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Complete Sequence of Three α-Tubulin cDNAs in Chinese Hamster Ovary Cells: Each Encodes a Distinct α-Tubulin Isoprotein

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The genome of Chinese hamster ovary (CHO) cells contains a complex family of approximately 16 α-tubulin genes, many of which may be pseudogenes. We present here the complete cDNA sequences of three expressed α-tubulin genes; one of these genes has been identified only in CHO cells. The noncoding regions of these three CHO α-tubulin genes differed significantly, but their coding regions were highly conserved. Nevertheless, we observed differences in the predicted amino acid sequences for the three genes. A comparison of the CHO α-tubulin sequences with all of the sequences available for mammals allowed assignment of the α-tubulin genes to three classes. The proteins encoded by the members of two of these classes showed no class-specific amino acids among the mammalian species examined. The gene belonging to the third class encoded an isoprotein which was clearly distinct, and members of this class may play a unique role in vivo. Sequencing of the three α-tubulin genes was also undertaken in CM^{R}795, a colcemid-resistant clonal CHO cell line which has previously been shown to have structural and functional alterations in its tubulin proteins. We found differences in the tubulin nucleotide sequence compared with the parental line; however, no differences in the α-tubulin proteins encoded in the two cell lines were observed.

The tubulin gene families are ubiquitous and are highly conserved within the eucaryotes (1, 8, 29). In mammalian cells there are about 15 to 20 different genomic members each for the α- and β-tubulin families (11, 14, 25, 30, 31, 58). The majority of these sequences are apparently pseudogenes (28, 29, 31, 45, 59, 60), and only a small subset, perhaps three or four each for α- and β-tubulin, are expressed (13, 15, 31, 46).

In clonal lines of Chinese hamster ovary (CHO) cells, three differentially expressed α-tubulin genes, genes I, II, and III, have been identified (13). The purpose of the present study was to determine whether these genes encode different tubulin isoproteins which could potentially confer functional specificity to the microtubules in which they are incorporated. Previously, preliminary sequence data have shown that, while genes I and II are similar, gene III encodes a distinct tubulin isoform which had not been identified previously (13). Thus, complete sequence data were required to reveal differences in the gene I and II products and the extent of the difference between these genes and the gene III α-tubulin isoprotein.

We previously isolated colcemid-resistant CHO cell line CM^{R}795, which possesses tubulin with reduced colcemid-binding ability and an altered α-tubulin protein, as determined by two-dimensional gel electrophoresis (2, 9, 26, 33). Sequences of the α-tubulin genes expressed in CM^{R}795 cells were determined and compared with sequences of the parental CHO cell line.

MATERIALS AND METHODS

Culture conditions and cell lines. The procedures used for maintaining CHO cells in culture have been described previously (12, 56). The present CHO cell karyotype is relatively stable and has 21 chromosomes, whereas the Chinese hamster karyotype has 22 chromosomes; nevertheless, most of the hamster genome can be accounted for in the rearranged chromosomes (61). The cell lines used in this study were isolated previously and have all been cloned. $E_{29}Pro^+$ is an auxotroph which requires adenosine and proline for growth. This auxotroph is the parental line from which colcemid-resistant cell line CM^{R}795 was isolated (2, 9, 26, 33).

Oligonucleotides. Oligonucleotides (18 to 35 nucleotides long) were synthesized by using an Applied Biosystems model 380A DNA synthesizer. They were purified by gel electrophoresis prior to use for priming M13 sequencing reactions or for hybridizing with cDNA clones.

Isolation of cDNA clones. Two CHO cDNA libraries, one each from cell lines $E_{29}pro^+$ and CM^{R}795, were constructed by using the pcD eucaryotic expression vector system (43, 44). The methods used for library construction, screening, DNA purification, blotting, and hybridization with α-tubulin nick-translated probes and oligonucleotide probes have been described previously (13). The initial screenings produced full-length cDNA clones (clones containing sequences extending from the polyadenylate tail to and including the 5′ noncoding region) from each library for the most highly expressed genes, genes I and III (13). Further library screening was undertaken to isolate full-length cDNA clones for gene II. These clones represented only 5% of all of the tubulin clones present. Duplicate library filters were created (36). One set of filters was hybridized with a gene II-specific oligonucleotide probe derived from the 3′ noncoding sequence (13). The other set of filters was hybridized with a coding region genomic α-tubulin probe under conditions which detected all three α-tubulin transcripts (13, 14). DNA from colonies positive in both assays was isolated, dotted onto nitrocellulose, and hybridized to an oligonucleotide probe which was derived from a conserved stretch of the extreme 5′ coding regions of genes I and III. The expectation that the homology would extend to gene II and thus identify full-length clones was realized. The presence of a 5′ noncoding sequence in all of the clones was confirmed by sequencing (see below).

