

METABOLISM

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



MID – Lecture 9

Oxidative Phosphorylation (Pt.3)

وَإِن تَتَوَلَّوْا يَسْتَبَدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَلَكُمْ

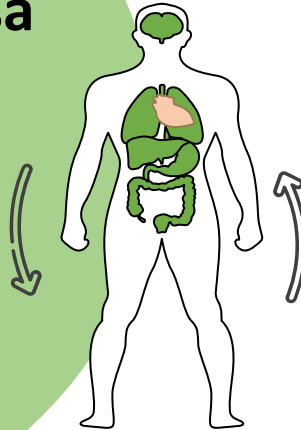
اللهم استعملنا ولا تستبدلنا

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REVISION: just before studying this lecture there are some bullet points that needs to be known to fully understand it.

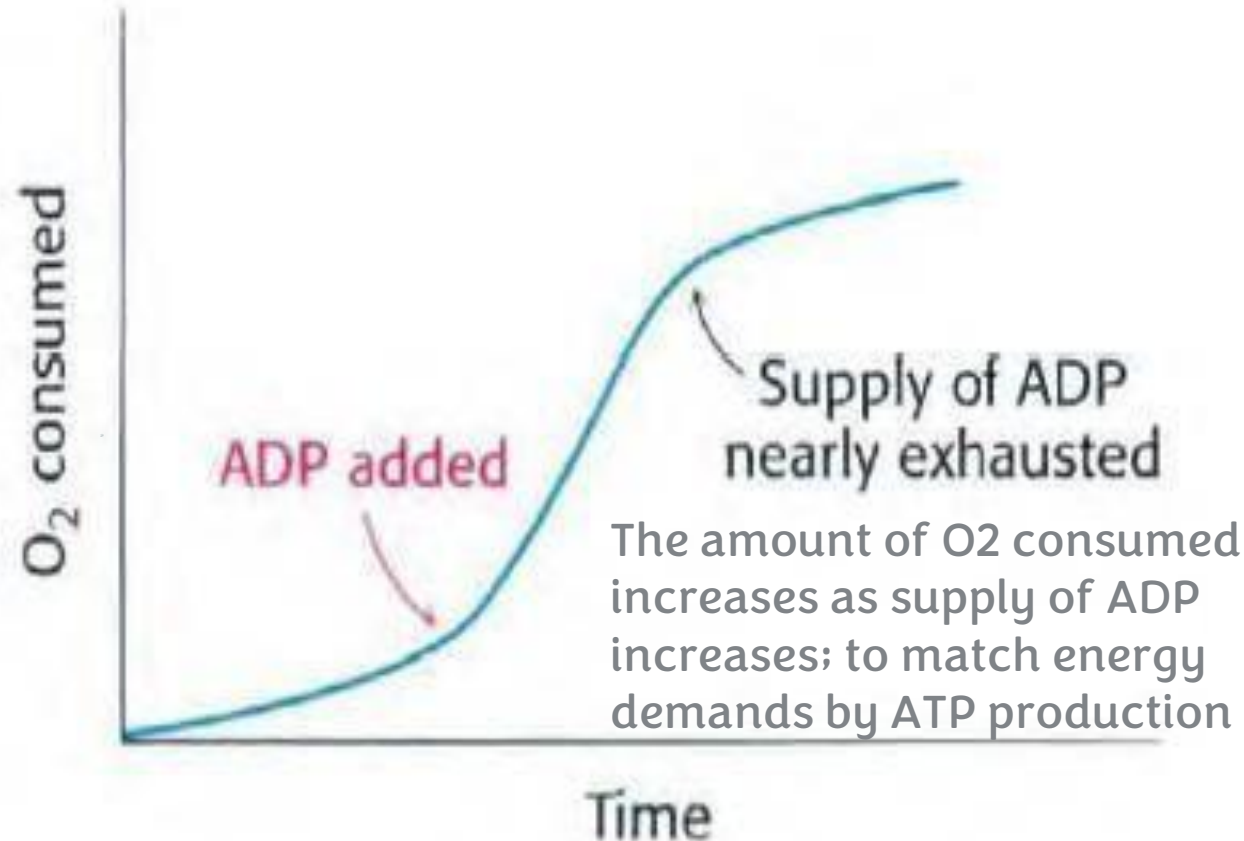
- NADH: electron donor, providing electrons that move through ETC (from low E° to higher E°).
- O₂: last electron acceptor; essential for the continuation of the process (aerobic respiration).
- ADP and Pi: imp. for ATP synthesis by ATP synthase.
- Complex I or NADH-Q oxidoreductase: takes electrons of NADH by its NADH dehydrogenase activity.
- Cytochrome C is a movable carrier between complexes III & IV.
- Cytochrome C oxidase or complex IV: has an enzymatic activity that reduces O₂ to H₂O.
- Mt comes only from the mother, whether defective or not.
- Non-shivering thermogenesis: generating Heat instead of ATP production without shivering.
- tRNA is used in translation process to produce proteins.
- recessive inheritance (وراثة الصفات المتنحية): the need of two copies of the gene (alleles) one from the mother and the other is from the father (both of them have to have the gene even if just carried).

