BioCore Exploring Scientific Community

International Journal of Biotechnology and Bioengineering

Research Article ISSN 2475-3432

GTPases: a significant signalling molecule in TB infection.

Laxman S. Meena

CSIR-Institute of Genomics and Integrative Biology, Mall Road, Delhi-110007, India

Abstract

Tuberculosis is major threat in today's society. Also, there is rapid increase in multi-drug resistant cases so there is urgent requirement of novel therapeutic drug target. Several molecules are present in mycobacterial cell that are crucial in host pathogen interaction thus recognition and simultaneous expression of such molecules might show some upregulation or downregulation of those molecules inside host. Understanding of whole signalling cascade involved behind receptor-molecule interactions would be key in finding some novel targets for drug designing. Recently, many promising target receptors like Toll like receptor (TLR-2 and 4), G-protein coupled receptor (GPCRs); adhesion GPCRs (aGPCRs), CXCR1, CXCR2, etc and molecules like GTPases; Calcium (Ca2+); PknA, PknB, PknG, NAD kinase, NAD synthetase; etc, are likely to be studied that can be used as target but yet proper mechanistic understanding of these receptor : pathogen interactions are obscure. G-proteins are important class of protein present in eukaryotes. But in prokaryotes these proteins had remain long ignored however in recent time targeting these G-proteins in prokaryotes is a major interest in research and detailed study could be used as a drug target which could be a promising diagnostic approach. In our study we have shown that conserved GTP binding protein like era, obg, elongation factor, engA, ftsY, etc consists of GTP binding or hydrolysing property and thus disruption of these genes is directly coefficient to the survival and pathogenecity of the organism. So we hypothesize that if we interrupt or manipulate binding of these genes with the receptor present in eukaryotes via use of some manipulative signal transduction then it could be beneficial for check of this disease.

Keywords: Mycobacterium tuberculosis; GTPases; GPCRs; G- protein; Drug target

Corresponding author: **Laxman S. Meena**

CSIR-Institute of Genomics and Integrative Biology, Mall Road, Delhi-110007, India.

E-mail: Laxmansm72@yahoo.com

Citation: Laxman S. Meena (2018), GTPases: a significant signalling molecule in TB infection. Int J Biotech & Bioeng. 4:6, 124-129.

Received: June 15, 2018
Accepted: July 12, 2018
Published: August 21, 2018

Abbreviations

Tuberculosis (TB); Mycobacterium tuberculosis (M. tb); Mycobacterium smegmatis (M. smegmatis); Mycobacterium bovis (M. bovis); Mycobacterium africanum (M. africanum); G-protein coupled receptors (GPCRs); adhesion-GPCRs (aGPCRs); GTP (Guanosine triphosphate); GDP (Guanosine diphosphate); Toll like receptor (TLR); Calcium (Ca2+); Nicotinamide adenine dinucleotide kinase (NAD kinase); multi-drug resistant tuberculosis (MDR-TB); extensively multi-drug resistant (XDR-TB); World Health Organisation (WHO); seven transmenbrane (7TM); extra cellular domains (ECD); GPCRs proteolysis site (GPS); Interferon gamma (IFNγ)

Introduction

In today's world mortality due to disease rates highest in case of tuberculosis and the pathogen responsible belongs to Mycobacterium complex. In recent times, diagnostic and treatment of TB is still a major issue in era of modernization. Since, the emergence of multi-drug resistant tuberculosis (MDR-TB) and extensively multi-drug resistant (XDR-TB) is a major threat to the society and it's treatment available is either ineffective or resulting in poor efficacy [1] so there is apparent need to find out some alternate molecule that could be detrimental to pathogen in host-pathogen interaction. According to WHO report TB is ninth cause of death worldwide as single infectious agent and approx 10.4 million people fell ill in 2017 [2].

Mycobacterium complex consists of pathogenic strains of mycobacterium genus such as Mycobacterium tuberculosis (M. tb), Mycobacterium bovis (M. bovis), Mycobacterium africanum (M. africanum), etc. However in human M. tb as causal organism is more prevalent. M.tb is an acid-fast, gram-positive, slow growing obligate bacterium which consists of glycolipids such as mycolic acid, lipoarabinomannan (LAM), manosylated LAMs (Man-LAM), various sulfolipids, etc [3,4].

