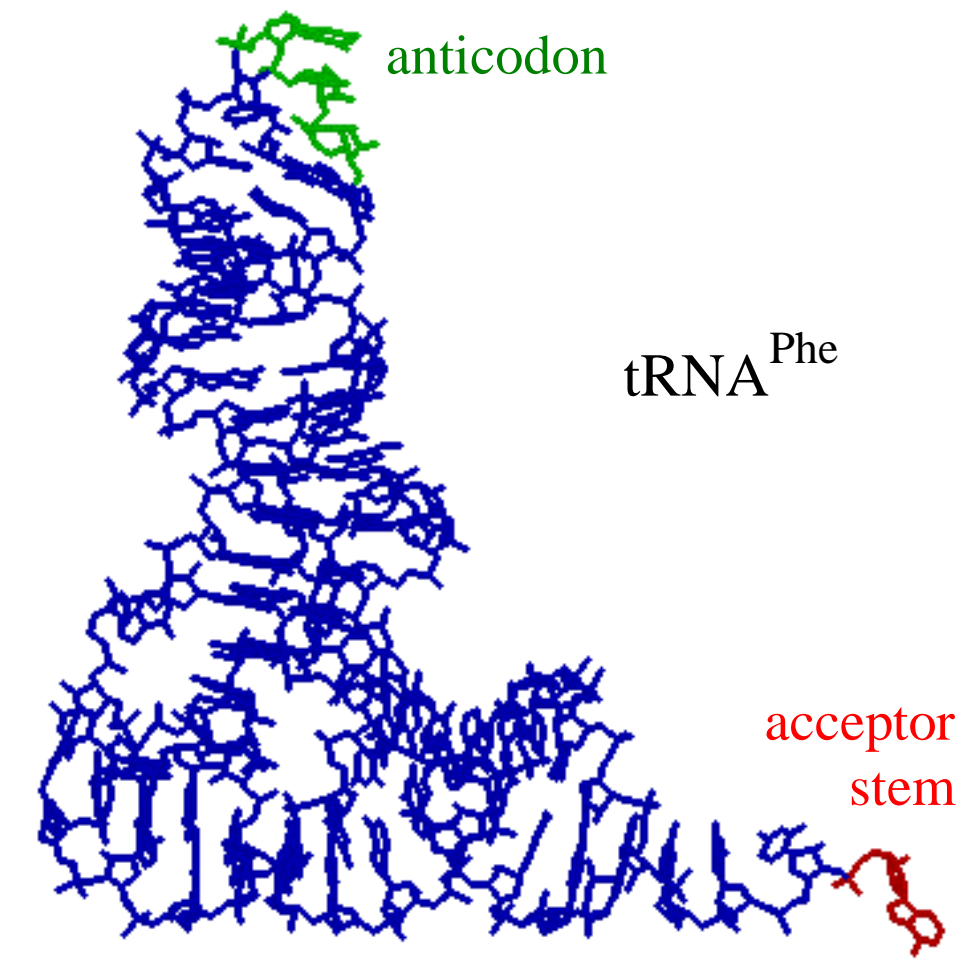
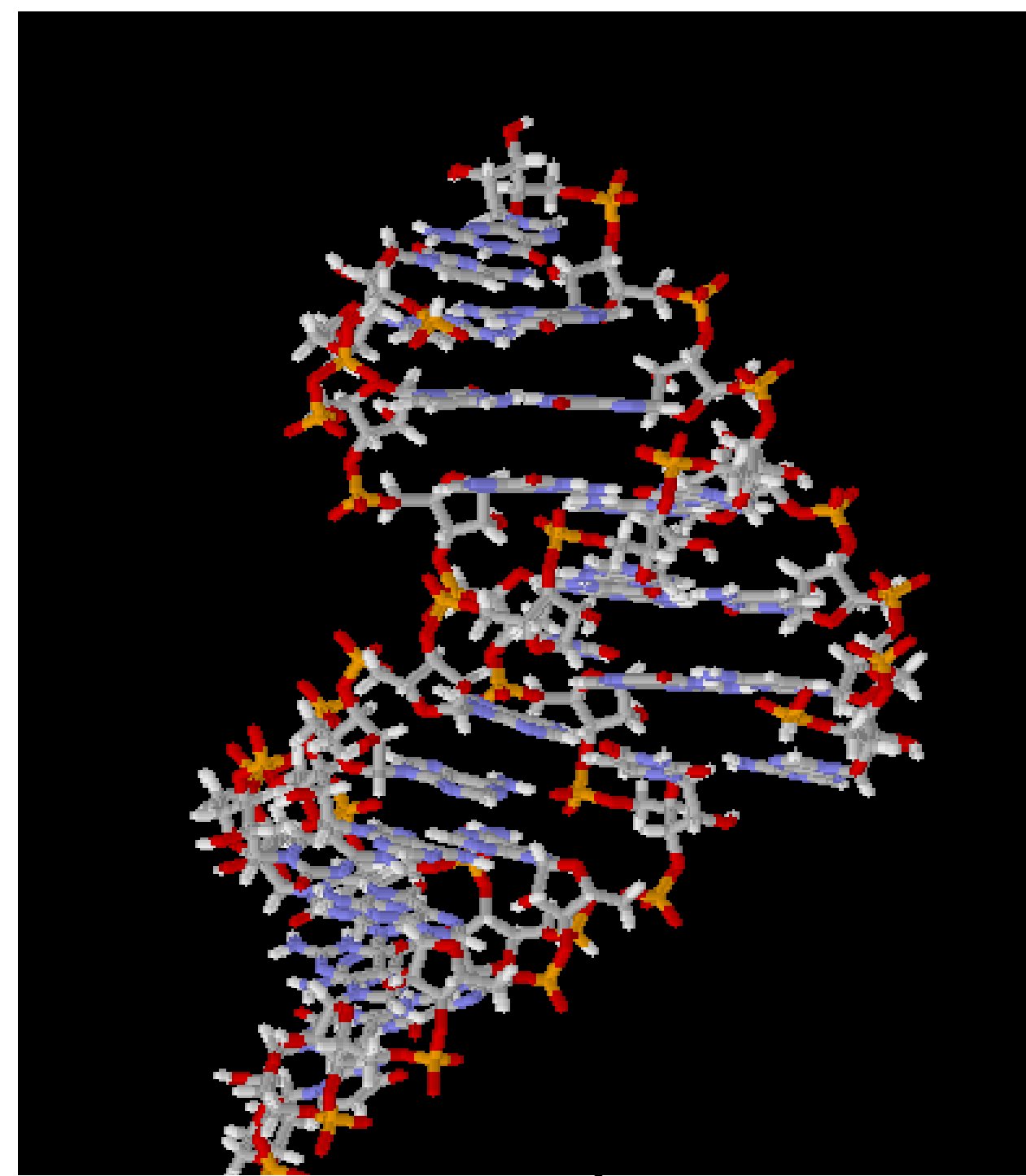
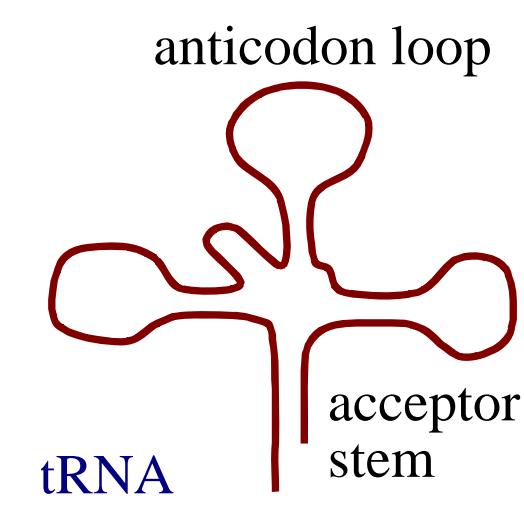


