# Differential expression of α-tubulin mRNA in rat cerebellum as revealed by in situ hybridization

I. Ginzburg, A. Teichman, W.S.T. Griffin+ and U.Z. Littauer

*Department of Neurobiology Weizmann Institute of Science, Rehovot 76100, Israel* and +*Department of Cell Biology, University of Texas Health Science Center, Dallas, TX 75235, USA*

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Nucleotide sequence analysis of two rat α-tubulin cDNA clones showed a marked divergence in their 3'-untranslated regions. However, each of the α-tubulin isotypes shows a high interspecies homology in this region, when compared with an isotubulin sequence from human and Chinese hamster. In situ hybridization of rat cerebellum with α-tubulin cDNA revealed differential expression in various cell layers. The mitotically active cells in the external granular layer show the highest level of α-tubulin mRNA, while lower levels are observed in the migrating cells in the molecular layer and in the differentiating cells in the internal granular layer. Very low levels of the mRNA are observed in the prenatally differentiated Purkinje cells.

*cDNA cloning* *3'-Noncoding region* *Nucleotide sequence* *Hybridization*

## 1. INTRODUCTION

Microtubules are cytoskeletal filaments present in all eukaryotic cells and are involved in various cellular functions such as cell division, cell migrations, cell shaping and secretion [1]. The major protein component of microtubules is tubulin, which is a heterodimer composed of $\alpha$- and $\beta$-subunits. It appears that different microtubule types are involved in several neuronal functions which may be controlled by the presence of various tubulin isotypes and microtubule-associated proteins (MAPs). Our results [2–4] and those of others [5,6] show that the expression of mRNAs coding for isotubulin and for TAU factors [7] are modulated during brain development. These results could be a consequence of either changes occurring similarly in each cell type or that microtubule composition is regulated independently in different cell populations upon assumption of a specific task.

Tubulin, like many other eukaryotic genes, is encoded by a multigene family [3,8]. In this report we describe the isolation of two rat $\alpha$-tubulin cDNA clones and their use for in situ hybridization studies to identify cells expressing specific gene transcripts.

This paper is dedicated to Professor S.P. Datta

## 2. MATERIALS AND METHODS

### 2.1. *cDNA cloning and identification of tubulin cDNA clones*

ds-cDNA was synthesized according to Gubler and Hoffman [9] and inserted into lambda gt10 phage vector [10]. DNA sequence analysis of selected clones was carried out by the chemical sequencing method [11] or by the chain terminator method [12].

### 2.2. *In situ hybridization of cDNA probes*

The cerebellum of 10-day-old rats was fixed by immersion in Boiun's solution for 24 h and then washed with 70% ethanol. Following fixation the tissue was embedded in paraffin. 5 $\mu$m sections were cut out and hybridized with $10^5$ cpm/section of nick translated $^3$H-labeled pT25 cDNA or pBR322 probe in 25% formamide, 10 mM Tris (pH 7.6), 200 mM NaCl and 500 $\mu$g/ml sheared *Escherichia coli* DNA. Following hybridization at 37°C for 18 h the sections were washed with low salt buffer of 50 mM Tris-HCl (pH 7.6), containing 10 mM KCl at 4°C, dipped in NTB-2 Kodak emulsion and exposed for 5–7 davs.

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## 3. RESULTS

### 3.1. *Sequence analysis and interspecies homology of two rat α-tubulin cDNA clones*

A more precise insight into the involvement of the multiple tubulin genes in microtubule function requires the construction of specific DNA probes for the individual gene transcripts. From the limited data available it appears that the various isotubulin mRNAs are distinguished by their highly divergent 3′-untranslated regions while the coding regions show extensive sequence homology [13–16].

Fig.1 shows the sequence of the 3′-noncoding regions of two rat α-tubulin clones. pT26 is a cDNA clone identical to our previously described pT25 clone coding for α-tubulin [14] except that it contains the complete 3′-noncoding regions up to and including the poly(A) tail (fig.1A). The second cDNA clone isolated and sequenced is pTA1 which is similar to the clone previously described by Lemischka et al. ([15]; fig.1B).

