Lezione 10

Il codice genetico -2
Due interrogativi

1. In che modo l’informazione passa dal nucleo al citoplasma? Infatti, il DNA della cellula eucariotica è quasi interamente confinato nel nucleo, mentre le proteine sono sintetizzate nel citoplasma.

2. In che rapporto stanno una determinata sequenza nucleotidica del DNA e una determinata sequenza amminoacidica di una proteina?
Un po’ di storia

- Jean Brachet in Belgium and Torbjörn Caspersson in Sweden, who in the 1940s had reported that RNA was found primarily in the cytoplasm, where protein synthesis took place, and that RNA levels increased in cells that were actively synthesising proteins.

- This leads Crick to think that protein synthesis did not directly involve chromosomal DNA, but instead took place in the cytoplasm and required RNA, although it was not at all clear how that process occurred, or what the form or the function of RNA was.
Andre’ Boivin, 1947, “Sur le role possible des deux acides nucléiques dans la cellule vivante”:

“the macromolecular desoxyribonucleic acids govern the building of macro-molecular ribonucleic acids, and, in turn, these control the production of cytoplasmic enzymes”
Francis Crick – The adaptor hypothesis

I shall not discuss this further here. In the second, each amino acid would combine chemically, at a special enzyme, with a small molecule which, having a specific hydrogen-bonding surface, would combine specifically with the nucleic acid template. This combination would also supply the energy necessary for polymerisation. In its simplest form there would be 20 different kinds of adaptor molecule, one for each amino acid, and 20 different enzymes to join the amino acid to their adaptors. Sydney Brenner, with whom I have discussed this idea, calls this the "adaptor hypothesis", since each amino acid is fitted with an adaptor to go on to the template.
Ribosomes

• 1955: George E. Palade discovered ribosomes and described them as small particles in the cytoplasm that preferentially associated with the endoplasmic reticulum membrane and were later named ribosomes. Along with other scientists, Palade discovered that ribosomes performed protein synthesis in cell. Crick assumed that each ribosome consisted of a common protein structure together with a unique sequence of RNA, which acted as a template for the synthesis of a particular protein ("one gene one ribosome one protein").

There had to be at least two kinds of RNA in the cytoplasm — what he called ‘template RNA’ located inside the ribosome, and ‘metabolic’ or ‘soluble RNA’, which he suspected was synthesised by each type of ribosome, and corresponded to the code on the template RNA.

Neither of these kinds of RNA corresponded in form, function or location to what we now call mRNA.
The assumption that ribosomal RNA (rRNA) was the messenger conflicted with other findings:

- In 1958, Volkin and Astrachan found that while radioactive RNA appeared rapidly in bacteria after phage infection, if the isotope was added later, then more radioactivity was found in DNA than in RNA [32]. Their interpretation of these results again focused on how RNA might act as a precursor to the synthesis of DNA.
The assumption that ribosomal RNA (rRNA) was the messenger conflicted with other findings:

- the main sections of rRNA occurred in only two lengths, whereas the polypeptide chains for which this RNA supposedly coded differed greatly in length
- Arthur Pardee, François Jacob, and Jacques Monod in their famous "PaJaMo-experiment" had produced evidence that protein synthesis commenced soon after the introduction of a gene into a cell and that it proceeded at a fast, steady rate. By contrast, the theory that ribosomal RNA was the messenger predicted that protein synthesis would start up gradually, as the newly-introduced gene first had to produce the ribosomes at which protein synthesis was to occur.
A famous meeting between the Pasteur and the Cambridge group

As the group chatted, Jacob explained the latest results from Paris, focusing on the puzzle of how the $z^+$ gene that enabled the cell to produce $\beta$-galactosidase was able to synthesise such high levels of the enzyme so soon after it was introduced into a cell. One of the possibilities that the Paris group had considered was that the gene coded for a very efficient type of ribosome, which then churned out the enzyme at a high rate. But, as Jacob explained, Pardee had recently done an experiment showing that the gene did not produce a stable ribosome, but only the transitory messenger molecule ‘$X$’. “At this point,” recalled Crick, “Brenner let out a loud yelp — he had seen the answer”
1961 Two teams rushing to find mRNA

François Gros, Howard Hiatt, Charles Kurland
Wally Gilbert, Watson and Risebrough vs Crick, Brenner and Meselson
1961 Jacob and Monod propose the 5 criteria for the messenger

• it was a polynucleotide
• its molecular weight should vary from case to case
• its base composition should reflect that of the DNA that produced it
• it should at least temporarily be associated with ribosomes
• it should have a very high rate of turnover.
1961 Nirenberg and Matthaei spent the next few years on the first part of his task: creating experiments to show that RNA could trigger protein synthesis.

Nirenberg and Matthaei set up a cell free system containing among others:

- Ribosomes
- Low MW RNAs (tRNAs)
- ATP energy system

They demonstrate that when tobacco mosaic virus RNA was added to their system, proteins were churned out at an amazing rate
Cellule di *E. coli* in rapida crescita vengono raccolte, raffreddate a 0 °C e lisate per ottenere un lisato cellulare grezzo. Questo viene trattato con l’enzima DNasi per degradare il DNA cellulare.

