Chapter 1

Background and Perspective

Two decades ago RNA's role in biology was simple. The central dogma (Figure 1.1) dictated that RNA's major role was an information carrier between DNA and protein. How things have changed. RNA is increasingly recognized to play a role in many cellular processes (1) including catalysis (2), chromosome maintenance (3), transcriptional control (4), and the synthesis of proteins (5).

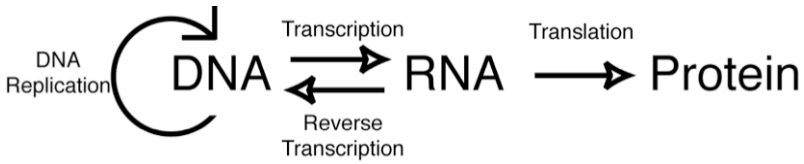

Figure 1.1. The central dogma of biology. DNA is transcribed into RNA, which is then translated into protein. RNA can be reverse transcribed into DNA and DNA can be replicated.

As RNA's role in biology is better understood, it becomes a more attractive target for drug design (6, 7). However, rational drug design is difficult since RNA often changes structure upon ligand binding (8), electrostatics and ion interactions must be accurately modeled (9), and obtaining structures by crystallography is difficult (10).

Genetic approaches (11, 12) and selection methods (13, 14) are powerful methods enabling the isolation of RNA binding proteins and peptides. However, these systems are often subject to biases and libraries sizes of 10^6-10^9 molecules since an *in vivo* step is required. Thus, these methods often target known binding sites on RNA, or limit the amino acid alphabet used in the selection.

The work described in this thesis represents initial efforts to develop methodologies that enable the isolation of RNA binding peptides and proteins to any RNA target. These proteins and peptides would be useful both as tools in biochemistry

and molecular biology and as potential therapeutics. mRNA display is the key experimental technique that enables the isolation of RNA binding peptides. mRNA display is performed entirely *in vitro*, allowing libraries of up to 100 trillion independent sequences to be synthesized (15). Thus, mRNA display will reduce the biases inherent in technologies requiring an *in vivo* step, as well as enable the selection from totally random libraries composed of all twenty amino acids.

The thesis is divided into chapters describing different aspects of the methodology development. Each chapter is described below:

Chapter 2

Chapter 2 is a published review of the mRNA display field, and describes successful selections resulting from mRNA display. Several conclusions can be drawn from mRNA display experiments including the fact that many sequences of nanomolar affinity can be isolated form mRNA display selections, in contrast with the results from phage display selections where micromolar affinities are more typical. Applications unique to mRNA display, including unnatural amino acid incorporation and protein chips are also discussed.

Chapter 3

This published chapter describes efforts to optimize the mRNA display selection cycle. Although we optimized the cycle for the selection of RNA binding peptides, the protocols described in this chapter are generally applicable to any mRNA display selection.

Chapter 4

Part of this chapter has been previously published and describes the initial experiments to use mRNA display for the selection of RNA binding peptides. We began with a control selection based on the λ N peptide system to show that the wild-type sequence could be enriched from a randomized pool, then extended the methodology to select for novel mutants of the λ N peptide. Characterization of one of these mutants implies the mutant binds in a different conformation from the wild-type peptide.

Chapter 5

This published chapter describes the further characterization of the λ N peptide mutants isolated in Chapter 4. The mutants all possess nanomolar affinities and different *in vivo* activites, however, affinity does not correlate with activity. Instead, activity is dependent upon binding in the correct structural conformation.

Chapter 6

Chapter 6 describes the selection of RNA binding peptides from totally random libraries to RNA targets for which no natural ligand is known. A strategy for the isolation of RNA binding peptides is described and used to isolate peptides with nanomolar affinity and high specificity that bind to human telomerase RNA (hTR). Selections targeted two important regions of telomerase RNA. Modification of the selection protocols enabled the selection of D-peptides.

Chapter 7

Chapter 7 discusses the attempts to improve the affinity of some of the peptides isolated in Chapter 6 through the introduction of mutagenic PCR into the selection cycle. Additionally, attempts to improve the specificity of the selected peptides as well as change the specificity toward a similar RNA target from the Hepatitis C Virus internal ribosome entry site are also described.

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