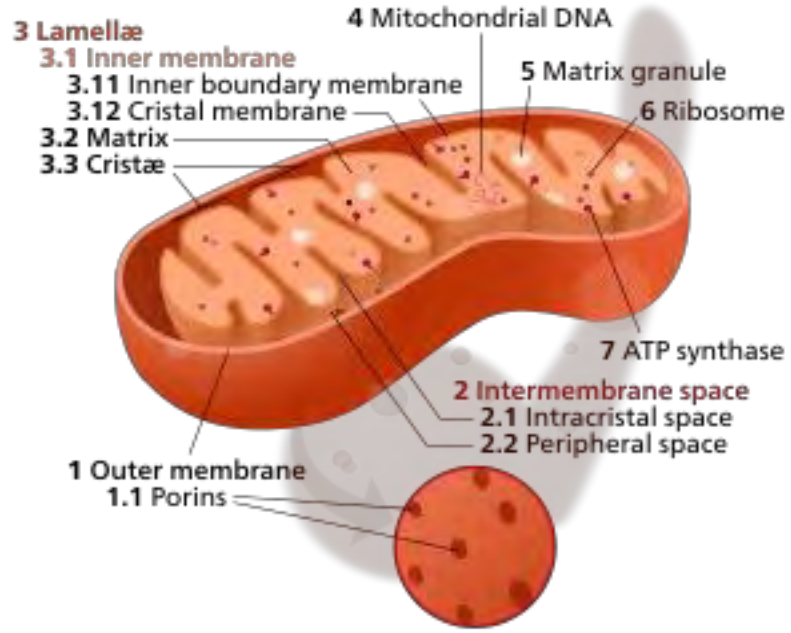
mitochondria I:
structure and respiration

mitochondria
give you
ENERGY



respiration isn't just breathing

Plus some chapter 2.4!!

Lodish chapter 12 (mitochondria)
and a bit on **peroxisomes**

Research Article
Cell Biology, Genetics and Genomics

Cytoprotection by a naturally occurring variant of ATP5G1 in Arctic ground squirrel neural progenitor cells

Neel S Singhal, Meirong Bai, Evan M Lee, Shuo Luo, Kayleigh R Cook, Dengke K Ma

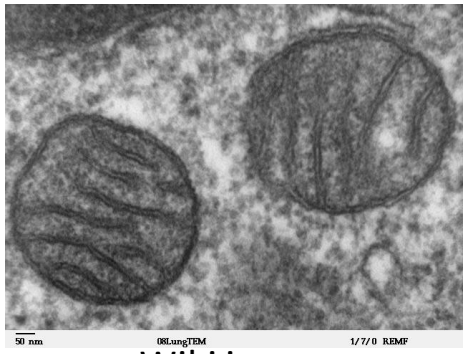
Department of Neurology, University of California-San Francisco, United States; Cardiovascular Research Institute, University of California-San Francisco, United States; Department of Physiology, University of California-San Francisco, United States; Innovative Genomics Institute, United States

Oct 14, 2020 • https://doi.org/10.7554/eLife.55578

Discussion week 5

DOI: 10.7554/eLife.55578

learning goals



Wiki image

by the end of today's topic, you should be able to:

- describe key features of mitochondria (ie, how they are organized, what functions they have, how they reproduce, and what metabolic pathways occur here)
- know which (subsets of) metabolic reactions take place in mitochondria, peroxisomes, or cytosol, and what the molecular starting and endpoints are for the subsets
- explain the advantages of aerobic metabolism (v anaerobic)
- explain why the citric acid cycle is central in oxidative metabolism
- recognize the chemical structures for key metabolites and know the pathways in which they are required
- compare and contrast mitochondria and peroxisomes
- relate several diseases to organelle dysfunction

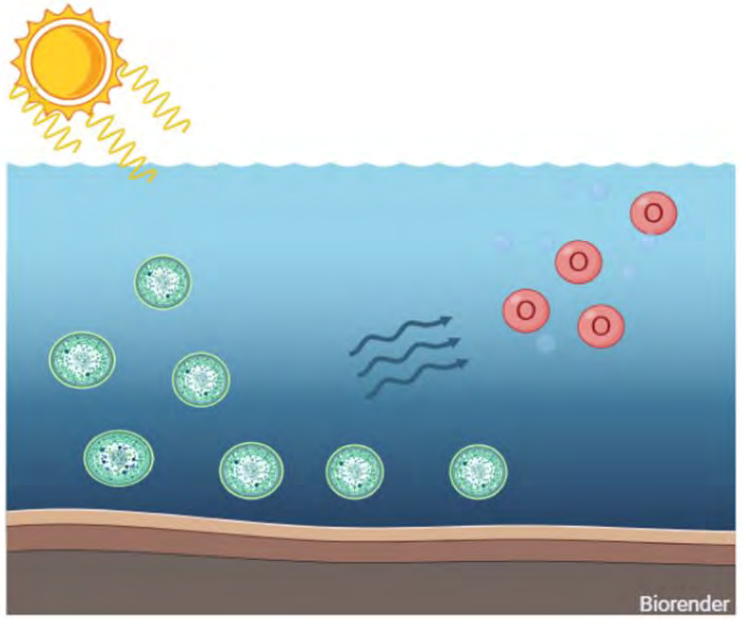
Warm up poll:

best way to get fast energy in the fall?

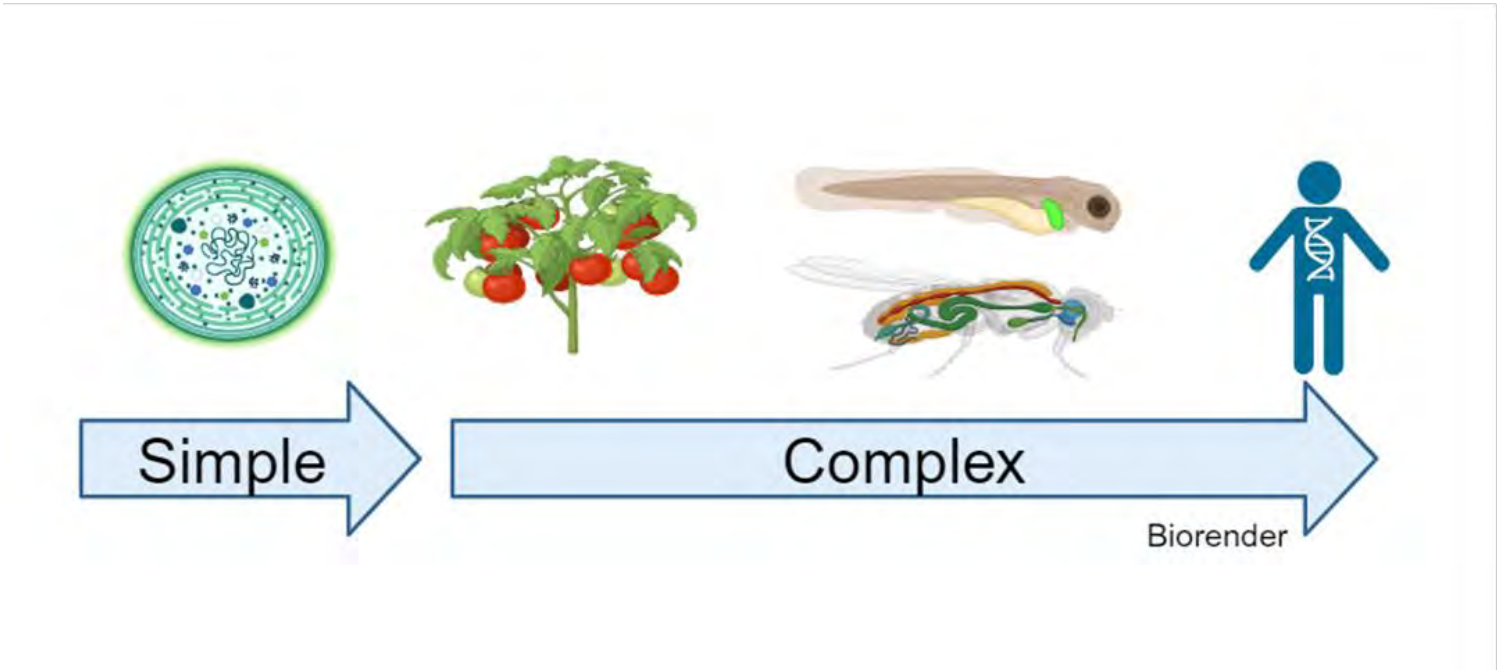
PollEv.com/msg303
Send **msg303** to **22333**

1. Pumpkin pie
2. Apple cider
3. Candy corn
4. Chocolate anything
5. Starbucks PSL
6. Creatine powder

link

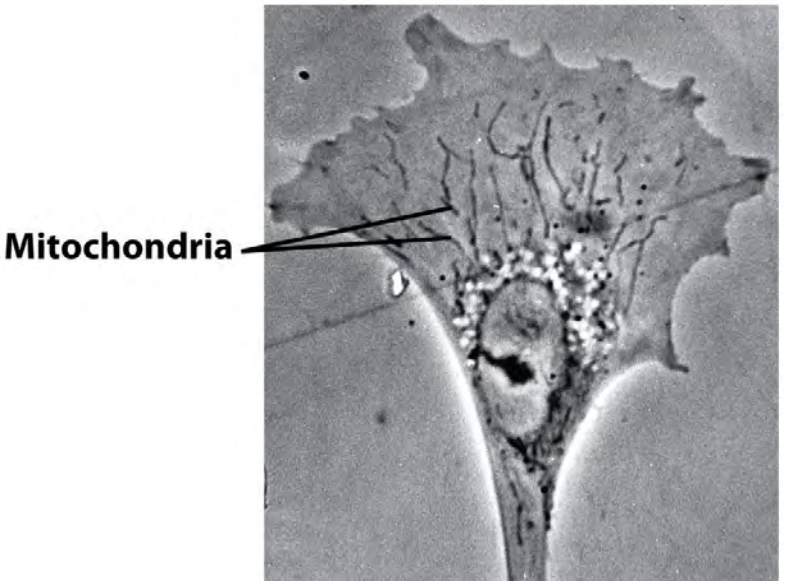


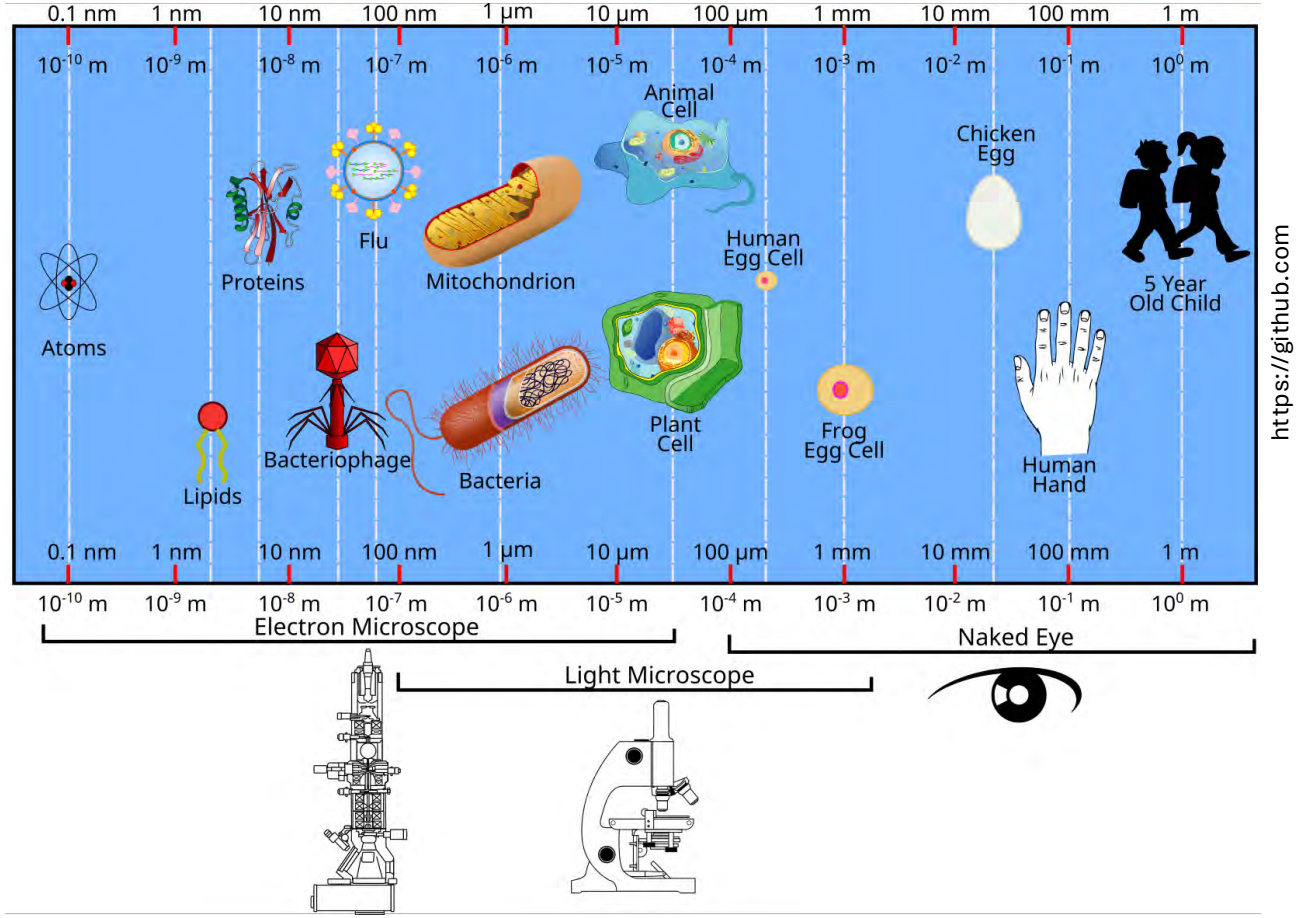
Development of more complex organisms

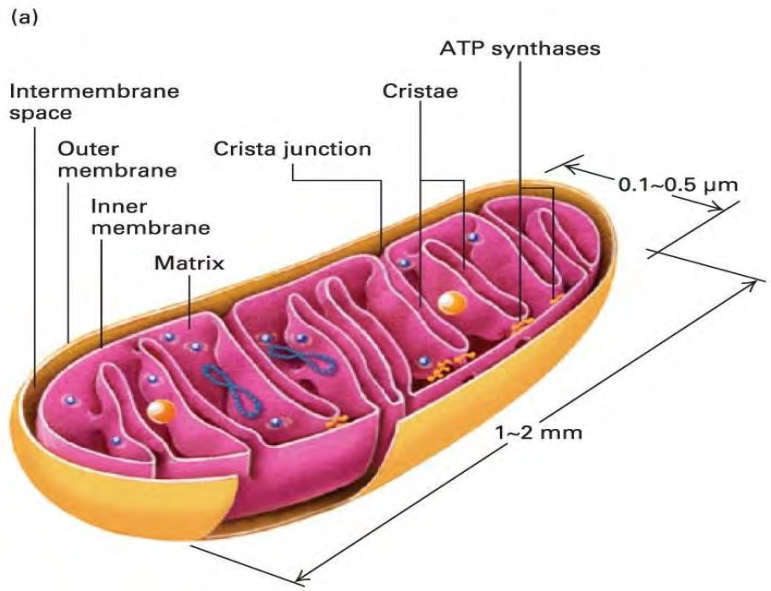


- early Earth was populated by **anaerobes**
- cyanobacteria appeared --> oxygen.
- **aerobes** evolved to use oxygen to create energy, adenosine triphosphate (ATP)
- in eukaryotes, aerobic respiration takes place in the **mitochondrion**.

