ABSTRACT We describe five mouse tubulin cloned cDNAs, two (Ma1 and Ma2) that encode α-tubulin and three (Mβ2, Mβ4, and Mβ5) that encode β-tubulin. The sequence of these clones reveals that each represents a distinct gene product. Within the sequence common to the two α-tubulin cDNAs, the encoded amino acids are identical, though the 3' noncoding regions are wholly dissimilar. In contrast, the three β-tubulin cDNAs show considerable carboxy-terminal heterogeneity. Two of the β-tubulin isotypes defined by the cloned sequences are absolutely conserved between mouse and human, and all three β-tubulin isotypes are conserved between mouse and rat. This result implies the existence of selective constraints that have maintained sequence identity after species divergence. This conclusion is reinforced by the near identity between a third mouse β-tubulin isotype and a chicken β-tubulin isotype. The significance of the interspecies conservation of tubulin isotypes is discussed in relationship to microtubule function.

We have used non-cross-hybridizing 3' noncoding region probes from the five cloned mouse tubulin cDNAs to study the developmental expression of each isotype in various mouse tissues. Ma1 and Mβ2 are expressed in an approximately coordinate fashion, and their transcripts are most abundant in brain and lung. Ma2 and Mβ5 are ubiquitously expressed and to a similar extent in each tissue, with the greatest abundance in spleen, thymus, and immature brain. In contrast, Mβ4 is expressed exclusively in brain. Whereas the expression of the latter isotype increases dramatically during postnatal development, transcripts from all four other tubulin genes decline from maximum levels at or before birth. Tissue-specific developmental changes in the abundance of tubulin isotype-specific mRNAs are discussed in relationship to organogenesis in the mouse.

Microtubules function in various ways in eukaryotic cells, playing a crucial role in morphogenesis, the mitotic and meiotic spindle, cilia, flagella, and axonal transport. Except for yeast (23), all species thus far examined contain multiple genes encoding α- and β-tubulin, the principle subunits from which microtubules polymerize, and it has been shown that more than one α- and one β-tubulin gene can be expressed in a given cell (14, 16). An important question with regard to the biology of microtubules is the extent to which different tubulin gene products may influence microtubule function. Genetic evidence in Drosophila (18, 19) and Aspergillus (24) suggests that alterations in a single tubulin gene can have wide-ranging effects on functionally different microtubules. However, the role of multiple α- and β-tubulin genes in the diversity of microtubule function in vertebrates is uncertain.
lated region probes from each cDNA clone to measure changing RNA levels in a wide variety of tissues.

**MATERIALS AND METHODS**

**Library Construction and Screening:** Polyadenylated mRNA from the brains of 15-21-d-old Swiss Webster mice and from 18-d-old mouse embryos was prepared by the method of Berk and Sharp (2). Sizc double-stranded cDNA flanked by synthetic oligonucleotide linkers was prepared from each of the two mRNA samples by methods previously described (17, 22) and ligated into the plasmid vectors pUC8 and 9. The bacterial transformation procedure used in library construction was that of Hanahan (15). The libraries were replicated onto nitrocellulose discs (1), and the replicas were probed with chicken α- and β-tubulin cDNA inserts (6), labeled with $^{32}$P by nick translation (27). Positively hybridizing colonies were picked and amplified, and the inserts contained in recombinant plasmids were subcloned into the replicative form (27). Positively hybridizing colonies were picked and amplified, and the inserts contained in recombinant plasmids were subcloned into the replicative form of bacteriophage M13 for sequencing by the Sanger dideoxy chain terminator method (29). Such clones were classified by the sequence of their 3′ untranslated regions, and the longest clone contained in each class was fully sequenced by the use of overlapping M13 subclones generated by Bal 31 exonuclease digestion of the original clones (21). For each class, an M13 subclone containing exclusively 3′ noncoding region sequences was selected and amplified, and the insert was excised by restriction digestion and purified on a 5% polyacrylamide gel.

