SELECTION OF RNA-BINDING PEPTIDES FROM COMBINATORIAL LIBRARIES

Kazuo Harada, Shelley Martin, and Alan D. Frankel
Department of Biochemistry and Biophysics
University of California, San Francisco
San Francisco, CA 94143-0448

The arginine-rich RNA-binding motif (ARM) is a short RNA-binding peptide motif (15-20 amino acids in length) which has been identified in viral proteins such as HIV Rev, HIV and BIV Tat, as well as a number of bacteriophage (λ, $\phi21$, and P22) antiterminator N proteins. Studies of the interactions of ARMs and their RNA sites have shown that both the structure of the peptide and the RNA targets that they recognize are diverse. Many of the amino acid determinants of these interactions have also been elucidated, and it appears that other than arginine, very few other amino acids (predominantly hydrophilic amino acids) are required for tight and specific binding. This low sequence complexity and versatility of the ARM lead us to hypothesize that arginine-rich RNA-binding peptides may have arisen readily early in evolution and played a role in the transition from an RNA world to an RNA-protein world.

In order to test how readily RNA-binding arginine-rich peptides may have been selected from random peptide mixtures, we created a number of relatively small combinatorial libraries and attempted to identify peptides that bind to the HIV RRE hairpin. We used a bacterial two plasmid system based on λ N antitermination, that consists of an N-expressor plasmid and a reporter plasmid containing the RNA site and transcriptional termination elements upstream of LacZ, so that peptide-RNA binding and resulting antitermination can be visualized by β-galactosidase activity. We were able to identify HIV Rev-like peptides from one library consisting of four amino acids (R, S, N, and H), and novel peptide sequences from a different library consisting of three amino acids (R, S, and G), therefore demonstrating the low sequence complexity needed to select specific RNA-binding peptides. The possible evolution and function of such ribonucleopeptide complexes in a predominantly RNA-based world will be discussed.