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Chapter 32
The Genetic Code

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Nature of the Genetic Code

- Gene and protein structures are colinear
  - Tryptophan synthetase mutations (Yanofsky)
- A cipher
  - Nucleotide sequence specifies amino acid sequence
- Size of code must be 3 nucleotides/AA
  - 2 is not enough ($4^2 = 16$ "codewords")
  - 3 is more than enough ($4^3 = 64$ "codewords")
  - Insertion and deletion mutations support 3
    - 3 insertions restore the reading frame
    - 3 deletions restore the reading frame

Yanofsky in 1964 studied many mutations in tryptophan synthetase, mapping the distance of the mutations in the gene. He showed that the distances between the mutations were proportional to the distances between the amino acid substitutions in the protein.

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Nature of the Genetic Code, con't.

- Code is neither **overlapping** nor **punctuated** (See Fig 32.2).
  - Point mutations cause only 1 AA change.
  - 3 insertions or deletions restore reading frame.
- Code is **degenerate.**
  - An amino acid can have more than one code word.
- All codewords have meaning.
  - 3 of 64 stand for "terminate", the remaining 61 code for one of the 20 amino acids.

Also, the code is **unambiguous**, each codeword codes for only one amino acid, and it is **universal**, with minor exceptions (Page 1074), most of which involve mitochondria DNA.

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Adapter Hypothesis

- Proposed by Francis Crick to provide the bridge between nucleotide sequences and amino acid sequences.
- Adapter function served by **transfer RNA**s, one or more for each amino acid.
  - See Fig 32.1 for "cloverleaf" tRNA structure
  - See also Fig 12.37 and 12.38

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Adapter Hypothesis, con't.

- Specific **synthetases** recognize a tRNA and attach a specific amino acid.
- The tRNA then recognizes the nucleotide codeword.
  - **Anticodon** sequence in the tRNA base pairs with the **codon** sequence in the code.

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Deciphering the Genetic Code

- Cell free extract of E. coli that catalyzed incorporation of radioactive amino acids into protein, stimulated by added RNA.
  - (Nirenberg and Matthei)
- Synthesis of polyribonucleotides
  - Random (polynucleotide phosphorylase)
    - Ochoa
  - Block copolymers
    - Khorana

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Deciphering the Code, con't.

- Early results:
  - Poly U stimulated poly **phe** synthesis
  - Poly C stimulated poly **pro** synthesis
  - Poly A stimulated poly **lys** synthesis
  - Poly UCUCUCUCU
    - Stimulated poly **ser-leu-ser-leu**
  - Poly AAGAAGAAG
    - Stimulated poly **lys**, poly **glu**, and poly **arg**

A number of other block copolymers showed similar specific stimulation that gave hints to the genetic code. For example, UUU should stand for phe, CCC should stand for pro, AAA should stand for lys, UCU and CUC would stand for ser and leu, AAG, AGA, and GAA would stand for lys, glu and arg.

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Deciphering the Code, con't.

- Niremberg and Leder found that trinucleotides promote binding of aminoacyl-tRNA's to ribosomes.
  - See Fig 32.3
- This allowed not only the composition but the sequence of the codewords to be determined.

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Features of the Genetic Code (Table 32.1)

- Most degeneracy is in the third base.
- Codons for similar amino acids are similar.
  - A pyrimidine in second position codes for hydrophobic amino acids.
  - A purine in second position codes for a polar or charged amino acid.
- Many mutations will not change the amino acid, and many will substitute a similar amino acid.

As a corroboration of the code, many known amino acid substitutions were analyzed, and almost all of them could be accounted for by a single base substitution. It is rare for a single mutation to involve more than one base change.

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The Wobble Hypothesis

- Explains degeneracy in 3rd position
  - See 32.14

5' of anticodon	3' of codon
C	G
A	U
U	A or G
G	U or C
I	U, C, or A

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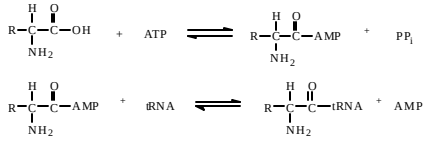
The "Second" Genetic Code

- Recognition by the aminoacyl-tRNA synthetase of the correct amino acid and the correct tRNA.
- Attachment of the amino acid is in two steps:



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Aminoacyl-tRNA Synthetases

- Two levels of specificity.
- Deacylase activity "edits" and hydrolyzes misacylated aminoacyl-tRNAs.
- Two different classes of t-RNAs (Fig 32.5).
  - Class I attaches AA to 2'OH.
  - Class II attaches AA to 3'OH.
- The two classes bind to opposite faces of tRNA (Fig 32.6)

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Recognition of tRNA's

- AA recognition is by the synthetase
- Recognition of the codon is by the tRNA
  - Von Ehrenstein experiment (Fig 32.14)
- Synthetase recognition of tRNA varies.
  - See Fig. 32.8 and 32.9

Von Ehrenstein loaded $tRNA^{Cys}$ with cysteine, then reduced the cysteine to alanine with Raney nickel. The alanine was incorporated into cysteine positions in cell free protein synthesizing systems.

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Codon Usage

- Varies among species
- Correlates with tRNA abundance
- Minor tRNA's responsible for **nonsense suppression**
  - A mutation producing a **stop** codon can be "suppressed" by a mutation in anticodon of a minor tRNA that can read that codon.

Amber mutations produce UAG, *ochre* mutations produce UAA, and *opal* mutations produce UGA. The amber mutation was named as a pun for its discoverer **Bernstein**, which is German for amber.