J Biosci (2019) 44:24
DOI: 10.1007/s12038-018-9828-4

Review

Interrelation of Ca^{2+} and PE_PGRS proteins during *Mycobacterium tuberculosis* pathogenesis

LAXMAN S MEENA

CSIR–Institute of Genomics and Integrative Biology, Delhi 110 007, India

(Email, meena@igib.res.in, Laxmansm72@yahoo.com)

MS received 9 March 2018; accepted 30 October 2018; published online 7 February 2019

In today's era tuberculosis is a major threat to human population. The lethality of this disease is caused by very efficiently thrived bacteria *Mycobacterium tuberculosis* (*M. tuberculosis*). Ca^{2+} plays crucial role in maintenance of cellular homeostasis. Bacilli survival in human alveolar macrophages majorly depends on disruption in Ca^{2+} signaling. Bacilli sustainability in phagosome lies in the interruption of phagolysosomal fusion, which is possible because of low intracellular Ca^{2+} concentration. Bacilli contain various Ca^{2+} binding proteins which help in regulation of Ca^{2+} signaling for its own benefit. For the survival of pathogen, it requires alteration in normal Ca^{2+} concentration in healthy cell. In this review we aim to find the various Ca^{2+} binding domains which are present in several Ca^{2+} binding proteins of *M. tuberculosis* and variety of roles played by Ca^{2+} to survive bacilli within host cell. This manuscript emphasizes the Ca^{2+} binding domains present in PE_PGRS group of gene family and their functionality in *M. tuberculosis* survival and pathogenesis.

Keywords. Ca^{2+} signaling; Ca^{2+} binding domains; Ca^{2+}; dormant stage; *M. tuberculosis*; PE_PGRS

Abbreviations used: Ca^{2+}, calcium ions; CAMLP, calmodulin-like proteins; *M. tuberculosis*, *Mycobacterium tuberculosis*; PE, Pro-Glu sequence; PE_PGRS, proline-glutamic polymorphic GC-rich repetitive sequence; TB, tuberculosis.

1. Introduction

Tuberculosis (TB) has become a serious disease for the human population as it infests and persists successfully in host. Till date the global scenario of cause of TB worldwide is chaotic (Meena and Rajni 2010; Kumari and Meena 2014). According to a WHO report, TB is the ninth leading cause of death worldwide and approximately 10.4 million people fell ill in 2017 (WHO 2017). According to previous studies, whole genome sequencing of *M. tuberculosis* provides better understanding of microbial pathogenesis and etiology (Cole 1999). A distinct multigene family found in *M. tuberculosis* genome consists of two closely related subfamilies PE (Pro-Glu sequence) and PE_PGRS (Proline-glutamic polymorphic GC-rich repetitive sequence), which form 5% of total *M. tuberculosis* genome (Meena 2015). PE subfamily consists of 38, while PE_PGRS contains 61 homologous genes which are distributed throughout *M. tuberculosis* genome (Tekaia *et al.* 1999). The PE_PGRS has conserved N terminal PE domain, which shows high similarity to PE family members and variable C terminal PGRS domain. PGRS domain contains repeats of Gly-Gly-Ala and Gly-Gly-X, where X can be any amino acid (Poulet and Cole 1995). PE_PGRS genes encrypt proteins of multiple lengths, widely varying from range of 175 to 1901 amino acids. The presence of many related genes in *M. tuberculosis* infers that the encoded proteins must play role in survival and growth of mycobacterial cell within its chosen environment. PE_PGRS is still very unique to *M. tuberculosis* as it is not found in genome of any other organisms (Brennan and Delogu 2002). So, it makes PE_PGRS genes an exciting field of research in *M. tuberculosis* pathogenesis. Previous studies on these genes showed that they aid as virulence factor of *M. tuberculosis* during infection and expressed in macrophage during granuloma formation in host (Ramakrishnan *et al.* 2000). PE_PGRS group of subfamily has the potential to bring strong immune response within host cell and promote apoptosis and necrosis in eukaryotic cell (Dheenadhalayan *et al.* 2006). During invasion of any pathogen inside the host cell, core atmosphere of the cell changes leads to the cascade of abnormal events. These cells require messenger molecules which can act as notable indicator of manipulative events (Shaw and Meena 2016). For this purpose, Ca^{2+} plays a crucial role by behaving as a

secondary messenger in the host cell (Clapham 2007). To effectively endorse a variety of functions by PE_PGRS gene family, they need a binding domain for Ca^{2+}. Numerous binding domains are available which effectively control Ca^{2+}concentration. Abundant host factors affect the varied functionality of PE_PGRS group of genes during infection. This article deals with the role and interaction of Ca^{2+} with PE_PGRS genes in the pathogenesis of *M. tuberculosis*.

