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This report summarizes the results of a graduate student fellowship award which resulted in the awarding of a Ph.D. in the emerging research area of theoretical molecular biology. The report includes a description of the student's research work on the comparative sequence analysis of structural RNA. This work has resulted in three publications to date and is detailed in the student's dissertation.

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Comparative Sequence Analysis of Structural RNA

FINAL REPORT

George E. Fox
and
Timothy Haselman

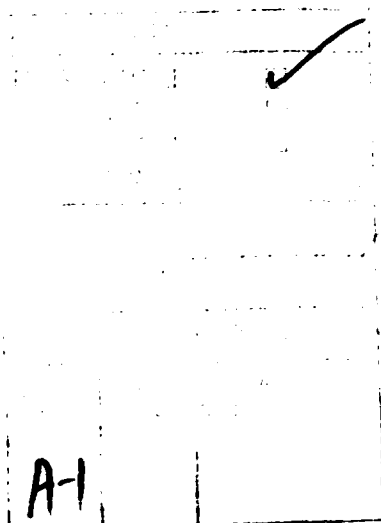
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University of Houston
4800 Callhoun
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The purpose of grant/contract DAAL03-86-6-0031 was to train a student at the Ph.D. level in the emerging discipline of theoretical molecular biology. The student who was recruited to this program was Mr. Timothy Haselman who received a B.S. degree in biology from Brigham Young University. Mr. Haselman participated in the program for its duration and was provided a stipend as well as tuition support until August of 1989. At that time funds were largely expended and although Mr. Haselman continued to receive tuition support from the fellowship his living stipend was transferred to other funds available to Dr. Fox for the period 9/1/89-8/31/90. Mr. Haselman successfully defended his dissertation entitled "Comparative Sequence Analysis of Structural RNA" in August of 1990 and following minor revision in language that dissertation was finally accepted by his committee in December of 1990. Dr. Haselman began attending medical school in September of 1990 at the University of Texas Medical School in Galveston. He plans to pursue a career in medical research following receipt of his M.D.

During his participation in the program Mr. Haselman took coursework and did research relating to the application of theoretical ideas in modern molecular biology. From several possibilities available to him he selected the detection of secondary and tertiary interactions in structural RNA as his thesis topic. His work in this area was successful and has to date resulted in three publications:

- (1) Haselman, T. Chappellear, J.E. & Fox, G.E. (1988) "Fidelity of Secondary and Tertiary Interactions in tRNA". Nucl. Acids Res. 16: 5673-5684.
- (2) Haselman, T., Camp, D.G. & Fox, G.E. (1989) "Phylogenetic Evidence for Tertiary Interactions in 16S like Ribosomal RNA." Nucl. Acids Res. 17: 2215-2221.
- (3) Haselman, T., Gutell, R.R., Jurka, J. & Fox, G.E. (1989) "Additional Watson-Crick Interactions Suggest a Structural Core in Large Subunit Ribosomal RNA." J. Biomol. Struct. Dyn. 7:181-186.

It is expected that additional publications will arise from results included in Mr. Haselman's dissertation. The remainder of this report will summarize the results of Mr. Haselman's thesis work.

The goal of Mr. Haselman's research was to develop the methods and insights needed to identify base-base interactions in structural RNAs. The availability of crystallographic information on tRNA tertiary structure made that molecule an ideal place to begin seeking the required insights. A database of all tRNA sequences was obtained and broken into subclasses according to minor variations in apparent secondary structure. The variational

pattern associated with positions known to be involved in tertiary interactions (ie from x-ray data) in yeast phenylalanine tRNA was observed in each tRNA subclass. Subsequently an algorithm known as matrix reduction was developed which can detect the types of variational pattern associated with secondary and tertiary structure from background noise. When applied to tRNA it was found that as many as six of the known tertiary interactions could be detected. Subsequently the approach was applied to other structural RNAs.

The major portion of the effort focused on the large rRNAs. In the case of 16S rRNA, 27 basepairs that are frequently included in secondary structure models were shown to be inconsistent with existing comparative evidence. Most of these are putative extensions of established helices that had been previously proposed from thermodynamic considerations. In addition a three base extension to the helix at E. coli position 65 was found. Thirteen strongly supported candidate tertiary interactions were identified by extensive searching. The location of these are summarized in Figure 1. In addition a possible conformational switch near the 3' end of the 16S rRNA was detected.

Exmination of the large subunit rRNAs began with the 5S rRNA. Within the 5S rRNA a four base irregular helix was detected as well as two isolated, but local, tertiary interactions. In the case of 23S rRNA strong comparative support was detected for nineteen candidate tertiary interactions despite a relatively modest data set. An additional five interactions, U1072/A1099, U1082/A1086, G1093/C2394, G2110/C2179 and U2111/A2147 were tentatively identified. In addition an important new two base pair helical element was detected that appears to define a central core structure of the molecule that may be involved in orienting domains II, III and IV. The 23S rRNA results are summarized in Figure 2.