**RNA Blot Transfer Experiments:** RNA was prepared (2) from 10 different tissues dissected from mice of various ages (see legend to Figs. 3-5), as well as from several stages of whole embryo (see legend to Fig. 6). RNA concentrations were determined by absorbance at 260 nm, and 10- or 20-μg aliquots (see figures legends) were electrophoresed on 1% agarose gels containing 2.2 M formaldehyde (3). The RNA was transferred to nitrocellulose (31) and the blots were probed with one of seven different probes, labeled with $^{32}$P by nick translation (27) to a specific activity of $10^6$ cpm/μg DNA. The seven probes were (1) the insert excised from the chicken β-tubulin cDNA clone pT1 (6); (2) the insert excised from the chicken α-tubulin cDNA clone pT2 (6); (3) the insert excised from the chicken α-tubulin cDNA clone pT1 (6) and (3-7) the insert excised from the subclones containing the 3′ noncoding regions of Ma1, Ma2, Mβ2, Mβ4, and Mβ5, respectively, self-ligated before nick translation to increase the size of substrate in the nick translation reaction. Seven identical blots were prepared for each time course, and for the adult RNA (Figs. 3-6), so that no blot had to be reused. After hybridization overnight in 50% formamide, 5× standard saline citrate buffer (SSC), 20 mM phosphate buffer, pH 6.5, 1× Denhardt’s, at 42°C, blots were washed to a final stringency of 2× SSC, 0.1% SDS at 68°C, and exposed for 6 h (probes 1 and 2) or 16 h (probes 3-7) with an intensifying screen.

**RESULTS**

Isolation of Five cDNA Clones, Each Encoding a Distinct Mouse α- or β-Tubulin Isoform

Two cDNA libraries were constructed in plasmid pUC8 (22) using polyadenylated mRNA, one from 15-21-d-old mouse brain and a second from 18-d-old whole mouse embryos. This choice was based on the known abundance of microtubules in the developing brain, and the prospect that the complex array of differentiating cell types present in the late embryo would provide the potential for isolating cDNAs encoding isotypes that might be expressed in a tissue-specific fashion during early development. We screened ~10⁶ colonies in each case, using (in separate experiments) cloned inserts excised from the chicken α- or β-tubulin specific probes pT1 or pT2 (6) labeled with $^{32}$P by nick translation (27). In each experiment, ~0.8% of the recombinants present in each library yielded a positive hybridization signal. 30 such clones were selected at random for further analysis. In each case, the plasmid DNA was amplified and subcloned into bacteriophage M13 for sequence analysis (20). Examination of each sequence and, in particular, the sequence of the 3′ untranslated regions showed that all the clones analyzed could be classified into five groups, two encoding α-tubulin and three encoding β-tubulin.

**Interspecies Conservation of α- and β-Tubulin Isotypes**

The DNA sequence of the longest member of each class of cDNA clone is shown in Figs. 1 and 2, together with the predicted amino acid sequences. The two α-tubulin cDNA clones, Ma1 and Ma2, are entirely homologous at the amino acid level within the region common to both clones; indeed, within 594 nucleotides of coding region, there are only eight nucleotide substitutions, in each case located in the third codon position (Fig. 1). In contrast, the 3′ untranslated regions are entirely dissimilar, with no significant homology between Ma1 and Ma2 throughout their length. However, there is extensive interspecies homology between the 3′ untranslated regions of Ma1 (mouse), ba1 (human) (7), and pILaT1 (rat) (20); and between Ma2 (mouse), ka1 (human) (7), and pT25 (rat) (10).

Unlike the α-tubulins encoded by Ma1 and Ma2, the amino acid sequences encoded by Mβ2, Mβ4, and Mβ5 (Fig. 2) show a significant degree of divergence in the carboxy-terminal region. Thus, within the carboxy-terminal 15 amino acids, Mβ2 and Mβ4 differ at seven positions, Mβ4 and Mβ5 at five positions, and Mβ2 and Mβ5 at nine positions. The possible functional significance and the interspecies conservation of these isotype-specific differences are discussed below. As in the case of the α-tubulin-encoding clones, the sequences within the 3′ untranslated regions of the three β-tubulin cDNAs are entirely unrelated. However, in contrast to the α-tubulin 3′ untranslated regions, for β-tubulin 3′ untranslated regions there is no evidence for interspecies conservation.

**Regulated Expression of Five Mouse Tubulin Isotypes**

To determine the pattern of developmental expression of the five tubulin isotypes described above, a probe containing exclusively 3′ untranslated region sequences was subcloned from each tubulin-specific cDNA clone. Because each 3′ untranslated region consists of non-cross-hybridizing sequences, these subclones could be used as labeled probes to uniquely detect mRNA sequences transcribed from each of the five mouse tubulin genes. We monitored the expression of each isotype in blot transfer experiments using total RNA isolated from brain, heart, kidney, lung, liver, muscle, spleen, testis, and thymus.

