Structural features and restricted expression of a human α-tubulin gene

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ABSTRACT

The nucleotide sequence of a human α-tubulin gene (bal) is described. This gene is extensively homologous to a rat α-tubulin gene in its coding regions, 3'-untranslated region and, indeed, in segments of its largest intron. However, with the exception of three short conserved blocks of homology, the 5' flanking regions of the rat and human genes are unrelated. Hence, these genes each encoding an identical protein are transcribed under the influence of divergent promoters.

Blot analyses using RNA from a variety of transformed cells derived from different tissues indicate that expression of the human α-tubulin gene is restricted to cells of neurological origin. Among neurological cell types bal expression is further restricted to adherent cells that are morphologically differentiated. The data presented suggest that the bal gene encodes a prominent neuronal and glial α-tubulin and that bal expression is a function of the differentiated state of these cells.

INTRODUCTION

Microtubules are filamentous structures that are present in virtually all eukaryotic cells. They are principal components of the cytoskeleton, of the mitotic spindle, of cilia and flagella, and of neuronal processes. They are involved in numerous functions including maintenance of cell shape, mitosis, cell movement, and intracellular transport (1). The major components of microtubules are the α- and β-tubulins. Heterodimers of these proteins form subunits that are polymerized in microtubules.

In mammals, the genes that encode α- and β-tubulin proteins are members of two distinct multigene families (2). These families each contain 15-20 members and include pseudogenes as well as functionally expressed sequences. Analysis of the human β-tubulin gene family has revealed that pseudogenes, most of which are of the processed type, account for the majority of sequences in the family (3,4). The high ratio of pseudogenes to functional genes that is characteristic of the β-tubulin gene family is likely to apply to the human α-tubulin gene family as well. The ongoing task of identifying
all of the functional human tubulin genes has resulted in the complete characterization of three β-tubulin genes (4-6), and, with this report, the characterization of a single α-tubulin gene.

Because tubulins are ubiquitous in eukaryotic cells and because of their role in diverse cellular functions, an important question concerns the pattern of tubulin gene regulation. There is evidence for multiple controls of tubulin gene expression. Experiments using drugs that depolymerize microtubules have indicated that the level of unpolymerized tubulin itself regulates the level of tubulin mRNA (7,8). Studies of tubulin gene expression in the rat and chicken employing 3'-untranslated region gene-specific probes have revealed that tubulin genes are differentially regulated during development (9,10). Of particular interest are a variety of experiments that demonstrate regulation of tubulin gene expression at the level of tissue specificity. These experiments, based on RNA blot transfer analysis as well as on genetic analysis of Drosophila melanogaster, indicate the following: 1) testis-specific expression of a Drosophila β-tubulin gene (11,12); 2) dominant expression in the testis of a mouse α-tubulin gene (13) and a chicken β-tubulin gene (14); and 3) occurrence of two neural specific β-tubulin mRNAs in the rat (9). In humans, RNA blot analyses using 3'-untranslated region gene-specific probes have revealed co-expression of at least two β-tubulin genes in many transformed cell lines and the restricted expression of a third β-tubulin gene to cells of neurological origin (6). Here we report the isolation and characterization of a human α-tubulin gene that also appears restricted in its expression to cells of neurological origin. Comparison of this gene with a closely related rat α-tubulin gene (15) reveals extensive homologies not only within the coding region, but within untranslated regions and intervening sequences as well. By contrast, the promoter regions of these two genes are highly divergent. The evolutionary implications of these observations are discussed.

MATERIALS AND METHODS

Library Screening, Cloning, and DNA Sequencing

The 3'-untranslated region probe of the bal cDNA was subcloned as described (16). This probe, labeled with 32P by nick-translation was used to screen a partial Hae III/Alu I recombinant human genomic library (17). The regions within the isolated phage containing α-tubulin sequences were identified by restriction digestion and Southern blotting using an α-tubulin coding region probe derived from the kal cDNA (16). α-tubulin hybridizing
regions, consisting of three Eco RI fragments, were subcloned into the pKY2700 vector, a plasmid that can be positively selected for Eco RI inserts when it is used to transform NS872 cells (18).

DNA sequencing was performed by the dideoxy chain terminator method of Sanger et al. (19) using fragments subcloned into bacteriophage M13 mp8 or mp9 as templates (20). Fragments for M13 subcloning were generated by either of two methods: 1) direct restriction digestion at appropriate sites for insertion at the M13 polylinker; 2) treatment of a linear fragment with exonuclease Bal 31 to shorten the fragment, followed by a second restriction digestion and ligation into M13. The times of Bal 31 digestion were varied from 2 to 15 minutes to generate overlapping clones. A set of clones was selected on the basis of their dideoxy A tracks such that their sequences would encompass the entire fragment.

