Making gene circuits sing

Arthur Prindle[a] **and Jeff Hasty**[b,c,1]

[a]*Department of Bioengineering,* [b]*BioCircuits Institute, and* [c]*Molecular Biology Section, Division of Biological Science, University of California at San Diego, La Jolla, CA 92093*

How many tries did it take to get that to work? The double-edged question, which arises regularly at synthetic biology meetings, succinctly summarizes a major hurdle for the field; even with the successes and enormous potential for the construction of genetic circuits in living cells, it is commonly understood that cloning and computational expertise do not typically lead to circuits that function as anticipated on the first try. Most of us are familiar with the concept of tuning. In music, it can transform an instrument from one of torture to one of beauty. It is what we do when something is close but not quite, as, in many instances, close is insufficient. In electrical engineering, an "impedance mismatch" describes circuit elements that are not tuned to interact properly. Genetic circuits are cellular networks "wired" via interactions between "parts" such as proteins, mRNA, and secondary signaling molecules. During the past decade of genetic circuits research, it has become painfully clear that the properly tuned (i.e., "impedance-matched") region for interacting gene circuits is often miniscule compared with the full range of possible behaviors. In PNAS, Egbert and Klavins (1) describe an elegant and general method for finding the sweet spot to make gene networks "sing."

The engineering of gene circuits spawned the modern field of synthetic biology with two small transcriptional networks that produced switchable (2) and oscillatory (3) gene expression. These early successes led to the construction of more ambitious circuits at an accelerating pace (4). Sophisticated logic gates capable of interpreting environmental signals have been constructed in multiple cell types (5, 6). Genetic circuits controlled by light yielded tunable genetic programs in live animals (7). Oscillatory transcriptional clocks were developed to function at the single-cell (8), colony (9), and multicolony (10) level in growing bacterial populations, and even in much more complex mammalian cells (11). Accommodating technical strategies have been developed, including directed evolution (12), high-throughput cloning (13), and combinations of the two (12, 14). These strategies aim to overcome design uncertainties by sampling parameter values across a wide range. The need for such strategies arises from the fact that fine-tuning is necessary to achieve proper function in genetic circuits.

Tuning Knobs for Genetic Circuits

Egbert and Klavins (1) introduce a method to tune gene expression by using variable-length repeating DNA spacers. They call them ribosome binding site (RBS) spacer region-associated simple sequence repeats (SSR), or more simply rbSSRs. These segments lie between the ribosome binding site and the start codon of the target gene. A longer spacer means less output, as the bound ribosome must travel further to begin translation and therefore has a greater chance of failing to reach the start codon. Although ribosome binding site modification is a common tuning method in gene circuits (15–17), the strategy of Egbert and Klavins (1) stands out for its quantitative simplicity: to evaluate the strength, simply count the number of bases.

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In an example application, the authors use this platform to generate a library of 36 toggle switches. They observe the full spectrum of possible behaviors: OFF, ON, and functional bistable switches, a finding that beautifully illustrates the necessity of tuning. Imagine the original toggle switch (3), one of the seminal circuits of synthetic biology, being one of these 36 versions. If the researchers in that study had constructed only one design, and it had not been one of the nearly tuned versions, they may not have arrived at a functional circuit.

Even more eye-opening is the fact that only 3 of 36 circuits worked properly in a second strain, compared with 25 of 36 in the first, despite the fact that both were closely related strains of *Escherichia coli.* This disparity is an illustration of host context dependence (18): a circuit may work in one host and work differently or not at all in another, often without clear reason. This issue is extremely important as we move beyond *E. coli* toward the programming of other microbes (19). A promising solution described by Egbert et al. is to explicitly build tuning knobs into the circuits and adjust them to compensate for host-specific differences.

The rbSSR motif of Egbert and Klavins (1) is also well suited to address this point. Because repeating sequences are preferentially targeted for mutation, each rbSSR is a specific tuning point that becomes active in mutator strains. An example workflow would include placing the circuit in a mutator strain, screening for the appropriately tuned circuit, then transferring back to the stable strain for use. As an added benefit, when performed in novel hosts, this compensation would serve as an indirect measure of strain parameters.

In Pursuit of Selection

Although it is effective at the small scale, screening becomes impractical for larger circuits because the required library size increases exponentially with each component. As the authors suggest, an attractive next step is to move toward selection: screening where only cells containing the properly tuned circuit can grow effectively. Natural selection, the workhorse of evolution, uses this tactic with growth rate as the optimized variable. Instead, the designer of a synthetic circuit has the luxury of manipulating genetics and the environment to specify an arbitrary growth advantage for the trait(s) of interest.

However, the complexity of selected behavior is limited by the sophistication of available selection strategies. Although we know well how to select for static properties like antibiotic resistance or metabolite consumption, selecting for dynamic behaviors like counting, filtering, and oscillating is likely to be more complicated. One approach might involve using a microchemostat to subject cells to a dynamic environment, as certain metabolic networks may have evolved this way (20).

The rbSSR platform of Egbert and Klavins (1) represents an impressive step forward in the design of quantitatively tunable genetic circuits. By using this platform, one can envision compensating for strain-specific differences to move our current designs into new microbial hosts. The next steps will involve harnessing the power of evolution to accelerate circuit

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[1]To whom correspondence should be addressed. E-mail: hasty@bioeng.ucsd.edu.

design via selection. Can we use hypermutable tuning regions to tune other aspects of gene expression, such as transcription, protein maturation, or protein degradation? In addition to mutating individual elements, can we shuffle connections between larger modules? In performing these studies, what new insights can we gain about natural selection? Perhaps answers will lead to large-scale circuits that behave as predicted, and, just maybe, questions that imply a lack of understanding will begin to subside from our scientific meetings. However, we are not holding our breath on that one.

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