The complete sequence of a frog α -tubulin gene and its regulated expression in mouse L-cells

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A frog α -tubulin cDNA and a frog α -tubulin gene, closely related to the cDNA, were cloned and sequenced and the structure of the gene deduced. The gene was introduced into mouse L-cells in order to investigate the transcription and regulation of the gene. The gene was transcribed and there was processing of the transcripts. Furthermore, the gene displayed the correct autoregulatory feedback control.

INTRODUCTION

Microtubules are filamentous protein polymers, the basic monomeric subunit of which is a heterodimer composed of one α - and one β -tubulin protein molecule. Microtubules are present in nearly all eukaryotic cells and have a wide variety of functions (for reviews, see Cleveland, 1983; Cleveland & Sullivan, 1985). Consistent with these multiple functions, tubulin genes display a wide variety of regulatory phenomena.

Of particular interest is the fact that tubulin gene expression is subject to autoregulatory feedback control by the level of unpolymerized tubulin monomers. This was demonstrated by use in cultured cells of the drug colchicine, which causes the depolymerization of microtubules (Taylor, 1965; Inoue & Sato, 1967). Colchicine therefore causes an increase in the concentration of unassembled tubulin monomers and results in a specific repression of new α - and β -tubulin proteins (Ben Ze'ev *et al.*, 1979). This response is not due to a non-specific effect of colchicine, since the rate of synthesis of other proteins is not affected by the drug. Subsequently it was shown that this regulation is mediated through decreased levels of both α - and β -tubulin mRNA (Cleveland *et al.*, 1981). This response is not due to a general effect of colchicine on all mRNA levels, since actin mRNA levels are unaffected by the drug. The response to colchicine addition is very rapid: the levels of α - and β -tubulin mRNA decay with a half-life of about 1 h after addition of the drug. The rate of loss of α - and β -tubulin mRNA is similar to the rate of loss of new α - and β -tubulin protein synthesis. The autoregulation occurs in a variety of cell types from diverse species, including mouse L-cells. As one would expect, increasing tubulin concentration by microinjection of purified α - and β -tubulin in equimolar quantities into cultured mammalian cells also causes a specific repression of α - and β -tubulin protein synthesis (Cleveland *et al.*, 1983). In order to investigate this feedback control of tubulin genes, a frog α -tubulin gene and a closely related cDNA were cloned and sequenced, and the gene was introduced into mouse L-cells. The transcription, processing of the transcripts and autoregulatory feedback control of the frog α -tubulin gene in the heterologous environment of the mouse L-cell was analysed.

MATERIALS AND METHODS

DNA sequencing

DNA sequence analysis was performed as described by Bankier & Barrell (1983). DNA sequences were determined entirely on both strands, except for the 5' 9 bp of the cDNA sequence, which were determined on the anti-message strand only. This was due to sequencing artefacts caused by the oligo(dG·dC) linker at the 5' end of the cDNA insert. Problems due to similar artefacts at the 3' end of the insert were caused by the poly(dA·dT) linker. These problems were overcome by priming DNA sequencing reactions from the poly(dA) tail as described below.

DNA sequencing from poly(dA)

Three separate sequencing reactions were performed by using an M13 clone containing a poly(dA) tract in the single (+)-strand DNA form of the clone. The first reaction was primed with dT₁₂C, the second with dT₁₂G and the third with dT₁₂A. The reactions were performed as described by Bankier & Barrell (1983), except that 1.5 μ l of dT₁₂C, dT₁₂G or dT₁₂A primer, each dissolved in TE [10 mM-Tris/HCl (pH 8.0)/0.1 mM-EDTA] at 0.4 ng· μ l⁻¹, was used instead of the universal M13 sequencing primer, and the annealing step was performed by incubation at 68 °C for 30 min, immediately followed by incubation at 42 °C for 1 h instead of incubation at 68 °C for 1 h. Either [α -³²P]dATP or [α -³⁵S]thio-dATP was employed in these sequencing reactions, as described by Bankier & Barrell (1983). One of the three primers, that is, either dT₁₂C, dT₁₂G or dT₁₂A, annealed correctly to the M13 clone and an acceptable sequence was therefore obtained.

