BIOL 230: Cell & Molecular Biology

Fall 2019 17-205 M, Oct. 7

http://accounts.smccd.edu/staplesn/biol230/

1. Pre-Lab writeups due each Mon. (for both M&W!!) at the start of lab. (*briefly*, **What**? **Why**? **How**? for each expt.). Question & **Hypothesis**?!

2. **LAB this week: DNA Extraction!!!**
3. **Ch. 10, PHOTOSYNTHESIS lecture will be posted online this week!!**
4. **Native PAGE data** is posted under **"Add'l Materials."**
   - ❖ (Enzyme/Tyrosinase Rpt. Due 10/11 Online); Rough draft due TODAY!!!
5. **Extra Credit: STEM SPEAKER SERIES,** Weds. @ 5pm-6pm, Sept. 11- Nov. 6. (NOT Oct. 9) in 6-102. *Write 1 page summary by the following week, and upload to CANVAS*. Extra-Extra credit: Ask the speaker a scientific question, and write about the answer.
6. **NEXT Wed.: Review for Midterm #2!! (Ch. 8, 9, 10, 13a?)**

1

REVIEW

1. List and describe the effects of 5 factors that can **regulate** enzyme activity.
2. Diagram and describe the **forms in which energy** may be transferred between molecules and reactions in cells.
3. Define **Glycolysis &** describe how **redox** reactions & **phosphorylations** drive the process.

TODAY's Objectives: Students should be able to....

1. Diagram and describe the **forms in which energy** may be transferred between molecules and reactions in cells.
2. Outline or diagram the **energy** inputs and outputs of Glycolysis and Cellular Respiration. What types of **cofactors** and biomolecules are involved in these processes?
3. Diagram the inputs & outputs of **carbons** during Glycolysis and Cellular Respiration.
4. Explain how ATP is synthesized in mitochondria, including the electron transport process. Define **substrate-level phosphorylation, chemiosmosis, & oxidative phosphorylation.**
5. **Ch. 13:** Describe the separate experimental processes by which **Griffith, Avery et al., and Hershey/Chase** proved the identity of the Genetic Material.

❖ ***Objectives and Study Guide Questions are your HOMEWORK between classes!!! DUE WED. at the end of Lecture!!***

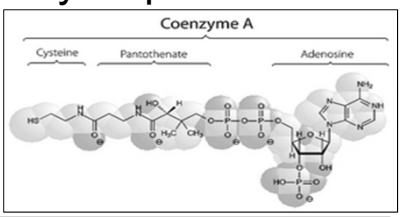
2

C. The Citric Acid Cycle (TCA)

1. Energy in <u>acetyl CoA</u> drives rxn:
   - **<u>Acetate (2C) + oxaloacetate (4C) → citrate (6C)</u>.**

2. TCA Cycle = series of rxns that *oxidize CITR and regenerate OAA (OXAL)*

3. For <u>each acetyl CoA</u>, TCA Cycle produces:
   - **2 CO_2**
   - **3 NADH** (52 kcal/mol)
   - **1 $FADH_2$** (43 kcal/mol)
   - **1 ATP** (12 kcal/mol)



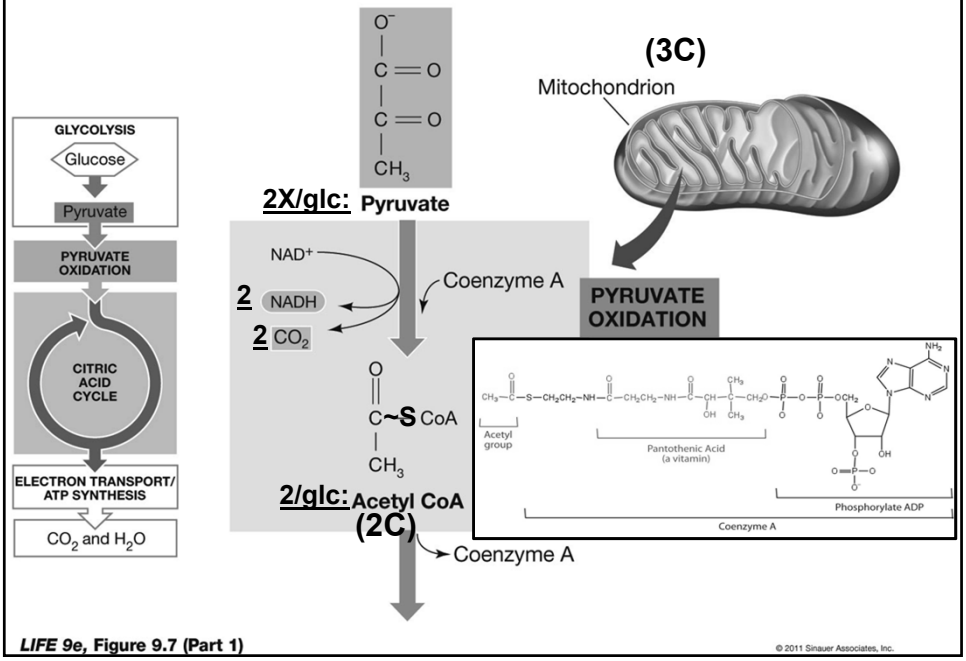
http://www.science.smith.edu/departments/Biology/Bio231/krebs.html

