The eukaryotic-like Ser/Thr protein kinases of *Mycobacterium tuberculosis*

Yossef Av-Gay and Martin Everett

In bacteria, extracellular signals are generally transduced into cellular responses via a two-component system. However, genome sequence data have now revealed the presence of 'eukaryotic-like' protein kinases and phosphatases. *Mycobacterium tuberculosis* appears to be unique among bacteria in that its genome contains 11 members of a newly identified protein kinase family. These *M. tuberculosis* eukaryotic-like protein kinases could be key regulators of metabolic processes, including transcription, cell development and interactions with host cells.

Y. Av-Gay is in the Divn of Infectious Diseases, University of British Columbia, 2733 Heather St, Vancouver, BC, Canada V5Z 3J5;*
M. Everett is in the Glaxo Wellcome Research and Development Medicines Research Centre, Stevenage, UK SG1 2NY.
**tel: +1 604 875 4588, fax: +1 604 875 4013, e-mail: yossi@interchange.ubc.ca*

Protein phosphorylation is the principal mechanism by which extracellular signals are translated into cellular responses. This process is carried out by specific protein kinases and is coupled to dephosphorylation reactions, which are carried out by protein phosphatases. In bacteria, the molecular system responsible for stimulus–response coupling involves a two-component system, consisting of histidine kinase sensors and their associated response regulators[1]. In eukaryotes, by contrast, protein phosphorylation mainly results in phosphorylated serine, threonine or tyrosine residues, and the eukaryotic protein kinases and phosphatases are the backbone of signal transduction pathways. Previously, serine, threonine and tyrosine kinases, and their coupled phosphatases, were thought to be unique to eukaryotes; however, recent evidence arising from the accumulation of bacterial genome sequencing data and the use of anti-phosphoprotein antibodies has revealed that some prokaryotes also contain phosphoester kinases and phosphatases[2–5].

To date, Ser/Thr protein kinases (STPKs) have been shown to be involved in three different processes in prokaryotes, namely regulation of development, stress responses and pathogenicity. Bacteria capable of differentiating into a new developmental state, including *Streptomyces*[6–8], *Anabaena*[5,9,10], and *Myxococcus xanthus*[11–13], have many STPK-encoding genes. In these bacteria, kinases are involved in the control of the late stages of development, sporulation or secondary metabolite production; for example, Pkn1 of *M. xanthus* is expressed exclusively during sporulation and its inactivation lowers the level of spore formation in this bacterium[13]. Additionally, STPKs have been shown to be involved in the survival of human pathogens within the host, as typified by the *Yersinia pseudotuberculosis* plasmid-encoded protein kinase YopO (YpkA, Ref. 14) or the *Pseudomonas aeruginosa* STPK (Ref. 15). Both kinases have been shown to be required for full virulence of these pathogens in mouse models. More recent studies have shown that multiple STPKs are present in *P. aeruginosa*, but provided no further evidence in support of a role for these proteins in virulence[16,17].

It is well established that some bacteria, for example, *Shigella*, *Salmonella*, enteropathogenic *Escherichia coli* (EPEC) and *Yersinia*[18], interact actively with mammalian cells. They either secrete proteins or directly inject proteins into host cells using the type III secretion system[19]. Several examples exist whereby microbial kinases promote infection by disrupting the eukaryotic cytoskeleton or internal cellular communication systems[19]. For example, YopO of *Y. pseudotuberculosis* is translocated to the inner surface of the host cell plasma membrane[10], where it interferes with host cell signalling pathways. *Listeria monocytogenes* invades mammalian cells and alters host signalling by directly stimulating mitogen-activated protein (MAP) kinase upon attachment to epithelial cells[20]. Another example of an active interaction between invasive bacteria and the host is provided by the human gastric epithelial pathogen *Helicobacter pylori*. *H. pylori* induces cytoskeletal rearrangements following attachment to gastric cells, as well as tyrosine phosphorylation of at least two host cell proteins[21].

Early studies using monoclonal antibodies showed that 'eukaryotic-like' protein phosphorylation occurs in *M. tuberculosis*[22]. Later work using a variety of molecular techniques such as Southern analysis, PCR and genome database screening demonstrated that *M. tuberculosis* encodes eight eukaryotic-like protein kinases[23]. Furthermore, we have shown that at least six proteins are phosphorylated *in vitro*, suggesting the presence of functional kinases in *M. tuberculosis*[23]. The completion of the *M. tuberculosis* genome

0966-842X/00/$ - see front matter PII: S0966-842X(00)01734-0

Table 1. Summary of *Mycobacterium tuberculosis* protein kinases properties[a]

Name	ORF[b]	MW[c]	Adjacent/nearby genes	TM[d]	Unique features	*M. leprae* homologue	Auto-P[e]	Proposed regulatory role
PknA	Rv0015c	45 598	*oriC/pbp*	+	−	+	−	Cell elongation/division
PknB	Rv0014c	66 511	*oriC/pbp*	+	PonA domain	+	+	Cell elongation/division
PknD	Rv0931c	69 514	Phosphate-uptake operon	+	β-propeller, PQQ domain	−	+	Phosphate transport
PknE	Rv1743	60 513	ABC transporter	+	−	−	−	Membrane transport
PknF	Rv1746	50 669	ABC transporter	+	−	−	+	Membrane transport
PknG	Rv0410c	81 579	*glnH*	−	Trx motif, TPR motif	+	−	Amino-acid uptake, stationary-phase metabolism
PknH	Rv1266c	66 755	*embR*	+	AfsK like	−	+	Arabinan metabolism
PknI	Rv2914c	61 806	*ffh, ftsY*	+	Asn in active site	−	−	Cell division
PknJ	Rv2088	61 564	Transposon	+	−	−	+	?
PknK	Rv3080c	119 420	*luxA* like	−	PDZ and AAA domains	−	−	Transcription, secondary metabolites
PknL	Rv2176	42 803	Transcriptional regulator	+	−	+	−	Transcription?

