Metabolism & ATP

(Part 2 of 2)

Lecture 14

Objectives

- Describe the structure, conformational states and reproduction of mitochondria. Compare between inner & outer membranes structure of the mitochondria
- Describe BRIEFLY the Krebs cycle and list its products
- Describe BRIEFLY the electron transport system/chain and list its product
- Describe the breakdown of ATP production per one glucose usage (the total ATP accounting)

Metabolism Re-Overview

- Glucose breakdown & ATP production (cellular respiration) shown in equation in previous lecture is complex process involving three main pathways:

1. Glycolysis, occurring in cell cytoplasm
2. TCA (Krebs) cycle, occurring in mitochondria
3. Electron transport system/chain & oxidative phosphorylation, also occurring in mitochondria

- From ONE glucose molecule, the three pathways will produce a total of 36 ATP molecules:
  - 2 from glycolysis
  - 2 from Krebs cycle
  - 32 from electron transport system

Mitochondria

- The "powerhouse of the cell": that is, where most ATP production occurs
- 0.5-1 μm diameter, about 7 μm long (RBC diameter) but variable in size and shape
- Range from 100 to several thousand in number, depending on cell's metabolic activity

Mitochondria As "Organism"

- Mitochondria have a single, circular ds DNA similar to bacteria, including the DNA polymerases to replicate the DNA and the RNA polymerases to transcribe the few genes
- Mitochondria also have ribosomes, so they can translate the proteins of the transcribed genes
- They are not autonomous organisms however, since they must import proteins from the proteins coded by cell's nucleus to function
- Mitochondria were once thought to be intracellular symbionts of the first eukaryotic cell
- All mitochondria are inherited from the mother, since sperm do not infuse the fertilized egg with the organelle

Mitochondrial Structure

1. matrix

   has all soluble enzymes for Krebs cycle as well as DNA and ribosomes of mitochondria

2. inner membrane

   contains all the electron transport system components to generate ATP

3. intermembrane space

   space between outer and inner membrane

   it will accumulate protons to drive ATP generation

4. outer membrane

   a highly porous membrane that encloses the organelle

5. crista

   inward invaginations of the inner membrane that increase surface area to permit large amounts of ATP production

This figure shows

- mitochondria relative to size and location in the cell
- the basic cross section of the organelle
  - schematically
  - in an electron micrograph

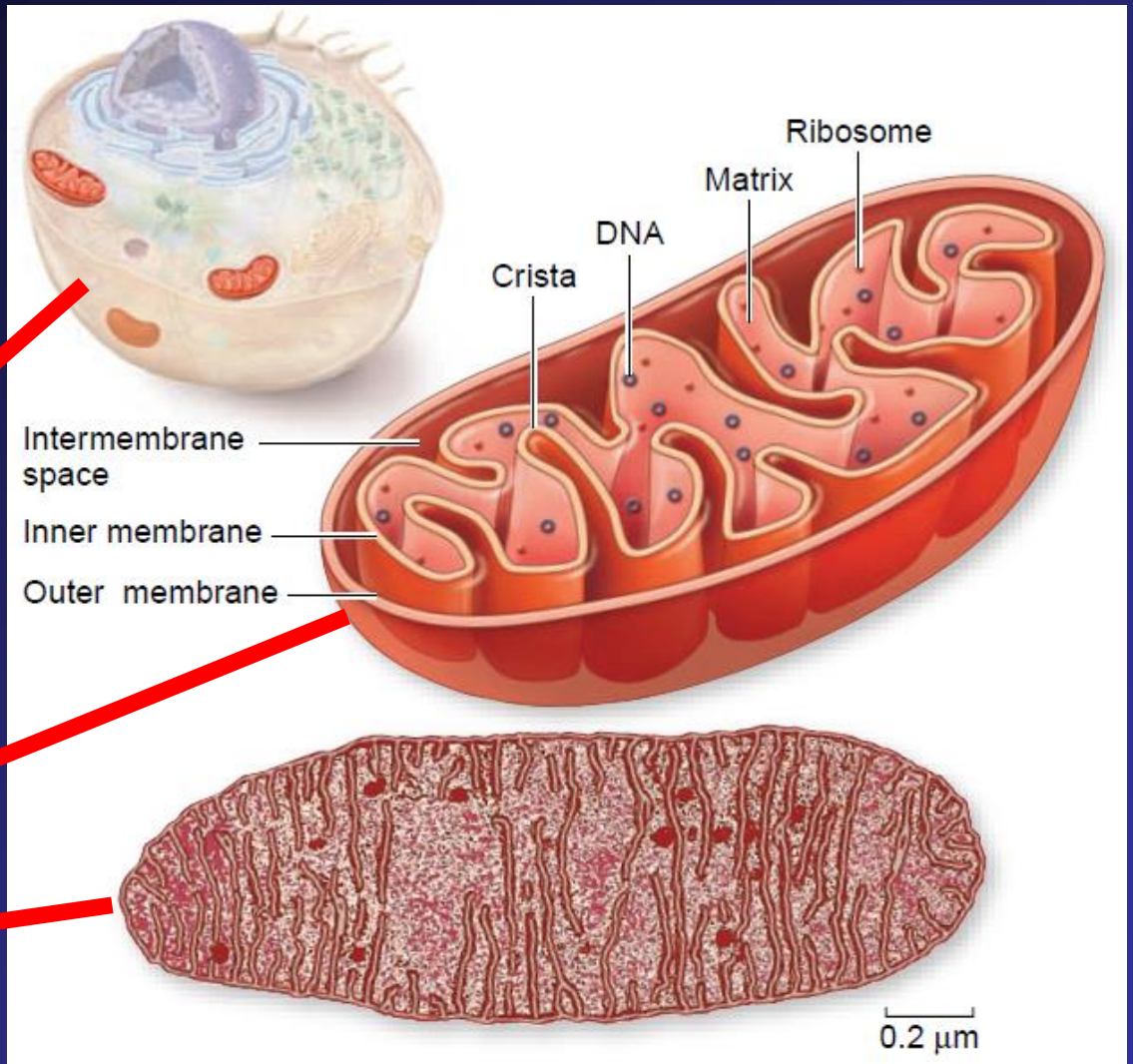


Figure 4.16 Mitochondria. The inner membrane of a mitochondrion is shaped into folds called cristae that greatly increase the surface area for oxidative metabolism. A mitochondrion in cross section and cut lengthwise is shown colored red in the micrograph.