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

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

3

Pyruvate Oxidation & the Citric Acid Cycle



LIFE 9e, Figure 9.7 (Part 1)

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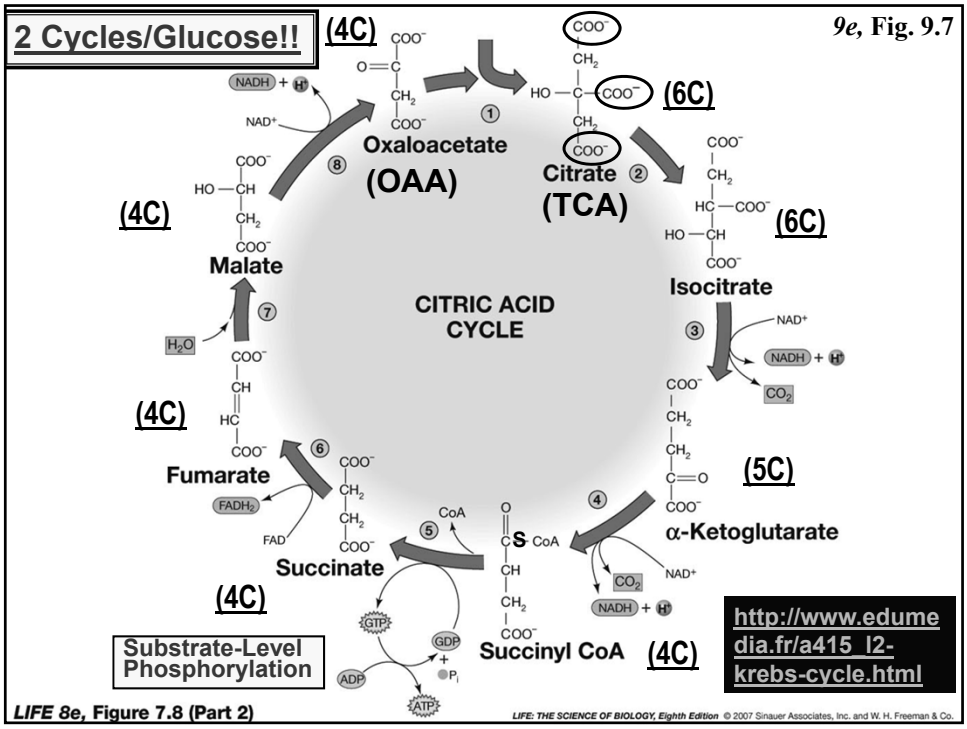
2 Cycles/Glucose!!
9e, Fig. 9.7
(4C)
Oxaloacetate
(OAA)
(6C)
Citrate
(TCA)
(6C)
Isocitrate
(5C)
α-Ketoglutarate
(4C)
Succinyl CoA
(4C)
Succinate
(4C)
Fumarate
(4C)
Malate
CITRIC ACID CYCLE
Substrate-Level Phosphorylation
http://www.edumedia.fr/a415_l2-krebs-cycle.html
LIFE 8e, Figure 7.8 (Part 2)
LIFE: THE SCIENCE OF BIOLOGY, Eighth Edition © 2007 Sinauer Associates, Inc. and W. H. Freeman & Co.

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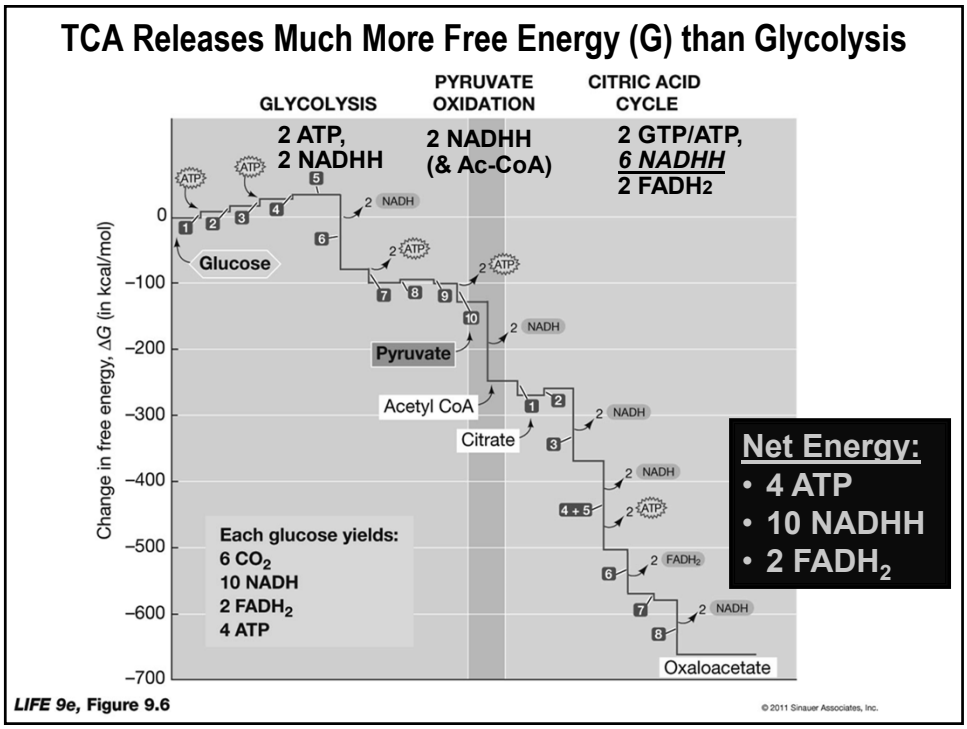
TCA Releases Much More Free Energy (G) than Glycolysis
GLYCOLYSIS
2 ATP,
2 NADHH
PYRUVATE OXIDATION
2 NADHH
(& Ac-CoA)
CITRIC ACID CYCLE
2 GTP/ATP,
6 NADHH
2 FADH2
Change in free energy, ΔG (in kcal/mol)
0
−100
−200
−300
−400
−500
−600
−700
Glucose
Pyruvate
Acetyl CoA
Citrate
Oxaloacetate
Each glucose yields:
6 CO2
10 NADH
2 FADH2
4 ATP
Net Energy:
• 4 ATP
• 10 NADHH
• 2 FADH2
LIFE 9e, Figure 9.6
© 2011 Sinauer Associates, Inc.