mitochondria vary in shape and number by cell type

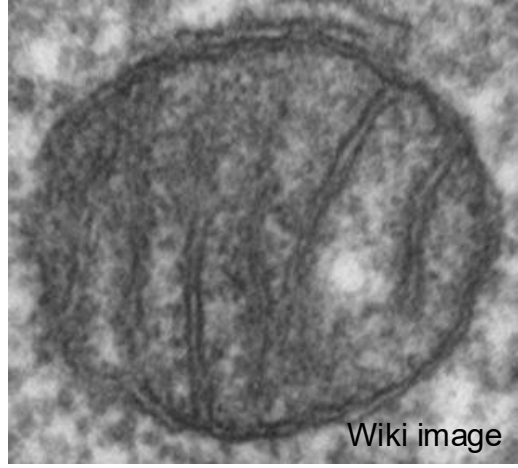






- can be bean-shaped, round, or stringy;
- tend to be around 0.2-1-micron x 1-4 microns
- cells that need more energy have more mitochondria

Lightning round poll: genetics flashback! where did you get YOUR mitochondria?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A. From mom
B. From dad
C. Half from mom, half from dad
D. I made them myself (*de novo*)

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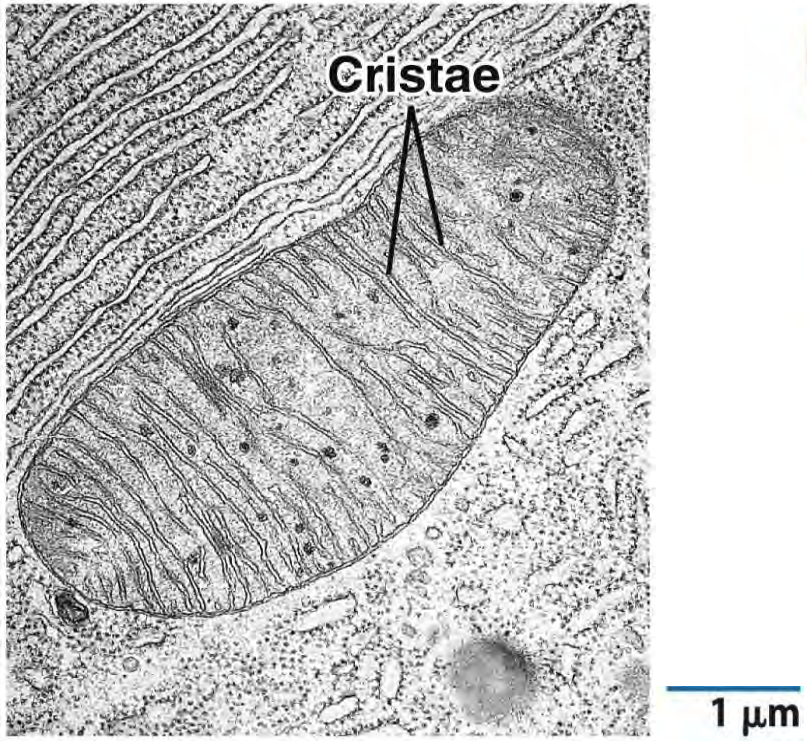
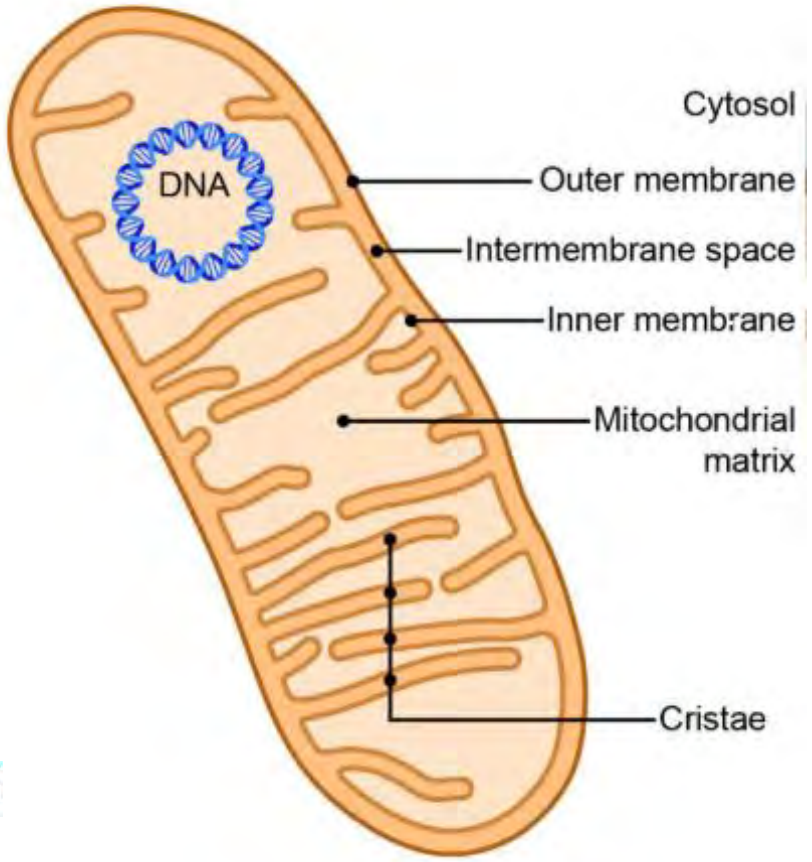
link

Feature 1: Double Membrane Structure

Mitochondria have an outer membrane and a highly folded inner membrane. The folds, called **cristae**, increase surface area for energy-producing reactions.

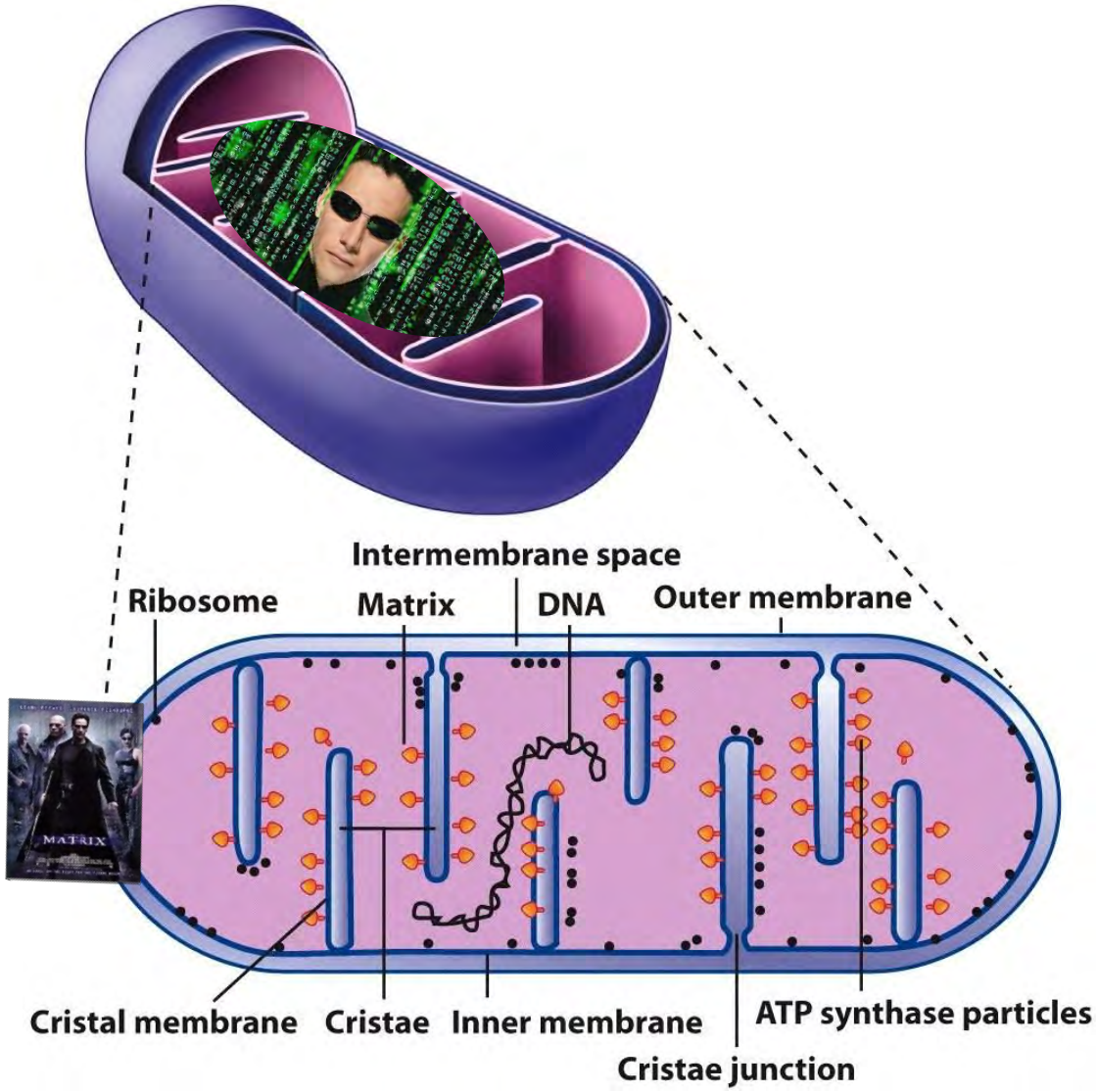




The **outer mitochondrial membrane** is about 50% lipid and contains enzymes for fatty acid elongation and other functions.

The **inner membrane** has a very high protein-to-lipid ratio, is rich in **cardiolipin** (important for the ETC and ATP synthesis), and lacks cholesterol.

Feature 2- The mitochondrial matrix



Mitochondrial DNA (mtDNA)
Holds genetic information for some mitochondrial proteins.

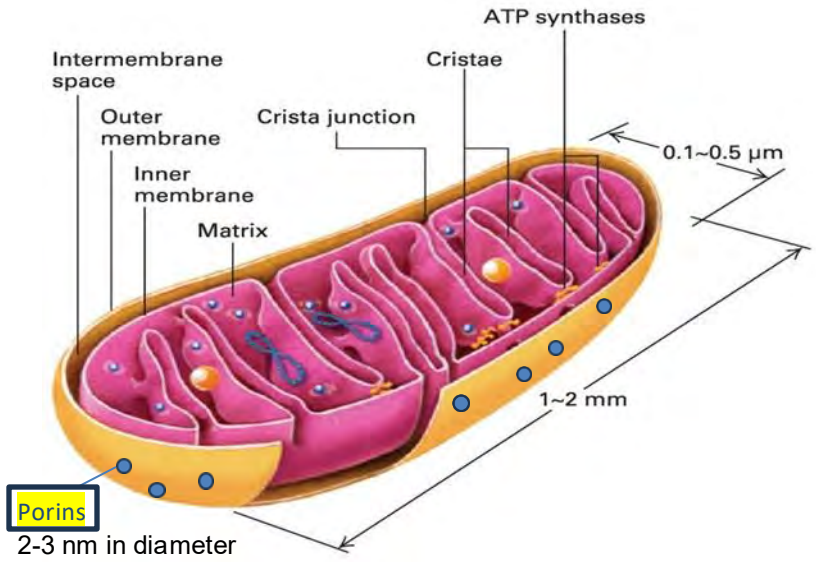
Ribosomes:
Produce proteins encoded by mtDNA.

Enzymes: Involved in the citric acid cycle and other metabolic processes.

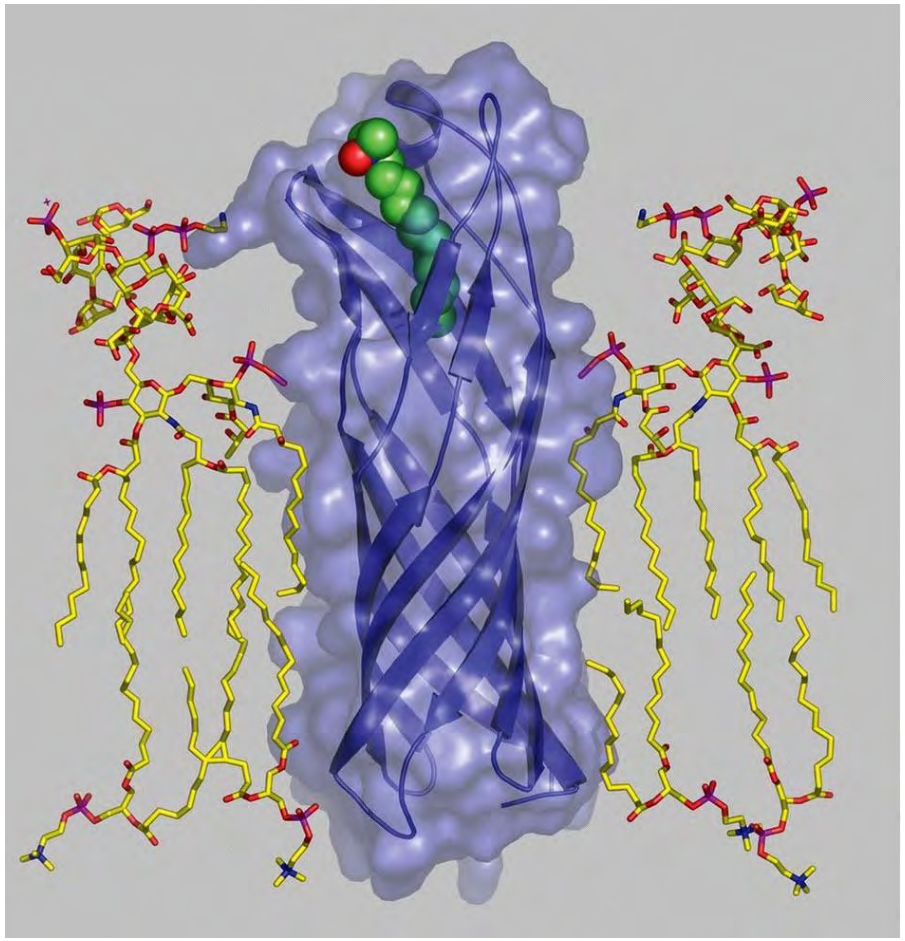
Many additional proteins and components are imported from the cytoplasm into the matrix.

ATP production machinery is located at **cristae**

Feature 3: Outer Membrane Porins



The porins can open or close depending on signals and concentration differences and cellular needs.



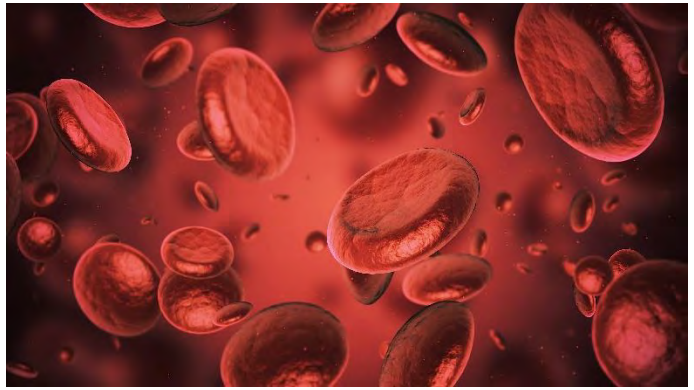
what kind of structure does this transmembrane protein have?

other kinds of transporter proteins are also found in the mitochondrial membranes

Porins are selective channels in the outer membrane that let small molecules like Coenzyme A and ATP

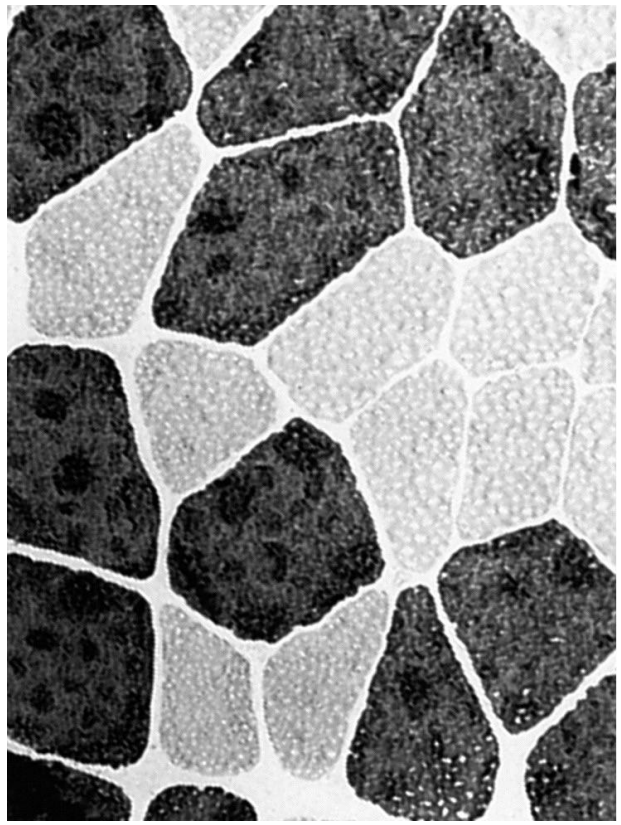
which of these cells would you expect to have the most mitochondria?

