A 28 kDa major immunogen of *Chlamydia psittaci* shares identity with Mip proteins of *Legionella* spp. and *Chlamydia trachomatis* – cloning and characterization of the *C. psittaci* *mip*-like gene

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***Chlamydia psittaci* strain guinea-pig inclusion conjunctivitis (GPIC) produces a self-limiting ocular infection of guinea-pigs, and this condition is a representative animal model of ocular chlamydial disease. Convalescent guinea-pigs, which are resistant to reinfection, produce antibodies to several elementary-body proteins, including an uncharacterized antigen of 28 kDa. Convalescent guinea-pig sera were used to identify, from a lambda expression library, two overlapping GPIC genomic clones that produced the 28 kDa antigenic protein. Nucleotide sequence analysis revealed that the gene coding for the 28 kDa protein was similar to the *mip* (macrophage infectivity potentiator) genes from *Legionella pneumophila* and *Chlamydia trachomatis*. The GPIC gene and its product were accordingly designated *mip* and Mip, respectively. Analysis of the regions flanking *mip* identified three tightly linked open reading frames coding for predicted products with sequence similarity to asparagine tRNA ligase (*AspS*), rRNA methylase (*SpoU*), and thioredoxin (*TrxA*). The arrangement of these genes in GPIC was *aspS-mip-spoU-trxA*. Sequence analysis of PCR products produced using genomic DNA from an ovine abortion strain of *C. psittaci* and from *C. trachomatis* strain LGV-434 demonstrated that the arrangement of *mip*, *spoU* and *trxA* is common among these chlamydiae.**

Keywords: *Chlamydia*, Mip-like protein, chlamydial antigen, intracellular pathogen, intracellular survival

INTRODUCTION

The *mip* (macrophage infectivity potentiator) locus was first identified in *Legionella pneumophila*; it is associated with enhanced intracellular survival of legionellae in human macrophages and other cells (Cianciotto *et al.*, 1989; Cianciotto & Fields, 1992; Engleberg *et al.*, 1989;

Abbreviations: EB, elementary body; FKBP, FK506-binding protein; GPIC, guinea-pig inclusion conjunctivitis; Mip/*mip*, macrophage infectivity potentiator protein/gene; LMip, *Legionella pneumophila* Mip protein; CtMip, *Chlamydia trachomatis* Mip protein; MOMP, major outer-membrane protein.

The GenBank accession number for the *C. trachomatis* L2 partial *spoU* and *trxA* sequences is L40369. That for the GPIC *aspS*, *mip*, *spoU* and *trxA* sequences is L39892.

O'Connell *et al.*, 1995). The *L. pneumophila* *mip* gene codes for a 24 kDa protein (LMip) which is localized to the bacterial cell surface (Engleberg & Eisenstein, 1991). Sequence comparisons and *in vitro* enzymic analyses have shown that LMip is structurally and functionally related to a family of peptidyl-prolyl cis/trans isomerases that bind the macrolide antibiotics FK506 and rapamycin, compounds that are therapeutically important as suppressors of T cell activation (Hacker & Fischer, 1993). Although the Mip protein is associated with enhanced infectivity by the legionellae, its role in the infectious process is not understood.

A *mip* homologue from *Chlamydia trachomatis* was recently cloned and expressed in *Escherichia coli* (Lundemose *et al.*, 1991, 1992). Subsequent reports have shown that the *C.*

Fig. 1. Preparative immunoblot of GPIC EB probed with sera from individual convalescent guinea-pigs. Lanes: 1 and 2, sera from two naive guinea-pigs; 3–11, sera from nine individual guinea-pigs that recovered from two serial challenges with GPIC. The positions of chlamydial lipopolysaccharide (LPS) and major outer-membrane protein (MOMP) are shown, and molecular masses are indicated in kDa.

trachomatis Mip protein (CtMip) is a lipoprotein (Lundemose *et al.* 1993b), has enzymic activity characteristic of other FK506-binding proteins (FKBP) (Lundemose *et al.*, 1993a), and may be exposed at the bacterial cell surface (Lundemose *et al.*, 1992). While no direct role of CtMip in the chlamydial infectious process has been demonstrated, FK506 added between 0 and 16 h post-infection has been shown to interfere with the developmental cycle of *C. trachomatis* (Lundemose *et al.*, 1993b).