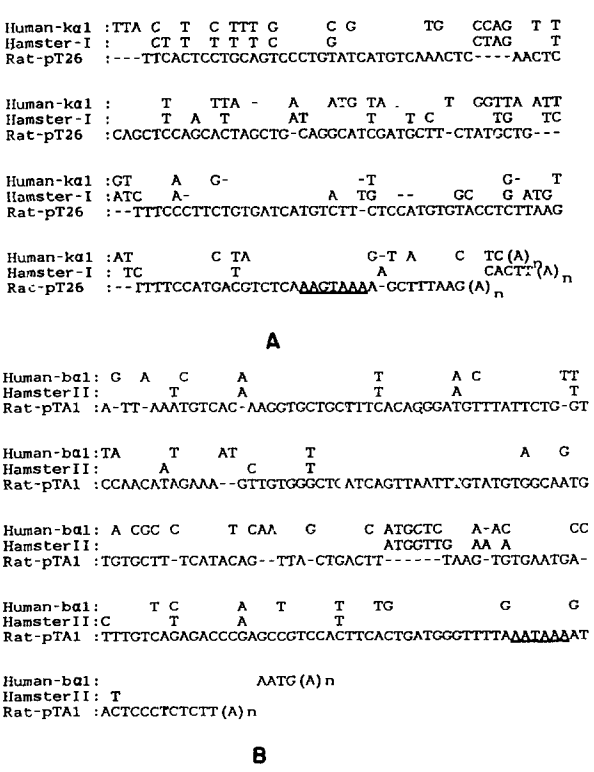


Fig.1. Nucleotide sequence of the 3′-noncoding regions of rat α-tubulin cDNA clones pTA26 (A) and pTA1 (B) as compared to human and CHO sequences.

While there is a close homology along the coding region between these clones there is high divergence in the 3′-noncoding regions. The 3′-noncoding region of clone pT26 contains 156 base pairs and its polyadenylation signal is AAGTAAA, which precedes by 9 bases the poly(A) tract. On the other hand, the 3′-noncoding region of clone pTA1 is longer and contains 194 base pairs, its polyadenylation signal is AATAAA and is located 14 bases before its poly(A) tract.

It has previously been noted that while within a given species individual isotubulin genes are totally dissimilar, each in turn shares very high interspecies homology [13,15–17]. The homology in this region is evident when the polyadenylation signal is used as an alignment point for mammalian isotubulin sequences. Clearly, the rat-pT26, human-kα1 [16] and hamster-I genes [17] are closely related, having AAGTAAA as polyadenylation signal and are probably derived from a common ancestral gene (fig.1A). The homology between these species ranges between 64 and 69%. On the other hand, the rat-pTA1, human-bα1 and hamster-II genes have AATAAA as polyadenylation signal and are derived from a second different gene (fig.1B). The homology in this group ranges between 75 and 88%. Moreover, it seems that the changes in nucleotide sequence along the 3′-noncoding regions appear to be nonrandom. A higher conservation is observed towards the 3′-end of the noncoding region around the polyadenylation signal. Whether this extensive interspecies homology is related to any function remains to be determined.

### 3.2. *In situ hybridization studies*

We have previously shown that the tubulin microheterogeneity found in brain is developmentally determined, increasing from 5 to 6 isotubulins prenatally to 7 isotubulins postnatally and reaching a value of 9–11 distinct components, during early brain maturation [18–20]. The question therefore arises as to whether changes occur in the relative proportion of the isotubulins upon assumption of different roles within the same nerve cell. Alternatively, the increase in tubulin microheterogeneity might arise from changes in the brain cell population during brain development, which unlike other organs is composed of

many cell types. The only way to differentiate between the above possibilities is to use the in situ hybridization technique which measures both aspects at the cellular level, i.e. changes in gene expression, cell type and number. Fig.2 shows the in situ hybridization of $^{3}$H-labeled pT25 rat $\alpha$-tubulin cDNA clone to cerebellum slices of 10-day-old rats. It was observed that there is relatively more of the total $\alpha$-tubulin mRNA in mitotically active external granular layer cells (EGL) than in the internal granular layer cells (IGL). These results show that migration and differentiation of the granule cells are accompanied by a decrease in their $\alpha$-tubulin mRNA level. Furthermore, nearly no signal is observed in the prenatally formed Purkinje cells and relatively low levels of $\alpha$-tubulin mRNA are observed in the postnatally formed stellate cells, in the molecular layer. Fig.2C shows the control for the above experiment where no signal is observed when the vector pBR322 is used for hybridization.