**Overall Levels of α- and β-Tubulin mRNAs in Adult Mouse Tissues**

To determine the overall level of α- and β-tubulin expression in mouse, a blot transfer experiment was performed in which RNA from the above tissues was detected with a coding region probe subcloned from cDNAs encoding either chicken α- or β-tubulin. The choice of heterologous (i.e., chicken) coding region probes was made so as to encourage indiscriminate hybridization between sequences encoding different mouse isotypes and to avoid the possibility that a selected homologous (i.e., mouse) coding region probe might emphasize the expression of one particular isotype. Fig. 3 (panels α...
Figure 1. The complete sequences of two cloned mouse α-tubulin cDNAs, Ma1 and Ma2, are shown together with the encoded amino acids. Spaces indicate identity with the sequence of Ma2; a vertical bar indicates the 5′ extremity of Ma2; a polyadenylation addition signal in the 3′ untranslated region of the Ma1 is underlined.

---

**Figure 2.** Complete sequences of three cloned mouse β-tubulin cDNAs, Mb2, Mb4, and Mb5, and the encoded amino acids are shown. Spaces indicate identity with the sequence of Mb4; asterisks denote deleted bases. Where the amino acids encoded by Mb2 and Mb5 differ from Mb4, the appropriate amino acid substitution is shown in the same order as that of the nucleotide sequences. The 5′ extremities of Mb5 and Mb2 are indicated by vertical bars. Polyadenylation addition signals in Mb5 and Mb2 are underlined.
and β) shows the pattern of α- and β-tubulin gene expression in various tissues of the adult mouse. The overall pattern of α- and β-tubulin gene expression is broadly similar, in that expression is most abundant in brain and testis, and least abundant in muscle. Though only a single α-tubulin mRNA species of 1.8 kilobases (kb) is evident (Fig. 3, panel α), three β-tubulin mRNA species were detected (Fig. 3, panel β): a 1.8-kb species that appears in all tissues where β-tubulin gene expression is evident; a 2.5-kb species that is unique to brain; and a 2.8-kb species that appears preponderantly in thymus, though it is also weakly detectable in other tissues.

Isotype-specific mRNAs in Adult Mouse Tissues

The pattern of individual α- and β-tubulin gene expression for each of the isotypes represented by the five cDNA clones was determined using RNA from the tissues of adult mice. The relative levels of the mRNAs encoded by Ma1 and Ma2 are shown in Fig. 3. Both isotypes are abundantly expressed in brain. Unexpectedly, the isotype encoded by Ma1 is also abundantly expressed in adult lung at a level only marginally lower than that in brain. Except for the testsis, which expresses Ma1 at an intermediate level, all other tissues examined show a much lower level of Ma1 expression.

The pattern of expression of Ma2 differs from that of Ma1 in three significant ways. First, Ma1 is expressed only weakly in spleen and thymus, whereas the level of expression of Ma2 in these tissues is relatively high. Indeed, expression of the latter isotype is greatest in thymus, exceeding that in brain. Second, the level of Ma1 expression in testis is second only to that in brain and lung, whereas Ma2 is relatively weakly expressed in testis. Finally, the level of expression of Ma1 in heart, kidney, liver, and stomach is lower (relative to brain) than that of Ma2.

The pattern of expression of Mβ2 is broadly similar to that of Ma1 in every tissue: the mRNAs that encode each isotype are most abundant in brain and lung. The genes encoding these isotypes therefore appear to be coordinately expressed in adult tissues. In contrast, the expression of Mβ4 is exclusive to brain. Finally, the expression of Mβ5 closely resembles that of Ma2, with preponderant mRNA levels in brain, lung, spleen, and thymus. Thus, like Ma1 and Mβ2, Ma2 and Mβ5 are apparently coordinately expressed in the adult.

Developmental Changes in α- and β-Tubulin Isotype-specific Gene Expression

To examine changes in tubulin isotype-specific gene expression as a function of development, blot transfer experiments were repeated using RNA derived from tissues dissected from mice aged 3, 6, 10, 15, 22, and 32 d (Figs. 4 and 5). In brain, where expression of both α- and β-tubulin mRNAs is most abundant throughout early development, the overall level declines in each case by a factor of from five to ten. In contrast, the total amounts of α- and β-tubulin mRNAs in all the other somatic tissues examined do not change greatly as a function of age. However, in testis, the corresponding levels of both α- and β-tubulin mRNAs increase significantly towards adulthood.

