Regulatory architectures and dynamics of bacterial gene expression

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Related organisms often express the same set of genes in response to a particular environmental signal. However, the regulatory mechanisms promoting the expression of these genes can be drastically different. One emerging class of regulatory designs depends on small proteins – termed connectors – which connect bacterial genetic regulatory systems at the post-translational level. We now have identified a novel connector-mediated regulatory architecture that structurally resembles one of the most abundant network motifs – the feedforward loop – but possesses different dynamic properties. This architecture, which we termed the feedforward connector loop (FCL), is used by the Gram-negative bacterium *Klebsiella pneumoniae* to activate the polymyxin B resistance gene *pbgP* under low Mg^{2+} conditions. In the related species *Yersinia pestis* and *Salmonella enterica*, this regulatory function is carried out by a direct regulation circuit and a cascade-like connector-mediated pathway, respectively. Our computational and experimental analyses suggest that the FCL, both functionally and evolutionarily, is the intermediate stage between the direct regulation circuit and the connector-mediated pathway.

***Keywords*: mathematical modeling, gene expression dynamics, signal transduction, post-translational regulation**

The architecture of genetic regulatory circuits influences the dynamics of gene regulation [1]. While the properties of a number of widely-encountered regulatory designs – termed network motifs – have been extensively investigated [1], those of newly discovered regulatory circuits are not easily predicted [2]. Network motif research traditionally focused on gene expression control at the level of transcription [1]; however, it is becoming increasingly clear that post-translational mechanisms play a critical role in bacterial gene regulation.

One recently discovered regulatory design – the connector-mediated pathway (CMP) – involves a small protein, PmrD, which connects two-component signal transduction systems in *Salmonella enterica* [2]. This pathway allows the bacterium to activate genes modulating resistance to the antibiotic polymyxin B in response to low Mg^{2+} conditions. A related species, *Yersinia pestis*, performs this function using a direct regulation circuit, whereby the primary regulatory protein of low Mg^{2+} response binds to the promoter of the target gene thus activating its transcription. By measuring the mRNA levels of target genes, we have determined that the CMP displays larger deactivation delays than the direct activation circuit (we termed this phenomenon persistence of expression) [2]. In addition, the CMP promotes small activation delays and heightened induction ratios (signal amplification). Mathematical modeling demonstrated that these phenomena are general properties of the CMP architecture, and allowed us to identify critical factors responsible for signal amplification [2]. Modeling studies involved model design (based on the formalism of deterministic chemical kinetics), development and application of a novel model fitting algorithm, parameter randomizations, and analytical studies.

We have discovered that *Klebsiella pneumoniae* uses a regulatory architecture (termed feedforward connector loop, or FCL) that involves a direct regulation branch and an indirect regulation branch that is similar to the CMP. The presence of two regulation branches also characterizes the feedforward loop (FFL), whose indirect branch is a transcriptional cascade [1]. Modeling of the FCL, FFL, CMP, and direct regulation predicted that the FCL displays persistence of expression, but no considerable activation delays. The FFL also demonstrates fast activation; however, its ability to promote large deactivation delays is significantly less pronounced than that of the FCL. We obtained a general mathematical result stating that the presence of two regulation branches should lead to a higher signal output level than that of a system with only one branch of regulation. Our modeling predictions were verified experimentally with *S. enterica* strains engineered to harbor the FCL, CMP, and direct regulation architectures. In computational and analytical studies, we demonstrated that the FCL can promote signal amplification, and identified the factors contributing to this property.

Our results shed light on the general dynamic features of post-translational mechanisms of gene expression control, and provide a methodological foundation for further explorations in this area.

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