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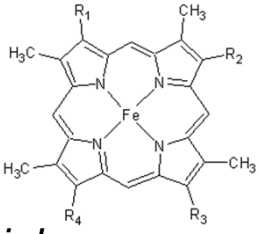
D. The Respiratory Chain (ETC): Electrons, Proton Pumping, and ATP

1. ***NADH + H^+*** and ***$FADH_2$*** (Glyc, Pyr Ox, TCA)
   - **oxidized by the respiratory chain (ETC)**
   - **regenerating NAD^+ and FAD.**



Porphyrin Ring

2. Most of the enzymes and other electron carriers of ETC are part of the *inner mitochondrial membrane*.
3. ***O_2 is the final acceptor*** of electrons and protons, forming H_2O.

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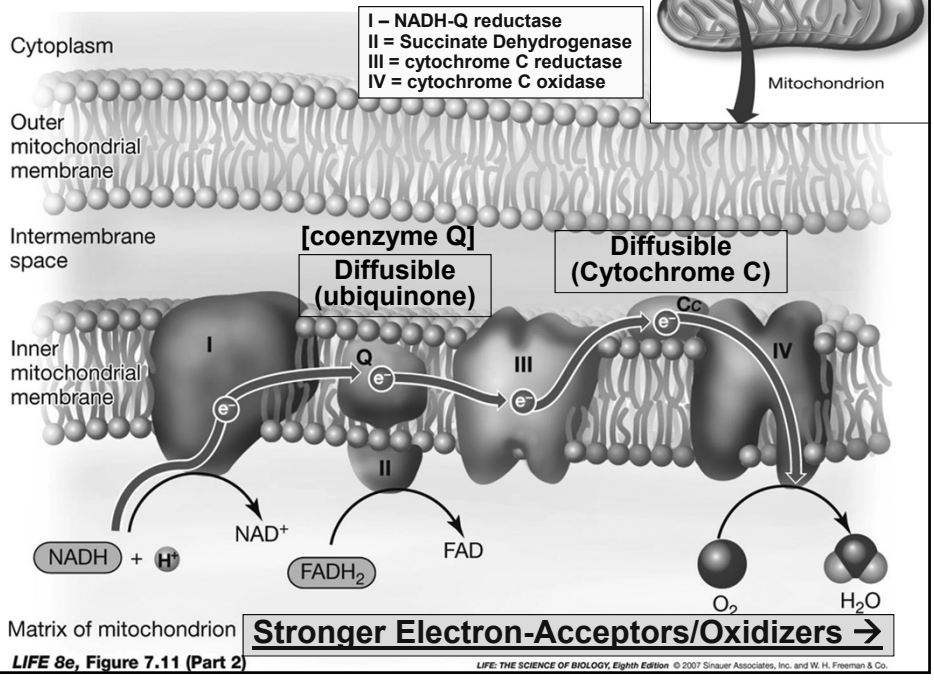
9.11 The Oxidation of NADH + H+



LIFE 8e, Figure 7.11 (Part 2)

