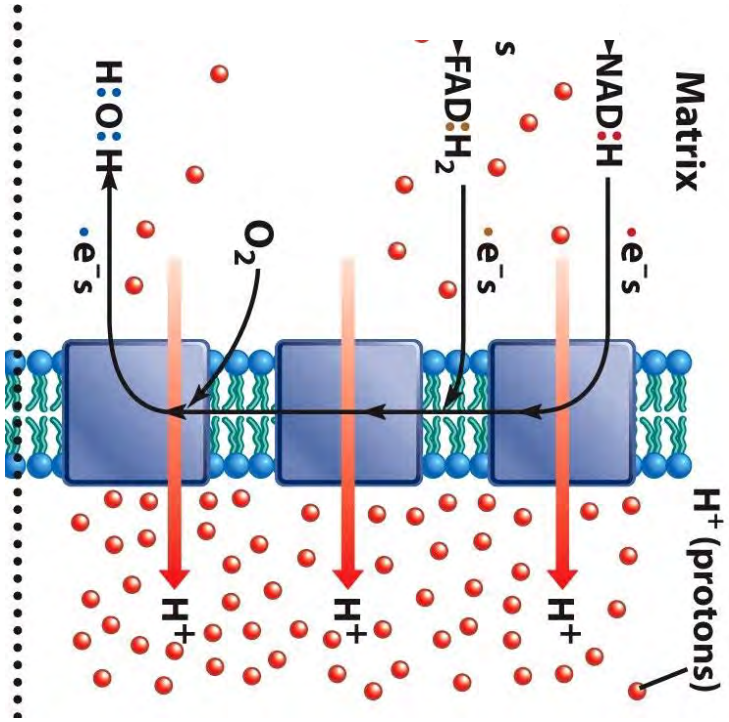
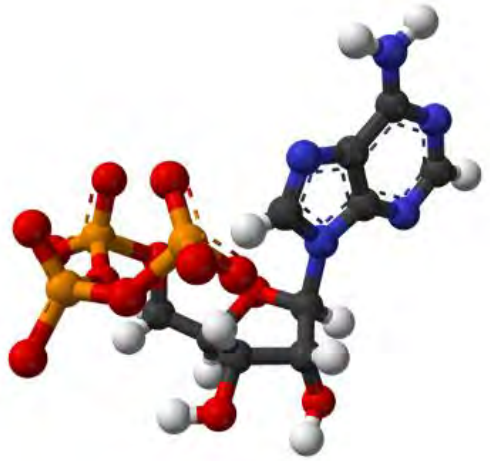
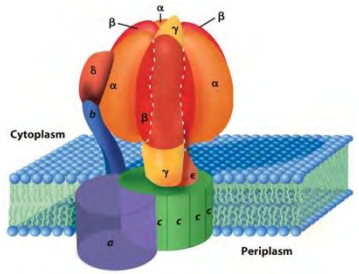
mitochondria II:
oxidative phosphorylation: the electron transport chain and ATP synthesis





Lodish chapter 12 (middle)

now playing:
you spin me round
(like a record)
by Dead or Alive



learning goals

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

- explain which metabolic reactions take place in which part of the mitochondria
- describe how energy is transferred (or stored) during oxidative phosphorylation
- explain the importance of redox potentials, and be able to calculate (estimate) free energy changes using the redox potentials of two couples
- know the major complexes of the electron transport chain, their primary functions, and the types of electron carriers they use
- describe the mitochondrial ATP synthase, and how the proton-motive force drives rotational catalysis, ATP synthesis, and contributes energy to other functions

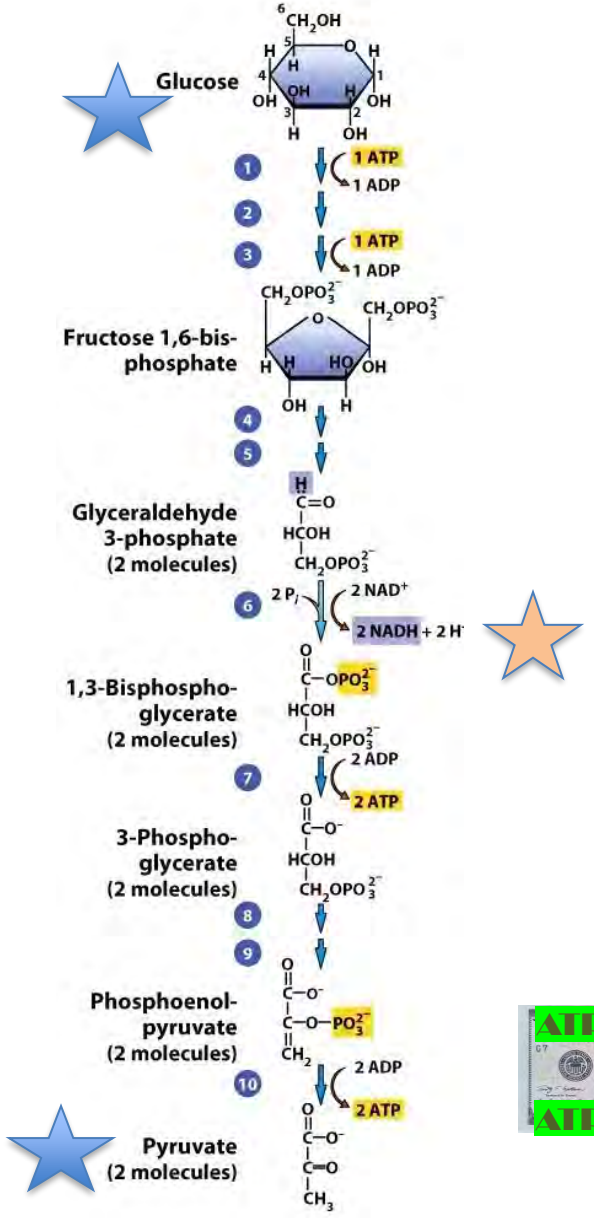
announcements

- Exam 1 average – grades will post tomorrow. Keys will be posted next week. If you do not have a score, you may have mis-coded your ID- email me: starz@umbc.edu

- Poll Ev grades will be updated over the weekend ; see the syllabus for details on weighted categories

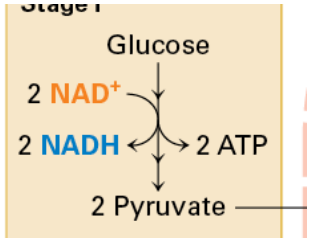
*Meanwhile, back in the **cytosol**….* glycolysis yields 2 NADH and 2 ATPs

this is the first part of oxidative metabolism – Glucose to PYRUVATE

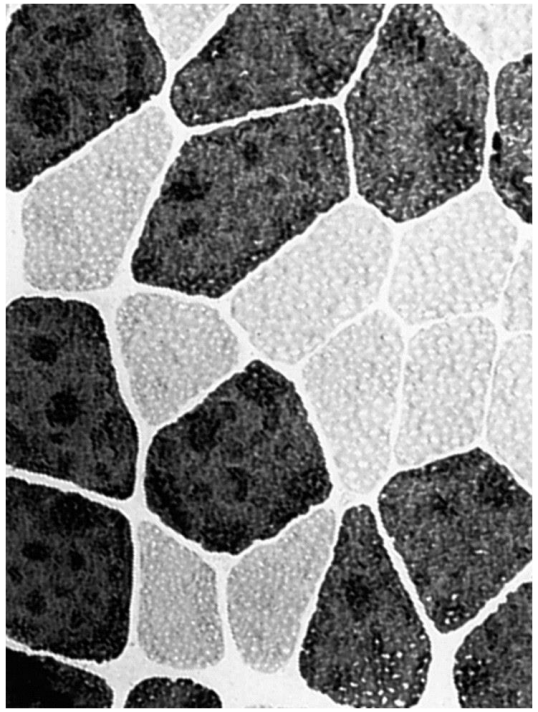


This requires some energy (ATP) and addition of NAD+

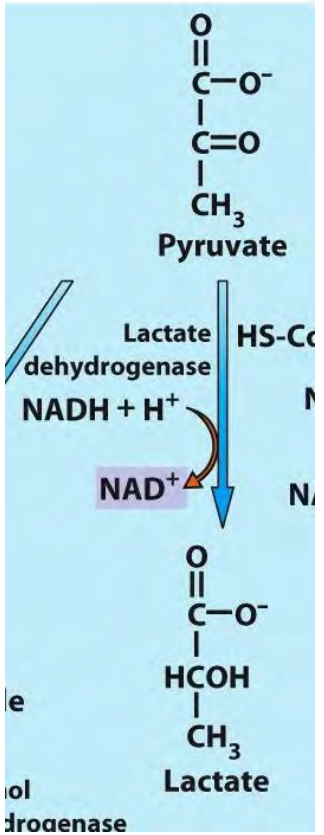
anaerobic ATP production is fast because glycolysis can occur quickly



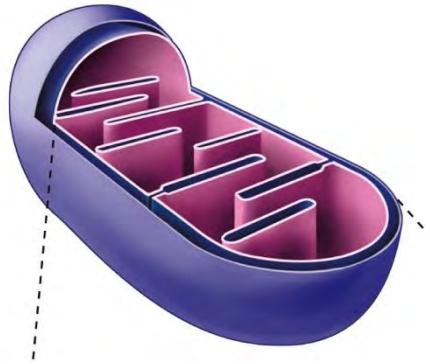
in anaerobic metabolism, cells make enough NAD+ to continue with glycolysis …



– **from fermentation of pyruvate to lactic acid**



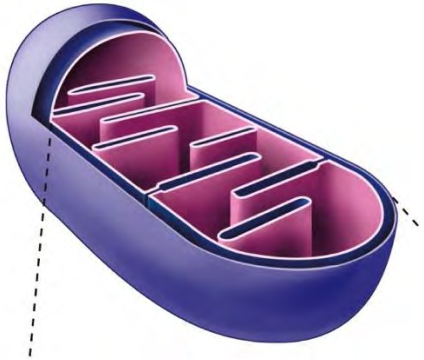
BUT aerobic metabolism is better
(more efficient)!!
why? Can't create energy- so
Where is the energy coming from and going to?



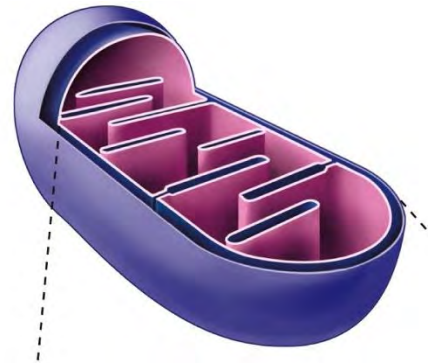
& O_2

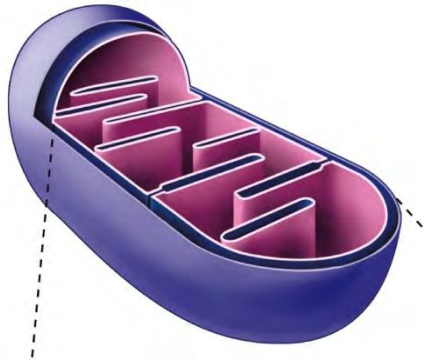
Poll warm up:

mitochondria are inherited into a cell, and change number and size by fission and fusion



But- how do you get rid of them?

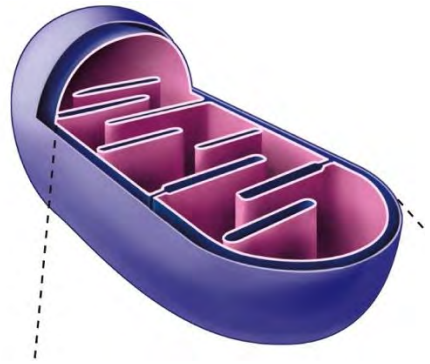




A. Removed by aliens
B. They explode periodically
C. They never go away
D. Autophagy

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

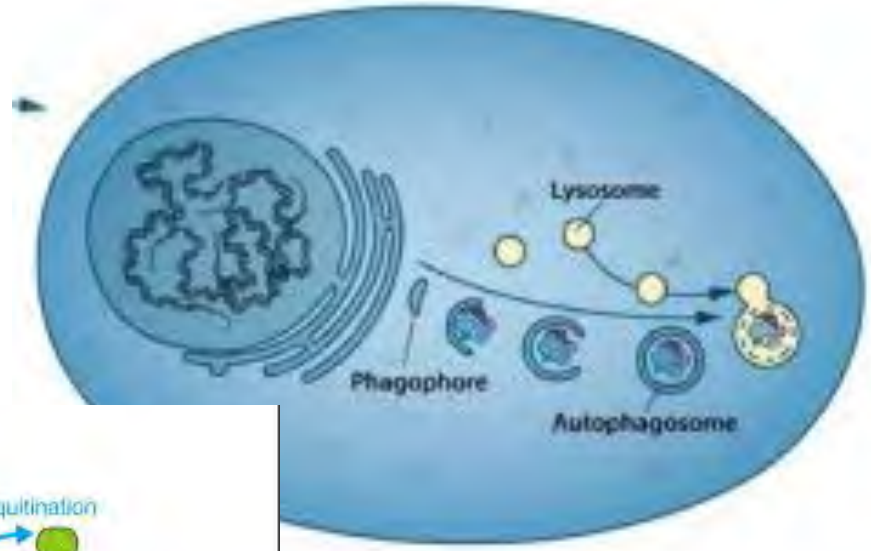
link

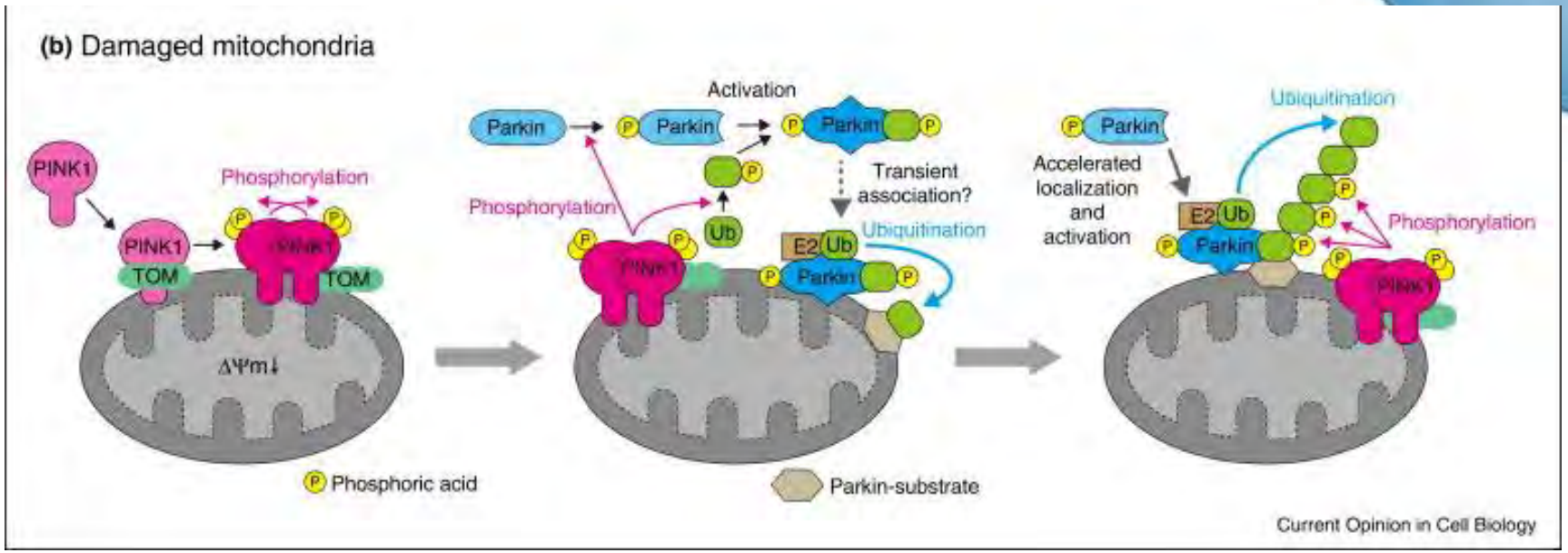


The 2016 Nobel prize for physiology and medicine was awarded to Yoshinori Ohsumi for his discoveries of mechanisms for autophagy "self-eating"

Nobelförsamlingen
The Nobel Assembly at Karolinska Institutet

Scientific Background
Discoveries of Mechanisms for Autophagy



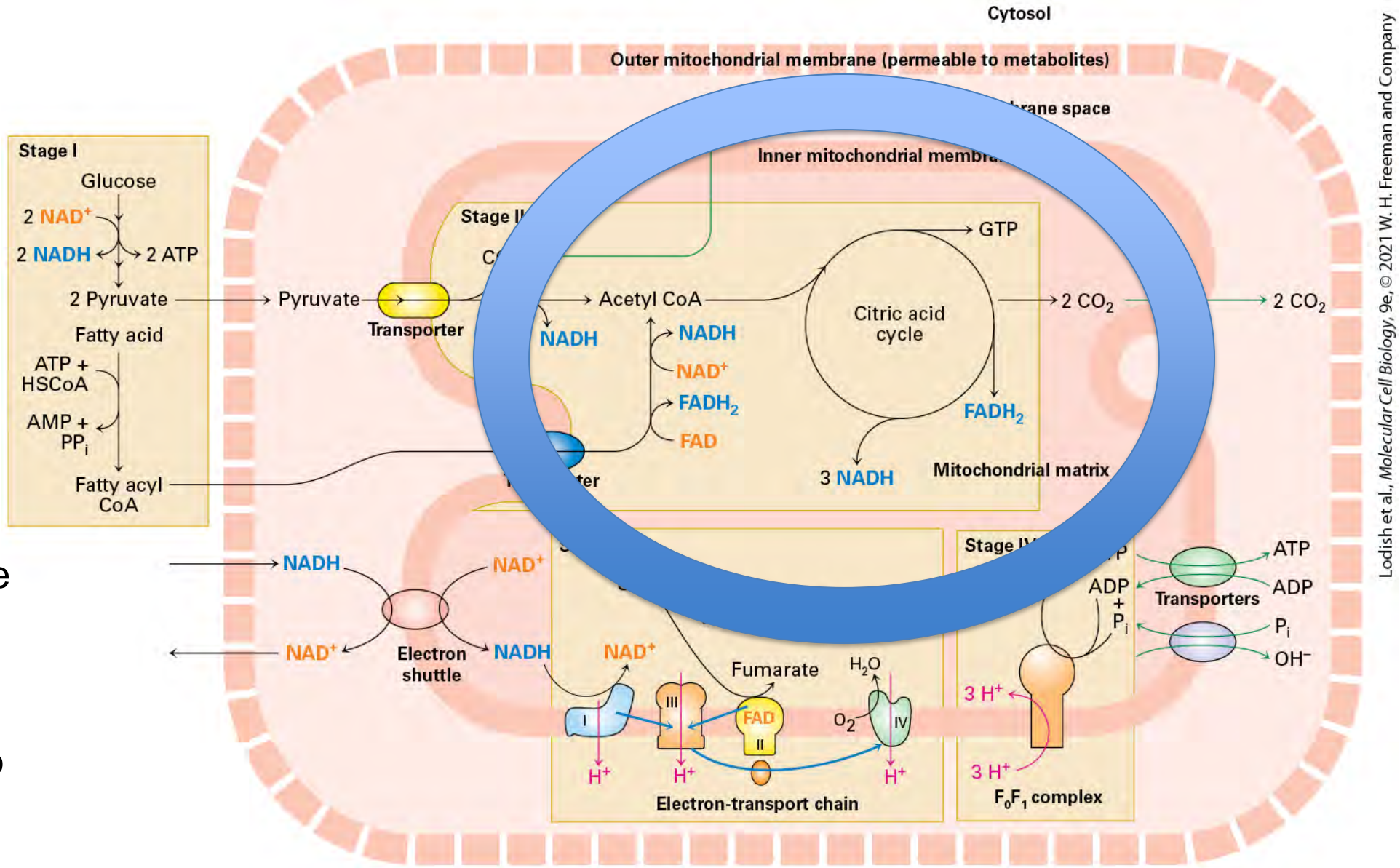


PINK1 and Parkin, linked to Parkinson's disease, regulate this process

Eiyama and Okamoto (2015)

metabolic focus on mitochondria

Look – here are two things you could add to purified mitochondria to get ATP



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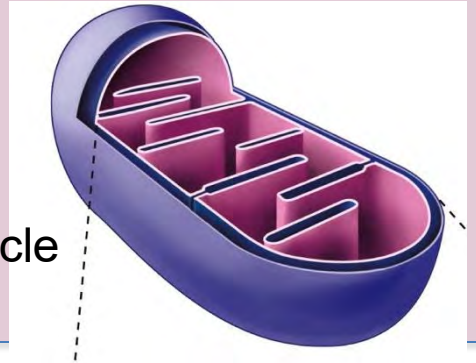
the Citric Acid (or TCA)* cycle in the matrix nets 5 reduced co-enzymes