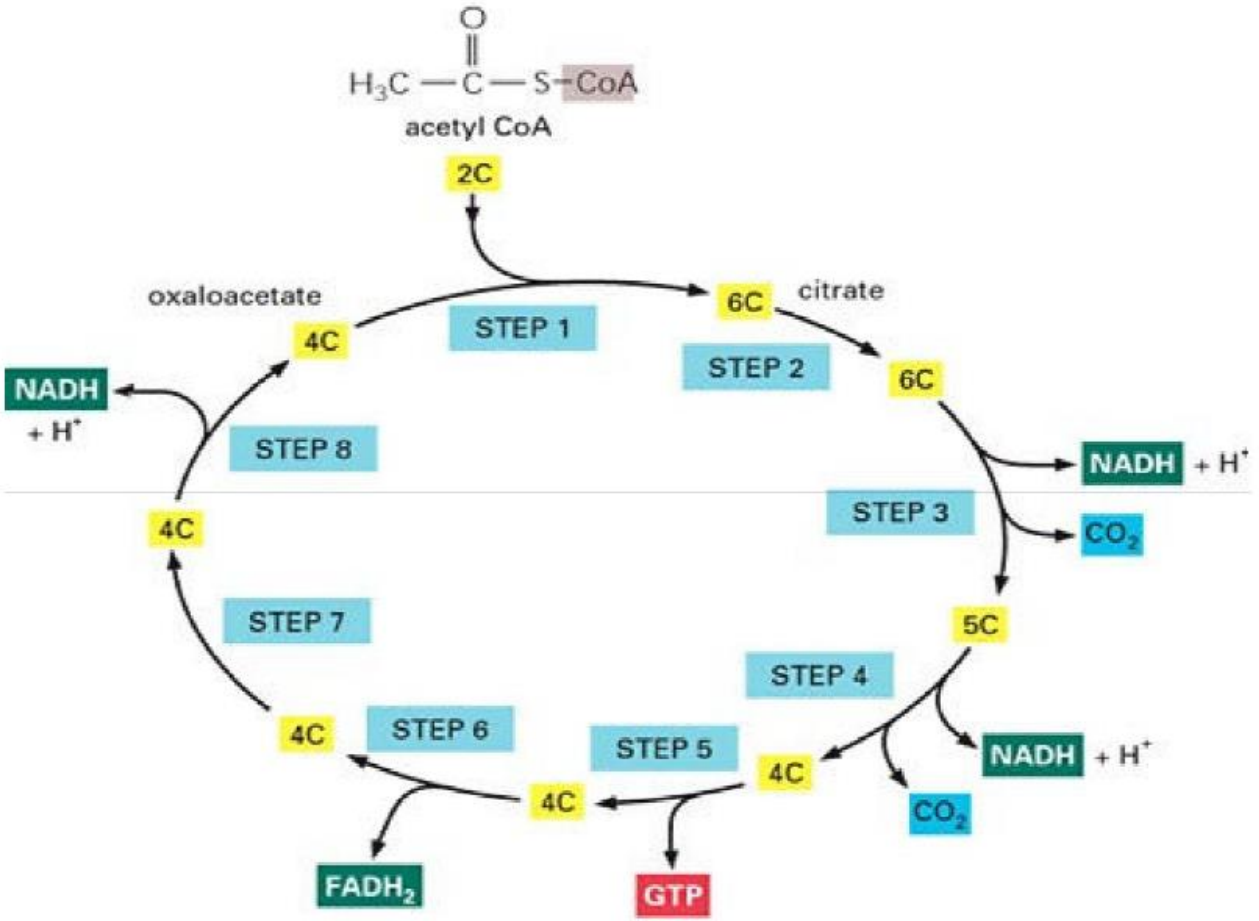
TCA (Krebs) Cycle Important Steps 1 of 2

- Reaction 1—2-carbon acetyl-CoA formed from pyruvate oxidation enters the cycle and combines with 4-carbon oxaloacetate coming out of the end of the cycle to form 6-carbon citrate
  - the energy for condensing these two compounds comes from Coenzyme A bond breaking; CoA goes back to PDHase reaction
- Reactions 3-4—one CO_2 molecule is generated in each of these oxidative decarboxylation reactions (total two CO_2 molecules): 6C → 5C → 4C
  - NAD^+ is reduced to NADH + H^+ in each of these two reactions, which will later make ATP

TCA (Krebs) Cycle Important Steps 2 of 2

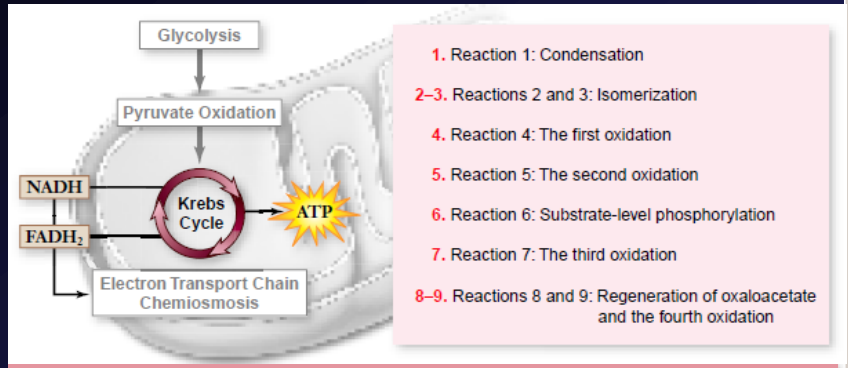
- Reaction 5—a substrate-level phosphorylation step: GDP is phosphorylated to GTP; GTP can phosphorylate ADP to make ATP
  - consider this to be a substrate-level phosphorylation reaction to make ATP
- Reaction 6—Another oxidation (dehydrogenation) step. The redox potential of (energy from) this step is not great enough to use NAD as electron acceptor: FAD is electron acceptor instead, reduced to $FADH_2$ (has lower redox potential)
- Reaction 8—Another NADH-generating oxidation step, and the final step of the cycle, generating oxaloacetate to go back to step 1

