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EDITORIAL

Bacterial Regulatory Mechanisms for the Control of Cellular Processes: Simple Organisms' Complex Regulation

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Bacteria employ a diverse array of cellular regulatory mechanisms to successfully adapt and thrive in ever-changing environments, including but not limited to temperature changes, fluctuations in nutrient availability, the presence or absence of electron acceptors such as oxygen, the availability of metal ions crucial for enzyme activity, and the existence of antibiotics. Bacteria can virtually modulate any step of gene expression from transcriptional initiation to posttranslational modification of a protein for the control of cellular processes. Furthermore, one gene regulator often controls another in a complex gene regulatory network. Thus, it is not easy to fully understand the intricacies of bacterial regulatory mechanisms in various environments. In this special issue, while acknowledging the challenge of covering all aspects of bacterial regulatory mechanisms across diverse environments, seven review articles are included to provide insight into the recent progress in understanding such mechanisms from different perspectives: positive regulatory mechanisms by secondary messenger (cAMP receptor protein), two-component signal transduction mechanisms (Rcs and Cpx), diverse regulatory mechanisms by a specific environmental factor in specific bacteria (oxygen availability in *Mycobacterium* and manganese ion availability in *Salmonella*), diverse regulatory mechanisms by a specific environmental factor (temperature and antibiotics), and regulatory mechanisms by antibiotics in cell wall synthesis.

Bacteria, as ubiquitous organisms that can be found in almost every environment, carry out complex cellular processes that allow them to survive and thrive in a variety of different conditions despite their small size and relative simplicity. One of the key factors that allows bacteria to carry out these complex processes is their ability to regulate gene expression through various mechanisms. Gene expression is a fundamental biological process by which the genetic information encoded in a gene is transcribed into an RNA molecule and subsequently translated into a functional gene product, often a protein. Furthermore, the activity levels of proteins may further be altered by posttranslational modification. Regulation of gene expression refers to the control of the amount and timing of gene expression, and thus it can be divided into transcriptional, translational, and posttranslational levels.

Negative and Positive Regulatory Mechanisms of Inducible and Repressible Genes

Induction and repression of gene expression, which provided the first models for gene expression, often involve the action of regulatory proteins. These proteins are capable of binding to specific regions of DNA and control the initiation of transcription in response to various internal and external signals. There are two types of regulatory proteins: repressor proteins for negative transcriptional control and activator proteins for positive transcriptional control. Since both the inducible and repressible genes can be controlled through negative and positive control mechanisms, the action of bacterial regulatory proteins can be classified into four types.

In the negative control of inducible genes, the repressor blocks transcription by binding to the operator region of a gene. When an inducer binds to the repressor, it no longer binds to DNA, and transcription occurs. One well-known example is the regulation of the lactose operon by the *lac* repressor protein (Lewis, 2013). In the absence of lactose, transcription of the lactose operon is blocked by binding of the *lac* repressor to operator sites. When lactose is available, the inducer (allolactose, which is converted from lactose) of the operon binds to the *lac* repressor. This binding causes

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a conformational change in the *lac* repressor, rendering it unable to bind to any operator site.

In the negative control of repressible genes, the repressor (aporepressor) is unable to bind DNA, and transcription occurs. When a corepressor binds to the aporepressor, it binds to the DNA and blocks transcription. This type of regulation is widely used in bacterial metal ion homeostasis, particularly by canonical Fur family proteins (Sevilla et al., 2021). Fur family proteins are named after the first member of the family, Fe-sensing Fur (*f*erric *u*ptake *r*egulator), and include Zn-sensing Zur, Ni-sensing Nur, and Mn-sensing Mur. When intracellular Fe concentrations are low, Fur is an inactive repressor and allows the transcription of genes involved in Fe-uptake. Conversely, when Fe concentration is high, the active Fe-bound Fur binds to the target DNA sequence known as Fur box leading to the transcriptional repression of Fe-uptake genes. A Mn-sensing transcription factor, MntR, which belongs to the DtxR family proteins rather than Fur family proteins, can also use this type of mechanism (Waters, 2020).

In the positive control of inducible genes, the activator alone is unable to bind to DNA, and no transcription occurs. When an inducer binds to the activator, it binds to the DNA and activates transcription. The cAMP receptor protein (CRP), also called catabolite activator protein (CAP), uses this type of regulation. As stated above, in the case of lactose operon the *lac* repressor only regulates gene expression in response to the presence or absence of lactose not in response to glucose. CRP functions in a global regulatory network that allows bacteria to preferentially use glucose via a mechanism called catabolite repression. When glucose is present, CRP alone is inactive in DNA binding, and the lactose operon is not activated even in the presence of lactose. However, in the absence of glucose, a signaling molecule, cAMP, is increased, which binds to CRP and increases its DNA binding affinity. This leads to subsequent transcriptional activation of the lactose operon in the presence of lactose. In this special issue, Youn and Carranza (2023) review the mechanism of CRP activation by cAMP. The transfer of the cAMP-binding signal to the DNA-binding F-helix is believed to occur through a series of conformational changes since the cAMP-binding site is far away (> 30 Å) from the DNA-binding site. The authors compared the structures of apo-CRP and cAMP-bound CRP. More importantly, to provide further insight into CRP activation by cAMP, the authors compare a group of CRP mutants, referred to as CRP*, which possess CRP activity without cAMP-binding. OxyR, a transcription factor that plays an important role in the regulation of oxidative stress response in bacteria, is an another example of this type of regulation. The reduced form of OxyR is inactive. When activated by reactive oxygen species (ROS) such as hydrogen peroxide, OxyR binds to the target sequence and activates the transcription of genes involved in ROS protection such as catalases, peroxidases, and superoxide dismutases (Imlay, 2015).

