In TCA we I stated that the NADH count be converted to 2.5 ATP, and the FADH\(_2\) could yield 1.5 ATP. We will now examine how this is accomplished, Will skip photosynthesis this year.

This is a process that occurs in mitochondria. A related process called photophosphorylation occurs chloroplasts.

Oxidative phosphorylation: \(O_2\) reduced to \(H_2O\) using electrons donated by NADH or FADH\(_2\) - spontaneous

Photophosphorylation: just the reverse, \(H_2O\) oxidized to \(O_2\) with electrons accepted by NADP\(^+\) not spontaneous, \(E\) must be supplied by light.

Both processes highly efficient and similar mechanism

Based on 1961 hypothesis by Peter Mitchell, that trans membrane difference in H\(^+\) concentration could be used to store and generate energy

Called the **Chemiosmotic Theory**. Has worked well and been a unifying principle for many diverse energy using or making processes.

Both phosphorylation and photophosphorylation have these same characteristics

1. Both involved flow of electrons through membrane bound carriers

2. “Downhill” electron flow is used to transport protons through a proton impermeable membrane against a concentration gradient. So \(E\) is conserved not in chemical bonds but in an electrochemical potential

3. Protons are then allowed to flow down their concentration gradient through specific channels, and it is here that ATP is synthesized

Will look at oxidative phosphorylation, starting with flow of electrons to make proton gradient. Then will look at how ATP synthase catalyzes the phosphorylation of ADP using the proton gradient. If we have time then we will look at how the flow electrons makes the proton gradient in a chloroplast for photophosphorylation, and then see if there is much of a difference between the chloroplast ATP synthase and the mitochondrial ATPase
19.1 Oxidative Phosphorylation

A. Mitochondrial architecture

Mitochondria as site of oxidative Phosphorylation was discovered in 1948 by Albert Lehninger (The author of the 1st edition of this text)

Need to start with a mitochondria anatomy lesson

Figure 19-2

Is covered with a double membrane

Outer relatively smooth, and permeable to small molecules (<5000) and ions because has lot’s of porin channels

Inner membrane, highly convoluted, impermeable to most small molecules and ions, including H⁺

Inner membrane has proteins for electron transport and ATP synthase

Matrix inside inner membrane contains enzymes of pyruvate dehydrogenase complex, most of TCA cycle, most of Fatty acid catabolizing, and many of amino acid oxidation.

Since the glycolysis is performed in the cytosol, as well as most of the ATP consuming reactions, this means there are also a host of membrane transport protein in the inner membrane to bring pyruvate, fatty acids and amino derivatives, ADP and Pi into the mitochondria, and to let ATP out.

B. Universal electron carriers (have essentially already done so skip)

We have already seen these NAD⁺ or NADP⁺ and FMN or FAD

NAD/NADP Nicotinamide nucleotide-linked dehydrogenases
Define enzymes that use NAD⁺ or NADP⁺ as dehydrogenases hence define as the reaction:

Reduced substrate + NAD⁺ = oxidized substrate + NADH + H⁺
Reduced substrate + NADP⁺ = oxidized substrate + NADPH + H⁺

Interestingly NAD⁺ enzymes are almost always used in to carry electrons in anabolic metabolism (breaking down compounds to get E), where the reaction occurs as written

NADP⁺ enzymes are almost always used in catabolic metabolism (using E to make new compounds) and thus usually seen in reaction going in the
reverse direction.

Electrons can be shuffled from one to the other using the enzyme nicotinamide nucleotide transhydrogenase

\[ \text{NADPH} + \text{NAD}^+ \rightleftharpoons \text{NADP}^+ + \text{NADH} \]

In both cases the NAD or NADP is a water soluble factor the binds reversibly with the enzyme so it can float around the cell as needed

In both cases transfer 1 H as H⁺ released to solution and the 2 electrons are associated with the other H, so you can think of it as a hydride (H⁻) ion. So ALWAYS a 2 e⁻ transfer

Neither NAD or NADP can cross the inner mitochondrial membrane
But electrons can be shuttled across indirectly

Many of the catabolic enzymes are in the only mitochondria, other NAD/NADP enzymes are in the cytosol, still others have two distinct isozymes, one for each compartment.