2. Ca^{2+} binding domains in *M. tuberculosis*

Ca^{2+} shows its effect by changing its intracellular concentration, and these alterations are generated by various Ca^{2+} binding molecules, which in turn decline free Ca^{2+} concentration within the cell. Ca^{2+} has been reported to have a major role in phagocytosis of the pathogen through various receptor-mediated events (Meena *et al.* 2016). Ca^{2+} transmits the information via phosphorylation event, protein interaction or change in gene expression (Hashimoto and Kudla 2011). These proteins are predominantly found in eukaryotes and are well studied, but in prokaryotes, their role is still not clear (Permyakov and Kretsinger 2009). According to recent studies, some common bacteria like *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas* have shown various gene expression and cellular processes by varying extracellular Ca^{2+} concentration, thus indicating the importance of Ca^{2+} in prokaryotic life (Patrauchan *et al.* 2007; Naseem *et al.* 2009; Oomes *et al.* 2009; Bilecen and Yildiz 2009; Dominguez 2011). Further, whole genome sequencing showed the presence of Ca^{2+} binding domains in *M. tuberculosis*. Ca^{2+} binding proteins have different domains like EF-hand domain, helix-loop-helix, Greek key motifs of βγ-crystalline, etc. (Yang 2001; Michiels *et al.* 2002; Rigden *et al.* 2003; Zhou *et al.* 2006; Aravind *et al.* 2009). There is a need to further investigate and find the role of Ca^{2+} binding proteins in prokaryotes. Calmodulin (CaM) is a widely known Ca^{2+} binding protein family which plays a major role in Ca^{2+}-mediated signaling in various cellular responses. CaM is a sensor protein which has 4 EF-hand motifs and binds to Ca^{2+} whenever its concentration increases, thus simultaneously altering various enzymatic activities (Cheung 1982). Increase in cytoplasmic Ca^{2+} is known to bring structural change in CaM by altering the structure of CaM-dependent protein kinase-II (CamKII) (Harmon *et al.* 1985). In prokaryotes, calmodulin-like proteins (CAMLP) were discovered in *Bacillus subtilis* (*B. subtilis*) and *Escherichia coli* (Fry *et al.* 1986). Later, the presence of CAMLP was shown in various strains of *M. tuberculosis,* like *Mycobacterium bovis (M. bovis)*, *Mycobacterium smegmatis (M. smegmatis)*, *Mycobacterium phlei (M. phlei)* and *M. tuberculosis* (Burra *et al.* 1991; Burra *et al.* 1995; Falah *et al.* 1988; Sharma and Meena 2017). Further, it was revealed that bacterial CAMLP have homology with eukaryotic CaM in terms of binding domain, like EF-hand domain with different binding sites. Recently, it was discovered that the product of gene Rv1211 of *M. tuberculosis* H_{37}Rv is an effective CAMLP, as it shows the Ca^{2+}-dependent activation of PDE (phosphodiesterase) and NAD (Nicotinamide adenine dinucleotide) kinase, which is exact feature of eukaryotic calmodulin (Reddy *et al.* 1992; Koul *et al.* 2009). Ca^{2+} signaling pathway is one of the major tracks other than MAPK (mitogen-activated protein kinase) and IFN-γ (interferon-gamma), which is used by *M. tuberculosis* bacilli to prevent phagosome–lysosome fusion within host (Koul *et al.* 2004). Phagolysosome formation, phagosome acidification, recruitment of lysosomal hydrolases, etc., are results of alteration in cytoplasmic Ca^{2+} concentration (Harmon *et al.* 1985). In eukaryotic cells, Ca^{2+} concentration is low comparted to the extracellular matrix because several cellular organelles like endoplasmic reticulum, mitochondria, etc., sequester the Ca^{2+} ions and maintain a sharp gradient of Ca^{2+} along the wall of the organelles (Gilabert 2012; Shaw and Meena 2016). In similar manner prokaryotic cells also uphold the gradient of cytosolic Ca^{2+} ions, but the mechanism behind it is still ambiguous (Torrecilla *et al.* 2000; Jones *et al.* 1999; Zhou *et al.* 2006). Possibly, some kind of CAMLP is required by bacterial cells to maintain the transient change in Ca^{2+} concentration. Proteomic analysis of *B. subtilis* by autoradiography suggested that various protein components bind with Ca^{2+} (Dominguez 2011). In prokaryotic cells, Ca^{2+} is stored in periplasmic space, which functions as barrier to Ca^{2+} entry and plays a major role in Ca^{2+} homeostasis across the membrane (Gangola and Rosen 1987).

3. Ca^{2+} binding motif

PE_PGRS genes family is one of the unique gene sequences in genome analysis, as it is confined to only *M. tuberculosis* H_{37}Rv strain (Copin *et al.* 2014). RTX (repeats in toxin) superfamily is a group of exoproteins that act as cytolysins and cytotoxin secreted by bacteria. These family proteins possess two distinct features, a unique way of exporting across the bacterial wall, and they have tandem repeats of nonapeptide GGXGXD/NXUX, where X represents any amino acid and U is non-polar or hydrophobic large residue which binds to Ca^{2+} (Coote 1992; Baumann *et al.* 1993). These nonapeptide repeats craft a Ca^{2+} binding motif popularly known as parallel β-helix or β-roll (Lilie *et al.* 2000). Due to the presence of these nonapeptides, various PE_PGRS gene family members are also stated as cell surface exposed adhesion molecules (Delogu *et al.* 2004; Sachdeva *et al.* 2005) which further involve in pathogenesis (Bachhawat and Singh 2007). Oddly, 56 of 61 PE_PGRS genes consists of these nonapeptide sequence; however, their functional characterization is still ambiguous (Michiels *et al.*

Table 1. List of various Ca^{2+} binding domains and proteins in *M. tuberculosis*

