Session 8

A

Metabolism = Linked set biochemical reactions by which we obtain and use free energy (ΔG) for life

see metabolic chart

Use ΔG for:
1. Mechanical work
2. Generate [gradients] (e.g., of ions)
3. Biosynthesis

Metabolism divided into:

B

1. Catabolism ($\Delta G < 0$)
   - energy yielding pathways
2. Anabolism ($\Delta G > 0$)
   - consumption of energy and reducing equivalents to finance biosynthesis

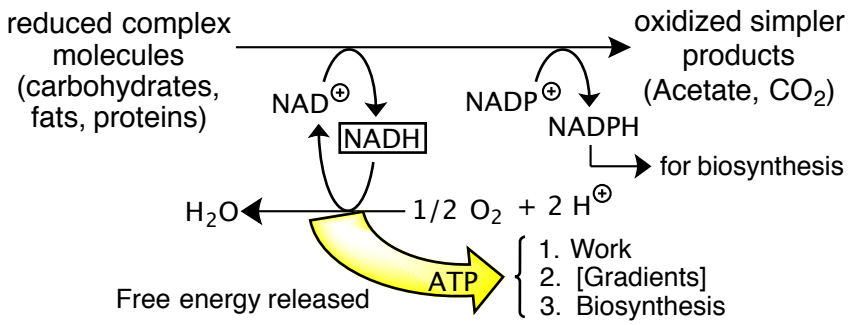
ATP

NADPH

Catabolism Paradigm

C

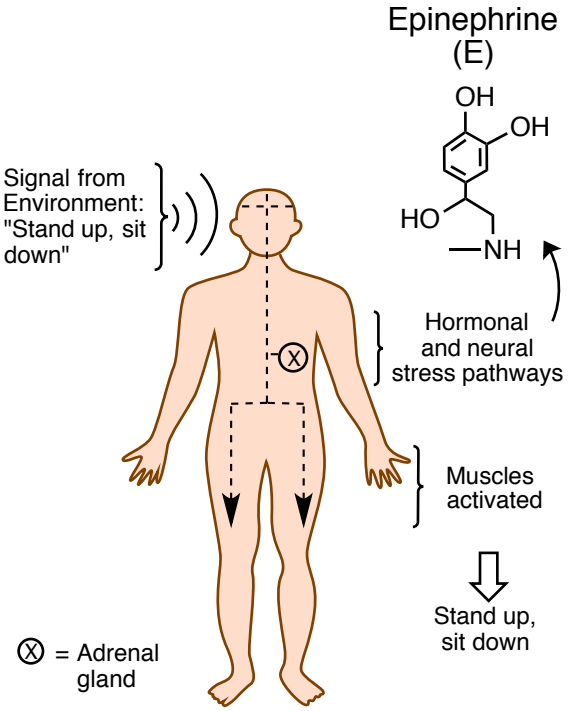
1



Physiological Scenario

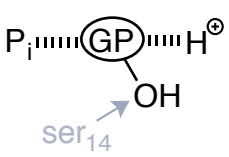
D

The professor tells a student to stand up and then sit back down. What happens in the student's body?



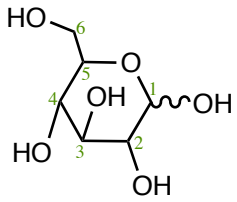
Biochemical Players

E

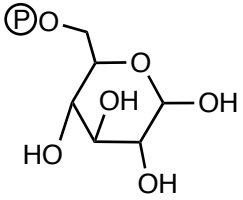


Glycogen Phosphorylase

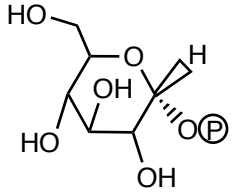
GPα = active ($Serine_{14}$ Phosphorylated)
GPβ = inactive
P_i = inorganic phosphate



Glucose (G)

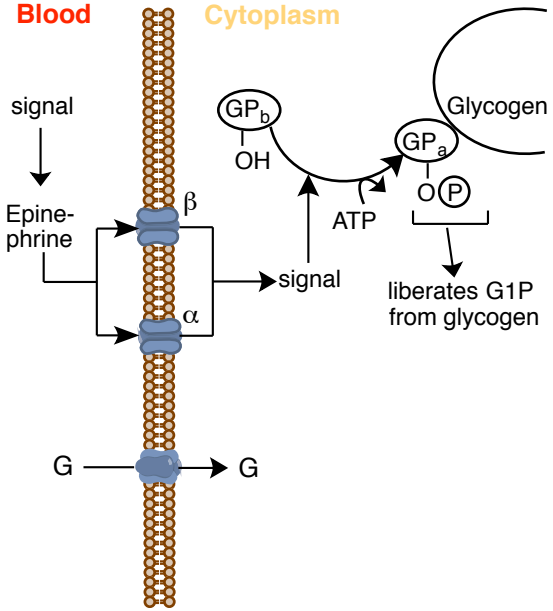


Glucose-6-Phosphate (G6P)



Glucose-1-Phosphate (G1P)

F



Signal ("stand up, sit down") causes epinephrine release that, in turn, causes the activation of (GP), which liberates G1P for metabolism.

Session 9 & 10

Carbohydrate Catabolism

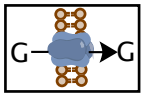
A

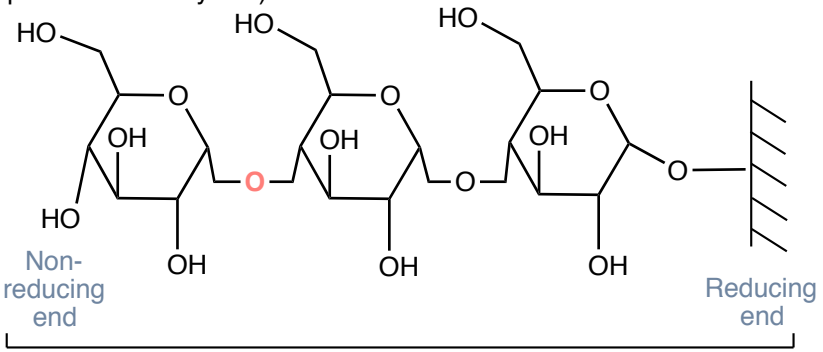
Two sources of glucose:

1.) G from blood via G transporter

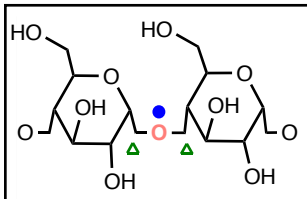
2.) G as G1P from glycogen

(animals and bacteria make glycogen, plants make amylose)

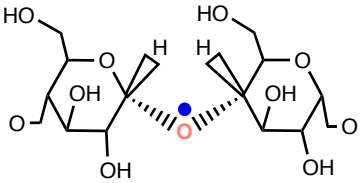




Glycogen (n units of glucose)



The △'s are not carbons. This is a convention used to denote the following stereochemistry:

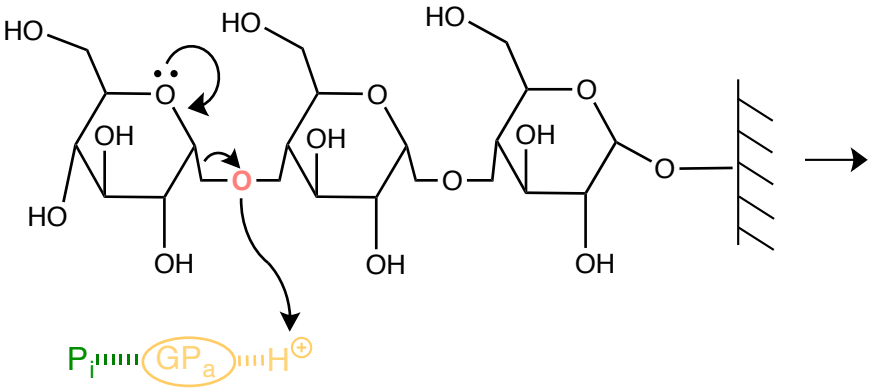


B

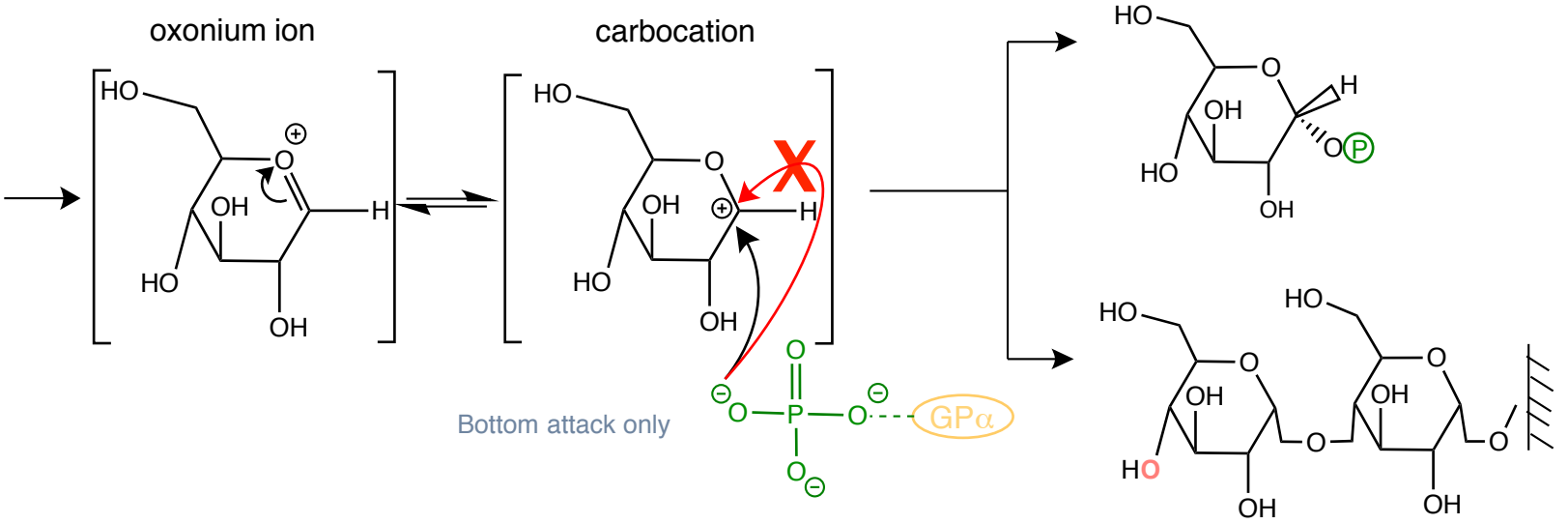
2

GP Mechanism

Glycogen = [glucose (α1➔4) glucose] with some (α1➔ 6) branches



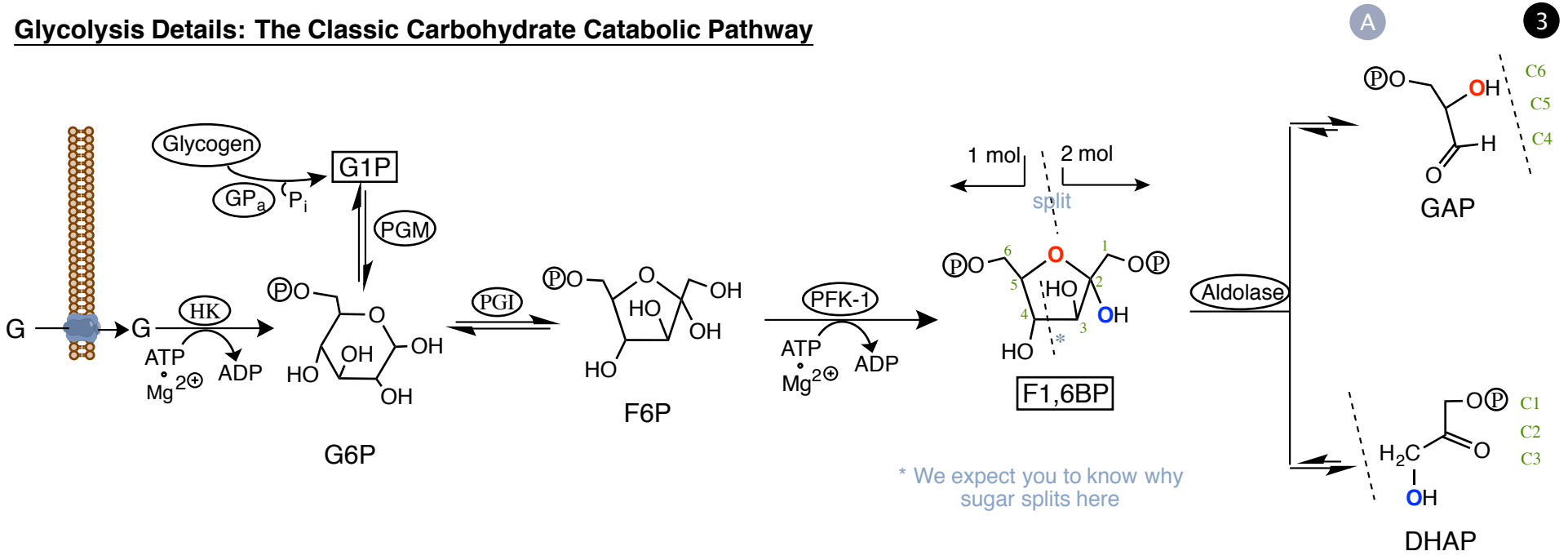
C

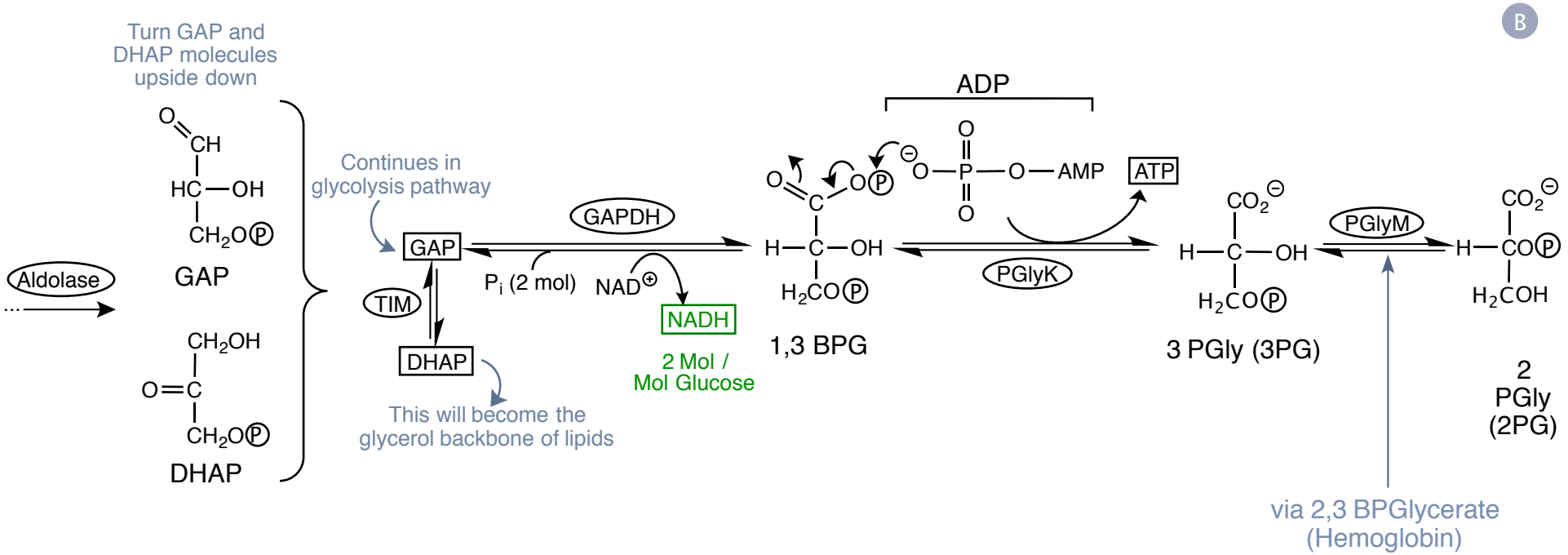


G1P

Glycogen (n-1 units of glucose)

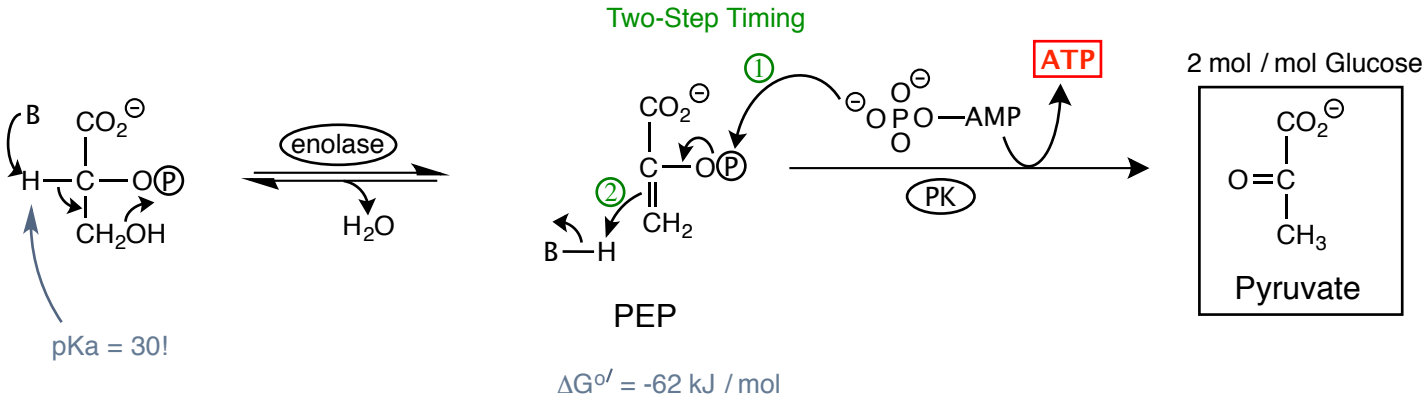
Glycolysis Details: The Classic Carbohydrate Catabolic Pathway





A

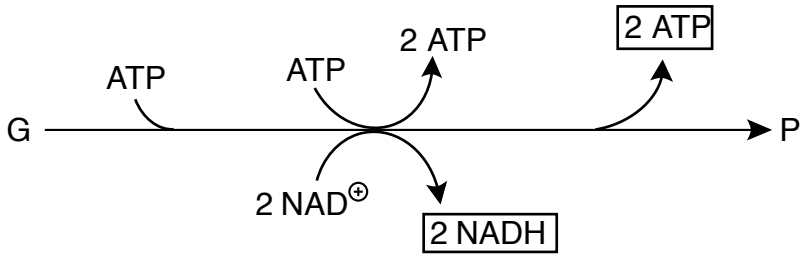
Glycolysis Details (continued)



$\Delta G^{o\prime}$ = -62 kJ / mol

B

Summary



-- note - we do not have a lot of $NAD^{\oplus}$
-- Needs to be regenerated
-- See notes on "shuttles"

Regulation

Glycolysis is regulated at the three irreversible steps:

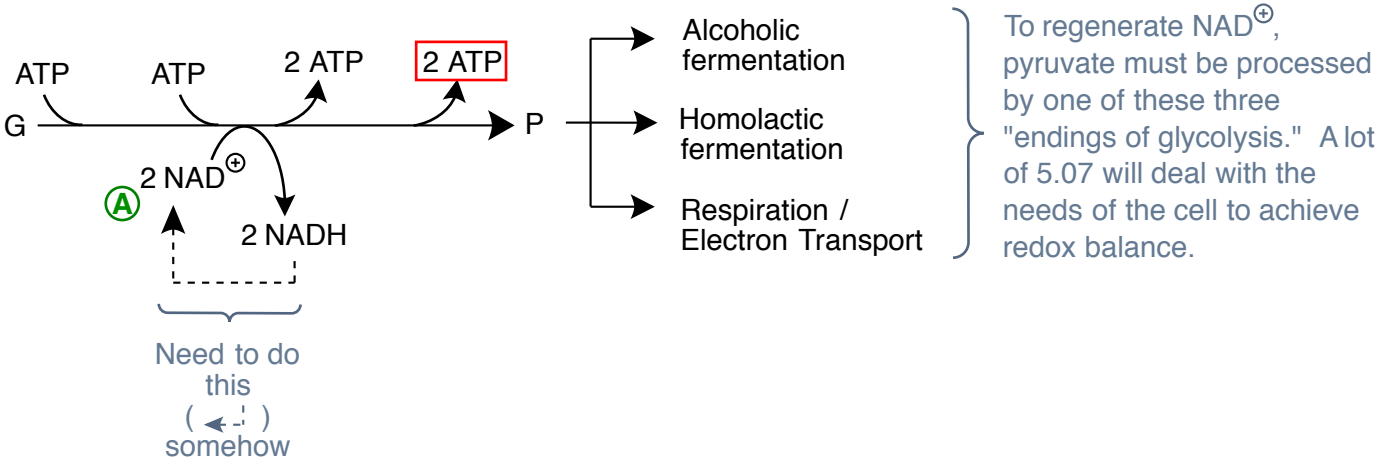
1. HK
2. PFK-1
3. PK

} control is both allosteric and covalent (enzyme activity altered by covalent modification)

4. As well as GP, which is upstream of glycolysis.

Nature's problem:

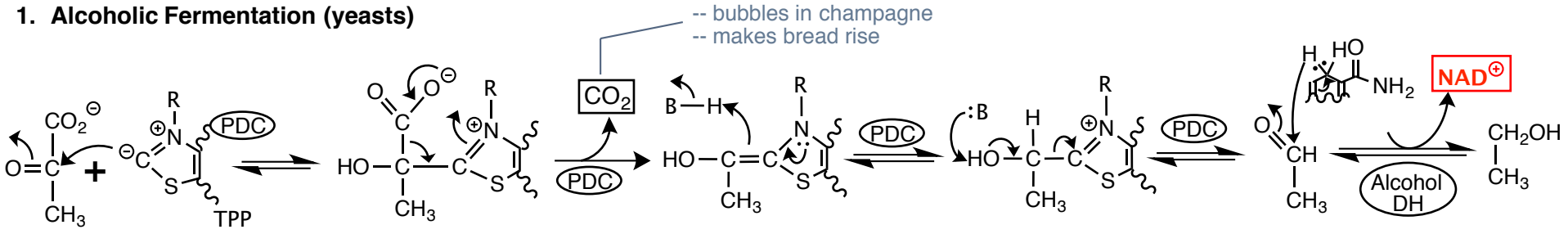
If you do glycolysis as above, you get (2) ATP but you will run out of $NAD^{\oplus}$. Must regenerate it from NADH.



Three ways to achieve redox balance AFTER glycolysis

C

1. Alcoholic Fermentation (yeasts)



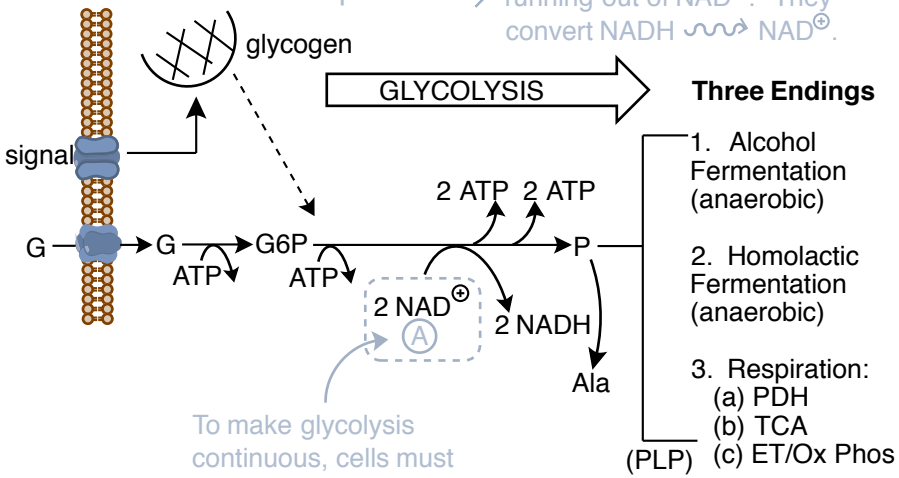
Pyruvate PDC = Pyruvate decarboxylase Acetaldehyde Ethanol

The $NAD^{\oplus}$ produced goes to (A) [previous panel] to keep overall process redox neutral.

