2022 BCDB Qualifying Exam Part I: Day 1

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Please remember as you answer the questions that you have approximately 1 1/2 hour per question. This means that we expect in-depth answers unless otherwise noted. You may use diagrams to illustrate your answer as needed. Although each question is worth the same number of points, not all questions will require the same amount of time. Allocate your time wisely. We recommend that you first make an outline of your answer, rather than just making up your answer as you write. Some questions ask you to propose experiments. Choose the most direct and realistic approaches and explain your experimental rationale as clearly as possible. Be sure to include controls, expected outcomes, and possible problems and solutions.

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Indicate the number of images you are including with each of your answers (add more columns as needed)

Question	# of Images

Contact Cell phone numbers (if you have other questions).

Andreas Fritz: 404 668 3753
Anita Corbett: 404 421 9061
Amy Walker 404 556 1883 (OneDrive or logistical questions)

Question 1

You recently learned that many genes in the mammalian genome are imprinted, meaning they are expressed, or not expressed, in specific tissues depending on the sex of the transmitting parent. For example, if gene W is maternally imprinted in liver, the copy of gene W inherited from the mother is epigenetically "shut down" and not expressed in liver, whereas the copy of gene W inherited from the father is expressed in liver. If gene W were paternally imprinted, the expression pattern would be reversed, so that only the copy inherited from the mother would be expressed in liver.

1. Assume gene W, which encodes a highly conserved enzyme essential for hepatocyte homeostasis, is maternally imprinted in liver.

a. What would be the consequence to a mouse pup of being heterozygous for an allele of gene W that carries a pathogenic mutation if that allele was transmitted by the mother? Explain. **(2 points)**

b. What would be the consequence to a mouse pup of being heterozygous for an allele of gene W that carries a pathogenic mutation if that allele was transmitted by the father? Explain. **(2 points)**

2. Intrigued by what you have learned about gene W, you want to find out what other genes are maternally, or paternally, imprinted in mouse liver. Assume you have at your disposal a well-equipped lab as well as male and female breeder mice of any inbred strains you might want.

a. Design an experiment to identify genes that are maternally imprinted versus paternally imprinted versus not imprinted in mouse liver. Explain your strategy and include appropriate controls. **(8 points)**

b. You are delighted that you found several genes in your experiment that you suspect are imprinted. Picking one of these genes, describe an experiment to validate that your candidate gene is actually subject to imprinting. **(8 points)**

Question 2:

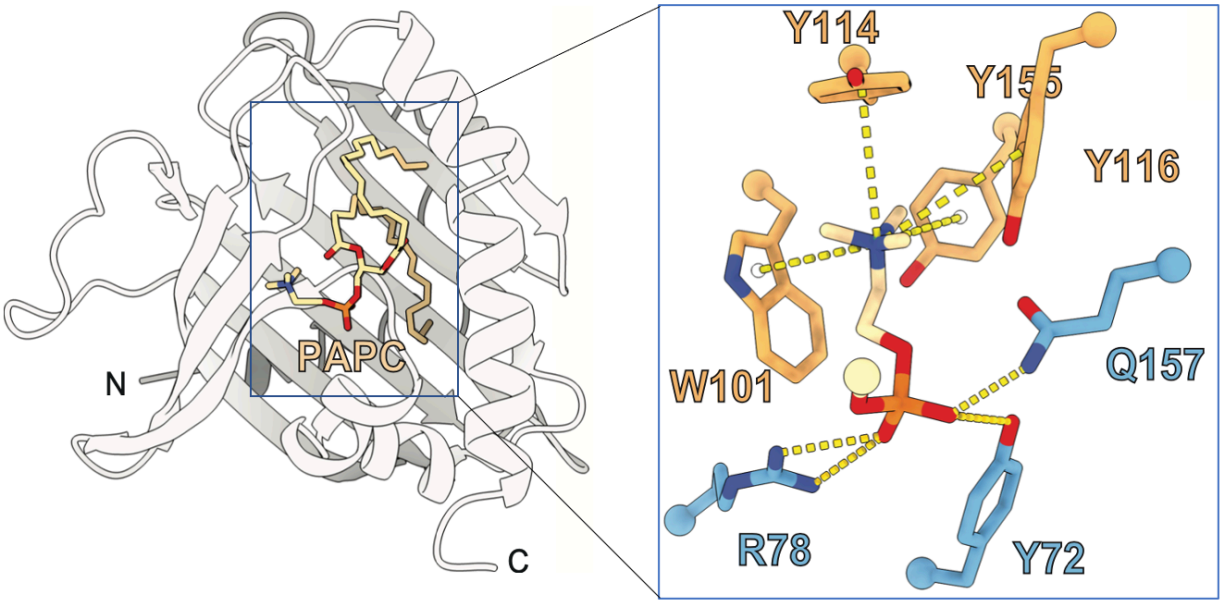
Lipid transfer proteins play a critical role in maintaining cellular lipid homeostasis and defects in proper lipid trafficking can lead to disease. For example, Barth Syndrome results from a defect in the production of cardiolipin in the mitochondria. This in turn impairs the function of the respiratory chain eventually leading to cardiac failure. Increasing mitochondrial cardiolipin synthesis may provide a treatment for this disease. Your lab has identified a soluble protein, StardX, that may function to shuttle phosphatidylcholine (PC) from the endoplasmic reticulum to the mitochondria where is may be used for cardiolipin synthesis. Your project in the lab is to study the function of StardX and develop additional tools such as chemical inhibitors and potentiators of StardX protein function.