The computer programs of Staden were used for compilation of the DNA sequences (21). The DNA sequence analysis programs of Pustell were used for the matrix homology plots (22).

Cell Cultures, RNA Preparation, and Blot Analyses

Human retinoblastoma (Y79) and neuroblastoma (IMR 6, CHP 126, CHP 134d) cells were provided by Dr. Fred Gilbert of the Mount Sinai School of Medicine. The SKN-SH and IMR 32 neuroblastoma cell lines were obtained from Dr. June Beidler of Sloan-Kettering Institute. Cells were cultured in RPMI 1640 containing 10% fetal calf serum, 150 units/ml penicillin, and 100 μg/ml streptomycin.

RNA was prepared according to the procedure of Cleveland et al. (2). For RNA blot transfer experiments, 5 μg samples of polyA+ RNA were run on denaturing agarose gels containing 2.2M formaldehyde (23) and the gel contents transferred to nitrocellulose by the method of Southern (24). Blots were prehybridized for 4 hrs in 10 x Denhardt's solution, 5 x SSC, 20 mM PO4, pH 6.5 (1 x SSC = .15M NaCl, .015M sodium citrate) at 42° and hybridized for 14 hrs at the same temperature in 50°/o formamide, 1 x Denhardt's solution, 5 x SSC, 20 mM PO4. Probes were 32P-labeled by nick-translation (25). Blots were washed to a final stringency of 0.2 x SSC, 0.1°/o SDS at 68°.

S1 Protection

A single-stranded 5'-end labeled fragment for S1 protection was prepared as follows. A 522 bp Eco RI-Sma restriction fragment was purified by polyacrylamide gel electrophoresis and recovered from the gel by electroelution. This fragment was incubated with Exo III under conditions
specified by the manufacturer (Bethesda Research Laboratories, Inc.). The fragment was $^{32}$P-labeled with polynucleotide kinase and $3 \times 10^5$ cpm were incubated for 16 hrs in a reaction containing 5 \( \mu \)g of IMR 6 polyA$^+$ RNA, 0.3M NaCl, 0.2 mM EDTA, 0.1M Tris-HCl (pH 7.5). After annealing, the reaction was diluted into 0.4 ml of 0.3M NaCl, 0.1M sodium acetate (pH 4.5), 3 mM ZnCl$_2$ and digested with 2000 or 3000 units of S$_1$ nuclease. The products of this digestion were analyzed on a 5% polyacrylamide sequencing gel.

RESULTS

Isolation and Structural Features of the Human \( \alpha \)-Tubulin Gene \( \text{ba}1 \).

A human \( \alpha \)-tubulin cDNA clone (\( \text{ba}1 \)) that was isolated from a human fetal brain cDNA library has been previously described (16). The subcloned 3'-untranslated region of this cDNA, which detects a subfamily containing only two of the 15-20 \( \alpha \)-tubulin sequences present in the human genome, was used to screen a human genomic library cloned in bacteriophage lambda. Two types of positively hybridizing clones were obtained and analyzed. One set of clones contained a 1.2 kb region that hybridized with an \( \alpha \)-tubulin coding region probe. This segment was subcloned and sequenced. Comparison of this sequence with the sequence of the \( \text{ba}1 \) cDNA revealed the presence of numerous base substitutions and at least one insertion. This insertion constitutes a frame-shift mutation and results in the occurrence of several termination codons in the new reading frame. These termination codons, which would interfere with the translation of a functional \( \alpha \)-tubulin polypeptide, are characteristic of pseudogenes. The second set of overlapping clones contained a more extensive region of \( \alpha \)-tubulin hybridizing sequences which were identified in a 4.1 kb segment that was subcloned and sequenced according to the strategy diagrammed in Fig. 1. Comparison of this sequence with the \( \text{ba}1 \) cDNA sequence revealed complete homology, and enabled a complete definition of the structure of the \( \text{ba}1 \) gene.

