The RNA Code and Protein Synthesis

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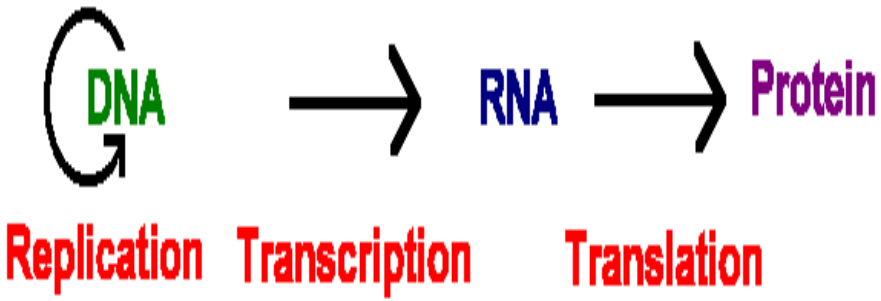
February 10, 2014

The Central Dogma

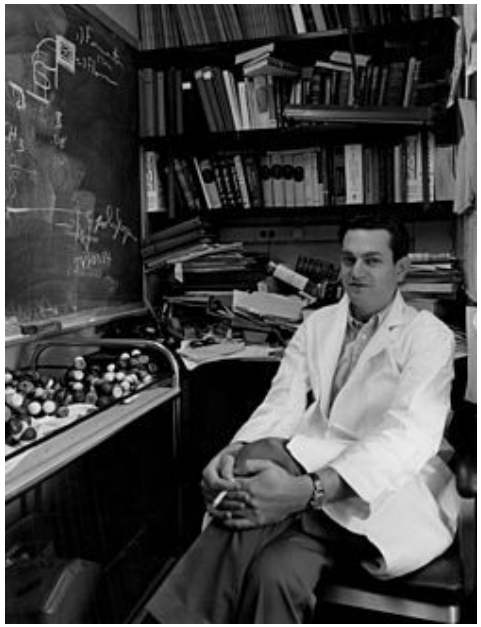


- Step 1: **Replication**: Duplication of a double-stranded DNA molecule
- Step 2 : **Transcription**: Synthesis of messenger RNA (mRNA)
- Step 3 : **Translation**: Synthesis of proteins

Our focus today will be on Step 3 and the making of Genetic Code Table.

Some facts about Nirenberg

- A Biochemist and Geneticist
- Completed Ph.d degree in Biological Chemistry from the University of Michigan in 1957
- Affiliated with the National Institute of Health, Bethesda, Maryland USA.
- Got the Nobel Prize with two others in 1968



Experimental approaches to protein synthesis

Two possible approaches to resolve the problem of the role of "genes" in protein synthesis back then.

- To reason on the basis of the structure of DNA molecule (discovered in 1953 by Watson and Crick) when looking at the action of genes in the cell
- To create in vitro systems that could synthesize proteins biochemically

What we knew about the properties of Genetic Code by 1962

- Most if not all codons consist of three (adjacent) bases.
- Adjacent codons do not overlap.
- The message is read in the correct groups of three by starting at some fixed point.
- The code sequence in the gene is co-linear with the amino acid sequence, the polypeptide chain being synthesized sequentially from the amino end.
- In general more than one triplet codes each amino acid.
- It is not certain that some triplet codes may not code more than one amino acid, i.e. they may be ambiguous.

- Triplets which code for the same amino acid are probably rather similar.
- It is not known whether there is any general rule which groups such codons together, or whether the grouping is mainly the result of historical accident.
- The number of triplets which do not code an amino acid is probably small.
- Certain codes proposed earlier, such as comma-less codes, two- or three-letter codes, the combination code, and various transposable codes are all unlikely to be correct.
- The code in different organisms is probably similar. It may be the same in all organisms but this is not yet know

