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Regulation of three β-tubulin mRNAs during rat brain development

I.Ginzburg, A.Teichman, H.J.Dodemont, L.Behar and U.Z.Littauer

Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel

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The nucleotide sequence of a complete rat brain β-tubulin T β15 has been determined from three overlapping cDNA clones. The overall length of the T β15 sequence is 1589 bp and shows between 84.5% and 88.6% homology within the coding region as compared with chick and human β-tubulin sequences. On the other hand, the 3'-non-coding region is highly divergent. Comparison of the derived amino acid sequences from different species demonstrates that the amino acid changes are not randomly distributed, but rather there are several conserved and two highly variable regions common to β-tubulin polypeptides from various sources. The T β15 sequence encodes a dominant neuronal 1.8-kb β-tubulin mRNA species. Two other minor β-tubulin mRNA species of 2.6 and 2.9 kb are present in rat brain. By using two synthetic oligonucleotide probes complementary to the carboxyl-terminal divergent region and to the amino-terminal conserved region, we have shown that the three mRNAs are distinct species, which are developmentally regulated. The level of the 1.8-kb mRNA species increases till the age of 12 days thereafter its level decreases. The 2.9-kb mRNA is an early neuronal mRNA species, while the 2.6-kb mRNA is a late neuronal species which is detected at 30 days of rat brain development. The data illustrate that there is a differential expression of the β-tubulin multigene family during rat brain development which may suggest different functions for the various β-tubulin isotopes.

Key words: cDNA sequences/synthetic oligonucleotides

Introduction

Microtubules are ubiquitous eukaryotic organelles participating in various cellular functions such as cell division, cell migrations, cell shaping and secretion (Bray, 1973; Dustin, 1984; Bray and Gilbert, 1981; Roberts and Hyam, 1979; Littauer and Ginzburg, 1985). The major protein component of microtubules is tubulin, which is a heterodimer composed of an α- and β-subunit each having a mol. wt. of ~50 kd. Early during brain development, microtubules participate in spindle formation in dividing cells (Dustin, 1984; Nunez *et al.*, 1980). At later stages of brain differentiation, microtubules are also involved in cell migration (Olmsted and Borisy, 1973) and are essential in creating the basic asymmetry of neurons, that distinguishes between axons and dendrites (Matus *et al.*, 1983; De Camilli *et al.*, 1984). In mature neurons axon outgrowth, synapse formation and axoplasmic transport depend on mcirotubule integrity (Daniels, 1972; Marchisio *et al.*, 1980; Tytell *et al.*, 1984). It appears that diverse microtubule types are involved in several neuronal functions which may be controlled by the presence of various tubulin isotypes and microtubule-associated proteins (MAPs). In spite of the overall decrease in tubulin synthesis during rat brain maturation (Schmitt *et al.*, 1977; Gozes *et al.*, 1977), more tubulin isotypes are expressed in the mature than in the pre-natal brain (Gozes and Littauer, 1978). Some of the age-dependent increase in tubulin and TAU factor microheterogeneity is controlled at the mRNA level (Gozes *et al.*, 1980; Ginzburg *et al.*, 1982).

Tubulin, like several other eukaryotic genes, is encoded by a multigene family. In *Drosophila* four α- and four β-tubulin genes have been identified which have been shown to be functional (Kalfayan and Wensink, 1982; Natzle and McCarthy, 1984). In the mammalian genome the tubulin gene family is more complex and, due to the high homology within the members of the family, the analysis of a unique member becomes more difficult. Using a rat α-tubulin cDNA probe, we have shown that ~20 copies of tubulin genes exist in the rat genome (Ginzburg *et al.*, 1983b). However, the number of functional genes is unknown, and indeed several rat α-tubulin pseudogenes were identified and sequenced (Ginzburg *et al.*, 1983b; Lemischka and Sharp, 1982). Here we describe the analysis of a complete rat β-tubulin sequence and its use for studying specific gene transcripts. The determination of the β-tubulin sequence allowed us to select specific regions for the synthesis of synthetic oligodeoxynucleotide probes. Using these probes, we could reveal the presence of three distinct mRNA species whose expression changes during rat-brain development.

Results

Isolation of overlapping β-tubulin cDNA clones

Poly(A)-containing mRNA was isolated from 12-day-old rat brains and fractionated by sucrose gradient centrifugation. The fractions above 16S encoding for tubulin mRNA, were pooled and used as a template for double-stranded cDNA synthesis which was subsequently inserted into pBR322 as previously described (Ginzburg *et al.*, 1980). About 2500 transformants were screened using the ^{32}P-labeled *Pst*I insert isolated from a chicken β-tubulin cDNA clone (Valenzuela *et al.*, 1981; kindly supplied by M.W.

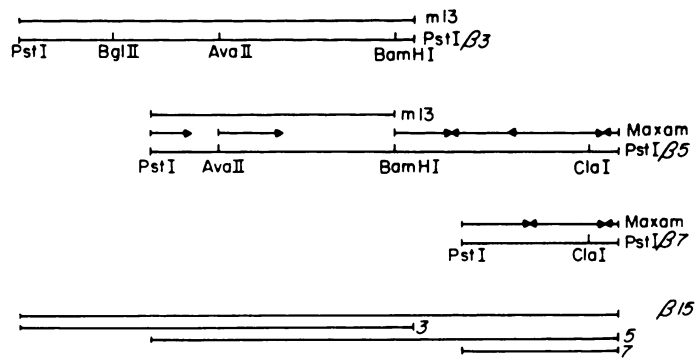

Fig. 1. The organization and sequencing strategy of rat β-tubulin cDNA clones. The partial restriction map of p β3, p β5 and p β7 is presented. The sequencing method for the respective DNA fragments is denoted.