Review

A

Important ⇒ These endings prevent cell from running out of $NAD^{\oplus}$. They convert NADH ⇝ $NAD^{\oplus}$.



To make glycolysis continuous, cells must find a way to replace the $NAD^{\oplus}$ consumed here.

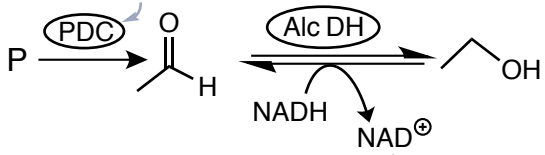
* Respiration is coupled with "shuttles" - later

B

(1.) Alcohol Fermentation (anaerobic)

yeasts

Refer to J. Stubbe notes



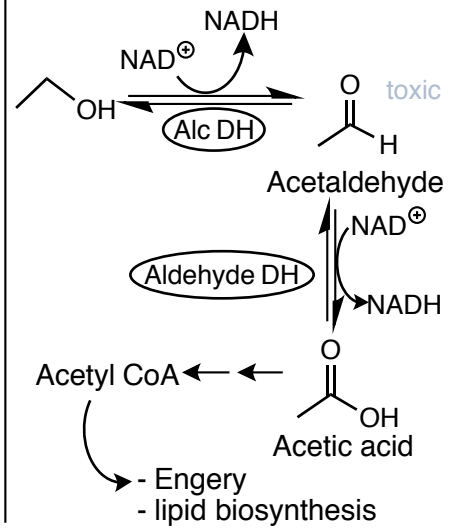
In summary to left } A

East Asians have an active (very) Alcohol Dehydrogenase, (Alc DH), but many have a relatively sluggish (Aldehyde DH). Hence they suffer from acetaldehyde toxicity (hangover) if they drink too much.

C 6

Relevant Digression

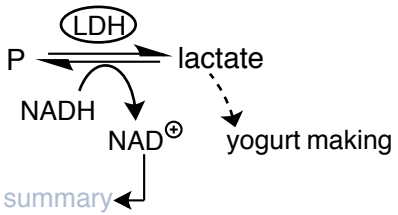
Metabolism of ethanol in mammals (and yeasts)



2. Homolactic Fermentation (anaerobic)

D

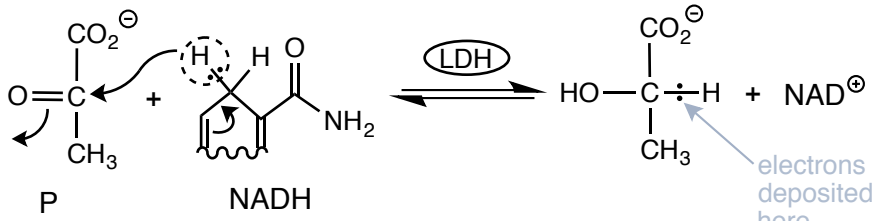
lactic acid bacteria; animals



A in summary (above)

- While fermentations are anaerobic, they can occur in the presence of O_2 - they just do not use O_2

- They are electronically balanced (redox neutral)



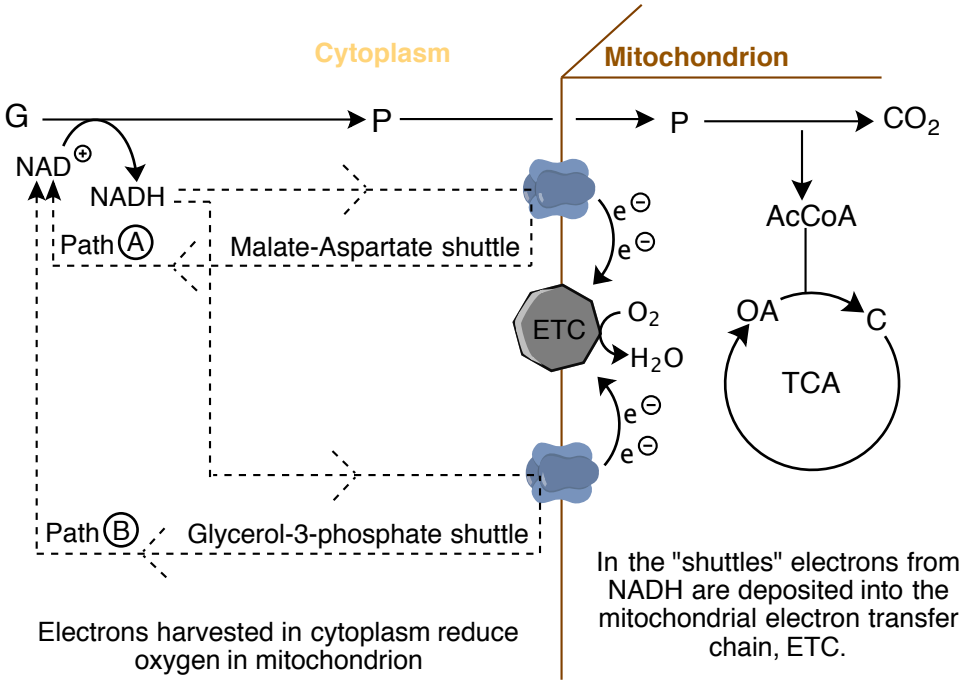
There is a similarity to path B to right

Animals working hard (anaerobically) do lactic acid fermentation. But the lactate from muscles can be re-built into glucose by process of gluconeogenesis in the liver. More on this later.

3. Respiration

E

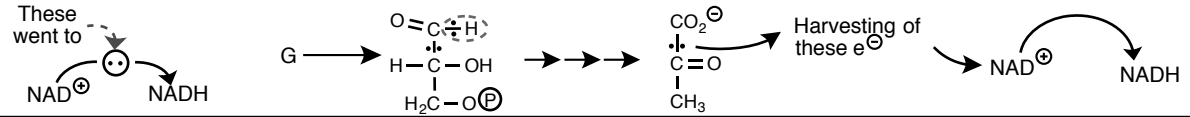
shuttles will be discussed after we do TCA cycle.



Electrons harvested in cytoplasm reduce oxygen in mitochondrion

In the "shuttles" electrons from NADH are deposited into the mitochondrial electron transfer chain, ETC.

Think retro-synthetically



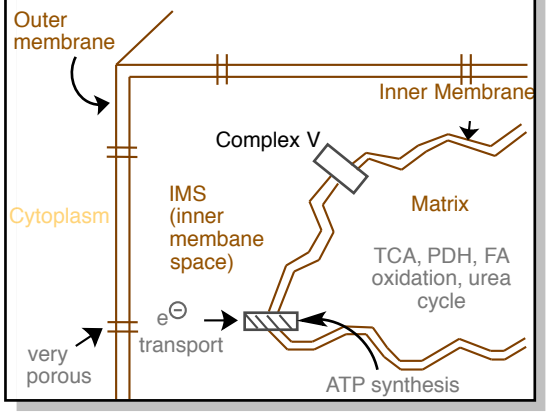
A Respiration: Oxidative metabolism of all metabolic fuels (carbohyrates, fats) via Acetyl CoA

-- Mitochondrial reactions
-- Require O_2 (or another $e^{\ominus}$ acceptor)
-- We can metabolize carbohydrates anaerobically or aerobically
-- We can only metabolize lipids aerobically

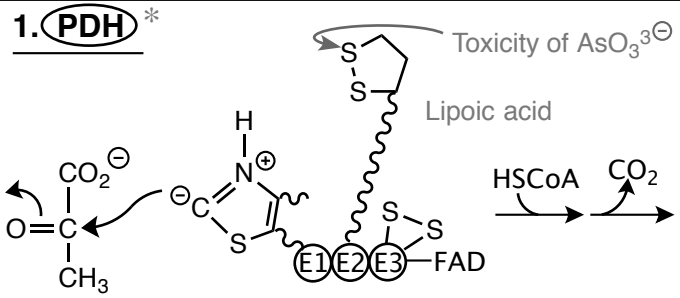
Stages:

1. PDH: P ⟶ AcCoA + NADH
2. TCA: AcCoA ⟶ CO_2 + ATP/GTP + NADH + $FADH_2$
3. Electron transport and oxidative phosphorylation } Oxidation of $FADH_2$ and NADH ⇨ Energy ⇨ ATP

Introducing: The Mitochondrion



B 1. PDH *

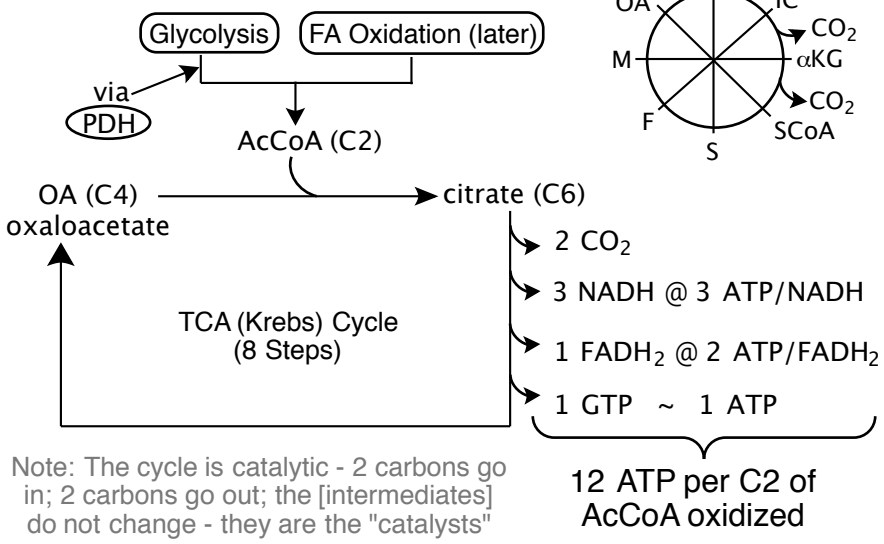


* Reaction mechanism is strikingly similar to αKGDH (below).

Basically, the pair of electrons move left to right across PDH (E1 E2 E3) and reduce FAD ⟶ $FADH_2$. Then, in a redox-challenged last step, $FADH_2$ gives its electrons to $NAD^{\oplus}$ to yield NADH. The NADH is oxidized by the electron transfer chain (later.)

C 2. TCA Cycle - Overview

-- receives Acetyl CoA from PDH



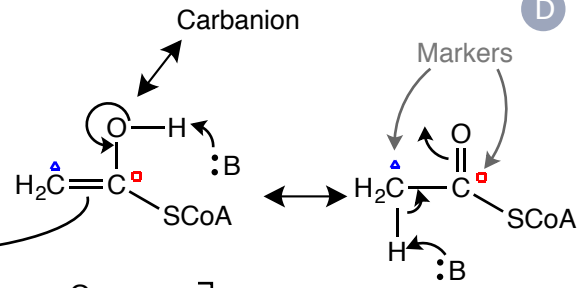
Note: The cycle is catalytic - 2 carbons go in; 2 carbons go out; the [intermediates] do not change - they are the "catalysts"

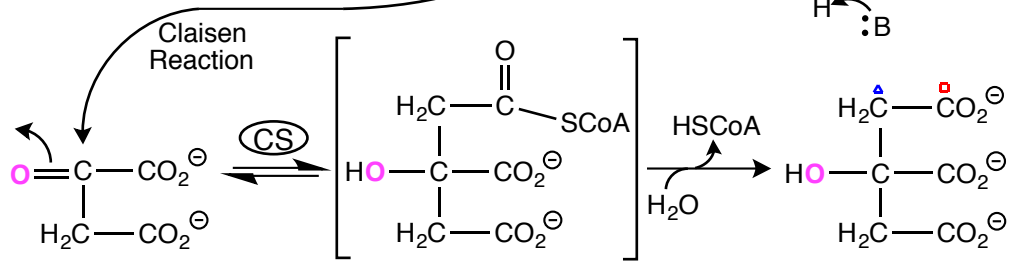
The Chemistry

Citrate Synthase

CS is stereospecific. AcCoA attacks from top (S_i) face only. More detail on this later.

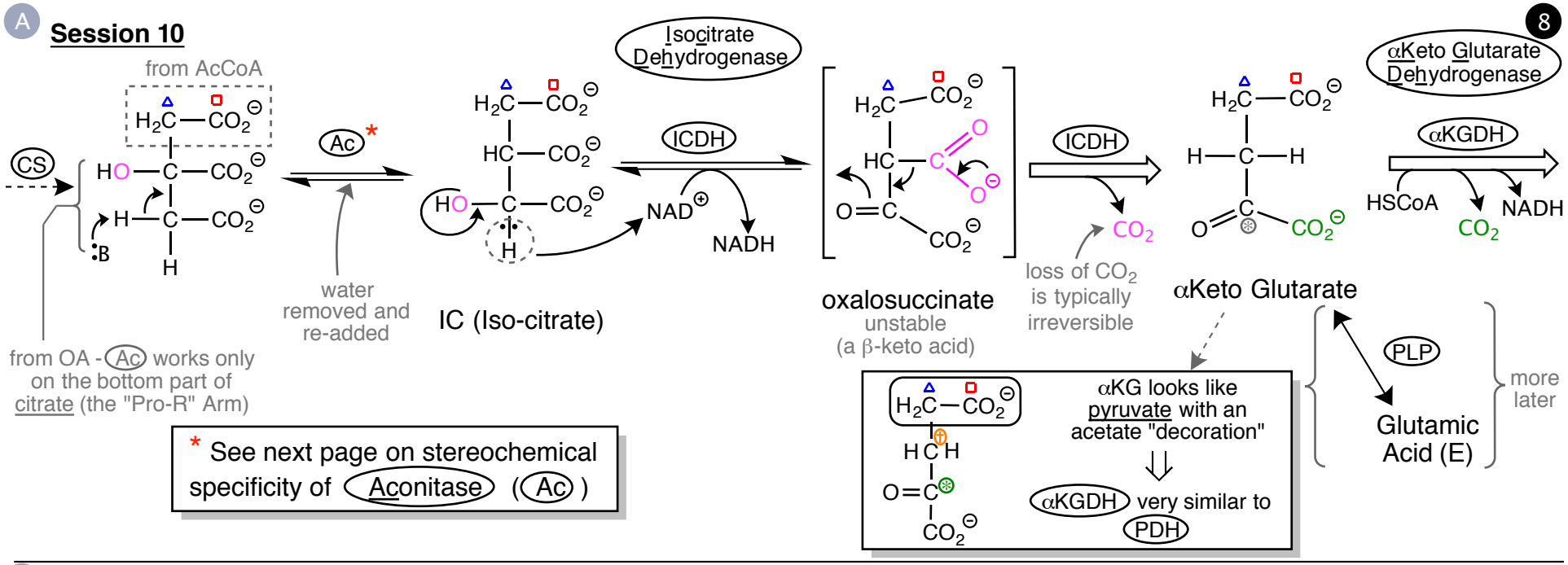
D



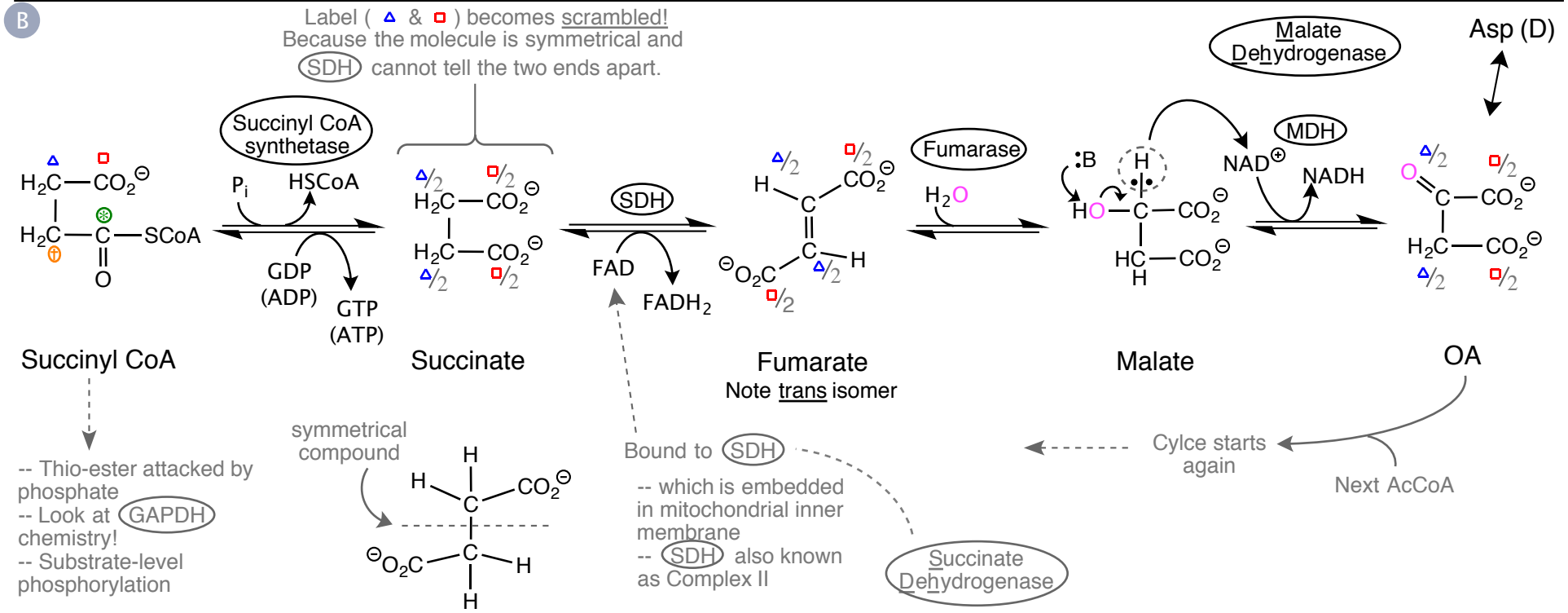


A

Session 10



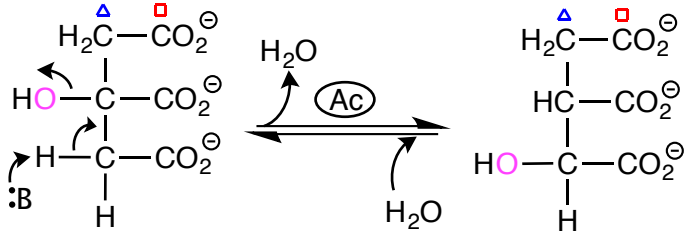
B



Detail on Stereochemical Specificity of (Aconitase)

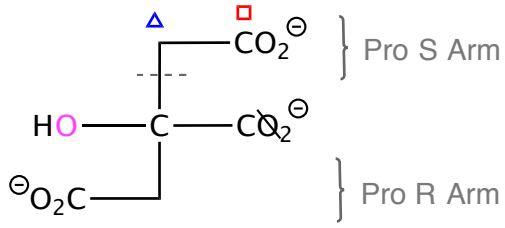
A

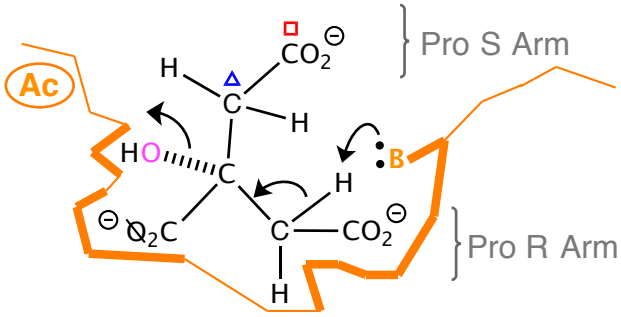
-- The hydroxyl group always moves to the ProR arm and never to the ProS, even though they are chemically identical, because (Ac) can distinguish the two arms (based on pro-chirality).



C (Citrate) IC (Iso-citrate)

B





-- The stereochemistry defined by (CS) generates only one isomer - where the -OH, $C\mathbb{O}_2^{\ominus}$ and $\wedge CO_2^{\ominus}$ fit in a specific way in three docking locations on (Ac).

-- The -OH, $C\mathbb{O}_2^{\ominus}$ and $\wedge CO_2^{\ominus}$ make contact with (Ac) at three sites.

Session 11 - Shuttles and Redox Neutrality

Now that we have done glycolysis, PDH and TCA - we can see how shuttles allow the cytoplasm to stay in redox balance.

Path of electrons →

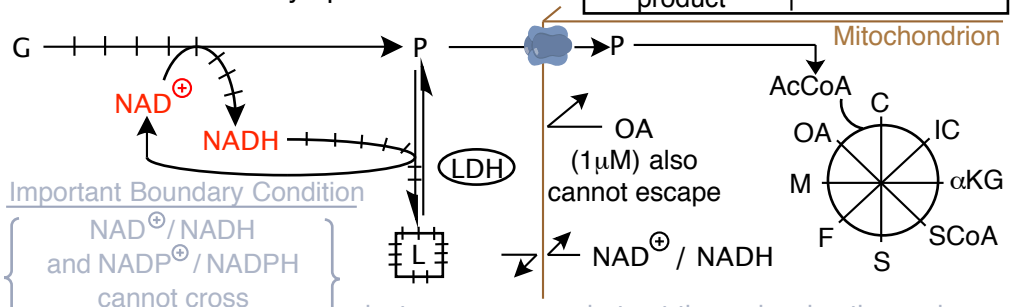
1. Lactate Dehydrogenase (LDH)

-- "Lactic Acid Fermentation"

-- We did this redox loop earlier, which was confined to the cytoplasm

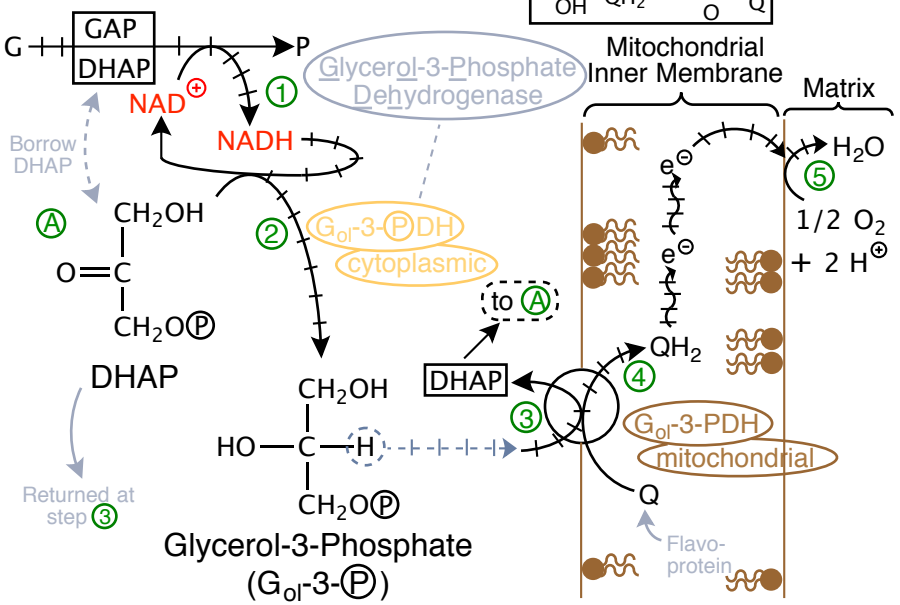
P = Ketone (C=O) / Alcohol (HC–OH, more reduced)

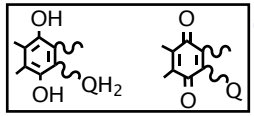
Lactate = "waste product"



2. Glycerol-3-Phosphate Shuttle

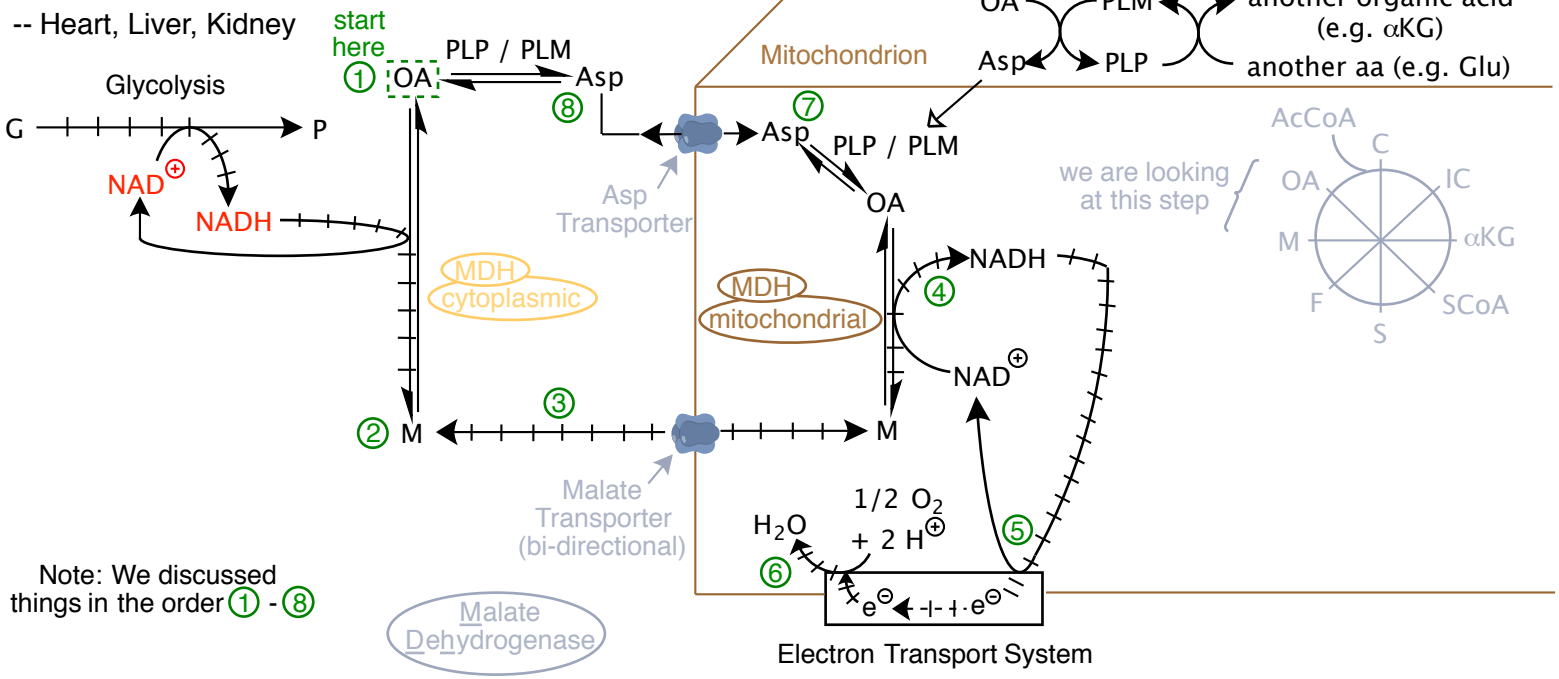
-- Brain and Skeletal Muscle



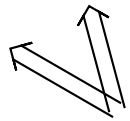


3. Malate-Aspartate Shuttle

-- Heart, Liver, Kidney



Note: We discussed things in the order ① - ⑧



So, all of this was done to achieve redox neutrality in glycolysis.

