Question 1

You are studying a putative nucleic acid binding protein that you hypothesize binds to and regulates the function of a 125 nucleotide noncoding RNA (ncRNA125). The sequence of this ncRNA125 is known and you have a specific antibody against the protein.

(A) You have purified the endogenous protein from cells using a native protocol that maintains its interactions with other molecules. How would you:

A.1 examine the chemical and conformational purity of your isolated protein? (**4 points**)

A.2 confirm the identity of your purified target protein? [Note: unfortunately, your mass spec facility is down for the unforeseeable future so you can't use this technique]. (**1 point**)

A.3 determine whether the protein has co-purified with an endogenous nucleic acid and the type of this nucleic acid (DNA vs. RNA)? (**3 points**)

(B) You would like to perform quantitative binding assays to demonstrate the direct interaction of your protein and ncRNA125, and to determine their binding affinity.

B.1 Describe how you would generate your RNA for these experiments. [Note: at 125 nucleotides the RNA cannot be chemically synthesized.] (**2 points**)

B.2 Select **ONE** potential method and describe your binding experiment being sure to include any necessary controls. Illustrate the data you expect to obtain and explain how you can use these data to determine a quantitative measure of binding. (**6 points**)

(C) Subsequent experiments indicate that the protein binds to multiple noncoding RNAs with almost the same affinity but only specifically affects the activity of the ncRNA125. Describe an approach you could use to investigate the role of binding kinetics that might explain the ability of your protein to discriminate between ncRNA125 and other non-regulated RNAs. (**4 points**)

Question 2

(A) What is Beer's law? Define each term and give units where appropriate. **(3 points**)

(B)

B.1 What is the utility of measuring a sample's ratio of absorbance at 260 nm light to its absorbance at 280 nm light? **(2 points)**
B.2 Describe what is measured at each wavelength and what the ratio represents. **(2 points)**

(C) What is the major drawback of using the 260:280 ratio and why? **(3 points)**

(D) You have a dilute cell extract that you think contains a standard bacterial plasmid.

D.1 Describe how you can purify the plasmid. If you must employ any commercial 'kits', explain how they work as specifically as possible. **(2 points)**
D.2 After purification your sample is still dilute. Describe ONE method you could use to concentrate nucleic acid. **(2 points)**
D.2 Describe how you would verify its purity. **(2 points)**

(E) You have overexpressed a protein and purified the protein using affinity chromatography. Describe how you would:

E.1 Confirm its chemical homogeneity. **(2 points)**
E.2 Determine its concentration. **(2 points)**

Question 3

Your lab is working on the influenza polymerase complex that is responsible for transcription and replication of the influenza virus. The complex comprises three proteins: PB1, PB2, and PA at a 1:1:1 molar ratio. PB1 has RNA-dependent RNA polymerase activity, PB2 binds the cap of mRNAs, and PA has an RNA-endonuclease activity. Assays for all three biological activities are available in the lab. cDNAs for all three components are available to you. The lab uses, or has access to, a variety of structural/biophysical instruments.

The crystal structure of PB1 in complex with PB2 has been determined (**Figure 1A**). The structure of the N-terminus of PA has also been determined providing insights into the RNA-endonuclease active site (**Figure 1B**; top). However, the structure of the C-terminal domain of PA (C-ter) is unknown (**Figure 1B**; bottom) and the interactions between the PB1-PB2 complex with PA remain elusive.

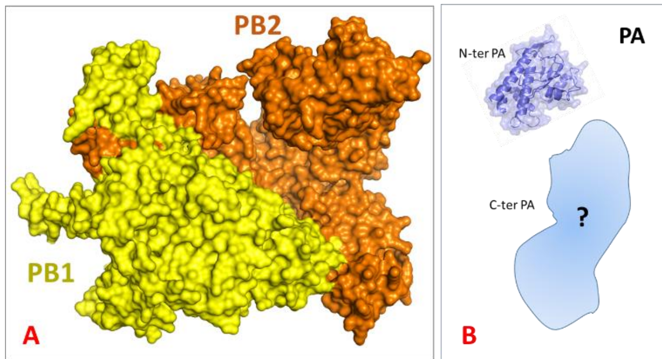


Figure 1: Structures of components of the influenza polymerase complex. (A) Crystal structure of PB1 (yellow) in complex with PB2 (orange). (B) The PA is composed of the N-terminal domain (N-ter PA; crystal structure is shown) and the C-terminal domain (C-ter PA, structure unknown).

