BIOL 240: General Microbiology

Spring 2020 Rm. 23-203 MW, Mar. 9-11

http://accounts.smccd.edu/staplesn/biol240/

1. **Pre-Lab Writeup #9 due!: ALL of Expt. 10.** Prepare before each Monday's labs (for BOTH Mon. & Wed.)!! ***(What? Why? How? are we doing in the lab? Question? HYPOTHESIS?)***
2. ***0.5-1 hour of OPEN LAB *expected* each week. Goal: 8-16h by May! ☺***
3. **LAB: Bacteriophages!!**
4. **QUIZ #4 DUE Wed. night!!!**
5. **Study Guides & Objectives (see slides) due THIS WEEK!! (Chs. 5b, 6, 7)!**
6. **Read Chs. 6 & 7!! See Chs. 6 & 7 lecture ONLINE!!**
   Objs. due THIS week! (MT2 Review Sheet is UPDATED!!)
7. **Midterm #1 REVIEW SESSIONS THIS WEEK: Mon/Wed 2-3pm…. in LAB. Wed. 8:15am-9:35ish. ☺ Come PREPARED with questions!**
8. ***Extra Credit Opportunity: Wed. evenings, 5-6 pm, (2/4-3/25/2020) in Bldg. 6, Room 102 – STEM SPEAKER Series. 1 page summary & reflection due (on CANVAS) the following week. (see also: YOUTUBE)***
   - **https://www.canadacollege.edu/stemcenter/speaker-series.php**
   - **NOTE: YOU may upload MULTIPLE Speaker Summaries, but only I can see all of them. You can only see the last upload! It's OK!! ☺**

1

REVIEW:

1. Diagram the **investment and release of Energy** and **Carbon** atoms from Glycolysis (through the Citric Acid Cycle).
2. Diagram or outline the process by which **high energy electron carriers** are produced by glycolysis, pyruvate oxidation, and the Krebs/TCA cycle.

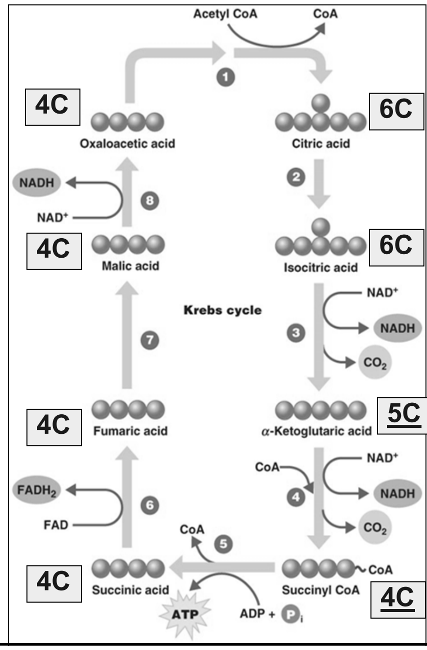
OBJECTIVES: Students should be able to: …..

1. Diagram how high energy **electrons are used to produce ATP** in the mitochondrial inner membrane (or bact. plasma mem).
2. Compare and contrast the **energy inputs and outputs of Fermentation & Aerobic Respiration/Oxidative** phosphorylation.
3. Explain how **lipids and proteins are catabolized** and energy harvested thru pathways shared with glucose metabolism.
4. Distinguish the **carbon and energy sources** for all of the **trophisms**: ***chemo, photo, hetero, auto***, & comb'ns of each.
5. Diagram how **catabolic** and **anabolic** pathways can share intermediates to efficiently regulate energy storage, energy usage, and biosynthesis.
6. **Ch. 8:** Describe the **Central Dogma** of molecular genetics and the three processes that drive the flow of genetic information in an organism.
7. Describe several **properties of DNA** and the process of **DNA replication** that contribute to DNA's central role as the hereditary material.
8. Draw a **replication fork** and label **5 enzymes involved in DNA replication**. Describe the function of each enzyme.

- ***These questions are your HOMEWORK between classes!!!***
  - ***DUE (and/or Study Guide questions) WED. at the start of Lab!!***

2

Krebs Cycle (2x/glc)



http://www.science.smith.edu/departments/Biology/Bio231/krebs.html

http://highered.mcgraw-hill.com/sites/0072507470/student_view0/chapter25/animation__how_the_krebs_cycle_works__quiz_1_.html

Figure 5.13.2

3

D. The Electron Transport Chain

- A series of carrier molecules that are, in turn, oxidized and reduced as electrons are passed down the chain.

- Energy released can be used to produce ATP by ***<u>ChemiOsmosis</u>***.

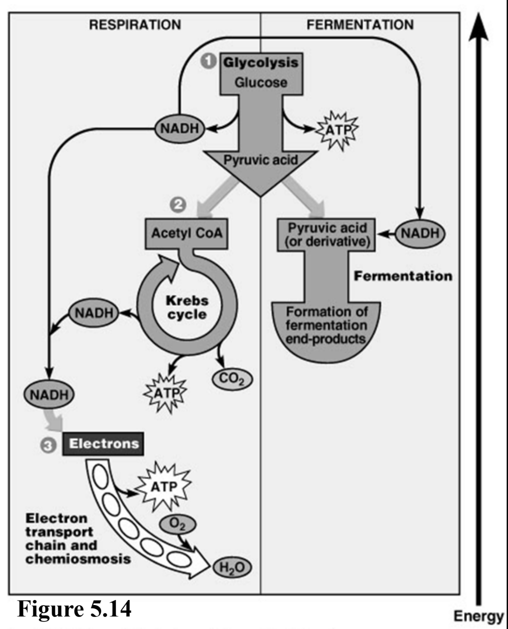
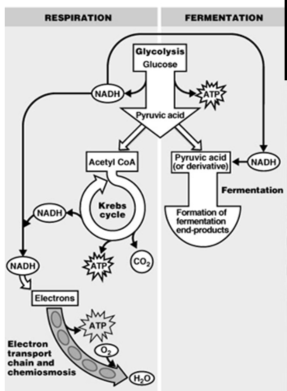


Figure 5.14

4

E. *Chemi-osmosis*

- **Each NADH → ~3 ATP**
- **Each $FADH_2$ → ~2 ATP**



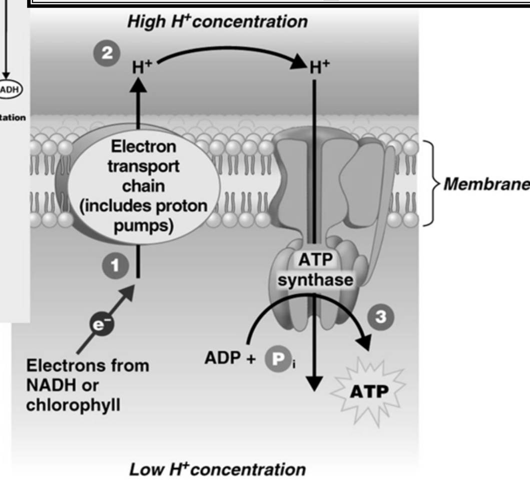


Figure 5.15

➢ ***Create <u>gradient of [H+]</u> and <u>electric charge</u> — the <u>Proton-Motive Force</u>.***

