Nucleotide Sequence and Expression of Two β-Tubulin Genes in *Stylonychia lemnae*

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The gene-sized macronuclear DNA of the hypotrichous ciliate *Stylonychia lemnae* contains one size class of DNA molecules of 1·85 kb (1 kb = 10^3 base-pairs) coding for β-tubulin. These DNA molecules consist of two different β-tubulin genes, β_1 and β_2, which are amplified to about 150,000 (β_1) and 30,000 (β_2) copies per macronucleus. Both genes were cloned and sequenced entirely. The coding sequences of the two molecules (1329 base-pairs including TGA) predict identical amino acid sequences for the proteins and show a nucleotide homology of 97·2%. The nucleotide as well as the encoded amino acid sequences are highly conserved, when compared to β-tubulin genes from vertebrates. The ciliate-specific codon TAA specifying glutamine is present only in the β_2-tubulin gene, whereas glutamine is encoded soley by CAA in the β_1-tubulin gene. The 5′ and 3′-non-coding regions of both β-tubulin genes are similar in length, but differ extremely in nucleotide sequence. Both β-tubulin genes are transcriptionally active in *S. lemnae*, although not all putative transcription-regulatory sequences known from higher eukaryotes can be detected within the non-coding regions. The two transcription products localized by S_1-mapping experiments show a similar length of about 1·40 kb and transcription seems to be regulated differently for β_1 and β_2.

1. Introduction

Microtubules are filamentous structures and represent principal components of the cytoskeleton, the mitotic spindles, centrioles, cilia, flagella and of neuronal processes (Kirschner, 1978). The major protein of microtubules is tubulin, a heterodimer of two distinct polypeptides designated α and β (Bryan & Wilson, 1971). Each tubulin has a molecular weight of about 50,000 and each is encoded in a distinct set of genes (Cleveland *et al.*, 1980). Tubulins are highly conserved proteins and the number of genes coding for both α and β-tubulin varies extremely among different species. In mammals the α and β-tubulin genes are members of two multigene families, mainly representing pseudogenes (Cleveland *et al.*, 1980; Cowan *et al.*, 1981; Lemischka & Sharp, 1982). The number of functional genes in these multigene families vary in different organisms: in human for example three α and four β-tubulin genes are known to be expressed (Cowan *et al.*, 1983; Dobner *et al.*, 1987; Hall & Cowan, 1985; Lewis *et al.*, 1985*a*); in mice six functional α and β-tubulin genes were found (Lewis *et al.*, 1985*b*; Villasante *et al.*, 1986; Wang *et al.*, 1986). At least five out of seven to nine β-tubulin genes are expressed in chicken (Lopata *et al.*, 1983; Sullivan *et al.*, 1986) and in *Drosophila* the four existing α and β-tubulin genes all represent functional genes (Raff, 1984). In contrast to these higher eukaryotic examples the genomes of lower eukaryotes, such as the slime mould *Physarum polycephalum* and *Chlamydomonas*, contain only two sequences coding for α or β-tubulin, which are both transcribed (Brunke *et al.*, 1982; Monteiro & Cox, 1987). Only one β-tubulin gene has been found in the yeast genome (Neff *et al.*, 1983).

The hypotrichous ciliate *Stylonychia lemnae* possesses two morphologically and functionally different nuclei, the macro- and the micronuclei. Micronuclei contain chromosome-sized, transcriptionally inactive DNA, whereas DNA in macronuclei is arranged in "gene-sized" molecules of 400 bp† to 20,000 bp in length. Each molecule is amplified to a specific copy number per macronucleus ranging from a few to more than 100,000. Gene expression occurs only in macronuclei (Ammermann *et al.*, 1974; Helftenbein, 1985; Lipps & Steinbrück, 1978; Nock, 1981; Steinbrück *et al.*, 1981; Helftenbein, unpublished results). Two α-

† Abbreviations used: bp, base-pair(s); kb, 10^3 bases or base-pairs.

tubulin genes were detected in the macronuclear genome, both representing actively transcribed genes. They are encoded by two size classes of macronuclear DNA molecules of 1·85 (α_1) and 1·73 kp (α_2), which are amplified to about 100,000 and 20,000 copies per macronucleus for α_1 and α_2, respectively (Helftenbein, 1985; E. Helftenbein & E. Müller, unpublished results). These molecules, coding for α-tubulin, show the typical properties of all macronuclear DNA molecules from hypotrichous ciliates, such as a centred continous coding region flanked by A+T-rich non-coding sequences of different length terminating in telomeres consisting of 5′-C_4A_4 repeats at both ends (Helftenbein, 1985; Kaine & Spear, 1982; Klobutcher *et al.*, 1981).

In this report we demonstrate that two different β-tubulin genes are present in the macronuclear genome of *S. lemnae*, and that both are expressed.

2. Materials and Methods

(a) *Cell cultivation and isolation of macronuclear DNA and total RNA*

Cells of *S. lemnae* strain DO were cultivated in Pringsheim solution as described by Ammermann (1965). DNA was purified from isolated macronuclei as described (Ammermann *et al.*, 1974; Steinbrück *et al.*, 1981). Total RNA was isolated by the procedure of Glisin *et al.* (1974) and contaminating DNA was eliminated by DNase I digestion with 3000 units/ml at 37 °C for 40 min.

(b) *Construction of recombinant plasmids*

Preparative agarose gel electrophoresis was carried out as described (Helftenbein, 1985) and DNA was eluted electrophoretically from agarose gels as described by Maniatis *et al.* (1982). Macronuclear DNA molecules were treated by exonuclease *Bal*31 with 0·02 unit/μg DNA at 0 °C for 1·5 min and the ends were filled in by Klenow polymerase as suggested by the manufacturer. *Pst*I linkers were attached in a 5 M excess to the resulting blunt-end DNA molecules by 1 unit of phage T_4-ligase at 20 °C for 4 h in a final volume of 20 μl (60 mM-Tris · HCl (pH 7·0), 6 mM-$MgCl_2$, 0·6 mM-dithiothreitol; 0·01 mM-ATP). After digestion with *Pst*I, unligated *Pst*I linkers were separated from macronuclear DNA molecules by Sephadex G20 column chromatography as described by Maniatis *et al.* (1982). Ligation of macronuclear DNA molecules into the *Pst*I site of the vector pUC12 was carried out in a 2 M excess of insert DNA at 12 °C under the same conditions as described for ligation of linkers. Cells of *Escherichia coli* strain JM83 were used for transformation according to Yanisch-Perron *et al.* (1985).

