Characterization of the *Mycobacterium tuberculosis* *erp* gene encoding a potential cell surface protein with repetitive structures

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Using the *phoA* gene fusion methodology adapted to mycobacteria, several *Mycobacterium tuberculosis* DNA fragments encoding exported proteins were recently identified. In this paper, the molecular cloning, genomic positioning, nucleotide sequence determination and transcriptional start site mapping of a new *M. tuberculosis* gene, identified by this methodology, are reported. This gene was called *erp* (for exported repetitive protein) and has a sequence similar to that of the *Mycobacterium leprae* 28 kDa antigen *irg* gene. *M. tuberculosis* *erp* gene contains a putative iron box close to the mapped transcriptional start site. The predicted Erp protein displays a typical N-terminal signal sequence, a hydrophobic domain at the C-terminus and harbours repeated amino acid motifs. These structural features are reminiscent of cell-wall-associated surface proteins from Gram-positive bacteria. We found that these repeats are conserved among *M. tuberculosis* isolates, and are absent from the published *M. leprae* *irg* gene sequence. In addition to being present in *M. leprae*, *erp* sequences were found in other members of the *M. tuberculosis* complex, but not in other mycobacteria tested. These results suggest that *erp* might encode a cell surface component shared by major pathogenic mycobacteria.

Keywords: *Mycobacterium tuberculosis*, *erp*, exported repetitive protein

INTRODUCTION

Mycobacterium tuberculosis and *Mycobacterium bovis*, the etiologic agents of tuberculosis, are facultative intracellular bacteria. The ability to invade, survive and multiply within macrophages contributes to their pathogenicity. For other intracellular bacterial pathogens, proteins compartmentalized on the outer surface mediate the interaction with the host cells. Examples include the 103 kDa invasin of *Yersinia pseudotuberculosis* (Isberg *et al.*, 1987) or the 80 kDa internalin from *Listeria monocytogenes* (Gaillard *et al.*, 1991). Similarly, proteins exported by pathogenic mycobacteria are likely to be involved in the infection of macrophages and intracellular survival.

The adaptation to *M. tuberculosis* of a genetic methodology for the identification and phenotypic selection of exported

proteins was recently described by Lim *et al.* (1995) and Timm *et al.* (1994). This method uses the *Escherichia coli* periplasmic alkaline phosphatase (PhoA) (Hoffman & Wright, 1985; Manoil *et al.*, 1990) as a heterologous reporter for exportation in mycobacteria. A plasmid vector allowing gene fusions between a truncated *phoA* gene and mycobacterial genomic DNA was constructed and used to select translational fusions with PhoA activity. Using this system, a *M. tuberculosis* DNA fragment showing sequence similarities with a *Mycobacterium leprae* gene (*irg*) encoding a 28 kDa exported protein antigen (Lim *et al.*, 1995; Cherayil & Young, 1987), was identified. This *M. leprae* protein has been previously reported to be a major target of the humoral immune response in the lepromatous form of leprosy. The predicted protein sequence contains a potential signal peptide at its amino terminus and two long hydrophobic domains, suggesting that it might be targeted for localization across the bacterial plasma membrane or the cell wall. The corresponding gene was referred to as the iron-regulated gene (*irg*), because a potential iron box is present in the

Abbreviation: PhoA, alkaline phosphatase.

The GSDB accession number for the sequence reported in this paper is L38851.

regions likely to control its expression (Dale & Patki, 1990; Young *et al.*, 1992). The *M. tuberculosis* gene, like the *M. leprae* *irg* gene, encodes an exported repetitive protein and is called *erp*. In this paper we report the cloning, genomic positioning, nucleotide sequence determination, distribution among mycobacterial species and transcriptional start site mapping of the complete *M. tuberculosis* *erp* locus.

METHODS

Bacterial strains, plasmids and culture media. The bacterial strains and plasmids used in this work are listed in Table 1. *E. coli* and *Mycobacterium smegmatis* were grown routinely on solid or liquid Luria Bertani (LB) medium (Miller, 1972). For RNA isolation, *M. smegmatis* cultures were grown in Middelbrook 7H9 liquid medium (Difco) supplemented with glycerol (0.2%). Transformants were selected and plasmids were maintained on media containing 100 µg ampicillin ml⁻¹ or 20 µg kanamycin ml⁻¹. PhoA activity was detected on LB plates containing 5-bromo-4-chloro-3-indolyl phosphate (40 µg ml⁻¹).

