Scientists

Recognizing the Site

Bill Jack

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Bill Jack's research on DNA polymerase function 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 made you choose to pursue a career in molecular biology?

Originally, I planned on becoming a biophysical chemist and studying membrane proteins. Once I was accepted to graduate school at Duke University, I asked if there were any labs where I could work during the summer before starting the program. I received a few replies, including an offer from Paul Modrich who had just arrived at Duke to study *EcoRI* endonuclease. I chose his lab to broaden my background, thinking it was far afield of my interests.

At the time, molecular biology tools and techniques were primitive, so I focused on kinetics. I loved doing those experiments, graphing the data, and then modeling the results. Along the way, I fell in love with the developing field of molecular biology and with Paul as a mentor.

Thus far, what do you think has been your most influential contribution to the field?

During graduate school, I studied how DNA-binding enzymes find their particular recognition sequences. One set of experiments focused on how the *EcoRI* endonuclease reduces its search for a certain site from three dimensions to one. We used long and short pieces of DNA, each with a recognition site in the middle, and asked which of the fragments was preferentially cleaved. The surprising result was that the

enzyme found the site on the long piece of DNA much more readily than on the short piece. By comparing the behavior of a range of fragment sizes, we were able to provide a rigorous kinetic characterization of site recognition, showing that *EcoRI* first bound non-specifically to DNA and then scanned the DNA looking for its site.

Another contribution I feel good about was our analysis of protein splicing here at New England Biolabs. Some colleagues cloned a 90kDa archaeal DNA polymerase, but DNA sequencing results repeatedly indicated a coding region reflective of a 180 kDa protein. My group set out to investigate RNA splicing scenarios that would rectify this discrepancy by making a number of mutations at the splice junction. These mutations had no effect on the observed polymerase, which meant that RNA splicing was not the explanation. At the same time, we read reports suggesting that protein splicing occurred in a yeast ATPase. Together with colleagues, we identified presumptive protein splicing junctions and my group created specific amino acid substitutions that blocked this splicing function. It was exciting to be involved in identifying and elucidating the mechanism of this novel protein splicing event.

What brought you to NEB?

As a graduate student and postdoctoral fellow, I expected to pursue an academic position. When I finished my postdoctoral work, I received job offers from an academic institution, an industrial company, and NEB. At the time, I had three sons, the youngest of whom was 2 years old. When making my decision, I looked at my sons and realized that if I took the academic position, in six years, I might have tenure, which would allow time to get to know them. But there was a real possibility that I would miss a large part of six formative years in those boys' lives. While I admire those who can succeed in academia while maintaining a balanced life, for me, that seemed a daunting task. NEB allowed me to pursue science while also being a member of the larger community.

What are you working on now?

Right now, my lab is working on thermostable DNA polymerases, studying their abilities to incorporate modified nucleotides, characterizing them kinetically, and identifying mutations that improve function. The most obvious application for these polymerases is in next generation sequencing platforms where terminators labeled with dyes are incorporated. Other labeling techniques incorporating modified nucleases have also been developed; we hope these methods will give us insights into DNA replication itself and how to alter amplification conditions to react with templates carrying modified bases. Our focus is on the basic science, and we collaborate with others for technology and platform development.

What do you think is the most important open question in molecular biology today?

We have come a long way in developing techniques for DNA sequencing and are now faced with vast challenges in using this data to determine cause and effect. Epigenetic modification makes this task even more difficult and is clearly a key player that will be explored more fully, but carbohydrate modification of proteins is another layer of complexity that has been largely neglected. We are just now developing the enzymes to identify specific carbohydrate structures on proteins, complementing mass spectrometry, HPLC, and labeling methods required for analysis. With this new toolbox, we finally have a way to better define the role of carbohydrate modifications in protein trafficking, stability, activity, and specificity. Epigenetics and glycobiology are rich areas for exploration and we are actively pursuing both.

Interviewed by Kristie Nybo, Ph.D. Image courtesy of Kim Indresano.

BioTechniques 52:225 (April 2012)

doi 10.2144/000113834

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