Cyclic AMP (cAMP) has emerged as a pivotal molecule for signalling in all life forms. Mycobacterial genomes have been found to encode for numerous proteins that are involved in cAMP generation, degradation and utilization. Many of these proteins have domain organizations unique to mycobacteria. This review summarizes recent advances in mechanisms of cAMP synthesis and degradation, focusing on the processes by which cAMP modulates mycobacterial signalling. We explore its impact on the physiology of the organism and on the discourse between cAMP and its host.

**Keywords:** Adenylyl cyclase, cyclic nucleotide binding protein, cyclic AMP, mycobacteria, phosphodiesterase.

**Introduction**

Cyclic nucleotides are small diffusible molecules that exhibit high stability to changes in pH and temperature, properties that have led to their evolution as universal second messengers. The discovery of cyclic AMP (cAMP) and cyclic GMP (cGMP) in the mid-1900s heralded the importance of this group of molecules in cellular signalling, following which a myriad of other cyclic nucleotides have been identified including, cyclic CMP, cyclic UMP, cyclic di-GMP, cyclic di-AMP and cyclic GMP–AMP. Of these, cAMP appears to be the most pervasive across the domains of life and its roles in eukaryotes have been well characterized.

In prokaryotes, cAMP was initially characterized to be a mediator of catabolite repression in *E. coli*. Since then, it has been found to be involved in numerous prokaryotic signalling cascades such as antibiotic production and phototaxis. Microorganisms also profit from the near-universality of this molecule and use it as a virulence strategy to manipulate the host as exemplified by *Vibrio cholerae* and the heat-labile toxin from *Escherichia coli*.

Mycobacterium is a genus of Actinobacteria which contains a number of widespread soil-dwelling organisms in addition to the widely recognized obligate parasites *Mycobacterium tuberculosis* and *Mycobacterium leprae*. Following the advent of genome sequencing, studies indicated that these organisms encode for an inordinately large number of adenylyl cyclases in their genomes and prompted several new forays into understanding the role of cAMP in mycobacteria and the enzymes that regulate its metabolism. These include adenylyl cyclases that synthesize cAMP from ATP, cAMP-binding proteins which bind to cAMP, therein modulating their downstream outputs and phosphodiesterases (PDEs), which mediate degradation of cAMP. Some of these studies are summarized below with information obtained from our laboratory as the main focus.

**Adenylyl cyclases**

The adenylyl cyclases of mycobacteria belong to the Universal Class of adenylyl cyclases, which encompasses cyclases from bacteria and eukaryotes. They typically function as head-to-tail homodimers using a catalytic mechanism requiring two-metal ions. Bioinformatic predictions and experimental data on the adenylyl cyclases from *M. tuberculosis* H37Rv indicate that the organism encodes for 16 putative adenylyl cyclases which have a variety of domain topologies, most of which are unique to mycobacteria. For example, *M. tuberculosis* H37Rv encodes for three putative adenylyl cyclases that contain an ATPase, a tetratricopeptide repeat (TPR) and a helix-turn-helix (HTH) domain. A knockout of one of these, Rv0386, has been shown to have impaired virulence in mice. Several mycobacterial adenylyl cyclases have been characterized in vitro and show distinct biochemical properties with over 1000-fold difference in activity, as assessed by *V*_{max} (refs 31 and 32). Interestingly, these cyclases can also exhibit divergence in the contribution of key residues to catalytic function. For example, when we mutated the predicted substrate specifying residues of the adenylyl cyclase, Rv1625c, the enzyme lost...
Figure 1. Model of cAMP metabolism within a mycobacterial cell. On stimulation by various factors, mycobacterial adenylyl cyclases (AC) synthesize cAMP. The cAMP formed is degraded to AMP by a phosphodiesterase Rv0805, which also shows additional cell-wall remodelling functions. Cyclic-AMP binds to effector proteins leading to post-translational modifications and transcriptional regulation. Cyclic AMP-bound KATmt acetylates substrate proteins such as USP and ACS (shown is a modelled structure of acetylated USP from \textit{M. smegmatis}) leading to their activation/inactivation. Acetylation serves as a regulatory mechanism for ACS activity thus modulating energy homeostasis in the cell. Cyclic AMP also binds to and activates the CRPs, CRP Mt and Cmr leading to transcription of genes essential for serine biosynthesis, resuscitation and development. Intracellular and extracellular cAMP may also have additional effects as illustrated.

the ability to dimerise, thus asserting the uniqueness of the active site of this cyclase\textsuperscript{3,34}.