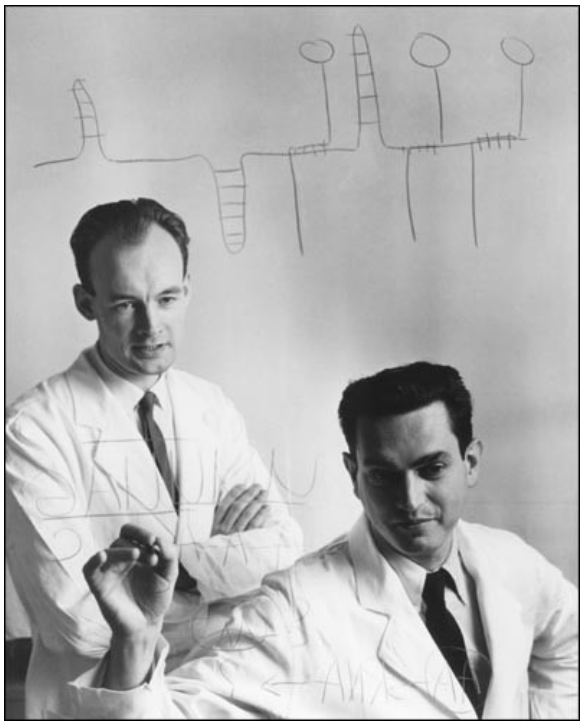
The properties of Genetic code confirmed by Nirenberg et al by 1966 can be summarized as follows.

- Codes are specific.
- Codes are degenerate.
- Codes are nonoverlapping.
- Codes are almost universal.

Outline of the RNA code and Protein synthesis

1. The fine structure of the RNA code
2. Factors affecting the formation of codon-ribosome-AA-sRNA complexes
3. Patterns of synonym codons for amino acids and purified sRNA fractions
4. Mechanism of codon recognition
5. Universality
6. Unusual aspects of codon recognition as potential indicators of special codon function

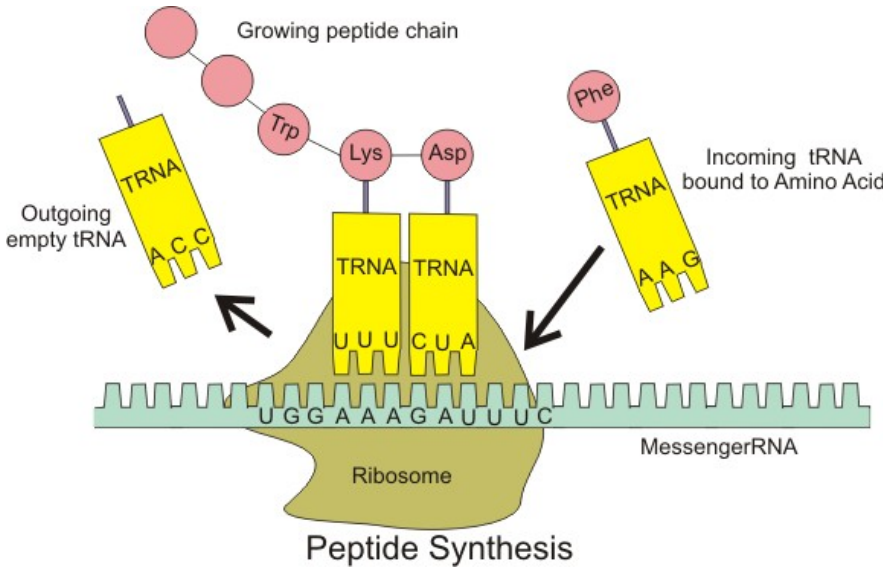
Experimental procedure



- Nirenberg said and I quote " would not have to get synthesis very far to break the coding problem. Could crack life's code"
- Nirenberg and Mathaei set out in 1960 to show that RNA was responsible for protein synthesis

How did the create the system

- Protein synthesis was driven using a base solution of E.coli Ribosomes,trisacetate buffer, magnesium acetate, and potassium chloride
- The E. Coli cells were lysed using a mortar and pestle and then rinsed with DNase to remove any remaining E. coli DNA .
- The cell-free extract provided all the necessary components needed to synthesize protein from RNA.



- After incubation the samples were diluted with standard buffer and then poured over a cellulose nitrate filter using light suction filtration.
- The filter was washed twice with standard buffer to remove any unbound C14-AA-sRNA complex. Only the ribosomes and bound sRNA remained on the filter.
- The filters were then dried and placed into small vials which contained scintillation fluid.
- Afterwards the vials were counted in a scintillation spectrometer.