*Flavoproteins* (FMN or FAD enzymes)

Unlike NAD/NADP Redox reaction can take place in 1 or 2 e⁻ steps

FMN or FAD very tightly liked to enzyme, sometimes covalently linked, so does not float off into the surroundings.

Reduction potential of FMN or FAD is determined by protein environment so varies from protein to protein (NAD/NADP always floated off, so once dissociated form the protein is had a constant potential)

**C. Electrons pass through a series of membrane bound carriers**

*(New Material, restart here)*

Respiratory chain consists of a series of sequentially acting electrons carriers
Most are integral membrane proteins
May be 1 or 2 electron reactions

Three types of electron transfers
1. Direct e⁻ transfer (Fe³⁺ + e⁻ → Fe²⁺)
2. Transfer as a hydrogen atom (or H⁺and e⁻)
3. Transfer as a hydride (:H⁻)
Use term reducing equivalent to refer to transfer of a single electron

In respiratory chain find 3 electron carriers other than FAD and NAD
- Ubiquinone (Coenzyme Q or Q)
- Heme-type iron containing proteins
- Fe-S type iron containing proteins

**Ubiquinone** (or **coenzyme Q** or **Q**)

- **Figure 19-3**
- A benzoquinone with a long isoprenoid tail
- Diffuses freely in membrane
- Definitely only soluble in membrane
- Can work in 1 or 2 e⁻ steps
- (Q, QH⁻, or QH₂)
- Closely related to plastoquinone in plants
- Carries both electrons and protons so couples electron and H flow across membrane

**Heme type iron containing proteins - cytochromes**

- Have strong absorbance in vis range
- Used to classify into three main groups, a, b, and c
  - a’s absorb about 600 (lowest E)
    - Heme has long hydrophobic tail
  - b’s about 560
    - Standard heme
  - c’s about 550 (highest E)
    - Covalently attached heme
- Have subtypes, so b₅₆₂ is a b cytochrome with an absorbance specifically at 562nm

Hemes of a and b type closely associated but not covalently bound to protein

c’s are covalently bound through cys linkages

Most cytochromes are integral membrane proteins

- **BUT**-

Cyto c of mitochondria is peripheral protein bound to outer surface of inner membrane

**Iron-Sulfur proteins**

- Fe not in heme but bound by S
- Simple to complex Fe-S centers **Figure 19-5**
Always used in 1 e⁻ transfers with one Fe in cluster getting hit
At least 8 Fe-S proteins known in mitochondria electron transport

Potential vary form -.65 to + .45

How do we know the determine sequence of electron carriers?
Tell you right now, general flow of electrons is:
(NADH or succinate) to Flavoproteins
(I.E. proteins containing FMN/FAD)
Flavoproteins to Ubiquinone
Ubiquinone to iron-sulfer proteins
Iron sulfur proteins to cytochromes
cytochrome to O₂

How do we know this?
1. can guess from potentials (but is only a guess, remember standard potential are for standard conditions, and cells aren’t standard
2. Can do experiments
   A. Exhaust O₂ supply so everything stops
      And everything stuck in reduced from
      Add O₂ and watch each species become oxidized
         Quick oxidation at O₂ end
         Slow oxidation at beginning
   2. Inhibition
      Certain substances can inhibit certain points in flow
      Add inhibitor and see what thing get backed up in reduced form, and what still get oxidized
      (Figure 19-6)

D. Electron carriers
   most of protein in respirations in membrane embedded complexes
   gently treat mitochondria inner membrane with detergent
   Isolate 4 different enzyme complexes
   Each complex can do its part of pathway by itself