A) A skin cell from your hand
B) A muscle cell in your leg while running a marathon
C) A lens fiber and corneal epithelial cells of a cat
D) A red blood cell carrying oxygen



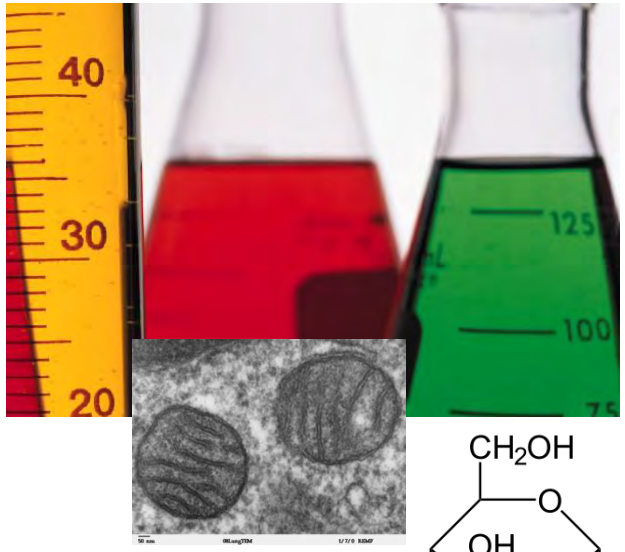
an ATP production experiment

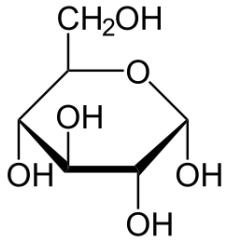
poll: you purify mitochondria from muscle cells, put them in a test tube and add glucose. will ATP be produced?



A. YES

B. NO





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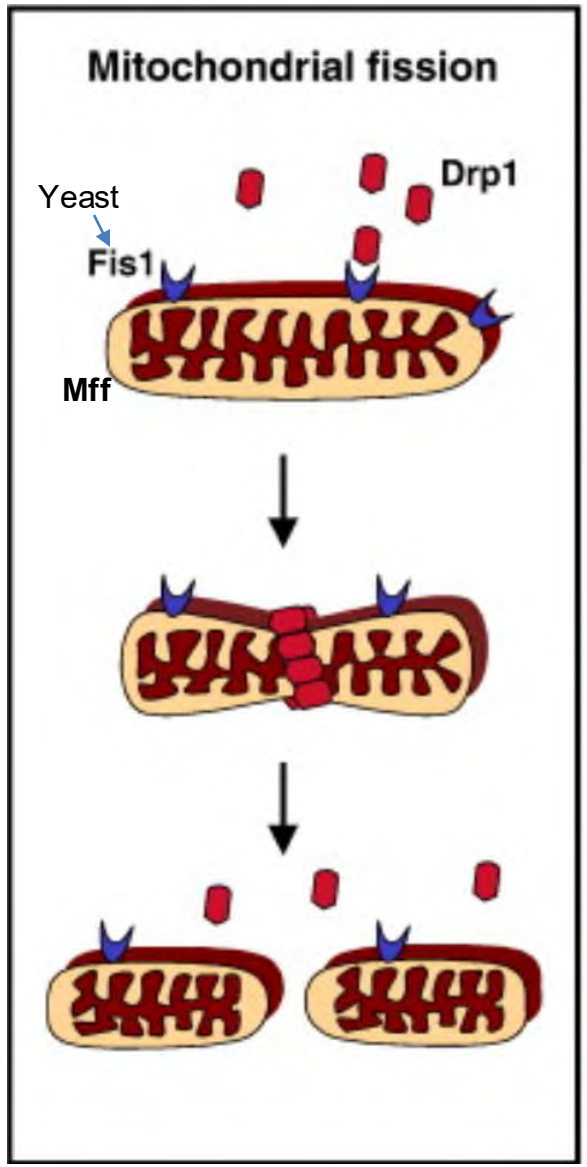
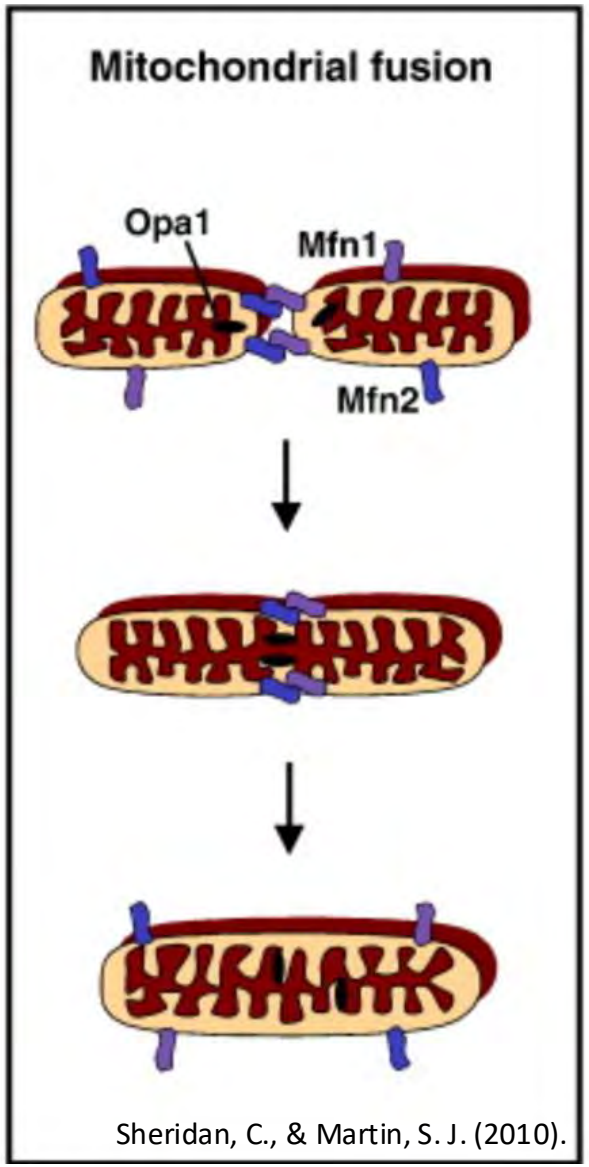
Feature 4: Dynamic Inheritance and Morphology

Mitochondria are passed to new cells and can change shape and number depending on the cell's needs.



fission:

- ✓ Driven by Drp1 (protein), which is mostly in the cytoplasm.
- ✓ Drp1 recruitment to mitochondria is not fully understood.
- ✓ Drp1 forms spirals around mitochondria at fission sites.
- ✓ Spirals promote mitochondrial constriction → followed by fission.

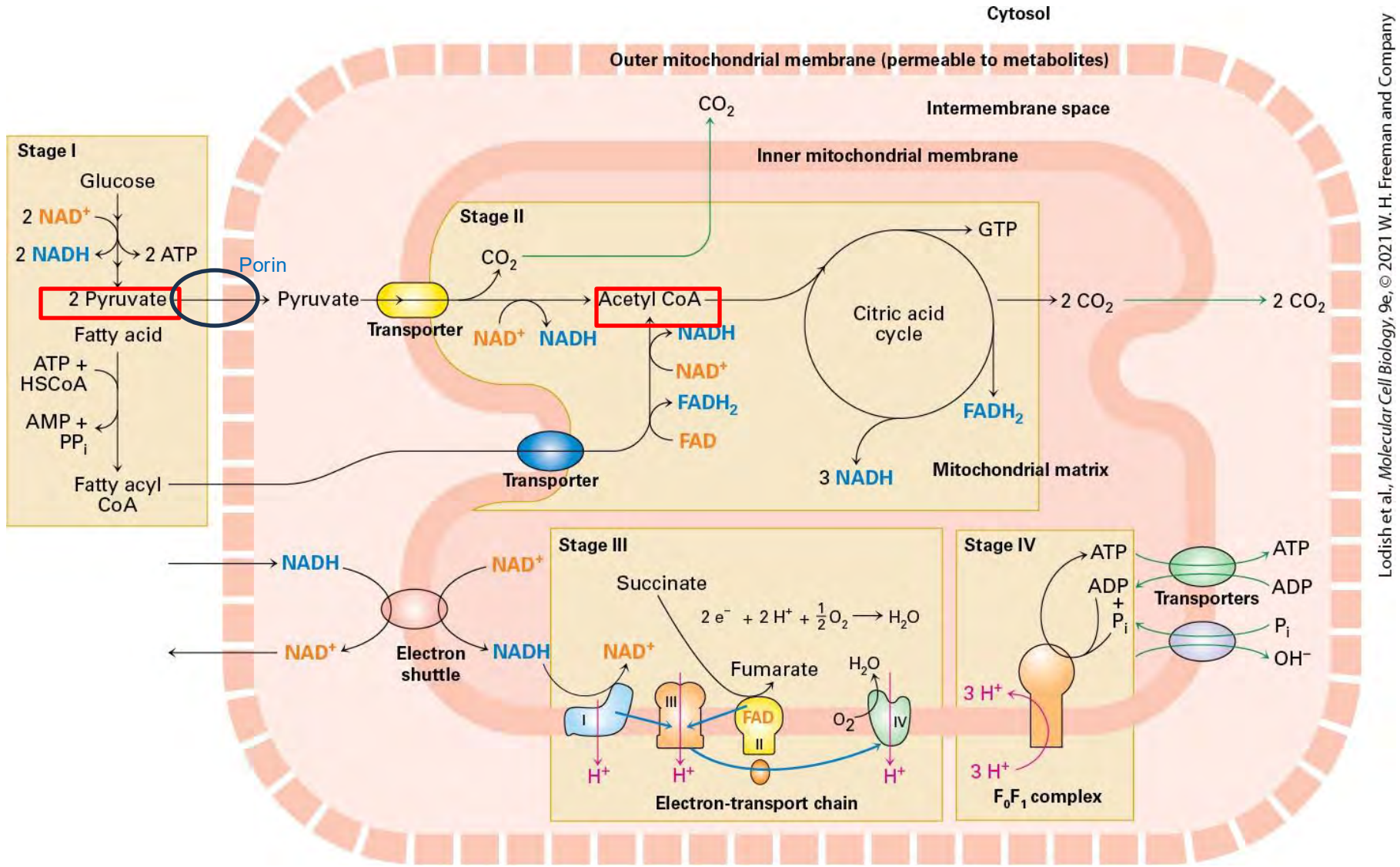


Sheridan, C., & Martin, S. J. (2010).

fusion:

- ✓ Driven by Mfn1 and Mfn2 on the outer mitochondrial membrane.
- ✓ Mitofusins tether adjacent mitochondria together.
- ✓ Mitofusins mediate outer membrane fusion.
- ✓ Opa1 mediates inner membrane fusion.

Feature 5: metabolic focus on mitochondria



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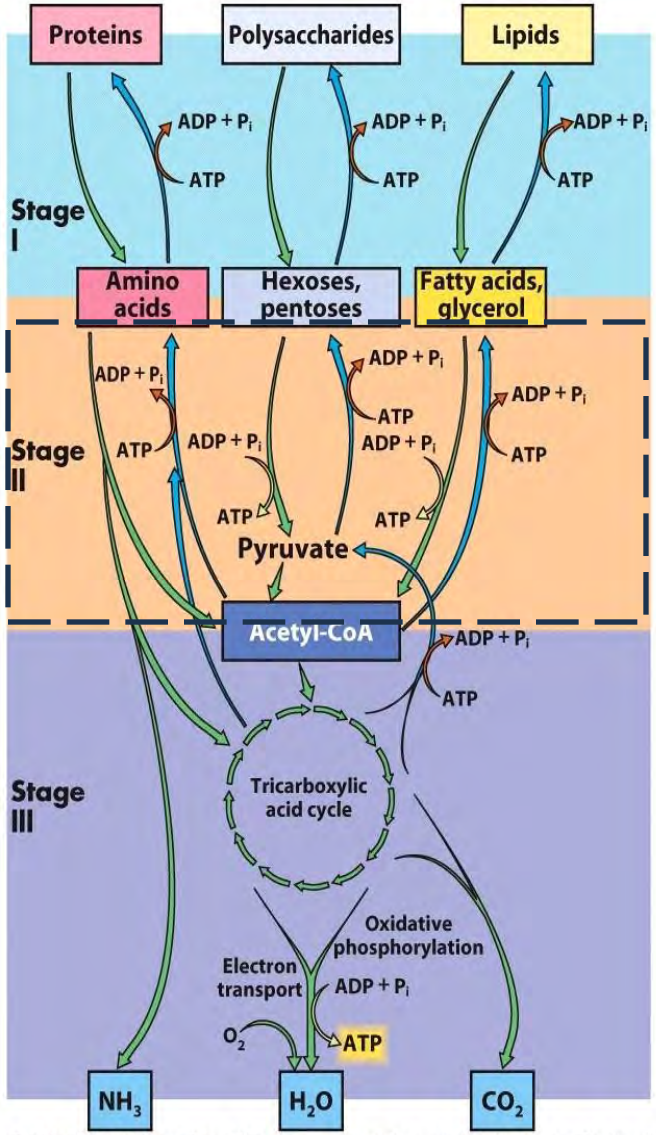
Metabolism in Action: Anabolic and Catabolic Pathways



Anabolic reactions **build** molecules up (using stored energy)

Gluconeogenesis making glucose from smaller molecules like pyruvate.