Recent studies in our laboratory have focused on the immune response of guinea-pigs following ocular infection with *Chlamydia psittaci* (strain GPIC). The resulting clinical condition, called guinea-pig inclusion conjunctivitis, is used as an animal model for ocular chlamydial disease in humans (Monnickendam *et al.*, 1980; Morrison *et al.*, 1989; Watkins *et al.*, 1986). Guinea-pigs that resolve a primary infection are resistant to reinfection (Rank *et al.*, 1988) and these animals produce antibodies against a variety of chlamydial antigens (Batteiger & Rank, 1987; Rank *et al.*, 1990; Rockey *et al.*,

1995; Rockey & Rosquist, 1994; Treharne & Shallal, 1991). One of the chlamydial antigens consistently recognized by convalescent guinea-pig sera is an uncharacterized 28 kDa protein. In this study, these convalescent sera were used to identify the gene coding for the 28 kDa protein from an expression library of GPIC DNA. The predicted amino acid sequence encoded by this gene shared identity with the Mip-like family of FKBP. The nucleotide sequence of the GPIC *mip*-like gene and three surrounding open reading frames (ORFs) is presented, and a similar gene arrangement is shown to be present in another strain of *C. psittaci* and in *C. trachomatis* serovar L2.

METHODS

Bacterial strains, genomic library manipulation, and nucleotide sequence analysis. *Chlamydia psittaci* strain GPIC, ovine abortion strain B577, and *C. trachomatis* strain LGV-434 (serovar L2) were cultured in HeLa cells. Original stocks of chlamydiae were purchased from the American Type Culture Collection. Chlamydial culture and elementary body (EB) purification were performed using described methods (Rockey & Rosquist, 1994; Caldwell *et al.*, 1981). Construction, screening and manipulation of a GPIC genomic library were conducted using methods described by Rockey *et al.* (1995). Phagemid rescue of pBlue-script plasmids from purified bacteriophage was performed using the Exassist helper bacteriophage and SolR strain of *Escherichia coli*, with methods provided by the supplier (Stratagene Cloning Systems). *E. coli* DH5 α was used for routine plasmid propagation and immunological analysis of expressed Mip. Nucleotide sequencing was done using the Sequenase system (US Biochemical) with the M13 forward and reverse primers, and internal primers synthesized on a Milligen/Biosearch Cyclone Plus DNA synthesizer. ORFs were identified and translated using the MacVector Sequence Analysis Software (International Biotechnologies). Deduced amino acid sequences were compared with those in the computer databases using the BlastP program available from the National Center for Biotechnology Information (e-mail address: blast@ncbi.nlm.nih.gov). Amino acid sequences were aligned using the 'lineup' program contained in the University of Wisconsin Genetics Computer Group sequence analysis package (Devereux *et al.*, 1984).

Polyacrylamide gel electrophoresis and immunoblotting.

Electrophoresis and immunoblotting were performed using described procedures (Rockey & Rosquist, 1994), with the following modifications. Comparisons of the reactivity of a collection of antisera with a single antigen were conducted using a slot-blotting device (Miniblotter-25, Immunetics). Lysates of GPIC EBs or DH5 α (pGP14.1) were loaded and electrophoresed through preparative 12% (w/v) polyacrylamide gels and transferred to nitrocellulose. These filters were placed into a blocking solution consisting of phosphate-buffered saline (PBS, 150 mM NaCl, 10 mM sodium phosphate, pH 7.2) plus 0.1% Tween-20 (TPBS) and 2% (w/v) bovine serum albumin (BSA-TPBS). After blocking, the filters were placed into the slot-blot device. Individual antisera were diluted 1:10 in BSA-TPBS and added to independent slots. After a 2 h incubation with gentle rocking, blots were washed five times with TPBS using the MF-2 washing device (Immunetics), and incubated in ¹²⁵I-protein A (3700–5550 Bq ml⁻¹; New England Nuclear) for 1 h. Blots were washed three times in TPBS, dried, and placed in contact with autoradiography film at room temperature for 16 h. To reduce background reactivity in immunoblots using *E. coli* lysates as