(c) *Screening for β-tubulin genes*

Colonies containing recombinant plasmids were screened for β-tubulin genes with a ^{32}P-labelled DNA probe of a cloned β-tubulin cDNA from chicken (Valenzuela *et al.*, 1981) according to the colony-hybridization procedure of Grunstein & Hogness (1975). The cloned cDNA probe was kindly given to us by D. Cleveland.

(d) *DNA-labelling and sequencing procedures*

Nick-translation of DNA, 5′ end-labelling with polynucleotide kinase and 3′ end-labelling with Klenow polymerase and terminal deoxynucleotidyl-transferase were all done according to Maniatis *et al.* (1982). DNA sequencing was performed as described by Maxam & Gilbert (1977). For enzymatic sequencing (Sanger *et al.*, 1977) DNA fragments were subcloned in the double strand sequencing vector pGEM-3 (Promega Biotec) and sequencing reactions were carried out as suggested by the manufacturer.

(e) *S_1 nuclease mapping*

S_1 mapping was done essentially according to Weaver & Weissmann (1979). Hybridization of 50 to 100 ng of 5′ or 3′ end-labelled double-stranded DNA fragments to 10 μg of total RNA was carried out at 50 °C (5′ ends) and 46 °C (3′ ends) for 12 to 16 h in a total volume of 20 μl. S_1 nuclease digests were performed as described by Miller & Sollner-Webb (1981) with 600 units nuclease/ml in a final volume of 220 μl. The reaction was terminated by the addition of ammonium acetate and EDTA to 0·4 M and 0·01 M, respectively, followed by 2 extractions with phenol/$CHCl_3$. Nucleic acids were precipitated with ethanol, dried, dissolved and denatured in 5 μl of loading buffer for 10 min at 100 °C (80% (v/v) formamide, 1 mM-EDTA, 50 mM-Tris · HCl (pH 8·3), 0·05% (w/v) bromphenol blue, 0·05% (w/v) xylene cyanol) and analyzed on 8% (w/v) denaturing polyacrylamide gels (Maxam & Gilbert, 1977).

(f) *Denaturing agarose gel electrophoresis and hybridization reactions*

For denaturing gels RNA and DNA were treated with glyoxal as described by McMaster & Carmichael (1977) and electrophoresed on 1·5% (w/v) agarose gels. After transfer of the nucleic acids to nitrocellulose membrane filters (Schleicher and Schüll, BA85) by the method of Thomas (1980) hybridization reactions were carried out at 60 °C in 5 × SSC, 10 × Denhardt's solution and 0·1% (w/v) SDS for 24 to 48 h. Filters were washed in 2 × SSC, 0·1% SDS at 60 °C for 2 h. (SSC is 0·15 M-NaCl, 0·15 M-sodium citrate, pH 7·0.)

(g) *Materials*

Exonuclease *Bal*31 was obtained from Bethesda Research Laboratories, while all other enzymes, *Pst*I linkers and Sephadex G20 were from Pharmacia. The enzymatic sequencing kit was purchased from Promega Biotec. Radioactive nucleotides were obtained from Amersham.

3. Results

(a) *Identification and cloning of macronuclear DNA molecules coding for β-tubulin*

To identify macronuclear DNA molecules coding for β-tubulin, total macronuclear DNA was separated according to size by gel electrophoresis and hybridized with a radioactively labelled heterologous cloned β-tubulin cDNA probe from chicken (Valenzuela *et al.*, 1981). The probe hybridizes only to one size class of macronuclear DNA molecules of 1·85 kb, consisting of two types of DNA molecules differing by restriction enzyme sites (Figs 1 and 2). Mating experiments showed that the two types of molecules encoding β-tubulin do not represent two alleles of one gene (data not shown).

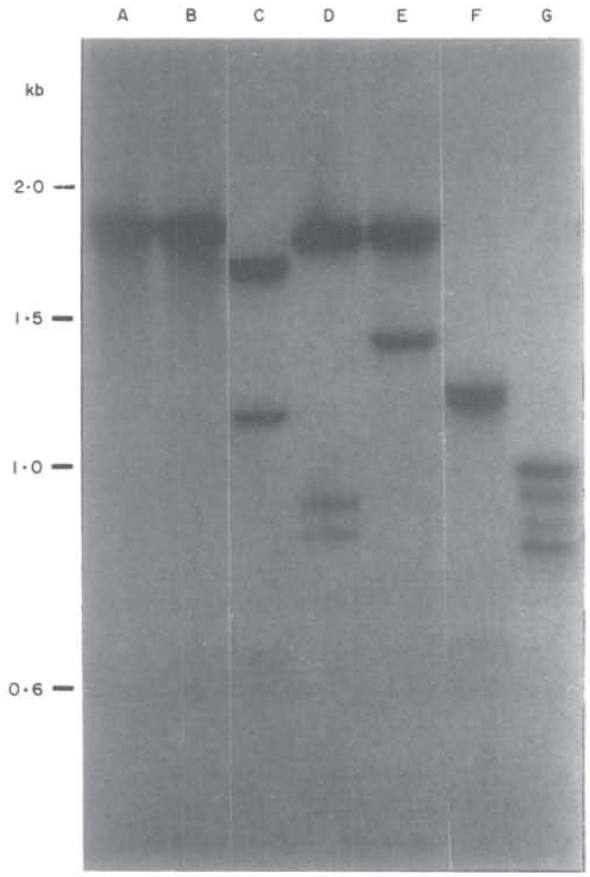

Figure 1. Identification and characterization of macronuclear DNA molecules coding for β-tubulin. Native (lane A) and restriction enzyme-digested macronuclear DNA (lanes B to F) were separated in a 2% (6 μg each) agarose gel according to size. After drying, the gel was hybridized to a ^{32}P-labelled heterologous cloned β-tubulin cDNA probe from chicken (Valenzuela *et al.*, 1981) and the radioactivity visualized by autoradiography. Native macronuclear DNA contains one size class (1·85 kb) of DNA homologous to the β-tubulin probe (lane A) consisting of 2 types of molecules as shown by restriction enzyme digests. In lane D one of the types (β_2) is cut by the enzyme *Hin*dIII (the 2 lower bands of 0·9 and 0·95 kb), while the other one exhibits no restriction site for this enzyme and remains uncut. Both molecules possess a restriction site for *Pvu*II (lane G) producing each 2 bands of 1·0+0·85 (β_1) and 0·95+0·9 kb (β_2). Lane A, native macronucleus DNA; lane B, *Pst*I; lane C, *Sal*I; lane D, *Hin*dIII; lane E, *Ava*II; lane F, *Bam*HI; lane G, *Pvu*II-digested DNA.