[a]Abbreviations: ABC, ATP-binding cassette transporter; MW, molecular weight; ORF, open reading frame; PQQ, pyrroloquinoline quinone; TPR, tetratricopeptide; Trx, thioredoxin.
[b]*M. tuberculosis* H37Rv ORF designation.
[c]Molecular weight is given in Daltons.
[d]Predicted transmembrane-spanning region.
[e]Experimental evidence of auto-phosphorylation

sequencing project has now provided a complete list of 11 putative eukaryotic-like protein kinases that form the *M. tuberculosis* STPK family[24] (Table 1). This review summarizes the current experimental- and bioinformatics-based knowledge concerning the members of this protein family, thereby providing a basis for experimental approaches to identify their role in the cellular metabolism of *M. tuberculosis*.

STPK structure and homology analysis

The protein kinase 'signature', including all 11 domains that are conserved according to Hanks[25], is present in all *M. tuberculosis* STPKs and was used as the criterion for their annotation during the genome sequencing project. An additional gene, *pknM*, which was previously annotated as a putative STPK-encoding gene, does not possess any recognizable kinase signatures. The homology between PknM and the carboxy-terminal region of PknH probably explains the original annotation. Alignment of the STPK family members revealed that 15 residues are absolutely conserved across the group (Fig. 1). All kinases other than PknI possess a lysine in the active site [Hanks domain VIb (DXKPXN, where X is any amino acid)], which is characteristic of STPKs. PknI has an asparagine at this position, which is unusual for an STPK; however, the other conserved residues in the domain indicate that PknI is a Ser/Thr kinase.

Sequence database comparison using several alignment tools including BLAST, Fasta and Clustal algorithms reveals strong homology to numerous known and putative kinases. The closest homologues are other prokaryotic eukaryotic-like STPKs. Not surprisingly, the homologues with highest sequence similarity were identified in the accumulating sequence data from *Mycobacterium leprae* and other actinomycetes such as *Streptomyces coelicolor*. Figure 2 shows a dendrogram of a multiple sequence comparison (compiled using ClustalX and Treetool of the Wisconsin GCG 10.0 package) of the kinase domains from all the *M. tuberculosis* STPKs, other prokaryotic kinases identified from the BLAST searches and eukaryotic STPKs representative of the various classes of cAMP- and Ca^{2+}/calmodulin-regulated kinases. All but two of the *M. tuberculosis* STPKs are present in a large cluster with most of the other prokaryotic sequences. PknG and PknK do not cluster with the rest of the *M. tuberculosis* STPKs and appear to be most similar to the eukaryotic STPKs, although no association with any particular enzyme class is apparent. At least four *M. tuberculosis* STPKs, namely PknA, PknB, PknG and PknL, have close homologues in *M. leprae*, with amino acid identity ranging from 74–87%. None of the members of the *M. tuberculosis* STPK family is present in the missing chromosomal regions in bacillus *Mycobacterium bovis* Calmette–Guérin (BCG)[26].

Functional predictions

Bioinformatics analyses have provided a number of clues to possible STPK functions, which, together with locational information from the genome, have