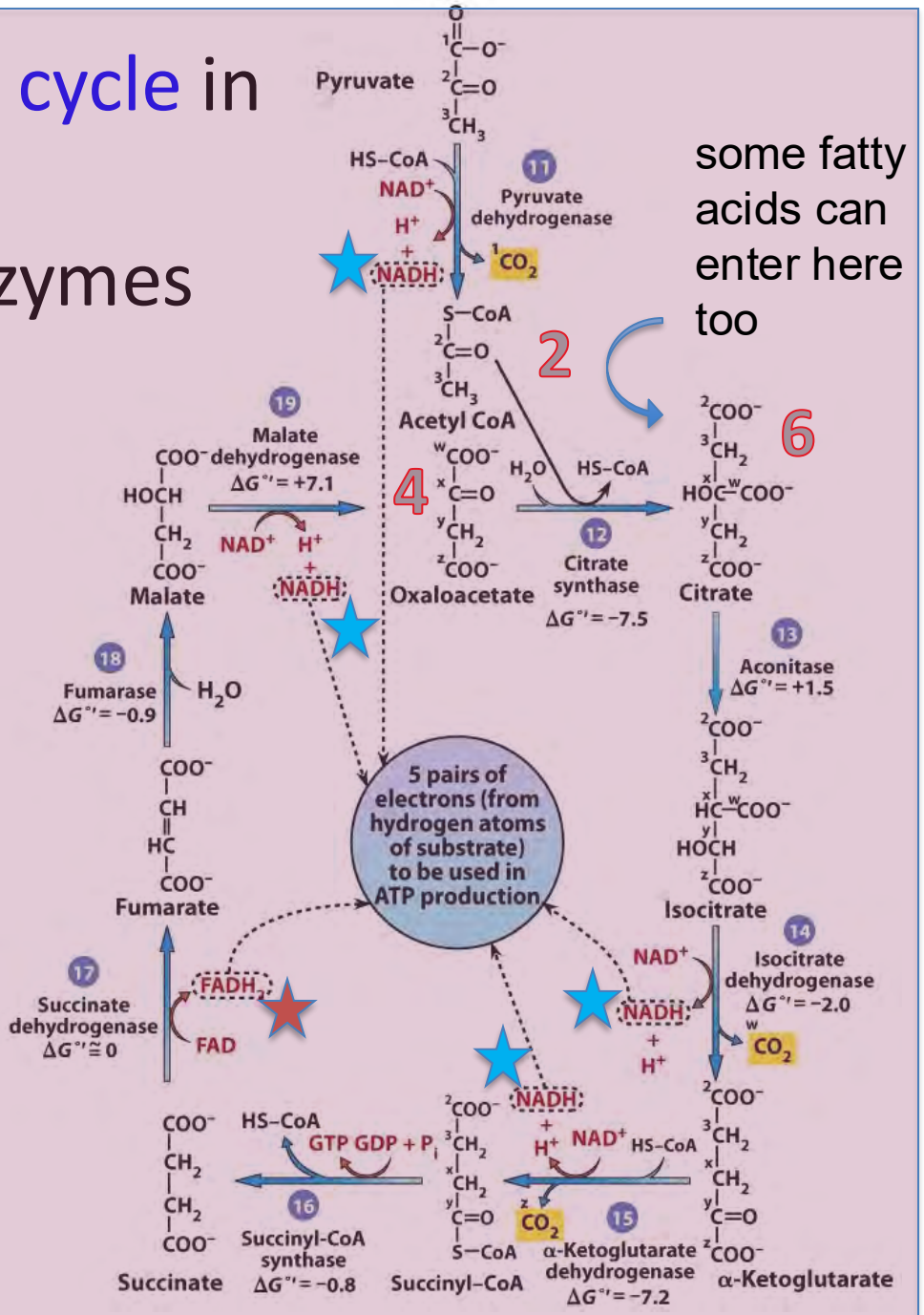
In the matrix

4 NADH and one $FADH_2$ molecules are produced from one pyruvate *(one NADH is prior to the cycle)*

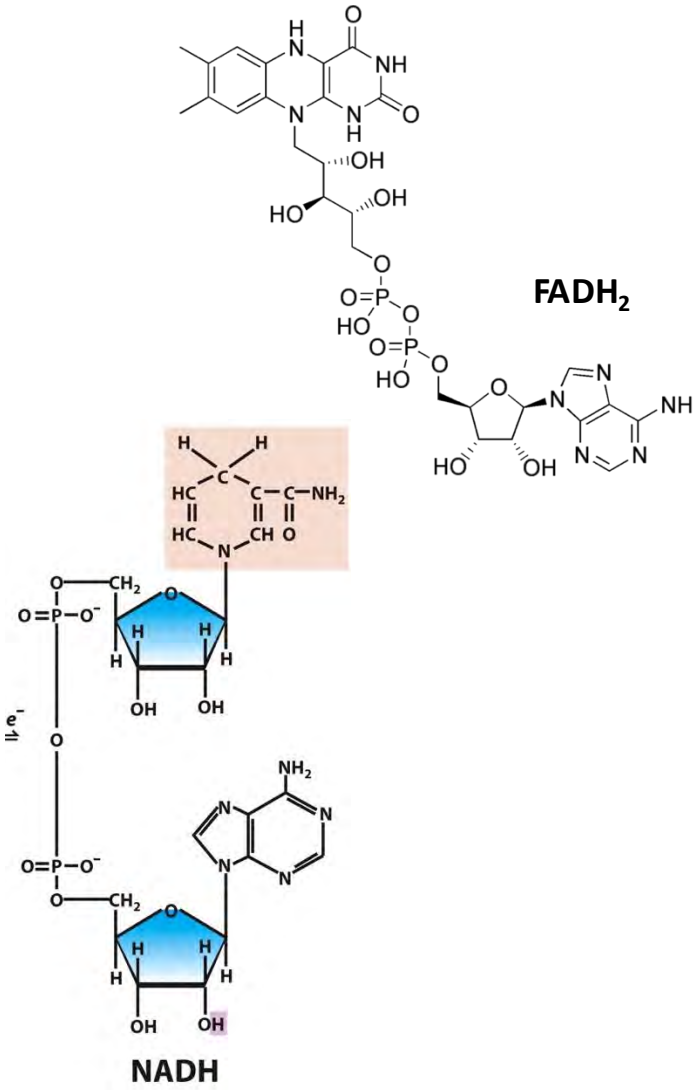
*TCA=
Krebs cycle
Citric acid cycle

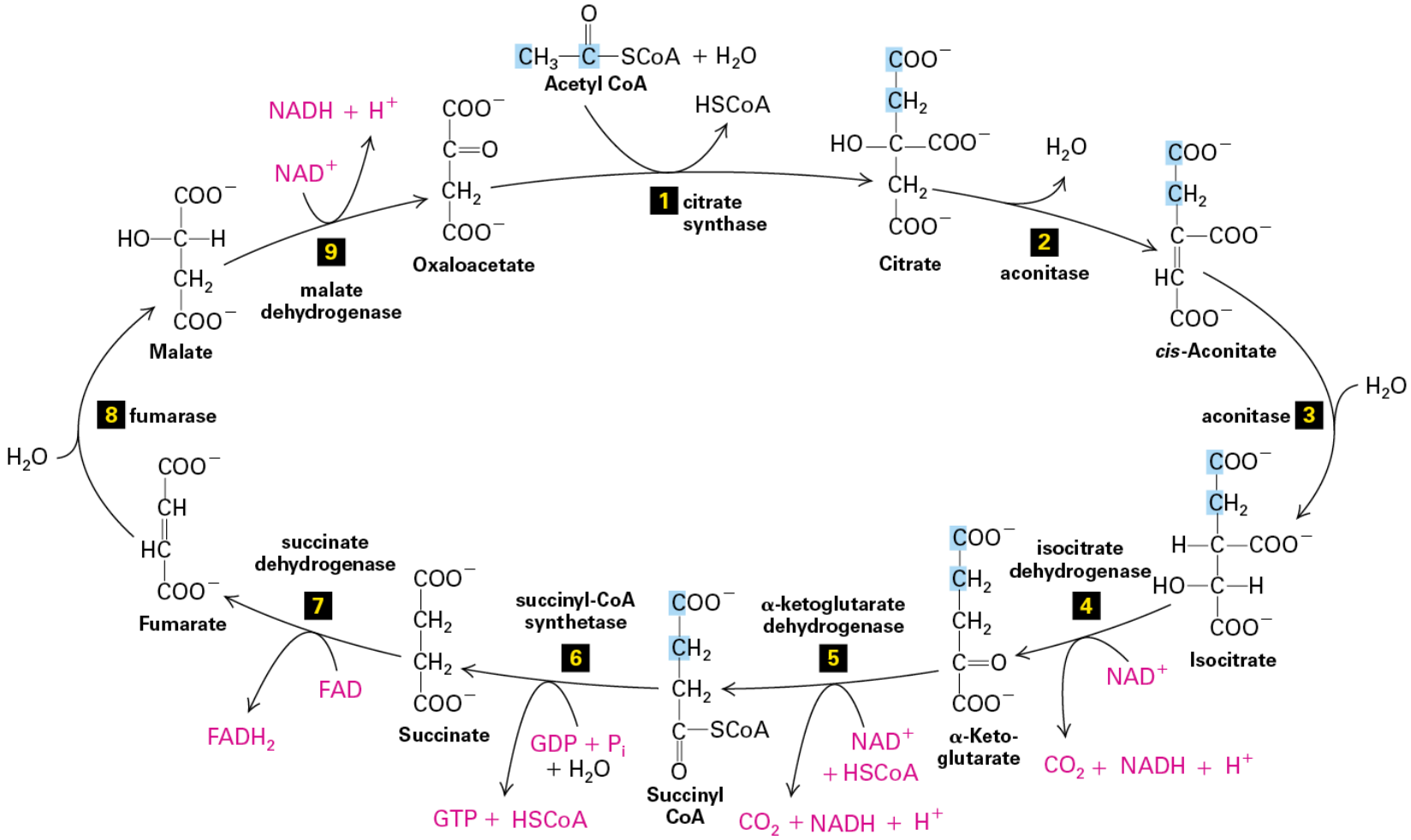


some fatty acids can enter here too



the TCA cycle oxidizes carbon-based substrates and reduces co-enzymes





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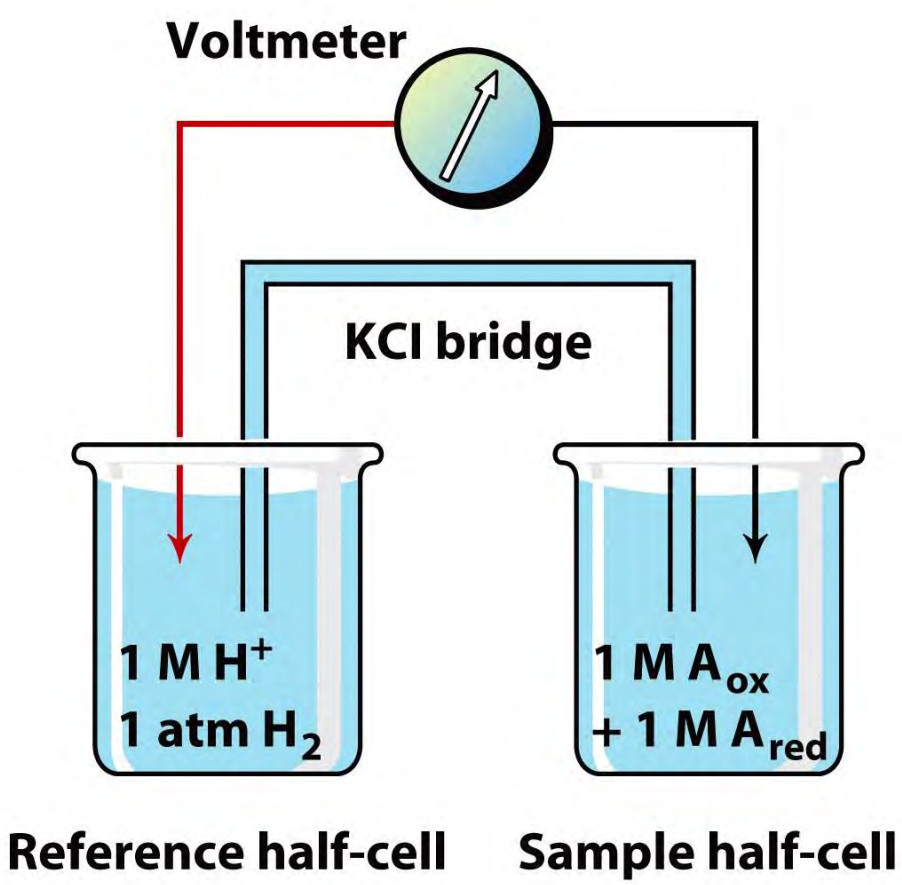
oxidation-reduction potential (redox potentials)

- strong oxidizing agents have a high affinity for electrons; strong reducing agents have a weak affinity for electrons
- redox reactions are accompanied by a decrease in free energy.
- transfer of electrons causes charge separation that can be measured as a redox potential.



measured against the H+ - H_2 standard

redox potentials and free energy change

for the reaction

$$A_{(ox)} + B_{(red)} \leftrightarrow A_{(red)} + B_{(ox)}$$

$$\Delta G_0' = -nF\,\Delta E'_0$$

where n is the number of electrons transferred,

F is the Faraday constant (23.063 kcal/V*mol) and

E is the difference in volts between the standard redox potentials of the two couples.

redox potential of some reaction couples

TABLE 5.1 Standard Redox Potentials of Selected Half-Reactions

Form: oxidant + e- → reductant

Electrode equation	E'_0(V)
Succinate + CO_2 + 2 H^+ + 2 e^- ⇌ α-ketoglutarate + H_2O	−0.670
Acetate + 2 H^+ + 2 e^- ⇌ acetaldehyde	−0.580
2 H^+ + 2 e^- ⇌ H_2	−0.421
α-Ketoglutarate + CO_2 + 2 H^+ + 2 e^- ⇌ isocitrate	−0.380
Cystine + 2 H^+ + 2 e^- ⇌ 2 cysteine	−0.340
NAD^+ + 2 H^+ + 2 e^- ⇌ NADH + H^+	−0.320
$NADP^+$ + 2 H^+ + 2 e^- ⇌ NADPH + H^+	−0.324
Acetaldehyde + 2 H^+ + 2 e^- ⇌ ethanol	−0.197
Pyruvate + 2 H^+ + 2 e^- ⇌ lactate	−0.185
Oxaloacetate + 2 H^+ + 2 e^- ⇌ malate	−0.166
FAD + 2 H^+ + 2 e^- ⇌ $FADH_2$ (in flavoproteins)	+0.031
Fumarate + 2 H^+ + 2 e^- ⇌ succinate	+0.031
Ubiquinone + 2 H^+ + 2 e^- ⇌ ubiquinol	+0.045
2 cytochrome $b_{(ox)}$ + 2 e^- ⇌ 2 cytochrome $b_{(red)}$	+0.070
2 cytochrome $c_{(ox)}$ + 2 e^- ⇌ 2 cytochrome $c_{(red)}$	+0.254
2 cytochrome $a_{3(ox)}$ + 2 e^- ⇌ 2 cytochrome $a_{3(red)}$	+0.385
$\frac{1}{2}O_2$ + 2 H^+ + 2 e^- ⇌ H_2O	+0.816

The substance that is oxidized (loses electron) is the reducing agent

better oxidizing agents (ie, better electron acceptors)

better reducing agents (ie, better electron donors)

- strong reducing agents (like NADH) are coupled to weak oxidizing agents (like NAD)
- negative E'_O -> good e- donor (like NADH) & positive -> good e- acceptor (O_2)
- standard redox potential of the two couples is equal to the difference of standard E'_o

an anaerobic example

Form: oxidant + e- → reductant

$Pyruvate + 2H^+ + 2e^- \rightarrow lactate \quad E'_0 = -0.19\ V$

$NAD^+ + H^+ + 2e^- \rightarrow NADH \quad E'_0 = -0.32\ V$

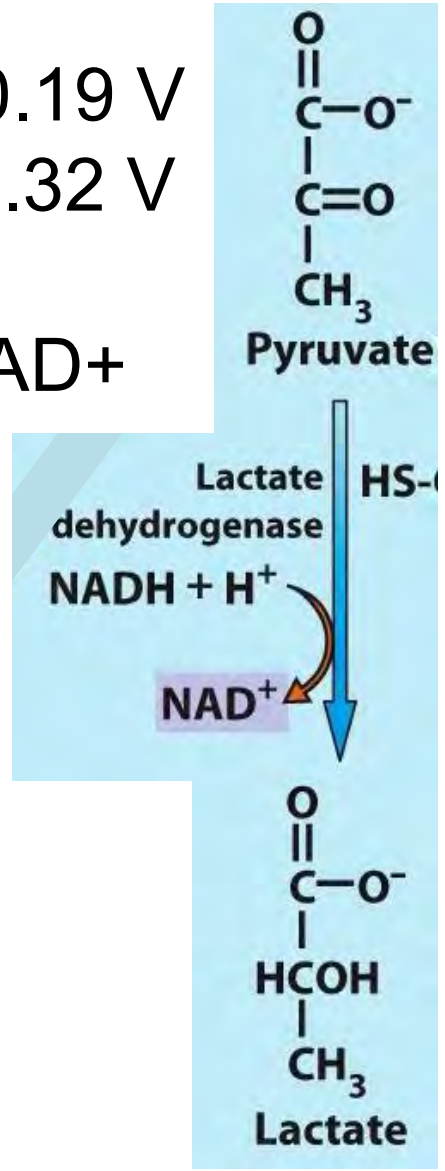
<u>Overall:</u>

$Pyruvate + NADH + H+ \leftrightarrow lactate + NAD+$

$$\Delta E'_0 = (-0.19) - (-0.32) = +0.13V$$

$$\Delta G^{0'} = -nF\,\Delta E'_0$$

$$\Delta G^{0'} = -6kCal/mol$$



(this is fermentation, as is pyruvate conversion to EtOH)

molecular O_2 has the highest affinity for electrons- it is the best electron acceptor

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The substance that is oxidized (loses electron) is the reducing agent

Poll 2: of the following substrates, which is the best electron donor (reducing agent)?