In order to clone the macronuclear DNA molecules coding for β-tubulin, DNA molecules of 1·7 kb to 2·0 kb were eluted from a preparative agarose gel and cloned as described in Materials and Methods. Five positive clones were identified by screening the colonies with the heterologous β-tubulin gene probe from chicken. Restriction maps of the recombinant DNA from these colonies showed that both types of β-tubulin genes were represented by the inserted DNA molecules. The inserts of pmacA6, B3, B8 and C4 were defined as β_1 and that of pmacF4 as β_2.

(b) *Copy numbers per macronucleus of the two different β-tubulin genes*

Copy numbers were estimated as 150,000 for β_1 and as 30,000 for the β_2-tubulin genes per macronucleus by quantitative measurement of radioactivity retained in hybridization experiments (Fig. 3).

(c) *Sequence analysis of the cloned macronuclear DNA molecules coding for β_1 and β_2-tubulin*

One representative of the cloned β_1-tubulin genes (pmacA6) and the cloned β_2-tubulin gene (pmacF4) were sequenced in full-length on both strands by chemical or enzymatic sequencing as described in Materials and Methods. The coding nucleotide sequences and the predicted amino acid sequences of both genes are compared to a β-tubulin gene from chicken in Figure 4. The coding regions of both β-tubulin genes from *S. lemnae* have an identical length of 1329 bp (including TGA). The β_2-tubulin gene differs from the β_1-tubulin gene in 90 silent nucleotide exchanges (97·2% homology), maintaining the amino acid sequences of both genes identical. One of the 90 different bases within the β_2-tubulin gene is located in the ciliate-specific codon TAA specifying glutamine at nucleotide position 571, where the β_1-tubulin gene contains the codon CAA for glutamine. Interestingly, codons with G in the third position are avoided in the two genes (only 5 out of 16 possible codons are used), whereas codons ending with C are preferred.

Comparison of the predicted amino acid sequence encoded by the two genes with β-tubulins from other organisms reveals a high conservation of the *Stylonychia* β-tubulin. The homology to other protozoal and even mammalian β-tubulins is approximately 90% (Fig. 6). A larger divergence is found between the *Stylonychia* and fungal β-tubulins (70 to 80% homologous). The divergent amino acids of the *Stylonychia* β-tubulin are mainly located in regions known to be highly variable, especially at the carboxy terminus of the protein. In regions exhibiting high interspecies conservation the sequence of the *Stylonychia* β-tubulin is nearly identical with those of the other proteins, especially the region between amino acids 388 and 428, known to be involved in polymerization of tubulins (Serrano *et al.*, 1984; Fig. 6).

The 5′ and the 3′-non-coding regions of the β_1 and the β_2-tubulin genes differ slightly in length, but dramatically in their nucleotide sequences (Fig. 5(a) and (b)). The β_1 5′-non-coding region (126 bp) is 44 bp smaller than that of the β_2-tubulin gene (170 bp), whereas the 3′-non-coding region of the β_1-tubulin gene (343 bp) is larger than that of the β_2-tubulin gene (284 bp). The sequences are terminated by C_4A_4 repeats of 20 or 28 nucleotides.

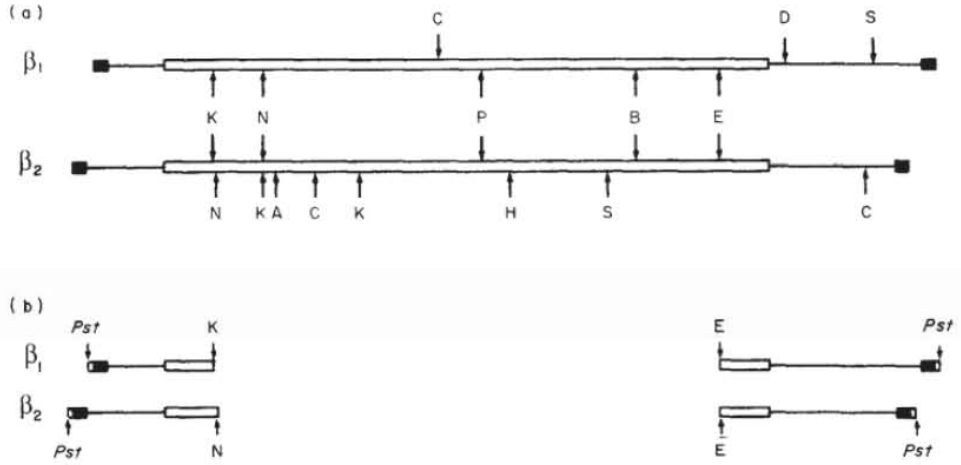

Figure 2. (a) Restriction maps of the macronuclear DNA molecules coding for β-tubulin. The 2 molecules are aligned to their coding regions (double lines). Single lines show the 5′ and 3′-non-coding regions, filled boxes represent the terminal C_4A_4 repeats. (b) Fragments of the cloned molecules used in S_1 protection analysis. The *Pst*I sites are created by cloning the molecules into the *Pst*I site of pUC12 after linker addition. B, *Bam*HI; C, *Cla*I; D, *Nde*I; E, *Eco*RI; K, *Kpn*I; N, *Nco*I; P, *Pvu*II; S, *Sal*I; *Pst*, *Pst*I.

The macronuclear DNA molecules coding for β_1-tubulin thus include 1846 bp, and are slightly larger than those coding for the β_2-tubulin genes with 1831 bp.

