MACQUARIE University

Tutorial 5

Sugar Metabolism: Citric Acid Cycle, Electron Transport Chain & Oxidative Phosphorylation

Amino Acid Metabolism

BMOL2201/6201

Tutorial 5 Aims

- Understand how the carbon atoms in **pyruvate** can be burnt off in the **Citric Acid Cycle**
- Describe the aerobic pathways in cellular respiration: **Electron Transport Chain** and **Oxidative Phosphorylation**
- Understand **Amino Acid Metabolism**:
  - How we can use dietary proteins to provide energy
  - How amino acids are made to make our proteins
- Learn how to answer a **Short Answer Question** – exam prep

Mitochondrial membranes

- **Two bilayers**
- **Outer membrane** allows free diffusion of large molecules: similar to plasma membrane
- The **intermembrane space** is similar to the cytosol.
- **Inner membrane** is almost ~75% protein! And almost impermeable (except for O_2, CO_2 and H_2O).

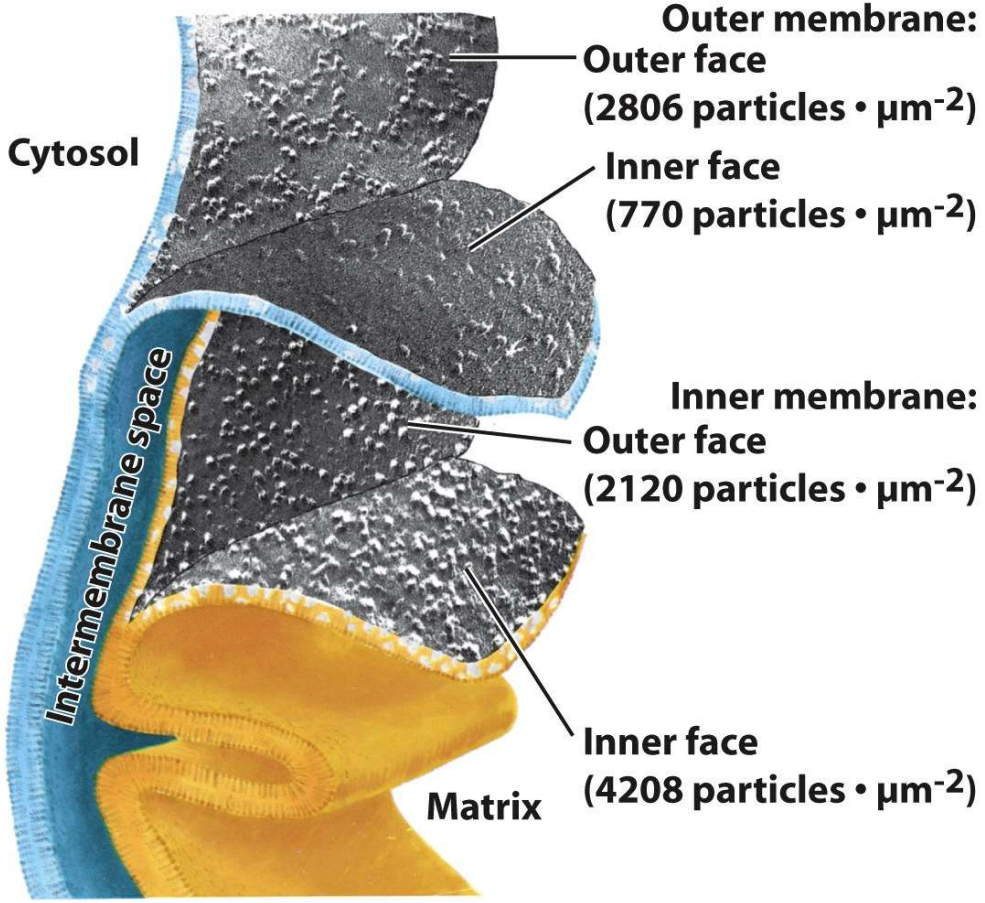


Figure 18-4
Courtesy of Lester Packer, University of California at Berkeley

- Need transport proteins for moving ATP, ADP, pyruvate, Ca^{2+} and phosphate
  - ➢ Concentration gradients!

Citric Acid Cycle follows Glycolysis

- The **citric acid cycle** (CAC) is a cyclical process in the mitochondria, that converts acetyl groups derived from:
  - ➢ carbohydrates,
  - ➢ fatty acids, and
  - ➢ amino acids

  to
  - ✓ **CO_2**, and
  - ✓ **NADH, $FADH_2$**, and **GTP**.
- The entry point is **acetyl-CoA** derived from **pyruvate** at the end of glycolysis, or other sources (amino acid or fatty acid breakdown)

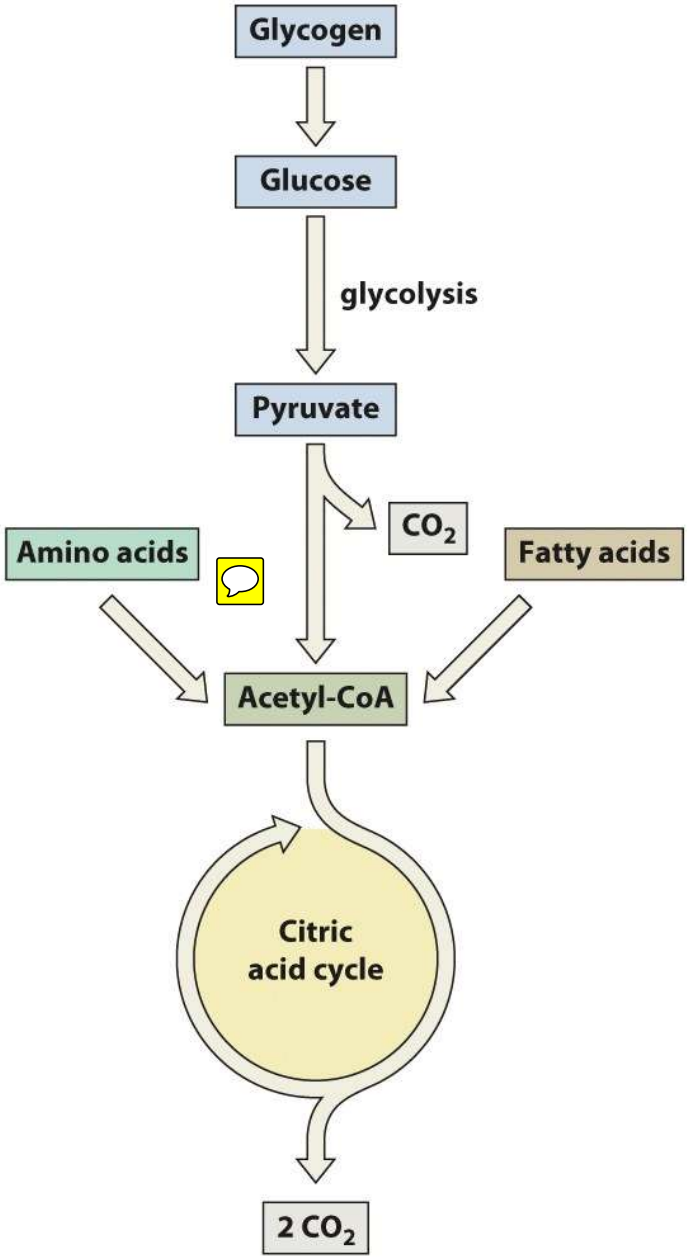
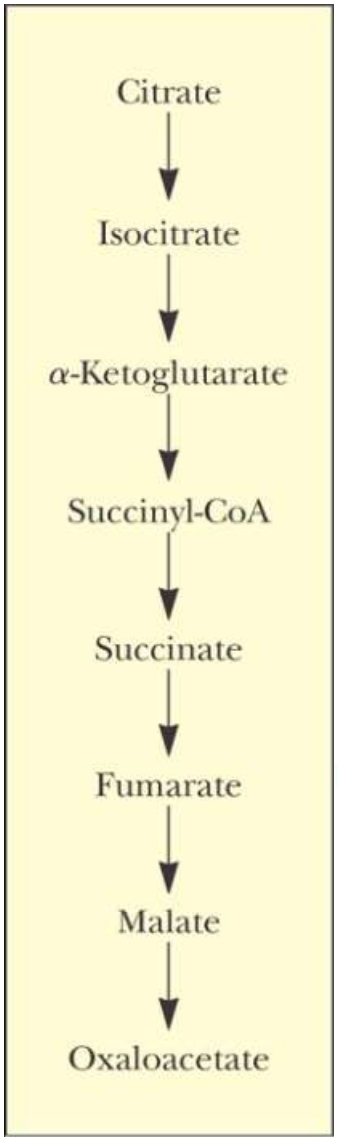
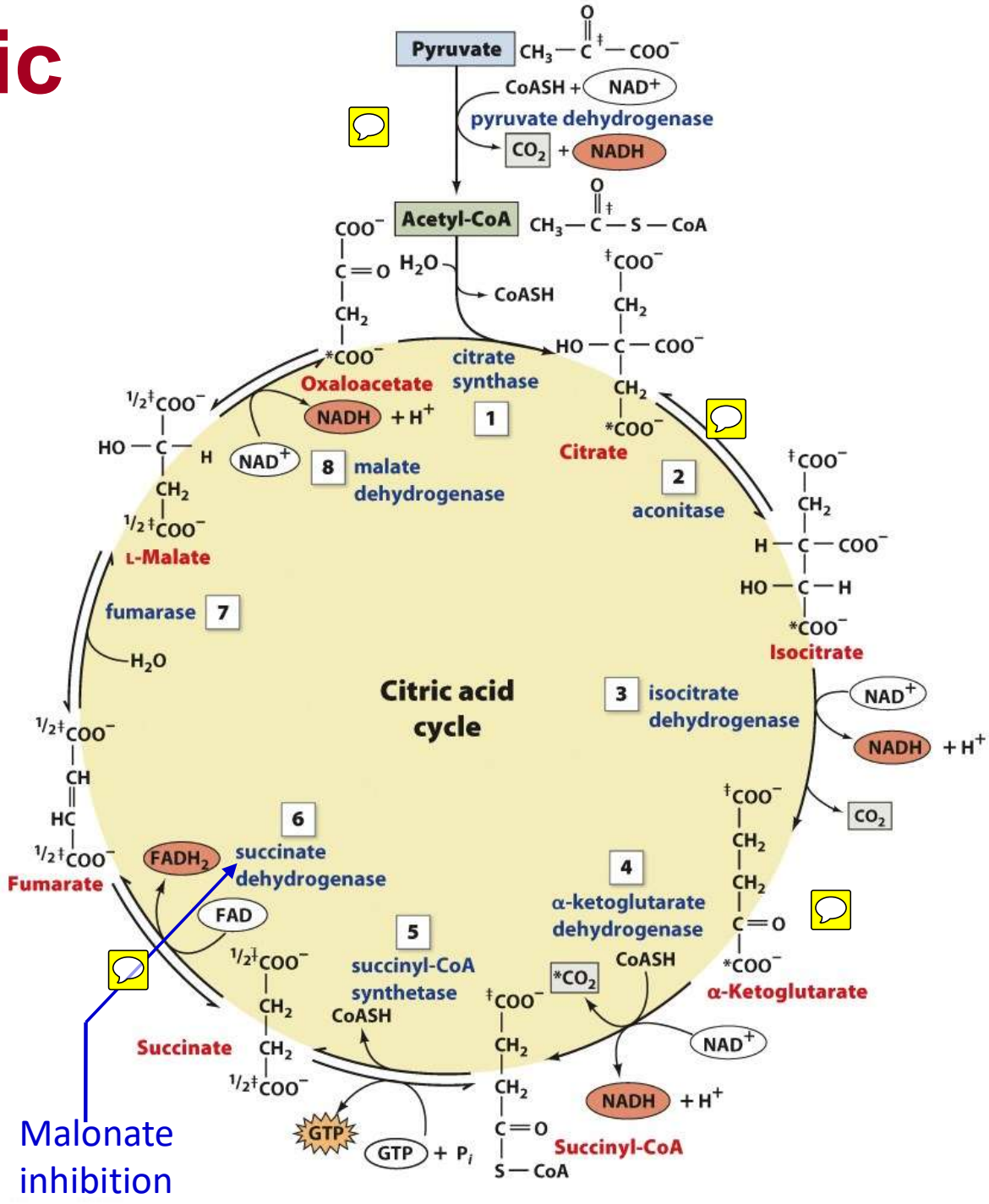


Figure 17-1

Reactions of Citric Acid Cycle

- **8 reactions** in the cycle itself
- **1 reaction** pre-cycle
- **9 enzymes**!
- ***Succinate dehydrogenase*** is a membrane protein complex, shared with ETC.
- **3 CO_2** produced – 1 from pyruvate and two from the cycle itself – not from the entering acetyl group.
- **4 NADH and 1 $FADH_2$**
- **1 GTP (= 1 ATP)**





Energetics of CAC reactions

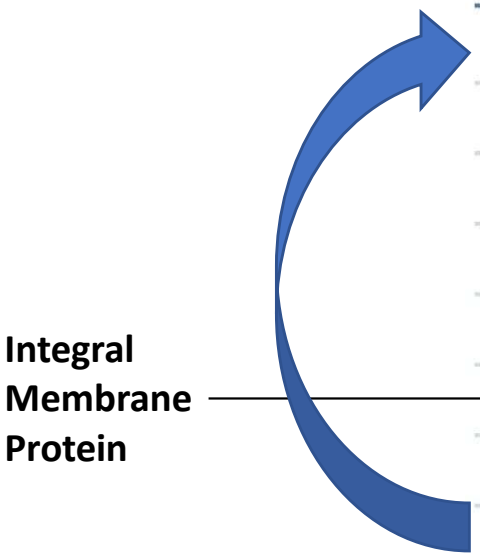


TABLE 17-2 Standard Free Energy Changes ($\Delta G^{\circ\prime}$) and Physiological Free Energy Changes (ΔG) of Citric Acid Cycle Reactions

Reaction	Enzyme	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})	ΔG (kJ · mol^{-1})
1	Citrate synthase	−31.5	Negative
2	Aconitase	~5	~0
3	Isocitrate dehydrogenase	−21	Negative
4	α-Ketoglutarate dehydrogenase	−33	Negative
5	Succinyl-CoA synthetase	−2.1	~0
6	Succinate dehydrogenase	+6	~0
7	Fumarase	−3.4	~0
8	Malate dehydrogenase	+29.7	~0

- **3 enzyme reactions** control the rate of the CAC.
- However, all CAC metabolites are present both in the mitochondria and the cytosol – so equilibrium conditions are assumed within the compartments.
- Note that the enzyme **succinate dehydrogenase** is membrane-bound, shared with Electron Transport Chain (as Complex II)
- **The last reaction is unfavourable**. However the next one (**1** in CAC) is highly favourable and drives CAC, with net $\Delta G < 0$.

