tubulin isotypes. Amino acid sequences of Mβ5, Mβ4, Mβ3, Mβ2, and Mβ1, are derived from the data shown in Fig. 1. Spaces denote sequence identity with respect to Mβ5. Asterisks have been introduced in the carboxy-terminal regions to indicate single amino acid gaps introduced so as to maintain maximum homology in this region. Probable residues involved in GTP binding (19) are underlined.

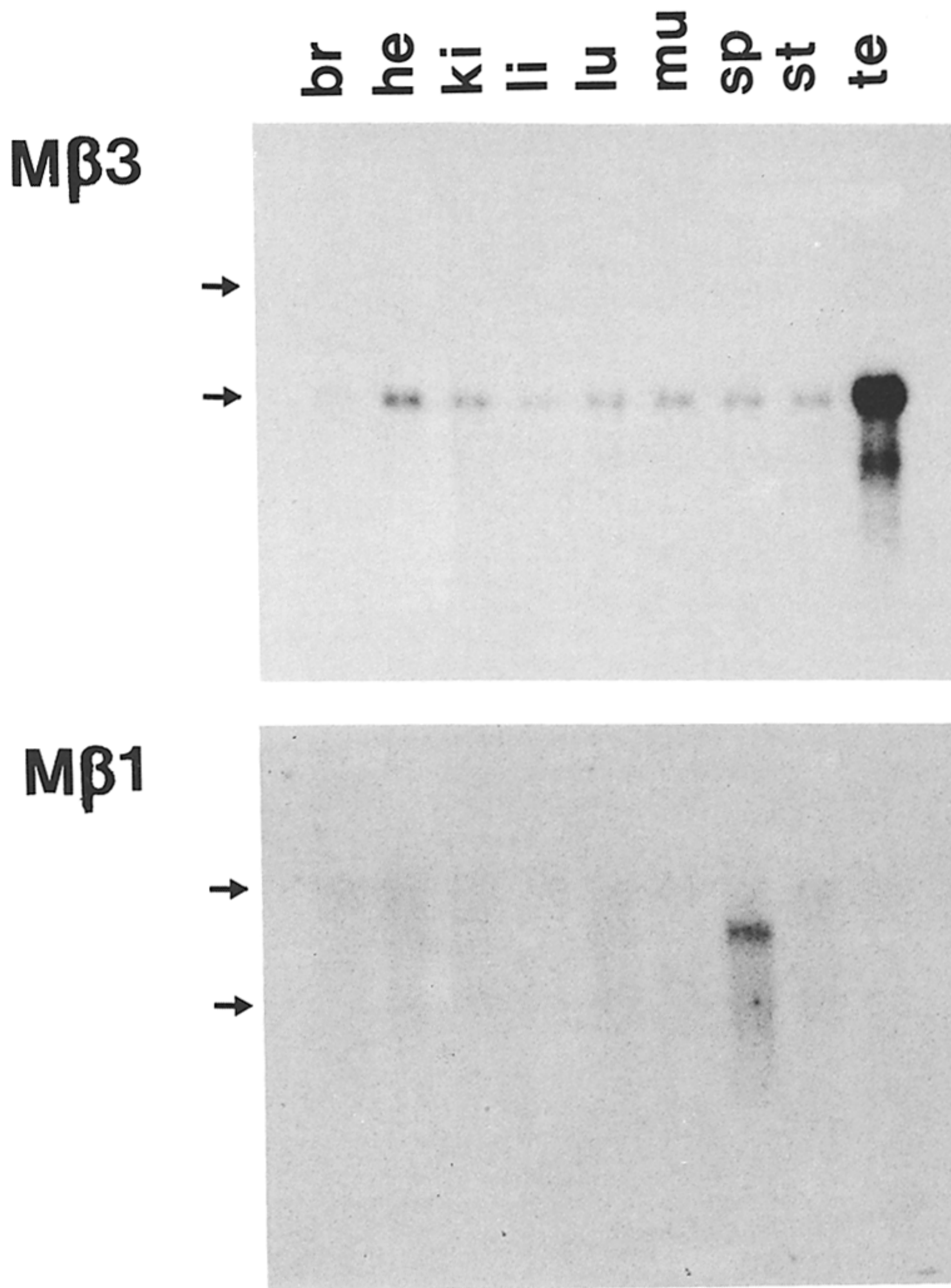

Figure 3. Expression of Mβ3 and Mβ1 in adult mouse tissues. Total RNA was prepared from brain (*br*), heart (*he*), kidney (*ki*), liver (*li*), lung (*lu*), muscle (*mu*), spleen (*sp*), stomach (*st*), and testis (*te*) of adult mice. 20-μg aliquots of each sample were resolved on denaturing 1% agarose gels. After transfer to nitrocellulose (26), the blots were hybridized either with a subcloned 3′ untranslated region probe ^{32}P-labeled by nick translation (23) derived from Mβ3 (*top*), or with a synthetic antisense oligonucleotide (24-mer) corresponding to a heterologous portion of the coding region of Mβ1 ^{32}P-labeled with polynucleotide kinase (*bottom*) (see Fig. 1). After hybridization in 50% formamide, 5× SSC at 42°C for the nick-translated fragment and in 20% formamide, 5× SSC at 42°C for the oligonucleotide, the blots were washed to a final stringency of 60°C, 2× SSC. The blot shown in the top panel was exposed to film for 20 h; that in the lower panel was exposed for 6 d. Arrows indicate the positions of 28S and 18S ribosomal RNA.

examined in these and other developing tissues. The data (Fig. 4) show that there is indeed weak but detectable expression of Mβ1 in the spleen of mice of all ages, as well as in the liver and developing lung of young mice. No expression of Mβ1 was observed in any of the other developing tissues examined.

Discussion

In this paper we describe the structure and expression of two novel mouse β-tubulin isotypes, Mβ1 and Mβ3. The amino acid sequences of these isotypes are compared to the extended amino acid sequences of three previously isolated β-tubulins (17) in Fig. 2, and the widely differing expression patterns of all five β-tubulin isotypes are summarized in Table I. Together with our work on mouse and human α-tubulin isotypes (summarized in reference 31), these data give a general (though not necessarily complete) picture of mammalian tubulin gene expression.

Genes encoding four of the five β-tubulin isotypes described here have been isolated from human genomic libraries (see Table I); three corresponding isotypes from rat have also been described (6). However, the patterns of expression of these human genes are to a large extent unknown because of the difficulty involved in studying human tissue, and because of the problem of sorting out functional genes from the large number of pseudogenes present in mammalian genomes (5, 13). A comparison of the sequences of the four human genes with those of the corresponding mouse