Part A

1. Using a cell culture model, how you would determine the function of StardX on mitochondrial lipid composition? For this question, assume you do not have access to a synthetic inhibitor. Include a rationale and controls needed. (**8 points**)

2. Design an assay to screen a pre-existing library of compounds to identify novel compounds that inhibit PC binding to StardX. You can assume that you have access to any reagents in your fully stocked lab. Describe the rationale for the selected assay, including controls required to interpret the data you obtain. (**6 points**)

Part B



You were fortunate enough to determine the crystal structure of StardX in complex with a phosphatidylcholine species (PAPC). A cartoon representation of the StardX-PAPC complex is shown on the left and a close-up view of PAPC is shown as an inset on the right (red=O, dark blue=N, orange=P). Non-covalent interactions that contribute to the recognition of the phosphocholine moiety on PAPC are highlighted with dashed lines.

3. Describe two molecular interactions contributing to the recognition of PC. (**2 points**)

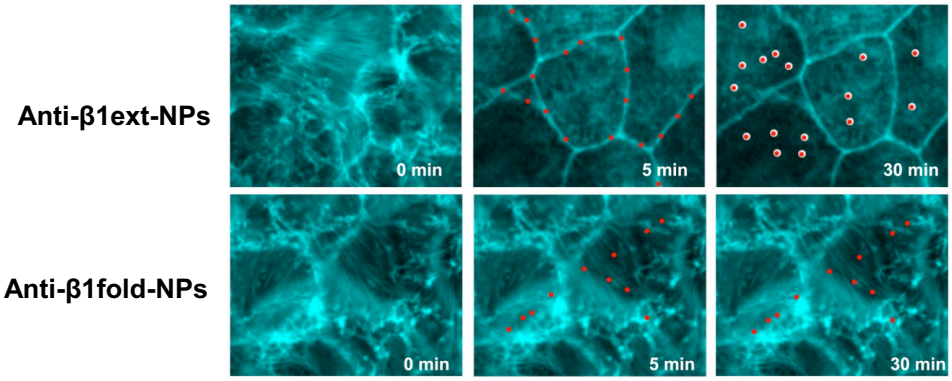
4. Propose a point mutation that would disrupt binding to all glycerophospholipids and explain your rationale. (**2 points**)

5. Propose a point mutation that would disrupt the recognition of PC species. For example, design a mutation that would prevent StardX from distinguishing PC from other phospholipids. Be sure to explain your rationale. (**2 points**)

Question 3

You are interested in how nanoparticles (NPs) coated with anti-integrin antibodies interact with cells. You create two types of fluorescently tagged NPs, one type coated with antibodies that recognize β1 integrin in an extended form (Anti-β1ext-NP) and another type that recognizes β1 integrin in a folded conformation (Anti-β1fold-NP)

You set up cultured intestinal epithelial cells in transwells and once they become confluent, you stain the actin filaments with a Silicon-Rhodamine fluorophore tagged compound that binds to actin (SiR-actin; cyan) then incubate them with either Anti-β1ext-NPs or Anti-β1fold-NPs (red) and follow them by live cell microscopy and obtain the following results:



At t=30 minutes after NP incubation, Anti-β1ext-NPs appear to be internalized (red contents) and in vesicles that are coated with actin (cyan surfaces). On the other hand, Anti-β1fold-NPs appear to remain associated with the plasma membrane. There is also a significant rearrangement of actin, where Anti-β1ext-NPs cause actin to become associated with the plasma membrane (cortical actin).
You measure transepithelial resistance (TER) at t=30 minutes and find that cells treated with Anti-β1ext-NPs have a TER of 1523 $\pm$ 121 Ohm x cm^2 and Anti-β1fold-NPs have a TER of 347 $\pm$ 94 Ohm x cm^2. Untreated cells have a TER of 532 $\pm$ 103 Ohm x cm^2

1. You are considering using anti-integrin NPs as an approach to facilitate intestinal drug delivery. Based on these results, how would you use these NPs as a drug delivery approach. (**4 points**)

2. Provide a hypothesis for the effects of Anti-β1ext-NPs on actin organization and epithelial barrier function. The hypothesis should specifically address why Anti-β1ext-NPs and Anti-β1fold-NPs have different effects on the cells. (**4 points**)

3. Provide an experimental approach to test your hypothesis. Make sure you address all the components affected by the NPs and also include both positive and negative controls. (**6 points**)

4. What do you predict would happen to actin and TER if you incubated cells with a 50:50 mixture of Anti-β1ext-NPs and Anti-β1fold-NPs? Explain the logic behind your prediction and include an experimental approach to elucidate a possible mechanism. (**6 points**)

Question 4

In your research group, you are studying a particularly aggressive and hard to treat form of breast cancer termed Triple Negative Breast Cancer (TNBC). In your studies, you have been trying to identify potential targets that might be highly expressed in the TNBC cells compared to normal breast cancer cells. For these studies, you have obtained several TNBC cell lines and you have control breast cell lines. In addition, you have a number of patient samples.

Performing a comparative proteomic study identified a number of candidate proteins that are detected at higher levels in the TNBC cells as compared to controls. With an interest in targeting these pathways for therapeutics, you have focused efforts on a kinase (MFK) that you hypothesize is important to drive a signaling pathway critical to cell transformation in TNBC. MFK is a classical kinase, which uses a catalytic lysine residue to mediate phosphorylation of target substrates. In your studies, you find that when you compare control non-transformed breast cells (Control) to those the TNBC cells (TNBC Tumor), there is an increase in the steady-state level of the MFK protein in the Tumor cells compared to the Control cells (**Figure 1**).