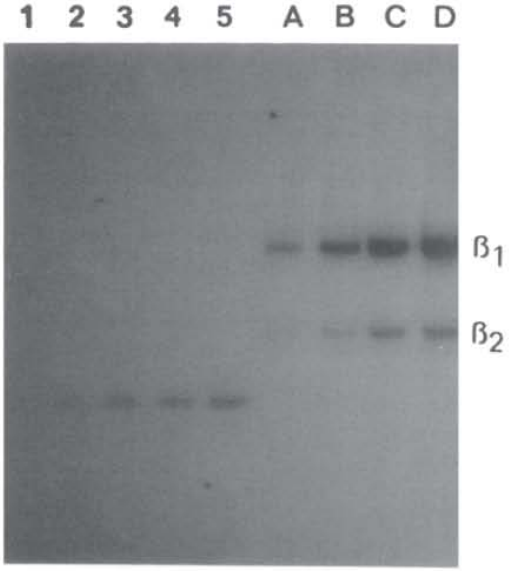

Figure 3. Estimation of the copy numbers of the β-tubulin genes in the macronucleus. Macronuclear DNA was digested with *Ava*II to separate the β_1 and β_2 genes by electrophoresis in a 1·5% agarose gel (lane A: 1·25 μg; lane B, 2·5 μg; lane C, 5 μg; lane D, 7·5 μg). As reference an *Eco*RI–*Ava*II fragment of the cloned β_2-tubulin gene was run in the same gel (lanes 1 to 5: 0·125, 0·25, 0·5, 0·75 and 1 ng of DNA). The gel was dried and hydridized to the same fragment labelled with ^{32}P. The radioactive regions were cut out after localization by autoradiography and the amount of radioactivity was measured by liquid scintillation counting. Taking into account the length of the reference fragment and a DNA content of 780 pg/macronucleus (Ammermann *et al.*, 1974) the copy numbers of β_1 and β_2 were calculated to be 150,000 and 30,000, respectively. Corresponding experiments using a fragment of the cloned β_1 gene as reference and hybridization probe provided the same results.

An homologous sequence of 18 nucleotides is found in the 5′-non-coding region of the two β-tubulin genes (β_1: position -2 to -19 and β_2: position -11 to -28; Fig. 5(a)). The 3′-non-coding regions contain a common octanucleotide beginning at position TGA+203 of the β_1 and at position TGA+240 of the β_2-tubulin genes (Fig. 5(b)). The non-coding regions of the two β-tubulin genes show the low G+C contents (23·8% and 25·3% on the 5′ and of 23·3% and 25·7% on the 3′-flank), typical for macronuclear DNA molecules of hypotrichous ciliates.

(d) *Putative control signals for transcription*

A TATA-box-like sequence, which is generally believed to be a transcriptional promoter (Baker *et al.*, 1979), is located at nucleotide -67 in the 5′ non-coding region of the β_2-tubulin gene. The 5′ non-coding region of the β_1-tubulin gene, however, does not contain such a sequence. Sequences homologous to the pentanucleotide CCAAA, also believed to be involved in the transcription initiation process (Koszak, 1984), are not present in either β-tubulin genes. The highly conserved nucleotide sequence AATAAA functioning as a polyadenylation signal in eukaryotes (Manley *et al.*, 1985; Proudfoot & Brownlee, 1976; Wickens & Stephenson, 1984) is only present in the 3′-non-coding region of β_1 at positions TGA+175, +271 and +281, but not in that of β_2. No similarities to other sequences involved in polyadenylation, 3′ RNA processing and transcription termination in several eukaryotes (Birnstiel *et al.*, 1985; Berget, 1984; Conway & Wickens, 1985; Gil & Proudfoot, 1984; Grass *et al.*, 1987; McLauchlan *et al.*, 1985) are found in both genes.

(e) *Transcription of the β_1 and β_2-tubulin genes*

To identify the β-tubulin gene transcription products, total RNA was separated on denaturing

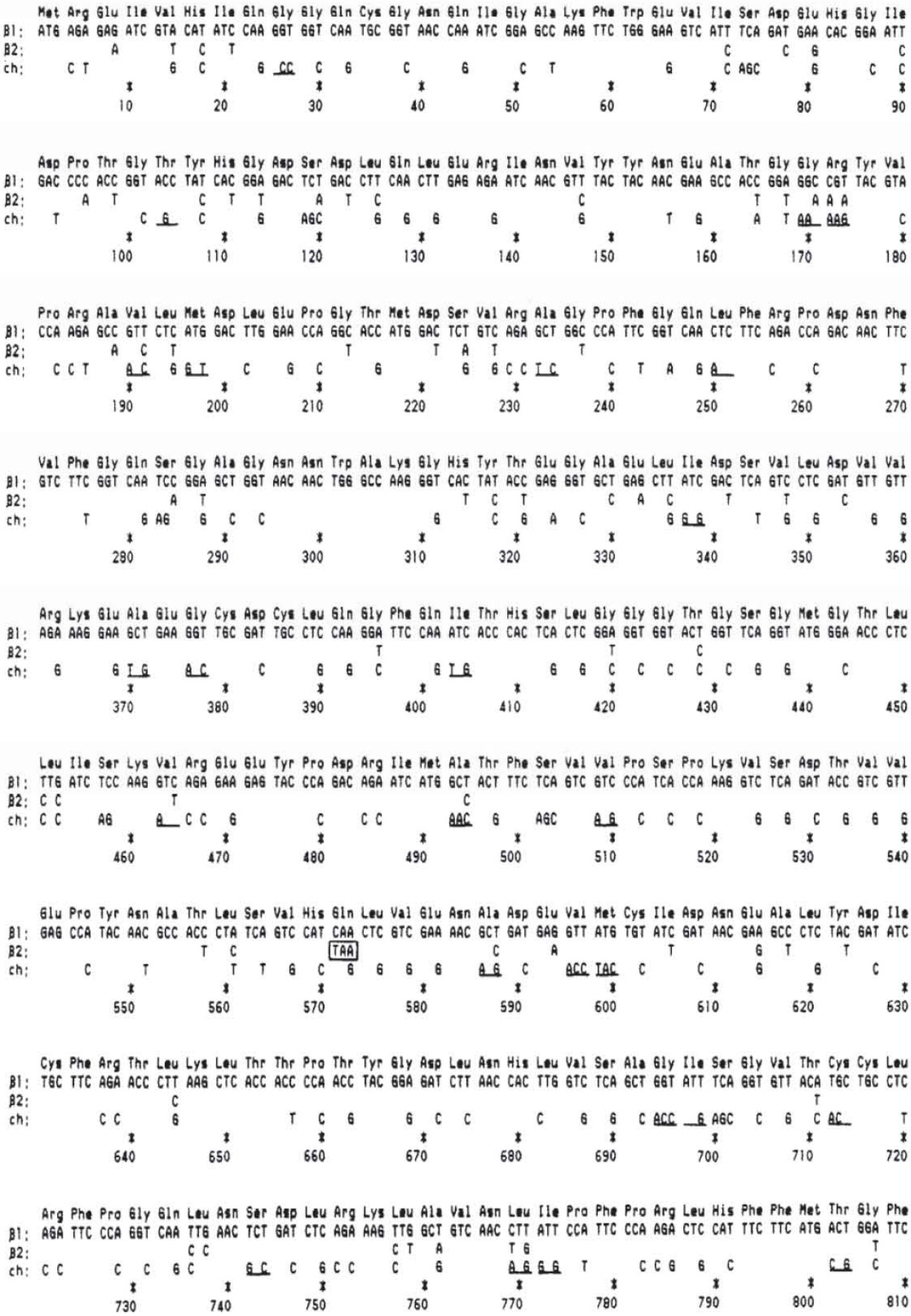

Fig. 4.

