MUTATIONS & REPAIR

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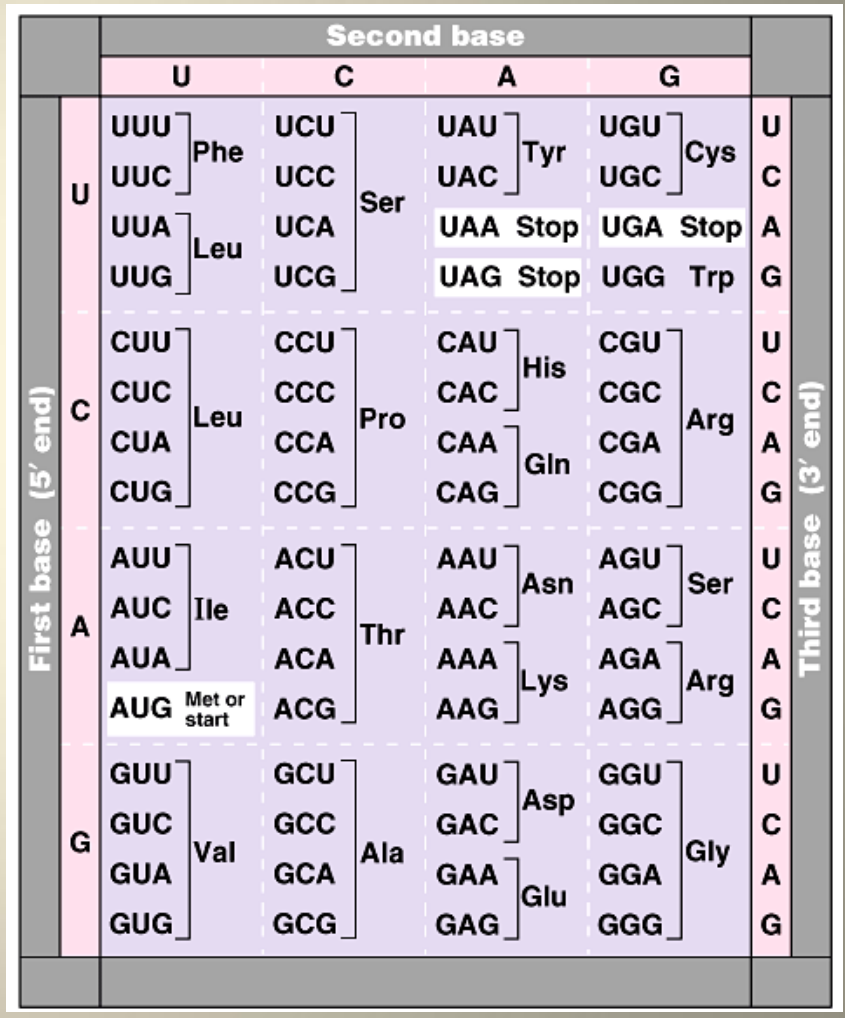
Characteristics of the Code

- Universal
- Redundant
- Non-ambiguous

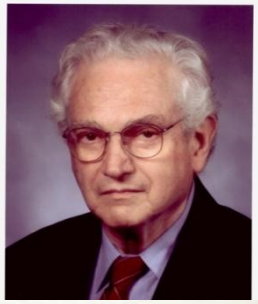
The Genetic Code is Universal

- All living things use the same 4 bases
- All living things use the same code: codons code for the same amino acid no matter what the organism



First base (5′ end)	Second base: U	Second base: C	Second base: A	Second base: G	Third base (3′ end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met or start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Marshall Nirenberg (1961)



- Deciphered first codon
- Awarded Nobel Prize in 1968 for the interpretation of the genetic code
- Discovery Channel 100 Greatest Discoveries – History of Genetics (Nirenberg @ 25:25-28:49) http://www.youtube.com/watch?v=0qgMd0obEkc

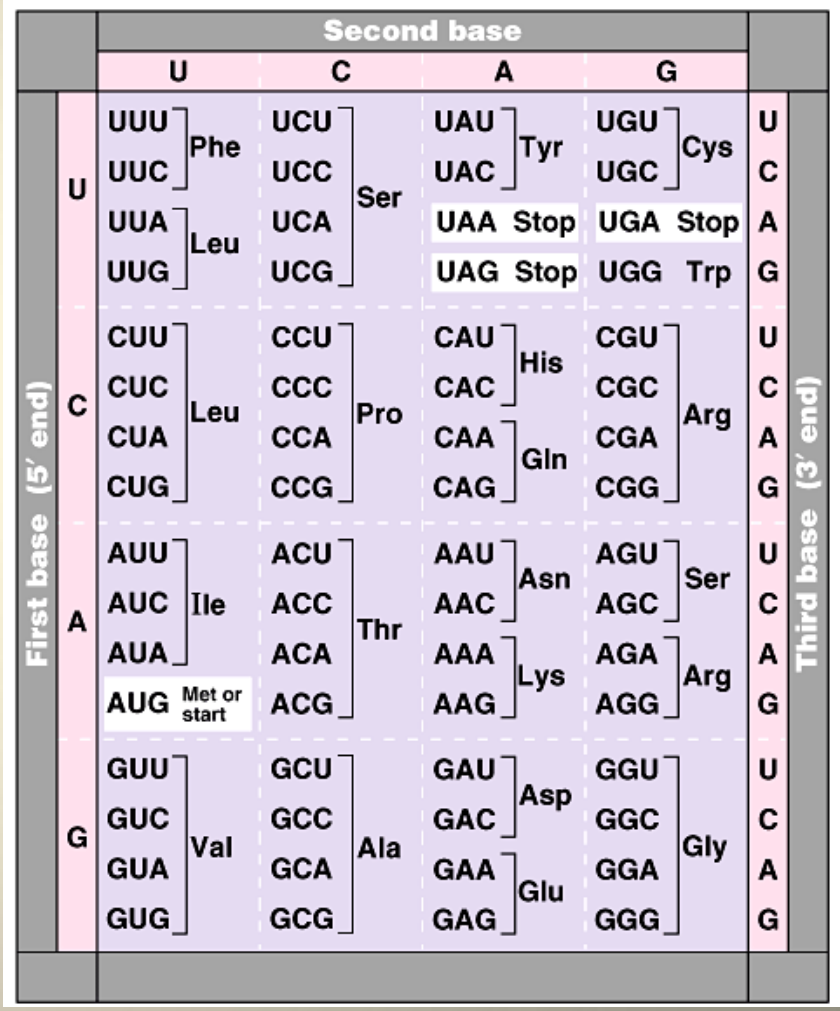
Nirenberg's Experiment

- Synthesized artificial mRNA with identical RNA nucleotides
  - E.g. UUUUUUUUUUUUUUU
  - Thus only 1 type of codon (e.g. UUU)
  - Resulted only in one type of amino acid in the polypeptide (e.g. phenylalanine)
- Repeated with other nucleotides
- Other techniques used to decode mixed triplets

The Genetic Code is Redundant

- 64 different codons on mRNA
- But only 20 different amino acids
- Conclusion?

More than one codon can code for the same amino acid.



First base (5′ end)	Second base: U	Second base: C	Second base: A	Second base: G	Third base (3′ end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met or start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Characteristics of the Code

- Degenerate / Redundant
  - There are 64 codons, but only 20 amino acids
  - The same amino acid may be coded by more than one codon
  - E.g. GCU and GCC both specify alanine
- No ambiguity
  - Each codon only specifies one amino acid

The Genetic Code is Redundant

- 64 different codons on mRNA
- But only 45 different tRNA molecules
- Conclusion?

Some tRNAs recognize more than one codon.