Various signalling molecules and their respective receptors are involved in transmission of signals throughout the body. Cells express various receptors among which some are involved in distant signal transduction while some are localized [5]. In past few decades extensive work had been done in understanding the role of GTPases in growth,

development, survival and pathogenesis of the bacterium. GTPases are large group of protein that are involved in binding or hydrolyzing of GTP to GDP molecules and the receptors for attachment of this protein is recognized as G-protein coupled receptor (GPCRs) are seven trans-membrane (7 TM) helices which are abundantly present in human genome.

GPCRs; Catalytic receptors & aGPCRs: crucial receptors present in eukaryotes

Various receptors like GPCRs, CXCR1, CXCR2, etc are known to play significant role in TB infections [6]. GPCRs are largest group of known trans-membrane receptor proteins in eukaryotes which are thought to be evolved as structurally signalling molecules to regulate complex interactions [7] and thus play significant role in host-pathogen interaction. Presences of G proteins are abundant in case of both eukaryotes and prokaryotes and GTPases are enzyme that acts as molecular switch between hydrolyzing of GTP to GDP thus are important group of enzymes regulating cellular processes (Fig:1a). Moreover, CXCL8 (IL-8) activates CXCR1 and CXCR2 to arbitrate neutrophil recruitment to enhance cytotoxic effect. Likely, GPCRs these receptors after release of chemokines gets phosphorylated and influx G-α subunit and hence get inactivated [8] (Fig: 1b)

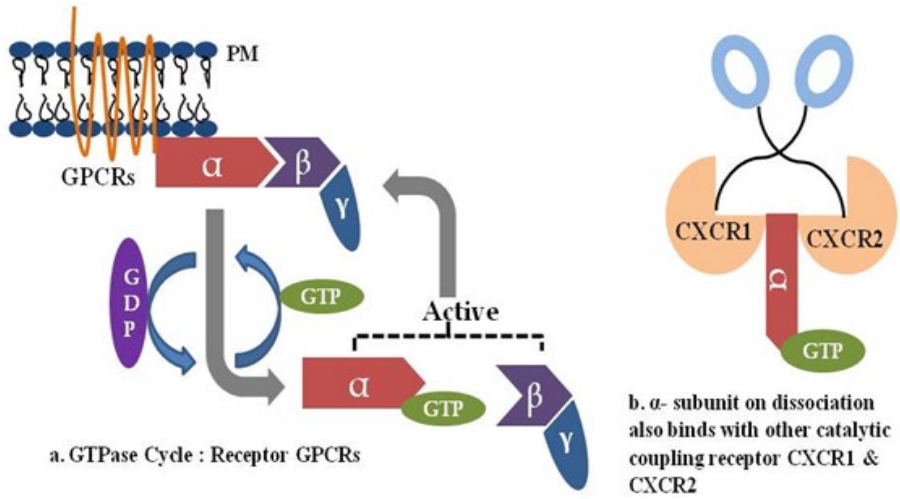

Figure 1: G-protein binding with their receptors

a] G-protein binding with GPCRs. On binding of receptor G protein undergoes conformational changes causing GDP to dissociate. GTP binds with α subunits in place of GDP and due to further conformational change leads to activation of both α & βγ complex. α- subunits dissociate from βγ complex and signalling cascade begins. Further, on binding of arrestin, a kinase protein results in phosphorylation of receptor and thus inhibits the interaction of G-protein with its receptor.
b] Chemokine receptors CXCR1 & CXCR2 gets activated due to CXCR8. These receptors couples with α- subunits which induces recruitment of leukocytes at the sites of infections.

Adhesion GPCRs (aGPCRs) are yet another sub-family of GPCRs enclosing large extra cellular domains (ECD) with double-barrelled structure that aids in interaction with extracellular proteins. They are subfamily of whole GPCRs consisting group of 33 member protein [9] ECDs have unique property of binding with cell itself and also the surrounding cells present around the receptors. aGPCRs get cleaved at a L□T/S/C proteolytic site of GPCRs proteolysis site (GPS) [10,11]. Mutations of these GPS site leads to impaired trafficking to the cell surface and are cause of aGPCRs dysfunction [12]. Now the bipartite structures of aGPCRs are of two different beneficiary importances; first that it can be tapered in cell-cell adhesion and secondly it can be used to manipulate intercellular signal transduction. Thus, these can be targeted to derive novel pharmacological methods.

