Molecular Biology of RNA: New Perspectives

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Preface

RNA is full of surprises. An RNA acting as an enzyme, an RNA capable of self-splicing, and a branched RNA covalently linked to DNA are a few examples of new and previously unexpected roles for RNA. It is apparent that RNA is a highly complex class of molecules, with a wide variety of cellular roles, from which we have much to learn. We believe that it is appropriate at this time to take stock of recent discoveries in this rapidly developing field and to look at the approaches, strategies, and methodologies used. It is for this reason that we have undertaken, with much joy, the task of editing this book. We have been extremely fortunate in being able to present chapters written by the leaders in this field. We believe that this book will help provide new direction and insight for those already working on the subject and will serve as a useful guide to those about to start research in the molecular biology of RNA. Most of the contributing authors participated in the Fifth Stony Brook Symposium held in May 1986 on "New Perspectives on the Molecular Biology of RNA."

We wish to thank Janet Koenig for her invaluable assistance throughout this project.

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Bernard S. Dudock

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Cleavage of RNA by RNase P from *Escherichia coli*

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I. INTRODUCTION

RNase P performs a function in cells that is very simple in comparison to the functions performed by other ribonucleoproteins (RNPs), such as the small nuclear (sn) RNPs involved in mRNA splicing. It removes, with great accuracy, the extra nucleotides from the transcripts of tRNA genes to yield the correct 5′ terminus of the mature tRNAs (Altman *et al.*, 1982). Figure 1 shows a linear transcript of three genes: The gene in the center codes for a tRNA and, in *Escherichia coli,* the flanking genes code for other tRNAs, ribosomal RNA, or protein. In many cases in prokaryotes, the first processing event is an endonucleolytic cleavage that occurs at, or near, the 3′ end of the sequence for the mature tRNA. Simultaneously, or very shortly thereafter, a second cleavage occurs to generate the correct 5′ terminal nucleotide of the mature tRNA, and it is this reaction that will be discussed in more detail below. In eukaryotes, the order of processing events seems to be less rigidly specified (Rooney and Harding, 1986). After these two endonucleolytic cleavages, there is exonucleolytic cleavage of the extra nucleotides at the 3′ end of the tRNA and, at the same time, the nucleotide-modifying enzymes function to generate the mature tRNA molecule. The nature of the enzymatic events in eukaryotes is

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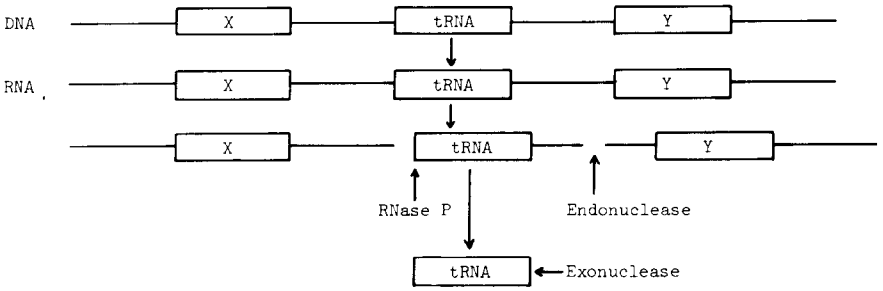

Fig. 1. A generalized scheme for the processing of tRNA gene transcripts by nucleases. X and Y denote sequences coding for protein and tRNA, rRNA and tRNA, tRNA alone, but not protein and rRNA. (Reprinted, with permission, from MIT copyright © 1981.)

similar, but most of the tRNA genes are transcribed as monomers and it is only the few extra nucleotides on either side of the mature sequence that must be removed.

Although RNase P was first identified over 15 years ago (Robertson *et al.*, 1972) in crude extracts of *E. coli*, it was only several years later that Stark *et al.* (1978) verified that the enzyme had very unusual chromatographic properties and could be inactivated by treatment with ribonucleases as well as proteases. We now know that these unusual properties were the result of the presence of an essential RNA component in the enzyme. Subsequent biochemical and genetic experiments have also demonstrated the essential nature of both RNA and protein subunits for growth of *E. coli* (Kole and Altman, 1979; Kole *et al.*, 1980).

II. ASPECTS OF THE RNase P REACTION

The reaction catalyzed by RNase P can be performed, *in vitro*, under two distinct sets of reaction conditions (Guerrier-Takada *et al.*, 1983). Most remarkably, we showed that the RNA component of RNase P can function by itself as a catalyst in the cleavage of precursors to $tRNA^{Tyr}$. Such a reaction, in the absence of protein requires the presence of 100 m*M* Mg^{2+}, although the reaction can also proceed with decreasing efficiency in the presence of lower concentrations of magnesium and added spermidine. It was shown subsequently (Gardiner *et al.*, 1985) that the RNA moiety of RNase P from *Bacillus subtilis* can act in a similar manner