The sequence data show (Fig. 2) that the gene consists of four exons interrupted by three introns. The largest intron which is 1.5 kb in length occurs between the first and second exons. This intron is located between the first and second codons of the gene and so demarcates a protein domain consisting of the initiator methionine residue alone. The second intron is located between the first and second nucleotides of codon 76 and is 148 bp long. The third intron is located between codon 125 and 126 and is 304 bp long. All exon-intron junctions contain the expected consensus splice signal.
Figure 1. Sequencing strategem. Horizontal arrows show the direction and extent of sequences. Restriction sites at which DNA segments were subcloned into M13 mp8 or mp9 are indicated by single letters. H, Hind III; K, Kpn-I; P, Pst; R, Eco RI; S, Sma I.

dinucleotides GT (upstream) and AG (downstream).

The 3' end of the gene contains a TAA termination codon at position 452 and an AATAAA polyadenylation signal 192 bp further downstream. The characteristics of the 5' end of bα1 are discussed below.

The first intervening sequence of bα1 contains an arrangement of (GT) dinucleotide repeats. Two hundred and sixty five base pairs into the intron there are 18 (GT) dinucleotides flanked by a 26 bp direct repeat which is perfect except for a single base mismatch. An additional stretch of 12 (GT) pairs plus the sequence CTGCTGTCTC is adjacent to the downstream 26 bp repeat and the entire arrangement of two poly(GT) sequences and the 26 bp repeat is itself encompassed by a 10 bp direct repeat that is also perfect except for a single base mismatch (Fig. 7A).

bα1 is Closely Related to a Rat α-Tubulin Gene

Several observations can be made on the basis of a structural comparison between the human bα1 gene and a rat α-tubulin gene to which it is related by extensive 3'-untranslated region homology (15,16). The number and position of the introns are identical in the rat and human genes, while the relative size of the introns is similar. The coding region sequences of the human and rat genes have been stringently conserved, and are 95%/ homologous at the DNA level. The 5%/ nucleotide sequence divergence occurs primarily in codon third positions and does not affect the encoded proteins which are absolutely identical in amino acid sequence. The 80%/ homology in the 3'-untranslated regions of the rat α-tubulin gene designated α-T14 and the human gene bα1 is depicted in a homology matrix plot in Fig. 3A.

Further comparison of the sequences of the rat and human α-tubulin genes revealed additional unexpected homologies. These homologies were found in the first intervening sequences of the two genes and are represented in a homology matrix plot in Fig. 3B. The homology occurs in a series of segments interrupted by non-homologous regions resulting in the broken diagonal seen...
in the matrix plot. One block of homology (A) occurs between the beginning of IVS 1 in the human gene and a region 370 bp downstream from the beginning of IVS 1 in the rat gene. The sequences then diverge through a region

| GAATTCACTGCCGTTGGAATCGAAGGCCCCGCTACAGCTGAAAGACCTTTACGCTTTCTCTCTTTCTG | 1 | Met |
|------------------|----|----|
| TTTGGCTTTTGGGAAGATACACACAACTTTCCGGAAAAACGCGGGGTTGCAAGGCTCGAG | 2 | Arg |

The homology between the human and rat sequences diverges at nucleotides 1 and 2. The homology is marked as (A) in the matrix plot.

The sequences are as follows:

Human sequence:

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GAATTCACTGCCGTTGGAATCGAAGGCCCCGCTACAGCTGAAAGACCTTTACGCTTTCTCTCTTTCTG
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Rat sequence:

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TTTGGCTTTTGGGAAGATACACACAACTTTCCGGAAAAACGCGGGGTTGCAAGGCTCGAG
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The divergence is marked as (A) in the matrix plot.

The homology continues through a region of the gene sequences.

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**Nucleic Acids Research**

212
Figure 2. Sequence of the bal gene derived by the strategem shown in Figure 1. The cap site and poly(A) addition signal are boxed; poly(GT) elements and direct repeats in IVS 1 are dashed; three short sequences in the 5' flanking region with homology to sequences in the 5' end of a rat gene (a-T14, ref. 15) are underlined.
Figure 3. Homology matrix plots generated by the Pustell forward homology matrix computer program (22). The abscissa corresponds to the human βαl sequence; the ordinate corresponds to the rat α-T14 sequence. Numbers on the axes represent the positions of nucleotides in each sequence. Homologies between the sequences over a range of 10 bp are indicated by a letter. Each letter signifies a percent homology (e.g., A = 100%, F = 90-91%, K = 80-81%). In order to plot homologies across regions of DNA that contain more bases than the number of columns on a page, the sequences are compressed. The compression factor refers to the number of bases assigned to each coordinate in the plot. Figure A depicts homology between the 3' untranslated regions of βαl and α-T14; the minimum percent homology plotted is 64%. The compression factor is 2. Figure B depicts homology between the first intervening sequence of βαl and α-T14. Blocks of homology are designated A, B, C, D, and E. The minimum value plotted is 66%. The compression factor is 10.
containing a A9CA7 tract in the human, but no corresponding oligo A tract in the rat. Homology resumes briefly (B) and then is lost again in the region of the human intron containing the (GT) dinucleotide repeats. The (GT) repeats are not present in the section of the rat intron that has been sequenced. Intermittent homology (C,D,E) continues 900 bp into the human and rat introns. Whether the homology persists throughout the remainder of the introns could not be established because the complete sequence of the rat intron was not available.