Role of Ras GTPases in tuberculosis infection

Ras families are well known group of important proteins in humans consisting of more than 150 members and have five very significant subfamilies named as Ras, Rab, Rho, Arf and Ran which are key regulators in cell proliferation, differentiation, survival, etc [13,14]. Intercellular pathogen like mycobacterium survives and replicate inside host by restabilising an intercellular niche for themselves thus escaping phagolysosome formation, the process where internalized bacteria are destroyed [15]. Rab GTPases are largest group of Ras superfamily proteins involved in signalling trafficking in human genome. These intercellular pathogens results in evading these trafficking events and develop some unique strategies that specifically target these molecules. According to a report Rab5 and Rab7 sequentially blocks phagosome-lysosome fusions [3, 16]. Yet another report suggests that in mouse macrophages M. tb phagosome undergoes conventional maturation, including an early EGFP-Rab5 to EGFP-Rab7 transition followed by dissociation. Thus, association suggests that M. tb undergoes late endosomal stages during initial hours of infection, which suggests that slow replication of M. tb inside host is important in its growth or restriction [15]. On another hand, Rab20 which is Interferon gamma (IFNγ) associated GTPases remains associated with M. tb phagosomes thus resulting in membrane influx and formation of spacious phagosomes [17] (Fig 2). Another report showed that silencing of Arf GTPase-activating protein1 (ArfGAP1) has effect on cytoskeleton rearrangement which is responsible for uptake and replication of M. tb thus indicating that it can act as a novel factor in host-pathogen interaction [18].

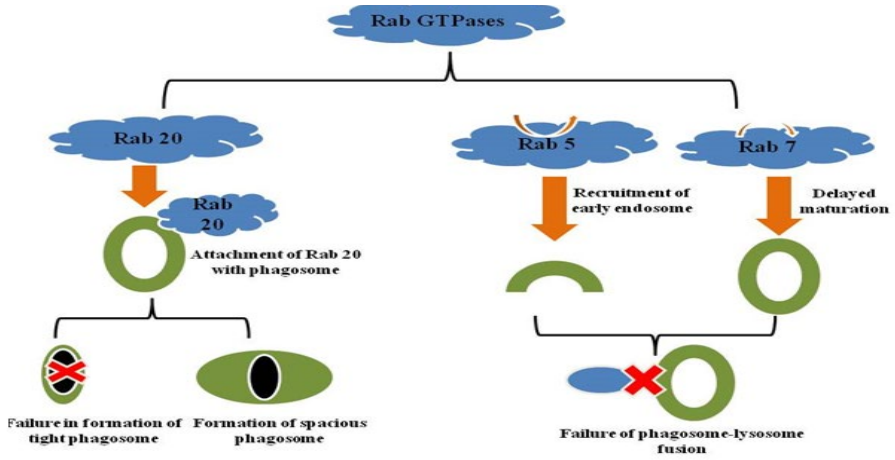

Figure 2 : Role of different Rab- GTPases in internalization of mycobacterium inside host

Rab is the largest group of GTPases involved in signal trafficking. Rab20 which is also called Interferon γ (IFN γ) gets attached with phagosomes which results in membrane influx and formation of spacious phagomes. Rab5 gets upregulated and results in formation of early endosome whereas Rab 7 gets downregulated thus endosome maturation gets delayed. Both of these events prevent phagosome-lysosome formation.

GTPases and its significance in prokaryotes

GTPases in eukaryotes had long been studied for its importance in cell signalling, protein synthesis, vesicular trafficking, differentiation, cell proliferation, etc. GTPases are responsible for binding and hydrolyzing GDP/GTP and this process continues in cyclic manner [19]. According to the previous studies mutation in these GTPases are important in cancer formation and infectious diseases. However, the importance of GTPases in prokaryotes has been rapidly taken in focus in recent time. Basically, GTPases in prokaryotes are involved as essential ribosomal assembly factors. However, there exact role is still elusive. Although prokaryotic GTPases are involved in recruitment of ribosomal proteins in ribosomal, rearrangements, recruitments, etc (Fig 3).