PCR conditions and primers. All PCR amplifications were performed in a final volume of 100 µl containing 50 mM KCl, 10 mM Tris/HCl, pH 8.3, 1.5 mM MgCl₂, 0.01% (w/v) gelatin, 20 µM of each dNTP, 0.2 mM of each primer, 10% (v/v) DMSO and 2 U Taq polymerase (Perkin Elmer Cetus). The 177 bp *erp* fragment used for Southern blotting experiments was generated by PCR amplification of nt 451–618 with 53Bw (5'-GCTCTCGACAGCCATGA-3') and 53Fw (5'-GGACAACCC-CTGCGATA-3') oligonucleotide primers. Oligonucleotides 53Bw2 (5'-TGACCGACCTGCCGCGC-3') and 53Fw5 (5'-GCCAACTCGTTGGCCACCTG-3') were used for amplifying the 539 bp fragment from various *M. tuberculosis* isolates. Both PCR reactions consisted of one cycle of denaturation (94 °C, 5 min) followed by 30 cycles of amplification consisting of denaturation (94 °C, 1 min), annealing (55 °C, 1 min) and primer extension (72 °C, 2 min).

DNA hybridization. For Southern blotting experiments, 10 µg genomic DNA was digested with 20 U of restriction enzymes and separated by electrophoresis through 1.0% (w/v) agarose gels. Gels were processed and DNA was transferred to a Hybond N⁺ nylon membrane (Amersham) as described by Sambrook *et al.* (1989). The DNA was immobilized by UV illumination at 365 nm for 3 min. Membranes were pre-hybridized at 65 °C for 2 h in Rapid-hyb buffer (Amersham) supplemented with denatured *E. coli* DNA (0.25 mg ml⁻¹, Sigma). The labelled probe (10⁶ c.p.m. ml⁻¹) was denatured, added to the membrane and incubated at 65 °C for 2 h. The membranes were washed once at 42 °C with 2 × SSC for low stringency conditions and three times for 10 min each at 65 °C with 2 × SSC, 1 × SSC and 0.1 × SSC for high stringency conditions.

Construction and screening of a plasmid genomic library. *M. tuberculosis* Mt103 genomic DNA (3 µg) was digested to completion with *Pst*I and then separated through 1.0% agarose gel. Restriction fragments ranging from 2 to 6 kb were excised from the gel, purified and ligated to a dephosphorylated pBluescriptII KS–vector (Short *et al.*, 1988) linearized with *Pst*I. The ligation mixture was used to transform *E. coli* XL1-Blue yielding 2000 ampicillin-resistant transformants. Plasmid DNA was prepared from ten clones and all contained an insert. Colonies were transferred to Hybond N⁺ nylon filters (Amersham) and hybridization-screened with a α-³²P-labelled 177 bp PCR fragment probe, using the conditions described above for Southern blotting under high stringency.

DNA sequencing and analysis. Sequences of double-stranded plasmid DNA were determined by the dideoxy chain-termination method (Sanger *et al.*, 1977) using the T7 sequencing kit (Pharmacia), according to the manufacturer's instructions, or using the Taq DyeDeoxy Terminator Cycle sequencing kit (Applied Biosystems), on a GeneAmp PCR System 9600 (Perkin Elmer), and run on a DNA Analysis system model 373 stretch (Applied Biosystems). The sequence was assembled and processed using DNA strider (CEA) and the University of

Table 1. Strains and plasmids used in this study

Strain/plasmid	Relevant characteristics	Source/reference
Strain		
<i>E. coli</i> XL1-Blue	<i>supE44 hsdR17 recA1 gyrA96 thi relA1 lac F'</i>	Sambrook <i>et al.</i> (1989)
<i>M. smegmatis</i> mc ^a 155	High-transformation mutant of <i>M. smegmatis</i> ATCC607	Snapper <i>et al.</i> (1990)
Plasmid		
pJEM11	<i>E. coli</i> –mycobacterial shuttle vector carrying a truncated <i>phoA</i> gene	Lim <i>et al.</i> (1995)
pExp53	pJEM11 carrying part of a <i>M. tuberculosis</i> <i>erp</i> gene similar to the <i>M. leprae</i> 28 kDa antigen gene (<i>irg</i>)	Lim <i>et al.</i> (1995)
pIPX412	pBluescriptII KS– vector carrying the full-length <i>M. tuberculosis</i> <i>erp</i> gene	This study
T74	<i>M. tuberculosis</i> cosmid clone containing the <i>fbpA</i> and <i>erp</i> genes	Poulet (1994)
T760	<i>M. tuberculosis</i> cosmid clone containing the <i>erp</i> gene	Poulet (1994)
T865	<i>M. tuberculosis</i> cosmid clone containing the <i>sodA</i> gene	Poulet (1994)