TCA Cycle

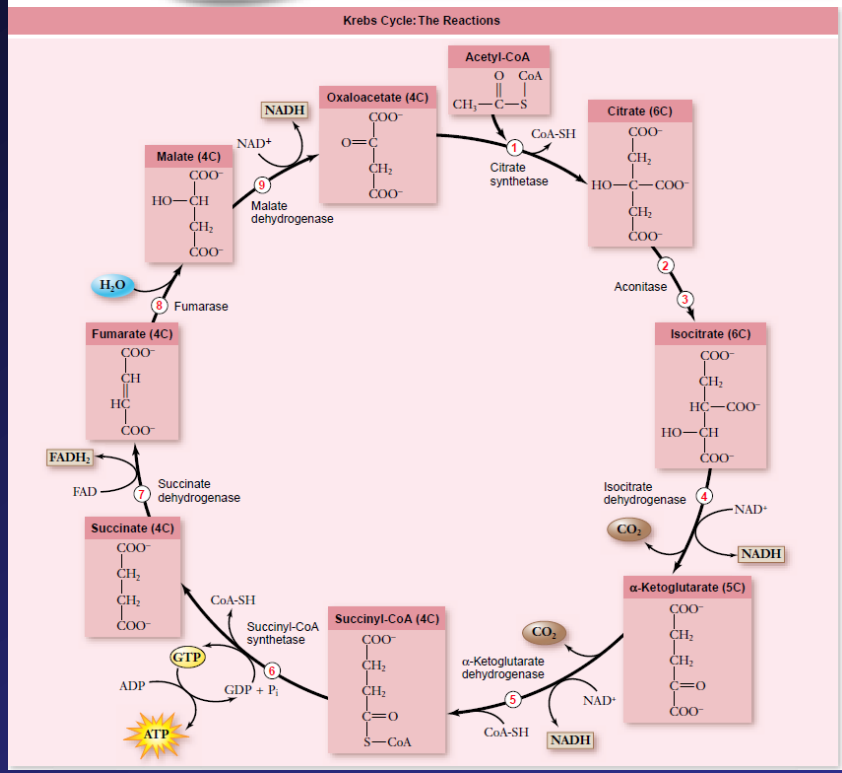


NET: 3 NADH, 1 ATP, 1 $FADH_2$, & 2 CO_2

(Double the above figures per one glucose molecule)



1. Reaction 1: Condensation
2–3. Reactions 2 and 3: Isomerization
4. Reaction 4: The first oxidation
5. Reaction 5: The second oxidation
6. Reaction 6: Substrate-level phosphorylation
7. Reaction 7: The third oxidation
8–9. Reactions 8 and 9: Regeneration of oxaloacetate and the fourth oxidation