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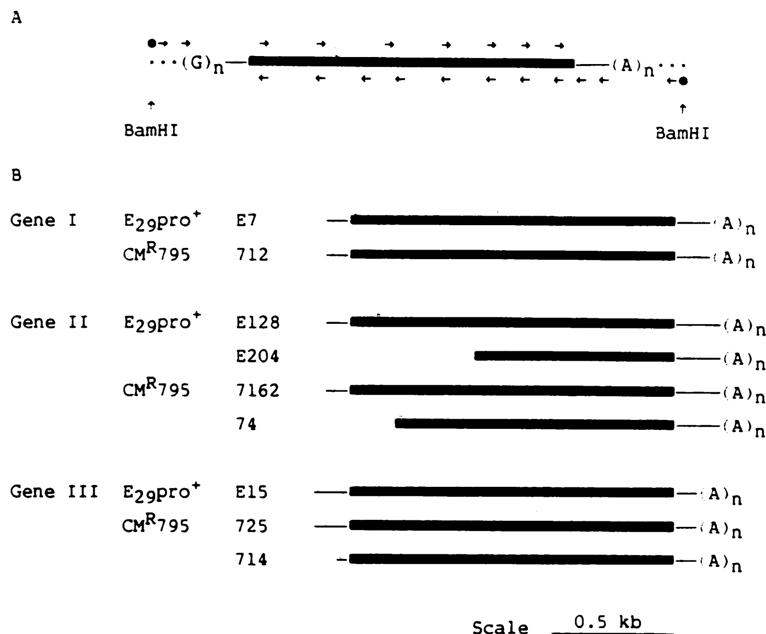


FIG. 1. Sequencing strategy and cDNA clones. (A) The DNA sequence of both strands was determined by a combination of chemical cleavage (37) and dideoxy sequencing (50). ←● and ●→ indicate the start and direction of chemical cleavage sequencing. ← and → indicate the location and direction of dideoxy sequencing from synthetic oligonucleotides primers. In each case sequencing continued until overlap with the next sequencing start location was achieved. (B) cDNA clones. Nine independent α-tubulin cDNA clones were used for sequencing both strands from parental and mutants libraries for the three different mRNA species (genes I, II, and III). The dotted lines indicate the vector; the thin lines indicate noncoding regions, and the thick lines indicate coding regions kb, Kilobases.

Sequencing strategy. Figure 1 shows the DNA sequencing strategy which we used. Initial sequencing was by the chemical cleavage method of Maxam and Gilbert (37). The tubulin insertions, none of which had internal *Bam*HI sites, were removed from the vector by *Bam*HI digestion. The isolated fragments containing the cDNA flanked by short vector sequences were then end labeled and cut internally with *Cla*I. For every clone, sequencing proceeded from the polyadenylate tail to the coding region of α-tubulin. For some clones chemical cleavage sequencing into the 5′ noncoding region was also done. In Fig. 1 we indicate the start and direction, but not the extent, of chemical cleavage sequencing. Sequencing was extended until overlap was achieved with the next sequencing.

Subsequently, the *Bam*HI fragments were cloned in their entirety (size range, 1.6 to 1.8 kilobases) into M13mp9 and sequenced by using the dideoxy method (50) and synthetic oligonucleotide primers. Initial data for the construction of the oligonucleotide primers were obtained by sequencing small regions of *Sau*3A gene II M13mp9 subclones which were constructed for this purpose. Throughout the coding region, primers for each strand were located about 200 base pairs apart at sites where the gene III sequence and the sequence of rat clone pILαT1 (30) were identical. Because of the high degree of coding region homology, this set of 15 coding region primers proved to be useful for sequencing both strands of clones from all three genes. Sequencing from each of these primers and an additional six noncoding region oligonucleotides (Fig. 1A) continued for approximately 250 base pairs until overlap was achieved with the start of sequencing from the next primer.

Special sequencing strategies were used at the extreme ends of the cDNA insertions. Sequencing from the universal M13 primer (27) through the 60 to 120 nucleotides of polyadenylate tail into the 3′ noncoding region was not possible. Therefore, 3′ noncoding sequences were derived from chemical cleavage sequencing and from gene-specific oligonucleotide primers, as indicated in Fig. 1A. Sequencing of the other strand of the 3′ noncoding region proceeded from an oligonucleotide primer located in the coding region, through the 3′ noncoding sequence, and into the polyadenylate tail.