Wisconsin Genetics Computer Group (UWCGC) packages. The BLAST algorithm (Altschul *et al.*, 1990) was used to search protein data bases for similarity.

RNA isolation and primer-extension analysis. Total RNA was isolated using the macaloid extraction procedure as previously described (Servant *et al.*, 1994). The *M. tuberculosis erp* transcriptional start site was mapped by primer extension using the TSM2 primer (5'-TGCACAAGGACTTGCAACTGCCAGG-GCGGCGAC-3') complementary to nucleotides 463-495. Unphosphorylated TSM-2 primer (15 pmol) was 5' labelled using T4 polynucleotide kinase (New England Biolabs) in the presence of [γ - 32 P]dATP (100 μ Ci; 3.7 MBq), and unincorporated radioactivity was eliminated on a SEP-PAK C18 column (Waters Millipore). RNA (10 μ g) and labelled TSM-2 primer (0.1 pmol) were mixed in a volume of 7 μ l containing 0.05 M Tris/HCl (pH 8.3) and 0.1 M KCl. The reaction mixture was incubated for 1 min at 94 °C, 10 min at 65 °C and 15 min on ice. The annealing mix was adjusted to 12 μ l by addition of 1 μ l of dATP, dGTP, dCTP, dTTP mix (2.5 mM each), 0.5 μ l (6 U) RNasin (RNAguard, Pharmacia), 2.2 μ l 5 $\times$ reverse transcriptase buffer [0.25 M Tris/HCl (pH 8.3), 0.2 M KCl, 0.036 M magnesium acetate, 0.01 M dithiothreitol], 0.8 μ l diethyl pyrocarbonate-treated H₂O and 0.5 μ l avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim). Reverse transcription was performed for 45 min at 42 °C and stopped by addition of 5 μ l T7 sequencing kit stop buffer. Primer extension mixtures were electrophoresed in 6% polyacrylamide gels containing 8 M urea, in comparison with a sequence ladder of the *erp* gene generated using the TSM-2 oligonucleotide, and then analysed by autoradiography.

RESULTS

A single copy of *erp* is present in the genome of *M. tuberculosis*

Lim *et al.* (1995) described the construction of a plasmid vector (pJEM11) allowing gene fusions between a truncated *phoA* gene and mycobacterial genomic DNA fragments. A *M. tuberculosis* DNA *phoA* fusion library was constructed and expressed in *M. smegmatis*. pExp53 (Fig. 1) is the plasmid harboured by one of the PhoA⁺ clones selected from this library. Detection of enzymically active PhoA protein indicated that the pExp53 insert contains functional expression and exportation signals. Restriction analysis showed that pExp53 carries a 2 kb insert, and its sequence was partially determined. It contained a 211 nt

Fig. 1. Schematic diagram of pExp53. This plasmid is an *E. coli*-mycobacteria shuttle replicon (*ori E. coli* *ori* mycobacterium) and carries the *aph* gene conferring resistance to kanamycin. It contains a 2 kb *M. tuberculosis* DNA insert (light grey) fused to the truncated alkaline phosphatase (*phoA*) gene (striped). The 177 bp PCR fragment used as a probe in this study is underlined.

Fig. 2. Southern blot analysis using a probe derived from pExp53. Blot of *M. tuberculosis* genomic DNA digested with five different restriction enzymes and hybridized with the 177 bp *erp* PCR fragment.

ORF fused in-frame with *phoA*, exhibiting both nucleotide (77%) and deduced peptide (68%) sequence similarities with the *M. leprae irg* gene encoding a 28 kDa protein antigen (Cherayil & Young, 1987). The presence of the ORF in the *M. tuberculosis* genome was confirmed by Southern blot analysis. A 177 bp PCR fragment corresponding to nt 23-200 of the pExp53 plasmid was used as the probe. The genomic DNA from *M. tuberculosis* Mt103, the clinical isolate used for constructing the PhoA fusion library, was digested with five restriction enzymes chosen because they do not cut within the probe sequence. A single hybridization signal ranging from 2.8 to 6 kb was detected for each of the enzymes tested (Fig. 2), suggesting that *erp* is present in a single copy in the genome of *M. tuberculosis*.