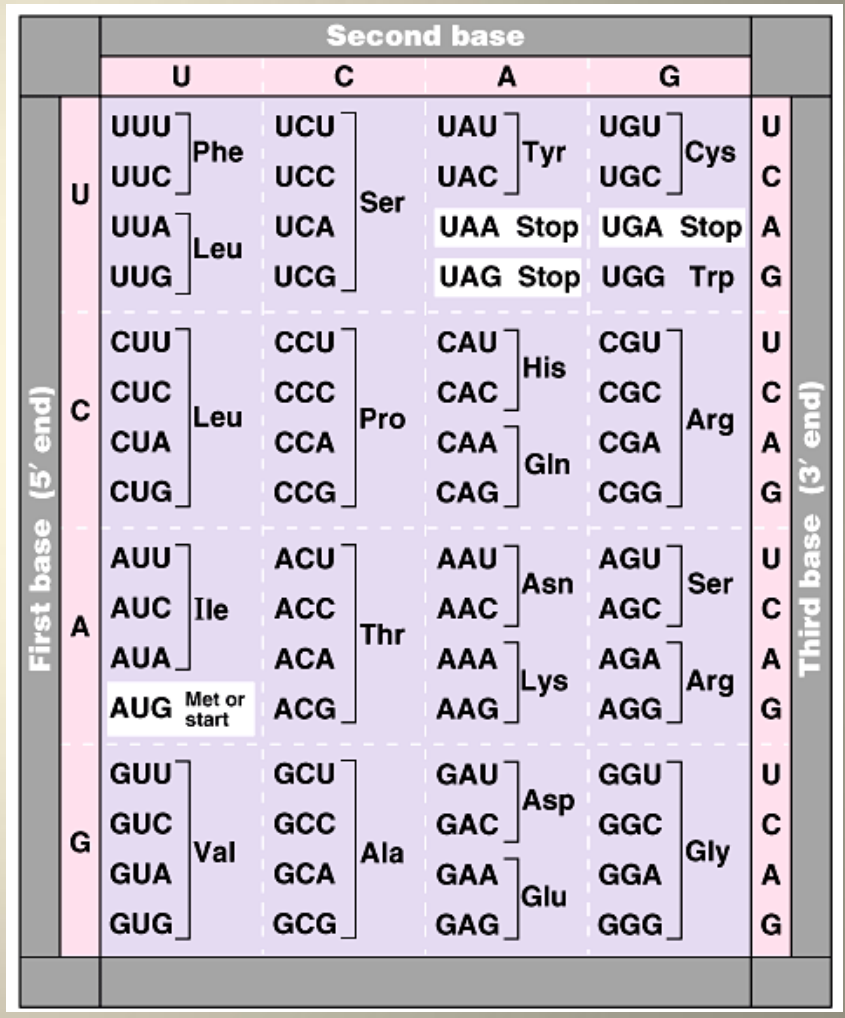


Fig. 17.4

Wobble Hypothesis

- Base pairing rules are flexible in the wobble position
- **Wobble position**: third base of the mRNA codon and its corresponding tRNA anticodon

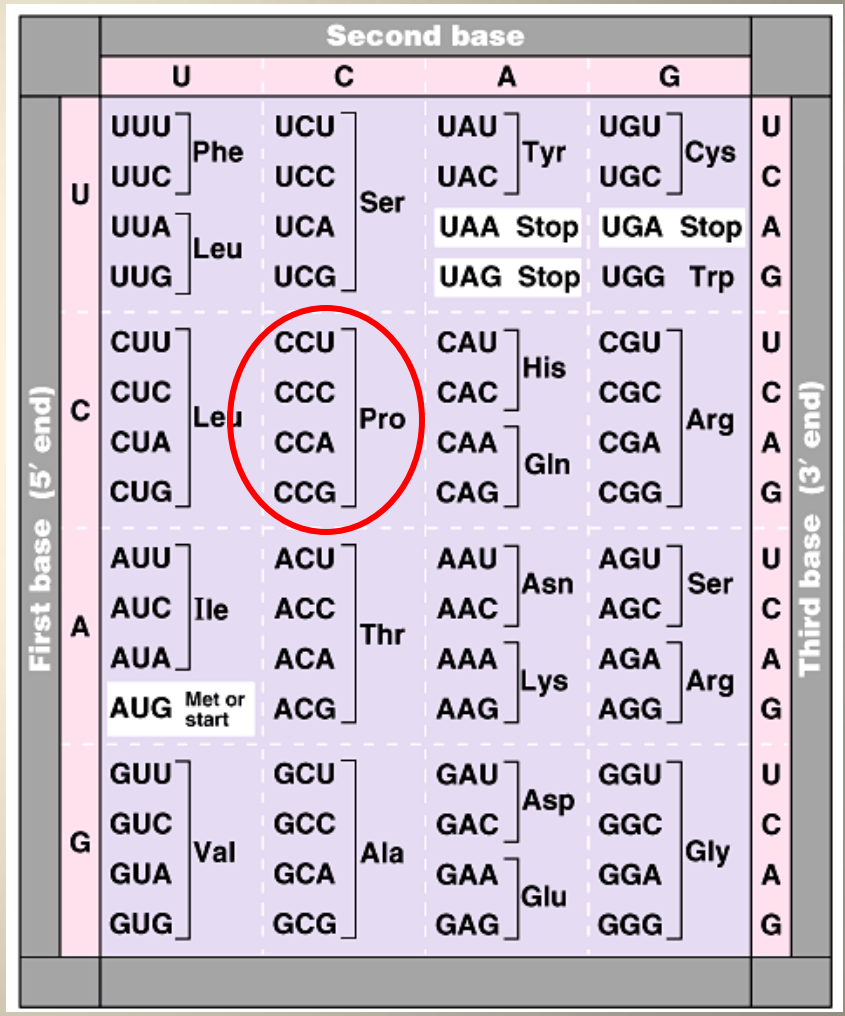


Fig. 17.4

tRNA Structure

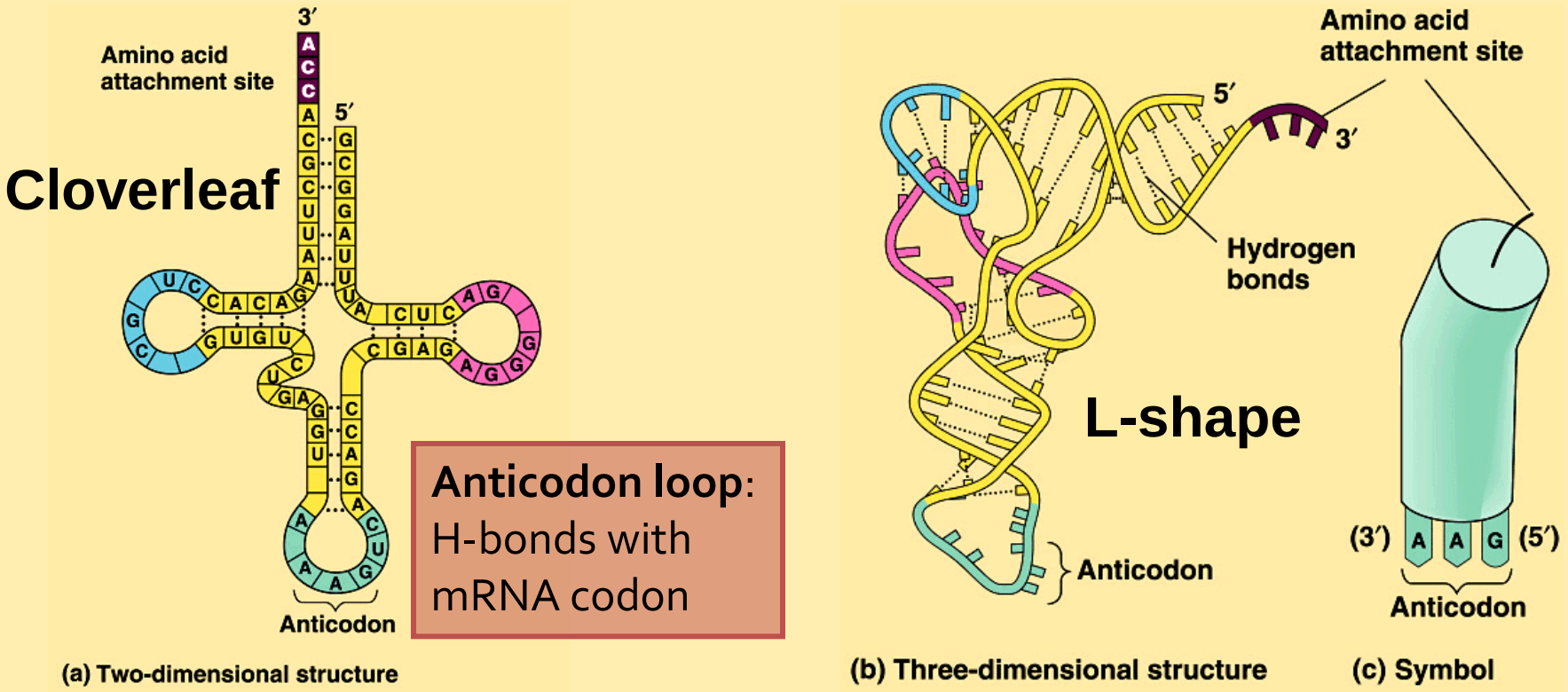
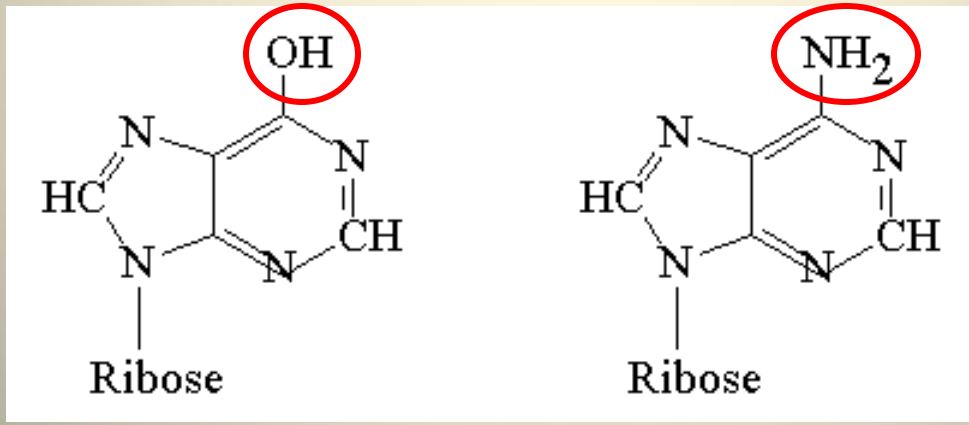