# FUNCTIONAL ANALYSIS OF A THERMOPHILIC BACTERIAL RIBOZYME

Susan B. Klein and Norman R. Pace

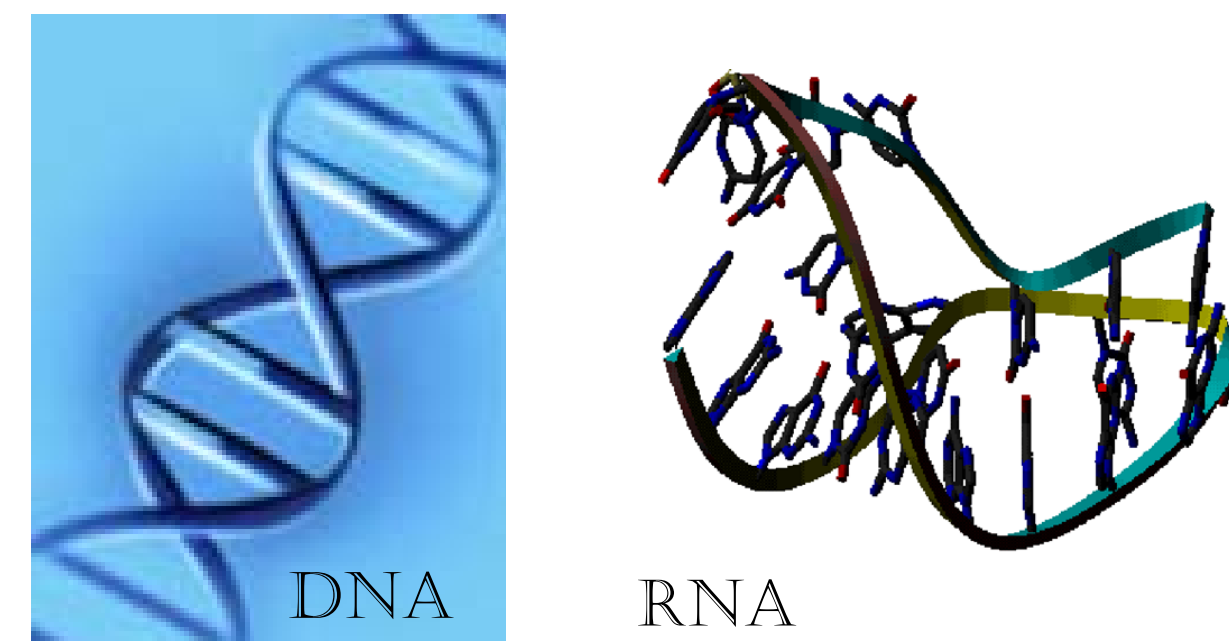
Indiana University Cyclotron Facility & University of Colorado Center for Astrobiology

