SYNTHETIC BIOLOGY

Synthetic polarization

Simple two- and three-component circuits induce polarization in yeast cells.

Unlike human engineers, nature does not work from the top down. We build structures mostly according to a blueprint of the final product; but biology takes a bottom-up approach and uses local molecular interactions to construct global structures and order. Wendell Lim from the University of California in San Francisco and Chao Tang from Peking University wanted to get at the underlying rules that biological systems play by.

"We wanted to look at biological regulation from an inverse approach," explains Lim, "not asking how one particular system performs a function of interest, but what are all the possible ways a function of interest can be accomplished." He adds that in the post-genomic era, scientists have identified the molecular parts for many systems, but how the parts work together to accomplish a given function is much less well understood.

In particular, Lim and Tang focused on cell polarization, which Tang, a physicist, describes as "symmetry breaking via self-organization." Their goal was to find the simplest network that accomplishes the task. Spatial organization in a cell is the key for many downstream behaviors such as motility.

Rather than go straight to the bench, the team started with a computational framework that allowed them to screen a large number of one- and two-node circuits. In their setup, one node represented a polarity marker, the other a regulatory element that altered the behavior of the first node. The researchers simulated different combinations of node self-regulation (positive, negative or no feedback) in conjunction with three different ways the two nodes can interact (positively, negatively or not at all). For each of the possible 81 networks, they also determined the magnitude of the interactions by varying parameters such as the concentrations of the two nodes or their diffusion rate. These measurements led to a polarization score indicating the robustness of the network with respect to a change in parameters. After removing nonpolarizing circuits and redundant ones, the scientists were left with eight core networks that displayed three minimal motifs: positive feedback of each of the two nodes or their mutual inhibition.

The simplest motif that could achieve polarization was a positive feedback loop, but it could do so only within a narrow range of concentrations. When two or three motifs were combined into a more complex architecture, polarization became much more robust over a large range of parameters.

To move these theoretical findings into a budding yeast cell, Lim's team chose phosphatidylinositol 3,4,5-triphosphate (PIP_3) as a polarization marker and the lipid PI3 kinase (PI3K) and the lipid phosphatase PTEN as regulators that, respectively, make and dismantle PIP_3. To exert spatial control, the researchers needed to target the enzymes to the plasma membrane where the lipids are found, and they did so by fusing PI3K to a PIP_3-binding pleckstrin homology (PH) domain and PTEN to a domain that bound the active, membrane-bound form of the GTPase Cdc42. The PI3K-PH fusion represents a simple positive feedback loop: it binds to existing PIP_3 and makes more of it. Combinations with localized PTEN, or a protein that inactivated Cdc42, created inhibitory links. The success of a circuit in polarizing a cell was tracked with a PH-GFP fusion marker that lit up areas of PIP_3 accumulation indicating a pole.

The researchers saw results similar to the computer simulation outputs. "This dramatic increase in robustness as you went from primitive motifs to combination motifs was probably the biggest surprise," remarks Lim.

The next challenges for the teams are to map the behavior of different circuits to natural polarization systems and to tease apart different sub-behaviors such as dynamic or static polarization or the occurrence of single or multiple poles.

"Simple design principles underlie seemingly complex and diverse biological networks," says Tang, "and these principles can not only help us to make sense of natural systems but also guide the design of synthetic circuits."

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RESEARCH PAPERS

Chau, A.H. *et al.* Designing synthetic regulatory networks capable of self-organizing cell polarization. *Cell* **151**, 320–332 (2012).