(Figure 19-8)
Complex I goes from NADH to Q
Complex II goes from Succinate in TCA to Q
Complex III goes from Q to cyto c
Complex IV from cytoc to O₂
Details

**Complex I: NADH to Ubiquinone**

*Figure 19-9*

Also called NADH:unbiquinone oxidase
42 different polypeptide chains
1 FMN flavoprotein
At least 6 Fe-S centers
By electron microscopy can see is L shaped
   One end sticks into inside of mitochondria
Chemical reaction is:
\[
\text{NADH} + \text{H}^+ \text{ (from matrix)} + \text{Q}^- \rightarrow \text{QH}_2 \text{ in membrane} + \text{NAD}^+
\]
As this happens 4 additional protons pumped from matrix to intermembrane space

So is a vectorial proton pump
   Pumps protons in 1 direction
   Generate both a conc gradient and a charge gradient

Can write reaction in following way:
\[
\text{NADH} + 5\text{H}^+_n + \text{Q}^- \rightarrow \text{QH}_2 + 4\text{H}^+_p + \text{NAD}^+
\]
Where $\text{H}^+_n$ means on negative side of membrane (inside)
And $\text{H}^+_p$ means on the positive side, (periplasmic space)

Amytal (a barbiturate) rotenone (a plant product used as an insecticide) and piericidin (an antibiotic) are all drugs that inhibit this reaction, and, as saw earlier would inhibit entire ox/phos system from the start

\[\text{QH}_2\] generated free to diffuse in membrane to complex III its next stop.

**Complex II: Succinate to Ubiquinone**

*Figure 19-10 & 19-8*

Actually already seen this puppy
Succinate dehydrogenase from the TCA cycle
Remember, it was the one membrane bound complex in the entire system
Smaller and simpler that complex I
At least 4 different proteins
   A & B in matrix
      Have three 2FE-2S centers
FAD
Binding site for Succinate
C & D integral membrane proteins
Five prosthetic groups
Electron move from succinate to FAD to FeS centers to ubiquinone

Heme b in complex II not in direct path of electron transfer seen above
May serve to reduce electron ‘leakage’
Sometimes electrons don’t follow path
Can react with water to form H₂O₂
Or oxygen to make ·O₂
These are referred to as Reactive Oxygen Species (ROS)
And are very damaging to the cell
More details in a few pages

Some point mutations in Complex II release more ROS and get benign tumors of head and neck

*Other complexes that pass electrons to Ubiquinone*
Still figure 19-8
In fatty acid oxidation first step catalyzed by acetyl-CoA dehydrogenase
Take 2E to oxidize a fatty acid and put on FAD of enzyme
Transfer e to electron transferring flavoprotein (ETF)
ETF passes to ETF:uniquinone oxidoreductase

Glycerol-3-P in *cytosol*
Figure 17-4
Go over fast, details later
Comes from glycerol of triacylglycerols
Of reduction if dihydroxyacetone phosphate in glycolysis
Enzyme glycerol-3-P dehydrogenase
On *outer* face of inner mitochondria membrane
Transfers electrons to Ubiquinone
Used to shuttle reducing equivalent between NADPH in cytosol and NAD in mitochondria (more later)
**Complex III: Ubiquinone to cytochrome c**
Also called
- Cytochrome bc₁ complex
- Ubiquinone:cytochrome c oxidoreductase
Electrons from QH₂ transferred to cytochrome c
More vectorial transport of protons
250,000 MW
11 subunits
Both hemes and Fe-S centers
X-ray structure known, done in 1995-1998 Figure 19-11
Net equation:
\[ \text{QH}_2 + 2 \text{cyt c}_i(\text{oxidized}) + 2 \text{H}^+_\text{N} - \text{Q} + 2 \text{cyt c}_i(\text{reduced}) + 4 \text{H}^+_\text{p} \]
Note cyto c is a 1 electron carrier
So 1 QH₂ does two cytoC
Path of electrons through complex is complicated
- Figure 19-12
  - Including taking 2 electrons off QH₂ to pump 2 proton to exterior and 1 e⁻ to cytoC
  - Then putting one electron BACK on Q to make \( \cdot \text{Q}^- \)
  - Then using \( \cdot \text{Q}^- \) to generate a second QH₂ and a second cyto c
  - And taking 2 more electrons off a second QH₂
Won’t worry about details here!! (hurray)