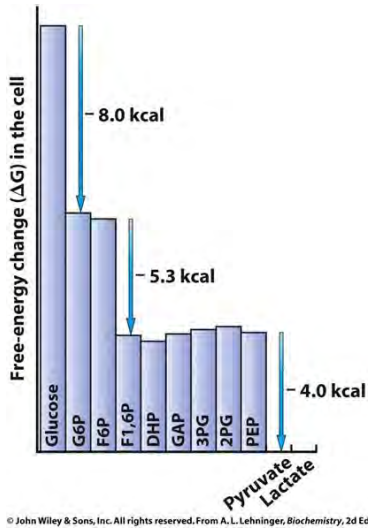
Catabolic reactions **break** molecules down (releasing energy)

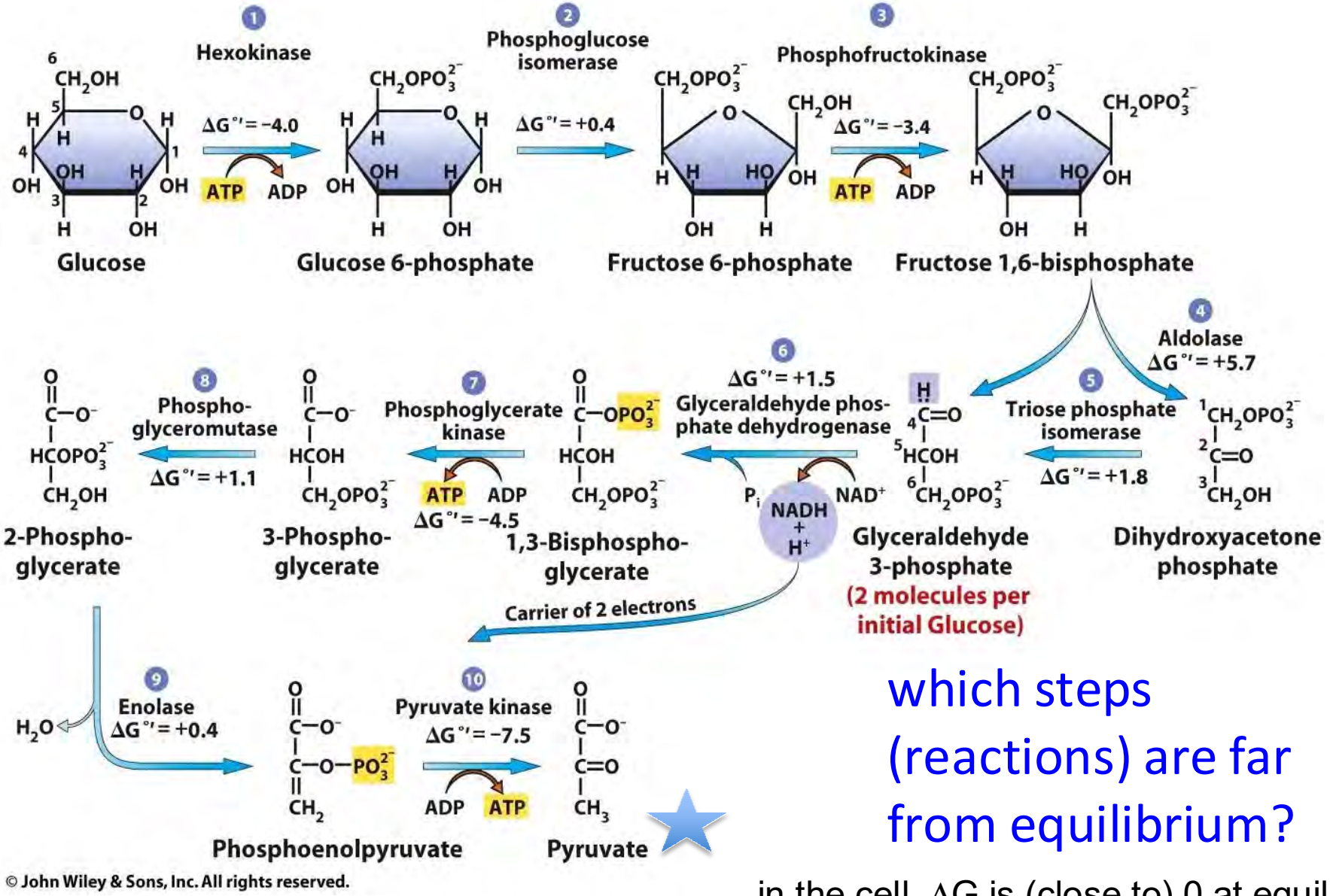
Glycolysis breaking glucose into pyruvate to release energy.

free energy and glycolysis

MAJOR CELLULAR REACTIONS ARE CATALYZED BY ENZYMES!

(enzymes are named "ase" - under the numbers)





which steps (reactions) are far from equilibrium?

in the cell, ΔG is (close to) 0 at equilibrium

irreversible steps act as control points in glycolysis

Glucose molecule

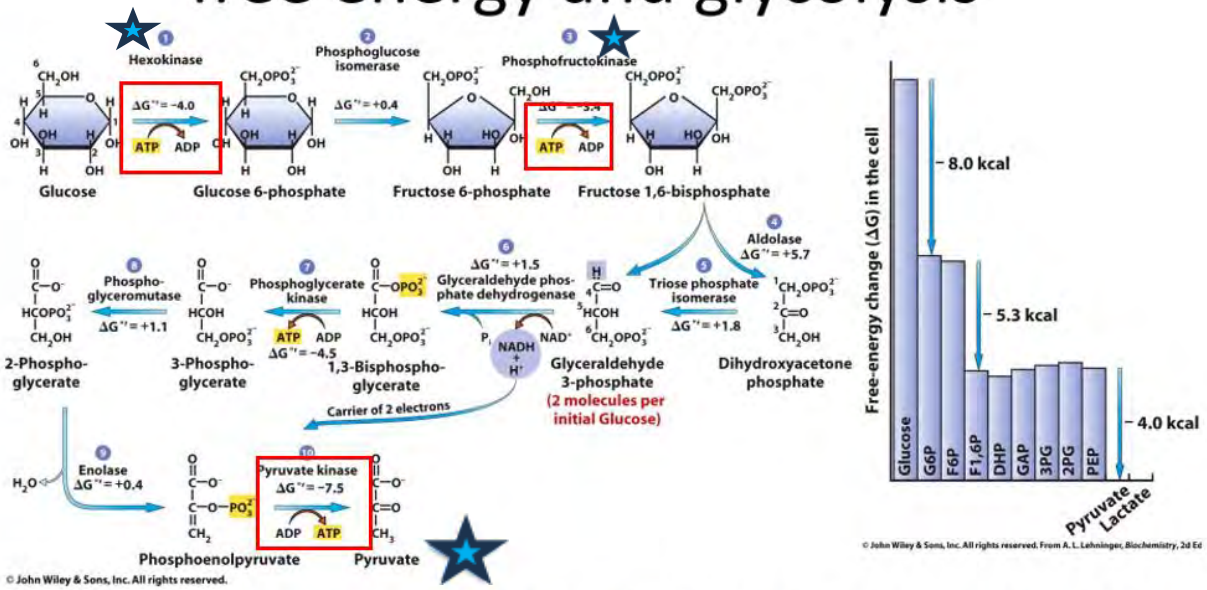
Steps far from equilibrium have a negative ΔG, making it favorable and one-way

positive ΔG

The reaction won't happen on its own. Thermodynamically unfavorable

ADP ATP ← Hexokinase

free energy and glycolysis



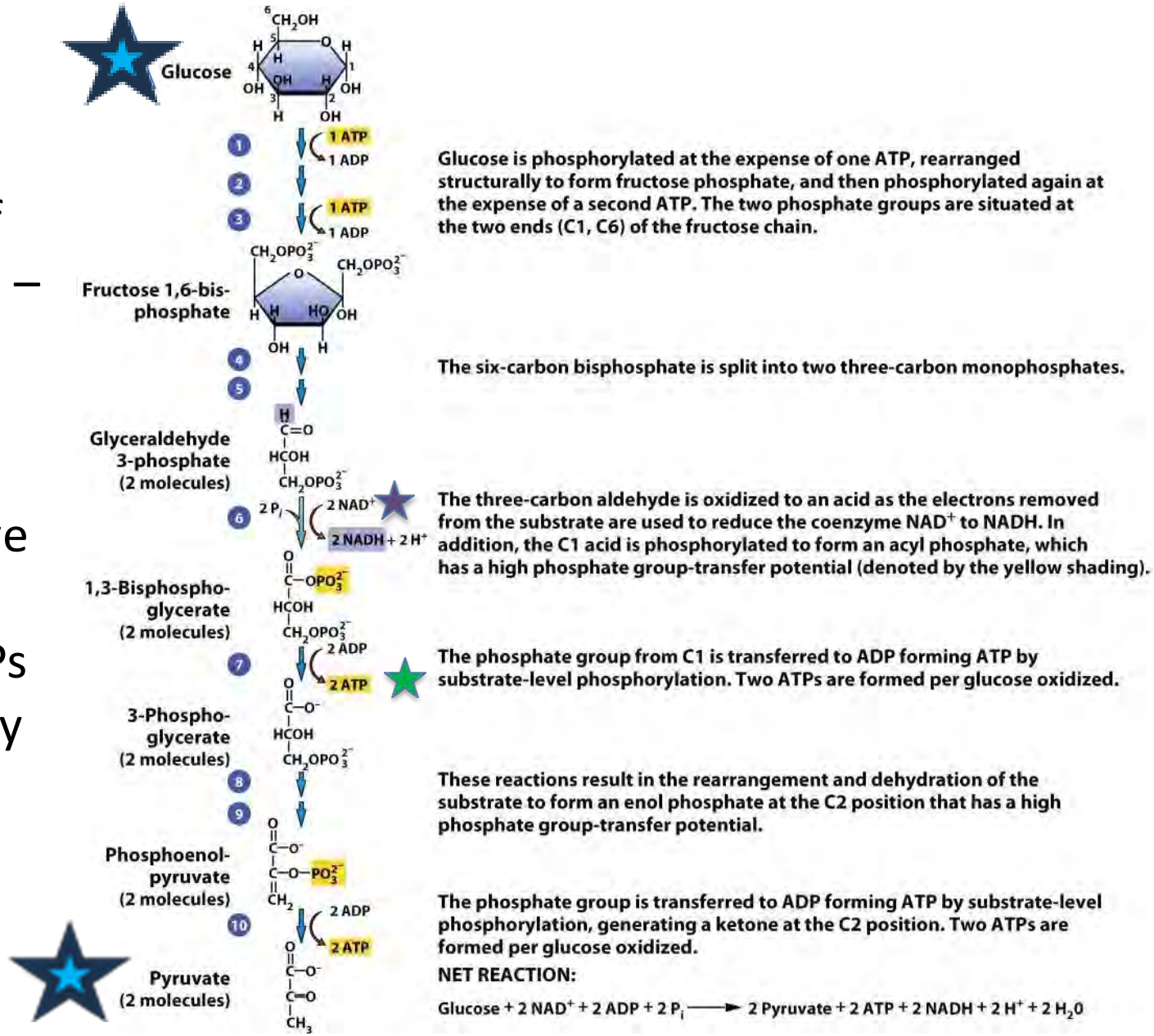
MAJOR CELLULAR REACTIONS ARE CATALYZED BY ENZYMES!

(enzymes are named "ase" - under the numbers)

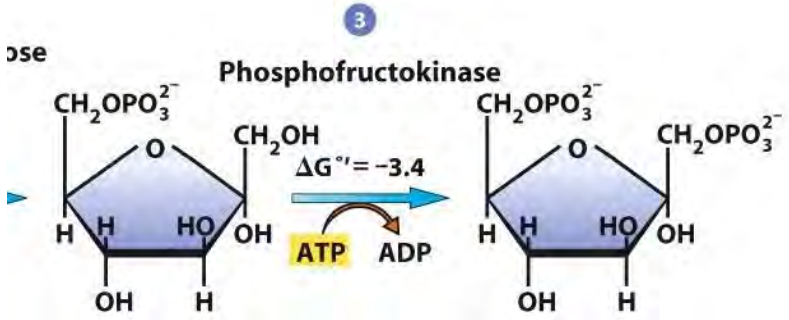
glycolysis is pretty good –net 2 NADH and 2 ATPs

this is the first part of oxidative metabolism – in the cytosol up to PYRUVATE

later steps of oxidative metabolism will produce 30 more ATPs (try to find them all by the end of next lecture!)



Gluconeogenesis is not just glycolysis run backwards



In glycolysis: catalyzed by phosphofructokinase

In gluconeogenesis: fructose 1,6-bisphosphatase

Hexokinase
And
Pyruvate kinase
are also
allosterically
regulated

AMP(Adenosine Monophosphate) and ATP are inversely proportional

High AMP activates phosphofructokinase

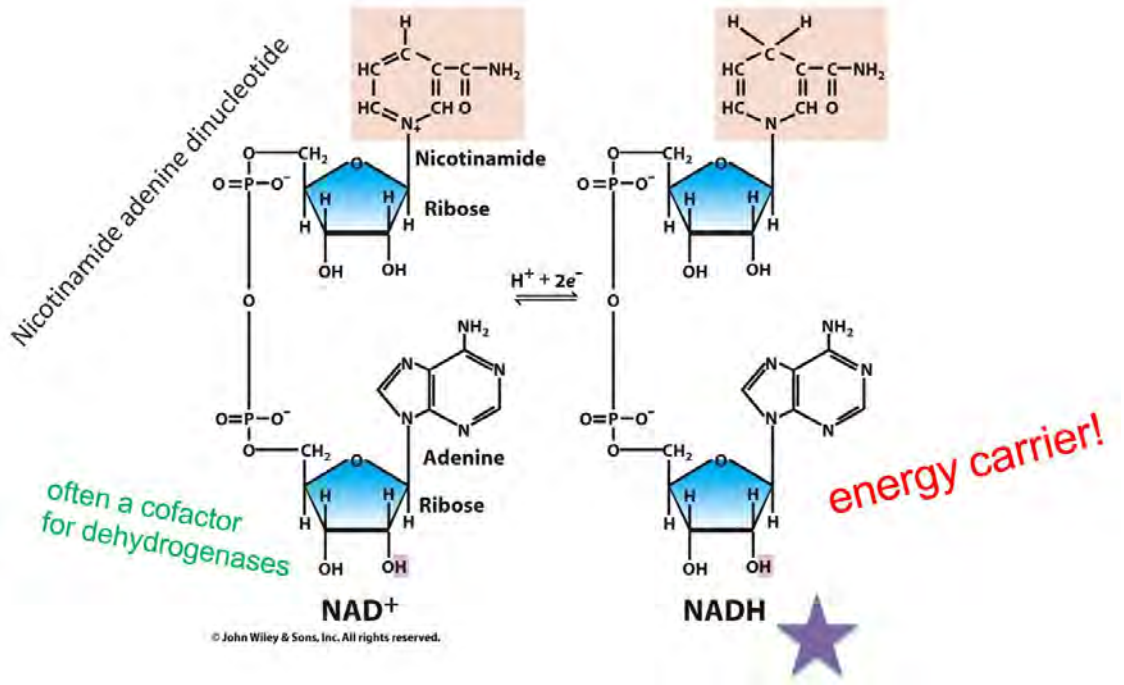
(to promote energy production)

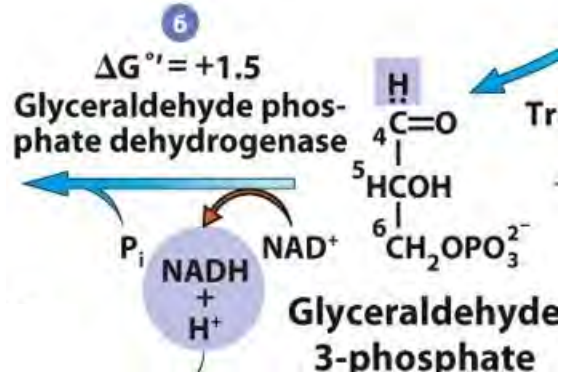
and inhibits fructose 1,6-bisphosphatase

(High ATP allosterically inhibits phosphofructokinase)

co-enzyme NAD can be reduced to NADH

NADH = Nicotinamide Adenine Dinucleotide + H (hydrogen/electron).