Nick translation

Nick translation of DNA (Rigby *et al.*, 1977) was performed as described by Maniatis *et al.* (1982).

Prime cut probes

Prime cut probes are single-stranded DNA probes (Bentley, 1984) and were prepared as described by Bienz (1984a).

Abbreviations used: 20 $\times$ SCP, 2 M-NaCl/0.6 M-Na₂HPO₄/0.02 M-EDTA, adjusted to pH 6.2 with conc. HCl; DMEM, Dulbecco's modified Eagle's medium; HAT, 100 μ M-hypoxanthine/0.5 μ M-aminopterin/16 μ M-thymidine; bp, base-pair(s); kb, kilobase(s); HSV-1, Herpes simplex virus 1; tk, thymidine kinase; SV40, simian virus 40.

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These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00713.

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Fig. 1. Comparison between the DNA and amino acid sequences of the frog α -tubulin gene and those of the cDNA insert of pDS α T1

The message-strand DNA sequence of the gene is shown above the message-strand DNA sequence and amino acid sequence of the cDNA insert. The poly(A) tail of the cDNA insert is indicated by A(n). 'Padding' characters (-) have been introduced into the DNA sequences in order to keep them in register. Differences between the DNA sequences are indicated by a relevantly positioned asterisk (*) placed above the message strand of the gene. Differences between the α -tubulin protein sequences deduced from the DNA sequences of the gene and the cDNA insert are shown.

cDNA library screening

cDNA libraries were screened as described by Maniatis *et al.* (1982).

λ -Phage library screening

λ -Phage library screening was performed as described by Maniatis *et al.* (1982), using pre-hybridization in $5 \times \text{SCP}/1\%$ Sarkosyl at room temperature for 15 min, hybridization in $5 \times \text{SCP}/1\%$ Sarkosyl at 68°C for 16–20 h, and washing in $1 \times \text{SCP}/1\%$ Sarkosyl at room temperature, at 68°C and at room temperature; each incubation was for 20 min and fresh wash solution was employed.

Maintenance of mouse L-cells

Mouse L-cells (Kit *et al.*, 1963) were maintained at 37°C in DMEM supplemented with 5% (v/v) foetal-calf serum under an atmosphere of CO_2/air (1:9).

Transfection of mouse L-cells

Transfection of mouse L-cells was by use of DNA/calcium phosphate co-precipitation and was performed as described by Wigler *et al.* (1979) and Graham & Bacchetti (1981). When the transfection was complete, the cells were fed with selective medium, which consisted of the normal growth medium supplemented with HAT (Littlefield, 1964).

Preparation of cytoplasmic RNA from tissue-culture cells

Cytoplasmic RNA from tissue-culture cells was prepared as described by Pelham (1982).

Preparation of frog oocyte total RNA

Frog (*Xenopus laevis*) oocyte total RNA was prepared as described by Bienz (1984a).

S₁ mapping

S₁ mapping analysis (Berk & Sharp, 1977) was performed as described by Bienz (1984a), except that hybridization was in 80% (v/v) formamide/0.4 M-NaCl/10 mM-Pipes (pH 6.5)/1 mM-EDTA.

RESULTS

Isolation and DNA sequence analysis of a frog α -tubulin cDNA

A cDNA library was constructed by using RNA from the frog blastula embryonic stage, using a method which preferentially selects for full-length cDNA inserts (Okayama & Berg, 1982). The 1125-bp endonuclease-*Pst*I fragment of the insert of the chicken cDNA clone pT1 (Valenzuela *et al.*, 1981) was labelled by nick translation and used to screen the library. This fragment contains 1002 bp of the coding region of the chicken cDNA insert, and, since α -tubulin is highly conserved, should hybridize with the desired tubulin clones in the frog cDNA library (Cleveland, 1983; Cleveland & Sullivan, 1985). A positively hybridizing frog α -tubulin cDNA clone, pDS α T1, was isolated from the library and subjected to DNA sequence analysis. The whole plasmid was sonicated and sequenced, and vector sequences were discarded by using the programs SCREENV and DBAUTO (Staden, 1982). Elucidation of DNA sequence from the anti-message strand of the cDNA insert near the 3' end of the insert was obtained using sequencing reactions from poly(dA) as described in the Materials and methods section.

