

Short communication

## mp23, a *Theileria parva* transmembrane protein with homology to the protein disulfide isomerase family<sup>☆</sup>

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Received 9 August 2001; received in revised form 1 February 2002; accepted 5 February 2002

**Keywords:** *Theileria parva*; Signal-sequence; Membrane protein; Protein disulfide isomerase family

The protozoan parasite *Theileria parva* (Apicomplexa) causes the bovine disease East Coast Fever in endemic areas in Sub-Saharan Africa. The intralymphocytic schizont stage is largely responsible for the pathogenicity and induces a transformed phenotype in host cells [1]. Current evidence supports a model in which the schizont perturbs the immune response by inducing production of cytokines and stimulating the growth of parasitized cells [2]. We were interested to identify parasite proteins involved in parasite–host interaction and have described earlier a screening procedure for identification of schizont stage-exported proteins based on cell-free expression of cDNA and testing for translocation of protein products across endoplasmic reticulum (ER)-derived membranes [3].

A cDNA library of the schizont stage of *T. parva* was established in plasmid pBluescript II SK+ (Stratagene) essentially as described [3]. In order to identify cDNAs encoding secretory and membrane proteins, which can be translocated across ER-derived microsomal membranes (rough microsomes (RM)), we screened 200 individual clones by an in vitro transcription/translation/translocation assay. Protection against subsequently added protease has been used as a criterion to identify those protein products that have crossed RM. Following this procedure, we found that clone 17 transcribed from the T7 RNA polymerase promoter

encodes a protein which fulfills this criterion (Fig. 1A). In the absence of microsomal membranes a 25 kDa protein, termed p25, is synthesized. A smaller product sized about 17 kDa is most likely due to premature termination. Synthesis in the presence of RM leads to an additional product of 23 kDa, termed mp23. Posttranslational treatment of RM with proteinase K (PK) resulted in complete digestion of the 25 and 23 kDa products and appearance of a protein fragment sized 21 kDa. When PK digestion was carried out in the presence of detergent Nonidet P-40 this protected fragment was digested. In contrast, when translation assays without RM were treated with PK and detergent the 21 kDa fragment did not occur. These data showed that the primary 25 kDa translation product is membrane processed resulting in mp23 and that mp23 appears only partially translocated across the membrane leaving a cytosolic portion sensitive to posttranslational protease digestion. Taken together with the partial membrane protection, the RM processing to a form with a decrease in apparent molecular mass of about 2 kDa strongly suggests signal peptide cleavage.

Since mp23 behaved like a transmembrane protein in protease protection analysis, we used carbonate extraction [4] to separate integral membrane proteins from soluble and peripheral membrane proteins (Fig. 1B). Clone 17 was expressed in a cell-free system in the absence (lane 1) or presence of RM (lane 2). RM were subsequently salt-washed and subjected to carbonate extraction as described [5]. Mp23 was predominantly found in the pellet fraction (lane 5), as expected for a transmembrane protein. A minor fraction of p25 present in the pellet can be explained by membrane translocation without concomitant signal peptide cleavage in the cell-free system. We also performed carbonate extrac-

**Abbreviations:** ER, endoplasmic reticulum; PDI, protein disulfide isomerase; PK, proteinase K; RM, rough microsomes.

<sup>☆</sup> **Note:** Nucleotide sequence data reported in this paper are available from the EMBL database under accession number AJ300678.

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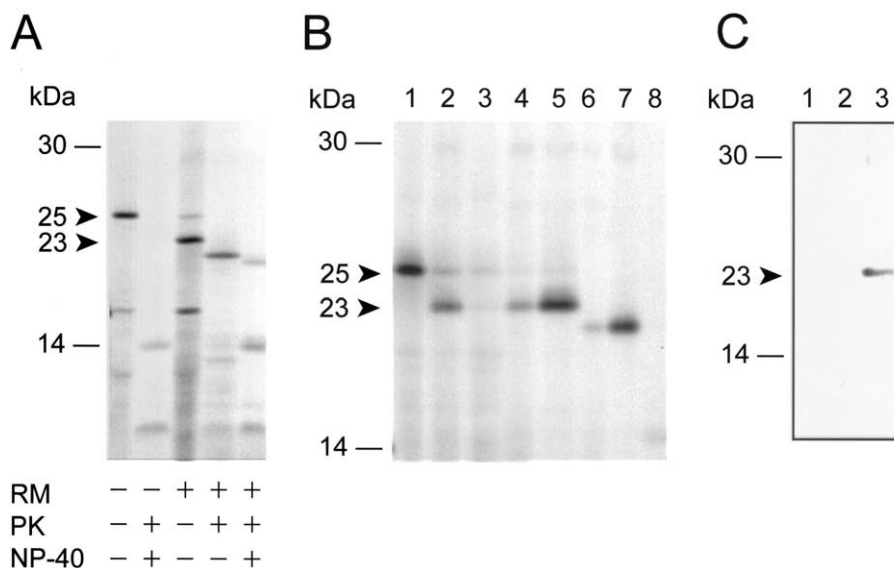