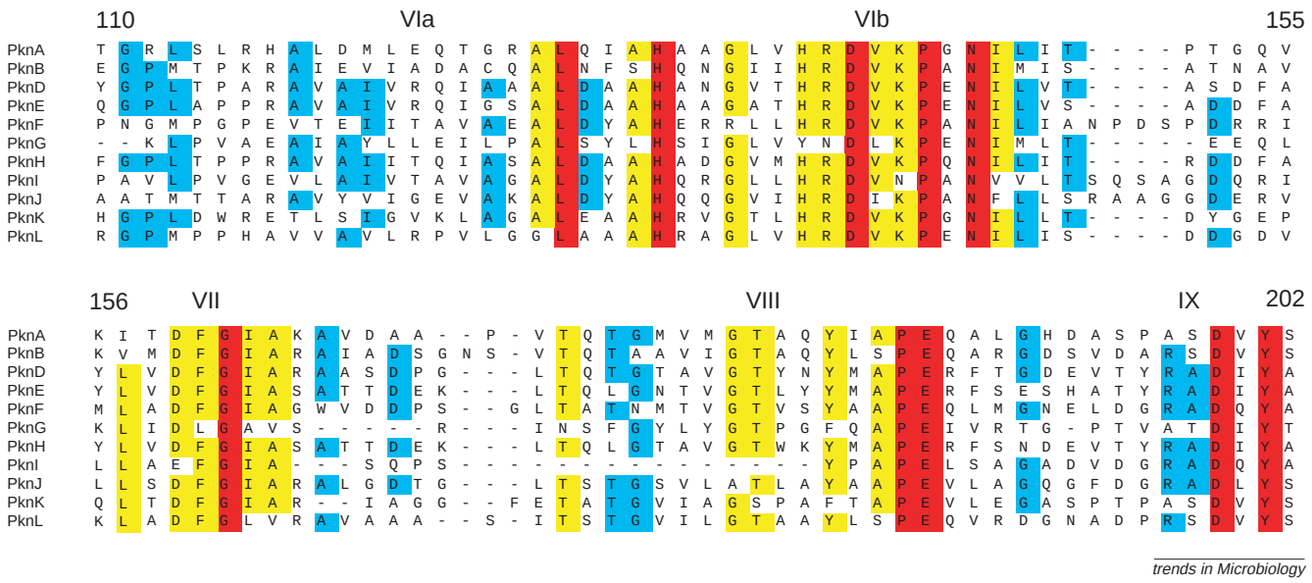

Fig. 1. Alignment of *Mycobacterium tuberculosis* STPKs from Hank domains VI to IX. The alignment was performed using ClustalW (Gap opening 10; gap extension 0.2) and ShadyBox programs of the Wisconsin GCG10.0 Seqlab package. Red represents 100% identity, yellow represents 75–99% identity and blue represents 50–75% identity.

allowed us to assign putative roles to certain STPKs. For example, all the proteins in the large cluster in Fig. 2 appear to contain a single transmembrane helix (Fig. 3). With the exception of PknL, this transmembrane region is located proximal to the amino-terminal kinase domain and is followed by a carboxy-terminal region of variable length. The topology of these membrane-spanning proteins, as predicted using the program HMMTOP, is 'N-in, C-out', consistent with these proteins acting as signalling molecules between the cell exterior and interior. Table 1 summarizes our present knowledge of the *M. tuberculosis* STPKs, obtained from both experimental and bioinformatic analyses.

PknA, PknB and PknD

Both *pknA* and *pknB* reside within an operon with *pbpA* (penicillin-binding protein 2; Rv0016c) and *rodA* (Rv0017c), which control the switch between peptidoglycan elongation and septum formation in other bacteria[27,28]. Interestingly, a putative Ser/Thr phosphatase gene, *ppp* (Rv0018c), is encoded by the same operon, suggesting regulation of this process by a phosphorylation/dephosphorylation cascade. This type of regulation of cell elongation has not previously been described in other bacteria, although it is noticeable that both the *pknA* and *pknB* homologues in *M. leprae* are also adjacent to a penicillin-binding-protein gene, suggesting a similar mode of regulation in this organism. The *M. tuberculosis* PknB protein also shows carboxy-terminal homology to another penicillin-binding protein, PonA (Rv0050). Experimental evidence clearly shows that PknB is autophosphorylated on a serine residue, possesses kinase activity and is continuously transcribed *in vitro* as well as in animal models and patient samples[29]. This, together with its localization near the origin of replication, suggests that PknB is important for normal growth of *M. tuberculosis*. The third member of the *M. tuberculosis* STPK family, PknD, was identified prior to release of the genome sequence, and was shown to be autophosphorylated on a serine residue and able to phosphorylate artificial substrates[30]. A striking feature of PknD is that the carboxy-terminal domain contains six imperfect tandem repeats homologous to those that form β-propeller structures in a variety of proteins, including lipoprotein and scavenger receptors, extracellular matrix components and tyrosine kinases[31]. The funnel-shaped β-propeller structure is thought to act as the receptor module in these proteins. Interestingly, this structure also encompasses a pyrroloquinoline quinone (PQQ)-binding site. PQQ is a redox coenzyme that serves as a cofactor for a number of enzymes, including some bacterial dehydrogenases[32]. A similar PQQ-containing β-propeller structure is present in the carboxyl terminus of the *S. coelicolor* Ser/Thr kinase AfsK, which phosphorylates AfsR, a transcriptional regulator of secondary metabolism[6]. In *M. tuberculosis*, *pknD* appears to be co-transcribed with *pstS* (Rv0932c), which encodes a component of a phosphate-uptake system, and several other components of the same system are encoded by nearby genes. Thus, a probable role for PknD could be regulation of phosphate transport.

PknE, PknF and PknH

The *pknF* gene is in an operon with an ATP-binding cassette (ABC) transporter gene (Rv1747) and is only three open reading frames distant from *pknE*, prompting a possible role for PknF, and perhaps also PknE, in regulation of this transport system. Similarly, the *pknH* gene is downstream of genes encoding ABC-transporter components and also *embR* (Rv1267c). EmbR is a transcriptional regulator of the *embA* and

embB genes, encoding cell wall arabinosyltransferases, which are the targets of the antimycobacterial drug ethambutol[33]. EmbR is homologous to AfsR in *S. coelicolor,* which, as already discussed, is phosphorylated by the Ser/Thr kinase AfsK.

PknI, PknJ and PknL

PknI is part of a cluster of genes including *dacB* (Rv2911), which encodes the D-amino acid hydrolase PBP5 (Ref. 34), another D-amino acid hydrolase (Rv2913c), and *ffh* (Rv2916c), which encodes a signal recognition particle. Another nearby gene, *ftsY* (Rv2921c), encodes the membrane receptor for Ffh (Ref. 35). Thus, a possible role for PknI could be to regulate the secretion of proteins such as PBP5 via the Ffh/FtsY pathway. As such, PknI could also be involved in regulation of cell division, as both PBP5 and FtsY have been implicated in this process in *Escherichia coli*[35].