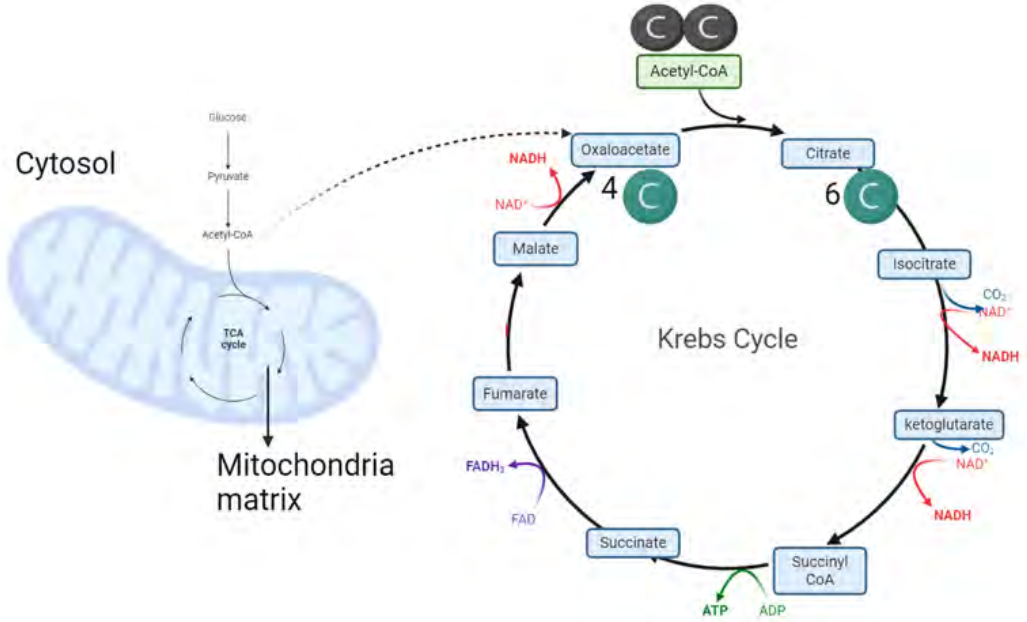
When you eat food, your cells break it down and make **NADH and $FADH_2$**, which act like little **trucks carrying energy**. NADH carries electrons from glycolysis and other pathways, while $FADH_2$ mostly carries electrons from the mitochondria. Both deliver energy to the mitochondria, where it's used to make ATP

the Citric Acid cycle (aka tricarboxylic acid cycle, TCA, aka Krebs cycle)

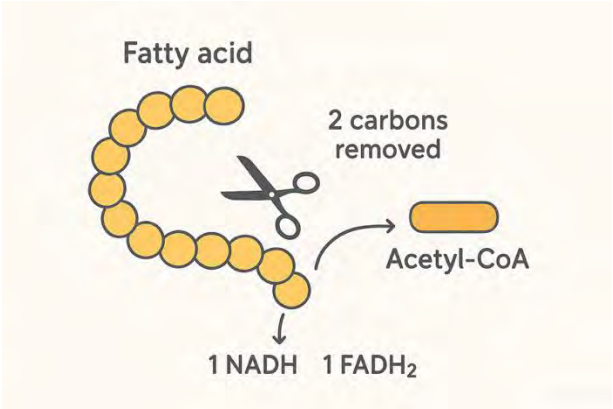
- nets 5 reduced co-enzymes which fuel ATP synthesis (30+ ATPs)
- Pyruvate is transported across the inner membrane (*into the matrix*) and decarboxylated to form *acetyl-CoA,* which enters the TCA cycle.
- 4 NADH and one $FADH_2$ molecule are produced from one pyruvate *(one NADH is before the cycle)*

– four reactions of the citric acid cycle transfer pairs of electrons

- (NAD+ to NADH, FAD+ to $FADH_2$)

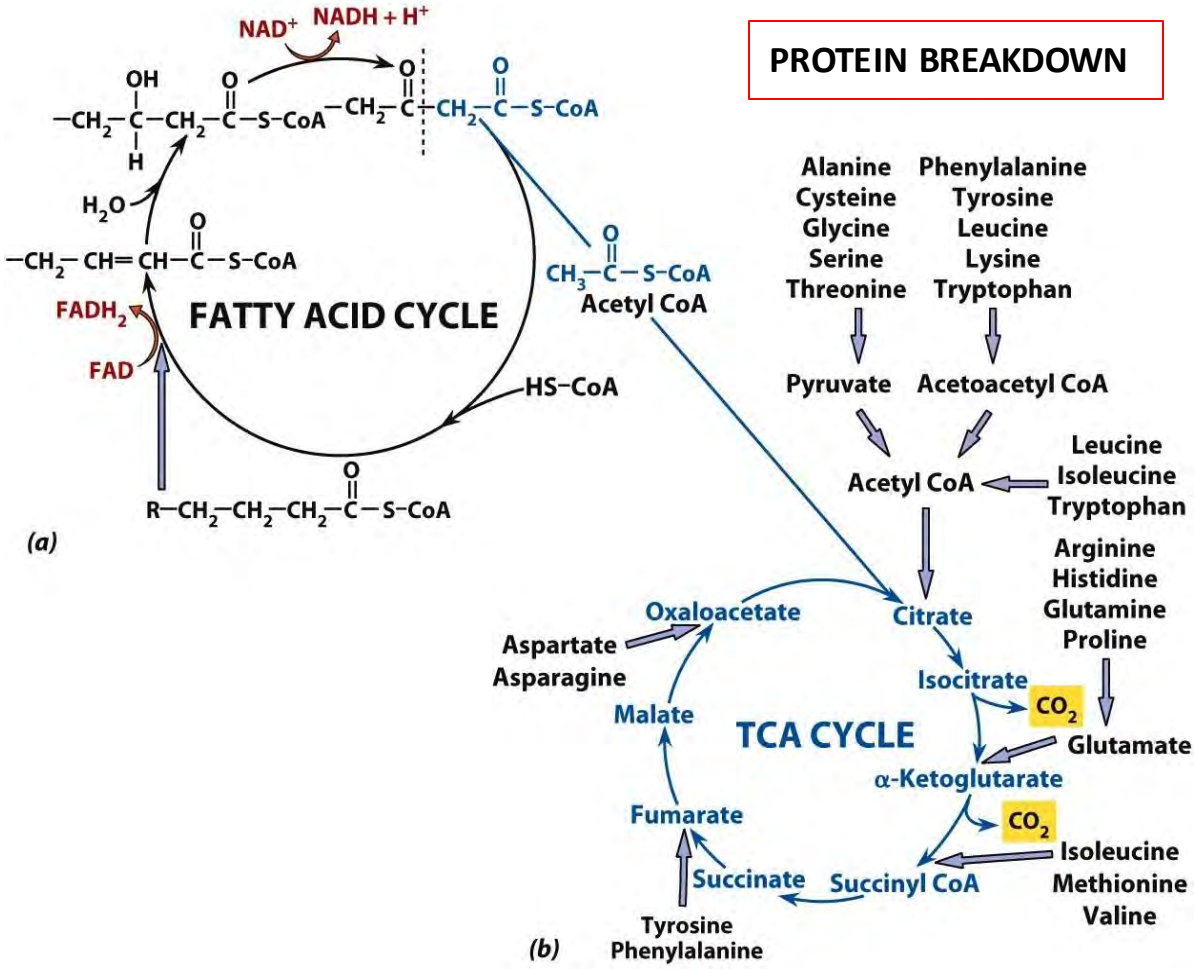


the TCA cycle integrates energy from sugars, fats, and proteins to generate NADH, $FADH_2$, and ultimately **ATP**

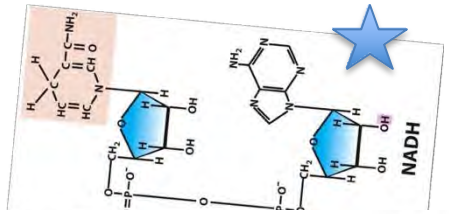


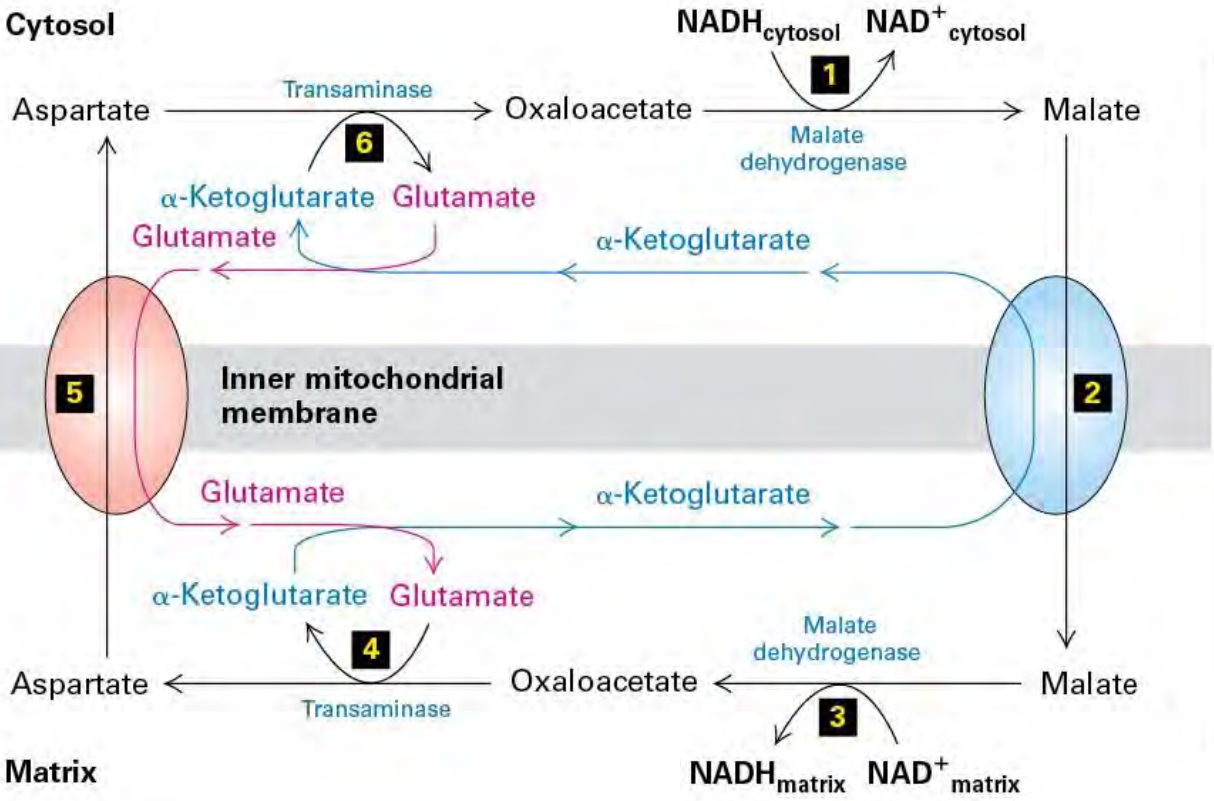
Fatty acids are broken down in a spiral process called **beta-oxidation.**

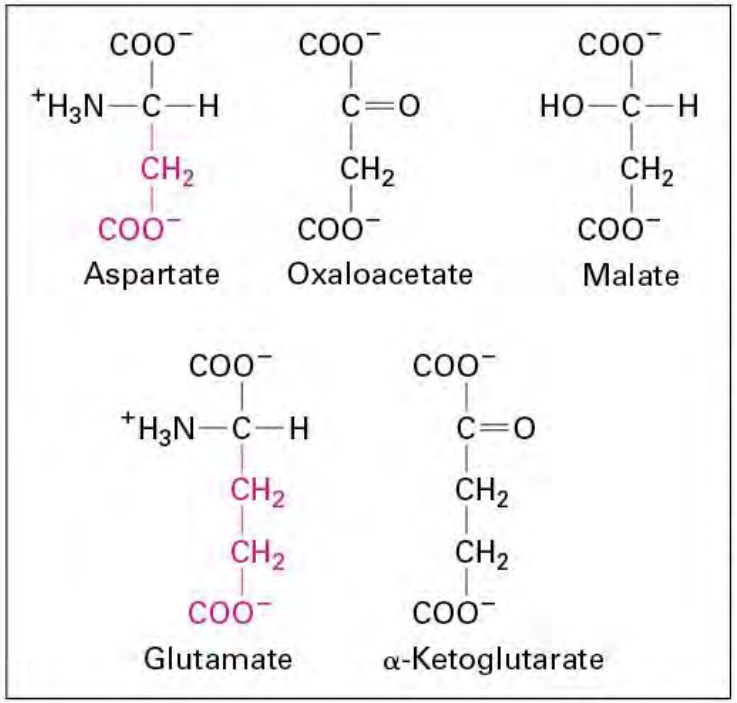
β-oxidation happens inside the mitochondrial matrix



the energy stored in NADH made in glycolysis can enter the mitochondria via the malate-aspartate shuttle or via transfer of electrons to FAD by glycerol phosphate shuttle







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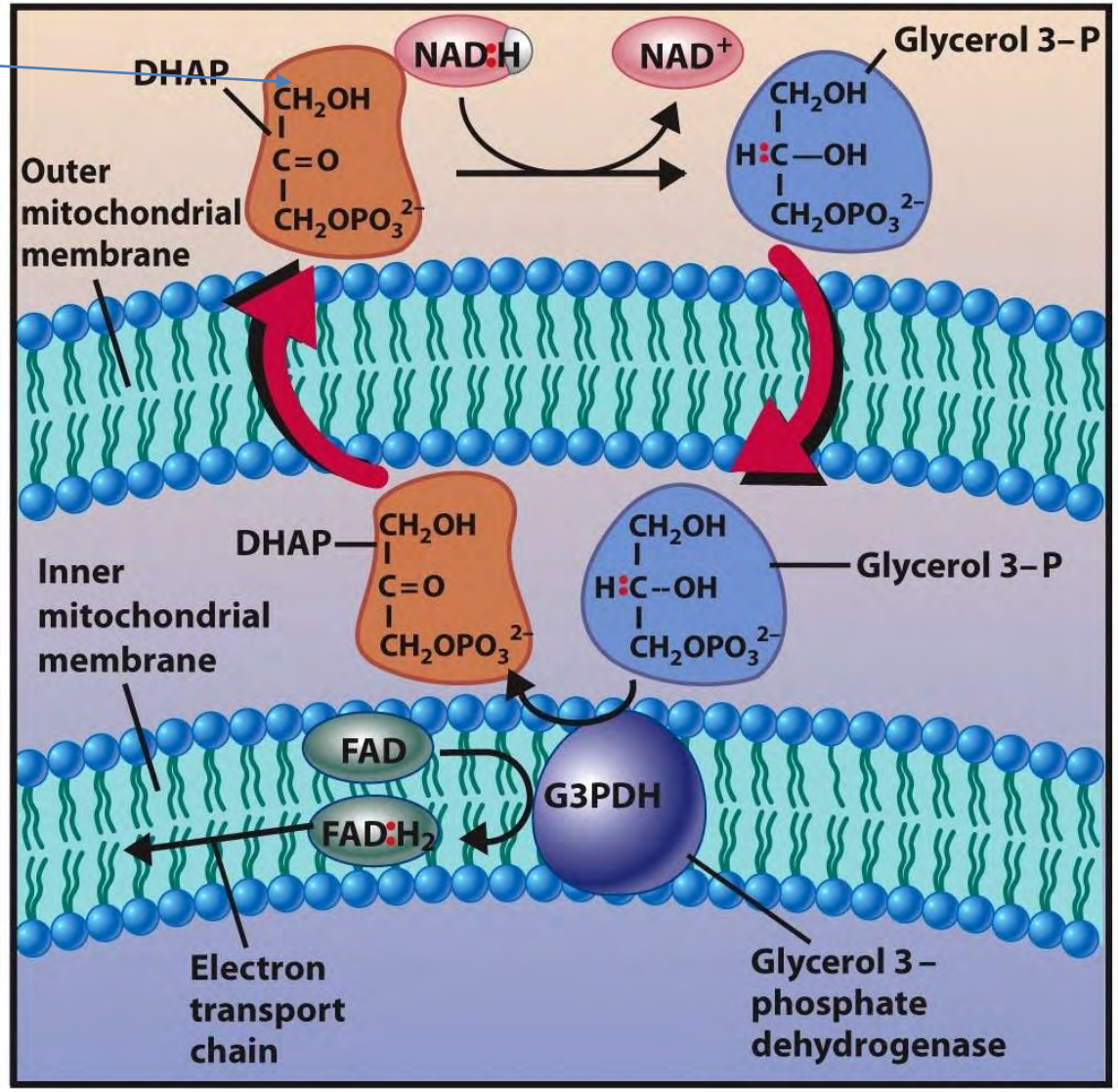
the glycerol phosphate shuttle

DHAP
dihydroxyacetone
phosphate



DHAP is reduced (gains electrons) to G3P in the cytosol.

