Published in final edited form as:

Curr Opin Microbiol. 2014 April ; 18: 23–29. doi:10.1016/j.mib.2014.01.007.

Allostery and compartmentalization: old but not forgotten

Hyungjin Eoh¹ and Kyu Y. Rhee^{1,2,*}

¹Division of Infectious Diseases, Department of Medicine

²Department of Microbiology & Immunology, Weill Cornell Medical College, NY, NY 10065, USA

Abstract

Homeostasis is an essential capability of all cells mediated by complex and diverse regulatory networks. Despite this complexity, many of the fundamental regulatory mechanisms used by cells have been evolutionarily conserved. It is thus somewhat surprising that the apparent physiologic significance of these mechanisms has been experimentally neglected. Here, we review 2 widely recognized regulatory mechanisms, allostery and compartmentalization, which exemplify this dissociation in our current understanding of the microbial pathogen, *Mycobacterium tuberculosis*.

INTRODUCTION

Regardless of size or evolutionary stature, all cells face the challenge of adapting to a complex but fundamentally shared environment. Growing evidence has further suggested that the regulatory mechanisms used by microbes to meet this challenge differ neither in constitution nor complexity from those of their nucleated counterparts. It is thus somewhat surprising that the physiologic scope and evolutionary conservation for some, if not many, of these mechanisms remain incompletely defined. The causes of this shortfall are multifactorial but, in large part, reflect the historically disproportionate impact of technological advances on molecular over physiologic, or systems level, disciplines.

Two widely recognized regulatory mechanisms which exemplify this deficiency are those mediated by allostery and subcellular compartmentalization (FIG. 1). Allostery is a fundamental principle in enzymology that describes the regulation of a given protein's activity by binding of a ligand to a site other than its active site and was first described for the control of O₂ binding to hemoglobin by protons and activation of glycogen phosphorylase by AMP [1,2]. From a physiologic perspective, allostery offers the potentially most direct, rapid and efficient regulatory mechanism to sense and respond to changes in the chemical environment of a cell. This is because it can operate at up to diffusion limited rates in a potentially reversible and energy-free manner. Yet, while considerable work has expanded our understanding of the molecular details of such regulation at the level of isolated proteins, considerably less progress has been made toward the more global

* to whom correspondence should be addressed; Tel) 1-212-746-4547, kyr9001@med.cornell.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

discovery of allosteric effectors, their target proteins and contribution to cellular homeostasis. Allostery has thus failed to penetrate many physiologic models of cellular regulation [3].

Subcellular compartmentalization conversely represents a cornerstone of eukaryotic cell biology that has failed to penetrate the thinking of most microbiologists due to the apparent lack of lipid bound organelles. However, all cells face the challenge of catalyzing thousands of metabolic reactions at a given instant in time; many of which are mutually competitive, if not incompatible with one another. Accordingly, growing evidence has suggested that bacteria may, in fact, achieve the same functional compartmentalization through the regulation formation and/or activity of protein localization and pseudo-organelles, such as protein microcompartments and periplasm. Cell anatomy is thus an organizing principle of cell chemistry, rarely considered in the absence of lipid bound organelles.

Here, we review existing knowledge of allosteric regulation and subcellular compartmentalization and consider their relevance in the human pathogen *Mycobacterium tuberculosis* (Mtb). Mtb is the causative agent of tuberculosis (TB) and leading bacterial cause of deaths worldwide. A major barrier to control of the TB pandemic is the lack of rapid and simple treatments. Current TB chemotherapies are longer and more complex than for virtually any other bacterial infection, and thus associated with rates of treatment non-compliance and failure that have paradoxically given rise to the emergence of multidrug-resistant (MDR) and extensively drug resistant (XDR) disease itself [4]. From a biological perspective, Mtb's pathogenicity is due in large part to its ability to exit cell cycle and enter an antibiotic-tolerant, but genetically susceptible, non-replicating state. Following infection, Mtb is believed to reside chiefly within macrophages where it is exposed to heterogeneous microenvironments that restrict replication and lead to a clinically asymptomatic infection in approximately one third of the world's population. In roughly 10% of immunocompetent hosts, some subpopulations re-enter cell cycle leading to approximately 8 million new cases and 2 million deaths each year, while others persist. Treatment for both active and latent Mtb infections thus requires prolonged treatments of 6-12 months duration due to a lack of antibiotics with activity against non-replicating Mtb [5-8].