We needed to convert OA in mitochondrion (step 7) to Asp (PLP reaction) because OA cannot escape the mitochondrion.

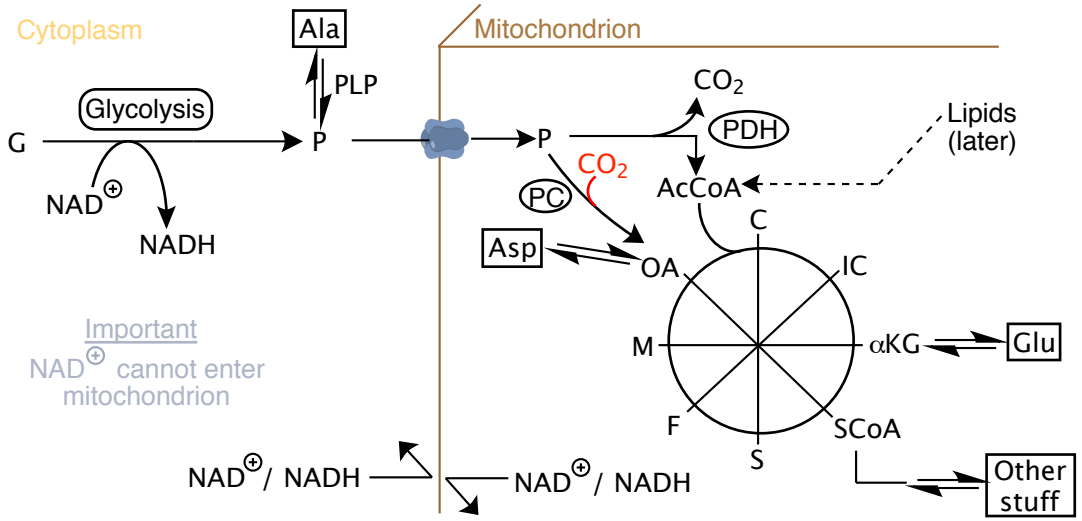
Anapleurotic Pathways

-- We know three Pathways -- look at Interactions

-- Definition: anapleurotic ≡ "filling up"

-- Pathways that maintain catalytic amounts of TCA cycle intermediates

- Today we'll add more detail to this network
- Start with problem of how different life forms avoid running out of cytoplasmic $NAD^{\oplus}$



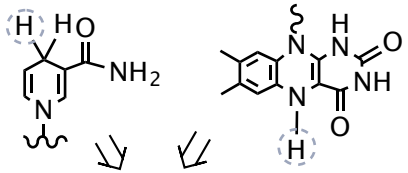
Session 12 - Back to the stages of Respiration

- ✔ 1.) PDH
- ✔ 2.) TCA
- 3.) ET/Ox Phos

NOW

ET / Ox Phos (Oxidative Phosphorylation)

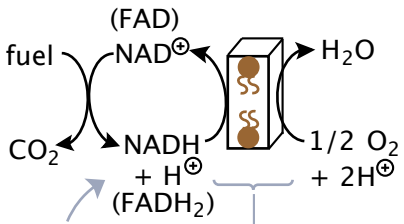
-- We want to convert the electron transfer potential of NADH and $FADH_2$ into the phosphate transfer potential of ATP



$\Delta G^{o\prime} \sim -32$ kJ/mol

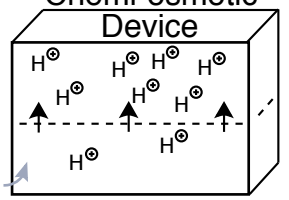
Outline:

1.) The big picture
2.) Mobile $e^{\ominus}$ carriers
3.) Integral Membrane Proteins
4.) Q-cycle (and other proton pumps)
5.) ATP Synthase



a.) Mitochondrial Inner Membrane (eukaryotic)
b.) Plasma Membrane (prokaryotic)

Oxidation liberates free energy ⟹ ΔG



Powers Pumps

⇓

Proton Gradient

This is like damming a lake

⇓

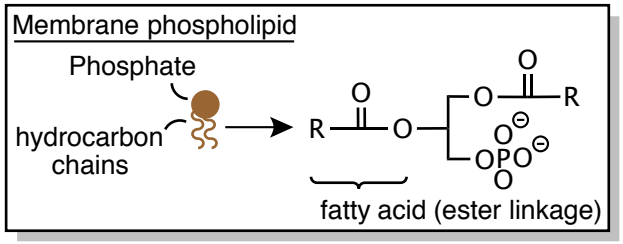
Release Gradient

Power Plant

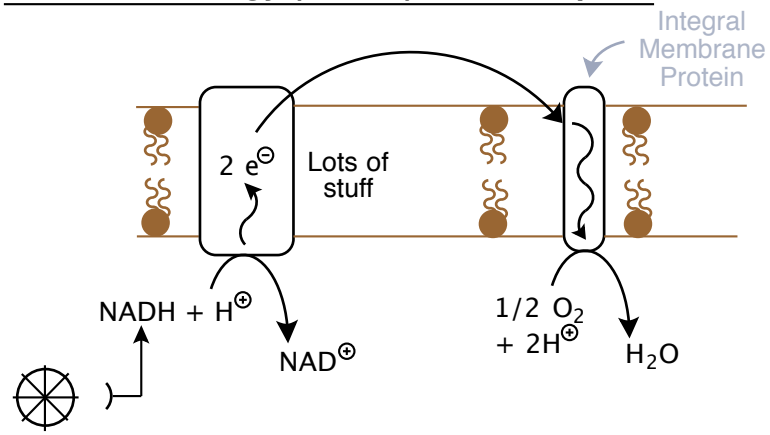
⇓

Use energy for:

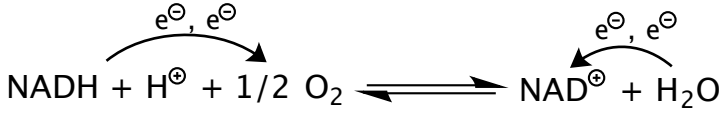
1.) ATP synthesis
2.) Heat Generation
3.) Movement (flagellar motor)



How much Energy (or ATP) can we expect?



Overall Reaction:



In which direction is this reaction favorable? (i.e., $\Delta G < 0$)

To determine the direction in which Rxn is favorable, write the half reactions in the direction of reduction

$NAD^{\oplus} + 2H^{\oplus} + 2e^{\ominus} \longrightarrow NADH + H^{\oplus}$ $\quad E^{o\prime} = -0.32$ V

$1/2\ O_2 + 2H^{\oplus} + 2e^{\ominus} \longrightarrow H_2O$ $\quad E^{o\prime} = +0.82$ V

Use a variant of the Nernst Equation:

$\Delta G^{o\prime} = -n\ F\ \Delta E^{o\prime}$

96.4 kJ / mol * V

no. electrons transferred

$\Delta E^{o\prime} = (E^{o\prime}_{e^{\ominus}\ \text{acceptor}} - E^{o\prime}_{e^{\ominus}\ \text{donor}})$ as the overall equation is written

$\Delta E^{o\prime} = (+0.82 - (-0.32))$

$\Delta E^{o\prime} = 1.14$ V

$\Delta G^{o\prime} = -2\ (96.4\ \text{kJ / mol * V})\ (1.14\ \text{V})$

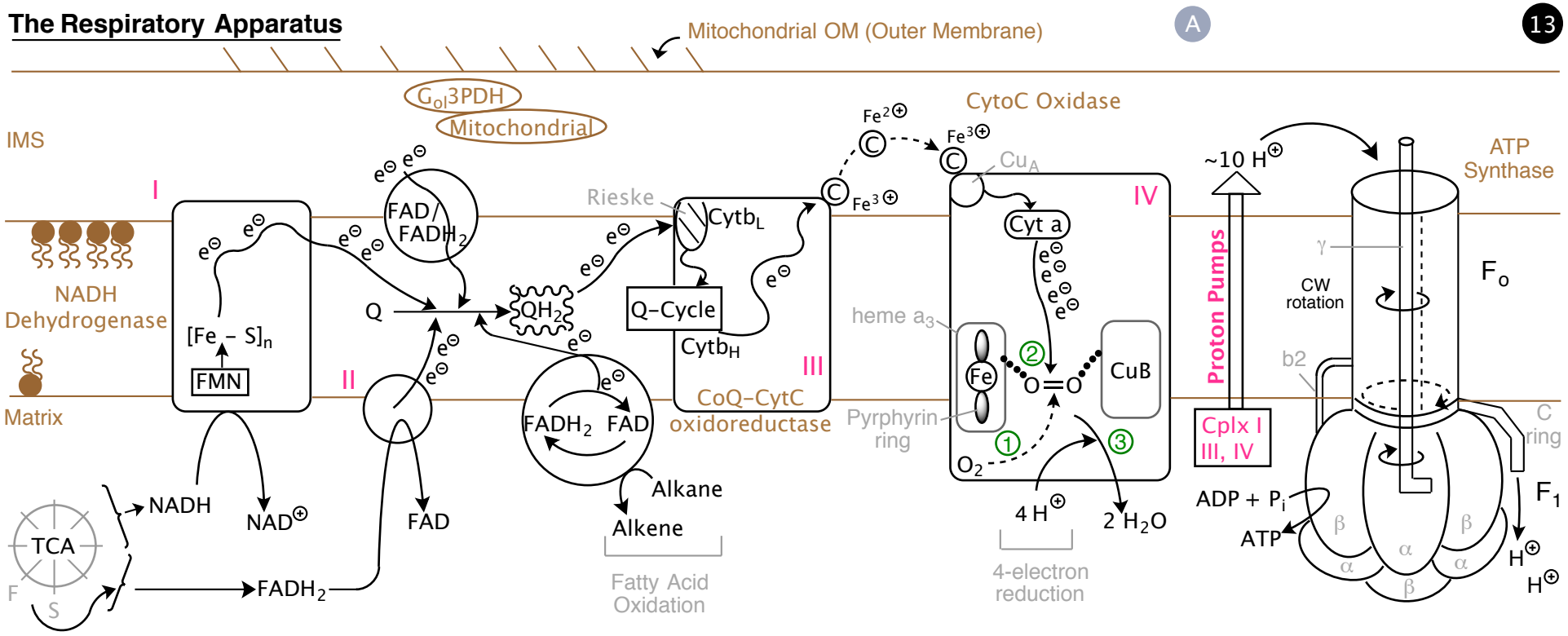
$\Delta G^{o\prime} = -220$ kJ / mol ⟹ Reaction favorable in direction written

How much ATP is this?

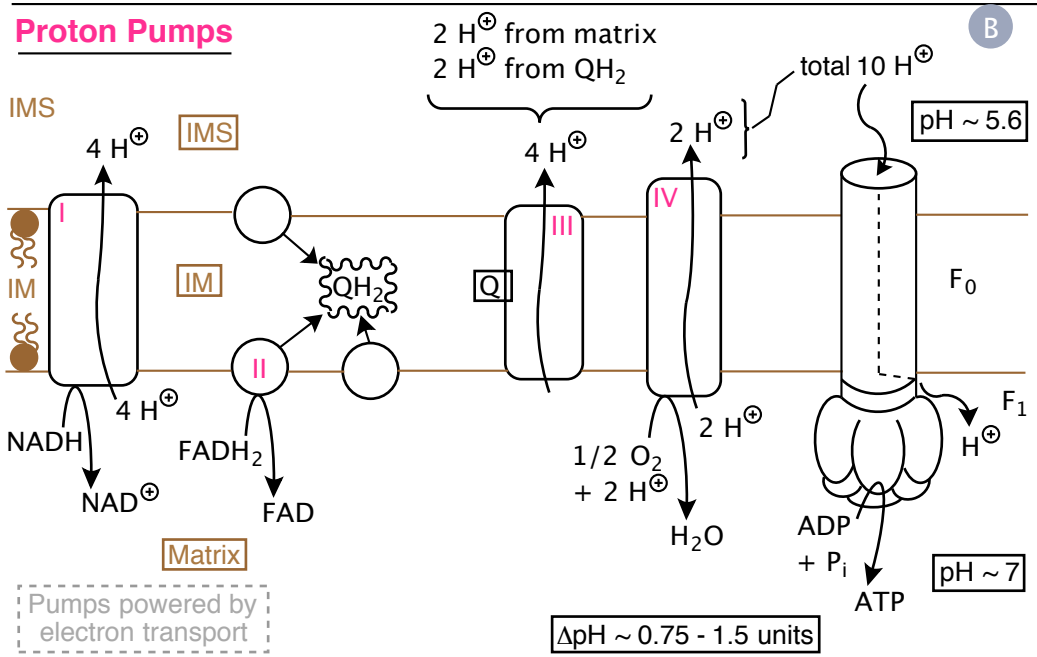
$\frac{220}{32} \cong 7.4$ ATP

In reality, get 2.5 – 3 ATP (remaining ΔG goes to heat)

The Respiratory Apparatus



Proton Pumps



Summary Points

C

-- NADH ⟶ oxidation pumps 10 H^+ ⟶ 3 ATP (~22 kJ/proton)

-- $FADH_2$ ⟶ oxidation pumps 6 H^+ ⟶ 2 ATP

-- System is reversible [ATP ⟶ ADP + P_i pumps H^+ into IMS]

-- In Complex IV (Cplx IV), the arrival of e^- reduces Fe^{3+} and Cu^{2+} ⟶ O_2 binding conformationally allowed

-- 4 e^- reduction of O_2 ⟶ 2 H_2O

Chemi-Osmotic Hypothesis for Synthesis of ATP (P. Mitchell):

D

-- Energy of e^- transport is conserved via the pumping of H^+ - creating an electro-chemical (change + chemical) gradient

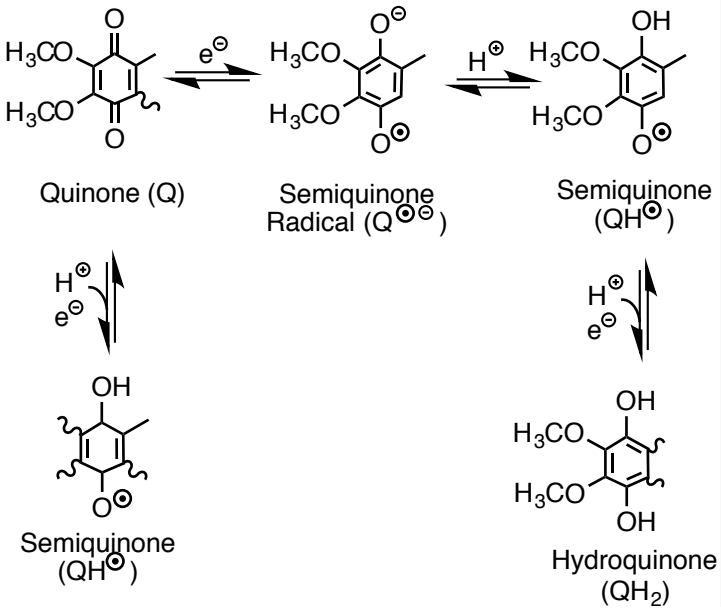
-- Use the stored electro-chemical potential to ADP + P_i ⟶ ATP (otherwise endergonic)

Session 13 - The Q-Cycle - A Proton Pump

A

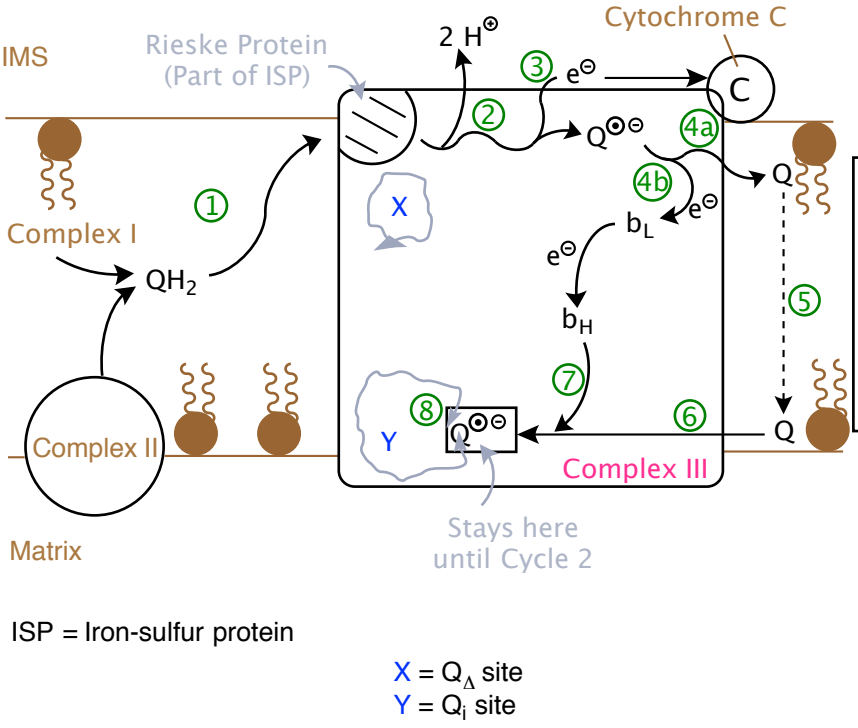
-- Works by a redox-loop mechanism

Players



Cycle 1

B



ISP = Iron-sulfur protein

X = Q_Δ site
Y = Q_i site

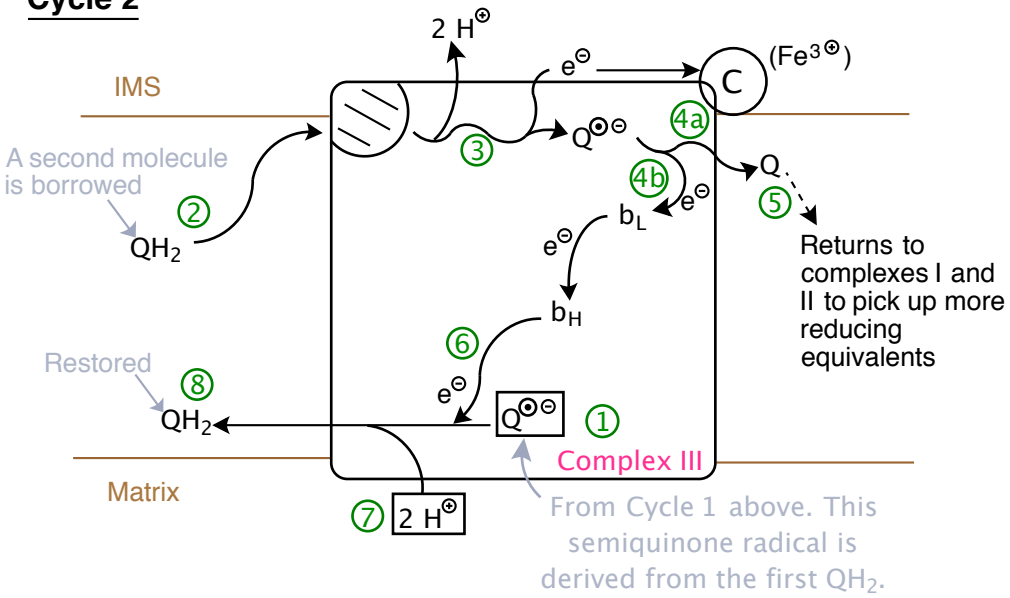
net 1st half-reaction

-- Two $H^{\oplus}$ translocated

-- One CytoC-($Fe^{3\oplus}$) reduced

Cycle 2

C



net 2nd half-reaction

-- Another two $H^{\oplus}$ translocated (Step ③)

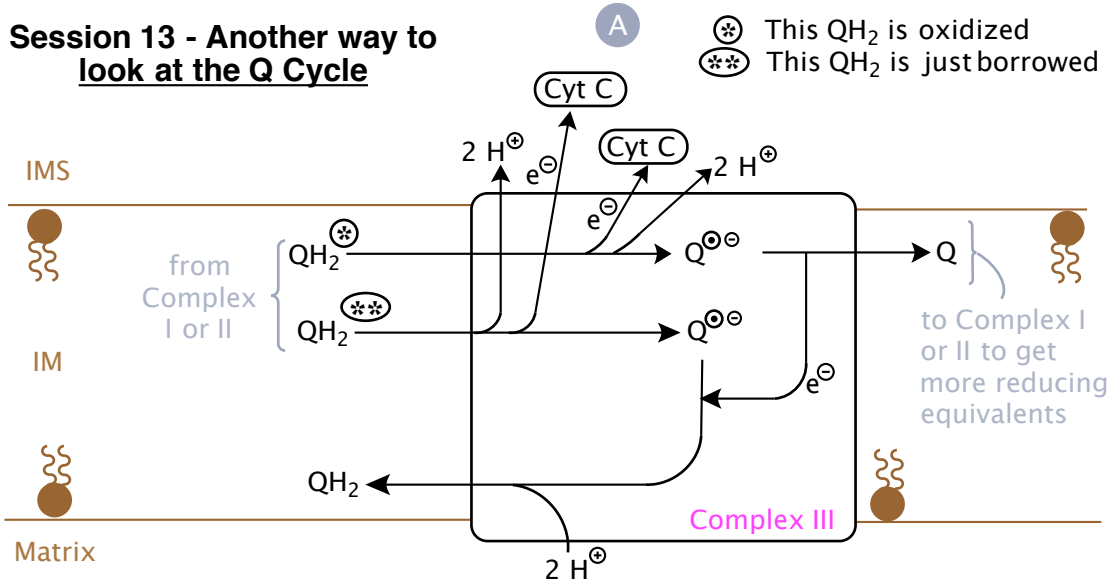
-- Another One CytoC-($Fe^{3\oplus}$) reduced (Step ③)

D

So, for each QH_2 oxidized, you translocate 4 $H^{\oplus}$ -- and move two electrons via CytC($Fe^{2\oplus}$) to Complex IV.

