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SHORT COMMUNICATION

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α-Tubulins of *Tritrichomonas mobilensis* are encoded by multiple genes and are not posttranslationally tyrosinated

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Abstract Reverse-transcriptase polymerase chain reaction cloning of the 3′-ends of the α-tubulin cDNAs of *Tritrichomonas mobilensis* in combination with Southern-blot analysis identified seven to eight distinct α-tubulin genes. All seven lack a carboxy-terminal tyrosine and the corresponding sequence compatible with posttranslational tyrosination. This indicates that whereas tyrosination of α-tubulin has been found in most species, including humans and trypanosomes, it is absent in trichomonads.

Introduction

Trichomonads are interesting for two reasons: (1) they include important human and animal parasites, and (2) they belong to the earliest diverging eukaryotes known. At the base of the eukaryotic evolutionary tree, three important groups of parasites are found: the diplomonads, the trichomonads, and the trypanosomatids (Cavalier-Smith 1993; Leipe et al. 1993; Sogin et al. 1989). Diplomonads are considered to be the oldest eukaryotic branch, whereas trichomonads seem to reflect a somewhat later branch that is clearly more ancient than the trypanosomatids. All of these parasitic protozoa have pronounced microtubule-dominated pellicular cytoskeletons as well as one or several flagella. These structures have proved to be excellent systems for study of the many different posttranslational modifications of tubulin that have been described (MacRae 1997).

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Materials and methods

The 3′-ends of α-tubulin cDNAs were cloned by a 3′-RACE procedure using a kit (Boehringer Mannheim, Germany). The following degenerate α-tubulin-specific primers were used: Tub1 (5′TT[C/T]GTICA[C/T]TGGTA[C/T]GT3′) and Tub2 (5′GA[G/A]GGIATGGA[A/G]GA[A/G]GC3′; *I* specifies inosine). They were designed using sequence information (FVHWYVRAFVHWYVGEGMEEAE) from the penultimate carboxy-terminal tryptic peptide of α-tubulin. The 3′-RACE was performed according to the manufacturer's procedure and the resulting polymerase chain reaction (PCR) fragments were cloned into pGEM-T. Plasmids were isolated from 16 randomly picked colonies, and their inserts were sequenced using the DNA-sequencing service provided by Microsynth (Balgach, Switzerland).

Results and discussion

In this study we focused on one type of posttranslational modification of α-tubulin: tyrosination (Raybin and Flavin 1975). The terminal tyrosine in certain α-tubulins participates in a detyrosination/tyrosination cycle based on a carboxy-peptidase activity and the tubulin-tyrosine ligase. Tyrosination of α-tubulin has been found in most species, including humans and trypanosomes (Ersfeld et al. 1993; Schneider et al. 1997; Sherwin et al. 1987). However, it has been shown to be absent in some ciliates (Redeker et al. 1994) and in the diplomonad *Giardia* (Weber et al. 1996). To determine whether tyrosination occurs in trichomonads, we focused on the species *Tritrichomonas mobilensis*. To investigate whether in this species the carboxy-termini of α-tubulins are subject to posttranslational tyrosination, we determined the sequences of the 3′-ends of α-tubulin mRNAs as well as the protein sequence of the major α-tubulin found in

isolated cytoskeletons. The carboxy-terminal peptide of α-tubulin was subjected to Edman degradation and provided the sequence EDLALLEKDYDEVAAESVEGD for the first 21 residues.

To extend the sequence information we determined the corresponding cDNA sequences. This seemed particularly important, as all three α-tubulin DNA sequences established for *Parabasalia* (Keeling and Doolittle 1996) lack the 3′-parts of the genes. Cloning and sequencing of the reverse-transcriptase PCR (RT-PCR)-amplified region of *T. mobilensis* α-tubulin provided the carboxy-terminal 36 amino acid residues. The sequence information obtained indicates that *T. mobilensis* α-tubulins lack a carboxy-terminal tyrosine and the corresponding sequence compatible with posttranslational tyrosination. Their terminal sequence DDGQQ also explains the lack of reactivity with antibodies specifically recognizing the carboxy-terminal epitope EEY (Monteiro-Leal et al. 1995), which is thought to be a potential indicator for the tyrosination cycle (Rüdiger et al. 1994; Wehland et al. 1984). The tyrosination cycle is also absent in *Giardia* (Weber et al. 1996). Our data therefore show that the detyrosination/tyrosination of α-tubulin is a modification that was acquired later in eukaryotic evolution. It is first observed in *Trypanosoma* (Schneider et al. 1997; Sherwin et al. 1987) which is clearly a younger eukaryotic radiation than *Giardia* or the trichomonads.