Microarray data and biochemical evidences have uncovered several physiologically relevant activators of these enzymes including low pH\textsuperscript{35} and fatty acids\textsuperscript{36}, conditions that mycobacteria are known to sustain within the host macrophage\textsuperscript{37}. Indeed, we found that the promoter of an adenylyl cyclase, Rv1647, was acid-sensitive and studies on its \textit{M. smegmatis} orthologue indicated that reduced transcription of this cyclase was responsible for the reduction of cAMP levels upon acid stress of \textit{M. smegmatis}\textsuperscript{38}. These results argue against the functional redundancy of mycobacterial cyclases and suggest that in spite of the large number of adenylyl cyclases, they may have distinct roles to play in the cell.

**Phosphodiesterases**

In stark contrast to the multitude of cyclases, only a single PDE has been identified thus far in \textit{M. tuberculosis} H37Rv, Rv0805 (ref. 39). It belongs to the Class III family of metallophosphoesterases and its orthologues are found exclusively in slow-growing mycobacteria. Studies from our laboratory indicated that the enzyme is an active PDE with the active site being occupied by two metal ions, namely, Mn\textsuperscript{2+} and Fe\textsuperscript{2+} (refs 39 and 40). Strikingly, Rv0805 appeared to be a poor catalyst of 3',5'-cAMP degradation \textit{in vitro} and overexpression of the enzyme in \textit{M. smegmatis} reduced cAMP levels by 30% (ref. 39). This suggests that there may be additional PDEs in mycobacteria that keep cAMP levels under check or that secretion of cAMP from the cell may serve as the predominant mechanism of abolishing cAMP signalling, a hypothesis that is supported by the high levels of extracellular cAMP measured in mycobacterial cultures\textsuperscript{28,38,41}. In addition, it also hinted towards cAMP-independent ‘moonlighting’ functions of Rv0805. Indeed, on over-expressing Rv0805 in \textit{M. smegmatis}, we observed that the C-terminus of this protein is responsible for anchoring it into the cell envelope where it can alter properties of the cell wall\textsuperscript{42}. The structure of Rv0805 finds the C-terminus
of the protein to be flexible indicating that it may serve as a floppy surface for binding to lipids/proteins of the cell envelope. This apparent cell wall remodelling function of Rv0805 is independent of its cAMP hydrolysing activity and precise mechanistic details as to how this is achieved remain to be determined.

Regulation of cAMP levels in mycobacteria

The intracellular and extracellular levels of cAMP in mycobacteria are several fold higher than those seen *E. coli* (summarized in Bai *et al.*\(^{43}\), table S3). We followed the levels of cAMP across growth in *M. smegmatis* and found that cAMP levels peak during the logarithmic phase of growth of the cells\(^{38}\). Further, the levels of cAMP were enhanced when the cells were grown in SDS but declined on exposure to acid\(^{38}\). In contrast, cAMP levels increased when the slow-growing *M. bovis* BCG or *M. tuberculosis* H37Rv were grown in acidic media\(^{44}\). In a striking departure from other microorganisms, mycobacteria do not exhibit classical carbon catabolite repression\(^{55}\). Instead, mycobacteria have been found to co-catabolize multiple carbon sources\(^{46}\), suggesting that one should be cautious when drawing parallels between cAMP functions in mycobacteria and other microorganisms.