**Complex IV cytochrome c to O₂**
Also called cytochrome oxidase
**Figure 19-13**
Large, 13 subunits, MW 204,000
Bacterial from much simpler
By comparison think 3 major subunits in mammalian
- I, II, III
Subunit I (Yellow)
  - 2 heme’s a and a₃
  - 1 Cu (called Cuₒ)
Subunit II (Purple)
  - 2 Cu complexed by cys (called Cuₐ)
  - Looks like an FeS center
Subunit III (Blue)
  - No special groups?
Subunit (Green) 10 proteins
  - Scaffolding to hold together?
  - Binding site for cyto C?
Figure 19-14
Electron passed from 4 cytochromes c to Cuₐ (unit II)
From Cuₐ to heme a to heme a₃ to Cuₐ subunit I
From Cuₐ to O₂
Every 4 electrons makes 2H₂O
  Using H⁺ from inside
  Also pumps 4 H⁺ from in to out

NET
4Cyt c(red) + 8H⁺ + O₂ → 4cyt(ox) + 4H⁺ +2 H₂O

Does this in four 1-electron steps
Yet no intermediates like OH-, HO· or peroxide releases
So must be tightly bound intermediates

E. Mitochondrial Complexes may associate in Respirasomes
Respirasomes - functional combinations of two or more electrons-transfer complexes
  Relatively new theory - Added in 5th edition
  Complex I and III can be isolated together if purification done gently
  Complex III and Complex IV can be observed in a complex by EM
  Kinetics support transfer of electrons through a tightly linked solid state
  The lipid Cardiolipin that is especially abundant in inner mitochondrial memberane may be important to integrity of Respirasome

F. Overall reaction is efficient
net pathway for 1 NADH shown figure 19-16

Net reaction:
NADH + 11H⁺ + ½ O₂ → NAD⁺ + 10H⁺ + H₂O

Looking at the energy releasing half of the reaction
NADH + 1H⁺ + ½ O₂ → NAD⁺ + H₂O
  This reaction has a ΔE“ of 1.14 (0.816-(−0.320))V
  And using ΔG= -nFE
  I get ΔG = -220 kJ.mol
  Actually E much higher due to real concentrations
If start Succinate get ΔG = -150 again much higher in cell
Let’s compare to energy stored in the proton gradient you have established
Back in chapter 12 (membrane transport)
We had the equation

\[ \Delta G = RT \ln \left( \frac{C_{\text{out}}}{C_{\text{in}}} \right) + ZF \Delta \psi \]

For a mitochondria, proton \( C_{\text{out}} \) is .75 pH units lower (H⁺ higher) than the matrix \( C_{\text{in}} \)
\( \Delta \psi \) is about .15-.2 V (outside + inside negative)

Net E about 20 kJ/proton
NADH transported 10 proton out so this is
10(20) = 200 kJ of E

So look like most of the E is stored in potential gradient
All we have to do is let the proton slide back in and get the energy back

G. Reactive Oxygen Species are generated during Oxidative Phosphorylation
Several step in above pathway have potential to produce highly reactive free radicals
Both passage if electrons from QH₂ to Complex III and Complex I & II to QH₂ involve the radical \( \cdot Q^\bullet \) as an intermediate
\( \cdot Q^\bullet \) has a low but measurable probability to pass electron to \( O_2 \) to form \( \cdot O_2^\bullet \), the superoxide free radical. This in turn produces the even more reactive hydroxyl free radical \( \cdot OH \)
The hydroxyl free radical can then attack and damage anything it touches, proteins, lipids and DNA

It is estimated the between .1-4% of \( O_2 \) used in respiration forms these radicals - More than enough to severely damage cell.