ALLOSTERIC REGULATION

Though primarily studied as a molecular, rather than physiologic, phenomenon, allostery was first described by Monod and colleagues as a regulatory mechanism capable of actively redirecting biochemical reactions according to physiologic need, rather than passive mass action and/or thermodynamic equilibration. They specifically hypothesized that allosteric regulation of protein activity represented an essential and characteristic biological control mechanism which enabled cells to respond immediately and reversibly to specific chemical signals, effectors, which may be totally unrelated to their specific substrates, cofactors or products and thus govern and control, that is to say to *modify*, the distribution of building blocks or chemical potential according to the requirements of remote pathways, or to chemical alterations of the environment, or to the physiological meaning of chemical signals issued by other cells; because the physiologic requirements [of the cell] could not be satisfied otherwise, certainly not by simply obeying mass action [1]. From a mechanistic

perspective, allosteric regulation offered a biologically flexible, kinetically rapid, and energy-free, mechanism of cellular control.

Work over the intervening decades has since fulfilled many of these predictions. Allosteric regulation has been reported as a property of proteins of diverse function, ranging from metabolic enzymes to cell surface receptors to DNA binding proteins to structural scaffolds, and evolutionary phylogeny, spanning all 3 kingdoms of life [9,10]. Structural and biochemical studies have further provided molecular insights into its underlying physicochemical principles. Perhaps most significantly however, this work has provided broader insight into several previously unrecognized physiologic regulatory principles that include the control of O₂ delivery by hemoglobin in response to changes in CO₂, pH and levels of the glycolytic intermediate 2, 3-bisphosphoglycerate, each of which reports on a distinct facet of the metabolic state of the cell. Studies of glycogen phosphorylase and AMP kinase similarly established ATP and AMP levels as regulatory indicators of the energetic status of the cell [11,12]. From a translational perspective, allosteric regulation has expanded both the chemical and functional nature of potential drug targets. Allosteric sites have evolved in response to selective pressures distinct from those of the primary functional site of a protein and thus represent orthogonal sources of therapeutic selectivity with the potential to either achieve species selectivity and/or overcome mechanism-based (orthosteric) resistance [13]. Notable examples of clinical drugs which have successfully exploited this difference include the benzodiazepines which allosterically modulate the activity of central nervous system-specific GPCRs, and the HIV drugs efavirenz and maraviroc which target its reverse transcriptase and the human chemokine receptor CCR5, respectively [14-16].

Unlike most microbial pathogens, Mtb resides within humans as both host and reservoir, and within humans, the macrophage phagosome as its quantitatively largest reservoir [8]. Mtb has thus evolved in an ultranarrow ecologic niche apart from other microbes in which, both unrestrained replication and perfect symbiosis threaten its existence as a species.

Relieved of the requirement to replicate continuously, Mtb occupies the majority of its decades-long lifecycle in a prolonged state of non- or slowly replicating physiology. However, even non-replicating Mtb face the challenge of preserving the integrity of essential cellular components and resuming replication at a given moment to ensure transmission to a new host and completion of its lifecycle [6,7,17,18]. Thus, while Mtb has been shown to encode adaptive transcriptional programs which may facilitate some of these transitions, their kinetics and bioenergetic requirements mitigate against their adequacy as Mtb's primary mode of adaptation. In fact, early studies by Wayne and colleagues [19], using ³H uracil, demonstrated that Mtb induced into a viable state of non-replication by prolonged oxygen depletion exhibited minimal, if any, transcriptional activity. Subsequent work established that such non-replicating Mtb underwent an 80% reduction in ATP levels while transcriptional profiling studies have provided only fragmentary views of its physiologic state [20,21]. Bioenergetics aside, Mtb has adapted its metabolic network to traverse a diverse but specific range of chemically stringent microenvironments associated with its lifecycle, which include, but are not limited to, the intracellular phagosomal environment,

cytosol, acellular center of caseating granulomas, and sputum. Allosterity thus represents a potentially critical but understudied biochemical regulatory property of Mtb.

Existing knowledge of allosterity in Mtb derives chiefly from *in vitro* studies of purified recombinant proteins and thus focused on its molecular, rather than physiologic, significance. Nonetheless, even such molecular studies have begun to uncover the existence of Mtb-specific allosteric controls. Three proteins which exemplify the varying degrees of species-specific adaptation of allosteric regulation are the Mtb orthologs of: (i) D-3-phosphoglycerate dehydrogenase (PGDH) involved in the biosynthesis of essential amino acid L-serine; (ii) 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAH7P) synthase, which catalyzes the first step of the shikimate pathway required for the biosynthesis of aromatic amino acids; and (iii) 2-hydroxy-3-oxoadipate (HOA) synthase, an putative enzyme of the Mtb TCA cycle.