Fig. 17.13

Inosine in the wobble position

- **Inosine**:
  - a modified form of adenine that is found in tRNA (anticodon)
  - can form H bonds with U, C, or A on the mRNA



Inosine Adenosine

From http://bio.winona.msus.edu/berg/ChemStructures/Inos-ade.gif

Inosine in the wobble position

Example:

- tRNA anticodon CGI
- Can bind to codons GCU, GCC and GCA
- All result in the addition of the amino acid alanine

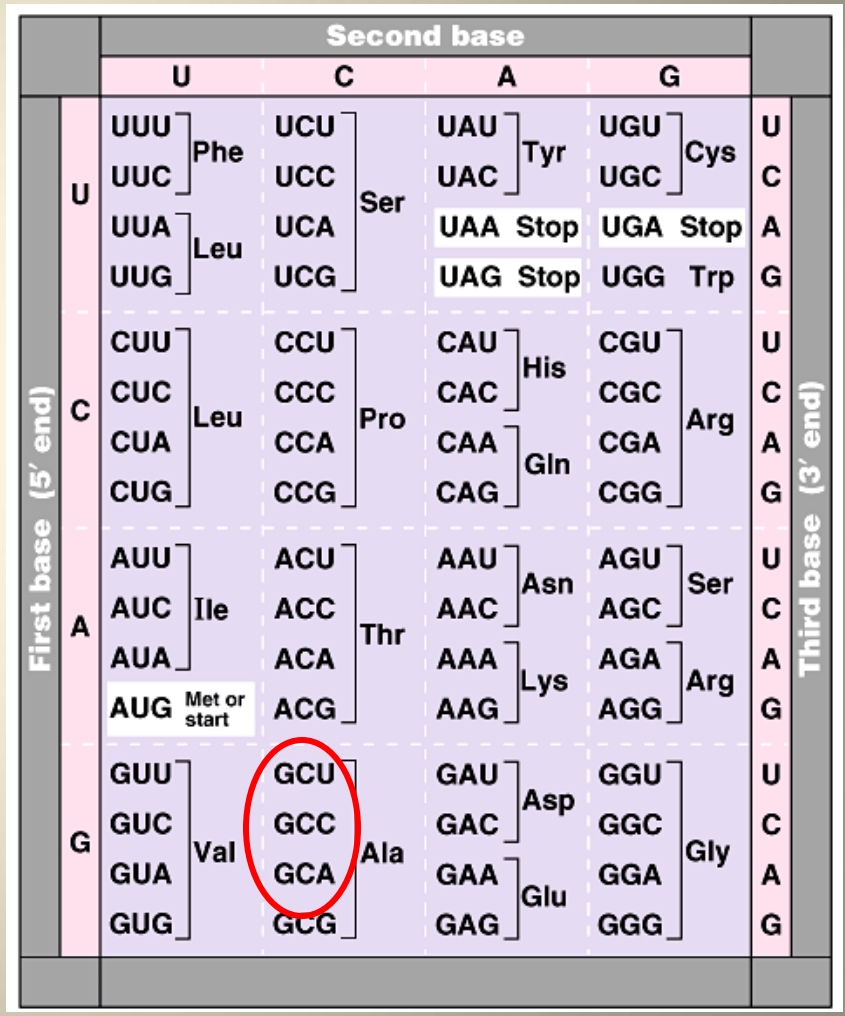


Fig. 17.4

Mutation

- a change in the genetic material of an organism
- genetic disorder or hereditary disease: harmful mutations in gametes that are passed onto the next generation

Origin/Cause of Mutation

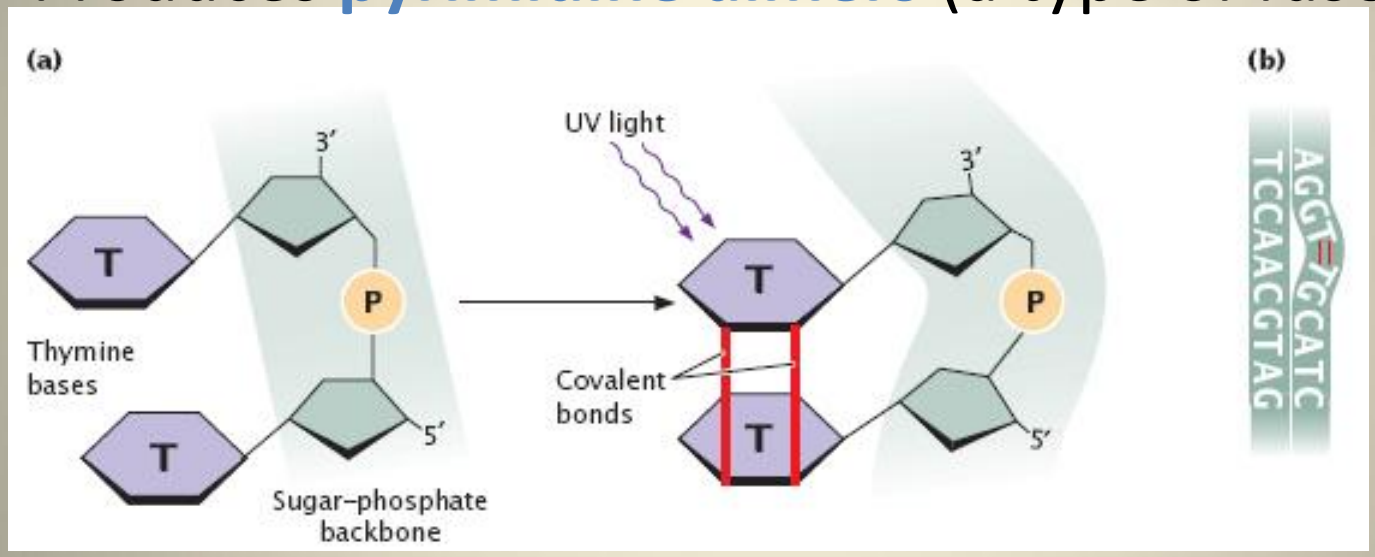
- **Spontaneous**:
  - errors in the genetic machinery during DNA replication
  - due to enzymes
- **Induced**: arising from exposure to mutagenic agents
- Transposable elements:
  - errors during recombination (crossing over)
  - transposons

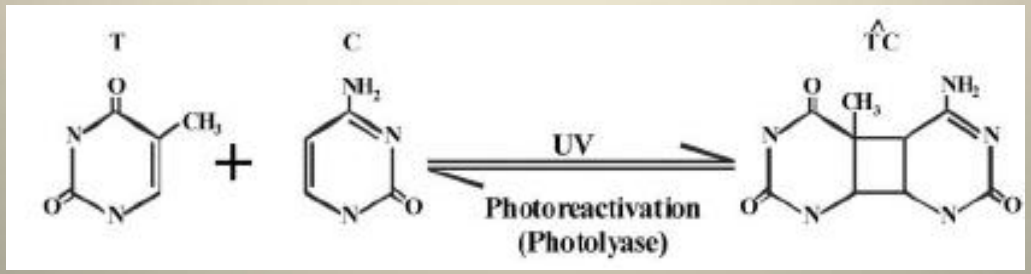
Types of Mutagens

- Mutagen: a substance that can cause mutations
- **Physical** mutagen
  - Radiation, UV light, x-rays
- **Chemical** mutagen
  - Base analogues
  - Intercalating agents
  - Base-Modifying agents