The developmental changes observed using probes specific for Ma1 and Mβ2 (Figs. 4 and 5) are broadly similar to the overall picture obtained using coding region probes, with small (though significant) differences. In both cases, expression in brain declines with increasing age, though the extent of reduction (by a factor of at least 10) is greater in Ma1 than in Mβ2, and begins somewhat earlier (around day 10). Both isotypes are expressed at a stable and broadly similar low level in heart, kidney, and stomach. Though Ma1 is weakly detectable in early muscle tissue, Mβ2 is undetectable in muscle at any developmental stage, and neither isotype is significantly expressed in adult muscle tissue. Expression of these isotypes is correspondingly weak in spleen of all ages. Whereas expression of Ma1 is undetectable in liver, a stable level of Mβ2 mRNA persists in this organ throughout development. Conversely, in lung, there is a stable abundance of Ma1 mRNA, and much less Mβ2 mRNA.

Although the expression of Ma2 and Mβ5 in adult tissues is largely similar (Fig. 3), the developmental time courses for each isotype in individual tissues reveal some minor differences. Both isotypes are strongly expressed in the thymus, reaching a peak at 10-16 d and declining thereafter. In brain, however, expression of Ma2 remains relatively constant throughout development, whereas expression of Mβ5 undergoes a rapid decline from day 3 onwards, reaching less than one-tenth of the day 3 level by day 32. Both isotypes are expressed in heart, kidney, liver, lung, spleen, and stomach at a level that either is maintained or declines slightly during development; in muscle, neither isotype is detectably expressed beyond day 15. Finally, in testis, expression of both Ma2 and Mβ5 rises somewhat until day 22, declining thereafter. The extent of expression of the 2.8-kb Mβ5 mRNA parallels that of the 1.8-kb Mβ5 mRNA at all stages and in all tissues; however, the relative abundance of the 2.8-kb species (as compared with the 1.8-kb mRNA) is conspicuously lower in brain.

The isotype encoded by Mβ4 differs dramatically in its pattern of developmental expression from the four isotypes described above in two respects. First, its expression is exclusive to brain (Fig. 5); and second, its level of expression in brain increases steadily throughout development, reaching a maximum in the adult (Fig. 3).

The experiments described above reveal several conspicuous and unexpected changes in specific α- and β-tubulin isotype expression as a function of postnatal development. To extend these observations to prenatal developmental stages, we performed blot transfer experiments with isotype-specific probes using RNA isolated from whole developing embryos at E8, E10, E14, and E18 (Fig. 6). The overall level of α- and β-tubulin mRNAs as measured using coding region probes shows that expression increases significantly between E8 and E10, and thereafter declines. Except for that of Mβ4, the expression of isotypes detected by all the gene-specific probes follows this pattern, with maximum expression at about E10.

DISCUSSION

Interstespecies Conservation of Distinct Tubulin Isotypes

This paper presents cDNA sequence data that define five distinct expressed tubulin genes in the mouse. Each cDNA differs from the others somewhat within the coding regions and entirely in the 3′ untranslated regions, thus defining each as representing a transcript from a distinct gene.

The α-tubulin genes represented by Ma1 and Ma2 can be identified with their counterparts in human, rat, and chicken
FIGURE 3 Total RNA was prepared from brain (br), heart (he), kidney (ki), liver (li), lung (lu), muscle (mu), spleen (sp), stomach (st), testis (te), and thymus (th) of adult mice. 20-μg aliquots of each sample were resolved on 1.0% agarose gels containing 2 M formaldehyde (3), and the gel content was transferred to nitrocellulose (30). The resulting blots were prehybridized and hybridized with the following probes labeled with 32P by nick translation: α, the cloned chicken α-tubulin cDNA pT1 (6); β, the cloned chicken β-tubulin cDNA pT2 (6); α1 and α2, the inserts excised from the subcloned 3' noncoding regions of Ma1 and Ma2, respectively; β2, β5, and β4, the inserts excised from the subcloned 3' noncoding regions of Mβ2, Mβ5, and Mβ4, respectively. The specific activities of the 3' noncoding region probes were adjusted to be approximately equal.
FIGURE 4  Total RNA was prepared from brain (br), heart (he), kidney (ki), liver (li), lung (lu), spleen (sp), stomach (st), and thymus (th) of mice aged 3, 6, 10, 15, 22, and 32 d. Total RNA was also prepared from muscle (mu) and testis (te) of male mice aged 10, 15, 22, and 32 d. 10-μg aliquots of each sample were resolved on 1% agarose gels containing 2 M formaldehyde, and the gel content was transferred to nitrocellulose. The resulting blots were prehybridized and hybridized with the following 32P-labeled probes: α, the cloned chicken α-tubulin cDNA pT1 (6); α1 and α2, the inserts excised from the subcloned noncoding regions of Mr1 and Mr2, respectively.
Figure 5  RNA blot transfer experiments were performed as described in the legend to Fig. 4, except that the following \(^{32}\)P-labeled probes were used: \(\beta\), the cloned chicken \(\beta\)-tubulin cDNA pT2 (6); \(\beta_2\), \(\beta_5\), and \(\beta_4\), the inserts excised from the subcloned noncoding regions of M\(\beta_2\), M\(\beta_5\), and M\(\beta_4\), respectively.
human, rat, and chicken have maintained significant sequence homology in these 3' noncoding regions implies that these sequences are functional, possibly playing a posttranscriptional role, and have therefore been maintained by selective pressure. Such selective constraints do not seem to extend to the other nontranslated regions of α-tubulin genes, since there is little discernible interspecies homology between, for example, the intervening sequences (13). Interspecies conservation of 3' noncoding regions has also been observed in vimentin (26) and actin (25) genes, but not in β-tubulin genes.

