BIOSENSORS

DNA makes GFP shine

An oligomerization-dependent system comprising two fragments of green fluorescent protein (GFP) fused to engineered zinc fingers could be a means to detect virtually any double-stranded DNA sequence.

Several tried-and-tested methods can be used to detect single-stranded DNA, but there are no reliable techniques to detect specific DNA sequences in their double-stranded configuration. To meet this need, Indraneel Ghosh and David Segal at the University of Arizona put together their respective expertise in split protein assembly and zinc finger–DNA interactions.

Split versions of reporter proteins such as GFP, which are inactive until the proteins to which they are fused oligomerize, have been used to detect interactions between proteins. In their present work, described in the *Journal of the American Chemical Society*, Ghosh and Segal took this approach a step further by creating split protein–DNA binding domain fusions in which the two halves of the reporter reassemble only when the two proteins bind adjacent sites on DNA, thus allowing detection of specific DNA sequences.

The researchers fused two zinc fingers, the most widely used DNA-binding motif in the human genome, to GFP fragments that were known to be capable of functional reassembly. They used two well-characterized zinc fingers that recognize unique 9–base–pair sequences, and designed a double-stranded oligonucleotide with these recognition sites. In an *in vitro* reaction containing this template and the two purified fusion proteins, the researchers observed an increase in fluorescence, indicating the formation of a ternary complex.

This approach, called sequence-enabled reassembly, has the potential to be developed for use in many applications. "We want to make a kit that will allow us to detect, in a reagent-based fashion, any kind of viral DNA or any other double-stranded DNA," says Ghosh. To make this a reality, however, the system has to be modified to increase signal intensity, because now the zinc fingers bind DNA tightly and only a single GFP reassembles. "So if we can get the zinc fingers to have lower affinity but the same selectivity, then they can bind DNA and rapidly turn over different GFPs or… other enzymes that we could detect," says Ghosh, and he adds that it may even be possible to detect a single mutation within the cell. That will be possible, of course, only when the method is optimized *in vivo*, and will require a good signal-to-noise ratio of the reporter activity in a cell.

Ghosh hopes it will eventually be possible to use this method to selectively target cells that have a certain mutation. "What we hope to do is take this another step and actually reassemble toxins that can detect a [specific] mutation in cancerous cells, resulting in their elimination."

Irene Kaganman

RESEARCH PAPERS
Stains, C.I. *et al.* DNA sequence–enabled reassembly of the green fluorescent protein. *J. Am. Chem. Soc.* **127**, 10782–10783 (2005).

MOLECULAR LIBRARIES

Finger pointing

A screen based on a library of randomly assorted zinc finger–containing transcription factors points out unique genes responsible for specific phenotypes of interest.

Transcription factors are among the most influential of proteins; they regulate gene expression that in turn determines the phenotype of cells. Harnessing this power, scientists have engineered these proteins to modulate gene expression.

The zinc finger family of transcription factors is especially amenable to modification because these proteins consist of defined domains, or zinc fingers, which specifically bind particular DNA sequences, thus selecting the genes they regulate. By shuffling three or four of these domains, a library of artificial transcription factors can easily be generated. Jin-Soo Kim from ToolGen in South Korea has a long history of working with such libraries, and he has demonstrated in eukaryotic systems that artificial transcription factors can modulate gene expression and change phenotypes. One open question, however, is how to achieve the direct identification of the target gene that causes a given phenotype. In a recent paper in the *Journal of Bacteriology*, Kim and his team present a method to directly link artificial transcription factor, target gene and phenotype.

To stack the cards in their favor, the scientists started with the less complex genome of bacteria rather than eukaryotic cells, expressing their zinc finger library in *Escherichia coli*. They then analyzed thermostable bacteria that managed to grow at 50 °C and characterized one of the artificial transcription factors that induced this desired phenotype. To find the target gene they cross-linked transcription factor and DNA *in vivo*, immunoprecipitated the protein and sequenced the bound DNA. By comparing their results with *in silico* predictions of target sites for a protein with this particular configuration of zinc fingers, they identified the target gene and thus demonstrated that these artificial transcription factors could be used to directly identify a gene responsible for a phenotype of interest.

The applications of this screen are numerous—Kim foresees many different phenotypes that could be generated with this library approach. He sees his work as bridging the gap between industry and academia. He says, "People in industry usually focus on interesting phenotypes,… in academia they look for genes [that] can induce the phenotypic change. We address the question of how to improve the phenotype and then we can identify the target genes."

All that remains to be shown now, to get a large number of people in industry and academia really excited, is proof that this direct linking between gene and phenotype also works in eukaryotic systems, something Kim may well deliver in the near future.

Nicole Rusk

RESEARCH PAPERS
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