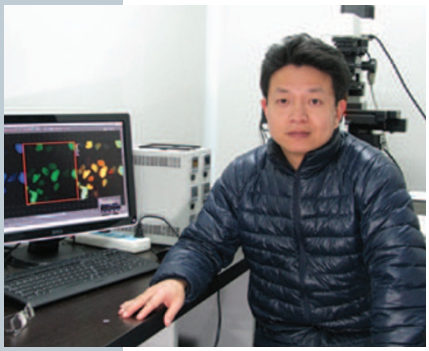
THE AUTHOR FILE

Yi Yang

Engineering light-induced gene expression

When Yi Yang was recruited back to China to build better bioreactors for making drugs and industrial chemicals, school officials asked him to look for unusual strategies. For biochemical engineering, standard tools to control cells in a bioreactor involve adding antibiotics and hormones. Yang's goal was to get away from such additives, which are difficult to calibrate and can pollute the environment.



Yi Yang

Ultimately, inspiration came from a hobby. Yang, an amateur astronomer, was working with lenses and mirrors in deconstructed telescopes when he decided that he would try to control gene expression with light. The advantages of light over chemical additives are clear, says Yang, a synthetic biologist at East China University of Science and Technology. "Light is easy to control. And light is ecological and economical."

Yang, a protein engineer by training, combed the scientific literature for instances of light-induced gene expression, reading nightly after his children were asleep. For a system to be easy to use and robust, he reasoned, it should be regulated by a single gene and work without additional chemicals.

He remembers one night in particular: he learned about a protein in a filamentous fungus with an apparent molecular weight that increased upon exposure to blue light. "My eyes lit up; I realized that this represented dimerization." Dimerization can transform a protein that binds DNA weakly as single units into double units that bind tightly. As Yang read more, he found the protein had other favorable properties: it could easily be targeted to the nucleus, and only low levels of light were required to initiate the dimerization reaction. He realized such a system could be applied more broadly than just to bioreactors.

But Yang had this idea over winter break. His labs were closed and he had to wait a few frustrating weeks before he could explain his goals to his graduate student and the first author on the paper, Xue Wang. "She understood very quickly and agreed to get started," Yang recalls.

Soon, Yang and his students had engineered cultured human cells and were ready for the first experiment. The effects of light on gene expression were weak, but real. "We were surprised and lucky," Yang says. "I knew [then] the technique would eventually work."

Next began a long slog of optimization. Yang had combined VIVID, the protein he had read about, with the well-studied GAL4 system, which uses a yeast protein to activate transcription. In addition to the promoter that is inserted before the gene of interest, the system had several components—the light sensor, the DNA-binding domain, and the domain that activates gene expression—with linkers between the domains. Each of these required considerable improvement.

The most frustrating component was the activation domain—the part of the protein that would turn on gene expression once bound to a desired sequence. It initially yielded just 1% of the expression that would be expected with a strong promoter. Yang and his students tried round after round of genetic alteration and selection experiments, and then hit upon a simple solution: they simply swapped one activation domain for another. Light-induced gene expression increased almost 100-fold.

Yang and his students began preparing results for publication at the beginning of the Chinese Year of the Rabbit (February 2011). The timing has special significance for Yang because it was in the previous Year of the Rabbit (August 1999) that he first left China to work abroad, as a postdoc at Harvard Medical School. Yang paid homage to the year in the way he depicts the protein dimer. "We drew it so that it looked like a bunny," he explains.

Yang paid another sort of homage when choosing a name for the system. 'LightON' mirrors the name of the popular and convenient gene induction system Tet-On, in which expression is activated with the antibiotic tetracycline. Compared with existing light-activated gene expression systems, LightON offers several potential advantages, not least that it works in mammalian cells and whole animals with no added chemicals. LightON uses a single exogenous protein that is orthogonal to mammalian cell-signaling pathways. Expression of the gene of interest increases 200-fold after exposure to light. And the length, placement and duration of light can both tune levels of gene expression and target expression to desired organs. This, plus the fact that the dimer disassociates over several hours, should allow researchers to use the same mouse for multiple experiments, says Yang, and so reduce the number of animals necessary for research.

Yang predicts that getting started with LightON should be inexpensive. "We bought the lamps and controllers on Taobao [China's equivalent to Ebay] and we assembled them ourselves. For less than $50 we can have a setup to control the lighting illumination." It should also be easy, he says. "We were amateurs when we started doing the first experiments. So the experiment can be done in every lab."

Monya Baker

Wang, X., Chen, X. & Yang, Y. Spatiotemporal control by a light-switchable transgene system. *Nat. Methods* **9**, 266–269 (2012).