Session 13 - Another way to look at the Q Cycle

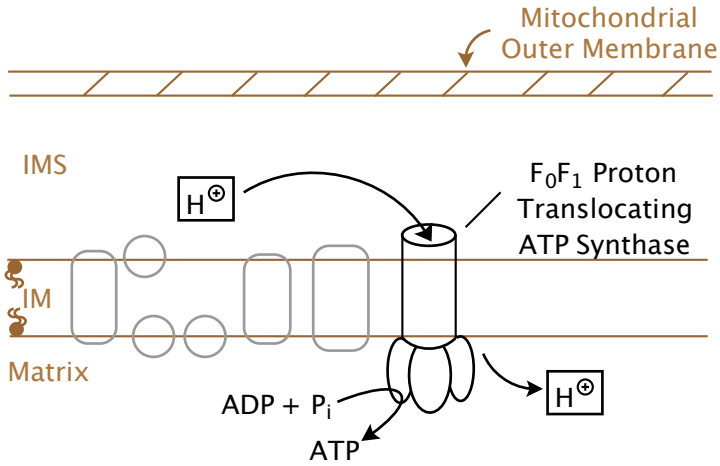
A



Note: The previous 2-cycle presentation is more chemically accurrate - but this way of looking at the cycle might help. Look at both versions but the previous page is the operative mechanism.

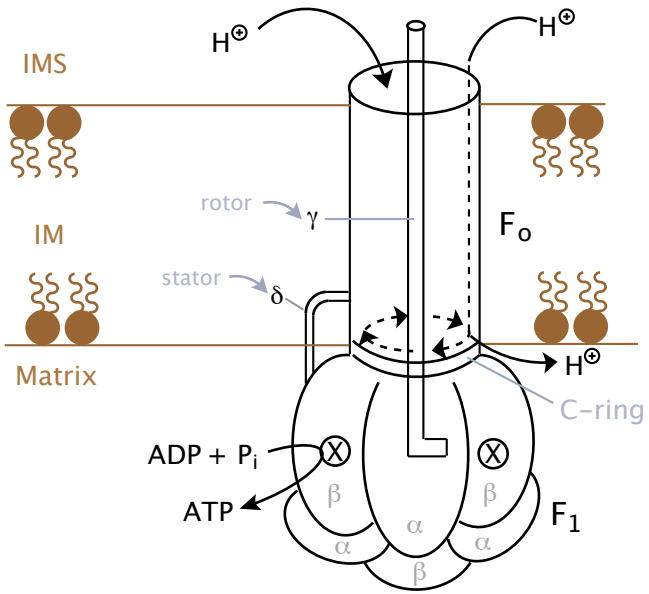
B

We have converted the $e^{\ominus}$ transfer potential of $NADH/FADH_2$ into a proton gradient -- Next we want to convert the proton gradient into the phosphate transfer potential of ATP ⟹ "oxidative phosphorylation"



Coupling Proton Transport with ATP Synthesis

C



As drawn here:

-- shaft rotates CW when it pumps $H^{\oplus}$

-- CCW when it hydrolyzes ATP (see book)

-- $\alpha 3\beta 3$ stays fixed (by δ) while γ rotates

-- C-ring rotates

⊗ = Active Site

Making ATP

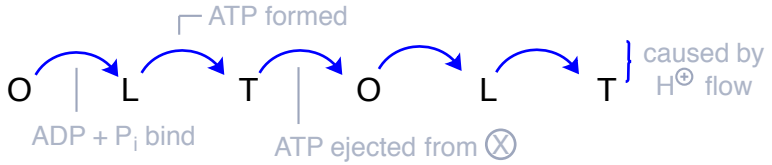
D

-- The C-Ring and γ spin when $H^{\oplus}$ are pumped

-- This causes conformational change in β subunit active site (⊗) that favors ADP + P_i ⟶ ATP

-- Three conformations at ⊗:

O = Open (nothing bound)
L = Loose (ADP + P_i bound)
T = Tight (ATP bound)



Key Point - flow of protons starts with binding of ADP

Physiological Scenario

A

1.) Stress-muscle intensive situation

2.) ATP ⟶ ADP + P_i [ADP]↑ in matrix

3.) ADP binds to ⊗ ⟶ protons flow

4.) T → O → L ∿∿∿ } ATP released and continuously made

5.) pH in Inner Membrane Space ↑ because of lost protons

6.) Electron Transport Chain responds by oxidizing NADH at elevated rate - trying to maintain ΔpH across the mitochondrial IM

B

7.) Concentration of NADH drops in matrix

8.) Note that NADH "product inhibits" the TCA cycle + PDH steps that make it (there also is an allosteric component)

9.) The ↑ [NADH] boots up the TCA cycle to make more of it - letting you continue to make ATP

10.) It all starts with ADP production. This is called "acceptor control" where ADP is the "acceptor" of P_i

11.) Eventually with a persistent dog, you become O_2 limited

C

16

12.) Glycolysis boots up

13.) The Lactate-Pyruvate (homo-lactic fermentation) shuttle boots up

14.) Lactate acidifies the blood

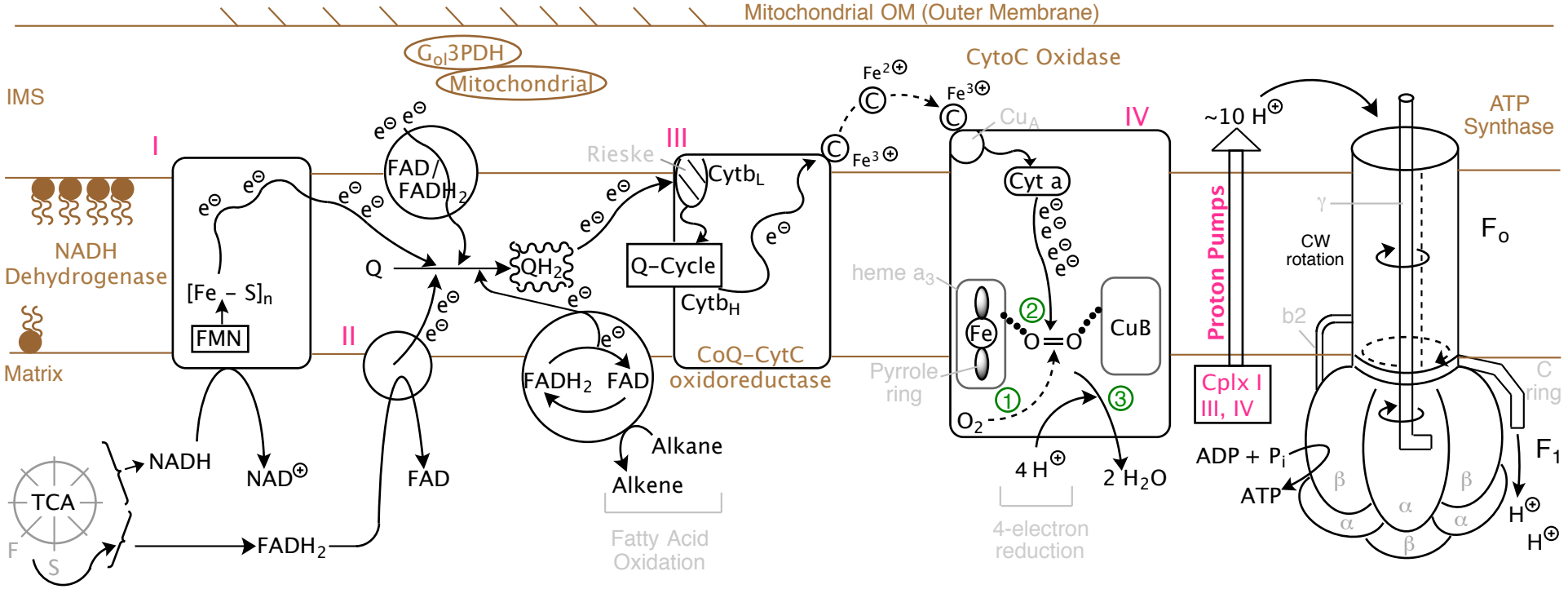
15.) Bohr effect reduces affinity of Hb for O_2

16.) More O_2 delivered to tissues

17.) Respiration boots up again, because O_2 is available

The Respiratory Apparatus

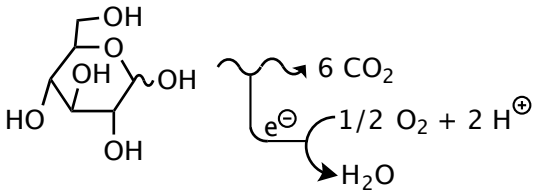
D



A

-- So far in 5.07 - carbohydrate metabolism

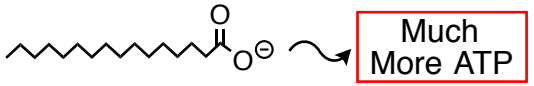
-- Get 36-38 ATPs



Session 16 - Lipid Catabolism

-- Lipids = Small hydrocarbons (often amphiphilic)

-- Lipids: Sometimes made of Fatty Acids (FA)



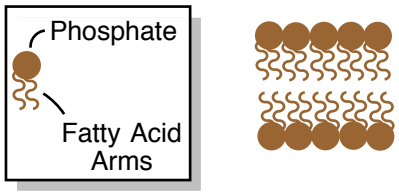
Fatty Acid (16:0) = Palmitate

B

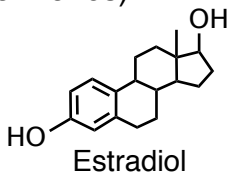
Roles of Lipids

1.) Energy storage (FAs) - our primary reserve

2.) Biological Membranes



3.) Signaling (e.g., steroid hormones)



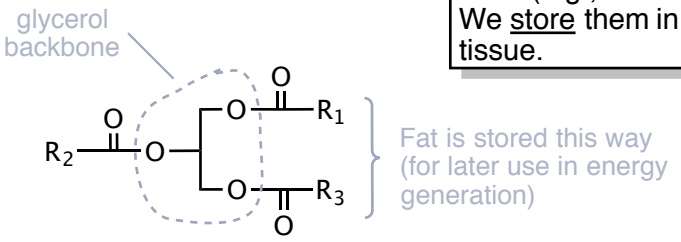
Estradiol

C

Fats

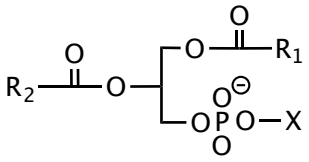
Complex Lipid
e.g., Triacylglyceride

See slides on how we acquire lipids from diet or how we manufacture them (e.g., in the liver). We store them in adipose tissue.



Fat is stored this way (for later use in energy generation)

or a Phospholipid (Membrane Lipid)



X = H ≡ Phosphatidic Acid

X = sugar ≡ Glycolipid

D

Stages of FA Catabolism

1.) FA → FAcyl CoA (cytoplasm)

2.) FAcyl CoA ⇝ Mitochondrion (site of β-oxidation)

3.) β-oxidation ⇝ to Acetyl CoA

4.) Special Endings of FA Catabolism

a.) Odd chain numbered FAs

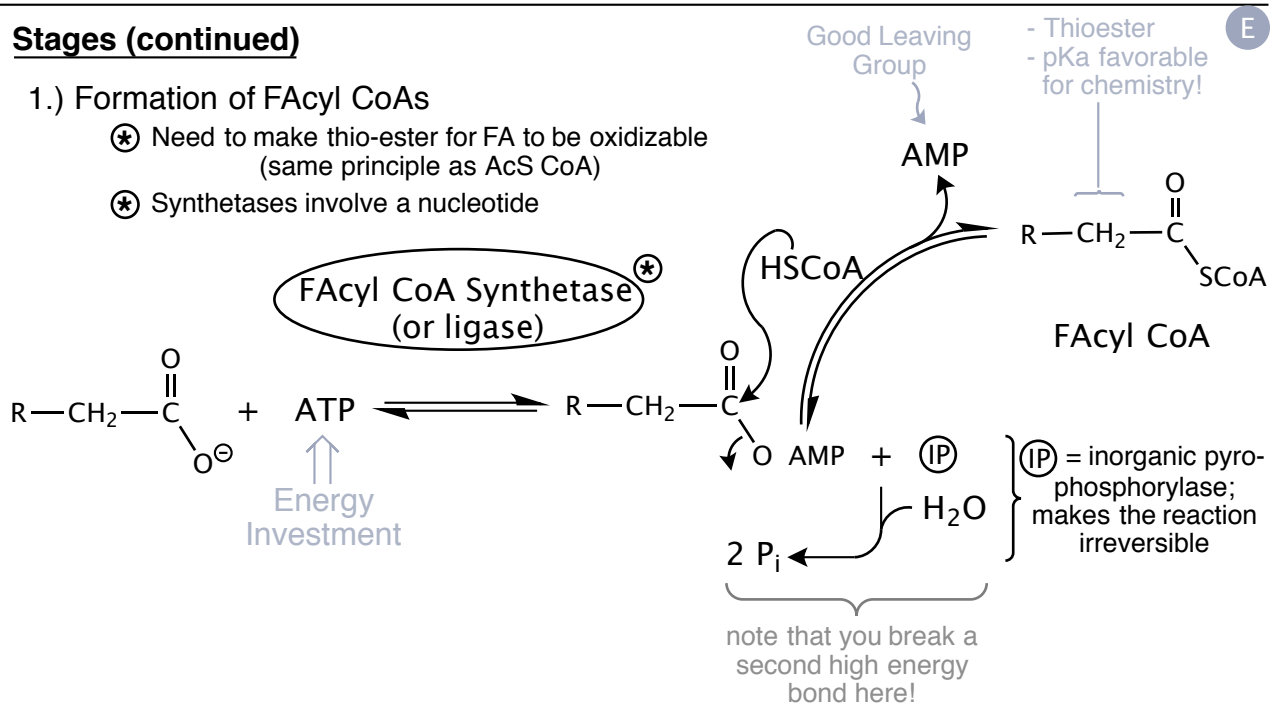
b.) Unsaturated FA (getting double bond in the right place for oxidation) - tricky because most unsaturated FA have cis-double bonds

Stages (continued)

1.) Formation of FAcyl CoAs

- ⊛ Need to make thio-ester for FA to be oxidizable (same principle as AcS CoA)
- ⊛ Synthetases involve a nucleotide

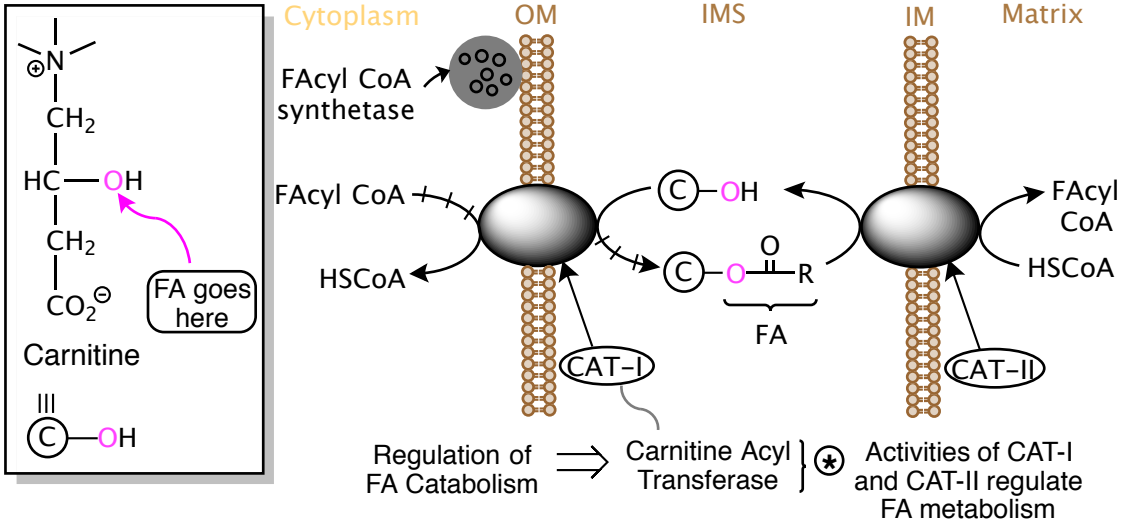
E



2. Entry of FAcyl CoA into Mitochondrion

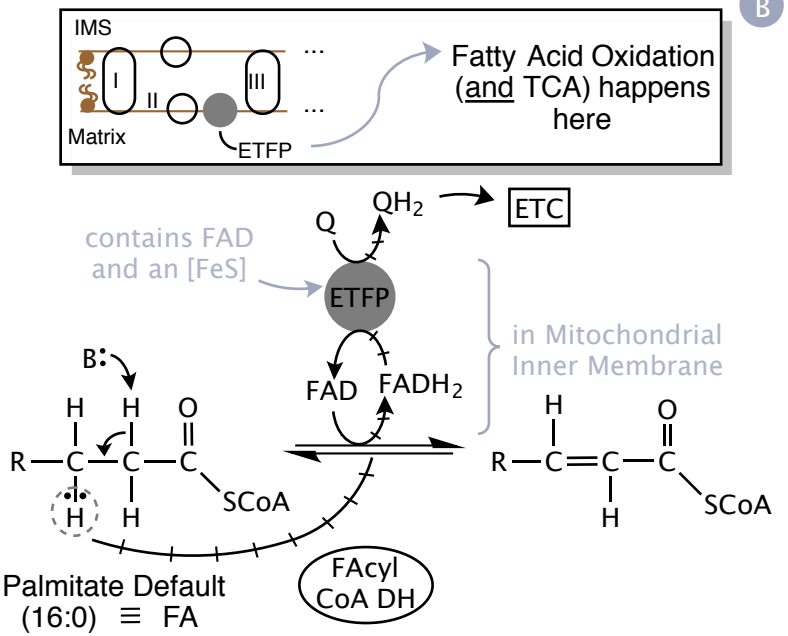
A

-- mitochondrion is the site of β-oxidation



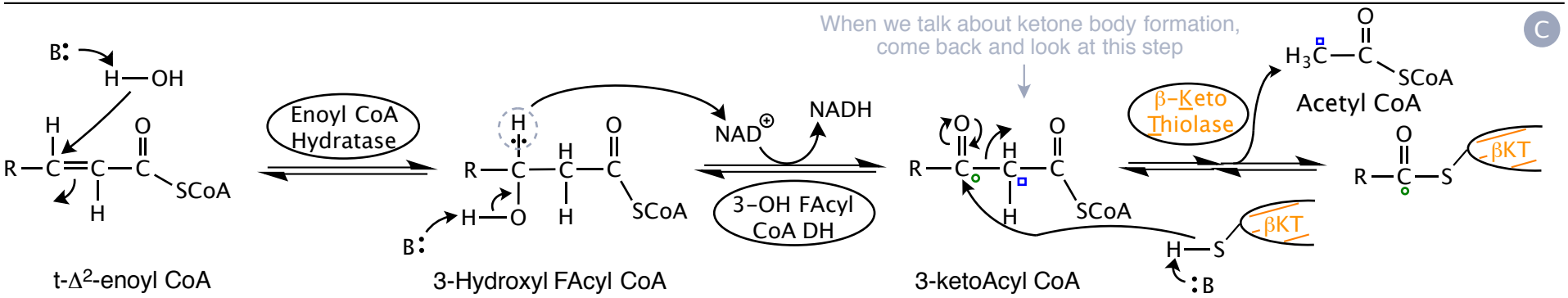
3. β-Oxidation (Mitochondrial Matrix Reactions)

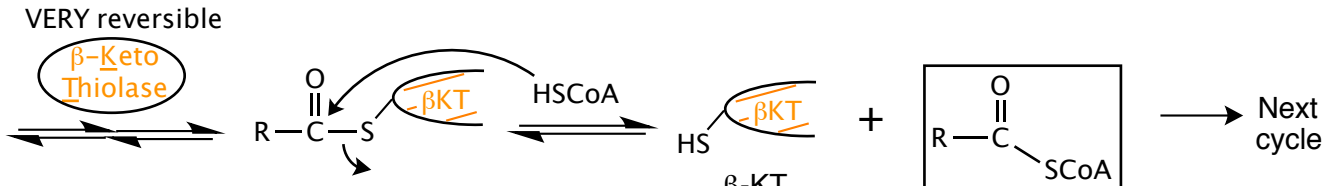
B



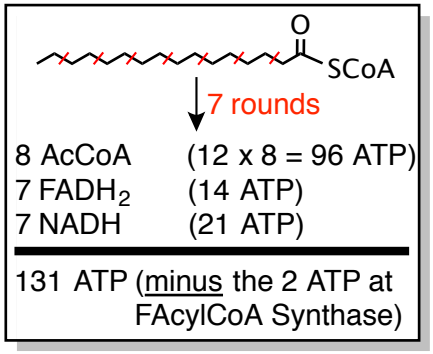
C

When we talk about ketone body formation, come back and look at this step





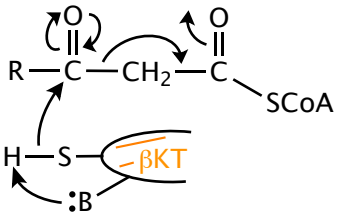
With this written out -- look at the TCA cycle reactions, starting with:
Succinate ⇝ OA ⇒ same chemistry!

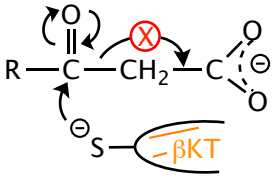


Session 15 - Chemical Interlude - Why did we have to use a thioester (FAcyl CoA)?

A

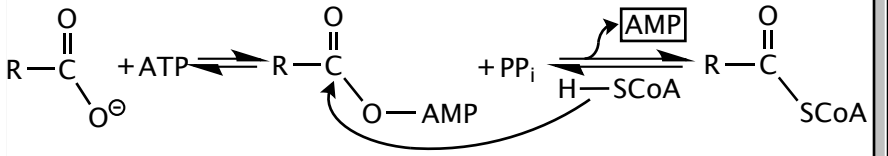
-- The reverse reaction (decarboxylation of β-keto acid) is VERY favorable.