Few functional clues are available for PknJ or PknL. The gene for PknJ is situated directly downstream of several transposon genes, whereas the gene for PknL, which is unique among the *M. tuberculosis* STPKs in having a carboxy-terminal transmembrane region, is in the same operon as a putative transcriptional regulator gene.

PknG and PknK

The last two members of the family, PknG and PknK, have no apparent transmembrane regions and are therefore predicted to be soluble proteins. PknG is the only STPK that has an amino-terminal region preceeding the kinase domain, and in this respect is similar to the secreted YopO kinase of *Yersinia*. This amino-terminal region contains a short double cysteine motif, which is a signature of disulfide reductases and isomerases such as the thioredoxins[36]. Members of this family of proteins are involved in maintenance of conformation of other proteins through oxidation and reduction of their disulfide bridges[36]. The presence of this motif in PknG suggests it could be activated by, or respond to, the redox status of the cell. In the carboxy-terminal region of the protein there is a pyridoxal phosphate-binding motif, a characteristic of class V amino acid transferase enzymes. Additionally, this region also contains a single tetratricopeptide (TPR) sequence, which, in higher eukaryotes, is repeated to form a signalling domain[37]. The *pknG* gene is located in an operon with *glnH* (RV1411c), which encodes an extracellular glutamine-binding protein with a lipid-attachment site to anchor it to the cell membrane. In other bacteria, GlnH is part of a high-affinity glutamine-uptake system, which is induced under nitrogen-limiting conditions. Thus, it seems likely that PknG has a role in glutamine uptake and could be co-transcribed with *glnH* under nitrogen-limiting conditions. Indeed, green fluorescence protein transcriptional fusion studies have demonstrated that *pknG* expression is upregulated upon entry into stationary phase[38]. The possible involvement of PknG in glutamine metabolism is interesting in the light of recent findings showing that inhibition of extracellular glutamine synthetase resulted in bacteriostasis of *M. tuberculosis,* both *in vitro* and in macrophages, whereas no effect on growth was seen with non-pathogenic mycobacteria[39]. Furthermore, treatment of *M. tuberculosis* with antisense oligonucleotides to glutamine synthetase mRNA leads to inhibition of bacterial replication[40]. This argues that glutamine metabolism, and perhaps also PknG, are important in the pathogenicity of *M. tuberculosis*.

The carboxy-terminal region of PknK shows homology to the regulatory regions of transcriptional regulators of the LuxR family, such as *Klebsiella*