Cloning and genomic mapping of *erp*

To isolate the full-length *erp* gene, a plasmid library, with *M. tuberculosis* DNA *Pst*I inserts of 2-6 kb, was constructed and screened with the 177 bp *erp* probe. Five strongly hybridizing transformants were isolated and restriction analyses of their plasmid DNA content indicated that all carried an identical 4.1 kb insert. One of them, pIPX412, was used for further studies. To map the *M. tuberculosis erp* locus with respect to other genomic markers, hybridization screening of an ordered *M. tuberculosis* cosmid library was performed with labelled pIPX412. Three overlapping cosmids hybridized with the *erp* probe (Fig. 3). The *M. tuberculosis erp* gene was thus mapped to roughly 10 kb from the *fbpA* gene encoding the fibronectin-binding protein A from the antigen 85 complex (Wiker & Harboe, 1992), and about 30 kb from the *sodA* gene encoding the superoxide dismutase (Zhang *et al.*, 1991).

Fig. 3. Genomic mapping of the *erp* gene. Genetic markers (black triangles) flanking *erp* are the fibrinogen-binding protein (*fbpA*) and superoxide dismutase (*sodA*) genes. The size of cosmid clones is estimated from other hybridization results.

erp encodes a 28 kDa polypeptide precursor with characteristic tandem repeats

The nucleotide sequence of a 1536 bp region covering the *M. tuberculosis* *erp* gene, and the deduced amino acid sequence of the *erp* coding region are shown in Fig. 4. A 852 bp ORF starting with a potential GTG initiation codon at position 418 includes the ORF fused in-frame to *phoA* in pExp53. There is a potential Shine–Dalgarno motif (GAGGAG) at position –7 upstream of this GTG codon. This ORF ends with a TAA stop codon in position 1270 and potentially encodes a 284 amino acid polypeptide precursor with a calculated molecular mass of 27.6 kDa. The first 22 N-terminal amino acids exhibit structural similarities with signal sequences of exported proteins (Izard & Kendall, 1994; Pugsley, 1993). The putative site of cleavage by signal peptidase is indicated with an arrow in Fig. 4. The degree of sequence identity between *M. tuberculosis* *erp* and *M. leprae* *irg* is 64% at the nucleotide level and 60% at the peptide level.

The central region of the predicted *M. tuberculosis* 28 kDa protein contains two sets of six tandem repeats of five amino acids based on the motif P(G/A)LTS. The positions 1, 3 and 5 of this motif are the most conserved, particularly in the first set of repeats, from residue 92 to 125 of the predicted peptide sequence. The second set of repeats, from residue 144 to 173, is more degenerate. The functional significance of these repeats is unknown. Interestingly, matrix alignment indicated that these repeats are absent from the published *M. leprae* *erp* gene product which contains only one copy of the PGLTS motif (data not shown). Therefore the conservation of these repeats between various *M. tuberculosis* isolates was investigated. A 539 bp DNA fragment, encompassing the two repeated domains, was amplified from ten epidemiologically and geographically unrelated *M. tuberculosis* isolates. Both RFLP analysis and partial DNA sequencing (data not shown) indicated that the number and the sequence of these repeats was well conserved in all the isolates tested.

Based on the Kyte–Doolittle plot, an hypothetical organization of the *M. tuberculosis* *erp* gene product is

proposed in Fig. 5. In addition to the N-terminal region corresponding to the predicted signal peptide, there is a C-terminal hydrophobic region. This region may anchor the protein in the cytoplasmic membrane or interact with other hydrophobic surface components. A stretch of four glycine residues upstream of this hydrophobic region could give flexibility to the protein.

erp is present in the genome of other mycobacteria of the *M. tuberculosis* complex

