SACRAMENTO STATE
Leadership begins here.

Chapter 7:

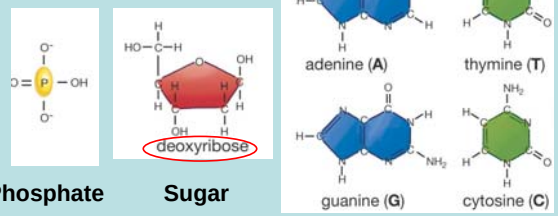
DNA Replication, Transcription & Translation; Mutations & Ames test

Dr. Amy Rogers
Bio 139 General Microbiology

DNA is the genetic material

- Hereditary information is carried by DNA
  - Griffith/Avery transformation experiments
  - Hershey-Chase viral labeling experiment
- DNA is organized into ***chromosomes***
  - In bacteria, *generally* one per cell, circular in shape
  - Humans: 46 linear chromosomes (23 homologous pairs)
- Each chromosome contains many ***genes***
  - Gene is a functional unit, like a word in a sentence.
  - One definition: one gene carries info to make one protein

Components of DNA

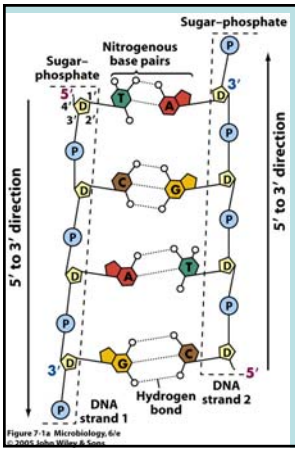


Phosphate group **Sugar**

Purines Pyrimidines

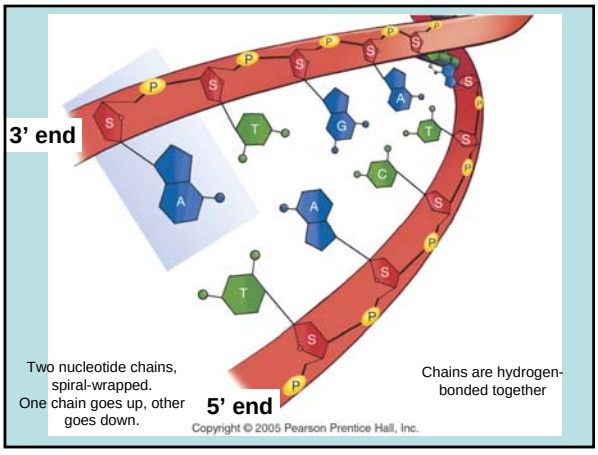
Base

All three together = a ***nucleotide***



DNA structure

- **Double stranded**
- Backbones:
  - Alternating sugar (deoxyribose) / phosphate
- Strands held together by hydrogen bonds
  - between the bases
- Adenine / Thymine
- Guanine / Cytosine
- **Antiparallel**
  - 5' & 3' ends
- Wrapped in a **helix**



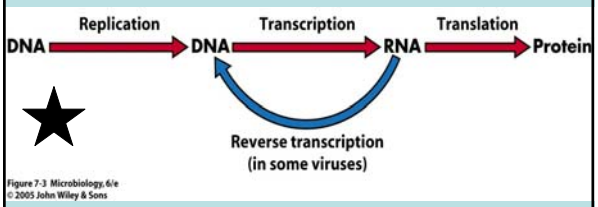
DNA structure linked to DNA function

- Information is encoded in *sequence* of the bases
- Complementary base pairing of strands that are NOT covalently bound: suggests mechanism for REPLICATION

DNA's jobs (information transfer)

1. Direct its own replication so each daughter cell gets an exact copy of the parent's genome
2. Direct all cellular activity by expressing genes as RNA
   - Transcription into messenger RNA (mRNA)
   - Translation of mRNA into protein

Information transfer: "The Central Dogma"



- DNA to DNA = ***replication***
- DNA to RNA = ***transcription***
- RNA to protein = ***translation***

DNA Replication

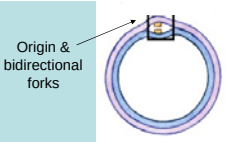
Replication is ***Semiconservative***:
- One strand from the original molecule is always "conserved" in each new DNA copy

1. DNA double helix denatures (strands separate)
2. Each strand serves as the ***template*** for synthesis of a new second strand
3. ***DNA polymerase*** adds complementary nucleotides
   - 1,000 per second!!!
   - Corrects its own mistakes (proofreading)