PGDH catalyzes the first step of the highly conserved biosynthetic pathway for L-serine from 3-D-phosphoglycerate. In this pathway, termed the phosphorylated pathway, PGDH converts 3-D-phosphoglycerate (originated from the glycolysis) into phosphohydroxypyruvate (PHP), which in turn is converted to phosphoserine, and finally dephosphorylated to generate L-serine [22]. The Mtb genome is annotated to encode 2 putative genes (*Rv2996c/serA1* and *Rv0728c/serA2*) for PGDH. However, biochemical evidence for PGDH activity has only been reported for *serA1* which has also been crystallized and shown to be genetically essential for *in vitro* growth [22]. Like most bacterial PGDHs, Mtb's PGDH is also allosterically regulated by the pathway end product, L-serine, that in *E. coli* and other bacteria is mediated by binding of L-serine to a regulatory, so-called ACT, domain located adjacent to the substrate-binding domain. Mtb's PGDH however contains an additional domain, termed "allosteric substrate binding (ASB) domain", which is inserted between the substrate-binding and ACT domains, that modulates or potentially co-regulates the impact of L-serine binding to ACT domain allowing Mtb to maintain PGDH catalytic activity at unusually high L-serine concentrations and perhaps in response to additional allosteric signals [23,24].

Like PGDH, DAH7P synthase catalyzes the first step of the pathway for shikimate biosynthesis, which is essential in bacteria and plants and is responsible for the production of aromatic amino acids (phenylalanine, tyrosine, and threonine), folic acid, an essential cofactor for many enzymatic processes, and salicylate [25]. DAH7P synthase specifically catalyzes the stereospecific aldol condensation of phosphoenolpyruvate (PEP) and erythrose 4-phosphate (E4P) to produce DAH7P, which is, in turn, converted to chorismate, a shared precursor of the aromatic amino acids. As the first enzyme in the shikimate pathway, DAH7P synthase provides a major point of regulation for nutrient flux to the shikimate pathway that, like other metabolic enzymes, is negatively regulated by downstream pathway end products. Because the shikimate pathway serves multiple downstream pathways, several microbial species have evolved multiple DAH7P synthase orthologs with differing allosteric sensitivities. For example, both *E. coli* and *Neurospora crassa* encode three separate DAH7P synthase isozymes, each of which is specifically inhibited by either Phe, Tyr, or Thr [26,27]. In contrast, *Saccharomyces cerevisiae* encodes only 2 DAH7P synthase isozymes, each of which is sensitive to either Phe or Tyr [28]. Mtb however encodes only a single annotated

DAH7P synthase (Rv2178c/AroG) that exhibits a unique pattern of allosteric regulation by dual combinations of Trp and Phe (or Trp and Tyr to a lesser extent), that appears distinct from those of Trp, Phe, or Tyr alone consistent with crystal structures showing simultaneous binding of Pre and Trp to distinct allosteric sites [29,30].

Though still emerging, the Mtb protein perhaps subject to the most species-specific allosteric regulation is its annotated α -ketoglutarate dehydrogenase (KDH) E1 subunit, Rv1248c, that has subsequently acquired additional annotations as an α -ketoglutarate decarboxylase and HOA synthase [31,32]. Many microorganisms modulate the TCA cycle flux by actively regulating the activity of their KDH for biosynthesis of intermediates of oxidative and reductive TCA cycle branches. However, *in vitro* studies of Mtb lysates and purified recombinant Rv1248c failed to detect KDH activity, revealing that Mtb has only an α -ketoglutarate decarboxylase activity. More recent studies using a method termed activity based metabolomic profiling (ABMP) identified a more robust activity as a carboligase that condensed α -ketoglutarate and glyoxylate to generate HOA, identifying yet a third potential catalytic activity to this protein [31]. Physiologic function notwithstanding, both *in silico* and experimental studies have identified a number of unusual and unexpected allosteric regulatory properties associated with this protein. For example, *Rv1248c* encodes an N-terminus acetyltransferase like domain not present in E1 orthologs from other species and renders its α -ketoglutarate-dependent reactions amenable to activation by binding of the metabolically related species acetyl-CoA [32,33]. Looking beyond direct ligands, Rv1248c has independently been found to be regulated by binding of the forkhead domain containing protein GarA, that itself is regulated by phosphorylation by PknB and PknG, 2 of 11 eukaryotic serine-threonine protein kinases that are expressed on the Mtb surface and thought to transduce environmental signals [34,35]. Binding of GarA to Rv1248c inhibits HOA synthase activity through a binding interaction both structurally and functionally independent of that of acetyl-CoA [32,33]. Future studies of Rv1248c and its allosteric properties thus promise to deliver key insights into the regulation of Mtb central carbon metabolism.