Calcium binding motif	Binding sequence	Description	Calcium binding proteins in *M. tuberculosis*	References
Parallel β-helix or β-roll	GGXGXD/NXUX	X is any amino acid, U is unpolar or hydrophobic large residue	PE_PGRS family binding proteins	Gilabert (2012), Jones *et al.* (1999), Torrecilla *et al.* (2000), Gangola and Rosen (1987), Copin *et al.* (2014), Coote (1992), Baumann *et al.* (1993)
EF-hand binding motif/ domain	Dx[DN]xDGx[ILV][DSTN]x DXDXDG	Two α helices which consist of acidic amino acids	Rv1211 contains one prototypic calcium binding EF-hand motif	Lilie *et al.* (2000), Delogu *et al.* (2004), Sachdeva *et al.* (2005), Koul *et al.* (2009)
Greek key motif	N/D-N/D-#-I-S/T-S, #	Unique ser/thr hydroxyl group	Calmodulin-like proteins	Rigden *et al.* (2003), Bachhawat and Singh (2007), Michiels *et al.* (2002)
Big domain	DNSNKDITSAVTDxSNxDxxSxVT	Immunoglobulin-like Ca^{2+} binding domains	Leptospira immunoglobulin-like protein LigA and LigB	Yeruva *et al.* (2016), Kretsinger (1976), Rigden and Galperin (2004)
Ca^{2+} dodecin	Rv0379 of *M. tuberculosis*	Like copper binding domain	Calcium dodecin (Rv0379)	Wistow (1990), Srivastava *et al.* (2014)

2002). Another domain, namely, EF-hand, is present, which is about 70 to 184 amino acids in length, possessing high phenylalanine/tyrosine ratio (Yeruva *et al.* 2016). Ca^{2+} binding domain in this superfamily is flanked by two α-helices which consist of acidic amino acids (Kretsinger 1976). Dx[DN]xDGx[ILV][DSTN]x is the sequence pattern which typically constitute Ca^{2+} binding loop with minor changes in others (Rigden and Galperin 2004). Another widely found Ca^{2+} binding domain is Greek key motif of βγ-crystalline (Wistow 1990) in which Ca^{2+}coordination is conserved as N/D-N/D-#-I-S/T-S, in which # residue offers the carbonyl chain and I residue provides the hydrophobic core of the domain (Srivastava *et al.* 2014). This motif requires unique ser/thr hydroxyl group for Ca^{2+} binding (Lin *et al.* 2008). Yet another Ca^{2+} binding domain is Big domain, which is related to a group of proteins having immunoglobulin-like Ca^{2+} binding domains (Halaby *et al.* 1999). It is recognized in several enzymes, transporters, molecular chaperons and other membrane-bound proteins (Raman *et al.* 2011). Although the nature of binding domain is not identified, tandem repeats of DNSNKDITSAVTDxSNxDxxSxVT sequences are present (Arockiasamy *et al.* 2011). A very unique and distinctive Ca^{2+} binding domain is reported in *M. tuberculosis* gene Rv0379 which has 71 kDa protein and is thought to be involved in protein transportation as secE2 (Barnham *et al.* 2003). This protein is identified as Ca^{2+} dodecin by its crystal structure, which is not seen anywhere else in the genome. The folds of Ca^{2+} dodecin show similarity as copper binding domain of amyloid precursor protein of Alzheimer's disease (Ikura *et al.* 2002), as shown in table 1. Variety of Ca^{2+} binding domains are found in prokaryotic organisms with wide structural range, and binding of Ca^{2+} to these proteins confers various structural deformations, which affect structural or functional changes in cell.

4. Varied roles of Ca^{2+} in *M. tuberculosis* pathogenesis

Ca^{2+} is an important and multipurpose molecule which is used by cells in their growth and proper functionality, whether they are prokaryotic or eukaryotic. It is a key element to convey various cellular messages intracellularly and regulates normal routine tasks of cell (Sanders *et al.* 1999; Berridge *et al.* 2000; Berridge *et al.* 2003; Hashimoto and Kudla 2011; Zampese and Pizzo 2012). The amount of free Ca^{2+} within the cell rapidly changes according to stimulus and varied concentration of Ca^{2+}, which in turn is sensed by cell to respond in a particular direction. Ca^{2+} concentration is not solely enough to make change in cellular processes; it also depends on signal specifications like its speed, frequency and magnitude (Jayachandran *et al.* 2007). In response to stimulus initiated by Ca^{2+} signaling, a chain of downregulated Ca^{2+} binding proteins are activated, which further aids in progression of the signaling pathway. During aerosol transmission of bacilli, they get engulfed by alveolar macrophages through phagocytosis. The foremost step of *M. tuberculosis* survival within host is to inhibit the formation of phagolysosome (Ferrari *et al.* 1999). FnBPs binds with Fn through integrin receptor and initiates PKCα (protein kinase C alpha) pathway. This increases Ca^{2+} concentration in cell and initiates several signaling cascade. Coronin/TACO is activated by calcineurin, which is Ca^{2+}- and calmodulin-dependent ser/thr phosphatase. It is also an actin binding