G3P carries electrons to the mitochondria.

G3P is oxidized inside the mitochondria back to DHAP, and the electrons continue through the ETC to make ATP.

overall reaction of TCA cycle (one round)

Acetyl CoA + 2 H_2O + FAD + 3 NAD^+ + GDP + P_i —>

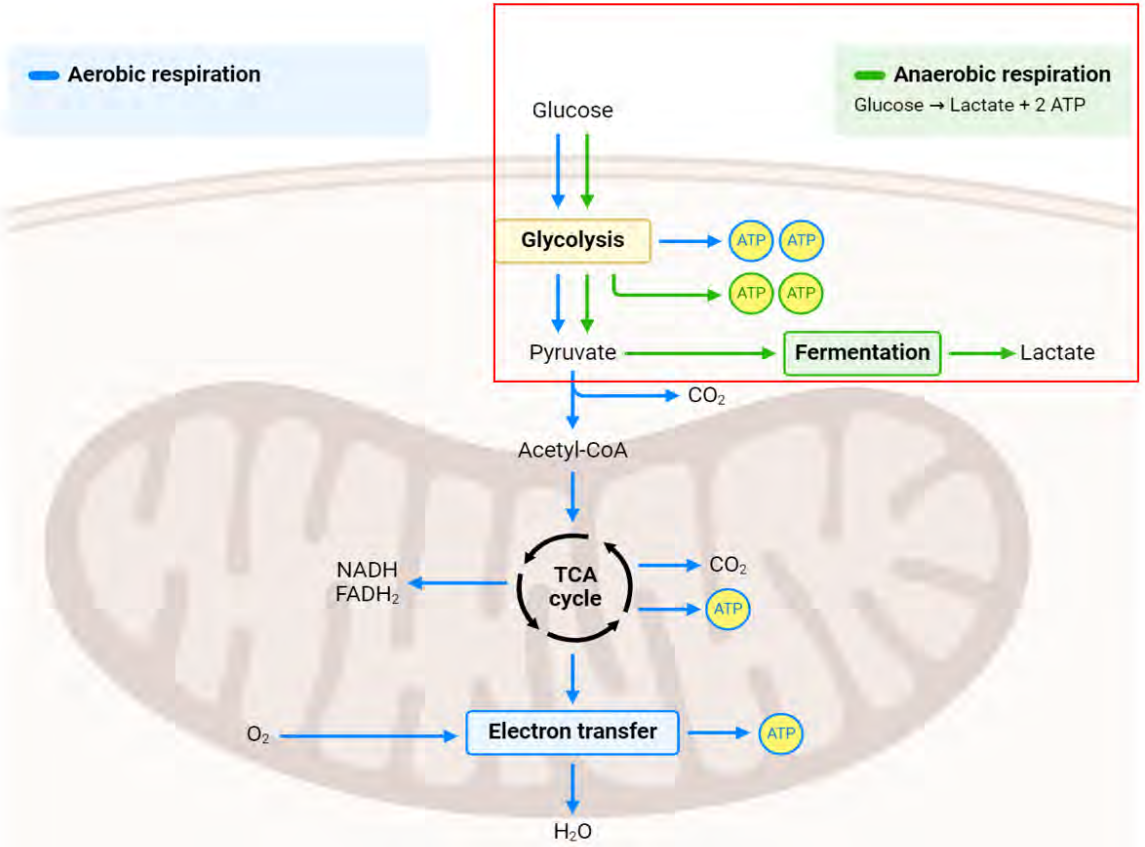
3 CO_2 + $FADH_2$ + 3 NADH + 3 H^+ + GTP + HS—CoA

Electron pair from NADH & $FADH_2$ supply energy to make ~3 ATPs & ~2 ATPs, respectively

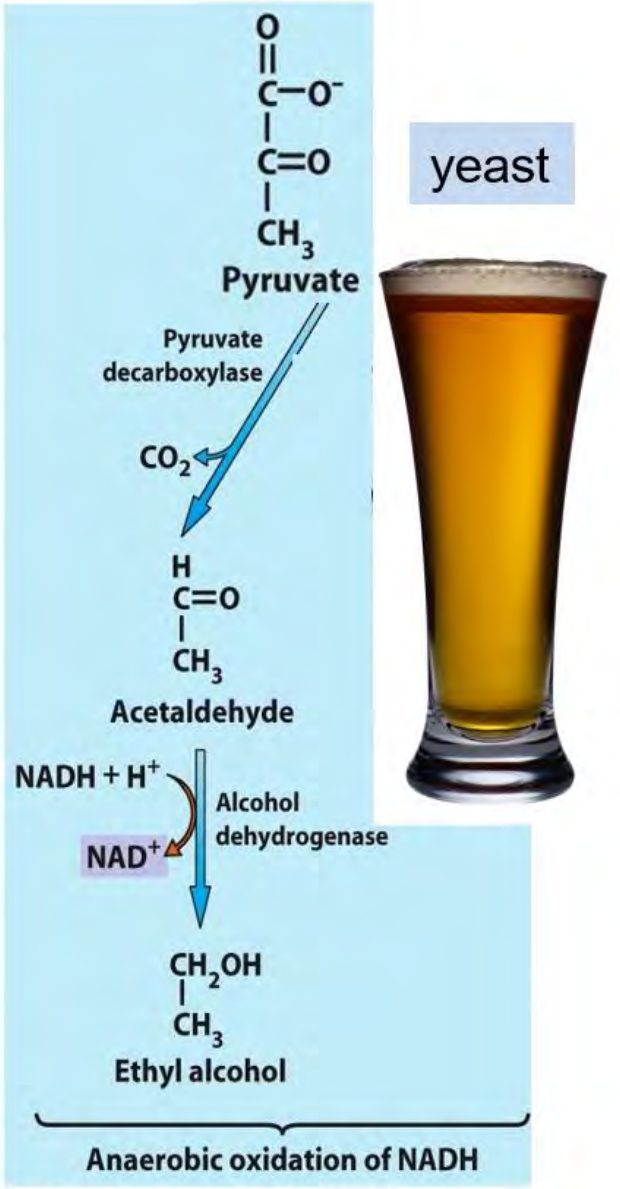
1. Maximal net gain of ATPs from one molecule of glucose is 36 (including GTP) (but often 30) made by each round of TCA cycle
2. Actual number of ATPs made/glucose depends on [ATP]/[ADP] ratio & actual cell activities at time

anaerobic vs aerobic oxidation

In the absence of oxygen; cells produce high levels of lactate

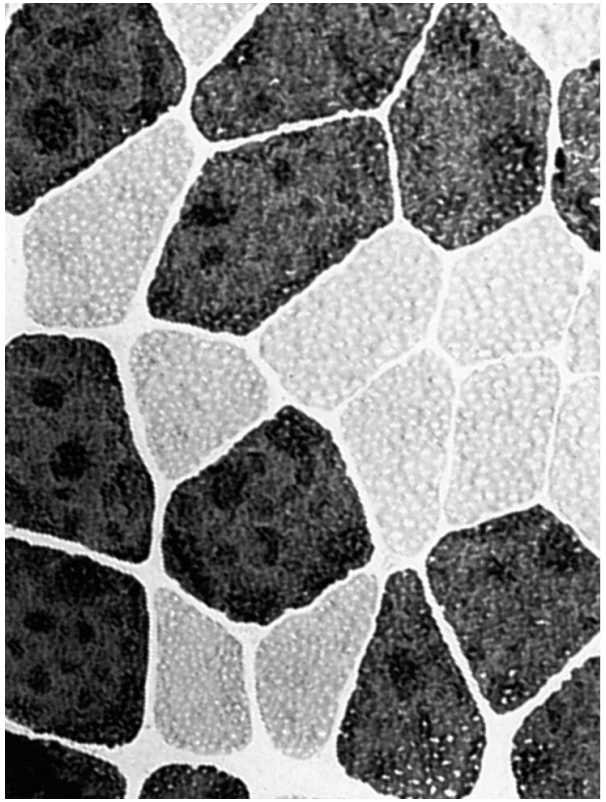


Biorender



what about when energy demand exceeds metabolism speed/input?

during skeletal muscle contractions, ATP hydrolysis increases 100x: how can the cells get enough energy?



- one way is through the use of stored creatine phosphate:

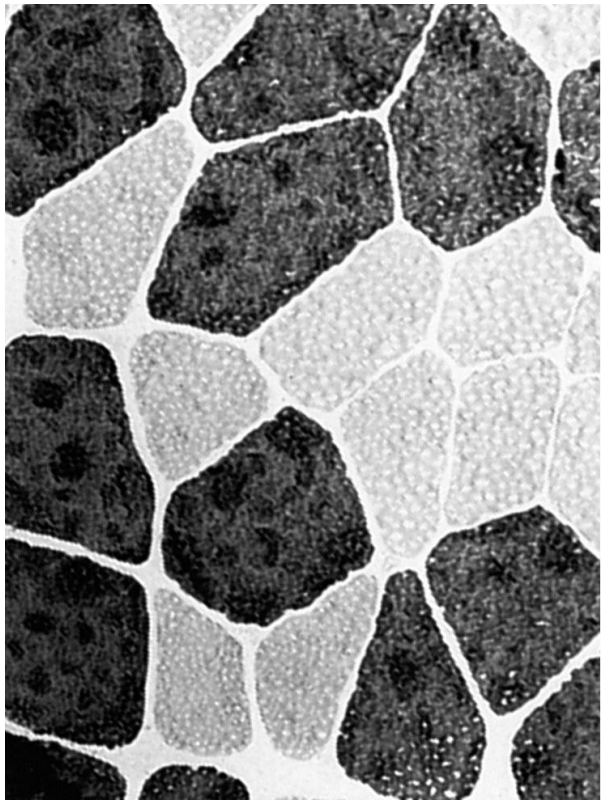
  CrP + ADP → Cr + ATP

- but this also gets depleted

- another strategy is to produce energy anerobically

anaerobic ATP production is fast because glycolysis can occur quickly

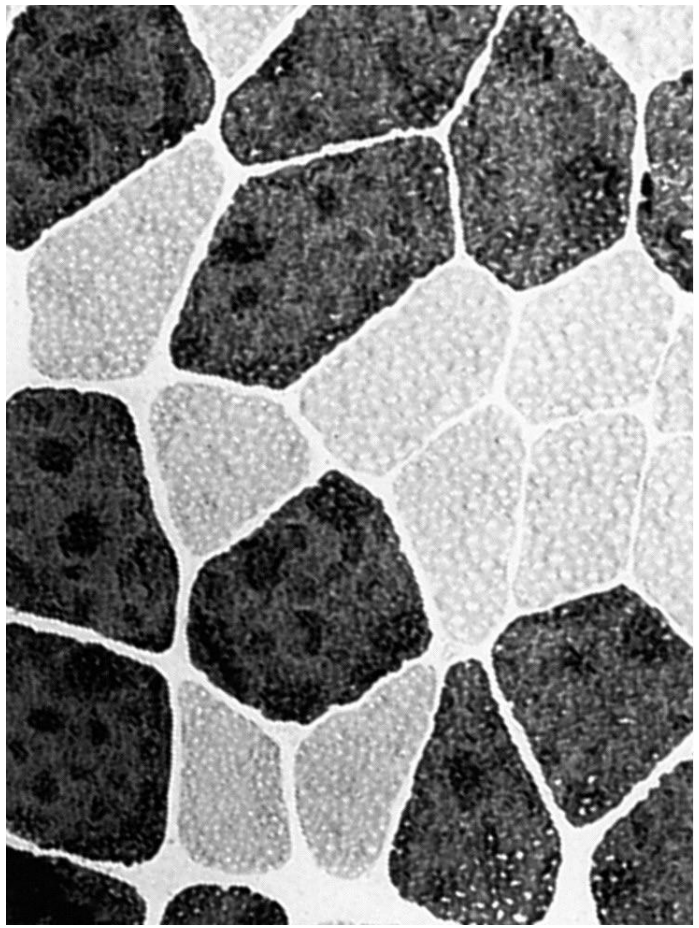
poll: in anaerobic metabolism, how do cells make enough **NAD+** to continue with glycolysis?



a) **from the TCA cycle**
b) **from fatty acid oxidation**
c) **from fermentation of pyruvate to lactate**

link

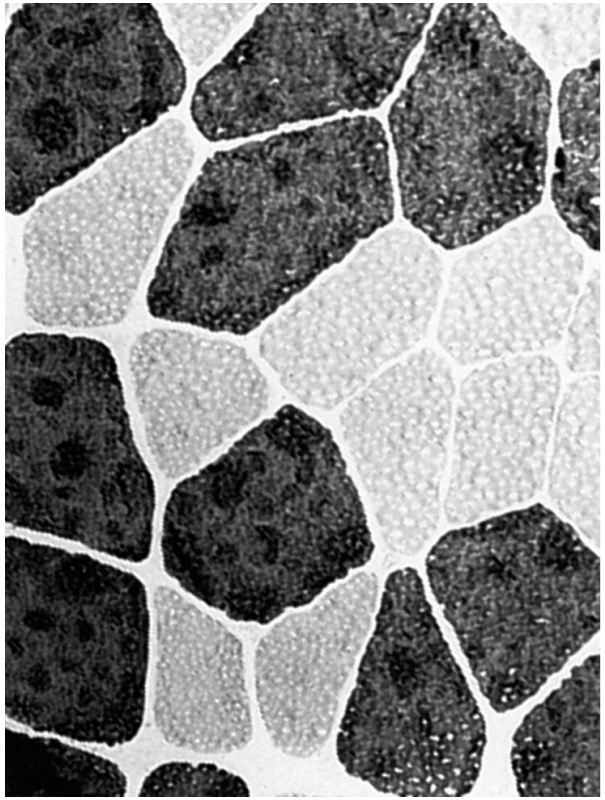
skeletal muscle is made up of two types of fibers – fast twitch and slow twitch



- fast twitch fibers contract quickly and use anaerobic metabolism (of glucose and glycogen)
- slow twitch fibers contract slowly and maintain aerobic metabolism (of glucose or fatty acids with O_2)

skeletal muscle is made up of two types of fibers – fast twitch and slow twitch.

poll: which do you predict have more mitochondria?