In the 1966 paper it was stated that the assays for base sequences of RNA codons depends on two variables:

- The ability of trinucleotides to serve as templates for AA-sRNA binding to ribosomes prior to peptide bond formation.
- That codon-ribosome-AA-sRNA complexes are retained by the cellulose nitrate filters.

On Saturday, May 27th, 1961, Matthaei combined Poly-U with C14-Phe-tRNA and found that it produced a chain of the repeating amino acid Phenylalanine.
UUUUUUUUUU = Phe-Phe-Phe
This was the breakthrough that they needed.
Nerinberg and Matthaei went on to apply the same method for Poly-A and Poly-C and found that:

- Poly-A = Lysine
- Poly-C = Proline
- No messenger activity was observed for Poly-G.
- What could be the reason for this?
- It was believed at the time that the very ordered structure of Poly-G was masking the template activity.

Nerinberg and Matthaei were now ready to tackle the task of cracking the code.

- To do this they would need to create more complex nucleotide polymers.
- They started by using simple polynucleotides composed of 2 different bases.
- Each poly-dinucleotide sequence contained 3 triplets, but no polymer had been found to code for anymore than 6 different amino acids.

Experimental procedure

- To the base solution, they added synthetic RNA that contained the nucleotide sequences they wished to study
- Poly U RNA, a polymer of Uracil = UUUUUUUUUUUUUUUU
- Poly A, a polymer of Adenine = AAAAAAAAAAAAAAA etc
- Nirenberg wanted to know which amino acid would be incorporated into a protein following the addition of a particular type of synthetic RNA.

- Each time the experiment was run, 20 test tubes would be filled with a different amino acid.
- Out of the 20 test tubes, 19 would be cold, and only 1 would be hot.
- The hot test tube would contain an amino acid transfer RNA that had been radioactively tagged using C^{14}-AA-sRNA

They would add an amino acid transfer RNA that had been radioactively tagged using C^{14}-AA-sRNA

- May 27th, 1961, Mathaei combined poly-U and C^{14}-phe-tRNA
- This produced a chain of the repeating amino acid: Phenylalanine
- This was the breakthrough they needed.
- UUUUUUUUUUU= Phe-Phe-Phe
- Nirenberg and Mathaei now understood the process for producing AA from RNA
- Replications of the experiment were carried out to get the other amino acids

- The analysis for base sequences of RNA codons depends on two factors
- The ability of trinucleotides to serve as templates for AA-sRNA binding to ribosome prior to peptide bond formation
- the observation that codon ribosome AA-sRNA complexes are retained by cellulose nitrate filters
- Amino acids are activated by ATP to form high energy AA-AMP complexes

- Activated amino acids are transferred to low molecular weight RNA molecules in an activated form
- Amino acyl -sRNA compounds function as the direct intermediate for peptide bond formation
- AA + ATP $\rightarrow$ AMP-AA + PP
- AMP-AA + sRNA $\rightarrow$ AA -sRNA + AMP

UNUSUAL ASPECTS OF CODON RECOGNITION AS POTENTIAL INDICATORS OF SPECIAL CODON FUNCTIONS

- Results of many studies shows that the RNA code is universal.
- However, translation of the RNA code can be altered in vivo by extragenic suppressors and in vitro by altering the components of the reactions of conditions of incubation
- Most codons correspond to amino acids but some serve in other capacities such as initiation, termination and elongation

- There is a probability that additional codons eventuallly may be found to serve special functions
- Unusual properties of codon recognition sometimes may indicate special codon functions
- An example is in the properties of initiator and terminator codons during codon recognition are quite distinctive

We can conclude from the following facts above that:

- A codon may have alternative meanings
  Example
  UUG at or near the 5'- terminus of mRnNA may correspond to n-formyl methionine whereas an internal UUG codon may correspond to leucine
- A codon may have multiple functions simultaneously
  Example
  A codon may specify both initiation and an amino acid , perhaps via AA-sRNA with high affinity for peptidyl -sRNA sites on ribosomes
- Codon function sometimes is subject to modification
- Degenerate codons for the same amino acid often differ markedly in template pr0perties

