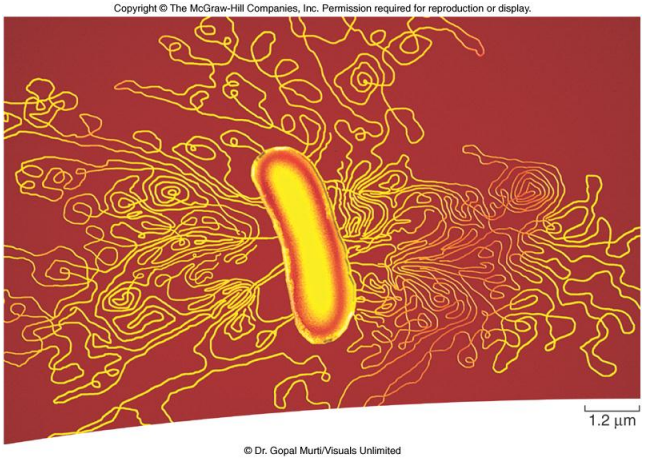
Genes & How They Work

Chapter 15



Nature of Genes

Early ideas to explain how genes work came from studying human diseases.

Archibald Garrod studied alkaptonuria, 1902

- Garrod recognized that disease inherited via a recessive allele
- Garrod proposed that patients with disease lacked a particular enzyme

These ideas connected genes to enzymes

Nature of Genes

Evidence for function of genes came from studying fungus

George Beadle & Edward Tatum, 1941

- studied *Neurospora crassa*
- used X-rays to damage DNA in cells of *Neurospora*
- looked for cells with a new (mutant) phenotype caused by damaged DNA

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Nature of Genes

Beadle & Tatum: fungal cells lacking specific enzymes

- Enzymes required for biochemical pathway producing amino acid arginine
- Identified mutants deficient in each enzyme of pathway

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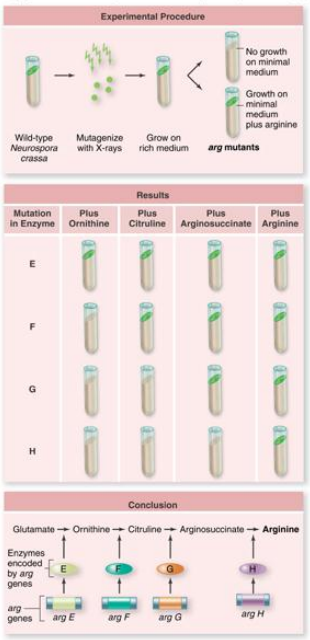

Nature of Genes

Beadle & Tatum proposed that each enzyme of arginine pathway encoded by a separate gene

Proposed **one gene – one enzyme hypothesis**

Today: **one gene – one polypeptide hypothesis**

The Nature of Genes

Central dogma of molecular biology states that information flows in one direction:

DNA ⟶ RNA ⟶ protein

Replication: DNA → DNA

Transcription: DNA to RNA

Translation: RNA to protein

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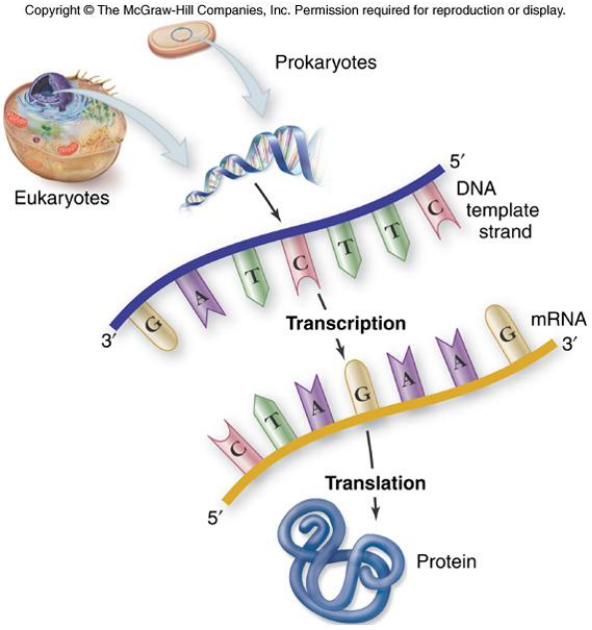