Formation of ROS favored when 2 things happen
Mitochondria are NOT making ATP because
Low on ADP
Low on \( O_2 \)
Have high NADH/NAD⁺ ratio
This is considered oxidative stress
More electrons enter the respiratory chain than can be passed to \( O_2 \)
Overproduction of ROS - Bad
Low levels ROS used as signal to cell to correct
Cell have evolved enzyme to prevent this, primarily superoxide dismutase, glutathione peroxidase, and glutathione reductase. Figure 19-18

Superoxide dismutase: \( 2 \cdot O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \)
Glutathione peroxidase:
\( H_2O_2 + \text{Glutathione (reduced)} \rightarrow H_2O + \text{glutathione (oxidized)} \)
Glutathione reductase:
\( \text{Glutathione (oxidized)} + \text{NADPH} \rightarrow \text{Glutathione (reduced)} + \text{NADP}^+ \)

**H. Plant mitochondria have alternative NADH oxidizing Mech**

Skip

19.2 ATP synthesis

Now let's look at the other end of the problem

Using the \( H^+ \) gradient to make ATP

Net reaction will be

\[ \text{ADP} + P_i + nH^+_p \rightarrow \text{ATP} + nH^+_N \]

See figure 19-19 for added last step

Let's skip pages 749 and 750 talking about coupling and come back in a bit

**A. ATP Synthase has two functional domains \( F_0 \) and \( F_1 \)**

F type ATPase, meaning that is really a synthase rather than an ATPase

similar structure for mitochondria, chromoplasts, and eubacteria

\( H^+ \) flows from P side to N side (down gradient)

\( \text{ADP} + \text{Pi} \rightarrow \text{ATP} \)

2 main structural components

- \( F_0 \) integral membrane protein
  - Technically F-O the letter not F zero
  - O stands for oligomycin sensitive
  - If put into a membrane alone, the membrane leaks protons

- \( F_1 \) peripheral protein
  - When isolated is an ATPase
  - Only when combined with \( F_0 \) and a properly oriented
  - \( H^+ \) gradient does it turn into an ATP synthase
B. ATP is stabilized relative to ADP on the surface of $F_\text{i}$

The first clue for how ATP synthase works comes from isotope exchange experiments.

Put an ATP with the O between P’s labeled with O$^{18}$

If you do this with plain ATP, it stays put.
If you do this with ATP bound to $F_\text{i}$, it quickly gets randomized to all potions.

Explanation? Figure 19-23 When Bound to $F_\text{i}$, ATP is hydrolyzed and resynthesized rapidly. I.e. ADP + P$\text{i} \rightarrow$ ATP is in equilibrium, $\Delta G$ near zero, not -30.5!!!

How can this be?

- ATP is very tightly bound ($K_{\text{dis}} < 10^{-12}$)
- ADP is weakly bound ($K_{\text{dis}}$ about $10^{-5}$)

So in solution at equilib [ADP] >> [ATP] due to $K_{\text{hydrolysis}}$ On the enzyme ATP is much more tightly bound than ADP so the relative concentration on the enzyme about equal. This makes [ADP]/[ATP] about 1 on the enzyme so $K$ is about 1

You have to be careful here. Remember one of the principles of enzyme catalysis is that you cannot change $\Delta G$ of a reaction, yet it appears that is just what we have done! The piece of information that you have to keep in mind is that we are talking about ATP on the surface of the enzyme, not yet back in solution.

Look at figure 19-24 the reaction diagram.
We have NOT changed the $\Delta G$ of the reaction, because the reaction is not complete. We have ADP and ATP in equilibrium on the enzyme, but if that was all that would happen, we would be stuck because we still need a big push of $E$ to get the tightly bound ATP if the enzyme again.

This is where the proton gradient comes in. It is going to give us the $E$ push.