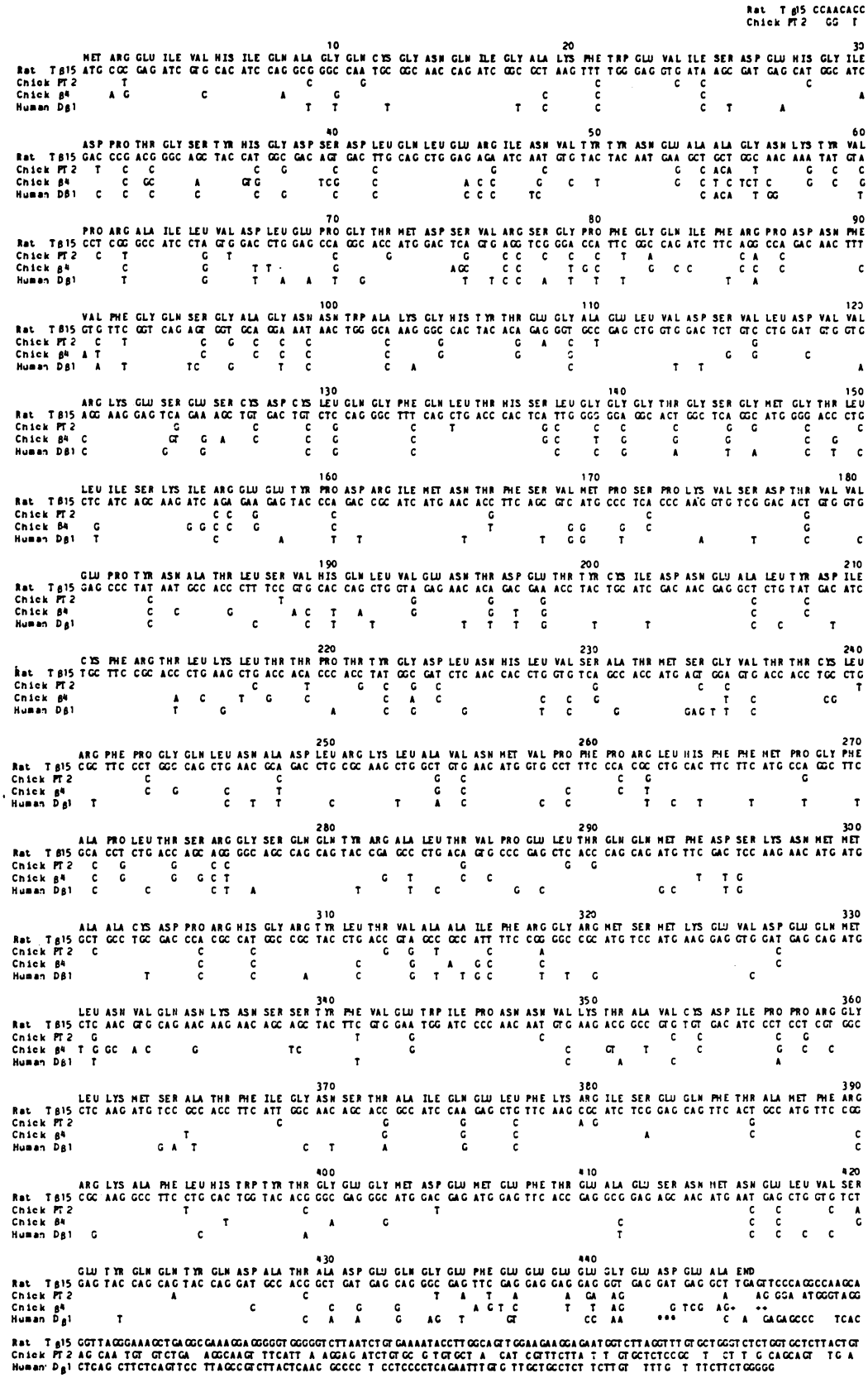

Fig. 2. The nucleotide sequence of rat β-tubulin T β15. Comparison of the sequence of T β15 with chicken pT2 (Valenzuela *et al.*, 1981). Chicken β4 (Sullivan and Cleveland, 1984) and human D β1 (Hall *et al.*, 1983). Only positions that differ from rat T β15 sequence are noted. * Indicates deletion of a nucleotide; + indicates insertion of a nucleotide; ++ indicates that data ends here.