The 1562-bp DNA sequence and 449-amino-acid translation of the frog α -tubulin cDNA insert of pDS α T1 is presented in Fig. 1. The reading frame of the frog α -tubulin shown in this Figure was established by comparison of the six-phase amino acid translations of the cDNA nucleotide sequence with α -tubulin amino acid sequences from other organisms (Lemischka *et al.*, 1981; Ponstingl *et al.*, 1981; Valenzuela *et al.*, 1981). Fig. 1 shows that the cDNA insert contains the entire coding sequence for the frog tubulin protein, plus 112 nucleotides of 5' untranslated region and 101 nucleotides of 3' untranslated region. The cDNA contains the usual 3' cleavage signal sequence (Wickens & Stephenson, 1984; Conway & Wickens, 1985), in this case AATAAA (Proudfoot & Brownlee, 1976), at an appropriate distance, in this case 24 nucleotides, 5' to the poly(A) tail.

Cloning of a frog α -tubulin gene closely related to the cDNA

Approx. 5×10^6 plaques of a frog genomic λ -phage library (Bienz, 1984b) were screened with a prime cut probe from the coding region of the frog α -tubulin cDNA insert of pDS α T1. This probe contains approx. 320 nucleotides of sequence complementary to the

message strand of the cDNA insert coding region, commencing from nucleotide 732 and extending to approximately nucleotide 413 of the insert. In all, 20 positively hybridizing λ -phages were isolated as a result of the screening and were designated ' λ DS α T1- λ DS α T20'. These λ -phages were then screened with a prime cut probe from the 3' untranslated region of the frog α -tubulin cDNA insert of pDS α T1. This probe contains sequences complementary to the entire 101 nucleotides of the message strand of the 3' untranslated region of the cDNA insert, but lacks any α -tubulin coding region sequence. Although the coding regions of tubulin genes are highly conserved, the untranslated regions of these genes are not subject to the same evolutionary constraints as the coding region and thus may display considerable divergence (Cowan *et al.*, 1983; Gwo-Shu Lee *et al.*, 1983, 1984; Bond *et al.*, 1984). Hence positively hybridizing plaques should contain frog α -tubulin genes closely related to the frog α -tubulin cDNA insert of pDS α T1. Of the 20 frog genomic clones, λ DS α T1- λ DS α T20, that were subjected to this screening, four gave a positive hybridization signal. There were λ DS α T8, λ DS α T10, λ DS α T14 and λ DS α T19. λ DS α T14 was selected for further study and DNA sequence analysis of its frog α -tubulin gene.

DNA sequence of the frog α -tubulin gene

The DNA sequence of the frog α -tubulin gene was determined by using three restriction fragments. These were (from 5' to 3'): a 2.8-kb *Pvu*II fragment, a 2.8-kb *Eco*RI fragment and a 1.6-kb *Eco*RI fragment. The 2.8-kb *Pvu*II and the 2.8-kb *Eco*RI fragments overlapped by 361 bp. The 2.8-kb *Eco*RI fragment and the 1.6-kb *Eco*RI fragment were proved contiguous by sequencing a restriction fragment that bridged them. The DNA sequence is presented in Fig. 1. This Figure compares the 6896-bp DNA sequence and 449-amino-acid sequence of the frog α -tubulin gene with the 1562-bp DNA sequence and 449-amino-acid sequence of the cDNA insert of pDS α T1. The DNA sequence of the gene reveals that it contains a 5' untranslated region, a coding region and a 3' untranslated region which are highly homologous with their equivalent regions in the cDNA insert and that the gene contains all of the cDNA insert sequence. Furthermore, the gene encodes for a complete α -tubulin protein which is identical, apart from the one amino acid described below, with the α -tubulin protein encoded by the cDNA insert. Thus the gene, as expected from the way it was isolated, is closely related to the cDNA insert.