To facilitate sequencing of the 5′ noncoding region of the α-tubulin clones, an oligonucleotide complementary to the pcD vector sequence present in the *Bam*HI fragments and just 5′ to the polyguanylate linker created during cDNA library construction was synthesized. This primer was used to sequence through the 12 to 19 nucleotides of linker, the 5′ noncoding region, and into the coding sequence. However, when the polyguanylate region was 20 nucleotides long or longer, dideoxy sequencing could not be depended upon, and chemical cleavage sequencing was used. Sequencing of the other strand in this region proceeded from the coding region through the 5′ noncoding DNA to the linker.

Two artifacts were observed during sequencing. In one case a four-base pair deletion was present in one M13 clone but not in the clone of the opposite strand or in either strand of a second independent isolate. Thus, we concluded that this deletion occurred during M13 cloning. Such deletions have been observed previously for M13 (39). A second artifact was the substitution of a single base. Again, this change was observed in only one of two complementary M13 phages derived from a single library clone and was not observed for either orientation of an independent clone.

cDNA clones. All nine cDNA clones shown in Fig. 1B were the result of independent cloning events. For clones having different transcripts or clones from different cell lines this fact is self-evident. When two clones belonging to the same class from the same library were used, they were considered the result of independent cloning events only if they differed in the amount of 5′ noncoding sequence present. To facilitate