In contrast to the two α-tubulin cDNAs that encode identical proteins, the three β-tubulin cDNAs described here each encode a distinct tubulin isotype varying at ten of the carboxy-terminal 15 amino acid positions. Fig. 7 compares these carboxy-terminal sequences from mouse with those of the same region from three human (21), four chicken β-tubulins (32, 33), and three rat β-tubulins (9). Two of the mouse tubulins, Mβ4 and Mβ5, are identical in amino acid sequence to two of the human tubulins, Sβ and M40, respectively, over this region. Indeed, the interspecies structural identity of these two β-tubulin isotypes is not restricted to the carboxy-terminal region of the protein: over the entire region covered by the sequence data (Fig. 2) mouse Mβ4 and human Sβ encode the identical protein. Similarly, mouse Mβ5 and human M40 share all isotype-specific amino acid changes over the region sequenced. This interspecies conservation extends also to rat β-tubulin sequences (see Fig. 7). Indeed, mouse Mβ2 and chicken β2 have identical carboxy-terminal amino acid sequences, except for the inversion of a glycine and a glutamic

**Figure 6**  Total RNA was prepared from whole mouse embryos aged 8, 10, 14, and 18 d. Aliquots (20 µg) were resolved on denaturing agarose gels and blotted onto nitrocellulose, and the blots were hybridized with the probes described in the legend to Fig. 3.

by virtue of the interspecies conservation of their 3' noncoding regions, which bear 82 and 72% homologies with the 3' noncoding regions of the human α-tubulin cDNAs bα1 and Kα1, respectively (7). The fact that species as divergent as

**Figure 7**  Comparison of the 15 carboxy-terminal amino acids encoded by three expressed mouse β-tubulin genes (this paper), three expressed human β-tubulin genes (22, 11, and 10, respectively), five expressed chicken β-tubulin genes (33), and three rat β-tubulin genes (9). A single amino acid "deletion" has been introduced in the sequence of Mβ4 (denoted by an asterisk) so as to maintain maximum homology with the other isotypes shown in the figure.

**Mouse**

| Mα2 | Mα4 | Mα5 |
|-----|-----|-----|
| DEQGELEELEEDEEA | E*EEGELEELEEDEEA | EEEEDGFEEAEAEAA |

**Human**

| α2  | α4  | Mα0 |
|-----|-----|-----|
| EEEGEEEEEEAEAEVA | E*EEGELEELEEDEEA | EEEEDGFEEAEAEAA |

**Chicken**

| α1  | α2  | α3  | α4  | α5  |
|-----|-----|-----|-----|-----|
| DEQGELEELEEDEEA | DEQGELEELEEDEEA | EEEGEEEEEEAEAEAE | EEEGEEGEEGEEGEEGEEGEEG | WDGEAAFEEDDEEIEWEB |

**Rat**

| RαT.1 | RαT.2 | RαT.3 |
|-------|-------|-------|
| DEQGELEELEEDEEA | E*EEGELEELEEDEEA | EEEEDGFEEAEAEAA |
acid residue at positions 440 and 441 (compare Figs. 2 and 7).