In the positive control of repressible genes, the activator binds to DNA and promotes transcription. When an inhibitor is present, the binding of the inhibitor to the activator prevents the activator from binding to DNA resulting in no transcription.

Although most gene expression regulation at the transcriptional level is mediated by a proteinaceous transcription factor, RNA can also regulate gene expression (Sherwood & Henkin, 2016). A riboswitch is a regulatory element typically located in the 5' untranslated region (UTR) of an mRNA molecule and can bind to a specific small effector molecule. Binding of an effector molecule to a riboswitch can cause a conformational change in the RNA structure and determines whether or not its target mRNA continues to be synthesized. Other riboswitches function at the translation level by regulating ribosome binding, as is the case in thiamine biosynthetic operons. In this special issue, Ha and Lee (2023) reviews Mn homeostasis by Mn-transporters and their regulators in *Salmonella enterica* serovar Typhimurium. As exemplified in this review, even the single gene *mntH* which encodes Mn-uptake transporter, is regulated by multiple transcription factors including OxyR, Fur, and MntR, and also by riboswitch. The *mntH* is positively controlled by OxyR in the presence of oxidative stress, and negatively by Fur and MntR in the presence of excess Mn. Riboswitch also negatively controls the transcription of *mntH* in the presence of excess Mn. In contrast, the expression of *mntP* coding for Mn-efflux transporter is positively controlled by MntR and Fur, and also by riboswitch at the translational level in the presence of excess Mn. This type of complex regulation by a single regulator protein is wide-spread in bacteria, and well-documented in Fur family proteins (Sherwood & Henkin, 2016). Although Fe-bound Fur typically acts as a repressor by directly binding to DNA, Fe-bound Fur can also activate target gene expression either directly by binding to target DNA or indirectly via sRNA. In addition, it is known that apo-Fur can function as an activator, while Fe-bound Fur can act as a repressor.

Global Regulatory Mechanisms

Compared with relatively small or simple stimuli such as the presence or absence of lactose, bacteria often encounter significant environmental changes. For example, when a pathogen enters a susceptible host, the host environment presents a range of challenges to the bacteria, including changes in temperature, pH, nutrient availability, and host defense mechanisms such as the immune system. The global regulatory systems in bacteria enable them to coordinate the expression of multiple genes and operons in response

to large-scale changes. The simplest approach is to use a single global regulatory protein to alter the expression of a group of genes and operons called a regulon. The above-mentioned proteins, CRP, Fur, and OxyR are examples of global regulatory proteins. Another example of which is the alternate sigma factor, which directs RNA polymerase to a specific subset of genes and simultaneously changes the expression level of many genes (Feklistov et al., 2014). In this special issue, Oh et al. (2023) provide a comprehensive overview of the complex regulatory systems of *Mycobacterium tuberculosis* and its relatives. *Mycobacterium tuberculosis*, the causative agent of tuberculosis, encounters a wide range of hostile environments in the granuloma, such as hypoxia, ROS, and nutrient deprivation. To introduce and explain complex regulatory systems, the authors focus on the adaptation to respiration-inhibitory conditions in which the functionality of the respiratory electron transport chain is decreased. The regulatory mechanisms involved in this adaptation process include not only CRP and sigma factors, which have been described above, but also the stringent response and two-component signal transduction systems which will be discussed below.

Many bacteria use secondary messengers such as cAMP, (p)ppGpp, cGMP, c-di-GMP, and c-di-AMP in global regulatory systems. As stated above, cAMP along with CRP is involved in catabolite repression. The second messengers, guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp), which are collectively called (p)ppGpp, respond to nutritional or antibiotic stress and are thus referred to as alamones (Irving et al., 2021). The concentration of (p)ppGpp remains low under good growth conditions. During the stringent response, the synthesis of (p)ppGpp increases, which in turn downregulates resource-consuming cell processes such as replication, transcription, and translation. As an extreme response to a slow growth rate, the role of (p)ppGpp is crucial in persister cell formation.