Form: oxidant + e- → reductant

1) ubiquinol
2) $FADH_2$
3) NADH
4) O_2
5) ethanol

link

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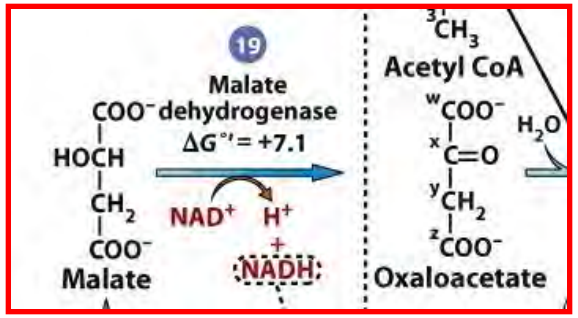
The substance that is oxidized (loses electron) is the reducing agent

Poll 3: using

$\Delta G^{0'} = -n\ (23\ kcal/V*mol)\ \Delta E'_0$

what is the free energy change of the TCA reaction that is catalyzed by malate dehydrogenase?

a) -1(23)(-0.154)
b) -2(23)(-0.154)
c) -2(23)(0.154)
d) -4(23)(-0.154)
e) -4(23)(0.154)



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Hint: $\Delta E'_0 = E'_0$ reduced (electrons gained) - E'_0 oxidized (electrons released)

using

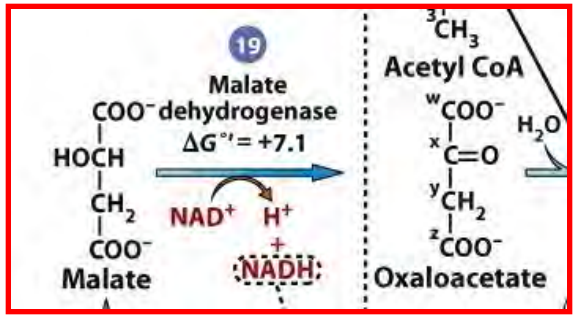
$\Delta G^{0'} = -n\ (23\ kcal/V*mol)\ \Delta E'_0$

what is the free energy change of the TCA reaction that is catalyzed by malate dehydrogenase?

$\Delta E'_0$ =E'_0 reduced (electrons gained)
- E'_0 oxidized (electrons released)

$= -2(23)(-0.154)$

$\Delta G^{0'} = \sim 28\ kJ$



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http://www.wiley.com/college/pratt/0471393878/student/review/redox/5_gibbs.html

for all of oxidative phosphorylation

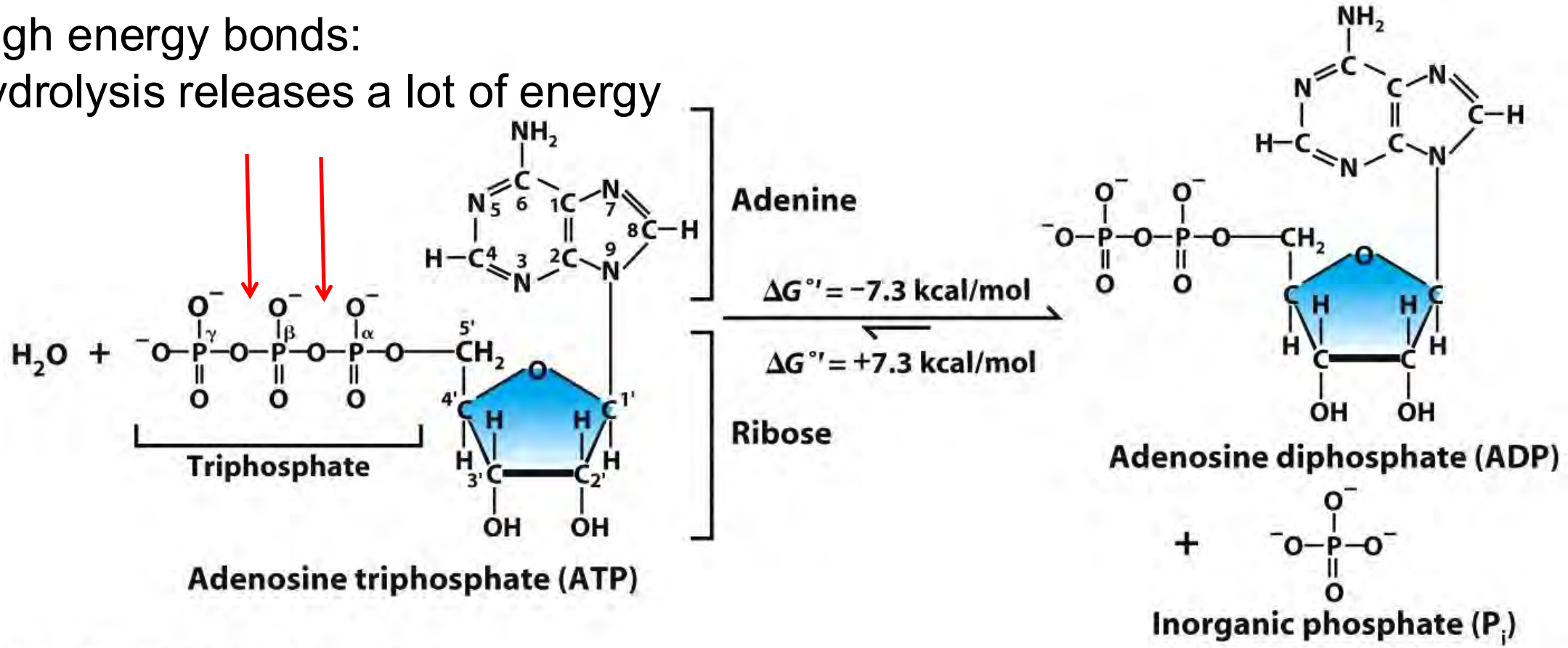
- $\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$ $E'_0 = +0.82$ V
- $NAD^+ + H^+ + 2e^- \rightarrow NADH$ $E'_0 = -0.32$ V
- Overall $\Delta G^{0'}$?

- $\Delta G^{0'} = -n\ F\ (23 kcal/V*mol)\ \Delta E'_0$

$$\mathrm{NADH} + \mathrm{H}^+ + {}^1\!/_2\mathrm{O}_2 \rightarrow \mathrm{NAD}^+ + \mathrm{H_2O},$$
$$\Delta G = -52.6\ \mathrm{kcal/mol}$$
$$\mathrm{FADH_2} + {}^1\!/_2\mathrm{O}_2 \rightarrow \mathrm{FAD} + \mathrm{H_2O},$$
$$\Delta G = -43.4\ \mathrm{kcal/mol}$$

compare to energy ”held” in ATP

High energy bonds:
hydrolysis releases a lot of energy



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this “free energy change” can be transferred to other reactions

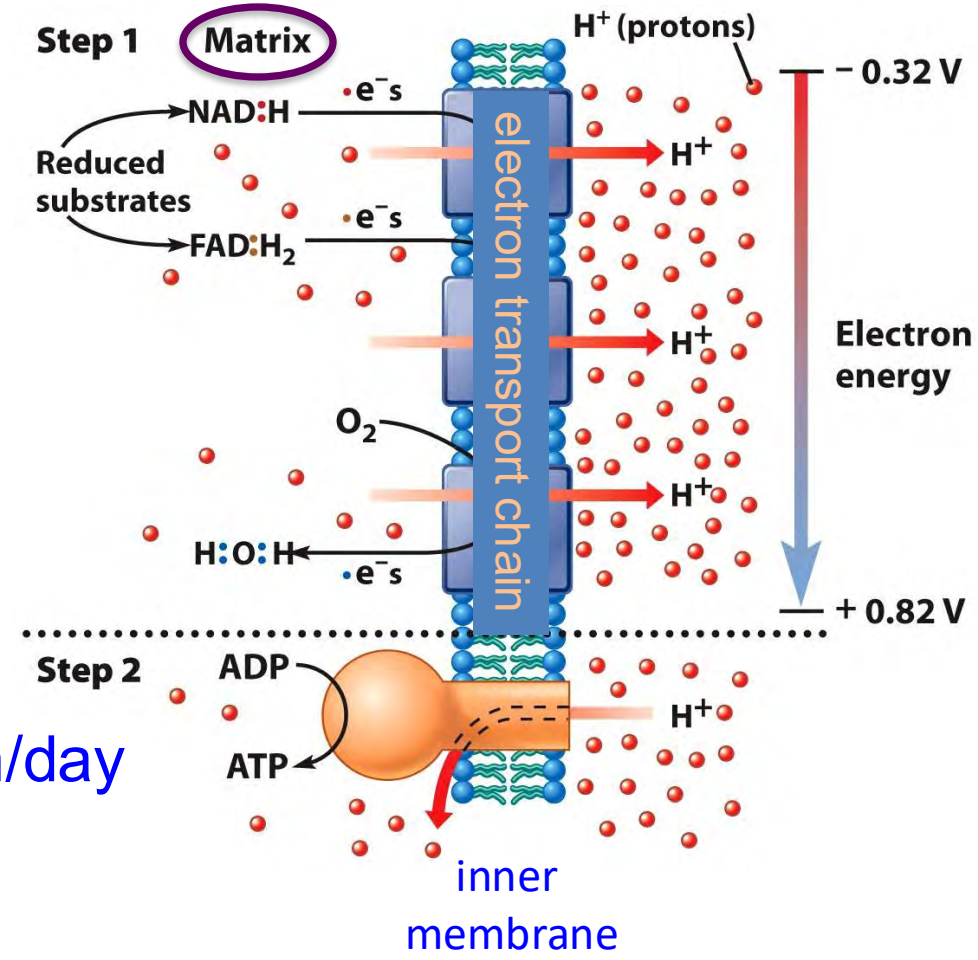
oxidative phosphorylation (ox phos)

ox phos - energy captured during substrate oxidation, and released by movement of electrons through the electron transport chain

powers ATP production

10^{26} (>60kg **ATP**) generated/person/day

(other phosphorylation events in the cell are mostly *substrate phosphorylations by kinases*)

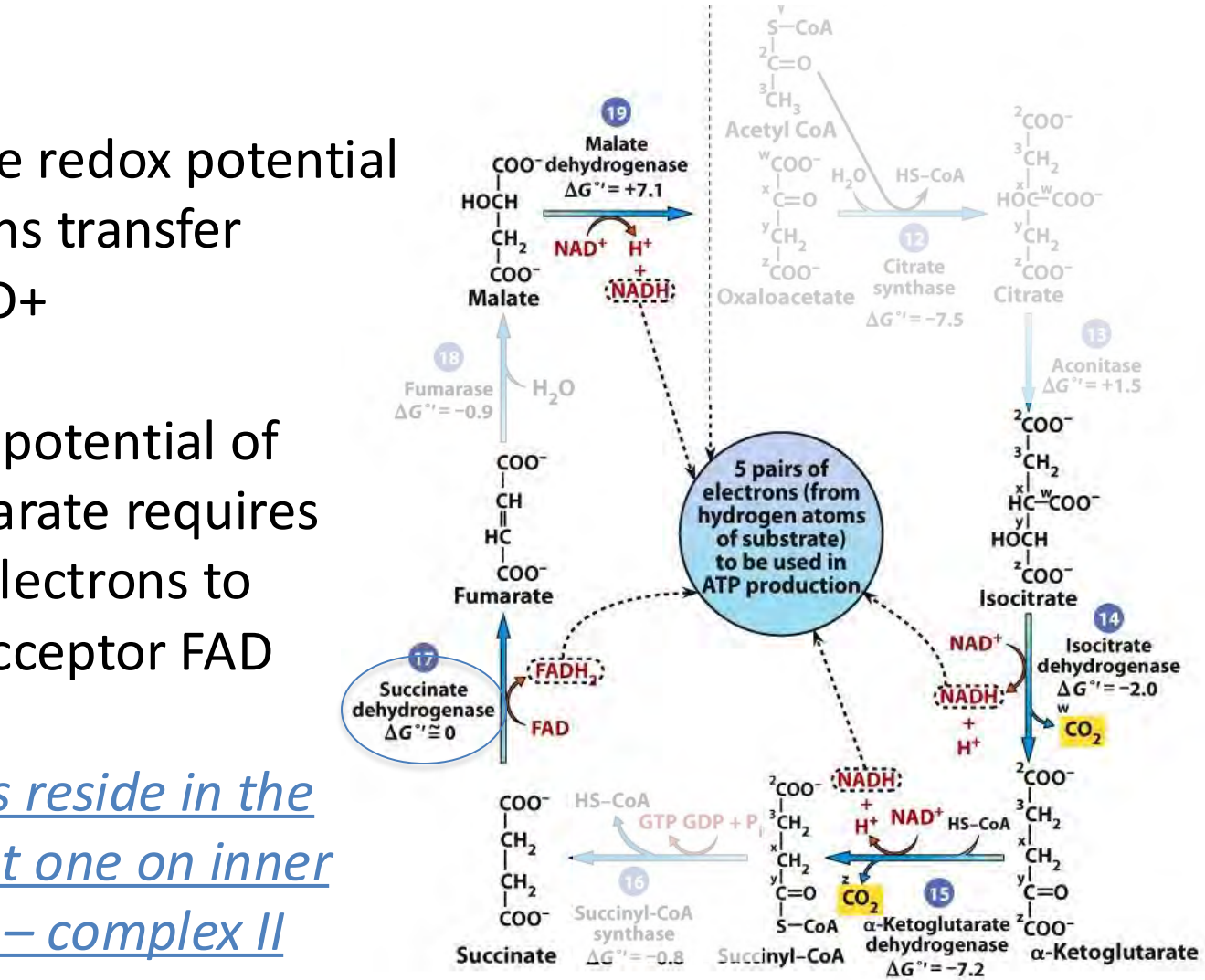


the citric acid cycle oxidizes carbon-based substrates and reduces co-enzymes

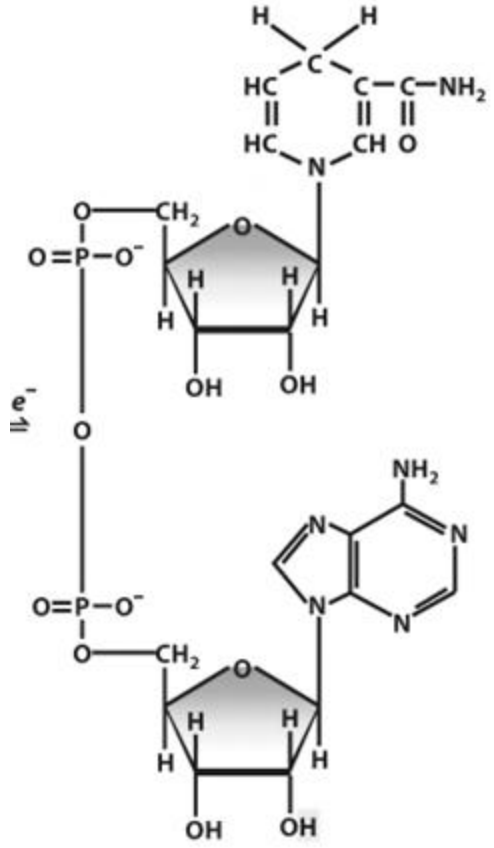
the high negative redox potential of 3 TCA reactions transfer electrons to NAD+

the lower redox potential of succinate – fumarate requires that it transfer electrons to higher affinity acceptor FAD

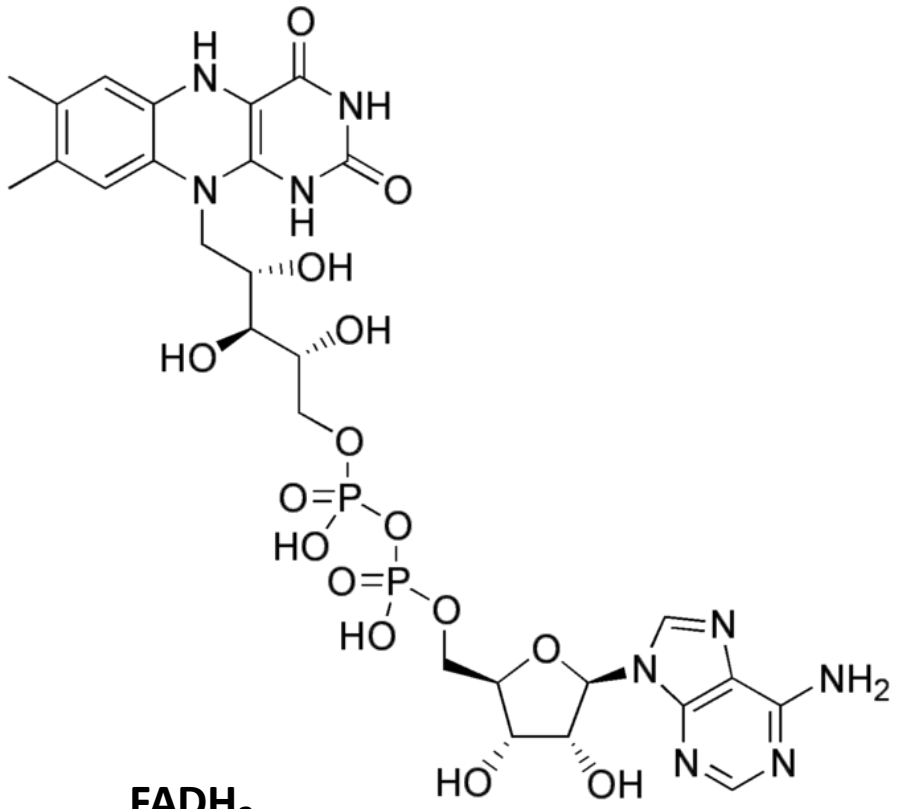
TCA enzymes reside in the matrix, except one on inner membrane – complex II



okay, we have lots of reduced co-enzymes. now what?