COMPARTMENTALIZATION

Most textbooks depict bacteria as unstructured bags of cytosol bounded by a cell wall-reinforced plasma membrane. However, bacteria, like all cells, face the challenge of catalyzing thousands of diverse chemical reactions at a given instant in time, many of which are mutually competitive, if not incompatible, with one another. Compartmentalization thus represents an essential but understudied facet of microbial physiology.

Growing evidence has established that, while lacking lipid bound organelles, bacteria possess functionally compartmentalized structures both within cell envelope and cytosol [36,37]. From a cellular perspective, these range from the membrane-delimited periplasm, to storage granules, to cytoskeletal structures and most recently discovered cytoplasmic protein-based microcompartments, each of which has been shown to enclose biochemically distinct processes. From a chemical perspective, the evolutionary ubiquity of compartmentalization was presaged by Atkinson's recognition of the limited solvation capacity (and effective aqueous volume) of cellular cytosol and accompanying need for

cellular mechanisms to propagate the catalytic activity of individual enzymes to their respective pathways. Work by Sreere and others helped to resolve this dilemma with the discovery of molecular channeling—a functional form of compartmentalization capable of insulating specific biochemical reactions from the bulk environment under locally optimized conditions— as an intrinsic property of multienzyme complexes [38–41]. Recent studies in yeast using GFP-fusions further showed that the formation of such assemblies was, in fact, subject to regulation by the environmental conditions of the cell that occurred in the absence of *de novo* gene expression [42].

As discussed above, a major unexplained of Mtb's pathogenicity is how it is able to traverse the diverse environments encountered across its lifecycle. Of particular significance is its ability to maintain viability for prolonged periods of time in noxious and nutrient-poor environments, yet preserve the capacity to resume replication and transmit itself to a new host [5,17,43]. Thus, while generally under-recognized, compartmentalization is emerging as an important regulatory property of Mtb. Traditional views of the Mtb envelope, for example, depict a chemically complex but functionally unitary barrier [44]. However, mounting biochemical and ultrastructural evidence has established that this chemical complexity is, in fact, linked to a structurally complex barrier that consists in phospholipid bilayer surrounded by a periplasmic space that is delimited by concentric layers of peptidoglycan and arabinogalactan polymers, galactan-linked and free mycolic acids, and a lush outer polysaccharide capsule nearly as thick as its underlying outer mycolate membrane [45,46]. This unusual structural organization thus serves as an efficient permeability barrier to hydrophobic solutes whose diffusion into Mtb periplasm and/or cytosol is slowed by the outer polysaccharide capsule and thickness and low fluidity of underlying mycolate-containing layers, and hydrophilic solutes whose access to cytosol is hindered by the hydrophobicity of the outer mycolate layer and undefined nature of its periplasmic space which is uniquely delimited by 2 lipid boundaries. Each layer of the Mtb envelope thus functions to effectively compartmentalize the stepwise transit of molecules between the extracellular environment and cytosol. For example, deletion of MmpL8 or LprG transporters abrogates biosynthesis of the virulence determining polyketides, phthiocerol dimycoserolate (PDIM) and sulfoglycolipid (SGL), even when biosynthetic enzymes are intact [47,48].

Looking beyond the cell envelope, recent work has provided evidence for the existence of protein-based microcompartments in Mtb [49]. Micro (and nano) compartments are structurally discrete organelles typically composed of functionally discrete protein complexes housed within an icosahedral protein-based shell. Such protein-based organelles are believed to enhance the: catalytic efficiency of enzymes within the cell by: (i) increasing the local concentration of enzymes for a given pathway, (ii) increasing the concentration of volatile substrates (gaseous metabolites), (iii) trapping the diffusion of possibly noxious toxic intermediates or products from the bulk cytosol, and (iv) providing a structural means of regulating potential interactions with proteins associated with related and unrelated pathways [50,51]. Though generally considered a phenomenon of highly specialized biochemical processes, such as CO₂ fixation in the case of RuBisCO or generation of noxious metabolites such as propanediol or ethanolamine, metabolic compartmentalization is emerging as a more ubiquitous property of microbial physiology [52,53]. Indeed,

orthologs of the shell proteins associated with these protein-based organelles have been found in all major bacterial lineages. For Mtb, sequence-based homology searches identified significant homology between Rv0798c and the encapsulin shell protein of the recently discovered nanocompartment of *Thermotoga maritima* which has been shown to assemble into an icosahedral shell of 12 homopentamers that encloses enzymes involved in iron metabolism [54]. Rv0798c has similarly been reported to form large multimers in solution with an apparent essentiality for optimal *in vitro* growth [49].

