

## Characteristics of Genetic Code

1. Composed of 4 nucleotides on mRNA (A,U,G,C)
2. Read in triplets (3 nucleotides / codon)
3. 64 possible codons  
Start: AUG (Also Met)  
Stop: UAG (Amber); UGA (Opal); UAA (Ochre)  
Amino Acids: 60 Codons + AUG
4. Code is Degenerate: >1 codon / amino acid
5. Code is non-overlapping  
Each nucleotide part of only 1 codon
6. AUG (Start) Defines Reading Frame

5' ----- AUG – (XXX)<sup>n</sup> – STOP ----- 3'

Open Reading Frame (ORF) between Start/Stop  
ORF lacks stop codons in frame; codes for polypeptide

## Experimental Evidence for Triplet Code

Frameshift mutations at rII locus of phage  
Proflavin (mutagen): Caused small deletions, insertions

+1 bp Altered reading frame and protein function

+2 bp Same as above

+3 bp Restore reading frame and protein function

5' ----- CAG CAG CAG ----- 3'    Normal mRNA

5' ----- CAG UCA GCA ----- 3'    1 bp insertion

5' ----- CAG UUC AGC ----- 3'    2 bp insertion

5' ----- CAG UUU CAG ----- 3'    3 bp insertion

## Cracking the Genetic Code:

### 1. Synthetic mRNA / in vitro translation:

Prepare cell extract with following components:  
Ribosomes, tRNAs, Enzymes, Cofactors, etc.  
Radioactive amino acids; "Random" synthetic mRNAs  
No template used for assembly  
NTPs ---> mRNAs; Control [NTPs]

### **1a. Homopolymer Synthetic mRNAs**

UTP ---> Poly (U) RNA  
20 tubes for in vitro translation  
Each tube: 1 \* Amino acid  
19 Unlabeled amino acids  
Same polypeptide synthesized in each tube  
Labeled (\*) polypeptide present in only 1 tube

UUU ---> UUU – UUU – UUU – UUU  
\* Phe ---> \*Phe - \*Phe - \*Phe - \*Phe  
Conclude: UUU codes for Phe

### **1b. Heteropolymer Synthetic mRNAs**

Use known mixture of different NTPs  
Example: 3:1 UTP : GTP  
Predict frequency of random codons  
Example: 27/64 UUU; 1/64 GGG; 3/64 UGG  
Determine: \*amino acid(s) in polypeptides as above

### **1c. Repeating Copolymers**

Repeating and predictable sequence of codons  
Example: ---- UGUGUGUGUGUG ----

## **2. Triplet Binding Technique**

Use synthetic triplet codons in place of mRNAs  
Synthesize codon - precise sequence known  
Produce charged tRNAs with labeled (\*) AA  
Codon, charged tRNA form ribosome complex  
Complex identified by specific binding to filter  
Determine which \*AA binds to filter for each codon

Complete Code Deciphered (Cracked) by mid 1960s

## **Overview of Translation**

### **Structure of tRNA:**

Short, ssRNA (70-95 nucleotides)  
About 30 different types (sequences) in *E. coli*  
Cloverleaf shape: paired “stems” / unpaired “loops”

*Modified bases in unpaired regions*  
*Examples: pseudouridine; methyl guanosine*  
*Post-transcriptional modifications*  
*Anticodon found in one unpaired loop region*  
*Amino acid attached to 3' end of tRNA*  
*Charged tRNAs have AA attached*  
*Process catalyzed by amino acyl synthetases*  
*Critical step in maintaining fidelity of genetic code*

***Simplified Structure of tRNA:***

5' *Amino Acid*

*Anticodon*

***Structure of Prokaryotic Ribosome***

*~ 10,000 ribosomes per bacterial cell*  
*Composed of large and small subunits*  
*Subunits dissociate in vivo and in vitro*

50S	<i>32 polypeptides</i> <i>2 rRNAs (5S, 23S)</i>
30S	<i>21 polypeptides</i> <i>1 rRNA (16S)</i>

*Eukaryotic ribosomes larger; 3 rRNAs in LS*

***Translation Process in Prokaryotes:***

*Initiation: SS binds to mRNA upstream of AUG*  
*Shine-Dalgarno sequence on mRNA*  
*Pairs with sequence on 16S rRNA*  
*tRNA-N-formyl-Met binds to AUG*  
*LS binds to SS / mRNA complex*

*Elongation: Ribosome moves 5' to 3' along mRNA  
Polypeptide begins to form  
N terminal end of polypeptide forms first*

*Termination: Polypeptide released from complex  
Ribosome subunits dissociate*

*Note: Many protein factors assist in translation*

***Translation Process in Eukaryotes:***

*Similar in many respects to prokaryotic process  
5' methyl (G) cap replaces Shine-Dalgarno sequence  
N-formyl-Met not used  
Special initiator tRNA charged with Met used  
More translation factors (helper proteins)*

***Overview of Translation in Prokaryotes:***

***A. The "BIG" Picture:***

**B. The “Detailed” Picture:**

**More Details on the Genetic Code:**

**1. Variable # codons / amino acid**

*trp* = 1 UGG  
*gly* = 4 (GG\_)

Maximum number codons / AA = 6  
1<sup>st</sup> 2 nucleotides critical for tRNA binding  
Wobble: 3<sup>rd</sup> nucleotide can mispair  
Result: 1 tRNA may pair with > 1 codon

**2. Genetic code is almost universal**

*Minor exceptions in mitochondria and protozoa*

Example: UGA = STOP (standard code)  
= TRP (rare exceptions)  
Change in recognition of 3<sup>rd</sup> base  
Significance uncertain

**3. Nonsense mutations**

*Produce internal STOP codon in frame  
Translation terminates prematurely  
Truncated (shortened) polypeptide made*

**4. Suppressor mutations**

*Compensate for defect in nonsense mutants  
Some alter tRNA structure  
Mutant tRNA binds to internal STOP  
Amino acid added to polypeptide*

**5. Frameshift mutations**

*Small insertions / deletions in gene  
Alter reading frame of mRNA  
Major disruption of protein function*