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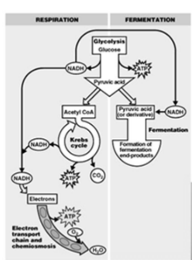
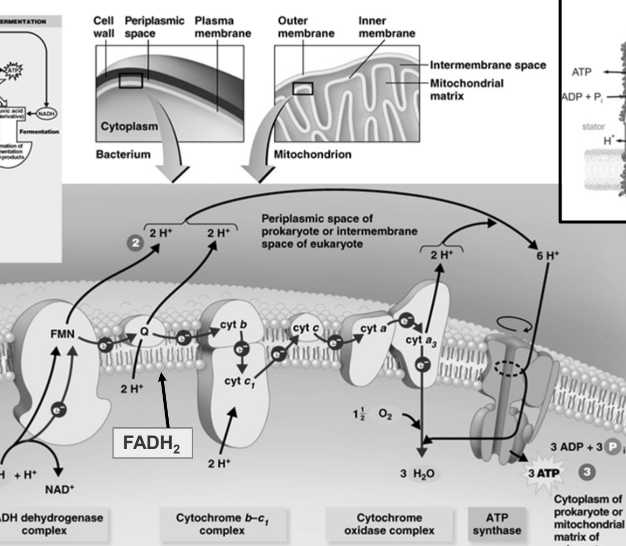
Chemiosmosis





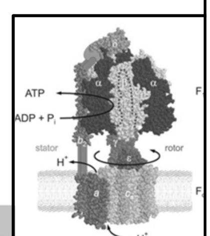


Figure 5.16

- **Stronger Electron-Acceptors/Oxidizers →→**

http://vcell.ndsu.nodak.edu/animations/etc/movie.htm

6

5.6) Respiration ★

- ***Aerobic respiration:*** The final electron acceptor in the electron transport chain is molecular oxygen (O_2).
- ******ETC + Chemiosmosis = Oxidative Phosphorylation!!******
- ***<u>Anaerobic</u> respiration:*** The final electron acceptor in the electron transport chain is not O_2.
  - Yields less energy than aerobic respiration
    - only part of the Krebs cycle operates w/out O_2.

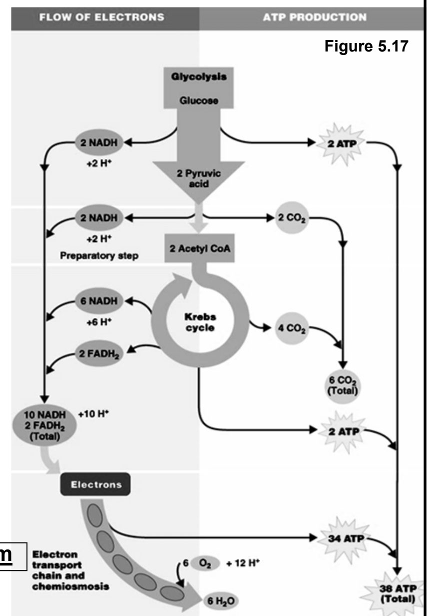


Figure 5.17

http://vcell.ndsu.nodak.edu/animations/etc/movie.htm

http://www.science.smith.edu/departments/Biology/Bio231/krebs.html

http://www.science.smith.edu/departments/Biology/Bio231/etc.html

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A. Aerobic Respiration

<u>Pathway</u>	<u>Eukaryote</u>	<u>Prokaryote</u>
Glycolysis	Cytoplasm	Cytoplasm
Intermediate step (Pyruvate Ox'n)	Mito. Inner Memb	Cytoplasm
Krebs cycle	Mitochondrial matrix	Cytoplasm
ETC	Mitochondrial inner membrane	**Plasma membrane**

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- **<u>Energy produced (types)</u> – from complete oxidation of 1 glucose using aerobic respiration**

Pathway	ATP produced	NADH produced	$FADH_2$ produced
Glycolysis	2	2	0
Pyruvate Oxidation	0	2	0
Krebs cycle	2	6	2
Total	**4**	**10**	**2**

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❖ **<u>ATP produced (chemical processes)</u>** from complete oxidation of **<u>1 glucose</u>** using aerobic respiration.

Pathway	By Substrate-Level Phosphorylation	By Oxidative Phosphorylation	
		<u>From NADH</u>	**<u>From $FADH_2$</u>**
Glycolysis	2	6	0
Pyruvate Oxidation	0	6	0
Krebs cycle	2	18	4
<u>Total</u>	**<u>4</u>**	**<u>30</u>**	**<u>4</u>**

- **38** ATPs are produced in ***prokaryotes***, **36** in ***Euk***.

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Aerobic Metabolic Yield, Summary (Eukaryotes):

NET:

1. Glyc = **2 ATP** (4 made, 2 invested)

2 NADHH → 4 ATP ***(use 2 ATP** on transport into **mito IM!!)***

2. Pyr.Ox.= 2 NADHH → 6 ATP

3. TCA = 6 NADHH → 18 ATP

2 GTP → 2 ATP

2 FADH2 → 4 ATP

36 ATP total NET from Glycolysis & Oxidative Respiration

http://vcell.ndsu.nodak.edu/animations/atpgradient/movie.htm

http://www.wiley.com/legacy/college/boyer/0470003790/animations/electron_transport/electron_transport.swf

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B. *Anaerobic Respiration*

= truly *RESPIRATION*, and NOT glycolysis/fermentation!

Electron acceptor	Products
NO_3^-	NO_2^-; N_2 + H_2O
SO_4^{2-}	H_2S + H_2O
CO_3^{2-}	CH_4 + H_2O

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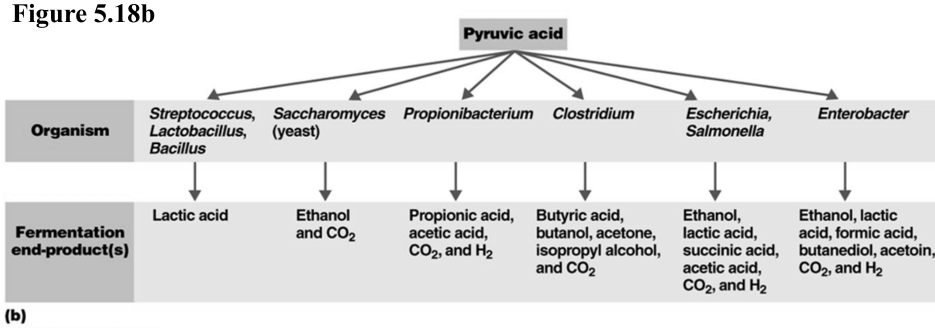
5.7) Fermentation

1. Releases energy from oxidation of organic molecules
2. Does not require oxygen
3. Does not use the Krebs cycle or ETC
4. Uses an organic molecule as the final electron acceptor

Figure 5.18b



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Types of Fermentation

A. ***Alcohol fermentation*** - Produces ethyl alcohol + CO_2

B. ***Lactic acid fermentation*** - Produces lactic acid.

- ***Homolactic fermentation*** - Produces lactic acid only.
- ***Heterolactic fermentation*** - Produces lactic acid and other compounds (eg: acetoin).