From a functional perspective, metabolomic tracing studies have begun to provide orthogonal evidence of metabolic compartmentalization. Recent studies specifically showed that, unlike the model bacterium *E. coli*, Mtb is capable of metabolizing multiple carbon sources simultaneously and to distinct metabolic fates [55]. Moreover, these studies showed that Mtb could co-catabolize glucose and acetate through gluconeogenic and glycolytic pathways simultaneously. The specific mechanisms by which this compartmentalization are achieved however remain yet unidentified.

CHALLENGES AND OPPORTUNITIES

Allostery and compartmentalization are evolutionarily ubiquitous, but understudied, regulatory mechanisms of potentially considerable significance to Mtb. Unlike the case for their genetically-based counterparts, tools for the systematic identification and study of allosteric regulators remain technologically underdeveloped. Studies of complex and potentially dynamic protein assemblies similarly remain limited by a historical dependence on the use of *in vitro* systems or genetically-based measures of individual protein-protein interactions. However, recent technological advances in the areas of mass spectrometry and high content microscopy have overcome these limitations. Orsak et al., for example, reported mass spectrometry integrated with equilibrium dialysis for the discovery of allostery systemically (MIDAS) to identify novel protein/metabolites interaction while Narayanaswamy et al. have developed high throughput methods for studying protein-protein interactions under various stresses [42,56,57]. Looking ahead, it will thus be of particular importance to ensure that the development of such new technologies revives the study of allostery and compartmentalization and their specific relevance to disease states such as tuberculosis.

ACKNOWLEDGEMENTS

We apologize that many papers could not be discussed owing to the lack of space. The authors gratefully acknowledge the support of the Bill and Melinda Gates Foundation Grand Challenges Exploration Program, NIH, and Stony Wold Herbert Fund.

REFERENCES

1. Monod J, Changeux JP, Jacob F. Allosteric proteins and cellular control systems. *J Mol Biol.* 1963; 6:306–329. [PubMed: 13936070]
2. Sprang SR, Acharya KR, Goldsmith EJ, Stuart DI, Varvill K, Fletterick RJ, Madsen NB, Johnson LN. Structural changes in glycogen phosphorylase induced by phosphorylation. *Nature.* 1988; 336:215–221. [PubMed: 3194008]