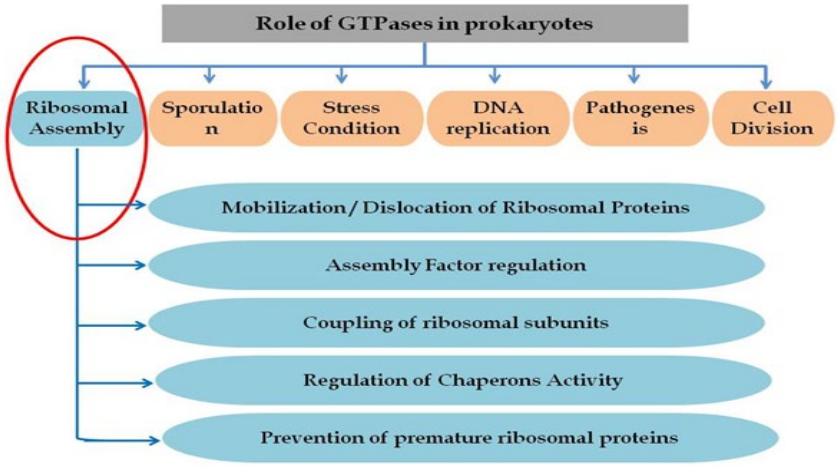

Figure 3 : Role of GTPases in prokaryotes

Mainly GTPases in prokaryotes is involved in ribosomal assembly in various manners. Various other factors like sporulation, stress condition, replication, cell division and pathogenesis are also affected due to this.

Other than that many of them are involved in stress response, sporulation, cell division, pathogenesis, etc. GTPases comprises of large family of protein that acts as molecular switches by hydrolyzing GTP to GDP which are essential in cellular processes [20]. They can be further subdivided into TRAFAC (translational factor related) and SIMIBI (signal recognition particle) in which TRAFAC consists of hetrotrimeric G-protein [21] which consists of three subunits namely alpha (α), beta (β) and gamma (γ) [22]. According to a report it had been shown that bacterial proteins amend GTP synthesis through complex formation in eukaryotic nucleoside diphosphate kinase (Ndk). Several genes of M.

smegmatis and P. aeruginosa had shown to form complex with Ndk thus modulating the synthesis of nucleoside diphosphate (NDP) [23]. Three other genes from P. aeruginosa namely Ras like Pra [24], Ef-Tu [25] and pyruvate kinase [26] had been demonstrated in to form complexes with Ndk thus manipulating its substrate specificity. Similarly another study showed that four other proteins of M. smegmatis P50, P60, P65 and P70 had shown complex formation with Ndk [23]

Importance of universally conserved G-protein in M. tb

The G-domain is conserved by presence of conserved amino acid sequence [GXXXXGK(S/T)] which is popularly known Walker motif or P-loop which is the site for binding of G-protein. There are a number of universally conserved GTPases [27] among which Elongation Factor (EF-Tu), EF-G, If-2 (fus A1 and fus A2 in case of mycobacterium tuberculosis), YihA, EngA, Ffh, FtsY, YchF HflX are found in all form of life, while Era, Der, Obg are conserved in prokaryotes and eukaryotes but absent in case of archea [21] and LepA and MnmE belongs to prokaryotic origins as are conserved in bacteria and in eukaryotes are found in mitochondria and chloroplast in plants [28, 29]. Many of these conserved genes had been reported essential for survival of mycobacterium and are basically cytoplasmic proteins (Table 1). Obg is yet another important target as it had been studied to get regulated in various functions of bacterium like ribosomal assembly, stress condition, sporulation, etc [23].