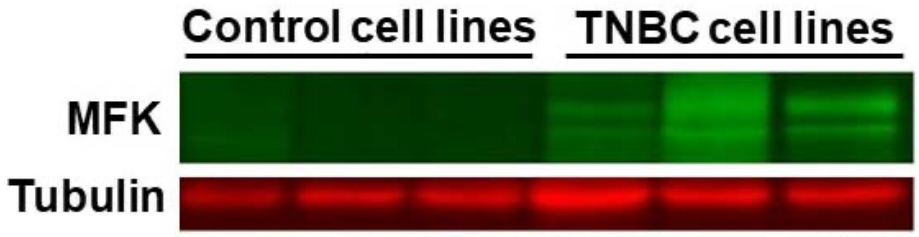


Figure 1. An immunoblot to detect the kinase MFK in a Control cell line and TNBC cells lines. Tubulin serves as a loading control.

You extend your work into patient samples and find a strong correlation between the level of MFK protein and the aggressiveness of the tumor (more aggressive tumors have more MFK).

Assume you have access to all tools required for any studies you would like to perform including an antibody directed against MFK, clones to express MFK in cultured cells, a clone to express recombinant MFK, and anything else that you might need for the proposed studies.

1. You decide to define the mechanism(s) that regulate(s) MFK protein levels in the TNBC cell lines you are studying, taking into consideration different steps within the central dogma of gene expression that could be impacted to alter the steady-state levels of MFK protein. Describe the first experiment you would perform to address the mechanism(s) regulating MFK in breast tumor cell lines (TNBC Tumor) as compared to the Control. Be sure to include appropriate controls and describe the results you obtain. (**2 points**).

2. Drawing on the results that you obtained in (**1**), what is the next experiment you would perform to understand how MFK levels are regulated in the TNBC breast tumor cell lines, making reference to the relevant central dogma step. Clearly state your hypothesis describing how you propose that MFK levels are regulated (**2 points**) and then provide an experimental approach to test the hypothesis (**6 points**). Be sure to include appropriate controls. Describe both the experimental outcome you would obtain if the data support your hypothesis and the outcome if the data refute your hypothesis. (**3 points**). (**11 points total**)

3. Based on the observation that MFK protein levels are increased in more aggressive TNBC tumor cell lines, you set out to test the hypothesis that MFK catalytic function is necessary to drive oncogenesis. You can choose a tumor phenotype of your choice such as anchorage-independent growth. Using the tools available to you, describe an experimental approach to test this hypothesis. (**5 points**).

4. In **Figure 1**, you note that not only is the steady-state level of MFK increased in the TNBC cells lines compared to Control cell lines, but there are two bands with an increase in the top band detected in the TNBC tumor cell lines. Provide a hypothesis that could explain why there could be two bands detected for this kinase. (**2 points**).

Question 5

In your first meeting with your thesis mentor, she explains some new preliminary data in the lab suggesting an uncharacterized transcript, *spirofibrillogen* (*sfg*) mRNA, is enriched in cultured mouse axon growth cones. She wants you to study the transport mechanisms of *sfg* mRNA.

1. Design an experiment that would allow you to monitor *sfg* mRNA in <u>live</u> cells. Briefly explain the key components of the assay and what they do. Include a simple labeled schematic with your response. (**5 points**)

2. While imaging on the microscope, you observe processive 'runs' of *sfg* mRNA directed toward the axon growth cone (anterograde transport) with average velocities around 1μm/s. You hypothesize a role for the cytoskeleton. Based on papers read during Foundations, articulate what filament system is involved and how you might perturb mRNA transport pharmacologically. What type of drug would you add to your culturing medium to compile kymographs of your mock versus drug treated samples (see Figure) and what cytoskeletal element does it antagonize? (**1 point**)

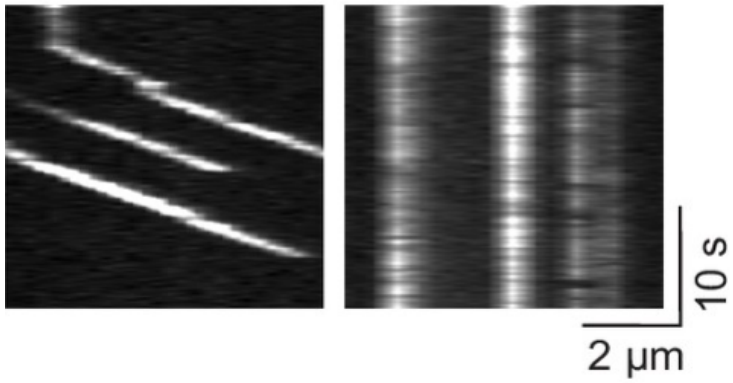


Figure: Image shows kymographs of the mock treated control (left) and drug treated (right) cultured mouse axon expressing fluorescently labeled *sfg* mRNA.

3. With respect to the motility of *sfg* mRNA, describe what is observed in the Figure. (**1 point**)

4. Based on your above observations, propose a hypothesis that would explain the molecular mechanism of *sfg* mRNA localization to the axon growth cone. Name the key proteins involved in this process. (**3 points**)

5. Propose one alternative strategy to test your hypothesis. Describe how your chosen approach works, how you will know if your hypothesis is true or null, and be sure to mention necessary controls. (**5 points**)

6. After discussing your exciting data during group meeting, your advisor suggests you examine whether *sfg* mRNA is locally translated in the axon growth cone. Propose one experiment that would test where *sfg* mRNA is translated. Describe how your chosen approach works, how you will know if your hypothesis is true or null, and be sure to mention necessary controls. (**5 points**)

Question 6

1. *In vivo* lineage tracing remains the gold standard for demonstrating stem cell function in adult tissues. Define and explain the two basic mutant alleles required to conduct a lineage-tracing experiment in a mouse model (**5 points**).