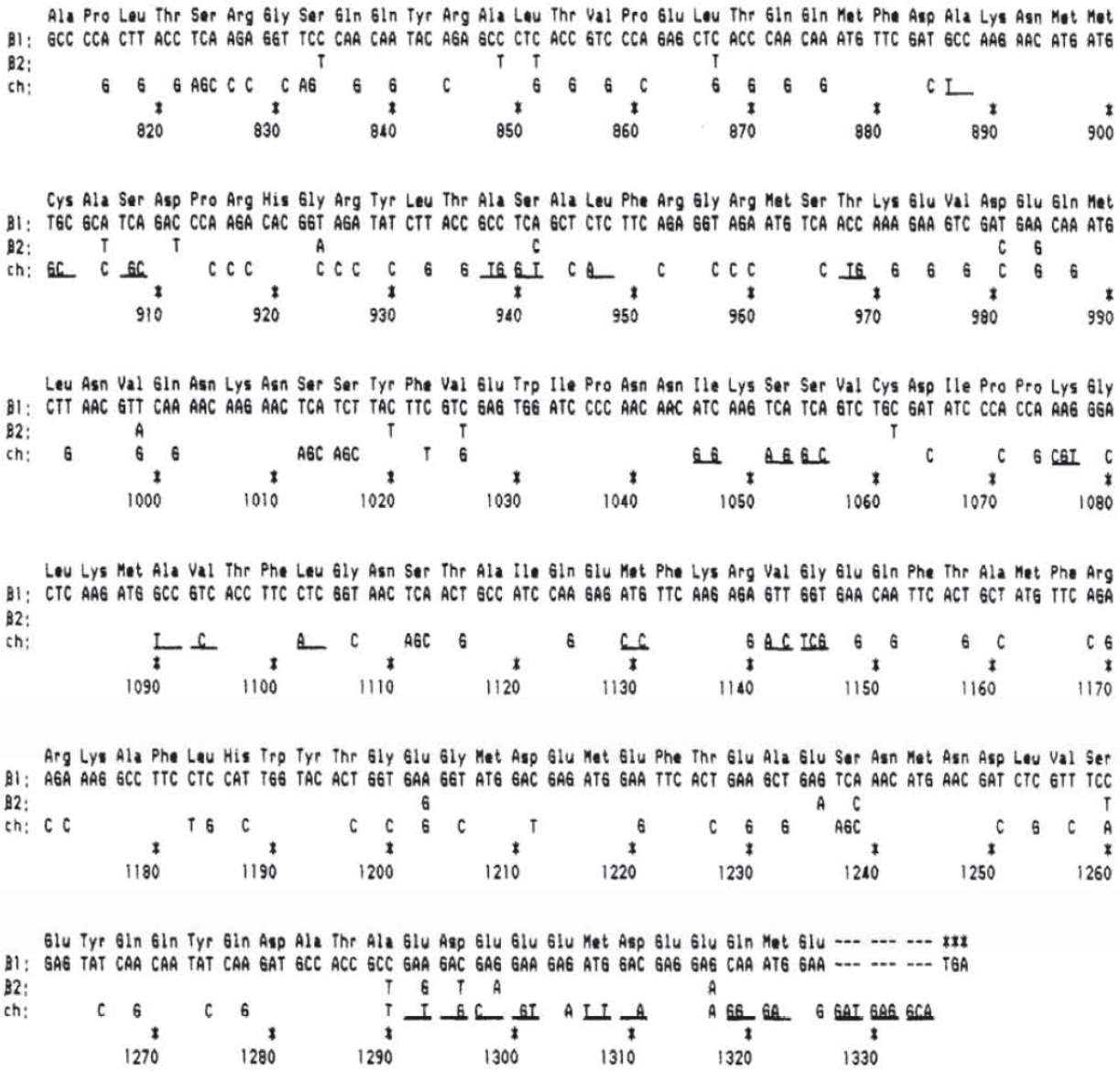

Figure 4. Comparison of the coding nucleotide sequences of the 2 β-tubulin genes from *S. lemnae* and of a chicken β-tubulin gene (ch; Valenzuela *et al.*, 1981). The complete DNA sequence is shown only for β_1. In the β_2 and chicken tubulin sequences only deviations from β_1 are given. The nucleotide exchanges leading to an amino acid substitution between the *Stylonychia* and chicken β-tubulins are underlined. The deduced amino acid sequence of the *Stylonychia* β-tubulin is given in the upper line. The ciliate-specific codon TAA encoding glutamine in the β_2-tubulin gene is boxed (position 571).

gels and hybridized with the homologous cloned macronuclear β_1 (pmacA6) and β_2-tubulin genes (pmacF4). Transcripts of only one size class of about 1·6 kb were found, which are smaller than the macronuclear DNA molecules (Fig. 7(a)).

In order to identify the non-translated sequences of the RNA transcripts, S_1 mapping experiments were carried out with both cloned β-tubulin genes separately. To estimate the length of the 5′ non-translated region, a *Pst*I–*Kpn*I DNA fragment of the cloned β_1-tubulin gene and a *Pst*I–*Nco*I fragment of the β_2-tubulin gene were used (Fig. 2(b)). The *Pst*I–*Kpn*I fragment is 260 bp in length and spans 104 nucleotides of the coding region, and the *Pst*I–*Nco*I fragment contains 300 bp and includes 112 coding nucleotides. DNA fragments of 150 nucleotides are protected in both experiments (Fig. 7(b)). Transcription thus starts at nucleotide −46 in the β_1 and at nucleotide −38 in the β_2-tubulin genes (Fig. 5(a)). To detect the 3′ non-translated regions, *Eco*RI–*Pst*I fragments of the cloned β-tubulin genes were used, which are 470 bp (β_1) and 420 bp (β_2) in length and span 108 nucleotides of the 3′-coding regions (Fig. 2(b)). DNA fragments of 181 nucleotides of the β_1 and of 165 bases of the β_2-tubulin genes are protected from the nuclease (Fig. 7(c)). Thus transcription terminates at position TGA+73 in the β_1 and at position TGA+57 in the β_2-tubulin genes (Fig. 5(b)).