Two-component signal transduction systems, typically consisting of a sensor kinase and a response regulator, are important for linking extracellular environmental signals to intracellular gene regulation (Kenney & Anand, 2020). The sensor kinase is a membrane-spanning protein that has one part exposed to the extracellular environment (periplasm in the case of Gram-negative bacteria) and another part exposed to the cytoplasm. The sensor kinase is autophosphorylated upon signal sensing and then transfers phosphate to the response regulator. The phosphorylated response regulator binds to DNA and regulates gene expression. Some response regulators interact with enzymes and other proteins in the global network. In a phosphorelay system, the transfer of phosphoryl groups typically involves the participation of multiple proteins rather than the direct transfer between two proteins. In this special issue, Cho et al. (2023) review the stress signal sensing mechanisms of Rcs and Cpx two-component systems which are important in defending cells from envelope stress. In the Rcs two-component system, RcsC is a sensor kinase, and RcsB is a response regulator. In addition, RcsD, a structural homolog of RcsC, is involved in phosphate transfer between RcsC and RcsB, and IgaA, which acts as a negative regulator of Rcs by interacting with RcsD, is also involved in this system. More importantly, RcsF, which is located where stress is generated, is proposed to be an outer membrane stress signal-sensing lipoprotein for the Rcs two-component system. Cho et al. (2023) introduce and intensively evaluate two models, RcsF-OMP and BAM sensor models, for the stress-sensing mechanism of RcsF. They also discuss the stress-sensing mechanism via NlpE for the Cpx two-component system in comparison with that via RcsF.

Posttranslational Regulatory Mechanisms

Posttranslational regulation is the modification of proteins that can alter their structure, activity, stability, or interactions, and, ultimately, their function. The function of proteins can be altered by noncovalent ligand binding, or by covalent modifications that can be either reversible (e.g. phosphorylation/dephosphorylation and disulfide bond formation) or irreversible (e.g. removal of amino acid residues and irreversible oxidation). The ultimate posttranslational modification is the regulated protein degradation by proteases. For example, caseinolytic protease P (ClpP), a serine protease, is the proteolytic core of the protease complex (Mabanglo & Houry, 2022). ClpP generally forms a larger complex through the direct binding of one or more cognate ATPase chaperones, such as ClpA. ClpA uses ATP hydrolysis energy to unfold and translocate substrates into the proteolytic chamber of ClpP for degradation. ClpS is an adaptor or recognin that recognizes the N-terminal amino acid sequence of the substrate, N- degron, and thus can recruit specific substrates for degradation by ClpAP protease. In this special issue, the review by Yee et al. (2023) provides various regulatory mechanisms by the membrane proteins involved in antibiotic persistence. Antibiotic persistence is a phenomenon in which a subpopulation of bacteria, known as persisters, exhibits extreme tolerance to antibiotics. Antibiotic tolerance and resistance refer to the slow killing of the bacterial population and the ability to grow in the presence of antibiotics, respectively. The authors introduce various regulatory mechanisms, including the proteome homeostasis by the ClpP protease system, the stringent response, and the cytosolic ATP levels. Moon et al. (2023) also provide a wide range of cellular regulatory mechanisms in response to changes in temperature, which affect the structure and composition of nucleic acids, proteins, and membranes. The topics covered in this review include structural changes in

nucleic acids and proteins by temperature shifts, diverse intracellular responses mediated by various heat- and cold-shock proteins, and phenotypic changes induced by temperature change. Cho (2023) discusses the relationship between surface glycopolymers in terms of the common lipid carrier used for their assembly. Sharing a lipid carrier provides a way to coordinate the synthetic processes of peptidoglycan and other surface glycopolymers based on the needs of the cell. The author also discusses the potential of developing pathway-driven screening strategies for novel antibiotics that target peptidoglycan and other surface glycopolymers by utilizing their biosynthetic pathways.

Bacteria typically do not involve cellular differentiation and morphogenesis, which are common in multicellular organisms; instead, they adapt and thrive in various fluctuating environments. Thus, despite the relative simplicity of bacteria, the regulatory mechanisms that sense signals and control cellular processes are not simple. As discussed by Youn and Carranza (2023), even for CRP, which is one of the best-studied transcription factors, many questions remain unanswered. Also for Fur family proteins, originally Fur was described as an Fe-sensing repressor protein; however, subsequent studies have revealed that the regulations by Fur are much more complex than initially expected (Sevilla et al., 2021). In addition, the archetypal EnvZ-OmpR two-component regulatory system can function non-canonically (Kenney & Anand, 2020). All of these indicate that our understanding of even the individual regulatory components of complex bacterial regulatory systems may still be incomplete. Given the multitude of bacterial regulatory mechanisms and signal types, it is crucial to investigate these regulatory mechanisms from various perspectives to uncover new insights into the regulation of bacterial gene expression. Finally, I express my gratitude to all the authors who have contributed to this special issue, and I am confident that their contributions will undoubtedly advance this field and inspire others.

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Data availability Since this is an editorial, I have no data available upon request.

Declarations

Conflict of interest The author declares that he has no conflicts of interest.

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