antigen, the antisera were first incubated for 30 min at room temperature in an equal volume of an *E. coli* extract (Promega) prior to dilution in BSA-TPBS and incubation with the blot.

Polymerase chain reaction (PCR). PCR was used to amplify relevant regions from genomic DNA of *C. psittaci* strain B577 and *C. trachomatis* strain LGV-434. Deoxyribonucleotides employed in PCR (Table 1) were derived from the nucleotide sequence of the GPIC genomic DNA insert of pGP14.1, from the published sequence of *C. trachomatis* LGV-434 *mip* gene (Lundemose *et al.*, 1992), or from the nucleotide sequences generated in these experiments. Amplifications were conducted with Amplitaq DNA polymerase and the 'Hot Start' procedure, using Ampliwax beads, as described by the manufacturer (Perkin-Elmer Cetus). All PCR was performed with a denaturing temperature of 94 °C, an annealing temperature of 50 °C, and a 2 min extension at 72 °C. Following amplification, reaction products were directly cloned into the pCRII vector (Invitrogen). Plasmids were purified and the ends of the PCR products were sequenced with M13 forward and reverse primers. If needed, internal primers were generated to extend and confirm the resulting sequences. The sequences were translated where appropriate, and compared to the respective GPIC sequences and to those within the computer databases.

Fig. 2. Immunoblots of lysates of *C. psittaci* GPIC EBs, and of *E. coli* DH5 α expressing the 28 kDa GPIC protein. Identical blots were probed with either pooled convalescent sera (a) or serum directed at formalin-fixed GPIC (b). Lanes: 1, GPIC-infected HeLa cells; 2, DH5 α (pGP14.1); 3, DH5 α (pGP14.2); 4, negative control *E. coli*. The position of MOMP is shown, and molecular masses are indicated in kDa.

RESULTS

Recognition of a 28 kDa EB protein by sera from convalescent guinea-pigs

Immunoblotting with sera from 10 guinea-pigs that had recovered from two serial ocular challenges with GPIC demonstrated that each produced antibodies to several chlamydial antigens, including the previously characterized 39 kDa major outer-membrane protein (MOMP), lipopolysaccharide, and the genus-common 60 kDa antigens (Batteiger & Rank, 1987; Morrison *et al.*, 1989). Other antigens that were consistently recognized by convalescent animals were a protein of 28 kDa and a group of proteins at approximately 90 kDa (Fig. 1). Convalescent sera from animals that had recovered from a single experimental infection also produced antibodies against this collection of antigens (not shown).

Cloning and sequencing of the *C. psittaci* *mip* gene

To identify the genes coding for the uncharacterized antigens detected in these immunoblots, a genomic expression library of GPIC DNA was constructed in bacteriophage lambda. Screening of this library with convalescent guinea-pig antiserum identified four clones which coded for two uncharacterized *C. psittaci* proteins. Two clones coded for a 39 kDa chlamydial protein (IncA)

Fig. 3. Preparative immunoblot of *E. coli* DH5 α (pGP14.1) probed with six individual convalescent sera. Lanes: 1 and 2, preimmune guinea-pig antisera; 3-8, sera from individual guinea-pigs which had recovered from two serial ocular challenges with strain GPIC. Molecular masses are indicated in kDa.