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Fermentation

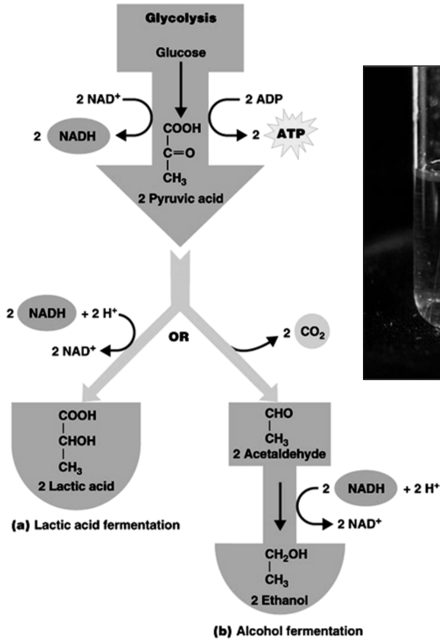
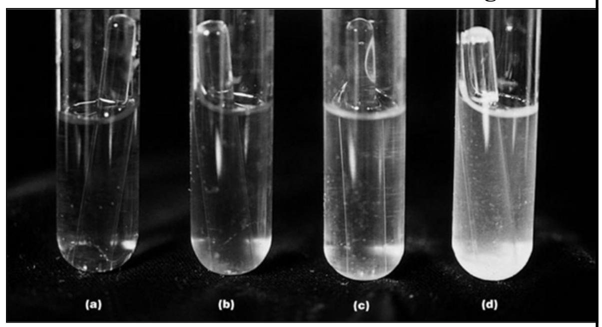


Figure 5.23



The Glucose song!!! …
https://youtu.be/jJvAL-iiLnQ

Figure 5.19

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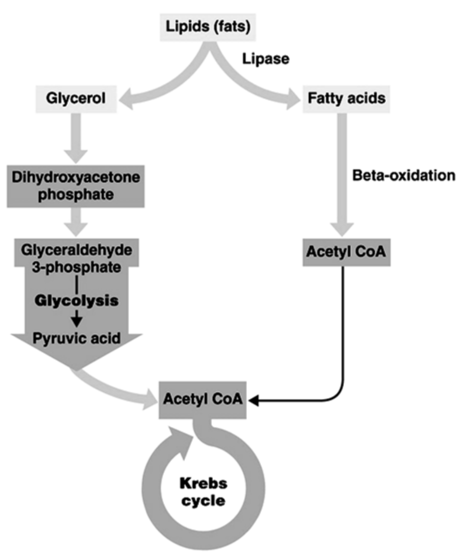
5.8) Other Catabolism: A. Lipid Catabolism



http://www.wiley.com/college/pratt/0471393878/student/animations/citric_acid_cycle/index.html

Figure 5.20

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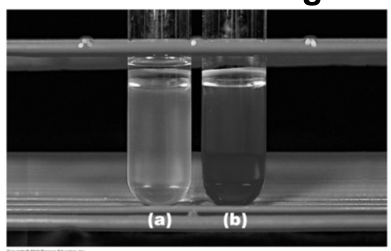
B. Protein Catabolism

Protein —*Extracellular proteases*→ **Amino acids**

—*Deamination (→ acid), decarboxylation (→ amine), dehydrogenation*→ **Organic acid** → Krebs cycle



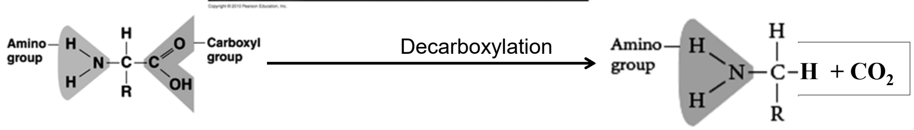


Figure 5.22

http://www.wiley.com/legacy/college/boyer/0470003790/animations/tca/tca.htm

17

** Biochemical tests **

- **Used to identify bacteria.**

Dichotomous Key:

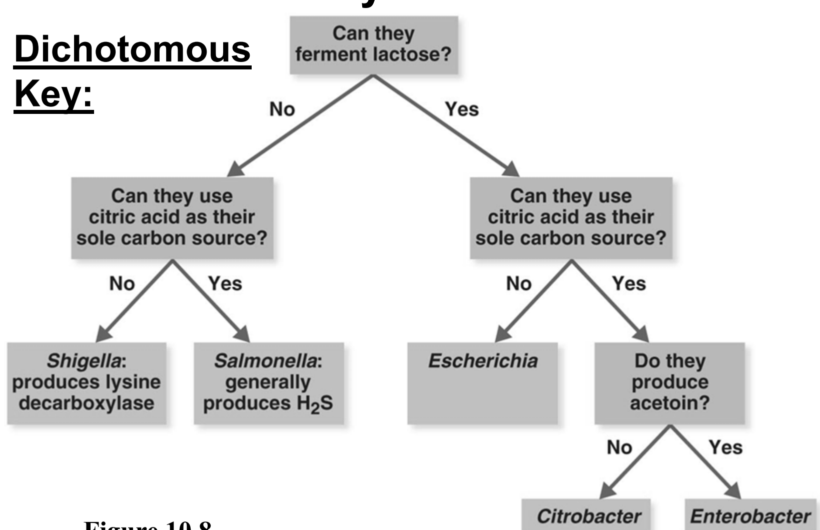


Figure 10.8

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5.9) Photosynthesis

- ***Photo:*** Conversion of light energy into chemical energy (ATP).
  - Light-dependent (light) reactions

- ***Synthesis:*** Fixing carbon into organic molecules.
  - Light-independent (dark) reaction, Calvin-Benson cycle
  - ***Oxygenic:***
    $6\ CO_2 + 12\ H_2O$ + Light energy $\rightarrow C_6H_{12}O_6 + 6\ O_2 + 6\ H_2O$
  - ***Anoxygenic***:
    $CO_2 + 2\ H_2S$ + Light energy $\rightarrow [CH_2O] + 2\ S + H_2O$

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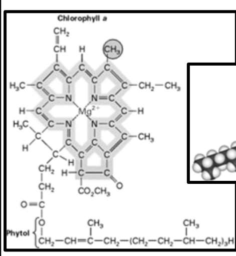
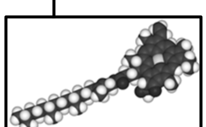
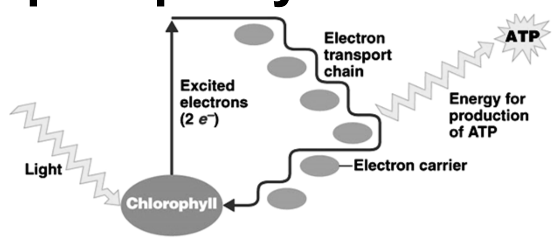
A. Cyclic Photophosphorylation

ATP ONLY!!!! (No NADPH)







(a) Cyclic photophosphorylation

B. Noncyclic Photophosphorylation

ATP AND NAD<u>P</u>H made!!!!