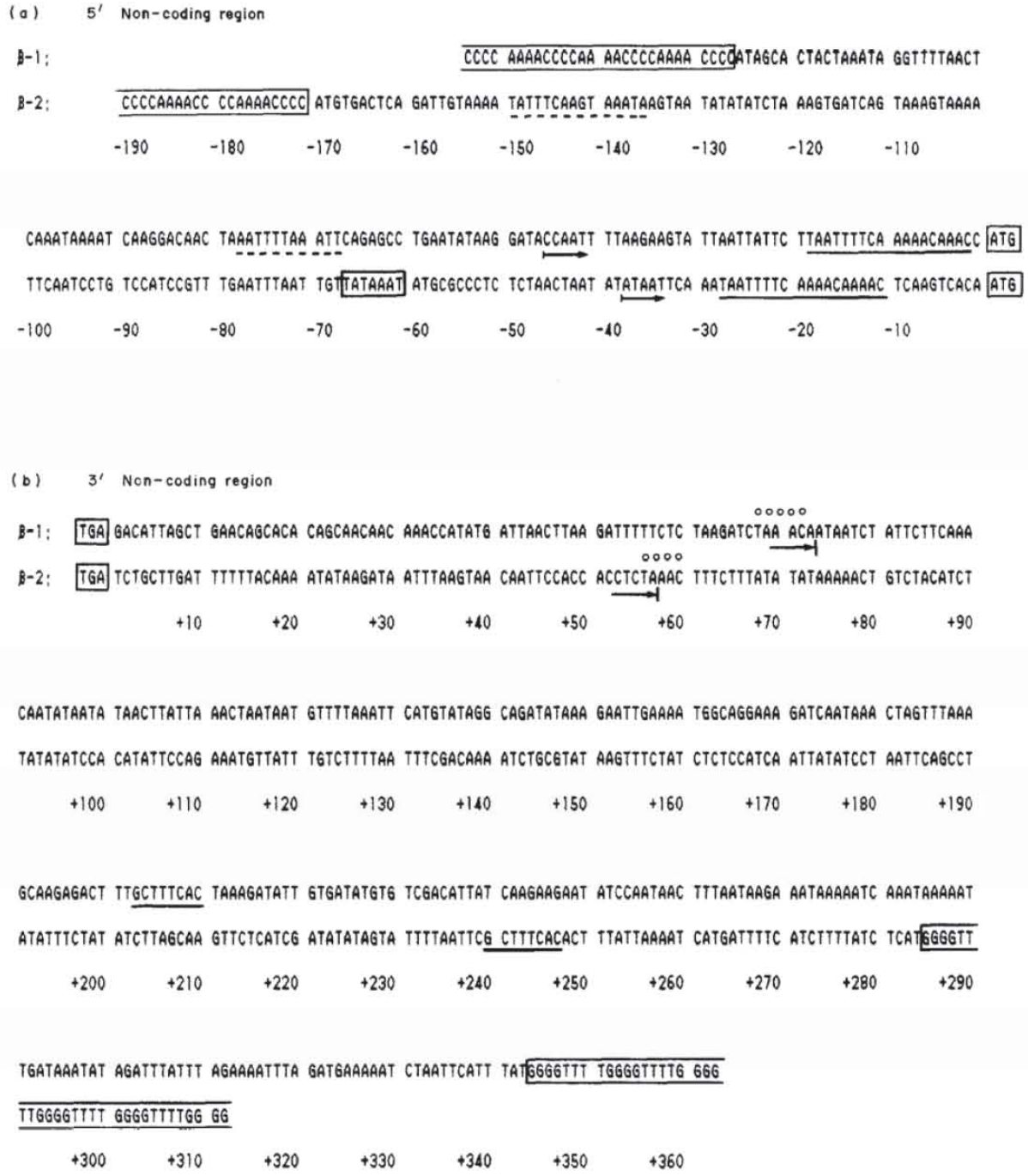

Figure 5. (a) 5′ and (b) 3′ non-coding sequences of the β-tubulin genes from *Stylonychia*. The start and termination sites of transcription are marked by arrows, the TATA box of β_2 is boxed, homologous sequences are underlined. Terminal C_4A_4 repeats are characterized by open boxes. The pentanucleotide TAAAC, presumably involved in 3′-processing of the RNA is indicated by circles and presumptive replication origins are marked by broken lines.

4. Discussion

The finding of an identical general structure with a continuous coding region flanked by A+T-rich non-coding regions of different lengths and nucleotide sequence in the two β-tubulin genes of *S. lemnae* is a strong indication that both molecules represent functional genes. The two genes showing a nucleotide homology of 97·2% in the coding regions encode identical polypeptides. In contrast, most of the organisms investigated so far possess different β-tubulin isotypes, supposed to be necessary for different functions within the cell or in different tissues (Rudolph *et al.*, 1987; Sullivan *et al.*, 1986; Wang *et al.*, 1986). The existence of only one β-tubulin gene in yeast and two β-tubulin genes encoding identical proteins in *Chlamydomonas* (Youngblom *et al.*, 1984), however, show that at least in lower eukaryotes a single β-tubulin polypeptide should be sufficient for all cellular