Products of Citric Acid Cycle

- While CAC itself produces only **1 GTP**, the **reduced cofactors** pass on their electrons to the **electron transport chain** for generating ATP by **oxidative phosphorylation**
  - ❖ 1 NADH ~ 2.5 ATPs
  - ❖ 1 $FADH_2$ ~ 1.5 ATPs
- So **CAC** results in overall **10 ATPs**!

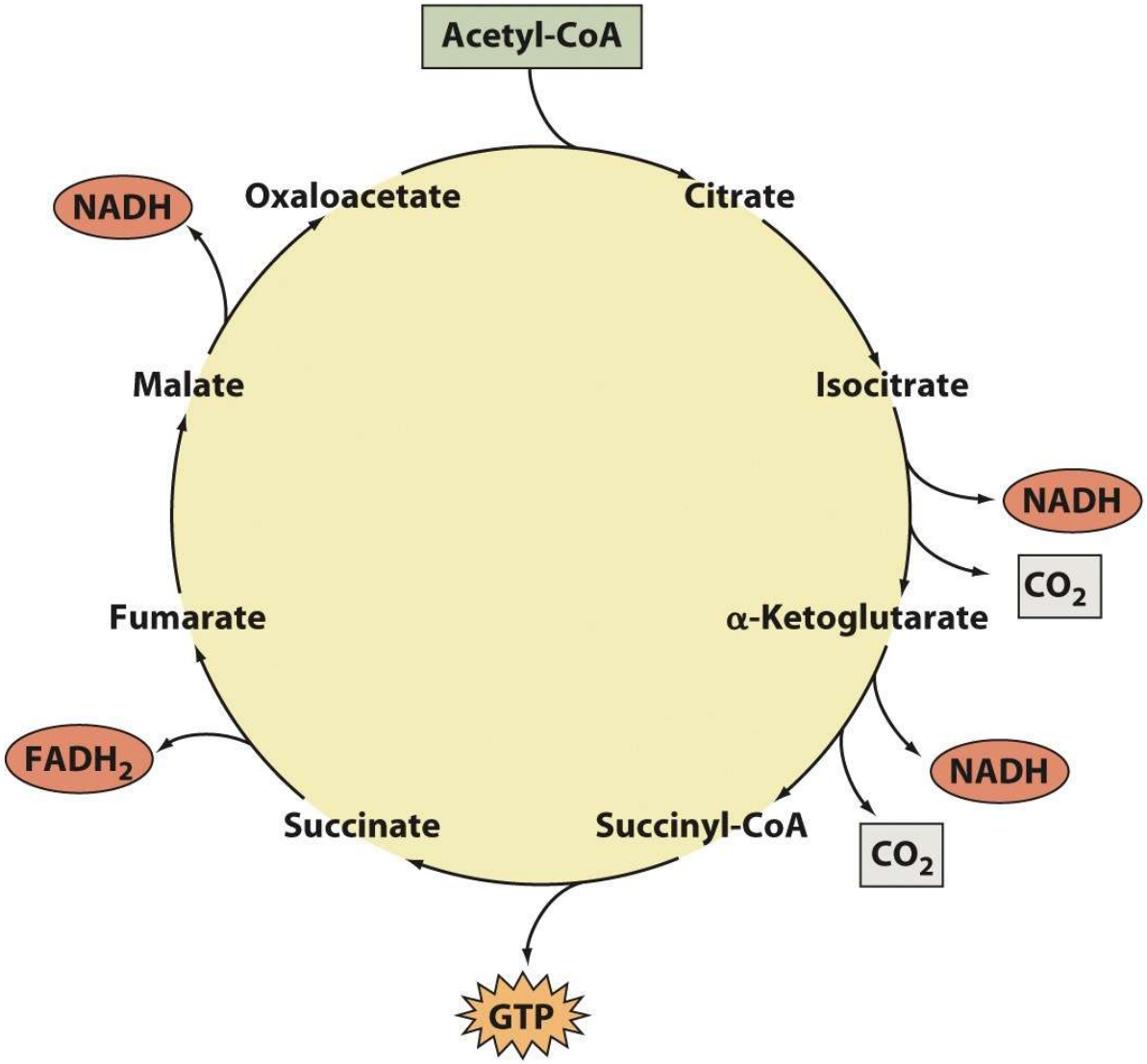
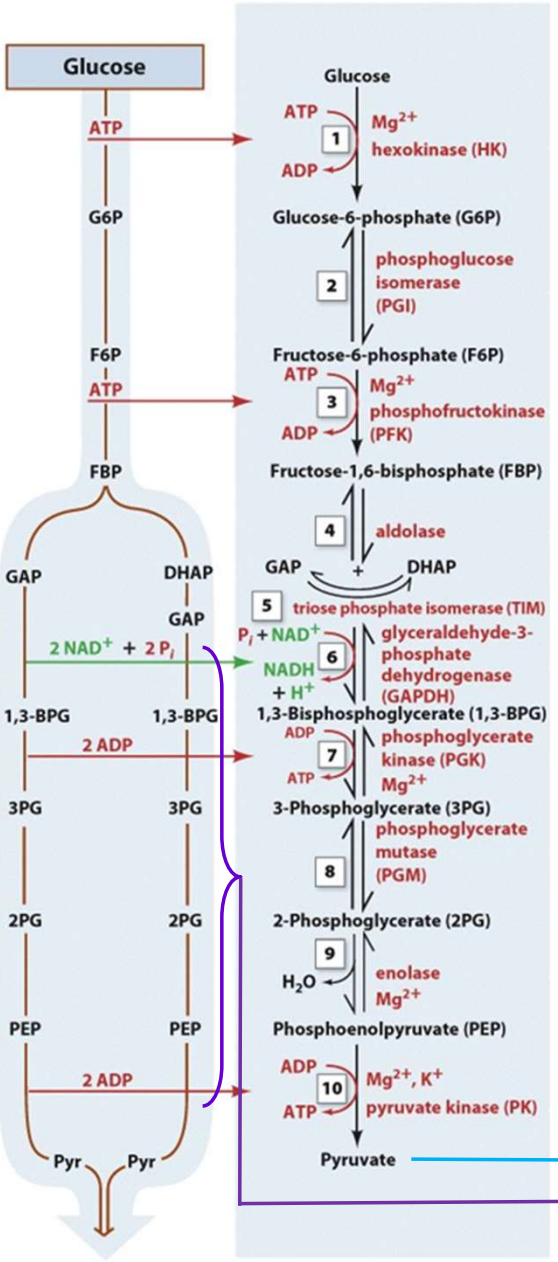
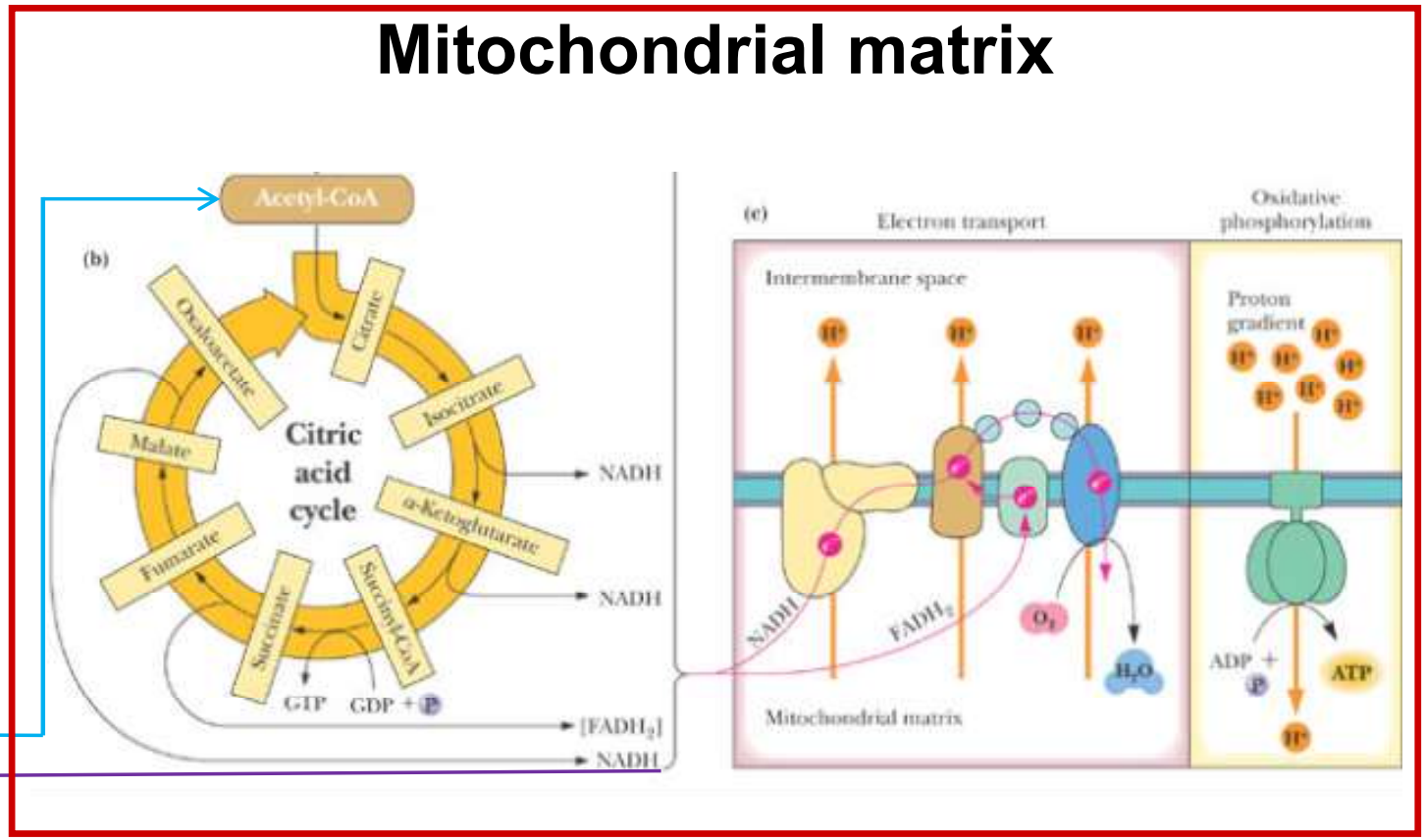


Figure 17-14

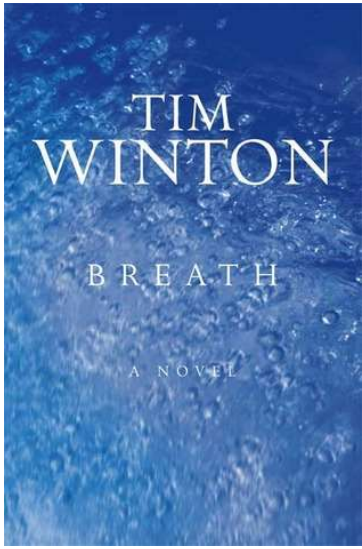
CAC links glycolysis to oxidative phosphorylation





CAC and ETC/OxPhos

1. CAC (completely anaerobic)
   - C atoms in pyruvate degraded to CO_2 producing reducing equivalents (NADH and $FADH_2$)
2. ETC/Oxidative phosphorylation (aerobic)
   - The electrons carried by NADH and $FADH_2$ (produced in glycolysis and CAC) are transferred to oxygen – breathing in oxygen!