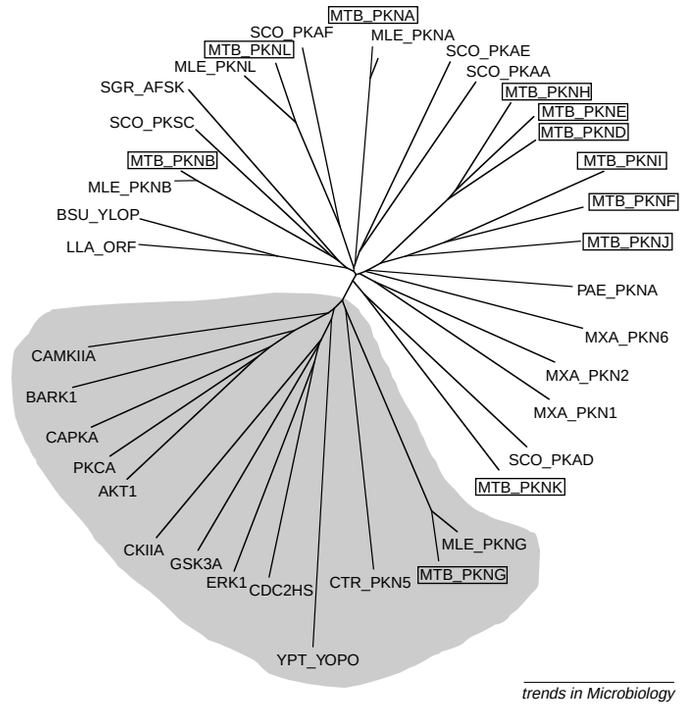

Fig. 2. Dendrogram of a multiple sequence comparison of kinase domains from all the *Mycobacterium tuberculosis* STPKs, other prokaryotic kinases identified from the BLAST searches and eukaryotic STPKs representative of the various classes of cAMP- and Ca^{2+}/calmodulin-regulated kinases. Sequences from the Swiss-Prot/EMBL database were aligned using ClustalW (Gap opening 10; gap extension 0.2) and displayed using Treetool from the Wisconsin GCG10.0 Seqlab package. *M. tuberculosis* STPKs are boxed and the eukaryotic kinases and their closest prokaryotic homologues are shaded. Accession numbers and abbreviations: MTB_PKNA, *Mycobacterium tuberculosis* (P71585); MTB_PKNB, (P71584); MTB_PKND, (O05871); MTB_PKNE, (P72001); MTB_PKNF, (P72003); MTB_PKNG, (P96256); MTB_PKNH (Q11053); MTB_PKNI, (Q10964); MTB_PKNJ, (Q10697); MTB_PKNK, (P95078); MTB_PKNL, (O53510); MLE_PKNA, *Mycobacterium leprae* (P54743); MLE_PKNB (P54744), MLE_PKNG (CAA22703); MLE_PKNL, (O69568); SCO_PKSC *Streptomyces coelicolor* (AAC64406); SCO_PKAA, (P54739); SCO_PKAF (BAA34200); SCO_PKAD, (O83032); SCO_PKAE, (BAA34340); SCO_PK3, (Q53839); SGR_AFSK, *Streptomyces griseus* (P54742); PAE_PKNA, *Pseudomonas aeruginosa* (AAD03499); BSU_YLOP, *Bacillus subtilis* (O34507); MXA_PKN1 *Myxococcus xanthus* (P33973); MXA_PKN2, (P54736); MXA_PKN6, (P54738); LLA_ORF, *Lactococcus lactis* (CAA10713); YPT_YOPO, *Yersinia pestis* (Q05698); CTR_PKN5, *Chlamydia trachomatis* (O84680); CAMKIIA, *Rattus norvegicus* (P11275); BARK1, *Bos taurus* (P21146); CAPKA, *Homo sapiens* (P17612); PKCA, *Homo sapiens* (P17252); AKT1, AKT8 murine leukemia virus (P31748); CKIIA, *Homo sapiens* (P19138); GSK3A, *Rattus norvegicus* (P18265); ERK1, *Rattus norvegicus* (P21708); CDC2HS, *Homo sapiens* (P06493).

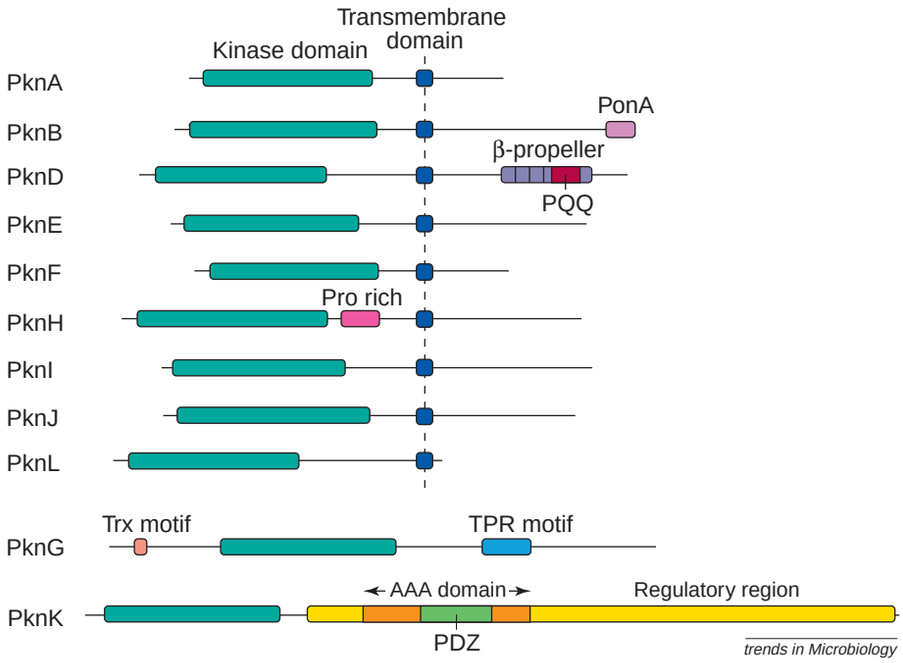

Fig. 3. Structural analyses of *Mycobacterium tuberculosis* STPKs. The programs Profile Scan (`http://www.isrec.isb-sib.ch/software/PFSCAN_form.html`), Easymotif (Glaxo Wellcome in-house package incorporating PFAM, BLOCKS and PRINTS searches) and the Pedant database (`http://pedant.mips.biochem.mpg.de/mtb/`) were used to search peptide sequences for known structural or function motifs. The motifs were drawn on a schematic representation of the linear peptide sequence. Abbreviations: PQQ, pyrroloquinoline quinone; TPR, tetratricopeptide; Trx, thiorexdoxin.

pneumoniae AcoK and *E. coli* MalT, and contains an ATP-binding motif (AAA) characteristic of molecules with ATPase activity. This AAA domain also encompasses a PDZ domain, which is involved in targeting signalling molecules to sub-membranous sites[41]. Although PknK does not show homology to the DNA-binding regions of MalT or AcoK, the *pknK* gene is situated close to that encoding a putative transcriptional regulator *virS* (Rv3082c) which has been described as a potential virulence gene in *M. tuberculosis* H37Rv (Ref. 42). The *pknK* gene is also located directly upstream of one of several *M. tuberculosis* genes (Rv3079c) that are homologous to members of the LuxA family of flavin mononucleotide (FMN)-dependent monoxygenases. In other bacteria, these enzymes are involved with the production of secondary metabolites, such as the antibiotic lincomycin in *Streptomyces* species. Hence, PknK might regulate the production of a secondary metabolite(s) in *M. tuberculosis*.