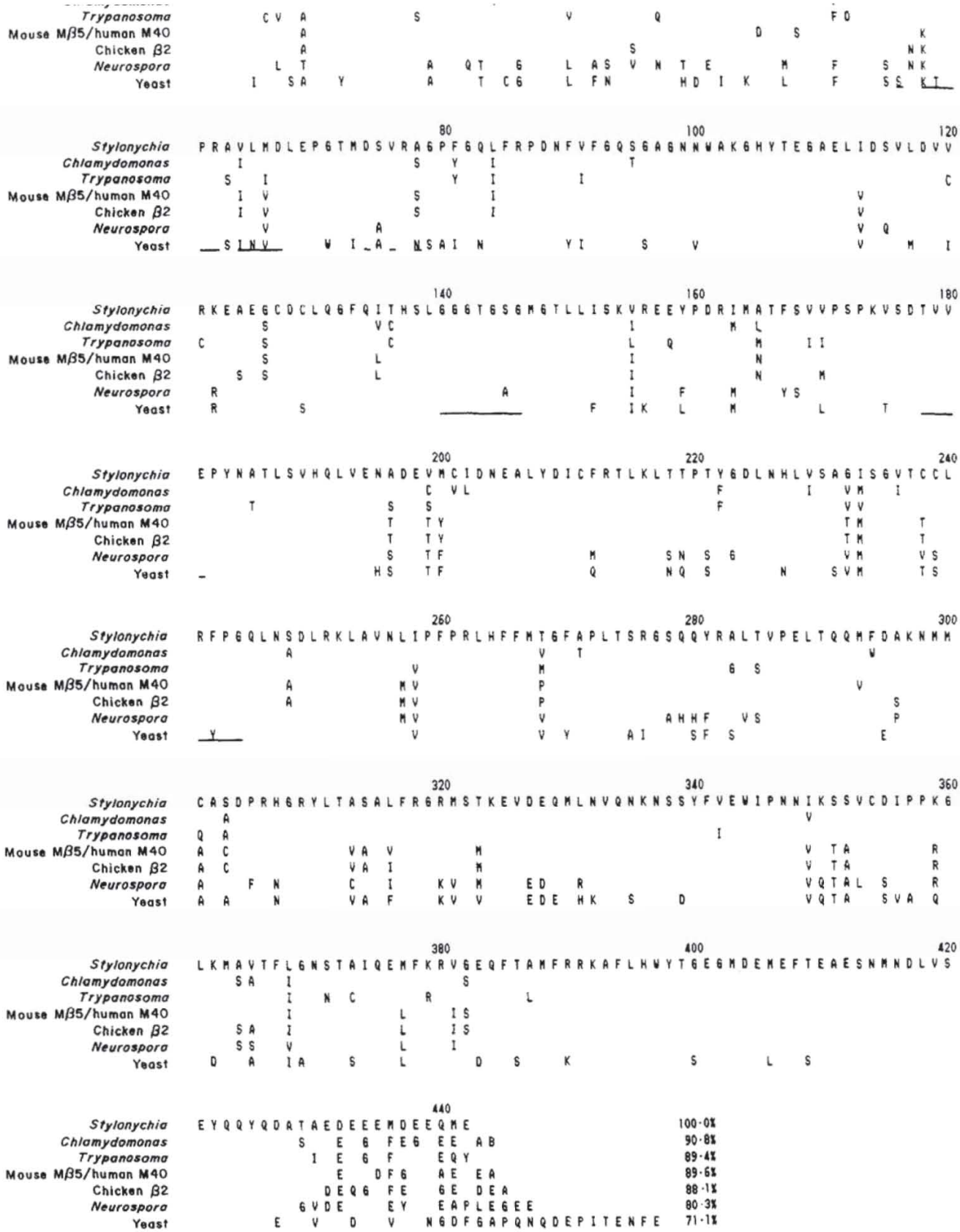

Figure 6. Comparison of the *Stylonychia* β-tubulin with those from other species. The complete predicted amino acid sequence of the *Stylonychia* β-tubulin is given on the top line in the single-letter amino acid code. For the other β-tublins only differing amino acids are shown. Homology to the *Stylonychia* β-tubulin is indicated for each protein. For mouse, human and chicken the isotypes with the highest homology to the *Stylonychia* protein were chosen. Sequence data are from Youngblom *et al.* (1984) *(Chlamydomonas reinhardtii)*, Kimmel *et al.* (1985) *(Trypanosoma brucei rhodesiense)*, Wang *et al.* (1986) (mouse isotype Mβ5, corrected human isotype M40), Valenzuela *et al.* (1981) (chicken isotype β2), Orbach *et al.* (1986) *(Neurospora crassa)* and Neff *et al.* (1983) *(Saccharomyces cerevisiae)*. The regions probably involved in GTP binding (Mandelkow *et al.*, 1986) are underlined.