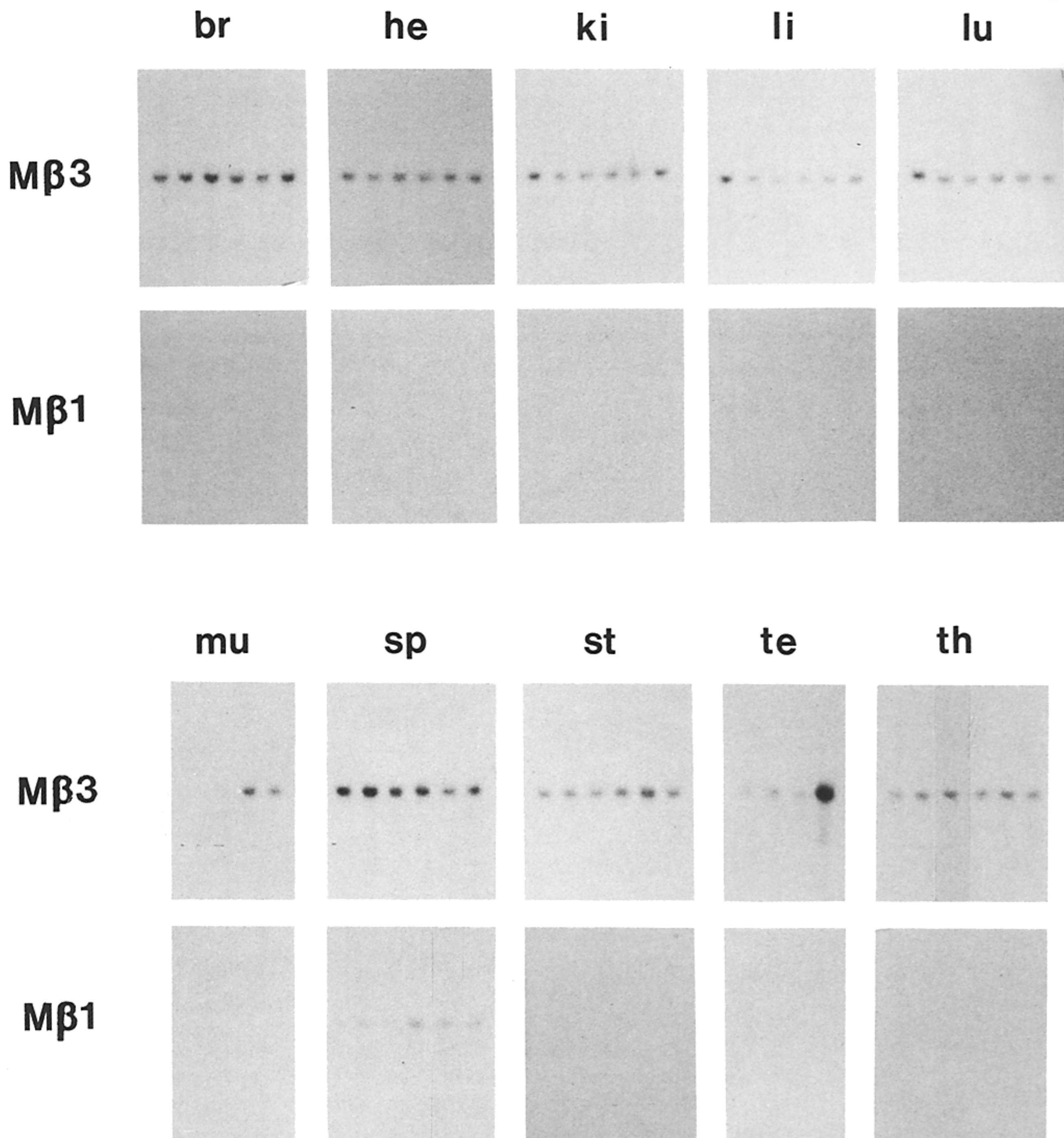

Figure 4. Developmental expression of Mβ3 and Mβ1. Total RNA from brain (*br*), heart (*he*), kidney (*ki*), liver (*li*), lung (*lu*), spleen (*sp*), stomach (*st*), and thymus (*th*) was prepared from mice of ages 3, 6, 10, 15, 22, and 32 d (left to right). RNA was also prepared from muscle (*mu*) and testis (*te*) of mice aged 10, 15, 22, and 32 d (left to right). Samples (10 μg) were resolved on denaturing agarose gels and transferred to nitrocellulose (26). Duplicate sets of blots were probed with ^{32}P-labeled subcloned probes spanning the 3′ untranslated regions of either Mβ3 or Mβ1. After hybridization, the blots were washed and exposed as described in the legend to Fig. 3.

cDNAs shows that the amino acid sequence of each isotype is absolutely identical between the two species. (At a small number of residues in the human genes 5β (14) [at amino acids 269, 283, 365] and M40 (13) [at amino acid 288] there were apparent interspecies amino acid differences; however, upon reexamination, these apparent differences proved to be the result of sequencing errors.) In view of this very surprising observation, namely, the absolute interspecies conservation of distinct tubulin amino acid sequences over a period of 100 My (i.e., since the mammalian radiation), it seems likely that each of the four isotypes, Mβ2, Mβ3, Mβ4, and Mβ5, has evolved to fulfill a specialized functional role. This conclusion implies that the expression pattern of each isotype is identical in all mammalian species. Such data as are available for the expression of human genes (13, 16) and rat cDNAs (6) encoding isotypes corresponding to Mβ3, Mβ4,

*Table I. Summary of Mammalian β-Tubulin Isotypes and Their Expression in Mouse**

	br	he	ki	li	lu	mu	sp	st	te	th	Corresponding human gene‡	Corresponding rat cDNA§
Mβ1				(∼)	(∼)		∼					
Mβ2‖	+++	∼	+	+	++	∼	∼	+	∼	+	Hβ9	RβT.1
Mβ3	+	+	+	+	+	+	+	+	++++	+	Hβ2	
Mβ4‖	+++										H5β	RβT.2
Mβ5‖	+++	+	+	+	++	+	++	+	+	+++	HM40	RβT.3

* Tissue abbreviations are the same as those in Figs. 3 and 4.
‡ Data from references 13, 14, and 16; and for Hβ9, Gu, W., and N. J. Cowan, unpublished observation.
§ Data from reference 6.
‖ Data from reference 17.