Questions for future research

- What are the specific metabolic pathways controlled by *M. tuberculosis* STPKs?
- What are the protein substrates of the STPKs?
- Which STPKs, if any, are involved in the switching mechanism that controls entry/exit of *M. tuberculosis* to/from a dormant state?
- Do members of the STPK family interact with host cell signalling elements?
- What roles do these STPKs fulfil in pathogenicity or the progress of the disease?

Conclusions

The aim of this review was to provide insights into the roles of the *M. tuberculosis* STPKs in the metabolism of the tubercle bacillus and its interaction with the host. In other bacteria, STPKs have been shown to be involved in cell development, stress response and pathogenesis. Some of the functions we have putatively assigned to the *M. tuberculosis* STPKs fall into similar categories (see Table 1).

From the available evidence, it appears that at least three STPKs could be involved in cell growth and development, namely PknA, PknB and PknI, all of which appear to regulate aspects of cell division/elongation. Regulation of this crucial step in the cell cycle by STPKs has not been previously described and this mode of regulation could be important with respect to the characteristically slow growth of *M. tuberculosis* and its ability to enter an extreme stationary phase or dormant state, which is associated with prolonged latent infection[43].

The role of *M. tuberculosis* STPKs in pathogenicity is difficult to predict without available mutants; however, the involvement of intra- and/or extracellular phosphorylation mechanisms in *M. tuberculosis* infection is likely. Altered protein phosphorylation could mediate *M. tuberculosis* inhibition of both lysosome fusion with the phagosome and exclusion of the vacuolar proton-ATPase from the phagosome, preventing its acidification[44]. Interference with the host's normal immune response during mycobacterial infection could also be mediated by disabling host signalling pathways. Indeed, it has recently been shown that the binding of *M. tuberculosis* to human macrophages via complement receptor 4 transduces a signal, resulting in tyrosine phosphorylation of macrophage proteins[45]. Superficially, PknG is the most likely candidate to participate in extracellular phosphorylation events. It is the most eukaryotic-like, has a similar structure to *Yersinia* YopO (Fig. 3), and does not contain any transmembrane signals, suggesting that it is a soluble protein. Additionally, it is implicated in pathogenicity via its possible involvement in extracellular glutamine metabolism.

Surprisingly, the complete genomes of other bacteria, including those similar in size to *M. tuberculosis*, such as *E. coli* and *Bacillus subtilis*, do not include any members of this family of regulatory proteins.

Instead, both *B. subtilis* and *E. coli* encode >30 members of the classical bacterial two-component system histidine kinases, compared with 11 in *M. tuberculosis*. The total number of kinases (STPKs and those of the two-component system) in these species seems to be the same. Thus, in addition to the proposed roles in pathogenesis and development, it is likely that other *M. tuberculosis* STPKs simply fulfill the role of the classical bacterial two-component system regulatory proteins. It is acknowledged that the functional predictions proposed in this article for the *M. tuberculosis* STPKs are speculative. Nevertheless, they provide hypotheses that can then be tested in the laboratory. Future experiments will aim to probe the function of these STPKs.

Acknowledgements
Research in Y.A.'s laboratory is supported by grants from the Glaxo Wellcome Action TB programme, British Columbia TB Veterans association and British Columbia Lung Association. We thank Julian Davies and Ken Duncan for their support, advice and comments.