Regulation: The need for ATP

Molecules important for ATP generation:

- What OxPhos needs? (NADH, O₂, ADP, and Pi) Inorganic phosphate ↪ Oxygen availability
- ET is tightly coupled to phosphorylation (simultaneously)
- ADP is the most important factor in determining the rate
- The regulation of the rate of oxidative phosphorylation by the ADP level is called respiratory control or acceptor control

Ex. Increasing ATP demand ($\uparrow[\text{ADP}]$), reflects on the rate (increase) of oxidative phosphorylation.

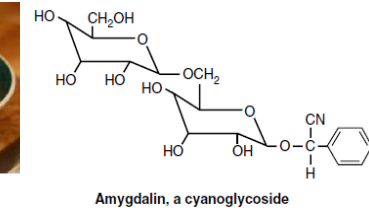


The rate of oxygen consumption by mitochondria increases markedly when ADP is added and then returns to its initial value when the added ADP has been converted into ATP

Notes regarding the previous slide:

- ETC is tightly coupled to oxidative phosphorylation means that they have to work with each other and the ETC has to be followed by ATP synthesis by ATP synthase (so if one process stops the other is disrupted)
- How much ATP is needed by the cell is an important factor to drive the krebs cycle first then the ETC followed by oxidative phosphorylation
- Lack of ADP or inorganic phosphate will inhibit this process
- Adding ADP will increase the rate of oxygen consumption over time (the rate of the reaction and producing ATP)
- Availability of oxygen is important, but the ADP is more important to drive the ATP synthase

Regulation: Inhibition (coupling)

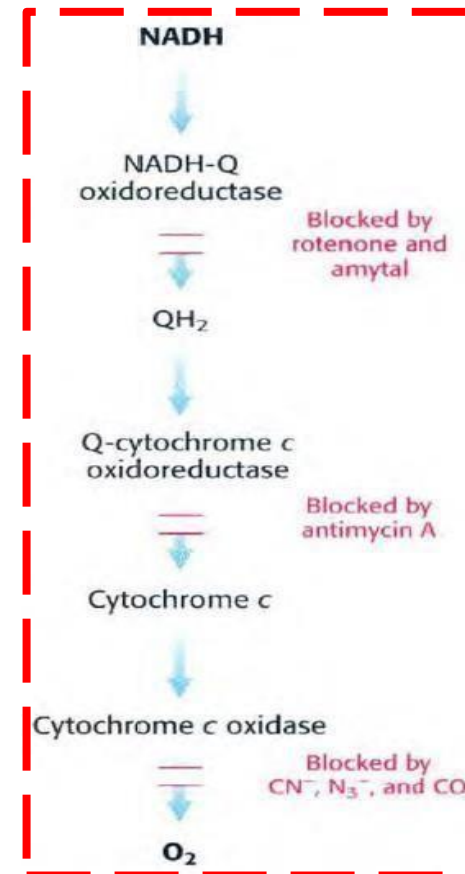


Anti-cancerous drug

Some of these inhibitors can be used to treat cancer.