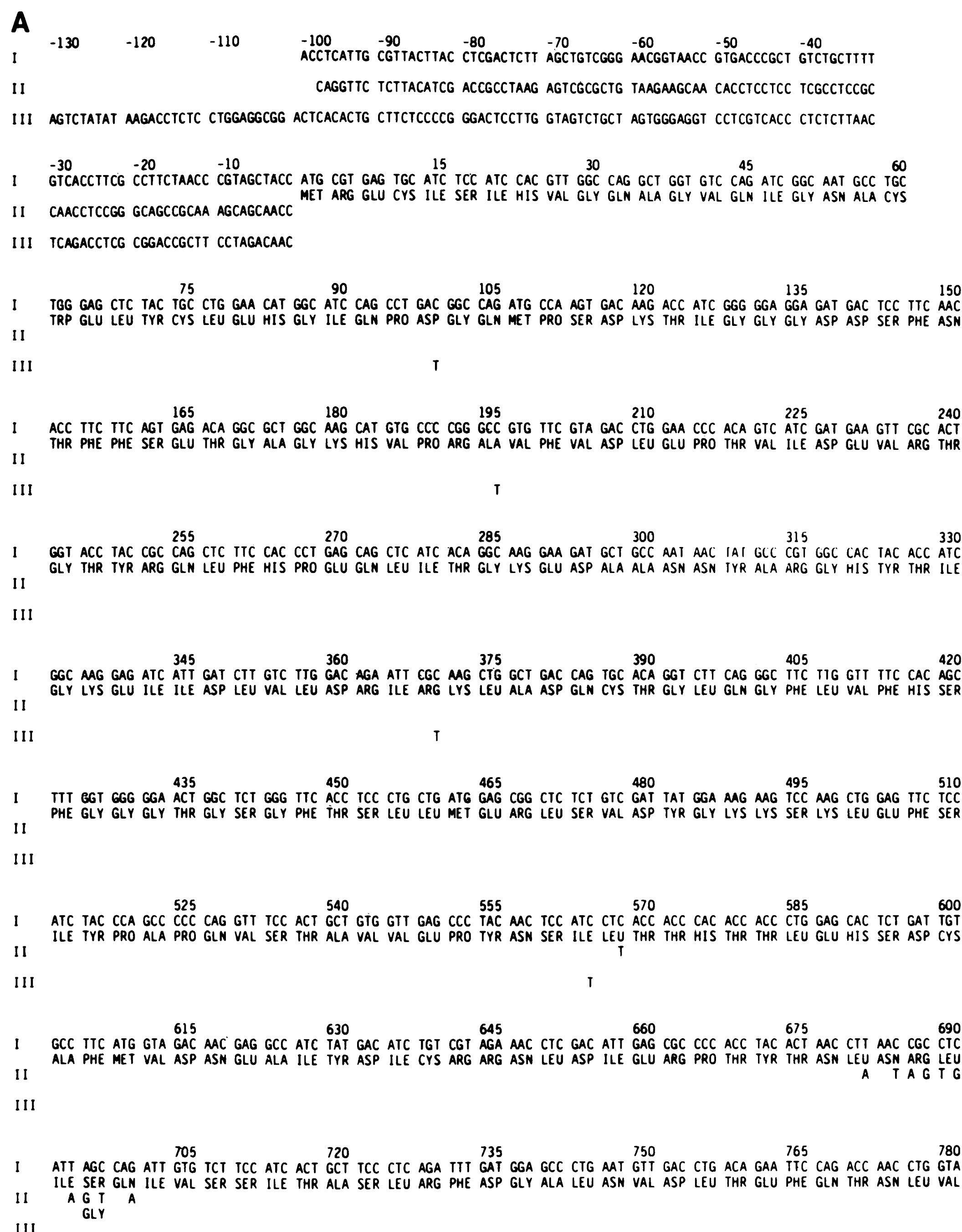


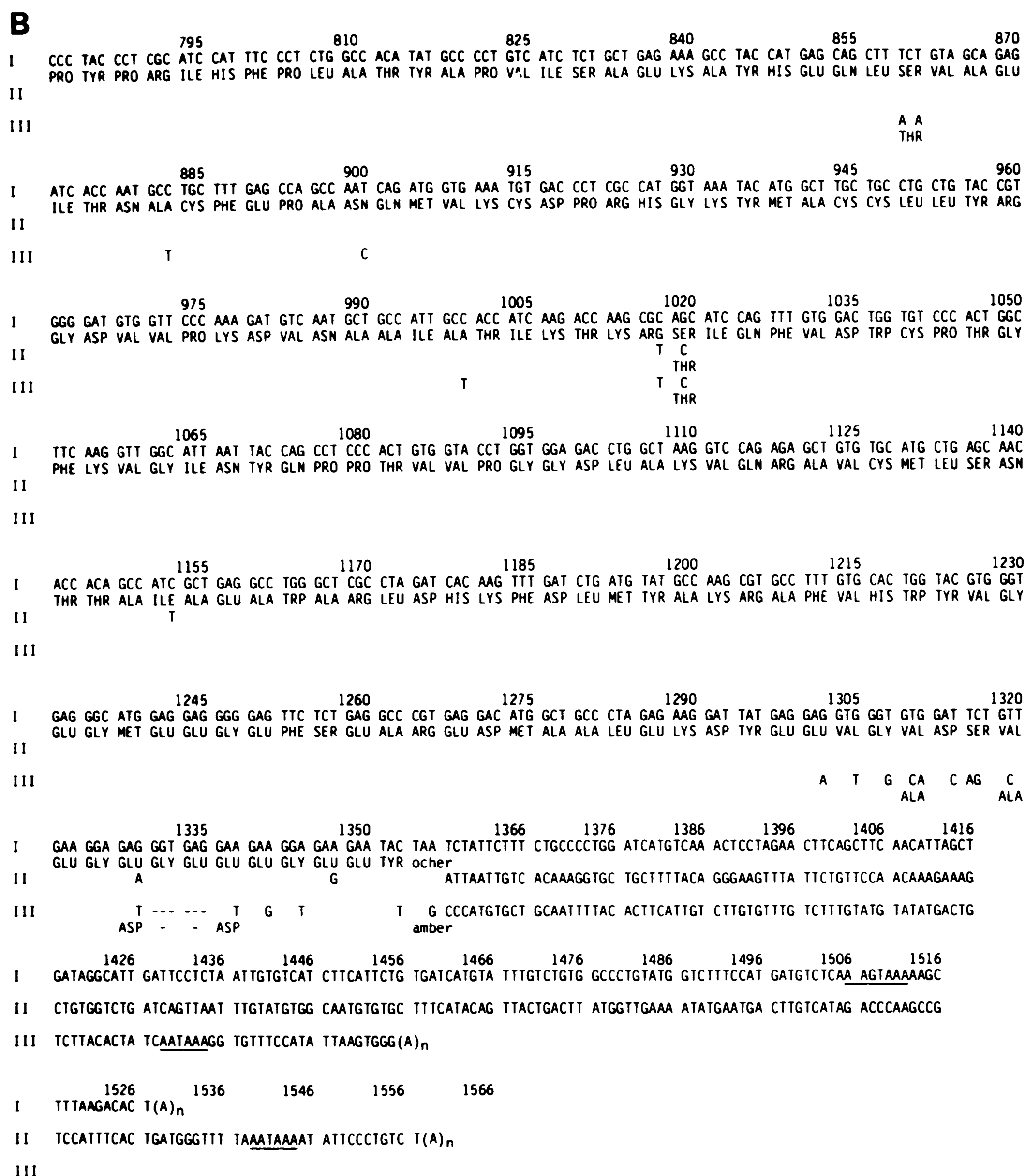
FIG. 2. Nucleotide and predicted amino acid sequences of CHO genes I, II, and III. The sequence data for each gene were derived from at least two independent cDNA clones (Fig. 1). The complete 5′ and 3′ data are shown. Within the coding region the sequence of gene I is shown; data for gene II and gene III are shown only where these data differ from the gene I data. The nucleotides are numbered from the first position of the initiator ATG. The polyadenlylation signals are underlined. Where necessary, deletions were introduced (dashes) to maintain maximum homology.