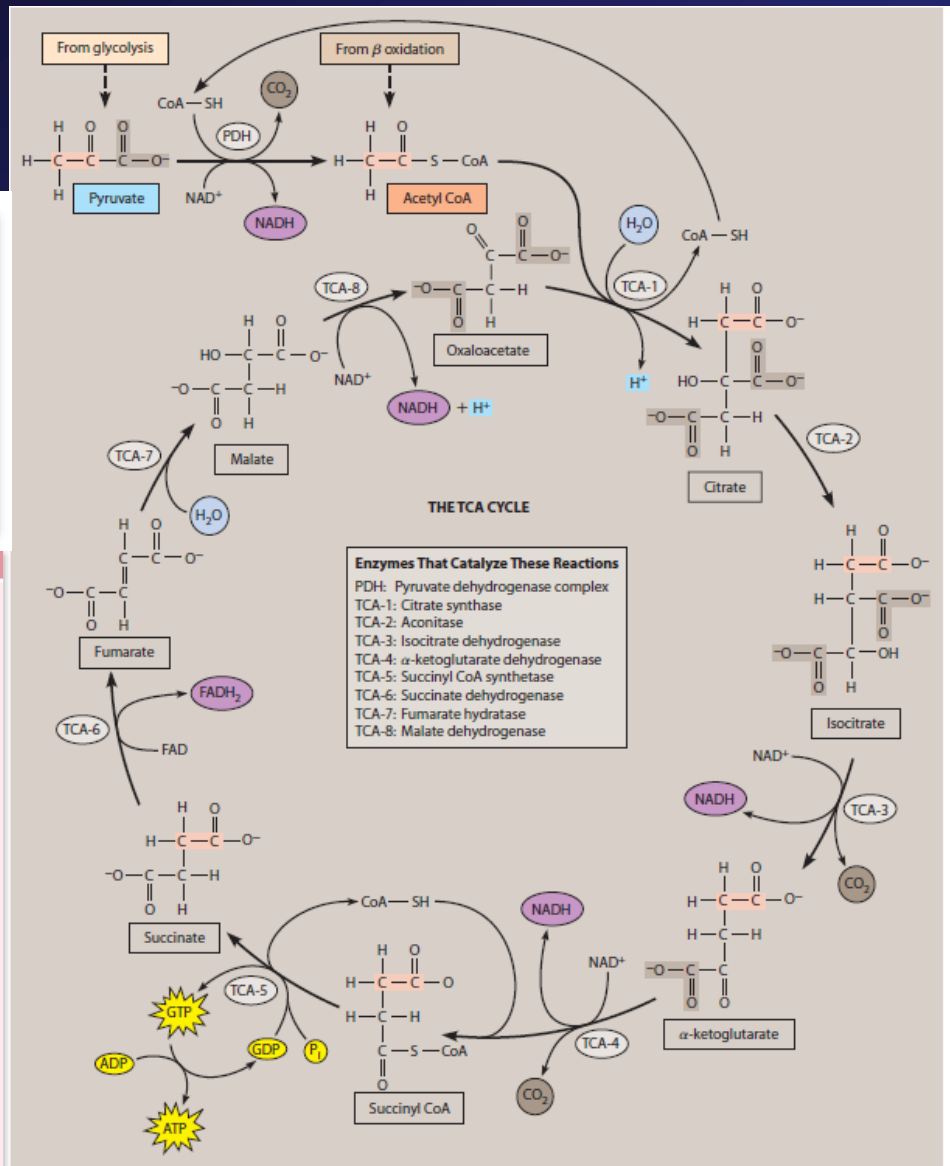


FIGURE 10-8 The Tricarboxylic Acid (TCA) Cycle. The two carbon atoms of pyruvate that enter the cycle via acetyl CoA are shown in pink in citrate and subsequent molecules until they are randomized by the symmetry of the fumarate molecule. The carbon atom of pyruvate that is lost as CO_2 is shown in gray, as are the two carboxyl groups of oxaloacetate that give rise to CO_2 in Reactions TCA-3 and TCA-4. Five of the reactions are oxidations, with NAD^+ as the electron acceptor in four reactions (PDH, TCA-3, TCA-4, and TCA-8) and FAD as the electron acceptor in one case (TCA-6). The reduced form of the coenzyme is shown in purple in each case. Note that when CO_2 is released, no H^+ is given off during NAD^+ reduction, thereby maintaining the charge balance of these reactions. The generation of GTP shown in Reaction TCA-5 is characteristic of animal mitochondria. In bacterial cells and plant mitochondria, ATP is formed directly.

Summary

- Oxygen is required for TCA cycle to proceed
- This is a cyclical series of reactions that give off CO_2 and produce one ATP in the cycle itself
- Two turns of the cycle per glucose molecule→ produces two ATP molecules from the cycle itself
  - ATP is produced by substrate level phosphorylation

Krebs Cycle Summary

- takes place in matrix of mitochondria.
- Each turn of the Krebs Cycle also produces three (3) NADH, one (1) $FADH_2$, and two (2) CO_2 molecules, so two turns double these numbers for one (1) glucose molecule

Electron Transport System

- Also called electron transport chain
- These are complexes of proteins and lipophilic molecules that are embedded in the mitochondrial inner membrane
- They will take the reduced coenzymes, NAD and FAD, produced from glycolysis and Krebs cycle and will oxidize (take electrons) from them
- A series of oxidation-reduction reactions will take place—electrons being passed from one component to another
- These succession of oxidation-reduction reactions will vectorially move protons (H^+ ions) across the mitochondrial inner membrane, generate a concentration difference that will drive ATP generation

Oxidation-Reduction Reaction

- red = reduced, ox = oxidized
- here's an example reaction
- $ETS\text{-}1_{red} + ETS\text{-}2_{ox} \rightarrow ETS\text{-}1ox + ETS\text{-}2red$
- Now here's how the reaction is broken into two half-reactions

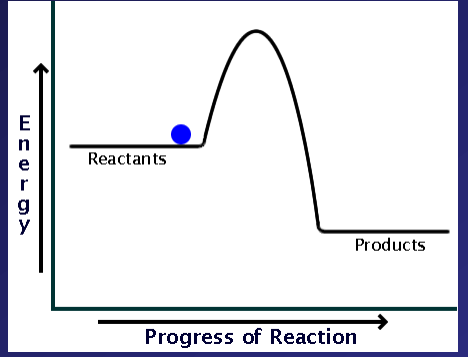
1. $ETS\text{-}1_{red} \rightarrow ETS\text{-}1_{ox} + 2\ e^- + 2\ H^+$
2. $ETS\text{-}2_{ox} + 2\ e^- + 2\ H^+ \rightarrow ETS\text{-}2_{red}$

- These half-reactions importantly show how protons are released and consumed in the process
- In oxidation, with release of H^+, H^+ are released from the inner membrane into the intermembrane space
- In reduction, with consumption of H^+, H^+ are taken from the matrix into the inner membrane