However, the frog α -tubulin gene contains three introns. The first intron is 3500 bp long, interrupts the coding sequence between the initiation codon ATG and codon 2 and delineates an exon coding for one amino acid, that is, the initiator methionine. The second intron is 1025 bp long and interrupts the coding sequence in codon 76 between the first and second nucleotide of this codon. The third intron is 184 bp and interrupts the coding sequence between codon 125 and codon 126. All three introns of the frog α -tubulin gene possess, at the 5' end of the intron, the invariant 5' consensus splicing sequence GT..., and possess, at the 3' end of the intron, the invariant 3' consensus splicing sequence ...AG (Breathnach & Chambon, 1981; Sharp, 1981). The number and positions of the introns of rat (Lemischka & Sharp, 1982) and human (Hall & Cowan, 1985) α -tubulin

genes are exactly conserved when compared with the frog α -tubulin gene.

There are a small number of nucleotide changes between corresponding regions of the frog α -tubulin gene and the cDNA insert. These changes lead to only one amino acid change: leucine at position 153 (nucleotide 572 of the cDNA insert) is replaced by methionine at position 153 (nucleotide 5843 of the gene). This is a conservative amino acid substitution. Since the frog genomic clone λ DS α T14 and the frog cDNA clone pDS α T1 were isolated from libraries constructed from different individuals, these changes may represent polymorphisms in allelic genes. Alternatively, these changes may occur because the two clones represent different, very highly related, non-allelic genes.

The DNA sequence analysis of the frog α -tubulin gene commences from 559 bp upstream of the nucleotide corresponding to the 5' nucleotide of the cDNA insert and extends to 63 bp downstream of the poly(A) tail addition site of the gene. The probable transcriptional initiation site of the gene was assigned by S₁ mapping to nucleotide 550 (see the text and Figs. 4 and 5 below; T. J. Mohun & D. J. Smith, unpublished work). This shows that the cDNA insert is very nearly full length, except for 10 bp. At 30 bp upstream of the transcriptional initiation site of the frog gene is found the sequence CATAGAA, which agrees fairly well with the TATA box consensus sequence

which may be found about 31 bp upstream of the transcriptional initiation site in many promoters (Breathnach & Chambon, 1981; Pelham, 1982). Similarly, at 64 bp upstream of the transcriptional initiation site of the frog gene is the sequence GGCCAATAG, which agrees fairly well with the CCAAT box consensus sequence

found about 70 bp upstream of the transcriptional initiation site in some promoters (Breathnach & Chambon, 1981; Pelham, 1982). The sequence ATTAAAAATAAA is found 29 nucleotides 5' to the poly(A) tail addition site of the frog α -tubulin gene. This sequence consists of the 3' cleavage signal sequences (Wickens & Stephenson, 1984; Conway & Wickens, 1985) ATTAAA (Ahmed *et al.*, 1982) and AATAAA (Proudfoot & Brownlee, 1976), in a head-to-tail arrangement, at an appropriate position.

The frog genomic insert fragment of λ DS α T14 is resectable as a single 15-kb *Xho*I fragment and possesses 4.7 kb of genomic sequence upstream of the transcriptional initiation site of the gene and 4.2 kb of genomic sequence downstream of the poly(A) tail addition site of the gene.

Introduction of the frog α -tubulin gene into mouse L-cells

The plasmid used in this study, pDS5, is depicted diagrammatically in Fig. 2. pDS5 was constructed by cloning the 15-kb *Xho*I frog genomic insert fragment of λ DS α T14 containing the frog α -tubulin gene into the *Sa*II site of pXTK10 (Pelham, 1982). pXTK10 contains the HSV-1 tk gene.