(A) Describe **ONE** strategy for expression and purification of this heterotrimeric polymerase protein complex for structure determination. Include how you would assess the composition (stoichiometry) of the complex. (**7 points**)

(B) Previous efforts in the lab to crystallize the full-length PA (molecular weight of ~75 kDa) have failed because of difficulties in obtaining this protein at high concentrations. Describe **ONE** alternative method that can be used to obtain some structural information on the shape of PA. Describe how this method works including both advantages and disadvantages of this alternative method? (**6 points**)

(C) Describe **ONE** experimental approach that will allow you to obtain information regarding amino acid residues that are involved in the interface of PB1-PB2 with PA. Include appropriate controls and describe advantages and disadvantages of your proposed approach. **(7 points)**

Question 4

MCF10A cells are epithelial cells that grow as an epithelial monolayer (**Figure**, top panels). When MCF10A cells are transformed by expression of an active form of Ras, their morphology becomes more mesenchymal (or fibroblastic) (**Figure** , bottom panels). In these cells, actin filaments were visualized by staining with rhodamine-phalloidin (**Figure**, left panels), and paxillin (a focal adhesion protein) was visualized by immunofluorescent staining by a specific antibody (**Figure**, right panels).

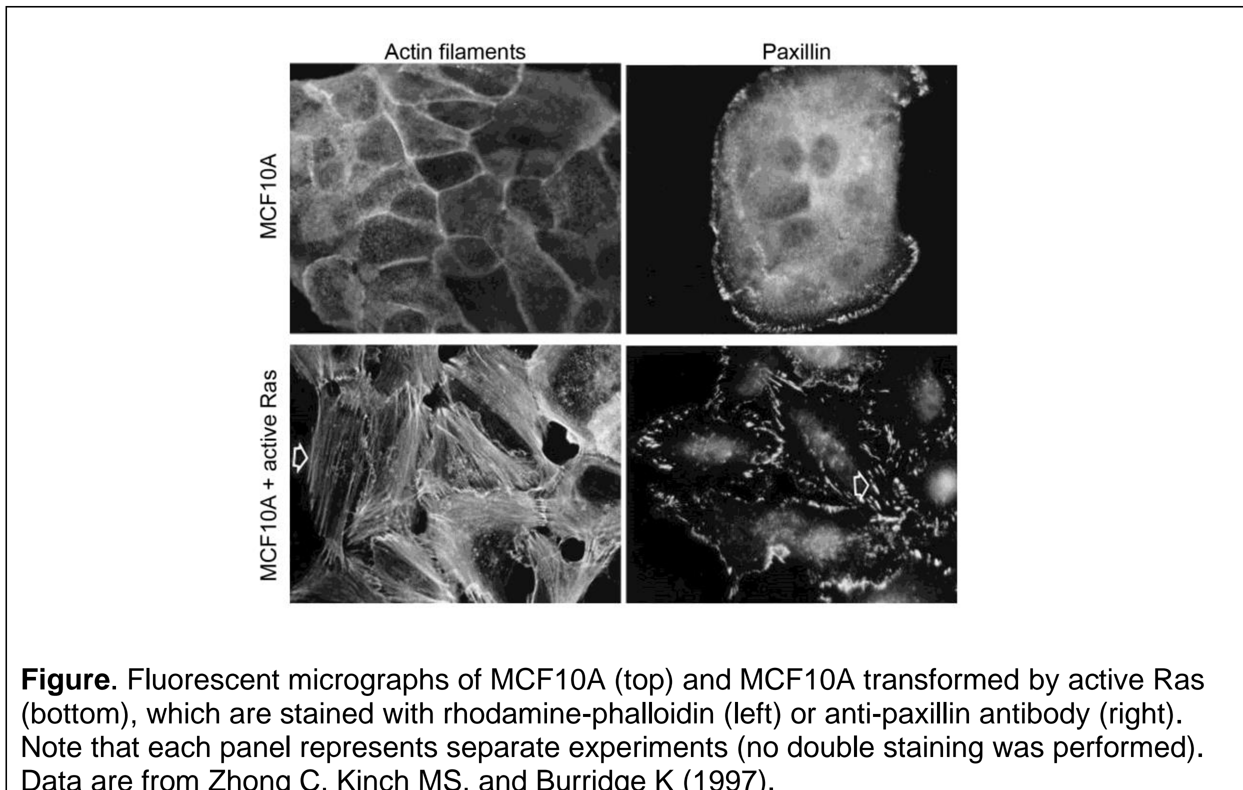


Figure. Fluorescent micrographs of MCF10A (top) and MCF10A transformed by active Ras (bottom), which are stained with rhodamine-phalloidin (left) or anti-paxillin antibody (right). Note that each panel represents separate experiments (no double staining was performed). Data are from Zhong C, Kinch MS, and Burridge K (1997).