Cannot do this with a carboxylic acid - unfavorable

Recall how to make a thioester from an acid:



Step I

Could phosphorylation work? - Probably.
AMP (and phosphate) are good leaving groups

Special Case 1 - FA has a Cis-Double bond

B

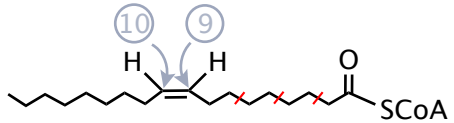
-- Cis-Double bonds promote membrane plasticity

-- trans-double bonds have a slight reduction in overall energy yield

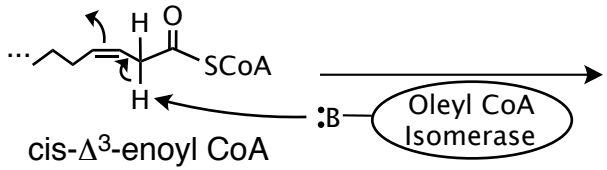
-- But, Cis-double bonds present a biochemical challenge to digestion

-- For example, Oleic Acid (18:1)Δ9

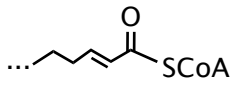
-- The word "oil" comes from oleic (olive oil = oleic)



Oleyl CoA



cis-Δ^3-enoyl CoA



trans-Δ^2 enoyl CoA

Hydratase

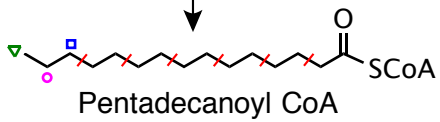
regular β-oxidation

Special Case 2 - Odd Chain FA: Introduction to Carboxylases

C

Pentadecanoic acid (15:0) = 1.2% of milk fat

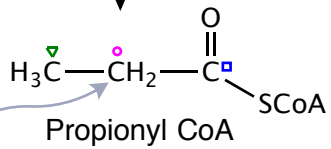
HSCoA
ATP



Pentadecanoyl CoA

6 Acetyl CoA

6 rounds of β-oxidation

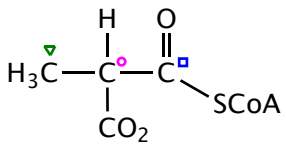


Propionyl CoA

"CO_2" inserted CH α-carbon; must activate CO_2 for it to add to Cα

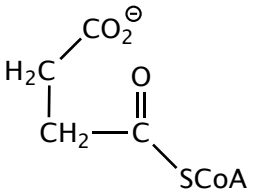
(Biotin)

Propionyl CoA Carboxylase



several steps

carbon skeleton rearrangement (Vitamin B_{12})



Succinyl CoA

TCA cycle

D

-- Odd chain FA in diet → Acetyl CoA (many) → Propionyl CoA (C–3)

Propionyl CoA (C–3) → "CO_2" → Succinyl CoA (C–4)

-- They are anapleurotic (increase rate of TCA cycle)

-- They can be gluconeogenic (later) ⇒ can result in net synthesis of glucose from this part of the FA chain (the Acetyl CoA-derived units are typically not gluconeogenic unless glyoxylate cycle (later) is operative)

-- The carboxylase family does much more than metabolize odd chain FA

More General View of Carboxylases

A

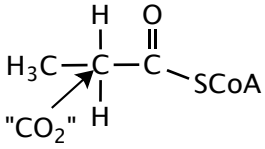
-- Require biotin (Vit. B_7), CO_2 and ATP

-- Increase size of molecule by one carbon (as "CO_2")

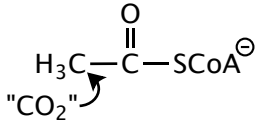
-- This is a kind of carbon fixation

-- Play a role in:

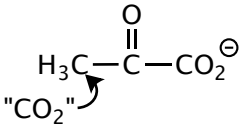
(a) Odd chain FA metabolism
Example: Propionyl CoA Carboxylase
* We'll look at this in detail later (this is also anapleurotic)



(b) FA biosynthesis
Example: Acetyl CoA Carboxylase



(c) Anapleurosis and Gluconeogenesis
Example: Pyruvate Carboxylase



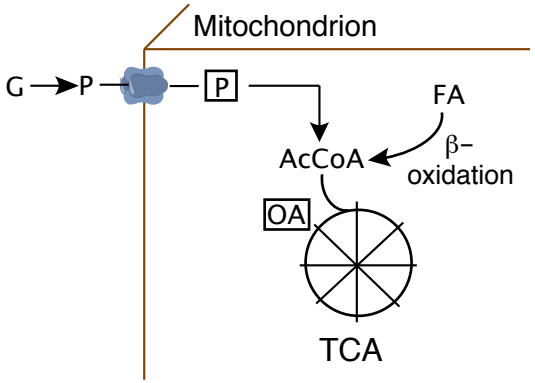
Start with (c) - Pyruvate Carboxylase (PC)

B

20

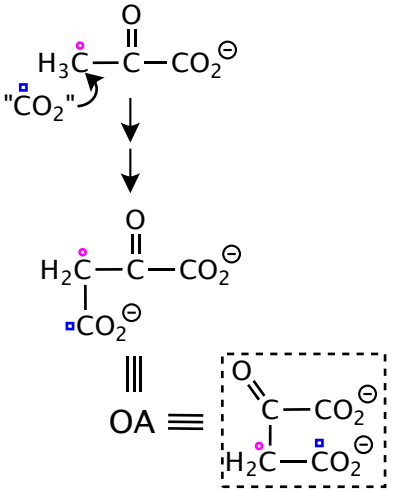
Role: To ↑ [Oxaloacetate] in mitochondrion → ↑ rate of TCA cycle

always limiting



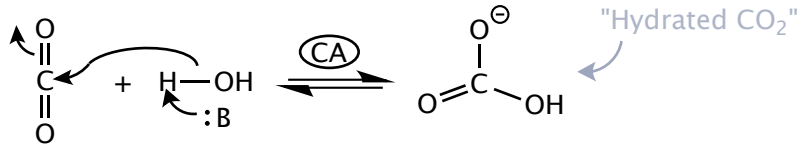
-- PC stimulated by AcCoA

-- This regulatory mechanism keeps rate of TCA matched to rate of generation of AcCoA

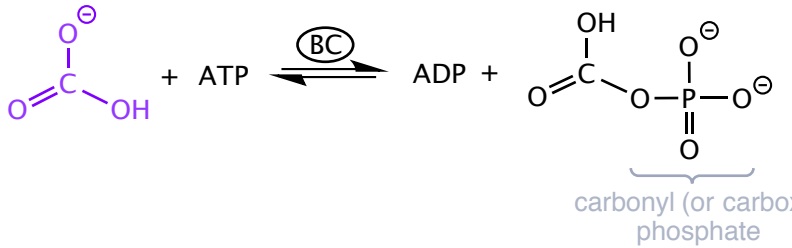


Steps (most are common to all carboxylases)

1.) Synthesis of Bicarbonate via Carbonic Anhydrase (CA)

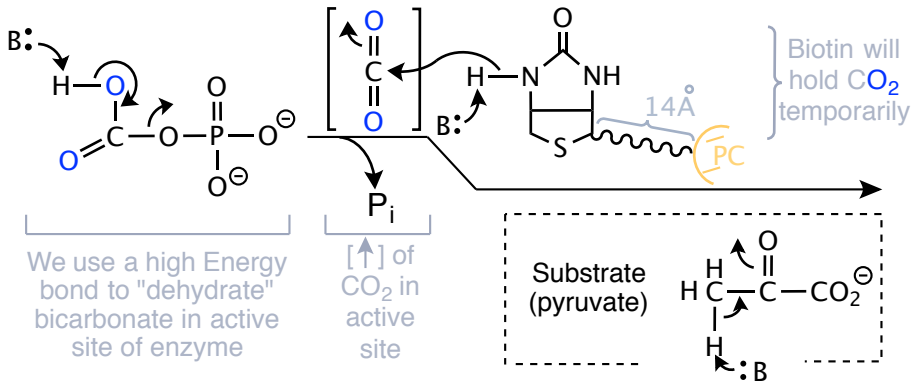


2.) Biotin Carboxylase (BC) Reaction of Pyruvate Carboxylase (PC)

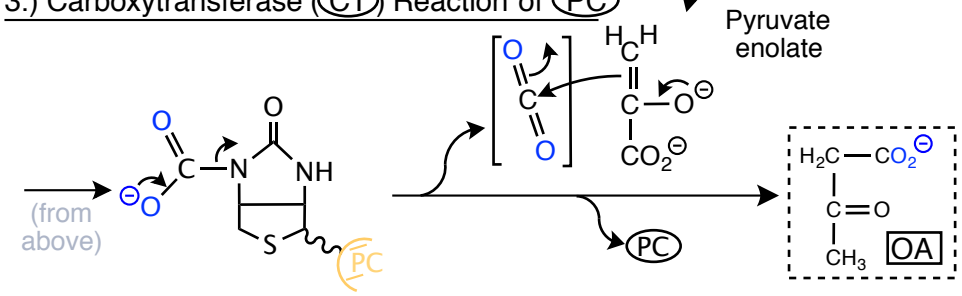


B

2 cont.) Biotin Carboxylase Reaction of Pyruvate Carboxylase (PC)



3.) Carboxytransferase (CT) Reaction of PC

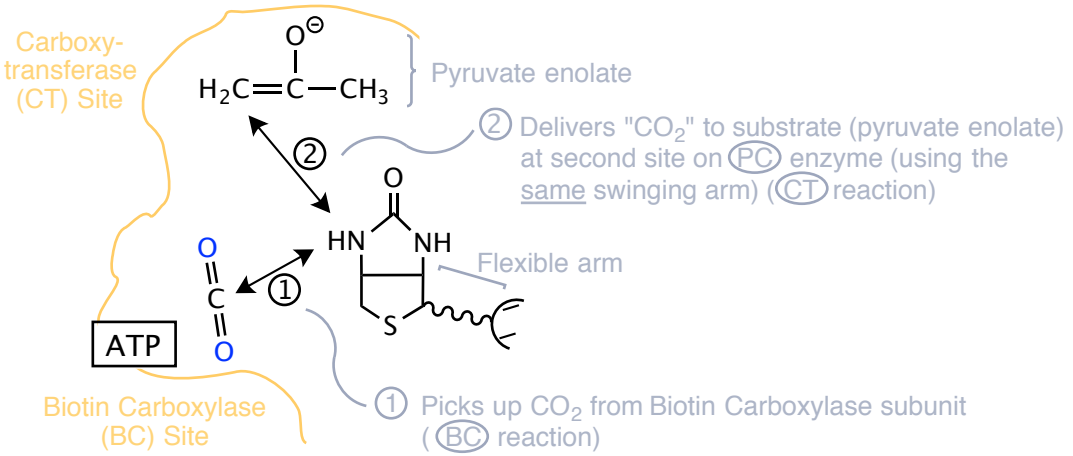


How to Visualize Carboxylase Chemistry

A

-- (CT) and (BC) are ~55Å apart

-- swinging arm does the "CO_2" transfer

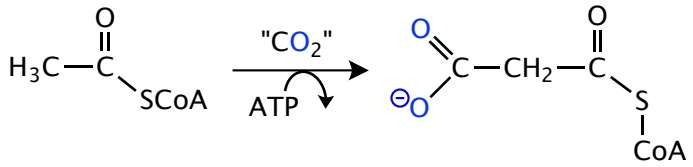


Example (b) - Acetyl CoA Carboxylase

B

22

-- Exactly the same chemistry, but acetyl CoA receives the CO_2



Malonyl CoA

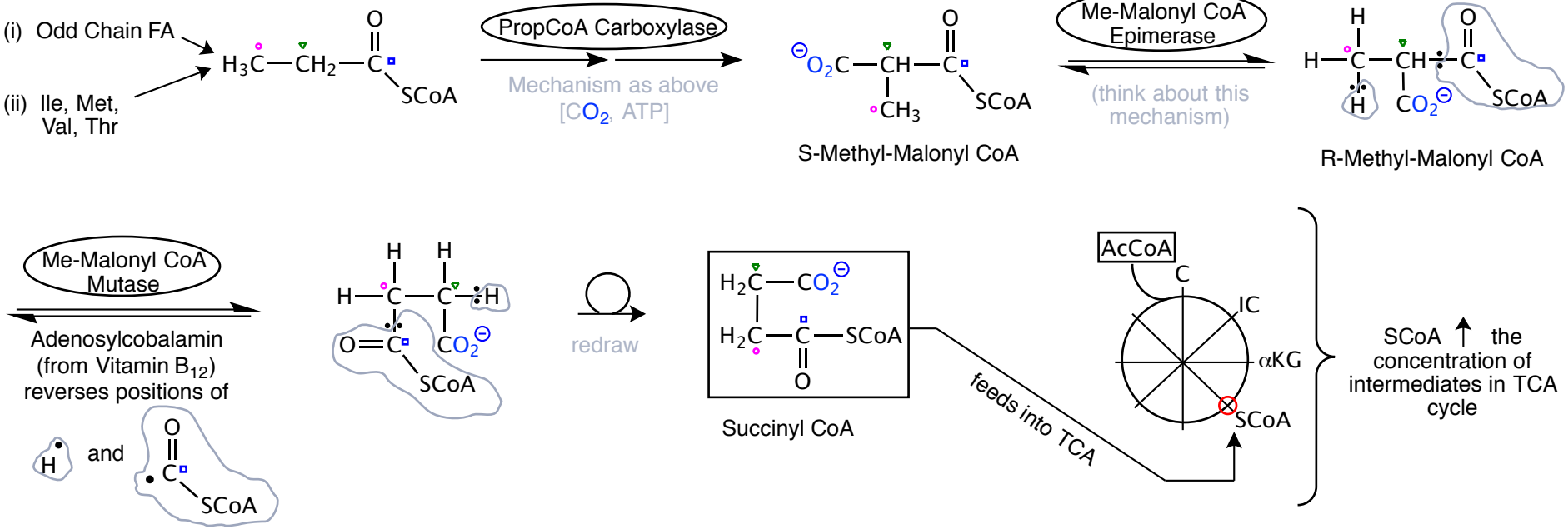
-- Malonyl CoA is the precursor to most of the ethylene units in FAs

-- We'll see this again soon when we talk about FA biosynthesis

Example (c) - Propionyl CoA Carboxylase - followed by synthesis of Succinyl CoA (2 more steps)

Introduced 2 pages back

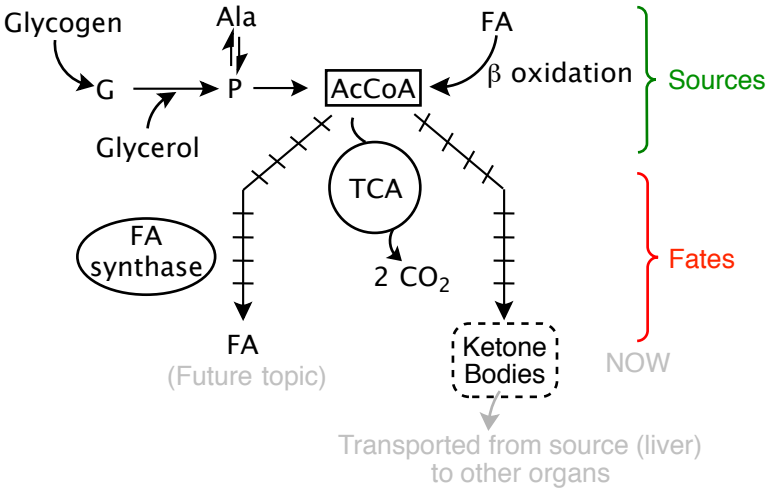
C

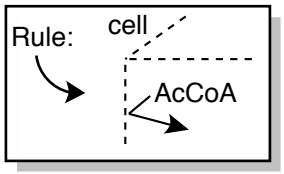


A

Session 16 - Ketone Bodies (KB)

-- Sources (so far) and fates of Acetyl CoA:





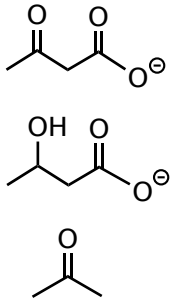
B

KB Facts

1. Produced by liver (mainly) - when OA becomes limiting
2. Primary (or very important) metabolic fuels of *heart* & *skeletal muscle*
3. Used by all organs (even brain) in times of starvation
4. Produced in excess in Diabetes Mellitus (also in Type I diabetes)
5. Ketogenesis = Mitochondrial reaction

C

Typical KBs



Acetoacetate

β-Hydroxybutyrate *

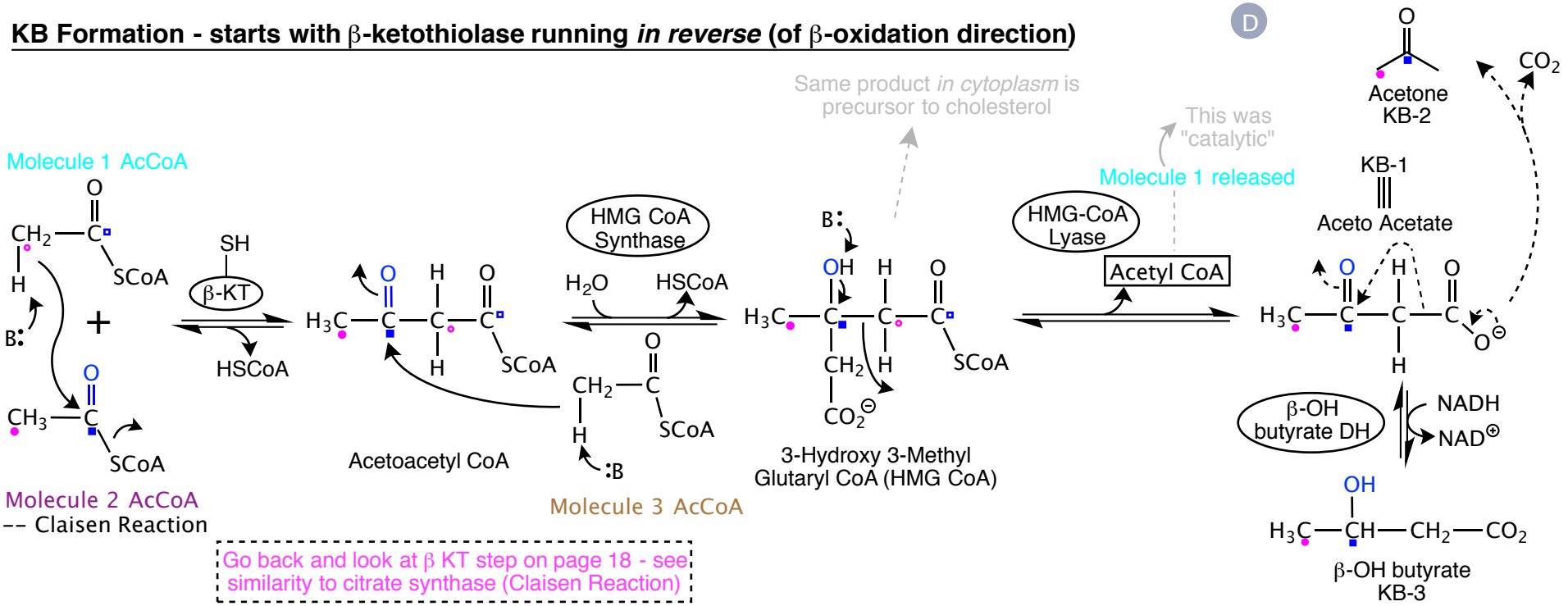
Acetone **

* Not actually a ketone
** Fruity breath in diabetes

They can ↓ pH of blood from 7.4 to < 7 (e.g., 6.8) in diabetics

D

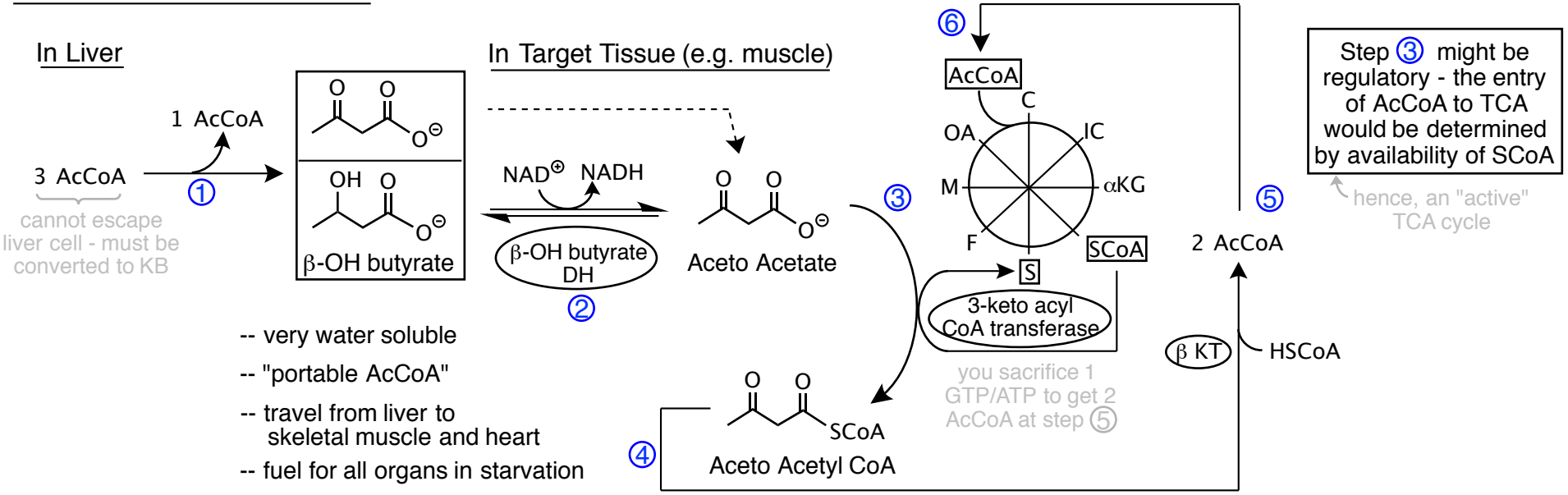
KB Formation - starts with β-ketothiolase running *in reverse* (of β-oxidation direction)



Go back and look at β KT step on page 18 - see similarity to citrate synthase (Claisen Reaction)

KB Formation and Utilization

Follow numbers ①-⑥



-- very water soluble

-- "portable AcCoA"

-- travel from liver to skeletal muscle and heart

-- fuel for all organs in starvation

Diabetes Scenario ⟶ technical state of starvation

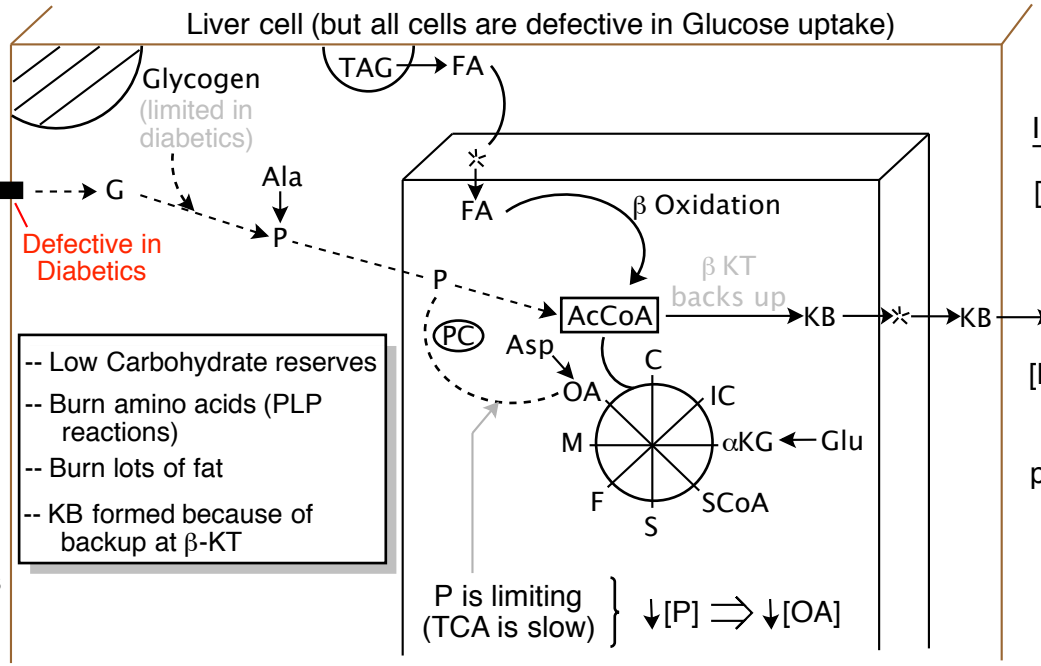
B

-- Pancreas β cells release insulin in response to food

-- Insulin triggers Glucose uptake

-- Insulin signaling defective:

- Glucose not taken up from blood
- Glycogen reserves very small



BLOOD

In "out of control" diabetes

[G] = 300+ mg / 100 mL (normal = 100 mg / 100 mL or 5 mM)

[KB] = 15 - 25 mM (normal = <0.2 mM)

pH as low as 6.8 (from 7.4)

Acidosis

Kidney tries to excrete H^+, [G]

Thirst ↑ to replace lost H_2O

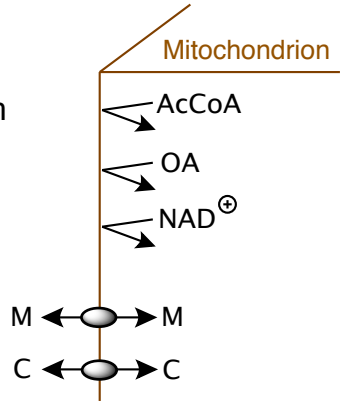
Session 17
5.07

So far: CATABOLISM | Next: ANABOLISM

Fatty Acid and Lipid Biosynthesis

Some Rules

Cytoplasm ||| Site of FA Biosyn



Stages of FA Biosynthesis

1.) FA biosynthesis = cytoplasmic reaction, but precursor (AcCoA "packaged" as citrate) is in mitochondrion; must get citrate to cytoplasm.)