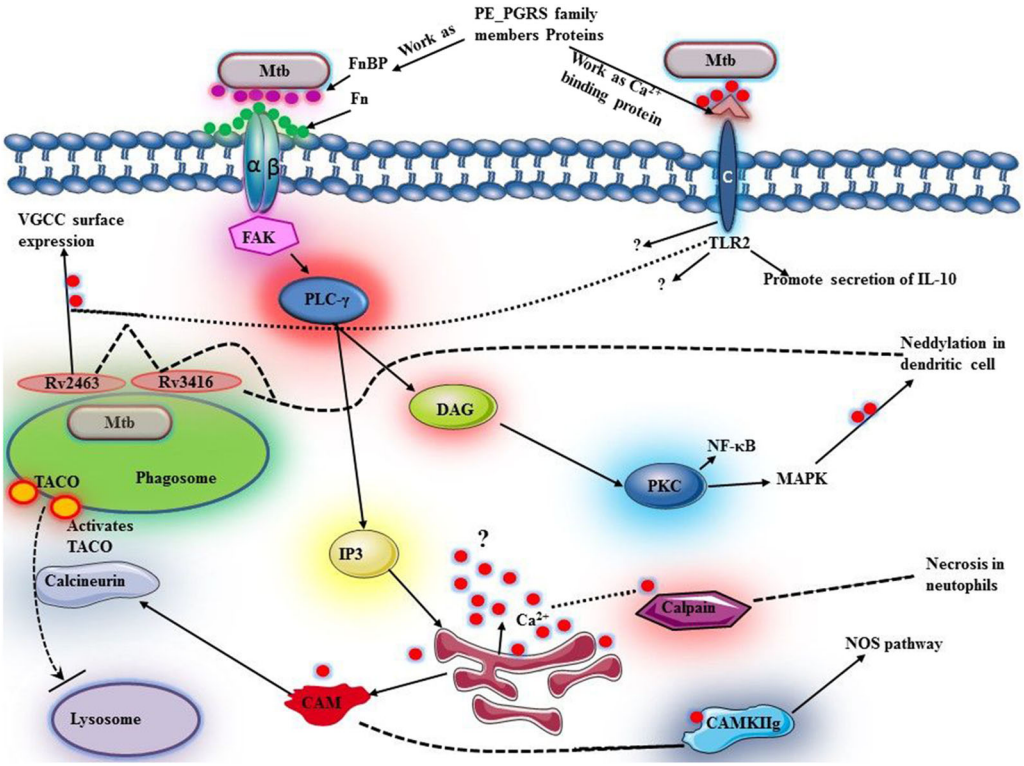

Figure 1. Schematic representation of multiple roles played by Ca^{2+} in context to Mtb survival and pathogenesis inside host cell. Invasion of bacilli in healthy macrophage cell initiates after binding of FnBP proteins with integrin receptor present on cell membrane. This binding initiates Plc-γ pathway, which dissociates its substrate PIP2 (Phosphatidylinositol 4,5-bisphospahte) into DAG (Di-acyl glycerol) and IP3. IP3 activates Ca^{2+} channel present on ER, which enhances intracellular Ca^{2+} level. This increased level of Ca^{2+} is necessary for phagocytic action of macrophage. Mtb manipulates this signaling by using its various Ca^{2+} binding proteins. Ca^{2+} binds to CAM, which activates CAMKIIg and Calcinuerin. Calcinuerin activates TACO or coronin protein, which binds with the membrane of phagosome inhibits lysosome. Another Ca^{2+}-dependent protease calpain activates and initiates necrosis in neutrophils. The other component of PIP2 is DAG, which initiates PKC pathway, which in turn activates NF-κB and MAPK pathway. MAPK pathway induces neddylation in dendritic cells. Rv2463 and Rv3416 are Mtb proteins which increased the surface level expression of VGCC (Voltage gated calcium channel). There is another way to infect macrophage through PE_PGRS genes having Ca^{2+} binding domain. These proteins interact with TLR2 receptor and change the IL-10 signaling in favor of bacilli survival. There are yet undiscovered paths which are activate by PE_PGRS proteins.

protein which adheres at phagosome membrane and prevents the maturation of phagosome containing bacilli (Majeed *et al.* 1998; Trimble and Grinstein 2007). Generally, higher Ca^{2+} concentration is required in phagocytosis process. Therefore, elevated level of Ca^{2+} ion is used by bacilli in favor of its own sustainability (Malik *et al.* 2000; Vergne *et al.* 2003). Higher concentration of Ca^{2+} minimizes severe immune response during Mtb infection and also inhibits heavy inflammation. Majorly, calmodulin is the molecule which binds Ca^{2+} and initiates PKCα and CAMKIIg (Ca^{2+}/calmodulin-dependent protein kinase II gamma) (Yadav *et al.* 2004; NYU 2015). According to a recent study, use of siRNA and inhibitors of CAMKIIg depicts that it is pro-apoptotic and critical for activation of ERK1/2 (extracellular signal regulated kinases). By inhibiting ERK1/2, production of NOS2 (nitric oxide synthase 2) and NO (nitric oxide) also decrease. In *Mycobacterium fortuitum,* it had been reported that ERK1/2 and NOS2 axis activation through PKCα pathway is important for caspase-dependent apoptosis (Banerjee *et al.* 2014). Earlier studies also revealed that Rv2463 and Rv3416 genes of *M. tuberculosis* involve in neddylation, which is a process similar to ubiquitination in which NEDD8 protein binds to its target proteins having its own E1 and E2 enzymes (Datta *et al.* 2016). These genes modulate NEDD8 and cullin-1 association. This connotation also belongs to Ca^{2+} and MAPK-dependent pathway which suppresses host immune system via modulating neddylation process in dendritic cells (Huang *et al.* 2004; Chadha *et al.* 2015). One of the genes *Rv0805* is dinuclear metallohydrolase and involves in signal transduction by binding of two divalent metal ions (Podobnik *et al.* 2009). Ca^{2+} also binds with *Rv0805* but the binding sites are different. Here Ca^{2+} acts as an activator of enzymatic activity and also promotes the hydrolysis of substrate even in the absence of metal ions (Shenoy *et al.* 2007). Some studies also suggest the involvement of VGCC (voltage-gated Ca^{2+} channel) in regulation of *M. tuberculosis* survival and pathogenesis inside host. VGCC are the Ca^{2+}-permeable channels found