## TRANSFER RIBONUCLEIC ACID (tRNA)

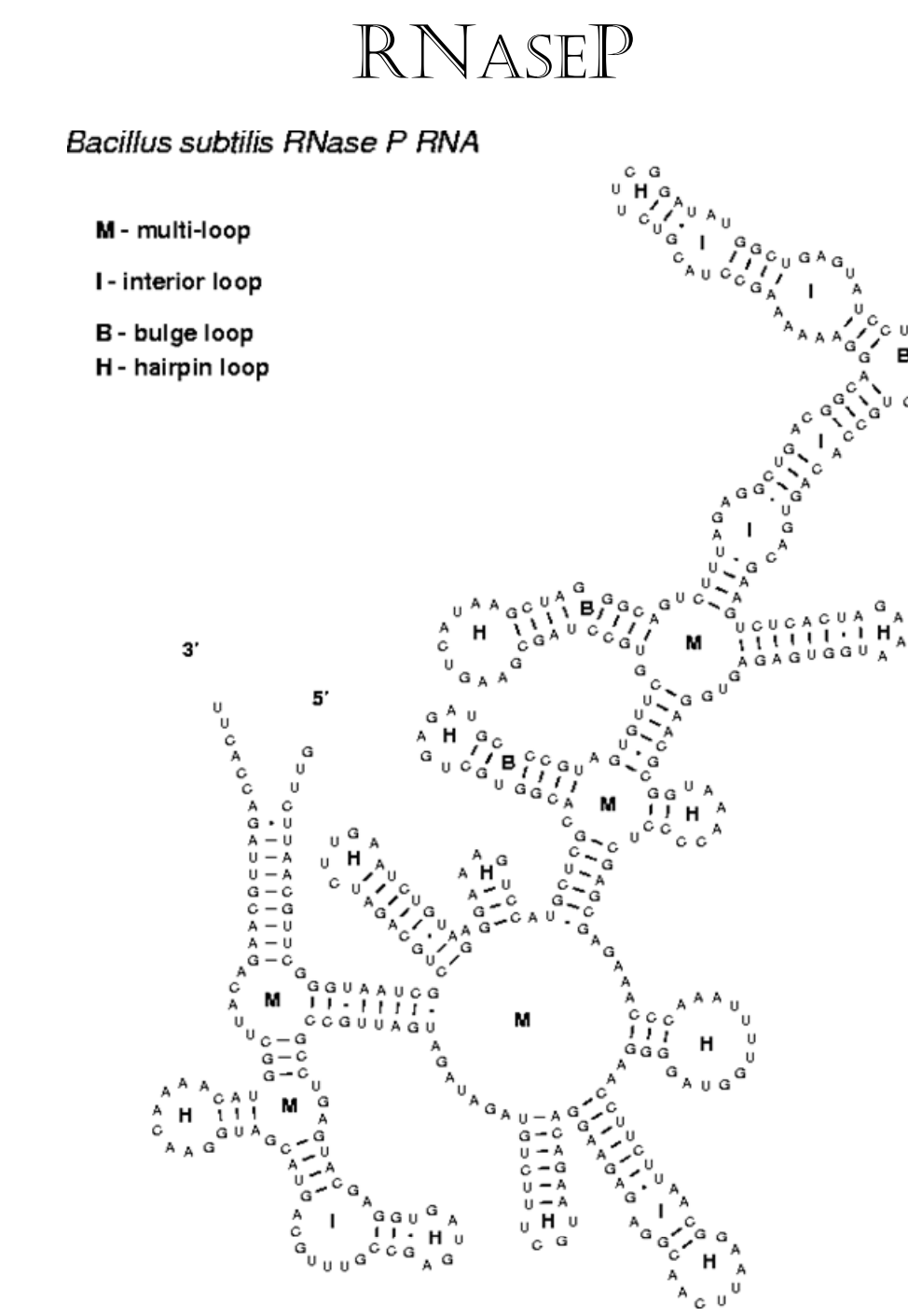


Each tRNA binds to a specific amino acid at the codon loop and a specific mRNA base at the acceptor stem.

## NUCLEIC ACID STRUCTURE



Unlike DNA that exists in paired strands, RNA is a single stranded macromolecule that is free to fold back