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The N-terminal region of the α-subunit of the TRAP complex has a conserved cluster of negative charges

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Abstract

The α-subunit of the TRAP complex (TRAPα) is a single-spanning membrane protein of the endoplasmic reticulum (ER) which is found in proximity of nascent polypeptide chains translocating across the membrane. Here, we demonstrate the widespread occurrence of TRAPα in eukaryotes as indicated by its existence in man, fish and plants. Despite the fact that the sequence homology is much lower than for other proteins in the translocation site, the overall topology, the location of the glycosylation sites and, most interestingly, the distribution of charges are conserved. These data indicate that the TRAP complex has a ubiquitous function.

Key words: Translocation; Endoplasmic reticulum; Membrane protein; TRAP

1. Introduction

The transport of secretory and membrane proteins across the mammalian endoplasmic reticulum is thought to be mediated by a complex protein structure in the membrane – the so-called translocon [1]. Cross-linking methods have been used to identify constituents of the translocon. A limited number of integral membrane proteins were found to be in close proximity of translocating nascent polypeptide chains [2–9]. At present, three of them have been identified, among them the α-subunit of the translocon-associated membrane protein (TRAPα, formerly called SSRα) [6,7,10].

Canine TRAPα is a glycosylated single-spanning membrane protein of 34 kDa [11]. It could be cross-linked to nascent chains only at late stages of translocation, when the signal sequence of the nascent chain had been cleaved off [9]. It is part of a protein complex of four subunits [12]. The complex is present in the ER membrane in equimolar amounts compared with membrane-bound ribosomes [11]. Further evidence for the location of the TRAP complex in the translocon comes from the observation that TRAPα can be cross-linked to membrane-bound ribosomes [13]. In addition, polypeptide translocation in vitro was found to be blocked by Fab fragments produced from anti-TRAPα-antibodies [11]. Although these data demonstrate that TRAPα is located in the translocation site, its function remains unclear.

If the TRAP complex plays an important general role, one would expect its widespread occurrence in different organisms. We indeed report here that TRAPα exists in man, trout and *A. thaliana*. Our results indicate a ubiquitous function of the TRAP complex.

2. Materials and methods

The canine cDNA-clone coding for TRAPα [14] was digested with *Hin*dIII and the 564 bp fragment obtained was labelled with [α-^{32}P]dATP by random oligonucleotide priming. The probe was used to screen cDNA-libraries from human HeLa cells and from trout liver. One positive clone from each library was sequenced in both strands. Employing the HUSAR program, a homolog of TRAPα was found in the GenBank database as a previously unidentified cDNA tag from *A. thaliana*. Since only partial sequence information was available, we determined the complete sequence of the coding region of the clone, kindly provided to us by M. Raynal, Univ. de Perpignan. Analysis of the amino acid sequences derived from the nucleotide sequences was carried out with the PCGENE computer program.

The sequences of man, trout and *A. thaliana* are deposited in the GenBank database under the Accession numbers Z12830, Z12831 and L32016, respectively.

3. Results

Using as a probe canine TRAPα cDNA, we isolated cDNA-clones from libraries of human HeLa-cells as well as from trout liver. In addition, homologs of TRAPα in *Arabidopsis thaliana* and rice were detected by searching the GenBank database. We determined the complete sequence of the *A. thaliana* clone and found that it lacks the 5-prime end including the codons for the first amino acids of the signal sequence.

Fig. 1 shows the multiple alignment of the deduced protein sequences. The human sequence shows 96% identity with that from dog whereas TRAPα of trout shows only 58% and that of *A. thaliana* only 29% identical amino acid residues. Comparison by multiple alignment of the coding regions following the signal sequence