- Can occur at any stage
- Specific inhibitors: **Non-physiological**
- ✓ Cyanoglycosides such as amygdalin (misnomer B17) are present in edible plant pits
- ✓ Oligomycin prevents the influx of H⁺ through ATP synthase (tight coupling)

Specific inhibitor	Target
Rotenone (insecticide) & Amytal (sedative)	NADH-Q Complex 1 oxidoreductase
Antimycin A (antibiotic)	Complex 3 Q-cytochrome c oxidoreductase
Cyanide (CN ⁻), Azide (N ₃ ⁻), & (CO)	Cytochrome c oxidase → Acts on the last step , interfere with the transfer of electrons to the oxygen
Oligomycin (antibiotic)	Complex 5 ATP synthase



الصفحة الرئيسية > محليات

أشهر جرائم القتل العائلية في المملكة

جراسا نيوز -

جراسا-تعرض فيما يلي قائمة بأشهر جرائم القتل العائلية التي حدثت في الأردن خلال السنوات الماضية ، والتي كان لكل منها وقع الصدمة حين وقوعها لما تمثله من فعل غريب على المجتمع وأعرافه ، فضلا عن مخالفتها للشرائع السماوية والقوانين النافذة والطبيعة الإنسانية بعامه.

قصة السيانيذ

أول جريمة من نوعها يرتكبها أب ضد ولديه ، إذ قام الأب بوضع مادة السيانيذ في كأس الحليب وطلب من طفليه ان يشربا منه ، حيث فارقا الحياة بعد 10 دقائق من مغادرة الام المنزل لتعود وتجدهما جثتين هامدتين.

وقد ادين الاب بعقوبة الاعدام شنقا الا ان والده اسقط الحق الشخصي كونه وليا عن الطفلين وحكم عليه بالاشغال المؤبدة.

Oligomycin (antibiotic) Complex 5 ATP synthase

Notes regarding previous slide :

- We can use synthetic inhibitors to inhibit different complexes or different stages of ETC or during ATP synthase → interruption of the coupling process between ETC (by complexes 1 to 4) and ATP synthesis (by complex number 5)
- Inhibitors can also be derived from natural compounds such as fruits
- all of the inhibitors mentioned at the previous slide (in the table) will hinder the process of ATP production , even if it doesn't act directly on the ATP synthase
- Vitamin b-17 Is not a vitamin , it's an inhibitor of ETC and acts on the last step of ETC on complex 4 (it is a cyanoglycoside so it is similar to cyanide inhibitor)

Regulation: Regulated Uncoupling Proteins (UCPs)

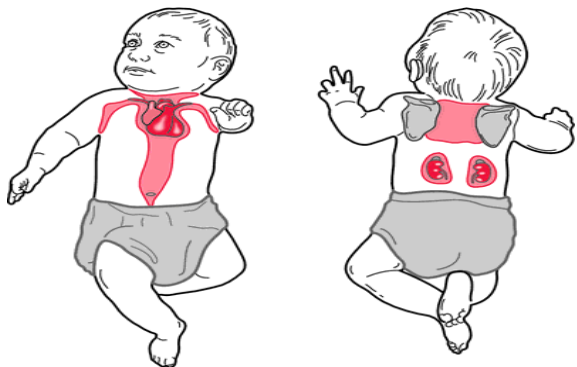
UCPs: proteins that prevent the coupling by allowing protons to bypass ATP synthase, therefore no ATP generation, and release of the gradient energy in the form of HEAT.

Hypothermia: The reduction of body temperature.