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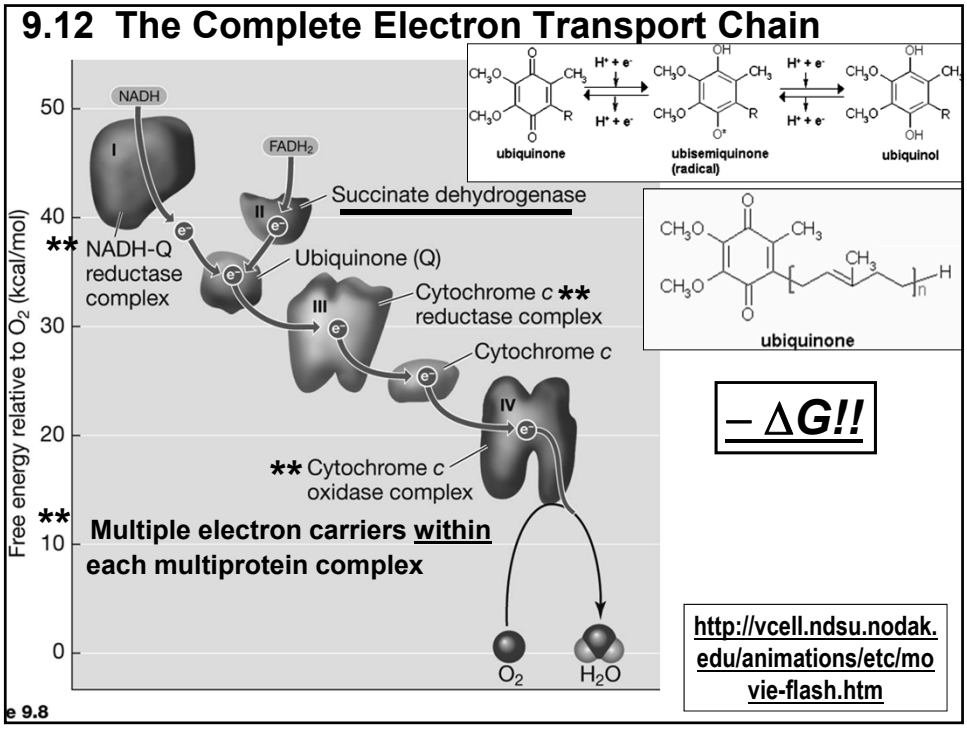

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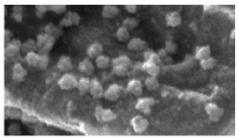
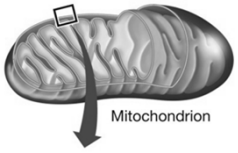
1. CHEMIOSMOSIS

- The **chemiosmotic mechanism** *couples* proton transport to **oxidative phosphorylation**.
  - (PMF → ATP synthesis)





- Electrons moving down the ETC release energy:
  - captured by proton (H^+) pumps:
    - *actively transport* H^+ out of the mitochondrial matrix (Ch. 5)

❖ *create gradient of [H+] and electric charge — the **Proton-Motive Force**.*

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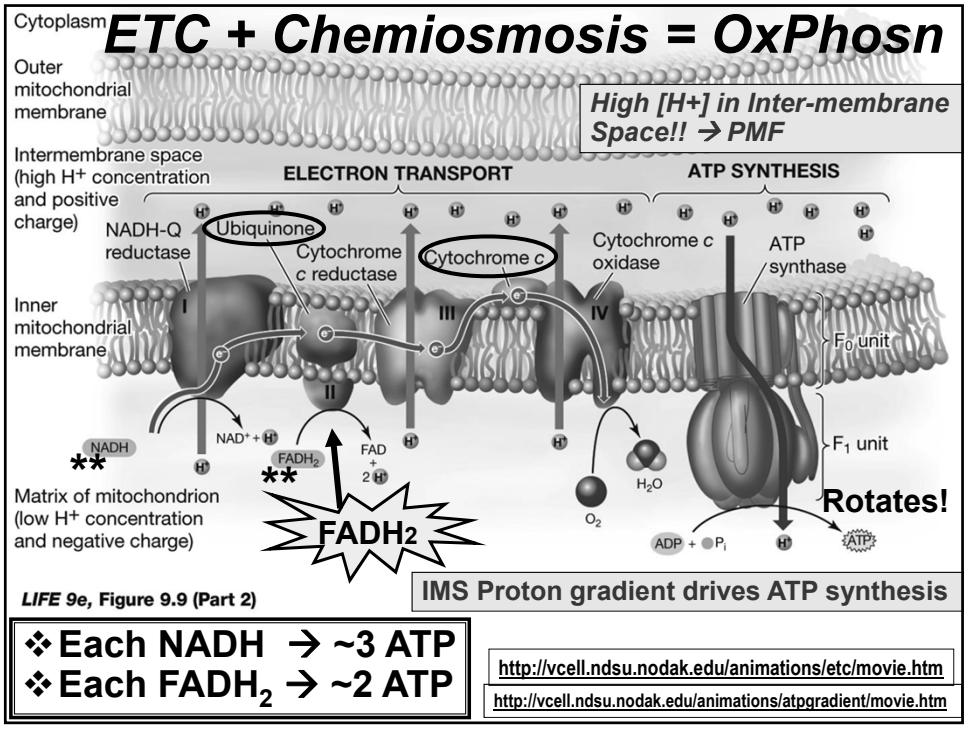