and Mβ5 support this hypothesis. Indeed, data on the expression of several chicken β-tubulin isotypes (10) suggests that this correspondence may also extend to lower vertebrate species.

The simplest explanation for the absolute interspecies conservation of the amino acid differences that distinguish the four most homologous β-tubulin isotypes is that these differences have functional significance. As noted previously (9, 17, 28) many isotype-specific amino acids are clustered at the carboxy terminus (see Fig. 2), a portion of the tubulin protein which is thought to be exposed when the tubulin is polymerized into microtubules (32), and which probably interacts with microtubule-associated proteins (25). On the other hand, transfection of a chimeric chicken/yeast β-tubulin gene into mouse NIH 3T3 cells results in the incorporation of a bizarre chimeric β-tubulin isotype into an array of microtubule structures in the host cells with no apparent effect on growth rate or cell morphology (3). This result could be explained in terms of functional distinctions between different microtubules being dependent on the relative abundance (rather than an absolute segregation) of heterodimers containing particular tubulin isotypes. Alternatively, the incorporation of chimeric tubulin into diverse microtubules may reflect the functional interchangeability of most, if not all, β-tubulin isotypes. In that event, the absolute interspecies conservation of isotypes noted here would require some explanation that is not based on the selection of functional differences. For example, the tubulin molecule, because of its many functional interactions, may be under such severe constraints that any single amino acid change would be likely to be deleterious, and thus several independent and compensating amino acid changes might be required in order to generate a new functional molecule. Since multiple mutation events are very rare, tubulin isotype amino acid differences, once generated, would tend to be retained. However, while such a scenario could account for the conservation of tubulin isotypes in the absence of selection for functional differences, it does not explain their widely different but nonetheless conserved patterns of expression.

