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Characterisation of single domain ATP-binding cassette protein homologues of *Theileria parva*

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Abstract Two distinct genes encoding single domain, ATP-binding cassette transport protein homologues of *Theileria parva* were cloned and sequenced. Neither of the genes is tandemly duplicated. One gene, *TpABC1*, encodes a predicted protein of 593 amino acids with an N-terminal hydrophobic domain containing six potential membrane-spanning segments. A single discontinuous ATP-binding element was located in the C-terminal region of *TpABC1*. The second gene, *TpABC2*, also contains a single C-terminal ATP-binding motif. Copies of *TpABC2* were present at four loci in the *T. parva* genome on three different chromosomes. *TpABC1* exhibited allelic polymorphism between stocks of the parasite. Comparison of cDNA and genomic sequences revealed that *TpABC1* contained seven short introns, between 29 and 84 bp in length. The full-length *TpABC1* protein was expressed in insect cells using the baculovirus system. Application of antibodies raised against the recombinant antigen to western blots of *T. parva* piroplasm lysates detected an 85 kDa protein in this life-cycle stage.

Nucleotide sequence data reported in this paper are available in the GenBank, EMBL, and DDBJ databases under the accession numbers, AF 255047 (TpABC1 cDNA), U88584 (TpABC2 cDNA) and AF255046 (TpABC1 genomic DNA).

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Introduction

The ATP-binding cassette (ABC) superfamily of transport proteins comprises perhaps the largest and most diverse group of macromolecules that facilitate selective translocation of an equally large and heterogeneous group of ligands across biological membranes. Members of this superfamily of proteins share characteristic functional domains that are contained within distinct, heteromeric polypeptides or are expressed in larger multi-domain proteins. These features include transmembrane domains, which determine the selectivity of the transporter’s channel, and cytosolic, nucleotide-binding sites, which through ATP hydrolysis facilitate ligand transport. Each transporter is relatively specific for a given substrate or group of related substrates, which can be either small molecules, such as ions, amino acids and sugars, or large molecules, such as proteins and complex polysaccharides (Ames et al. 1990; Higgins 1992). A unique structural feature that distinguishes members of the ABC superfamily of membrane transporters from other nucleotide-binding proteins is the high degree of conservation of the ATP-binding domains amongst the transporters from prokaryotes, eukaryotes and plants (Ames et al. 1990; Dudler and Hertig 1992; Higgins 1992). Across such diverse species, the nucleotide-binding domains of ABC transporters exhibit 30–50% sequence identity over a cassette of about 200 amino acids (Hyde et al. 1990; Mimura et al. 1991).

P-Glycoproteins, a subgroup within the ABC transport protein superfamily, are phosphorylated, membrane-bound transporters that are thought to provide a mechanism of exocytosis of anionic, toxic metabolites and other endobiotics, as well as afford the cell protection against environmental carcinogens and other cytotoxins. In different cell types, certain isoforms of P-glycoproteins have been shown to act as energy-dependent, efflux pumps that confer resistance to multiple drugs in cultured cells in vitro, as well as in cancer cells
in vivo (Endicott and Ling 1989; Gottesman and Pastan 1993). The mammalian P-glycoproteins are encoded by the multidrug resistance (MDR) gene family, with two and three genes reported in the human and mouse genomes, respectively. In both of these species, one or two of the P-glycoproteins (170 kDa) are products of tandemly duplicated genes (Chen et al. 1986). The structural organization of these genes is similar as they each occupy a large locus and exhibit a conserved organization, with each of the genes containing 28 exons (Raymond and Gros 1989; Chen et al. 1990). Recent reports have shown that P-glycoprotein gene homologues are found in pathogenic protozoa, including *Plasmodium* and *Leishmania*, and have been implicated in multidrug resistance in these parasites (Foote et al. 1989; Wilson et al. 1989; Ouellette et al. 1990). The *Plasmodium falciparum* MDR1 has undergone gene duplication and a structure similar to mammalian P-glycoproteins, with 12 predicted transmembrane domains and two ATP-binding sites (Foote et al. 1989).

