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Previews

Bacteria as Control Engineers

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Bacteria encounter fluctuations in both their external and internal environments, and to manage these conditions, they employ various control mechanisms. In this issue of *Molecular Cell*, Hart et al. (2011) investigate how *E. coli* robustly controls nitrogen assimilation.

Residents of New England are used to rapidly changing weather conditions—it can be 70°F and sunny one day and snowing the next. Most of us rely on engineered control systems to maintain comfortable temperatures in our living rooms, offices, and laboratories, in spite of such fluctuations outdoors. These control systems operate through a simple but effective mechanism: the indoor temperature is detected by a thermostat, which then regulates heat flow to achieve a set, desired temperature. The robustness of this automated mechanism to changing weather conditions provides us comfort and efficiency and, most would agree, is preferable to other temperature-control schemes, such as shoveling coal, chopping wood, or shivering.

In this issue of *Molecular Cell*, Hart et al. (2011) investigate how the bacterium *E. coli* robustly controls nitrogen metabolism in the face of fluctuations. The study of robustness in bacterial metabolic and transcriptional networks has grown over the last decade, with an increasing awareness of noise in these systems (Barkai and Shilo, 2007; Shinar et al., 2009; Shinar and Feinberg, 2010). Noisy gene expression leads to bacterial cells that behave differently, despite being the same genetically. This phenomenon accounts for numerous phenotypes that are not described by genetics alone (Avery, 2006; Kaern et al., 2005). An important question that has arisen from bacterial noise research is how bacteria avoid possible negative consequences associated with stochastic gene expression, such as a lack of necessary molecules or an overabundance of toxic ones. The answer increasingly appears to be that bacteria employ a number of robust control strategies—they sense various chemical signals, both inside and outside their membranes, and adjust activity on transcriptional and enzymatic levels to maintain efficiency and safety.

Hart and colleagues add to this study of robustness by examining nitrogen utilization in *E. coli*. Nitrogen is an essential component of all life, being required for information storage and processing (DNA, RNA, and protein), among other cellular functions. For nearly all processes in *E. coli*, nitrogen is donated by the amino acids glutamate and glutamine. Nitrogen, in the form of ammonium, is enzymatically added to an α-ketoglutarate backbone to produce glutamate. An additional ammonium is then added to glutamate by glutamine synthetase (GlnA) to produce glutamine. The activity of GlnA is controlled by its adenylation state: the adenylated form is inactive; the unadenylated form is active. Both adenylation and deadenylation of GlnA are carried out by the bifunctional enzyme glutamine synthetase adenylyltransferase (GlnE). The activity of GlnE is allosterically regulated by α-ketoglutarate and glutamine levels (Jiang et al., 2007). Hence, by sensing both an input (α-ketoglutarate) and output (glutamine) of nitrogen assimilation, GlnE acts as a bacterial "thermostat" of sorts and maintains a robust ratio between glutamine and α-ketoglutarate (Figure 1).

To investigate this mechanism, Hart et al. begin by introducing a simple model of nitrogen assimilation and show that this model cannot account for the robustness of the glutamine-to-α-ketoglutarate ratio. The mass-action kinetics model suggests that the rate of glutamine production depends linearly on the concentration of GlnA, the enzyme that produces it. Hence, the rate of glutamine production would change with different concentrations of GlnA, and glutamine production would not be robust.

To improve the model, the authors introduce the concept of *avidity*, increased binding of a protein to a substrate due to multiple binding sites. The bifunctional adenylation enzyme GlnE is reasoned to *avidly* bind two GlnA monomers simultaneously, as GlnE has two catalytic sites, and GlnA monomers, which form dodecamers, are organized in close proximity to one another. The authors use the increased stability associated with the ternary complex, in which both catalytic sites of GlnE are bound to monomers of GlnA, to simplify the adenylation reaction kinetics. Under steady-state conditions, where the adenylation and deadenylation rates catalyzed by GlnE are equivalent, the concentration of ternary complexes can be factored out; as a result, the rate constants for the forward and reverse reactions are equivalent. Given that both rate constants depend primarily on glutamine and α-ketoglutarate concentrations and do not depend on GlnA concentration, the model suggests that the ratio of glutamine to α-ketoglutarate is independent of and robust to GlnA concentration.