Physical Mutagen: UV light

- A common cause of DNA damage
- Produces **pyrimidine dimers** (a type of fused base)





http://www.nature.com/scitable/nated/content/19185/pierce_17_24_FULL.jpg

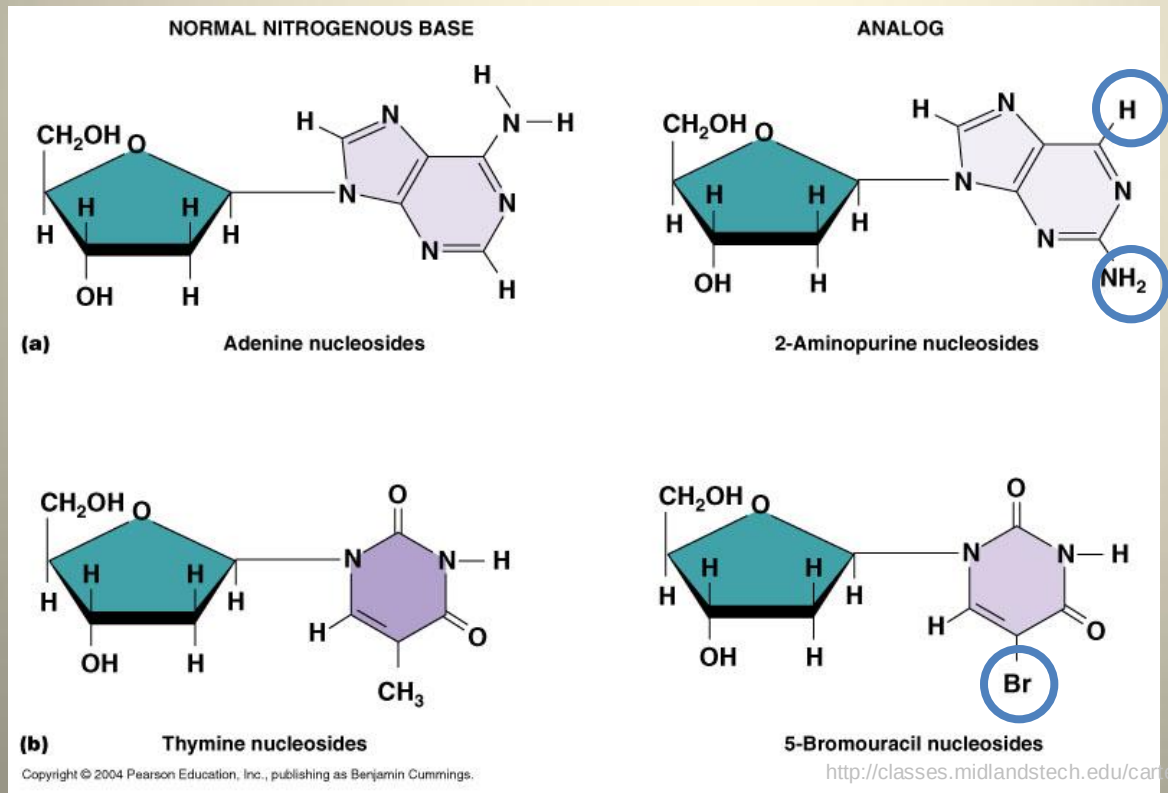
Xeroderma Pigmentosum (XP)

- Definition
  - Xeroderma = dry skin
  - Pigmentosum = change in pigmentation
- An autosomal recessive genetic disorder
  - unable to repair damage caused by UV light
  - mutation in an enzyme in the NER
- Individuals may need to avoid sunlight completely ("children of the night")
- Leads to early skin cancer

http://www.casesjournal.com/content/1/1/254/figure/F1

Chemical Mutagen: Base Analogs

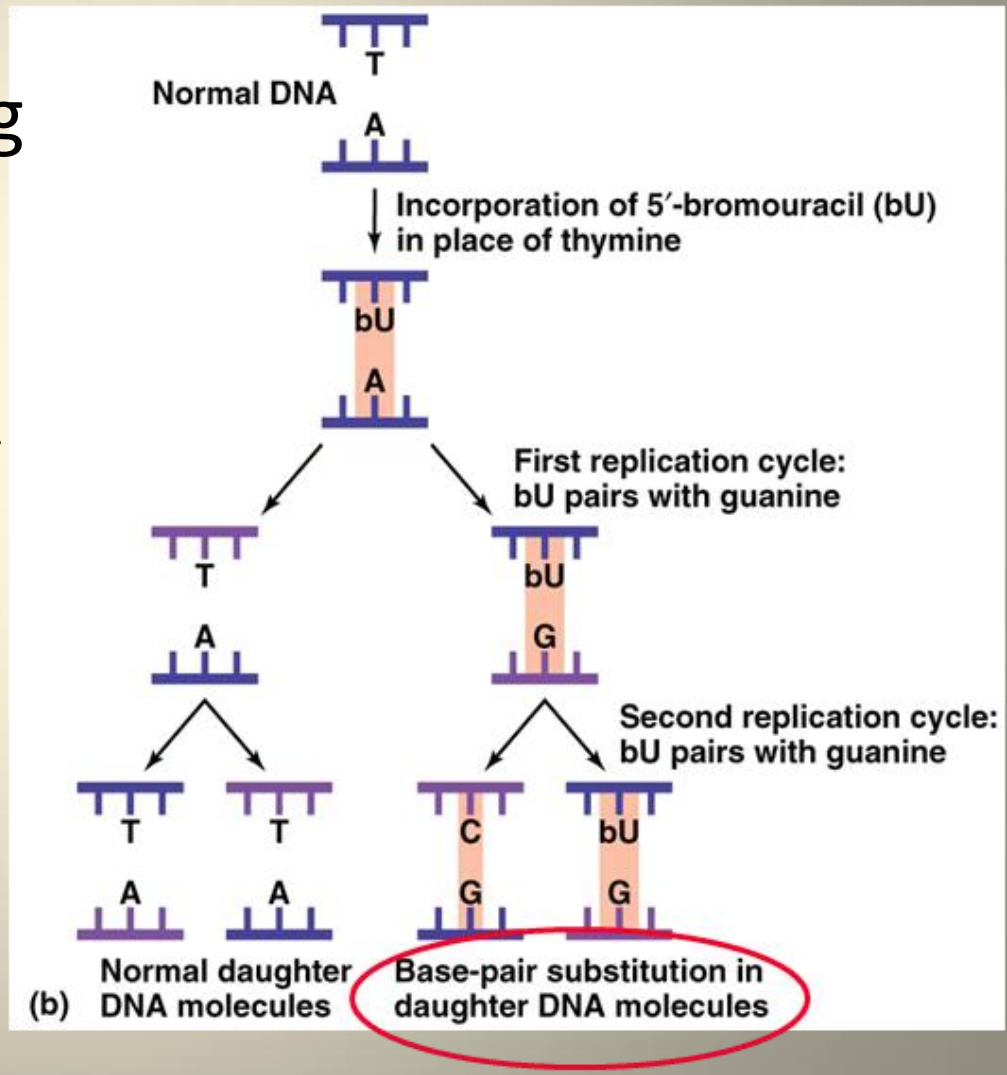
- Structurally similar to normal DNA bases (mimic)
- May get incorporated instead of the normal base
  - E.g. thymine is replaced with 5-bromouracil



http://classes.midlandstech.edu/carterp/Courses/bio225/chap08/08-19_Nucleoside_1.jpg

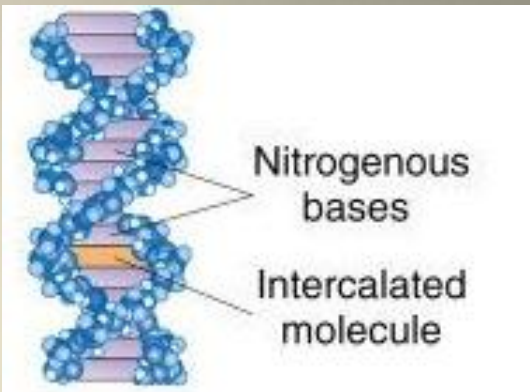
Chemical Mutagen: Base Analogs

- Pairs incorrectly during DNA replication
  - E.g. 5-bromouracil can pair with G instead of A
- Used in a drug for treating HIV