Semiconservative Replication

- DNA strands separate
- DNA polymerase (enzyme) synthesizes complementary (new) strands, using the nucleotide sequence of the original molecule as a template
- Two DNA molecules result
  - Each double stranded (ds) DNA has one old and one new strand

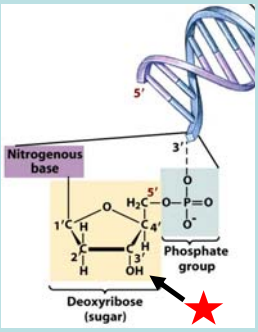
- DNA replication in prokaryotes begins at a specific location: ***origin of replication***
  - Plasmids must have an "origin" to survive
- Replication proceeds simultaneously in both directions away from the origin
  - The moving point where replication is actually occuring is called the replication fork

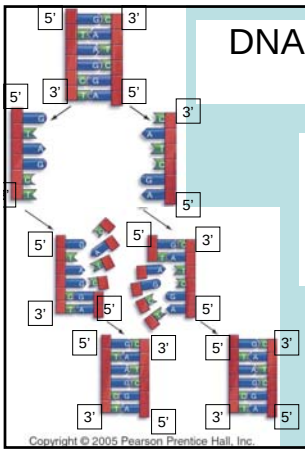


When forks meet on opposite side of the circle, replication of the chromosome is complete and the two DNAs separate

Nucleotides can only be added to the 3' end

- 3' carbon on deoxyribose has –OH group to which new nucleotide gets attached ★
- 5' carbon has phosphate group, no extension of the DNA strand there ★

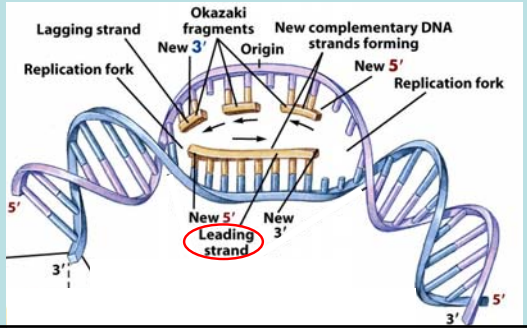




DNA is synthesized 5' ➡ 3'

- Remember the strands run antiparallel
- Replication "up" one strand, "down" the other

To copy the 3' ➡ 5' strand: Polymerase makes a continuous long "***Leading Strand***" in the antiparallel 5' ➡ 3' direction

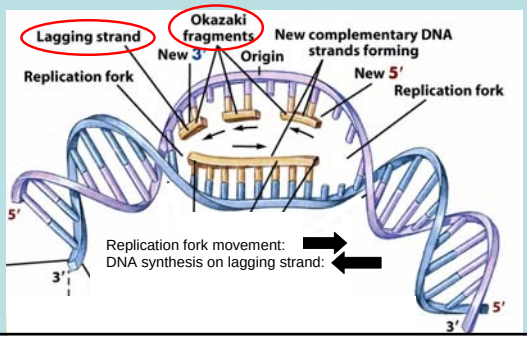


To copy the 5' ➡ 3' strand:
Polymerase must make a 3' ➡ 5' strand (IMPOSSIBLE)

Solution:

- ***Lagging strand*** DNA synthesis:
  - DNA polymerase must jump forward and backward as the helix unwinds
  - Short, discontinuous fragments of DNA are made 5' to 3' even though the DNA is unwinding in the other direction
- These are called ***Okazaki fragments***
  - Lagging strand synthesis is enzymatically complex (many steps involved)

Lagging strand synthesis & Okazaki fragments



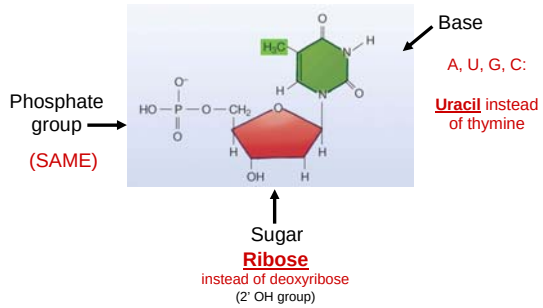
DNA ➡ RNA Transcription

- Unwound regions of DNA can also be "copied" as RNA
- **RNA polymerase**

★Properties of RNA:

- **Single stranded** (but often folded up)
- ***Uracil*** instead of thymine (U still pairs with A)
- **Ribose instead of deoxyribose in backbone**

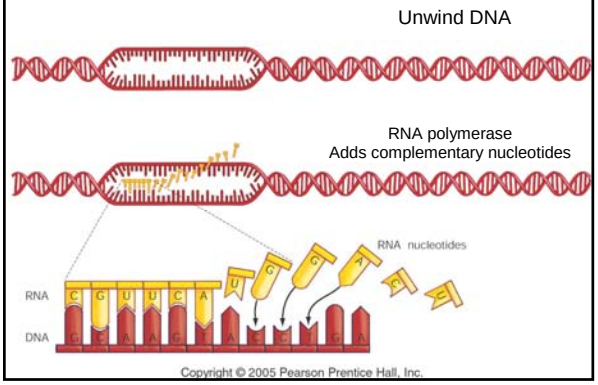
★Nucleotides: DNA vs. RNA



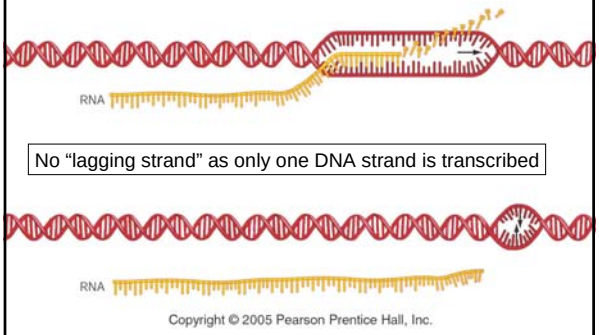
★ RNA types

1. ***rRNA (<u>ribosomal</u>):*** rRNAs bind to proteins to form the protein-producing ribosome "factories" (70S in prokaryotes)
2. ***mRNA (<u>messenger</u>):*** mRNAs carry the protein-coding information from DNA to the ribosomes. mRNA sequence determines amino acid sequence of protein.
3. ***tRNA (<u>transfer</u>):*** tRNAs translate the coded message of mRNAs into a different chemical polymer: proteins!

<u>Transcription:</u> DNA → RNA



Transcription



Prokaryotic Transcription

- In eukaryotes, transcription is more complex: noncoding regions called introns must be spliced (cut) out of RNAs before they are finished
- **<u>Prokaryotes DO NOT have introns</u>**

<u>Prokaryotes</u>: transcription & translation both occur in **<u>cytoplasm</u>**

<u>Eukaryotes</u>: transcription in nucleus; translation in cytoplasm

All 3 kinds of RNA are involved in translation

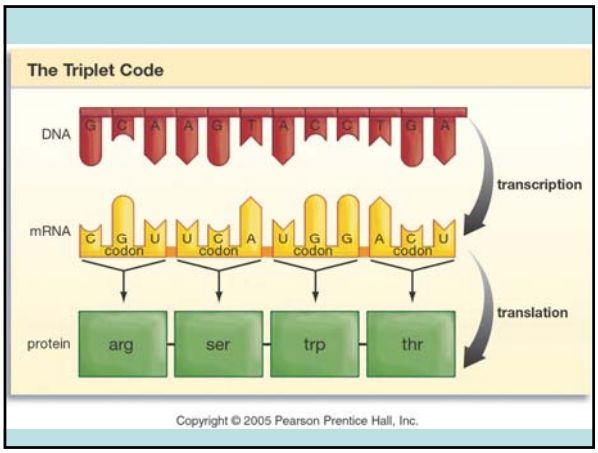
1. **<u>rRNA</u>**: crucial part of the ribosome which catalyzes synthesis of new proteins
2. **<u>mRNA</u>**: the blueprint; information-carrying molecule which dictates the amino acid sequence of a new protein
3. **<u>tRNA</u>**: tRNA (transfer RNA) translates nucleotide sequences into amino acid sequences

tRNA recognizes the Genetic Code

mRNA sequences code for amino acids:

<u>Three nucleotides = One amino acid</u>

<u>Codon</u> = 3 nucleotides



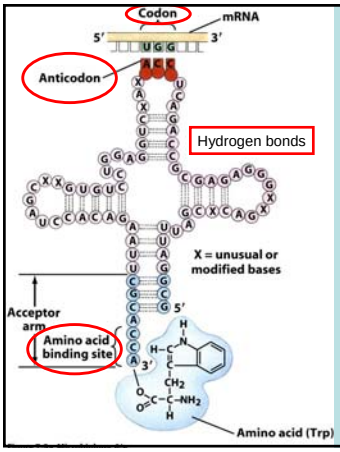
The Genetic Code

First position	Second position U	Second position C	Second position A	Second position G	Third position
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	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

- Each amino acid may have multiple codons ★ ***(degenerate)***
- Some codons do NOT code for an amino acid; they mean **STOP** (end of protein)
- ★ **AUG = methionine = START** (all mRNAs start being translated with AUG)

The Genetic Code

- ★Nearly the same in ALL organisms (from bacteria to humans)
- This makes genetic engineering possible
  - DNA from one organism can be put into another and will be expressed normally



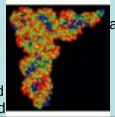
tRNA

tRNAs "read" the genetic code

Twisted 3D "cloverleaf" structure (hydrogen bonds)

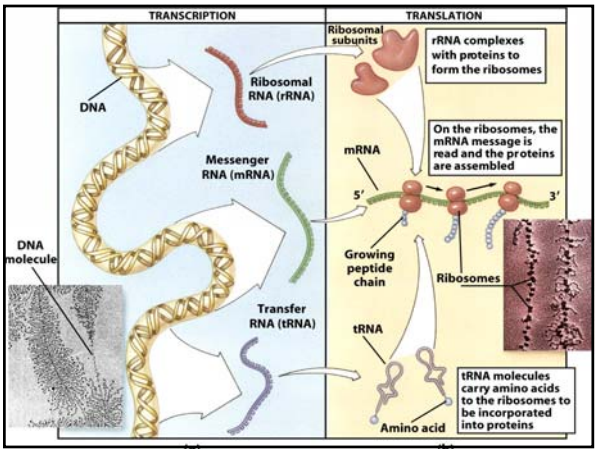
One end: binds a single amino acid

Other end: anticodon



tRNA

- Amino acid bound specifically matches the sequence of the ***anticodon***
- Accurate base pairing of the anticodon with the codon of mRNA brings correct amino acid to the growing polypeptide (protein) chain



PROKARYOTES:

Many ribosomes can simultaneously be acting on the same mRNA molecule (***polyribosomes***)

AND

transcription and translation in cytoplasm; can occur simultaneously

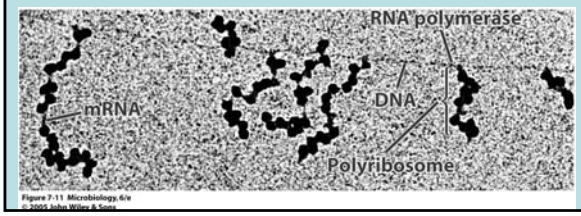


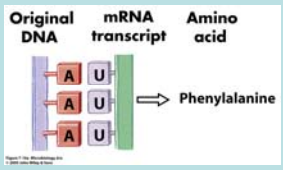
TABLE 7.1

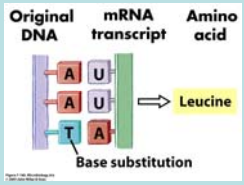
Properties of the Different Kinds of RNA

Kind of RNA	Properties
Ribosomal	Combines with specific proteins to form ribosomes.
	Serves as a site for protein synthesis.
	Associated enzymes function in controlling protein synthesis.
Messenger	Carries information from DNA for synthesis of a protein.
	Molecules correspond in length to one or more genes in DNA.
	Has base triplets called codons that constitute the genetic code.
	Attaches to one or more ribosomes.
Transfer	Found in the cytoplasm, where they pick up amino acids and transfer them to mRNA.
	Molecules have a cloverleaf shape with an attachment site for a specific amino acid.
	Each has a single triplet of bases called an anticodon, which pairs complementarily the corresponding codon in mRNA.