Cyclic AMP effectors

cAMP in the cell is recognized by two structurally different domains, the GAF (mammalian cGMP-binding PDEs, *Anabaena* adenylyl cyclases and *E. coli* Fhl) domain and the cyclic nucleotide-binding (CNB) domain\(^{47-50}\). The GAF domain is an ancient small molecule binding domain which has evolved to bind to a versatile set of ligands including, but not confined to, the cyclic nucleotides\(^{41,52}\). In mammals, GAF domains are associated primarily with PDEs where they are present as two tandem copies and can allosterically modulate cGMP hydrolysis\(^{53}\). For example, in human PDE5, cGMP binding to the GAF domain increases the catalytic activity of the adjacent cGMP-specific PDE domain, thereby allowing it to precisely control cellular cGMP levels\(^{54,55}\). In prokaryotes, on the other hand, this domain is present in a wide variety of proteins including adenylyl cyclases\(^{56}\) and has been shown to reduce methionine sulphoxide\(^{47,58}\).

The CNB domain was first identified in 1982, through comparisons between the crystal structure of catabolite repressor protein (CRP) from *E. coli* and the polypeptide sequence of protein kinase A (PKA) type II from humans\(^{59}\). Subsequent studies have elucidated that the CNB domain has a conserved topology consisting of an eight-stranded \(\beta\)-sandwich flanked by two helical regions\(^{60,62}\). A set of 12 amino acids between two strands of the \(\beta\)-sandwich define the phosphate binding cassette (PBC) of the domain which interacts with the phosphate-sugar component of the cyclic nucleotide\(^{63}\). Post binding, structural rearrangements in the C-terminal hinge and lid regions stabilize the cAMP-bound form\(^{64}\).

Mycobacterial genomes encode for several CNB proteins as shown in Figure 2. Similar to the cyclases, the CNB domain in these organisms is found to make unique associations with other domains, reiterating that the evolution of these novel domain architectures may be a consequence of the adaptation of mycobacteria to different environmental niches. Two of these associations, one of a CNB domain with an HTH domain and the other of a CNB domain with an acetyltransferase domain, are conserved in *M. leprae*, a pathogen that has undergone extensive genome downsizing and pseudogenization, thus reiterating the importance of these proteins in fundamental mycobacterial physiology. *M. marinum* encodes for 13 putative CNB-domain containing proteins, some of which have domain organizations absent in *M. tuberculosis* H37Rv. This is in concordance with its ability to be free living and within a host, which has led to the preservation of several genes otherwise eliminated from the pathogenic *M. tuberculosis* and *M. leprae* via reductive genome evolution\(^{65}\).

*M. tuberculosis* H37Rv is predicted to encode 10 proteins containing the CNB domain and three containing the GAF domain\(^{77,37}\). Of the three GAF domain containing proteins, one is known to bind to haem and is therefore unlikely to be a cNMP-binding protein, leaving 12 putative cNMP-binding proteins in the organism\(^{66}\). Of these, three CNB-domain containing proteins have been studied, viz. Rv3676, Rv1675c and KATmt (Rv0998). Rv3676 and Rv1675c contain an N-terminal CNB domain followed by a C-terminal HTH domain and are orthologues of the well-studied CRP from *E. coli*. They mediate the direct transcriptional roles of cAMP. KATmt, on the other hand, encodes for a cAMP-dependent protein lysozyme acetyltransferase, which allows post-translational regulation of protein function by cAMP. Each of these proteins will be discussed in brief below.

**cAMP receptor protein (CRP)**

Rv3676 (or CRP\(_{58}\)) was the first CNB-domain containing protein characterized from *M. tuberculosis* H37Rv and has been shown to regulate the expression of several genes in the organism\(^{67,68}\). The consensus DNA-binding site of this protein was elucidated using a genomic systematic evolution of ligands by exponential enrichment (SELEX) approach and was found to be similar to that of the *E. coli* CRP\(^{69}\). This motif was then used to computationally predict potential sites for CRP\(_{58}\) binding across the genome of *M. tuberculosis* H37Rv and other mycobacteria\(^{69,70}\). Interestingly, it appeared that the predicted CRP\(_{58}\)-regulated genes were more highly conserved in the pathogenic mycobacteria than in the non-pathogenic...
Figure 2. Distribution and domain compositions of CNB domain-containing proteins in mycobacteria. CNB domain-containing proteins and their domain compositions were identified using the InterPro database\(^1\). CNB = Cyclic nucleotide-binding domain; HTH = Transcription regulator HTH, Crp; GNAT = Gcn5-related N-acetyltransferase domain; ABC = ABC transporter-like domain; PL = Patatin/phospholipase A2-related domain; NAD = NAD(P)-binding domain; ATP = ATPase-like, ATP-binding domain; CDB = CopG-like DNA-binding domain; MFS = Major facilitator superfamily domain; MS = Mechanosensitive ion channel domain; LSM = Like-Sm domain.

