ONR Augmentation Award Final Technical Report

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The ONR Augmentation Award was used to support Scott Baskerville, a graduate student in my lab. Scott's research was directed towards increasing our understanding of how selected RNA molecules can recognize target ligands. As a model system, Scott studied how arginine-rich motifs (ARMs) frequently found in viral proteins can recognize RNA molecules. This model system was chosen because we had previously observed that RNA molecules that bind to ARMs frequently contained a discrete arginine-binding site (Ellington et al., 1995; Ellington et al., 1996). We had hypothesized that residues adjacent to the arginine-binding site were important in determining the specificitiy of ARM:RNA interactions. In other words, we believed that we had identified a potential 'code' for at least one class of peptide:RNA interactions. By deciphering this code, we hoped to better understand whether and how molecular sequence could be directly mapped to molecular recognition.

Scott originally attempted to discern how the ARM of the Rex protein of the human T-cell leukemia virus interacted with its RNA ligand, the Rex-binding element (XBE). Scott partially randomized the XBE and selected variants that could still bind to Rex. By sequencing selected clones, he was able to map the sequences and secondary structural features that were critical for Rex-binding function. As a result of these experiments, Scott found that the XBE contained an arginine-binding site similar to those we had previously observed in other RNA molecules, bolstering our hypothesis for molecular recognition. Subsequent structural studies carried out in collaboration with Dinshaw Patel at the Sloan-Kettering Institute have confirmed the existence of this arginine-binding domain. These results are described in greater detail in Baskerville et al. (1995).

Scott then attempted to describe the breadth of RNA molecules that could interact with Rex. Starting from a completely (as opposed to partially) randomized sequence population, Scott again selected aptamers (binding species) that could bind to Rex. By sequencing selected clones, he was able to identify three unique classes of RNA sequences and structures. One of these classes (Class II) contained the arginine-binding site previously observed in the wild-type XBE and in other ARM-binding RNAs. Another

class (Class III) had a variant of the arginine-binding site that has been observed in natural BIV-1 TAR. Scott was able to model structural relationships between the Class III anti-Rex aptamers and BIV-1 TAR. Overall, these results confirmed and expanded the nascent 'rules' for the recognition of ARMs by RNAs. These results are described in greater detail in Baskerville et al. (1997).

Although he is not listed as an author, Scott also provided substantial intellectual contributions to the analysis of a different ARM:RNA interaction. A post-doctoral fellow in my lab, Xu Wei, selected RNA molecules that could bind to the isolated ARM of Rev, a regulatory protein from HIV-1. The selected RNAs again contained the arginine-binding site observed in other ARM-binding RNAs. These results are described in greater detail in Xu and Ellington, 1996).

My lab continues to study molecular recognition using ARM:RNA interactions as a model system. In particular, we now feel that we understand the rules for sequence interactions well enough that we can 'swap' domains from different ARMs and ARM-binding RNAs and generate hybrid and novel interactions. The success or failure of these predictions should continue to aid in understanding how nucleic acid sequence can be mapped directly to nucleic acid recognition.

Scott Baskerville has since graduated from my lab. His extremely productive period as a graduate student has allowed him to become a post-doctoral fellow at the prestigious Whitehead Institute in Boston. He is currently working with David Bartel on the selection of novel RNA catalysts and the development of a 'coupled translation' system. The latter project builds on discussions begun in my lab, and is again an outgrowth of ONR funding (a Young Investigator award).