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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. Fluorescent substrates have been developed that can be used for transient kinetics experiments. The ultimate goal is identification and understanding of each step in the mechanism for splicing. <i>splicing, molecular biology, (K)</i>			
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PRINCIPAL INVESTIGATOR: Douglas H. Turner
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RESEARCH OBJECTIVE

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

PROGRESS (Year 1)

Analysis was made of previously obtained kinetic data on the reaction of dinucleoside monophosphates, rCrU, rCdU, dCrU, and dCdT, with the covalently closed circular form of the intervening sequence of the precursor rRNA of *Tetrahymena thermophila*. The results suggest the 2' OH of the 5' nucleotide is involved in a hydrogen bond to the intervening sequence, and the 2' OH of the 3' nucleoside is involved in binding Mg^{2+} .

Three fluorescent substrates for model splicing reactions were synthesized. One, ϵ ACUCU, is an analogue of the 5' exon in the self-splicing reaction of the intervening sequence. The other two G ϵ A and ϵ AG, are expected to mimic the 3' intron-exon junction. Fluorescence titration protocols that require small amounts of RNA were worked out with tRNA as a model system. With the intervening sequence, the fluorescence intensity of ϵ ACUCU is reduced about a factor of two when it binds to the covalently closed form. A fluorescence titration has provided a binding constant for this interaction. Problems have been encountered, however, with decomposition of ϵ ACUCU.

Plasmid DNA has been isolated that is suitable for producing large quantities of a shortened intervening sequence. Transcription with T7 RNA Polymerase gave large amounts of intervening sequence. A column protocol was developed for purification. This eliminates the necessity for purification by denaturing gels. Different methods of renaturing the RNA, however, gave different populations of species on non-denaturing gels.

WORKPLAN (Year 2)

Renaturation protocols will be developed that give a single conformational form of the intervening sequence. Attempts will also be made to fluorescently label the intervening sequence. These RNAs will be used in fluorescence titration experiments to provide binding constants and thermodynamic parameters for the ribozyme-substrate interactions. These parameters will permit design of transient kinetics experiments. Initial experiments will employ stopped-flow methods to determine the time scale of the reaction. Depending on the time scale, either a stopped-flow or a laser temperature-jump apparatus will be built that is suitable for the proposed studies.

INVENTIONS

NONE

89 2 23 010

PUBLICATIONS AND REPORTS

1. Partial support for: N. Sugimoto, M. Tomka, R. Kierzek, P. C. Bevilacqua, & D. H. Turner, "Effects of substrate structure on the kinetics of circle opening reactions of the self-splicing intervening sequence from *Tetrahymena thermophila*: evidence for substrate and Mg^{2+} binding interactions," *Nucleic Acids Res.* 17, 355-371 (1989).
2. This work was presented at the UCLA-Keystone meeting on the Molecular Biology of RNA, and at the Biopolymers Gordon Conference.

TRAINING ACTIVITIES

Dr. Sean Moran, a postdoctoral fellow, and Mr. Philip Bevilacqua, a first year graduate student, are working on this project. Both are native born American citizens.

AWARDS/FELLOWSHIPS

Mr. Bevilacqua is a Sproull Fellow.



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