The fact that β-tubulin isotypes resembling those in mouse occur in vertebrate species as evolutionarily distant as chicken and human implies that the distinctive and highly acidic carboxy-terminal regions have important and specific roles to play in the diversity of vertebrate microtubule function. Individual β-tubulin isotypes could be polymerized either exclusively or preponderantly into distinct microtubules and/or interact differently with microtubule associated proteins or organelles. In this regard, it is interesting to note that the carboxy-terminal domains of both α- and β-tubulins have been shown to modulate microtubule assembly and are thought to interact with the assembly-stimulating microtubule-associated protein MAP2 (30). Because of the probable importance of unique tubulin isotypes in microtubule function, it is of interest to establish the patterns of expression of each isotype in a wide variety of cells and tissues; indeed, due to the conservation of these isotypes across species boundaries, information about the expression and function of these sequences in mouse can in all probability be extended to other mammals, including humans.

**Developmental Regulation of Five Tubulin Genes**

The non-cross-hybridizing 3' noncoding regions of the five mouse tubulin cDNAs were used as probes to study the developmental pattern of expression of the corresponding tubulin genes. Ma1 and Ma2 are expressed in a roughly coordinate fashion in almost all tissues studied, and most strongly in brain and lung. Similarly, Ma2 and Mg5 are expressed ubiquitously, and approximately in parallel, most strongly in spleen, thymus, and immature brain (see Figs. 3–5). In contrast, Mg4 is expressed exclusively in brain: its expression increases dramatically after birth, reaching a maximum in adulthood, whereas brain transcripts from the other four tubulin genes all decline in abundance to a varying extent from maximum levels reached at or before birth (see below). A β-tubulin mRNA that increases in abundance has also been described in rat brain (4, 5) and encodes the same carboxy-terminal sequence as Mg5 (Fig. 7). The existing expression data from humans (7, 13, 21) and rat (4, 5, 9) are consistent with the above inference that the five tubulin genes described here have their exact counterparts in other mammalian species.

Whether this identity can be extended to nonmammalian vertebrates is not clear. Cleveland and co-workers have extensively studied β-tubulin gene expression in the chicken (for example, reference 16). Mouse Mg2 encodes a carboxy-terminal region similar to that encoded by chicken β2 (Fig. 7), and the two genes have a similar expression pattern in adult animals. Similarly, the expression of mouse Mg5 is comparable to that of chicken β4 in adult and embryonic tissues, though data are not at present available for the comparison of isotype-identifying carboxy-terminal sequences. However, Mg4 seems to have no counterpart in chicken. Many of the chicken isotypes have not been described in mammals (Fig. 7), and, on balance, the current data suggest that avian and mammalian tubulin isotype and/or gene requirements may differ.

The α-tubulins are all encoded by 1.8-kb mRNAs in mouse, whereas the β-tubulins are encoded by mRNAs of 1.8, 2.5, and 2.8 kb. The 3' noncoding region of Mg5 hybridizes to both a 1.8- and a 2.8-kb mRNA. These two mRNA species are probably transcribed from a single gene, and differ in size as a result of two alternative polyadenylation sites, as is the case with the corresponding gene in human, M40 (11). It is curious that although the 3' untranslated regions of these two genes show no homology, they both appear to use two polyadenylation sites in the same relative position. A β-tubulin gene yielding 1.8- and 2.8-kb mRNAs that encodes the same carboxy-terminal amino acids as Mg5 has also been described in rat (9).

The developmental changes in tubulin gene expression in tests are of interest in relationship to developmental changes in this organ. In mouse, spermatogenesis begins at around day 8, the prostate and seminiferous tubules enlarge at about day 17, and sexual maturity is reached at 7 to 10 wk (28). Overall α- and β-tubulin mRNA levels rise throughout development, reaching an adult level as high or higher than in brain (Figs. 3–5). However, Ma1 and Mg2 are expressed at very low constant levels in tests, whereas Ma2 and Mg5 mRNAs peak at puberty and then fall with the onset of sexual maturity. Thus, it seems likely that none of the five genes described in this paper plays a dominant role in spermatogenesis, and that one or more different testis-specific or testis-abundant α- and β-tubulins exist. In this context, it is noteworthy that a mouse α-tubulin mRNA expressed in spermatids has been reported (8). In addition, a testis-abundant β-tubulin isotype has been described in chicken (16) and a multifunctional, testis-specific α-tubulin has been characterized in Drosophila (18, 19).