Whether the unusually divergent β-tubulin isotype represented by Mβ1 is as rigidly conserved between mammalian species as the other four β-tubulin isotypes described here is an open question. Murphy and co-workers (21, 22) have purified and studied a unique and divergent β-tubulin protein that is specific to chicken erythrocytes and thrombocytes. Because Mβ1 is specific to hematopoeitic tissue (Figs. 3 and 4), we feel it is likely to be the mammalian equivalent of this unique chicken isotype. However, comparison of the sequence of Mβ1 with limited protein sequence data for the chicken erythroid β-tubulin (D. B. Murphy, personal communication) reveals many differences between these two proteins. This may not be surprising, in view of the differences between hematopoeisis in mammals and lower vertebrates. In lower vertebrates marginal bands composed of microtubules are found in the nucleated erythrocytes and thrombocytes of the blood, whereas in mammals marginal bands are found only in nucleated primitive erythrocytes (8), erythroblasts of the definitive erythroid line (30), and in the anucleate platelets. The mammalian tissue distribution of marginal bands correlates with our data on the expression of Mβ1. However, to address the question of whether the β-tubulin isotype encoded by Mβ1 indeed participates in mammalian marginal band formation, it will be necessary to raise a specific antiserum to a cloned fusion protein.

The amino acid differences between Mβ1 and the other four β-tubulin isotypes are scattered throughout the polypeptide chain, with a concentration of differences in an extended and divergent carboxy terminus (Fig. 2). About half of these differences are nonconservative. However, those residues thought to be involved in GTP binding (19) are completely conserved in all five isotypes (see Fig. 2) and all five have a highly acidic carboxy terminus. The divergent nature of Mβ1 could reflect the absence of severe selective constraints on a β-tubulin molecule whose only function is to form the marginal band. In this regard, it is noteworthy that calf brain microtubules are capable of forming marginal bands in detergent-extracted cytoskeletons prepared from chicken erythrocytes (29). However, the absence of a similarly divergent α-tubulin isotype (31) and the unique biochemical properties of the chicken erythroid β-tubulin (21, 22) are consistent with the existence of a specialized erythropoietic β-tubulin.

Although microtubules form part of a large variety of unique organelles in testis (such as the flagellum and manchette of spermatids, and the meiotic and mitotic spindles), there is almost certainly no β-tubulin isotype specific to testis. This conclusion is based on the fact that as a result of exhaustive analysis of 2×10^5 cDNA clones from the testis cDNA library, no sequences encoding β-tubulin isotypes other than Mβ3, Mβ2, and Mβ5 were isolated. Mβ3 is by far the most abundant β-tubulin in this organ, and therefore must contribute to many of its unique structures. There exists, however, an α-tubulin isotype that is unique to testis (31) and, in addition, posttranslational modifications may form functionally distinct pools of tubulin (18).

The five β-tubulin cDNAs described here, together with the six α-tubulin cDNAs we characterized previously (31) encode a total of 10 mouse tubulin isotypes. In addition, we have isolated and sequenced functional human tubulin genes

encoding most of these isotypes (13, 14, 16, 31, Gu, W., and N. J. Cowan, unpublished observation). Based on our analysis of about 20 human genes and pseudogenes and our thorough examination of mouse cDNA libraries from bone marrow, brain, testis, and embryo, we conclude that these eleven cDNAs represent most of the expressed tubulin genes in mammals. From these data, certain patterns emerge. For example, although tubulin is a heterodimer of α- and β-subunits, many α- and β-tubulin genes do not appear to be expressed in pairs. Whereas pairs of widely occurring tubulin isotypes (Mβ5 and Mα2, Mβ2 and Mα1) (17) are expressed in a parallel fashion, the tissue-specific tubulins encoded by Mβ1 (Fig. 4), Mβ4 (17), and Mα3 and Mα7 (31) have no coordinately expressed subunit counterparts. Therefore the incorporation into a given microtubule of either specialized α- or β-subunits may well be sufficient to confer functional specificity on that microtubule. The existence of these specialized tubulins and the absolute interspecies conservation of mammalian tubulin isotypes strengthens our previous conclusion (17, 31) that the encoded heterogeneity in α- and β-tubulins is likely to contribute to the diversity of microtubule function.

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