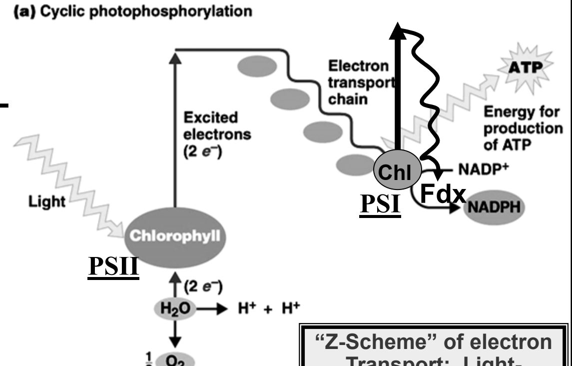


Figure 5.25 (b) Noncyclic photophosphorylation

"Z-Scheme" of electron Transport: Light-energized TWICE!!

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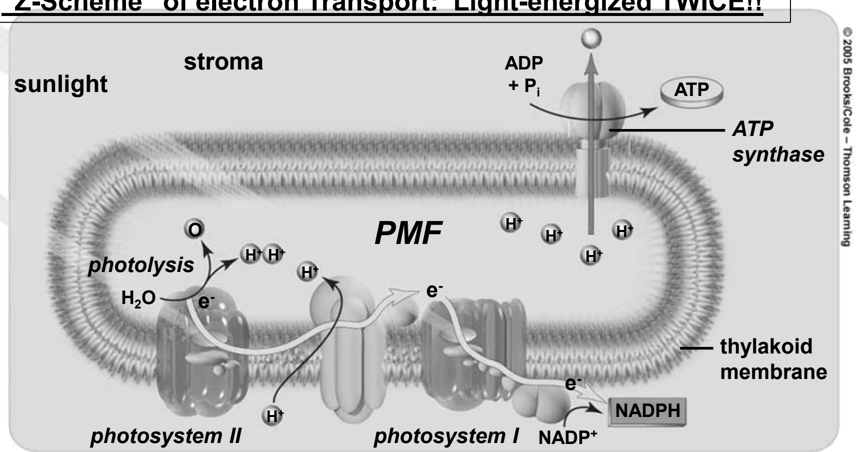
Noncyclic Pathway

"Z-Scheme" of electron Transport: Light-energized TWICE!!



http://vcell.ndsu.nodak.edu/animations/photosynthesis/movie.htm

http://www.science.smith.edu/departments/Biology/Bio231/ltrxn.html

http://www.fw.vt.edu/dendro/forestbiology/photosynthesis.swf

http://www.stolaf.edu/people/giannini/flashanimat/metabolism/photosynthesis.swf

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C. Calvin-Benson Cycle: *Carbon-Fixation!*

- SYNTHESIS!!! = "Dark Reactions"
  - Using ATP & NADPH made in the light reactions!!

Step-By-Step Narration: http://www.cells.de/cellseng/1medienarchiv/Zellfunktionen/Memb_Vorg/Photosynthese/Dunkel_u_Staerke/Calvin-Benson-Zyklus/index.jsp

**http://www.science.smith.edu/departments/Biology/Bio111/calvin.html

Figure 5.26

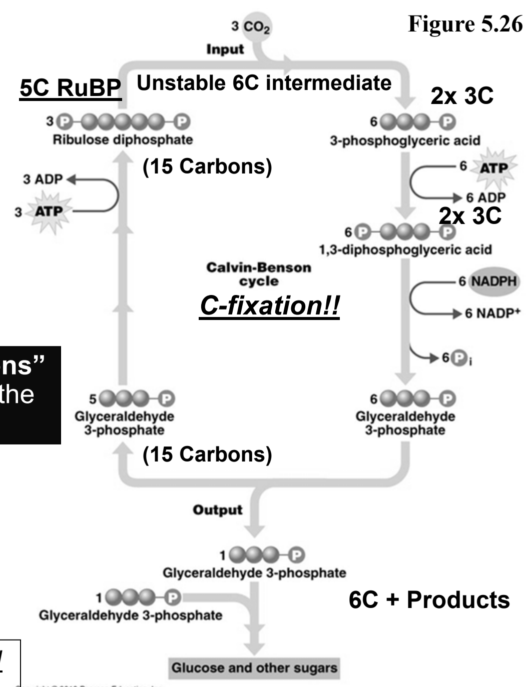

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Table 5.6 Photosynthesis Compared in Selected Eukaryotes and Prokaryotes

Characteristic	*Eukaryotes*	*Prokaryotes*		
	Algae, Plants	**Cyanobacteria**	**Green Bacteria**	**Purple Bacteria**
Substance That Reduces CO_2	H atoms of H_2O	H atoms of H_2O	Sulfur, sulfur compounds, H_2 gas	Sulfur, sulfur compounds, H_2 gas
Oxygen Production	Oxygenic	Oxygenic (and anoxygenic)	Anoxygenic	Anoxygenic
Type of Chlorophyll	Chlorophyll *a*	Chlorophyll *a*	Bacteriochlorophyll *a*	Bacteriochlorophyll *a* or *b*
Site of Photosynthesis	Chloroplasts with thylakoids	Thylakoids	Chlorosomes	Chromatophores
Environment	Aerobic	Aerobic (and anaerobic)	Anaerobic	Anaerobic

****Study figure 5. 28!! ** -- nutritional flow chart of organisms****

- ***Halobacterium*** uses **bacteriorhodopsin**,
  - not chlorophyll,
  - to generate electrons for a *chemiosmotic proton pump*.
    - Different pools drying, with different salinities.
      - Different pigmented species of *Halobacteria* grow in diff't [salt]s.

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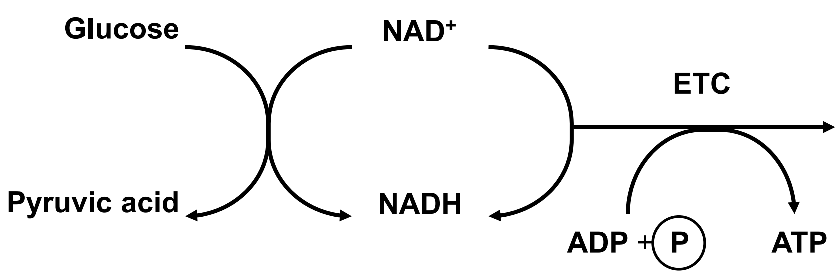
5.10) Chemotrophs

- Use energy from chemicals.
  - ***Chemoheterotroph.***



- Energy is used in anabolism.

