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How to Regulate a Gene: To Repress or to Activate?

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Gene-expression responses to an input can depend on growth conditions; in this issue, Sasson et al. (2012) show that this dependence is lower when the input results in a high degree of promoter occupancy.

All biological regulatory processes involve intermolecular interactions. Strong binding between the interacting molecules, for example a transcription factor (TF) and its cognate DNA promoter, can result in high specificity of signaling, since the TF is tightly bound to its cognate promoter and, thus, excluding other nonspecific interactions, which can result in condition-specific influences on the signal transduction (Shinar et al., 2006). Such tight binding, however, limits the dynamical range of the regulatory effect only to a regime of high-promoter occupancy by the cognate TF. Thus, signal transduction is constrained by a trade-off between the dynamical range of the input signals (TF) and the conditions-dependent difference in the input-output response function (which quantifies the relation between input and downstream gene expression). Sasson et al. (2012) combine well-controlled experiments and a simple model to demonstrate an elegant solution to this trade-off in *E. coli*.

Sasson et al. (2012) construct *E. coli* strains in which a fluorescent reporter (output) is transcribed under the control of cAMP-receptor protein (CRP) that depending on the promoter acts either as an activator or as a repressor (input). Since the activity of CRP is modulated by cAMP, the input in these strains can be controlled easily by growing the strains across different concentrations of cAMP, and the resulting fluorescent signal can be quantified accurately. This experimental design allows answering fundamental questions: Does the input-output response function depend on the growth conditions? Is this dependence affected by the input level (cAMP concentration) or by the mode of regulation, activation versus repression? The authors found that the input-output response function was less dependent on the growth conditions, for both the activating and the repressing CRP, in the regime when the CRP promoter occupancy was high. This result is consistent with earlier theoretical predictions (Shinar et al., 2006) and corroborates the idea that high-promoter occupancy by its cognate TF may prevent nonspecific binding—and thus result in more similar input-output response functions across different growth conditions.

Such high fidelity of the input-output response function, however, is limited only to input levels that result in high-promoter occupancies, raising another intriguing question: Is it possible to overcome this limitation and make the input-output function robust to changes in the growth conditions over a wider dynamical range? One possibility is to place a gene under the control of both a repressor, which improves fidelity when the gene is lowly expressed, and an activator, which improves fidelity when the gene is highly expressed. As an example of a promoter regulated by two regulators, Sasson et al. (2012) studied the regulation of a classical system, the lac operon. By modulating both an activator of the lac promoter, CRP, and a repressor, LacI, the authors were able to analyze the differences in promoter activity across equiexpression lines—that is, combinations of activator and repressor activities resulting in equal promoter activity. This is a particularly ingenious aspect of the experimental design that allows separating promoter occupancy (fraction of bound binding sites) from other confounding variables and obtaining a clear result: Controlling for other variables, the higher the promoter occupancy, the higher the similarity in the input-output function across conditions.

The work of Sasson et al. (2012) not only provides a concrete and compelling example for a design principle that can reduce undesired condition-dependent influences on transcription, but also has numerous broader implications that open avenues for further research. One such implication is that the principles suggested by Sasson et al. (2012) may not be limited to the interactions between TFs and their cognate promoters but likely extend to other regulatory interactions, such as protein-protein, protein-small molecule, or RNA-microRNA interactions. Indeed, the idea that the tight binding of a ligand to its cognate regulatory site can prevent no-specific interactions by exclusion is quite general, and it seems likely that such tight binding among regulatory proteins contributes to the fidelity of signaling in other contexts. Another important implication concerns the nonredundant function of multiple regulators that operate in parallel. Each regulator may be optimized to increase the signaling fidelity over a part of the dynamical range, low or high level of signaling, and thus contribute to nonredundant functions. The results of Sasson et al. (2012) also raise the question of what the effect and significance of promoter occupancy is when the input signal is oscillating, such as oscillating nuclear localization of transcription factors or genome-wide transcriptional oscillations (Cai et al.,

2008; Slavov et al., 2011). The mode of gene regulation affects the variability in single-cell responses (Munsky et al., 2012), raising another exciting question: Can high-promoter occupancy also reduce variability among the input-output responses of single cells? These implications and questions provide a fertile ground for further work characterizing the design principles of signal transduction.

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When Death Was Young: An Ancestral Apoptotic Network in Bacteria

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In this issue of *Molecular Cell*, Dwyer et al. (2012) characterize a RecA-dependent and ClpXP-regulated pathway that controls the acquisition of several apoptotic markers upon bactericidal treatment of prokaryotes, placing the hypothetical origin of apoptosis further downstream in evolution.

In metazoans, the life span of individual cells is regulated by an integrated suicide system (programmed cell death, PCD) that can be activated when cells become superfluous, accumulate damage, or menace organismal fitness. Among the distinct subroutines constituting PCD, apoptosis represents the best-studied one. Apoptotic death is a structurally and functionally conserved process in thus far that it is also observed in unicellular eukaryotes, such as protozoan parasites or yeast (Carmona-Gutierrez et al., 2010; Madeo et al., 1997). Dwyer et al. (2012) provide phenotypic and mechanistic evidence that may expand the evolutionary conservation frame of apoptosis into the realm of prokaryotes.

The authors demonstrate that bacterial cell death induced by treatment with different bactericidal antibiotics is accompanied by several biochemical markers of apoptosis, including DNA fragmentation, chromosomal condensation, exposure of phosphatidylserine to the outer leaflet of the plasma membrane, and dissipation of membrane potential (Dwyer et al., 2012). These results add to previous work by the same group (Dwyer et al., 2007; Kohanski et al., 2007) showing that bactericidal antibiotics promote the generation of reactive oxygen species (ROS), which are crucial apoptotic regulators in multicellular as well as in unicellular eukaryotes (Herker et al., 2004; Simon et al., 2000). In bacteria, ROS seem to play a similar role, since suppressing their formation reduces drug-induced cell death (Dwyer et al., 2007) as well as DNA fragmentation (Dwyer et al., 2012).

Now, Dwyer et al. (2012) identify and characterize RecA, a multifunctional protein crucial for DNA maintenance and repair, as an additional player involved in the antibiotic-triggered apoptotic demise of bacteria. Consistent with this finding, RecA plays a critical role in the recently described apoptosis-like death (ALD) pathway of *E. coli* (Erental et al., 2012). Dwyer et al. (2012) extend these observations by showing that the cell stress-triggered conversion of RecA into its active form is a prerequisite for its contribution to cell-death induction (Dwyer et al., 2012). The lethal activity of active RecA is thereby negatively regulated by the ClpP protease complex ClpXP. These factors also dampen the LexA-regulated bacterial DNA-damage (or SOS) stress response, which is necessary for the efficient induction of apoptosis in response to cellular stress (Dwyer et al., 2012).

In this network of interacting regulators, RecA seems to function in a similar fashion as do caspases, the central executionary cysteine proteases in many scenarios of mammalian apoptosis. Indeed, RecA can bind and hydrolyze synthetic caspase substrates and appears to be the only bacterial enzyme to