ones, highlighting the relevance of this protein in virulence. Other attempts to define the regulon of CRP\(_{Mt}\) came from microarray analysis of \emph{M. tuberculosis} strains deleted for this gene\(^6\). Crucial genes that were downregulated in the knockout strain included the resuscitation promoting factor A, a secreted protein that reverses dormancy in mycobacteria\(^7\) and whiB1, an essential NO-oxide sensing DNA-binding protein\(^7\). Although efforts to determine the DNA-binding sites of CRP\(_{Mt}\) have centred on transcriptional regulation at specific promoters, studies on the \emph{E. coli} orthologue have uncovered thousands of weaker binding sites for CRP on the genome, suggesting it may act as a chromosome shaping protein. This hypothesis may hold true for \emph{M. tuberculosis} as well based on the ability of CRP\(_{Mt}\) to bend DNA\(^6\), the large number of non-coding regions that bound to CRP\(_{Mt}\) in the SELEX experiment\(^6\), and the emerging trend of reassigning classical mycobacterial transcription factors as global nucleoid-associated proteins\(^7,74\).

Regulation of whiB1 transcription by CRP\(_{Mt}\) has been studied in detail and shows an uncommon mechanism of regulation by CRP\(^7,74\). The whiB1 promoter has two CRP\(_{Mt}\) binding sites, CRP1 and CRP2. CRP1 is located upstream of the –35 region and binding to this site activates whiB1 transcription. Conversely, CRP2, which encompasses the –35 region, is a weaker site for CRP\(_{Mt}\) binding and negatively regulates whiB1 expression. Thus the expression of whiB1 is titrated by the cell such that whiB1 is upregulated at low concentrations of CRP\(_{Mt}\)–cAMP while high concentrations of CRP\(_{Mt}\)–cAMP lead to cessation of whiB1 expression. Interestingly, whiB1 is self-inhibitory, that is, it represses its own transcription\(^7\).
leading one to speculate that whiB1 may directly/indirectly upregulate CRP<sub>Mt</sub> expression, thus potentiating the existing negative feedback loop.

The knockout of CRP<sub>Mt</sub> is impaired in growth in vitro, in the macrophage and also shows attenuated virulence in mice<sup>69</sup>. This attenuation has been partly attributed to the downregulation of the CRP<sub>Mt</sub>-regulated serC gene in the knockout<sup>77</sup>. This gene codes for a phosphoserine aminotransferase and supplementation of bacterial media with serine restores the growth rate of the knockout. However, a similar supplementation in the macrophage does not rescue this defect, indicating that dysregulation of serC can only partially explain the phenotypes of the knockout and alteration of the expression of other genes, such as whiB1, may also contribute to the growth defect.<sup>77</sup>

The dependence of CRP<sub>Mt</sub> for cAMP appears to be less absolute as compared to the <i>E. coli</i> CRP. This is apparent in electrophoretic mobility shift assay (EMSA) experiments which indicate that though cAMP improves the affinity of CRP<sub>Mt</sub> for its cognate DNA site, it is not essential for DNA-binding<sup>68,69,76</sup>. Moreover, reporter assays demonstrated that exogenous addition of dibutyryl-cAMP, a diffusible cAMP analogue, increased CRP<sub>Mt</sub> promoter activity in <i>M. tuberculosis</i> by a mere 2.5-fold<sup>79</sup>. This is in contrast to the several thousand-fold increase in DNA binding affinity seen for the <i>E. coli</i> CRP in presence of cAMP<sup>76</sup>. These observations suggest that the existing high levels of cAMP in growing mycobacterial cells may have necessitated the adaptation of CRP<sub>Mt</sub> from a cAMP-dependent transcription factor as seen in <i>E. coli</i> to a cAMP-regulated one.