3. Changeux JP. Allosterity and the Monod-Wyman-Changeux model after 50 years. *Annu Rev Biophys.* 2012; 41:103–133. ** This review provides a contemporary re-evaluation of the Monod-Wyman-Changeux model of allosterity first proposed in 1965. [PubMed: 22224598]
4. Raviglione M, Marais B, Floyd K, Lonnroth K, Getahun H, Migliori GB, Harries AD, Nunn P, Lienhardt C, Graham S, et al. Scaling up interventions to achieve global tuberculosis control: progress and new developments. *Lancet.* 2012; 379:1902–1913. [PubMed: 22608339]
5. Barry CE 3rd, Boshoff HI, Dartois V, Dick T, Ehrh S, Flynn J, Schnappinger D, Wilkinson RJ, Young D. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat Rev Microbiol.* 2009; 7:845–855. [PubMed: 19855401]
6. Nathan C. Taming tuberculosis: a challenge for science and society. *Cell Host Microbe.* 2009; 5:220–224. [PubMed: 19286131]
7. Nathan C, Gold B, Lin G, Stegman M, de Carvalho LP, Vandal O, Venugopal A, Bryk R. A philosophy of anti-infectives as a guide in the search for new drugs for tuberculosis. *Tuberculosis (Edinb).* 2008; 88(Suppl 1):S25–33. [PubMed: 18762150]
8. Russell DG, Barry CE 3rd, Flynn JL. Tuberculosis: what we don't know can, and does, hurt us. *Science.* 2010; 328:852–856. [PubMed: 20466922]
9. Nussinov R, Tsai CJ, Ma B. The underappreciated role of allosterity in the cellular network. *Annu Rev Biophys.* 2013; 42:169–189. [PubMed: 23451894]
10. Huang Z, Mou L, Shen Q, Lu S, Li C, Liu X, Wang G, Li S, Geng L, Liu Y, et al. ASD v2.0: updated content and novel features focusing on allosteric regulation. *Nucleic Acids Res.* 2013
11. Kahn BB, Alquier T, Carling D, Hardie DG. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metab.* 2005; 1:15–25. [PubMed: 16054041]
12. Yonetani T, Kanaori K. How does hemoglobin generate such diverse functionality of physiological relevance? *Biochim Biophys Acta.* 2013; 1834:1873–1884. [PubMed: 23643742]
13. Nussinov R, Tsai CJ. Allosterity in disease and in drug discovery. *Cell.* 2013; 153:293–305. ** This minireview offers a more cellular perspective on the physiologic relevance of allosteric regulation in the context of disease pathogenesis and drug development. [PubMed: 23582321]
14. Adkins JC, Noble S, Efavirenz. *Drugs.* 1998; 56:1055–1064. discussion 1065–1056. [PubMed: 9878993]
15. Smith NJ, Milligan G. Allosterity at G protein-coupled receptor homo- and heteromers: uncharted pharmacological landscapes. *Pharmacol Rev.* 2010; 62:701–725. [PubMed: 21079041]
16. Wood MR, Hopkins CR, Brogan JT, Conn PJ, Lindsley CW. "Molecular switches" on mGluR allosteric ligands that modulate modes of pharmacology. *Biochemistry.* 2011; 50:2403–2410. [PubMed: 21341760]
17. Boshoff HI, Barry CE 3rd. Tuberculosis - metabolism and respiration in the absence of growth. *Nat Rev Microbiol.* 2005; 3:70–80. [PubMed: 15608701]
18. Rustad TR, Sherrid AM, Minch KJ, Sherman DR. Hypoxia: a window into *Mycobacterium tuberculosis* latency. *Cell Microbiol.* 2009; 11:1151–1159. [PubMed: 19388905]
19. Wayne LG, Hayes LG. An in vitro model for sequential study of shutdown of *Mycobacterium tuberculosis* through two stages of nonreplicating persistence. *Infect Immun.* 1996; 64:2062–2069. [PubMed: 8675308]
20. Rao SP, Alonso S, Rand L, Dick T, Pethe K. The protonmotive force is required for maintaining ATP homeostasis and viability of hypoxic, nonreplicating *Mycobacterium tuberculosis*. *Proc Natl Acad Sci U S A.* 2008; 105:11945–11950. [PubMed: 18697942]
21. Weinstein EA, Yano T, Li LS, Avarbock D, Avarbock A, Helm D, McColm AA, Duncan K, Lonsdale JT, Rubin H. Inhibitors of type II NADH:menaquinone oxidoreductase represent a class of antitubercular drugs. *Proc Natl Acad Sci U S A.* 2005; 102:4548–4553. [PubMed: 15767566]
22. Grant GA. *Arch Biochem Biophys.* 2012; 519:175–185. [PubMed: 22023909]
23. Burton RL, Chen S, Xu XL, Grant GA. Role of the anion-binding site in catalysis and regulation of *Mycobacterium tuberculosis* D-3-phosphoglycerate dehydrogenase. *Biochemistry.* 2009; 48:4808–4815. [PubMed: 19388702]