(A) Describe how actin filaments and focal adhesions are organized in MCF10A and MCF10A + active Ras with an emphasis on changes induced by active Ras. **(4 points)**

(B) These morphological changes are generally described as the epithelial-mesenchymal transition (EMT) and are associated with changes in cell migration.

B.1 Based on these data, state a hypothesis for how cell migration might be altered by EMT. **(2 points)**
B.2 Describe **ONE** experimental strategy to test your hypothesis. **(6 points)**

(C) The authors found that EMT could be blocked by treatment with either an inhibitor of the Rho GTPase (C3 exoenzyme) or an inhibitor of myosin (2,3-butanedione 2-monoxime [BDM]) and hypothesized that Rho and myosin are in the same signaling pathway to induce EMT. Describe **ONE** experimental strategy to determine the relationship between Rho and myosin in EMT induction (upstream, downstream, or parallel). Feel free to utilize lysophosphatidic acid (LPA: an activator of Rho), calyculin A (an activator of myosin), and reagents to determine activities of Rho and myosin. **(8 points)**

Question 5

Overexpression of the pro-apoptotic *Drosophila* gene Hid specifically in the developing fly eye results in a small adult eye due to excessive apoptosis.

(A) In a mutagenesis screen for suppressors of the GMR-Gal4 [GMR is an eye-specific promoter], UAS-Hid small-eye phenotype, you have identified two new loss-of-function mutants that dominantly (i.e. m/+) suppress this "small eye" phenotype. Both mutants affect the same gene which you name "gene X".

A.1 Based only on your recovery of mutant gene X alleles as suppressors of GMR-hid "small eye" phenotype, do you predict that wildtype protein X is normally pro- or anti-apoptotic? **(2 points)**
A.2 Would eye cells homozygous (m/m) for one of the new gene X mutant alleles be alive (and hard to kill) or dead? **(2 points)**