- Each NADH → ~3 ATP
- Each $FADH_2$ → ~2 ATP

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

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

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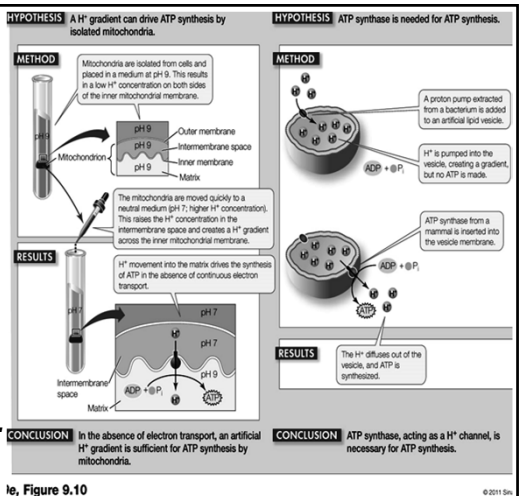
2. PMF

- The **Proton-Motive Force** causes **H+ to diffuse** back into the mitochondrial interior (MATRIX) **thru ATP synthase**, which
  - ***couples H+ diffusion to the production of ATP.***



- ***Uncoupling → Heat!!***
  (***thermogenin*** in newborns)
  - Proton pore, without ATP synthase;
  - also in ***brown fat***
- *Voodoo Lily* – (***calorigen***) early pollination, aromatic amines & indoles

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E. Fermentation: ATP from Glucose, without O_2

- Anaerobic organisms
  - energy from ***glycolysis & fermentation.***
  - partly oxidize glucose
    - generate energy-containing products
      - (Lactate, EtOH)
  - anaerobically oxidize the NADH + H^+ produced in glycolysis → ***recycle NAD+.***

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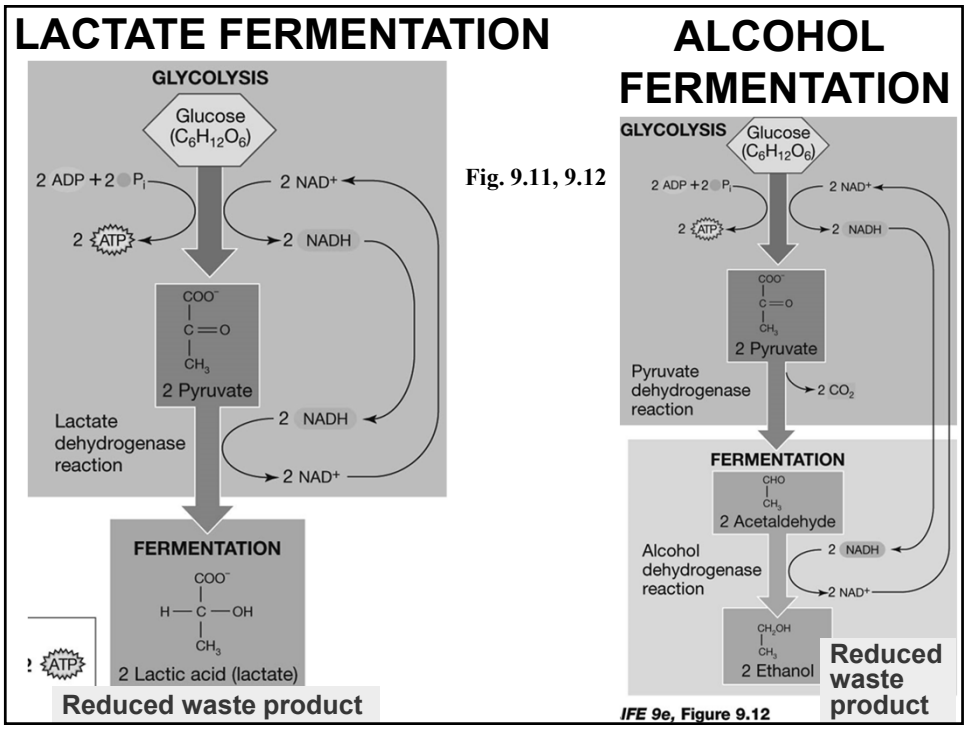

Fig. 9.11, 9.12

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9.3) Contrasting Energy Yields

- **For each GLUCOSE:**
  - fermentation yields 2 ATP
  - Glyc/Pyr Ox/TCA/ETC yield up to 36 (or 38) ATP