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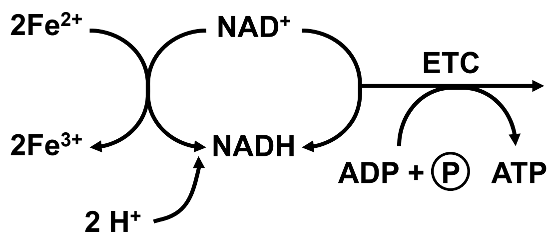
Chemotrophs

- Use energy from chemicals.
  - **Chemo*<u>auto</u>*troph**, *Thiobacillus ferroxidans*



- Energy used in the **Calvin-Benson** cycle to **fix CO_2**.

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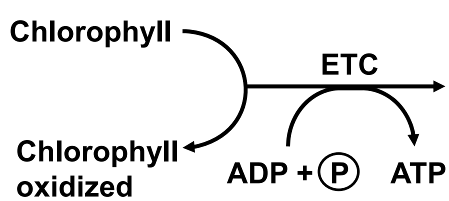
5.11) Phototrophs

- Use light energy.



- **Photo<u>auto</u>trophs** use energy in the Calvin-Benson cycle to fix CO_2.
- **Photo<u>hetero</u>trophs** use energy.

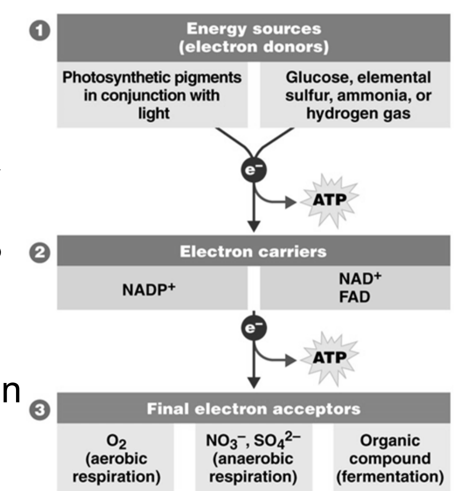


Figure 5.27

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Metabolic Diversity Among Organisms

Nutritional type	Energy source	Carbon source	Example
<u>Photo</u>autotroph	Light	CO_2	Oxygenic: Cyanobacteria plants. Anoxygenic: Green, purple bacteria.
Photo<u>hetero</u>troph	Light	Organic compounds	Green, purple nonsulfur bacteria.
Chemo<u>auto</u>troph	Chemical	CO_2	Iron-oxidizing bacteria.
<u>Chemo</u>heterotroph	Chemical	Organic compounds	Fermentative bacteria. Animals, protozoa, fungi, bacteria.

**** Study figure 5.28!! ****

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5.12) Anabolism: Metabolic Pathways of Energy Use