S.No.	Name of gene	Gene Identifier in M.tb	Category	Essentiality Call	Reference No.
1	Ef-Tu	Rv1406	TRAFAC	GF	21, 32, 33, 35
2	If-Tu/Ef-G	a. fusA1(Rv0684) b. fusA2(Rv0120c)	TRAFAC	ES/NE	21, 32, 33, 36, 37
3	YihA	----------	TRAFAC	Ab	21, 32, 33
4	YchF	Rv1112	TRAFAC	NE	21, 32, 33, 38
5	HflX	Rv2725c	TRAFAC	NE	21, 32, 33, 39
6	EngA	Rv1713	TRAFAC	ES	21, 32, 33, 40
7	Ffh	Rv2916c	SIMIBI	ES	21, 32, 33, 41
8	FtsY	Rv 2921c	SIMIBI	ES	21, 32, 33, 42
9	Era	Rv2364c	TRAFAC	GF	21, 32, 33, 43
10	Der	Rv2711	TRAFAC	ES	21, 32, 33, 44
11	Obg	Rv2440c	TRAFAC	ES	21, 32, 33, 45
12	LepA	Rv2404c	TRAFAC	NE	21, 32, 33, 46
13	MnmE	Rv3024c	TRAFAC	NE	21, 32, 33, 47

•Mnm is a large class of protein and its another form MnmA is present in M.smegmatis while homolog of MnmA is trmU in M.tb, which is probable tRNA(5-methylaminomethyl-2-thiouridylate)-methyltransferase TrmU
•GF= Involoved in growth factor; NE= Non-Essential Gene; ES= Essential Gene; Ab=Absent

Table 1: Conserved GTPases in prokaryotes and in case of mycobacterium tuberculosis

In M. tb phosphorylation of Ef-Tu by PknB reduces its interaction with GTP thus showing reduced level of protein synthesis [30]. Another mutation study in E. coli suggested that Ef-G is important in interaction with specific ribosomal assembly [31]. Point mutation in conserved GTP binding motif (AspXXGly) from Asp to Ala in Era at position 258 and in Obg at position 212 had shown loss of GTPases activity in M. tb [27]. Thus, important protein EngA on subjected to GTPases assay showed its intrinsic GTPases activity thus indicating its importance in M. tb pathogenesis [20]. According to a recent study antisense knockdown of ffh and FtsY had shown more significant decrease in in-vitro growth due to ffh then due to FtsY [32]. Thus, these studies showed their importance of conserved GTPases gene in ribosomal assembly and pathogenesis of M. tb and other prokaryotic organisms.

GTPases: a novel drug target

Translation factor Ef-Tu and Ef-G have come up as a target for many new antibiotics. Also interaction of protein with their ligands yet remains another loop hole that could be targeted for new drug designing. Prior to molecular approaches predictive study on inhibiting association of adhesive molecules with their receptors by using efficient drug would be very much beneficial for therapeutic purpose. Also, a way of detection of downstream factors of such molecules inside body would be beneficial in development of biomarker for early detection of the disease. Further more detailed understanding of interaction of bacterial GTPases is necessary and identifying the whole signalling mechanism still remains with wide gap. Fulfilling this gap area could be beneficial in future development of some tough drug that could finally bring end to this deadly disease.

Summary

The study here depicts that GTPases in M. tb plays a crucial putative role in various cellular processes while a wide class of receptor molecules are present in host. But due to uncertainty of the protein involved in infection of tuberculosis targeting a single molecule as marker or for diagnostic purpose is very much challenging. Another problem lies in modulation of bacterium genome according to the host environment and its persistent dormancy makes it more challenging. So a deeper understanding of these molecules and their interaction with respective receptors would surely provide a chance to exploit these molecules as new therapeutic targets.

Acknowledgement

The authors acknowledge economic sustain from the project GAP0145 of the Department of Science and Technology (SERB). We also acknowledge the support from scientific and administrative staff of CSIR-IGIB.

Conflict of interest

None

References

1. Campaniço A, Moreira R & Lopes F (2018) Drug discovery in tuberculosis. New drug targets and antimycobacterial agents. Eur J Med Chem 150, 525-545

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

3. Rajni & Meena L S (2009) Guanosine triphosphatases as novel therapeutic targets in tuberculosis. Int J Infect Dis 14(8), e682-687

4. Meena LS & Rajni. (2010) Survival mechanisms of pathogenic Mycobacterium tuberculosis H37Rv. FEBS J 277(11), 2416-2427

5. Cooper GM (2000) The Cell: A Molecular Approach. (2000); 2nd edition; Cooper GM.; Sunderland (MA): Sinauer Associates; 2000