Though the crystal structures of the apo and the holo forms of CRP<sub>Mt</sub> have been determined, they have been unable to clearly explain the mechanism of cAMP-induced activation of CRP<sub>Mt</sub>. On the other hand, the activation of <i>E. coli</i> CRP by cAMP-binding has been well studied<sup>60,61,76-81</sup>. CRP is a homodimer and cAMP-binding to each protomer leads to the reorientation of the DNA-binding F-helices. This positions the F-helices at an optimum distance relative to each other to fit into adjacent major grooves of the palindromic CRP site. CRP<sub> Mt</sub> was first crystallized in the cAMP-free form and showed extensive asymmetry between the two subunits of the dimer<sup>82</sup>. One subunit appeared to be in the active form, but the other adopted an inactive ‘off’ state. This asymmetry was not confined simply to the DNA-binding region, as seen in the <i>E. coli</i> apo-CRP, but was apparent even in the internal protein core. A conflicting report on the crystal structure of apo-CRP<sub>Mt</sub> showed that the structure does not exhibit extreme asymmetry<sup>83</sup>. Instead, the authors suggested that the ‘off’ state of the protein could be attributed to the strong hydrophobic interactions between the N and C-terminal domains which restrain the protein into a closed inflexible form. They further suggested that the asymmetry observed in the initial structure may be an artifact of crystal packing effects, a view supported by NMR studies of this protein in solution<sup>84</sup>. A third study crystallized holo-CRP<sub>Mt</sub> in which both subunits were symmetric and showed re-orientation of the F-helices in a manner similar to the <i>E. coli</i> orthologue<sup>85</sup>. Thus the precise interpretation of cAMP-induced activation of CRP<sub>Mt</sub> is consequent on which structure of the apo-protein is true. However, in both structures of the apo-protein, the F-helices are oriented in a manner unsuitable for DNA-binding, an observation not entirely in agreement with the ability of CRP to mediate its functions in the absence of cAMP, as seen in vitro<sup>68,69,76</sup> and in vivo<sup>75</sup>.

**cAMP-responsive transcription factor**

In an attempt to identify cAMP-regulated genes in mycobacteria, Gazdik and McDonough<sup>86</sup> grew <i>M. bovis</i> BCG cells in the presence of dibutyryl-cAMP under different environmental stresses followed by a proteome analysis. This led to the identification of five proteins which were upregulated in cAMP and hypoxia-dependent manner. However, none of these appeared to be targets of CRP<sub> Mt</sub>. A later study then demonstrated that three of these proteins, viz. GroEL2, Mdh and Rv1265 were targets of a second CRP-like protein, Rv1675c, also known as cAMP and macrophage regulator (Cmr)<sup>84</sup>. This was confirmed using EMSA and also shown within macrophages. However, Cmr showed two important differences from CRP<sub> Mt</sub>. First, the knockout of Cmr did not have any overt phenotype, including growth defects. Second, though the initial identification of this regulation was through cAMP, in vitro assays failed to show cAMP-dependent regulation of Cmr-binding to DNA. This suggests that other factors in addition to cAMP may be required for Cmr-mediated gene regulation. Though CRP<sub> Mt</sub> and Cmr are the most thoroughly studied transcriptional targets of cAMP, newer findings have also highlighted the ability of cAMP to regulate non-coding RNAs in <i>M. tuberculosis</i> H37Rv, arguing for non-canonical roles of cAMP in mediating transcriptional regulation<sup>87</sup>.