C. Proton gradient drives the release of ATP

Note: these crystal structure only done ~ 10 years ago!! These detailed explanations I am giving you today weren’t even around when I was in graduate school so really late breaking news scientifically!
D. Each β unit of ATP synthase can assume three conformations

Figure 19-25
Better yet, animations on dnatube
www.dnatube.com ATP synthase parts I...IV

F₁ is α₃β₃γδε
αβ form 3 sets of structures, like section of an orange
ATP binding site is at αβ interface, but mostly on β
γ is a central shaft that extends down into the F₀
δε no visible in X-ray coordinates

In X-ray observe 3 physical state of αβ (conformations) one with ATP bound, one with ADP&Pi bound and one with nothing bound
All three present at any one time

F₀ has 3 subunits

ab₂c₁₀₋₁₂
C is small, 8000 AA very hydrophobic, 2 membrane spanning helixes and a loop on the matrix side

Attached to F₁ by γε so F₁ stand on top of F₀ cylinder
α and β are to the side and anchor to δ on side of F₁

E. Rotational movement of F₀ changes F₁ structure

Figure 19-26 and animations on dnatube
As H⁺ streams through core of F₀, F₀ appears to rotate relative to α and β rotation of c’s of F₀ makes the γ part of F₁ move
as γ of F₁ interacts with each αβ dimer in F₁, get conformational changes that accept ADP, and Pi, then force them into ATP, then change binding so releases ATP (Note protons located in 19-25 c & d)

Each rotation of 120° takes 3H⁺ and generates 1 ATP from ADP and Pi

As shown in text can actually see this motion if attach a fluorescent label to F₀ and watch in a fluorescent microscope (19-27)
In fact saw rotate one direction when makes ATP and the other when is an ATPase
F. How does proton flow in $F_o$ complex produce rotary motion?

One model shown in Figure 19-28. 

Individual c subunits in $F_o$ arranged in a ring. 

Probably lipids in middle. 

A subunit on side. 

There are two proton channels at c/a interface. 

Each channel only goes ½ the length. 

Ends at a key asp (on c subunit) in middle that can hold or release a $H^+$. 

Start at bottom of diagram! 

P side (side with high conc of protons- cytosol). 

Proton enters channel. 

When proton gets to asp, it displaces and Arg. 

(I believe the Arg is on the a unit). 

Arg moves aside and tries to form interaction is asp on adjacent c subunit. 

As binds to adjacent asp, it displaces the $H^+$ on that asp and that proton moves down the other ½ channel into the mitochondria. 

Overall the protons move from high concentration to low concentration, and the arg acts as a ratchet, keeping the c subunits rotating relative to the a. 

Number of c’s varies with organism (e coli 10, animals 8, etc). 

Rate of rotation estimated at 6000 rpm - 100 revs/sec! 

G. Chemiosmotic coupling allows non-integral stoichiometries of O$_2$ and ATP. 

When thought was a chemical reaction it was assumed that an integral # of P’s would be made form one NADH to 1 FADH$_2$. 

Much work was do to establish a P/O ratio. 

How many P’s for each O? 

Experiments were difficult, both ATP and O$_2$ are being used by mitochondria for other purposes. 

But accepted figure was. 

Thought was 2-3 P for an NADH and 1-2 P for an FAD. 

(Numbers weren’t exact, but they rounded since they expected integral values). 

Now measure $H^+$ fluxes instead. 

Have seen 1 NADH moved 10 $H^+$ 

FAD missed 1$^{st}$ 4 $H^+$ so only moved 6$H^+$. 

Have seen that ATP synthase itself uses 3$H^+$ 

Will need additional $H^+$ for ATP, ADP, and Pi transport.
So end up with about 2.5 ATP/NADH and 1.5 ATP/FAD
Non-integral values!