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Genetic Code

Deciphering genetic code required determining how 4 nucleotides (A, T, G, C) could encode more than 20 amino acids

Francis Crick & Sydney Brenner determined that DNA is read in sets of 3 nucleotides for each amino acid

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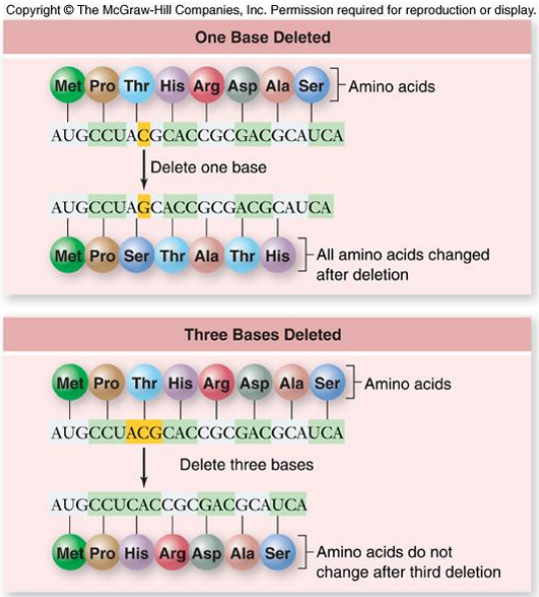

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Genetic Code

codon: set of 3 nucleotides that specifies a particular amino acid

reading frame: series of nucleotides read in sets of 3 (codon)

- only 1 reading frame correct for encoding correct sequence of amino acids

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Genetic Code

Marshall Nirenberg identified codons that specify each amino acid

RNA molecules of only 1 nucleotide & of specific 3-base sequences used to determine amino acid encoded by each codon

Amino acids encoded by all 64 possible codons determined

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TABLE 15.1 The Genetic Code

First Letter	SECOND LETTER U		C		A		G		Third Letter
U	UUU	Phe Phenylalanine	UCU	Ser Serine	UAU	Tyr Tyrosine	UGU	Cys Cysteine	U
	UUC		UCC		UAC		UGC		G
	UUA	Leu Leucine	UCA		UAA	"Stop"	UGA	"Stop"	A
	UUG		UCG		UAG	"Stop"	UGG	Trp Tryptophan	G
C	CUU	Leu Leucine	CCU	Pro Proline	CAU	His Histidine	CGU	Arg Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile Isoleucine	ACU	Thr Threonine	AAU	Asn Asparagine	AGU	Ser Serine	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys Lysine	AGA	Arg Arginine	A
	AUG	Met Methionine; "Start"	ACG		AAG		AGG		G
G	GUU	Val Valine	GCU	Ala Alanine	GAU	Asp Aspartate	GGU	Gly Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu Glutamate	GGA		A
	GUG		GCG		GAG		GGG		G

A codon consists of three nucleotides read in the sequence shown. For example, ACU codes for threonine. The first letter, A, is in the First Letter column; the second letter, C, is in the Second Letter column; and the third letter, U, is in the Third Letter column. Each of the mRNA codons is recognized by a corresponding anticodon sequence on a tRNA molecule. Many amino acids are specified by more than one codon. For example, threonine is specified by four codons, which differ only in the third nucleotide (ACU, ACC, ACA, and ACG).

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Genetic Code

stop codons: codons (UUA, UGA, UAG): terminate translation

start codon: codon (AUG): start of translation

Remainder of code is **degenerate** meaning that some amino acids are specified by more than one codon

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Gene Expression Overview

template strand: strand of DNA double helix used to make RNA

coding strand: strand of DNA that is complementary to the template strand

RNA polymerase: enzyme that synthesizes RNA from DNA template

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Gene Expression Overview

Transcription proceeds through:

- initiation – RNA polymerase identifies where to begin transcription
- elongation – RNA nucleotides added to the 3' end of new RNA
- termination – RNA polymerase stops transcription when it encounters terminators in the DNA sequence

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Gene Expression Overview

- **Translation** proceeds through
  - initiation – mRNA, tRNA & ribosome come together
  - elongation – tRNAs bring amino acids to the ribosome for incorporation into polypeptide
  - termination – ribosome encounters a stop codon & releases polypeptide