2.) Must maintain OA mass balance between cytoplasm and mitoplasm.

3.) Activation of Acetyl CoA → MalCoA

4.) Formation of ACP (acyl carrier protein) derivatives

5.) FAS (Fatty Acid Synthase) reactions to make palmitate (16:0)

Post FAS Reactions

1) Elongation, Desaturation, Branching

2) 2 FA + $G_{ol}3P$ → Phospholipid (PL)

Constituent of biological membrane

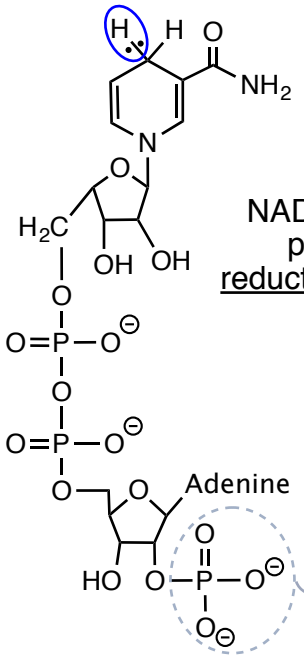
P_i FA

TAG

Energy storage

3) Polyketide biosynthesis

Introducing NADPH (Biosynthesis is reductive)



NADPH is our principal reductive cofactor

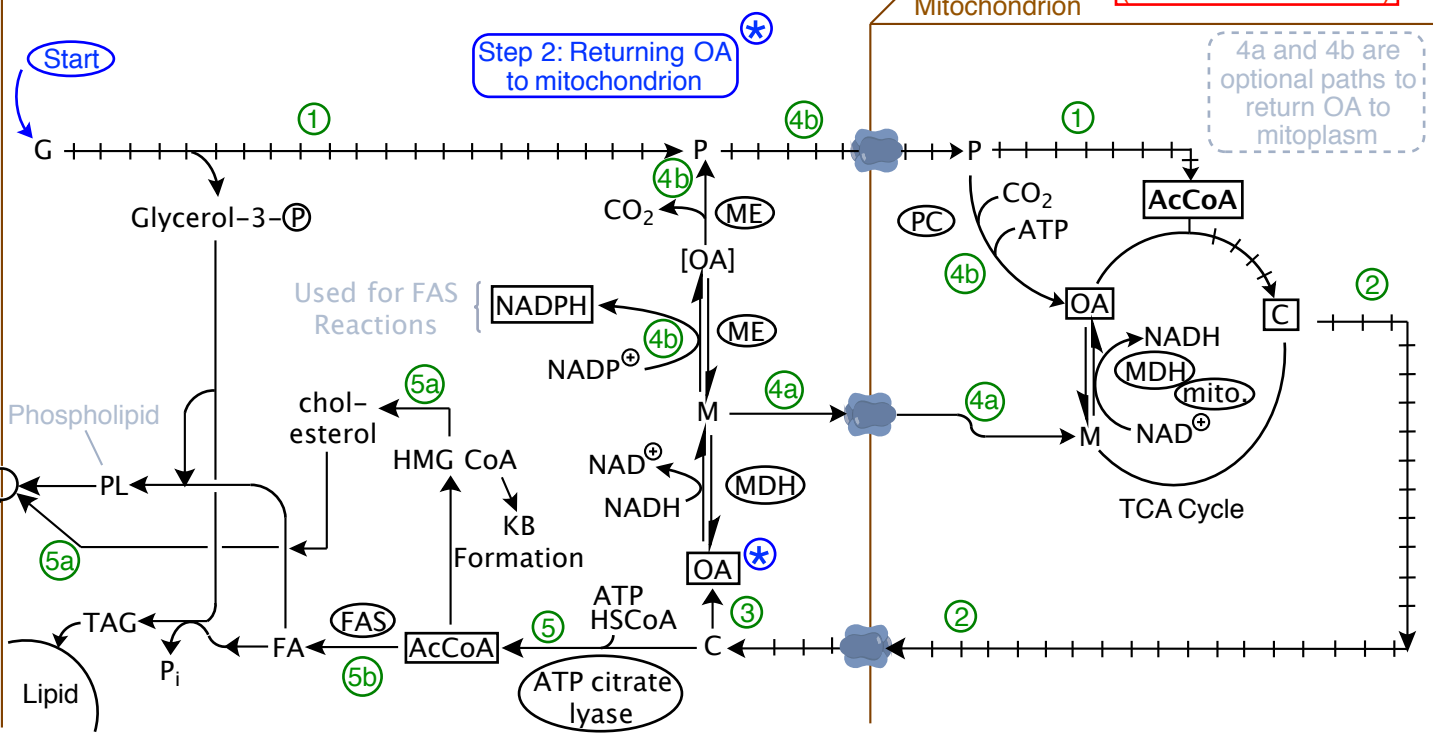
Differentiates NADPH from NADH

Step 1: Getting Acetyl CoA (as citrate) into Cytoplasm (location of FAS)

Physiological Scenario: eat sugar → get fat

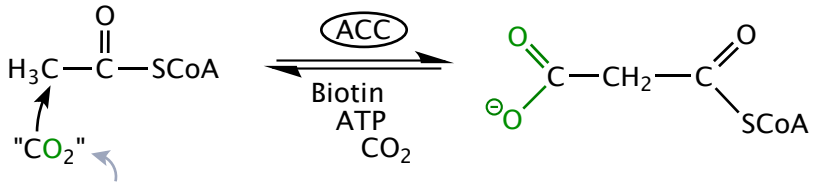
ME = Malic enzyme (uses NADP+/NADPH)

4a and 4b are optional paths to return OA to mitoplasm



A

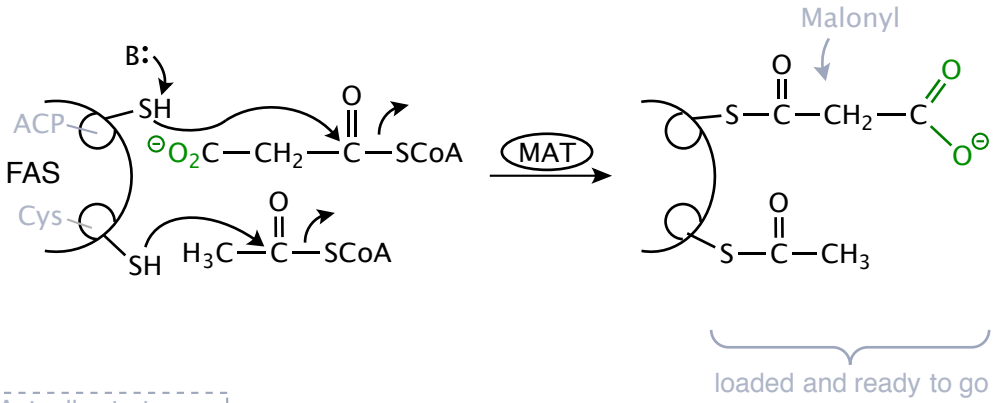
Step 3 Synthesis of Malonyl CoA (the precursor to all but (2) carbons of the FA)



Acetyl CoA Carboxylase

B

Step 4 Synthesis of ACP derivatives

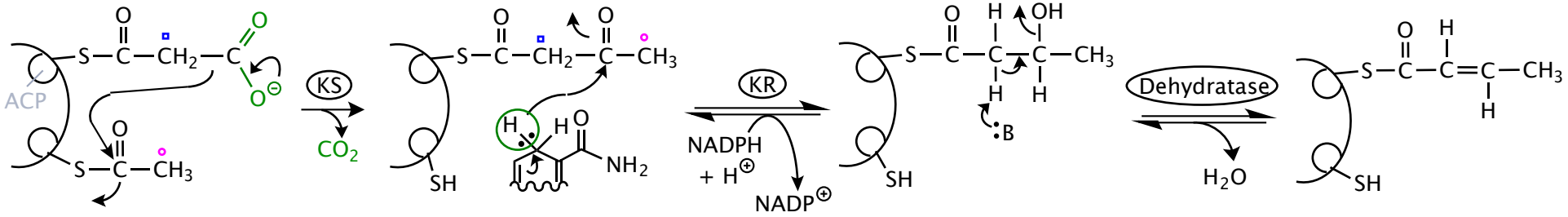


Actually starts on ACP and is transferred to Cys

Malonyl/Acyl Transferase

C

Step 5 FAS Reactions



ACP = Acyl Carrier Protein

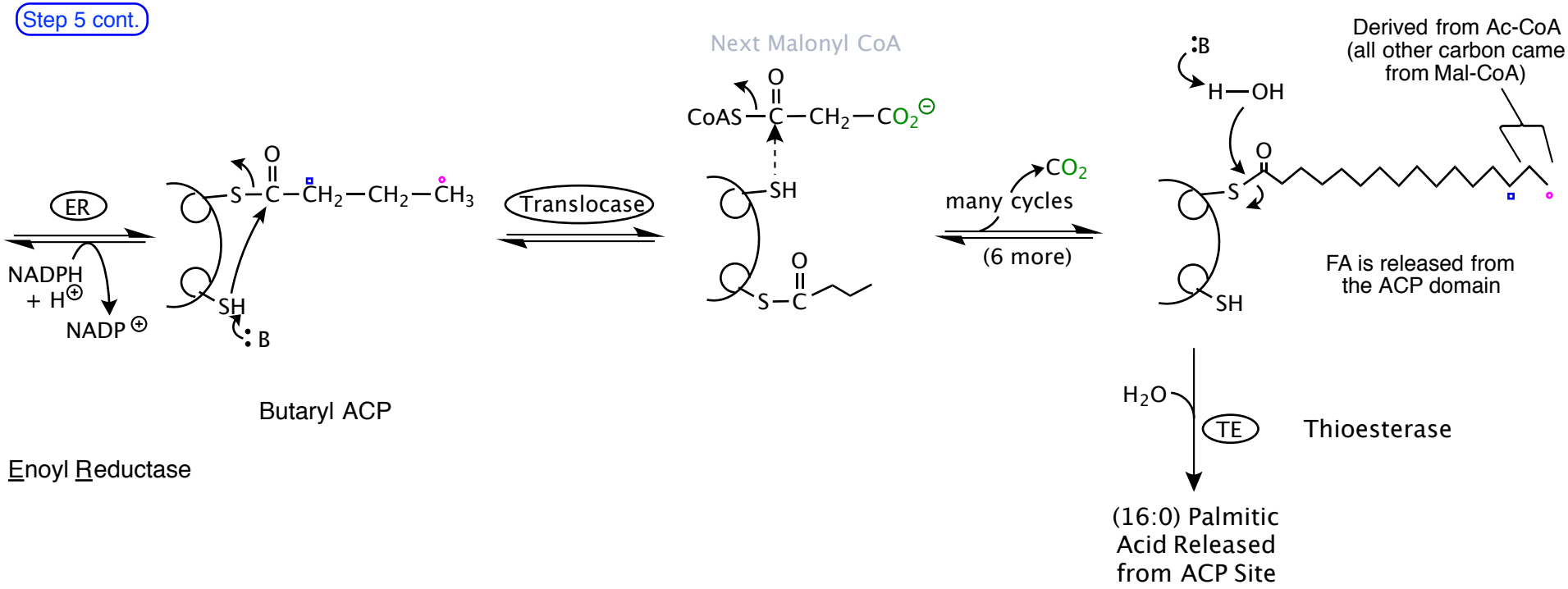
= long arm transports substrate among different catalytic domains

β-ketoacyl ACP

β-Hydroxy-Acyl ACP

Δ^2 enoyl ACP

Step 5 cont.

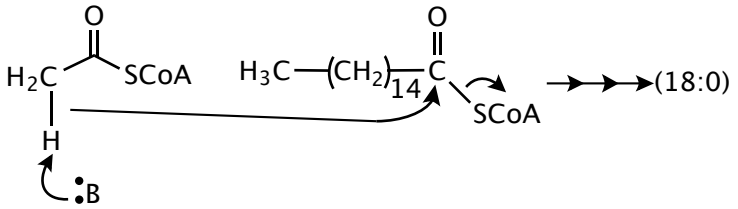


Elongation

-- FA released from FAS in cytoplasm

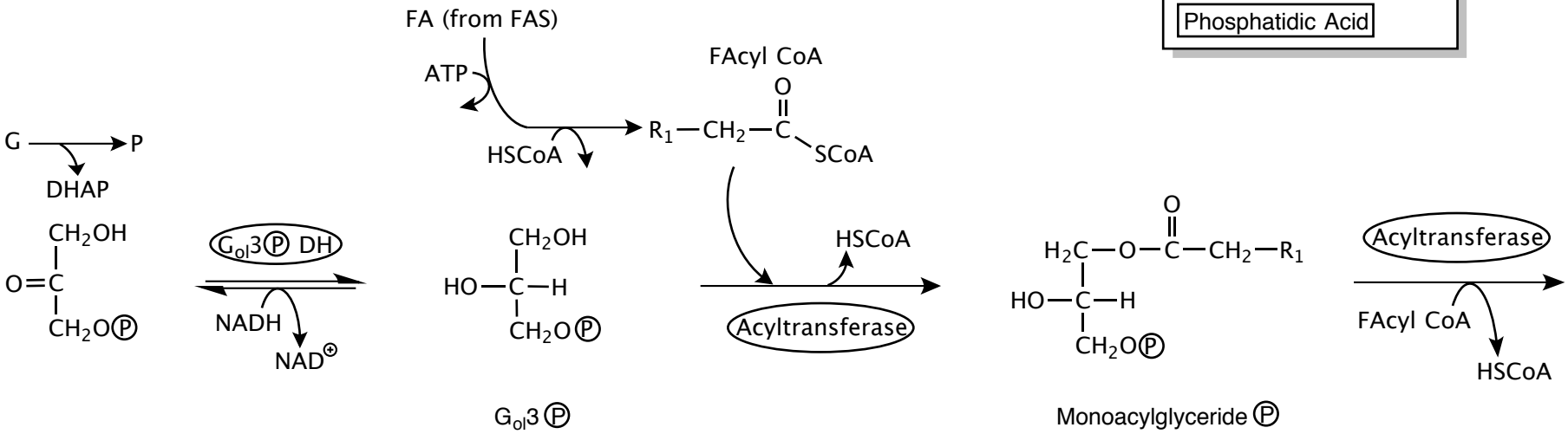
-- If it needs to be elongated - it is transported (as HSCoA ester) to mitochondrion or endoplasmic reticulum

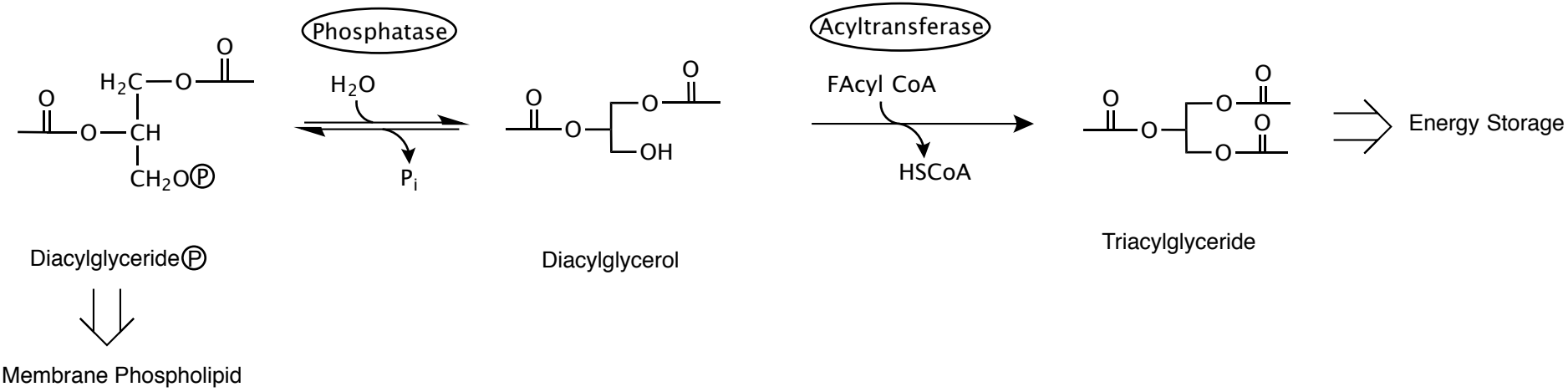
-- See book for mechanisms of elongation



Converting FA to Phospholipid and TAG

Membrane lipid
Phospholipid
Phosphatidic Acid





Session 18 - Carbohydrate Synthesis

1.) Glycogen synthesis

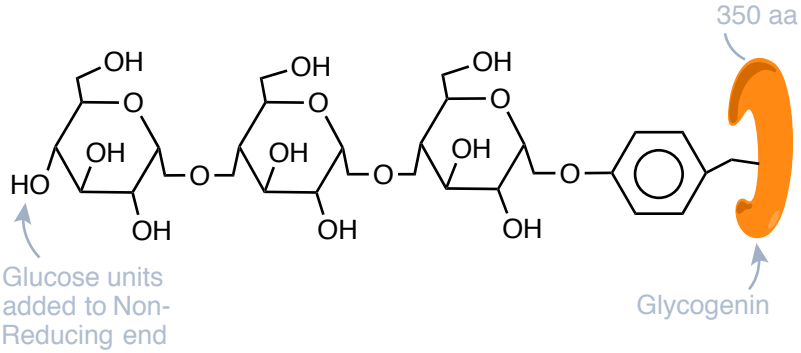
2.) Gluconeogenesis

A

B

Recall the structure of glycogen and how it is degraded

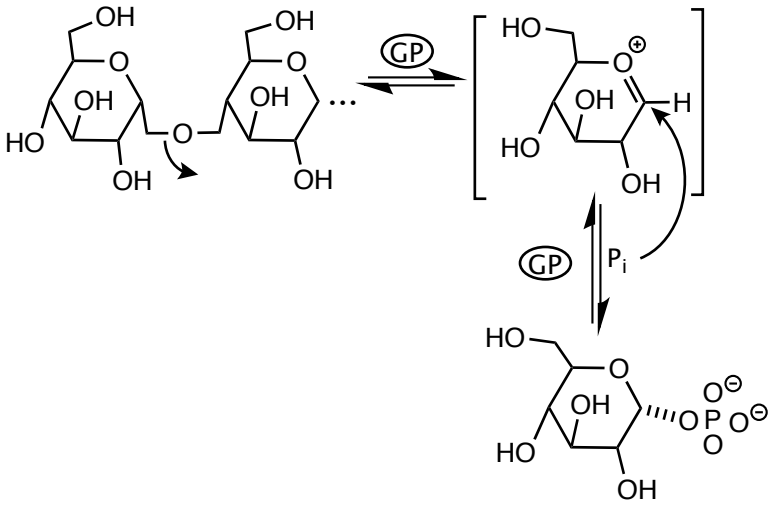
Glycogen Structure



Glycogenin = enzyme, adds 1st 4-7 molecule of glucose (via UDP-glucose) to its Y-194. Then Glycogen Synthase (GS) takes over.

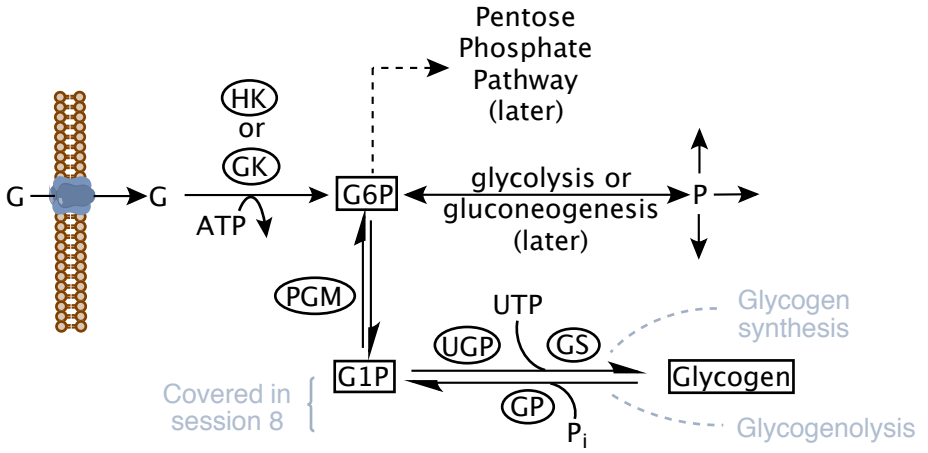
C

Glucogenolysis

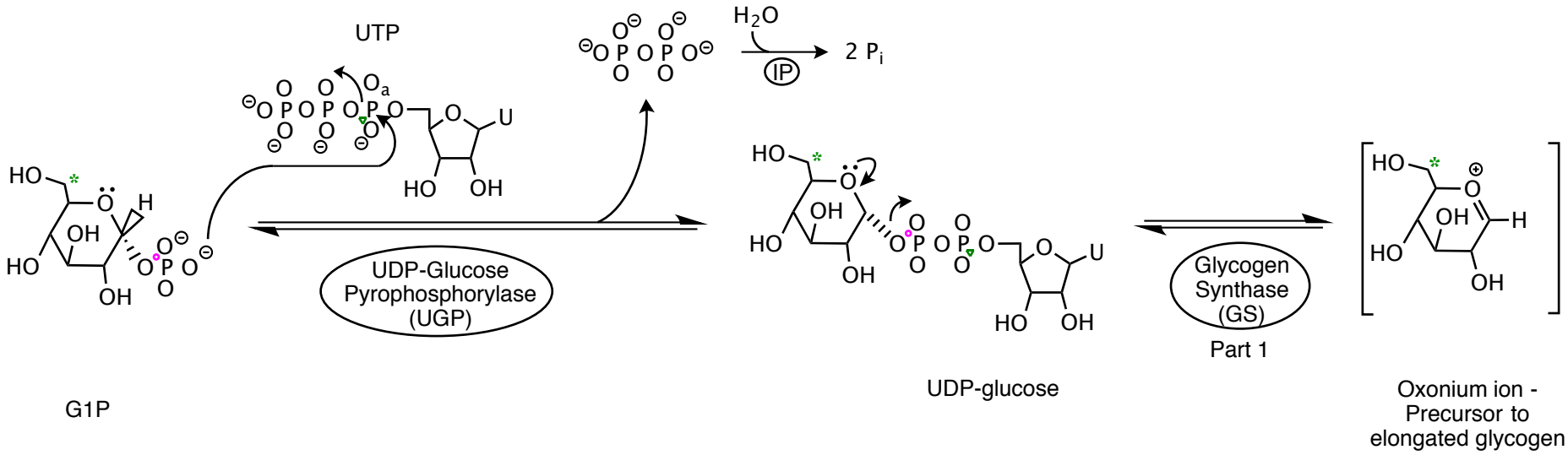


D

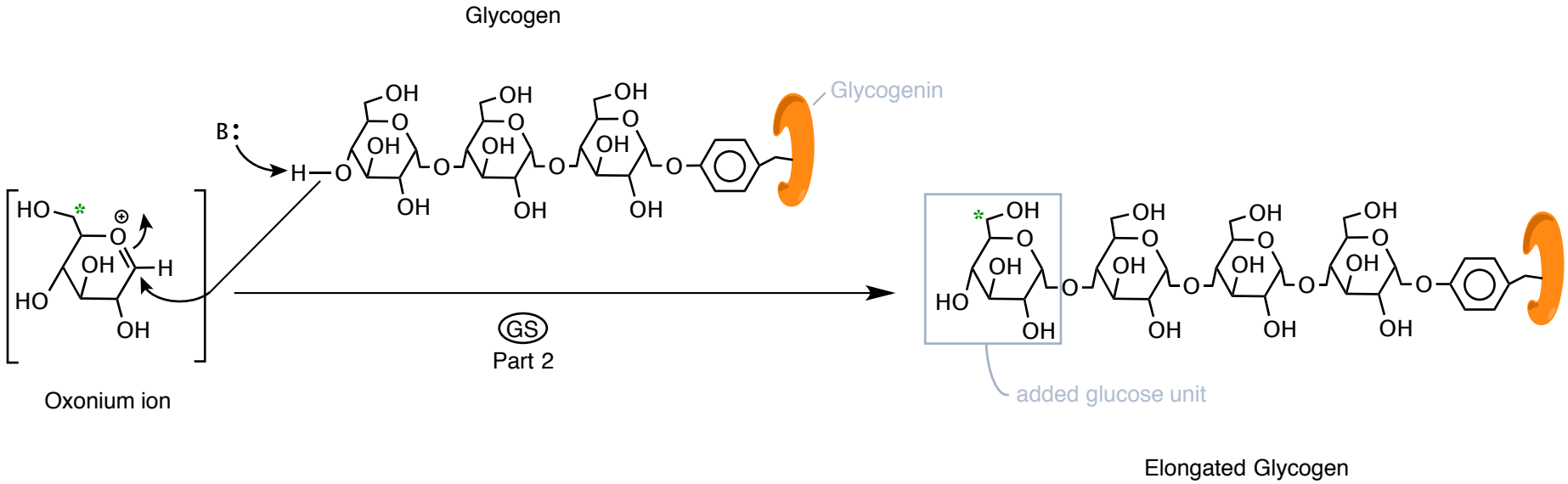
Now, recall how G1P interfaces with the mainstream of metabolism



Glycogen Synthesis



B



Session 19 - Gluconeogenesis

"New" synthesis of glucose from noncarbohydrate precursors = GNG (A)

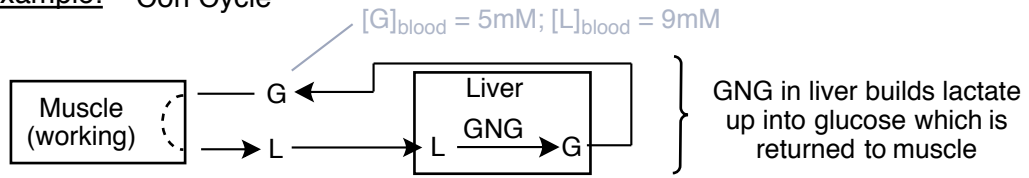
Problem: Brain, Renal Medulla, Erythrocytes, Testis } Require glucose as their primary metabolic fuel

-- Brain uses 120 g / day
-- Whole body uses 160 g / day
-- Total [glucose + glycogen] reserves = 190 g ⇒ Not much!