H. Proton-Motive Force also used for active transport
One last puzzle piece. If you make ATP in the mitochondria how do you get it into the cytosol, and vice versa with ADP and Pi

Figure 19-30

**Adenine nucleotide translocase** exchanges ATP\(^{-4}\) on inside with ADP\(^{-3}\) on outside, integral membrane proteins
In this process inside loses a negative charge and outside gains a negative charge so net is moving a negative out
Outside is already + (excess H\(^{+}\)) so this is favored by gradient and nothing special has to be done

Can be inhibited by atractyloside (a toxic glycoside) that inhibits this transported and keep the mitochondria ATP form getting out

**Phosphate Translocase** Brings H\(_2\)PO\(_4\)^{-} in the cell (P). If outside of membrane already +, bringing a - in from outside would NOT be a favored process

Bring and H\(^{+}\) in with the P\(_{i}\). In a symport process, to make energetically feasible

Net: takes 1 more H\(^{+}\) for every ATP synthesized

A complex of ATP synthase and both translocases called the **ATP synthasome** can be isolated if you are very gentle!

Worked example 19-2 in text adds another wrinkle. If the animal ATP synthase has 8 c units, then it only takes 8 protons for a complete rotation and 3 ATPs. On the other hand the e coli ATP synthase has 10 c units so it takes 10 protons/3ATPs. If you add this your numbers get a little different, but you are still within the experimental error of the actual experiments

I. Shuttle systems are required to oxidize cytosolic NADH in mitochondria

Figure 19-31

Not only does ATP need to get transported, but the NADH generated in glycolysis also must be transported to mitochondria so it can get oxidized and NAD\(^{+}\) regenerated
Malate-aspartate shuttle is used in liver, kidney, and heart.

1. (Cytosol) oxaloacetate reduced by cytosolic NADH to make malate and regenerates NAD+: Malate dehydrogenase Note: this is the REVERSE of the reaction that normally occurs in the mitochondria.

2. In an antiport system, malate goes into mitochondria, and \( \alpha \)-ketoglutarate comes out.

   \textit{Malate \( \alpha \)-ketoglutarate transporter}

3. In normal TCA reaction, malate oxidized to \( \alpha \)-ketoglutarate to generate NADH, malate dehydrogenase thus the cytosolic NADH has appeared in the mitochondria. But have also increased the amount of oxaloacetate in the TCA cycle, and this would throw off the balance, unless something is done to removed this excess material.

4. Change oxaloacetate into aspartic acid but pulling NH\(_2\) off a glutamic.

   \textit{Aspartate aminotransferase}

   This change glutamic into an alpha ketoglutarate that can be used in the \( \alpha \)-ketoglutarate transporter.

5. Aspartate transported out and Glutamate transported in using \textit{glutamate aspartate transporter} (so that where the Glu came from!)

6. Aspartate converted back to oxaloacetate by removing its NH\(_2\).

\textit{Aspartate amino tranferase} again, but this time in the cytosol.

\( \text{NH}_2\) transferred to \( \alpha \)-ketoglutarate to make glutamate.

Have regenerated everything!

Other systems used in muscles and in plants.

Muscle and brain system (figure 19-32)

- Not as complicated.
- Goes to ubiquinone Q using a mitochondria dehydrogenase on OUTSIDE of mitochondria.
- Only get 1.5 ATP.

19.3 Regulation of Oxidative Phosphorylation

Table 19-5

- Final yield 30 or 32 ATP/glucose (depend on NADH Shuttle used).

\textbf{A. Oxidative phosphorylation regulated by cellular needs}

- \( O_2 \) consumption tightly regulated, limited by availability of ADP and Pi called \textbf{acceptor control}
- Rate can increase over 10 fold over basal rate when ADP is around
In general cell maintains high [ATP] and low [ADP][P]

Any E using process in cell increases amount of ADP then respiration takes off to get back down again

Overall control is so good, that very little change in [ATP], [ADP], or [P] is ever observed