**A cAMP-regulated protein lysine acetyl transferase (KATmt)**

KATmt is the only non-CRP-like cAMP-binding protein that has been characterized from <i>M. tuberculosis</i> H37Rv. It presents a protein with a novel fusion of a CNB domain attached to Gcn5-related N-acetyltransferase-like (GNAT-like) domain. Our initial characterization of this protein, and its orthologue in <i>M. smegmatis</i> (KATms), showed that both bind cAMP and mutation of the conserved arginine to alanine in the phosphate binding cassette (PBC), abolishes binding<sup>88</sup>. We also identified an interacting partner and a putative substrate for the enzyme,
MSMEG 4207, a Universal Stress Protein (USP), through a pull-down experiment, where GST-KATms was interacted with *M. smegmatis* lysates. Further experiments confirmed that KATms acetylates USP at K104 and in a deletion mutant of KATms, USP is not acetylated. This was the first report of protein lysine acetylation in mycobacteria. KATms is able to acetylate USP even in the absence of cAMP though acetylation increased by 2.5-fold when cAMP was added, thereby drawing parallels with the cAMP-mediated regulation of CRP<sub>M</sub>. On the other hand, KATmt shows strict cAMP dependence for USP acetylation and has a 1000-fold lower affinity for cAMP than KATMs. Given the high levels of intracellular cAMP in *M. tuberculosis*<sup>41</sup>, these results imply that KATmt is more fine-tuned to cAMP levels as not only is its activity contingent on cAMP binding, its threshold for binding is higher, thus allowing it to adapt to the elevated cAMP levels in the cell. However, the absence of an orthologue of USP in *M. tuberculosis*<sup>90</sup> argued for the presence of additional unknown substrates of KATmt. One of these was identified to be acetyl CoA synthase<sup>89</sup>.

Acetyl CoA synthase (ACS) was shown to be acetylated by KATMs at a conserved lysine residue leading to its inactivation<sup>89</sup>. This inactivation was reversible as addition of a deacetylase from *M. tuberculosis*, Rv1151c restored catalysis by ACS. Because deacetylases require NAD<sup>+</sup> as a co-factor, this suggested that the acetylation status of ACS may be a function of the energy status of the cell. Upon nutrient starvation, the accumulation of NAD<sup>+</sup> could lead to activation of Rv1151c and consequently activation of ACS leading to the synthesis of acetyl CoA. Note that in these conditions, ATP, and therefore cAMP, are also likely to be scarce. Conversely, under nutrient replete conditions, the high levels of cAMP would activate KATmt leading to inhibition of ACS.

We elucidated the structural rearrangements that occur in KATmt post binding of cAMP using a combination of bioluminescence resonance energy transfer (BRET) and amide hydrogen/deuterium exchange mass spectrometry (HDXMS)<sup>90</sup>. Cyclic AMP binding to KATmt led to a conformational change that was detectable by BRET and was lost on mutation of the conserved arginine in PBC. We also performed HDXMS analysis of the *M. smegmatis* orthologue, KATms, which permitted monitoring of the dynamics of cAMP-binding and led to the following observations: First, binding of cAMP resulted in decreased deuterium exchange, indicating that this leads to increased ordering of the CNB domain on cAMP-binding. Second, this reduction in exchange was confined to the CNB domain and the predicted helical linker region of the protein, with no change in the acetyltransferase domain. This highlighted the importance of the linker in transmitting the activation signal to the acetyltransferase domain. When we performed BRET assays with a protein lacking the linker or a mutant protein with a disrupted linker, it was unable to mimic the conformational change seen in the wild type protein. Finally, unlike PKA<sup>91</sup> and Epac (Exchange protein activated by cAMP)<sup>92</sup>, where cAMP-binding led to considerable rearrangement of residues binding to the exocyclic oxygen followed by complex allosteric relays, cAMP-binding to KATms showed no changes in this region, and exhibited shifts in the residues contacting the adenine moiety of cAMP instead<sup>90</sup>. These results signified that KATms functioned in a simple allosteric switch-like manner, wherein binding of cAMP to the CNB domain led to a relief of inhibition on the catalytic domain in a linker-dependent manner. This mechanism of cAMP induced allostery is unique to KATMs.

Crystal structures of KATmt in the cAMP-bound and free forms further illuminated our understanding of this protein’s catalytic mechanism<sup>93</sup>. The cAMP-free form of the protein is autoinhibited by two mechanisms. First, the C-terminus of the protein extends and fastens into the cAMP-binding site such that the terminal carboxyl group mimics a cyclic phosphate moiety. This leads to exclusion of cAMP from the regulatory domain. Second, a large loop formed by residues 161–203 folds over the acetyltransferase active site creating a lid which prevents substrate binding. This loop contains a His-173 which binds to the catalytic base E235 by imitating a lysine residue. Binding of cAMP induces a 40° rotation of the regulatory domain around the interdomain linker. This displaces the C-terminus from the CNB domain and also leads to refolding of the lid leaving the active site accessible to the substrate. Note that in concordance with the HDXMS data, there is minimal change in the acetyltransferase domain on activation<sup>93</sup>. Moreover, the helical linker region defined in the previous study is a component of the autoregulatory lid region observed in the structure, thus reiterating that the linker is essential for inter-domain communication. The linker acts as a crucial pivot for rotation between the two domains indicating that apart from increasing flexibility, the linker may pre-encode for successive conformational states, as suggested by a recent review<sup>94</sup>.