Fig. 1. (A) Clone 17 was in vitro transcribed and translated in a wheat germ lysate system in the absence or presence of dog pancreas microsomes (RM) as indicated. Following translation, PK was added to an aliquot of the reaction mixtures, either in the absence or in the presence of detergent Nonidet P-40 (NP-40) as indicated. (B) Clone 17 transcript was in vitro translated in the absence (lane 1) or presence of RM (lane 2) as in panel A. RM were subsequently salt-washed (lane 3 shows the wash solution), treated with carbonate buffer and fractionated into supernatant (lane 4) and pellet (lane 5). An aliquot of RM was first digested with PK and then fractionated likewise. Lanes 6, 7, 8 show the supernatant, the pellet, and the wash solution, respectively, from PK-digested RM. (C) Immunoprecipitation of mp23 from infected lymphocytes. TpM803 cells were metabolically labeled with L-[<sup>35</sup>S]-methionine and lysed with buffer containing 1% Nonidet P-40. The clarified lysate was extracted twice with 4  $\mu$ l preimmune serum (lanes 1, 2). The resultant cleared sample was extracted with 4  $\mu$ l immune serum (lane 3). Immunocomplexes were collected with protein A-sepharose, washed and eluted with electrophoresis buffer. All panels show fluorographs of reduced samples separated by SDS-PAGE. The mobility of standards is indicated. Specific products are marked by arrowheads and their apparent molecular mass is given.

tion analysis after PK-treatment of RM. As predicted for a membrane anchored digestion product the 21 kDa fragment was predominantly found in the pellet fraction (lane 7). Minor amounts of RM-processed mp23 in the carbonate extract (lane 6) are most likely due to carry over in the fractionation or disintegration of RM. Thus it can be concluded clearly that mp23 is a membrane-integrated protein. Taken together the in vitro expression analysis shows that clone 17 encodes a 25 kDa polypeptide, which becomes integrated into and processed by ER-derived membranes to a 23 kDa transmembrane protein.

We confirmed by Southern hybridization that cDNA clone 17 is derived from *T. parva* (data not shown). Northern analysis resulted in detection of a single transcript sized 0.8 kb, which was specific for schizont-infected cells (data not shown). We raised rabbit antiserum 31 to a bacterially expressed protein consisting of the C-terminal 147 residues of p25/mp23 fused C-terminally to  $\beta$ -galactosidase. This antiserum immunoprecipitated specifically an antigen with apparent molecular mass of 23 kDa from schizont-infected cell lysates (Fig. 1C). We conclude that probably this antigen is a form, which is intracellularly processed like in the cell-free system, and represents mp23.

Sequencing analysis revealed that clone 17 comprises a cDNA of 755 bp including a 38 bp poly-A tail. Only one open reading frame of clone 17 has sufficient length

to encode a polypeptide larger than 14 kDa. Starting with the first ATG codon the resulting 220 amino acid polypeptide, termed p25 (Fig. 2), has a calculated molecular mass of 24 682 Da, which is in agreement with the size of the in vitro synthesized polypeptide (Fig. 1A and B). We analyzed the primary protein sequence for the presence of potential signal sequences for ER translocation and membrane insertion sequences. The N-terminus contains a bona fide signal peptide for targeting to the ER (marked in Fig. 2) with a predicted cleavage after Ala 19 resulting in a decrease of molecular mass of about 2.1 kDa [6]. This agrees with the observed processing by RM (Fig. 1A and B). One hydrophobic stretch of 29 amino acids (Ala 174 to Phe 202, marked in Fig. 2) fulfils the criteria for a membrane-spanning segment and is located close to the C-terminus. Taken together with our in vitro expression data, it can be deduced that mp23 is a type I transmembrane protein, with a single membrane-spanning segment and a short C-terminal cytosolic tail. The cytosolic segment of mp23 is predicted to be highly basic and the last four amino acids constitute a potential ER retention and retrieval sequence for type I transmembrane proteins, the so called dilysine motif [7]. However, it is not known whether this motif functions in *T. parva* or other apicomplexan parasites.