**B. Inhibitory protein prevents ATP Hydrolysis during Hypoxia**

hypoxic cell - cell deprived of O$_2$

Can happen during heart attack or stroke

If no O$_2$ then proton pumping ceases

Under these conditions ATP synthase could start to run in reverse

Degrade ATP to ADP to pump protons out of cell

Prevented by protein inhibitor IF$_1$

84 residue protein

Bind to 2 ATP synthase molecules and inhibits activity

(Figure 19-33)

Only works when in dimer form

Only in dimer form at pH < 6.5

pH only <6.5 if pyruvic or lactic acid have built up because of O$_2$ debt

**C. Hypoxia Lead to ROS Production and several adaptive responses**

ROS Reactive Oxygen species (Superoxide radical and hydroxide radical)

In hypoxia imbalance between input electrons into the chain and O$_2$ to finish

Under these conditions start to build up ROS

In addition to superoxide dismutase-glutathione peroxidate-glutathione reductase system to remove ROS there are two additional controls Figure 19-34

1. Slow down Pyruvate dehydrogenase (PDH)
   Phosphorylated by a PDH kinase to inactivate

2. Swap out a subunit of Complex IV that is optimized for normal O$_2$ levels (COX4-1) with one better suited for hypoxic conditions (COX4-2)

Both of above controlled by Hypoxia Inducible Factor (HIF-1)

Part of another genetic control
D. ATP production Pathways are coordinately regulated

Looking at all of glycolysis
see all places where ATP, ADP and AMP regulate

19.4 Mitochondria in Thermogenesis, Steroid Synthesis and Apoptosis
Mito has other functions than just making ATP
Generates heat in adipose tissue
Make steroids in adrenal glands and gonads
In most or all tissues key participant in programmed cell death (apoptosis)

A. Uncoupled mitochondria in Brown Fat *(The real ‘Fat Burner’)*

Fig 19-36
one place normal control is subverted is ‘brown fat’
Found in newborn animal and hibernating animals
brown fat is brown because has unusually large amounts of mitochondria in fat cells
Also have protein called thermogenin
thermogenin allows H⁺ back into cell without generating ATP
Also called uncoupling protein
Thus FA degradation gets uncoupled, and literally burn the fat to make heat!

B. Mitochondrial P-450 Oxygenase catalyzes Steroid Synthesis
Most steroid hormones synthesized by hydroxylating Cholesterol
These reactions done by a family of heme containing enzymes called P-450s because absorb light at 450 nm.
These enzymes are located on inner membrane of mitochondria

May have also heard of P-450s in liver cells
Here p-450's are located in the endoplasmic reticulum
reactions are similar to one done by mitochondrial, but used as a general defense system to hydroxylate hydrophobic compounds to make them water soluble.

While this system is used to detoxify poisons
It also degrades and removes drugs, taking them out of the blood system and limiting their lifetime

C. Mitochondria are Central to the initiation of Apoptosis
Apoptosis - controlled or programmed cell death
used when individual cells die for the good of the organism
When a stressor or a signal tell the cell it is time to die
One early consequence is that mitochondria release cytochrome c from intermembrane space into the cytosol. I won’t go into any
19.5 *Mitochondrial Genes*

Mitochondria have their own double stranded circular DNA and their own ribosomes

*Map figure 19-40 if you want it*

All of 37 genes (16,569 bp)

Only a fraction of the proteins found in mitochondria

(And seem to be mostly the membrane proteins and a few rRNA and tRNA)

Bulk of proteins, about 900, are made in cell nucleus synthesized on cytosolic ribosomes and imported to mitochondria after synthesized

Current theory is the mitochondria were once free living bacteria that entered into a symbiotic relationship with eucaryotic cells that could only do fermentation

No proof, but having their own nuclear material and ribosome does suggest this also many bacteria have $F_0F_1$ complexes in their PM that work just like the mitochondria ATPases

Many bacterial flagella are run on $H^+$ gradients rather than on ATP

And that's all I will do for this chapter. A couple of pages on mitochondrial mutations and disease that could be interesting to a Med student, but I think I will pass for now.