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Assignment 1

a) The metabolic disorder is called Phenylketonuria (PKU).In this disorder the gene which produces enzyme phenylalanine hydroxylase(PAH) which converts phenylalanine to tyrosine is mutated, this renders PAH non functional. Due to which the amino acid Phenylalanine gets accumulated in the body , phenylalanine is toxic for the body. Phenylalanine is converted to Phenylypyruvate that is detected in the urine.

b) The gene that produces PAH is found on chromosome 12. In the disorder both the alleles of that gene are mutated. Mutations on both copies can mean the enzyme is completely inactive or inefficient. In cases where the patient has heterozygous chromosomes then a milder PKU is present. In a few cases a mild version might be present in PKU subjects this is known as hyperphenylalaninemia.

c) I will propose three mechanisms by which we can potentially cure the disease by gene modifications. Since it is not provided in the question whether or not we have to edit human genes or not. In the **first method** we will use recombinant DNA technology and modify a bacteria with low pathogenicity to produce PAH. We can do this by using viral vectors to insert the PAH gene into the plasmid of bacteria by restriction enzymes. We can use these transformed bacteria to produce PAH enzyme in a bioreactor to be used as syringes or tablets. In the **second method** we can use we can use CRISPR - Cas9 to repair the mutated PAH gene. For this we will use a virus vector to introduce Cas9 machinery and RNA with homology to the parts of the mutated gene(target). The Cas9 we use this gene to make incision in the target gene and the host mechanism will repair the mutations. Adeno associated virus (AAV) can be used as a potential vector to infect the embryo due to its low pathogenicity. In the **third method** we can use somatic gene therapy for curing the PKU. For this we will use a retro viral vector to insert a wild type gene in the place of a mutated gene on the chromosome 12 of the hepatic cells. Out of the three methods the first one is much safer as there is no direct change in the human genome. The second one has a significant chance of failure and may induce unrequired changes in the human genome. Thus this method is very dangerous and unethical. Similarly the third method is very dangerous as it may introduce some more unwanted and possibly irreversible changes.

Assignment 2

Oxidative phosphorylation is a process of producing energy(in the form of ATP) through chemical reactions. It occurs in the inner mitochondrial membrane and is a redox reaction where the glucose is oxidised and the oxygen is reduced. Oxidative Phoshphorylation has two features: the electron transport chain (ETC) and chemiosmosis.

The ETC is a chain of protein complexes embedded inside the inner membrane that help in transport the electrons of the above redox reaction. As the electrons go to the subsequent complex it loses some energy which helps transport hydrogen ions into intermembrane space. The ETC consists of four protein complexes with their corresponding proteins as shown below:

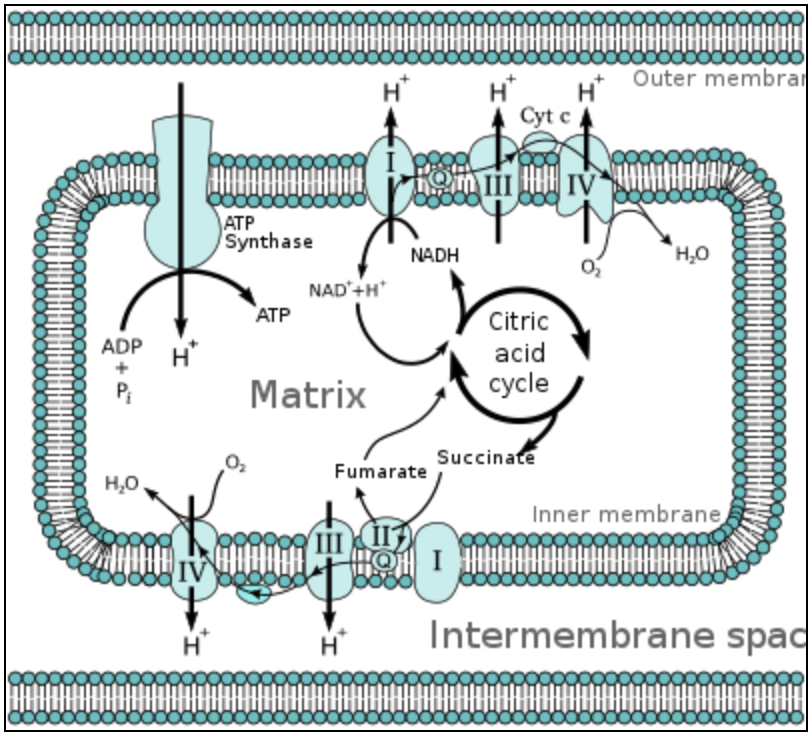

Fig 1: Mitochondrial ETC

The electron carriers in the complexes found are Ubiquinone or coenzyme Q, Cytocrome a, a3, b, c, c1 (these are heme complexes having an iron core), FMN(Flavin mononucleoside) and Fe-S(Iron SUlphur clusters). These electron carriers transport electrons and protons along with them. For example, we take the case of ubiquinone to ubiquinol. The ubiquinone takes two electrons and two protons(H+) to give ubiquinol as illustrated.

The citric cycle provides two sources of protons and electrons from FADH and NADH. The detailed process of ETC is as follows

1. COMPLEX I: The NADH deposits two protons and two electrons at complex I. The electrons are picked up by FMN which transfers them to the FeS clusters. The Ubiquinone at the end of the complex picks up the electrons and two protons. The electrons transfer from FMN to Ubiquinone generates enough energy to pump four electrons. Thus we can pump at least 4 protons across the membrane. The ubiquinol which is formed transfers the electrons to COMPLEX II

2. COMPLEX II: This step is parallel to the citric acid cycle. The succinate which is an intermediate in citric acid cycle is reduced by succinate dehydrogenase to form fumarate and the FAD in the COMPLEX is also reduced to form FADH that picks up the electrons from succinate to fumarate reduction. Similar to COMPLEX I, the FeS clusters pick up these electrons and transfer the protons and electrons to ubiquinone to form ubiquinol.
3. COMPLEX III: The two ubiquinol molecules formed in COMPLEX I and II are free to move between the membrane and transfer the electrons to complex III. THe cyt-b picks these electrons which are transferred to FeS centers to cyt-c1. The ubiquinol is oxideized to ubiquinol. The cyt-c1 transfers to cyt-c which is freely moving inside the membrane and transfers them to COMPLEX IV. The complex III pumps 4 protons in intermembrane space.
4. COMPLEX IV: In complex IV the copper ions take the electrons from cyt-c and transfer them to heme complexes cyt-a , cyt-a3 and then to copper ions. Finally these electrons are picked up by the oxygen forming water. COMPLEX IV pumps 4 protons in intermembrane space.

Finally we have created a high concentration of H+ ions (protons) in intermembrane space. A special protein is embedded in the inner membrane of mitochondria called the ATP synthase. The ATP syntase allows the protons to move across the membrane freely as they move across the membrane they create an electrochemical gradient called the proton motive force. The production of ATP is coupled by the movement of protons. The proton movement to a lower concentration gives the ATP synthase enough energy to produce ATP.

Peter D Mitchell first came out with his chemiosmotic theory which stated that the production of ATP is coupled with movement of protons across an electrochemical gradient formed by the energy NADH and FADH. Later Paul D Boyer gave his rotational catalysis theory and suggested that ATP synthesis happens to a conformational change in ATP synthase generated by the rotation of its subunit due the flow of protons. John E Walker got the crystallized F1 catalytic-domain of ATP synthase leading to confirmation of Boyer's theory. They shared a Nobel price for this discovery in 1997.