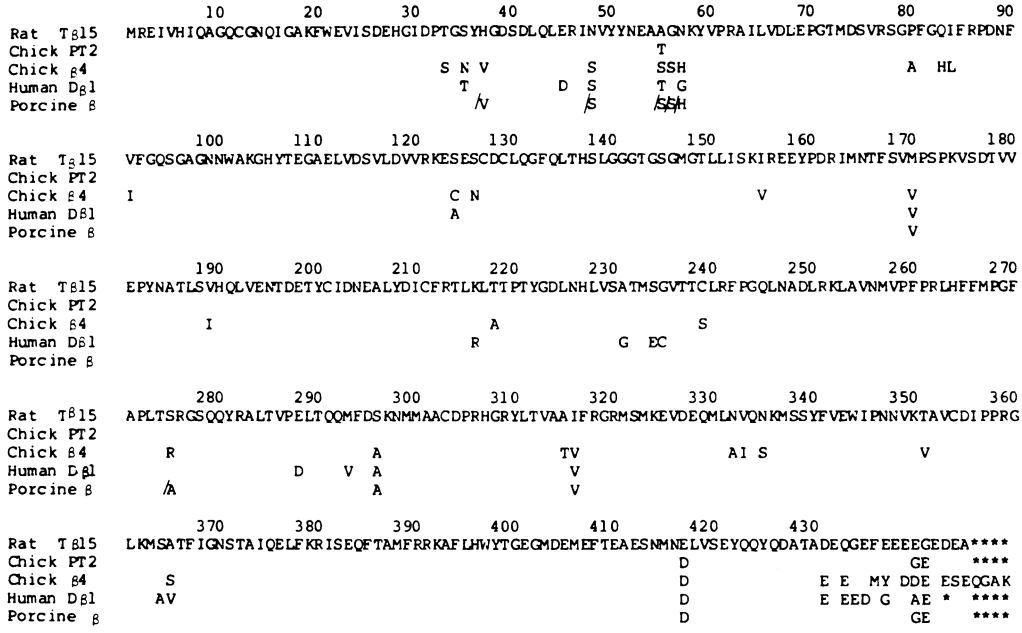

Fig. 3. The derived amino acid sequence of rat T β15 as compared with other vertebrate β-tubulins. The data are derived from Figure 2 together with the amino acid sequence of β-tubulin isolated from porcine brain (Krauhs *et al.*, 1981). Only positions that differ from rat T β15 sequence are noted. * Indicates a deletion; / indicates a site of microheterogeneity where either the amino acid of the top line or the slashed one may be present.

Kirschner). Twenty rat clones containing β-tubulin sequences were further identified by positive hybridization followed by translation of the selected mRNA in a reticulocyte lysate cell-free system (Ricciardi *et al.*, 1979). The labeled products were analyzed by SDS-polyacrylamide gel electrophoresis and shown to co-migrate with tubulin.

Figure 1 shows the restriction map analysis of three overlapping β-tubulin cDNA clones (p β3, p β5, p β7) that were chosen for further studies and their cumulative sequence was designated as T β15.

Sequence analysis of rat β-tubulin cDNA clones

The overall length of T β15 is 1589 bp and includes 1338 bp comprising the entire coding region and the termination codon as well as 8 bp from the 5′-non-coding region and 243 bp from the 3′-non-coding region. The nucleotide sequence strategy is illustrated in Figure 1 and included both the chemical modification method as well as subcloning into phage M13 and using the dideoxy termination method (Maxam and Gilbert, 1977; Sanger *et al.*, 1977).

The deduced amino acid sequence confirmed that these three clones carry β-tubulin sequences. Clones p β3, p β5 and p β7 contain inserts of 1064 bp (positions −8 to 1056), 1245 bp (positions 350 − 1595) and 419 bp (positions 1170 − 1589), respectively. The nucleotide sequence confirmed the absolute identity of the overlapping regions, suggesting that they are encoded by the same gene. The nucleotide sequences of T β15 is presented in Figure 2 in comparison with the sequence of three other available vertebrate β-tubulin sequences, namely chicken cDNA clone pT2, the chicken genomic clone β-4 (Valenzuela *et al.*, 1981; Sullivan and Cleveland, 1984) as well as human cDNA clone D β-1 (Hall *et al.*, 1983).

The overall calculated nucleotide divergence between the coding region of rat T β15 and chicken pT2 is 153 nucleotides of which 143 are silent substitutions, while the divergence of human D β-1 is higher and reaches 199 nucleotides of which 159 are silent substitutions. The chicken genomic β-4 is even more divergent when compared with the rat β-tubulin sequence. There are 207 nucleotide changes of which only 165 are silent substitutions that do not result in changes of amino acids. Thus, there is a high degree of nucleotide homology in the coding regions between the various β-tubulin clones ranging between 84.5% and 88.6%. On the other hand, there is little homology between the 3′-non-coding regions among these DNA clones, except for a region between positions 1471 and 1499 that is highly enriched with T residues and also appears with minor changes in the 3′-non-coding region of the human D β-1 sequence.

Comparison of the derived rat T β15 polypeptide with that of chicken, human and porcine β-tubulin sequences

Comparison of the derived rat T β15 β-tubulin amino acid sequence with that of chicken pT2 and the two porcine variants shows a high degree of homology (Figure 3) (Valenzuela *et al.*, 1981; Sullivan and Cleveland, 1984; Hall *et al.*, 1983; Krauhs *et al.*, 1981). All these sequences represent major brain β-tubulin isotypes, which have a chain length of 445 residues and differ by four, six and 12 amino acids, respectively, from rat brain T β15. On the other hand, the amino acid sequence derived from the human fetal brain clone p β-1 demonstrates 25 amino acid replacements and contains only 444 residues. The amino acid sequence encoded by the genomic chicken β-4 DNA shows even a higher divergence from the rat sequences with 42 amino acid changes and is longer with 449 residues.

Figure 3 also demonstrates that the amino acid changes are not randomly distributed, but rather there are distinct conserved and highly variable regions common to β-tubulin polypeptides from the various sources. A high degree of amino acid conservation is observed at the N-terminal region between residues 1 and 32 and in another region between residue 336 and 430. The amino acid conservation between residue 410 and 430 is interrupted only at one site, namely position 417 in which glutamic acid in the rat sequence replaces aspartic acid in β-tubulin from the other sources. This substitution, however, is conservative and does not change significantly the charge of the molecule. On the

