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Synthetic circuit allows dialing gene expression up or down in human cells

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Scientists who built a synthetic gene circuit that allowed for the precise tuning of a gene's expression in yeast have now refined this new research tool to work in human cells, according to research published online in *Nature Communications*.

"Using this circuit, you can turn a gene from completely off to completely on and anywhere between those two extremes in each cell at once. It's a nice tool if you want to know what happens at intermediate levels of gene expression. There has been no such system so far, but now it is available for mammalian cell research," said senior author Gábor Balázsi, Ph.D., associate professor in The University of Texas MD Anderson Cancer Center Department of Systems Biology.

Present options for altering gene expression in human cells are blunt instruments by comparison. Knocking out a gene eliminates its expression completely. Inhibiting it with RNA interference dials it partially down and can affect other genes. Inserting a gene expression vector into cells overexpresses the gene, but it's usually uncontrolled. Commercially available versions can switch a gene on or off, but cannot precisely dial between these extremes.

"For cancer research, the system will allow scientists to test the boundaries of a gene known to confer resistance to a drug in cancer cells by dialing its expression to different levels and treating the cells with the drug," said first author Dmitry Nevozhay, M.D. Ph.D., instructor in Systems Biology.

"Likewise, such a system would allow personalized gene therapy, by precisely tuning the therapeutic gene level expression depending on disease progression and the patient's need," Nevozhay said.

In microbial or yeast biology research scientists have started to understand and manipulate gene function quantitatively, almost like we understand electronic circuits, Balázsi said. "This makes research in those areas more amenable to engineering and mathematical characterization, - but that's not true for human cells, and part of the problem is that tools that tune gene expression have been lacking."

A step-by-step guide for others to build mammalian synthetic gene circuits

By refining their circuit to work in a human breast cancer cell line, the team demonstrated that their approach can be used in mammalian cells while offering a step-by-step guide that other researchers could follow to build other synthetic circuits for use with other genes.

"With all of our steps reported, if someone wants to build another type of gene expression switch, or oscillator, they could build the circuit in fast-growing yeast cells, where it can be engineered and optimized quickly and reliably," Balázsi said. "Once you know it works in yeast, you know the steps to make it function in human cells. This process is similar to extensive testing of NASA's space operations on Earth before actually carrying them out in space."

Synthetic biologists apply engineering principles to design and build new biological systems for predefined purposes.

In yeast, Balázsi and colleagues synthesized a gene circuit designed to control the level of gene expression precisely using the tetracycline

repressor.

They made the promoter for the repressor identical to the promoter for the reporter gene yEGFP encoding the green fluorescent protein. This caused a negative feedback loop, creating a linear dependence of the yEGFP level on the tetracycline analog in the growth medium.

Tunable control of gene expression in mammalian cells

The researchers modified the synthetic network, which initially did not work at all in human cells. A computational model suggested a strategy to optimize the network for mammalian cells.

Several modifications improving transcription, translation and intracellular localization of the regulator protein were added to the synthetic network one at a time. Each one bolstered the network's output in human cells, until it finally achieved a linear dose response of gene expression to the tetracycline analog doxycycline.

Among the additions made to the circuit:

- Addition of an intron (non-coding DNA), which when inserted into genes can increase their expression in mammalian cells.
- Codon optimization in the repressor and reporter genes.
- Introduction of a nuclear-localization sequence, to take the circuit into the cell nucleus, where it can influence gene expression.
- Addition of the Kozak sequence, which improves gene expression in mammalian cells by enhancing translation.
- Promoter optimization, which maximizes the gap between full and basal expression.

Finally, they used the same circuit to control expression of an additional red fluorescence protein gene called mCherry as proof of concept for regulating other genes.

His synthetic gene circuit research won Balázsi a National Institutes of Health New Innovator Award in 2009, one of only 54 such grants made nationally that year to fund bold ideas with the potential to quickly translate research into improved human health.

"This research is not possible without the New Innovator Award," Balázsi said. "It allows you to explore off the beaten path. We aren't looking directly at the next obvious step towards curing cancer or discovering new molecular interactions.

"Yet, we believe steps that don't seem obvious today are crucial for tomorrow's therapies. We've outlined a set of engineering steps that will help us better understand and control gene expression to improve cancer treatment or develop new approaches to gene therapy," he said. "Traditional funding mechanisms would not have done it."

Provided by University of Texas M. D. Anderson Cancer Center