It should be noted that the segments of homology in the introns of the rat and human genes are composed of relatively complex sequences. Regions of the introns that contain simple sequences or repeats are not homologous. In contrast to the first introns, comparison of the second and third introns of the rat and human genes revealed no significant homology.

Characteristics of the 5' end of bal

To determine the location of the 5'end of the bal gene an S1 nuclease protection experiment was performed (Fig. 4). A 5' end labeled restriction fragment spanning a 250 bp region upstream from the putative ATG was annealed.
Figure 5. Restricted expression of bal to cell lines of neurological origin. Approximately 5 µg of poly(A)+ mRNA from HeLa cells (Lane 1), Y79 retinoblastoma cells (Lane 2), 132 INL glioma cells (Lane 3), CHP 126 neuroblastoma cells (Lane 4), and IMR 6 neuroblastoma cells (Lane 5) were resolved on an agarose gel containing formaldehyde (23). The contents of the gel were transferred to nitrocellulose (24) and the blots were probed with nick-translated α-tubulin coding region (Figure A) or the bal 3' untranslated region (Figure B). A summary of this and other bal expression data (16) appears in Table 1.

to polyA+ RNA from a human neuroblastoma cell line that expresses the bal gene (see below). S1 digestion yielded a cluster of protected fragments approximately 201 bp in length. The 5' end label of the protected fragment is 8 bp from the ATG, so the 5'-untranslated region is approximately 209 bp long. This result places the cap site of bal transcripts at the position indicated in Fig. 2.

The bal 5' flanking sequence is unusual in that it does not contain a TATA box -35 bp upstream from the cap site. Surprisingly, the rat gene α-T14 5' end does contain a TATA box. Comparison of the 5' ends of bal and α-T14 reveals that the cap site of bal is approximately twice the distance from the initiation codon (209 bp) as the cap site of α-T14 (99 bp) and that, whereas the 5' flanking sequences are not closely related, there are distinctive homologies. These homologies are limited to three regions (underlined in Fig. 2 and shown in Fig. 7B). The regions are 13, 20, and 29 bp long and are respectively 92°/0, 80°/0, and 76°/0 homologous. Region I is 341 bp upstream from the cap site in the rat gene and 279 bp upstream from the cap
Table I
Expression of bal in Various Human Cell Lines and in Human Fetal Brain

| Cell Type                     | bal Expression |
|-------------------------------|----------------|
| HeLa cells                   | -              |
| Diploid fibroblasts           | -              |
| Epidermal cells               | -              |
| Squamous cell carcinoma SCC-15| -              |
| Fibrosarcoma                  | -              |
| Brain (fetal)                 | +++            |
| Myeloma CM 1500A              | -              |
| Hepatoma PLC/PRF 15           | -              |
| Retinoblastoma Y79            | -              |
| Glioma 132 1M1*              | +              |
| Neuroblastoma CHP 126         | -              |
| Neuroblastoma IMR 6*          | +              |
| Neuroblastoma IMR 32*         | ++             |
| Neuroblastoma CHP 134d*       | ++             |
| Neuroblastoma SKN-SH*         | +              |

Cell lines marked with an asterisk are adherent.

site in the human gene. Region II is 286 bp upstream from the cap site in the rat gene and 261 bp upstream from the cap site in the human gene. The third region of homology is 121 bp upstream from the cap site in the rat gene; in the human gene this sequence is transcribed.