sequence comparisons of the colcemid-resistant mutant and its parent, mutant and wild-type clones were electrophoresed on gels loaded in pairs such that the ladders of the sequencing lanes alternated between the two clones.

RESULTS

Sequences of three α-tubulin genes. Figure 2 shows the nucleotide and predicted amino acid sequences of CHO α-tubulin genes I, II, and III. These data are based on sequencing of both strands of at least two independent cDNA clones for each of the three genes (Fig. 1). The gene I sequence is presented in full. For genes II and III the 5′ and 3′ noncoding regions are shown fully, but the nucleotide and amino acid sequences of the coding regions are shown only where they differ from those of gene I.

The proteins predicted for genes I and II are 451 amino acids long, and the protein predicted for gene III is 449 amino acids long. All three sequences encode a carboxy-



terminal tyrosine. The polyadenylation signal of each α-tubulin sequence is underlined in Fig. 2 (4), and the polyadenylated tail sequence is indicated by $(A)_n$. A computer search for the presence of direct repeats, inverted repeats, hairpins, and inverted hairpins revealed examples of such structures (data not shown), but these structures had no perceivable functional significance.

Although the untranslated regions of the three transcripts are different, the coding regions are remarkably similar (Fig. 2). Genes I and II differ at only 16 nucleotide positions, resulting in two amino acid substitutions, one of which is not conservative (Ser-Gly at position 232) and one of which is conservative (Ser-Thr at position 340) (7, 24). Interestingly, 10 of the 16 base pair substitutions are clustered in a short stretch of 19 bases from nucleotide 681 to nucleotide 699.

Gene III is more divergent, but compared with gene I differs at only 23 base pairs and has 6-base pair deletion. These changes lead to a protein which differs by six conservative amino acid substitutions (Ser-Thr at positions 287 and 340, Val-Ala at positions 437 and 440, and Glu-Asp at positions 443 and 446) and is two amino acids shorter (Gly and Glu). Again, these changes are clustered; 14 of the base changes, four of the amino acid differences, and the deletion all occur in the carboxy-terminal 52 base pairs.

Comparison of the α-tubulins from cell lines $E_{29}pro^+$ and CM^R795. CHO cell line CM^R795 was isolated by three-step selection for resistance to the tubulin-binding drug colcemid (2, 9, 26, 33). The purified tubulins of this cell line demonstrate reduced colcemid binding and an altered α-tubulin protein, as determined by two-dimensional gel electrophoresis (26). A comparison of the tubulin genes of cell lines $E_{29}pro^+$ and CM^R795 was undertaken to determine whether DNA lesions which affect biological function could be found.

The only differences between the parental and mutant cell

lines in either the coding or noncoding regions of the three expressed genes were two silent substitutions in gene II. These differences were cell line specific; this was confirmed by sequencing both strands of two independent clones from each cell line. The sequences shown in Fig. 1 are the sequences determined for parental CHO cell line $E_{29}pro^+$. In cell line CM^R795 at position 999 the nucleotide changed from cytosine to thymine, and at position 1182 the change was from guanine to adenine. At these two bases the parental sequence in CHO cells agrees with the sequence determined for CHO gene I, both α-tubulins of rats (18, 29) and both α-tubulins of humans (10). Surprisingly, no replacement changes which resulted in protein alterations were detected in any of the three encoded α-tubulin proteins of cell line CM^R795.

Mammalian α-tubulin gene codon and nucleotide usage. In addition to the sequences of the three CHO α-tubulin genes described above, the sequences for two rat genes (18, 29), two mouse genes (32), and two human genes (10, 20) have been determined. Codon usage for all of these mammalian α-tubulin genes was not biased. This finding contrasts with the strongly biased codon usage observed in chicken α-tubulin (57).