in excitable membranes. One of the gene of *M. tuberculosis, Rv2463,* was reported to regulate VGCC surface expression in host cells (Nejatbakhsh and Feng 2011), where Ca^{2+} plays a key role in the appearance of *CACNA 1S* (a gene belong to a family which provide instructions for the formation of Ca^{2+} channel). This regulation is directed by MyD88-independent TLR pathway in which transcription factor pCREB and STIM 1 and STIM 2 (stromal interaction molecule 1 and 2), which are ER-associated Ca^{2+} sensors, are also involved. Fascinatingly, ROS (reactive oxygen species) plays negative role in the expression of *CACNA 1S* which revealed that ROS and pCREB cross-regulate and direct this pathway (Antony *et al.* 2015). PE_PGRS genes containing nona-domain have the ability to bind with Ca^{2+} and initiate infection in macrophage through TLR2 (toll-like receptor). This binding modifies IL-10, an anti-inflammatory interleukin, signaling. One recent study reported that these proteins binds TLR2 with lower affinity when Ca^{2+} concentration is depleted within cell. It revealed the importance of PE_PGRS with Ca^{2+} in *M. tuberculosis* pathogenesis (Yeruva *et al.* 2016), as shown in figure 1. As PE_PGRS family proteins possess both Fn and Ca^{2+} binding domains, they act as adhesion as well as signaling molecules (Meena and Meena 2016 and Monu and Meena 2016). According to another report, in *Leptospira* species, LigB protein containing Fn and Ca^{2+} binding domain revealed that its binding with Fn during adhesion is enhanced by the presence of Ca^{2+}. So, we can say that availability of Ca^{2+} in host cell boosts the attachment of pathogen with membrane (Lin *et al.* 2008). To know the role of Ca^{2+} further in the pathology of *M. tuberculosis,* we need detailed signaling inputs and outputs of bacilli infection in host cell.

5. Summary

Ca^{2+} is a multipurpose molecule in maintenance of cellular homeostasis. Ca^{2+} acts as an important signaling molecule in all aspects of cell growth. To modulate cell functionality, different Ca^{2+} binding proteins are involved in changing Ca^{2+} concentration. It is also a multifaceted molecule which enhances several pathways and regulates the immune cells during any pathogenic infection. To enhance the bacterial survival within host, low Ca^{2+} concentration is maintained. This is achieved by the presence of various Ca^{2+} binding proteins in *M. tuberculosis*. These Ca^{2+} binding proteins further manipulate the Ca^{2+} signaling so as to benefit bacilli survival. PLC-γ pathway is the basic signaling cascade of Ca^{2+}. Calcinuerin promote the inhibition of the basic phagocytic function of cell, i.e. fusion of phagolysosome. Varied Mtb proteins having variety of Ca^{2+} binding domains enhance the binding efficiency and produce different results, which help bacteria to remain inside the cell. The interconnection of $Ca^{2+}$and PE_PGRS proteins in the pathogenic aspect of bacilli has provided interesting facts: PE_PGRS family members of proteins are involved in adhesion and as signaling modulator by working as an FnBP and Ca^{2+} binding proteins. To know the deep molecular mechanism behind the survival of bacilli in host cells, we need to elucidate the variety of role played by Ca^{2+} and its binding proteins with major emphasis on PE_PGRS members.

Acknowledgements

The authors acknowledge financial support from GAP0145 of the Department of Science and Technology and the Council of Scientific and Industrial Research, India.

References

Antony C, Mehto S, Tiwari BK, Singh Y and Natarajan K 2015 regulation of l-type voltage gated calcium channel CACNA1S in macrophages upon *Mycobacterium tuberculosis* infection. *PLOS One* **10** e0124263

Aravind P, Mishra A, Suman SK, Jobby MK, Sankaranarayanan R and Sharma Y 2009 The beta gamma-crystallin superfamily contains a universal motif for binding calcium. *Biochemistry* **48** 12180–12190

Arockiasamy A, Holzenburg A, Aggarwal A, Savva CG and James C 2011 Sacchettini crystal structure of calcium dodecin (Rv0379), from *Mycobacterium tuberculosis* with a unique calcium-binding site. *Protein Sci*. **20** 827–833

Bachhawat N and Singh B 2007 Mycobacterial PE PGRS proteins contain calcium-binding motifs with parallel β-roll folds. *Genomics Proteomics Bioinformatics* **5** 236–241