- These two processes are intimately linked because the NAD^+ and FAD used to make NADH and $FADH_2$ must be regenerated to allow glycolysis and CAC to operate.

Short answer question: exam prep

- The final exam (80 marks: 40% of assessment) is in two parts:
  1. 4 short answer questions: each worth 10 marks
  2. 40 MCQs: each worth 1 mark.
- So far you have been doing MCQs.
- So we now have a Short Answer question for you to try out – you will need to provide complete sentences.
- The SA question is usually composed of several parts, so you can score lots of marks.

SA question: Citric acid cycle

a. What are the final products generated by one complete round of the citric acid cycle (also known as the tricarboxylic acid or Krebs cycle)? (2 marks)?

SA question: Citric acid cycle

b. The **last reaction** in the citric acid cycle:

$$\text{L-malate} + NAD^+ \rightarrow \text{oxaloacetate} + NADH + H^+$$

is **energetically unfavourable** ($\Delta G^{o\prime} = 29.7$ kJ mol^{-1}).

(i) How does the cycle **continue to function** in organisms, with oxaloacetate being continually produced? (1 mark)

(ii) Which **enzyme** in the citric acid cycle **uses** the **oxaloacetate** produced in the above reaction **as substrate**? (1 mark)

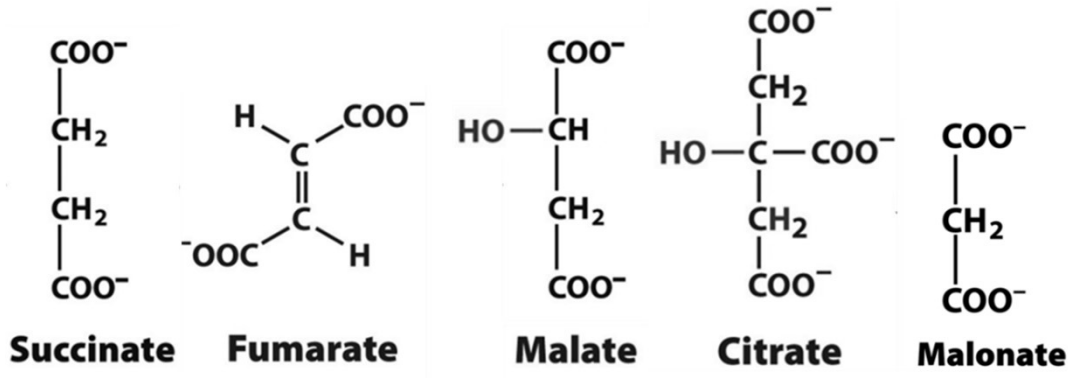
(iii) What is the **product** formed from **oxaloacetate**, by the **enzyme** in **b.(ii) above**? (1 mark)

SA question: Citric acid cycle

c. Under **normal cellular conditions**, the **concentrations** of the **metabolites** in the citric acid cycle **remain almost constant**. List **any one process** by which we can **increase** the **concentration** of the citric acid cycle intermediates. (2 marks)

SA question: Citric acid cycle

d. Albert Szent-Györgyi discovered that muscle tissue suspensions **oxidised** the **organic ions**: **succinate, fumarate, malate** and **citrate**, in the **citric acid cycle**. The oxidation was **blocked** by **malonate**. The **structures** of these **organic ions** are given below.



(i) Which **organic ion** in the citric acid cycle is **most similar** to **malonate**? (1 mark)

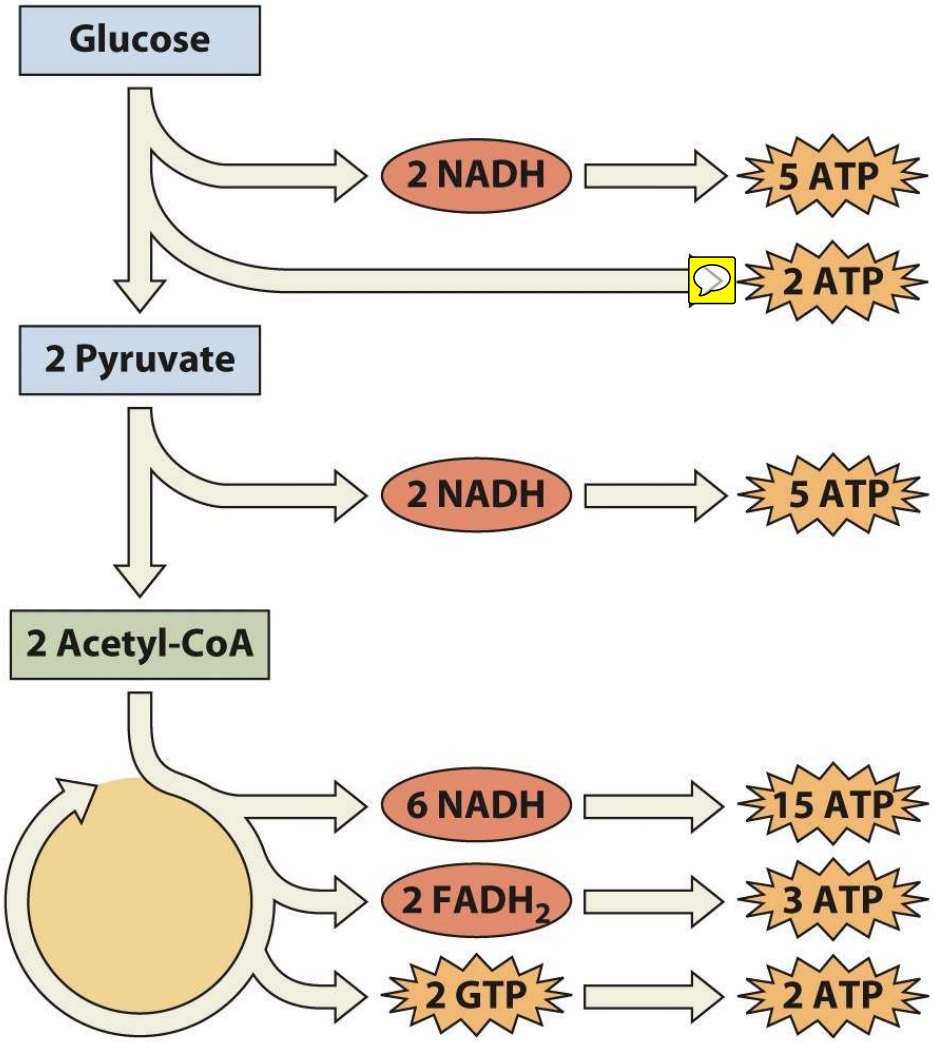
(ii) What kind of **inhibition** is caused by **malonate**? (1 mark)

(iii) Which **organic ion** would **accumulate** because of this **inhibition**? (1 mark)

Electrons are Funneled into ATP Synthesis from glycolysis

- 1 molecule of glucose going through glycolysis and CAC to complete oxidation in ETC-OxPhos, the theoretical amount of energy produced is:

  32 ATPs.
- Of these 20 ATPs (62.5%) come from CAC alone.
- So, although glycolysis and CAC are anaerobic, max. energy extraction comes for **aerobic reactions.**



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Amphibolic Functions of CAC

- CAC is both **catabolic** and **anabolic**: amphibolic!
- **Catabolic :** degradative; intermediates are required only in small quantities to maintain the cycle.
- **Anabolic** because many biosynthetic pathways use CAC intermediates:
  - Gluconeogenesis
  - Fatty acid biosynthesis
  - Amino acid biosynthesis

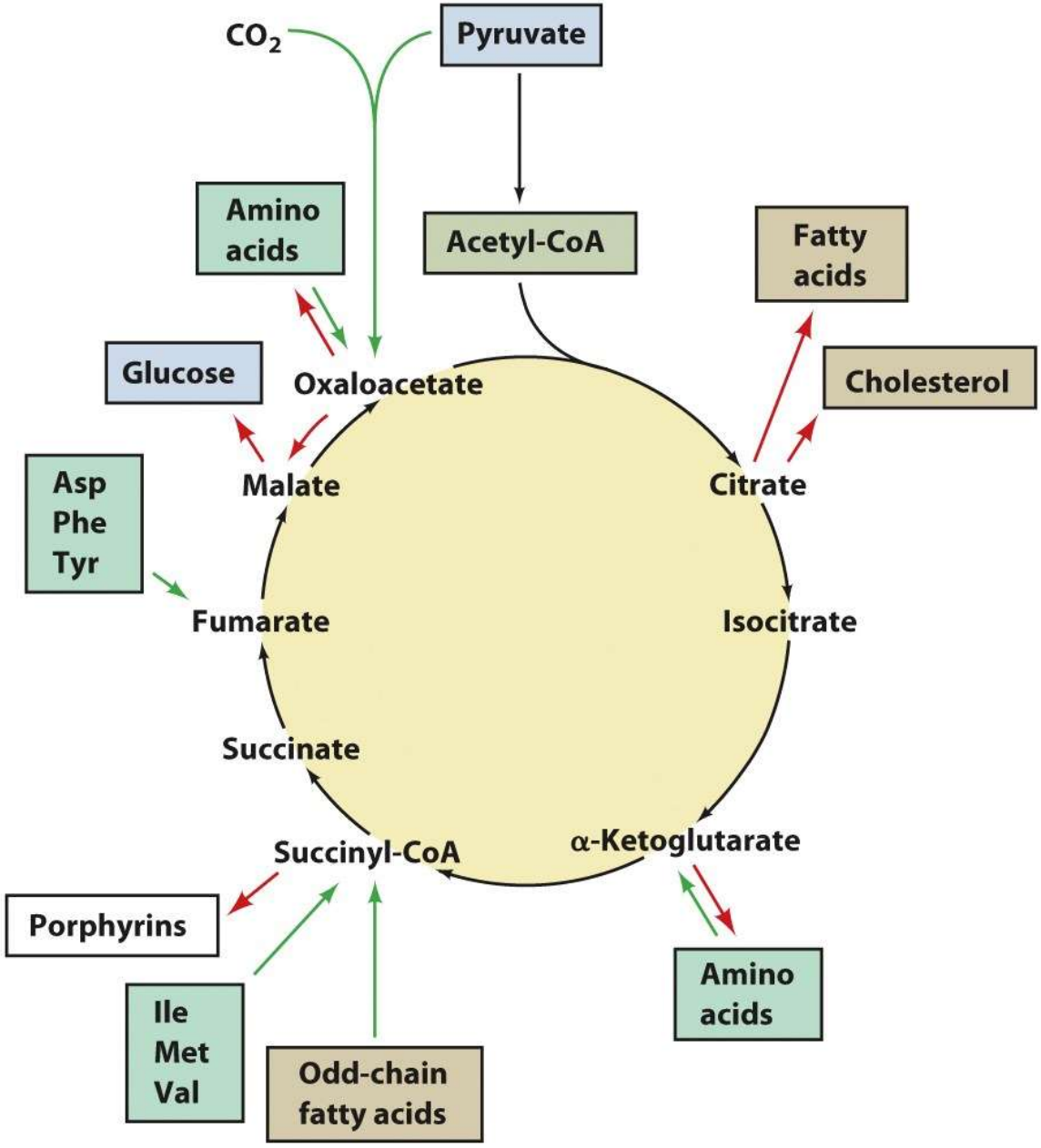


Figure 17-17

Q1:

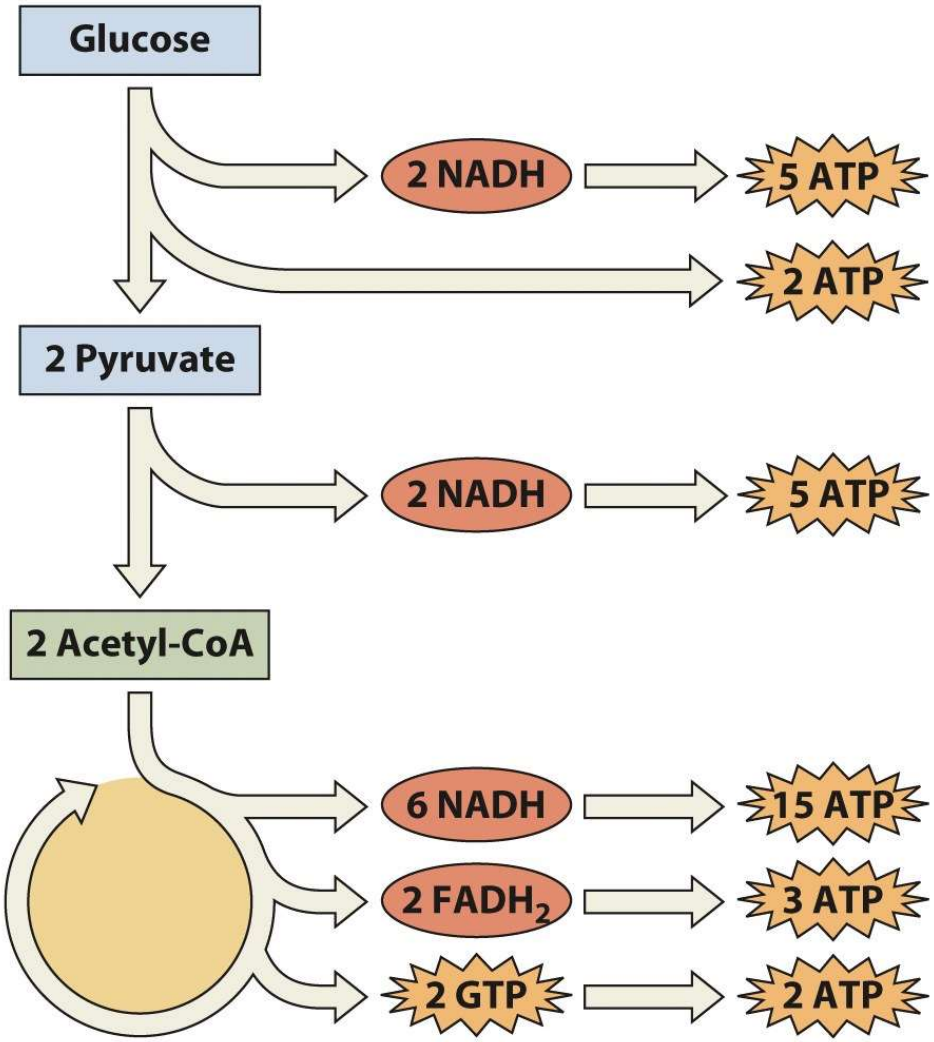
Which of the following statements about the CAC is TRUE?