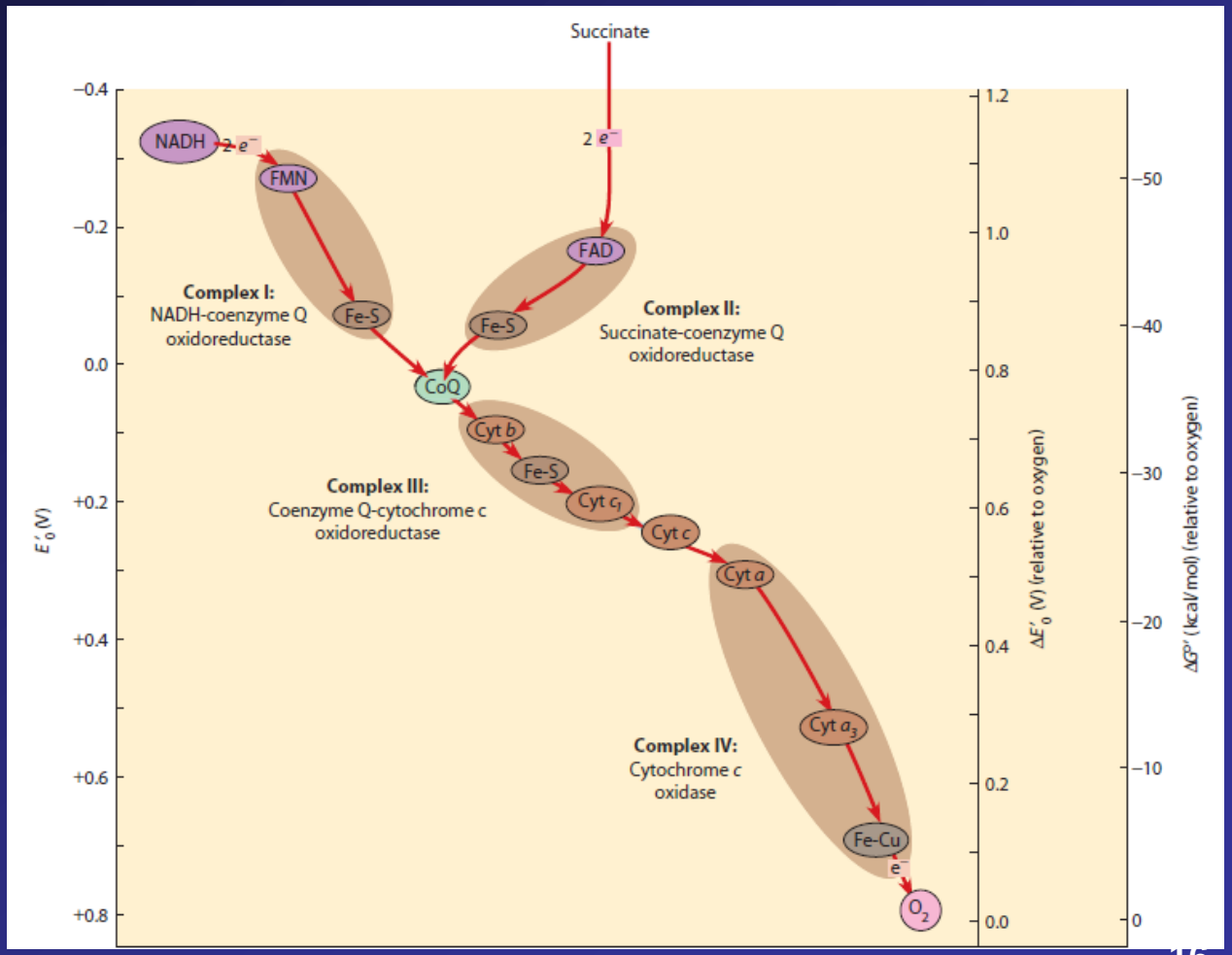
ETS Energetics

- Electrons from NADH are passed along a chain of electron acceptors in turn
- Each electron acceptor has a lower "redox potential" (potential energy) than one component before it in chain
- Certain series of reactions have enough energy to power production of 1 ATP molecule
- These ETS components, in order, are NAD → CoQ → cytochrome → oxygen (O_2): 3 ATP per NAD
- Other ETS path is $FADH_2$ → cytochrome → O_2: 2 ATP per $FADH_2$

ETS Energetics



This shows how the products how NAD and FAD from Krebs + glycolysis enter the electron transport system and how a chain of oxidation-reduction reactions occur with the relative change in energy between reactants and products