Banerjee C, Khatri P, Raman R, Bhatia H, Datta M and Mazumder S 2014 Role of calmodulin calmodulin kinase II, camp/protein kinase a and Erk 1/2 on *Aeromonas hydrophila*-induced apoptosis of head kidney macrophages. *PLoS Pathog.* **10** e1004018

Barnham KJ, McKinstry WJ, Multhaup G, Galatis D, Morton CJ, Curtain CC, Williamson NA, White AR, Hinds MG, Norton RS, Beyreuther K, Masters CL, Parker MW and Cappai R 2003 Structure of the Alzheimer's disease amyloid precursor protein copper binding domain. A regulator of neuronal copper homeostasis. *J. Biol. Chem.* **278** 17401–17407

Baumann U, Wu S, Flaherty KM and McKay DB 1993 Three-dimensional structure of the alkaline protease of *Pseudomonas aeruginosa*: a two-domain protein with a calcium binding parallel beta roll motif. *EMBO J.* **12** 3357–3364

Berridge MJ, Bootman MD and Roderick HL 2003 Calcium signalling: dynamics, homeostasis and remodelling. *Nat. Rev. Mol. Cell. Biol.* **4** 517–529

Berridge MJ, Lipp P and Bootman MD 2000 The versatility and universality of calcium signalling. *Nat. Rev. Mol. Cell. Biol.* **1** 11–21

Bilecen K and Yildiz FH 2009 Identification of a calcium-controlled negative regulatory system affecting *Vibrio cholera* biofilm formation. *Environ. Microbiol.* **11** 2015–2029

Brennan MJ and Delogu G 2002 The PE multigene family: a 'molecular mantra' for mycobacteria. *Trends Microbiol.* **10** 246–249

Burra SS, Reddy PH and Murthy PS 1995 Effect of some antitubercular drugs on the calmodulin content of *Mycobacterium tuberculosis*. *Ind. J. Clin. Biochem.* **10** 126–128

Burra SS, Reddy PH, Falah SM, Venkitasubramanian TA and Murthy PS 1991 calmodulin-like protein and the phospholipids of *Mycobacterium smegmatis*. *FEMS Microbiol. Lett.* **64** 189–194

Chadha A, Mehto S, Selvakumar A, Vashishta V, Kamble SS, Popli S, Raman R, Singh Y and Natarajan K 2015 Suppressive role of neddylation in dendritic cells during *Mycobacterium tuberculosis* infection. *Tuberculosis* **95** 599–607

Cheung W Y 1982 Calmodulin: an overview. *Fed. Proc.* **41** 2253

Clapham DE 2007 Calcium Signaling. *Cell* **131** 1047–1058

Cole ST 1999 Learning from the genome sequence of *Mycobacterium tuberculosis* H_{37}Rv. *FEBS Lett.* **452** 7–10

Coote JG 1992 Structural and functional relationships among the RTX toxin determinants of Gram negative bacteria. *FEMS Microbiol. Rev.* **8** 137–161

Copin R, Coscollá M,Seiffert SN, Bothamley G, Sutherland J, Mbayo G, Sebastien Gagneux S and Ernst JD 2014 Sequence diversity in the PE_PGRS genes of *Mycobacterium tuberculosis* is independent of human T cell recognition. *mBio* **5** e00960–13

Datta D, Khatri P, Banerjee C, Singh A, Meena R, Saha DR, Raman R, Rajamani P, Mitra A and Mazumder S 2016 calcium and superoxide-mediated pathways converge to induce nitric oxide-dependent apoptosis in *Mycobacterium fortuitum* infected fish macrophages. *PLoS One* **11** e0146554

Delogu G, Pusceddu C, Bua A, Fadda G, Brennan MJ and Zanetti S 2004 Rv1818c-encoded PE PGRS protein of *Mycobacterium tuberculosis* is surface exposed and influences bacterial cell structure. *Mol. Microbiol.* **52** 725–733

Dheenadhalayan V, Delogu G, and Brennan MJ 2006 Expression of the PE_PGRS 33 protein in *Mycobacterium smegmatis* triggers necrosis in macrophages and enhanced mycobacterial survival. *Microbes Infect.* **8** 262–272

Dominguez DC 2011 Proteome analysis of *B. subtilis* in response to calcium. *J. Anal. Bioanal. Tech.* https://doi.org/10.4172/2155-9872.s6-001

Falah AMS, Bhatnagar R, Bhatnagar N, Singh Y, Sidhu GS, Murthy PS and Venkitasubramanian TA 1988 On the presence of calmodulin-like protein in mycobacteria. *FEMS Microbiol. Lett.* **56** 89–93

Ferrari G, Langen H, Naito M and Pieters J 1999 A coat protein on phagosomes involved in the intracellular survival of mycobacteria. *Cell* **97** 435–447

Fry IJ, Villa L, Kuehn GD and Hageman JH 1986 Calmodulin-Like Protein from *Bacillus subtilis*. *Biochem. Biophys. Res. Commun.* **134** 212–217

Gangola P and Rosen BP 1987 Maintenance of intracellular calcium in *Escherichia coli*. *J. Biol. Chem.* **262** 12570–12574

Gilabert JA 2012 Cytoplasmic calcium buffering. *Adv. Exp. Med. Biol.* **740** 483–98

Global Tuberculosis Report 2017 World Health Organization http://www.who.int/tb/publications/global_report/MainText_13Nov2017.pdf?ua=1