In the 3′ untranslated regions of the nine different mammalian α-tubulin genes, thymidine dominates (34 to 42%), creating an adenine · thymine-rich region. Similarly, adenine · thymine-rich 3′ untranslated regions have been observed in other genes from various species (38, 42). Data are available for the 5′ noncoding regions of six mammalian α-tubulin genes, including those described in this paper (10, 20, 29). In five of six genes cytosine predominates in the 5′ noncoding strand (34 to 40%), resulting in an overall cytosine · guanine-rich sequence. The exception is the 5′ end of the human α-tubulin gene bα1 (10, 20).

Classification of the mammalian α-tubulin family members. As an initial step in understanding the evolution and function of the α-tubulin gene family, the noncoding regions were examined. Figure 2 shows that the 3′ noncoding regions of the three hamster α-tubulin genes have no sequence similarity to each other (13); however, the same regions of genes I and II do show a high degree of homology to specific α-tubulin genes isolated from other species (Table 1). Gene III has thus far been isolated only in CHO cells (13). A similar comparison of the 5′ noncoding regions indicates that there is no sequence identity between the 5′ noncoding regions of the three hamster α-tubulin genes (Fig. 2); however, between CHO gene I and its human equivalent, kα1 (10), the 5′ noncoding sequence is even more conserved than the 3′ region (Table 1). Likewise, CHO gene II is highly homologous in both its 5′ and 3′ noncoding sequences to rat pILαT1 (29). This pattern breaks down when the 5′ sequences of CHO gene II and human bα1 are compared (10, 20) (Table 1). Despite a level of homology of 80% for the 3′ noncoding sequences, the 5′ noncoding regions show only random similarity (24%).

The inconsistent homology pattern of the bα1 gene implies that the 5′ and 3′ noncoding regions may be altered such that they are no longer indicative of a relationship among the α-tubulin genes. One explanation could be that differential splicing may alter the 5′ or 3′ noncoding regions. We hybridized gene-specific 3′ noncoding region oligonucleotide probes (13) to a panel of 11 different α-tubulin genomic clones with insertions that were 13 to 20 kilobases long (14). In no case did a single genomic clone hybridize with more than one gene-specific probe (data not shown). This observation suggests that the alternative 3′ noncoding regions are not differentially spliced to coding sequences and hence that the three transcripts are derived from three different α-tubulin genes.

TABLE 1. Interspecies homology of 5′ and 3′ noncoding regions

Species	% Homology of α-tubulin genes to comparable genes in CHO cells[a]					
	Gene I			Gene II		
	Gene	5′ Region	3′ Region	Gene	5′ Region	3′ Region
Hamster[b]	I	100	100	II	100	100
Human[c]	kα1	78	64	bα1	24	80
Rat[d]	pT25		67	pILαT1	95	88
Mouse[e]	Mα2		62	Mα1		84

[a] Percent homology was calculated from the overlap regions of manually aligned sequences (data not shown). Gaps introduced into the sequences to obtain better alignments were conservatively considered to be regions of nonidentity. No significant noncoding region homology was found between noncomparable α-tubulin genes.

[b] Genes I, II, and III were identified in CHO cells (13; this study).

[c] kα1 was isolated from a keratinocyte cDNA library (10). The bα1 sequence was derived from a genomic clone (20) and from a partial brain cDNA clone which did not contain a 5′ noncoding sequence (10).

[d] Both pT25 (18) and pILαT1 (29) were isolated from brain cDNA libraries. pT1 contained a sequence coding for amino acid 297 to the translation stop and an incomplete 3′ noncoding region.

[e] Both Mα2 and Mα1 were isolated from cDNA libraries (32). Neither cDNA clone contained a 5′ noncoding sequence.

Another explanation for not finding homology to the 5′ sequence of human bα1 in the rodent genes may reside in the unusual 5′ genomic structure of mammalian α-tubulin genes. The initiating AUG codon is an exon by itself and is separated from the rest of the gene by an intron that is ≥1.4 kilobases long (20, 30). Thus, it is possible that the transcribed 5′ noncoding region of bα1 lies further upstream than the region presently identified. Unlike the other α-tubulin mammalian genes studied, for which a contiguous cDNA sequence is available, the original bα1 cDNA clone (10) contained sequence data only from amino acid 110 to the polyadenylate tail; the 5′ coding and noncoding data were derived from a genomic clone (20). Thus, the location of the lone AUG was assigned without prior knowledge of the 5′ noncoding sequence. Support for this interpretation of the data is also found in the fact that the currently assigned 5′ noncoding region for bα1 lacks a TATA promoter box (20). Thus, in this study we considered the human bα1 gene to be a class II gene.