The DNA/calcium phosphate co-precipitation

Fig. 2. Structure of plasmid pDS5

Frog insert and frog α -tubulin gene: wide black boxes, translated sequences; wide white boxes, untranslated sequences; narrow black boxes, upstream and downstream flanking sequences; thin black lines, introns; arrow, direction of transcription; start of arrow, transcriptional initiation site. pXTK10 vector sequences: diagonally striped boxes, pBR322 sequences; vertically striped boxes, pBR322 ampicillin-resistance (Ap^r) gene; stippled boxes, HSV-1 tk gene; black boxes, SV40 DNA; thin arrows, direction of transcription; start of arrow, transcriptional initiation site. The SV40 (SV40 Ori) and pBR322 (pBR322 Ori) origins of replication together with the directions of replication and also shown. The SV40 DNA contains neither an enhancer nor an efficient promoter. The ligated *Xho*I and *Sa*II sites of pDS5 are denoted by 'S/X'.

method was used to transfect pDS5 into tk-deficient (tk⁻) mouse L-cells (Kit *et al.*, 1963). Four separate DNA co-precipitations were set up. The first three each used 20 μ g of pDS5, and each was used to transfect a separate 25 cm² flask of mouse tk⁻ L-cells. The fourth used 40 μ g of pDS5 and was split into three equal parts, each of which was used to transfect a separate 25 cm² flask of mouse tk⁻ L-cells. Each of these flasks was maintained separately and eventually seeded into selective HAT media. Each 25 cm² flask eventually gave rise to about ten HAT-resistant mouse tk⁺ L-cell colonies, which were then allowed to grow to confluency. The three mouse tk⁺ L-cell pools which were thus created from the first three co-precipitations were designated 'pDS5-1', 'pDS5-2' and 'pDS5-3'. The three mouse tk⁺ L-cell pools that were created from the equal splitting of the fourth co-precipitation into three parts were designated 'pDS5-4a', 'pDS5-4b' and 'pDS5-4c'. The six mouse tk⁺ L-cell pools that resulted were all propagated in selective HAT medium.

Frog α -tubulin gene introduced into mouse L-cells is transcribed and regulated

Cytoplasmic RNA was prepared from each of the six mouse tk⁺ L-cell pools when the cells were 50–80% confluent, in parallel experiments. The mouse tk⁺ L-cell pools were either not treated with colchicine or else

Fig. 3. Expression and regulation of the frog α -tubulin gene introduced into mouse L-cells analysed by S_1 mapping

The gene-specific *Eco*RI prime cut probe of AT4.52 was hybridized to three-tenths of the cytoplasmic RNA (about 24 μ g of cytoplasmic RNA) from 75 cm² flasks of the mouse tk⁺ L-cell pools, digested with S_1 nuclease and analysed by electrophoresis through a 6%-(w/v)-polyacrylamide sequencing gel. The variations in the amount of RNA employed in each S_1 mapping analysis are negligible. The gel lanes are as follows. Cell pools had either not been treated with colchicine ('0') or else treated with 10 μ M-colchicine for 6 h ('6'). M, end-labelled *Msp*I-digested pBR322 to provide markers. The positions and sizes (bp) of the end-labelled pBR322/*Msp*I fragments are indicated. P, undigested prime cut probe (about 1000 Čerenkov c.p.m.). Oocyte, 20 μ g of frog oocyte total RNA. Control, no-RNA control. The protected fragment produced by genuine transcripts of the frog α -tubulin gene in frog oocyte total RNA is indicated by ◀.

incubated in their normal medium supplemented with 10 μ M-colchicine for 6 h before being harvested for the preparation of cytoplasmic RNA. This RNA was analysed by S_1 mapping employing the *Eco*RI prime cut probe of the M13mp8 clone AT4.52. This probe contains 354 nucleotides of sequence complementary to the message strand of the frog α -tubulin gene commencing from nucleotide 719 and extending to nucleotide 366, thus spanning the likely transcriptional initiation site suggested by the cDNA start site. The probe does not cross-hybridize to mouse tubulin message and only hybridizes to the 5' untranslated region of frog α -tubulin mRNA, and should map the 5' end of this message.