6. Alaridah N, Lutay N, Tenland E, Rönnholm A, Hallgren O, Puthia M, Westergren-Thorsson G & Godaly G (2017) Mycobacteria Manipulate G-Protein-Coupled Receptors to Increase Mucosal Rac1 Expression in the Lungs; J Innate Immun 9(3), 318-329

7. Cohen LJ, Esterhazy D, Kim SH, Lemetre C, Aguilar RR, Gordon EA, Pickard AJ, Cross JR, Emiliano AB, Han SM, Chu J, Vila-Farres X, Kaplitt J, Rogoz A, Calle PY, Hunter C, Bitok JK & Brady SF (2017) Commensal bacteria make GPCR ligands that mimic human signaling molecules; Nature 549(7670), 48-53

8. Raghuwanshi S K, Su Y, Singh V, Hayes K, Richmond A & Richardson R M (2012) The chemokine receptors CXCR1 and CXCR2 couple to distinct G protein-coupled receptor kinases to mediate and regulate leukocyte functions. J Immunol 189(6), 2824-2832

9. Hamann J, Aust G, Araç D, Engel F B, Formstone C, Fredriksson R, et al (2015) International union of basic and clinical pharmacology. XCIV. Adhesion G protein-coupled receptors, Pharmacol Rev 67(2), 338-367

10. Krasnoperov V, Lu Y, Buryanovsky L, Neubert T A, Ichtchenko K & Petrenko A G (2002) Post-translational proteolytic processing of the calcium independent receptor of alpha latrotoxin (CIRL), a natural chimera of the cell adhesion protein and the G protein-coupled receptor. Role of the G protein-coupled receptor proteolysis site (GPS) motif. J Biol Chem 277(48), 46518-46526

11. Lin H H, Stacey M, Yona S & Chang G W (2010) GPS proteolytic cleavage of adhesion-GPCRs. Adv Exp Med Biol 706, 49-58

12. Nijmeijer S, Vischer HF & Leurs R (2016) Adhesion GPCRs in immunology. Biochem Pharmacol 114, 88-102

13. Wennerberg K, Rossman K L & Der C J (2005) The Ras superfamily at a glance. J Cell Sci 118(Pt 5), 843-846

14. Vigil D, Cherfils J, Rossman K L & Der C J (2010) Ras superfamily GEFs and GAPs: validated and tractable targets for cancer therapy?. Nat Rev Cancer 10(12), 842-857

15. Fairn G D & Grinstein S (2012) How nascent phagosomes mature to become phagolysosomes; Trends Immunol 33(8), 397-405

16. Spanò S & Galán J E (2017) Taking control: hijacking of Rab GTPases by intracellular bacterial pathogens. Small GTPases 9(1-2), 182-191

17. Schnettger L, Rodgers A, Repnik U,Lai R P, Pei G, Verdoes M, Wilkinson R J, Young D B & Gutierrez M G (2017) A Rab20-Dependent Membrane Trafficking Pathway Controls M. tuberculosis Replication by Regulating Phagosome Spaciousness and Integrity. Cell Host Microbe 21(5), 619-628

18. Song O R, Queval C J, Iantomasi R, Delorme V, Marion S, Churlet R V, Werkmeister E, Popoff M, Ricard I, Jouny S, Deboosere N, Lafont F, Baulard A, Yeramian E, Marsollier L, Hoffmann E & Brodin P (2018) ArfGAP1 restricts Mycobacterium tuberculosis entry by controlling the actin cytoskeleton. EMBO Rep. 19(1), 29-42

19. Pei G, Schnettger L, Bronietzki M, Repnik U, Griffiths G & Gutierrez M G (2015) Interferon-g-inducible Rab20 regulates endosomal morphology and EGFR degradation in macrophages. Mol Biol Cell 26(17), 3061-3070

20. Meena L S & Meena R. (2011) Cloning and characterization of engA, a GTP-binding protein from Mycobacterium tuberculosis H37Rv. Biologicals 39(2), 94-99

21. Verstraeten N, Fauvart M, Verse´es W & Michiels J (2011) The Universally conserved prokaryotic GTPases. Microbiol Mol Biol Rev 75(3), 3507-3542

22. Shaw S & Meena L S (2016) GTPases: Prerequisite Molecular Target in Virulence and Survival of Mycobacterium tuberculosis. Inter J Mol Biol 1(1), 1-2

