Synthetic gene circuit allows precise dosing of gene expression

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Researchers have crafted a gene circuit that permits precise tuning of a gene's expression in a cell, an advance that should allow for more accurate analysis of the gene's role in normal and abnormal cellular function.

This gene "dosing effect" is achieved by installing a negative feedback loop in the synthetic gene circuit, a concept similar to signal distortion control in electronics, a team led by scientists at The University of Texas M. D. Anderson Cancer Center reports this week in the Online Early Edition of the Proceedings of the National Academy of Sciences.

"To understand what a gene does, you need to change its expression and observe the results. Present methods do not allow close control of gene expression," said senior author Gábor Balázsi, Ph.D., assistant professor in M. D. Anderson's Department of Systems Biology.

Knocking out genes is an all-or-nothing approach, and suppressing them with small interfering RNA has undesired effects. Transfecting cells with a gene expression vector overexpresses the gene, but still in an uncontrolled way, Balázsi noted. The synthetic gene expression system the researchers developed in a yeast model would allow more detailed investigation of a gene's effects.

"Say you have a gene that is involved in resistance to drugs, and you want to know how much protection the cell gets at different levels of expression," Balázsi said. "You place the gene circuit in the cells set at

first to fully repress the protective gene. You then tune gene expression to the desired level and add chemotherapy to the cell culture, to discover the relationship between the gene and cellular defense against the drug."

The gene expression circuit built by Balázsi and colleagues produces a linear relationship between the dose of an inducer that regulates the circuit and the level of gene expression.

Gene network built to repress

The team first synthesized a gene network designed to repress yEGFP, a fluorescent reporter gene whose presence can be detected in each cell in a culture by flow cytometry. The gene circuit was then added to the growth medium, where it diffused into cells and blended into the yeast's DNA.

The gene circuit starts with a promoter that launches the tetracycline repressor gene, which then blocks
yEGFP via a promoter for that gene, shutting down its expression. Adding anhydrotetracycline (ATc) to the cell culture in measured doses stifles the tetracycline repressor and permits expression of yEGFP.

When ATc was added to the culture, there was little or no response at low dosages, then a sudden, steep increase in yEGFP, which quickly hit a plateau at saturation. "This is called a sigmoidal response, which does not follow in a linear fashion the dose of ATc," Balázsi said.

Similar to distortion and an amplifier

The team then made the promoter for the repressor identical to that used for yEGFP. This, in effect, turns the tetracycline repressor back on itself, a negative feedback loop that results in automatic reduction of the

repressor when it reaches high levels and an increase when levels drop.

They found that adding negative autoregulation makes yEGFP linearly responsive to ATc dose. So a 20 percent increase in ATc yields a 20 percent rise in gene expression, and so on.

Balázsi compares this to dealing with distortion in electronic circuits. Amplifiers strengthen a signal, but also distort it. By distorting the signal before it enters the amplifier in the opposite way that the amplifier will distort it, the two distortions cancel each other out, resulting in a clear signal. Making both gene promoters identical has the same effect in the gene network.

This linear dose-response relationship works while it achieves similar expression levels of yEGFP in all cells in the culture. Cell-to-cell differences are large in cell cultures treated with the gene circuit that lacks negative feedback, with some cells expressing a great deal of yEGFP and others very little. The feedback loop leveled cell-to-cell differences, affecting them all at once, improving the dose effect.

After their first experiments with the synthetic gene circuit, the researchers constructed a mathematical model to predict gene expression response in the presence of negative autoregulation. The circuit with the negative autoregulation performed as predicted by the model.

Balázsi said the team is working on a gene circuit that will work as well in a mammalian cell model. Balázsi and colleagues create new gene sequences, or circuits, part of the emerging field of synthetic biology - the application of engineering principles to design and build new biological parts and devices

Source: University of Texas M. D. Anderson Cancer Center (news : web)