Fig. 4. Regulation of the frog α -tubulin gene in the mouse tk⁺ L-cell pool pDS5-4a analysed by S_1 mapping

The gene-specific *Eco*RI prime cut probe of AT4.52 was hybridized to 24.3 μ g of the cytoplasmic RNA from regulated cell pool pDS5-4a (equivalent to approx. three-tenths of a 75 cm² flask of cells), digested with S_1 nuclease and analysed by electrophoresis through a 6%-(w/v)-polyacrylamide sequencing gel. The cells were treated with 10 μ M-colchicine for 0, 1, 2, 4 or 6 h. The 'M', 'P', 'Control', 'Oocyte' and '◀' designations are as for Fig. 3.

The S_1 mapping analysis displayed in Fig. 3 shows that RNA from all the mouse tk⁺ L-cells which express the frog α -tubulin gene protects a single 128-nucleotide fragment of the probe, identical with the result obtained with frog oocyte RNA (and also frog RNA from other tissues; T. J. Mohun and D. J. Smith, unpublished work). This assigns the probable transcriptional initiation site of the gene to nucleotide 550. The protected fragment could conceivably represent an internal exon of the gene rather than the 5' exon. This is unlikely, since there is no invariant 3' consensus splice sequence near the transcriptional initiation site. Furthermore, although Northern-blot experiments (D. J. Smith, unpublished work) show multiple α -tubulin transcript sizes in frog tissues, one size class is 1.6 kb, which is close in size to the cDNA insert.

The S_1 mapping analysis presented in Fig. 3 implies that the gene is showing correct transcription initiation and, since the probe spans an exon/intron boundary, correct processing of the message in mouse L-cells. These results thus demonstrate that the frog α -tubulin gene is functional in mouse cells. Of the six HAT-resistant

Fig. 5. Response of actin mRNA levels during exposure of the mouse tk^+ L-cell pool pDS5-4a to colchicine, analysed by S_1 mapping

This S_1 mapping analysis used the cytoplasmic RNA samples from the experiment shown in Fig. 4. The *Bgl*II prime cut probe of B9.12X was hybridized to 12.15 μ g of the cytoplasmic RNA from regulated cell pool pDS5-4a (equivalent to approx. three-twentieths of a 75 cm² flask of cells), digested with S_1 nuclease and analysed by electrophoresis through a 6%-(w/v)-polyacrylamide sequencing gel. The cells were treated with 10 μ M-colchicine for 0, 1, 2, 4 or 6 h. The 'Oocyte', 'Control', 'P' and 'M' designations are as for Fig. 3. The protected fragment produced by actin mRNA is indicated by '►'.

mouse tk^+ L-cell pools, only pDS5-2 does not give rise to detectable frog α -tubulin gene transcripts, even though this pool is phenotypically tk^+ . Correctly initiated and correctly processed frog α -tubulin transcripts are detectable from pDS5-3 upon a longer exposure of the gel shown in Fig. 3.

In addition to displaying correct transcription of the frog α -tubulin gene and correct processing of the 5' splice site of intron 1, three of the mouse tk^+ L-cell pools, namely pDS5-3, pDS5-4a and pDS5-4c, also show correct regulation of the frog α -tubulin gene in the heterologous environment of the mouse L-cell. Thus the S_1 mapping analysis shows that these cell pools display a decrease in the level of frog α -tubulin mRNA in response to a 6 h incubation in their normal medium supplemented with 10 μ M-colchicine.

However, despite displaying accurate transcription of

the frog α -tubulin gene and correct processing of the transcripts of the gene, two of the mouse tk^+ L-cell pools, namely pDS5-1 and pDS5-4b, do not show correct regulation of the gene. The S_1 mapping analysis displayed in Fig. 3 shows that these cell pools constitutively express the frog α -tubulin gene and do not show a significant change in the level of frog α -tubulin mRNA in response to a 6 h incubation in their normal medium supplemented with 10 μ M-colchicine.

Rate of autoregulation of the frog α -tubulin gene in mouse L-cells

In order to investigate the rate of loss of frog α -tubulin gene transcripts in mouse L-cells treated with colchicine, cytoplasmic RNA was prepared from the regulated cell pool pDS5-4a that had been incubated for various lengths of time in its normal medium supplemented with 10 μ M-colchicine. The confluence of the cells was 50–80 % when the colchicine was added and was equal for each time point. The cytoplasmic RNA was then analysed by S_1 mapping using the *Eco*RI prime cut probe of AT4.52. The results of this S_1 mapping analysis are presented in Fig. 4. The concentration of frog α -tubulin mRNA falls by about 50 % in 1 h. This is similar to the result shown by endogenous tubulin mRNA in Chinese-hamster ovary cells (Cleveland *et al.*, 1981).