in on itself to form local paired strands. This pairing produces secondary structure.



The secondary structure of RNaseP has been predicted but the tertiary structure remains undetermined.

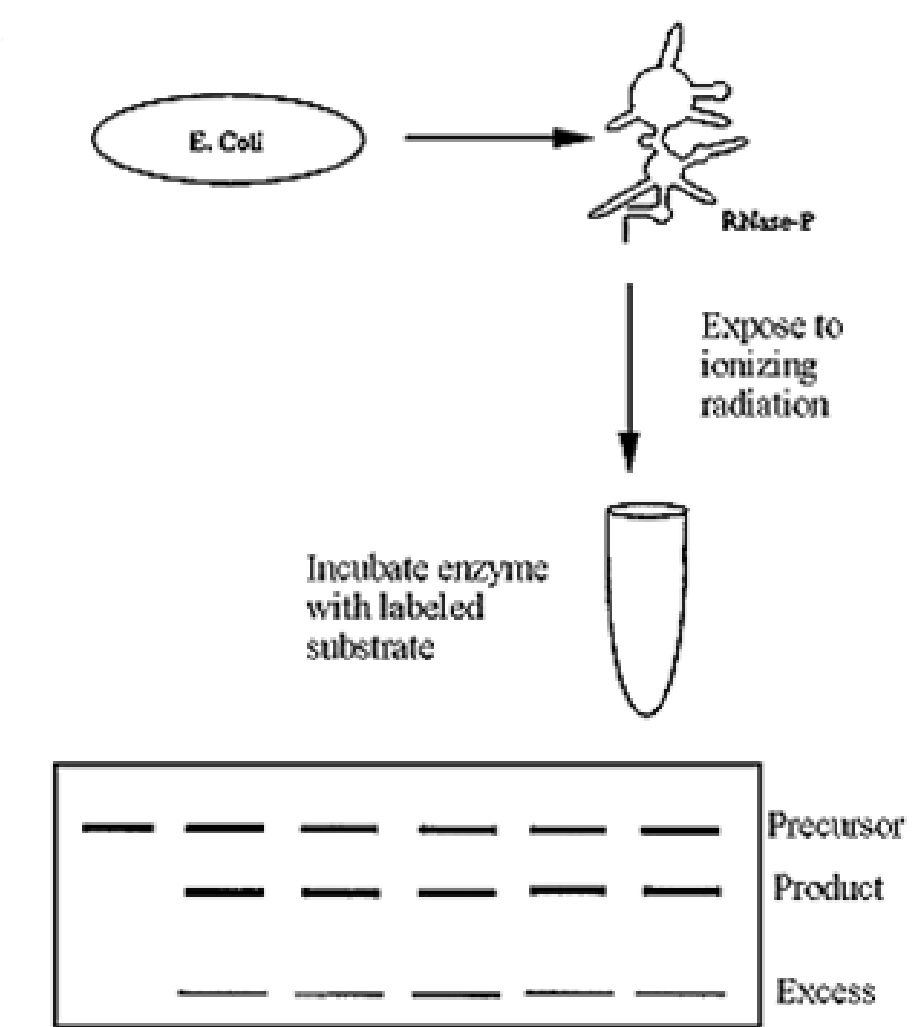
A KEY QUESTION FOR EVOLUTION: WHAT WAS THE FIRST MOLECULE THAT MADE EVOLUTION POSSIBLE?