A. Only one reaction in the TCA cycle directly produces the ATP equivalent, GTP.
B. There are 4 reducing equivalents produced in every round of the TCA cycle.
C. Each round of the TCA cycle produces two CO2 molecules.
D. One of the enzymes in the TCA cycle is membrane bound.
E. All of the above

Electrons are Funneled into ATP Synthesis from glycolysis

- From 1 molecule of glucose going through glycolysis followed by citric acid cycle to complete oxidation in ETC-OxPhos, the theoretical amount of energy produced is:

  32 ATPs.
- Of these 20 ATPs (62.5%) come from CAC alone.
- So, although glycolysis and CAC are anaerobic, max. energy extraction comes for aerobic reactions.



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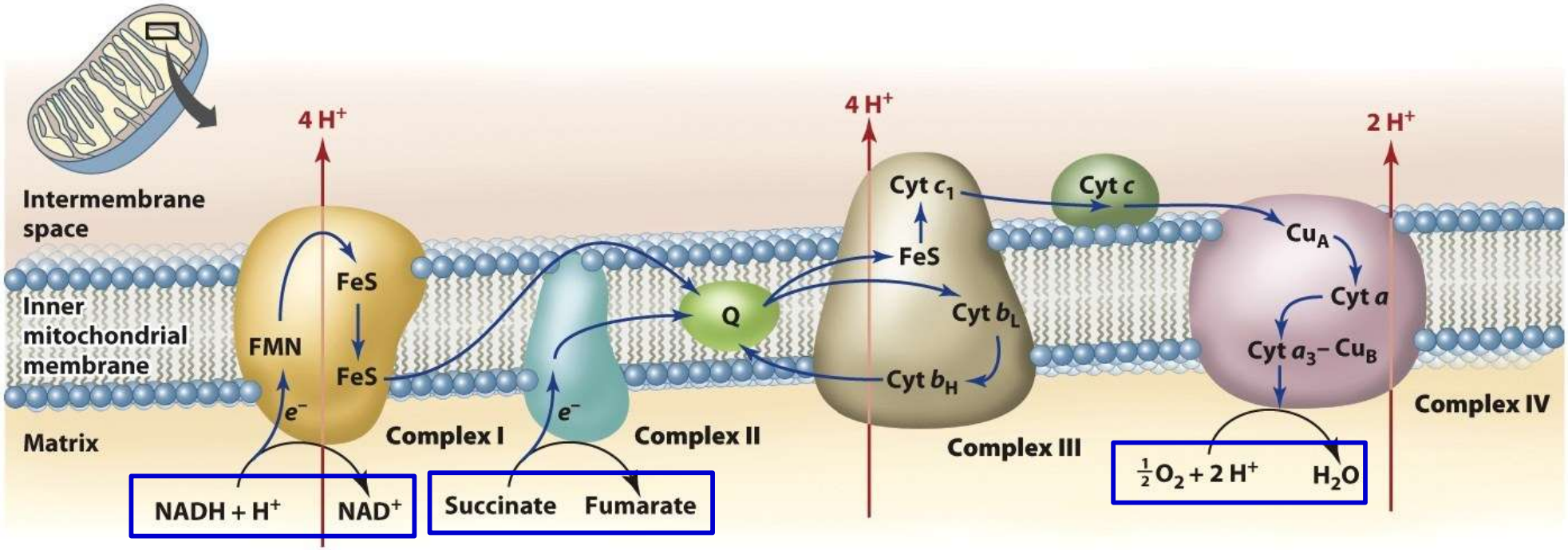
Mitochondrial Electron-Transport Chain

- **4 membrane-embedded redox proteins**: Complexes I, II, III, IV
- **2 mobile electron carriers:**
  - lipophilic coenzyme Q (CoQ or Q for ubiquinone) and
  - the peripheral membrane protein cytochrome c (Cyt C)
- **3 redox chemical reactions** occur here



Q2:

Which one of the complexes in the electron transport chain does not have a chemical reaction?

A. NADH dehydrogenase (complex I)

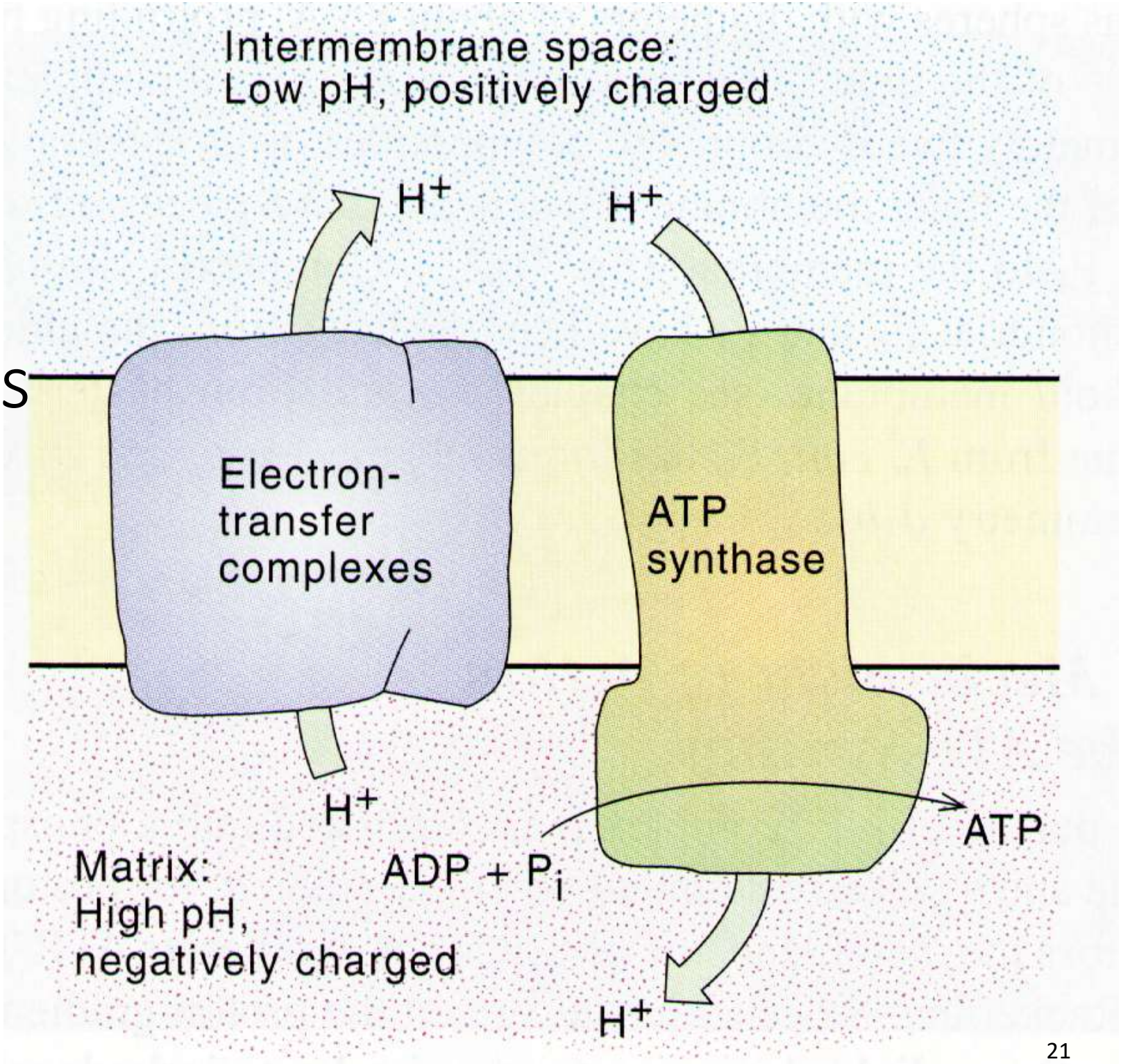
B. succinate dehydrogenase (complex II)

C. Ubiquinone: cytochrome c oxidoreductase (complex III)

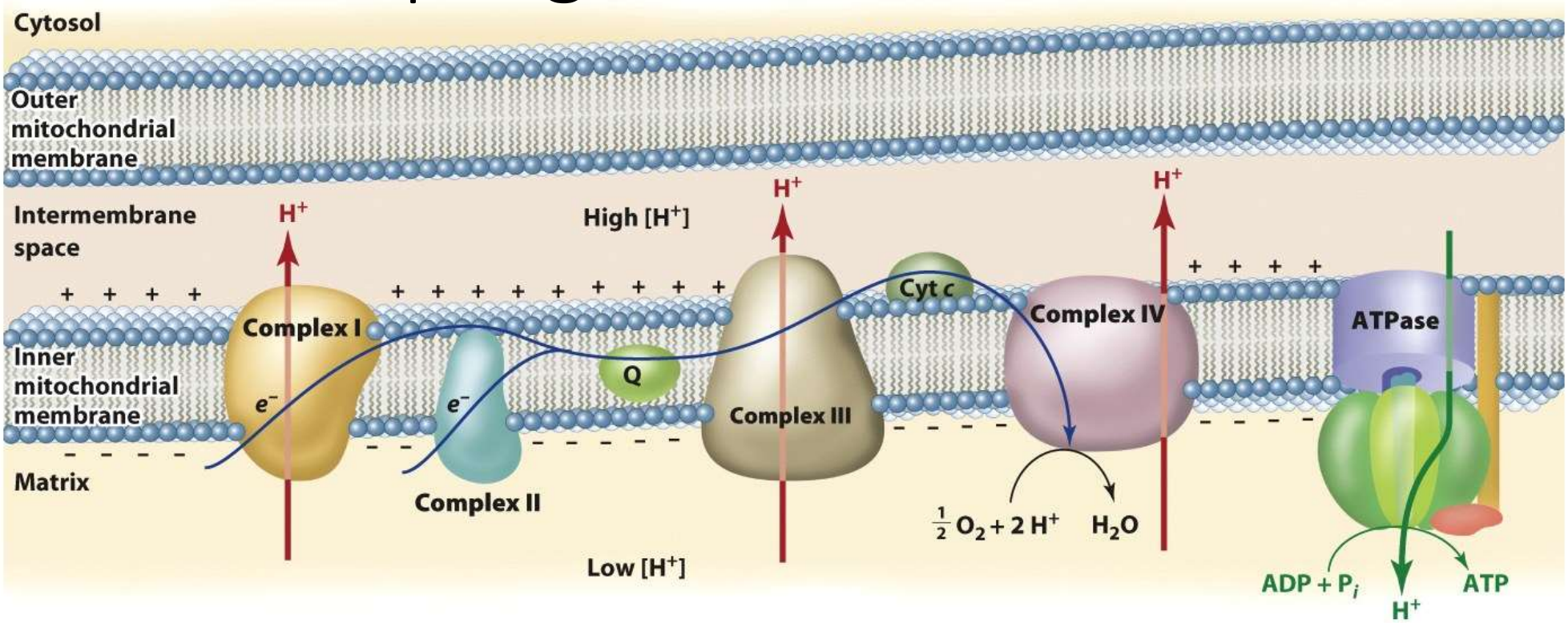
D. Cytochrome oxidase (Complex IV)

E. none of the above.

ETC works with OxPhos



Coupling of ETC and OxPhos



- **Complexes I, III and IV pump protons** into the **intermembrane space** – increasing [H^+] and positive charge
- The **matrix loses [H^+]** and accumulates negative charge
- ✓ **Charge difference** across the **inner mitochondrial membrane**: electrochemical gradient
- Powers **Complex V:** ATP synthase, **pumping out H^+**

Summary of electron and proton flow in ETC/OxPhos

Electron flow

- Complex 1 to CoQ
- Complex II to CoQ
- CoQ to Complex III
- Complex III to Cyt c
- Cyt c to Complex IV

Proton flow

- Matrix to inner membrane space
    1. Complex I
    2. Complex III
    3. Complex IV
- Inner membrane space to matrix
    - Complex V

Summary of ETC/OxPhos chemical reactions:

- Complex I: redox reactions between NADH and flavin mononucleotide

  $2\ NADH + FMN \rightarrow 2NAD^+ + FMNH_2$

- Complex II: redox reactions between succinate and FAD (flavin adenine dionucleotide)

  $succinate + FAD \rightarrow fumarate + FADH_2$

- Complex III: no chemical reaction
- Complex IV: redox reactions between reduced cytochrome c (red. CytC) and oxygen

  $red.\ CytC + ½\ O_2 \rightarrow CytC + H_2O$

- Complex V: redox reactions between ADP and phosphate (P_i)

  $ADP + P_i + 4H^+ \rightarrow ATP + 2\ H_2O$

Q3:

2,4-dinitrophenol functions as an uncoupler of oxidative phosphorylation. Which one of the statements best describes its function?