Mutations

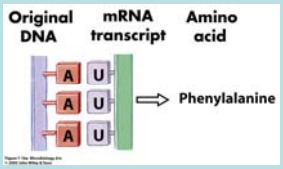
- Change in DNA
- ***Point mutation***: substitution of a single base

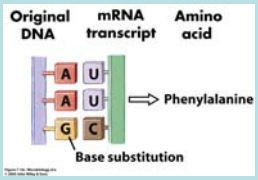




Point mutation can cause a change in amino acid sequence of a protein

Point mutations can be *silent* (no change in amino acid sequence) because the genetic code is degenerate



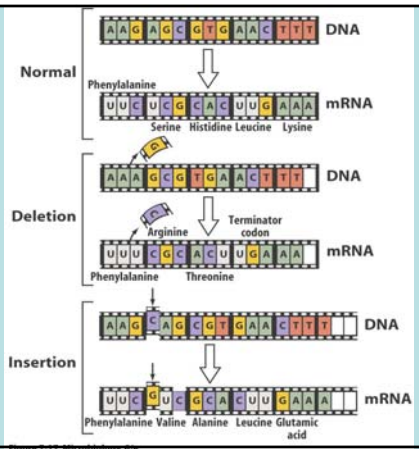


Point mutations can also produce a *stop* codon, making truncated protein

Mutations: ***Frameshift***

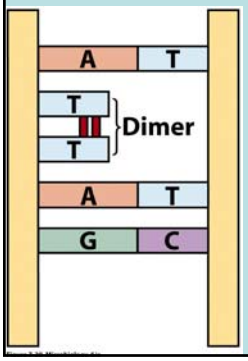
- Frameshift mutations: deletion or insertion of nucleotides
  - Alter the amino acid sequence of *entire protein* from that point on
- NOT if in multiples of 3
  - Insertion or deletion of 3/6/9 etc. nucleotides will insert or delete one/two/three amino acids but other amino acids will remain the same

FRAMESHIFTS



Types of Mutations	Effects on Organisms
Point Mutation	
Single base change in DNA with no change in the amino acid specified by the mRNA codon.	No effect on protein; a "silent" mutation.
Change in DNA with change in the amino acid sequence specified by the mRNA codon.	Change in protein by substitution of one amino acid for another; can significantly alter function of protein.
Change in DNA that creates a terminator codon in mRNA.	Produces polypeptide of no use to organism and prevents synthesis of normal protein.
Frameshift Mutation	
Deletion or insertion of one or more bases in DNA.	Changes entire sequence of codons and greatly alters amino acid sequence; can introduce terminator codon and produce useless polypeptides instead of normal proteins.

Ultraviolet radiation

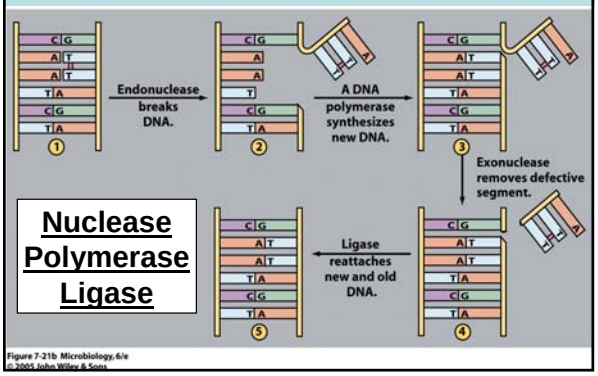


- Formation of pyrimidine dimers (such as T-T)
- Such damaged DNA cannot be replicated or transcribed normally

DNA repair

- *Some* bacteria are able to repair DNA damage & prevent permanent mutations
- Multiple mechanisms exist
  - **Light repair**: bonds between thymidine dimers are broken
    - Called "light" because expression of the enzyme involved is induced by light
  - **Dark repair**: see next slide

Dark repair



Nuclease
Polymerase
Ligase

★Enzymes that act on DNA

- **Nuclease**:
  - Cuts DNA backbone (breaks covalent bonds)
  - Many kinds, depends on where they work in a DNA strand, whether they cut one strand or both…
- **Polymerase**:
  - Synthesizes new DNA polymer (adds nucleotides)
- **Ligase**:
  - Pastes together broken DNA backbones (forms covalent bonds; opposite of nuclease activity)

Studying mutations: The Ames Test

- In eukaryotes, mutations can disrupt control of cell division, ultimately causing cancer
- Generally, mutagen = carcinogen

(a chemical that causes mutations may also cause cancer)

- Important question: how to determine if a chemical is a potential carcinogen?

Ames test

- One could expose lab animals to a chemical and wait years, looking for cancer to develop (expensive, slow)
- Or, screen for mutagenesis in bacteria! (fast, cheap)

Ames test

- Positive test: further study needed
- Negative test: chemical is probably safe