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Allostery and compartmentalization: old but not forgotten

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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*.

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Introduction

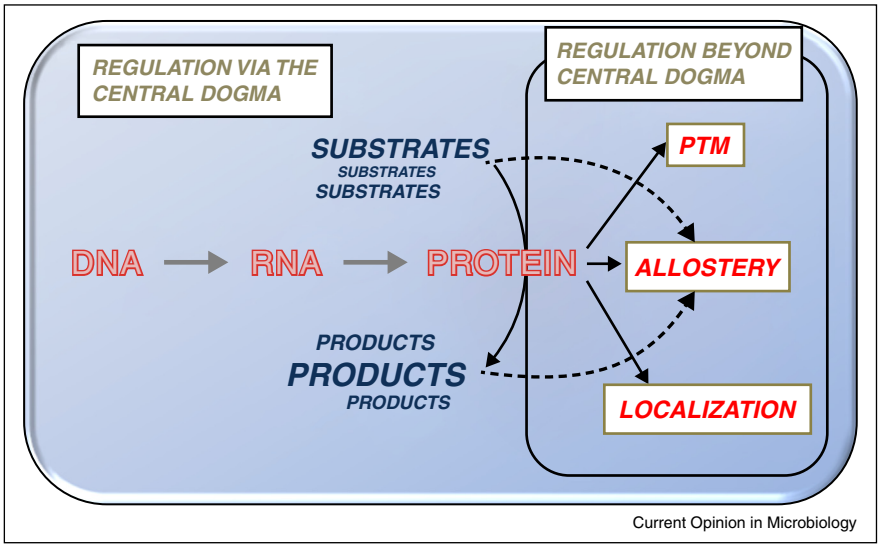
Regardless of size or evolutionary stature, all cells face the challenge of adapting to a complex but fundamentally shared environment. Growing evidence has further suggested that the regulatory mechanisms used by microbes to meet this challenge differ neither in constitution nor complexity from those 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 (Figure 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 O_2 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 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 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 of 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 the 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 the control of 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 multi-drug-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

Figure 1



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

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 treatment of 6–12 months duration due to 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 three 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 O_2 delivery by hemoglobin in response to changes in CO_2, 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 efarvirenz 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,