SP25 Group Activity #3 KEY

Your team is studying cellular metabolism, and you have run a series of experiments across various cell lines from patients with unknown metabolic diseases. Several of these cell lines exhibit reduced ATP production and you are trying to determine the cause.

You fluorescently label glucose and add it to Cell Line #1. These cells appear to be producing only ~4 ATP molecules per glucose. You further perform localization and activity analyses on the enzymes of the TCA cycle and find that while they are all localized to the mitochondrial matrix, they have very low activity.

Q1. Where do you expect your fluorescent signal to be present in your cells? Why? (10 points)

You would expect the signal to be present in the cytosol, as it appears glycolysis can occur without issue, but that pyruvate is prevented from entering the mitochondria, likely due to a mutated carrier protein.

Q2. What might you expect to happen to the pyruvate produced in this cell line? (10 points)

As pyruvate cannot enter the mitochondria, it would likely be instead further metabolized to lactic acid for an alternative energy source to ATP or into metabolites for other cellular processes.

Cell Line #2 similarly produces a greatly reduced amount of ATP, but here the activity analyses of the TCA cycle suggest normal function. You conclude there must be some issue with the electron transport chain. While Complexes II, III, and IV appear to have normal structures following x-ray crystallographic analysis, Complex I appear to have a mutation in the active site where it interacts with NADH. You decide to measure the redox potential of this mutant Complex I and discover it is greatly elevated, to a value even larger than that of oxygen!

Q3. Why would this elevated redox potential be a problem for ATP generation? (10 points)

This elevated redox potential would be a problem for ATP generation because Complex I would take the high energy electrons from NADH and keep them, because now coenzyme Q (and all subsequent members of the electron transport chain) would have a lower redox potential and so would not be a productive exchange partner.

This would lead to limited proton pumping from NADH molecules produced through the TCA cycle into the intermembrane space, greatly reducing the electrochemical gradient and proton-motive force driving ATP production.

Q4. Would you expect Cell Line #1 or Cell Line #2 to produce more ATP? Why? (10 points)

Cell Line #2 would produce more ATP because $FADH_2$ would still be able to function as normal, producing ATP via oxidative phosphorylation, and it is possible NADH would still have some contribution via Complex I alone. In contrast, Cell Line #1 cannot participate in oxidative phosphorylation at all due to pyruvate being prevented from entering the mitochondria.

The next cell line you examine appears to function similarly to Cell Line #2, except that when you perform x-ray crystallography Complex I appears to be completely absent from the electron transport chain. You perform an immunofluorescence experiment to locate Complex I and find it is sequestered

to the cytosol. A more detailed structural analysis of this cytosolic Complex I reveals a truncation of the N-terminus of the protein.

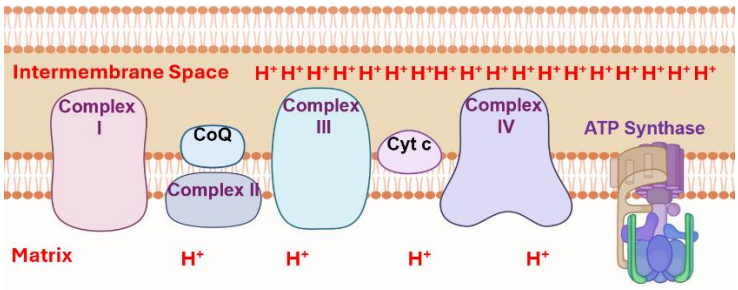
Q5. Why would this mutant Complex I appear in the cytosol? (10 points)

The N-terminus of Complex I must contain a mitochondrial targeting sequence that would normally be recognized and brought to TOM and TIM for mitochondrial import. Without this sequence, the translated protein would remain in the cytosol as it has nothing to indicate a specific cellular destination.

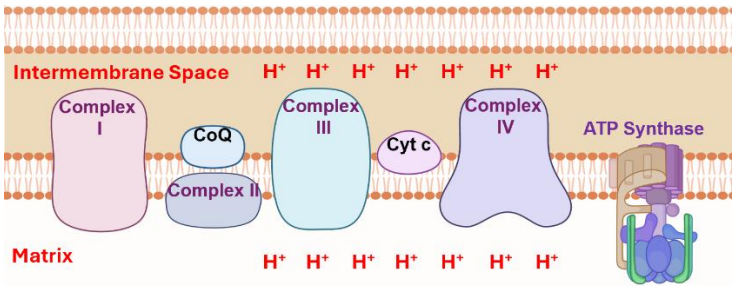
You decide to take a break from the lab work and move to the clinic, where a patient is complaining of hyperthermia (excessive body heat). They claim this all started when they began taking a weight loss drug promoted by their favorite fitness influencer. You investigate the mechanism of action of this drug and it appears that it works as an ionophore, binding protons and transporting them across lipid bilayers down their concentration gradients.

Q6. On the following images label the mitochondrial matrix and the intermembrane space, and draw in H^+ protons as you would expect in the absence or presence of this drug (20 points)

Absence of drug:



Presence of drug:



Q7. Why would this drug lead to hyperthermia? (10 points)

The drug would cause a dissolution of the proton gradient produced by the electron transport chain, so instead of the large amount of free energy produced through the pumping of protons being used to synthesize ATP it would simply be released as heat.

A separate patient has an aggressive tumor that you have identified as being caused by an overactive MAP kinase signaling pathway, which promotes cell growth. Further study reveals the receptor tyrosine kinases in these tumor cells are dimerizing and autophosphorylating each other even in the absence of ligand binding.

Q8. Why would the activity of these RTKs lead to tumor formation? (10 points)

Auto-dimerization and activation of these RTKs would lead to constitutive activation of the MAPK pathway via constant recruitment of Ras-GEF, leading to GDP-to-GTP exchange on Ras, causing activation of MAPKKK, etc., which would lead to uncontrolled cell growth.

Q9. Propose a targeted intervention that would help prevent further growth of this tumor. (10 points)

To prevent further growth of the tumor you would need to prevent this pathway from actively signaling. This could be done by inhibiting Ras-GEF, Ras, or any of the MAP kinases, depleting GTP, or preventing nuclear entry of downstream transcription factors.