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MIT Study Uses Zinc Finger Proteins to Detect Genes and Elicit Cellular Response

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Premium

NEW YORK (GenomeWeb) – Scientists from the Massachusetts Institute of Technology have created a zinc finger protein-based system for detecting DNA in a cell and eliciting a response, such as apoptosis, in that cell. The system could have a variety of applications such as confirming the presence of genome edits made with CRISPR/Cas9, homogenizing cell populations, and detecting chromosomal inversions and translocations.

James Collins and Shimyn Slomovic leveraged the DNA-binding properties of zinc finger proteins in two different ways to create the "sense and respond" protein modules. They published their study this week in *Nature Methods*.

"There was a lot of potential in using [zinc finger] proteins not just to do something to the gene that they detect, but to tap into the detecting capabilities," Slomovic told GenomeWeb. "This could be used to report the presences of that gene if it were a pathogen gene, or associated with cancer or a virus, and more importantly make a connection between the detection of that sequence and some sort of tailorable response."

To use the system, a researcher must choose both a target, like a retroviral gene, and a response, such as expression of GFP. Both involve zinc finger proteins.

"We're using the zinc fingers at the front end of this model to detect the target sequence and using zinc fingers to turn on the gene that we want to be turned on in response to the detection," Slomovic said.

Zinc fingers have long been known to be programmable and selectively bind to DNA targets. To ensure that the system created the response only in the presence of the target gene, the scientists leveraged the properties of inteins — short proteins that can be used to split larger proteins into two pieces, called exteins. Exteins only become functional once the intein removes itself while rejoining the two halves.

Collins and Slomovic decided to divide an intein in two and then attach each portion to a split extein half and a DNA-binding zinc finger protein. The zinc finger proteins are engineered to recognize adjacent DNA sequences within the targeted gene, so if they both find their sequences, the inteins line up and are then cut out, allowing the extein halves to rejoin and form a functional protein. The extein protein in this case is a zinc finger-based transcription factor designed and optimized to turn on any gene the researchers want.

The researchers were able to put the humpty-dumpty transcription factor back together again by attaching the extein halves to other zinc finger proteins designed to bind adjacent sequences in the target gene.

With the transcription factor reassembled, it can turn on just about any gene in the genome, Slomovic said. "It's a genetic circuit that is highly modular with response to what gene you can put into it." Regardless of the gene a researcher wants to turn on, the transcription factor is going to turn it on, he said. "There is not really a limit to what gene you can turn on in response to the detection."

That modularity lends itself to a suite of potential applications, including reporting, generally accomplished by activating a fluorescent protein, and winnowing, somehow activating cell death either through apoptosis pathways or otherwise.

Zinc finger proteins were one of the first programmable gene-editing methods, and while they're more expensive than using CRISPR/Cas9, they could be used to help confirm that CRISPR-based editing actually happened.

"To tweeze out those cells [that have been edited] is no easy process," Slomovic said. "Cells have to be grown out and individual cells have to be isolated and those clonal cell lines have to be subjected to PCR or sequencing in order to distinguish between the ones that have received the edit and those that have not." With zinc finger proteins designed to recognize the new, edited sequence, Slomovic said researchers could immediately distinguish and enrich the cell population that has received that CRISPR/Cas9 genome edit.

Collins offered another application in the realm of synthetic biology.

"There's increasing interest in having inducible gene expression systems," he said. "This could be the basis for a broad, diverse set of inducible systems where you can use DNA as the inducing agent." DNA could even be delivered on coated gold nanoparticles. "You could deliver the DNA, and when it's detected it would flip on your genes of interest."

The system could also be used to help better understand the biology of chromosomal translocations and inversions. Leveraging the fact that the detection zinc finger proteins need to be adjacent to elicit the desired response, the system could be designed so that it recognizes cells that have undergone these types of chromosomal defects.

While there are existing ways to detect these chromosomal defects, they don't allow researchers to study the live biology and dynamics of the cell because they require killing the cell and extracting its DNA. "If a cell line had this system in it, you could study the biology of these cells in real time, actually watch them and observe them while they were still alive," Slomovic said.

Researchers could even explore the three-dimensional chromosomal architecture using the proteins, confirming long-range interactions between two genes that come together when the chromosome folds.

There are even industrial applications. Mammalian cell bioreactors used to produce enzymes and medicines can become susceptible to viral infection, Collins said. "We envision equipping the cell lines used in these bioreactors with a sense and response system that would protect them from viral infection. If one did become infected we could program it to kill itself before the pathogen could spread to the rest of the population."

The authors hint that clinical researchers could one day find a use for their system as well. HIV and cancer are cases where it could be useful to kill off a cell. "You could program the cell to kill itself or to secrete proteins that would allow the immune system to identify it as an enemy cell so the immune system would take care of it," he said.

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