The developmental regulation of the various tubulin genes is most striking in brain (Figs. 3 and 4) and in whole embryo (Fig. 6). The level of mRNA from four of the five genes, and indeed the overall level of α- and β-tubulin mRNAs, increases greatly between days 8 and 10 of embryogenesis. At this time the mouse embryo undergoes accelerated organogenesis and nervous system cellular differentiation (28). Because neural tissue is the richest source of microtubules, its contribution to overall embryonic levels of tubulin mRNA is likely to be dominant, and the increase seen at embryonic day 10 is therefore accounted for largely by the growth and differentiation of neural tissue. Thus, expression of Ma1, Ma2, Ma2, and Mg5 probably peaks in brain at this time and declines thereafter in the growing embryo and throughout postnatal development. The decline in brain tubulin levels generally, and of the expression of the latter four genes particularly, may be due not only to a decrease in mitotic activity, but to decreasing axonal outgrowth.

The fact that one β-tubulin isotype (Mg4) increases postnatally as the two others decrease leads one to speculate that Mg4 may be glial, since glial differentiation is mainly postnatal. Conversely, the other two β-tubulin isotypes, Mg2 and Mg5, may be neuronal, since axon outgrowth is primarily an embryonic event. In situ hybridization experiments using gene-specific 3' encoding region probes on brain sections from mice of different ages will be necessary to address this point.

We thank Elizabeth Delgado for typing the manuscript, and Cynthia Loomis for technical assistance.

This work was supported by grants from the National Institutes of Health and the Muscular Dystrophy Association.

Received for publication 29 March 1985, and in revised form 13 May 1985.
REFERENCES