Response of actin mRNA levels during autoregulation of the frog α -tubulin gene in mouse L-cells

In order to control for the possibility that colchicine has a general effect on mRNA levels, an S_1 mapping experiment was performed in order to measure the levels of actin mRNA during exposure of the mouse tk^+ L-cell pool pDS5-4a to colchicine.

The prime cut probe used in this S_1 mapping analysis was prepared from the M13mp8 clone B9.12X (kindly donated by Dr. Tim Mohun) and endonuclease *Bgl*II. The probe contains 48 nucleotides of M13mp8 sequence together with 134 nucleotides of sequence complementary to the message strand of a frog cytoplasmic γ -actin cDNA coding region commencing from nucleotide 389 and extending to nucleotide 256 of the cDNA coding region. Since actins are highly conserved, this probe should cross-hybridize with mouse actin mRNA and hence give rise to a protected fragment of 134 nucleotides in an S_1 mapping analysis.

An S_1 mapping analysis was performed using this probe and part of the cytoplasmic RNA samples derived from the colchicine time-course experiment employing mouse tk^+ L-cell pool pDS5-4a shown in Fig. 4. The results of this S_1 mapping analysis are displayed in Fig. 5. This S_1 mapping analysis results in a single protected fragment of the expected size, that is, 134 nucleotides. Thus the prime cut probe does indeed cross-hybridize with the detect mouse L-cell actin transcripts.

It can be seen that the actin mRNA levels increase by about 2-fold within about 1 h of the addition of colchicine, and thereafter stay constant. The significance of this observation is unclear, but agrees with previous findings (Lau *et al.*, 1985). This demonstrates that the decrease in frog α -tubulin mRNA concentration seen in the presence of colchicine is not a non-specific response of the cells. This observation also makes it unlikely that the decrease in frog α -tubulin mRNA levels is due to an artefact in the RNA preparation, gel loading or other aspect of the experimental procedure.

DISCUSSION

A frog α -tubulin gene has been introduced into mouse cells in order to investigate the regulation of the gene in a heterologous environment. Not only can mouse cells produce authentic frog α -tubulin mRNA, but in some of the cell pools the effect of colchicine is to decrease the level of frog α -tubulin mRNA. Colchicine has similar effects on endogenous tubulin mRNA concentration, although the exact mechanism of this effect is not clear. Tubulin synthesis is regulated by a post-transcriptional mechanism, possibly via a direct effect on mRNA stability (Cleveland & Havercroft, 1983; Caron *et al.*, 1985; Pittenger & Cleveland, 1985), although other levels of control, for instance primarily at the translational level, have not been ruled out. Whatever the mechanism of control, it appears that sequences in the tubulin gene required for this regulation have been conserved between mice and frogs. Correct autoregulation of chicken and human β -tubulin genes in mouse L-cells has recently been described (Lau *et al.*, 1985). That study employed a transient assay in which the genes were present largely as replicating episomes, unlike the present study, which employed a stable assay system.

The reason why some of the cell pools in the experiments described here did not display correct regulation of the frog α -tubulin gene is unclear. One possibility is that the gene in these cell pools became mutated during transfection such that it has lost or incapacitated a regulatory site(s), thus causing loss of regulation of the gene.

The system described here may permit identification of these regulatory sites of tubulin genes by introduction of appropriate mutant genes into L-cells and investigation of their control. Tubulin genes display cell-cycle regulation (Cleveland & Sullivan, 1985), and it may be of interest to see whether the exogenously introduced frog α -tubulin gene displays correct cell-cycle regulation in addition to (or despite lack of) the correct autoregulatory feedback control. Finally, introduction of mutated tubulin genes back into L-cells may allow definition of some of the functional domains of these proteins.

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