Class-specific amino acids. Table 2 shows the 12 amino acids which are known to differ among the mammalian α-tubulin proteins, as predicted from DNA sequence data. The class I rodent proteins are identical throughout the length for which sequence data are available (Table 2). Only four amino acid substitutions have been observed between the class I rodent and human sequences; two of these are conservative (Asp-Glu at position 290 and Thr-Ser at position 340), and two are not conservative (Arg-Gly at position 131 and Gly-Arg at position 308). For the class II rodent genes, complete data are available for hamster and rat cells, and again the predicted amino acid sequences are identical. However, the mouse class II protein differs from the proteins of other rodents by one nonconservative substitution (Gly-Ser at position 232). The human type II protein differs from the rodent proteins at three amino acids; one change is conservative (Asp-Glu at position 290), and two changes are not conservative (Arg-Gly at position 131 and Gly-Arg at position 308). An examination of the predicted class I and II

TABLE 2. Comparison of amino acid sequences in mammalian α-tubulins

Gene	Species	Amino acid at position:[a]											
		131	232	287	290	308	340	437	440	443	444	445	446
I	Human[b]	Arg	Ser	Ser	Asp	Gly	Thr	Val	Val	Glu	Gly	Glu	Glu
	Hamster[c]	Gly	Ser	Ser	Glu	Arg	Ser	Val	Val	Glu	Gly	Glu	Glu
	Rat[d]	NA[e]	NA	NA	NA	Arg	Ser	Val	Val	Glu	Gly	Glu	Glu
	Mouse[f]	Gly	Ser	Ser	Glu	Arg	Ser	Val	Val	Glu	Gly	Glu	Glu
II	Human[g]	Arg	Gly	Ser	Asp	Gly	Thr	Val	Val	Glu	Gly	Glu	Glu
	Hamster[h]	Gly	Gly	Ser	Glu	Arg	Thr	Val	Val	Glu	Gly	Glu	Glu
	Rat[i]	Gly	Gly	Ser	Glu	Arg	Thr	Val	Val	Glu	Gly	Glu	Glu
	Mouse[j]	Gly	Ser	Ser	Glu	Arg	Ser	Val	Val	Glu	Gly	Glu	Glu
III	Hamster[k]	Gly	Ser	Thr[l]	Glu	Arg	Thr	Ala[l]	Ala[l]	Asp[l]	—[m]	—	Asp[l]

[a] The amino acids at other positions in α-tubulins are identical for all mammalian species for which data are available.
[b] kα1 was isolated from a keratinocyte cDNA library (10).
[c] CHO gene I (13; this study).
[d] pT25 (18) is a partial cDNA clone which was isolated from a brain library. It contains a sequence coding from amino acid 297 to the 3′ noncoding region.
[e] NA, Data not available.
[f] Mα2 (32) is a partial cDNA clone; it contains a squence coding from amino acid 100 to the 3′ noncoding region.
[g] The bα1 coding sequence was derived from a genomic clone (20) and a partial brain cDNA clone. The cDNA clone contains a sequence coding for amino acid 110 to the polyadenylate tail (10).
[h] CHO gene II (13; this study).
[i] pILαT1 (29) was isolated from a brain cDNA library.
[j] Mα1 (32) is a partial cDNA clone; it contains a sequence coding from amino acid 254 to the 3′ noncoding region.
[k] CHO gene III (13; this study).
[l] Amino acid characteristic of a gene class.
[m] —, Gap introduced into the sequence in order to acheive maximum homology.

protein sequences revealed no class-specific amino acids (Table 2).

Of the 12 variable α-tubulin amino acids encoded by mammalian α-tubulin genes, 7 are divergent only in hamster gene III. Since gene III is the lone identified member of its class, all of these differences are indicated in Table 2 as class III-specific amino acids. Six of these sites are clustered at the 3′ carboxy terminus of the protein. Five of the sites are conservative substitutions (Thr-Ser at position 287, Ala-Val at positions 437 and 440, and Asp-Glu at positions 443 and 446). The other changes are the loss of a Gly residue and a Glu residue, shortening the protein by two amino acids. Figure 2 shows that the carboxy-terminal tail of α-tubulin is rich in negatively charged Glu and Asp; the loss of one Glu residue reduces the negative charge of the carboxy terminus. Interestingly, the extent of the negative charge at the carboxy terminus has been shown to regulate the propensity of the tubulin protein to assemble in vitro (49, 53).