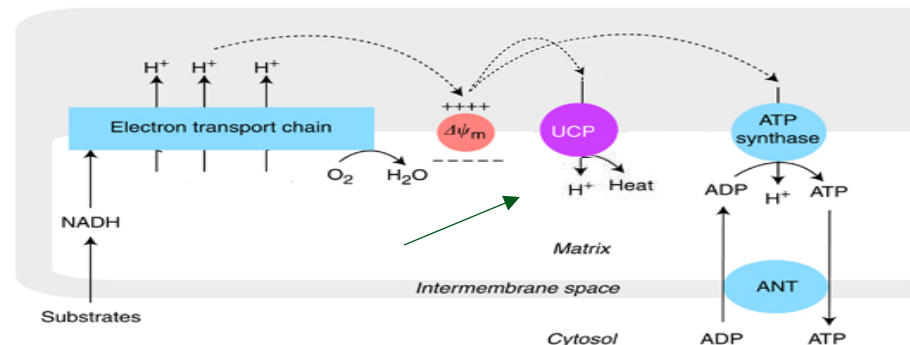
Brown Adipose	White adipose
Has high concentration of mitochondria (that's what gives it the brown color)	Lower mitochondrial content
Abundant in infants; to protect them from hypothermia; because they're more susceptible to it as they're incapable to move on their own when they feel cold.	Most abundant in adults
Has UCP1	UCP1 can be expressed when white adipocytes are exposed to stimuli.

- Short-circuiting ATP synthase
- UCP₁ (thermogenin):
 - ✓ Highly expressed in Brown adipose tissue, responsible for non-shivering thermogenesis.
 - ✓ Infants: neck, breast, around kidneys.
 - ✓ Fatty acids directly activates UCP₁
- UCP₂ (most cells); UCP₃ (skeletal muscle); {UCP₄, UCP₅} (brain)
- Obesity tendency in some populations

This inhibition (esp. By UCP1) is good in maintaining temperature (esp. In infants) and reduction of weight.



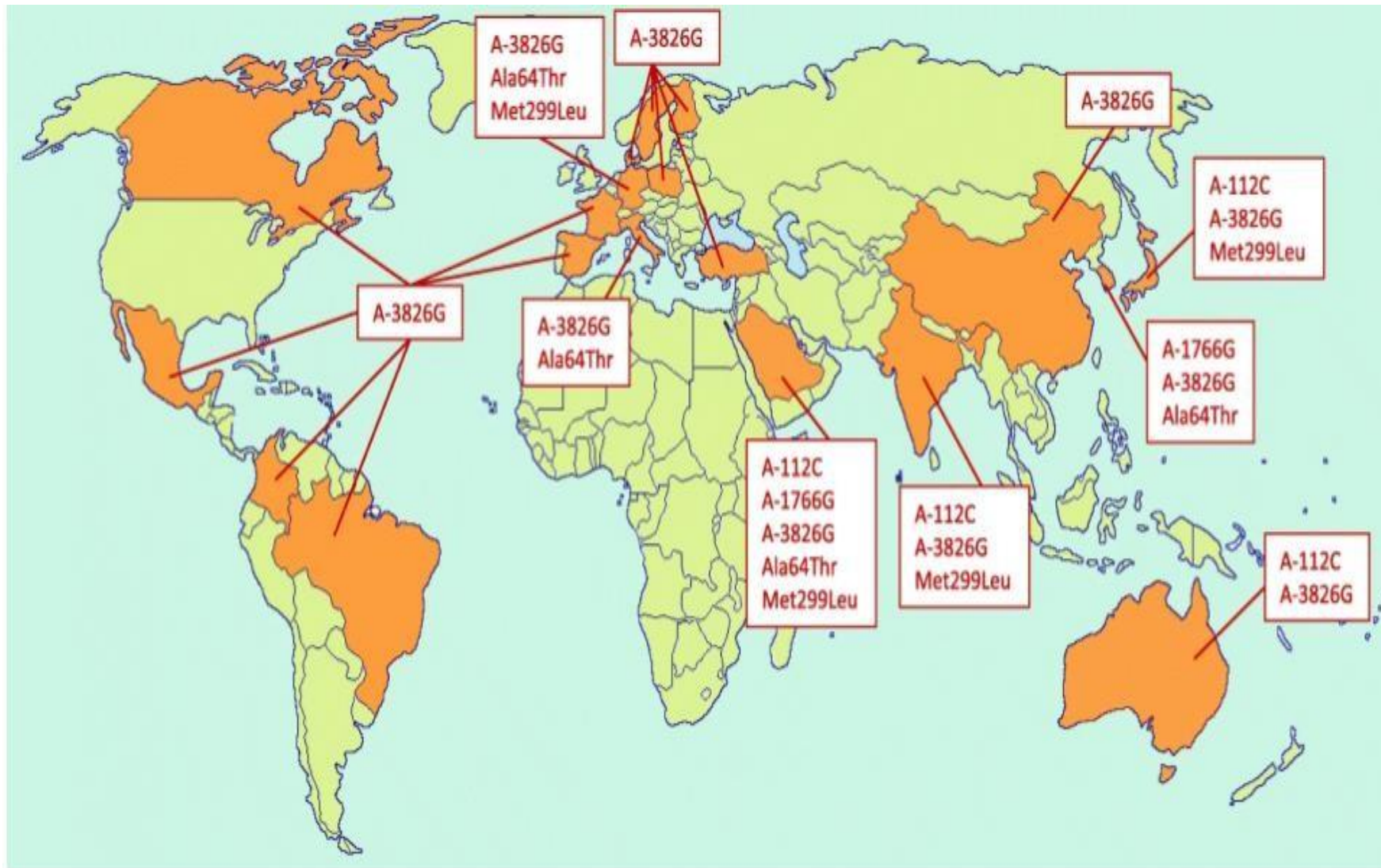
This image illustrates the locations in which BAT is located in infants.