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Gene Expression Overview

Gene expression requires participation of multiple types of RNA:

messenger RNA (mRNA) carries information from DNA that encodes proteins

ribosomal RNA (rRNA): structural component of ribosome

transfer RNA (tRNA) carries amino acids to ribosome for translation

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Gene Expression Overview

Gene expression requires participation of multiple types of RNA:

small nuclear RNA (snRNA): involved in processing pre-mRNA

signal recognition particle (SRP): composed of protein & RNA & involved in directing mRNA to RER

micro-RNA (miRNA): very small & their role ??

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Eukaryotic Transcription

RNA polymerase I transcribes rRNA

RNA polymerase II transcribes mRNA & some snRNA

RNA polymerase III transcribes tRNA & some other small RNAs

Each RNA polymerase recognizes its own promoter

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Eukaryotic Transcription

Initiation of transcription of mRNA requires a series of transcription factors

- **transcription factors** – proteins that act to bind RNA polymerase to promoter & initiate transcription

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Eukaryotic pre-mRNA Splicing

In eukaryotes, primary transcript must be modified by:

- addition of a **5' cap**
- addition of a **3' poly-A tail**
- removal of non-coding sequences (**introns**)

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Eukaryotic pre-mRNA Splicing

Spliceosome: organelle responsible for removing introns & splicing exons together

Small ribonucleoprotein particles (**snRNPs**) within spliceosome recognize intron-exon boundaries

- **introns** – non-coding sequences
- **exons** – sequences that will be translated

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tRNA & Ribosomes

tRNA molecules carry amino acids to ribosome for incorporation into a polypeptide

- **aminoacyl-tRNA synthetases** add amino acids to **acceptor arm** of tRNA
- **anticodon loop** contains 3 nucleotides complementary to mRNA codons

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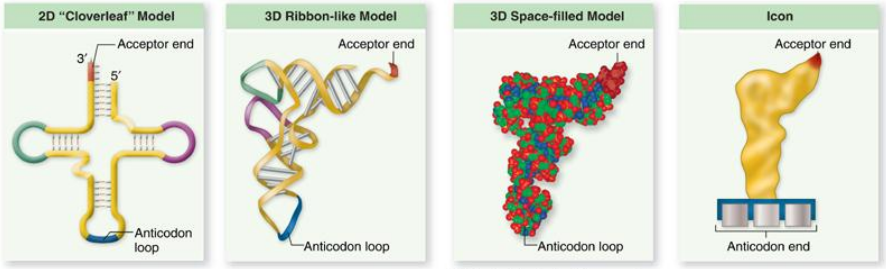

© Created by John Beaver using ProteinWorkshop, a product of the RCSB PDB, and built using the Molecular Biology Toolkit developed by John Moreland and Apostol Gramada (mbt.sdsc.edu). The MBT is financed by grant GM63208

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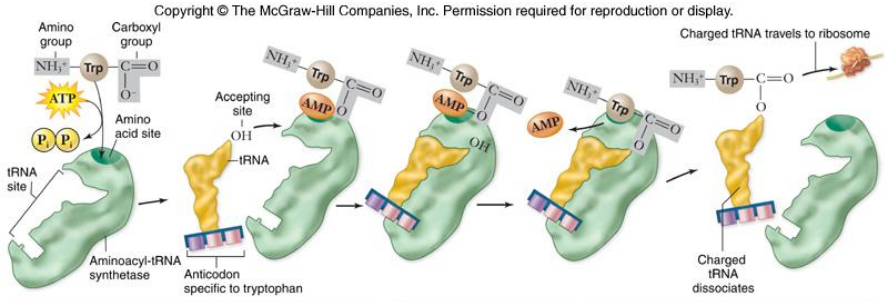

1. In the first step of the reaction, the amino acid is activated. The amino acid reacts with ATP to produce an intermediate with the carboxyl end of the amino acid attached to AMP. The two terminal phosphates (pyrophosphates) are cleaved from ATP in this reaction.