Interestingly, not just 1 but 11 slightly distinct α-tubulin cDNA sequences were found (Fig. 1). In all, 5 nucleotide substitutions were found within the last 111 nucleotides of the α-tubulin coding region. Three of these mutations are silent, whereas a mutation in one clone (Fig. 1, 1c) replaces a leucine by a histidine and one of the mutations in another clone (Fig. 1, 2c) replaces a leucine by a proline. These two amino acid replacements are not compatible with the protein-sequencing results and must reflect minor α-tubulins or represent artifacts due to misincorporation of nucleotides by the Taq polymerase during the 3′-RACE procedure. However, it is unlikely that much of the observed diversity of the α-tubulin cDNAs represents an artifact, since even if single nucleotide differences between the clones are disregarded, seven to eight distinct sequences remain that differ from each other by more than one point mutation. In addition, only short sequence fragments were amplified, reducing the chances for mistakes. Thus, it seems that *T. mobilensis* contains some seven to eight transcribed α-tubulin genes. These genes can be arranged into three groups (Fig. 1) according to the length and actual sequence of the 3′-untranslated region (UTR). Interestingly, the 3′-UTR of clones 2a–e and 3a, b includes the consensus sequence AATAAA, which is thought to direct polyadenylation in higher eukaryotes. The 3′-UTRs of clones 1a–d, however, do not contain such a sequence or a derivative thereof. Several clones were obtained that differed only in the lengths of their 3′-UTRs (Fig. 1, 1a). These might all have originated from the same transcript polyadenylated at different sites.

```
                                                                                                                                                  Number of clones

1a GAATTCCCAGAAGCTCGTGAAGATCTTGCCCTCCTCGAAAAGGACTACGACGAAGTCGCAGCCGAATCAGTCGAAGGCGACGAAGAAGAAGAAGACGATGGACAACAATAA ACAGCTTCACAAACC*C*ATC AAA    5
   E  F  P  E  A  R  E  D  L  A  L  L  E  K  D  Y  D  E  V  A  A  E  S  V  E  G  D  E  E  E  E  D  D  G  Q  Q

1b ---------------------------------------------------------------------------------------------------------------   ACAGCTTCACAAACCCATTG AAA    1
   -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -

1c ----------------------------------A----------------------------------------------------------------------------   ACAGCTTCACAAACCCATC AAA     1
   -  -  -  -  -  -  -  -  -  -  -  H  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -

1d --------G-----C------------------------------------------------------------------------------------------------   ACAGCTTCACAAACCC AAA        1
   -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -

2a ---------------------------------------------------------------------------------------------------------------   GCAATTTTTTAATAAACGTTCTTCCAATCCC AAA       1
   -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -

2b --------------C------------------------------------------------------------------------------------------------   GCAATTTTTTAATAAACGTTCTTCCAATCCC AAA       1
   -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -

2c --------------C-----------C------------------------------------------------------------------------------------   GCAATTTTTTAATAAACGTTCTTCCAATCCC AAA       1
   -  -  -  -  -  -  -  -  P  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -

2d --------------C------------------------------------------------------------------------------------------------   GCAATTTTTTAATAAACGTTCTTCCAATTCC AAA       1
   -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -

2e --------------C------------------------------------------------------------------------------------------------   GCAATTTTTTAATAAACGTTCTTCCAATCCTTG AAA     1
   -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -

3a --------------------------------------------T------------------------------------------------------------------   GCAATTTTTTAATAAATTTCCTTCAAATCCTC AAA      2
   -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -

3b --------------------------------------------T------------------------------------------------------------------   GCAATTTTTTAATAAATTTCCTTCAAGTCCTC AAA      1
   -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
```

Fig. 1 Sequences of the 3′-ends of α-tubulin cDNAs. The entire sequence of clone 1a is shown, whereas for all other clones, only the nucleotide (*upper line*) or amino acid (*lower line*) substitutions in the coding sequence are given. The stop codon is shown in **boldface**. The entire 3′-flanking sequence is shown for each clone. The poly A tail is indicated by the 3′-terminal AAA. Potential alternative polyadenylation sites were found for clone 1a and are indicated by *asterisks*. The clones were divided into three groups, indicated by the numbers, according to similarities in the 3′-flanking sequence. The members of each group are distinguished by slight differences in the coding or the 3′-flanking region (*underlined nucleotides*) and are indicated by *letters*

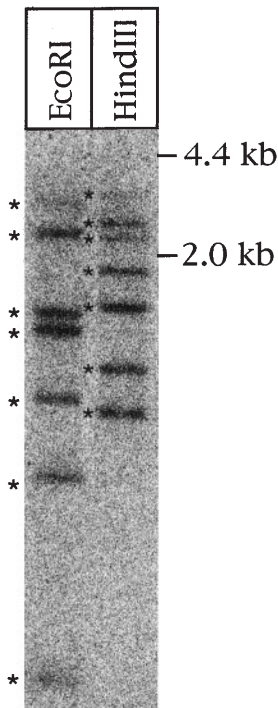

Fig. 2 Southern-blot analysis of trichomonad genomic DNA. In all, 5 μg of genomic DNA was digested by *Eco*RI or *Hind*III, respectively; separated on an agarose gel; and probed by pGEM-T vector containing the insert sequence 1a (See Fig. 1). The different bands are indicated by *asterisks*. Nick-translated pGEM-T vector containing the insert 1a was used as a probe

The diversity of the α-tubulin genes was confirmed by Southern-blot analysis using a cloned 3′-end of an α-tubulin cDNA as a probe (Fig. 2). In this experiment, seven distinct bands were detected on both *Eco*RI- and *Hind*III-digested genomic DNA, confirming that *T. mobilensis* contains at least seven distinct α-tubulin genes. Distinct tubulin genes may be a general feature of *Parabasalia*, as two distinct α-tubulins have also been observed in *Monocercomonas* (Keeling and Doolittle 1996). The different α-tubulin genes of *T. mobilensis* have identical or nearly identical carboxy-termini (Fig. 1). This is unexpected, as the very carboxy-terminal part is usually the least conserved region in tubulins (Luduena 1998). The 3′-flanking sequences of the different α-tubulin genes, in contrast, diverged much more, and they differed not only in multiple point mutations but also in length. Although we cannot compare the entire coding region of the different α-tubulin genes, protein-sequencing data indicate that all α-tubulins might be identical or nearly identical. The functional significance of multiple tubulin genes is unclear. It is noteworthy that multiple tubulin genes are also found in trypanosomes (Seebeck et al. 1983; Thomashow et al. 1983). However, in this case, identical α- and β-tubulin genes having identical 5′- and 3′-UTR are tandemly repeated.

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