Here you can see how UCPs inhibit coupling; by using the gradient to produce heat rather than ATP.

Just to know: there is ongoing research aimed at increasing the amount and activity of **brown adipose tissue (BAT)** in adults to enhance energy dissipation. The objective is to stimulate **non-shivering thermogenesis**, where energy from different fuels (such as glucose and fatty acids) is released as **heat** rather than being stored as fat or used for **ATP synthesis**.

UCP mutations and cardiometabolic diseases risk



People with different sets of mutations in UCPs might be associated with high risk of developing cardiometabolic diseases.

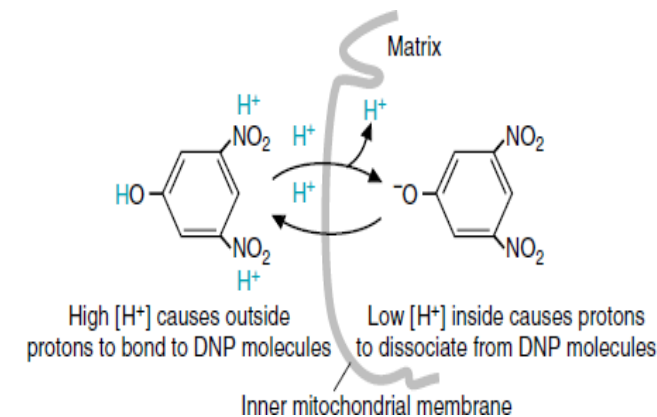
Regulation:

Unregulated Chemical Uncouplers (Non-Physiological)

- 2,4-dinitrophenol (DNP) & other acidic aromatic compounds
- DNP disrupts the tight coupling of electron transport and phosphorylation in mitochondria, **HOW?**
- **Disruption mechanism:** It carries/ Binds to protons across the inner mitochondrial membrane, returning them into the Matrix.
- How does it occur? Dissipation of PMF
- Results in increased oxygen consumption and oxidation of NADH but no ATP production; as no protons pass through ATP synthase. Also, excessive heat production causing hyperthermia.
- DNP was used to reduce weight, and it got used in some wars, but FDA banned DNP in 1938; because of the dangerous side effects.