Fig. 4. Nucleotide sequence and the derived amino acid sequence of the GPIC *aspS*, *mip*, *spoU* and *trx* genes, encoded by pGP14.1. The *aspS* sequence is a partial reading frame. The *Pst*I and *Hind*III sites that flank the *mip* coding sequence are indicated, and a possible ribosome-binding site upstream of *mip* is underlined. Amino acid sequences are indicated in boldface, below the first nucleotide in the codon. The *trx* gene is coded by the complementary strand, and is indicated with italics.

which we have recently described (Rockey *et al.*, 1995). The other two clones coded for a 28 kDa protein recognized by both convalescent antisera and sera from guinea-pigs immunized with formalin-fixed EBs (Fig. 2). The rescued pBluescript plasmid clones, designated pGP14.1 and pGP14.2, had inserts of 2.8 and 2.9 kbp, respectively, and were shown to be overlapping by restriction endonuclease analyses (not shown). The 28 kDa protein expressed by DH5 α (pGP14.1) and

(pGP14.2) comigrated precisely with the 28 kDa antigen in the EB lysates (Fig. 2). There were also lower molecular mass antigenic bands in lysates of both GPIC and the recombinant *E. coli*, which may represent common processing products of the 28 kDa protein (Fig. 2, Fig. 3). Additionally, all convalescent sera that recognized the 28 kDa protein in the EB lysates recognized the 28 kDa protein coded by pGP14.1 (Fig. 3). These results support the conclusion that the 28 kDa product coded by plasmids

	1		50
GPIC Mip	MKKR.WHLM I TMVVSFSIA S DSSSRQGN QIAQVEEKS ANDSQLSVNQ		
CtMip	MKN.ILSWML M.FAVALPIV G.DNG...GG SQTSAEKS VEDSALTDNQ		
LMip	MFMKLVTA AV MGLAMSTAMA ATDAT..... ..SIATDKD		
Consensus	MK-----m- m--avs--ia -cD-----g- -----eks- --ds-ltdnq		
	51		100
GPIC Mip	QISRTFGHLL ARQLRKSEDI MMDIAEVAKG LQGELECKSA PLTSEYEYEEK		
CtMip	KLSRTFGHLL ARQLSRTEDF SLDLVEVIKG MQSEIDGQSA PLTDTYEYEQ		
LMip	KLSYSIGADL GKNF.KNQGI DVNPEAMAKG MQDAMSGAQL ALTEQQMKDV		
Consensus	kLSrtfGhLL arql-k-edi --d--evakG mQ-e--g-sa pLTa-eye--		
	101		150
GPIC Mip	MAEIQKLVEF K.....KA KENLYLAERF LQENKNSGV VEVQADKLQY		
CtMip	MAEVQKASFE A.....KC SENLASAEF LKENKEKAGV IELEPNKLQY		
LMip	LNKFQKDLMA KRTAEFNKKA DENKVKGEAF LTENKPKGV VVL.PSGLQY		
Consensus	mae-QK--fe k-----Ka -ENl--aE-F L-ENK-k-GV vel-p-kLQY		
	151		200
GPIC Mip	RIVKEGTGRA ISGKPSALLH YKGTFIGDQV FSSSEANKEP ILLPLAQ TIP		
CtMip	RVVKEGTGRV LSGKPTALLH YTGSEIDGKV FDSSEKNEP ILLPLTKVIP		
LMip	KVINSNGGVK PGKSDTVTVE YTGRLIDGTV FDSSEKNEP ATTQVSQVIP		
Consensus	rvvkeGtGr- -sgkptallh YtG-fIDG-V FHSsEknep ILLpl-GrVP		
	201		250
GPIC Mip	GFALGQGMK EGETRILYIH PDLAYG...T SQGLPPNSLL IFEINLIETT		
CtMip	GFSQGMGMK EGEVRVLYIH PDLAYG...T AQGLPPNSLL IFEVKLIEN		
LMip	GWTEALQLMP AGSTWEIYVP SGLAYGPRSV GGPFGNETL IFKIHLLSVK		
Consensus	GF--gmQgMk eGetr-lyih pdlAYG---t -GqlpPnsll IFei-Lie--		
	251	267	
GPIC Mip	DDSVAAQNT E NENTAS		
CtMip	DDNVSVTE		
LMip	KSS		
Consensus	ddsv-----		