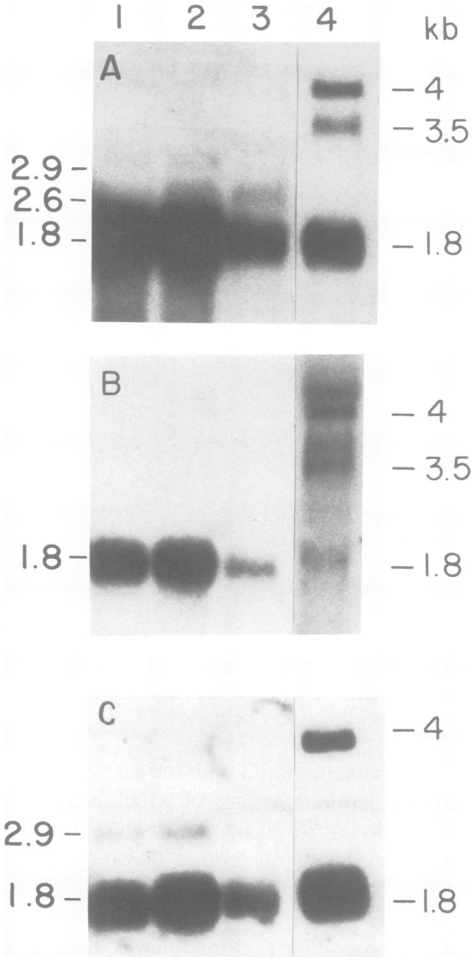

Fig. 4. Differential expression of β-tubulin mRNAs during rat brain development. Equal amounts of 5 μg of poly(A)-containing RNA were subjected to electrophoresis on denaturing agarose gels, transferred to nitrocellulose filter and hybridized to the following probes. **(A)** T β15 ^{32}P-labeled by nick-translation; **(B)** 3′ β oligonucleotide probe phosphorylated by T4 polynucleotide kinase; **(C)** 5′ β oligonucleotide probe phosphorylated by T4 polynucleotide kinase. Brain mRNA isolated from 0 (new-born). 12, 30-day-old rats (**tracks 1–3**, respectively) and chicken brain (**track 4**); rRNA markers were run and stained in a parallel slot. Before hybridization of **(B)** and **(C)** the previous probe was removed by treatment with 10 mM methylmercury hydroxide. In **panel B, track 10**, is shown following longer exposure.

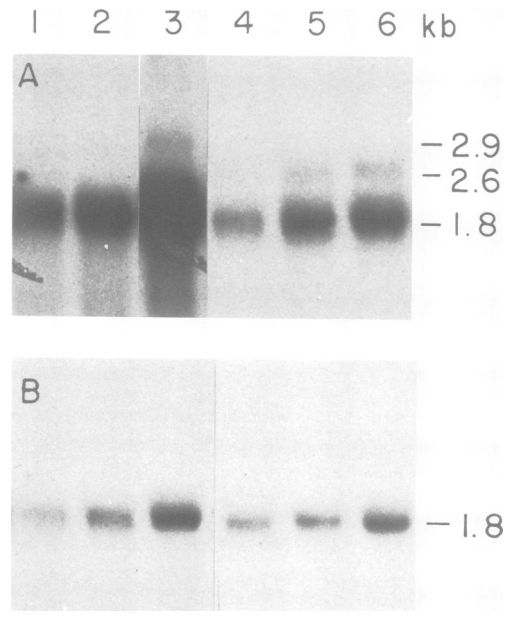

Fig. 5. Quantitative analysis of β-tubulin and α-tubulin mRNA. Increasing amounts of mRNA were subjected to analysis as described in Figure 4. **(A)** β-Tubulin T β15 cDNA. **(B)** Following removal of the probe used in **A** the blot was re-hybridized with labeled α-tubulin pT25 cDNA (Ginzburg *et al.*, 1981). 1, 2 and 5 μg of brain mRNA from 0-day-old rats (**tracks 1–3**, respectively). 1, 2 and 5 μg of brain mRNA from 30-day-old rats (**tracks 4–6**, respectively).

other hand, prominent divergence is apparent at the C-terminal region beyond residue 430. From residue 430 up to 445, there are between two and 10 amino acid substitutions. An additional divergent region is found between residues 33 and 57. This latter region has been shown to display internal microheterogeneity between the two porcine β-tubulin variants (Krauhs *et al.*, 1981).

T β15 sequences are prominently expressed in rat brain and are developmentally regulated

Several β-tubulin mRNA species which differ in their size distribution exist in various vertebrates. Thus, two mRNA species of 1.8 and 2.6 kb were detected in HeLa cells by using human cDNA D β-1 as a probe (Hall *et al.*, 1983; Lewis *et al.*, 1985). In addition, chicken pT2 cDNA revealed three β-tubulin mRNA species in chicken brain, namely an abundant 1.8-kb species as well as a 3.5-kb and 4-kb species (Lopata *et al.*, 1983). We sought to determine the size distibution and expression of β-tubulin mRNA at different stages of rat-brain development. As a labelled probe we employed the mixture of clone p β3 and clone p β5 that span together the coding and 3′-non-coding regions of T β15.

Equal amounts of RNA were subjected to gel electrophoresis under denaturing conditions, blotted onto nitrocellulose paper and then hybridized to the radiolabeled β-tubulin cDNA probe. Figure 4A shows that the most prominent hybridization was to a 1.8-kb mRNA species which is present in high abundance in brain tissue. As was previously described, there is a slight increase in the amount of hybridizable β-tubulin mRNA at 12 days of rat-brain development followed by a sharp decrease towards the age of 30 days to 30% of the level found in new-born brain (Ginzburg *et al.*, 1983a; Bond *et al.*, 1984). Two additional mRNA species are also observed in rat brain preparations although to a lesser extent than the major 1.8-kb species. A 2.9-kb species is prominent in mRNA from new-born and 12-day rat brain, the level of which decreases during later stages of brain development. The second β-tubulin mRNA species has a size of 2.6 kb, is not detected at earlier stages of brain development and starts to appear at the age of 12 days (Figure 4A). Quantitative analysis of mRNA from new-born and 30-day-old mRNA showed that the 2.6-kb and 2.9-kb species comprise 7% and 3%, respectively, of the total hybridizable β-tubulin mRNA (Figure 5A). Following removal of the previous cDNA probe, the same RNA blot used in Figure 5A was re-hybridized, with ^{32}P-labeled α-tubulin pT25 cDNA. Figure 5B shows that this probe only hybridizes to the 1.8-kb mRNA species. Thus, the 2.6- and 2.9-kb mRNA species are unique for β-tubulin.