Chemicals but not natural as those in slide 6.

Not from the body, from the outside.



This image shows the mechanism.

OxPhos Diseases (Genetic)

A. Mitochondrial DNA and OXPHOS Diseases

- ✓ **mtDNA:** Small (16,569) base pair, double-stranded, circular DNA
- ✓ Encodes 13 subunits of ETC: 7 (I), 1 (III), 3 (IV), 2 (Fo). As you know these aren't all subunits; so some of them are encoded by nDNA.
- ✓ Also encodes necessary components for translation of its own mRNA: a large and small rRNA and tRNAs
- ✓ mtDNA has a mutation rate ~10 times more than nuclear DNA
- ✓ Maternal inheritance, replicative segregation & heteroplasmy
- ✓ Accumulation of somatic mutations with age
- ✓ Highest ATP demands: CNS, heart, skeletal muscle, and kidney, liver are affected more

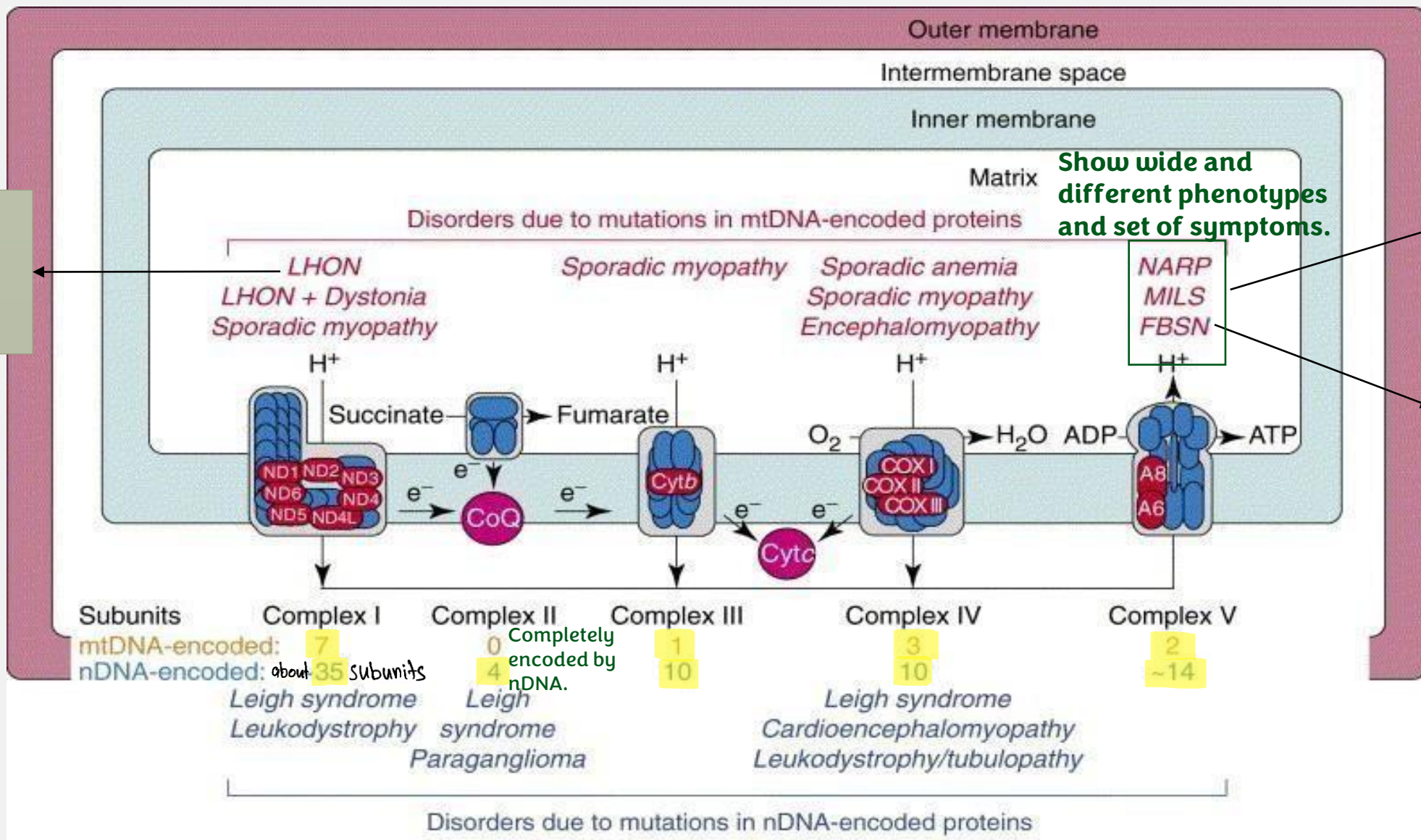
Replicative Segregation and Heteroplasmy (Acquired Mutations):