The *M. tuberculosis* *erp* genomic region covered by the 177 bp PCR fragment probe is similar (74% identity) to the corresponding *M. leprae* *irg* gene sequence, although these two species differ markedly in their G + C content. We used the same probe for investigating the distribution of *erp* homologues among other mycobacteria. Genomic DNA of 13 mycobacterial species was digested with *Pst*I, and analysed by Southern blotting (Fig. 6). At high stringency the probe hybridized specifically with the genomic DNA of bacteria belonging to the *M. tuberculosis* complex (Fig. 6b). At low stringency, a much weaker signal was also observed with DNA from *M. avium*, *Mycobacterium goodii* and *Mycobacterium scrofulaceum* (Fig. 6a). This may correspond to hybridizations with sequences unrelated to *erp*, perhaps due to the high G + C content of both the mycobacterial genomic DNA and the probe. Anyhow, hybridization signals were detected only with genomic DNA of slow-growing mycobacterial species.

Characterization of the *M. tuberculosis* *erp* transcriptional start site

The *M. tuberculosis* *erp* transcriptional start site was investigated by primer-extension analysis using RNA extracted from untransformed *M. smegmatis* mc²155 or from strains carrying either pExp53 or the control pJEM11 vector (Fig. 7). A single start, located 93 nt upstream of the GTG start codon (Fig. 4) was identified with the construct pExp53 but not with the control plasmid or untransformed bacteria. The predicted *erp* –10 box (TACCAT) shares some sequence similarities with the *E. coli* (σ^{70}) and *Bacillus subtilis* (σ^A) consensus (TATAAT) (Moran, 1993), whereas the corresponding *erp* –35 box (TCTAGT) is more atypical in this respect (TTGACA).

Dale & Patki (1990) suggested that a putative iron box may control the expression of the *M. leprae* *irg* gene. We found a similar motif upstream of the *M. tuberculosis* *erp* ORF, which overlaps with the predicted –35 box (Fig. 4). This observation is compatible with *M. tuberculosis* *erp* expression being regulated by iron.

DISCUSSION

The adaptation to *M. tuberculosis* of the *phoA* methodology for identification of exported proteins was recently described by Lim *et al.* (1995). This methodology relies on a plasmid vector and thus can identify genes the disruption of which would be lethal for the bacterium. In the present

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CGGCTTCGGAATAGGCATTGCCCCCGATGTGCGGGCGCCGCTCGAGGACGAGCAGCGCTTGTGAGTTGGGTGGCCACGC 81
GCTCGGCAATCGTCAGGCCGAAGAATCCTGAGCCGACGACGAAAAGGTCAAAACGAGCGGTTCATCGGTTGCATAGGGTAAC 162
CGACCTTGCTGGCAAAACCCGATTGTGGCAGCTCGTGGCGGTTCATGGCCCGAACGGGTTTCACCGCAGGTGCGCATGGCCGA 243

*****

CCAGTGTGGTTGGCCGGAGGTCGTTTGGTTCGCGATTGCCTCAGATTTCGATATAACCACtctagtCACATCAACCACACTC 324
                                     -35

GtaccatCGAGCGtGTGGTTCATGCCATGCACTCGCGACCCGGGAGCCGGCGAAACCCGGCGCCACACATAATCCAGATT 405
-10      +1

GAGGAGACTTCCGTGCCGAACCGACGCCGACGCAAGCTCTCGACAGCCATGAGCGCGGTTCGCCGCCCTGGCAGTTGCAAGT 486
1  RBS      M P N R R R R K L S T A M S A V A A L A V A↓S

CCTTGTGCATATTTTCTTGTCTACGAATCAACCGAAACGACCGAGCGGCCCGAGCACCATGAATTCAAGCAGGCGCGGTG 567
24 P C A Y F L V Y E S T E T T E R P E H H E F K Q A A V

TTGACCGACCTGCCCGGCGAGCTGATGTCCGCGCTATCGCAGGGGTTGTCCAGTTTCGGGATCAACATACCGCCGGTGGCC 648
51 L T D L P G E L M S A L S Q G L S Q F G I N I P P V P

AGCCTGACCGGGAGCGCGATGCCAGCACGGGTCTAACCGGTCTTGGCCTGACTAGTCCGGGATTGACCAGCCCGGGATTG 729
78 S L T G S G D A S T G L T G P G L T S P G L T S P G L

ACCAGCCCGGGCCTCACCAGCCCTGCCCTTACCAGTCCGGGCTGACGCCAACCCGCGCGATCACTCGCCGCGCCCGGC 810
105 T S P G L T D P A L T S P G L T P T L P G S L A A P G