Il lisato viene centrifugato per rimuovere i frammenti più pesanti di pareti e membrane cellulari. Il supernatante (estratto cellulare) contiene polisomi, ribosomi liberi, tRNA, mRNA, enzimi ecc. A esso vengono aggiunti amminoacidi radioattivi, ATP e GTP, e poi viene suddiviso in provette.

Le provette vengono poi incubate a 37 °C per tempi crescenti. Alla fine dell’incubazione la soluzione viene acidificata per precipitare le proteine; gli amminoacidi liberi rimangono in soluzione.

I precipitati sono raccolti e lavati e la radioattività misurata mediante un contatore di radiazioni. La quantità di radioattività nel precipitato dà la misura della quantità di amminoacidi incorporati (cioè della sintesi proteica).
THE DEPENDENCE OF CELL-FREE PROTEIN SYNTHESIS IN E. COLI
UPON NATURALLY OCCURRING OR SYNTHETIC
POLYRIBONUCLEOTIDES

BY MARSHALL W. NIRENBERG AND J. HEINRICH MATTHAEI*

NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

Communicated by Joseph E. Smadel, August 3, 1961

A stable cell-free system has been obtained from *E. coli* which incorporates
C\textsuperscript{14}-valine into protein at a rapid rate. It was shown that this apparent protein
synthesis was energy-dependent, was stimulated by a mixture of L-amino acids,
and was markedly inhibited by RNAase, puromycin, and chloramphenicol.\textsuperscript{1} The
present communication describes a novel characteristic of the system, that is, a
requirement for template RNA, needed for amino acid incorporation even in the
presence of soluble RNA and ribosomes. It will also be shown that the amino
acid incorporation stimulated by the addition of template RNA has many proper-
ties expected of *de novo* protein synthesis. Naturally occurring RNA as well as a
synthetic polynucleotide were active in this system. The synthetic polynucleo-
tide appears to contain the code for the synthesis of a "protein" containing only one
amino acid. Part of these data have been presented in preliminary reports.\textsuperscript{2, 3}
Figure 1 shows that incorporation of C14-L-valine into protein by Incubated-S-30 fraction was stimulated by the addition of purified E. coli soluble RNA.

![Graph showing stimulation of amino acid incorporation into protein by E. coli soluble RNA.](image1)

**Fig 1.—**Stimulation of amino acid incorporation into protein by *E. coli* soluble RNA. Composition of reaction mixtures is specified in Table 1. Samples were incubated at 35° for 20 min. Reaction mixtures contained 4.4 mg of Incubated-S-30 protein.

Figure 2 demonstrates that E.coli ribosomalRNA preparations markedly stimulated incorporation of C"-valine into protein even though maximally stimulating concentrations of soluble RNA were present in the reaction mixtures.

![Graph demonstrating stimulation of amino acid incorporation into protein by E. coli ribosomal RNA.](image2)

**Fig. 2.—**Stimulation of amino acid incorporation into protein by *E. coli* ribosomal RNA in the presence of soluble RNA. Composition of reaction mixtures is specified in Table 1. Samples were incubated at 35° for 20 min. Reaction mixtures contained 4.4 mg of Incubated-S-30 protein and 1.0 mg *E. coli* soluble RNA.
Phenylalanine incorporation was almost completely dependent upon the addition of polyuridylic acid. The data of Table 6 demonstrate that no other polynucleotide tested could re-place polyuridylic acid.

**TABLE 6**

**POLYNUCLEOTIDE SPECIFICITY FOR PHENYLALANINE INCORPORATION**

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Additions</th>
<th>Counts/min/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>+ 10 μg Polyuridylic acid</td>
<td>39,800</td>
</tr>
<tr>
<td></td>
<td>+ 10 μg Polyadenylic acid</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>+ 10 μg Polycytidylic acid</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>+ 10 μg Polynosinic acid</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>+ 10 μg Polyadenylic-uridylic acid (2/1 ratio)</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>+ 10 μg Polyuridylic acid + 20 μg polyadenylic acid</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Deproteinized at zero time</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>+ 10 μg UMP</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>+ 10 μg UDP</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>- 10 μg UTP</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>Deproteinized at zero time</td>
<td>6</td>
</tr>
</tbody>
</table>

Components of the reaction mixtures are presented in Table 1. Reaction mixtures contained 2.3 mg Incubated-S-30 protein. 0.02 μmoles U-C14-L-phenylalanine (~125,000 counts/minute) was added to each reaction mixture. Samples were incubated at 35° for 60 min.
by puromycin or chloramphenicol. One or more uridylic acid residues therefore appear to be the code for phenylalanine. Whether the code is of the singlet, triplet, etc., type has not yet been determined. Polyuridylic acid seemingly functions as a synthetic template or messenger RNA, and this stable, cell-free *E. coli* system may well synthesize any protein corresponding to meaningful information contained in added RNA.
By 1966 the chart had been completed.