NADH



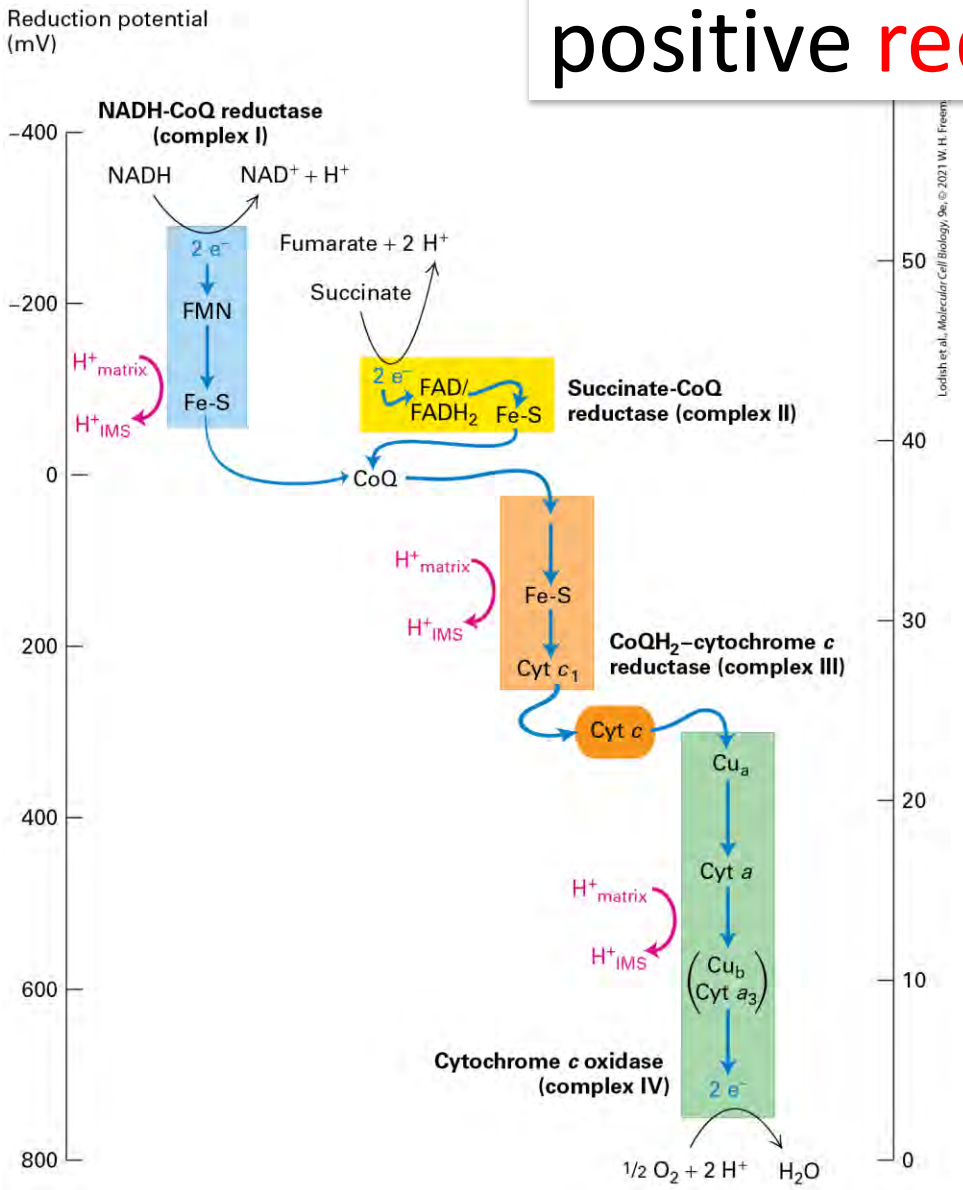
$FADH_2$

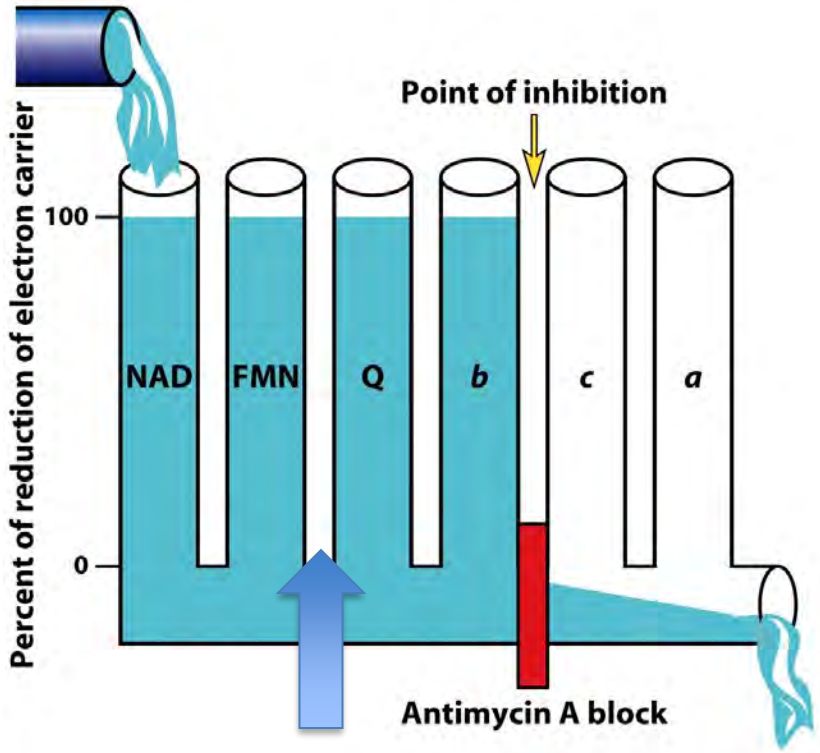
image from wikipedia

electron transport chain

- electrons associated with either NADH or $FADH_2$ are transferred through specific electron carriers that make up the **electron transport chain**.
- electrons move through the inner membrane via a series of carriers of more positive redox potential.

electron carriers are arranged in order of increasingly positive redox potential

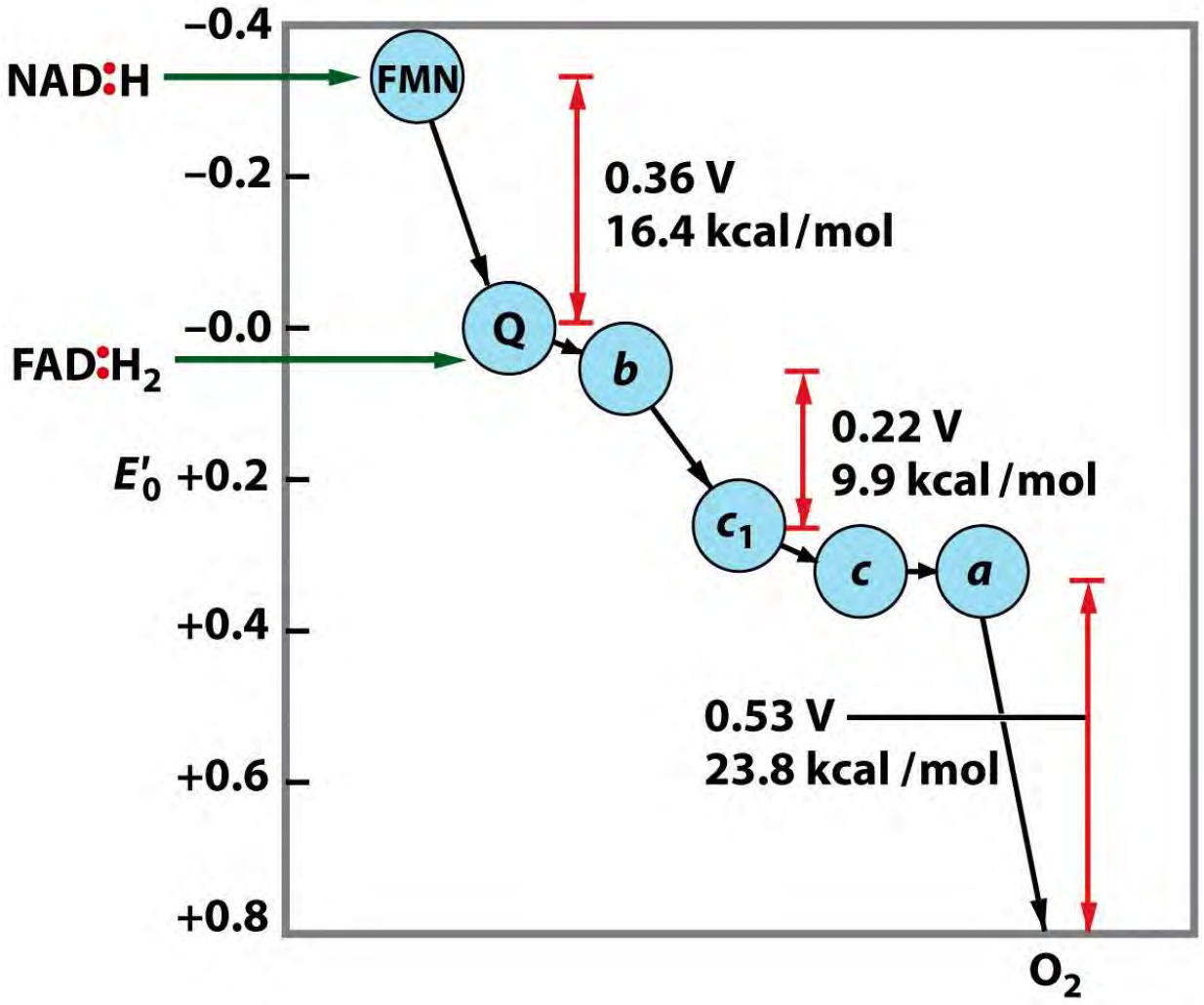




– Sequence of carriers determined by use of inhibitors (rotenone).

two electron entry points

but where in the cell are these coming from?



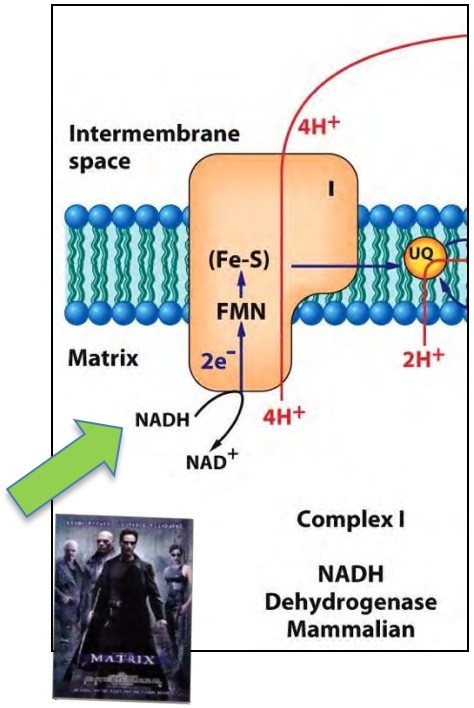
transfer energy into:

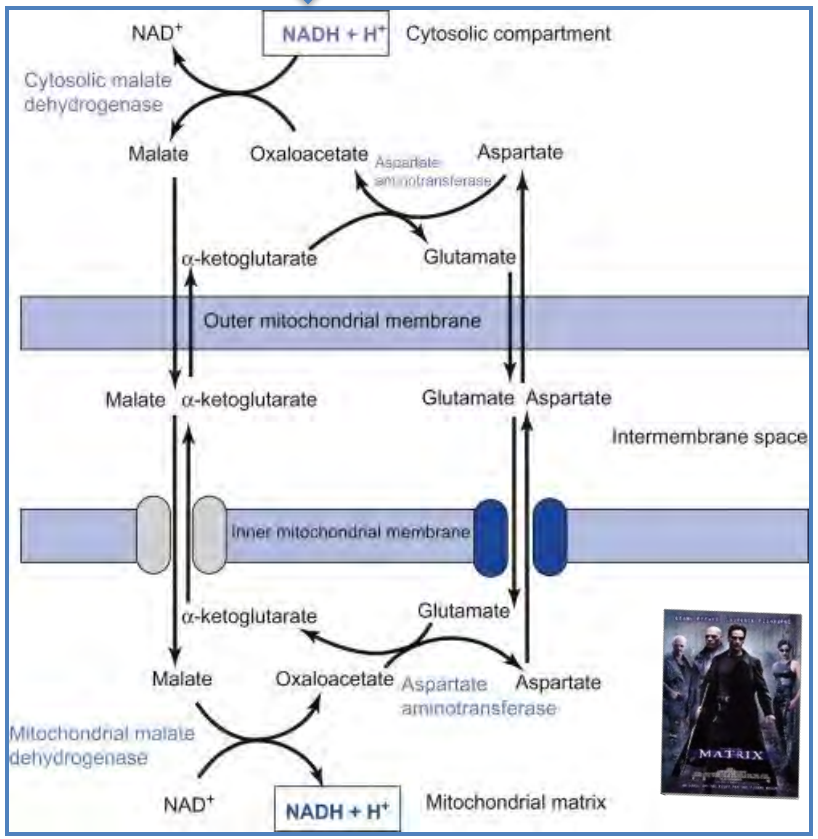
start of electron transport chain directly from within the matrix -

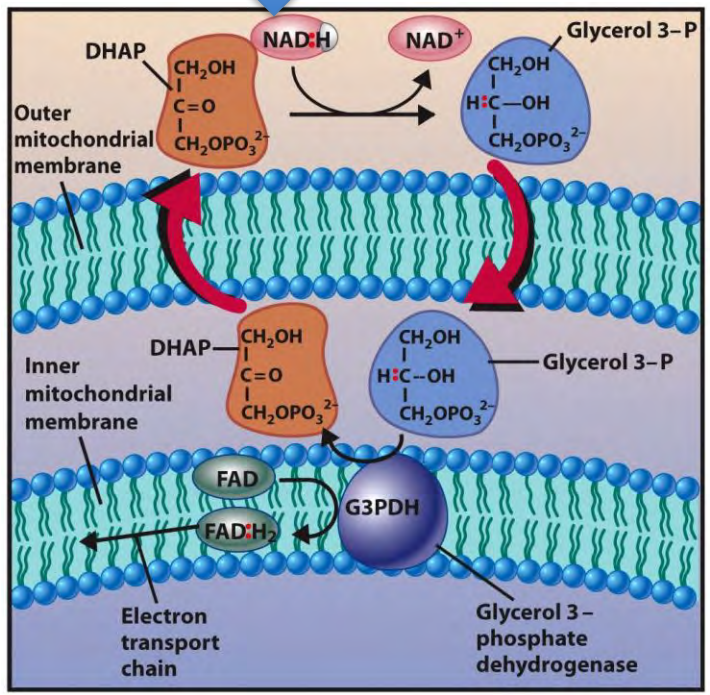
&/or...

through the malate-aspartate shuttle or

the glycerol phosphate shuttle



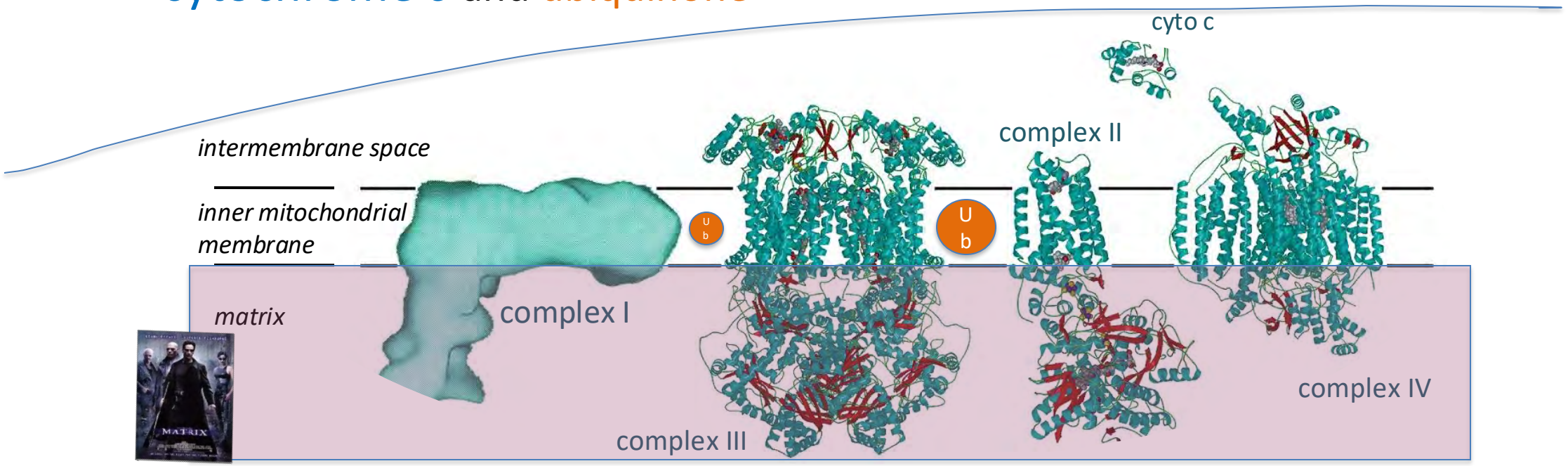




dihydroxy-acetone phosphate

4 electron-transport complexes and two helpers

- *Complex I (NADH dehydrogenase)*
- *Complex II (succinate dehydrogenase)*
- *Complex III (cytochrome bc_1)*
- *Complex IV (cytochrome c oxidase)*
- cytochrome *c* and ubiquinone