In parallel, we have examined the size distribution of chicken brain β-tubulin mRNA. The pattern obtained (Figure 4A) was identical to that previously described with the chicken pT2 DNA probe (Lopata *et al.*, 1983). Three mRNA species were evident, the most abundant one was a 1.8-kb species and, in addition,

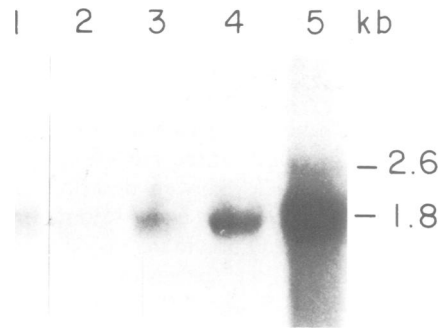

Fig. 6. The tissue distribution of β-tubulin mRNA. The methods used were as described in Figure 4. β-Tubulin ^{32}P-labeled T β15 cDNA was used as the probe. **Tracks 1–4** contained 15 μg of mRNA isolated from rat glioma C6BU-1; human neuroblastoma SK-N-AS; rat muscle and rat liver, respectively. **Track 5** contains only 1 μg of mRNA isolated from 12-day-old rat brain.

two minor species of 3.5 and 4.0 kb were also evident which were not present in the various mRNA preparations from rat tissues. Moreover, the 2.9- and 2.6-kb β-tubulin mRNA species which are present in rat brain were not detected in mRNA isolated from chicken brain. In contrast to the high level of T β15 mRNA expression in brain, considerably lower levels were observed in muscle, liver, rat glioma and human neuroblastoma cells. Figure 6 shows the hybridization of the labeled T β15 probe with 15 μg of poly(A)-containing RNA from the various sources as compared with 1 μg of brain mRNA.

Use of labeled synthetic oligodeoxynucleotides as probes to explore the expression of various β-tubulin mRNA species

To examine further the expression of the β-tubulin gene family, we have used as probes two synthetic oligodeoxynucleotides. The first oligodeoxynucleotide synthesized was complementary to the 3′ end of the rat T β15 β-tubulin gene, namely 3′-CTCAAGCTCCTCCTCCTCCCA-5′. This sequence (3′ β) corresponds to amino acid residues 435–441 and is located in the highly divergent region of β-tubulin (Figure 2). Following removal of the previous cDNA probe, the same RNA blot used in Figure 4A was re-hybridized, with ^{32}P-labeled oligodeoxynucleotide 3′ β. Figure 4B shows that the only species detected in the rat-brain mRNA preparations was the highly abundant 1.8-kb species. On the other hand, the 2.6- and 2.9-kb mRNA species did not hybridize to this probe. Most interestingly, the abundant chicken 1.8-kb species displayed poor hybridization with this probe, indicating that there is a high degree of sequence divergence in this region between the rat and chicken genes. In contrast, the two higher mol. wt. chicken mRNA species of 3.5 and 4.0 kb show significant hybridization, and appear in doublet form. The origin of these two subpopulations present in the 3.5- and 4.0-kb chicken mRNA species is not clear.

In contrast to the 3′ end of the mRNA, the region near the 5′ end which codes for the N-terminal region of the polypeptide, is highly conserved (Figure 2). We have, therefore, synthesized an oligodeoxynucleotide complementary to this region which corresponds to amino acids 4–10 of the β-tubulin sequence, namely 3′-TAGCACGTGTAGGTCCGCCCG-5′ (5′ β). This sequence was used to probe the same mRNA blot used in Figure 4A and B. The result of such an hybridization experiment is shown in Figure 4C. The 5′ β probe hybridized readily to the 1.8-kb abundant mRNA from either rat or chick brain. However, of the two minor rat mRNA species, it hybridized only to the 2.9-kb species and, in the chicken mRNA, to the 4.0-kb species.