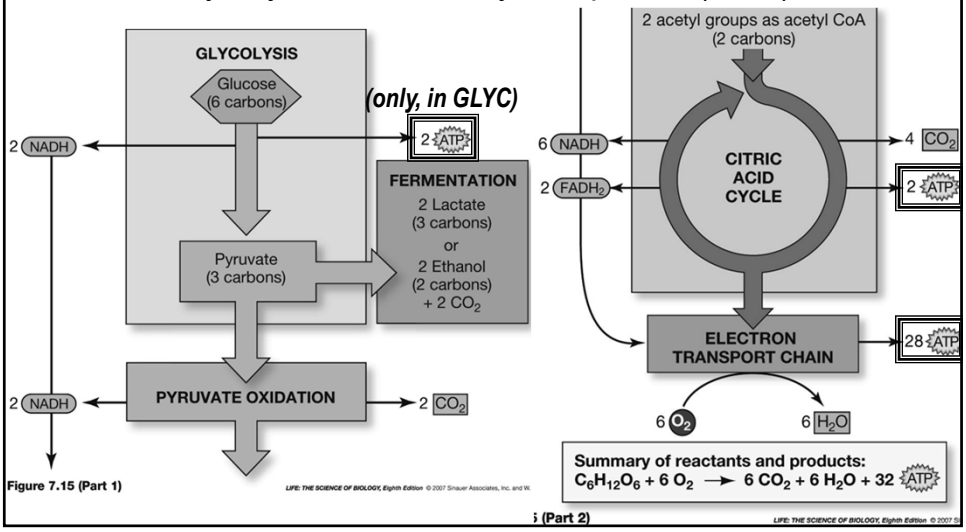

Figure 7.15 (Part 1) ... (Part 2)

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Oxidative Metabolic Yield, Summary:

NET:

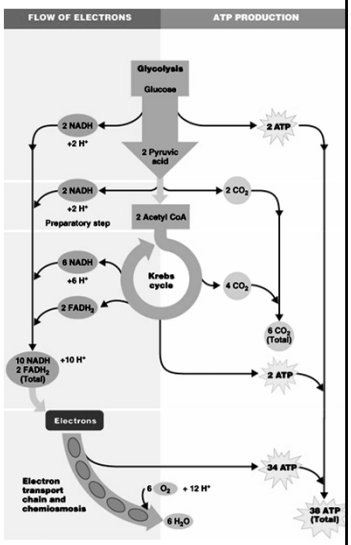
1. Glyc = 2 ATP
(4 made, 2 invested)
2 NADHH → 4 ATP
(use 2 ATP on transport into mito. I.M.!!)

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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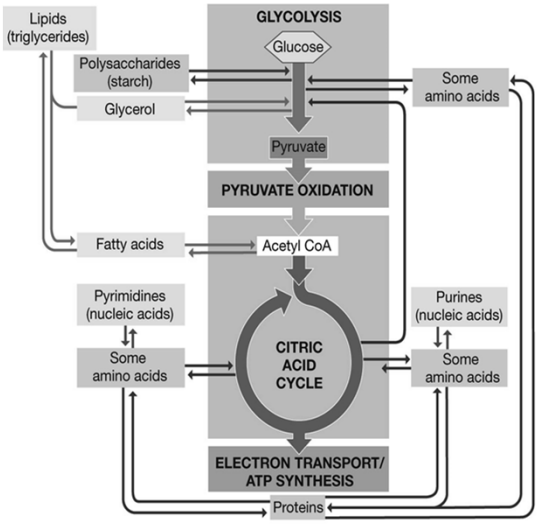
9.4) Metabolic Pathways: Catabolic pathways feed into the Respiratory pathways

1. **<u>Polysaccharides</u> → glucose**
   - enters *glycolysis*
2. **<u>Fats</u> → Glycerol**
   - enters glycolysis (DHAP)
3. **<u>Fatty acid</u> degradation →Acetyl CoA**
   - enters *TCA cycle*
4. **<u>Proteins</u> → amino acids**
   - enter glycolysis & TCA



igure 9.14

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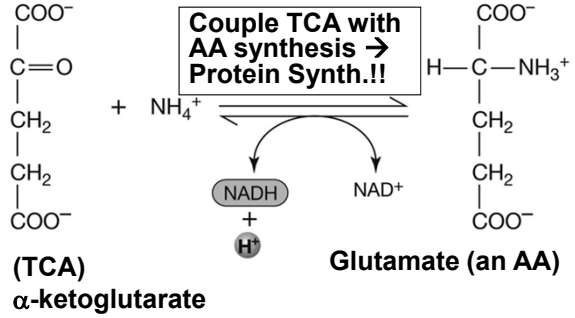
9.5) Anabolic pathways: ***<u>coupled</u>*** to catabolism by enzymes

- use <u>respiratory intermediate compounds</u> of metabolism.
  - fats, amino acids, and others
- synthesize <u>essential building blocks</u> for cellular structure & function.



(TCA) α-ketoglutarate

Glutamate (an AA)

LIFE 8e, Figure 7.17

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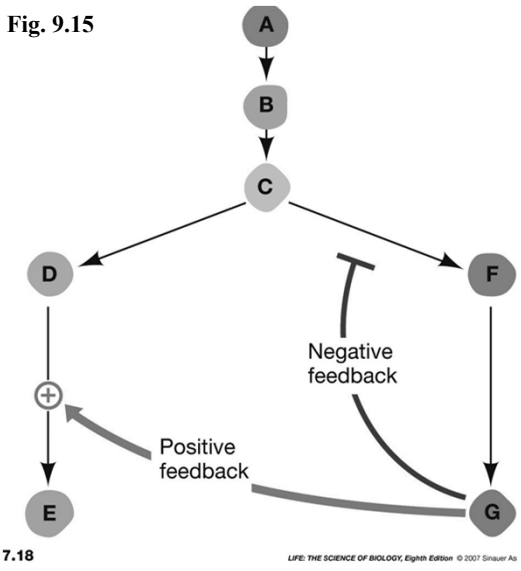
9.6) Regulating Energy Pathways