FOOD FOR THOUGHT; HERE IS A SYSTEM - ESSENTIAL TO EVOLUTION - COMPOSED ENTIRELY OF SUGAR MOLECULES.

## BACILLUS SUBTILIS RNaseP

How sturdy is this molecule? Could it survive the hostile prehistoric Earth environment? What is the active binding site for this ribozyme?

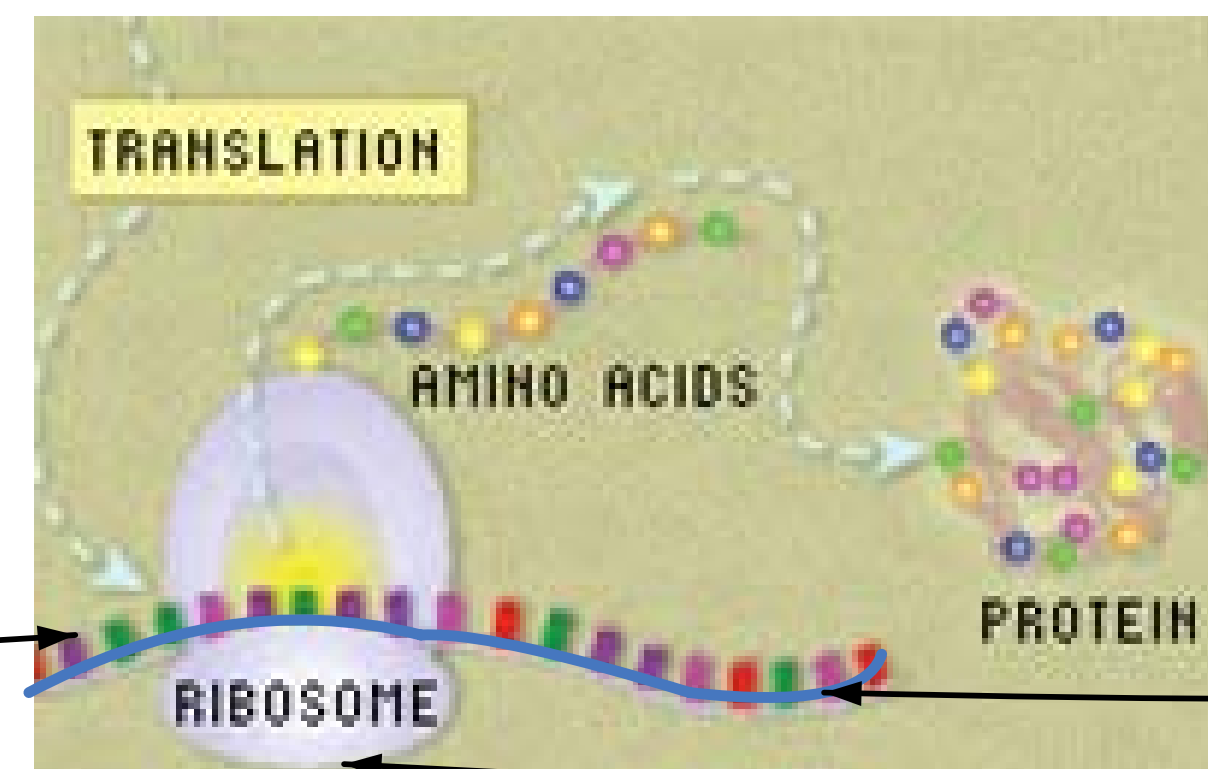
### EXPERIMENTAL PROTOCOL



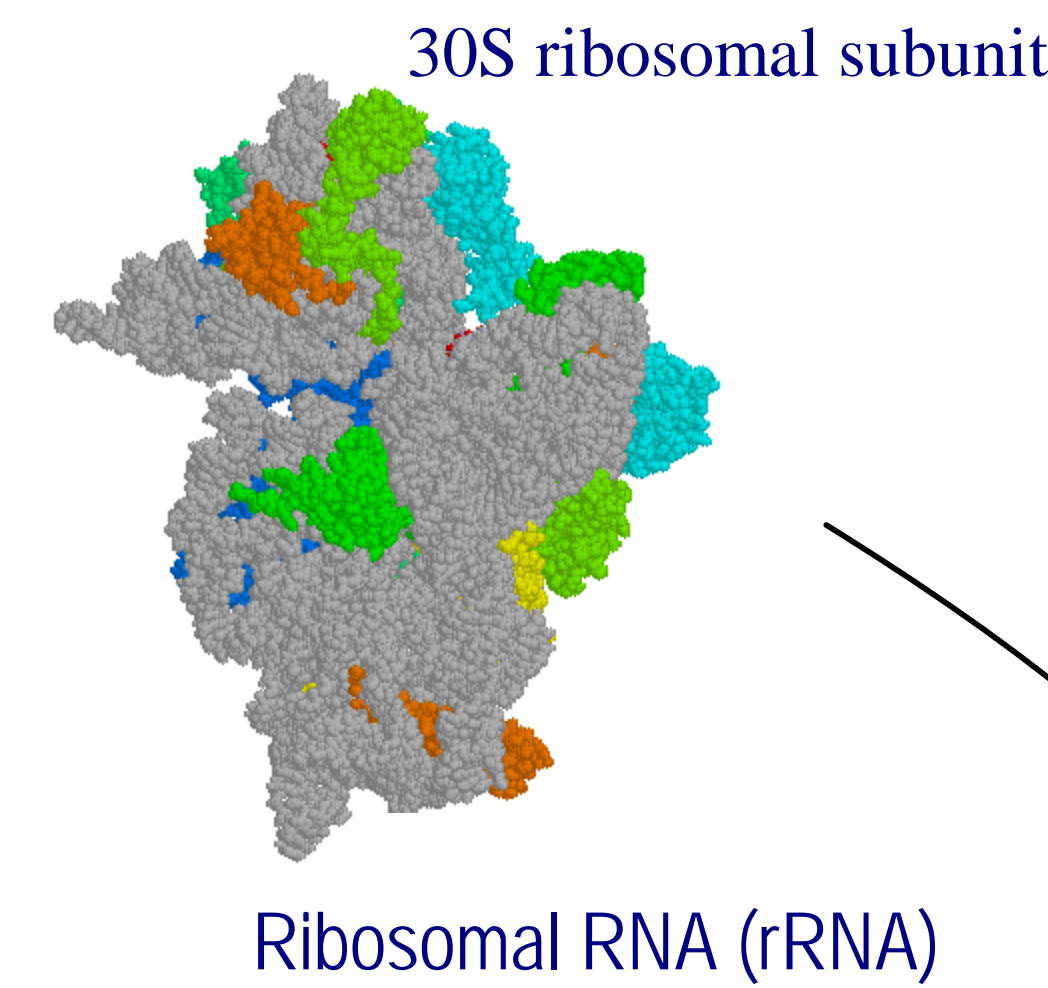
Milligram quantities of RNaseP were produced in *E. coli*. The product was isolated and exposed to proton radiation.