Restricted Expression of the bal Gene

In previous experiments, the bal 3'-untranslated region subclone was used as a probe for RNA blot transfer experiments using polyA+ RNA from the following human cell types: HeLa cells, diploid fibroblasts, epidermal cells, a squamous cell carcinoma, a fibrosarcoma, and fetal brain. The only sample in which bal RNA was detected was human fetal brain (16). In order to extend these experiments, we have examined a variety of cultured human cell lines of neurological origin for bal expression. The resulting RNA blot transfer experiments are shown in Fig. 5. In the first panel, polyA+ RNA from HeLa cells, a retinoblastoma (Y79), a glioma (1321N1), and two neuroblastomas (CHP 126 and IMR 6) were probed with an a-tubulin coding region sequence. In the second panel the same RNAs were probed with the bal 3'-untranslated region subclone. a-tubulin mRNAs are present in all the cell lines. In contrast, bal-specific mRNA is undetectable in HeLa cells, the retinoblastoma, and one of the neuroblastomas (CHP 126), but present in the glioma and the second neuroblastoma (IMR 6). The complete bal expression data, including results from three additional neuroblastomas are summarized in Table I.

DISCUSSION
Conserved Structure of a-Tubulin Genes

Southern blots of human DNA probed with the bal 3'-untranslated region subclone show that the human genome contains two copies of this sequence both of which are associated with a-tubulin specific sequences. Two distinct but
unrelated sets of overlapping recombinant clones containing each of these copies have been isolated. Sequence analysis of one of these clones revealed that within the α-tubulin coding region, homology to the bal cDNA is less than perfect and that point mutations in the sequence preclude the synthesis of a functional α-tubulin protein. Accordingly, this clone must be classified as a pseudogene. In the absence of complete sequence data, it is not clear whether this pseudogene contains intervening sequences, or is of the processed type. The only other genomic copy of the 3'-untranslated region is associated with α-tubulin coding region sequences that are completely homologous to the bal cDNA sequence. Hence, this copy belongs to a unique functionally expressed human α-tubulin gene.

The presence of α-tubulins with related functional and biochemical properties in all eukaryotic organisms is consistent with the evolutionary conservation of tubulin coding regions and protein sequences. The amino acid homology between the human bal and rat α-T14 tubulins is absolute. The human and rat sequences differ at only two residues from a pig α-tubulin sequence (26) and at three residues from a chicken α-tubulin sequence (27). The similarity between the rat and human genes extends to their structure as well and includes the conservation of both the number and placement of introns within the genes. This structural identity raises the question whether all vertebrate α-tubulin genes conform to the exon arrangement shared by bal and α-T14 (Fig. 6). Conservation of structure appears to be a feature of the β-tubulin genes of higher eukaryotes; all of the expressed human and chicken β-tubulin genes thus far characterized contain the same number of introns in the same locations (6). However, heteroduplex analysis of another human α-tubulin gene suggests that the carboxyterminal exon is split by an additional intervening sequence and therefore this gene represents an alternative to the structure shared by bal and α-T14 (28).

An interesting feature of the rat and human α-tubulin genes is the occurrence of an intervening sequence immediately after the first codon. This feature is not unique to α-tubulin genes. A single mouse α-amylase gene contains two 5' leader sequences both of which are spliced after the
initiator triplet (29). In this case the position of the first intron is associated with the flexible expression of the α-amylase gene. The two different leaders are employed in different tissues; one or the other leader is spliced onto the common coding region of the gene. There is no evidence for such a mechanism among α-tubulin genes, but the presence of an intervening sequence after the initiator ATG has the effect of isolating 5' regulatory sequences from the coding region of the gene and may provide the basis for other as yet undefined functions.

**Interspecies Conservation of Intron Sequences**

The substantial nucleotide sequence homology between the human β1 and rat α-T14 genes extends to non-coding regions. The 3'-untranslated regions of these genes are extensively homologous and there is segmental homology in the first intervening sequences. Interspecies homology of intron sequences has been reported among globin genes and proto-oncogenes. For example, the introns of the goat and human e-globin genes are 64-69%/ homologous (30); and the introns of the mouse c-fos proto-oncogene are 63-76%/ identical to the introns of the human cellular homologue c-fos (31). In light of the fact that intron sequences are not generally conserved even among members of a gene family within a species, these instances of interspecies homology of
Nucleic Acids Research

Intron sequences define a category of genes that are extensively conserved. It is not yet known how many genes belong in this category and it remains to be determined whether the homologies in IVS 1 of βα and α-T14 or the homologies in their 3′-untranslated regions are functionally significant.