A. Polysaccharide Biosynthesis

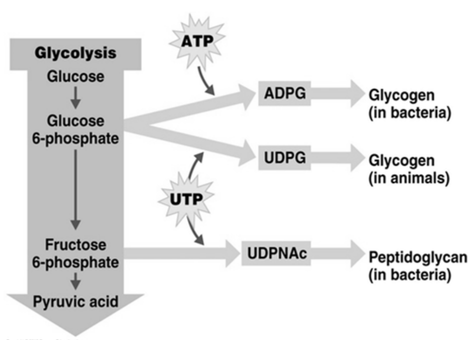

Figure 5.29

B. Lipid Biosynthesis

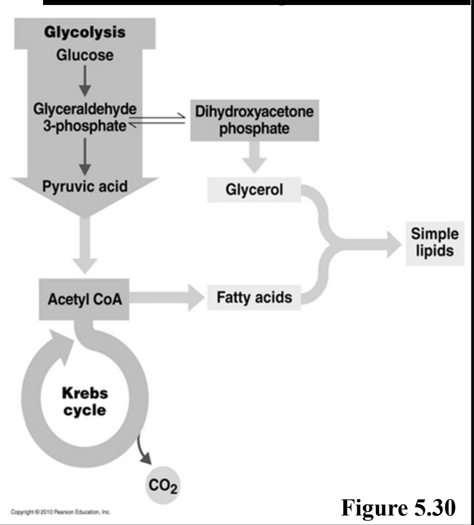

Figure 5.30

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Anabolism/Biosynthesis

C. Amino Acid and Protein Biosynthesis

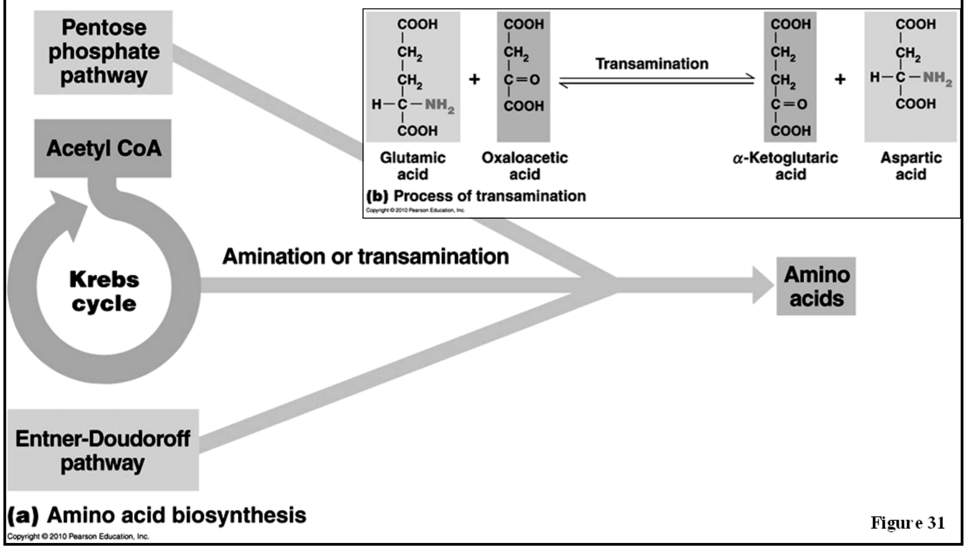

Figure 31

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Anabolism

D. Purine and Pyrimidine Biosynthesis

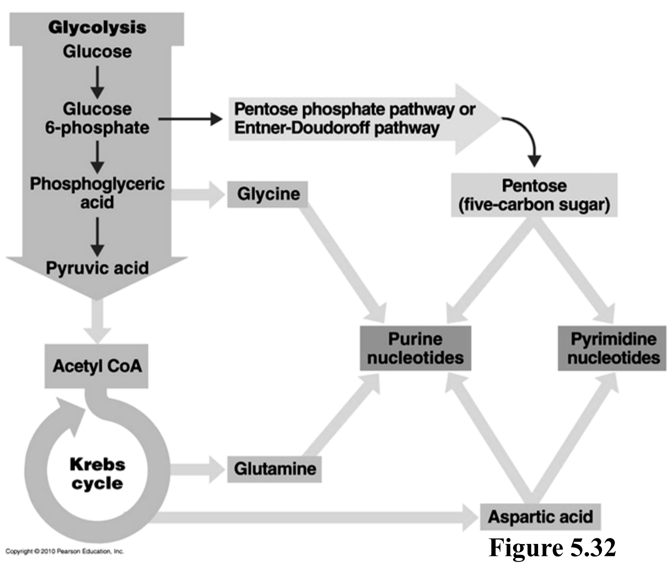
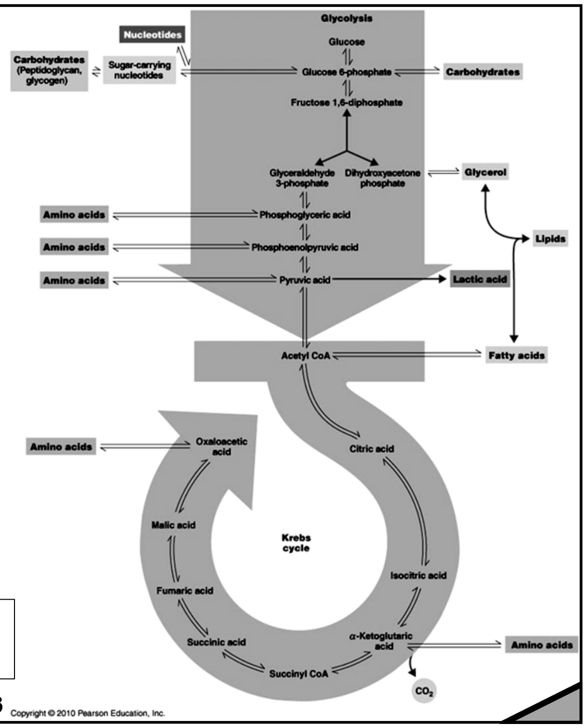


Figure 5.32

30

5.13) ***Amphibolic*** pathways

- Are metabolic pathways that have **both *catabolic* and *anabolic*** functions.



Glucose, Glucose!!
https://youtu.be/jJvAL-iiLnQ

Figure 5.33

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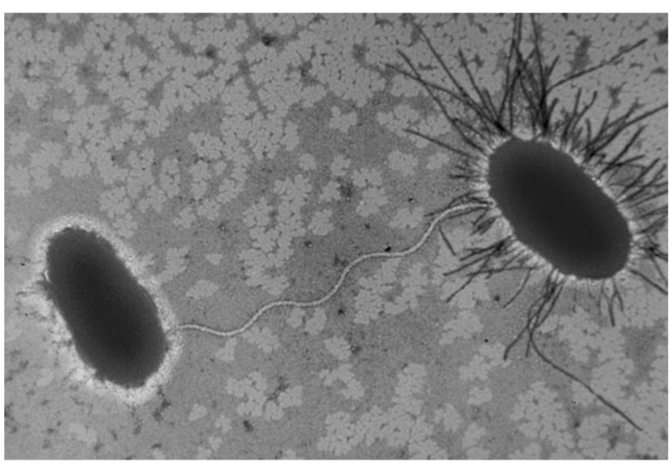
Chapter 8 Microbial Genetics



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Terminology

1. ***Genetics:*** Study of what genes are, how they carry information, how information is expressed, and how genes are replicated; "the science of heredity"
2. ***<u>Gene:</u>*** Segment of DNA that encodes a functional product, usually a protein
3. ***Genome*** = All of the genetic material in a cell
4. ***Genomics*** = Molecular study of genomes
5. ***<u>Genotype</u>*** = Specific forms of genes in an organism
   – Types of alleles present.
6. ***<u>Phenotype</u>*** = *physical characteristics resulting from expression of the genes*

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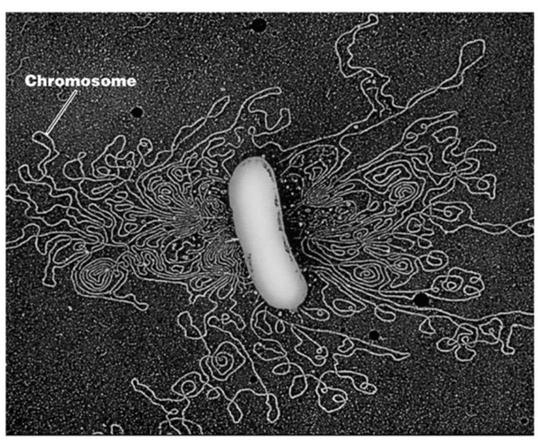
E. coli



(a) The tangled mass and looping strands of DNA emerging from this disrupted *E. coli* cell are part of its single chromosome.

The Model Organism for molecular biology.

Figure 8.1a

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Flow of Genetic Information: The Central Dogma

Replication

Transcription *Translation*

DNA → (m)RNA → Protein

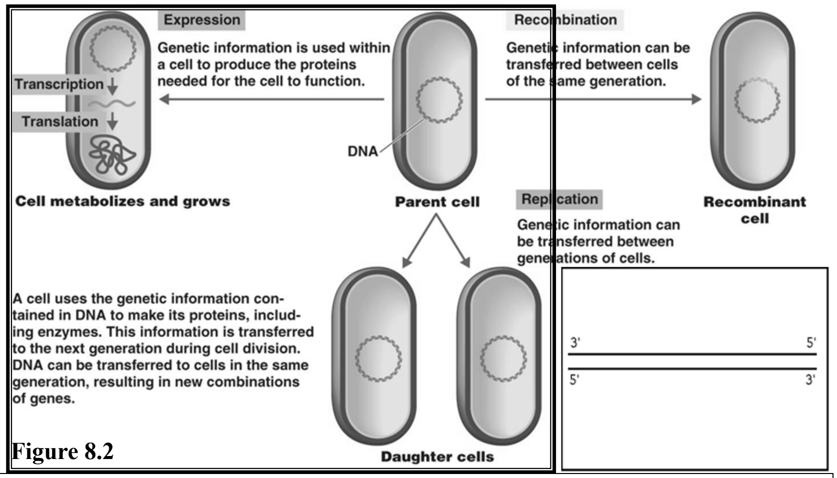

Figure 8.2

** ***Central Dogma*** **of Molecular Genetics** **

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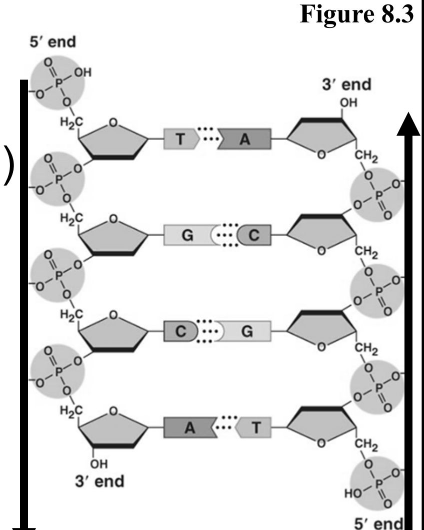
8.1) DNA

1. Polymer of nucleotides: adenine, thymine, cytosine, guanine (**ATGC**)
2. Double helix associated with proteins
3. "Backbone" is deoxyribose-phosphate
4. Strands held together by hydrogen bonds between **A=T** and **G≡C**
5. Strands are ***Antiparallel***
   - ***5' (PO_4)→ 3' (OH) polarity***

Figure 8.3



(b) The two strands of DNA are antiparallel. The sugar-phosphate backbone of one strand is upside down relative to the backbone of the other strand. Turn the book upside down to demonstrate this.