2. The intestinal epithelium has been referred to as a "prototypical" stem cell model, due in part to its rapid rate of homeostatic renewal throughout adult life and its diverse complement of post-mitotic cell types (**Figure 1**). Explain what the lineage tracing results shown in **Figure 2** indicate and why. (**10 points)**

   Make sure your answer:
   a. provides a brief explanation of how the results were obtained.
   b. highlights why the experiment likely included both early and late timepoints. Note that you are not expected to know the precise marker gene of the traced cells, but that your answer should demonstrate a strong conceptual understanding of the principles underlying lineage tracing.

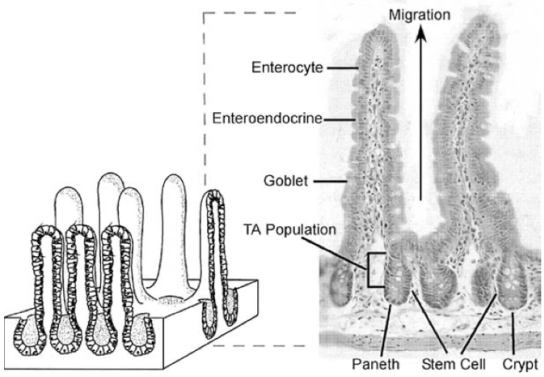


Figure 1: Morphology and cell types of the intestinal epithelium.

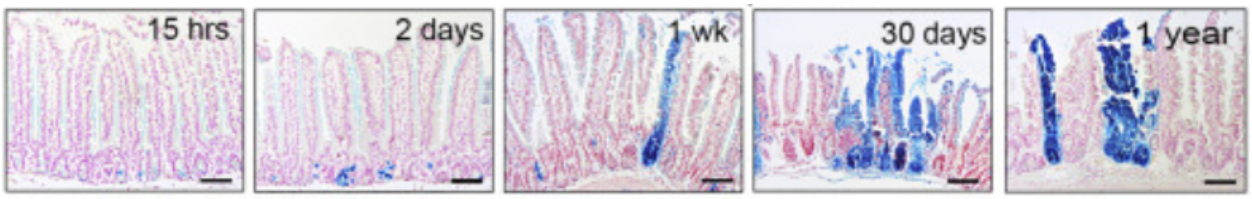


Figure 2: Results of an *in vivo* lineage-tracing experiment in the intestinal epithelium. Blue signal indicates X-gal staining.

3. Do the lineage tracing results above suggest that the traced cells are self-renewing? Make sure your answers explain why or why not. (**2 points**)

4. Describe an experiment that would test whether the traced cells are multipotent. (**3 points**).

2022 BCDB Qualifying Exam Part I: Day 2

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Question 7

Chronic itch (pruritis) is a significant unmet health problem that can be caused by liver disease or can be a side effect of medications, particularly chemotherapy. Identification of the receptors involved in sensing such non-histamine-mediated itch would be an important breakthrough. One of the drugs that can cause itch is codeine.

For your project in the lab, you have found that sensory neurons express a previously uncharacterized G-protein coupled receptor (GPCR). Because this GPCR is expressed at the highest levels in the sensory neurons that process the itch signal, you hypothesize that this GPCR (which you have named *Scratchy*) is involved. As pilot data, a previous student in the lab has shown that a sensory neuron-derived cell line used in your lab responds to codeine by increasing cytosolic levels of the second messenger calcium (Ca^{2+}). You hypothesize that *Scratchy* is a receptor for codeine.

For the following questions, assume that you have a fully stocked lab, including all the resources for cell culture and approaches such as cell transfection, a variety of cell lines, and all the equipment that you would need to measure cell signaling. You also have expression plasmids for *Scratchy*, G-protein alpha subunit family members, G-protein beta subunits, G-protein gamma subunits, and for different downstream effector proteins. This includes plasmids expressing these proteins as fusions with enzymes such as luciferase or with fluorescent proteins such as Green Fluorescent Protein.

1. Design a detailed experiment to test your hypothesis that *Scratchy* mediates the response (increase in Ca^{2+}) to codeine in the neuronal cell line. Describe the experimental approach that you will use, including any relevant controls needed to interpret the results. **(6 points).**

2. Congratulations. The results from your previous experiment support your hypothesis that codeine signals through *Scratchy*. **(8 points).**

The next question that you would like to answer is: Which of the G-protein alpha subunit family members does *Scratchy* couple with/signal through? Design a detailed experimental approach to answer this question. Your goal would be to identify understand how this signaling occurs in response to codeine. As with Part 1, include a description of the experimental approach that you will use, including any relevant controls needed to interpret the results.

3. A common cause of itch is histamine. Histamine binds to a different GPCR, the histamine H1 receptor, and signals specifically through Phospholipase C β3 (PLCβ3).

The next question that you would like to answer is: Does Scratchy also signal through PLCβ3? Design a detailed experiment to answer this question. As with Part 1, include a description of the experimental approach that you will use, including any relevant controls needed to interpret the results. **(6 points).**

Question 8

RNAz is a non-coding RNA (ncRNA) that is highly expressed in pathogenic bacteria during antibiotic exposure. RNAz appears to function in transcriptional regulation but its precise function is unclear. To become a mature ncRNA (40 nucleotides), RNAz is processed/cleaved from a precursor RNAz (pre-RNAz, 60 nucleotides) and this process is catalyzed by a protein-only ribonuclease (RNase) Q (PORQ). To date, many PORQs have been identified in bacteria by sequence analysis, but the molecular basis underlying substrate recognition by PORQs remains elusive. Johnny's dissertation focuses on the structural and biochemical characterization of the PORQ family of proteins and, in particular, aPORQ. Johnny has overexpressed and purified aPORQ from *E. coli* and his goal is to solve structures of apo- and pre-RNAz-bound structures of aPORQ to understand how the protein facilitates RNA processing, how the catalytic site is formed, and whether the catalytic site is conserved among aPORQ family members. Given that antibiotic-resistance mutations have arisen in PORQ protein family members, understanding how these mutations may affect protein function and RNA processing is important.