Halaby DM, Poupon A and Mornon J 1999 The immunoglobulin fold family: sequence analysis and 3D structure comparisons. *Protein Eng.* **12** 563–571

Harmon, AC, Prasher D and Cormier MJ 1985 High Affinity Calcium Binding proteins in *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **127** 31–36

Hashimoto K and Kudla J 2011 Calcium decoding mechanisms in plants. *Biochimie* **93** 2054–2059

Huang DT, Miller DW, Mathew R, Cassell R, Holton JM, Roussel MF and Schulman BA 2004 A unique E1-E2 interaction required for optimal conjugation of the ubiquitin-like protein NEDD8. *Nat. Struct. Mol. Biol.* **11** 927e35

Ikura M, Osawa M and Ames JB 2002 The role of calcium-binding proteins in the control of transcription: structure to function. *BioEssays* **24** 625–636

Jayachandran R, Sundaramurthy V, Combaluzier B, Korf H, Huygen K, Miyazaki T, Albrecht I, Massner J and Pieters J 2007 Survival of mycobacteria in macrophages is mediated by coronin 1-dependent activation of calcineurin. *Cell* **130** 37–50

Jones HE, Holland IB, Baker HL and Campbell AK 1999 Slow changes in cytosolic free Ca^{2+} in *Escherichia coli* highlight two putative influx mechanisms in response to changes in extracellular calcium. *Cell Calcium* **25** 265–74

Koul A, Herget T, Klebl B and Ullrich A 2004 Interplay between mycobacteria and host signalling pathways. *Nat. Rev. Microbiol.* **2** 189–202

Koul S, Somayajulu A, Advani MJ and Reddy H 2009 A novel calcium binding protein in *Mycobacterium tuberculosis*- potential target of trifluoperazine. *Ind. J Exp. Biol.* **47** 480–488

Kretsinger RH 1976 Calcium-binding proteins. *Annu. Rev. Biochem.* **45** 239–266

Kumari P and Meena LS 2014 Factors affecting susceptibility to *Mycobacterium tuberculosis*: a close view of immunological defence mechanism. *Appl. Biochem. Biotechnol.* **174** 2663–73

Lilie, H, Haehnel W, Rudolph R and Baumann U 2000 Folding of a synthetic parallel beta-roll protein. *FEBS Lett.* **470** 173–177

Lin YP, Raman R, Sharma Y and Chang YF 2008 Calcium binds to leptospiral immunoglobulin-like protein, LigB, and modulates fibronectin binding. *J Biol. Chem* **283** 25140–25149

Majeed M, Perskvist N, Ernst JD, Orselius K and Stendahl O 1998 Roles of calcium and annexins in phagocytosis and elimination of an attenuated strain of *Mycobacterium tuberculosis* in human neutrophils. *Microb. Pathog.* **24** 309–320

Malik ZA, Denning GM and Kusner DJ 2000 Inhibition of Ca(2+) signaling by *Mycobacterium tuberculosis* is associated with reduced phagosome-lysosome fusion and increased survival within human macrophages. *J. Exp. Med.* **191** 287–302

Meena LS 2015 An overview to understand the role of PE_PGRS family proteins in *Mycobacterium tuberculosis* H_{37}Rv and their potential as new drug target. *IUBMB* **62** 145–153

Meena LS and Meena J 2016 Cloning and Characterization of a novel PE_PGRS60 protein (Rv3652) of *Mycobacterium tuberculosis* H_{37}Rv, exhibiting fibronectin binding property. *Biotechnol. Appl. Biochem.* **63** 531

Meena LS and Rajni 2010 Survival mechanisms of pathogenic *Mycobacterium tuberculosis* H_{37}Rv. *FEBS J.* **277** 2416–2427

Meena PR, Monu and Meena LS 2016 Fibronectin binding protein and Ca^{2+} play an access key role to mediate pathogenesis in *Mycobacterium tuberculosis*; An overview. *Biotechnol. Appl. Biochem.* **63** 820–826

Michiels J, Xi C, Verhaert J and Vanderleyden J 2002 The functions of Ca in bacteria: a role for EF-hand proteins? *Trends Microbiol.* **10** 87–93

Monu and Meena LS 2016 Biochemical characterization of PE_PGRS61 family protein of *M. tuberculosis* H_{37}Rv reveals the binding ability to Fibronectin, *Iran. J. Basic. Med. Sci.* **19** 1105–1113

Naseem R, Wann KT, Holland IB and Campbell AK 2009 ATP regulates calcium efflux and growth in *E. coli*. *J Mol. Biol.* **391** 42–56

Nejatbakhsh N and Feng ZP 2011 Calcium binding protein-mediated regulation of voltage-gated calcium channels linked to human diseases. *Acta Pharmacol. Sin.* **32** 741–748

NYU Langone Medical Center/New York University School of Medicine 2015 unexpected role for calcium in controlling inflammation during chronic lung infection *www.sciencedaily.com/releases/2015/*

Oomes SJ, Jonker MJ, Wittink FR, Hehenkamp JO, Breit TM and Brul S 2009 The effect of calcium on the transcriptome of sporulating *B. subtilis* cells. *Int. J Food. Microbiol.* **133** 234–242

Patrauchan MA, Sarkisova SA and Franklin MJ 2007 Strain-specific proteome responses of *Pseudomonas aeruginosa* to biofilm-associated growth and to calcium. *Microbiology* **153** 3838–3851