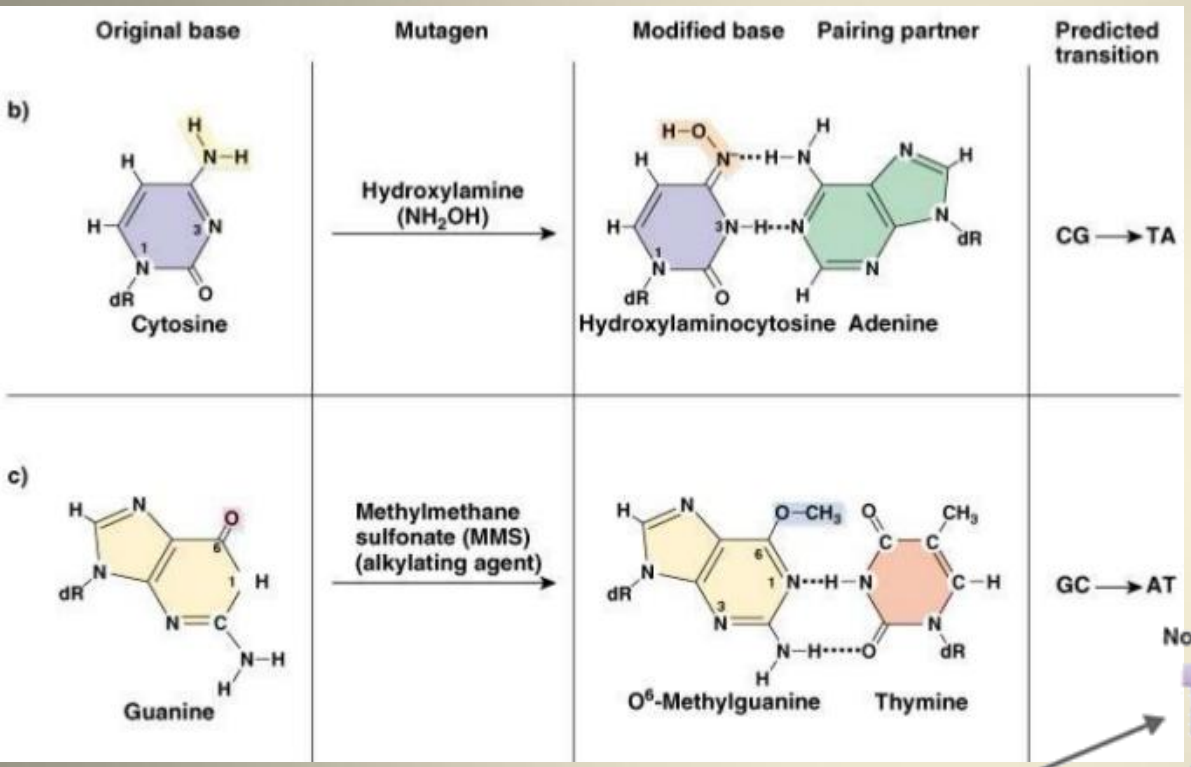
Chemical Mutagen:
Intercalating Agent

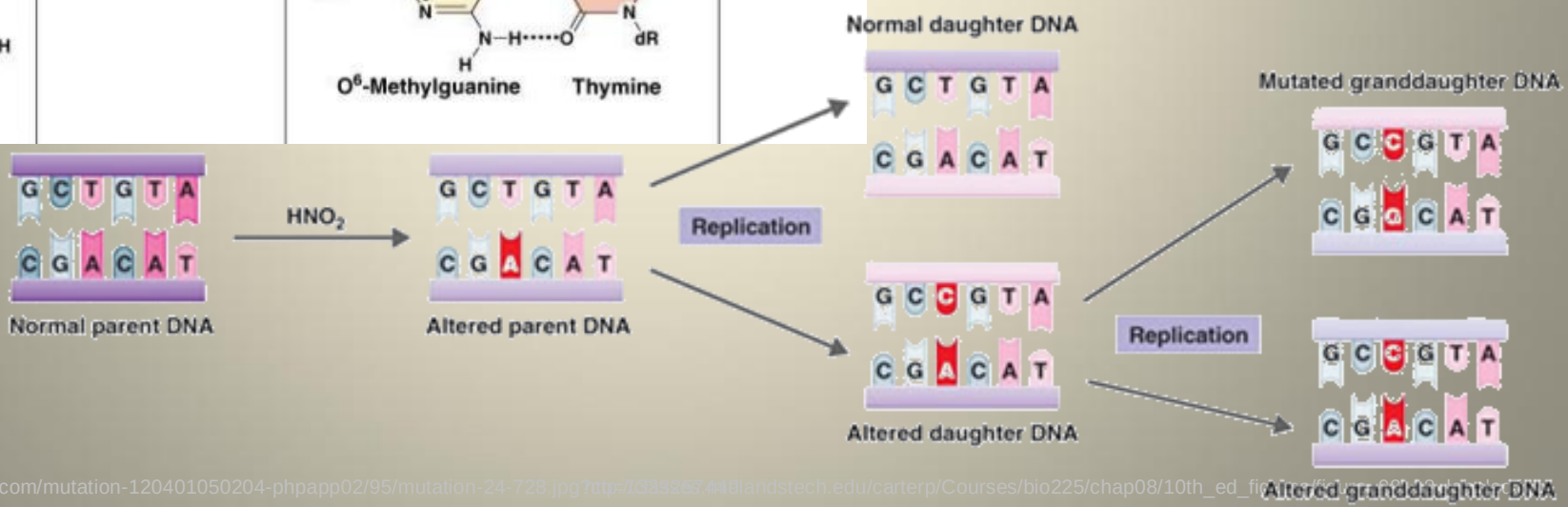
- Distort DNA helix intercalating between adjacent base pairs
- May cause DNAP to “stutter” and copy the mutagen as an extra base pair
- May interfere with replication
- Commonly used for staining and visualizing DNA in biotechnology (e.g. **ethidium bromide**)



Chemical Mutagen:

Base Modifying Agent





- Modifies base changing pairing properties

Types of DNA Mutations

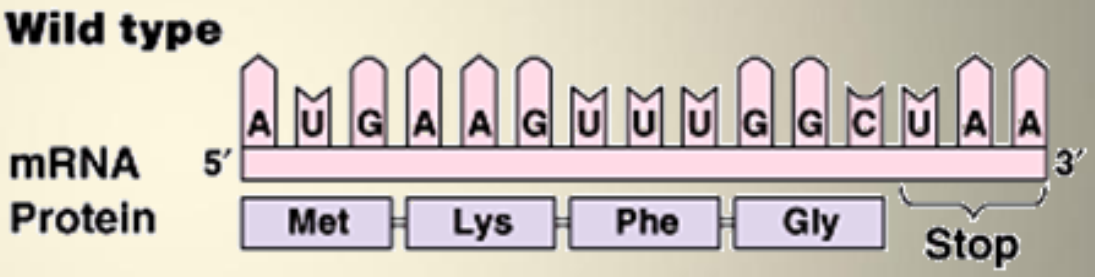
- Chromosomal mutations
  - Occurs during meiosis (e.g. crossing over)
- Missing bases 
- Fused bases 
  - Pyrimidine dimers (e.g. xeroderma pigmentosum)
- **Mismatch** mutations:
  - incorrectly paired bases
  - caused by **point** mutations
  - one of the most common types of errors during replication

Point Mutations

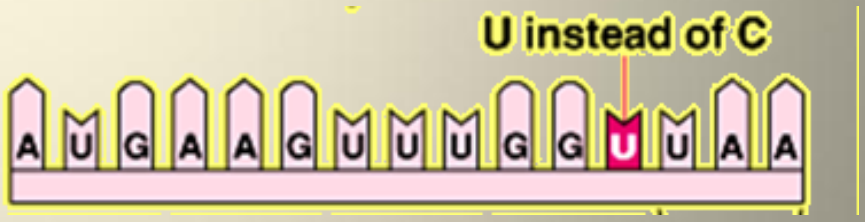
- One nucleotide (or a base pair) is altered
- Types of point mutations:
  - Substitution
    - Transition
    - Transversion
  - Frameshift
    - Insertion
    - Deletion