1. To investigate whether pre-RNAz cleavage activity is conserved in PORQs, Johnny overexpressed and purified two PORQs (aPORQ and bPORQ) from two different bacteria pathogens. Both PORQ proteins are ~15 kDa and share ~60% amino acid sequence similarity and ~30% amino acid sequence identity. Because of the high sequence similarity/identity, Johnny applied the same purification strategy for both proteins. During the final purification step on a size exclusion chromatography column (SEC), Johnny noticed that aPORQ and bPORQ behave differently from each other in a buffer containing 100 mM NaCl (**Fig. 1**). Provide an explanation for why aPORQ and bPORQ behave differently (**4 points**).

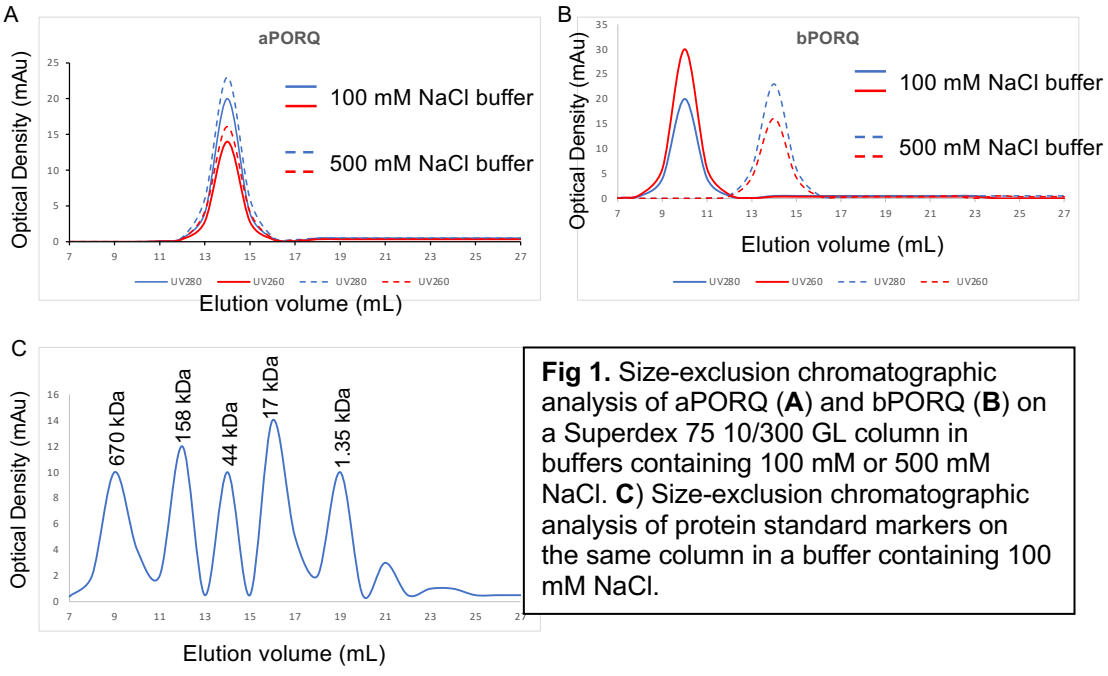


Fig 1. Size-exclusion chromatographic analysis of aPORQ (**A**) and bPORQ (**B**) on a Superdex 75 10/300 GL column in buffers containing 100 mM or 500 mM NaCl. **C**) Size-exclusion chromatographic analysis of protein standard markers on the same column in a buffer containing 100 mM NaCl.

2. To test if there are functional differences between the proteins at 100 mM NaCl, Johnny performs an *in vitro* cleavage assay using *in vitro* transcribed pre-RNAz with purified aPORQ and bPORQ in a buffer containing 100 mM NaCl. The PORQ protein concentrations are fixed at in all reactions. Johnny then analyzes the results of his experiment on a gel (**Fig. 2**).

a. What kind of gel was used for this experiment and why? (**2 points**)

b. How was the gel visualized? (**1 point**)

c. What do these results reveal? (**3 points**)

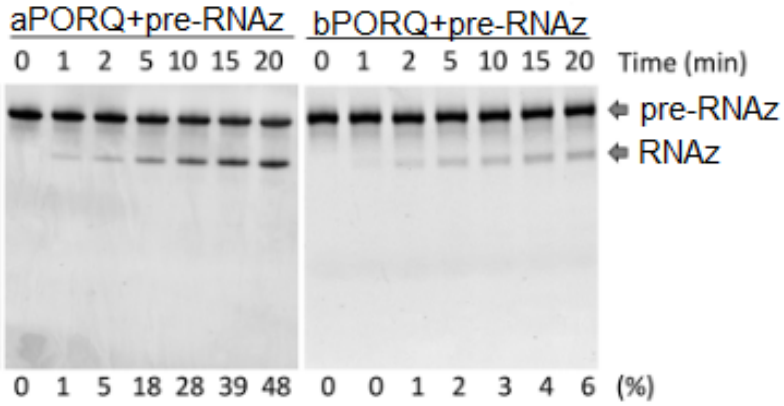


Fig 2. In vitro pre-RNAz cleavage assays catalyzed by PORQs. The substrate cleavage percentage (%) is shown at the bottom of the gels.

3. Puzzled by different behaviors of the two PORQ proteins, Johnny performs crystallographic studies for both proteins in their apo forms, but no crystals are obtained. He next tries to co-crystallize the proteins with pre-RNAz. To “trap” this complex, the cleavage of the pre-RNAz needs to be inhibited. Given that you have performed a protein sequence homology analysis, which amino acids would you predict would be important for this reaction? (**2 points**).