The Large Intron of βα Contains a Repetitive Element

A notable feature of the βα gene is the presence of the oligo (GT) repetitive element in the first intron. Members of this family of dispersed repetitive sequences have been found in the genomes of yeast, Xenopus, mouse, and human (32-34). In the latter species, there are estimated to be 30,000-50,000 copies per genome. The example cited here is interesting in that it contains two (GT) stretches and two sets of flanking direct repeats (Fig. 7A). The direct repeats associated with these (GT) dinucleotides are sufficiently complex to qualify as duplicated sequences rather than as simple sequences that happen to surround the oligo(GT) elements. Evidently, poly(GT) elements occur in two genomic environments: some are flanked by direct repeats whereas others are not (34,35). The presence of the flanking direct repeats suggests that these sequences are mobile genetic elements, and is consistent with the insertion of the (GT) tracts at sites generated by staggered chromosomal breaks.

Proposals regarding the function of these repeats are numerous (34): they may function at the ends of chromosomes, they may serve as recombination hotspots, they may be involved in gene conversion events, or they may influence the transcription of genes with which they are associated. The latter view includes the notion that poly(GT) sequences might have enhancer properties by virtue of their ability to form left-handed DNA helices or Z-DNA (36).

Evolution of the βα Promoter

Current theories of gene regulation have stressed the importance of relatively short sequences located upstream from the cap site of regulated genes (37). These sequences, which are distinct from the TATA box and the CAAT "boxes", have been demonstrated to be essential for the transcriptional activation of a variety of eukaryotic genes. In some cases, the regulatory sequences (which may be as short as 9 bp) are repeated several times in the 5′ flanking region of a gene. Their identification has been facilitated among genes that are coordinately expressed. For example, the family of genes that are induced by glucocorticoids all share a 23 bp upstream consensus sequence (38). A 16 bp consensus sequence is repeated in the 5′ flanking region of Chlamydomonas reinhardt α- and β-tubulin genes which are
also transcribed coordinately (39). Short regulatory sequences have also been identified upstream from genes that are not known to be coordinately induced (40).

From this perspective, the three short regions of homology in the 5' ends of bal and α-T14 (Fig. 7B) are candidates for regulatory sequences in α-tubulin gene expression. A segment of region 3 is particularly interesting. The sequence of this element is TTCTCTGGG. In both the rat and human it is followed by an AG-rich sequence. Interestingly, this element followed by an AG-rich sequence also occurs in the 5' end of the human β-tubulin gene designated 5b (5). This element is, therefore, a potential regulatory sequence for both α- and β-tubulin gene expression and could conceivably provide a basis for coordinate regulation of α- and β-tubulin gene expression. A demonstration of the influence of these short sequences on tubulin gene expression will require the construction of appropriate mutants, and the introduction of such constructs into host cells by DNA-mediated gene transfer.

Considering the overall similarity of the human bal and rat α-T14 genes, it is striking that bal does not contain a TATA box whereas α-T14 does. Comparison of these two genes suggests that bal, an otherwise remarkably conserved gene, has diverged in its promoter region. Pressure for a divergent promoter could not have come from a requirement for differential regulation that might be associated with the emergence of a new α-tubulin isotype, since the bal and α-T14 proteins are identical. Rather, there is the possibility that evolutionary pressure was applied to the 5' regulatory elements themselves. Accordingly, the divergent promoter of bal may be an example of the sort of evolution recently suggested for the tubulin gene families by Raff: separate regulatory mechanisms for functionally equivalent genes might evolve because of different requirements for tubulin transcripts in different cells or at different developmental stages (41).

bal Expression

The data presented here suggest that the expression of bal is regulated at the level of tissue specificity. Expression of bal has only been detected in cells of neurological origin. However, since our expression data are based on RNA blot transfer analysis we cannot rule out the possibility of extremely low levels of bal expression in other cell types. In contrast, transcripts of the rat gene α-T14 are prominent in fibroblasts as well as brain cells (15).

The variable expression of bal among the different cell lines of
neurological origin suggests a correlation between bal expression and cellular morphology. Cells in which bal mRNA levels are undetectable, such as the retinoblastoma Y79 and the neuroblastoma CHP 126, are small round cells that grow in suspension. Cells in which the bal gene is expressed are adherent neuroblastoma or glioma cells with characteristic cytoplasmic processes. The exclusive identification of bal transcripts in morphologically differentiated cells raises the question whether the differentiation of these cells involves the regulated expression of the bal gene. In order to address this issue, we are assessing transcription of the bal gene in retinoblastoma and neuroblastoma cells in which differentiation has been induced in tissue culture.

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