1. Benton, W. D., and R. W. Davis. 1977. Screening λgt recombinant clones by hybridization to single plaques in situ. Science (Wash. DC). 196:180-181.
2. Berk, A., and F. Sharp. 1978. Spliced early mRNAs of SV40. Proc. Natl. Acad. Sci. USA. 75:1274-1278.
3. Boedtker, H. 1971. Conformation independent molecular weight determination of RNA by gel electrophoresis. Biochim. Biophys. Acta. 240:448-453.
4. Bond, J. F., and S. R. Farmer. 1984. Differential expression of two neural cell-specific β-tubulin mRNAs during rat brain development. Mol. Cell. Biol. 4:1313-1319.
5. Cleveland, D. W., M. A. Lopata, N. J. Cowan, R. J. McDonald, W. J. Rutter, and M. W. Kirschner. 1980. Number and evolutionary conservation of α- and β-tubulins and cytoplasmic β- and γ-actin using specific cloned cDNA probes. Cell. 20:95-105.
6. Cowan, N. J., P. R. Dobner, E. V. Fuchs, and D. W. Cleveland. 1983. Expression of human α-tubulin genes: interspecies conservation of 3′ untranslated regions. Mol. Cell. Biol. 3:1738-1745.
7. Dietz, R. J., K. C. Kleene, and N. B. Hecht. 1984. Haploid expression of a mouse testis α-tubulin gene. Science (Wash. DC). 224:68-70.
8. Farmer, S. R., J. F. Bond, G. S. Robinson, D. Mbangkollo, M. J. Fenton, and M. E. Berkowitz. 1984. Differential expression of the rat β-tubulin multilineage family. In Molecular Biology of the Cytoskeleton. G. G. Borisy, D. W. Cleveland, and D. B. Murphy, editors. Cold Spring Harbor Publications, Cold Spring Harbor, NY. 333-342.
9. Ginzburg, J., L. Behar, D. Givol, and U. Z. Littauer. 1981. The nucleotide sequence of rat α-tubulin: 3′ end characteristics and evolutionary conservation. Nucleic Acids Res. 9:2691-2697.
10. Gwo-Shu Lee, M., S. A. Lewis, C. D. Wilde, and N. J. Cowan. 1983. Evolutionary history of a multilineage family: an expressed human α-tubulin gene and three processed pseudogenes. Cell. 33:477-487.
11. Gwo-Shu Lee, M., C. Loos, and N. J. Cowan. 1984. Sequence of an expressed human tubulin gene containing ten Alu family members. Nucleic Acids Res. 12:5823-5836.
12. Hall, J. L., and N. J. Cowan. 1985. Structural features and restricted expression of a human α-tubulin gene. Nucleic Acids Res. 13:207-223.
13. Hall, J. L., L. Dudley, P. R. Dobner, S. A. Lewis, and N. J. Cowan. 1983. Identification of two human β-tubulin isotypes. Mol. Cell. Biol. 3:854-862.
14. Hanash, D. 1983. Studies on transformation of Escherichia coli with plasmids. J. Mol. Biol. 166:557-580.
15. Havercroft, J. C., and D. W. Cleveland. 1984. Programmed expression of β-tubulin gene during development and differentiation of the chicken. J. Cell Biol. 96:927-1935.
16. Hellman, D. M., J. R. Feramisco, J. C. Fiddes, G. P. Thomas, and S. H. Hughes. 1983. Identification of clones that encode chicken tropomyosin by direct immunological screening of a cDNA expression library. Proc. Natl. Acad. Sci. USA. 80:31-35.
17. Kemphues, K. J., R. A. Raff, T. C. Kaufman, and E. C. Raff. 1979. Mutation in a structural gene for a β-tubulin specific to testis in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA. 76:3993-3995.
18. Kemphues, K. J., E. C. Raff, R. A. Raff, and T. C. Kaufman. 1979. Mutation in a testis-specific β-tubulin in Drosophila: analysis of its effect on meiosis and map location of the gene. Cell. 21:455-451.
19. Krek, K. C., J. L. Hall, and N. J. Cowan. 1985. Nucleotide sequence and evolution of a mammalian α-tubulin messenger RNA. J. Mol. Biol. 181:101-120.
20. Lewis, S. A., M. E. Gilchrist, J. L. Hall, and N. J. Cowan. 1983. Three expressed sequences within the human β-tubulin multigene family each define a distinct isotype. J. Mol. Biol. 162:11-20.
21. Lewis, S. A., J. M. Balcarek, V. Krek, M. L. Shelanski, and N. J. Cowan. 1984. Sequence of a cDNA clone encoding mouse gial fibrillary acidic protein: structural conservation of intermediate filaments. Proc. Natl. Acad. Sci. USA. 81:2743-2746.
22. Lewis, S. A., J. M. Balcarek, V. Krek, M. L. Shelanski, and N. J. Cowan. 1984. Sequence of a cDNA clone encoding mouse gial fibrillary acidic protein: structural conservation of intermediate filaments. Proc. Natl. Acad. Sci. USA. 81:2743-2746.
23. Neff, N. J., J. H. Thomas, P. Grisafi, and D. Botstein. 1983. Isolation of the β-tubulin gene from yeast and demonstration of its essential function in vivo. Cell. 33:211-219.
24. Oakley, B. R., and N. R. Morris. 1983. A β-tubulin mutation in Aspergillus nidulans that blocks microtubule function without blocking assembly. Cell. 12:561-571.
25. Ponte, P., P. Gunning, H. Blau, and L. Kedes. 1983. Human actin genes are single copy for α-skeletal and α-cardiac actin but multicopy for β- and γ-cytoplasmic actin. J. Cell Biol. 98:1687-1696.
26. Quan, W., Y. Quan-Keuen, W. Vree Egbets, R. Van den Heuvel, W. Hendriks, and H. Bloemendal. 1984. The genes for vimentin and desmin. In The Molecular Biology of the Cytoskeleton. D. W. Cleveland, D. Murphy, and G. Bursen, editors. Cold Spring Harbor Publications, Cold Spring Harbor, NY. 443-454.
27. Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg. 1977. Labeling DNA to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. 113:237-251.
28. Rugh, R. 1968. The mouse: its reproduction and development. Burgess Publishing Co., Minneapolis, Minnesota.
29. Sanger, F., A. R. Coulten, B. G. Barrell, A. J. H. Smith, and B. Roe. 1980. Cloning single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. 143:161-178.
30. Serrano, L., J. de la Torre, R. B. Maci, and J. Avila. 1984. Involvement of the carboxyterminal domain of tubulin in the regulation of its assembly. Proc. Natl. Acad. Sci. USA. 81:5989-5993.
31. Southern, E. 1975. Detection of specific sequences among fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-517.
32. Sullivan, K., and D. W. Cleveland. 1984. Sequence of a highly divergent β-tubulin gene reveals regulatory heterogeneity in the β-tubulin polyadenylate. J. Cell Biol. 99:1754-1760.
33. Sullivan, K. F., J. C. Havercroft, and D. W. Cleveland. 1984. Primary structure and expression of a vertebrate β-tubulin gene family. In Molecular Biology of the Cytoskeleton. Borys, G. G., D. W. Cleveland, and D. B. Murphy, editors. Cold Spring Harbor Publications, Cold Spring Harbor, NY. 321-332.