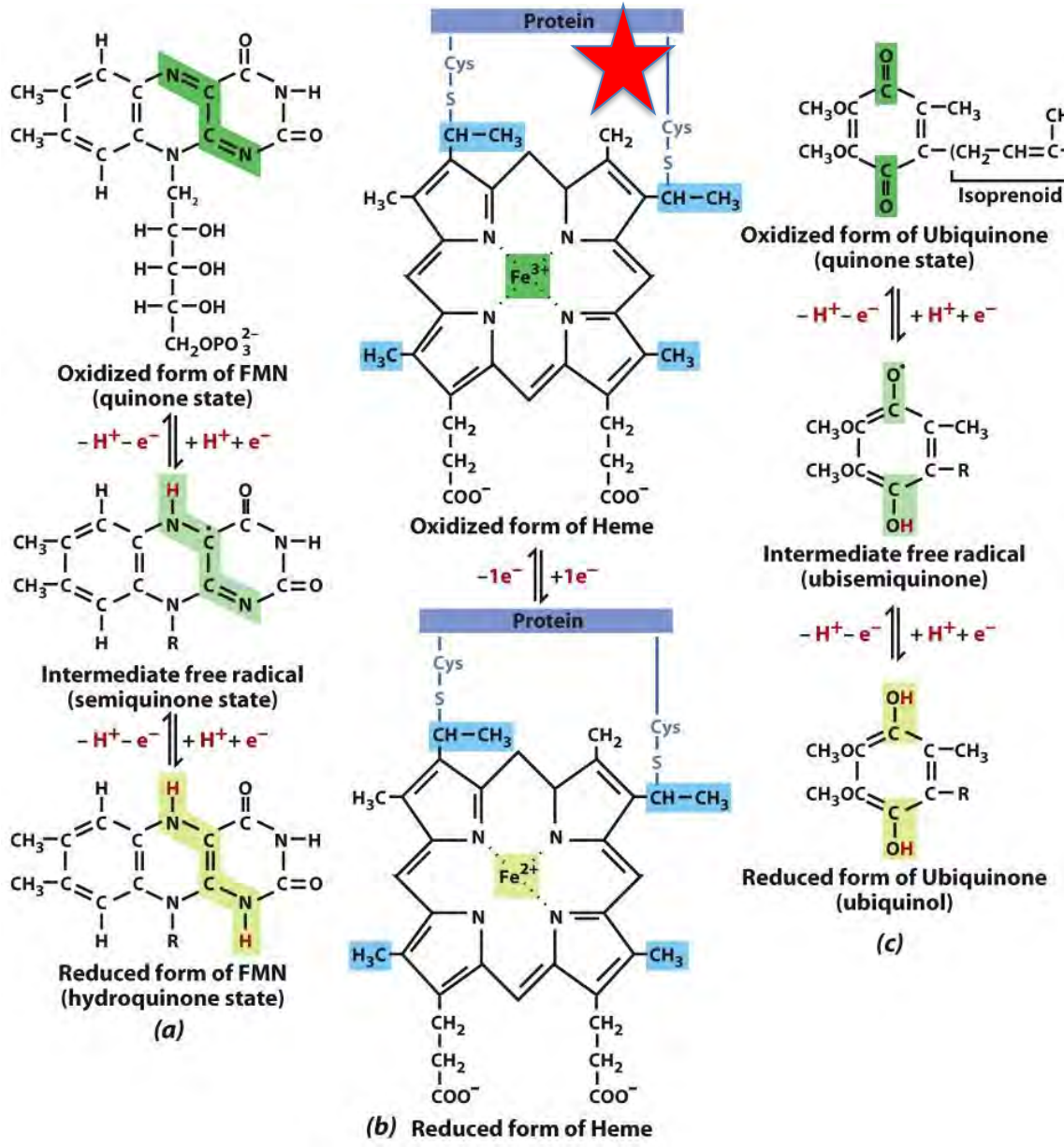


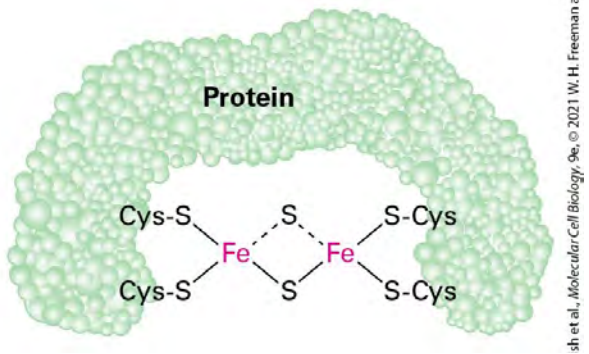
5 types of electron carriers

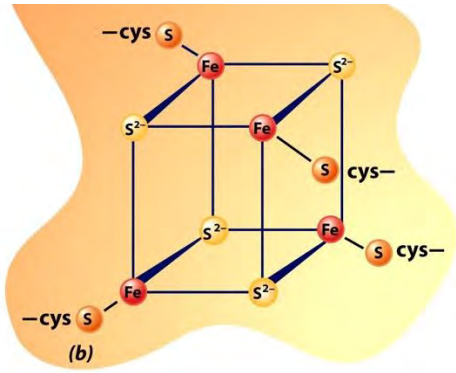
- **Flavoproteins** are *polypeptides* bound to either FAD (flavin adenine dinucleotide) or FMN (flavin mononucleotide).
- **Cytochromes** contain heme groups bearing Fe or Cu metal ions.
- **Three copper atoms** are located within a single protein complex and alternate between Cu^{2+}/Cu^{3+}
- **Ubiquinone** (coenzyme **Q**) is a lipid-soluble molecule made of five-carbon isoprenoid units.
- **Iron-sulfur proteins** contain Fe in association with inorganic sulfur.

structures of four electron carriers (prosthetic groups)

Flavin mononucleotide





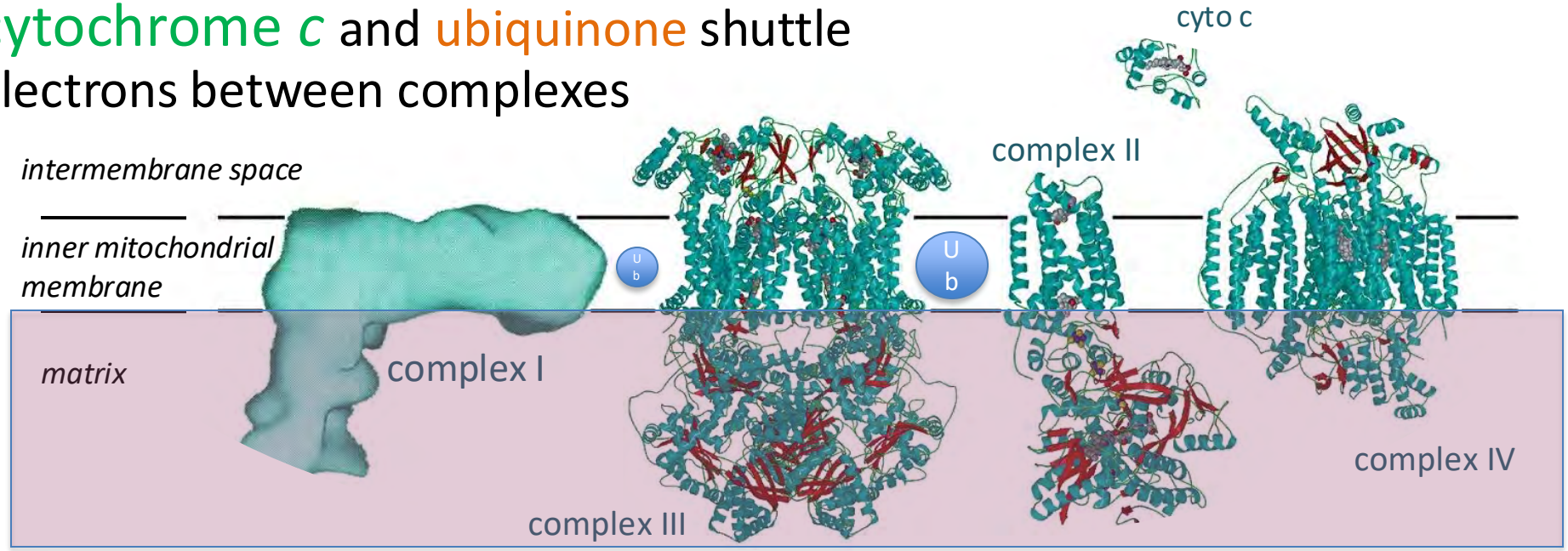


NADH dehydrogenase
cytochrome c
ubiquinone
Iron sulfur centers (various)

electron-transport complexes

(and carriers)

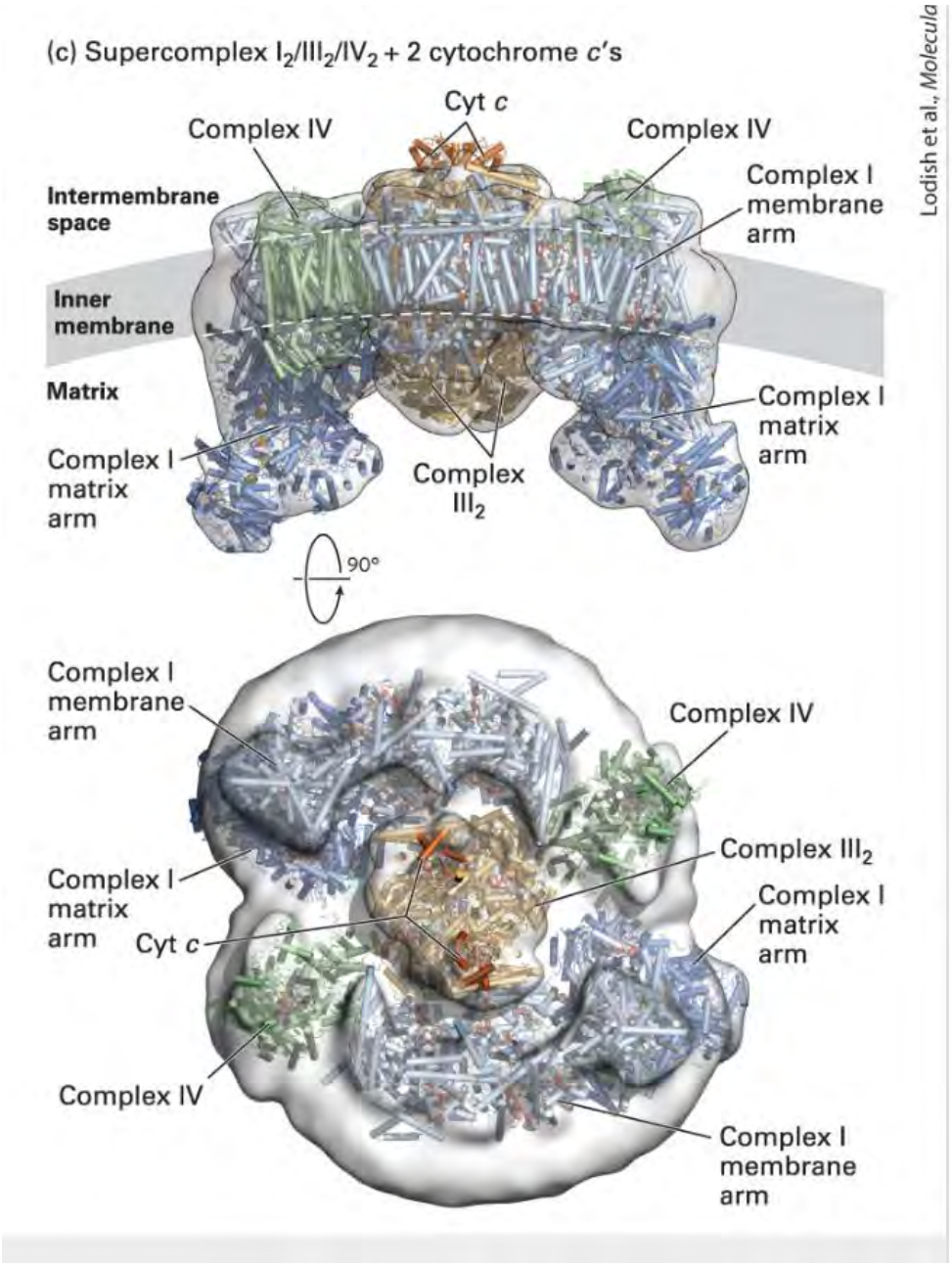
- *Complex I (NADH dehydrogenase)*(FMN, FeS) catalyzes transfer of electrons from **NADH** to ubiquinone and **transports** 4 H^+ per pair of e-.
- *Complex II (succinate dehydrogenase)*(FAD, FeS) catalyzes transfer of electrons from **succinate** to FAD to ubiquinone (no transport of H^+).
- *Complex III (cytochrome bc_1)*(FeS, heme) catalyzes the transfer of electrons from **ubiquinone** to cytochrome c and **transports 4 H^+** per pair of e-.
- *Complex IV (cytochrome c oxidase)*(Cu, heme) catalyzes transfer of electrons from **cyto c** to O_2 and **transports 2 H^+** across the inner membrane.
- cytochrome *c* and ubiquinone shuttle electrons between complexes

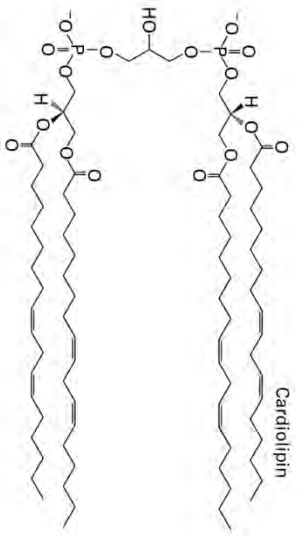


electron-transport super complexes structure

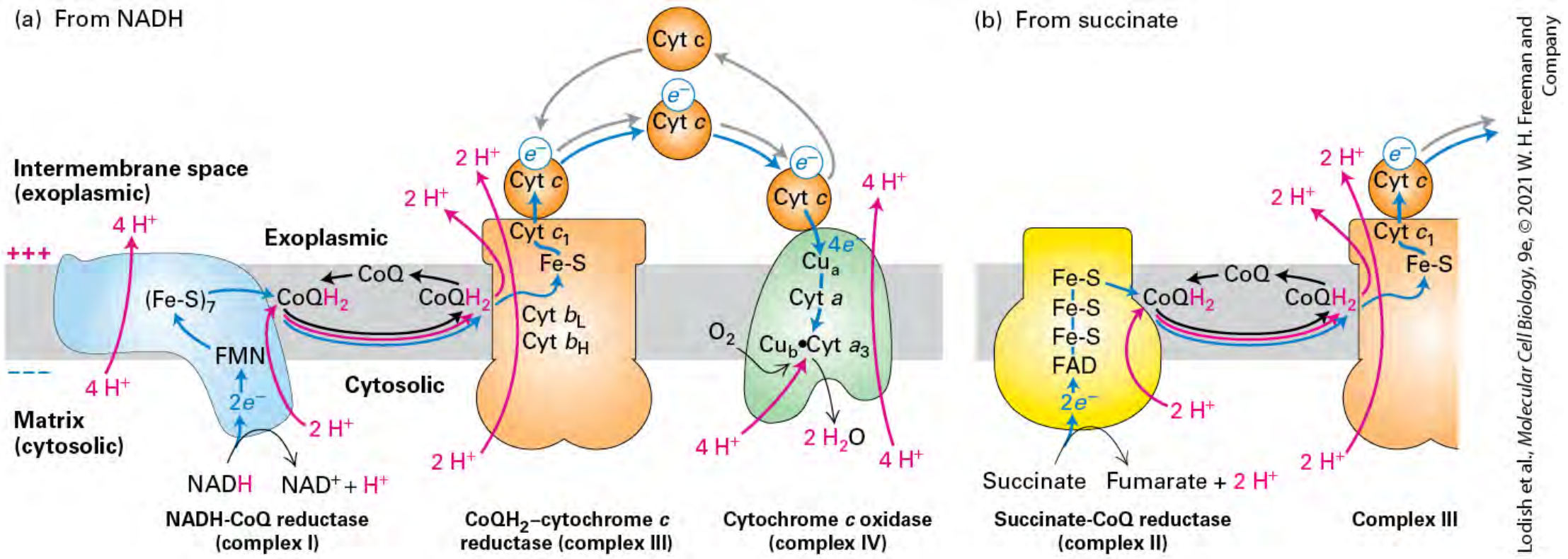
- *Complex I (NADH dehydrogenase)*(FMN, FeS)
- *Complex III (cytochrome bc_1)*(FeS, heme)
- *Complex IV (cytochrome c oxidase)*(Cu, heme)

Stabilized in the membrane by cardiolipin





the electron-transport chain -inner mitochondrial membrane



Lodish et al., *Molecular Cell Biology*, 9e, © 2021 W. H. Freeman and Company

SUBUNITS	NADH Dehydrogenase Mammalian	Cytochrome bc_1	Succinate dehydrogenase	Cytochrome c Oxidase
mtDNA	7	1	0	3
nDNA	39	10	4	10
TOTAL	46	11	4	13
Molecular mass (Da)	>900,000	~240,000	~125,000	~200,000

As electrons move, H+s are pumped

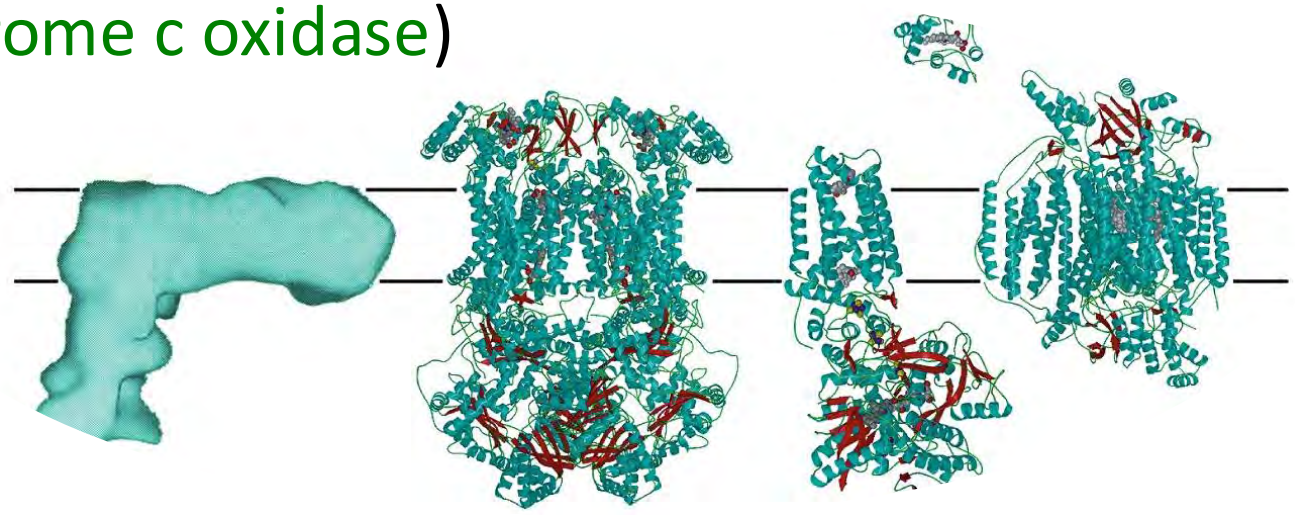
Poll: Which complex does move electrons but does not directly pump protons?

a) Complex I (NADH dehydrogenase)

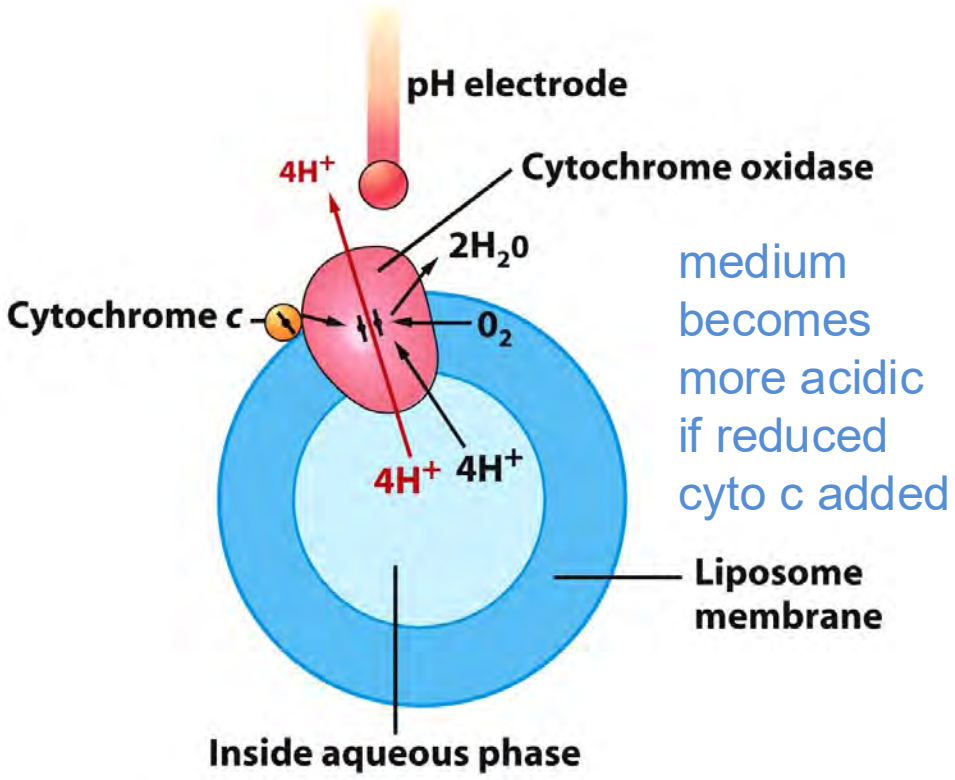
b) Complex II (succinate dehydrogenase)

c) Complex III (cytochrome bc_1)

