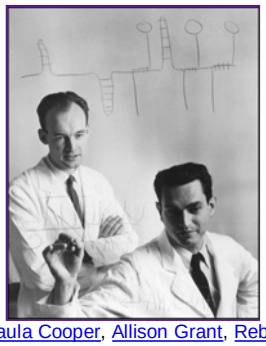
The Dependence of Cell-Free Protein Synthesis in *E. coli* Upon Naturally Occuring or Synthetic Polyribonucleotides

By Marshall W. Nirenberg and Johann Heinrich Matthaei



Ashley Collier, Paula Cooper, Allison Grant, Rebecca Mawhinney

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
  - Schlerin 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

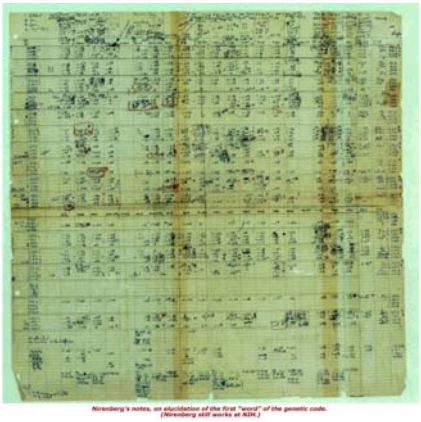
- Other requirements include ribosomes, supernatent solution, ATP and an ATP generating system

- Inhibited by puromycin, chloraphenicol and RNAase but NOT DNAase

- No significant incorporation of another amino acid occured 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-phenylalaine
  - Both showed the same characteristics

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



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!