

CELL SIGNALLING

RNA device rewires cellular networks



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Synthetic devices that translate molecular inputs into gene expression changes are valuable for manipulating cellular behaviour, but until now these circuits have been able to respond only to a limited set of inputs, such as transcription factors. However, a new synthetic device based on RNA can respond sensitively to alterations in the levels of endogenous signalling molecules, and so can potentially link disease-associated pathways to cell fate decisions.

At the heart of the new device is an RNA aptamer, an RNA molecule that alters its conformation and function upon binding to a particular protein ligand. In the new study, an RNA aptamer is inserted into the intron of a three-exon minigene. On binding of the aptamer to a cellular factor, the RNA alters its conformation, and in so doing affects the alternative splicing of the middle exon

of the minigene. This splicing event has crucial consequences: the middle exon contains a stop codon, and so its removal leads to the upregulation of an output gene that is under the control of the minigene product. This sensor-actuator construct therefore has the potential to link changes in intracellular protein concentration (via the sensor module, represented by the aptamer) to gene expression changes (via the actuator minigene and the output gene).

The flexibility of the synthetic device was shown by applying it in different settings in human cell lines. In the first application, cells were stably transfected with devices in which two aptamers responded, respectively, to changes in the levels of two subunits of nuclear factor- κ B (NF- κ B). In the second example, the aptamer was designed to respond to β -catenin, the main downstream effector of the

Wnt signalling pathway. In both cases, stimulation of cells with pathway activators led to the specific expression of reporter genes, at levels that correlated with the degree of alternative splicing of the minigene.

To illustrate the clinical utility of the circuit, the authors modified the design to connect upregulated signalling to particular cell fate decisions. The NF- κ B and the β -catenin aptamers were combined and linked to an output gene that induced apoptosis only in the presence of the pro-drug ganciclovir. Indeed, cells in which the two signalling pathways were stimulated showed only ~20% survival when ganciclovir was added.

RNA is an apt molecule for such devices, given its versatile sensing functions and the ease with which RNA molecules can be designed. As the examples above show, these particular devices can also easily be adapted for combinatorial use in clinical applications (such as gene or cellular therapy, or in diagnostics) or as drug discovery tools.

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ORIGINAL RESEARCH PAPER Culler, S. J., Hoff, K. G. & Smolke, C. D. Reprogramming cellular behavior with RNA controllers responsive to endogenous proteins. *Science* **330**, 1251–1255 (2010)

FURTHER READING Khalil, A. S. & Collins, J. J. Synthetic biology: applications come of age. *Nature Rev. Genet.* **11**, 367–379 (2010) | Lim, W. A. Designing customized cell signalling circuits. *Nature Rev. Mol. Cell Biol.* **11**, 393–403 (2010)