The irradiated ribozyme was incubated with precursor tRNA and the tRNA was run out on an acrylamide gel.

## THE TRANSLATION ENGINE



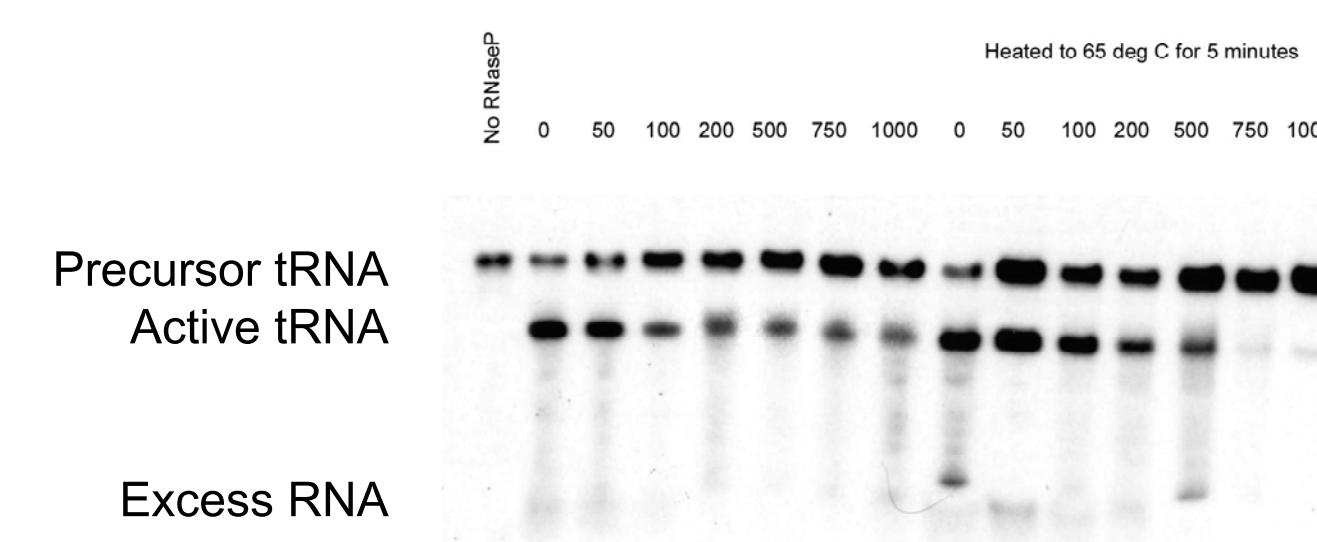
Messenger RNA (mRNA)



Ribosomal RNA (rRNA)

The primary base sequence (A, U, G, or C) of a RNA molecule determines the base pairing possibilities. As the bases form pairs, the molecule folds to form the secondary structure and twists to form the tertiary structure. In general, the primary sequence contains extra material to enforce, by the rules of thermodynamics, a particular tertiary form. This excess material is then removed in "post-processing" reactions.

**RNaseP IS THE RIBOZYME (ENZYME COMPOSED OF RNA) THAT POST-PROCESSES tRNA TO PRODUCE THE CORRECT ACCEPTOR STEM.**



The experiment was repeated, and this time half of the irradiated sample was heated before the tRNA substrate was added. Heating breaks the hydrogen bonding linking the strands of RNA and therefore disturbs the secondary and tertiary structure of the ribozyme. When the samples cool, the inter-strand bonds reform. If the primary structure is damaged, the tertiary structure will be affected.

**7.5 GY OF PROTON RADIATION CAUSES SIGNIFICANT DAMAGE TO THIS RNA ENZYME, BUT THE BUILT-IN RADIATION PROTECTION DERIVED FROM THE STRUCTURE ALLOWS THE MOLECULE TO REMAIN ACTIVE.**

**AMAZING! HIGH LEVELS OF PARTICLE RADIATION FAILED TO INACTIVATE THE RIBOZYME!**