Quiet Gene Circuit More Fragile Than Its Noisy Peer

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Why is a particular architecture for a pathway chosen over seemingly equivalent alternatives? Çağatay et al. (2009) use a synthetic biology approach to show that fluctuations—or noise—in protein levels may play a key role in determining which network design is selected during evolution.

A central question in systems biology is why a specific network design is used for a given function, when alternative designs would apparently yield identical outcomes. A powerful approach to address this question is to compare the behavior of alternative circuits designed to perform the same function. Indeed, recent research has focused on the theoretical analysis of alternative circuit designs and on the experimental comparison of different synthetic transcription regulatory circuits in terms of their static properties, dynamics, and stochastic behaviors (Guet et al., 2002; Guido et al., 2006; Igoshin et al., 2007; Kollmann et al., 2005; Ma et al., 2009; Shinar et al., 2007; Tsai et al., 2008). In this issue of *Cell*, Çağatay et al. (2009) take this approach to the physiological level. Focusing on the genetic pathway regulating competence for DNA uptake in *Bacillus subtilis*, they built and studied the behavior of an alternative genetic circuit designed to execute the same cellular function. While both alternative and endogenous circuits produced similar average behavior, the alternative circuit reduced variability among cells. The authors found that this decrease in "noise" made DNA uptake efficiency more sensitive to changes in environmental conditions.

B. subtilis can transiently switch to a "competent" cell state under stressful conditions. In this state, expression of the master regulator ComK enables DNA uptake via regulation of more than 100 genes. Individual cells switch from vegetative growth to the competent state at random times. Importantly, cells do not remain in the competent state indefinitely, instead returning to vegetative growth after a certain time.

Which types of circuits allow such stochastic entrance into competence and a deterministic return to the basal state? The genetic circuit controlling this transient cell differentiation exhibits the dynamic properties of an excitable system (Figures 1A and 1B), mediated through fast positive and slow negative feedback regulation of *comK* (Süel et al., 2006): ComK transcriptionally activates itself and indirectly represses the expression of ComS, which inhibits ComK degradation (Figure 1C). Basal *comK* expression is low during vegetative growth, but a sufficient stochastic fluctuation ("noise") can activate the positive feedback autoregulatory loop, amplifying the fluctuation, greatly increasing *comK* expression, and triggering entry into the competent state. After some delay, negative feedback regulation through *comS* leads to degradation of *comK*, returning cells to the vegetative state. Thus, the two key features in this circuit are fast positive feedback regulation that pushes the system away from the steady state when a sufficiently large fluctuation occurs, and slow negative feedback regulation that returns the system to its steady state after a delay (Figure 1A).

In principle, there are two ways to achieve delayed negative feedback regulation in excitable systems: negative regulation of an activator or positive regulation of a repressor (Figure 1B). The natural system implements the former strategy: ComK represses *comS*, which effectively acts as an activator of ComK. Çağatay et al. (2009) asked why the natural system uses this design rather than the alternative (Figure 1B).

To address this question the authors constructed a synthetic circuit ("SynEx") by removing the regulatory link between ComK and *comS* and introducing a new link between ComK and *mecA* (*mecA* plays a role in ComK degradation; Figure 1C). These modifications result in a functionally similar feedback loop of alternative design. The authors tuned the synthetic circuit so that its single-cell dynamics and competence frequency were similar to those of the wild-type. Additional modifications allowed the authors to match the median duration of the competent state in the synthetic network to that of the wild-type ("SynExSlow"). However, while the average dynamics of the natural and rewired circuits were similar, the cell-to-cell variability of their response was very different. Whereas wild-type cells spent variable amounts of time in the competent state, timing was in fact more uniform in the rewired cells (Figure 1D). On the basis of theoretical analysis, the authors predicted that the difference in variability was due to the role of the intermediate component ("Y" or "Z" in Figure 1B) in the circuit design: In the SynEx circuit, this intermediate component in the delayed negative feedback loop (MecA) is upregulated when the system is excited (right panel in Figure 1C), leading to relatively high numbers of MecA molecules and lower variability. The opposite is true in

the natural system, where the intermediate component (ComS) is repressed (left panel in Figure 1C), leading to low numbers of ComS molecules and higher variability.

The more precise timing of competence events in the synthetic circuit may appear desirable from an engineering point of view. So why was the more noisy circuit chosen by evolution? A plausible hypothesis is that the wild-type network may be more efficient in DNA uptake. Çağatay et al. (2009) show, however, that the SynExSlow network, in which the median duration of the competent state is equal to that in the wild-type, takes up DNA with wild-type efficiency. The authors then had the insight to compare the performance of the wild-type and synthetic circuits under different environmental conditions. By varying the extracellular DNA concentration, the authors revealed an interesting functional difference between the two network designs: the more noisy wild-type network leads to more efficient DNA uptake over a wide range of DNA concentrations, while the SynExSlow network is only efficient within a relatively narrow DNA concentration range.