Fig. 5. Comparisons of the predicted amino acid sequences of the *C. psittaci* Mip (GPIC Mip), *C. trachomatis* Mip-like protein (CtMip), and *L. pneumophila* Mip (LMip). A consensus sequence of 3 of 3 matches (capital letters) and 2 of 3 matches (lower-case letters) is included. The boxed sequences at the amino terminus of the chlamydial proteins are predicted signal sequences. The boxed amino acids in the consensus sequence are the locations of amino acids which are predicted to be important in the binding of FK506.

pGP14.1 and pGP14.2 is the same protein as the 28 kDa antigen in GPIC EBs.

Subcloning experiments demonstrated that the coding capacity for the complete 28 kDa protein was present on a 1100 bp *HindIII*-*PstI* fragment of insert DNA from pGP14.1 (not shown). Nucleotide sequencing of this fragment identified an ORF that coded for a predicted 28 kDa protein (Fig. 4). The deduced amino acid sequence of this protein was similar to the sequences of LMip and CtMip (25 and 58% identity respectively; Fig. 5). Sequence identity was highest in regions of the protein that are involved in the interaction between FKBP and FK506 (Hacker & Fischer, 1993). A 4 base sequence 9 bases upstream of the *mip* ATG (Fig. 4, position 377) resembles a consensus ribosome-binding site (Gold *et al.*, 1981). The predicted Mip polypeptide has a putative signal peptidase II leader sequence similar to the amino terminus of unprocessed CtMip (Figs 4 and 5; Lundemose *et al.*, 1992, 1993b). This suggests that *C. psittaci* Mip, like CtMip, is a lipoprotein.

Nucleotide sequence analysis of the genomic DNA flanking *mip*

The regions flanking *C. psittaci mip* on pGP14.1 were sequenced to determine if *mip* was adjacent to other genes coding for proteins with known function. These analyses demonstrated that *mip* was linked to three ORFs whose deduced amino acid sequences shared identity with a diverse collection of protein products (Figs 4 and 6).

These products include the carboxy terminus of asparagine tRNA ligase (*AspS*), a family of rRNA methylases (*SpoU*), and thioredoxin (*TrxA*). Each of the ORFs that flank *mip* has been named on the basis of their similarity to known *E. coli* gene products (Bachmann, 1990). The predicted product of the partial *C. psittaci aspS* exhibited 52% identity with the last 98 amino acids of aspartate tRNA synthase from *E. coli* (Sharples & Lloyd, 1991), the predicted product of *C. psittaci spoU* exhibited 39% identity with the *SpoU* homologue from *E. coli* (Koonin & Rudd, 1993; Mallinder *et al.*, 1992), and the predicted product of *C. psittaci trxA* exhibited 47% identity with thioredoxin from *Bacillus subtilis* (Chen *et al.*, 1989). The partial *C. psittaci aspS* is directly upstream of *mip* while the *spoU* and *trxA* homologues are downstream. The *trxA* gene is oriented with opposite polarity to *aspS*, *mip* and *spoU*. The intergenic region between *aspS* and *mip* is 74 bp, and the junctions between *mip/spoU* and *spoU/trxA* overlap. The sequence ATGA at position 1141-1144 contains the *mip* and *spoU* stop and start codons, while the sequence TTAA at position 1608-1610 contains the stop codons for both *spoU* and *trxA* (Figs 4 and 6).

