In Vitro Selection of RNA Binding Peptides

Thesis by

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Abstract

RNA is recognized to play an increasing number of roles in the cell: transcription regulation, translation, and catalysis. Peptides that bind RNA would therefore be useful as biochemical tools and lead compounds for therapeutics. Existing genetic methods of isolating RNA binding peptides are prone to biases and can only search millions of sequences. *In vitro* selections using mRNA display provide an avenue to discover specific, high affinity peptides that bind to any RNA target from libraries composed of trillions of molecules.

Here, we describe initial experiments to optimize the mRNA display selection cycle for the isolation of RNA binding peptides. We use this optimized cycle to show that enrichment of specific sequences is possible using mRNA display, and select mutants of the λ N peptide which bind in a different conformation than wild-type. Characterization of these peptides demonstrates that affinity is not enough for *in vivo* activity; binding in a correct conformation is also important.

Based on these experiments, we designed a strategy to isolate RNA binding peptides to targets for which no natural ligand is known. We test this strategy and isolate peptides that bind to functionally important domains of telomerase RNA with nanomolar affinity and high specificity. Using mutagenic PCR and additional rounds of selection, we increase the specificity of several peptides for telomerase RNA and also isolate other peptides which bind an important domain of the Hepatitis C Virus internal ribosome entry site.

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List of Abbreviations

DNA =	Deoxyribonucleic acid
RNA =	Ribonucleic acid
mRNA =	Messenger ribonucleic acid
cDNA =	Complementary deoxyribonucleic acid
RT =	Reverse transcription/reverse transcriptase
Tris =	Tris-(hydroxymethyl)-aminomethane
pmol =	Picomole
DTT =	Dithiothreitol
EDTA =	Ethylenediaminetetraacetate
ddH_2O =	Double distilled water
tRNA =	Transfer ribonucleic acid
Ni-NTA =	Nickel-nitriloacetic acid
RNase =	Ribonuclease
TMV =	Tobacco mosaic virus
Bsa =	Bovine serum albumin
HSQC =	Heteronuclear single quantum coherence
NMR =	Nuclear magnetic resonance
HCV =	Hepatitis C virus
IRES =	Internal ribosome entry site
hTR =	Human telomerase RNA
hTERT =	Human telomerase reverse transcriptase