Other less extensive searches focused on possible inter-ribosomal RNA interactions. Although not exhaustive, these searches suggest that no substantial number of such interactions exist. This implies that subunit/subunit interaction is primarily a protein and not an RNA phenomenon. Application of the approach to other structural RNAs, eg the U-RNAs or splicesomes, proved premature due to extremely limited availability of sequence information. It was possible to confirm secondary structure proposals for the U-RNAs but only one candidate tertiary interaction could be detected. The work performed by Mr. Haselman is described in detail in his thesis which has been submitted to the Office of ARMY Research in compliance with reporting requirements associated with DAAL03-86-6-0031. The dissertation is also available from the other usual sources.

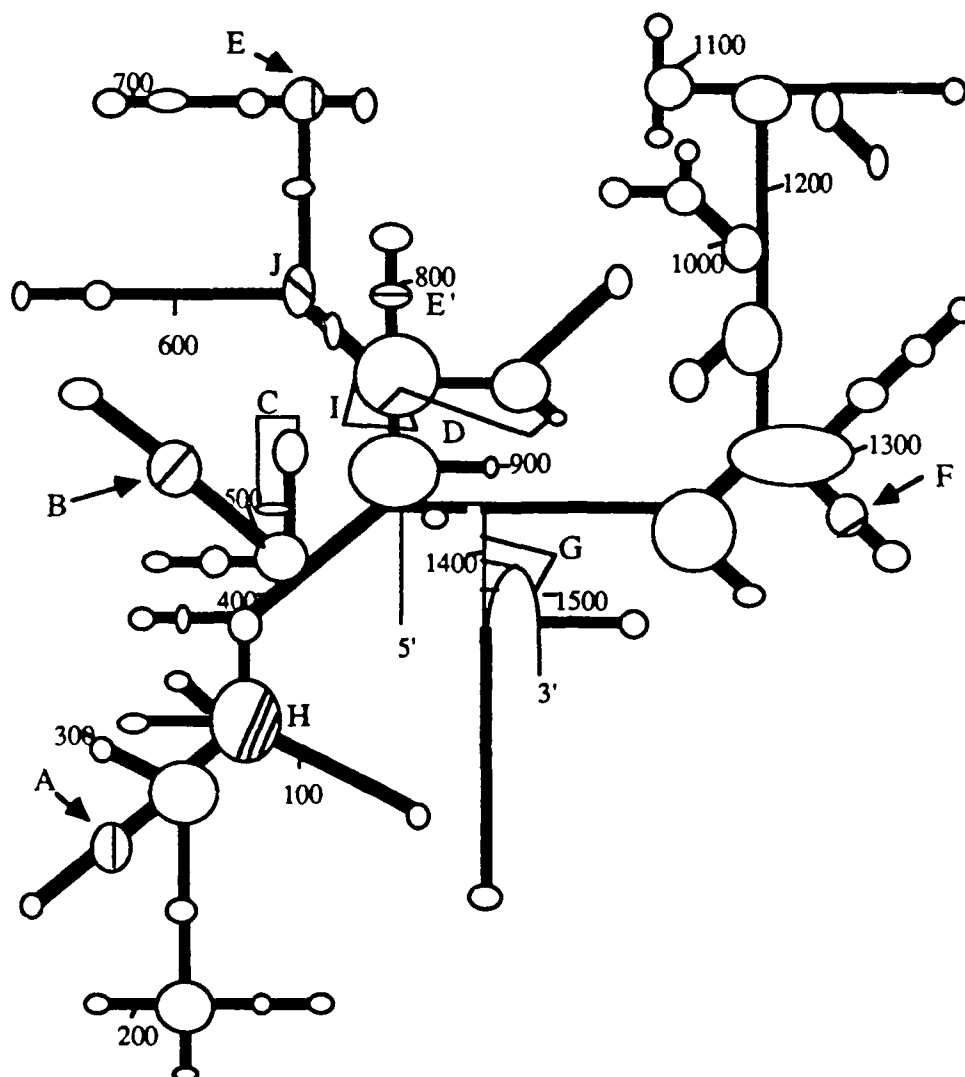


Figure 1:

Location of eleven known and five new tertiary interactions in 16S-like rRNA. The secondary structure of *E. coli* is shown in schematic format. The location of the tertiary interactions for which extensive phylogenetic evidence exists are indicated. The interactions are as follows: A) U245/U283, B) G450/C483, C) G505-6/C526-5, D) G570/C866, E) G722/G733, E') C779/G803, F) U1307/U1330, G) C1399/G1504, G1401/C1501 and G1405/C1496, H) G61/C106, U62/G105, C63/G104, I) G575/C880 J) G654/C754. *E. coli* numbering.