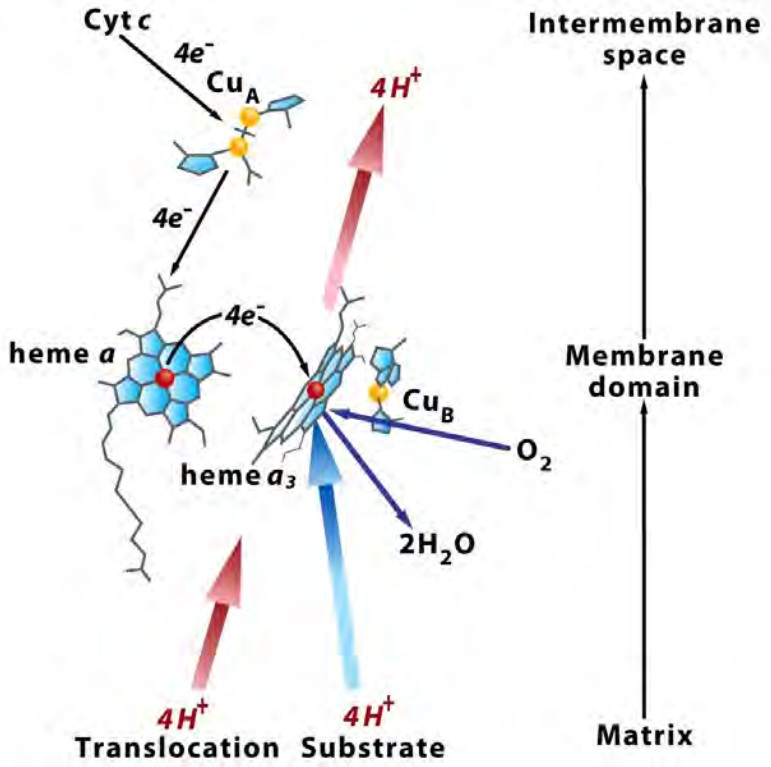
d) Complex IV (cytochrome c oxidase)



Cytochrome oxidase (complex IV) requires O_2 to finish the job

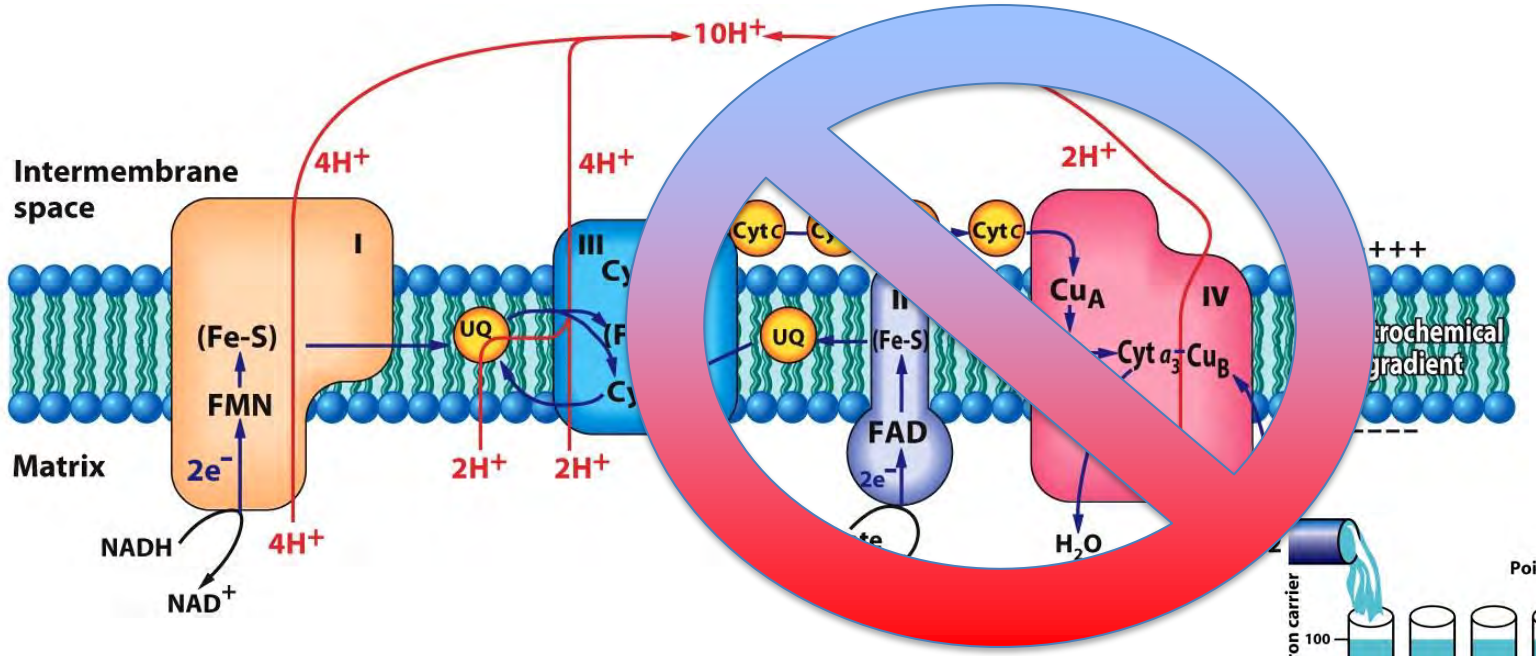


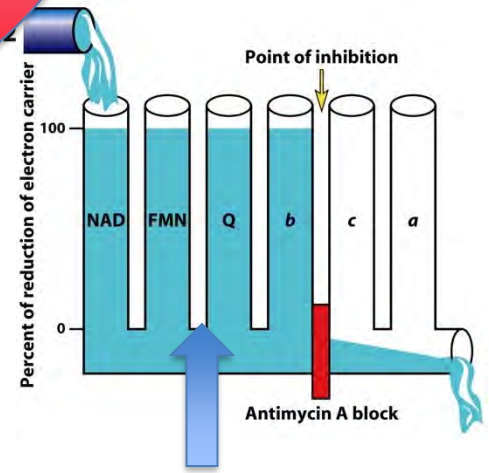
medium becomes more acidic if reduced cyto c added



Cytochrome c oxidase adds four electrons to O_2 to form two molecules of H_2O.
Electrons are transferred one at a time.
Energy released by O_2 reduction presumably drives conformational changes that promote the movement of H^+ ions through the protein.

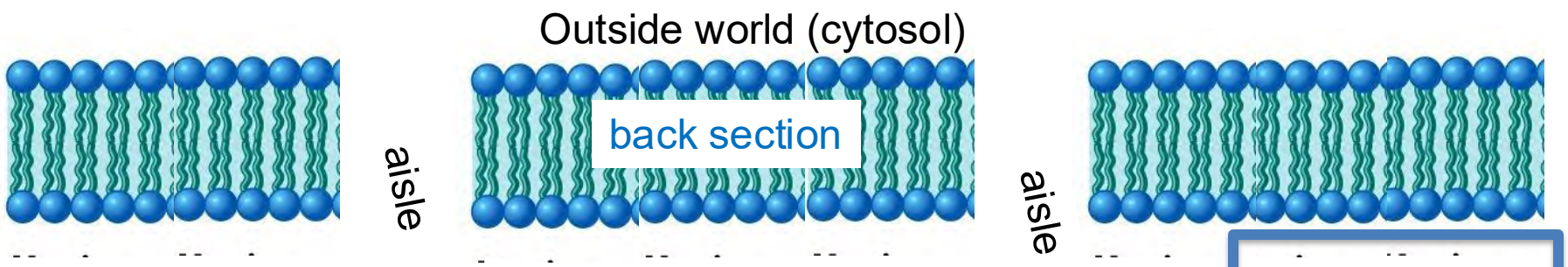
disruptions in the ETC are detrimental
back up the system and/or cause energy loss



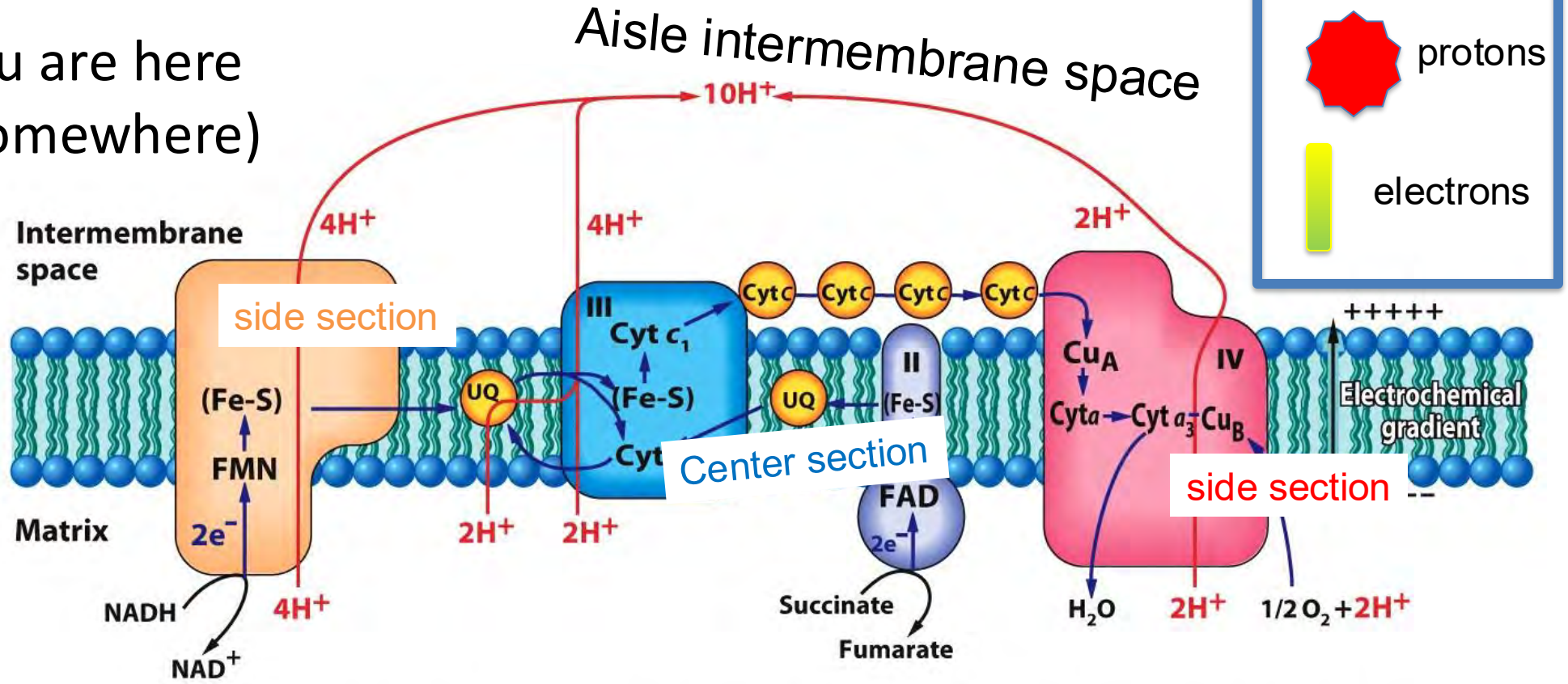


- Toxic levels of CO or cyanide bind and block **cytochrome oxidase**
- metabolic poisons CO, N_3^-, and CN^- bind catalytic sites in Complex IV
- some genetic defects linked to disease

rotenone



- You are here (somewhere)



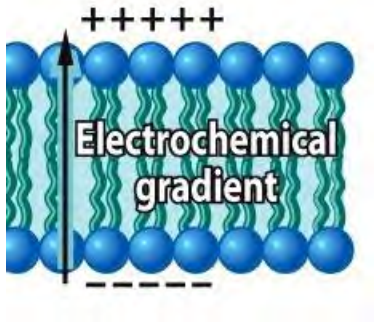
I am here -

a coenzyme

Complex I	Complex III	Complex II	Complex IV
NADH Dehydrogenase	Cytochrome bc_1	Succinate dehydrogenase	Cytochrome *c* Oxidase

MATRIX

Great - but where’s the ATP?



the proton-motive force

- The [H+] concentration gradient between the matrix and intermembrane space creates a **pH gradient (ΔpH)**.
- The separation of charge across the membrane creates an electric potential (psi, Ψ).

The energy present in both components of the gradients is the *proton-motive force* **(Δp)**.

$$\Delta p = \Psi - 2.3\,(RT/F)\,\Delta pH$$

$$\Delta p = \Psi - 59\,\Delta pH \text{ (at standard conditions)}$$

and…. $\Delta G = F\Delta p$

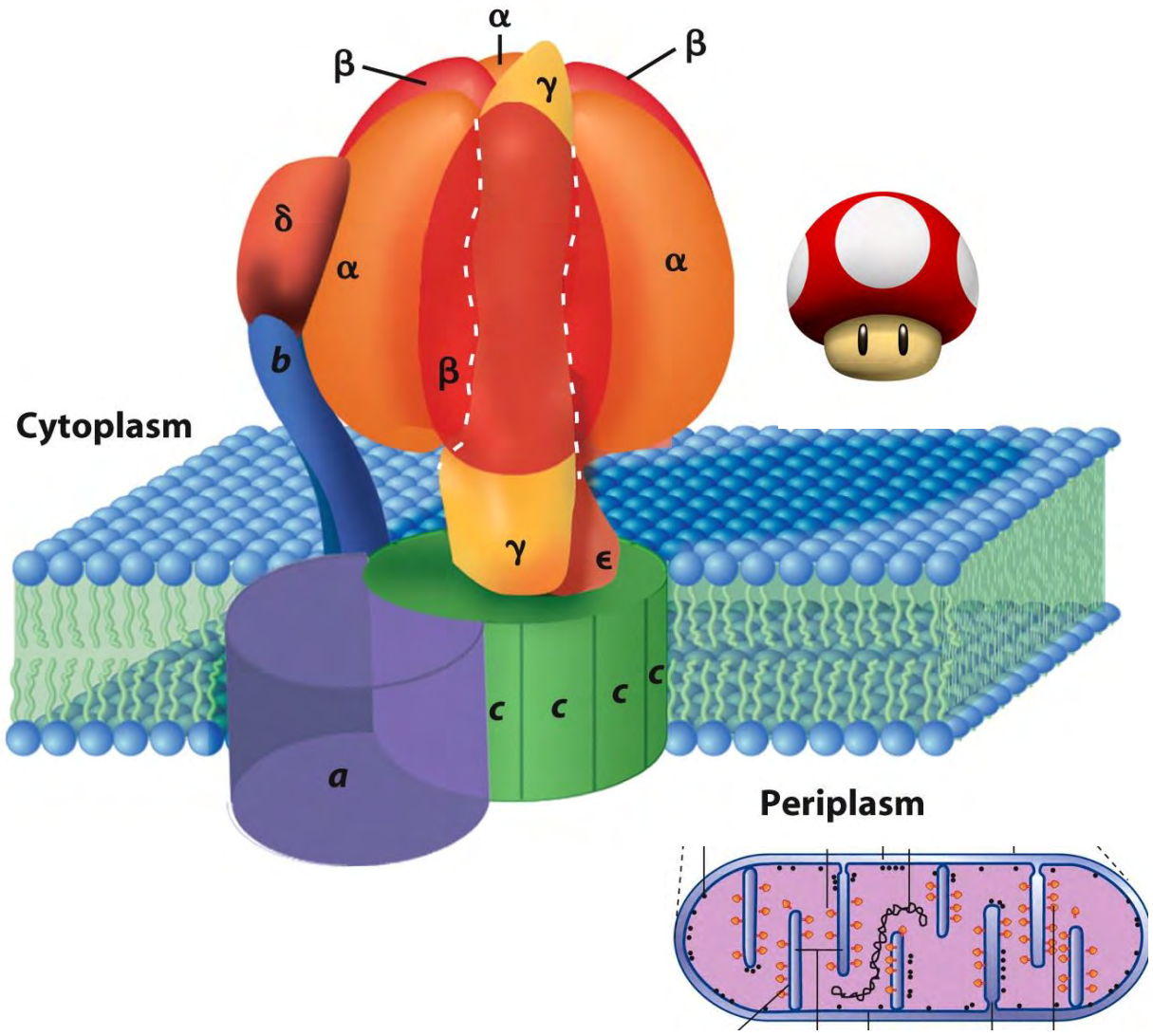
the proton-motive force holds energy

$$\begin{aligned}\Delta G(\mathrm{cal/mol}) &= -nF\Delta E = -(23{,}062\,\mathrm{cal}\cdot V^{-1}\cdot \mathrm{mol}^{-1})\Delta E \\ &= (23{,}062\,\mathrm{cal}\cdot V^{-1}\cdot \mathrm{mol}^{-1})(0.22\,\mathrm{V}) \\ &= 5074\,\mathrm{cal/mol, or}\ \ -5.1\,\mathrm{kcal/mol}\end{aligned}$$

Per mol protons

$\Delta G^{0'}$ = to make one ATP from ADP + Pi ~= +7.3 kcal/mol
(but maybe +10-12 in a cell)

bacterial ATP synthase



ATP synthase is very highly conserved

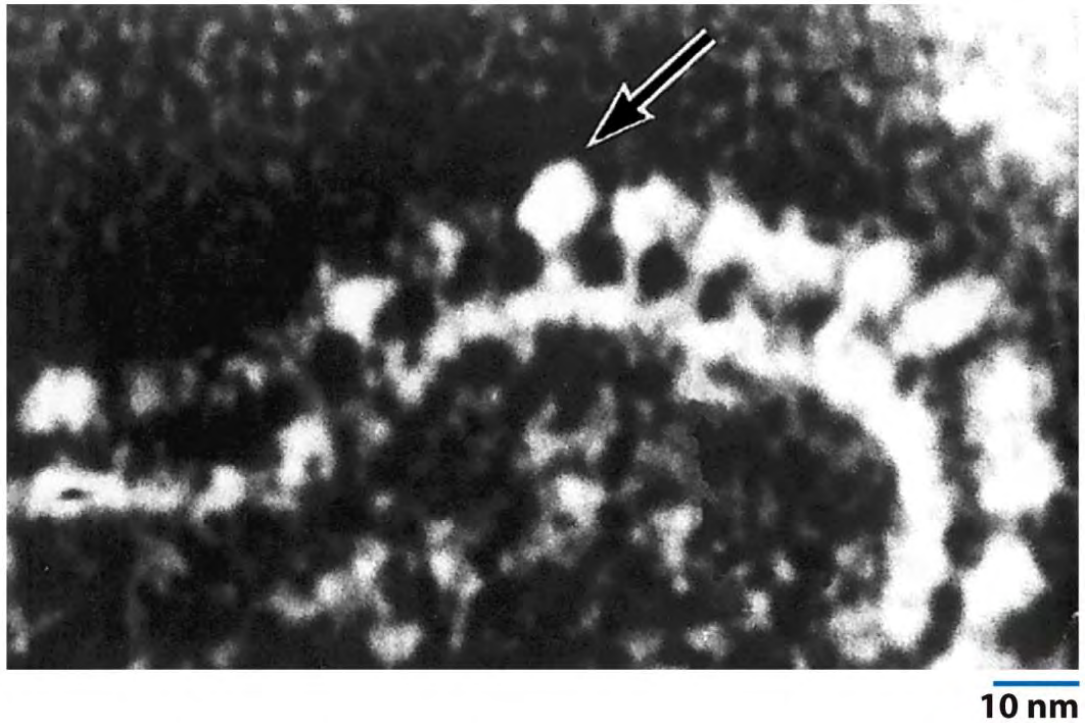
the F_1 particle is the catalytic subunit, with three catalytic sites for ATP synthesis.