Solution: GNG = efficient way to manufacture glucose to meet steady state needs

GNG happens in (a) Liver and (b) Renal Cortex

Example: Cori Cycle



Precursors to Glucose (GNG Substrates) (B)

1. Lactate
2. Ala
3. Glu
4. Asp
5. Odd Chain FA
6. Met, Ile, Val
7. Glycerol
8. (Ribose) (via Pentose Phosphate Pathway)

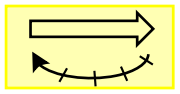
This actually is a carbohydrate but it can get converted to glucose via GNG

We'll map ① → ⑧ on detailed GNG Pathway (next page)

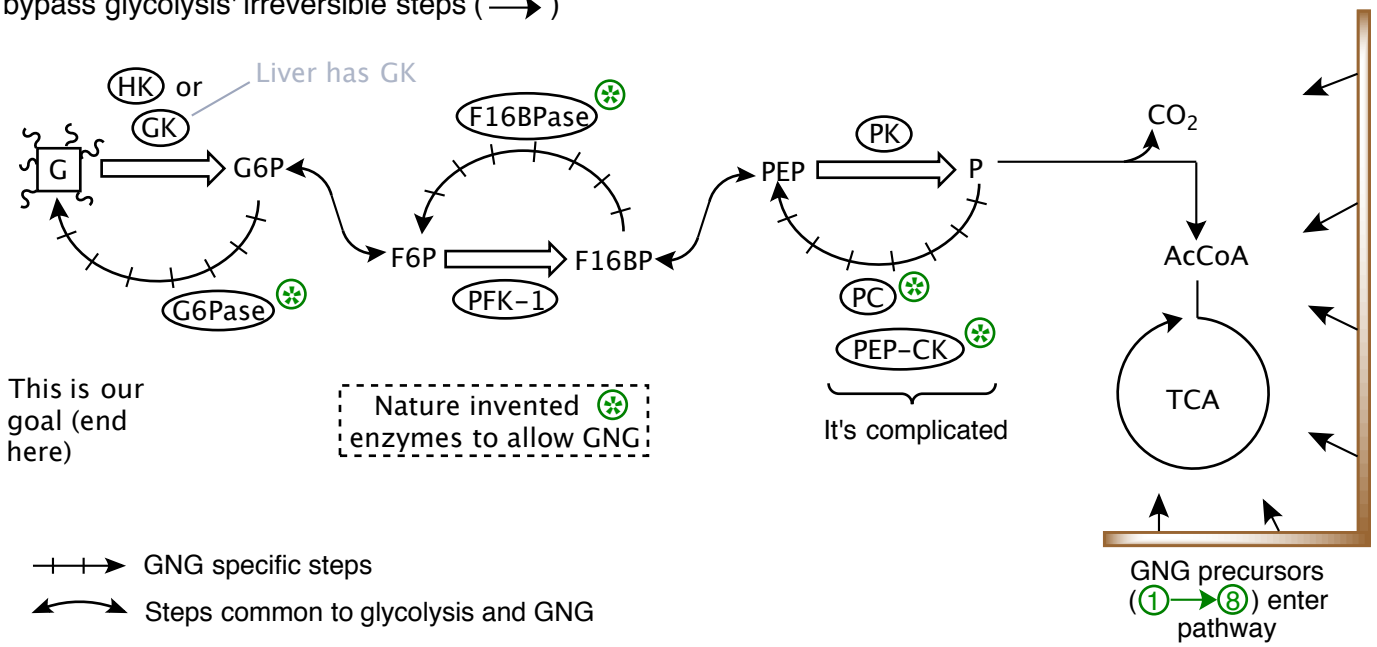
Pathway Overview

-- Looks like Glycolysis in Reverse
-- But must bypass glycolysis' irreversible steps (⟶)

(C)



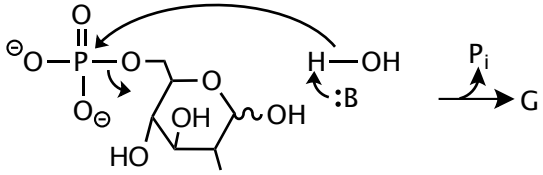
Sites of Pathway Control



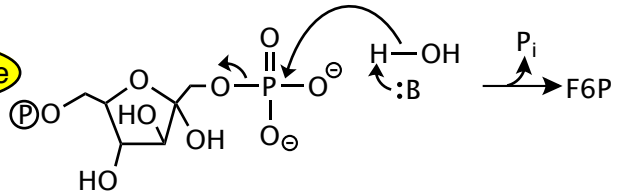
Mechanisms of GNG Enzymes

A

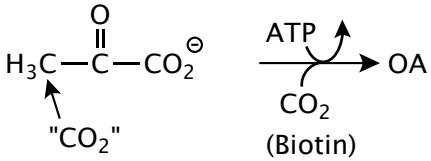
1.) G6Pase



2.) F16BPase



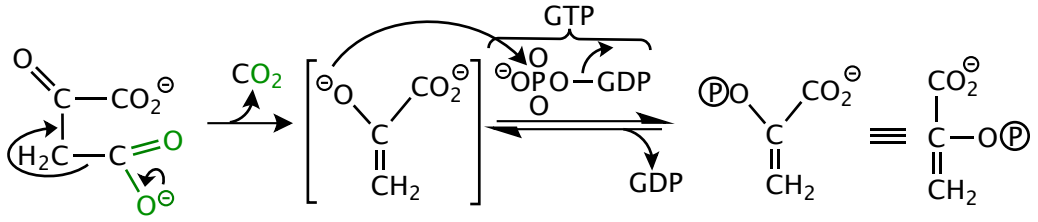
3.) PC



B

4.) Phospho Enol Pyruvate Carboxylase (PEP-CK)

-- The same CO_2 is lost that was put on by PC



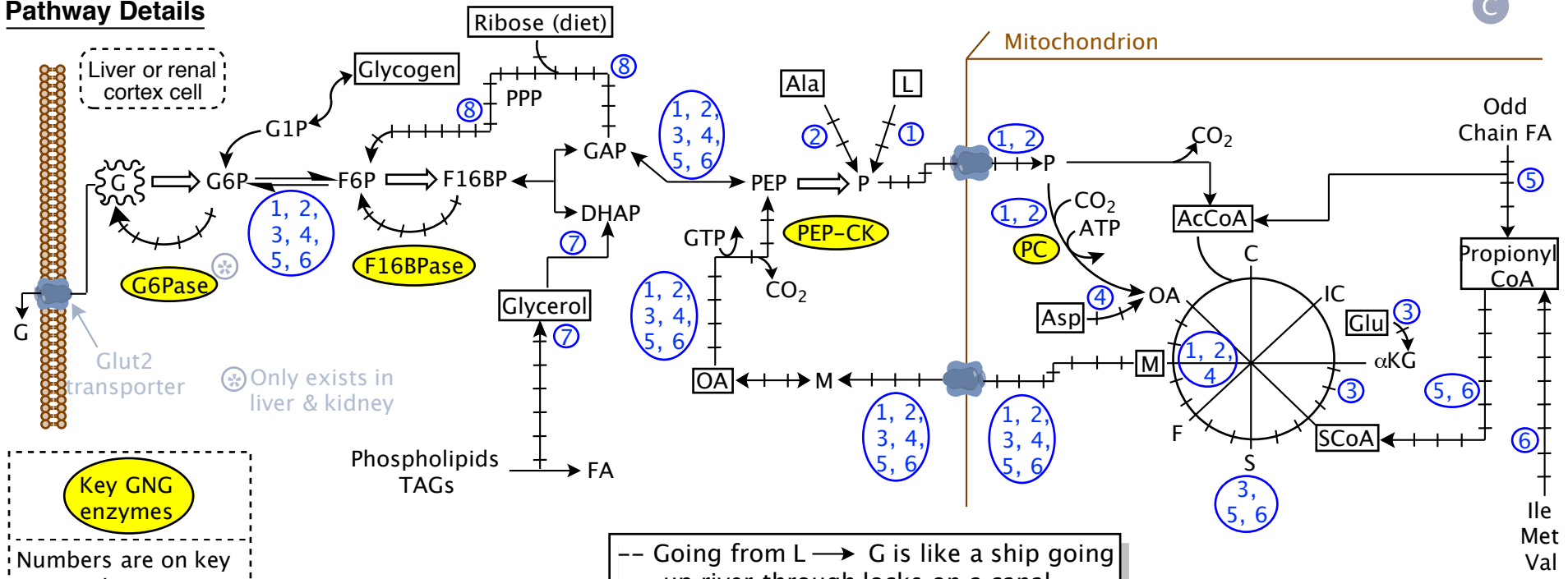
OA (β-keto acid)

PEP

-- PEP-CK can be cytosolic, mitochondrial, or both (depending on species)
-- If mitochondrial, PEP can freely go into cytoplasm via transporter to participate in GNG

Pathway Details

C



Key GNG enzymes

Numbers are on key on previous page – e.g., ① = lactate

-- Going from L ⟶ G is like a ship going up river through locks on a canal
-- Energy input is needed

Session 20 - Pentose Phosphate Pathway (PPP)

Roles:

1.) Cell's primary source of NADPH ≡ biosynthetic reductive cofactor (Malic enzyme = another source)

2.) Source of ribose for ribonucleotides (also, this is entry portal for metabolism of ribose from diet)

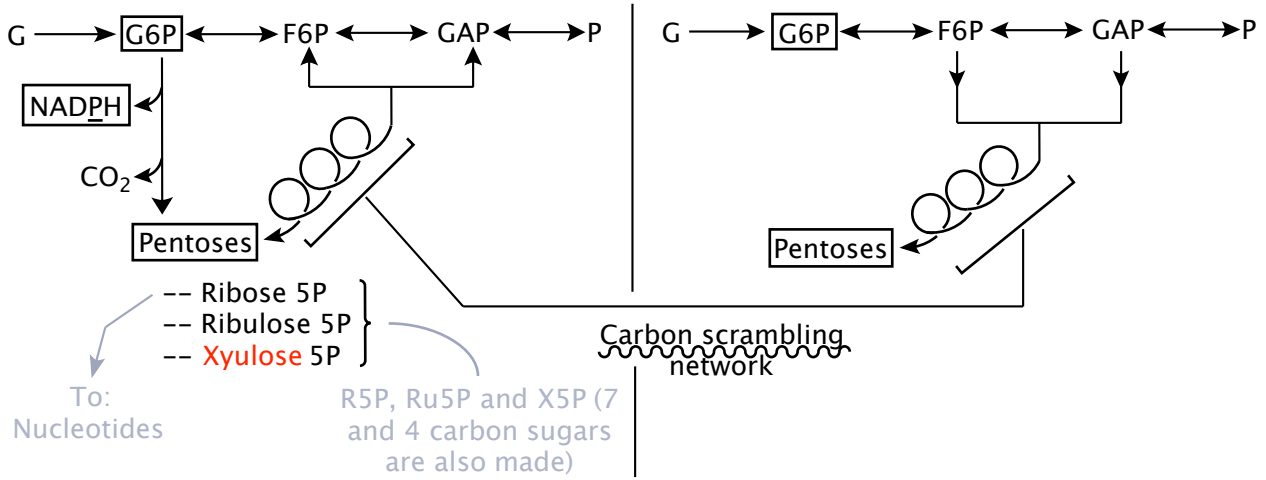
-- Highly expressed in tissues making lipid

-- Expressed in growing tissues (e.g. cancer)

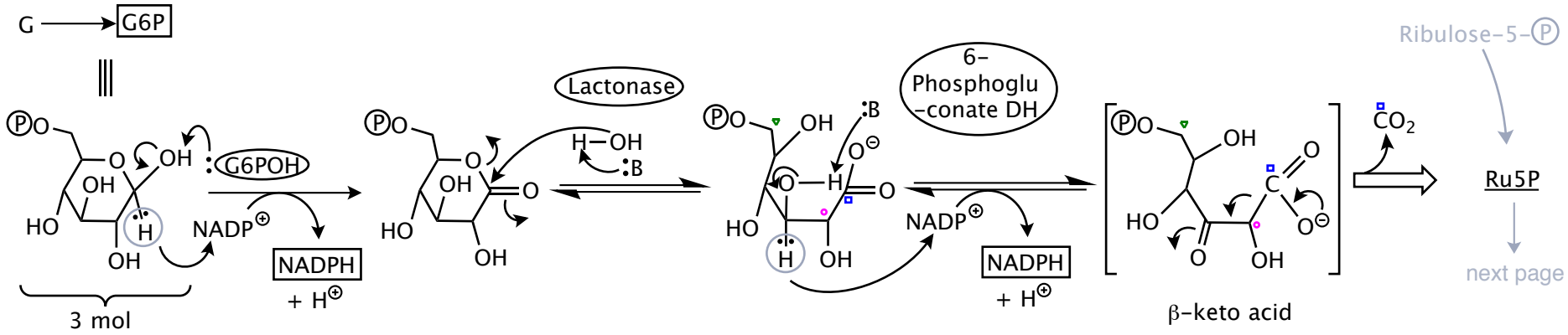
Pathway can run in either of two modes

A. Oxidative Mode: Need NADPH and Pentoses

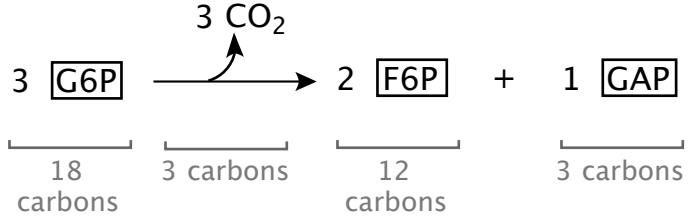
B. Non-oxidative Mode: Just need pentoses



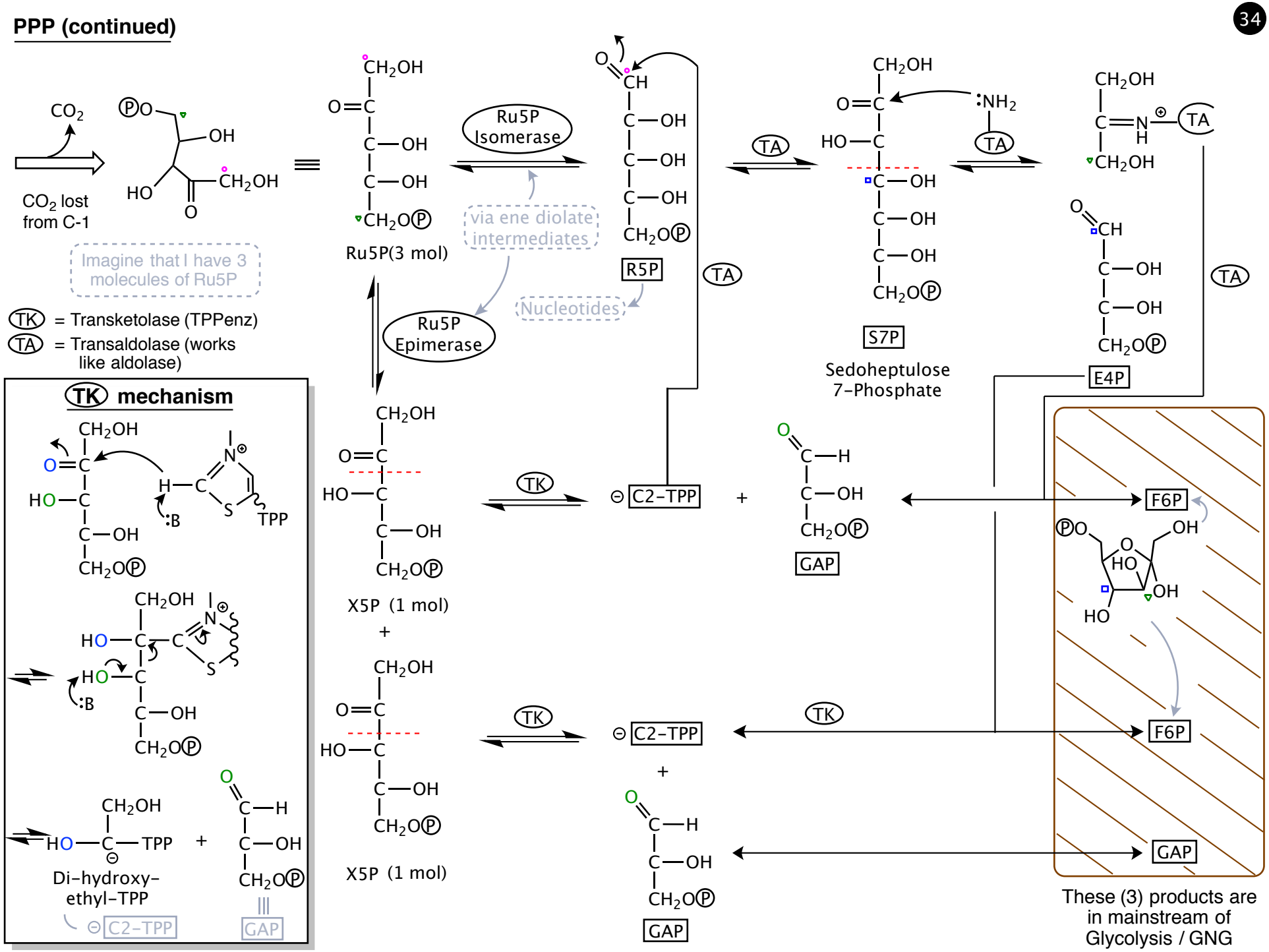
PPP Details (shorthand in 2 pages) -- This is cytosolic pathway



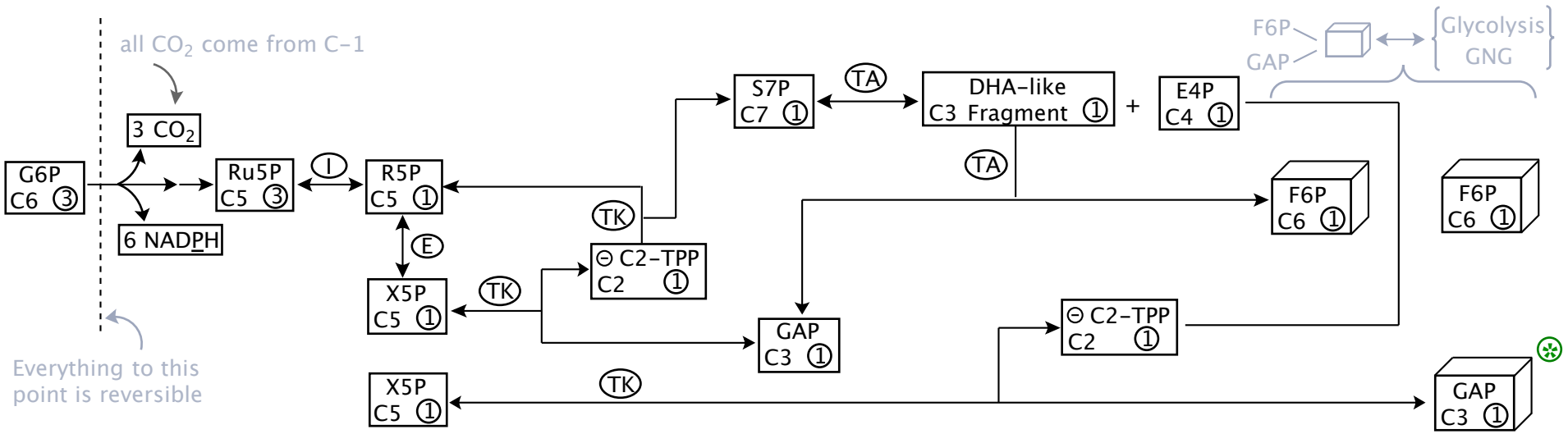
Typically the pathway is run by processing 3 mol of G6P



PPP (continued)



PPP Shorthand - Helps you see the mass balance



Summary Points on PPP

1.) Expressed in tissues when making lipid (and in growth)

2.) If run in oxidative mode, you could oxidize all carbons of glucose to CO_2 (if use GNG to get GAP and F6P back to G6P).