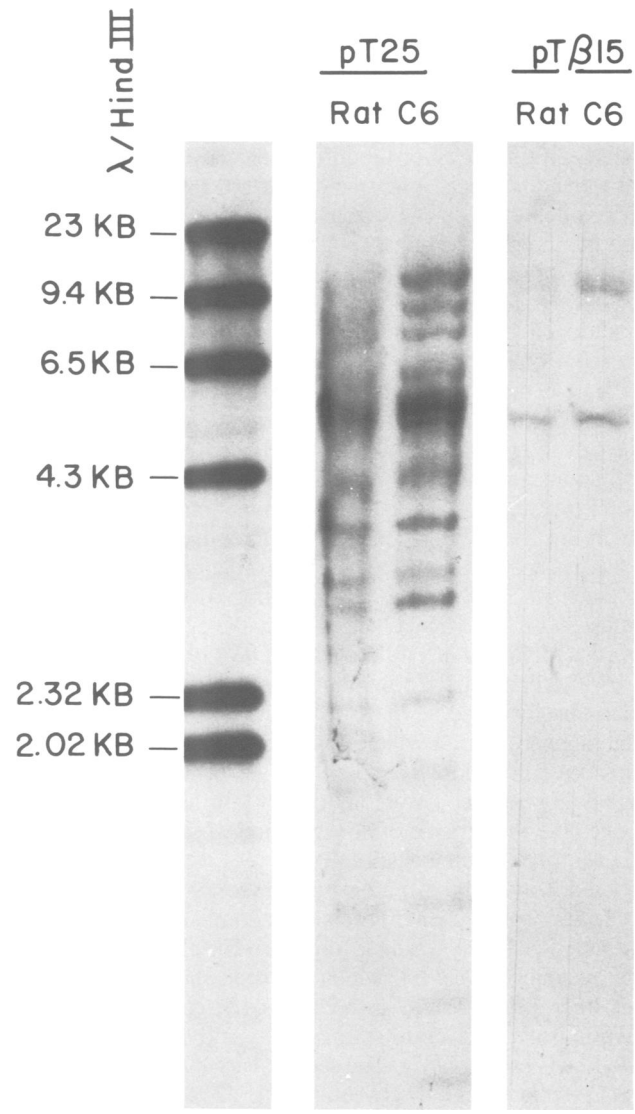

Fig. 7. Hybridization of tubulin cDNA probes to *Eco*RI-digested genomic DNA. 10 μg of rat brain and rat glioma C6 DNAs were digested with *Eco*RI, subjected to electrophoresis on 0.7% agarose gel, and transferred to nitrocellulose filters. The hybridization was carried out with ^{32}P-labeled pT25 or T β15 probes. Hybridization was performed at 42°C in buffer containing 50% formamide; 5 × SSC, 5 × Denhardt solution; 50 mM $NaPO_4$, pH 6.5; 25% dextran sulfate and 0.15 μg/ml salmon sperm DNA. Following the hybridization reaction, the filter was washed twice for 30 min at 50°C in a solution containing 1 × SSC, 0.1% SDS and once at 70°C in a solution containing 0.1 × SSC and 0.1% SDS.

Identification of the gene coding for β-tubulin

Southern blot analysis with labeled rat α-tubulin cDNA pT25 has allowed us to detect 10–20 tubulin genes in rat DNA (Figure 7). Such analysis will detect all the functional tubulin sequences as well as some non-functional pseudogenes. Indeed several α- and β-tubulin pseudogenes were identified and sequenced in genomic DNA clones from human (Cowan and Dudley, 1983) and rat brain (Ginzburg *et al.*, 1983b; Lemischka and Sharp, 1982). The hybridization pattern obtained with the rat β-tubulin T β15 sequence revealed only two genes in rat DNA and DNA isolated from rat glioma C6 cells (Figure 7). This pattern was observed under stringent conditions of hybridization and washing, thus indicating that the β-tubulin T β15 sequences detects only a limited number of genes.

Discussion

Among the possible different mechanisms leading to tubulin microheterogeneity one may involve the existence of multiple gene families. We have previously demonstrated the presence of 15–20 tubulin genes in the rat (Ginzburg *et al.*, 1983b). Similarly, multiplicity of tubulin genes was observed in DNA from other mammals (Cleveland *et al.*, 1980). However, the number of expressed genes remains to be determined. About half of the human or rat α- or β-tubulin genes so far sequenced appear to be pseudogenes (Lemischka and Sharp, 1982; Ginzburg *et al.*, 1983b; Cowan *et al.*, 1983). In the present work we have characterized a rat β-tubulin cDNA sequence and described its expression during brain development. The coding region of vertebrate β-tubulin appears to be highly conserved (Figure 1). Comparison of rat T β15 with chicken pT2 and human D β-1 sequences shows that most of the nucleotide differences are silent substitutions and only 0.7% and 2.9%, respectively, resulted in amino acid changes. The rate of accumulation of silent substitutions in the nucleotide sequence of DNA during the evolution process has been estimated to be of the order of 1% per 10^6 years (Miyata *et al.*, 1982). The accumulation of these changes has been used as a biological clock to study the evolutionary history of genes (Miyata *et al.*, 1982; Shen *et al.*, 1981; Efstratiadis *et al.*, 1980). The expected degree of homology between rat and human that diverged $\sim 75 \times 10^6$ years ago is $<50\%$ and between chicken and rat which diverged $\sim 300 \times 10^6$ years ago the expected homology is $<30\%$. However, the high degree of amino acid sequence homology observed in the coding regions indicates the importance of conservation of functional domains along the protein molecule.

Comparison of the β-tubulin species so far sequenced shows that the amino acid changes are not randomly distributed; rather there are two highly variable regions. The first is located between residues 33 and 64, which has been shown to display internal microheterogeneity in both porcine, chicken and human β-tubulin isotypes (Krauhs *et al.*, 1981; Sullivan and Cleveland, 1984; Lewis *et al.*, 1985). The second variable region is located at the C-terminal region between residue 430 and the C terminus of the polypeptide which varies in length for different vertebrate β-tubulin species. The variability in the amino acid sequence of these two domains may contribute to or change the specificity of interaction of β-tubulin isotypes with its various ligands (including MAPs), thus generating functionally different microtubules (Ginzburg and Littauer, 1984; Ginzburg *et al.*, 1985).

In contrast to the coding region, comparison of the nucleotide sequence of the 3′-untranslated regions of rat T β15, chicken pT2, β-4 and human D β1 cDNA clones reveals a high degree of divergence. It has been previously noted that, while within a given species the 3′-untranslated region of individual α-tubulin genes are totally dissimilar, each in turn shares very high interspecies homology (Cowan *et al.*, 1983; Ginzburg *et al.*, 1985; Elliot *et al.*, 1985). For the β-tubulin family such conservation of the 3′-untranslated sequence has not yet been observed, this could be due to the limited number of active β-tubulin genes so far sequenced.

To study the expression and size distribution of β-tubulin during brain maturation, we have used the rat T β15 sequence and two synthetic oligodeoxynucleotide probes which are complementary to the conserved and divergent regions located at the 5′ and 3′ end of β-tubulin sequence, respectively. Using the T β15 sequence as a probe, we have detected three β-tubulin mRNA species in rat brain, which are developmentally regulated. The most dominant mRNA species has a size of 1.8 kb and may be specific to neurons, as only very low levels were found in glioma cells. In addition to the major 1.8-kb β-tubulin mRNA species, two minor species of 2.9 and 2.6 kb were detected in rat brain mRNA. The 2.9-kb species appears early during brain development while the 2.6-kb mRNA species appears at later stages of brain development. Similarly, three mRNA species exhibiting size classes of 1.8, 2.6 and 2.9 kb were described in developing cerebellum (Bond *et al.*, 1984). In contrast to rat brain, chicken brain mRNA contains, in addition to the major neuronal species of 1.8 kb, two higher mol. wt. species of 3.5 and 4.0 kb while neither of the rat brain 2.6- and 2.9-kb, species are present.