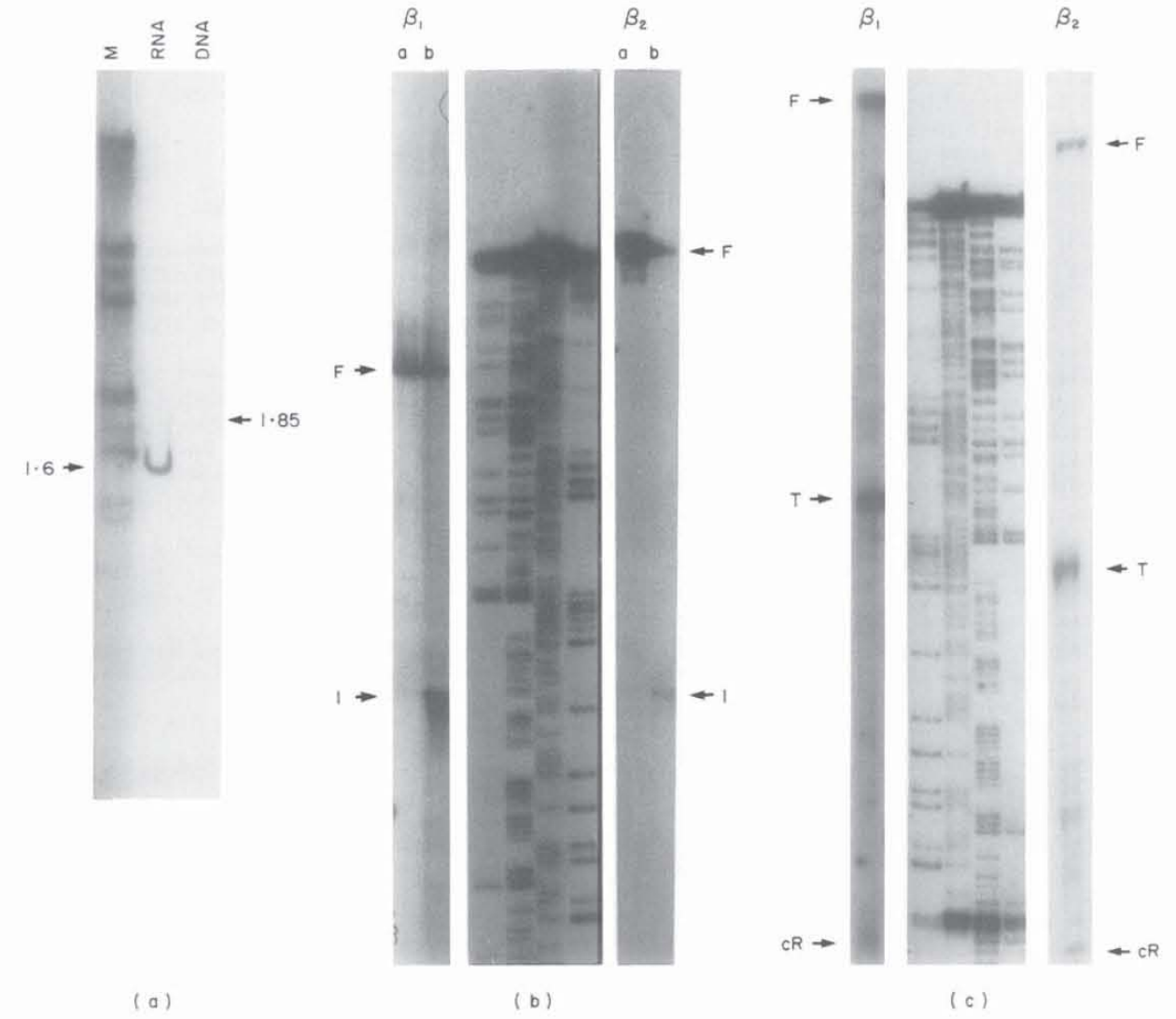

Figure 7. Detection of transcription products of the β-tubulin genes and localization of their untranslated sequences. (a) Total RNA (10 μg), macronuclear DNA (50 ng) and *Hin*dIII/*Eco*RI-digested λ DNA (100 ng) were denatured, electrophoresed, transferred to nitrocellulose and hybridized with the cloned macronuclear DNA molecules coding for β_1 (pmacA6) and β_2-tubulin genes (pmacF4) as described in Materials and Methods. Location of start and termination sites of the transcription products of β_1 and β_2-tubulin genes was done by S_1 mapping experiments as described in Materials and Methods. The protected DNA products were separated by polyacrylamide gel electrophoresis and the radioactive bands were identified by autoradiography. The gels were calibrated by using radioactive products of a sequencing reaction as molecular size markers. Autoradiographs of DNA fragments containing the 5′-transcribed regions are shown in (b) and the 3′-transcribed regions in (c). Lanes a, without RNA; lanes b with RNA. F indicates DNA fragments used in hybridization experiments; I indicates DNA fragments protected by RNA on the 5′ flank; T indicates DNA fragments protected by RNA on the 3′ flank; cR indicates DNA fragments of the coding region.

functions. This also seems to be true for the highly differentiated ciliates showing an elaborate, highly ordered anatomical structure (Nanney, 1980).

The presence of the ciliate-specific codon TAA for glutamine (position 571 to 573) in only one of the two β-tubulin genes (in contrast to its presence in both α-tubulin genes) might be an indication for the evolutionary development of the two β-tubulin genes from a common ancestral gene, which did not show the altered genetic code. This supports the hypothesis that the divergence of the genetic code occurred after the ciliates branched off from the ancestors of the eukaryotes (Horowitz & Gorovsky, 1985). The observed identical codon usage in both β-tubulin genes (codons ending with C are preferred, those ending with G are avoided) is very similar to that found in the α-tubulins and may be a characteristic feature of all hypotrichous ciliates.

Copy numbers of the two β-tubulin genes (150,000 β_1 and about 30,000 β_2 copies per macronucleus) are similar to those found for the α-tubulin genes (100,000 and 20,000 for α_1 and α_2, respectively; Helftenbein, 1985). These similarities and the relation of 1 : 5 for the copy numbers of both the α_2 and β_2 to the α_1 and β_1-tubulin genes might be an indication for the involvement of the

copy numbers in regulation of gene expression. Expression of the abundant α_1 and β_1 and the less abundant α_2 and β_2-tubulin genes might be correlated and might be regulated differently as it is found for several β-tubulin genes in other organisms (Sullivan *et al.*, 1986; Raff, 1984; Wang *et al.*, 1986). Gene-specific regulated expression of the tubulin genes in *Stylonychia* might be necessary for a quantitatively different tubulin synthesis, providing alternative post-translational modifiable subunit proteins of microtubules for specific purposes, such as mitotic spindles, cilia, centrioles or elements of the cytoskeleton (Cleveland & Sullivan, 1985; Cowan, 1985).

The hypothesis of a differential regulation of transcription of the two β-tubulin genes is strongly supported by the distinct non-coding regions of the β_1 and β_2-tubulin genes (Fig. 5). The presence of a TATA box 29 bp upstream from the transcription start site in β_2 suggests that transcription of this gene is initiated as in other eukaryotes (Baker *et al.*, 1979; Bucher & Trifonov, 1986). The nucleotide sequences involved in initiation of transcription within the β_1-tubulin gene missing a TATA box remain to be identified. It also remains to be investigated whether the tubulin mRNAs are polyadenylated or whether their poor enrichment by oligo(dT)-cellulose chromatography (data not shown) is due to the very high A content of the non-translated sequences. The eukaryotic polyadenylation signal AATAAA (Proudfoot & Brownlee, 1976), present in only one of the genes far downstream from the 3′ end of the corresponding mRNA molecule, may not be functional in *S. lemnae*. The only common sequence, located around the 3′ ends of the mRNAs, is the pentanucleotide TAAAC. This sequence is also found at similar positions in the α-tubulin genes of *S. lemnae* (E. Helftenbein & E. Müller, unpublished results) and may be involved in 3′ processing of the mRNA or transcription termination.

The homologous sequences of the 5′-non-coding regions of both β-tubulin genes located within the transcribed region (Fig. 5(a)) show similarities to those found in the 5′ non-translated regions of both α-tubulin genes. This sequence might represent conserved elements possibly involved in initiation of translation in *S. lemnae*.

A sequence capable of forming a hairpin loop with a stem consisting of A+T residues only is located 49 nucleotides downstream from the 5′-C_4A_4-terminal repeats in the β_1 and 21 nucleotides downstream from the 5′-C_4A_4 structures in the β_2-tubulin gene (Fig. 5(a)). Such sequences are found in similar distances from the 5′-terminal repeats of all cloned and sequenced macronuclear DNA molecules of *S. lemnae*. Probably they represent the replication origins of these molecules (Helftenbein, 1985; E. Helftenbein & E. Müller, unpublished results). These sequences are capable of initiating replication in an heterologous mouse L-cell system (F. Grummt & E. Helftenbein, unpublished results).

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