References

1 Stock, J.B. *et al.* (1989) Protein phosphorylation and regulation of adaptive responses in bacteria. *Microbiol. Rev.* 53, 450–490
2 Cozzone, A.J. (1993) ATP-dependent protein kinases in bacteria. *J. Cell. Biochem.* 51, 7–13
3 Kennelly, P.J. and Potts, M. (1996) Fancy meeting you here: a fresh look at 'prokaryotic' protein phosphorylation. *J. Bacteriol.* 178, 4759–4764
4 Potts, M.H. *et al.* (1993) A protein-tyrosine/serine phosphatase encoded by the genome of the cyanobacterium *Nostoc*. *J. Biol. Chem.* 268, 7632–7635
5 Zhang, C.C. (1996) Bacterial signalling involving eukaryotic-type protein kinases. *Mol. Microbiol.* 20, 9–15
6 Matsumoto, A.S. *et al.* (1994) Phosphorylation of the AfsR protein involved in secondary metabolism in *Streptomyces* species by a eukaryotic-type protein kinase. *Gene.* 146, 47–56
7 Nadvornik, R. *et al.* (1999) Pkg2, a novel transmembrane protein Ser/Thr kinase of *Streptomyces granaticolor*. *J. Bacteriol.* 181, 15–23
8 Urabe, H. and Ogawara, H. (1995) Cloning, sequencing and expression of serine/threonine kinase-encoding genes from *Streptomyces coelicolor* A3(2). *Gene* 153, 99–104
9 Zhang, C.C. *et al.* (1998) Molecular and genetic analysis of two closely linked genes that encode, respectively, a protein phosphatase 1/2A/2B homolog and a protein kinase homolog in the cyanobacterium *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* 180, 2616–2622
10 Zhang, C.C. and Libs, L. (1998) Cloning and characterization of the *pkn*D gene encoding an eukaryotic-type protein kinase in the cyanobacterium *Anabaena* sp. PCC7120. *Mol. Gen. Genet.* 258, 26–33
11 Hanlon, W.A. *et al.* (1997) Pkn9, a Ser/Thr protein kinase involved in the development of *Myxococcus xanthus*. *Mol. Microbiol.* 23, 459–471
12 Udo, H. *et al.* (1997) Biochemical characterization of Pkn2, a protein Ser/Thr kinase from *Myxococcus xanthus*, a Gram-negative developmental bacterium. *FEBS Lett.* 400, 188–192
13 Zhang, W. *et al.* (1996) Reciprocal regulation of the differentiation of *Myxococcus xanthus* by Pkn5 and Pkn6, eukaryotic-like Ser/Thr protein kinases. *Mol. Microbiol.* 20, 435–447
14 Hakansson, S.E. *et al.* (1996) The *Yersinia* YpkA Ser/Thr kinase is translocated and subsequently targeted to the inner surface of the HeLa cell plasma membrane. *Mol. Microbiol.* 20, 593–603
15 Wang, J.Y. *et al.* (1998) A novel serine/threonine protein kinase homologue of *Pseudomonas aeruginosa* is specifically inducible within the host infection site and is required for full virulence in neutropenic mice. *J. Bacteriol.* 180, 6764–6768
16 Mukhopadhyay, S. *et al.* (1999) Characterization of a Hank's type serine/threonine kinase and serine/threonine phosphoprotein phosphatase in *Pseudomonas aeruginosa*. *J. Bacteriol.* 181, 6615–6622
17 Motley, S.T. and Lory, S. (1999) Functional characterization of a serine/threonine protein kinase of *Pseudomonas aeruginosa*. *Infect. Immun.* 67, 5386–5394
18 Bliska, J.B. and Falkow, S. (1993) The role of host tyrosine phosphorylation in bacterial pathogenesis. *Trends Genet.* 9, 85–89
19 Menard, R. *et al.* (1996) The secreted Ipa complex of *Shigella flexneri* promotes entry into mammalian cells. *Proc. Natl. Acad. Sci. U. S. A.* 93, 1254–1258
20 Tang, P. *et al.* (1994) *Listeria monocytogenes*, an invasive bacterium, stimulates MAP kinase upon attachment to epithelial cells. *Mol. Biol. Cell.* 4, 455–464
21 Segal, E.D. *et al.* (1996). *Helicobacter pylori* attachment to gastric cells induces cytoskeletal rearangements and tyrosine phosphorylation of host cell proteins. *Proc. Natl. Acad. Sci. U. S. A.* 93, 1259–1264
22 Chow, K.D. *et al.* (1994) Protein tyrosine phosphorylation in *Mycobacterium tuberculosis*. *FEMS Microbiol. Lett.* 124, 203–207
23 Av-Gay, Y. and Davies, J. (1997) Components of eukaryotic-like signaling pathways in *Mycobacterium tuberculosis*. *Microbial Comp. Genom.* 2, 63–73
24 Cole, S.T. *et al.* (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 393, 537–544
25 Hanks, S. and Quinn, A.M. (1991) Use of degenerate oligonucleotide probes to identify clones that encode protein kinases. *Methods Enzymol.* 200, 38–62
26 Behr, M.A. *et al.* (1999) Comparative genomics of BCG vaccines by whole-genome DNA microarray. *Science* 284, 1520–1523
27 Henriques, A.O. *et al.* (1998) Control of cell shape and elongation by the *rodA* gene in *Bacillus subtilis*. *Mol. Microbiol.* 28, 235–247
28 Signoretto, C. *et al.* (1996) Modified peptidoglycan chemical composition in shape-altered *Escherichia coli*. *Microbiology* 142, 1919–1926
29 Av-Gay, Y. *et al.* (1999) Cloning, expression and characterization of *M. tuberculosis* protein kinase B. *Infect. Immun.* 67, 5676–5682
30 Peirs, P. *et al.* (1997) A serine/threonine kinase from *Mycobacterium tuberculosis*. *Eur. J. Biochem.* 244, 604–612
31 Springer, T.A. (1998) An extracellular beta-propeller module predicted in lipoprotein and scavenger receptors, tyrosine kinases, epidermal growth factor precursor and extracellular matrix components. *J. Mol. Biol.* 283, 837–862
32 Cozier, G.E. *et al.* (1995) The structure of the quinoprotein alcohol dehydrogenase of *Acetobacter aceti* modeled on that of methanol dehydrogenase from *Methylobacterium extorquens*. *Biochem. J.* 308, 375–379
33 Belanger, A.E. *et al.* (1997) The *embAB* genes of *Mycobacterium avium* encode an arabinosyl transferase involved in cell wall arabinan biosynthesis that is the target for the antimycobacterial drug ethambutol. *Proc. Natl. Acad. Sci. U. S. A.* 93, 11919–11924
34 Markiewicz, Z. *et al.* (1982) Spherical *E. coli* due to elevated levels of D-alanine carboxypeptidase. *Nature* 297, 702–704
35 Luirink, J. *et al.* (1994) An alternative protein targeting pathway in *Escherichia coli*: studies on the role of FtsY. *EMBO J.* 13, 2289–2296
36 Aslund, F. and Beckwith, J. (1999) The thioredoxin superfamily: redundancy, specificity and gray-area genomics. *J. Bacteriol.* 181, 1375–1379
37 Sikorski, R. *et al.* (1990) A repeating amino acid motif in CDC23 defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis. *Cell* 60, 307–317
38 Cowley, S. *et al.* (1999) Investigations of the eukaryotic Ser/Thr protein kinases of *M. tuberculosis*. *Proc. US-Japan Tuberculosis–Leprosy Research Conference* 34, 308