A. It dissipates the proton gradient across the mitochondrial inner membrane.

B. It inhibits the ATP synthase.

C. It makes holes in the mitochondrial outer membrane.

D. all of the above

E. none of the above

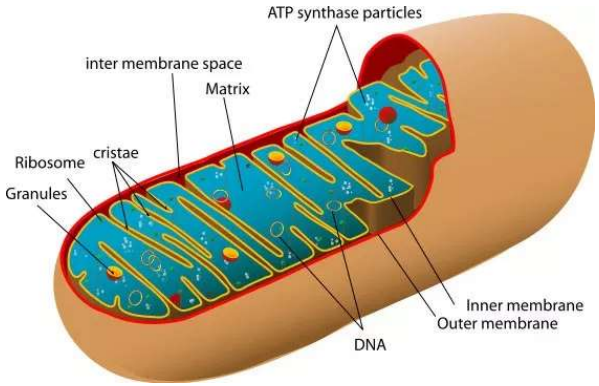
Case Study 5: Mitochondrial Disease

Team work time!

Case Study: Mitochondrial Disease

What is Mitochondrial Disease?

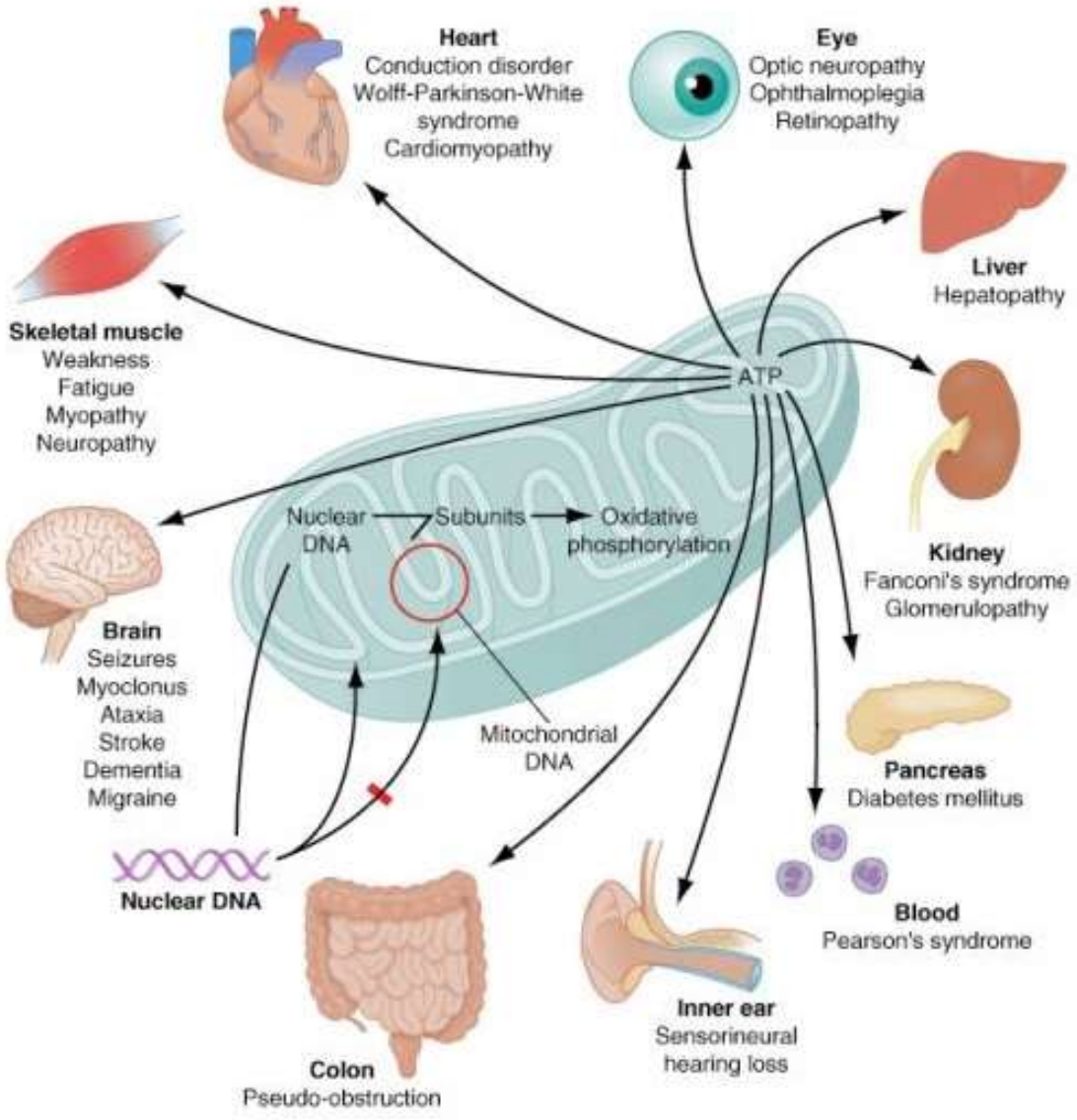
What is Mitochondrial disease?



- From failures of the mitochondria, specialized compartments present in every cell of the body (except red blood cells).
- Mitochondria are responsible for creating more than 90% of the energy needed by the body to sustain life and support organ function. When they fail, less and less energy is generated within the cell. Whole organ systems begin to fail. Cell injury and even cell death follow.
- The parts of the body, such as the heart, brain, muscles and lungs, requiring the greatest amounts of energy are the most affected. Symptoms can include seizures, strokes, severe developmental delays, inability to walk, talk, see, and digest food combined with a host of other complications. If three or more organ systems are involved, mitochondrial disease should be suspected.
- Although mitochondrial disease primarily affects children, adult onset is becoming more common.

Mitochondrial Disease

- Mitochondrial Disease is suspected when three or more organ systems are involved.
- Difficult to identify.
- Most likely inherited mutations.
- Multiple diagnostic tests required.



Fauci AS, Kasper DL, et al.: Harrison's Principles of Internet Medicine, 17th Edition

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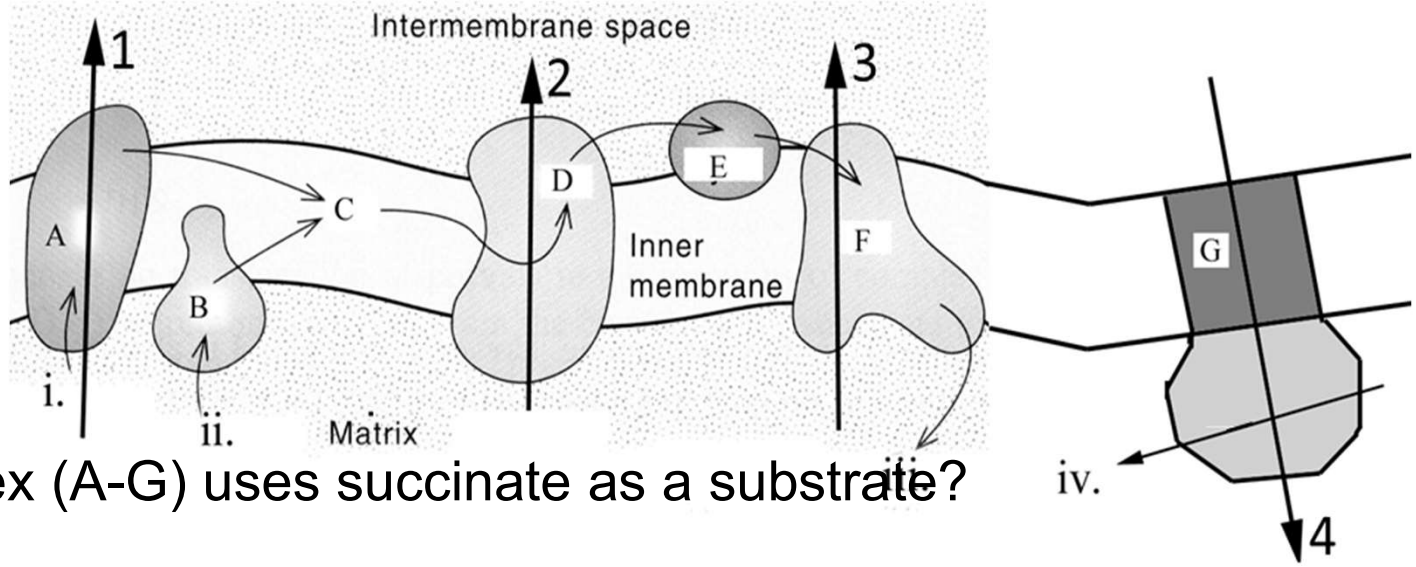
Clinical setting

- **A mutation** in a *mitochondrial gene* encoding a component of ***ATP synthase*** has been identified.
- People who have this mutation suffer from *muscle weakness, ataxia, and retinitis pigmentosa*.
- A tissue biopsy was performed on each patient, and submitochondrial particles were isolated that were **capable** of **succinate-sustained ATP synthesis**.

CS1: Succinate in ATP synthesis

In the figure below, the protein complexes involved the mitochondrial production of ATP are indicated by **A-G**. The chemical reactions that occur are numbered **i., ii., iii.** and **iv.** and chemical flux movements are numbered **1, 2, 3 and 4.**



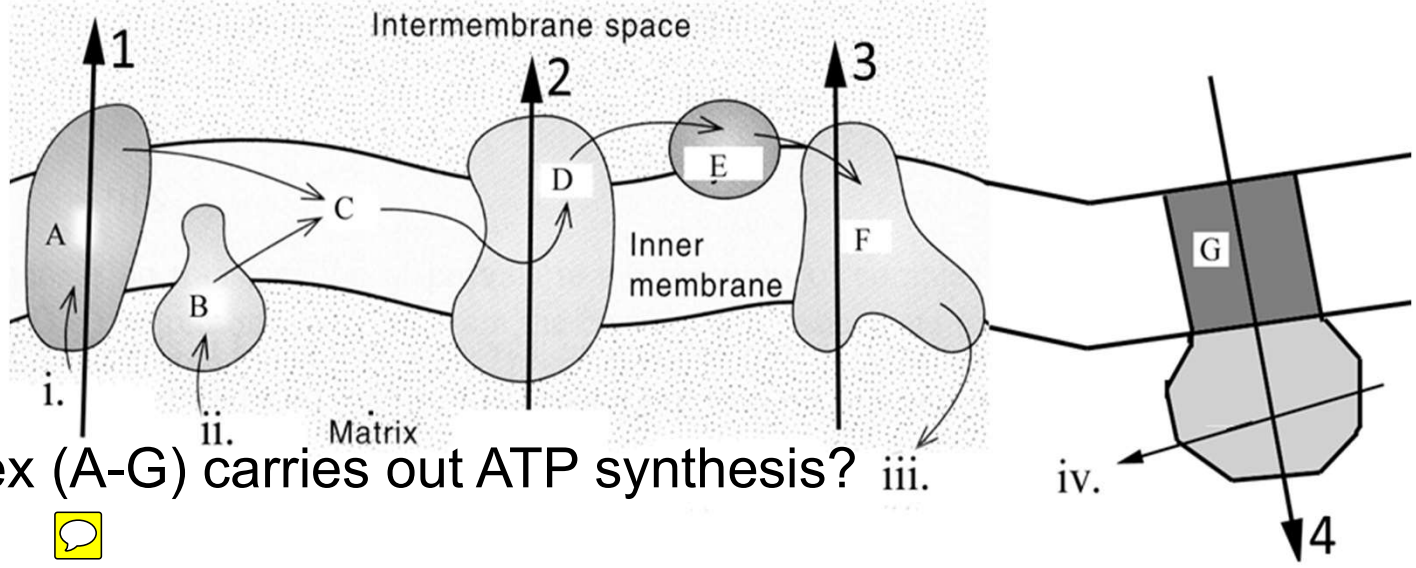
(a) Which complex (A-G) uses succinate as a substrate?

(b) Which chemical reaction (i. – iv.) occurs at this complex?

(c) What is succinate transformed into?

CS2: Where is ATP synthase?

In the figure below, the protein complexes involved the mitochondrial production of ATP are indicated by **A-G**. The chemical reactions that occur are numbered **i., ii., iii.** and **iv.** and chemical flux movements are numbered **1, 2, 3 and 4.**



(a) Which complex (A-G) carries out ATP synthesis?