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A. DNA Structure

- ***Semi-Conservative Replication:***
  - Each "parental strand" serves as ***template…***
  - for synthesis of a new "daughter strand"
  - Following ***complementary base-pairing*** rules
    - **A=T**
    - **G≡C**
  - *Rules allow one to predict 2nd strand sequence from 1st!!!*

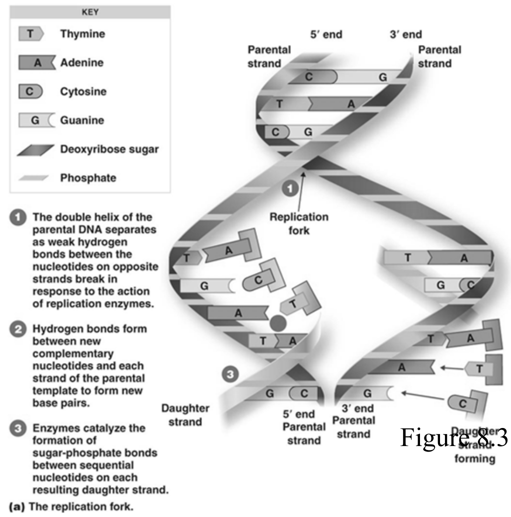

Figure 8.3

http://www.fed.cuhk.edu.hk/~johnson/teaching/genetics/animations/dna_replication.htm
http://www.uic.edu/classes/cmeng/cmeng521/DNA_replication.html

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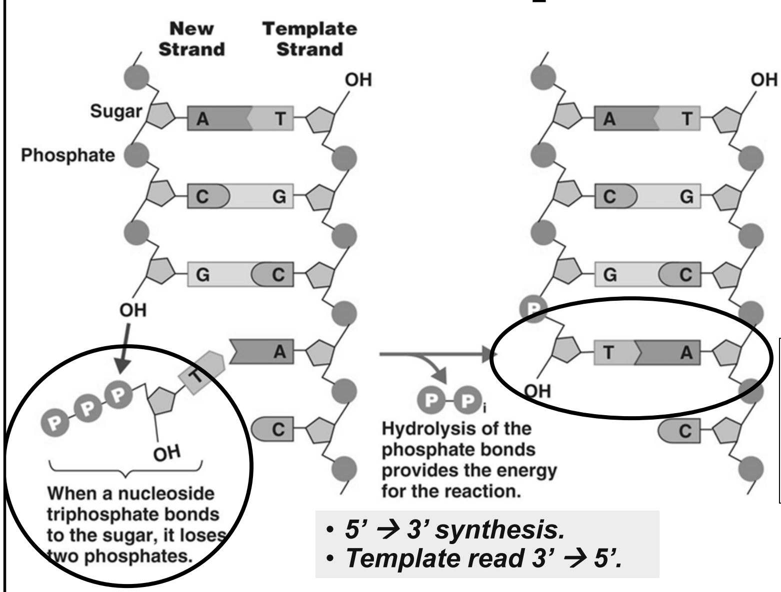
B. DNA Replication

= DNA-directed DNA synthesis.



- ***5' → 3' synthesis.***
- ***Template read 3' → 5'.***

http://www.fed.cuhk.edu.hk/~johnson/teaching/genetics/animations/dna_replication.htm

http://www.uic.edu/classes/cmeng/cmeng521/DNA_replication.html

http://www.courses.fas.harvard.edu/~biotext/animations/replication1.swf Figure 8.5

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DNA Replication Proteins

1. Open helix at ***Origin (Ori)***, lay-down primers:
   - ***a)*** ***DNA Helicase*** "melts" strands apart, breaking H-bonds
   - b) Single-Strand Binding Proteins (**SSB**) keep template strands apart.
   - ***c)*** ***RNA Primase*** lays down first several nucleotides (RNA!!) – gives "starting block" (free 3'-OH) to begin actual DNA synthesis. [RNA Primers are removed later!]
2. DNA = copied by ***DNA polymerase III*** (Dpol3)
3. In the 5′ → 3′ direction – new nucleotides added to the 3' hydroxyl (-OH) group on deoxyribose in the growing strand
   - Initiated by an ***RNA primer*** (*RNA Primase* enz.)
4. **Leading strand** synthesized continuously
   - follows fork (1/fork; 2/ "bubble")
5. **Lagging strand** synthesized discontinuously
   - Opposite to fork movement → ***Okazaki fragments*** *(unsealed lagging pieces)*
6. RNA primers are removed and Okazaki fragments joined by ***DNA polymerase I*** & ***DNA ligase*** → ***"fill and seal" to finish job!!***

AMOEBA SISTERS!!!!
https://youtu.be/5qSrmeiWsuc

- **http://www.stolaf.edu/people/giannini/flashanimat/molgenetics/dna-rna2.swf**

Detailed!: http://www.wiley.com/college/pratt/0471393878/student/animations/dna_replication/index.html

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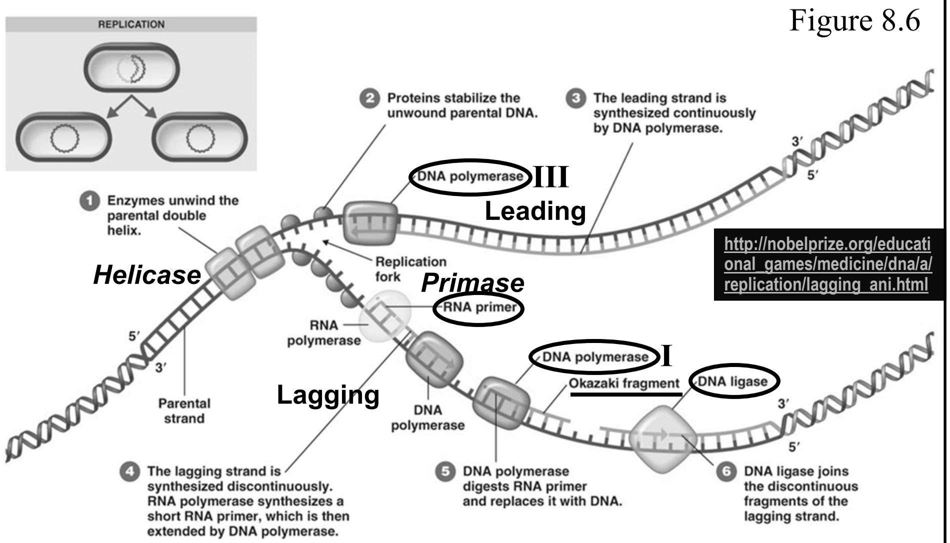
C. DNA Replication Fork