Substitution

- A small change in a DNA base pair where one nucleotide is replaced with another



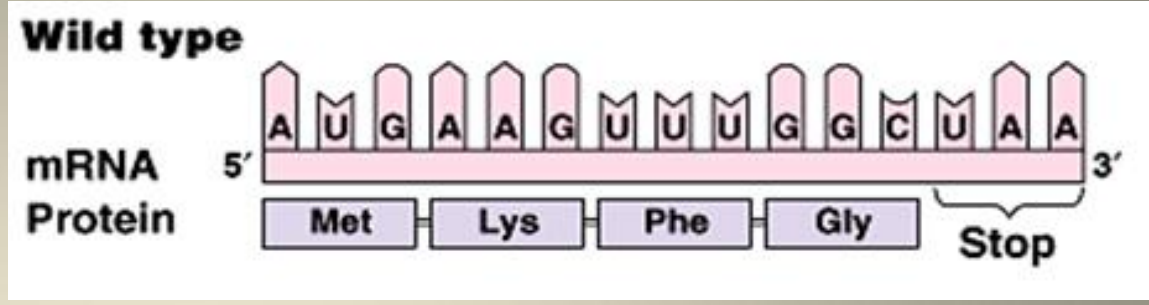
- Transition:
  - purine to purine
  - pyrimidine to pyrimidine
- Transversion:
  - purine to pyrimidine
  - pyrimidine to purine



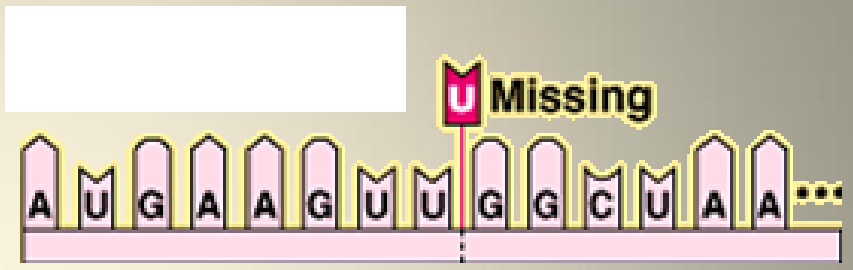
Frameshift Mutation

- **Reading frame**: triplet grouping (codons) of a genetic message
- **Frameshift mutation**: number of nucleotides added/lost is not a multiple of 3 thus altering the reading frame
  - **Insertion**: addition of one or more nucleotide pairs in a gene
  - **Deletion**: loss of one of more nucleotide pairs in a gene
- No frameshift: number of nucleotides added/lost is a multiple of 3
  - Leads to extra or missing amino acid

Frameshift Mutations



- Frameshift deletion



- Frameshift insertion

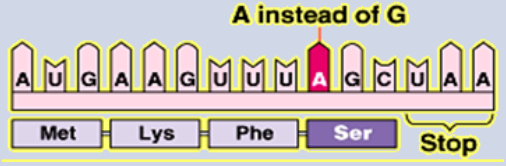
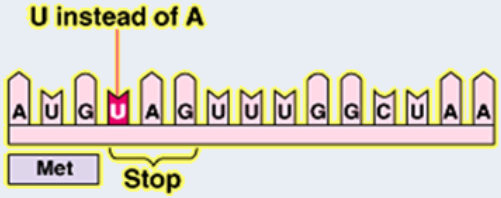
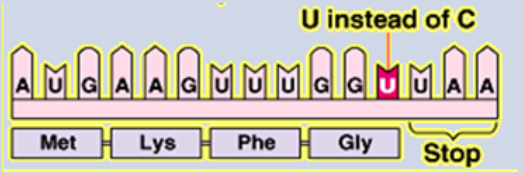
- No frameshift

Fig. 17.24

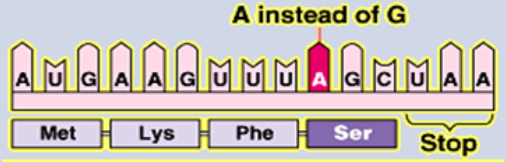
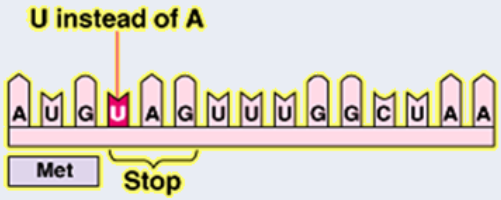
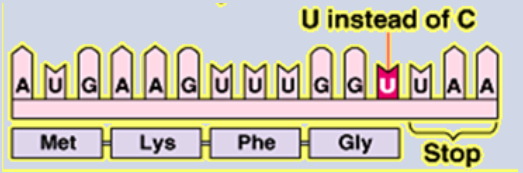
Functional Classification of Mutations

- Effects on amino acid sequence (polypeptide):
  - Missense
  - Nonsense
  - Silent
- Effect on protein function:
  - Negative
  - Positive
  - Neutral

Effects of mutations on polypeptide

Class	Effect on amino acid sequence	Effect on protein function	Example
Missense	▪ Codes for different amino acid		▪ Sickle Cell Anemia 
Nonsense	▪ Codes for a stop codon ▪ Results in truncated polypeptide		
Silent	▪ Codes for same amino acid		

Effects of mutations on polypeptide

Class	Effect on amino acid sequence	Effect on protein function	Example
Missense	▪ Codes for different amino acid	▪ May or may not change	▪ Sickle Cell Anemia 
Nonsense	▪ Codes for a stop codon ▪ Results in truncated polypeptide	▪ Most of these mutated proteins are digested by the cell. ▪ Mutations are often lethal at the embryonic stage.	
Silent	▪ Codes for same amino acid	▪ No change	

Missense Mutation

First base (5′ end)	Second base: U	Second base: C	Second base: A	Second base: G	Third base (3′ end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met or start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Fig. 17.4

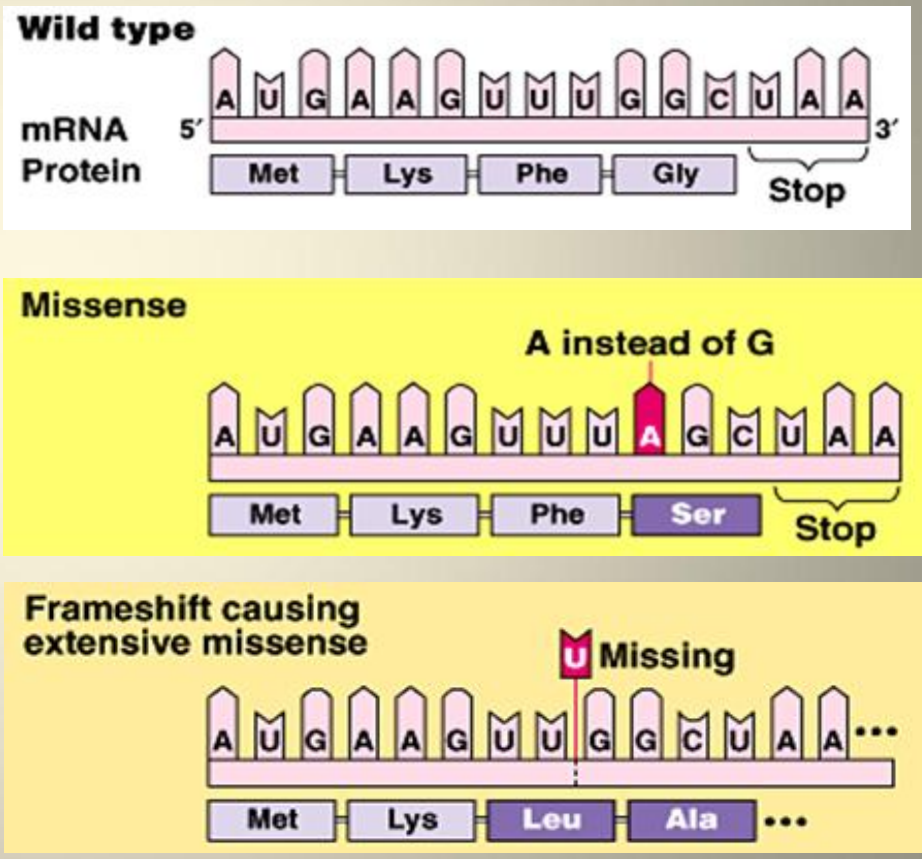
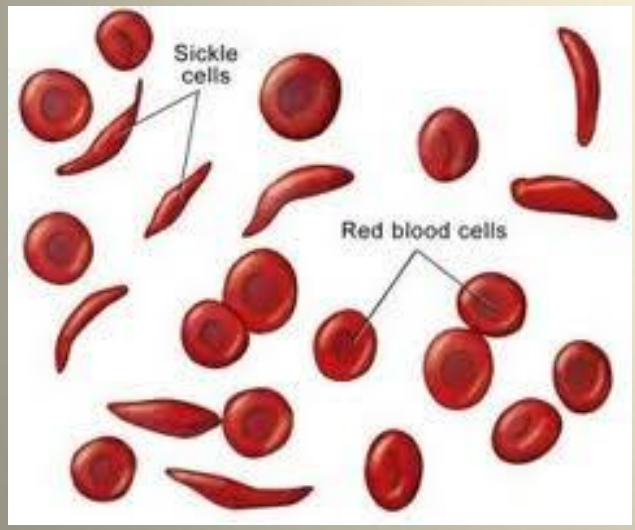