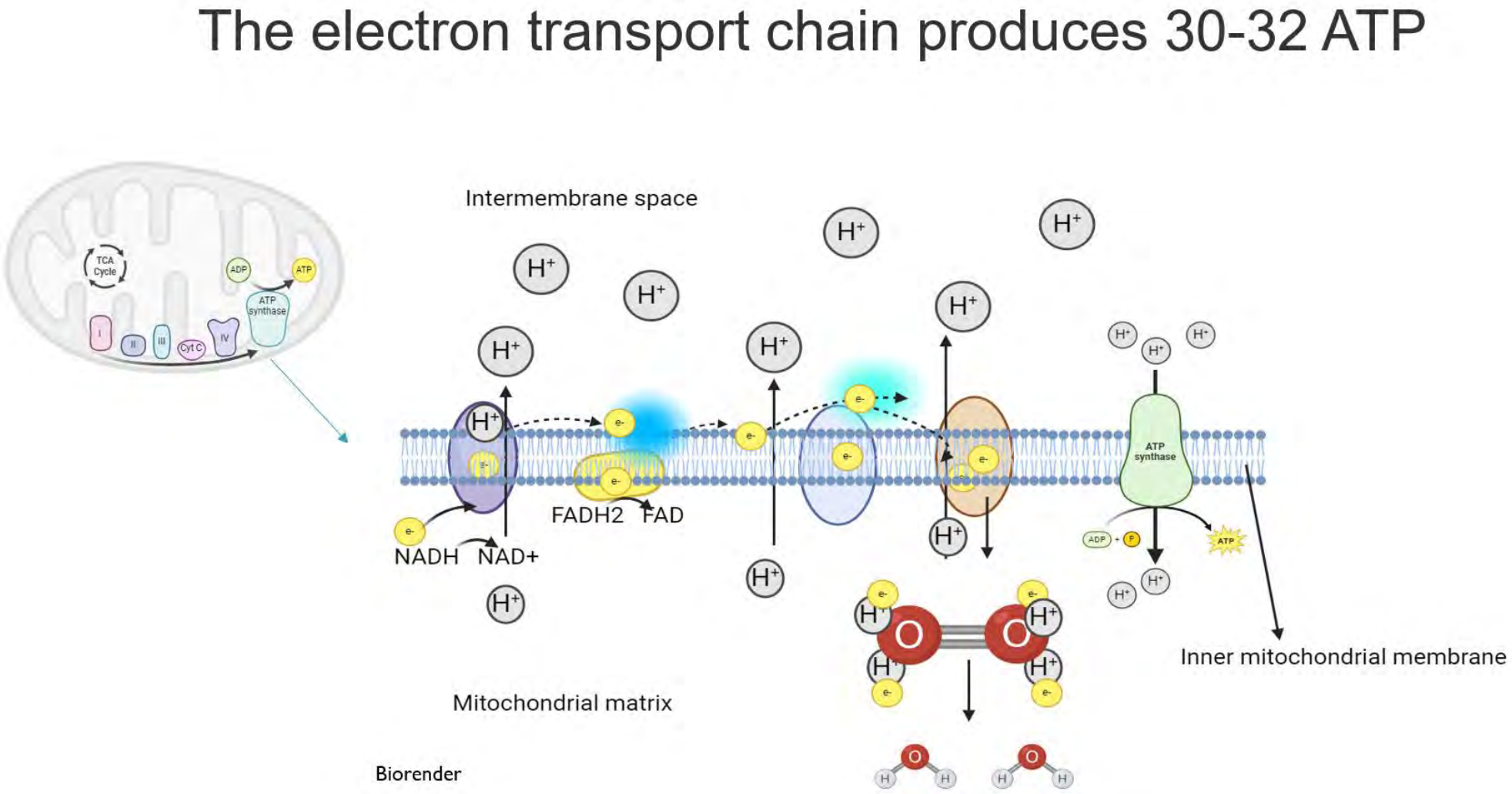
A. **Fast-twitch**
B. **Slow twitch**

- fast twitch fibers contract quickly and use anaerobic metabolism (of glucose and glycogen)
- slow twitch fibers contract slowly and maintain aerobic metabolism (of glucose or fatty acids)

link

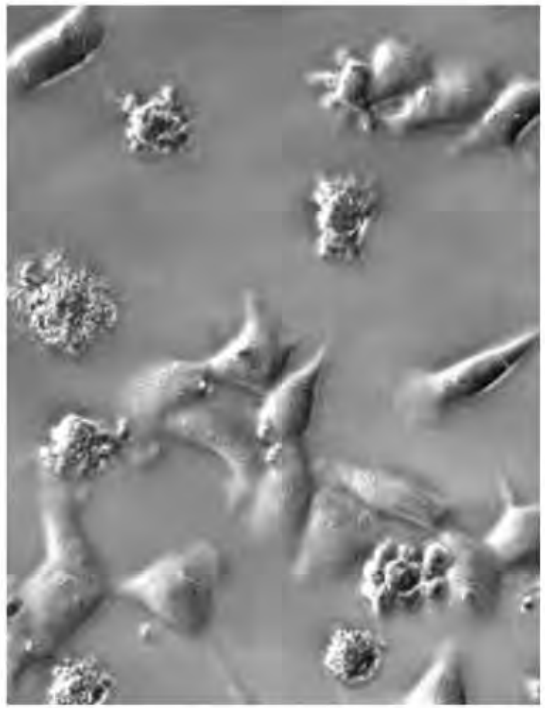
Preview of coming attractions:

summary of oxidative phosphorylation

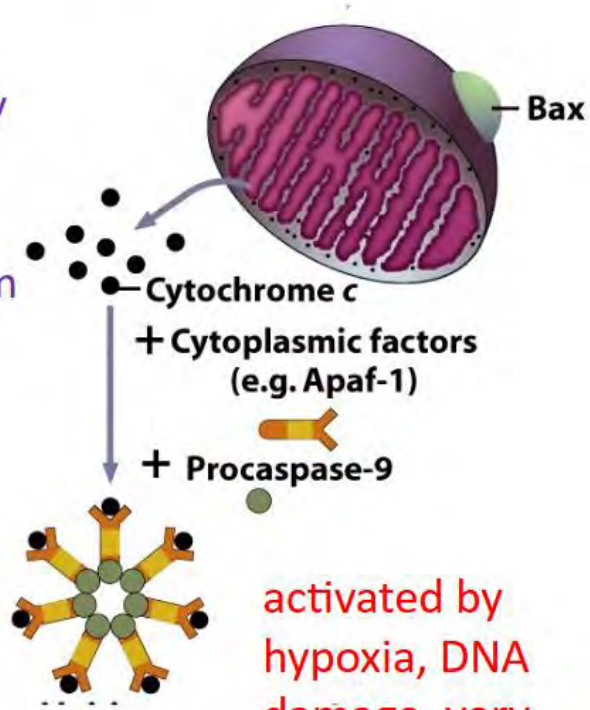


mitochondria also can trigger cell death (apoptosis) if there is damage

There are several types of deliberate cell death in multicellular organisms



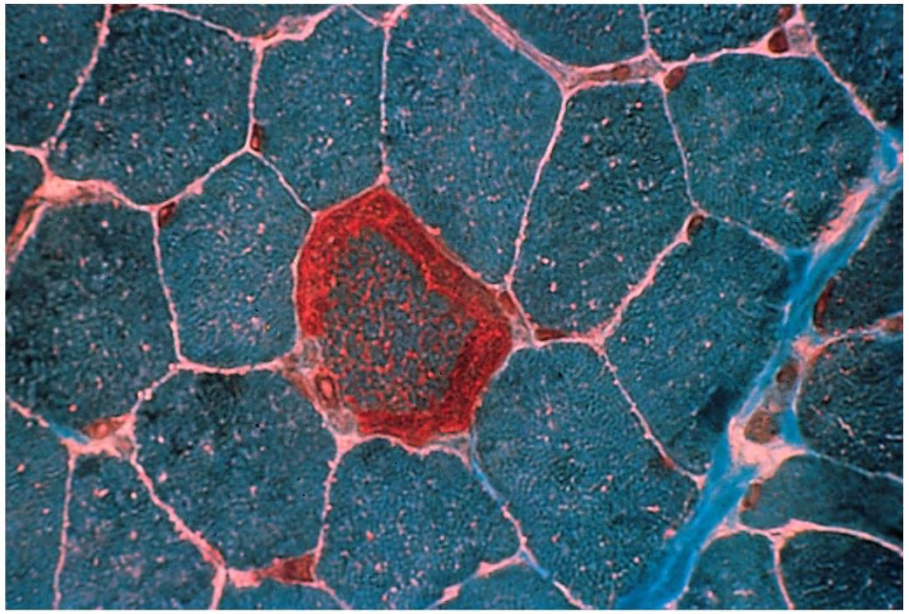
Mitochondria are needed for one intrinsic apoptosis pathway – leads to chopping up of contents and dead cells are removed by the immune system

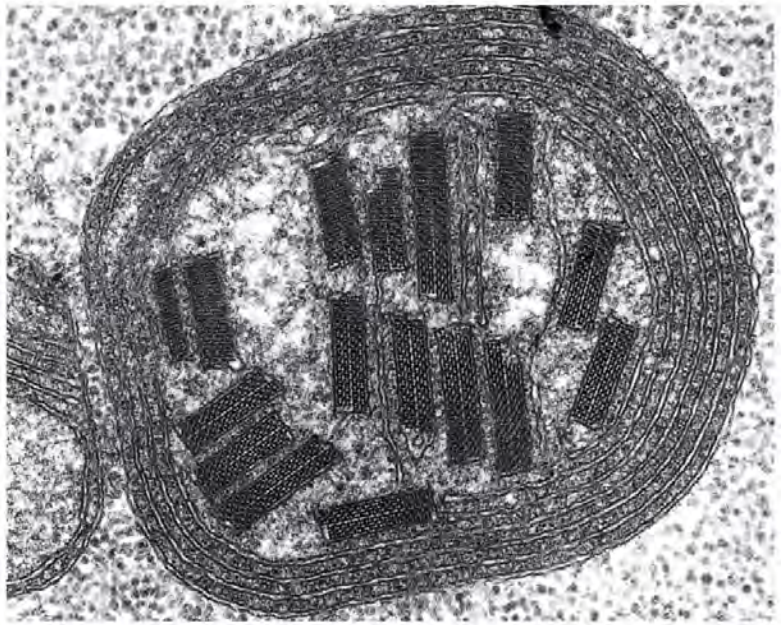


activated by hypoxia, DNA damage, very high Ca++, free radicals,

abnormal mitochondrial function can lead to disease/disorders

- majority of mutations linked to mitochondrial diseases are traced to mutations in mtDNA.
- mitochondrial disorders are inherited maternally.





these diseases predominantly affect muscle and/or brain tissue

abnormal mitochondrial function may contribute to aging

- some evidence suggests that accumulations of mutations in mtDNA is a major cause of aging.
- mutations in mtDNA may cause premature aging but are not sufficient for the *normal* aging process.

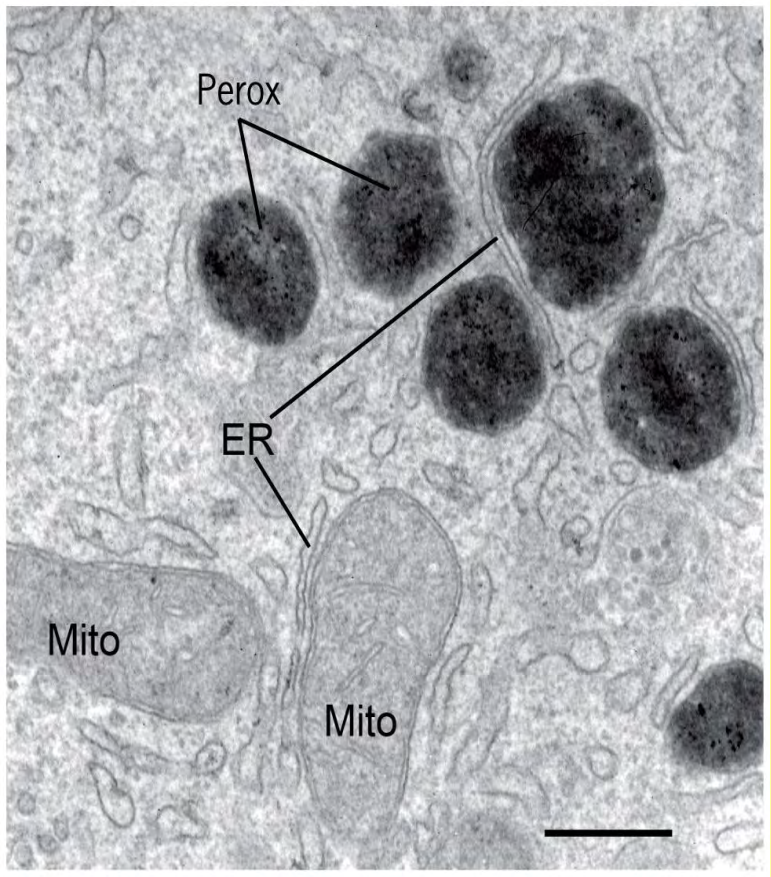
in mice carrying a mutation in their mtDNA, signs of premature aging develop

peroxisomes are another important organelle in metabolism (and for fireflies)

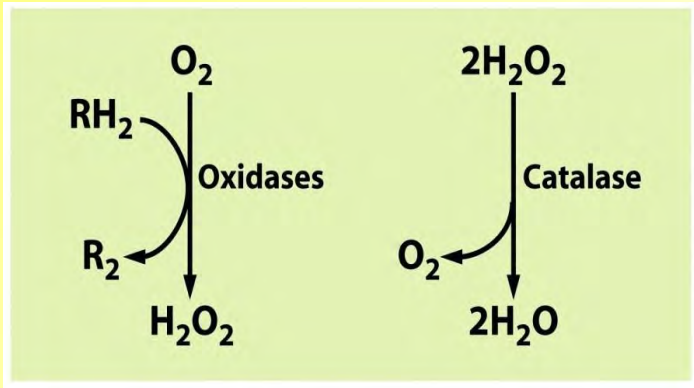
- **peroxisomes** are single membrane-bound vesicles that contain oxidative enzymes
- they form by splitting from pre-existing organelles, import preformed proteins, and engage in oxidative metabolism
- the site of **oxidization of very-long-chain fatty acids** (*typically 24-26 Carbons*), and synthesize plasmalogens (a class of phospholipids)

http://www.uknowhy.com/blog/u-know-why-the-fireflies-glow/

structure and function of peroxisomes

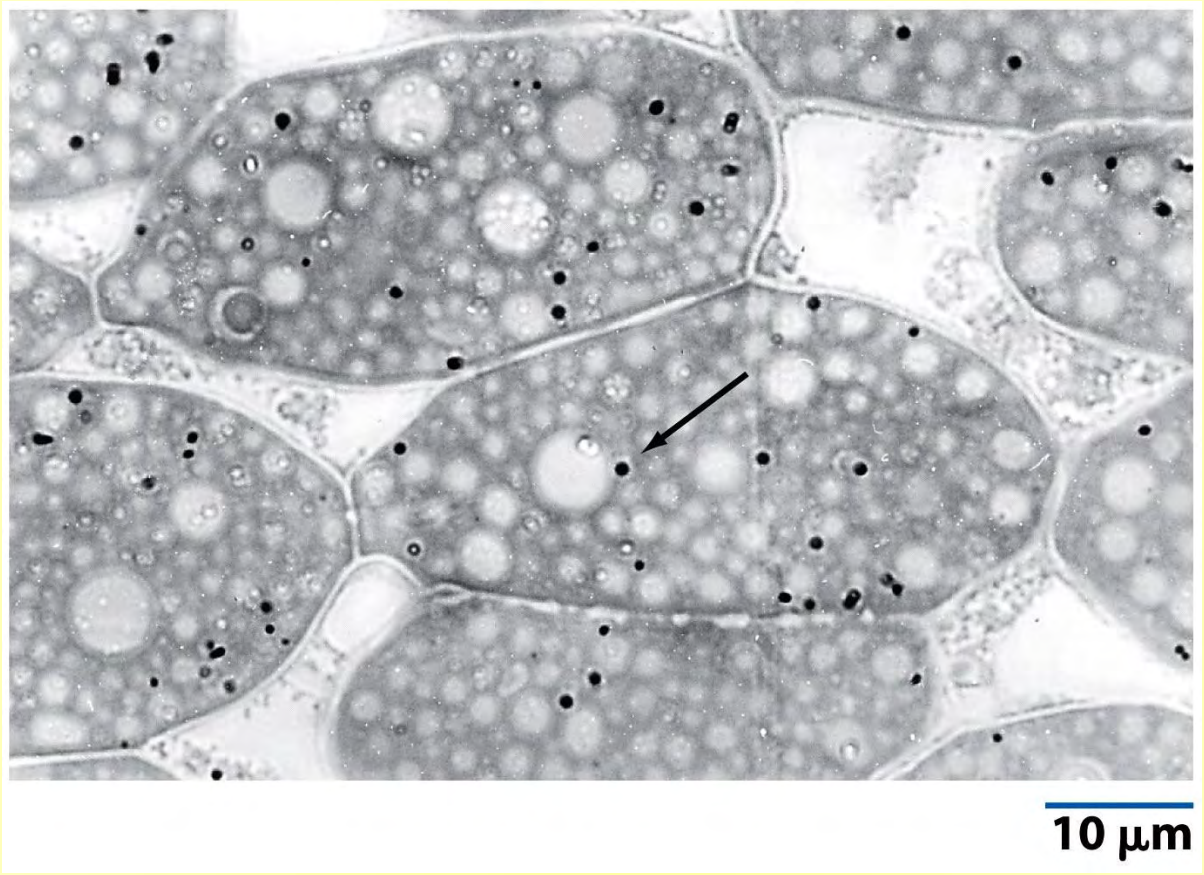


hydrogen peroxide (H_2O_2), a reactive and toxic compound, is formed in peroxisomes and is broken down by the enzyme catalase