Performing database searches, we found that the N-terminal region of p25 (residues 33–116) shows signifi-

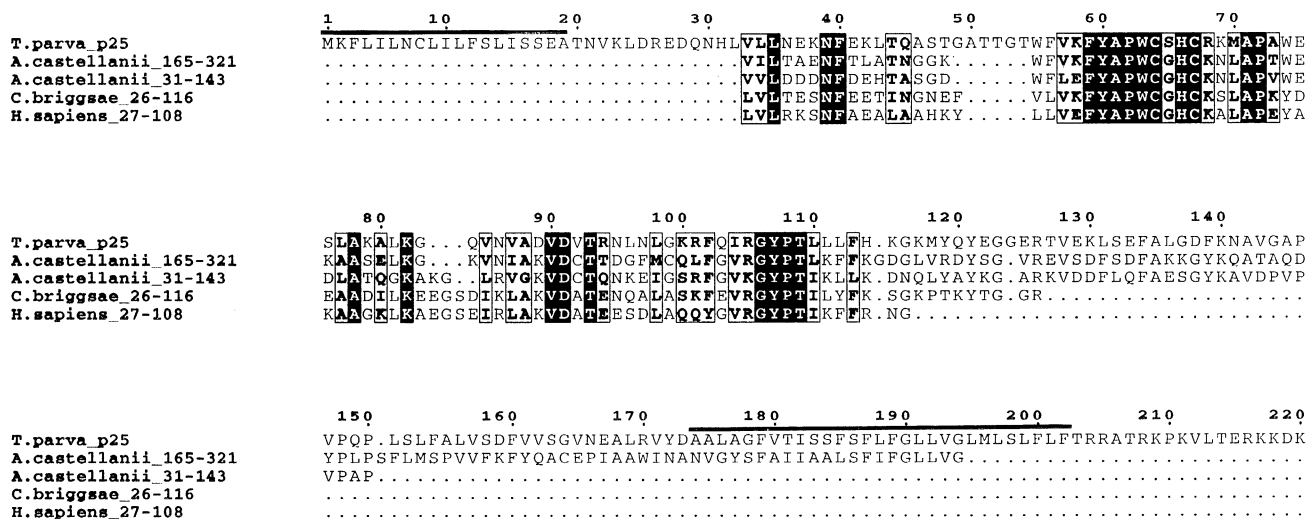


Fig. 2. Multiple alignment of *T. parva* p25 sequence (top) and partial sequences of PDI superfamily members (below with decreasing similarity) from *Acanthamoeba castellanii*, *Caenorhabditis briggsae*, and *Homo sapiens* (SWISS-PROT accession numbers Q16961, Cab40200.1, and P07237). The one letter amino acid code is used. The first and last residues of the polypeptide fragments used for alignment are given after the species name. Gaps introduced to maximize alignment are indicated by dots. Residues printed white on black are identical and columns boxed depict residues with a similarity score larger than 0.7 using the Risler matrix [12]. The putative N-terminal signal peptide and the hydrophobic region containing the membrane-spanning segment are marked with straight lines.

cant similarity to redox-active thioredoxin homology domains of members the protein disulfide isomerase (PDI) family (a type domains [8]; Fig. 2). P25 has essentially a complete a-type domain preceded by a single peptide. Notably, a highly conserved peptide stretch (position 64–67) containing the redox-active center motif (consensus CGHC) is present. Thioredoxin sequences have significantly lower similarity scores. Multiple alignment of relevant sequence fragments from p25 and proteins of the PDI family (Fig. 2) shows the partial similarity of p25/mp23 to them. The PDI-like protein of the protozoan *Acanthamoeba castellanii* contains two a-type domains, which both have higher similarity to mp23 than any other data base sequence. For the more C-terminal one of the *A. castellanii* protein fragments the similarity appears to extend from the a-type domain into a hydrophobic putatively membrane-spanning region. The other two proteins fragments aligned are from the PDI subfamily.

Although PDI-related sequences of other apicomplexans are found in databases, presently only one protein of *Plasmodium falciparum* is characterized [9]. It has the overall structure of the PDI or Erp57 subfamily and no closer similarity to mp23. Despite having relatively low similarity on sequence level compared with other PDI family members, in our opinion the recently characterized PDI-2 of the protozoan *Giardia lamblia* has the overall structure most similar to mp23. It has an N-terminal signal peptide, followed by a single a-type PDI domain and some 300 further residues including a putative membrane-spanning segment and, like mp23, a dilysine motif for putative ER retrieval [10].

As we have delineated above by sequence analysis, mp23 has partial homology to members of the PDI family. Typical PDI family members have a cleavable N-terminal signal peptide and several thioredoxin homology domains, both redox-active and inactive ones. Most family members lack transmembrane segments and are located in the ER due to a C-terminal retrieval motif for receptor-mediated sorting of soluble protein [8]. Based on the presence of a complete PDI-like thioredoxin homology segment and its signal peptide-mediated translocation into the lumen of ER-derived membrane vesicles, we regard *T. parva* mp23 as PDI-related protein.

It would be very interesting to know the physiological function(s) and subcellular location(s) of mp23. If it were a resident of the ER it would be the first characterized *T. parva* ER protein and could serve as marker for an organelle, which in the schizont stage, is not clearly visible by electron microscopy. For the multifunctional PDI itself subcellular localization in other compartments including cell surface has been described [11].

#### Acknowledgements

We thank M. Zsak for immunization of rabbits and serum collection. This study was funded by grant P11955-MED to Joachim Lipp from Fonds zur Förderung der wissenschaftlichen Forschung, Austria.

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