ACCACCCGCGCCAACGCCCGCGGTGGGGGCCAATCCGGCGCTACCAACCCCGCGCTGACCAGCCCGACCGGGGCGACG 891
132 T T L A P T P G V G A N P A L T N P A L T S P T G A T

CCGGGATTGACCAGCCCGACGGGTTTGGATCCCGCGCTGGGGCGGCCAACGAAATCCCGATTACGACCGCGGTGGATTG 972
159 P G L T S P T G L D P A L G G A N E I P I T T P V G L

GATCCCGGGGCTGACGGCACCTATCCGATCCTCGGTGATCCAACACTGGGGACCATACCGAGCAGCCCGCCACCACTCC 1053
186 D P G A D G T Y P I L G D P T L G T I P S S P A T T S

ACCGGCGGCGCGGTCTCGTCAACGACGTGATGCAGGTGGCCAAACGAGTTGGGCGCCAGTCAGGCTATCGACCTGCTAAAA 1134
213 T G G G G L V N D V M Q V A N E L G A S Q A I D L L K

GGTGTGCTAATGCCGTCGATCATGCAGGCCGTCCAGAATGGCGGCGCGTTCGCGCCGGCAGCCAGCCCGCGGTCCCGCCC 1215
240 G V L M P S I M Q A V Q N G G A V A P A A S P P V P P

ATCCCCGCGCGCGCGCGGTGCCACCGACGGACCCAATCACCGTGCCTAAGCCCCGGGTGCGCCGAAAACGCAC 1296
267 I P A A A A V P P T D P I T V P V A *

CCGCGGCCAAGGCGTCGGTCAATTGCTTCGGCCCGTCACAATTACTCGCCTAAGGGTCGCTAGGTGTTCTCGAGAGTTTAT 1377
CGCACCGATTCCGTGTCGTCTCATTAAATACCAATAGAAACACACGTAACATCAGCTGGTGGCGTCCCGCACCCGCGCGCG 1455
ACGACGCTGCTCACC CGATGGCAGCGACCGTCTCATCGTTCGCGTGGATAGCGAATCGTCCACCCGCGAGCTCCCAT

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Fig. 4. Nucleotide sequence of the *M. tuberculosis erp* gene and deduced amino acid sequence. The nucleotide sequence corresponding to a putative iron box is indicated by black stars. The mapped transcriptional start site, together with the predicted -10 and -35 boxes are represented in lower case letters. The potential RBS is underlined. Single-letter amino acid code is used. A potential signal peptidase cleavage site is indicated with an arrow. Repeated amino acid stretches in the central part of the *erp* gene product are underlined.

report, the characterization of the *M. tuberculosis erp* gene which has similarity with a *M. leprae* gene (*irg*) encoding a 28 kDa protein antigen is described. *M. tuberculosis erp* contains repetitive structures conserved among isolates and a putative iron box is located close to the mapped transcriptional start site.

The organization of the *M. tuberculosis erp* gene is similar to that of the *M. leprae* counterpart (Cherayil & Young, 1987). The C-terminal hydrophobic region could be involved in membrane anchorage of the protein (Davis *et al.*, 1985). However, the *M. tuberculosis erp* gene product is longer due to the presence of additional residues (amino

Fig. 5. Kyte-Doolittle plot and predicted domain organization of the *erp* gene product. Plots above the line indicate a hydrophobic nature. Tandem repeats numbered from R1 to R12 are represented as black arrowheads.

Fig. 7. Mapping of the *erp* transcriptional start site by primer extension using the TSM-2 oligonucleotide primer. Lanes 1-3 correspond respectively to primer-extension reactions performed with RNA extracted from *M. smegmatis*(pExp53), *M. smegmatis*(pJEM11) and non-transformed *M. smegmatis*. The end of the primer-extension product is indicated by a star.