**cAMP in mycobacterial pathogenesis**

Initial studies on the role of cAMP in mycobacteria established a strong correlation between the inhibition of phagolysosomal fusion and the increase in levels in cAMP<sup>95,96</sup>. Although they were unable to conclusively establish if this surge in cAMP was derived from the pathogen, the enhanced cAMP levels found in media harbouring actively growing mycobacteria<sup>28</sup>, and the observation that *M. microti* secretes 80% of the cAMP it produces<sup>95</sup>, suggested that the cAMP was bacterially derived. cAMP is known to be elevated in macrophages on mycobacterial passage and bacteria isolated from macro-
phages showed 50-fold higher levels of cAMP than those incubated in media alone. Studies have also shown that increasing cAMP levels mitigate mycobacterial sequestration to the lysosomes and this may be through the inhibition of phagosomal actin assembly by cAMP.

The number of adenylyl cyclase genes in mycobacteria are a deterrent to gene deletion studies, which are traditional options employed to illuminate the function of a molecule, in this case cAMP, in the cell. This is manifest in the lack of growth defects shown by deletion strains of adenylyl cyclases Rv1625c (ref. 98) and Rv1264 (ref. 99) in mice. However, a recent report has shown that loss of Rv0386, also an adenylyl cyclase, led to reduced survival of M. tuberculosis in mice. Macrophages infected with these bacteria were found to harbour reduced levels of bacterially derived cAMP, leading to decreased TNF-α production in the macrophage.

Conclusions

In spite of the continuing successful treatment of drug-sensitive tuberculosis (41% decrease in mortality rate since 1990), the alarming resurgence of multidrug resistant (MDR) and extensively drug resistant (XDR) TB are pressing issues in global health. After a 40-year gap in the availability of new drugs, the US Food and Drug Administration recently approved bedaquiline as part of combination therapy to treat adults with MDR-TB. Combating drug-resistant tuberculosis has been further complicated by the dearth of accurate biomarkers and the inadequate vaccination strategies. As most early antibiotics were derivatives of existing drugs it has become essential to discover novel drug scaffolds which can subvert the current bacterial resistance mechanisms.

This approach is incumbent on a deeper understanding of the signalling pathways and virulence strategies of mycobacteria. The central role that cAMP plays in cellular physiology places it in a key position in the macrophage, one that can be seized by invading mycobacteria. Though we have achieved a reasonable understanding of some of the components in the cAMP machinery, key issues remain to be addressed. Why do mycobacteria encode for so many adenylyl cyclases and are all required for pathogenesis? What are the downstream signalling pathways triggered by cAMP and what is the role of the other CNB-domain containing proteins? How is cAMP secreted into the macrophage? How does the macrophage respond to the cAMP elevation? Answering these questions will not only provide us with fundamental insights into cAMP signalling in mycobacteria but also with a greater appreciation of host–microbe communication and dynamics.

Note added in proof: Following the submission of this article, we have identified a number of FadD enzymes as being substrates for KATmt, and shown that acetylation inhibits their acyl CoA ligase activity (Nambi, S. et al., Cyclic AMP-dependent protein lysine acylation in mycobacteria regulates fatty acid and propionate metabolism. J. Biol. Chem., 2013, 288, 14114–14124). Transcriptional responses mediated by overexpression of Rv0805 in M. tuberculosis have also been described (Matange, N., Hunt, D. M., Buxton, R. S. and Visweswariah, S. S., Overexpression of the Rv0805 phosphodiesterase elicits a cAMP-independent transcriptional response. Tuberculosis, 2013, 93, 492–500).


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