The use of oligodeoxynucleotide probes complementary to regions located at the 5′ and 3′ end of β-tubulin coding sequence allowed us to conclude that there are three different β-tubulin mRNA species in brain and to ascribe some relationship between the different mRNA size classes. It is clear from Figure 4B that the 3′-terminal probe (3′ β) detected only the 1.8-kb major neuronal mRNA species and hence it differs in its 3′ sequence from the 2.6- and 2.9-kb mRNA chains. On the other hand, the 5′-terminal (5′ β) probe revealed that the 2.9- and 1.8-kb mRNA species share sequence homology at the 5′ end, while the 2.6-kb is not detected with this probe (Figure 4C). The data therefore suggest that the 2.6-kb mRNA species is a product of a different gene with unique 5′ and 3′ ends. On the other hand, it is not clear whether the 2.9- and 1.8-kb mRNA species which differ at the 3′ end are transcribed from two separate genes or are derived from a single gene by different splicing processes and therefore share homologous 5′ sequences. The latter hypothesis would be compatible with the genomic hybridization data (Figure 7) which showed that the T β15 sequence detects two genes in the rat DNA. In the case of chicken brain β-tubulin mRNA the 5′-terminal probe preferentially hybridized to the 4.0- and 1.8-kb mRNA species and did not show homology with the 3.5-kb species. On the other hand, the 3′-terminal probe showed low homology to the major 1.8-kb species but hybridized to the 4.0- and 3.5-kb species. The interrelationship of the three chicken β-tubulin species is not clear, but appears to differ from that found for the rat brain.

In neurons microtubules are essential in creating the basic asymmetry that distinguishes between axons and dendrites. It appears that neither genetic diversity nor any other single mechanism may account for cellular control over the enormous versatility of microtubule functions. Among the possible different mechanisms, one may invoke the presence of multiple genes coding for various tubulin isotypes and different MAP forms. Additional mechanisms which may yield several products may involve different splicing events, a read-through mechanism (Lee *et al.*, 1983) as well as post-translational modifications of these proteins. Finally, the different interactions that may take place between all these components may result in diverse microtubules involved in specific functions.

Materials and methods

Identification of tubulin cDNA

mRNA isolation, preparation of recombinant plasmids containing rat-brain tubulin sequences and positive identification was carried out as previously described (Ginzburg *et al.*, 1980).

Nucleotide sequence analysis of recombinant DNA

DNA sequence analysis was carried out by the chemical sequencing method (Maxam and Gilbert, 1977) or by the chain termination method (Sanger *et al.*, 1977) using M13 vectors mp10 and mp11 (Messing and Vieira, 1982).

Analysis of tubulin mRNA levels

RNA blots were carried out on the indicated poly(A)-containing RNA samples as previously described (Fellous *et al.*, 1982).

Oligodeoxynucleotide synthesis

Oligonucleotide probes were synthesized by Dr. O. Goldberg from the Weizmann Institute of Science. The synthesis was conducted in a step-wise manner essentially as described (Beaucage and Caruthers, 1981). The oligonucleotides were 5′ end-labeled by the procedure of Richardson (1965). In short, 10 pmol of oligonucleotide were labeled with 10 pmol of γ-labeled [^{32}P]ATP (7000 Ci/mol) in the presence of four units of T4 phage polynucleotide kinase. The probes were separated from free [^{32}P]ATP by chromatography on DE52 cellulose. Hybridization was performed in 4 × SSC; 5 × Denhardt's solution; 0.02 M EDTA, 0.1% SDS, 250 μg/ml salmon sperm DNA, 0.06% NaPPi at 55°C, which is 10°C lower than the calculated suitable temperature for the base composition and length of the oligonucleotide that were used (Dalbadie-McFarland *et al.*, 1982). Hybridization was performed for 18 h, and the hybridization was followed by two washes for 30 min in 1 × SSC containing 0.1% SDS and 0.06% NaPPi at 50°C.