Fig. 9.15



- Rates of GLYC and TCA are increased or decreased by:
  - **ATP, ↓**
  - **ADP, ↑**
  - **NAD^+, or ↑**
  - **NADH + H^+ ↓**
- (all act on ***allosteric*** enzymes)

Abundant product "G":
- Inhibits own production
  - (***neg. feedback***)
- Activates alternate branch
  - (***pos. feedback***)

http://www.science-groove.org/Now/Glucose.html

https://youtu.be/jJvAL-iiLnQ

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Chapter 13: DNA & Its Role in Heredity

1. DNA: The Genetic Material
2. The Structure of DNA
3. DNA Replication
4. The Mechanism of DNA Replication
5. DNA Proofreading and Repair
6. Practical Applications of DNA Replication

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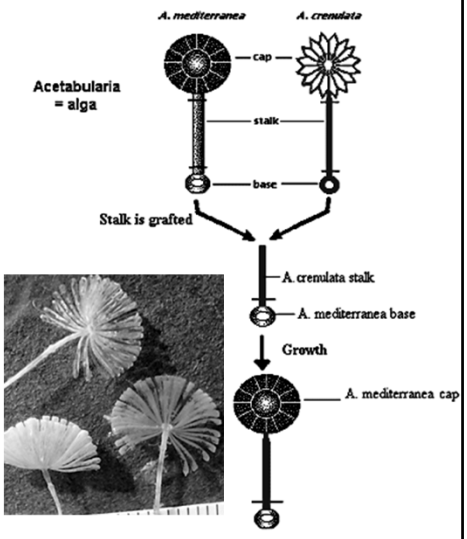
13.1) DNA: The Genetic Material

- **Nuclear transplantation experiments (1930s):**
  - **the nucleus carries the genes!!!**

❖**Joachim Hammerling:**

- **Algae - "giant cells"**
  - ***Acetabularia…..***

- **Animals –**
  - **frogs, sheep, cattle….**



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DNA: The Genetic Material

1. Nucleus from *somatic* ("body", udder) cell of Species 1

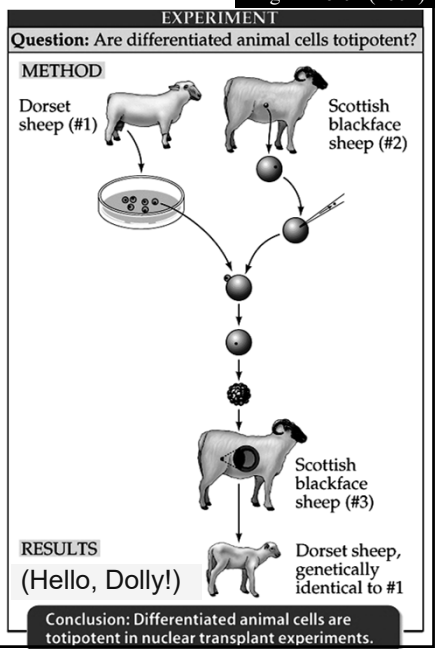
2. Implanted in enucleated, fertilized egg of Species 2

3. Offspring Genetically identical to species 1 !!

FYI: http://www.dnalc.org/cloning.html

http://learn.genetics.utah.edu/units/cloning/index.cfm

figure 16-04 (2004)



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DNA: The Genetic Material

- Nuclear transplantation showed that the nucleus carries the genes
- Staining reveals the "nucleic acids" within the nucleus (***Feulgen's Dye***)
  - Different amounts in different species
  - Half the amount in eggs and sperm
    - (***haploid*** gametes vs. ***diploid*** adult)
- *Three experiments then proved DNA is the genetic material: .....*

FYI:
http://www.wwnorton.com/college/biology/discoverbio3/core/content/ch12/animations.asp

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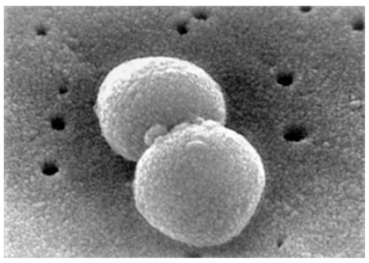
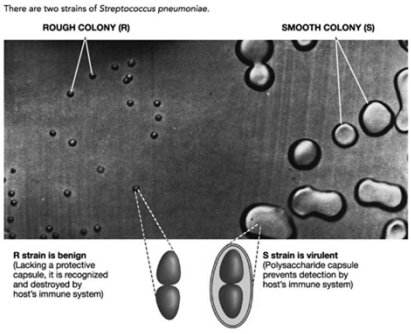
A. Griffith: The "Transforming Substance" is the Genetic Material