The aim of the studies reported herein was to isolate and characterize ABC transport protein homologues in *Theileria parva*. Such studies would provide a better understanding of the molecular elements of transport pathways engaged by *Theileria*, which is unique among protozoan parasites in inducing cellular transformation of bovid lymphocytes. The elucidation of the roles of membrane transporters in the biology of *Theileria* may also provide useful insight into the means employed by *Theileria* in the translocation of parasite molecules that elicit cytotoxic T-cell responses from the bovine host. We report herein the cloning and characterization of two single-domain, ABC protein homologues of *T. parva*. Comparison of the predicted amino-acid sequence with other prokaryotic and eukaryotic ABC transporters reveals that the *T. parva* ABC protein genes have no internal duplication, although one of the genes was present in four closely related copies on three different *T. parva* chromosomes.

**Construction of cDNA and genomic libraries**

A *T. parva* schizont cDNA library was constructed in Xg10 using poly (A)+ RNA isolated from *T. parva* (Muguga) -infected lymphocyte cell lines, as described by ole-MoiYoi et al. (1992). Construction of a *T. parva* piroplasm genomic library has been described previously (Bishop et al. 1997). *T. parva* piroplasm DNA was sheared to an average insert size of about 4 kb and used to prepare a genomic library in Xg11, as previously described (Kibe et al. 1994).

**Oligonucleotide synthesis, labelling and screening of libraries**

Two oligonucleotides (48-mer and 33-mer) were synthesized using a model 380A Applied Biosystems Synthesizer. The sequences of the two degenerate oligonucleotides used to screen the sheared *T. parva* genomic library were 5’ TTA TG(C/T) GG(A/T) GG(A/T) CAA GAA CAA AGA AT (A/T) GC(A/T) AT(A/T) GC(A/T) AGA GC(A/T) TTA GT(A/T)3’ and 5’ TTA TTA TTA GAT GAA GC(A/T) ACA TC(A/T) GC(A/T) TTA GAT 3’. The degenerate oligonucleotides were designed based on the amino acid sequences, LSGGKQRQIAARLV and LLDEATSLAD, respectively, found in the two nucleotide-binding sites of human MDR1, as well as *P. falciparum* MDR1 (Chen et al. 1986; Foote et al. 1989). Oligonucleotides were subjected to polynucleotide kinase labelling with [y-32P]ATP according to standard methods (Sambrook et al. 1989). Hybridization of oligonucleotide probes was performed at 45 °C and filters washed in 2× SSC/0.1% SDS for 1 h at 45 °C. The *T. parva* piroplasm cDNA library was screened by hybridization with a 270 bp *T. parva* sequence that was homologous to an M13 VNTR probe (Bishop et al. 1998). Filters were hybridized at 60 °C and washed in 2× SSC/0.1% SDS at 60 °C. The 2167 bp insert from one positively hybridizing bacteriophage was subcloned into pBluescript (Stratagene).

**Southern blot analysis**

DNAs made from piroplasms (1 µg) or *T. parva*-infected lymphocytes (20 µg), as well as plasmid and phage DNA, were digested with restriction enzymes according to the manufacturer’s instructions (New England Biolabs). The digested DNA was size-fractionated in 1% agarose gels, transferred to Hybond membranes (Amersham) and fixed onto the membrane using a UV Stratalinker (Stratagene). Probes were radiolabelled to a specific activity of 1–2×10⁶ cpm/g of DNA by random priming (Feinberg and Vogelstein 1983) using a commercial kit (Amersham). Filters were hybridized at 65 °C for 16 h with radiolabelled probes in hybridization buffer (4× SSC, 10× Denhardt’s/0.1% SDS/0.1% sodium pyrophosphate) and washed for 1 h at the same temperature using 0.1× SSC/0.1% SDS.

**Northern blot analysis**

Poly(A)+ RNA was isolated from total RNA using an oligo(dT) column as described by the manufacturer (Pharmacia). Piroplasm poly(A)+ (1 µg), *T. parva*-infected lymphocyte poly(A)+ RNA (10 µg) and uninfected lymphocyte poly(A)+ RNA (10 µg) were size-fractionated in 1.4% agarose gels as previously described (Pelle and Murphy 1993). The RNA was transferred to nitrocellulose filters, fixed by exposure to UV light and hybridized with radiolabelled probes at 60 °C. Washing was at the same temperature in 2× SSC/0.1% SDS.

**Nucleotide sequence analysis**

Nucleotide sequences of genomic and cDNA clones were determined by the dideoxy chain termination method (Sanger et al. 1977) using a Sequenase kit (United States Biochemicals) or the “Flm0” DNA sequencing system (Promega). Sequencing was done on double-stranded DNA, using specific oligonucleotides derived from acquired sequences. The deduced amino acid sequences were compared with other sequences in the GenBank Database.