the F_0 particle attaches to the F_1 and is embedded in the (inner) membrane.

the F_0 base contains a channel through which protons are moved

machinery for ATP synthesis on cristae

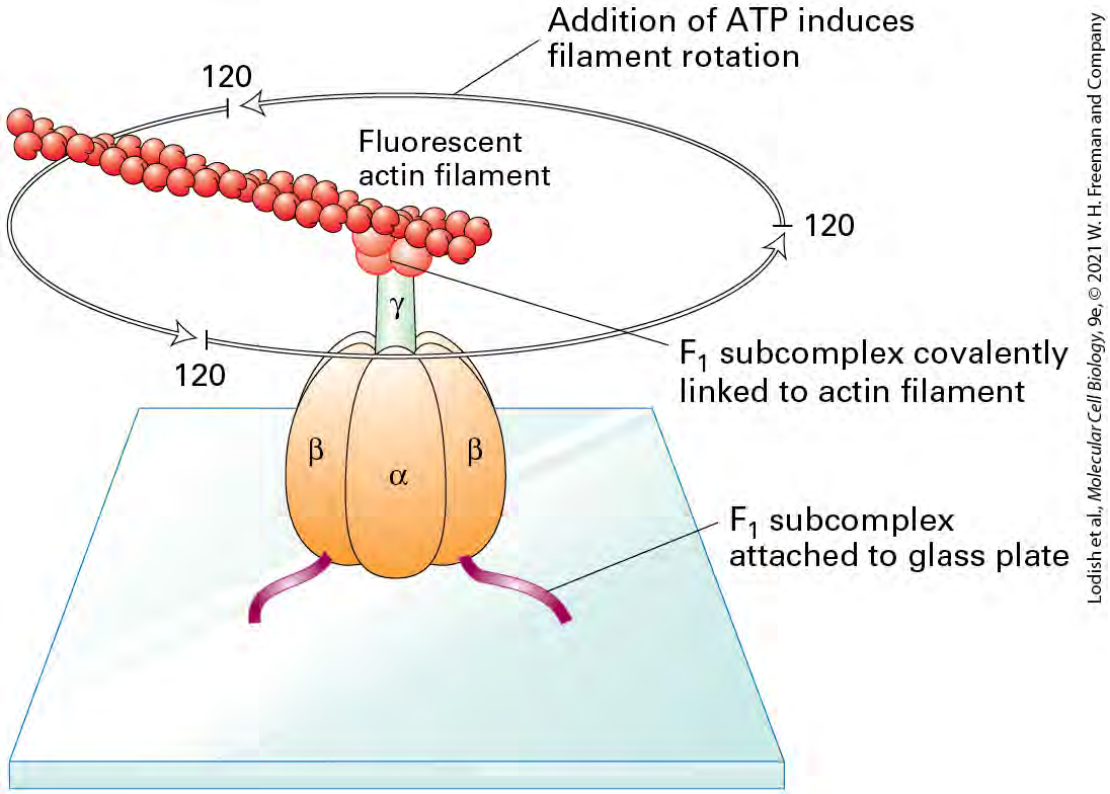
- Isolation of F_1 particle, showed that it hydrolyzed ATP – but this depends on the conditions.
- = F1 is the catalytic part of ATP synthase



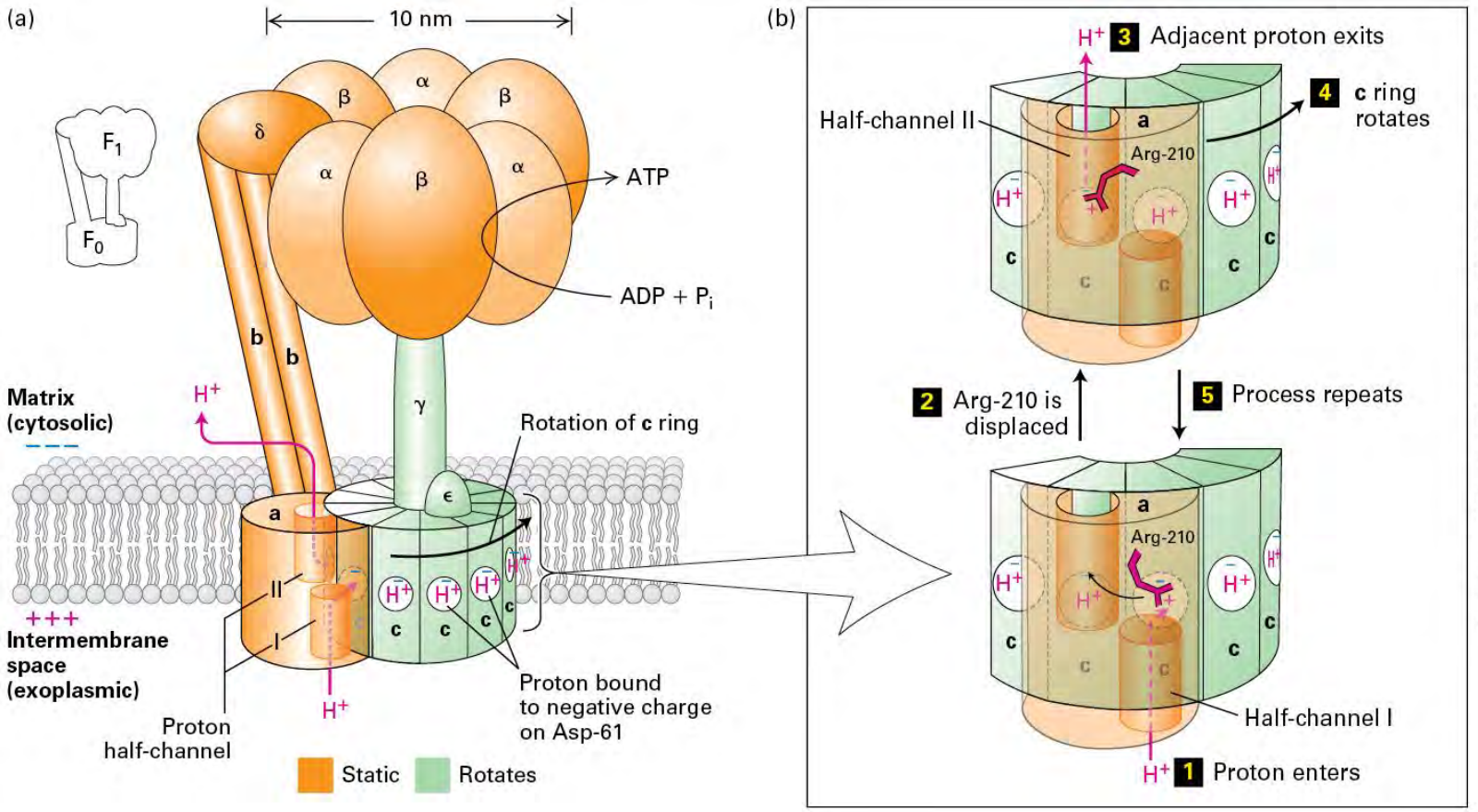
negatively stained inner membrane (matrix side)

rotational catalysis

- direct observation of rotational catalysis using purified parts of ATP synthase (F_0) + ATP
- actin “propeller” moves in 120^o steps



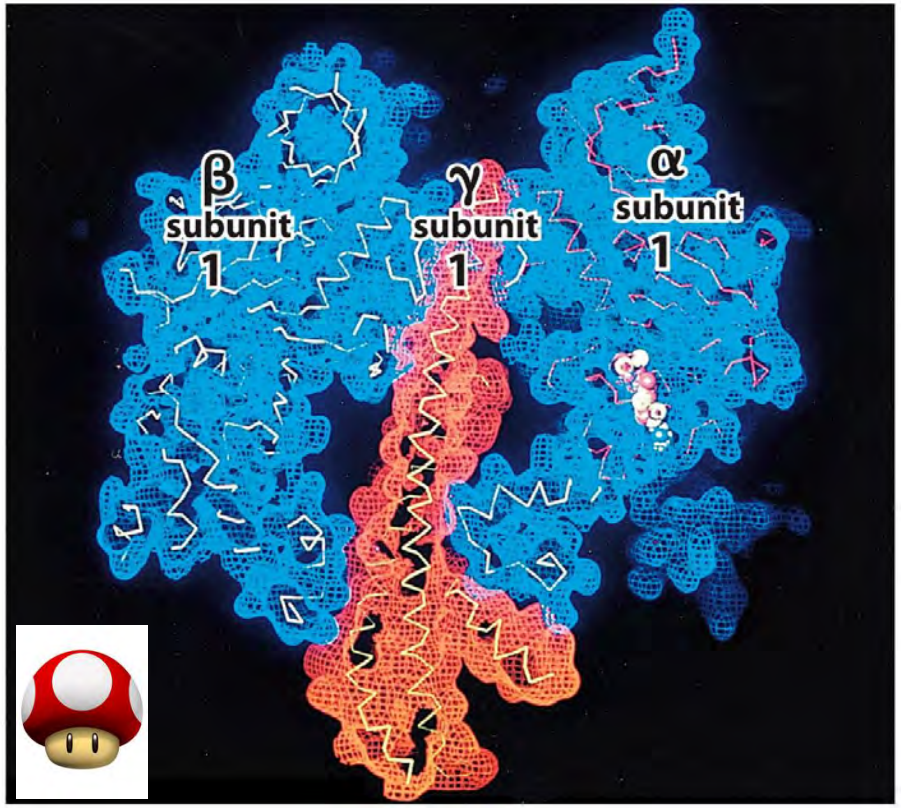
proton diffusion is coupled to rotation of the F_0 complex



Lodish et al., *Molecular Cell Biology*, 9e, © 2021 W. H. Freeman and Company

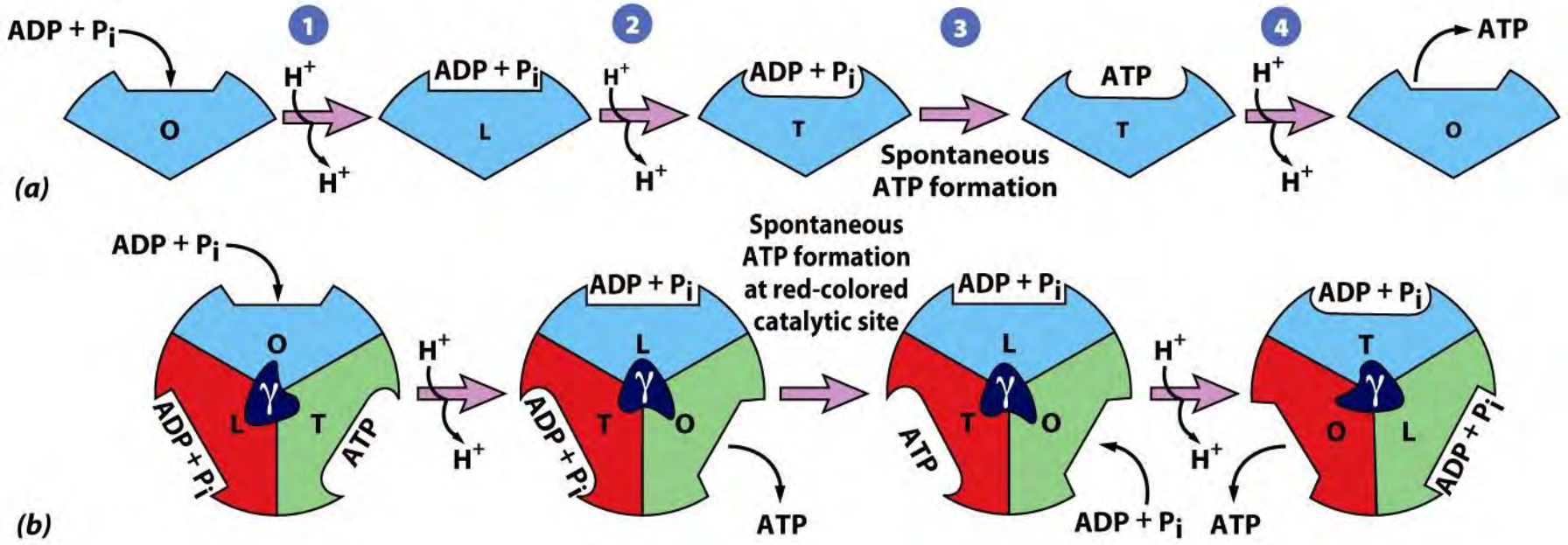
- The c subunits of the F_0 base form a ring.
- The c ring is bound to γ subunit of the stalk.
- Protons moving through membrane *rotate the ring*.
- Rotation of the ring provides twisting force that drives ATP synthesis.

conformation changes alter the binding sites



beta subunits contain the binding sites; association with the gamma subunit determines its conformation

the binding change mechanism for ATP synthesis



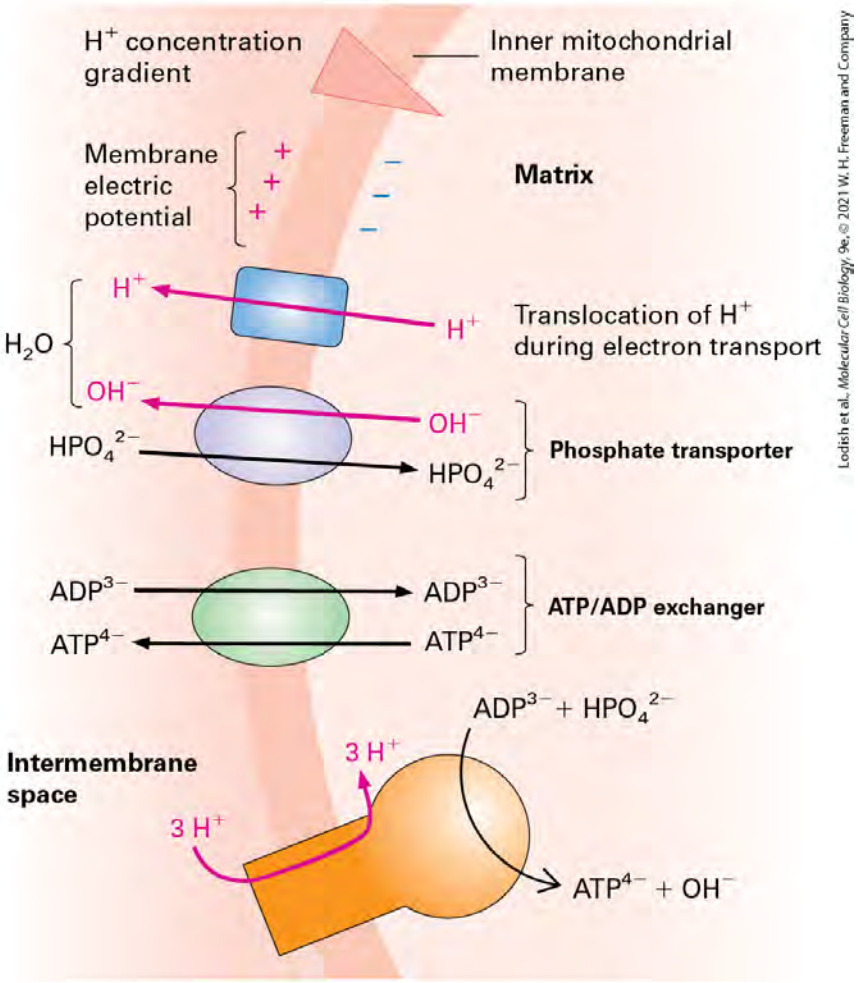
- binding site is Tight, Loose, or Open
- each active site goes through distinct conformations that have different affinities for substrates and product.
- movement of protons through ATP synthase alters the binding affinity of the active site.

ATP synthase in action

- I also recommend

https://www.youtube.com/watch?v=LQmTKxI4Wn4

the proton-motive provides energy for mitochondria as well as ATP synthesis



- the H+ gradient drives transport of ADP into and ATP out of the mitochondrion
- used for energy for Ca^{++} movement, mitochondrial fusion, protein import, etc…
- ADP (and ATP/ADP ratio) is the most important factor controlling the respiration rate.
- many factors influence the rate of respiration, but the pathways are poorly understood.

mitochondrial transporters in the membrane export ATP from matrix

- “carriers” or “translocase”
- Exchange for ADP to keep the cycle going
- import more Pi

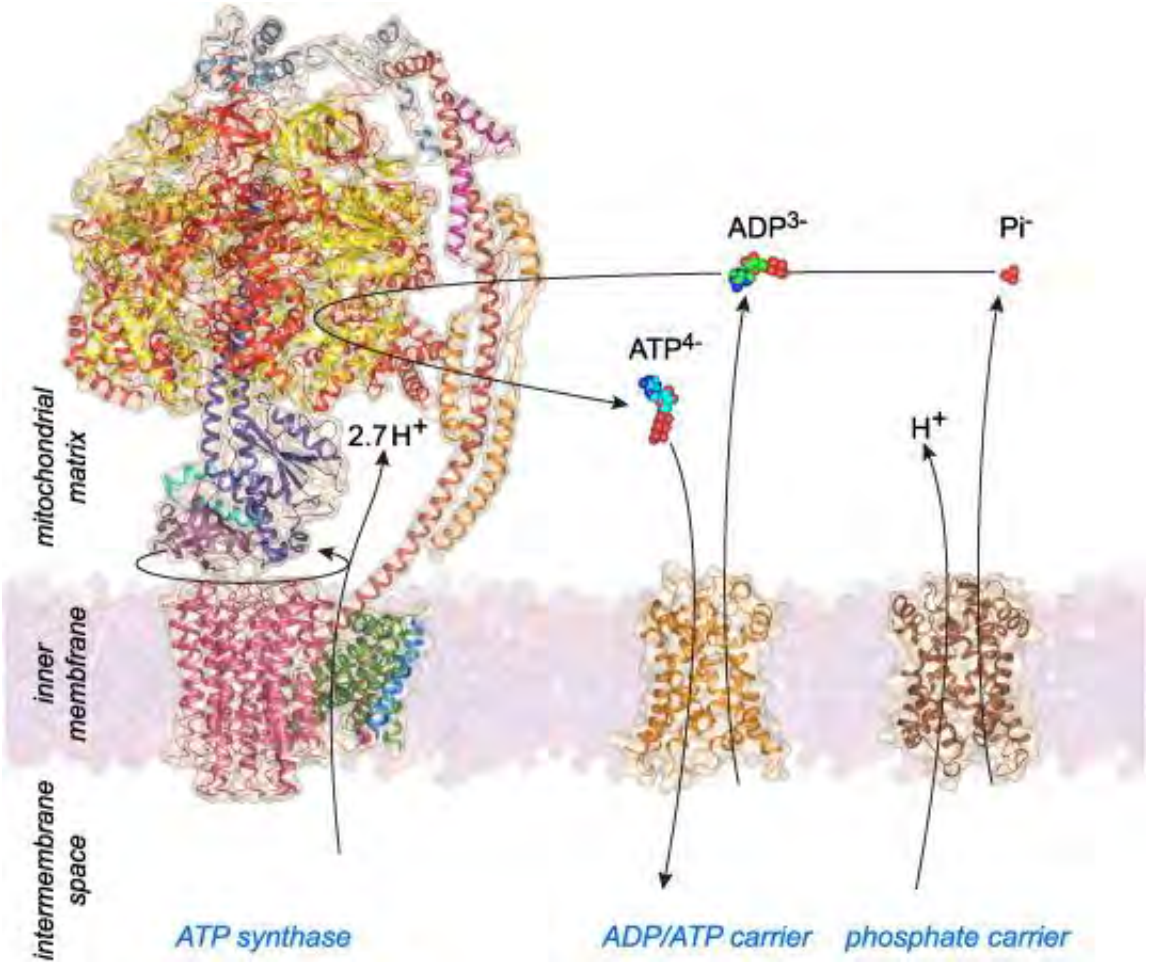


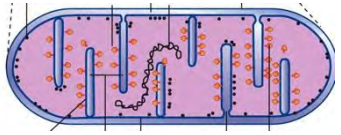
Figure from Kunji et al 2016

Warburg effect

- Cancer cells often skip oxidative phosphorylation and instead undergo high rates of glycolysis (and lactic acid fermentation).
- Why might cancer cells adopt this metabolic strategy?