- Round or oval.
- **Single lipid bilayer**,
- Dense interior packed with enzymes.
- No genetic material: They rely on the nucleus and cytoplasmic ribosomes to make their proteins.
- Often near mitochondria: because they work closely in metabolism, especially fatty acid breakdown

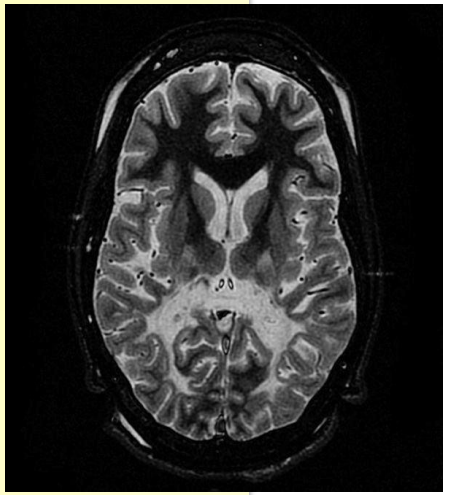
glyoxysomes are special peroxisomes in plants



glyoxysomes can convert fatty acids to glucose by germinating seedlings.

abnormal peroxisomal function can cause disease

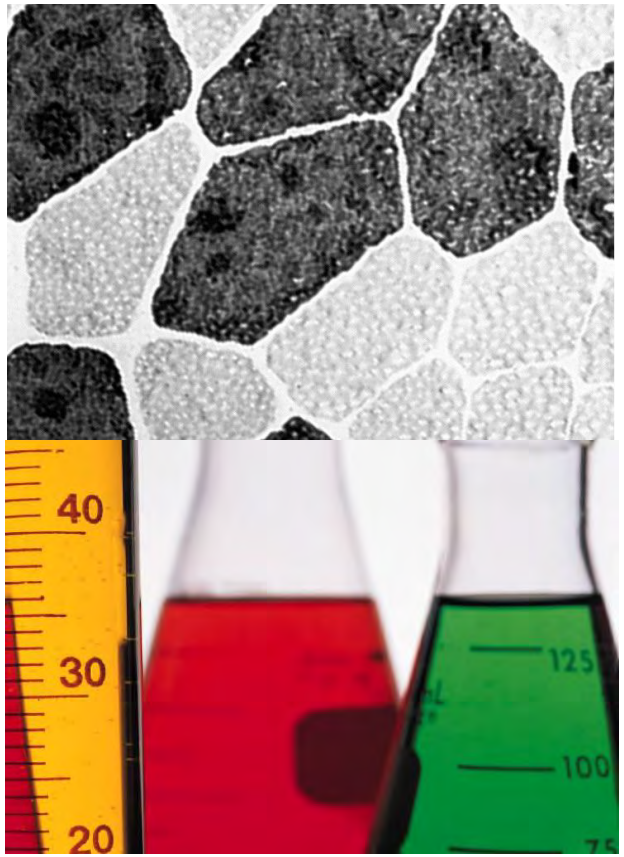
- patients with Zellweger syndrome lack peroxisomal enzymes due to defects in translocation of proteins from the cytoplasm into the peroxisome – affects mostly liver, kidney, brain
- Adrenoleukodystrophy is caused by lack of a peroxisomal enzyme, leading to fatty acid accumulation in the brain and destruction of the myelin sheath of nerve cells.

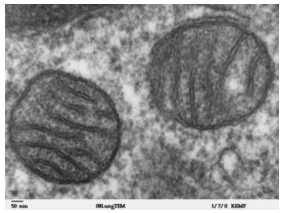


http://radiopaedia.org/images/318699

an ATP production experiment

which of the following could you add to your purified mitochondria to result in ATP production?





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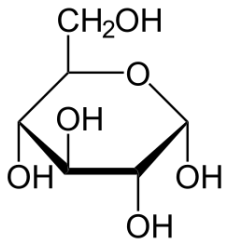


+

a) glycogen
b) lactic acid
c) pyruvate
d) short-chain fatty acids
e) two of the above

link

a Fall estimation problem:

poll: How many ATP can you make from ONE candy corn (optimally)

A. 2

B. 342

C. 1×10^{7}

D. 2×10^{23}

E. 4×10^{46}

PollEv.com/msg303
Send **msg303** to **22333**

you don't need a calculator!
one candy corn weighs 2g.
molecular weight of sucrose : 342g/mol
6×10^{23} molecules/mole

link

A Fall estimation problem

candy corn is 90% “sugar” (no fat) ~ sucrose, which can be broken down into fructose and glucose. Fructose can be converted to pyruvate via fructolysis with energy gain similar to glycolysis

How many ATP can you make from one candy corn (optimally)?

estimate!!

you don’t need a calculator!

one candy corn weighs 2g.

molecular weight of sucrose : 342g/mol

6×10^{23} molecules/mole

estimation

you don't really need a calculator!
one candy corn weighs 2g.
molecular weight of sucrose : 342g/mol
6 x 10^{23} molecules/mole

- 1.8g/342g/mol = 0.005mol
- x $6x10^{23}$ molecules/mole

$= 5x10^{-3} \times 6x10^{23}$

$= 3x10^{21}$ molecules sucrose

estimating 36 ATP for <u>each</u> glucose and fructose

$= 2\ (36 \times 3x10^{21})$

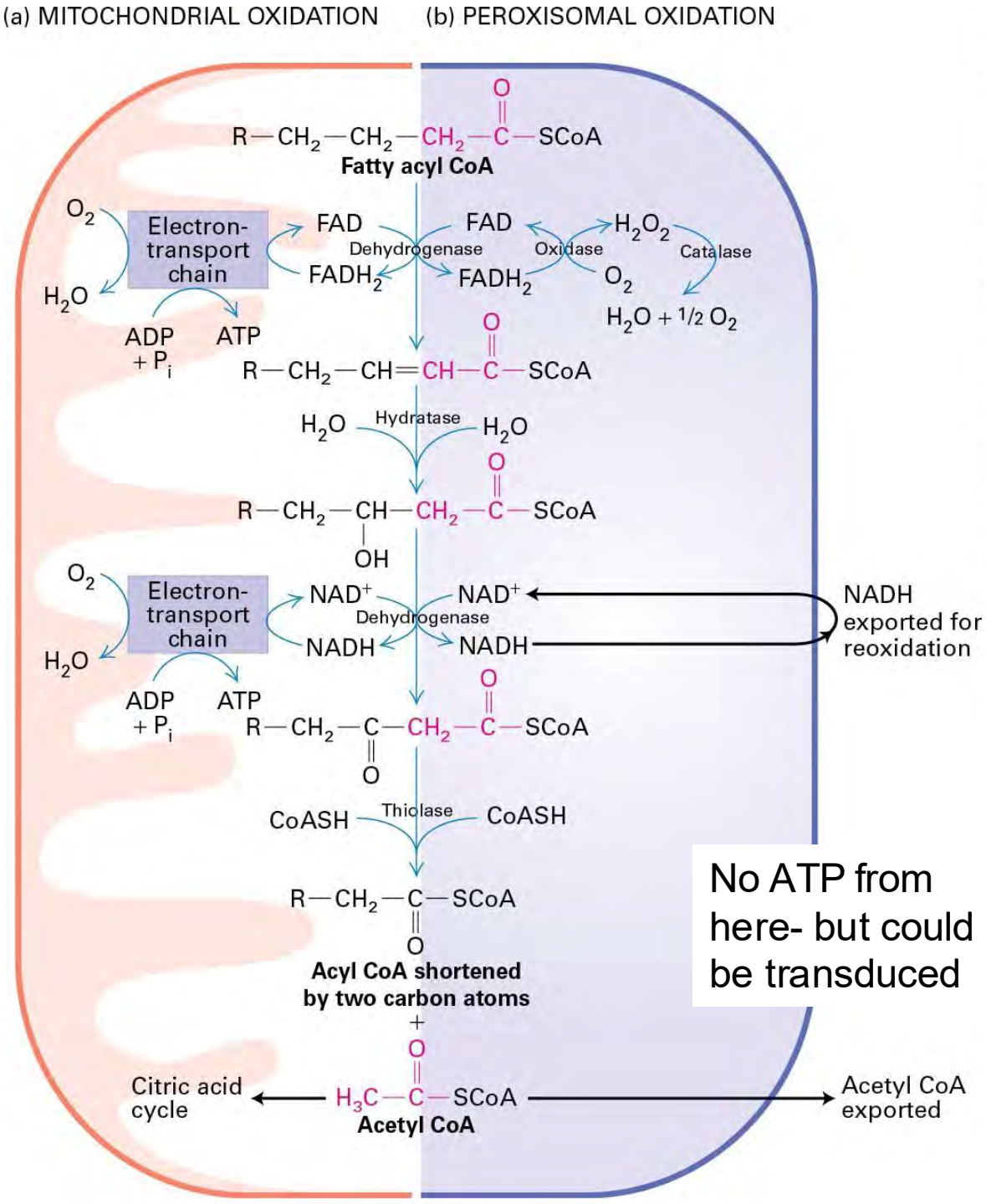
$= 2.16x10^{23}$ ATPs!!

Even a tiny 2-gram candy corn contains enough sugar to produce an astounding 216,000,000,000,000,000,000,000 ATP molecules!

Fatty acids are oxidized in mitochondria and/or peroxisomes

- Converted into acetyl CoA

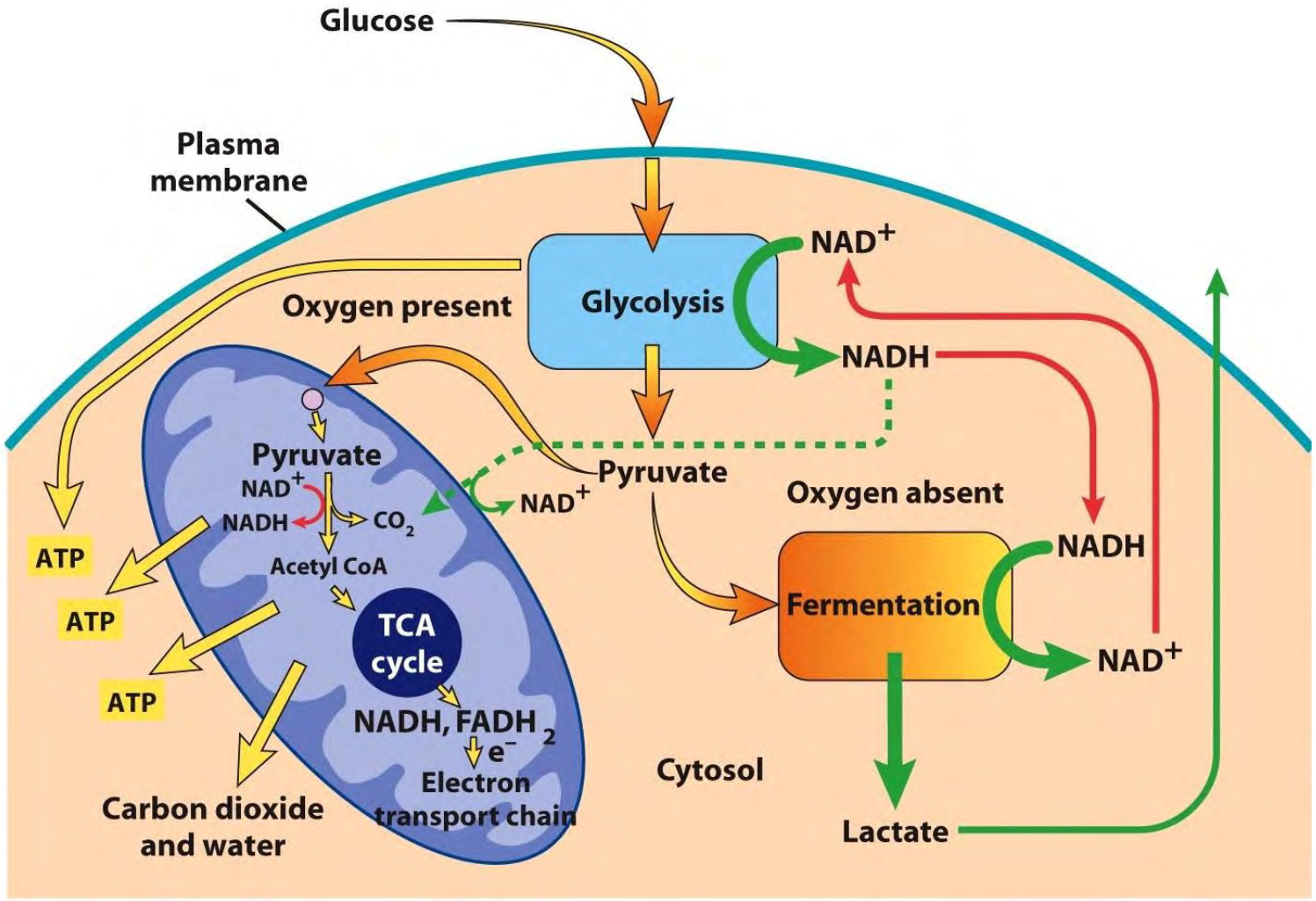
A fatty acyl CoA molecule is converted to acetyl CoA and a fatty acyl CoA shortened by two carbon atoms. One FAD molecule is reduced to $FADH_2$ and one NAD+ is reduced to NADH.



Maximal net gain of ATP*s from one molecule of glucose is 36 (*one is GTP) made by each round of citric cycle

MOST of the ATP comes from this -→

Lots more on that next time



… but sometimes cells need something faster or they don't have enough O_2

flashback

$\Delta G^{\circ\prime}$ = standard free-energy change

- Ideal conditions •
- Reactants = 1 mole/liter •
- $\Delta G = \Delta G^{\circ\prime} + RT \ln [X]/[Y]$ •
- R = gas constant •
- T = absolute temperature (Kelvin) •
- X and Y = reactants and products in moles/liter •
- ln = natural logarithm

for next time

- review oxidation/reduction reactions
- More of chapter 12

(but not photosynthesis)

- Discussion & related work