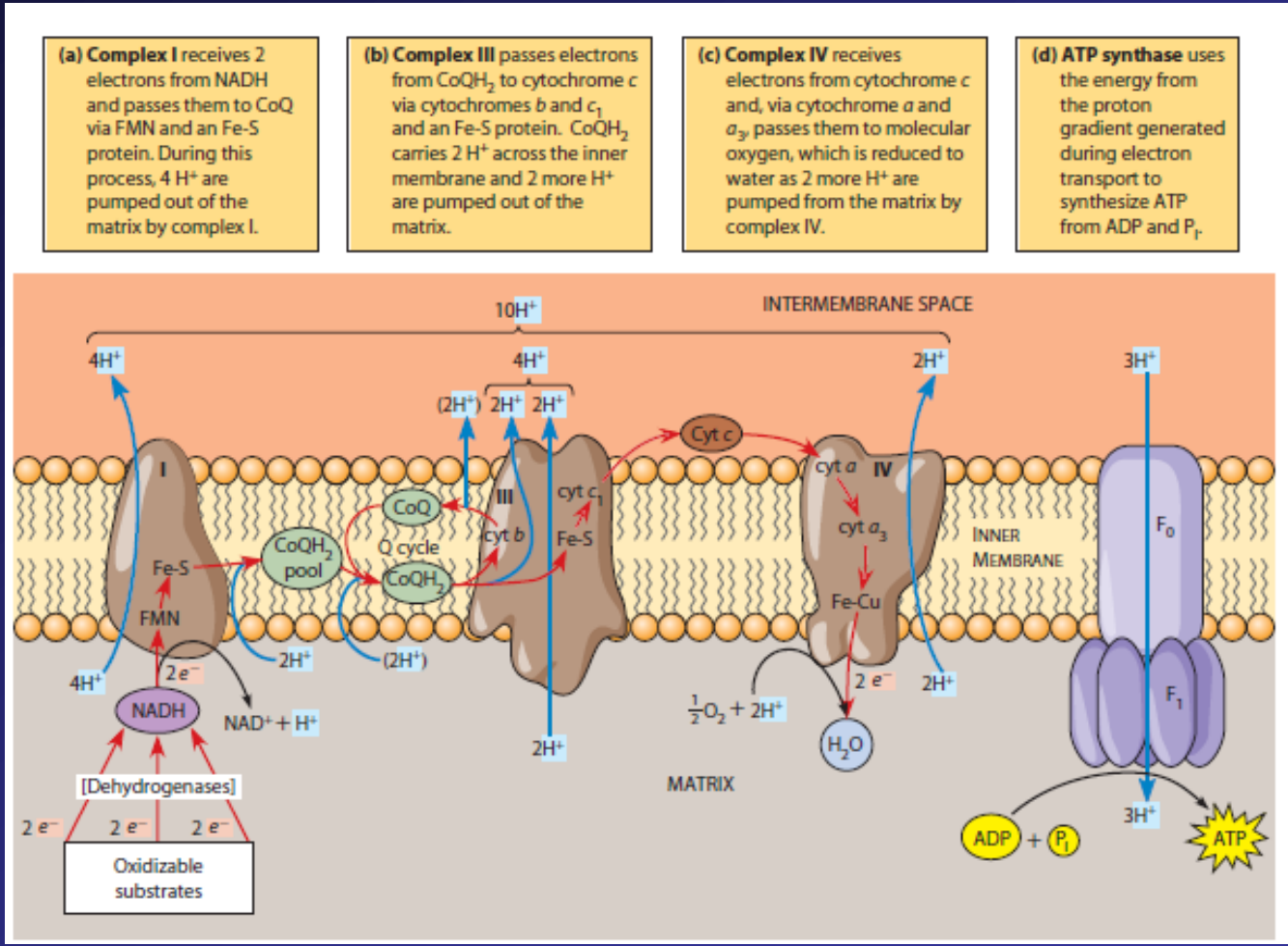


Chemiosmotic Gradient

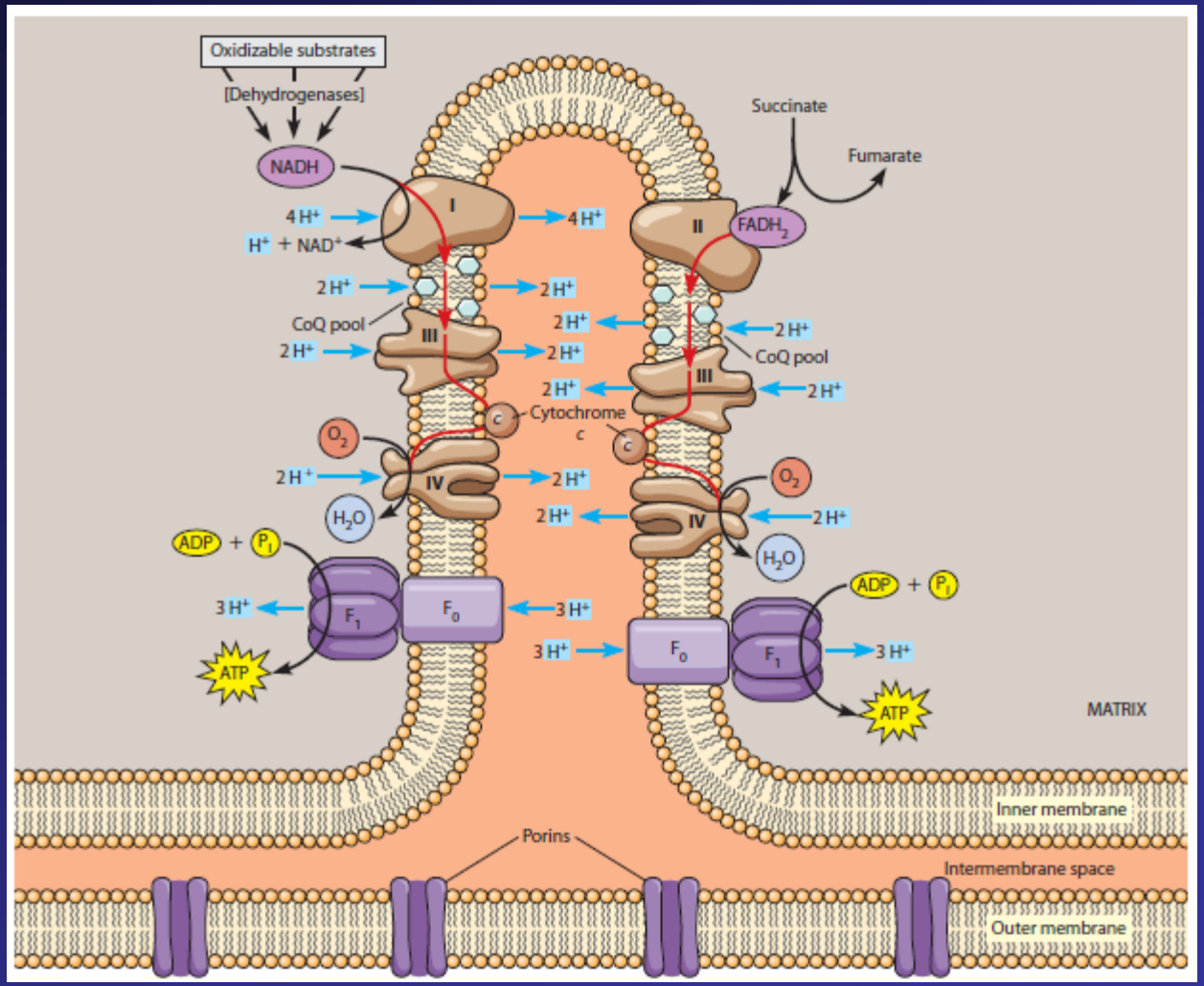
- The ETS therefore is pumping H^+ ions from one side of the inner membrane to the other (from matrix → intermembrane space), building up a H^+ concentration difference (also called the chemiosmotic gradient)
- Recall that Na^+ ion concentration differences across a membrane are produced by using ATP, and these Na^+ concentration differences can be used to do other kind of cellular work, particularly coupled transport
- This proton (H^+) concentration difference across the inner membrane will be used to produce ATP itself

Electron Transport System

- NADH produced in glycolysis and Krebs cycle is oxidized back to NAD by proteins in Complex I (NADH dehydrogenase)
- Electrons are then moved to the cytochromes and Fe-S proteins in Complex III through quinone (CoQ) molecules in the membrane
- Cytochrome c gets reduced (i.e., gets electrons) from last protein Complex III chain, and reduces in turn the Complex IV (cytochrome c oxidase)
- With each oxidation-reduction, H^+ gets moved across the membrane
- The buildup of H^+ is used by ATP synthase to produce ATP



- This is the same ETS as shown previously, but location of the complexes are shown as part of the larger mitochondrial structure



Inputs to ETS

- From Krebs Cycle (two turns for 1 glucose)
  - 6 NADH → 3 ATP / NADH = 18 ATP
  - 2 $FADH_2$ → 2 ATP / FADH2 = 4 ATP
- From pyruvate → acetyl-CoA
  - 2 NADH → 3 ATP / NADH = 6 ATP
- From glycolysis
  - 2 NADH → 3 ATP / NADH = 6 ATP

Summary of ETS

- 34 ATP produced by oxidative phosphorylation
  - Proton (H^+) concentration difference across inner membrane generated by oxidation-reduction reactions with vectorial movement from matrix → intermembane space
  - H^+ concentration difference drives ATP production with ATP synthase membrane protein
- O_2 accepts electrons at last step to make H_2O molecules
- Uses coenzymes NAD^+ and FAD to accept electrons from the breakdown of glucose

Full Accounting of ATP Made

- Grand total ATP from ONE glucose
  - +2 ATP from glycolysis
  - +2 ATP from Krebs cycle (via GTP)
  - + 34 ATP from ETS
- = 38 ATP

- HOWEVER, transport of NADH generated by glycolysis from cytosol → matrix of mitochondria requires using ONE ATP per NADH, so we lose 2 ATP for NET 36 ATP
- In reality only 30 ATP are generated from one glucose because of H+ leaking and other coupled energy processes