1. Frederick Griffith (1920s)

– DNA from a virulent strain of pneumococcus ***genetically transformed*** nonvirulent bacteria into virulent bacteria. *(Streptococcus pneumoniae)*





Tutorial: http://nortonbooks.com/college/biology/animations/ch12a01.htm

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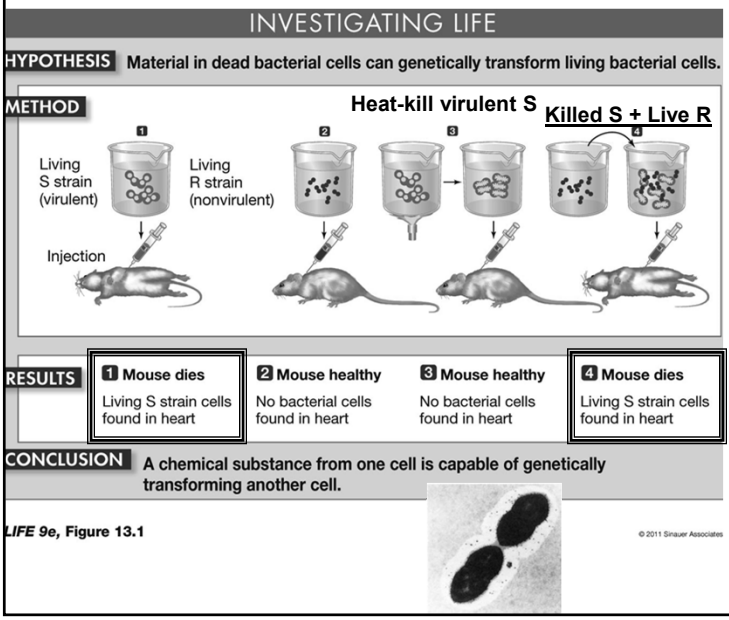
Griffith's Experiments



MOUSE INJECTIONS:

1. **Living S → death**
2. **Living R → healthy**
3. **Heat-killed S → healthy**
4. **Killed S + Live R → Dead!!**

- ***Something from the dead S cells TRANSFORMED the harmless R cells into Killers!!!***
- **(smooth with polysaccharide capsule)**

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What is the "Transforming Principle"?

1. **Griffith**: partially purified "***Transforming Substance***" caused the same transformation from R→S bacteria.
   - ***Was mostly DNA!***

2. Oswald **Avery,** Colin **MacCleod &** Maclyn **McCarty (1944):** treated "TS" with various, highly-specific, macromolecule-degrading enzymes
   - (Proteases, RNAses, DNAses, Glycosidases, Lipases)
   - ***None, except DNAses, removed the transforming ability of the Transforming Material***
   - ***Therefore, DNA Must be the Genetic Material!!!***

     - Still, many detractors remained!!!…("Still protein contaminant")….

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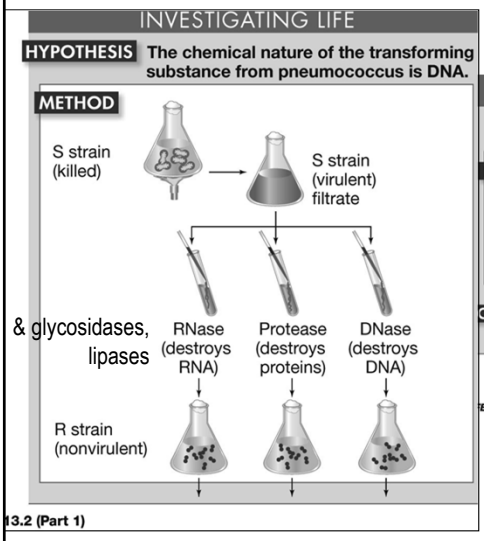
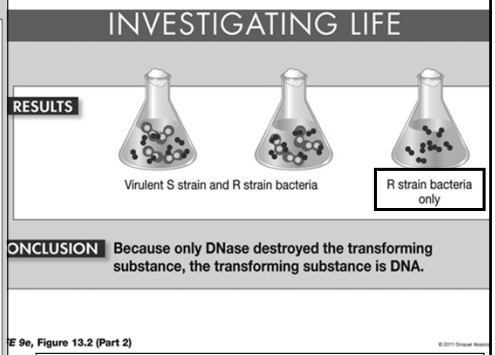
B. Avery, MacLeod, McCarty Experiment

- Individually treated the TS with different procedures to remove each known type of macromolecule…..





1. **TS = genetic material (Griffiths)**
2. **TS = DNA**
3. **Therefore, DNA = Genetic material!!**

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C. Hershey & Chase Blender Expt: Prove DNA = Genetic Material!

- **<u>Alfred Hershey, Martha Chase (1952):</u>**
  - Labeled viruses were incubated with host bacteria.
    - ***Labeled viral DNA entered host cells, while labeled virus protein did not.***
- Entry of the viral nucleic acids produced hundreds of label-bearing viruses.

<u>http://highered.mcgraw-hill.com/olc/dl/120076/bio21.swf</u>

Original paper: <u>http://www.jgp.org/cgi/reprint/36/1/39</u>

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