When cells divide, their mitochondria are randomly distributed between the two daughter cells during the process of cytoplasmic division. This random distribution is known as **replicative segregation**. During this process, somatic mitochondrial mutations can arise. As a result, the mutated mitochondria may not be evenly distributed between the daughter cells. One cell may end up with a higher proportion of mutated mitochondria than the other, leading to a condition called **heteroplasmy**.

B. Nuclear Genetic Disorders of Oxidative Phosphorylation

- ✓ nDNA contains genes encoding 1,000 proteins that are important for the function of the mt (OxPhos), and these genes can get mutated resulting in different diseases.
- ✓ Usually autosomal recessive inheritance; therefore 2 copies of the gene must be defective to develop these diseases.
- ✓ Expressed in all tissues; therefore affecting many types of cells and tissues.
- ✓ Phenotypic expression with high ATP demand, meaning: they get affected by these mutations more than cells with no or low demand.

OxPhos Diseases (Genetic)



Leber's hereditary optic neuropathy

Loss of central vision; because of mt dysfunction in optic cells.

Show wide and different phenotypes and set of symptoms.

Neuropathy, ataxia, and retinitis pigmentosa

Familial infantile bilateral striatal necrosis

Movement problems and developmental delays.

The regulator	Cyanoglycosides (amygdalin b17)	Rotenone (insecticide) +Amytal (sedative)	AntimycinA (antibiotic)	Cyanide+ Azide + CO	Oligomycin (antibiotic)	UCP1 (thermogenin)	dinitrophenol (DNP) +other acidic aromatic compounds	ADP
Site of act:	Complex 4 Cytochrome c oxidase	Complex 1 NADH-Q oxidoreductase	Complex 3 Q-cytochrome c oxidoreductase	Complex 4 Cytochrome c oxidase	Complex 5 ATP synthase	Inner membrane of mitochondria, brown adipose tissue	Across mitochondria	Many sites
Mechanism	Inhibits the ETC and acts on the last step of ETC on complex 4	Acts on first step of ETC (initially)	Blocks the transfer of electrons from cyt 3 - acts on the middle of the ETC	Acts on the last step , interfere with the transfer of electrons to the oxygen	electrons pass all complexes before this , but once they arrive here the process will stop and no ATP production will be	prevent the coupling by allowing protons to bypass ATP synthase→ no ATP generation, and release of the gradient energy in the form of HEAT	It carries/ Binds to protons across the inner mitochondrial membrane, returning them into the Matrix.	Feedback regulation
Extra info	are present in edible plant pits	-	-	-	-	responsible for non-shivering thermogenesis, Fatty acids directly activates UCP1	Lead to excessive heat production causing hyperthermia	ADP is the most important factor in determining the rate
Physiological or not	Non-physiological	Non-physiological	Non-physiological	Non-physiological	Non-physiological	Physiological	Non-physiological	Physiological

QUESTIONS.

