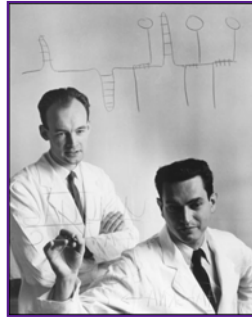


# The Dependence of Cell-Free Protein Synthesis in *E. coli* Upon Naturally Occurring or Synthetic Polyribonucleotides

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[Dr. Carr's Biol 4241 Course Webpage](#)

## History

### Introduction

- The start to cracking the genetic code

Two experiments:

- 1) - messenger RNA is required for protein synthesis
  - For this experiment, ribosomal RNA incorporates what we know as messenger RNA
- 2) - Synthetic polynucleotide -> the synthesis of a "protein"
  - Synthesis of a *specific* amino acid chain

- "Template RNA" = mRNA; "Soluble RNA" = tRNA

## Materials & Methods

### Results

Part 1: Stimulation by ribosomal RNA

#### Soluble RNA vs. Ribosomal RNA

- Maximal stimulation of C14-L-valine incorporation into protein obtained with very small amount of soluble RNA when not in presence of ribosomal RNA (~ 1mg soluble RNA)
- Greatly increasing concentration did NOT further stimulate system
- Incorporation markedly stimulated by addition of ribosomal RNA (more than a 3-fold)
- Linear relationship between ribosomal RNA & C14-valine incorporation when low concentrations of this RNA
  - Soluble RNA – catalytic relationship
  - Ribosomal RNA – stoichiometric relationship

#### C14-L-valine Incorporation

- Inhibited by Chloramphenicol, Puromycin, RNAase, and deproteinization

- Requires ATP, & ATP generating system
- Unaffected by boiling or addition of DNAase
- Further stimulated by addition of 20 L-amino acids (suggests **cell-free protein synthesis**)
- 4% radioactivity from C-terminal end, 1% from N-terminal end, remainder of C14-label was internal
- Presence of washed ribosomes required for effective stimulation of amino acid incorporation
- Both [ribosomes & supernatant](#) solution necessary for incorporation
- Incorporation of every amino acid tested **increased** with ribosomal RNA
- Effect NOT observed with [other polyanions](#)
  - RNA appears to be active principle
- Intact S-30, S-50, S-70 ribosomal subunits not observed
  - Schlieren optics show peaks of [23, 16 & 4](#) therefore subunits broken down
  - [Trypsin](#) - [No effect](#)
  - RNAase - [Loss of activity](#)

### Summary Part I: Template (Messenger) RNA is required for protein synthesis

#### Part 2: Stimulation of amino acid incorporation by synthetic polynucleotides

- Synthetic polynucleotides were added into the reaction mixture with phenylalanine
- Incorporation of C14-L-phenylalanine is almost completely dependent upon the addition of [polyuridylic acid](#), as seen in [Figure 6](#)
- Effects of polyuridylic acid on phenylalanine incorporation [could not be replaced](#) by any other polynucleotide
  - [Single strandedness](#) required
  - Other [requirements](#) include ribosomes, supernatant solution, ATP and an ATP generating system
  - Inhibited by puromycin, chloramphenicol and RNAase but NOT DNAase
  - [No significant incorporation](#) of another amino acid occurred in the presence of polyuridylic acid
    - Note: [Asparagine and glutamine](#) were excluded from the experiment
  - Product of the incorporation reactions were tested to ensure that it was poly-[L-phenylalanine](#)
    - Both showed the same [characteristics](#)

### Summary Part II: Polyuridylic acid is required and specific to the stimulation of phenylalanine incorporation



Nirenberg's notes, an elucidation of the first "word" of the genetic code.  
(Nirenberg still works at this.)

## Discussion

- Ribosomal RNA is different from soluble RNA since bases found in soluble RNA are not present in the other
- Ribosomes are not assembled exclusively from the rRNA
- At low [ribosomal RNA], rate of amino acid incorporation into protein was proportional to amount of rRNA added = stoichiometric
- Soluble RNA acts in catalytic fashion
- Discovered mRNA is required for cell-free protein synthesis
- mRNA is thought to serve as template for protein synthesis
- **Poly-U contains the information for the synthesis of a poly-L-phenylalanine "protein"**
- One or more uridylic acid residues appear to be the code for phenylalanine, functioning as a synthetic template
- Not sure if the code is a singlet, triplet, etc.
  - Direct evidence for the number of uridylic acid residues forming to code for phenalanine not yet established
- Footnote:
  - Polycytidylic acid specifically mediates the incorporation of L-proline

## Cracking the Code!