Arrangement of *aspS*, *mip*, *spoU* and *trxA* in other chlamydiae

PCR was used to examine if a similar arrangement of this gene cluster was present in another strain of *C. psittaci* (B577) and in *C. trachomatis* LGV-434. Different sets of

Fig. 6. Schematic representation of the gene organization of *aspS*, *mip*, *spoU* and *trxA*, and analyses of the homologous loci in *C. psittaci* B577 and *C. trachomatis* LGV-434 (serovar L2). Arrows indicate the direction of transcription/translation. The sequences presented at the bottom of the figure show the junctions of *mip/spoU* and *spoU/trxA* in the genomic sequences of strain GPIC and the amplified products from B577 and LGV-434. Predicted amino acid sequences surrounding the junctions are presented in boldface both above and below the nucleic acid sequences.

Table 1. PCR oligonucleotides used for the amplification of *mip* and linked genes from other chlamydiae

A summary of the results using these oligonucleotide pairs with, as template, genomic DNA of *C. psittaci* strains GPIC and B577, and *C. trachomatis* serovar LGV-434, is shown.

Region amplified	5' oligonucleotide	3' oligonucleotide	Homologous product*		
			GPIC	B577	LGV-434
Pair A <i>aspS/mip</i>	CAACGTATTCATAACG	CTCTTTATTAGCTTCTG	+	+	—
Pair B <i>mip/spoU</i>	GAATCCGAATATGAGG	CCATAGGAAGAGCCACGTC	ND	+	—
Pair C <i>mip/trxA</i>	GGAACAGCTGGACAATTACC	TATGGGGGAACCGGTAG	ND	ND	+
Pair D <i>spoU/trxA</i>	AGGGATGGATTACTG	TATGGGGGAACCGGTAG	+	+	+

* An amplification was considered positive if sequence analysis of the cloned PCR product demonstrated that the predicted ORF is contained within the fragment. A negative result indicates that the predicted product was not amplified using these oligonucleotide pairs and the indicated genomic DNA as template. ND, Not determined.

oligonucleotide pairs (Table 1) were used to amplify homologous sequences from genomic DNAs of B577 and LGV-434. The products resulting from these PCR reactions were cloned and sequenced, and the predicted products of ORFs present in these sequences were compared to proteins in the computer databases. The primers used in these experiments and the results of the amplifications are presented in Table 1. PCR reactions were scored as positive if nucleotide sequence analysis of the products identified ORFs with deduced amino acid sequences homologous to those identified in GPIC. These

studies demonstrated that the gene organization in B577 was identical to that in GPIC, and that a similar arrangement of *mip*, *spoU* and *trxA* was present in *C. trachomatis* LGV-434. We were unable to examine the sequences upstream of *mip* in LGV-434 because no combination of our primers amplified a product spanning that region. The sequencing also revealed that in the B577 genome both the junction between *mip* and *spoU*, and the junction between *spoU* and *trxA*, have an arrangement of overlapping codons identical to that seen in GPIC (Fig. 6). This is distinct from that seen for each of these gene

junctions in *C. trachomatis*; the LGV-434 *mip* and *spoU* genes are separated by 15 bp, while the *spoU* and *trxA* genes are separated by 53 bp (Fig. 6; Lundemose *et al.*, 1992).

DISCUSSION

Proteins which bind the immunosuppressant FK506 are present in several different prokaryotic species (reviewed by Hacker & Fischer, 1993). FKBP are either cytosolic, as with the *Neisseria* spp., or membrane-associated, as with *Legionella* spp. and *C. trachomatis*. Mip-like proteins or *mip*-like ORFs have also been identified in *Coxiella burnetii* (Cianciotto *et al.*, 1995), *Pseudomonas* spp. (Isaki *et al.*, 1990; Konyecsni & Deretic, 1990), and *E. coli* (Horne & Young, 1995). Functionally, LMip is associated with increased infection proficiency by *Legionella* spp. in several cell types (Cianciotto *et al.*, 1989; Cianciotto & Fields, 1992), and CtMip may have a role in the early stages of the *C. trachomatis* infectious process (Lundemose *et al.*, 1993a).