2. The amino acid-AMP complex remains bound to the enzyme. The tRNA next binds to the enzyme.

3. The second step of the reaction transfers the amino acid from AMP to the tRNA, producing a charged tRNA and AMP. The charged tRNA consists of a specific amino acid attached to the 3′ acceptor stem of its RNA.

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tRNA & Ribosomes

Ribosome: multiple tRNA binding sites:

- **P site** – binds tRNA attached to growing peptide chain
- **A site** – binds tRNA carrying next amino acid
- **E site** – binds tRNA that carried last amino acid

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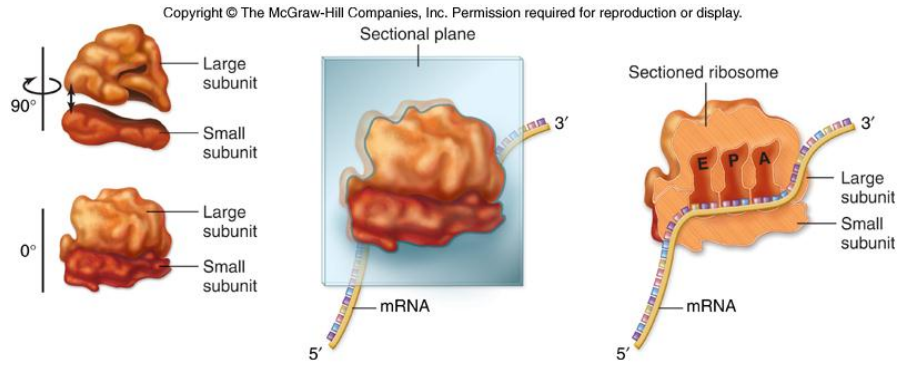

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tRNA and Ribosomes

Ribosome: two primary functions:

- decode mRNA
- form peptide bonds

peptidyl transferase: enzymatic component of ribosome which forms peptide bonds between amino acids

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Translation

Eukaryotes, translation may occur on ribosomes in cytoplasm or on ribosomes of RER

Signal sequences at beginning of polypeptide sequence bind to **signal recognition particle (SRP)**

Signal sequence & SRP recognized by RER receptor proteins

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Translation

Signal sequence/SRP holds ribosome on RER

As polypeptide synthesized, it passes through a pore into interior of endoplasmic reticulum

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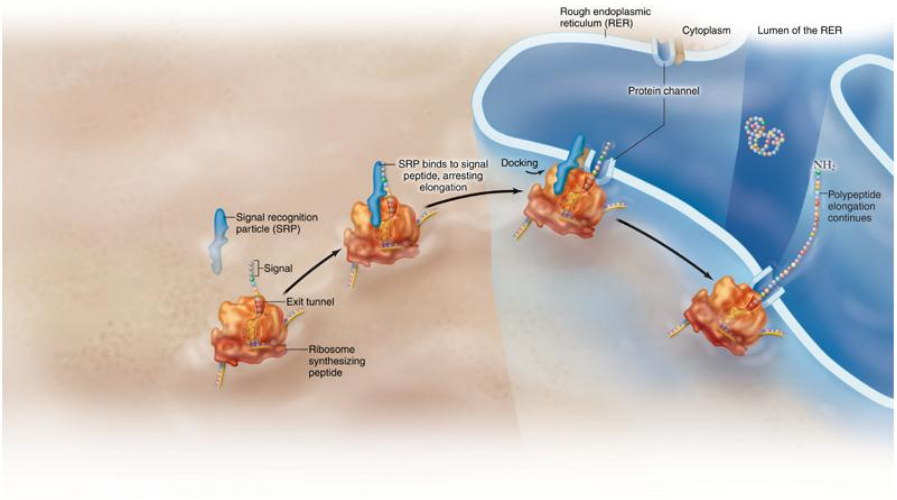

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Mutation: Altered Genes

Point mutations alter a single base

- **base substitution mutations** – substitute one base for another
  - **transitions** or **transversions**
  - also called **missense mutations**
- **nonsense mutations** – create stop codon
- **frameshift mutations** – caused by insertion or deletion of a single base