Figure 8.6



http://nobelprize.org/educational_games/medicine/dna/a/replication/lagging_ani.html

http://www.stolaf.edu/people/giannini/flashanimat/molgenetics/dna-rna2.swf

http://highered.mcgraw-hill.com/sites/0072943696/student_view0/chapter3/animation__dna_replication__quiz_1_.html

http://www.johnkyrk.com/DNAreplication.html **http://www.dnalc.org/resources/3d/DNAReplicationBasic_w_FX.html**

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DNA replication is ***Semiconservative*** & ***Bidirectional***

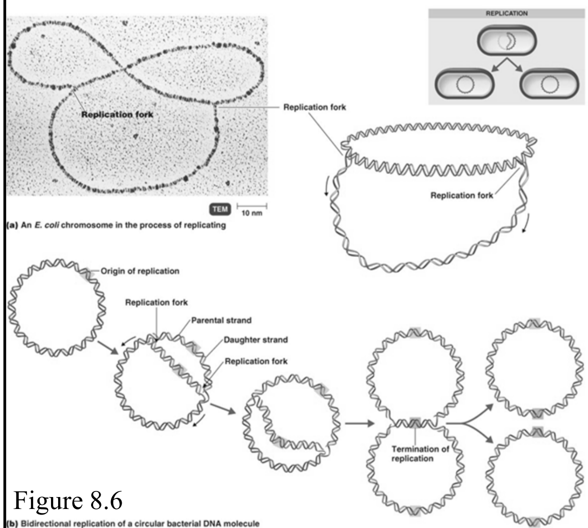

Figure 8.6

- ***= DNA-directed DNA synthesis!!!***
- ***2 forks: opposite directions!***
- Replication results in two daughter DNA duplexes,
  - ***each with one completely new strand, &***
  - ***one old strand (parental strand)***
  - ***= "SEMI-CONSERVATIVE"***
  - ***ONLY synthesize 5'→3'!!!***
- Two replication forks move in opposite directions =
- ***"Replication Bubble"***

http://www.dnalc.org/resources/3d/DNAReplicationBasic_w_FX.html
http://www.hhmi.org/biointeractive/dna/animations.html

http://www.wwnorton.com/college/biology/mbio/animations/dna_replication.asp
http://www.sciencemedia.com/website/demos/biochem/ecoliReplication.html

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8.2) Transcription: RNA Synthesis

1. DNA is transcribed to make RNA (**AUGC**)
   - **a) mRNA** = messenger RNA → translated to protein
   - **b) tRNA** = transfer RNA → brings amino acid to ribos.
   - **c) rRNA** = ribosomal RNA → makes up ribo; catalytic
2. Transcription begins when ***RNA polymerase*** binds to the ***PROMOTER*** sequence
   - *Tells Rpol which strand, and where to start transcribing!!*
3. Transcription proceeds in the ***5′ → 3′ direction*** (same in ALL nucleic acid synthesis!)
   - new nucleotides added to the 3' hydroxyl group on ***ribose*** in the growing strand
4. Transcription stops when it reaches the ***Terminator Sequence*** *(often many U's or "hairpin loop")*
   - New RNA and Rpol fall off of DNA template.

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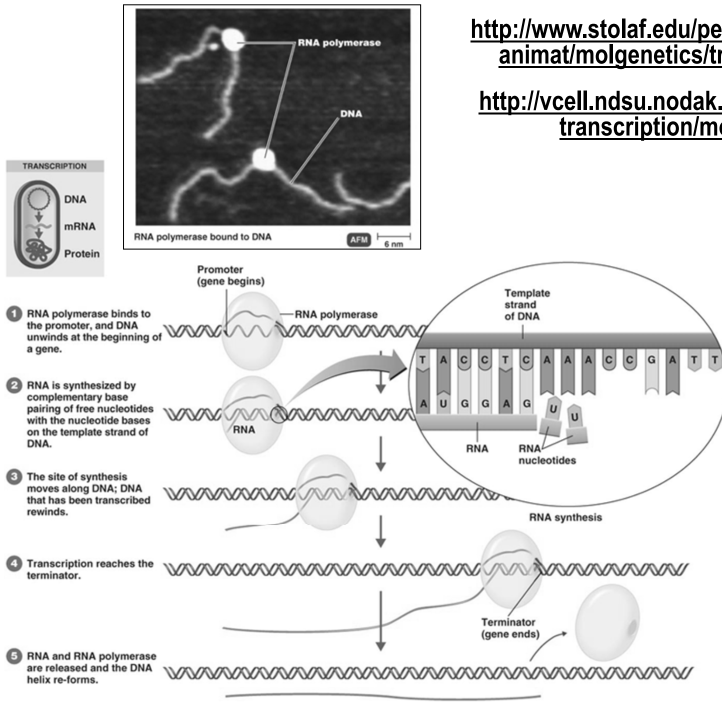

http://www.stolaf.edu/people/giannini/flashanimat/molgenetics/transcription.swf

http://vcell.ndsu.nodak.edu/animations/transcription/movie.htm

Termination: (eg:)
- **polyU sequence, or**
- **Stem-loop in RNA**

Figure 8.7

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8.3) Translation

1. ***= RNA-directed Protein synthesis!!!***
2. mRNA is translated in ***Codons.***
   - 3 nucleotide "words"
3. Translation of mRNA begins at the ***start codon*: AUG.**
   - ***Tells ribosome where to start translating,***
   - ***and sets the reading Frame!!!***
4. Translation ends at a ***STOP ("nonsense") codon*: UAA, UAG, UGA.**
   - ***Do NOT encode an amino acid!!!***

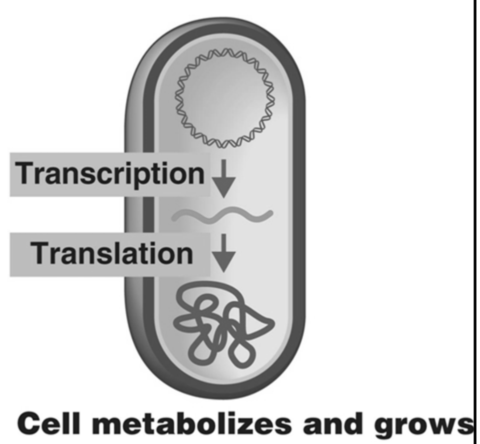

Figure 8.2

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