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References

Beaucage,S.L. and Caruthers,M.H. (1981) *Tetrahedron Lett.*, **22**, 1859-1862.
Bond,J.F., Robinson,G.S. and Farmer,S.R. (1984) *Mol. Cell. Biol.*, **4**, 1313-1319.
Bray,D. (1973) *Nature*, **224**, 93-96.
Bray,D. and Gilbert,D. (1981) *Annu. Rev. Neurosci.*, **4**, 505-523.
Cleveland,D.W., Lopata,M.A., McDonald,R.J., Cowan,N.J., Rutter,W.J. and Kirschner,M.W. (1980) *Cell*, **20**, 95-105.
Cowan,N.J., Dobner,P.R., Fuchs,E.V. and Cleveland,D.W. (1983) *Mol. Cell. Biol.*, **3**, 1738-1745.
Cowan,N.J. and Dudley,L. (1983) *Int. Rev. Cytol.*, **85**, 147-173.
Dalbadie-McFarland,G., Cohen,L.W., Riggs,A.D., Morin,C., Itakura,K. and Richards,J.H. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 6409-6413.
Daniels,M.P. (1972) *J. Cell Biol.*, **53**, 164-176.
De Camilli,P., Miller,P.E., Navoni,F., Theurkauf,W.E. and Vallee,K.B. (1984) *Neuroscience*, **11**, 819-846.
Dustin,P. (1984) *Microtubules*, published by Springer-Verlag, NY.
Efstratiadis,A., Posakony,J.W., Maniatis,R., Lawn,R.M., O'Connell,C., Spritz,R.A., DeRiel,J.K., Forget,B.G., Weissman,S.M., Slightom,J.L., Belchl,A.E., Smithies,O., Baralle,F.E., Shoulders,C.C. and Proudfoot,N.J. (1980) *Cell*, **21**, 653-668.
Elliot,E.M., Okayama,H., Sarangi,F., Henderson,G. and Ling,V. (1985) *Mol. Cell. Biol.*, **5**, 236-241.
Fellous,A., Ginzburg,I. and Littauer,U.Z. (1982) *EMBO J.*, **1**, 835-840.
Ginzburg,I. and Littauer,U.Z. (1984) in Borisy,G.G., Cleveland,D.W. and Murphy,D.B. (eds.), *Molecular Biology of the Cytoskeleton*, Cold Spring Harbor Laboratory Press, NY, pp. 357-366.
Ginzburg,I., De Baetselier,A., Walker,M.D., Behar,L., Lehrach,H., Frischauf,A.M. and Littauer,U.Z. (1980) *Nucleic Acid Res.*, **8**, 3553-3564.
Ginzburg,I., Behar,L., Givol,D. and Littauer (1981) *Nucleic Acid Res.*, **9**, 2691-2697.
Ginzburg,I., Scherson,T., Giveon,D., Behar,L. and Littauer,U.Z. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 4892-4896.
Ginzburg,I., Ryback,S., Kimhi,Y. and Littauer,U.Z. (1983a) *Proc. Natl. Acad. Sci. USA*, **80**, 4234-4247.
Ginzburg,I., Scherson,T., Rybak,S., Kimhi,Y., Neuman,D., Schwartz,M. and Littauer,U.Z. (1983b) *Cold Spring Harbor Symp. Quant. Biol.*, **48**, 783-790.
Ginzburg,I., Teichman,A. and Littauer,U.Z. (1985) *Ann. N.Y. Acad. Sci.*
Gozes,I. and Littauer,U.Z. (1978) *Nature*, **276**, 411-413.
Gozes,I., Walker,M.D., Kaye,A.M. and Littauer,U.Z. (1977) *J. Biol. Chem.*, **252**, 1819-1825.
Gozes,I., Baetselier,A. and Littauer,U.Z. (1980) *Eur. J. Biochem.*, **103**, 13-20.
Hall,J.L., Dudley,L., Dobner,P.R., Lewis,S.A. and Cowan,N.J. (1983) *Mol. Cell. Biol.*, **3**, 854-862.
Kalfayan,L. and Wensink,P.C. (1982) *Cell*, **29**, 91-98.
Krauhs,E., Little,M., Kempf,T., Hofer-Warbinek,R., Ade,W. and Ponstingl,H. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 4156-4160.
Lee,G.-S.M., Lewis,S.A., Wilde,C.D. and Cowan,N.J. (1983) *Cell*, **33**, 477-487.
Lemischka,I. and Sharp,P.A. (1982) *Nature*, **300**, 330-335.
Lewis,S.A., Gilmartin,M.E., Hall,J.L. and Cowan,N. (1985) *J. Mol. Biol.*, **182**, 11-20.
Littauer,U.Z. and Ginzburg,I. (1985) in Zomzely-Neurath,C. and Walker,W.A. (eds.), *Gene Expression in Brain*, John Wiley & Sons, Inc., NY, pp. 125-156.
Lopata,M.A., Havercroft,J.C., Chow,L.T. and Cleveland,D.W. (1983) *Cell*, **32**, 713-724.
Marchisio,P.C., Weber,K. and Osborn,M. (1980) in Giacobini,E., Vernadakis,A. and Shahar,A. (eds.), *Tissue Culture in Neurobiology*, Raven Press, NY, pp. 99-109.
Matus,A., Huber,G. and Bernhardt,R. (1983) *Cold Spring Harbor Symp. Quant. Biol.*, **48**, 775-782.
Maxam,A. and Gilbert,W. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 560-564.
Messing,J. and Vieira,J. (1982) *Gene*, **19**, 269-276.
Miyata,T., Hayashida,H., Hasegawa,M., Kobayashi,M. and Koike,K. (1982) *J. Mol. Evol.*, **19**, 28-35.
Natzle,J.E. and McCarthy,B.J. (1984) *Dev. Biol.*, **104**, 187-198.
Nunez,J., Fellous,A., Francon,J. and Lennon,A.M. (1980) in de Bradander,M. and de May,J. (eds.), *Microtubules and Microtubule Inhibitors*, Elsevier/North Holland Biomedical Press, pp. 264-279.
Olmsted,J.B. and Borisy,G.G. (1973) *Annu. Rev. Biochem.*, **42**, 507.
Ricciardi,R.P., Miller,S.J. and Roberts,B.E. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 4927-4931.
Richardson,C.C. (1965) *Proc. Natl. Acad. Sci. USA*, **54**, 158-165.
Roberts,K. and Hyams,J.S.,(eds.) (1979) *Microtubules*, published by Academic Press, NY.
Sanger,F., Nicklen,S. and Culsen,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463-5467.
Schmitt,H., Gozes,I. and Littauer,U.Z. (1977) *Brain Res.*, **121**, 327-342.
Shen,S.I., Slighton,J.L. and Smithies,O. (1981) *Cell*, **26**, 191-203.
Sullivan,K.F. and Cleveland,D.W. (1984) *J. Cell Biol.*, **99**, 1754-1760.
Tytell,M., Brady,S.T. and Lasek,R.J.(1984) *Proc. Natl. Acad. Sci. USA*, **81**, 1570-1574.
Valenzuela,P., Quiroga,M., Zaldivar,J., Rutter,W.T., Kirschner,M.W. and Cleveland,D.W. (1981) *Nature*, **289**, 650-655.

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