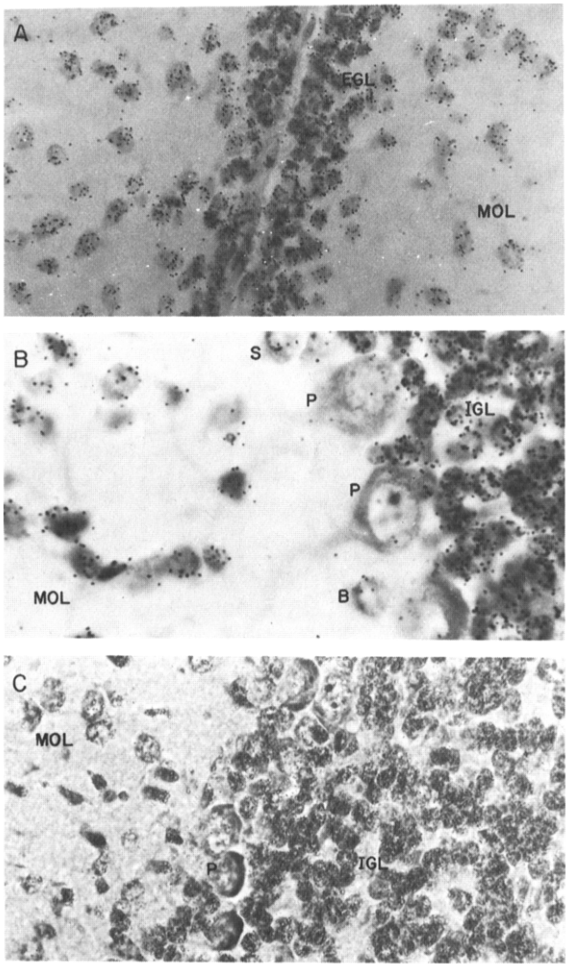


Fig.2. In situ hybridization of $^{3}$H-labeled pT25 cDNA with a section from 10-day-old rat cerebellum (A,B) and with pBR322 (C). (A) Hybridization over granule cells in the EGL and molecular layer. (B) Hybridization over Purkinje cells and IGL. (C) Hybridization with pBR322. EGL, external granular layer; IGL, internal granular layer; MOL, molecular layer; P, Purkinje cells; S, stellate cells; B, basket cells.

## 4. DISCUSSION

The dynamic nature of microtubules and the broad spectrum of cellular processes in which they are involved suggest that their assembly and growth are controlled not only temporally but also spatially in all cells. The detailed mechanisms by which microtubules regulate this process are not yet understood well [20–22]. The great variety of microtubule functions can only be explained by the large number of interactions between tubulin and other proteins such as MAPs and is possibly governed by the microheterogeneity of the microtubule proteins.

Here we describe the sequence of two $\alpha$-tubulin cDNA clones which differ markedly in their 3′-untranslated regions. In contrast to the high nucleotide divergence observed between the two rat species, a high interspecies conservation was observed when compared with 3′-untranslated sequences isolated from human [16] and Chinese hamster ovary cells [17]. The interspecies homology is also based on the polyadenylation signal which is different in the two species, i.e. AAGTAAA and AATAAA, as well as the size conservation of the 3′-untranslated regions being between 156–172 and 194–216 base pairs, respectively. The conservation of the unique AAGTAAA polyadenylation signal is noteworthy and may be of functional significance. The rate of accumulation of silent substitution in the nucleotide sequence of DNA during evolution has been estimated to be in the order of 1% per million years [23]. The accumulation of these changes has been used as a biological clock to study the evolutionary history of genes [23–25]. The degree of homology expected between rat and human that diverged about 75 million years ago is less than 50%. However, the high degree of sequence homology observed in the 3′-untranslated regions

between mRNA coding for tubulin isotypes in distantly related mammals indicates a very strong evolutionary constraint to preserve these sequences.

The nervous system consists of a vast network of cells, many of which are anatomically and functionally unique. This diversity suggests that characteristic cell groups express a distinct set of genes not expressed in other nerve cells. Our results [2–4] and those of others [5,6] show that the expression of tubulin mRNAs is regulated during brain development. Using the in situ hybridization technique and employing an $\alpha$-tubulin probe it was demonstrated that the decrease in tubulin mRNA level during brain maturation is not a result of a uniform decrease in all cerebellar cells. Rather, the results show that migration and differentiation is accompanied by a decrease in the $\alpha$-tubulin level, i.e. in the internal-granular layer and molecular layer cells. The mitotically active cells, in the extragranular layer, are rich in the $\alpha$-tubulin mRNA whereas the prenatally formed Purkinje cells show very little if any $\alpha$-tubulin mRNA signal. Using oligonucleotide probes specific for each tubulin isoform will allow one to characterize further the expression of the various isotypes during normal brain development and to compare them to cases of neurological disorders.

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