Codon frequency and distribution

- Multiple species of sRNA corresponding to the same amino acid recognize different synonym codons
- Degenerate codon usage in mRNA sometimes is nonrandom
- The possibility that different sets of sRNA may be required for the synthesis of two proteins with the same amino acid composition suggests that protein synthesis sometimes may be regukated by codon frequency and distribution coupled with differential recognition of degenerate codons
- The rates of synthesis of certain proteins may be regulated simultaneously by alterations which affect the apparatus recognizing one degeneracy but not another

Codon position

- The template properties of 5'-terminal and 3'-terminal and internal codons may differ
- Reading of mRNA probably initiated at or near the 5' terminus and proceeds toward the 3'-terminus of the RNA chain
- Not known whether mechanisms of 5' terminal and internal initiation in polycistronic messages are similar
- Internal and 3'-terminal mechanisms of termination need to be defined
- N-formyl-Met-sRNA may serve as an initiator of protein synthesis in E.coli (Clark and Marcker, 1966)

- Met -sRNA can be converted enzymatically to N-formyl-Met-sRNA and responds to UUG,CUG,AUG, and to some extent GUG
- Met-sRNA does not accept formyl moieties and responds primarily to AUG(Clark and Marker)
- In E.coli extracts, protein synthesis is initiated in at least two ways
  1. By initiator codons specifying N-formyl Met-sRNA
  2. At higher mg^{++} concentrations by another means probably not dependent upon N-formyl-Met -sRNA
  3. This is because of the fact that many synthetic polynucleotides without known initiator codons direct cell-free protein synthesis (Nakamoto, 1966)

Example

- Poly U directs di as well as polyphenylalanine synthesis(Arlinhaus et al) though it does not have any known initiator.
- Probably, codons for N-formyl-Met-sRNA initiate protein synthesis with greater accuracy than codons which serve as initiators only at relatively high mg^{++} concentrations
- UAA and UAG may function as terminator codons(Brenner et al, 1965), and they do not stimulate binding appreciably of unfractionnated E.coli AA-sRNA to ribosomes.
- However, sRNA fraction(s) corresponding to UAA and/ or UAG are not ruled out.

Template Activity

- There are trinucleotides with activity for AA-sRNA like UAA, UAG and UUA
- Others are active templates with AA-sRNA from one organism and not the other.
- A + sign means that the trinucleotide stimulates AA-sRNA at the magnesium concentration
- A − sign means it is relatively inactive as a template
- A () sign means not tested

TABLE 9. TEMPLATE ACTIVITY OF TRINUCLEOTIDES IN 0.01 OR 0.03 M Mg^{++}

	U	C	A	G	
U	PHE	SER	TYR	CYS	U
	PHE	SER	TYR	CYS	C
		(SER)			A
	F-MET	SER		(TRP)	G
C		PRO	HIS	ARG	U
		PRO	HIS	ARG	C
		(PRO)	GLN	ARG	A
	LEU	(PRO)	GLN	ARG	G
A	ILE	THR	ASN	SER, CYS	U
	ILE	THR	ASN	SER, CYS	C
		THR	LYS		A
	MET	THR	LYS		G
G	VAL	ALA	ASP	GLY	U
	VAL	ALA	ASP	GLY	C
	VAL	ALA	GLU	(GLY)	A
	VAL	ALA	GLU	(GLY)	G

Legend:

	0.01 M Mg	0.03 M Mg
□ =	+	+
No Box =	−	+
( ) =	not tested	

Codon specificity

- Often synonymn trinucleotides differ strikingly in template specificity
- These indicate that template specificities of terminal, and internal codons differ or that special function codons or suppresors are present
- Relative template activities of synonyms trinucleotides in reactions containing 0.01 or 0.03 m Mg^{++}
- Note that in some cases, only one or two trinucleotides in a synonym set are active templates at 0.01 whereas all degeneracies are active at 0.03m Mg^{++}
- This kind of data suggest that codon ribosome AAsRNA complexes formed with degenerate trinucleotides often differ in stability.

Thank you all :)