24. Burton RL, Chen S, Xu XL, Grant GA. A novel mechanism for substrate inhibition in *Mycobacterium tuberculosis* D-3-phosphoglycerate dehydrogenase. *J Biol Chem.* 2007; 282:31517–31524. [PubMed: 17761677]
25. Bentley R. The shikimate pathway--a metabolic tree with many branches. *Crit Rev Biochem Mol Biol.* 1990; 25:307–384. [PubMed: 2279393]
26. Tribe DE, Camakaris H, Pittard J. Constitutive and repressible enzymes of the common pathway of aromatic biosynthesis in *Escherichia coli* K-12: regulation of enzyme synthesis at different growth rates. *J Bacteriol.* 1976; 127:1085–1097. [PubMed: 8426]
27. Hoffmann PJ, Doy CH, Catcheside DE. The separation of three allosterically inhibitable 3-deoxy-D-arabino-heptulosonate 7-phosphate synthases from extracts of *Neurospora crassa* and the purification of the tyrosine inhibitable isoenzyme. *Biochim Biophys Acta.* 1972; 268:550–561. [PubMed: 4260319]
28. Hartmann M, Schneider TR, Pfeil A, Heinrich G, Lipscomb WN, Braus GH. Evolution of feedback-inhibited beta /alpha barrel isoenzymes by gene duplication and a single mutation. *Proc Natl Acad Sci U S A.* 2003; 100:862–867. [PubMed: 12540830]
29. Blackmore NJ, Reichau S, Jiao W, Hutton RD, Baker EN, Jameson GB, Parker EJ. Three sites and you are out: ternary synergistic allostery controls aromatic amino acid biosynthesis in *Mycobacterium tuberculosis*. *J Mol Biol.* 2013; 425:1582–1592. [PubMed: 23274137]
30. Webby CJ, Jiao W, Hutton RD, Blackmore NJ, Baker HM, Baker EN, Jameson GB, Parker EJ. Synergistic allostery, a sophisticated regulatory network for the control of aromatic amino acid biosynthesis in *Mycobacterium tuberculosis*. *J Biol Chem.* 2010; 285:30567–30576. [PubMed: 20667835]
31. de Carvalho LP, Zhao H, Dickinson CE, Arango NM, Lima CD, Fischer SM, Ouerfelli O, Nathan C, Rhee KY. Activity-based metabolomic profiling of enzymatic function: identification of Rv1248c as a mycobacterial 2-hydroxy-3-oxoadipate synthase. *Chem Biol.* 2010; 17:323–332. [PubMed: 20416504]
32. Wagner T, Bellinzoni M, Wehenkel A, O'Hare HM, Alzari PM. Functional plasticity and allosteric regulation of alpha-ketoglutarate decarboxylase in central mycobacterial metabolism. *Chem Biol.* 2011; 18:1011–1020. * The paper reports the previously unprecedented positive allosteric regulation of the multifunctional enzyme alpha-ketoglutarate decarboxylase (KGD) in *Mycobacterium smegmatis* and *M. tuberculosis* homolog, Rv1248c, by the entry point TCA cycle metabolite, acetyl CoA. [PubMed: 21867916]
33. Balakrishnan A, Jordan F, Nathan CF. Influence of allosteric regulators on individual steps in the reaction catalyzed by *Mycobacterium tuberculosis* 2-hydroxy-3-oxoadipate synthase. *J Biol Chem.* 2013; 288:21688–21702. [PubMed: 23760263]
34. O'Hare HM, Duran R, Cervenansky C, Bellinzoni M, Wehenkel AM, Pritsch O, Obal G, Baumgartner J, Vialaret J, Johnsson K, et al. Regulation of glutamate metabolism by protein kinases in mycobacteria. *Mol Microbiol.* 2008; 70:1408–1423. [PubMed: 19019160]
35. Villarino A, Duran R, Wehenkel A, Fernandez P, England P, Brodin P, Cole ST, Zimny-Arndt U, Jungblut PR, Cervenansky C, et al. Proteomic identification of *M. tuberculosis* protein kinase substrates: PknB recruits GarA, a FHA domain-containing protein, through activation loop-mediated interactions. *J Mol Biol.* 2005; 350:953–963. [PubMed: 15978616]
36. Ames GF. Bacterial periplasmic transport systems: structure, mechanism, and evolution. *Annu Rev Biochem.* 1986; 55:397–425. [PubMed: 3527048]
37. Cameron JC, Wilson SC, Bernstein SL, Kerfeld CA. Biogenesis of a bacterial organelle: the carboxysome assembly pathway. *Cell.* 2013; 155:1131–1140. [PubMed: 24267892]
38. Ovadi J, Srere PA. Macromolecular compartmentation and channeling. *Int Rev Cytol.* 2000; 192:255–280. * This paper provides a conceptual and experimental framework for the importance of molecular channelling and compartmentalization as a cellular regulatory mechanism. [PubMed: 10553282]
39. Morgunov I, Srere PA. Interaction between citrate synthase and malate dehydrogenase. Substrate channeling of oxaloacetate. *J Biol Chem.* 1998; 273:29540–29544. [PubMed: 9792662]
40. Srere PA, Ovadi J. Enzyme-enzyme interactions and their metabolic role. *FEBS Lett.* 1990; 268:360–364. [PubMed: 2200717]

