# CHAPTER 11
# RNA Folding Pathways

## INTRODUCTION

Elucidating the mechanisms by which macromolecules such as RNA fold into their three-dimensional biologically active conformations is among the most challenging research endeavors at the chemistry-biology interface. Because of the connection between structure and biological function, defining the factors that govern RNA folding should lead to a better understanding of how ribonucleic acids perform their functions in vivo. In practical terms, this understanding will also facilitate efforts to predict the three-dimensional structure of complex RNAs from primary sequence and to design RNA molecules that adopt stable structures possessing specific binding and catalytic properties. Such RNA molecules hold considerable promise as biochemical tools and diagnostic reagents, and as starting points for the development of therapeutics for human disease.

A complete understanding of folding requires knowledge of the structures and energetics of each conformational state of a given molecule along the pathway from the unfolded structure to the final native functional state. However, much of the research on RNA folding conducted over the last 25 years has focused on elucidating the structures and stability of folded RNA molecules. Recent advances in X-ray crystallography, coupled with improved methods for both the synthesis and analysis of RNA, have facilitated significant advances in efforts to elucidate RNA folding pathways. The goal of this chapter is to present the reader with a current understanding of the problems in the field of RNA folding and the most useful and cutting-edge approaches to solving these problems.

*UNIT 11.1* is an introductory commentary that addresses important considerations such as the questions that one wishes to ask about RNA structure, the difference between RNA and proteins in structure analysis, and the difference between folding and unfolding. *UNIT 11.2* focuses on the prediction of RNA secondary structure using RNAstructure and *m*fold. *UNIT 11.3* uses thermal analysis to study the tertiary structure of an RNA as it unfolds. In *UNIT 11.4*, different conformers of an RNA are separated based on structure-dependent electrophoretic properties and their activity is assayed in the gel, providing a simple means to correlate structure and function. *UNIT 11.5* describes the use of circular dichroism and urea to look at RNA structure transition, which can be used to accurately and rapidly determine thermodynamic parameters in a wide variety of conditions. *UNIT 11.6* presents an elegant use of X-rays for time-resolved hydroxyl radical footprinting of RNA. *UNIT 11.7* uses magnesium chelation to study real-time tertiary unfolding of RNA. *UNIT 11.8 & 11.9* discuss the use of fluorescence and chemical modification for studying the kinetics of RNA folding. *UNIT 11.10* complements *UNIT 11.8* by presenting the use of fluorescence resonance energy transfer to study the kinetics as well as the structural basis of a conformational change. Finally, *UNIT 11.11* presents methods for synthesizing large pyrene-labeled RNAs that can be used in fluorescence experiments.

Gary D. Glick

**Contributed by Gary D. Glick**
*Current Protocols in Nucleic Acid Chemistry* (2004) 11.0.1