39 Harth, G. and Horwitz, M.A. (1999) An inhibitor of exported *Mycobacterium tuberculosis* glutamine synthetase selectively blocks the growth of pathogenic mycobacteria in axenic culture and in human monocytes: extracellular proteins as potential novel drug targets. *J. Exp. Med.* 189, 1425–1435
40 Harth, G. *et al.* (2000) Treatment of *Mycobacterium tuberculosis* with antisense oligonucleotides to glutamine synthetase mRNA inhibits glutamine synthetase activity, formation of the poly-L-glutamate/glutamine cell wall structure, and bacterial replication. *Proc. Natl. Acad. Sci. U. S. A.* 97, 418–423
41 Ponting, C.P. *et al.* (1997) PDZ domains: targeting signalling molecules to sub-membranous sites. *Bioessays* 19, 469–479
42 Gupta, S. and Tyagi, A.K. (1993) Sequence of a newly identified *Mycobacterium tuberculosis* gene encoding a protein with sequence homology to virulence-regulating proteins. *Gene* 126, 157–158
43 DeMaio, J. *et al.* (1996) A stationary-phase stress-response sigma factor from *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. U. S. A.* 93, 2790–2794
44 Sibley, L.D. *et al.* (1988) Mycobacterial lipoarabinomannan inhibits gamma interferon–mediated activation of macrophages. *Infect. Immun.* 56, 1232–1236
45 Pollock, K. and Withnall, M.T. (1993) Protein tyrosine kinase but not protein kinase C inhibition blocks receptor-induced alveolar macrophage activation. *Mediators Inflamm.* 2, 373–377

Introducing...microbiology

Microbiology In Action
edited by J. Heritage *et al.*

Cambridge University Press, 1999.
£35.00/$54.95 hbk/
£12.95/$20.95 pbk (xv + 290 pages)
ISBN 0 521 62111 9/
ISBN 0 521 62912 8

This book is one of a series of introductory works in biology, published by Cambridge University Press in association with the Institute of Biology. The series is aimed at readers at first year undergraduate level, which presumably dictates a maximum size and price. This particular volume is presented as a companion to *Introductory Microbiology*, which is in the same series, by the same authors and, as they admit, this volume is based on the material that had to be omitted from the earlier volume owing to space constraints. Herein lies an irritation for me, and I suspect a far greater irritation for many other potential readers: I did not have the introductory text to hand. Although this did not cause major problems for me in understanding this book, I suspect it will mean that the undergraduate reader will often struggle to assimilate complex topics that cannot be introduced fully in the space available here. For example, quite detailed flow charts for the identification of medically important bacteria are given, but the related text is condensed into a few pages and cannot provide all the necessary explanations (why is gas–liquid chromatography used for anaerobes and what does it do?). Many readers will therefore need to read this book with other sources of information to hand to provide underlying basic information. Unfortunately, despite the space constraint, some space is squandered, for example on chemical sources of food poisoning.

The approach adopted by the authors to make this book readable and arouse interest in the target population (a notoriously difficult task in introductory microbiology, often taught to refractory 'general' biologists!), is to pose a series of questions as headings of numbered sections within each chapter. This approach does work. Critically, it depends on the questions asked, which must be answerable in the space provided and leave the reader feeling informed as a result. Rarely did I find such questions left unanswered, although I cannot resist mentioning that 'What are poliomyelitis and chronic fatigue syndrome?' left the second part not only unanswered but barely addressed. A large and difficult question indeed and it was not the best place to ask it! The style of writing is generally friendly, with an admirable use of short, informative sentences,which I wish I could emulate.

The topics chosen for exploration in the book are appropriate, and the authors have generally resisted the strong temptation for medically oriented microbiologists to focus too heavily on medical aspects of the subject. The positive aspects of the role of microorganisms in soil and elemental cycling are covered first, and their uses in food preparation are covered later in the book. Nevertheless, the average first year undergraduate does have a fascination with the downside of the subject, including the gruesome consequences of a variety of infections, and these topics are well covered. Infections are largely approached from the clinical viewpoint of the symptoms or syndrome rather than the microorganism, but the question and answer format does allow questions about the organisms to be interspersed effectively with the clinical issues.

One can usually find fault with the detail of books written by academics (specialists) for a general audience and this book is no exception. I was surprised to learn that viroids comprise RNA molecules much smaller than the genome of typical RNA viruses, being only about 200 kb! It is a pity to find that authors can be constrained by illogical definitions, which sometimes have widespread acceptance, and not explain or perhaps even recognize the problem. Thus, I thought initially that *Campylobacter enteritis* had been omitted from the book, as it did not appear with *Salmonella* and *Escherichia coli* infections in answer to the question 'What food poisoning is associated with bacterial infection?'. I found it several pages away under the heading 'What are food-borne infections?', and I challenge the reader to provide an explanation to the first year undergraduate for this idiosyncratic distinction. Worse, one of the most important bacterial pathogens of humans is covered only with a brief mention under 'Water-borne *Campylobacte*r infections': it is *Helicobacter pylori*! I was disappointed by the standard of proof reading; for example there are several errors within a page or two in the section on