Fig. 17.24

Example: Sickle Cell Anemia

- Substitution missense: A → T changes amino acid glutamine to valine



HBB Sequence in Normal Adult Hemoglobin (Hb A):

Nucleotide	CTG	ACT	CCT	GAG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Glu	Glu	Lys	Ser
	3			6			9

HBB Sequence in Mutant Adult Hemoglobin (Hb S):

Nucleotide	CTG	ACT	CCT	GTG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Val	Glu	Lys	Ser
	3			6			9

Nonsense Mutation

First base (5′ end)	Second base: U	Second base: C	Second base: A	Second base: G	Third base (3′ end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met or start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Fig. 17.4

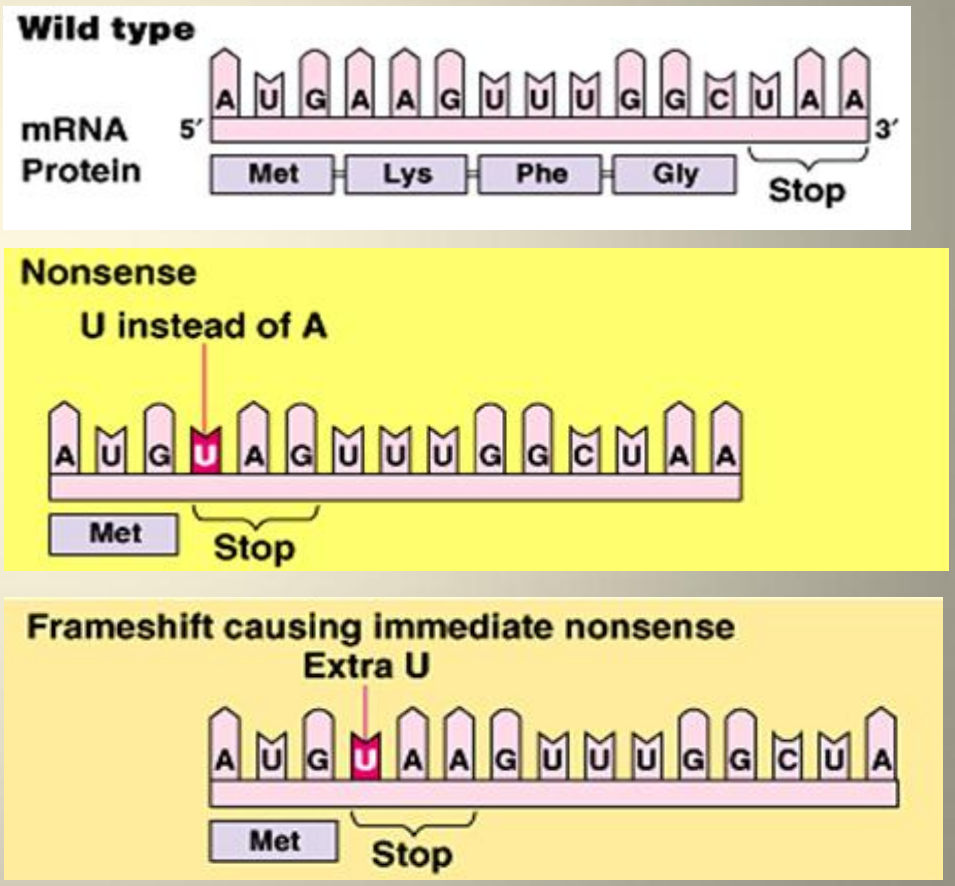
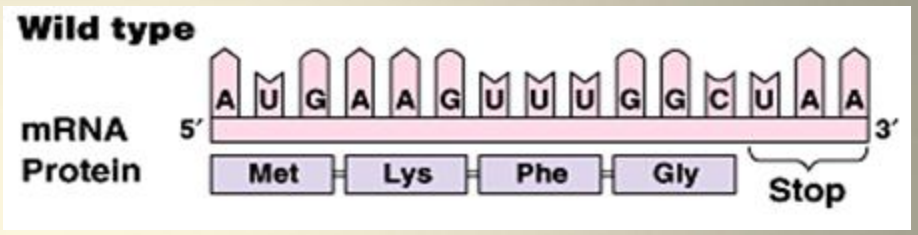


Fig. 17.24

Silent Mutation

First base (5′ end)	Second base: U	Second base: C	Second base: A	Second base: G	Third base (3′ end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met or start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Fig. 17.4



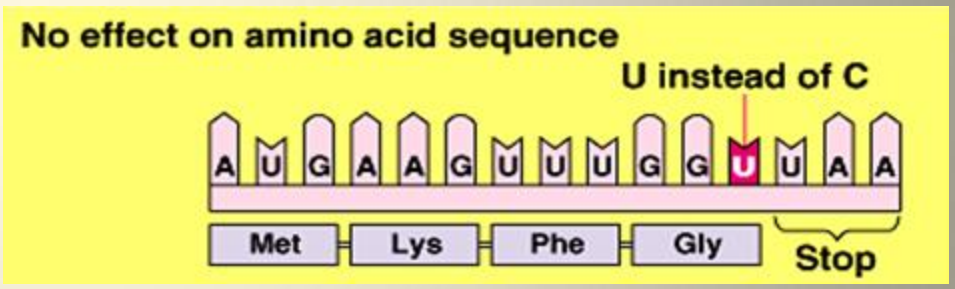


Fig. 17.24

Wild Type:
Two men sat and had hot tea

Classify these “mutations”:

- Two men **S**at and had hot tea
- Two men sat and had hot **s**ea
- Two me**.**
- Two men sat and had hot **t**te a
- Two mes ata ndh adh ott ea

Silent

Missense

Nonsense

Frameshift Insertion

Frameshift Deletion

Point/Frameshift Mutation Summary

Types	Missense effect	Nonsense effect	Silent
Point Mutation Substitution			
Frameshift Insertion or Deletion			
No Frameshift Insertion or Deletion			

Point/Frameshift Mutation Summary

Types	Missense effect	Nonsense effect	Silent
Point Mutation Substitution	✓	✓	✓
Frameshift Insertion or Deletion	✓ extensive	✓	✗
No Frameshift Insertion or Deletion	✓ (extra or missing amino acid)	✓	✗

Effect of mutation on protein function / organism

Effect	Protein function	Cause	Example
Negative	Detrimental to the organism	Missense or nonsense mutations	▪ most molecular biological research is related to this idea
Positive	Benefits the organism	Missense mutations	▪ back mutations / reversions that restore original sequence ▪ antibiotic resistance
Neutral	No change	Silent or sometimes missense mutations (change in amino acid but without changing protein function)	▪ mutations in non-coding regions (e.g. introns)

Proofreading and Repair

- Error Rate
- Repair methods
  - **Exonuclease**: Mechanisms in place to proofread errors as DNA is being replicated
  - **Endonuclease**: Cell also continuously monitors and repairs DNA outside of replication

Error Rate

- Average human chromosome has 150,000,000 bp
- Initial pairing error: 1 in 10,000 bp
  - = 15,000 errors per replication
- Final error: 1 in 1,000,000,000 bp
  - = 0.15 errors per replication
  - = 1 error in ~7 replication

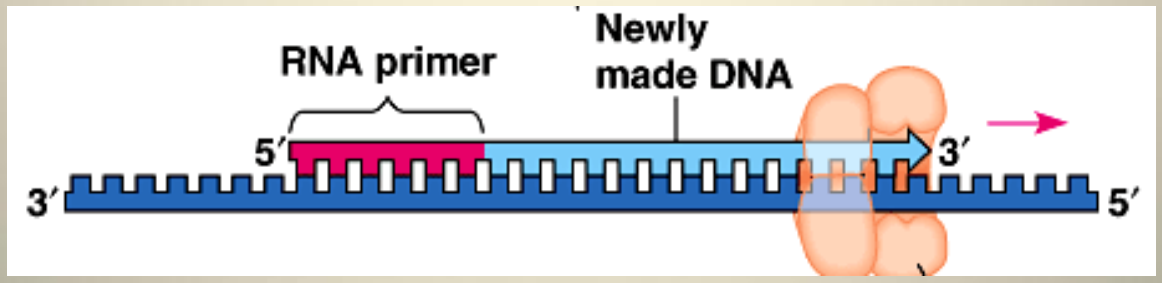
Thought question: What happened in between the initial and final error rate that could explain this difference?