(b) Which chemical reaction (i. – iv.) occurs at this complex?

(c) Which substrate is transformed into ATP?

Back to the lab!

- First, the activity of the ATP synthase was measured on the addition of succinate and the following results were obtained.

CS3: Why was **succinate added** in this assay?

Assay 1: ATP synthase activity (nmol of ATP formed min^{-1} mg^{-1})

Controls	3.00
Patient 1	0.25
Patient 2	0.11
Patient 3	0.17

Back to the lab!

- The activity of the ATP synthase has been measured on the addition of succinate. How can we interpret these results?

CS4: What is the **effect** of **this mutation** on **succinate-coupled ATP synthesis**?

Assay 1: ATP synthase activity (nmol of ATP formed min^{-1} mg^{-1})

Controls	3.00
Patient 1	0.25
Patient 2	0.11
Patient 3	0.17

ATP Synthase

- This is a reversible enzyme.

CS5: When there is no protein gradient, to drive ATP synthesis, what does it do?

Does the mutation affect ATP synthesis or its breakdown?

- Next, the ATPase activity of the enzyme was measured by adding ATP to the submitochondrial particles, in the absence of succinate.

Assay 2: ATP hydrolysis (nmol of ATP hydrolyzed min^{-1} mg^{-1})	
Controls	33
Patient 1	30
Patient 2	25
Patient 3	31

CS6: What is the **effect** of this **mutation** on **ATP hydrolysis**?

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CS7: What can you conclude from these two assays?

A. The mutation enhances succinate oxidation.

B. The mutation does not affect ATP synthesis.

C. The mutation affects the coupling of the electron transport chain to oxidative phosphorylation.

D. The mutation affects the active site in ATP synthase.

How are mitochondrial diseases treated?

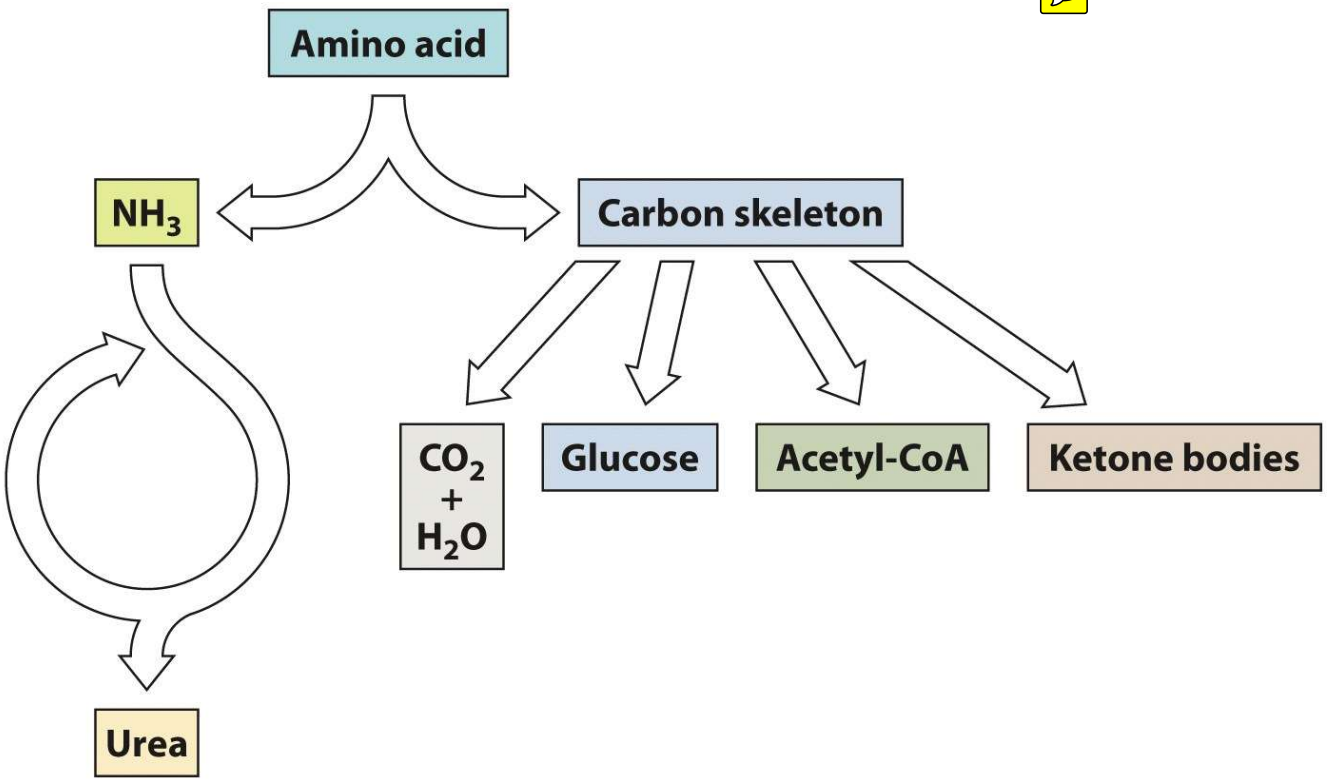
- There are no cures for mitochondrial diseases, but treatment can help reduce symptoms or slow the decline in health.
- Treatments for mitochondrial disease may include:
  - Vitamins and supplements, including Coenzyme Q10; B complex vitamins, especially thiamine (B1) and riboflavin (B2); Alpha lipoic acid; L-carnitine (Carnitor); Creatine; and L-Arginine.
  - Exercises, including both endurance exercises and resistance/strength training. These are done to increase muscle size and strength. Endurance exercises include walking, running, swimming, dancing, cycling and others. Resistance/strength training include exercises such as sit-ups, arm curls, knee extensions, weight lifting and others.
  - Conserving energy by not doing too much in a short period of time.
  - Other treatments: speech therapy, physical therapy, respiratory therapy, and occupational therapy.

Amino acids

- What do we do with the proteins we eat?
- Also, how to recycle any damaged/non-functional proteins we have?
  - ➢No storage for proteins/amino acids.
  - ➢Can we use them for energy?

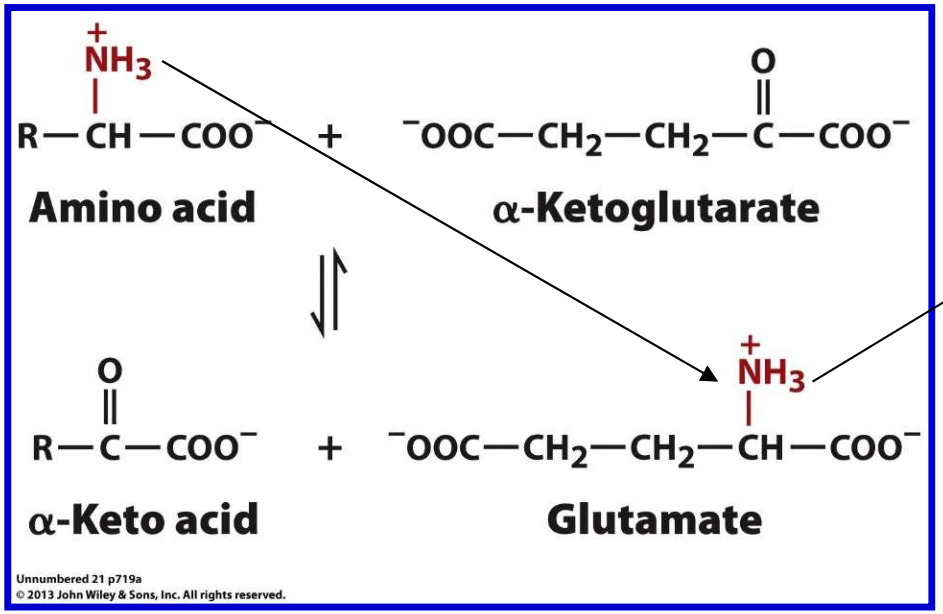
- How can we make amino acids we need?
  - ❖10 essential ones must come from food
  - ❖10 non-essential ones, we can make!

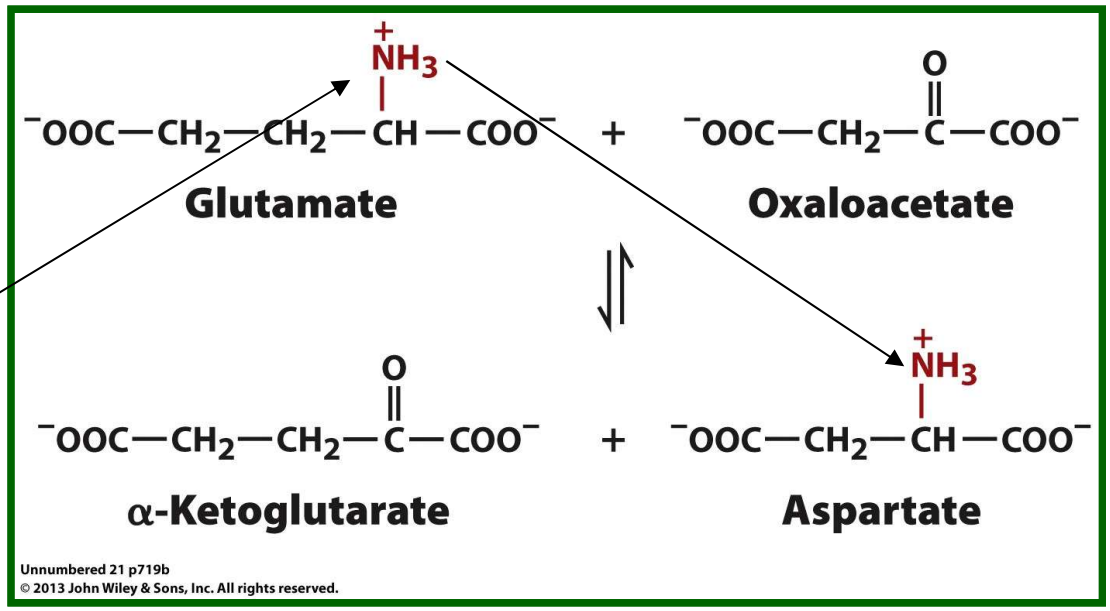
Breaking down amino acids



- Intracellular process
- Special treatment for N removal (in the mitochondria) as ammonia is toxic to the cell
- Recycling the C chain.