23. Shankar S, Kapatral V & Chakarbarty A M (1997) Mammlian hetrotrimeric G-protein like proteins in mycobacteria: implications for cell signalling and survival in eukaryotic host. Mol Microbiol 26(3), 607-618

24. Chopade B A, Shankar S, Sudin G W & Chakarbarty A M (1997) Characterization of Membrane-Associated Pseudomonas aeruginosa Ras-Like Protein Pra, a GTP-Binding Protein That Forms Complexes with Truncated Nucleoside Diphosphate Kinase and Pyruvate Kinase to Modulate GTP Synthesis. J Bacteriol 179(7), 2181-2188

25. Chakarbarty A M (1998) Nucleoside diphosphate kinase: role in bacterial growth, virulence, cell signalling and polysaccharide synthesis. Mol Microbiol 28(5), 875-882

26. Sundin G W, Shankar S, Chugani S A, Chopade B A, Kavanaugh-Black A & Chakrabarty A M (1996) Nucleoside diphosphate kinase from Pseudomonas aeruginosa: characterization of the gene and its role in cellular growth and exopolysaccharide alginate synthesis. Mol Microbiol 20(5), 965-979

27. Meena L S, Chopra P, Bedwal R S & Singh Y (2008) Cloning and characterization of GTP-binding proteins of Mycobacterium tuberculosis H37Rv. Enzyme Microb Technol 42(2), 138-144

28. Caldon C E, Yoong P & March P E (2001) Evolution of a molecular switch: universal bacterial GTPases regulate ribosome function. Mol Microbiol 41(2), 289-297

29. Caldon C E, Yoong P & March P E (2003) Function of the universally conserved bacterial GTPases; Curr Opin Microbiol 6, 135-139

30. Sajid A, Arora G, Gupta M, Singhal A, Chakraborty K, Nandicoori VK & Singh Y (2011) Interaction of Mycobacterium tuberculosis elongation factor Tu with GTP is regulated by phosphorylation. J Bacteriol 193(19), 5347-5358

31. Rao AR & Varshney U (2002) Characterization of Mycobacterium tuberculosis ribosome recycling factor (RRF) and a mutant lacking six amino acids from the C-terminal end reveals that the C-terminal residues are important for its occupancy on the ribosome. Microbiology 148(Pt 12), 3913-3920

32. Venkatesan A, Palaniyandi K, Sharma D, Bisht D & Narayanan S (2018) Characterization of FtsY, its interaction with Ffh, and proteomic identification of their potential substrates in Mycobacterium tuberculosis. Can J Microbiol 64(4), 243-251

33. Jeffrey P. Murry J P, Christopher M. Sassetti C M, James M. Lane J M, Xie Z & Rubin E J (2008) Transposon Site Hybridization in Mycobacterium tuberculosis; Microbial Gene Essentiality. Protocols

and Bioinformatics 416, 45-59
34. DeJesus M A, Gerrick E R, Xu W, Park S W, Long J E, Boutte C C, Rubin E J, Schnappinger D, Ehrt S, Fortune S M, Sassetti C M & Ioergera T R (2017) Comprehensive Essentiality Analysis of the Mycobacterium tuberculosis Genome via Saturating Transposon Mutagenesis. MBio 8(1), e02133-16
35. https://mycobrowser.epfl.ch/genes/Rv1406
36. https://mycobrowser.epfl.ch/genes/Rv0684
37. https://mycobrowser.epfl.ch/genes/Rv0120c
38. https://mycobrowser.epfl.ch/genes/Rv1112
39. https://mycobrowser.epfl.ch/genes/Rv2725c
40. https://mycobrowser.epfl.ch/genes/Rv1713
41. https://mycobrowser.epfl.ch/genes/Rv2916c
42. https://mycobrowser.epfl.ch/genes/Rv2921c
43. https://mycobrowser.epfl.ch/genes/Rv2364c
44. https://mycobrowser.epfl.ch/genes/Rv2711
45. https://mycobrowser.epfl.ch/genes/Rv2440c
46. https://mycobrowser.epfl.ch/genes/Rv2404c
47. https://mycobrowser.epfl.ch/genes/Rv3024c