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19 ABSTRACT (Continue on reverse if necessary and identify by block number) This project involves kinetic studies of RNA splicing reactions catalyzed by the intervening sequence from the precursor rRNA of Tetrahymena thermophila. The ultimate goal is identification and understanding of each step in the mechanism for splicing. Special attention is being focussed on the role of Mg ²⁺ .					
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ANNUAL REPORT: Contract N00014-88-K-0179
PRINCIPAL INVESTIGATOR: Douglas H. Turner
CONTRACTOR: University of Rochester
CONTRACT TITLE: Molecular Basis of RNA Catalysis
START DATE: 1 March 1989

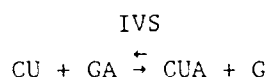
RESEARCH OBJECTIVE

To determine the mechanism for self-splicing reactions of intervening sequences in RNA.

PROGRESS (Year 2)

A shortened form of the intervening sequence (IVS) was fluorescently labeled, and titrated with Mg^{2+} . Two transitions are observed. The first is very cooperative as expected for Mg^{2+} induced folding of IVS. The second transition is broader and in the concentration range expected for Mg^{2+} binding to a hypothesized active site (N. Sugimoto, M. Tomka, R. Kierzek, P. C. Bevilacqua, & D. H. Turner, Nucleic Acids Res. 17, 355-371 (1989)).

Steady state kinetic studies were conducted with the shortened form of IVS. The substrates CU, UCU, and CUCU were used as analogues of the 5' exon with GA, UCCA, and ACUCCA as analogues of the 3' exon. The simplest overall reaction is:



In all cases, lengthening the substrate affects K_M and (k_{cat}/K_M) about as expected from nearest neighbor parameters for base pairing as previously measured in our lab (Fraier et al., Proc. Natl. Acad. Sci. U.S.A. 82, 9272-9277 (1986)). Thus the K_M 's of substrates can be tailored based on those parameters.

The secondary structure of the shortened form of the IVS was mapped with chemical modification reagents, and compared to results previously obtained in our lab for the covalently closed circular form of IVS.

WORKPLAN (Year 3)

Using appropriately tailored substrates, steady state kinetics and fluorescence titration experiments will be performed to further test the hypothesis that the reaction mechanism involves uptake of at least one loosely bound Mg^{2+} . The order of addition of substrates and Mg^{2+} will be determined. Attempts will also be made to determine if 1 or 2 Mg^{2+} are required.

The structure of a shorter form of the IVS will be mapped, and its reactivity determined.

INVENTIONS

NONE

PUBLICATIONS AND REPORTS

1. This work was presented in invited lectures at the University of New Hampshire (Mobay Lecture), Bowling Green State University, and at the Biophysical Training Grant Symposium at the University of Minnesota.

TRAINING ACTIVITIES

Dr. Sean Moran, a postdoctoral fellow, Mr. Philip Bevilacqua, a third year graduate student, and Mr. Aloke Banerjee, a second year student, are working on this project. The first two are native born American citizens. Mr. Banerjee was born in India, but received his college training in the U.S. at College of Wooster.

AWARDS/FELLOWSHIPS

Mr. Bevilacqua is a Hooker Fellow.

Accession For	
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BIO. CHEM.	✓
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