Transferring the amino group out

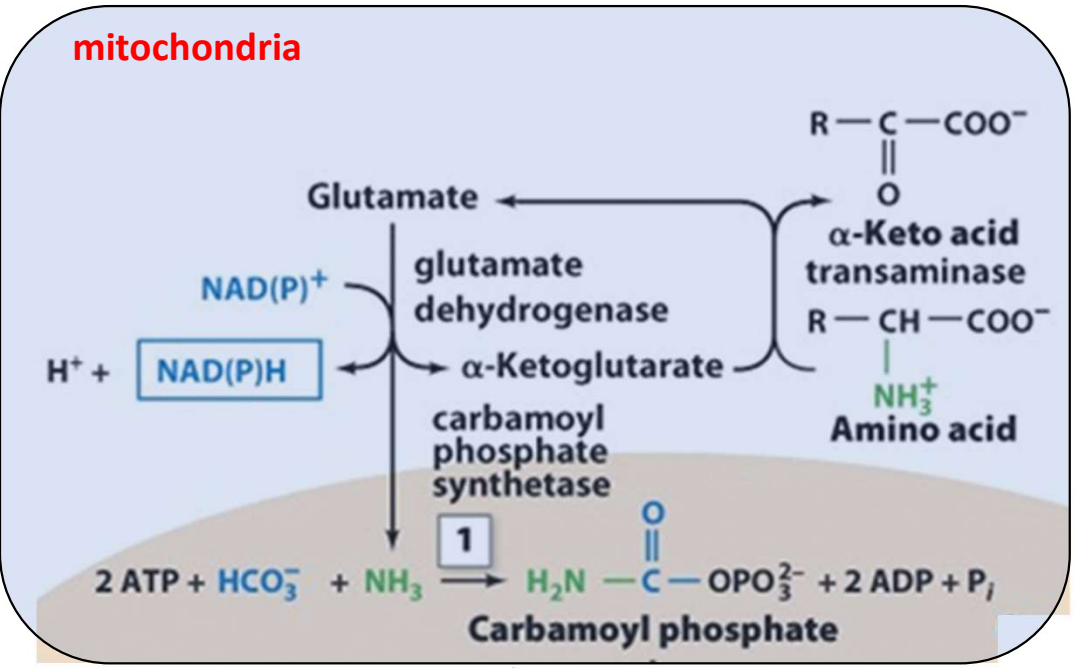


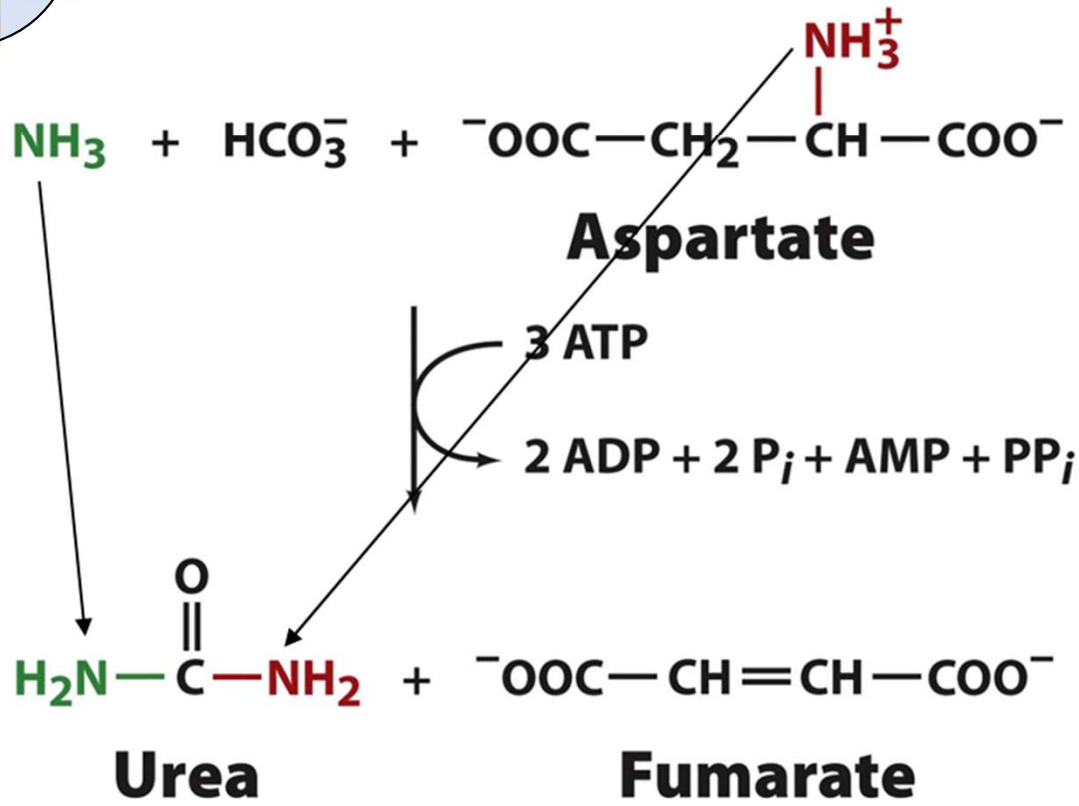


1. **Transamination** by **aminotransferases**: transfer amino group to α-keto acid (predominantly α-ketoglutarate) forming glutamate and an α-keto acid from all aa's except lysine (Lys or K). PLP (pyridoxal-5'-phosphate) cofactor required - from pyridoxine (Vitamin B_6)

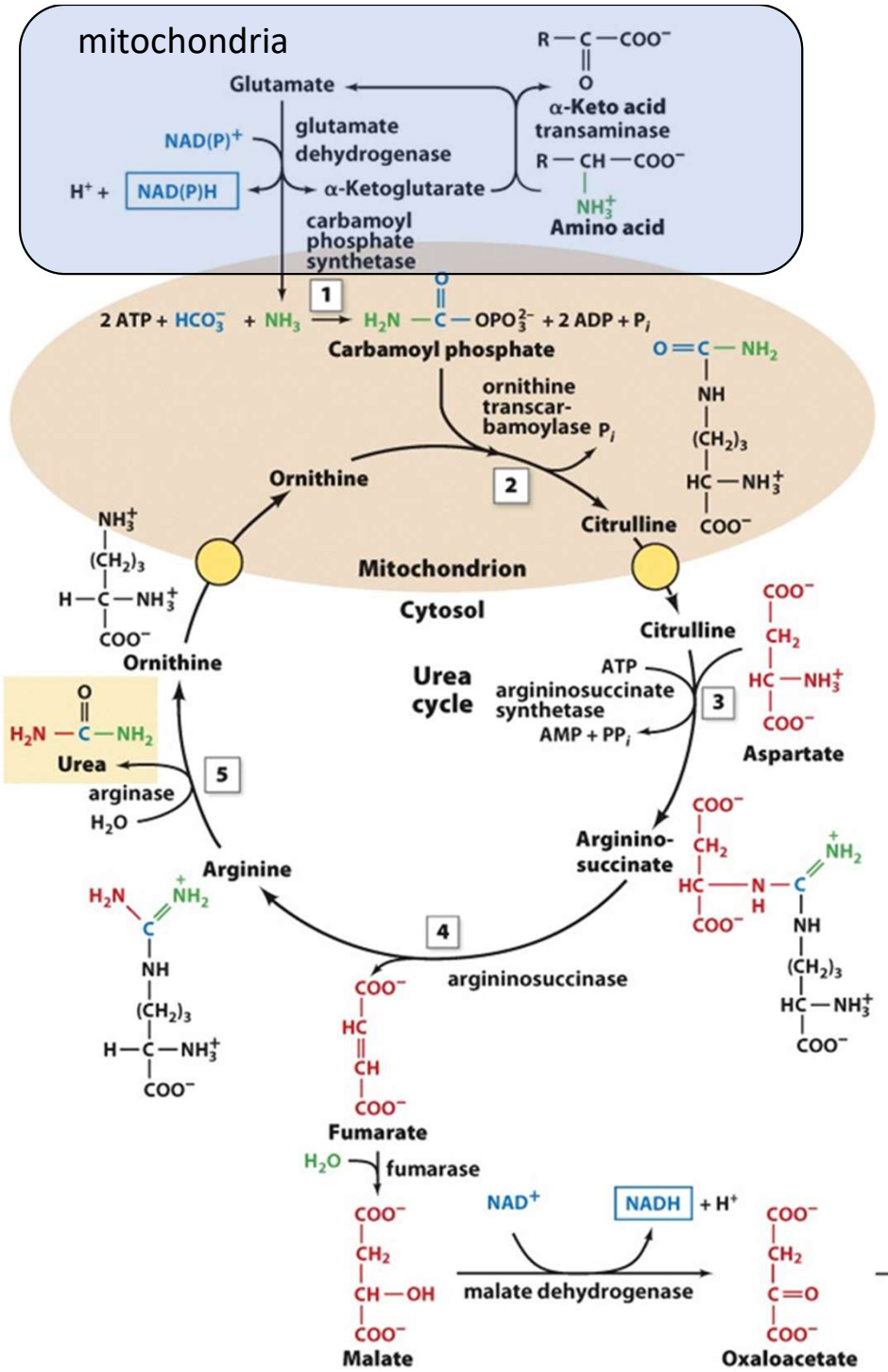
2. Preferred **α-keto acid** however is **α-ketoglutarate**, followed by ***oxaloacetate***, leading to **glutamate** and ***aspartate*** as the main products of aa degradation

Urea cycle: rendering N harmless





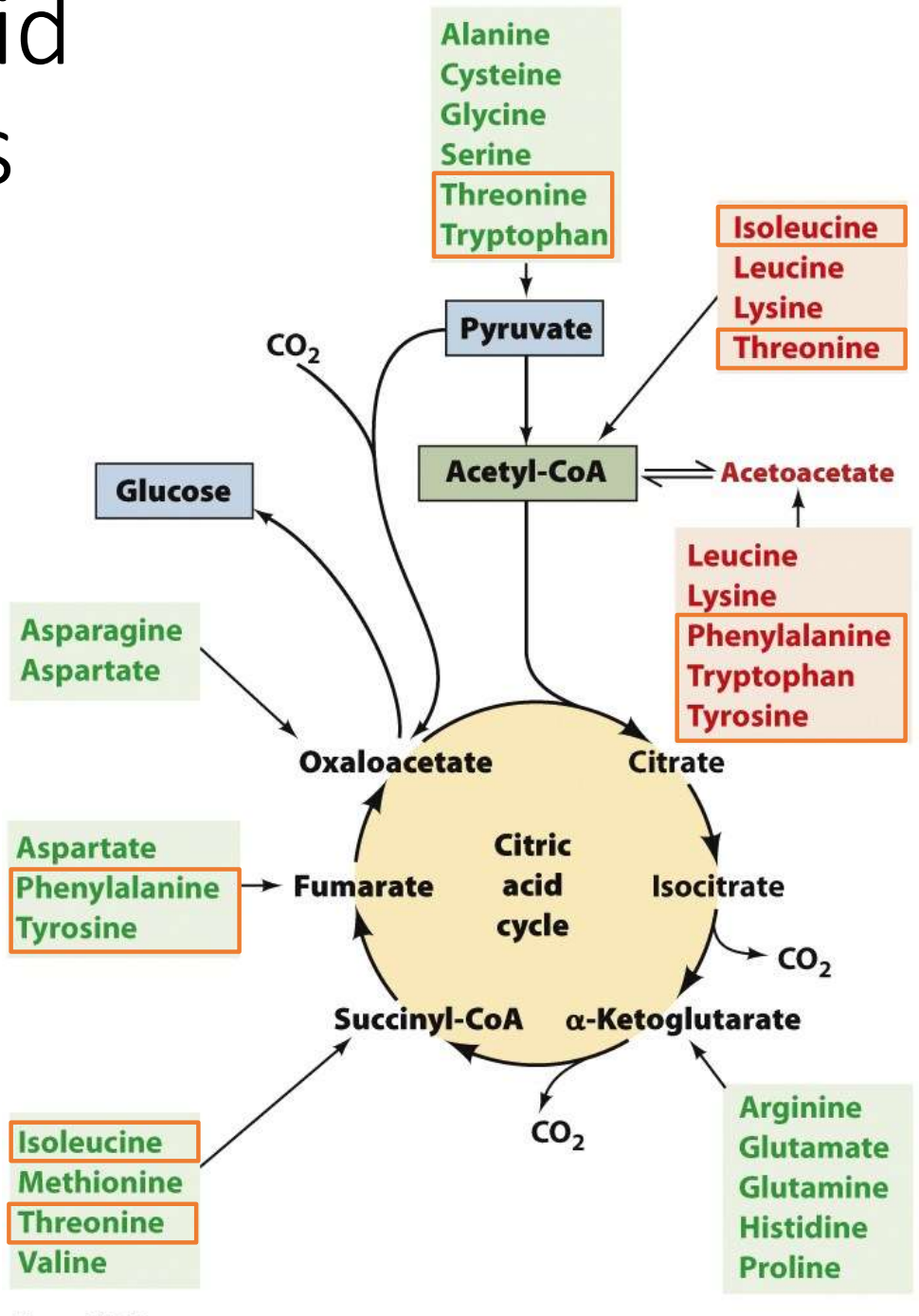
The urea cycle



- 5 enzymatic reactions: 2 in the mitochondria; 3 cytosolic
  - Step 1 is the committed step for the entire pathway.
- *Similar to CAC*
- Two intermediates: ornithine and citrulline must enter and exit the mitochondria
- Urea goes to the kidneys via the blood stream.
- <u>Arginine</u> is synthesized!

7 Common Amino Acid Degradation Products

- C skeleton is degraded to compounds metabolized to CO_2 (CAC) and H_2O (ETC-OxPhos).
- 10-15% of metabolic energy comes from aa's
  - Glucogenic aa's: pyruvate, α-ketoglutarate, succinyl-CoA, fumarate and oxaloacetate: (5) glucose precursors
  - Ketogenic aa's: acetyl-CoA and acetoacetate: (2) fatty acid or ketone body precursors
  - Some are both: Ile, Phe, Thr, Trp, Tyr (IFTWY)



7 metabolites linked to CAC

Diseases from defective amino acid degradation

- Best known is **phenylketonuria:**
  - phenylalanine breakdown affected
  - absence or deficiency of phenylalanine hydroxylase
  - Results in severe mental retardation a few months after birth

Making amino acids

- *Essential and nonessential amino acids*
  - Essential: 10 aa: from food – we cannot make these
  - Non-essential: 10 aa – we make these!

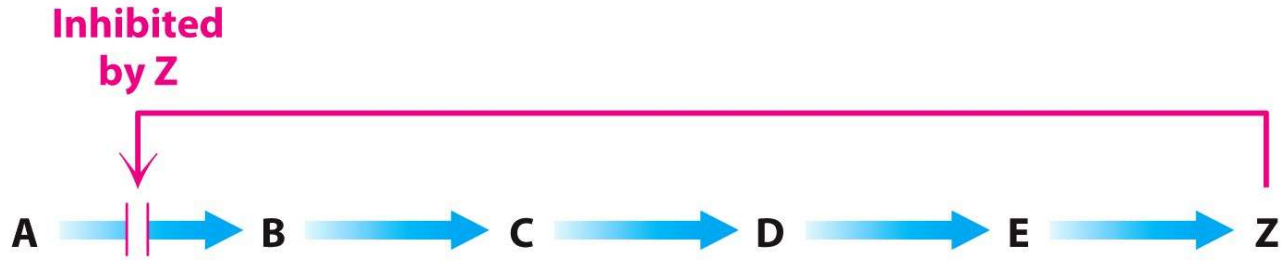
- *Transmination is an important step*
  - Same process as in amino acid breakdown.