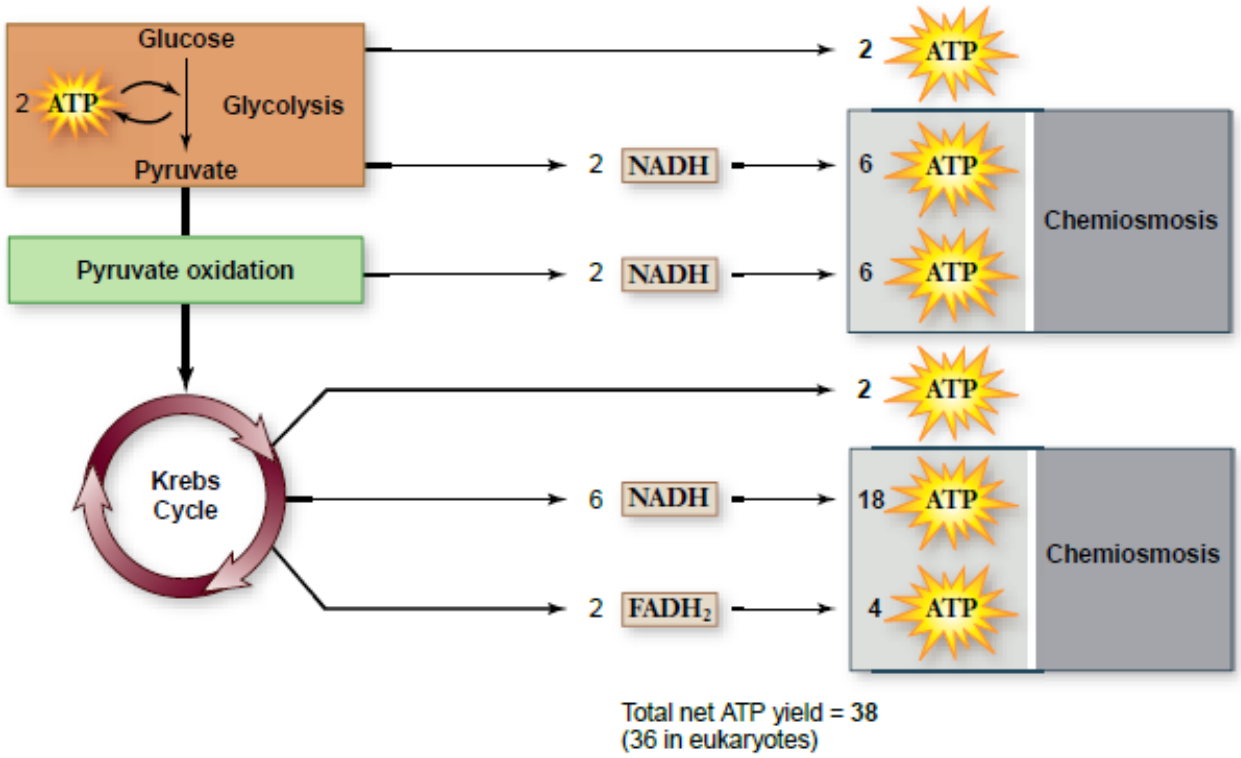
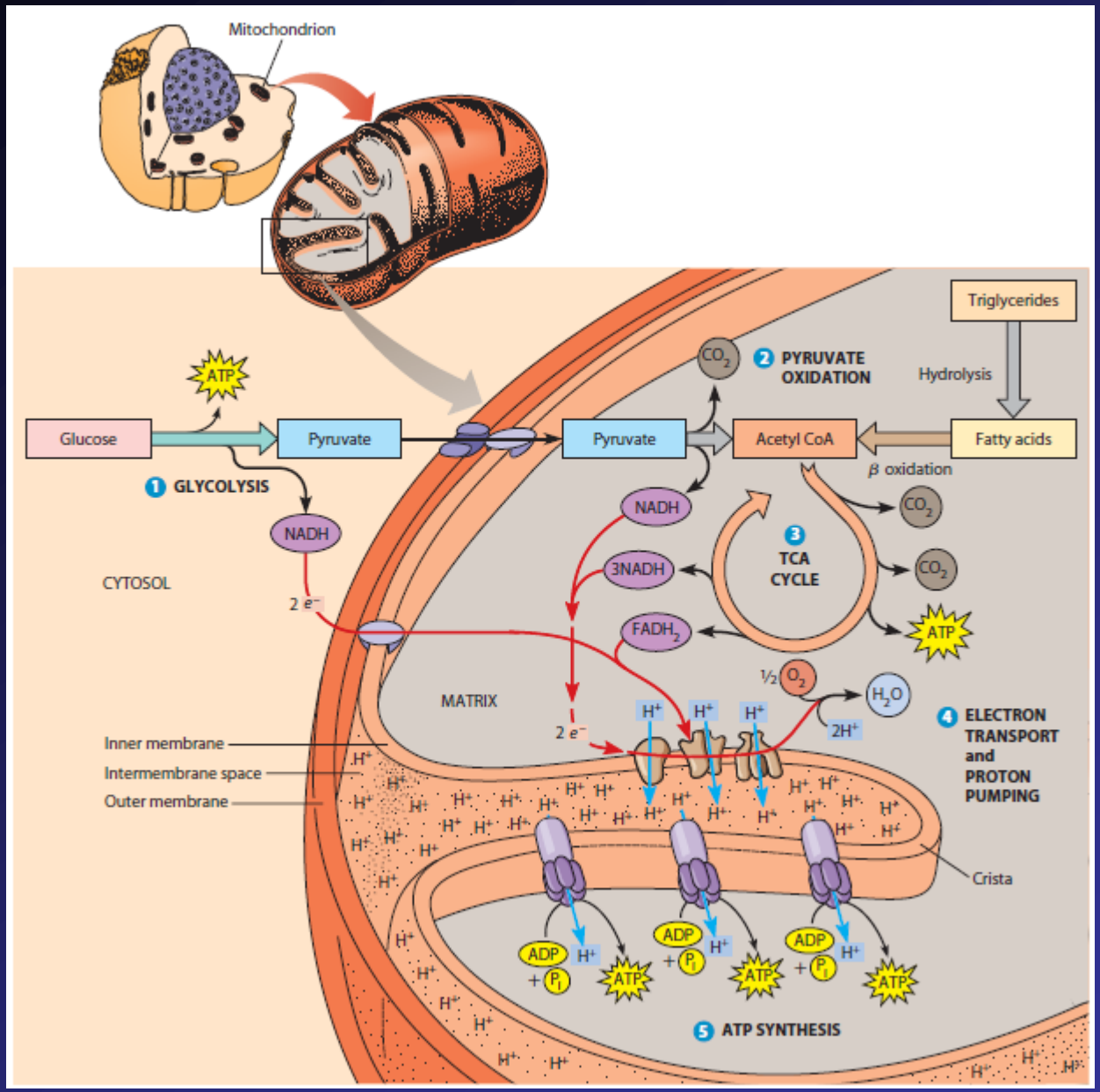
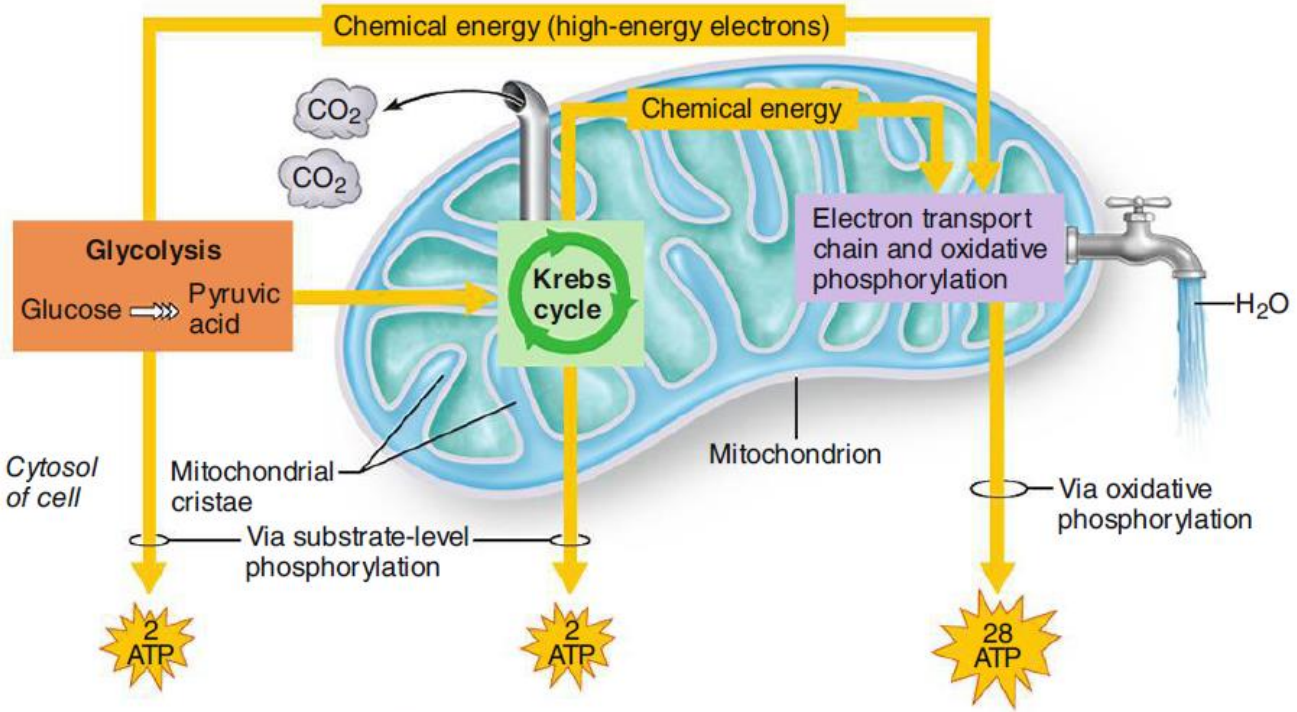