4. Johnny eventually succeeds in solving a 2.8Å structure of aPORQ bound to pre-RNAz (**Fig. 3A**). In this structure, aPORQ is found as a tetramer (dimer of dimers, labeled A-D) with one dimer solely interacting with the pre-RNAz (**Fig. 3B**). Using information obtained from direct contacts between aPORQ and pre-RNAz, design an experiment to test which aPORQ residues are important for activity. Include a rationale for your experiment, suggest appropriate controls and draw out the results from the experiment (**4 points**).

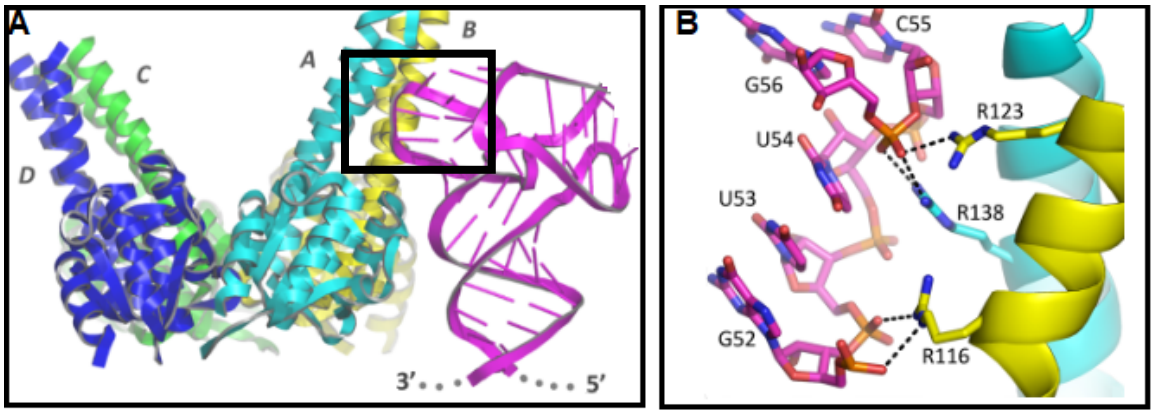


Fig 3. Co-crystal structure of aPORQ-pre-RNAz. **A**) aPORQ forms a dimer of dimers (tetramer) made up of protomers A, B, C and D. The RNA (magenta) is adjacent to one dimer. **B**) Zoomed in view of the region boxed in A showing the molecular interactions between aPORQ and pre-RNAz.

5. Next, Johnny would like to assess the significance of dimerization by aPORQ for its catalytic activity on pre-RNAz. For this experiment, Johnny needs to construct and purify an aPORQ mutant defective in forming a dimer (**Fig. 4**). Based on the structural information he obtained, design a mutational strategy to prevent dimerization including a rationale and experimental plan to test the hypothesis that dimerization is essential for the function of aPORQ in pre-RNAz cleavage. Include appropriate controls and draw out the results from the experiment (**4 points**).

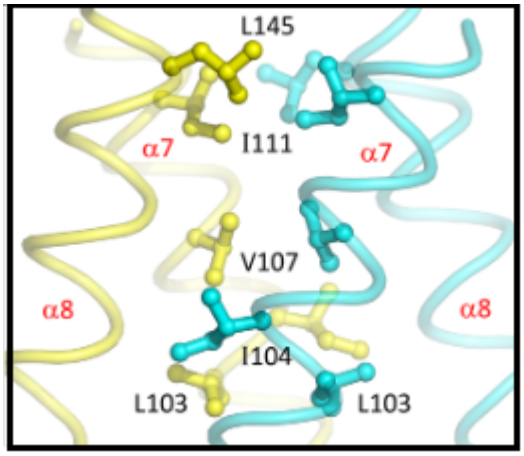
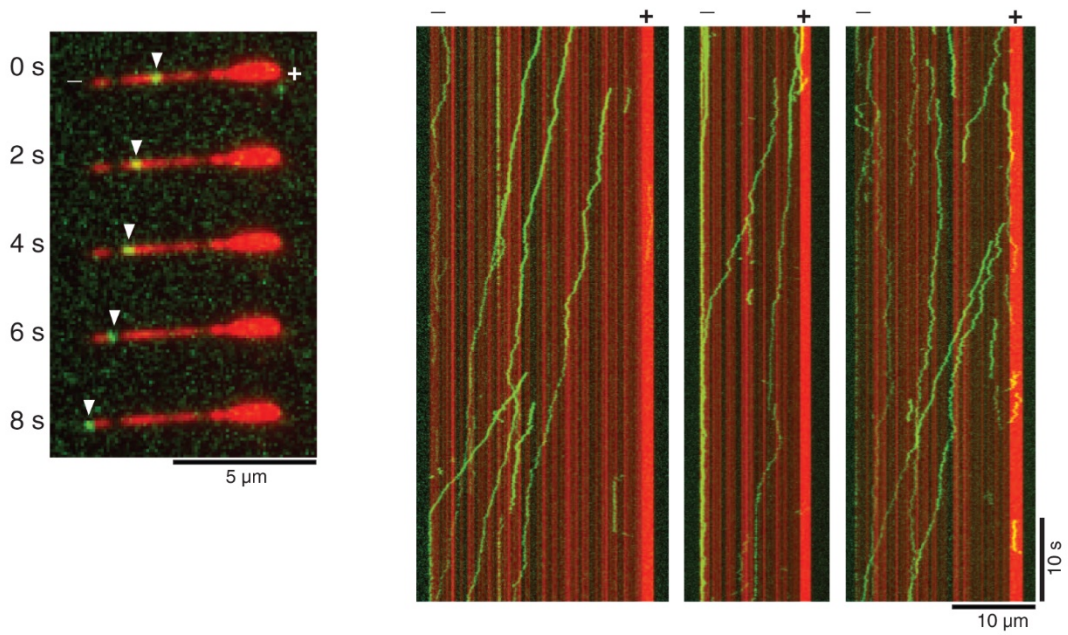


Fig 4. Interactions involved in aPORQ dimerization.

