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Short communication

The *Plasmodium vivax* homolog of the ookinete adhesive micronemal protein, CTRP ☆

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Abstract

The *Plasmodium* circumsporozoite protein/thrombospondin-related anonymous protein-related protein (CTRP) is expressed at the mosquito midgut ookinete stage and is considered to be a transmission-blocking vaccine candidate. CTRP is composed of multiple von Willebrand factor A (vWA) and thrombospondin type 1 domains in the extracellular portion of the molecule, and a short acidic cytoplasmic domain that interacts with the actomyosin machinery. As a means to predict functionally relevant domains within CTRP we determined the nucleotide sequences of CTRP from the *Plasmodium vivax* Sall and the *Plasmodium yoelii* 17XL strains and characterized the conservation of domain architectures and motifs across *Plasmodium* genera. Sequence alignments indicate that the CTRP 1st to 4th vWA domains exhibit greater conservation, and thereby are perhaps functionally more important than the 5th and 6th domains. This point should be considered for the development of a transmission-blocking vaccine that includes CTRP recombinant subunit. To complement previous cellular studies on CTRP, we further determined the expression and cellular localization of CTRP protein in *P. vivax* and *P. yoelii*.

Keywords: *Plasmodium vivax*; Ookinete; CTRP; von Willebrand factor A domain

Malaria parasites possess an apicomplexan-specific class of molecules, termed the TRAP/MIC2 family, which mediate adhesion onto host cell and tissue surfaces, gliding motility, and invasion of host cells. Members of this diverse family of transmembrane proteins are typically composed of one or more von Willebrand factor A (vWA) and thrombospondin type 1 (TSP1) domains in the extracellular portion of the molecule, and a short acidic cytoplasmic domain that interacts with the actomyosin machinery. Family members include the prototypic thrombospondin-related anonymous protein (TRAP) [1,2] that is expressed in *Plasmodium* sporozoite micronemes; the circumsporozoite protein/thrombospondin-related anonymous protein-related protein (CTRP) [3–7], localized to *Plasmodium* ookinete micronemes, and the *Toxoplasma gondii* tachyzoite micronemal protein, TgMIC2 [8]. TRAP/MIC2 family proteins are found across the apicomplexan clade, including NcMIC2 in *Neospora caninum*, Et100 in *Eimeria tenella*, and Em100 in *Eimeria maxima major* [9–11], and predicted homologs within the genome sequence of *Theileria annulata* (TA07755) and *Theileria parva* (TP04_0306). The *Cryptosporidium parvum* genome sequence lacks extracellular examples of the vWA domain and in this pathogen the predicted TRAP functional homolog (*Cp*TRAP-C1) is composed of Apple domains and TSP1 domains [12] (Fig. 1A).

Abbreviations: CTRP, circumsporozoite protein/thrombospondin-related anonymous protein-related protein; MIDAS, metal ion-dependent adhesion site; TSP1, thrombospondin type 1; vWA, von Willebrand factor A.

☆ Nucleotide sequence data reported in this paper are available in the GenBank™, EMBL, and DDBJ databases under the accession numbers: AB247368–AB247370.