Repair by Nuclease

- **Nuclease**: an enzyme that can break phosphodiester bonds in DNA thus **excising** out the nucleotide
  - **Exonuclease**: binds to ends of nucleotide chain (5’ or 3’)
  - **Endonuclease**: binds to the middle of a nucleotide chain

Exonuclease Proofreading

- Instantaneous repair:
  - Occurs as the DNA is replicating
  - Due to errors during elongation at the 3’ end
- DNAP III and DNAP I both have exonuclease activity



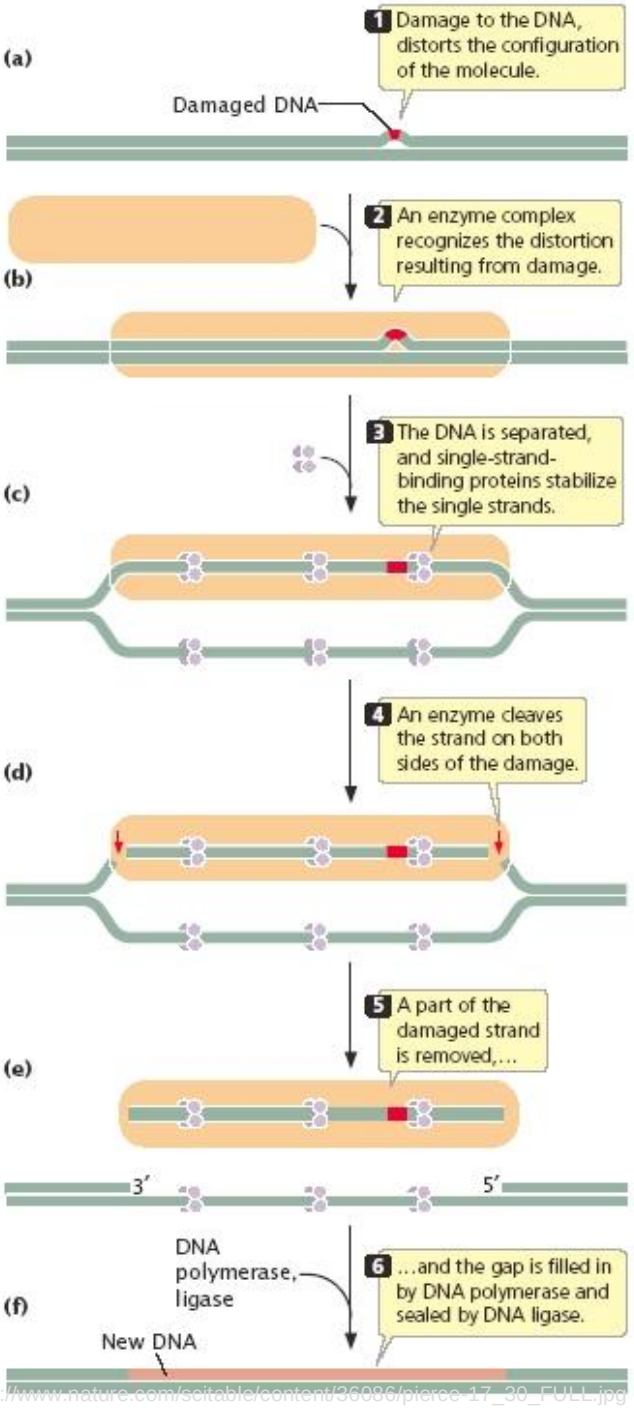
Exonuclease Proofreading

- Mechanism of repair:
  - DNAP instantly recognize mismatches during replication
  - hydrolyze the phosphodiester bond releasing the last nucleotide that was just added (exonuclease activity)
  - replaces with the correct nucleotide (polymerase activity)

- Note: one enzyme (DNAP) does both the nuclease and polymerase function

Endonuclease Proofreading

- Repair often occurs after DNA is already replicated
- Mechanism of repair known as **nucleotide excision repair (NER)**



http://www.nature.com/scitable/content/36086/pierce_17_30_FULL.jpg

Endonuclease Proofreading: NER

- Endonuclease:
  - recognizes and binds to error
  - nicks the strand by breaking phosphodiester bonds
  - error is excised (removed)
- Polymerase: replaces the gap with the correct nucleotides
- Ligase: seals the nick

- Video: Repair thymine dimers (1:11)
  https://www.youtube.com/watch?v=azszodOhXqk&index=54&list=PLXwnjgs_UWpJLSTT_BHTbJvZgRiYS5kf1

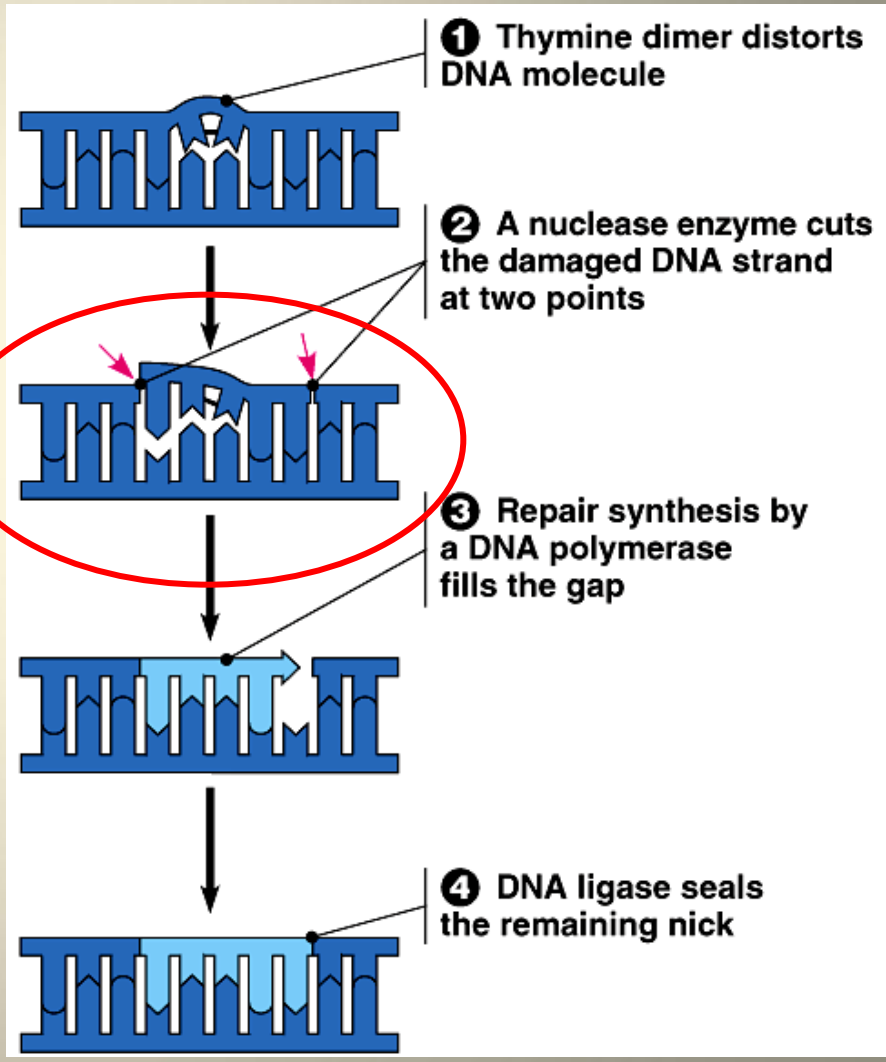


Fig. 16.17

HW Question

- The genetic code is redundant but not ambiguous. Explain what that means.