Substrates for making amino acids

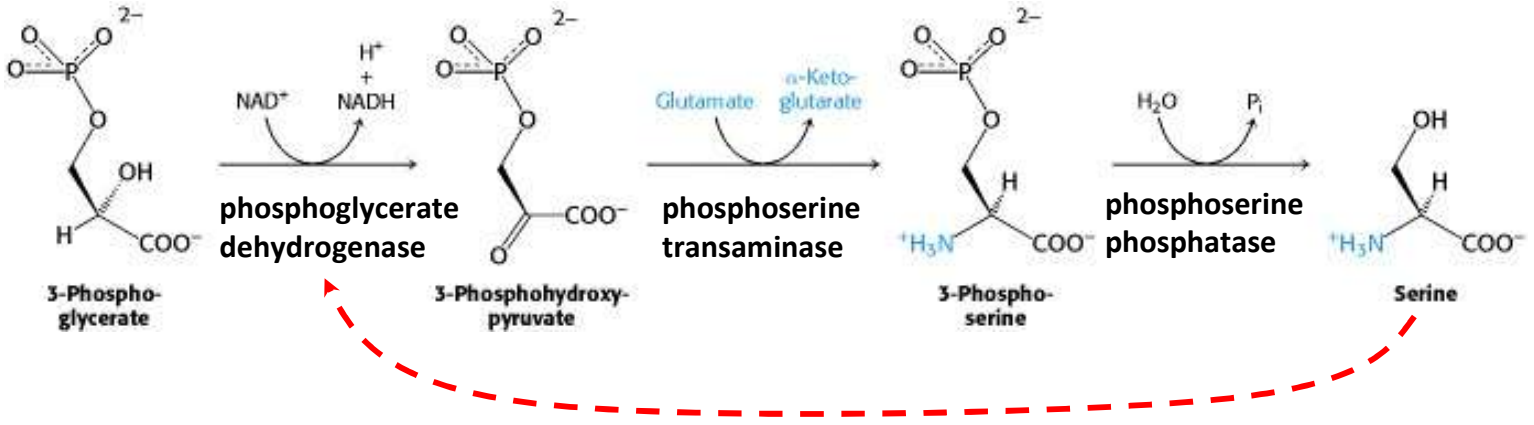
- *Glutamine and Glutamate: from external sources*
- *Most other amino acids from metabolites of:*
  - Glycolysis: pyruvate and 3-phosphoglycerate
  - CAC: α-ketoglutarate and aspartate
  - PPP: phosphoenolpyruvate and erythrose-4-phosphate; PRPP (phosphoribosyl pyrophosphate) from ribose-5-phosphate
  - "Amino acid families" are named after these substrates.
- Notes:
  - His: shares its synthetic pathway from PRPP with purine nucleotides
  - Arg: is made in the urea cycle, during the degradation of other amino acids

Regulation of amino acid synthesis -1

- For many amino acids, synthesis follows a linear set of reaction steps.
- **End-point inhibition:** Feedback inhibition of the first committed step, with the final product (Z) inhibiting the enzyme that catalyzes the first step.

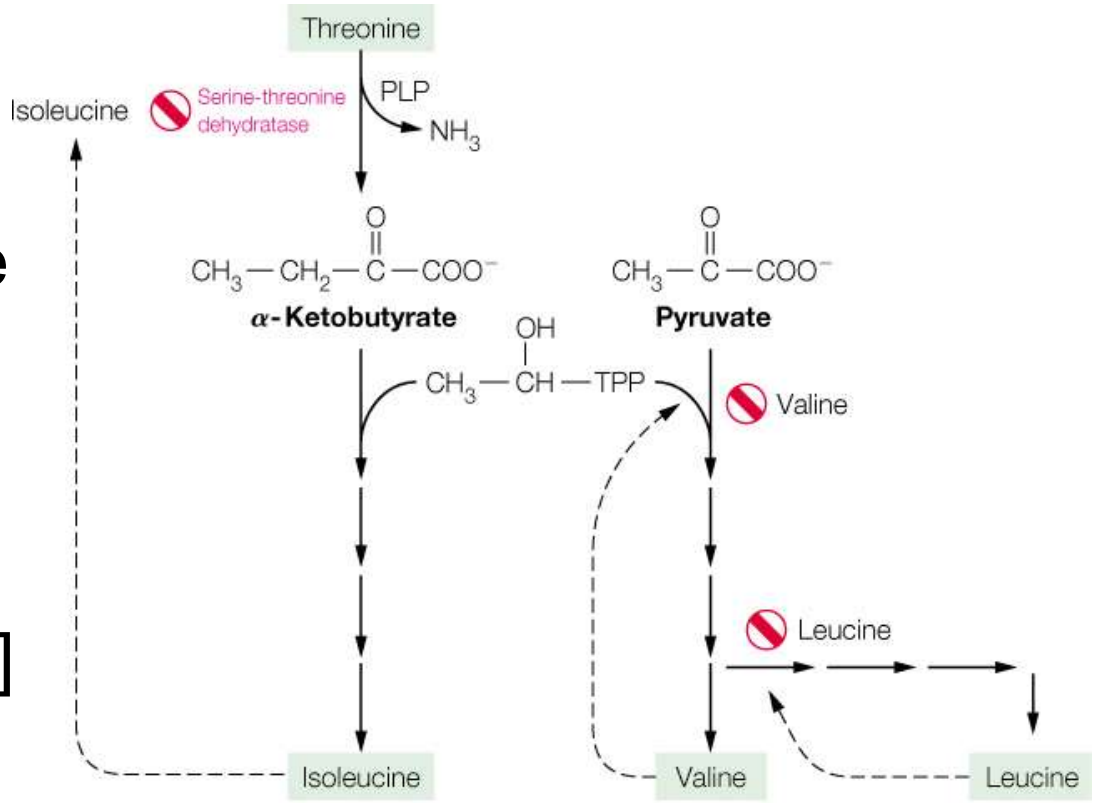


E.g. Serine biosynthesis: serine inhibits the enzyme for step 1



Regulation of amino acid synthesis - 2

- Feedback inhibition in branched pathway
- e.g. biosynthesis of Val, Le and Ile.
- Common intermediate: hydroxyethyl thiamine pyrophosphate
- [pyruvate]:[α-keto butyrate] = [Val/Leu]:[Ile]
- So, Val and Leu inhibit branches leading to their synthesis
- Ile inhibits inhibits Thr's oxidative deamination.



Lots of Valine: leads to Ile synthesis
Lots of Ile: leads to Val/Leu synthesis

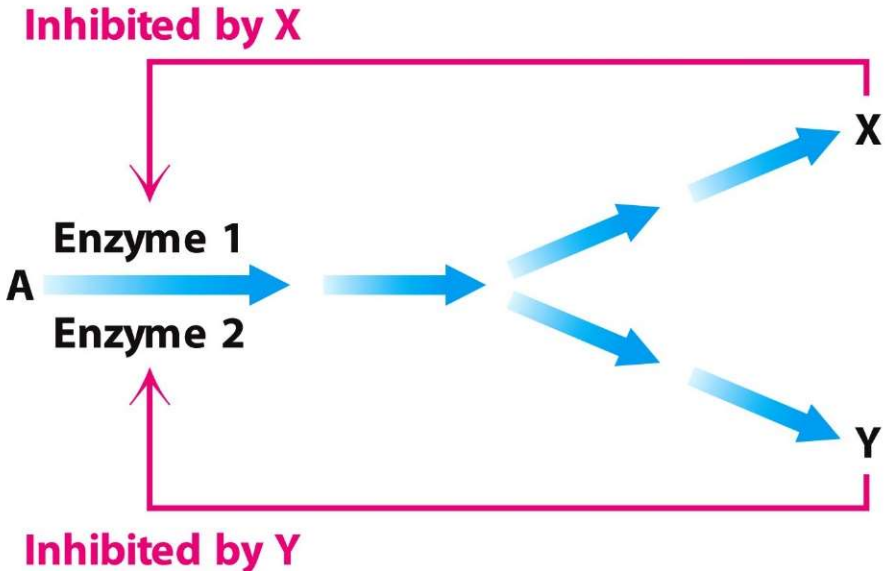
Regulation of amino acid synthesis - 3

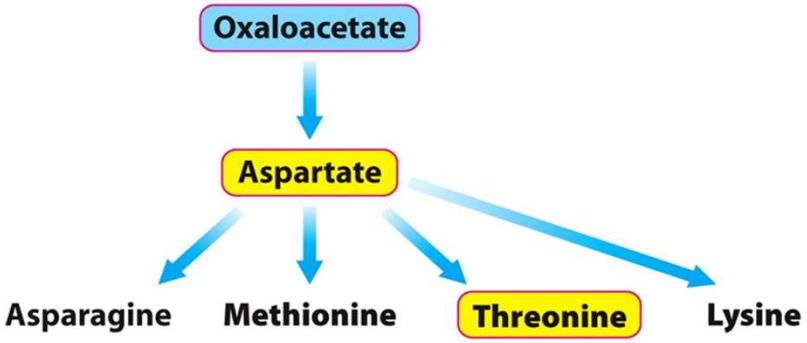
- Multiple enzymes catalyzing the committed step
- **E.g. Asp** leading to **Thr, Met and Lys.**
  - Three distinct **aspartokinases** (I, II and III) catalyze this step in *E. coli*.
  - **Same mechanism but regulation is different.**

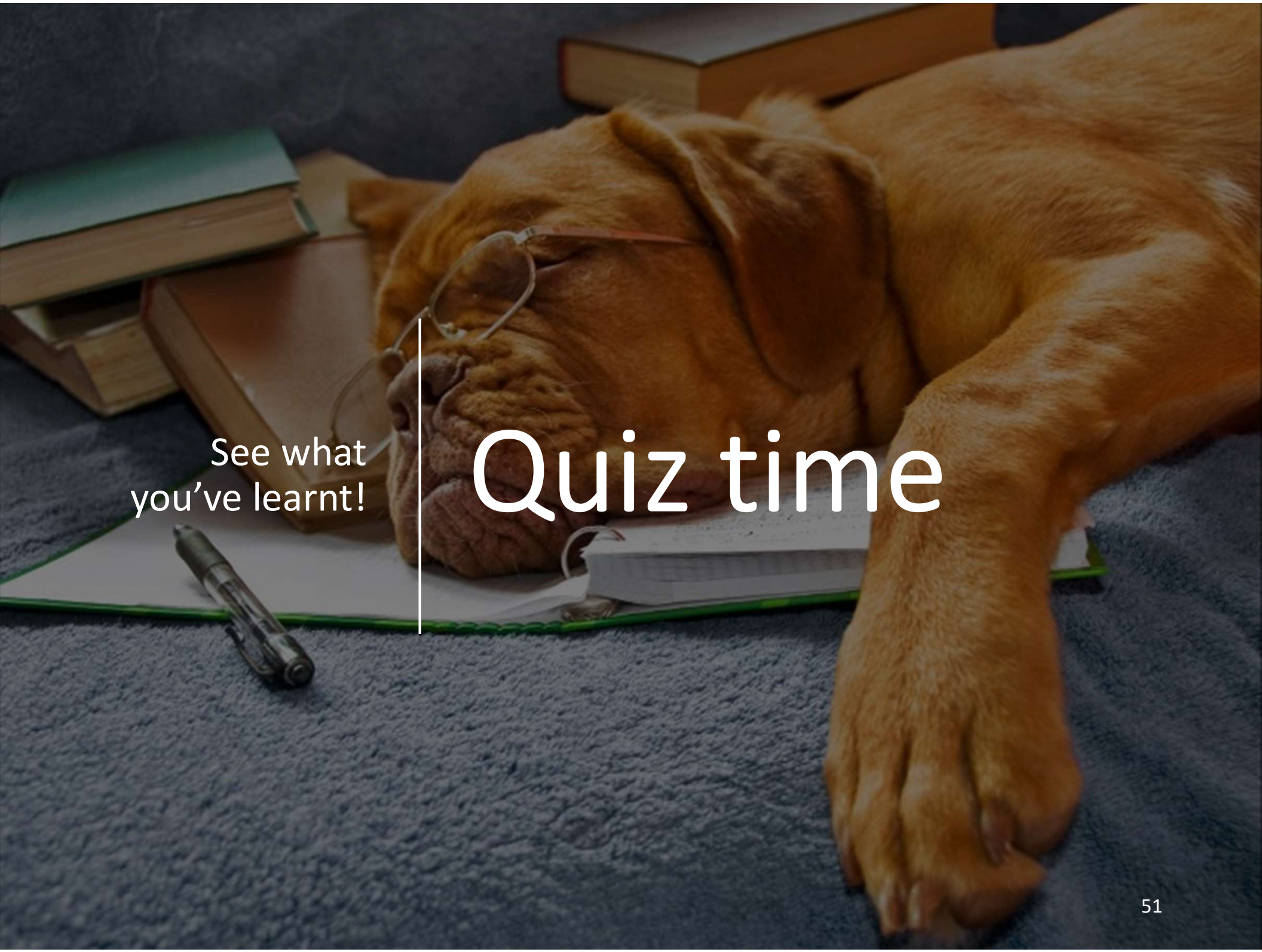
    I: Inhibited by Thr

    II: No inhibition by Met but enzyme synthesis repressed at the gene level by Met

    III: Inhibited by Lys.






See what
you've learnt!
Quiz time