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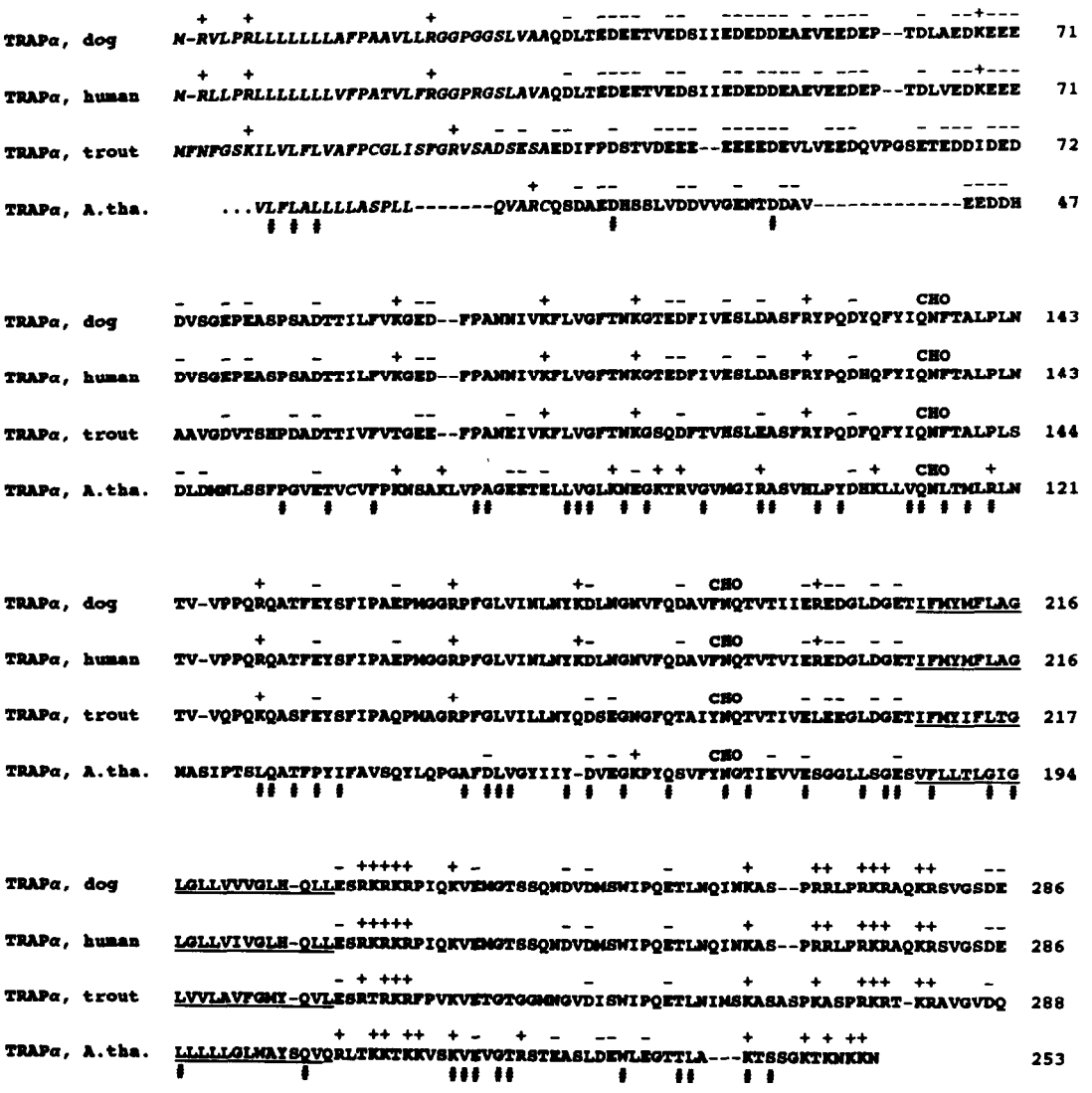

Fig. 1. Multiple alignment of TRAPα homologs. TRAPα amino acid sequences of dog, man, trout and *A. thaliana* were aligned using the computer program CLUSTALL. Negatively and positively charged residues are indicated by a '+' or '−', respectively. Amino acid residues identical between all four sequences are marked with a '#'. Membrane spanning regions are underlined and the putative signal peptides are set in italics. Glycosylation sites are indicated by a 'CHO' above the sequences. The TRAPα homolog of rice (accession no. D24822) is not included, because only a portion of the sequence is known.

indicates an identity of 21%. A region of higher conservation of about 30% identity is found in the central part of the protein that precedes the membrane anchor. The position of the two glycosylation sites relative to the membrane anchor is also conserved. In addition, there are regions in which not the actual amino acid sequence but the occurrence of charges is conserved. Particularly striking is the N-terminal region of 40–50 amino acids which contains 40–55% of acidic residues. Furthermore, there are two regions beyond the membrane anchor, one immediately at the border and the other at the extreme C-terminus, which have a cluster of basic amino acids. It should also be noted that the region flanking the membrane anchor in all cases shows a high charge difference between the cytosolic and lumenal domains.

4. Discussion

We report here the primary sequence of TRAPα from different eukaryotic organisms. A number of membrane protein complexes are located at the mammalian ER translocation site, such as the Sec61 complex [15], the signal peptidase complex [16], the TRAMp [6], the TRAP complex [12], the oligosaccharyl transferase complex [17] and the SRP–receptor complex [18]. For many of them homologs exist in other eukaryotic organisms [15,17,19–21], indicating a remarkable degree of conservation of the protein translocation machinery. The identification of TRAPα in mammals as well as in plants supports the idea that the TRAP complex is also a common part of the translocon in the ER.

Whereas the conservation of some proteins like Sec61α or Sec61γ is relative high (ca. 70% identity between mammals and plants) others like Sec61β show a higher divergence (37% identity between mammals and plants) [15,19]. The degree of conservation of TRAPα is even lower (ca. 30% identity between mammals and plants). However, TRAPα contains regions, which, although containing only few identical amino acid residues, have a similar distribution of charges in mammals and plants. Thus, the net charge pattern along the protein sequence is conserved. The N-terminal region with a high density of negative charges is the most obvious one. It could be responsible for the observation that in calcium overlay blots TRAPα is the major integral ER-membrane protein that binds calcium [22].

The function of the TRAP complex is unclear as yet. It is conceivable that it is involved in the recycling of the translocation apparatus after completion of the translocation process, similar to what has been suggested for SecD and SecF in bacteria [23], or that it functions as a membrane-bound chaperon, facilitating the folding of translocated proteins. It is also possible that the TRAP complex has an unidentified enzymatic activity. Whatever the function of the TRAP complex may be, it seems to have a role in the translocation site that is highly conserved during evolution.

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