We and others (Batteiger & Rank, 1987) have previously shown that sera from convalescent guinea-pigs recognize a 28 kDa GPIC protein. Because this protein was highly immunogenic in the context of infection, we pursued its identification and characterization. In this report we have described the cloning of a GPIC DNA fragment coding for a 28 kDa antigenic GPIC protein. Nucleotide sequencing of the insert in plasmid pGP14.1 identified four ORFs, one of which encoded a predicted 28 kDa protein that shared sequence identity with CtMip. The cloned product comigrated with the 28 kDa antigen in lysates of GPIC EB, and all sera that recognized the 28 kDa protein in EB recognized Mip expressed in *E. coli*. While other authors have discussed antigenic 28 kDa chlamydial proteins that are not Mip-like proteins (Comanducci *et al.*, 1994), our results support the conclusion that the *mip* gene described in this work codes for the 28 kDa EB protein recognized by convalescent guinea-pigs.

These experiments demonstrate that the Mip protein is one of several immunodominant proteins in the guinea-pig model of chlamydial disease. However, the immune response to chlamydial Mip-like proteins is not likely to be limited to this model system. There are reports in the literature that chlamydial infection of turkeys, mice and sheep stimulates the production of antibodies against a 28–30 kDa protein (Griffiths *et al.*, 1992; Jensen *et al.*, 1990; Pal *et al.*, 1993; Tan *et al.*, 1990). Additionally, humans that have recovered from pneumonia caused by *C. pneumoniae* produce antibodies against a 30 kDa protein (Puolakkainen *et al.*, 1993; Wilson *et al.*, 1994). While it is not known if each of these species is producing antibodies against Mip-like proteins, it is possible that Mip is a common immunodominant antigen following chlamydial infection.

The *mip* gene from *C. trachomatis* has been cloned and sequenced from three different serovars. Within this species the sequence is 99% identical, with only two amino acid differences present in the predicted sequences (Lundemose *et al.*, 1992). The predicted Mip product

from *C. psittaci* is 58% identical to CtMip and 25% identical to LMip. During the preparation of this manuscript the sequence of a *mip*-like gene from *Coxiella burnetii*, another obligate intracellular bacterial pathogen, was submitted to the GenBank (accession number U14170). The deduced amino acid sequence of *C. burnetii* Mip is 27% identical to GPIC Mip. Through the region of the protein that encompasses the FK506 binding function (amino acids 169–241) Mip from GPIC and CtMip are 80% identical, and the GPIC Mip protein is 37% and 39% identical to LMip and *C. burnetii* Mip, respectively.

The *mip* gene of *C. psittaci* is placed within a cluster of previously unidentified chlamydial genes coding for proteins with diverse functions, including asparagine tRNA ligase (AspS), rRNA methylase (SpoU), and thioredoxin (TrxA). The arrangement was conserved among *C. psittaci* strains GPIC and B577, and *mip*, *spoU* and *trxA* were shown to be linked in *C. trachomatis* LGV-434. It is difficult to propose any functional association among these apparently unrelated gene products or any reason why their genes are juxtaposed in the genome. None of these genes are closely linked in *E. coli*, although homologues to each have been mapped (Bachmann, 1990; Horne & Young, 1995; Sharples & Lloyd, 1991).

While there is no known function *in vivo* for any prokaryotic FKBP, the presence and possible surface localization of these proteins in several different intracellular pathogens suggests some role for the protein in the intracellular stage of the life cycle. In the eukaryotic cell, cytosolic FK506/FKBP exert their suppressive effects by interfering with calcineurin-mediated regulation of gene activation (Flanagan *et al.*, 1991; Liu *et al.*, 1991). Membrane-associated FKBP of intracellular bacteria may mimic host FKBP and subsequently influence intracellular signalling pathways in order to direct cellular events in favour of the pathogen. Additionally, because this protein is potentially surface-associated and is recognized by infected animals, it may be an important determinant in the immune response against the chlamydiae. Future analyses investigating the immune response to Mip or its function within infected cells are likely to further our understanding of the host–pathogen interaction following chlamydial infection.

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