CELL BIOLOGY

Signaling rewired

Synthetic signaling molecules can be designed to display complex gating properties and to reprogram signaling networks in cells.

If you know enough about a system, whether it is an airplane, an electronic circuit or a cellular signaling network, you can build it. And in the case of the latter, this synthetic approach may reveal something about how evolution generates biological complexity as well.

Many signaling proteins are known to have a modular structure, with a catalytic domain and one or more regulatory domains. In work carried out some years ago, researchers in the laboratory of Wendell Lim at the University of California at San Francisco showed that it is possible to build synthetic protein switches with sophisticated gating behaviors by tethering simple modular autoinhibitory binding domains to the protein of interest. “The surprising thing was that this was relatively easy,” says John Dueber, first author on the paper describing the work (Dueber *et al.*, 2003). “My gut instinct told me that reprogramming signaling behavior would be extremely difficult. But this is one of those rare cases where things turned out to be simpler to do than you expected.” But several questions remained unanswered, and two recent papers from the same group now take this forward engineering approach further (Yeh *et al.*, 2007; Dueber *et al.*, 2007).

“There were a few things that we wanted to know,” says Brian Yeh, first author on a recent paper demonstrating the engineering of synthetic guanine nucleotide exchange factors (GEFs; Yeh *et al.*, 2007). “First, would these synthetic proteins be able to reprogram signaling in cells? And second, how generalizable is the approach?” As the researchers had a long-standing interest in cell shape and motility, they tried to rewire the activity of Rho-GTPases, master regulators of the cellular cytoskeleton, by engineering Rho-GEFs responsive to non-native inputs, in this case to protein kinase A (PKA).

Figure 1 | Engineering synthetic signaling proteins. Modular recombination yields synthetic GEFs that are active upon phosphorylation (P) by PKA. DH-PH, *Dbl* homology–pleckstrin homology. Reprinted from *Nature*.

The scientists designed an autoinhibitory module based on a PDZ domain–peptide interaction pair, which could be disrupted as a result of phosphorylation by PKA. They tethered this module to seven different GEF catalytic domains, reasoning that it would result in inhibition of constitutive exchange factor activity unless phosphorylated by PKA (Fig. 1). *In vitro* nucleotide exchange assays showed that, indeed, all seven engineered GEFs were silenced by the autoinhibitory module, and four of them were reactivated after phosphorylation by PKA. “This suggests that the approach is at least reasonably generalizable,” says Yeh. “And we didn’t optimize the design at all. We could probably improve the properties of the other three GEFs that way.”

To ask whether these synthetic PKA-responsive GEFs would retain their engineered functions *in vivo*, the researchers microinjected the proteins into fibroblasts and examined their effects on cell shape. As expected, the cells changed shape in response to activation of PKA, showing a dose-dependent increase in the production of filopodia or lamellipodia, depending on the specific GTPase being activated. Moreover, synthetic GEFs could be linked into a dual system, and generated a cell shape response that required the consecutive action of both engineered proteins.

In work conducted in parallel, Dueber continued to follow up on his initial observation. “We had started out by taking a library-based approach to look for proteins that had complex switch behaviors,” he says. “Now we decided to rationally design one such response.”

Dueber and colleagues applied both computation and experiment to study the property of ultrasensitivity in signaling proteins, in which, above a certain detection threshold, the relationship between input and output is nonlinear (Dueber *et al.*, 2007). They simulated the behavior of a protein with multiple cooperative auto-inhibitory modular domains, reasoning that this could in theory give rise to ultrasensitivity. They derived a model based on these simulations and tested its predictions experimentally. They found that ultrasensitivity depended principally on the number of interaction domains as well as the cooperativity between them, and the experimental data correlated well with the simulations in the model. “The work shows that you can build a complex ultrasensitive switch from very simple modular components,” says Dueber, “and that the overall approach is scalable. You can increase the complexity through the addition of other interaction domains.”

Recombination of domains in a modular protein may be a mechanism by which new cellular functions evolve. Synthetic biology, the engineering of new proteins as well as networks and even, ultimately, of entire cells, is one way in which the contribution of such mechanisms to biological complexity will continue to be studied. The work of Lim and colleagues, in both furthering our understanding of the principles that govern molecular switches and demonstrating that these switches can be manipulated to change complex cellular behavior, may bring us closer to that goal.

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RESEARCH PAPERS

Dueber, J. *et al.* Reprogramming control of an allosteric signaling switch through modular recombination. *Science* **301**, 1904–1908 (2003).

Dueber, J. *et al.* Engineering synthetic signaling proteins with ultrasensitive input/output control. *Nat. Biotechnol.* **25**, 660–662 (2007).

Yeh, B. *et al.* Rewiring cellular morphology pathways with synthetic guanine nucleotide exchange factors. *Nature* **447**, 596–600 (2007).