The authors verify this prediction in vivo using mass spectrometry to measure individual metabolites. They find that the ratio of glutamine to α-ketoglutarate varies little in response to a range of GlnA concentrations. Using a GlnE knockout strain, they demonstrate that this insensitivity to GlnA concentration

requires the bifunctional adenylation enzyme GlnE, as the ratio drifts from its natural set-point when this bacterial controller is absent.

Hart et al. also demonstrate experimentally that robust glutamine-to-α-ketoglutarate ratios confer robustness in growth. By measuring the growth rate of *E. coli* that induce different levels of GlnA, the authors show that robustness in growth coincides with robustness in the ratio. This result highlights the necessity to control nitrogen assimilation, as a failure to do so results in inefficient growth.

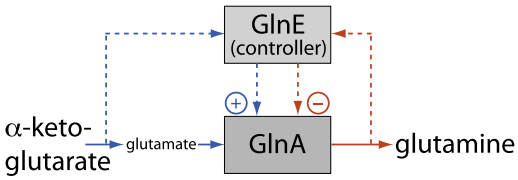

Figure 1. Control of Glutamine Synthetase
Glutamine synthetase (GlnA) is an essential component of nitrogen assimilation and converts α-ketoglutarate (blue), via glutamate, to glutamine (orange). Levels of the metabolic input and output (dashed lines) are sensed by glutamine synthetase adenylyltransferase (GlnE), which adjusts GlnA activity. GlnA activity is induced by α-ketoglutarate and repressed by glutamine. GlnE thereby ensures robust control of nitrogen assimilation.

This study provides a novel model and experimental validation for a robustly controlled process in *E. coli*. The number and diversity of bacterial processes under this type of control remain unclear. However, by investigating the types, levels, and extent of control bacteria exert over different processes, biologists and bioengineers will broaden our understanding of how organisms utilize both robustness and noise to protect vital functions and generate diversity in changing environments. Insights arising from work of this sort will also allow for the rational engineering of novel bacterial processes and enhance advances being made in synthetic biology (Gardner et al., 2000; Mukherji and van Oudenaarden, 2009; Khalil and Collins, 2010).

Our growing understanding of bacterial control mechanisms will likely benefit us in the reverse direction as well. Given that bacteria have been developing control mechanisms for millions of years, and we have been at it rigorously for less than two centuries, we may be able to learn a great deal from them, and perhaps future process engineers will look to bacteria for novel control strategies.

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P-Rex1, a Guanine Exchange Factor that Is Overexpressed in Breast Cancer, Is a Convergence Node for ErbB and CXCR4 Signaling

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In a recent issue of *Molecular Cell*, Kazanietz and colleagues (Sosa et al., 2010) show that P-Rex1, a Rac1 GEF, is overexpressed in ER+ and/or ErbB2+ breast cancers, suggesting that P-Rex1 might be a convergence node downstream of these receptors and an attractive therapeutic target.

Rho family GPTases are activated in response to diverse stimuli and control multiple cellular processes (Vigil et al., 2010). Members of the Rho family are deregulated in human cancer, often due to increased levels or altered activity of guanine nucleotide exchange factors (GEFs). These enzymes catalyze exchange of GDP for GTP, producing the active GTP-bound form that associates with effector proteins, thereby conveying intracellular signals. Sosa and colleagues show in a recent issue of *Molecular Cell* that the Rac1-specific GEF, P-Rex1, has a novel role in breast cancer (Sosa et al., 2010). Previous work from the Kazanietz lab showed that Rac1 is activated in breast cancer cell lines treated with