Previous work has shown that phenotypic variability caused by gene expression noise can be beneficial when dealing with unpredictable environmental changes (Kussell and Leibler, 2005). The synthetic circuit built by Çağatay et al. (2009) provides a striking example, where a network design that reduces noise, but is very similar to the wild-type in almost every other respect, appears to have functional disadvantages. This finding suggests that uncertainty in the environment may have been an important selective pressure in the evolution of the wild-type competence circuit of *B. subtilis*. The work further demonstrates that the topology of a genetic regulatory circuit can affect its noise characteristics and that two networks with identical deterministic outcomes can have profoundly different behaviors when noise is taken into account. More generally, the work by Çağatay et al. (2009) opens the door for a systematic investigation of the extent to which the design of gene regulatory circuits is optimized to perform certain functions. Genetic circuit design has two aspects: the topology of the regulatory links and their biochemical parameters. Combining the synthetic network approach with laboratory evolution experiments will allow us to disentangle these two design aspects and reveal the optimal behavior possible for each circuit topology. Combined with theoretical predictions, these types of experiments will provide insight into the evolutionary choice of specific network designs.

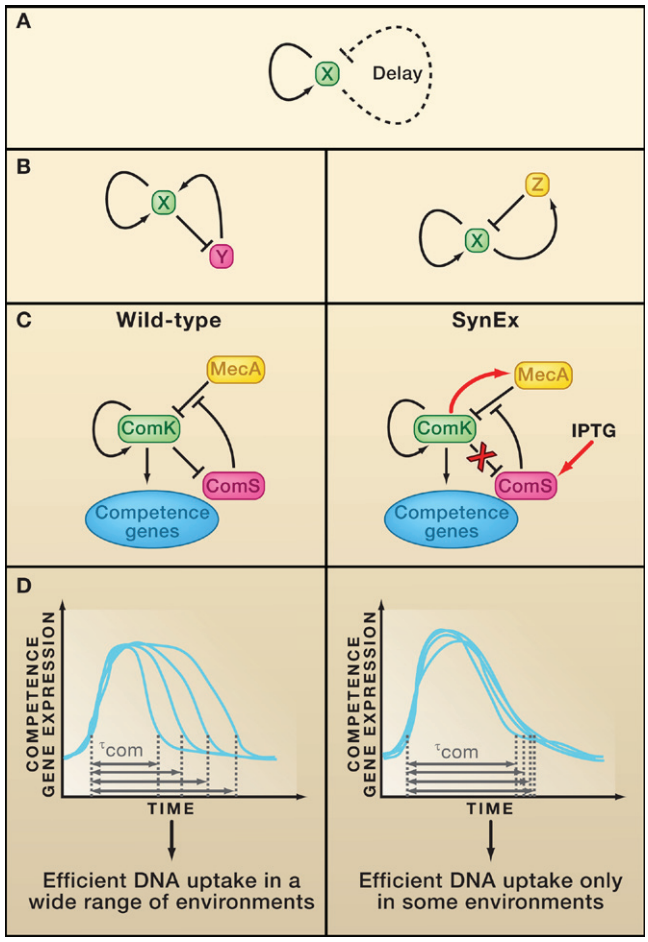

Figure 1. Rewiring the Competence Circuit in *B. subtilis*

(A) Schematic of a simple excitable system containing a fast positive feedback loop (solid) and a delayed negative feedback loop (dashed). Pointed arrows indicate activation, and blunt arrows indicate repression.

(B) Two different implementations of the general circuit design shown in (A): the delayed negative feedback loop can be achieved by repression of an activator Y (left) or by activation of a repressor Z (right).

(C) Simplified schematic of wild-type (left) and synthetic (right) gene regulatory circuits controlling DNA uptake competence in *B. subtilis*. In the wild-type, the competence master regulator ComK indirectly represses *comS*, which interferes with degradation of ComK by the MecA-ClpP-ClpC complex. This negative feedback loop mediates exit from the competent state. In the synthetic "SynEx" circuit (right), this feedback loop is abolished by removal of the regulatory link between ComK and *comS* and replaced with a functionally similar feedback loop of alternative design in which ComK activates *mecA* (red arrow). *comS* is under control of an IPTG-inducible promoter, which allows tuning of the frequency of competence events to match that of the wild-type.

(D) Competence events in single cells: In response to ComK activation, competence gene expression first increases sharply, and then, after a delay, decreases. Arrows indicate the duration τ_{com} of competence events in individual cells. The synthetic circuit (right) produces competence events with a more uniform duration than the wild-type circuit (left), even when other circuit properties are identical (e.g., frequency and median duration of competence events). The greater variability of competence durations in the wild-type network leads to more efficient DNA uptake under a wide range of environmental conditions.

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