Fig. 6. Distribution of the *erp*-like gene sequences among mycobacteria. Mycobacterial genomic DNAs were digested to completion with *Pst*I and analysed by Southern blotting under (a) low or (b) high stringency conditions.

acids 126-177) encompassing the second set of repeats, and encodes a predicted 28 kDa protein precursor. The molecular mass calculated from the published *M. leprae* '28' kDa antigen precursor sequence is actually 24 kDa. The presence of tandemly repeated sequences is a striking structural feature of many Gram-positive cell-wall-

associated proteins (Kehoe, 1994). Tandem repeats can vary in length from two to more than 100 residues. The mechanisms by which they are produced are not clearly understood. However, duplication and deletion of these repeats are frequent events. For instance, in group A streptococcal M proteins, the number of copies of tandem repeats differs among isolates (Fischetti *et al.*, 1986). Here, the number and the sequence of *erp* repeats was found to be conserved among *M. tuberculosis* isolates of various origins, and thus is not a source of polymorphism. They may thus be important for the function of the *erp* gene product.

Cell-wall-associated proteins with tandem repeats have been associated with binding domains for other proteins or polysaccharides (Talay *et al.*, 1994; Westerlund & Korhoen, 1993). In the serotype 5 M protein, the binding site for fibrinogen has been localized within the group B repeats. IgG Fc-binding proteins such as protein A, protein G and several group A streptococcal *emm*-like proteins also share the same general organization with an N-terminal signal sequence, tandemly repeated sequences and a hydrophobic charged C-terminal tail (Kehoe, 1994). Genes encoding wall-associated fibronectin-binding proteins have been cloned from *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus dysgalactiae* (Flock *et al.*, 1987; Hanski & Caparon, 1992; Lindgren *et al.*, 1992; Talay *et al.*, 1991). They contain a number of different types of tandemly repeated sequences. In mycobacteria, at least two other genes in addition to *M. tuberculosis erp*, have been reported to potentially encode proteins with tandem repeats: the *M. leprae* proline-rich antigen (*pra*) gene (Thole *et al.*, 1990), and an ORF found next to the *M. tuberculosis* 65 kDa antigen gene (Shinnick, 1987). However, no function has yet been ascribed to these repeats.

Localization of proteins at the bacterial cell surface is the

result of a complex targeting process, involving exportation and interaction with the peptidoglycan network. In most cases, cell-wall-associated proteins of Gram-positive bacteria have a characteristic hexapeptide motif LPXTGX, followed by the putative region of membrane anchorage (Fischetti *et al.*, 1990). This motif has recently been shown to be required for covalent linkage of the peptide chain to the peptidoglycan (Navarre & Schneewind, 1994). This signature is absent from the *M. tuberculosis* Erp protein. However, the *Listeria monocytogenes* gene *inlB*, encoding the cell-wall-associated internalin B, is also devoid of the LPXTGX motif (Gaillard *et al.*, 1991).

Iron has been shown to be involved in the regulation of virulence genes in many pathogenic bacteria (for reviews see Griffith, 1991, and Litwin & Calderwood, 1993). These regulatory mechanisms rely on a bacterial repressor protein controlling gene expression at the transcriptional level. In the presence of iron, this repressor interacts with a target DNA sequence referred to as the iron box, and blocks gene expression. Under iron-limited conditions, such as those existing during growth within mammalian tissues or cells, repression is relieved and the expression of iron-regulated genes is induced. Based on nucleotide sequence similarities, Dale & Patki (1990) have postulated the existence of such a putative iron box regulating the expression of the *M. leprae* *irg* gene. In the present paper, a similar motif overlapping the predicted -35 box of the *M. tuberculosis* *erp* gene was found. This result might be considered to support there being iron regulation of the *erp* gene. However, when the PhoA activity of the Erp-PhoA protein fusion was assayed in cell extracts of *M. smegmatis*(pExp53) grown under iron-sufficient or iron-limited conditions, no increase in Erp-PhoA expression was detected under iron-limited conditions (data not shown). Determining whether or not *erp* is iron-regulated will require a detailed analysis of its expression in *M. tuberculosis*.

It is known for other iron boxes described so far that they are preferentially located between the -10 and -35 regions. Additionally, both *M. leprae* *irg* and *M. tuberculosis* *erp* putative iron box motifs share similarities with the consensus sequence defined for binding the Gram-negative Fur repressor (Litwin & Calderwood, 1993). Recently, Fiss *et al.* (1994) cloned and functionally characterized an iron box involved in the regulation of the ferric exochelin biosynthetic pathway of *M. smegmatis*. Interestingly, the sequence fits with the consensus for the Gram-positive DtxR repressor binding site (Tao *et al.*, 1994). Collectively, these observations raise the possibility that iron may regulate gene expression by different mechanisms in mycobacteria.

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