Abstract

Mycobacterium tuberculosis profoundly exploits protein phosphorylation events carried out by Serine/Threonine Protein Kinases (STPKs) for its survival and pathogenicity. Genes encoding STPKs once thought to be unique to eukaryotes are now an established integral component of many prokaryotic genomes. The genome of *M. tuberculosis*, the causative agent for tuberculosis, has shown the presence of 11 genes that code for STPKs and one gene (Mstp) that codes for Ser/Thr phosphatase. Nine of these kinases are predicted to be localized to the cell membrane due to the presence of a transmembrane domain. The transmembrane domain connects the N-terminal kinase domain inside the cell to a C-terminal sensory component outside the cell. The C-terminal domain is presumed as a sensor of environmental signals which then transduce them within the cell for the generation of an adaptive response. These STPKs and Mstp have emerged as crucial players for environmental sensing and physiological signaling in prokaryotes. The co-ordinated action of these signaling modules leads to the formation of numerous signaling cascades essential for cell survival. Consequently, they have been implicated in diverse control mechanisms, including stress responses, developmental changes and host–pathogen interactions, in several microorganisms. Interestingly, these STPKs have also been proposed to mediate signaling between mycobacteria and host cells to establish an environment that is favourable for the replication and survival of mycobacteria.

Knowledge of the substrates of each of the *M. tuberculosis* STPKs is essential for understanding their function; however, a limited number of kinase-substrate cognate pairs have been characterized to date. The presence of 11 STPK genes in the *M. tuberculosis* genome suggests that Ser/Thr phosphorylation is an important mechanism for signal transduction in this organism but that the total phosphoproteome is likely to be substantially smaller than that of most eukaryotes. In *M. tuberculosis*, Ser/Thr Protein Kinases (STPKs) have been wired to large number of events concerning cell development, metabolism, cell wall synthesis, stress-response and virulence.

We have tried to establish the role of phosphorylation in the regulation of protein translational machinery through *M. tuberculosis* Elongation factor Tu (*Mtb*Ef-Tu), described in chapter 1. Ef-Tu and its homologs play a pivotal role in protein

biosynthesis in both prokaryotes and eukaryotes. It is a highly conserved, multi-domain protein which interacts with RNA, proteins and nucleotides. Ef-tu has been historically known to be regulated by multiple post-translational modifications including phosphorylation, acetylation and methylation in various species, especially *E. coli*. This intrigued us to find out the possible regulation of mycobacterial Ef-Tu by phosphorylation. Present study reveals *Mtb*Ef-Tu as the substrate of multiple STPKs of *M. tuberculosis* and phosphorylation by PknB inversely affects its interaction with GTP. The study is further detailed in terms of roles of Ef-Ts and kirromycin. Additionally, differential phosphorylation by PknA and PknB and the significance of *Mtb*Ef-Tu phosphorylation site Thr118 is discussed.

Till date a large number of mycobacterial proteins are shown to be the substrates of STPKs. These substrates also get dephosphorylated by the only known Ser/Thr phosphatase Mstp. On the basis of domain architecture, Ser/Thr phosphatases are divided into the PPP and PPM families. PP1, PP2A and PP2B phosphatases are grouped in the PPP family and PP2C is the classic member of the PPM family. PP2C phosphatases are dependent on the divalent cations Mg^{2+} or Mn^{2+} in addition to being resistant to standard PPP inhibitors (okadaic acid, sodium orthovanadate, calyculinA), which actually make it an independent class.

Mstp also belongs to the PP2C superfamily of phosphatases and requires Mn^{2+} for activity. It is a membrane localized enzyme with 237 residue long intracellular catalytic domain joined by a juxtamembrane region to 191 residue extracellular domain (196 aa) rich in proline and serine residues with single transmembrane helix. Genomic organization of Mstp in *M. tuberculosis* revealed that this gene is a part of 7.6 kb region that encodes five overlapping ORFs. This gene cluster consists of two Ser/Thr kinases (PknA and PknB) and other genes involved in cell wall synthesis.

In due absence of precise mechanisms, most of the membrane associated kinases and phosphatases are known or hypothesized to be regulated by external stimulus. In chapter 2, we have demonstrated an example of PknA and PknB mediated regulation of *M. tuberculosis* Mstp through inter-dependent phosphorylation-dephosphorylation reactions. Regulation of phosphatases by phosphorylation has been an important part

in cell signaling events. It is also associated with feedback phenomena in case the phosphatases are phosphorylated by kinases that are in turn dephosphorylated by the same phosphatase. Certain examples in literature illustrate the phosphorylation of PP2C phosphatases, but not detailed in terms of feedback regulation. A number of reports over the years have shown the role of Mstp in unanimous dephosphorylation of almost all the *M. tuberculosis* STPKs and their substrates. However, there is void of information about the conditions in which either kinase or phosphatases exert their effects, which are essentially opposite of each other. This study reveals the novel mechanism of Mstp being phosphorylated by PknA and PknB on multiple residues and its regulation is discussed further.