Scientists

Building with Biological LEGO

Keith Joung

Associate Chief of Pathology for Research; Associate Professor of Pathology; Massachusetts General Hospital, Boston, MA

Keith Joung's development of technologies to create specific DNA binding proteins caught our attention. Curious to know more, *BioTechniques* contacted him to find out about the ambition, character, and motivation that led to his success.

What do you consider to be your most significant scientific contribution?

I'm most proud of our efforts to develop publically available platforms and technologies for engineering DNA binding proteins using zinc finger proteins and, more recently, transcription activator-like (TAL) proteins. For zinc finger proteins, we developed two methods: oligomerized pool engineering (OPEN) and context-dependent assembly (CoDA). And we also developed a high-throughput automated method for making TALs called FLASH (fast ligation-based automatable solid-phase high-throughput).

What are some of the advantages these technologies offer?

A single zinc finger binds approximately 3bp of DNA, so it doesn't provide enough specificity to accomplish anything useful in the context of a cell. To create proteins that can be targeted, we must link together multiple fingers to achieve the specificity needed to recognize a unique sequence in a complex genome. The tools we designed address the various challenges of making multifinger proteins.

In the case of zinc fingers, we expect a protein containing three fingers to bind a 9bp sequence; an early idea was to preselect an archive of single zinc fingers which could

be modularly assembled into multi-finger proteins. While the best way to modify the function of a single zinc finger is to randomize around six positions in the finger known to mediate DNA recognition and create a library, as you can imagine, this creates unapproachably large combinatorial libraries of different zinc fingers. Another challenge is that fingers behave differently depending on their neighboring fingers, an effect we refer to as context dependent behavior. So, we needed to account for the context dependent behaviors of the fingers, but at the same time, screen all possible combinations to identify the best pattern of fingers.

OPEN accomplished this by selecting smaller pools of fingers with different amino acid sequences that recognize the same 3bp site. These publically available pools contain roughly 100 zinc fingers each. Now, it is much easier for other labs to take those pools and stitch together the components of interest to make their own libraries from which they can select zinc finger arrays.

CoDA contains information derived from many different selections we performed with OPEN to identify pairs of fingers that worked well next to one another. This allows researchers to design a zinc finger protein for a particular site, accounting for context dependence, based on the database that we created. So in practice, researchers simply search a sequence and if it is a site targetable using the CoDA approach, the program returns the sequence of the zinc fingers required. So CoDA accounts for context dependence and eliminates the requirement for combinatorial library construction and selection.

What are the most common applications for zinc finger proteins today?

Right now, a major focus is fusing them to nuclease domains to introduce targeted double strand breaks. If you introduce a double strand break into a cell, repair mechanisms are often error prone, leading to insertion or deletion mutations at the break site. In some cases, you can trick the cell undergoing repair into employing an exogenous piece of DNA that you introduce on a plasmid or a virus, allowing targeted mutations or even correcting a mutated

allele. I think this mechanism will be useful for reverting mutations that are responsible for disease and ultimately might be used in a therapeutic setting. For now, this presents a powerful new tool because there are a number of organisms and cell types where making targeted mutations is difficult, and this approach provides for high frequency mutagenesis in those difficult cell types.

In the course of this research, what has been your greatest surprise?

Recently, we have devoted much of our efforts to TAL technology. A single TAL domain binds a single base pair of DNA and different domains are known for each of the four nucleotides, suggesting that we can just string these together to make a multi-domain protein that recognizes an extended site. When I first heard this, I was skeptical because of our experience with zinc finger context dependent behaviors. Not to mention the fact that, in my experience with science and biology, things are rarely that simple. But we made the FLASH platform for generating TALENs (TAL effector nucleases) in high-throughput, which allowed us to do very large scale tests on nuclease function in human cells. And I was stunned by how well they worked. Unlike zinc fingers, TAL repeats do appear to be modular. They are like LEGO that we can literally assemble into functional proteins to target just about any DNA sequence. Almost everything works; the success rate is probably greater than 85-90% in our hands. So, I have to admit, I have been really surprised by these results.

Editors Note: Hear more from Dr. Joung and other researchers on emerging genetic engineering tools by listening to a BioTechniques podcast at www.biotechniques.com/multimedia/podcasts/

Interviewed by Kristie Nybo, Ph.D. Image courtesy of MGH Photography.

BioTechniques 52:351 (June 2012)
doi 10.2144/000113870

To purchase reprints of this article, contact: biotechniques@fosterprinting.com