Permyakov EA and Kretsinger RH 2009 Cell signaling, beyond cytosolic calcium in eukaryotes. *J. Inorg. Biochem.* **103** 77–86

Podobnik M, Tyagi R, Matange N, Dermol U, Gupta AK, Mattoo R, Seshadri K and Visweswariah SS 2009 A mycobacterial cyclic AMP phosphodiesterase that moonlights as a modifier of cell wall permeability. *J. Biol. Chem.* **284** 32846–32857

Poulet S and Cole ST 1995 Characterization of the highly abundant polymorphic GC-rich-repetitive sequence (PGRS) present in *Mycobacterium tuberculosis*. *Arch. Microbiol.* **163** 87–95

Ramakrishnan L, Federspiel NA and Falkow S 2000 Granuloma specific expression of mycobacterium virulence proteins from the glycine-rich PE-PGRS family. *Science* **288** 1436–1439

Raman R, Sharma Y and Chang YF 2011 Ca-binding and spectral properties of the common region of surface-exposed Lig proteins of leptospira. *Commun. Integr. Biol.* **4** 331–333

Reddy PH, Burra SS and Murthy PS 1992 Correlation between calmodulin-like protein, phospholipids, and growth in glucose-grown *Mycobacterium phlei*. *Can. J. Microbiol.* **38** 339–342

Rigden DJ and Galperin MY 2004 The DxDxDG motif for calcium binding: Multiple structural contexts and implications for evolution. *J. Mol. Biol.* **343** 971–984

Rigden DJ, Jedrzejas MJ, Moroz OV and Galperin MY 2003 Structural diversity of calcium-binding proteins in bacteria: single-handed EF-hands? *Trends Microbiol.* **11** 295–297

Sachdeva G, Kumar K, Jain P and Ramachandran S 2005 SPAAN: a software program for prediction of adhesins and adhesin-like proteins using neural networks. *Bioinformatics* **21** 483 491

Sanders D, Brownlee C and Harper JF 1999 Communicating with calcium. *Plant Cell* **11** 691–706

Sharma S and Meena LS 2017 Potential of Ca^{2+} in *Mycobacterium tuberculosis* $H_{37}Rv$ pathogenesis and survival. *Appl. Biochem. Biotechnol* **181** 762–771

Shaw S and Meena LS 2016 FnBPs: An effective adhesion molecule in *Mycobacterium tuberculosis* pathogenesis. *MRJMMS* **4** 448–450

Shenoy AR, Capuder M, Draskovic P, Lamba D, Visweswariah SS and Podobnik M 2007 Structural and biochemical analysis of the Rv0805 cyclic nucleotide phosphodiesterase from *Mycobacterium tuberculosis*. *J. Mol. Biol.* **365** 211–225

Srivastava SS, Mishra A, Krishnan B and Sharma Y 2014 Ca^{2+} binding motif of beta gamma crystallins. *J. Biol. Chem.* **289** 10958–10966

Tekaia F, Gordon SV, Garnier T, Brosch R, Barrell BG and Cole ST 1999 Analysis of the proteome of *Mycobacterium tuberculosis* in silico. *Tuberc. Lung. Dis.* **79** 329–342

Torrecilla I, Leganes F, Bonilla I and Fernandez-Pinas F 2000 Use of recombinant aequorin to study calcium homeostasis and monitor calcium transients in response to heat and cold shock in cyanobacterial. *Plant Physiol.* **123** 161–175

Trimble WS and Grinstein S 2007 TB or not TB: Calcium Regulation in Mycobacterial survival. *Cell* **130** 12–14

Vergne I, Chua J and Deretic V 2003 tuberculosis toxin blocking phagosome maturation inhibits a novel Ca^{2+}/calmodulin-PI3K hvps34 cascade. *J. Exp. Med.* **198** 653–659

Wistow G 1990 Evolution of a protein superfamily: relationships between vertebrate lens crystallins and microorganism dormancy proteins. *J. Mol. Biol.* **30** 140–145

Yadav M, Roach SK and Schorey JS 2004 Increased mitogen-activated protein kinase activity and tnf-production associated with *Mycobacterium smegmatis* but not *Mycobacterium avium* infected macrophages requires prolonged stimulation of the calmodulin/calmodulin kinase and cyclic amp/protein kinase a pathways. *J. Immunol.* **172** 5588–5597

Yang K 2001 Prokaryotic calmodulins: recent developments and evolutionary implications. *J. Mol. Microbiol. Biotechnol.* **3** 457–459

Yeruva VC, Kulkarni A, Khandelwal R, Sharma Y and Raghunand TR 2016 The PE_PGRS proteins of *Mycobacterium tuberculosis* are Ca^{2+} binding mediators of host-pathogen interaction. *Biochemistry* **55** 4675–4687

Zampese E and Pizzo P 2012 Intracellular organelles in the saga of Ca homeostasis: different molecules for different purposes? *Cell. Mol. Life. Sci.* **69** 1077–1104

Zhou Y, Yang W, Kirberger M, Lee HW, Ayalasomayajula G and Yang JJ 2006 Prediction of EF-hand calcium-binding proteins and analysis of bacterial EF-hand proteins. *Proteins* **65** 643–655

Corresponding editor: BJ Rao