Question 9



You are investigating a mechanism of mitochondrial motility in neurons. As a candidate protein that mediates the motility, you successfully purify a motor protein of unknown identity from brain extracts. To characterize this motor protein, you perform an in vitro reconstitution experiment in which mitochondrial fragments (labeled by green fluorescence) move along immobilized red-labeled microtubules in the presence of the motor protein. The left panels illustrate an example of successive frames of a total internal reflection fluorescence (TIRF) microscopy time-lapse movie showing locations of a mitochondrial fragment (green) at different time points (indicated by white arrowheads) on a surface-immobilized red-labeled polarity-marked microtubule with a bright plus end (+) and a dim minus end (-). The right panels show representative kymographs of the movement of mitochondrial fragments (green) on red-labeled polarity-marked microtubules. Note, for each kymograph, time zero is at the top edge.

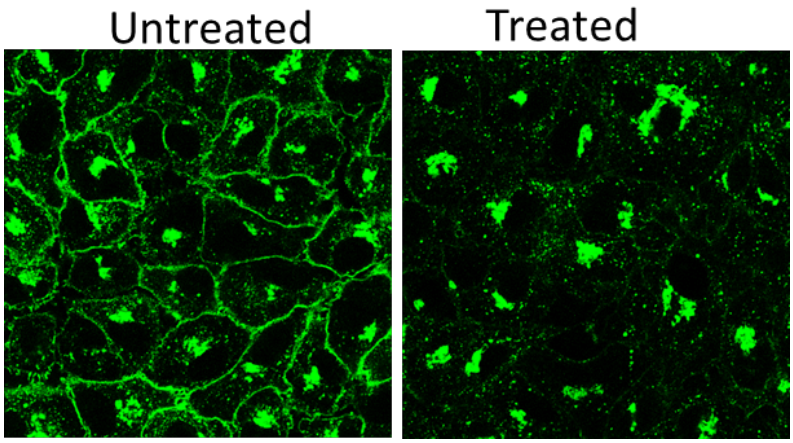
1. Based on this experiment, describe functional properties of this motor protein. Be sure to include the polarity of movement of this motor and hypothetical molecular features required to mediate microtubule-based movement of mitochondria. Illustrate as required. **(2 points)**.

2. Propose **TWO** experimental strategies including controls to test molecular features you proposed in (1). **(6 points)**.

3. The kymographs indicate that velocity and persistence of mitochondrial movement vary. State **TWO** hypothetical mechanisms causing the variability in the movement (excluding the possibility that the motor was partially denatured or degraded). **(6 points)**

4. For each of the two mechanisms you hypothesized in (3), design **ONE** experiment to test your hypothesis. Be sure to include controls. **(6 points)**.

Question 10



Immunostaining epithelial cells for protein X revealed that this protein is primarily localized at the cell periphery, with some notable perinuclear localization (left). Cell pretreatment with compound AG resulted in a marked loss of protein X from cell periphery and apparent accumulation at perinuclear sites (right).

1. Formulate a hypothesis regarding the initial localization and post-treatment localization of protein X. (**2 points)**

2. Design an experiment to test the hypothesis using an immunofluorescence assay. Assume you have antibodies to all cellular proteins of interest. Describe which microscopy technique you will use for this experiment, discuss appropriate controls, and include how you would interpret the results. (**8 points)**

3. You found that protein X is localized to the plasma membrane. How would you test if protein X forms nanodomains at the basolateral membrane (<200nm resolution is needed for this). (**8 points)**

4. You would like to observe the dynamics of protein X redistribution during treatment with AG. Design an experiment to visualize changes in subcellular localization of this protein. Which microscopy technique would you chose to monitor protein X dynamics in living cells? Why? (**2 points)**

Question 11

1. Your colleagues have discovered a new mouse line that they have named "*Largo*" mice. Largo mice are slow moving, have poor grip strength, are lethargic, and have reduced lifespans (only live to 8 months of age). Some of your colleagues believe that Largo phenotype is due to a dominantly inherited, single mutation in a nuclear DNA specified gene. Some other colleagues believe that it is due to a mitochondrial DNA (mtDNA) mutation. You have male and female Largo mice and wild type mice at your disposal. Detail genetic crosses (with a diagram of anticipated results) that would help you discriminate between the two possibilities. (**4 points**)

2. Dr. Luigi Sforzando, a visiting scientist, believes that Largo is a mitochondrial outer membrane protein that normally functions to tether mitochondria to the endoplasmic/sarcoplasmic reticulum.

    a) Describe two independent methods that you would use to show that Largo is mitochondrial protein localized to the outer membrane. (**4 points**)

    b) What effect would Largo mutations have on mitochondrial calcium influx and mitochondrial ATP synthesis if such mutations disrupt the connections between the mitochondria and the ER/SR? (explain your answers) (**4 points**)