DISCUSSION

In CHO cells three α-tubulin transcripts are derived from three functional genes which are differentially expressed (13). These genes are dissimilar in their noncoding sequences, and although their coding regions are highly conserved, each encodes a different α-tubulin isoprotein. The most distinct of these proteins, the gene III product, has thus far been observed only in CHO cells. It will be interesting to examine other cell types and species for the expression of this gene product.

The greatest extents of class-specific sequences for the mammalian α-tubulins are their 5′ and 3′ noncoding regions. These sequences are not similar within species but do show striking homology to specific α-tubulin genes between species. This kind of homology is observed in the 3′ noncoding regions of other genes but is not found in all multigene families (19, 62). The sequence homology was used in this study to identify different α-tubulin classes. The class members may be orthologous α-tubulin genes which diverged before mammalian radiation. The high degree of conservation of noncoding sequences among α-tubulin genes belonging to the same class since mammalian speciation suggests a functional significance for these sequences. Future experiments will address the question of whether these sequences are indicative of, or perhaps regulate, the pattern of expression of the α-tubulin genes.

There is a higher degree of coding region DNA conservation among the α-tubulin genes within species than among the genes in different species (Fig. 1). In addition, there are three amino acids (positions 131, 232, and 287) for which the two human α-tubulin genes are the same and differ from the five rodent sequences (Table 2). These data suggest that the tubulin multigene family may be undergoing concerted evolution (17, 21–23, 40).

The presence of different isoforms is a necessary but not sufficient requisite for the existence of functionally specific tubulin proteins, as suggested by the multitubulin hypothesis (16). In CHO cells three distinct α-tubulin isoproteins are encoded. However, in a comparison of all the α-tubulin DNA sequences known, gene classes I and II do not encode α-tubulin proteins with class-specific amino acid sequences, while the class III gene encodes an isoprotein with seven unique amino acids, six of which are clustered at the carboxy terminus. This carboxy-terminal difference is reminiscent of that observed among the β-tubulin genes (1, 32, 55). For the β-tubulins it was originally suggested that a cluster of changes may be seen in this region because the acidic terminus is less constrained functionally (1). However, more recent work suggests that this region may be the binding site of MAP2 and tau proteins (34, 52). In addition, the region may play a regulatory role in modulating the interactions responsible for tubulin self-association (49, 53). Thus, we speculate that in mammalian cells there may be at least two functionally distinct α-tubulin proteins.

The two silent substitutions found in colcemid-resistant cell line CM^R795 are likely due to ethyl methanesulfonate (EMS)-induced mutagenesis. EMS produces transitions (35, 47) of the type which we observed. During the three-step colcemid resistance selection, the CM^R795 cells were treated

with EMS prior to each step, reducing the level of cell viability to 25 to 50% each time (33). EMS treatment which reduces CHO cell viability to 50% produces one mutant protein per 482 loci, and treatment resulting in 10 to 20% viability produces one mutant per 192 loci, as determined by two-dimensional gel electrophoresis (54). Only about one-third of the amino acid substitutions would be detectable as charge changes which resulted in an electrophoretic shift (54). Extrapolating from these data, one protein alteration per 40 loci is expected in CM^R795 cells, along with an undetermined number of silent substitutions. Comparative sequencing, such as that described here, should reveal both protein-altering and silent substitutions. We found silent substitutions at a frequency of two in three genes or one substitution in 2,430 base pairs. This estimate of silent substitution is higher than that predicted for replacement substitutions and thus is consistent with the level expected for EMS-induced mutagenesis.

In a variety of different systems tubulin mutants have been selected which are similar to CM^R795 cells in that they possess an altered tubulin protein, as determined by two-dimensional gel electrophoresis (5, 6, 26, 41, 48, 51). Although the DNA lesion has not been identified for any tubulin mutant, it has been suggested that structural gene mutations may be responsible for the altered tubulin proteins observed. Therefore, it was surprising that no replacement substitutions were found in CM^R795 cells when the three expressed α-tubulin genes were sequenced. Our results do not rule out the possibility that both alleles of each α-tubulin gene are expressed and that we determined the structure of only one of the alleles. Alternatively, a fourth divergent α-tubulin gene may be expressed in these cells (3, 55). Nonstructural alterations (for example, posttranslational modification or a regulatory change) could also be responsible for the observed phenotype. Futher investigations of this cell line are underway to explore these various possibilities.

ACKNOWLEDGMENTS

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