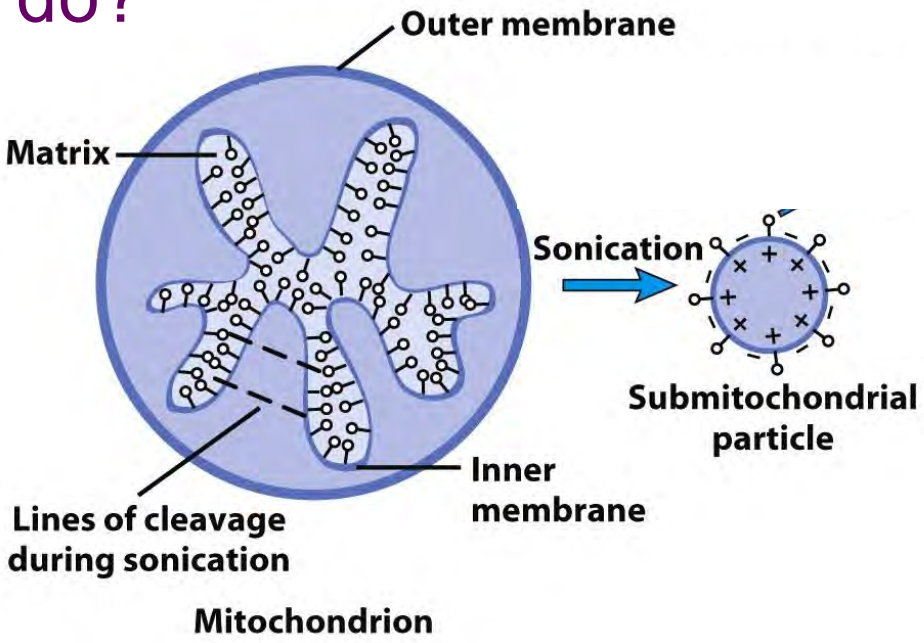
Heat transduction

- In brown fat, the inner mitochondrial membrane contains the uncoupler protein UCP1, a proton transporter that dissipates the proton-motive force into heat.
- Certain chemicals also function as uncouplers (e.g., DNP, FCCP) and have the same effect, uncoupling oxidative phosphorylation from electron transport.
- There are two distinct types of thermogenic fat cells: brown-fat and beige-fat cells.



poll 5 - experiments with purified submitochondrial particles

Which of the choices can your pure submitochondrial particle NOT do?



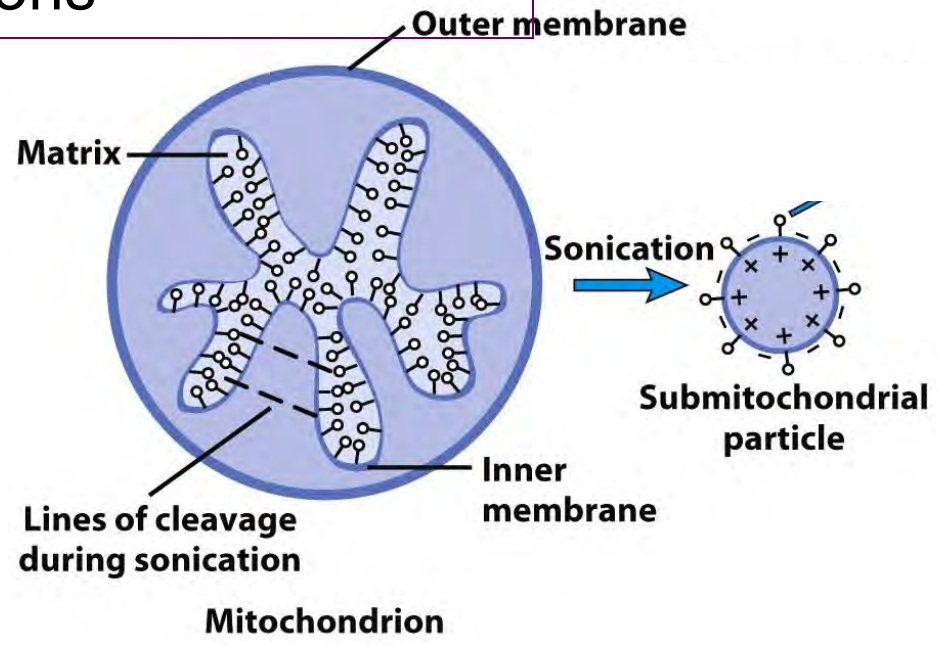
a) catalyze reactions of the TCA cycle
b) oxidize NADH
c) produce H_2O from O_2
d) generate a proton gradient
e) synthesize ATP

you next split your sample into 2 parts – one for expt 5, one for 6

poll 6 –more experiments with submitochondrial particles

you treat one sample with a **protophore**, which makes the membrane leaky to protons

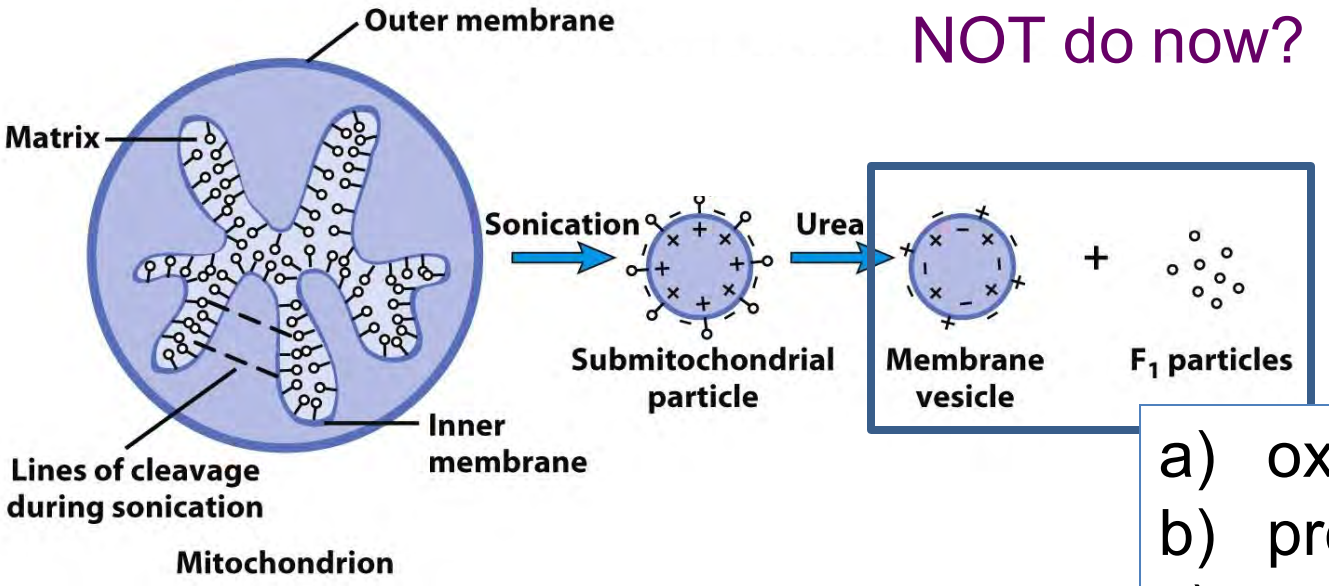
Which of the following can your pure submitochondrial particle NOT do now?



a) oxidize NADH
b) produce H_2O from O_2
c) synthesize ATP

poll 7- still more experiments with submitochondrial particles

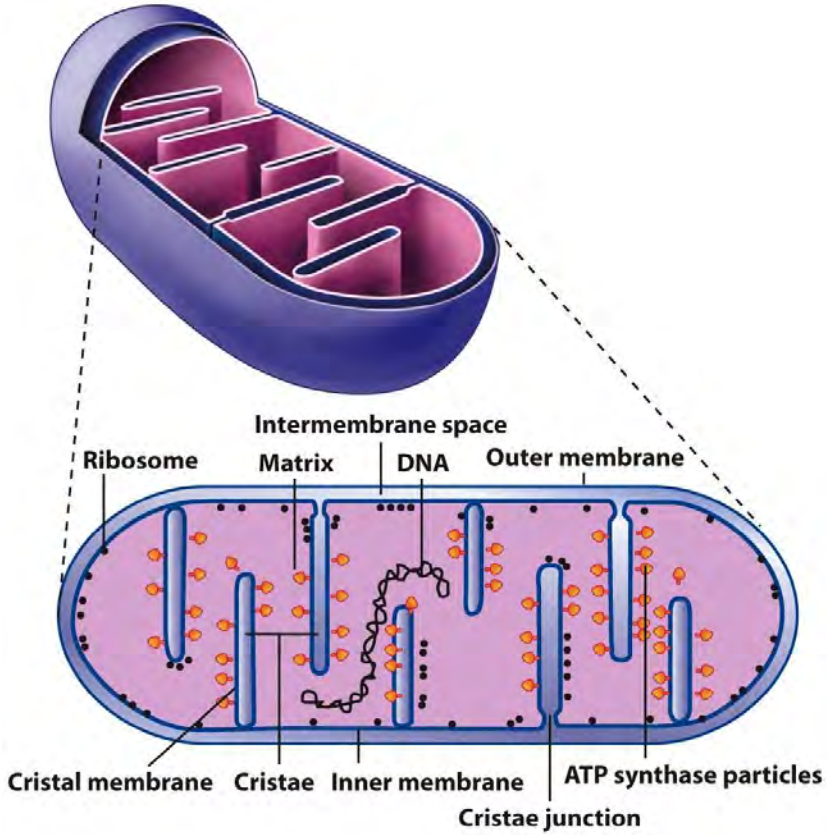
Next, you strip the particles of the F1 subunits by urea treatment…
(this expt does not have the protophore)

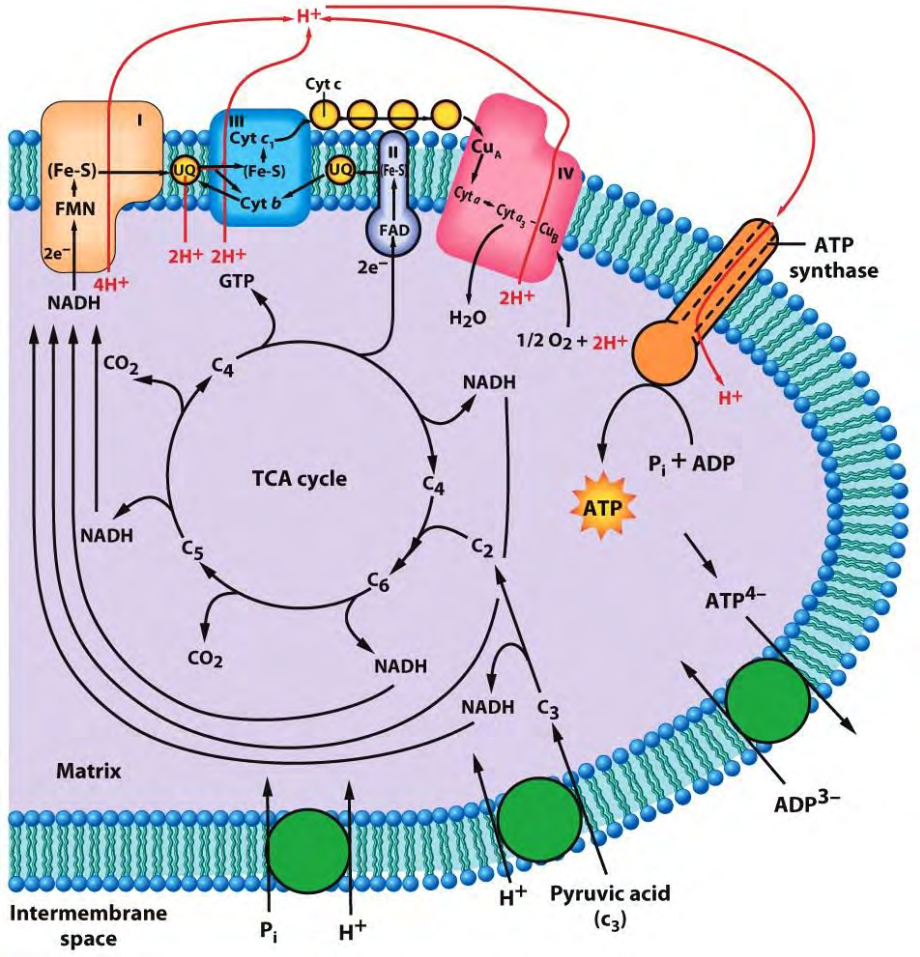


Which of the following can your pure submitochondrial particles NOT do now?

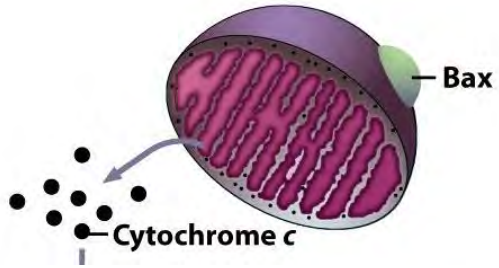
a) oxidize NADH
b) produce H_2O from O_2
c) generate a proton gradient
d) synthesize ATP
e) two of the above

summary of the major activities during aerobic respiration *in the mitochondrion*



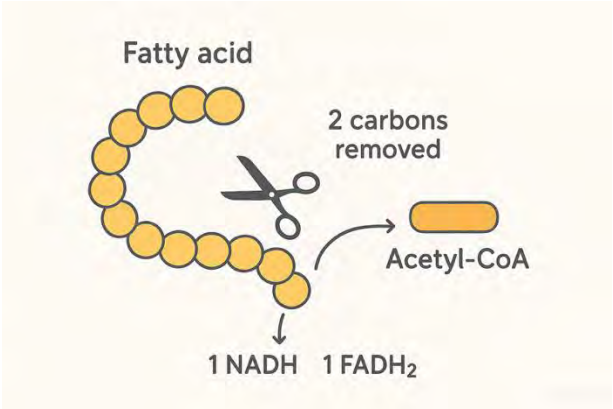


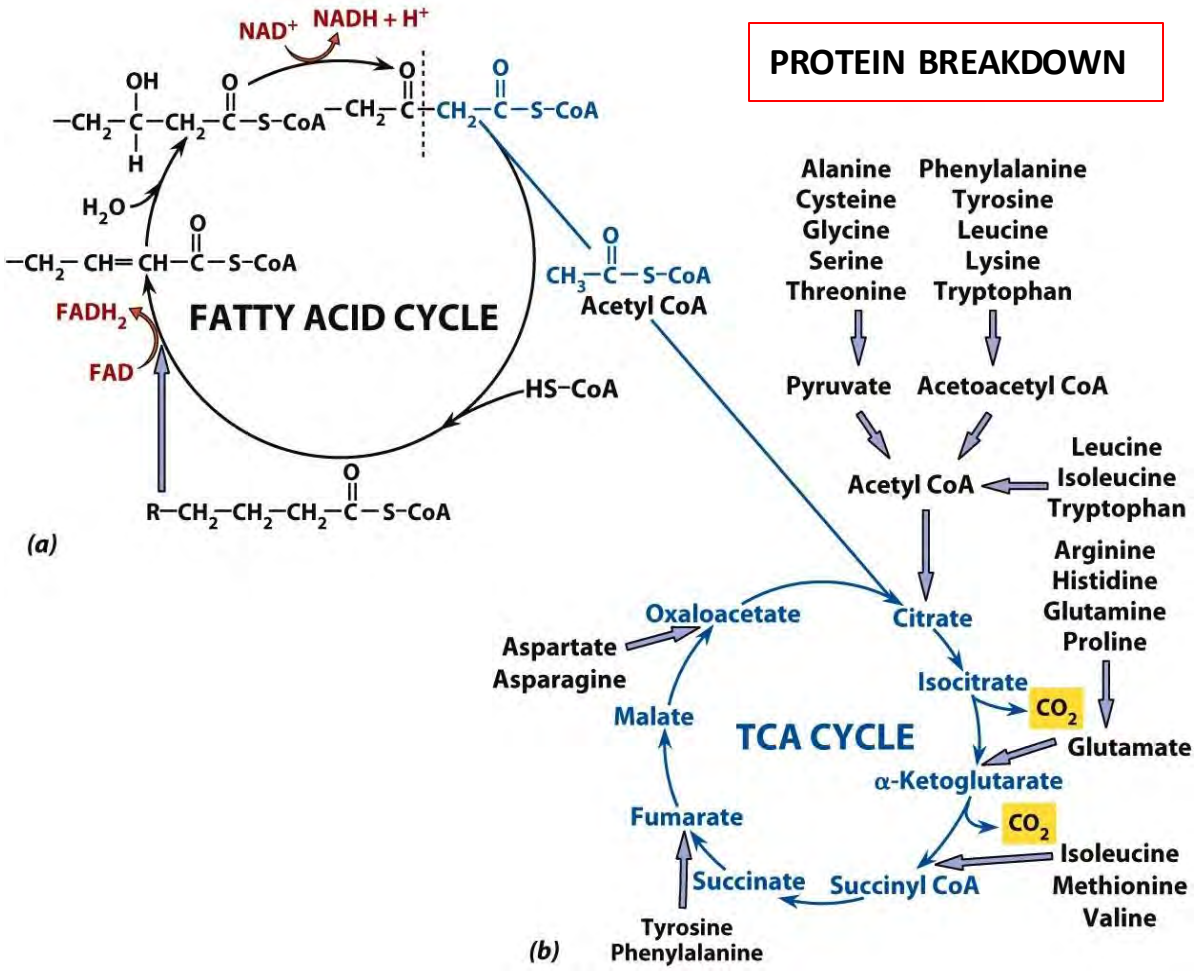
for next time



- In discussion: experiments on super mitochondria?
- Tuesday: ECM, junctions and adhesions (chapter 20)
- Achieve due Friday
- prework for discussion (after mitochondria)

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

mitochondrial matrix

Important aside- **cytochrome c** can also act as a death signal

Intrinsic cell death (apoptosis) signaling pathway