41. Srere PA. Channeling: the pathway that cannot be beaten. *J Theor Biol.* 1991; 152:23. [PubMed: 1753759]
42. Narayanaswamy R, Levy M, Tsechansky M, Stovall GM, O'Connell JD, Mirrieles J, Ellington AD, Marcotte EM. Widespread reorganization of metabolic enzymes into reversible assemblies upon nutrient starvation. *Proc Natl Acad Sci U S A.* 2009; 106:10147–10152. [PubMed: 19502427]
43. Gill WP, Harik NS, Whiddon MR, Liao RP, Mittler JE, Sherman DR. A replication clock for *Mycobacterium tuberculosis*. *Nat Med.* 2009; 15:211–214. [PubMed: 19182798]
44. Hett EC, Rubin EJ. Bacterial growth and cell division: a mycobacterial perspective. *Microbiol Mol Biol Rev.* 2008; 72:126–156. table of contents. [PubMed: 18322037]
45. Cook GM, Berney M, Gebhard S, Heinemann M, Cox RA, Danilchanka O, Niederweis M. Physiology of mycobacteria. *Adv Microb Physiol.* 2009; 55:81–182. 318–189. [PubMed: 19573696]
46. Nguyen L, Thompson CJ. Foundations of antibiotic resistance in bacterial physiology: the mycobacterial paradigm. *Trends Microbiol.* 2006; 14:304–312. [PubMed: 16759863]
47. Converse SE, Mougous JD, Leavell MD, Leary JA, Bertozzi CR, Cox JS. MmpL8 is required for sulfolipid-1 biosynthesis and *Mycobacterium tuberculosis* virulence. *Proc Natl Acad Sci U S A.* 2003; 100:6121–6126. [PubMed: 12724526]
48. Jain M, Cox JS. Interaction between polyketide synthase and transporter suggests coupled synthesis and export of virulence lipid in *M. tuberculosis*. *PLoS Pathog.* 2005; 1:e2. [PubMed: 16201014]
49. Rosenkrands I, Rasmussen PB, Carnio M, Jacobsen S, Theisen M, Andersen P. Identification and characterization of a 29-kilodalton protein from *Mycobacterium tuberculosis* culture filtrate recognized by mouse memory effector cells. *Infect Immun.* 1998; 66:2728–2735. [PubMed: 9596740]
50. Kerfeld CA, Heinhorst S, Cannon GC. Bacterial microcompartments. *Annu Rev Microbiol.* 2010; 64:391–408. * This paper provides a contemporary review of the structure and function of bacterial microcompartments. [PubMed: 20825353]
51. Tanaka S, Sawaya MR, Yeates TO. Structure and mechanisms of a protein-based organelle in *Escherichia coli*. *Science.* 2010; 327:81–84. [PubMed: 20044574]
52. Rae BD, Long BM, Badger MR, Price GD. Functions, compositions, and evolution of the two types of carboxysomes: polyhedral microcompartments that facilitate CO₂ fixation in cyanobacteria and some proteobacteria. *Microbiol Mol Biol Rev.* 2013; 77:357–379. [PubMed: 24006469]
53. Chen AH, Robinson-Mosher A, Savage DF, Silver PA, Polka JK. The bacterial carbon-fixing organelle is formed by shell envelopment of preassembled cargo. *PLoS One.* 2013; 8:e76127. [PubMed: 24023971]
54. Sutter M, Boehringer D, Gutmann S, Gunther S, Prangishvili D, Loessner MJ, Stetter KO, Weber-Ban E, Ban N. Structural basis of enzyme encapsulation into a bacterial nanocompartment. *Nat Struct Mol Biol.* 2008; 15:939–947. [PubMed: 19172747]
55. de Carvalho LP, Fischer SM, Marrero J, Nathan C, Ehrt S, Rhee KY. Metabolomics of *Mycobacterium tuberculosis* reveals compartmentalized co-catabolism of carbon substrates. *Chem Biol.* 2010; 17:1122–1131. [PubMed: 21035735]
56. Kuhner S, van Noort V, Betts MJ, Leo-Macias A, Batisse C, Rode M, Yamada T, Maier T, Bader S, Beltran-Alvarez P, et al. Proteome organization in a genome-reduced bacterium. *Science.* 2009; 326:1235–1240. [PubMed: 19965468]
57. Orsak T, Smith TL, Eckert D, Lindsley JE, Borges CR, Rutter J. Revealing the allosterome: systematic identification of metabolite-protein interactions. *Biochemistry.* 2012; 51:225–232. * The authors introduce a potentially promising method for identifying physiologically relevant, low-affinity metabolite-protein interactions using unmodified proteins and complex mixtures of unmodified metabolites, termed "mass spectrometry integrated with equilibrium dialysis for the discovery of allostery systematically" (MIDAS), [PubMed: 22122470]

HIGHLIGHTS

- Cellular homeostasis is a regulatory capability of all cells.
- Cells utilize mechanisms that lie within and downstream of the central dogma.
- Allostery and subcellular compartmentalization are evolutionarily ubiquitous.

FIGURE 1.

Cellular regulatory mechanisms both within and beyond the central dogma, which include post-translational modifications (PTM), allostery and localization (compartmentalization).