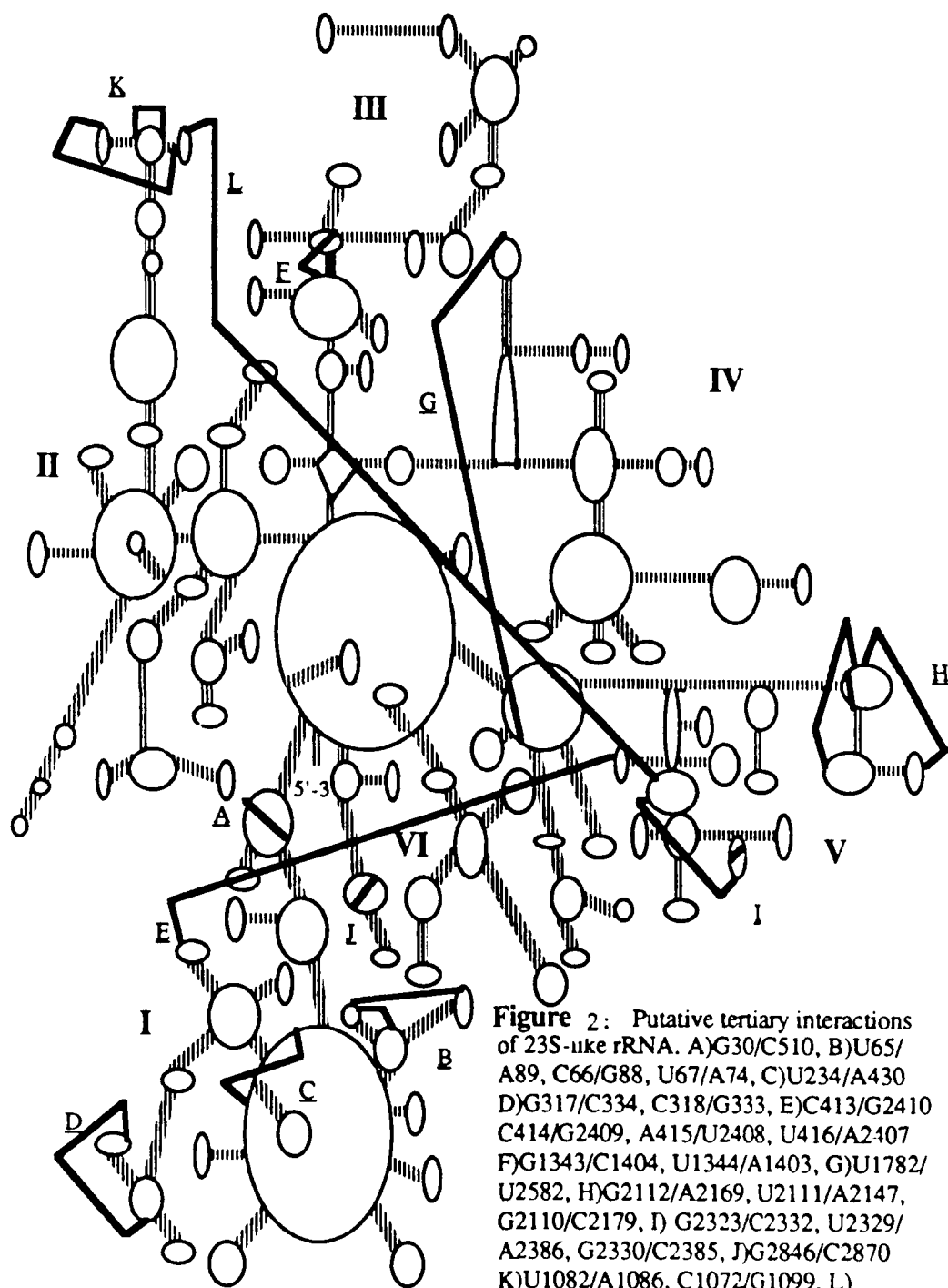


Figure 2: Putative tertiary interactions of 23S-like rRNA. A)G30/C510, B)U65/A89, C66/G88, U67/A74, C)U234/A430 D)G317/C334, C318/G333, E)C413/G2410 C414/G2409, A415/U2408, U416/A2407 F)G1343/C1404, U1344/A1403, G)U1782/U2582, H)G2112/A2169, U2111/A2147, G2110/C2179, I) G2323/C2332, U2329/A2386, G2330/C2385, J)G2846/C2870 K)U1082/A1086, C1072/G1099, L) G1093/C2394.