3.) PPP is entry point of dietary ribose into catabolism

4.) NADPH helps defend against oxidative stress (cofactor for glutathione reductase)

5.) Cytosolic pathway

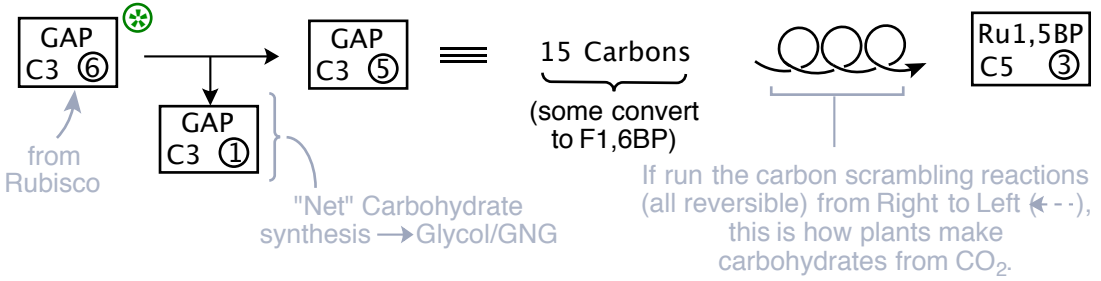
6.) G6PDH Rate determining step (Oxidative pathway) - stimulated by $NADP^{\oplus}$

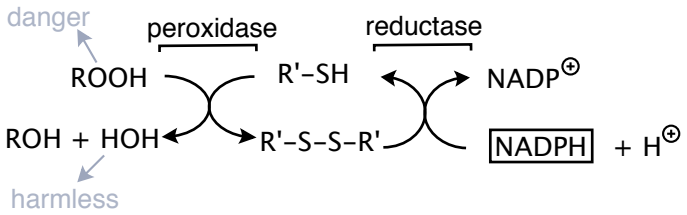
7.) Calvin Cycle = this series of reactions in reverse

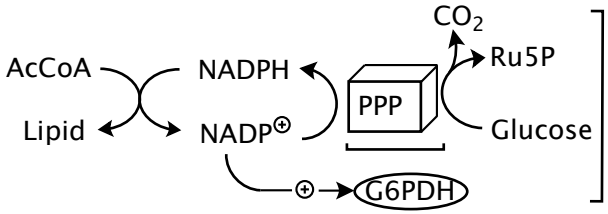
a.) Photosynthesis Ru1,5BP + CO_2 → → (2) PGA → → (2) GAP (Rubisco)

b.) Must regenerate catalytic molecule of Ru1,5BP (C5 sugar)

c.) Take 6 molecules GAP (18 carbons)







B

Cell regulates generation of NADPH via sensng need for FA biosynthesis (and other cytoplasmic NADPH regulated reactions)

= Glu Reductase
= RNR

Session 21 - Regulation of Metabolism

A

-- To now, 5.07 has been at the molecule to cell level

-- Now we consider cell to organ and organ to organism levels

Pathways (and sites of regulation)

B

1.) Glycolysis
(GK / HK, PFK-1 / F16BPase, PK)

2.) TCA
(PDH; all steps that make NADH)

3.) GNG
(PC, F16BPase via F2,6BP, GS / GP)

4.) FA Catabolism
(MalCoA ⊣ CAT-I)

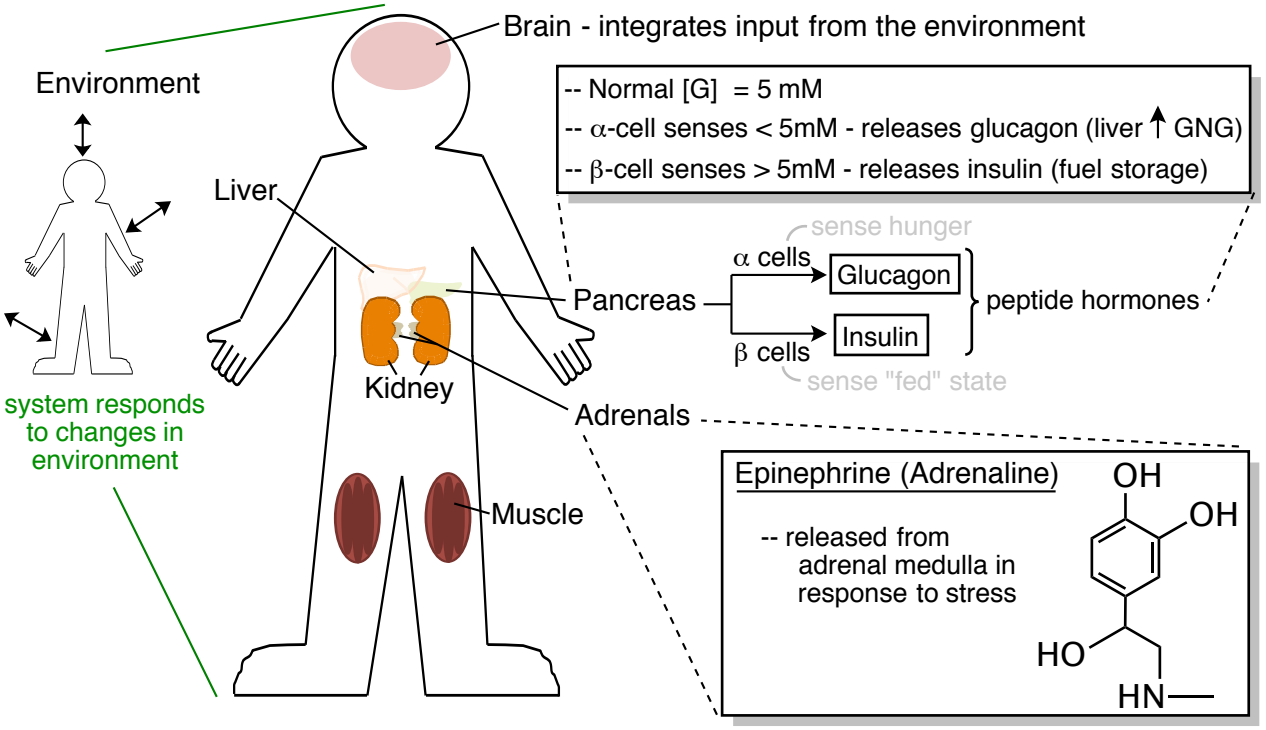
5.) FA Biosynthesis
(ACC activated by Insulin)

6.) PPP
(G6PDH stimulated by $NADP^{\oplus}$)

7.) ET/Oxidative Phosphorylation
(ADP [↑] needed for $H^{\oplus}$ flow; NADH or $FADH_2$ needed for $e^{\ominus}$ flow to oxygen)

Key organ systems and hormones

C



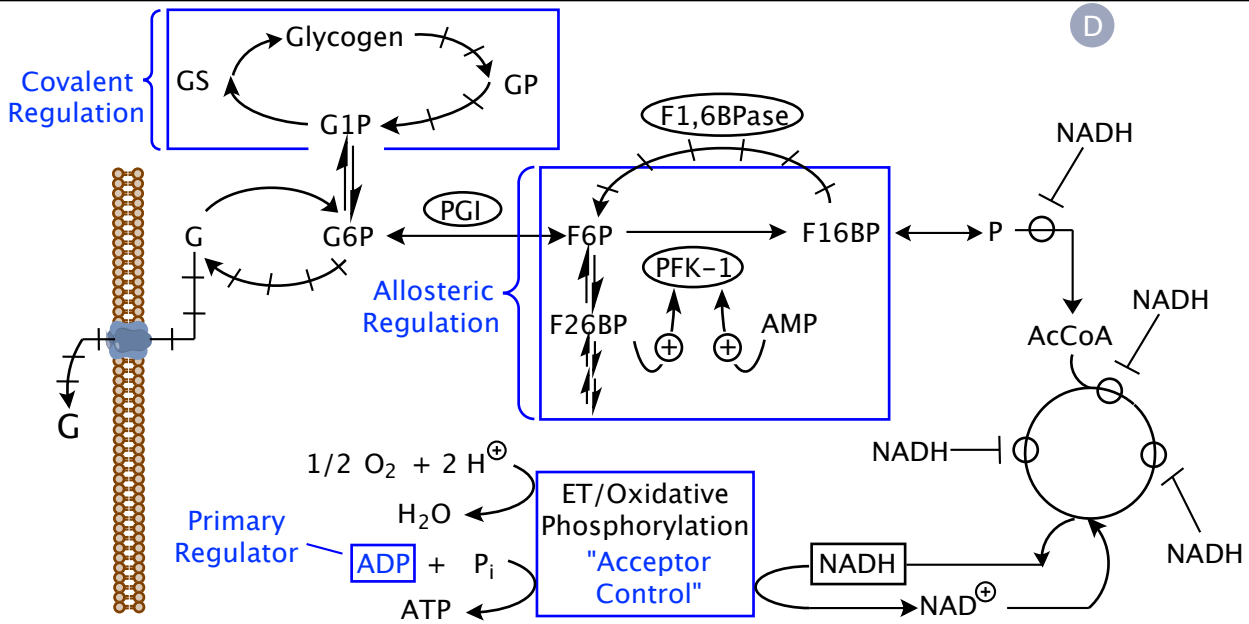
General Paradigms of Pathway Control

1.) Covalent ("Hormonal") Control
- covalent modification of enzyme affects activity
- e.g., as we saw with GP in session 8

2.) Allosteric Control
- model = Hemoglobin
- others: PFK-1 very sensitive to [AMP] (AMP ↑ activity) as well as F26BP

3.) "Acceptor Control"
- ADP = "Acceptor" of P_i in ET/Oxidative Phosphorylation
- we already covered this in detail

D



Paradigm I: Covalent Control

(A)

GS / GP Regulation = Primarily by covalent post-translational modification of the enzymes

GS—OP	inactive	Prevents "futile cycling"
GP—OP	active	

Scenario = Stress

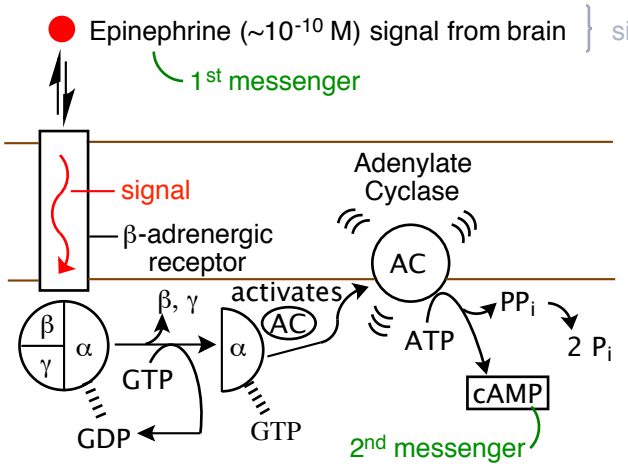
-- Liver (makes and stores fuel)
- instructed to liberate G from glycogen (G → other organs)

-- Muscle (run away or otherwise deal with stress)
- instructed to absorb glucose and liberate G from glycogen for local (in-muscle) use by glycolysis

Muscle or Liver Cell - Top part of pathway is similar

(B)

A.) Primary messenger (Epinephrine) to secondary messender (cAMP) ✻



1.) Hormone ⇌ β-adrenergic receptor
- signal transduced to G-protein

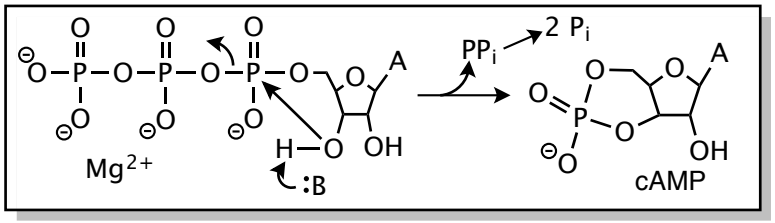
2.) α subunit released
- It is replaced by GTP
- It is now "active"

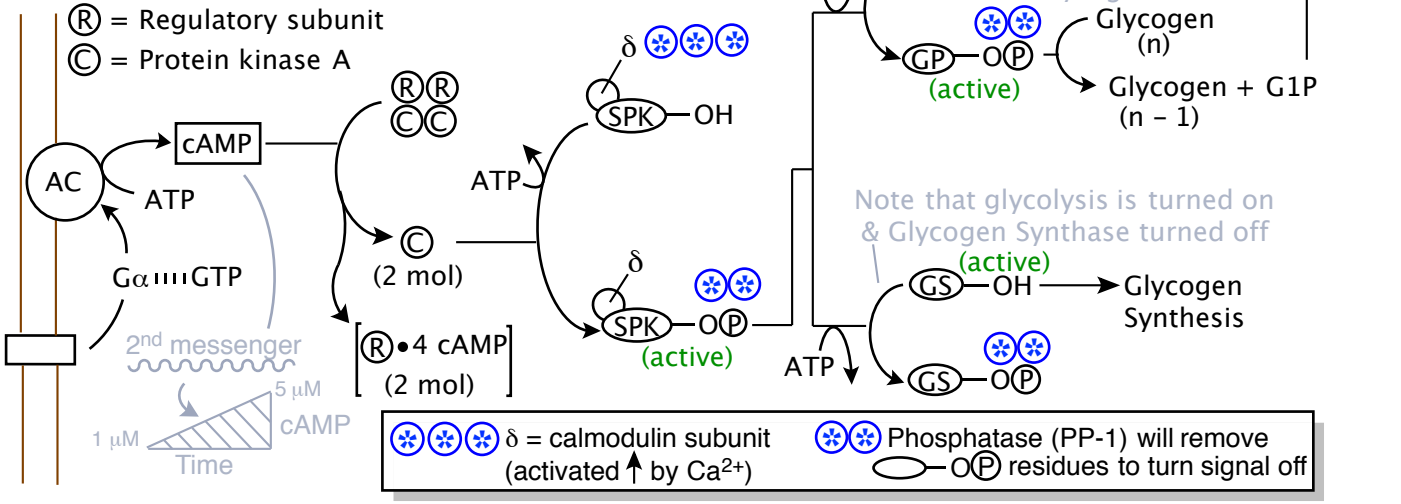
3.) α-GTP activates AC

4.) AC makes cAMP (second messenger)

✻ Glycogen (senses hunger) will do pretty much the same thing

B.) Second messenger initiates a "Kinase Cascade"





(C)

1.) Ⓒ ≡ cAMP dependent protein kinase ≡ PK-A
- It is inhibited by Ⓡ (its regulatory protein)

2.) PK-A phosphorylates SPK
SPK ≡ Synthase-phosphorylase kinase
(Synthase = GS; Phosphorylase = GP)

3.) SPK—OP = active kinase

4.) In liver - glycogen → G → other organs

5.) In muscle - use G for energy (run away from stressor)

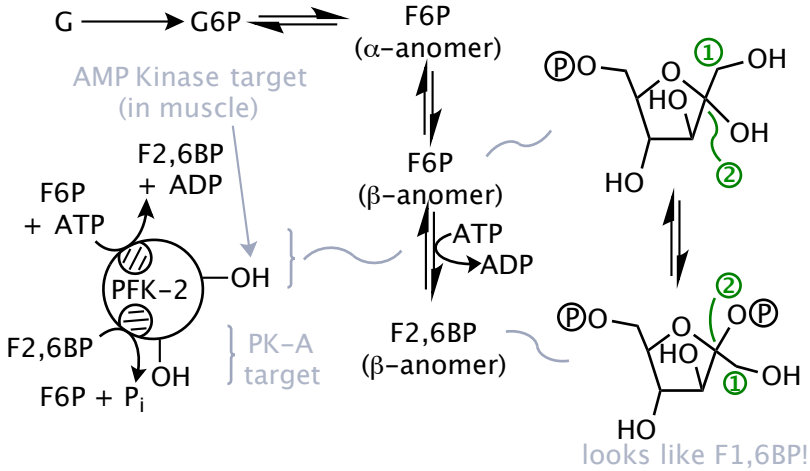
Paradigm II: Allostery (mostly)

A

PFK-1 / F16BPase Regulation = Primarily by small molecule allosteric effector (or competitive inhibitor)

-- F2,6BP = primary effector of glycolysis/GNG (AMP also has an effect)

-- Made by PFK-2 ≡ Complicated enzyme

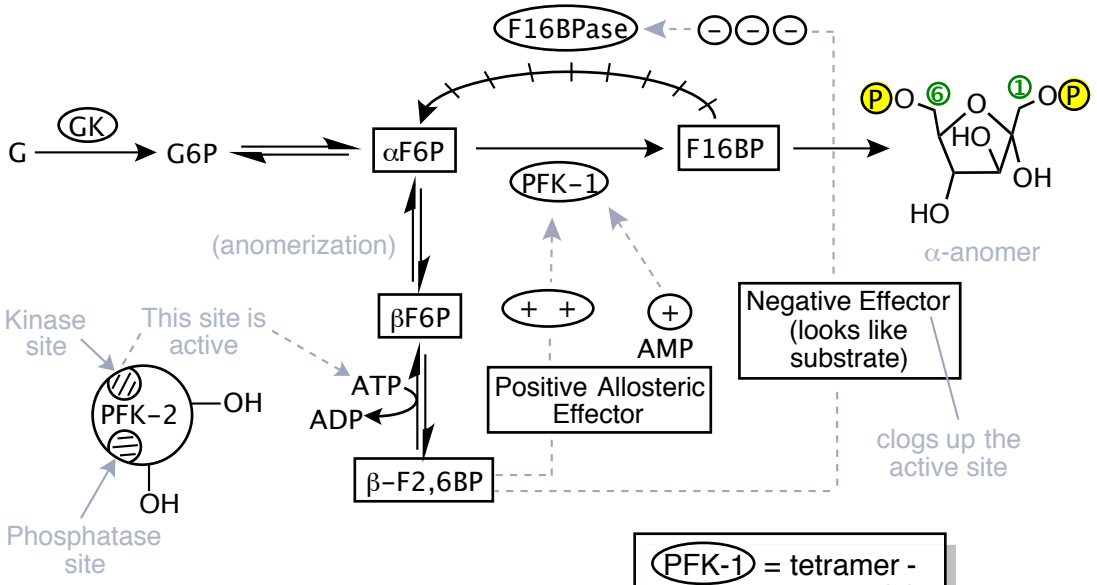


Scenario 1 (Liver, pre-stress state)

B

-- Net flux favors Glycolysis

Glycolysis ~ Net Flux



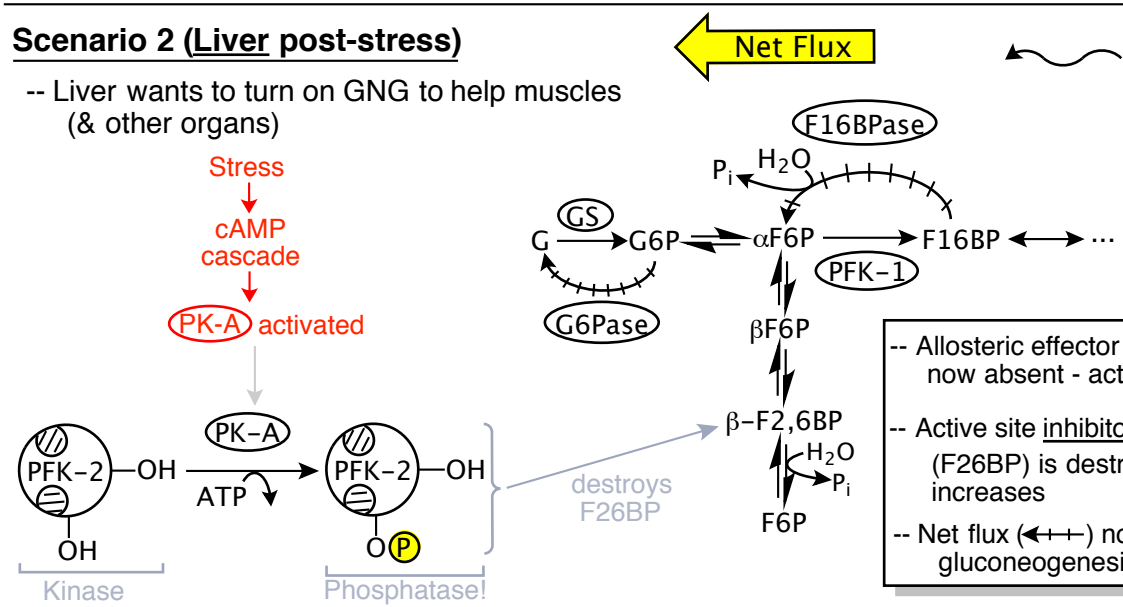
PFK-1 = tetramer - suggests cooperativity

Scenario 2 (Liver post-stress)

C

-- Liver wants to turn on GNG to help muscles (& other organs)

Net Flux ~ GNG



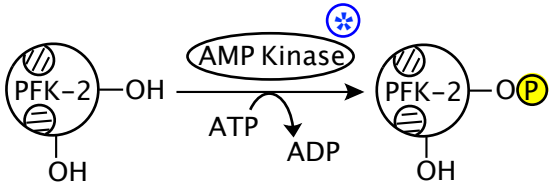
-- Allosteric effector of PFK-1 is now absent - activity drops

-- Active site inhibitor of F16BPase (F26BP) is destroyed - activity increases

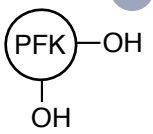
-- Net flux (←+→) now favors gluconeogenesis

Scenario 3 (Muscle post-stress)

D



This PFK does the same thing as in Panel A



but it is more active.

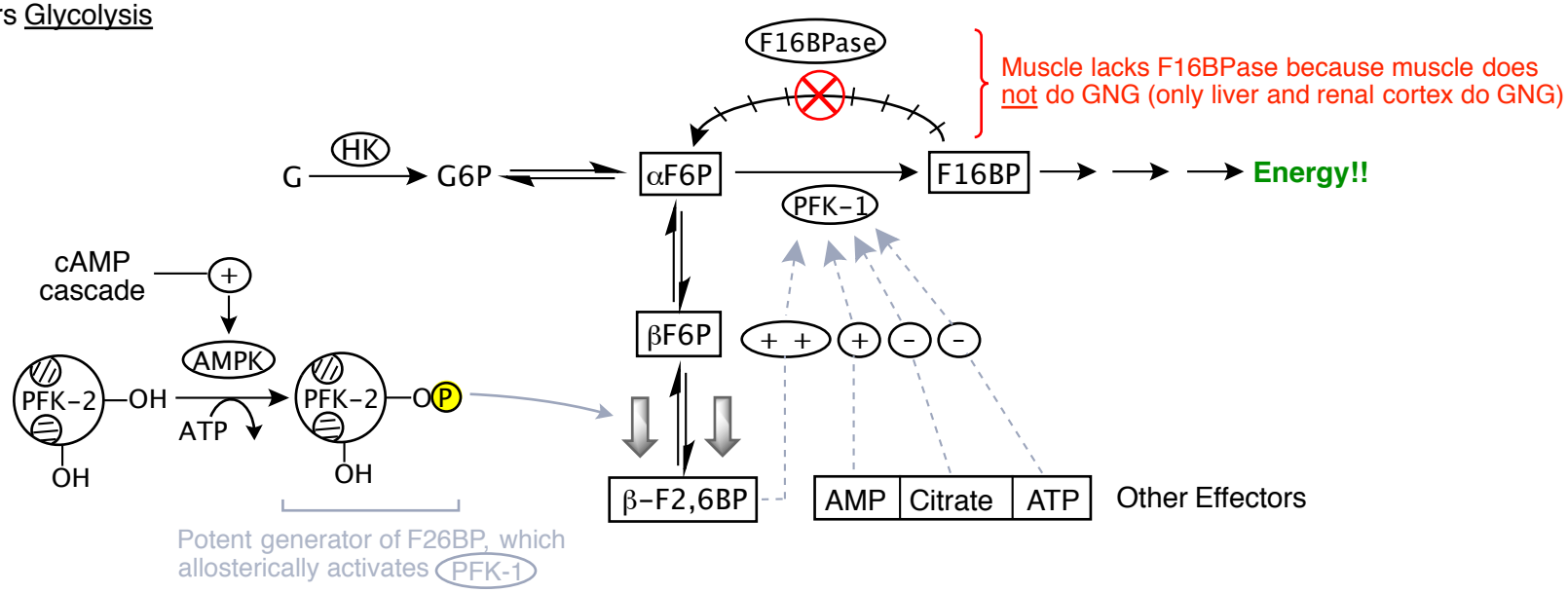
AMPK = energy sensing kinase

Scenario 3 (Muscle post-stress) - continued

Glycolysis ~~> Net Flux

A

-- favors Glycolysis



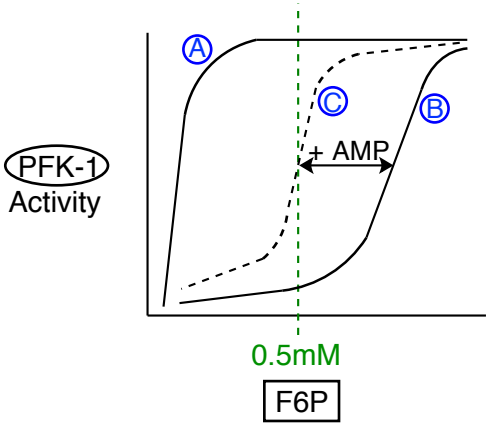
B

PFK-1

-- PFK-1 is a tetramer, is subject to allosteric regulation, as well as covalent regulation

-- PFK-1 = tetramer ⟹ cooperativity

-- A lot is known about its activity in presence of AMP (senses energy need) and ATP (energy surplus).

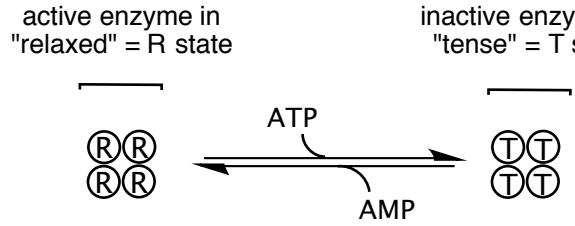


Ⓐ low or no ATP (not realistic)

Ⓑ 1mM ATP (typical = 1-10 mM)

Ⓒ condition Ⓑ plus 0.1mM AMP

C



-- AMP (allosteric activator) binds better to Ⓡ than to Ⓣ

-- Shifts ⇌ to more active protein

-- ATP binds better to T state