Q1: A patient was diagnosed with a mitochondrial DNA mutation that led to reduced complex I activity. This patient would have difficulties in which of the following electron transfers?

- (A) Succinate to complex III
- (B) Cytochrome c to complex IV
- (C) Coenzyme Q to complex III
- (D) Malate to coenzyme Q
- (E) Coenzyme Q to oxygen

Q3: Oligomycin is an inhibitor of which of the following processes in oxidative phosphorylation?

- (A) Electron transport chain
- (B) ATP synthase
- (C) Oxidative phosphorylation
- (D) All of the above

Q2: A researcher was studying oxidative phosphorylation in a suspension of carefully washed and isolated mitochondria. ATP, ADP, inorganic phosphate, lactate, lactate dehydrogenase, and oxygen were introduced to the suspension, and he was able to demonstrate ATP production within the mitochondria. The researcher then added oligomycin to the mixture, which stopped oxygen uptake. This occurred due to which of the following?

- (A) Inhibition of complex I
- (B) Inhibition of complex II
- (C) Inhibition of complex III
- (D) Inhibition of complex IV
- (E) Inhibition of the proton translocating ATPase

Q4: which of the following statements about uncoupling protein (UCP1) is **false**?

- (A) UCP1 is found in brown adipose tissue.
- (B) UCP1 can reduce weight.
- (C) UCP1 is found in most cells.
- (D) UCP1 is responsible for thermogenesis.

ANSWERS.

1) The answer is D: Malate to coenzyme Q. Complex I accepts electrons from NADH, and will transfer them to coenzyme Q. Malate dehydrogenase will convert malate to oxaloacetate, generating NADH in the process. The NADH will then donate electrons to complex I to initiate electron transfer. Succinate donates electrons at complex II (via succinate dehydrogenase, a component of complex II), which donates to coenzyme Q, thereby bypassing complex I. Cytochrome c transfers electrons from complex III to complex IV. Once electrons are carried by coenzyme Q, complex I is no longer required for electron transfer to oxygen.

2) The answer is E: Inhibition of the proton translocating ATPase. Oligomycin blocks the FO component of the proton-translocating ATPase, thereby blocking proton flow through the enzyme and ATP synthesis. Oligomycin does not affect any other complex of oxidative phosphorylation

3) The answer is B

4) The answer is c

For any feedback, scan the code or click on it.



Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	2	Cytochrome C oxidoreductase or complex IV: has an enzymatic activity that reduces O ₂ to H ₂ O.	Cytochrome C oxidase or complex IV: has an enzymatic activity that reduces O ₂ to H ₂ O.
V1 → V2	8	UCDs	UCPs

Additional Resources:

رسالة من الفريق العلمي:

Reference Used:
(numbered in order as cited in the text)

1. Lippincott's illustrated: Review of Biochemistry 7th edition
2. Marks Basic Medical Biochemistry 5th edition

Extra References for the Reader to Use:

1. [Uncoupling overview](#)
2. [regulation by Khan Academy](#)

يقول النبي صلى الله عليه وسلم: ما يُصِيبُ الْمُسْلِمَ، مِنْ نَصَبٍ وَلَا وَصَبٍ، وَلَا هَمٍّ وَلَا حُزْنٍ وَلَا أذى وَلَا غَمٍّ، حَتَّى الشُّوْكَةِ يُشَاكُهَا، إِلَّا كَفَّرَ اللَّهُ بِهَا مِنْ خَطَايَاهُ..
رواه البخاري.

سُورَةُ الطَّلَاقِ

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

ذَلِكَ أَمْرُ اللَّهِ أَنْزَلَهُ إِلَيْكُمْ وَمَنْ يَتَّقِ اللَّهَ يُكَفِّرْ عَنْهُ
سَيِّئَاتِهِ وَيُعْظِمْ لَهُ أَجْرًا

#ارحموا الافريج