Figure 7.16 Theoretical ATP yield. The theoretical yield of ATP harvested from glucose by aerobic respiration totals 38 molecules. In eukaryotes this is reduced to 36 because it takes 1 ATP to transport each molecule of NADH that is generated by glycolysis in the cytoplasm into the mitochondria.


Mitochondrion
Triglycerides
Hydrolysis
ATP
Glucose
Pyruvate
Pyruvate
Acetyl CoA
Fatty acids
CO_2
2 PYRUVATE OXIDATION
β oxidation
1 GLYCOLYSIS
NADH
NADH
3NADH
3 TCA CYCLE
CO_2
CO_2
CYTOSOL
2 e^-
$FADH_2$
ATP
$\frac{1}{2}$ O_2
H_2O
MATRIX
H^+
H^+
H^+
2 e^-
2H^+
4 ELECTRON TRANSPORT and PROTON PUMPING
Inner membrane
Intermembrane space
Outer membrane
Crista
ADP + P_i
ATP
ADP + P_i
ATP
ADP + P_i
ATP
5 ATP SYNTHESIS



(1) During glycolysis, each glucose molecule is broken down into two molecules of pyruvic acid as hydrogen atoms containing high-energy electrons are removed.

(2) The pyruvic acid enters the mitochondrion where Krebs cycle enzymes remove more hydrogen atoms and decompose it to CO_2. During glycolysis and the Krebs cycle, small amounts of ATP are formed.

(3) Energy-rich electrons picked up by coenzymes are transferred to the electron transport chain, built into the cristae membrane. The electron transport chain carries out oxidative phosphorylation, which accounts for most of the ATP generated by cellular respiration, and finally unites the removed hydrogen with oxygen to form water.

Figure 14.20 During cellular respiration, ATP is formed in the cytosol and in the mitochondria. The maximum energy yield per glucose molecule is 32 ATP.

Reading

The reading is the same for Lectures 13 & 14

- Becker's WotC: 224-236, 252-263, 267-272, 274-288
- Raven: pp 108-110, 112-113, 117-119, 123-138