SYNTHETIC BIOLOGY

Network countdown

Researchers lay the foundation for networks that will self-destruct after being exposed to a predetermined number of stimuli.

Synthetic biology is not met with unequivocal acceptance by the general public. As the engineering capabilities of biologists increase, there is a growing fear that the scientists may create something that will escape the lab and wreak havoc.

James Collins from Boston University, who has been working in the field of synthetic biology for over a decade, takes these concerns very seriously. "We engineer organisms with the intent of putting them into the environment as biosensors or into human bodies as therapeutic agents," he says. "The legitimate concern is how to get rid of them once they are released." As one potential answer, Collins and his collaborators propose the creation of synthetic gene networks designed to self-destruct after a certain number of discreet events. Collins explains: "After *n* number of cell divisions the cell would be programmed to express a toxic protein and commit cellular hara-kiri."

In a recent report in *Science*, the researchers describe two prototypes of so-called 'counting' networks. One is based on RNA switches; the other, embedded in DNA, is based on recombinases that can flip DNA.

The RNA switch, or riboregulated transcriptional cascade counter, can currently count up to three. It starts with a constitutive promoter that drives expression of T7 DNA polymerase that in turn drives T3 DNA polymerase that in the prototype triggers expression of GFP. Both genes are additionally regulated by *cis*- and *trans*-regulatory elements. The *cis* repressor is complementary in sequence to the ribosome-binding site and forms a stem-loop that inhibits translation. To relieve this inhibition, a *trans* regulator—a short noncoding RNA, expressed from an arabinose-dependent promoter—binds the repressor, which allows translation to proceed. This network is able to count brief arabinose pulses; after each pulse an additional protein in the cascade is expressed, with easily detectable GFP being the last.

The DNA switch, or DNA invertase cascade counter, uses a similar principle. Here each pulse of arabinose results in the expression of a recombinase, which inverts a memory module that itself contains an inverted promoter. After the inversion, this promoter is placed in the forward orientation and drives expression of the next recombinase, which will then flip and inactivate this module and trigger expression of GFP.

The RNA switch operates in the 15–40 minute range and is thus suited to count, for example, cell cycle proteins during cell division. The DNA counter operates on the order of hours and could respond to circadian rhythms and count days or nights.

The versatility of the networks is rooted in the fact that every component is tunable. The promoters can be made to respond to a wide range of stimuli, such as heavy metals, metabolites or environmental stimuli such as heat, osmotic pressure or light. Each protein expressed from these promoters is fused to a degradation tag, and the choice of this tag can tune the stability of the proteins.

For Collins, the main challenge was not in constructing the individual parts of the network but in getting them to perform as desired. "You want to balance the different properties of the components properly," he explains; "you need to have a good handle on the degradation and expression characteristics."

Though there currently is no shortcut to empirically test each component, Collins says that there are efforts underway to speed this process. These efforts include the generation of diverse parts that are properly characterized so that individual modules can be more rapidly assembled into a network.

Now that the prototype is out, Collins wants to focus on specific applications and design a network that expresses a toxic protein after a set number of cell divisions.

A cell that commits suicide at the count of three should help diffuse the specter of ogres coming out of synthetic biology labs.

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RESEARCH PAPERS
Friedland, A. E. *et al.* Synthetic gene networks that count. *Science* **324**, 1199–1202 (2009).

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