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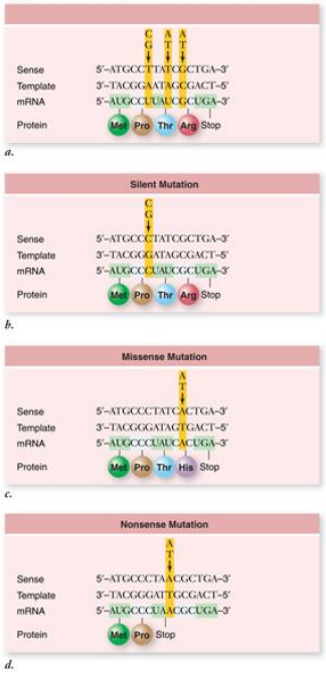

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Mutation: Altered Genes

triplet repeat expansion mutations involve a sequence of 3 DNA nucleotides that are repeated many times

triplet repeats are associated with some human genetic diseases

- abnormal allele causing disease contains these repeats whereas normal allele does not

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Mutation: Altered Genes

Chromosomal mutations change structure of a chromosome

- **deletions** – part of chromosome lost
- **duplication** – part of chromosome is copied
- **inversion** – part of chromosome in reverse order
- **translocation** – part of chromosome moved to a new location

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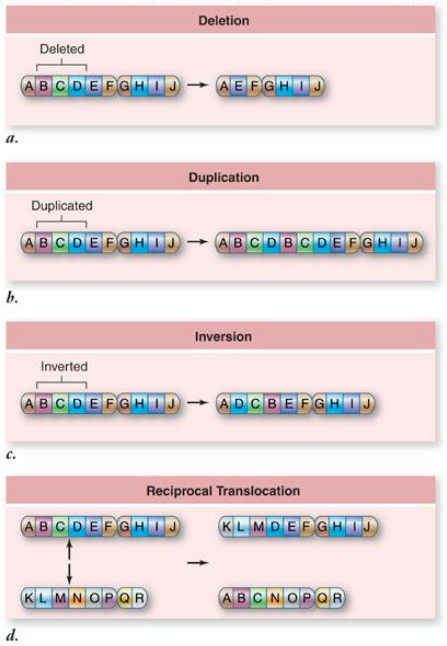

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Mutation: Altered Genes

Too much genetic change (mutation) can be harmful to individual

However, genetic variation (caused by mutation) ***necessary for evolutionary change*** of the species

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Table 2-1
Abbreviations for amino acids

Amino acid	Three-letter abbreviation	One-letter symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Asparagine or aspartic acid	Asn	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glutamine or glutamic acid	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

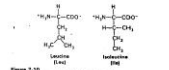


Figure 2-10
Amino acids having aliphatic side chains.

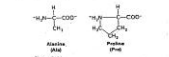


Figure 2-11
Proline differs from the other common amino acids in having a secondary amino group.

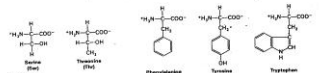
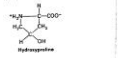


Figure 2-12
Serine and threonine have aliphatic hydroxyl side chains.

Figure 2-13
Phenylalanine, tyrosine, and tryptophan have aromatic side chains.



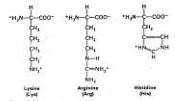


Figure 2-14
Lysine, arginine, and histidine have basic side chains.

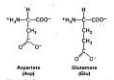


Figure 2-15
Aspartate and glutamate have acidic side chains.

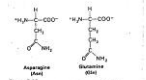


Figure 2-16
Asparagine and glutamine have amide side chains.

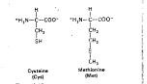


Figure 2-17
Cysteine and methionine have sulfur-containing side chains.

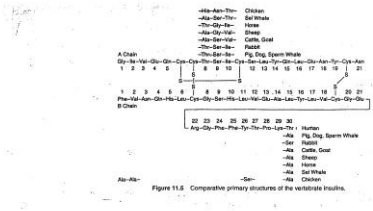


Figure 11.6 Comparative primary structures of the vertebrate insulins.

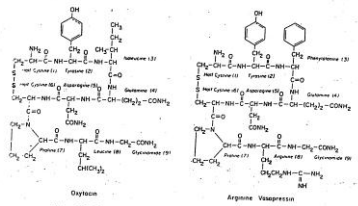


Figure 7.2 Primary structures of oxytocin and vasopressin. The individual amino acids are numbered by the conventional method.