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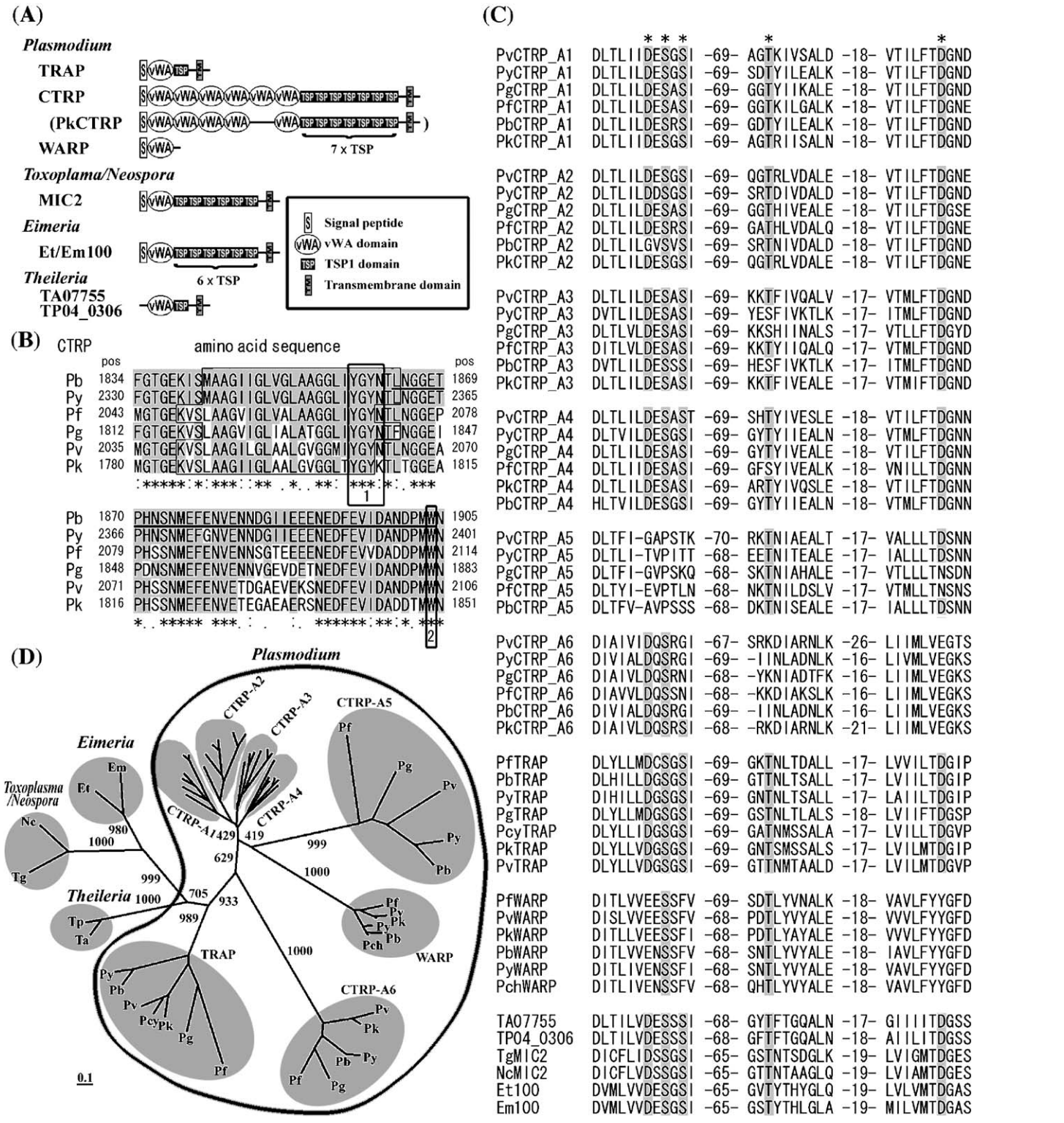

Fig. 1. (A) Schematic diagram of apicomplexan proteins containing vWA and TSP1 domains. Tg, *Toxoplasma gondii*; Et, *Eimeria tenella*; Em, *Eimeria maxima major*; TA, *Theileria annulata*; TP, *Theileria parva*. (B) Amino acid alignment of the transmembrane (boxed with thin line) and cytoplasmic regions of *Plasmodium* CTRP. The underlined region corresponds to that used to generate anti-*Pb*CTRP immune sera recognizing the cytoplasmic tail of *Pb*CTRP (amino acid position 1864–1904). Amino acids identical to those of *Pb*CTRP are shaded. Asterisks indicate the positions where amino acids are identical in all species, and similar amino acids are indicated with colons or periods under the alignments. The tyrosine-based motif involved in cellular trafficking (1) and the tryptophan residue that interacts with the motility actomyosin machinery (2) are boxed with thick lines. (C) Alignment of the MIDAS motif in the apicomplexan vWA domain superfamily. Numbers indicate intervening amino acids separating the three components of MIDAS (DxSxS, T and D, shaded). (D) Phylogenetic analysis of vWA domain of CTRP, TRAP, and WARP. Predicted amino acid sequences were aligned using the MUSCLE multiple sequence alignment program. Phylogenetic trees were constructed with deduced amino acid sequences by using neighbor joining method with Kimura's correction.