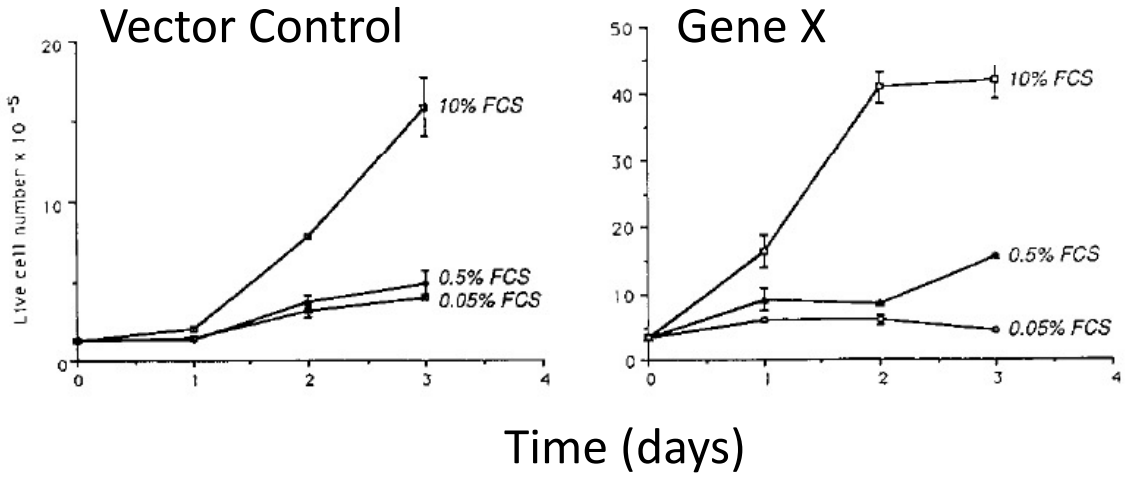
3. Dr. Nicola Pianissimo believes that the Largo mice have a mtDNA mutation, but is not sure exactly what mutation it might be. She thinks that it might be a mutation that impairs the transcription of either the mtDNA cytochrome b (mt-cyb) gene (encodes a component of ETC III) or the mtDNA encoded tRNA leucine gene.

    a) What would a Blue Native gel (BN-PAGE) of skeletal muscle mitochondria from Largo mice look like compared to wild type mice if Largo is a mt-cyb mutation? How would a BN-PAGE of skeletal muscle mitochondria isolated from Largo mice be compared to wild type mice be if Largo is a mt-tRNA-Lysine mutation? (**4 points**)

    b) If Dr. Pianissimo's lab only has access to respirometry (oxygen consumption experiments), design a set of experiments that would help her determine if the mutation is either in the mt-cytochrome b or in in the mt-tRNA lysine. (**4 points**)

Question 12

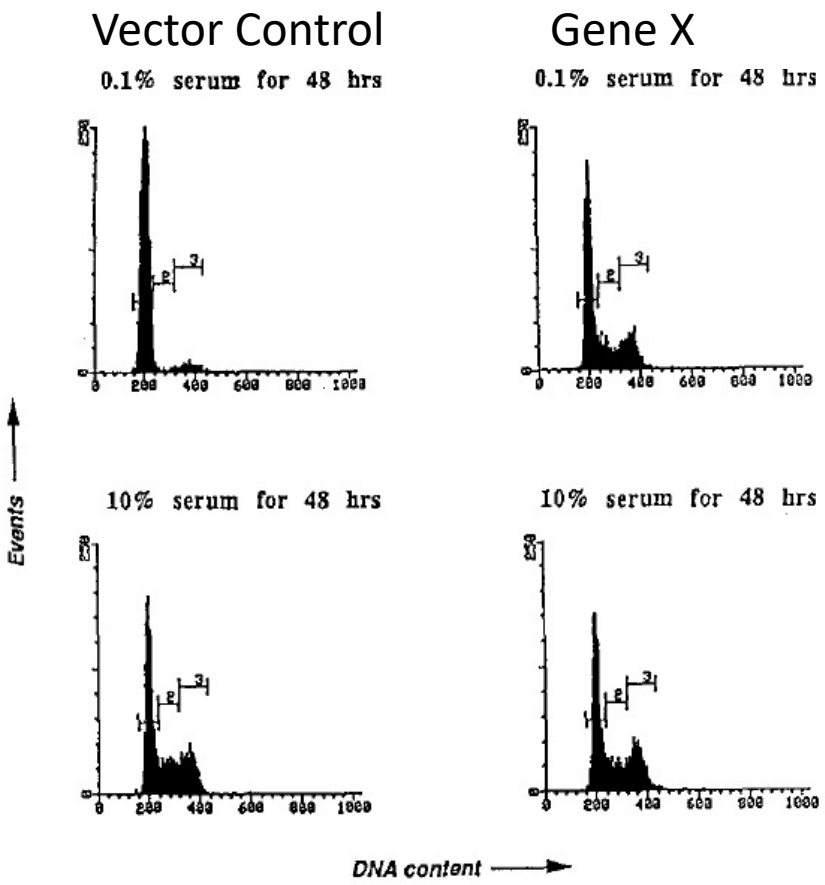
You are studying a gene (Gene X) that you think will drive cell proliferation of cells so you set up an experiment to determine if the expression of Gene X can sustain cell proliferation under low serum conditions. You over-express Gene X in an immortalized fibroblast cell line and measure live cell number after culture with the indicated serum level for 3 days. The results of your experiment are shown below.



(FCS: Fetal Calf Serum)

1. Based on these results can Gene X drive proliferation of the cells? Briefly explain your answer. **(5 points)**

To confirm your findings, you perform a cell cycle analysis under low and high serum conditions and these are your findings:



2. How does this assay measure cell cycle? Taken alone, what do these results suggest about the ability of Gene X to drive cell proliferation? (**5 points**)

3. What is your hypothesis as to what Gene X is doing based on the collective results of these two experiments? Design an experiment to test these results. Be sure to explain how the method you choose measures the activity you are testing. (**5 points**)

4. What is the pathway that is most likely induced by Gene X under low serum conditions in Part C and what are the likely changes that are being induced that result in the activation of this pathway? (**5 points**)