SYNTHETIC BIOLOGY

Cooperating on synthetic gene circuits

Biological gene networks coordinate complex behaviors through the interactions of activators and repressors with DNA. In eukaryotic gene networks, cooperative interactions between transcription factor and cofactors affect promoter binding and convert analog inputs into digital outputs. Until now, this type of cooperativity has been lacking from synthetic gene circuits that rely on one-to-one interactions between transcription factors and promoters. In a recent paper, Bashor, Patel et al.[1] engineer multivalent assembly into the yeast *Saccharomyces cerevisiae* and explore the theoretical and experimental frameworks for programming cooperativity into synthetic biology gene circuits.

Natural genetic circuits use the interactions in the core initiation machinery at a basal promoter to activate transcription. The authors' system uses synthetic zinc fingers fused to transcriptional activator domains. These synthetic transcription factors bind motifs upstream of a core promoter. Cooperativity is then mediated by the 'clamp'—a set of linked PDZ domains that bind the synthetic transcription factors. This enhances the binding of the factors to the DNA and allows researchers to manipulate the free energy of the complex by altering either the number of repeats in the clamp or the affinity of protein–protein or protein–DNA interactions (K_p or K_t, respectively).

"We wondered just how good a simple, first-principle model could be in predicting complex behaviors, if we could accurately measure as much as possible about our constituent molecular components. We discovered that, in many cases, we had fairly good predictive power for gene circuit behavior, and at the very least we could constrain the biochemical design space for a circuit from something extremely large to a much more narrowly defined space," says Ahmad Khalil, senior author on the paper and associate professor at Boston University. The work was not without its difficulties, as he explained: "However, particularly as we advanced to engineering dynamic behaviors and circuits with multiple nodes and positive feedback loops, we found that prediction was more difficult. In these cases, simple models of gene regulation may break down and may require more refinement."

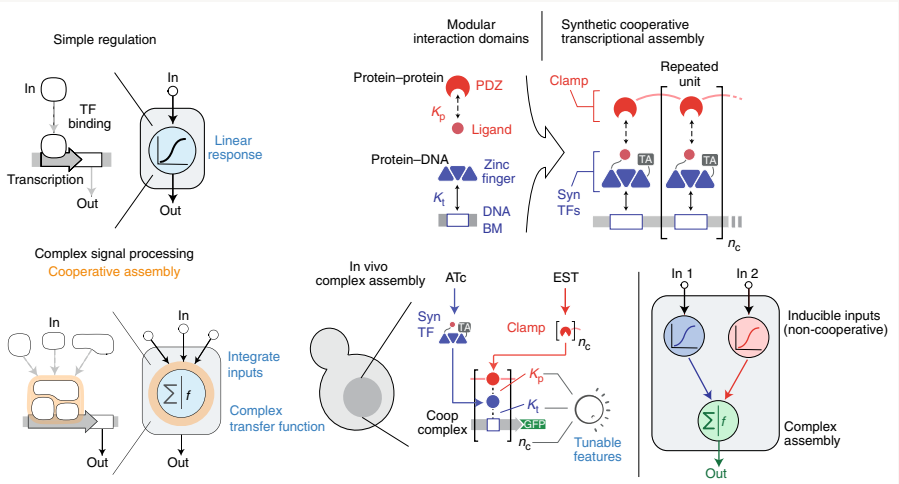
To help guide circuit engineering, the group built a statistical thermodynamic model of complex formation that relates component expression (synthetic transcription factors and the clamp) to promoter occupancy and reporter output. This allows them to model dose response by both circuit behavior and the 603 possible configurations of parts of the couples with variable numbers of clamp repeats, along with altering K_t and K_p. In line with experimental results, switch-like behavior was observed when the number of clamp repeats exceeded three. Exploration of configuration and behavior space enabled the authors to identify combinations of synthetic transcription factors that allowed AND or OR Boolean logic gate integration of inputs.

Next, the team wondered whether the rate of assembly configuration could be used to control circuit behavior. Pulses of the inducer doxycycline caused a cooperative circuit to display delayed activation and rapid decay of activity. Bringing this observation to multiple node cascades comprising synthetic transcription factors assembled in series, the researchers were able to predict and experimentally observe a range of temporal behaviors, including a stable memory configuration using positive feedback loops.

"From an engineering perspective, this is a powerful approach because you don't have to build, test and refine your designs (and testing and refining are labor intensive) to achieve new complex functions," says Jeff Tabor, associate professor of bioengineering at Rice University: "One cool result of their method is you can engineer lots of different behaviors with a relatively small number of parts. This modularity mimics what we see in biology and is appealing from an engineering perspective because designing new protein folds and functions is hard."

One of the co-lead authors on the paper, Caleb Bashor, who now heads a team at Rice University, says the approach provides a way of introducing nonlinearity into circuit designs. "This is something that has historically been a challenging engineering obstacle," he observes. "We show that building circuits using a cooperative assembly scheme grants access to classes of circuit behavior that would otherwise be very difficult to achieve."

Tabor predicts the system could be used to build a range of interesting devices: "One application is the design of a cellular 'radio' where you could reuse one input signal (e.g., anhydrotetracycline) to control four or five different cellular pathways by engineering genetic circuits that are tuned to the small molecule being delivered in a periodic fashion with different frequencies. ❐



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References

1. Bashor, C. J. et al. *Science* **364**, 593–597 (2019).