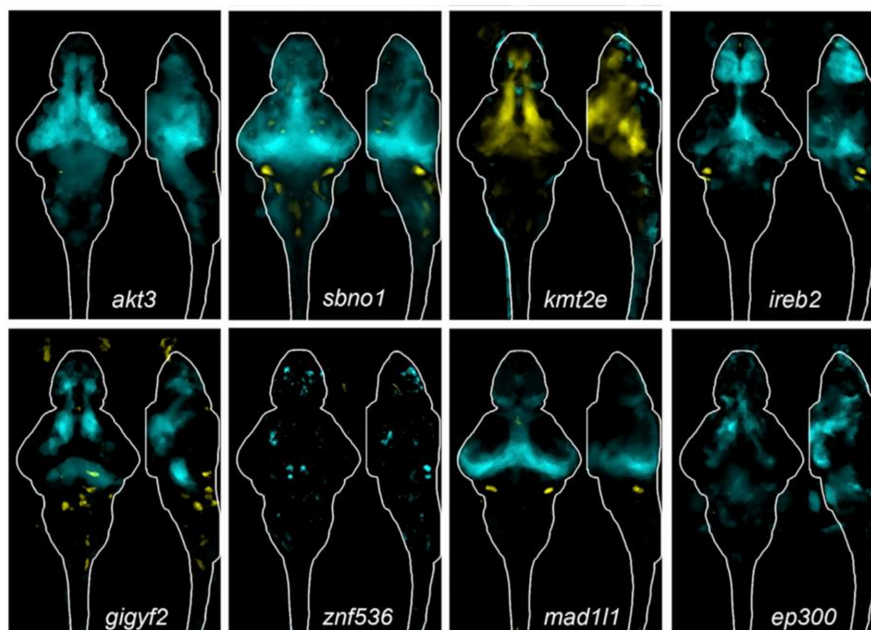
(B) Based on the sequence of protein X, you conclude that it is a homolog of the SMAC2/Diablo protein, which was identified by Xiaodong Wang in his biochemical purification of caspase regulators. You theorize that protein X can regulate apoptosis by displacing caspases from IAPs.

B.1 What are IAPs and how do they regulate apoptosis? **(2 points)**
B.2 Starting with the initiating methionine (M), the first 6aa's at the N-terminus of protein X are: MAVAFY…
Why does this feature suggest an interaction with Drosophila IAP (DIAP)? **(2 points)**
B.3 Provide **ONE** genetic test and **ONE** biochemical test of your hypothesis that protein X triggers apoptosis by acting upstream of IAPs and disrupting caspase-DIAP complexes. Recall that fly cells completely lacking DIAP die by apoptosis. **(8 points)**
B.4 Propose an experiment to test whether protein X can also regulate apoptosis in a cultured mammalian cell line. Be sure to identify your apoptotic stimulus and what metric you will use to measure apoptosis of these cells. **(4 points)**

Question 6

Images depict structural phenotypes in the brain of zebrafish carrying null mutations in different neuronal genes required for **endoplasmic reticulum protein synthesis and translocation.** These genes have also been associated to neurodevelopmental disorders in human genome-wide association studies.

The structural differences in mutants were calculated using deformation-based morphometry and displayed as sum-of-slices projections (Z- and X- axes). Brain images represent the significant differences in signals between homozygous mutants versus heterozygous siblings. Cyan color represents areas where the brain is decreased in volume while yellow marks brain regions that undergo increased in volume.



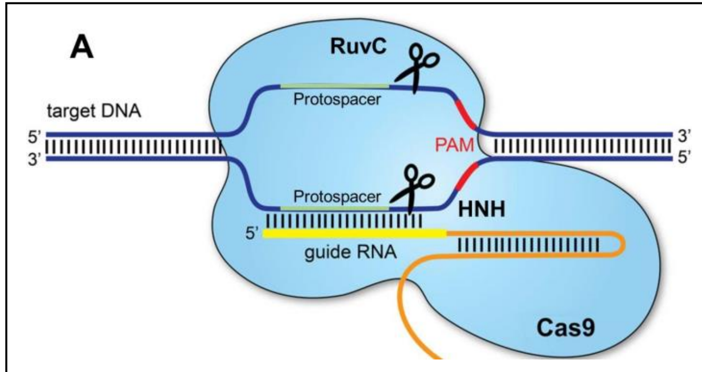
Based on these findings:

(A) Propose **TWO** hypotheses that would explain the different anatomical phenotypes caused by mutations in the same biochemical process, namely **endoplasmic reticulum protein synthesis and translocation.** Provide a brief rationale for each one of your hypotheses. **(6 points)**

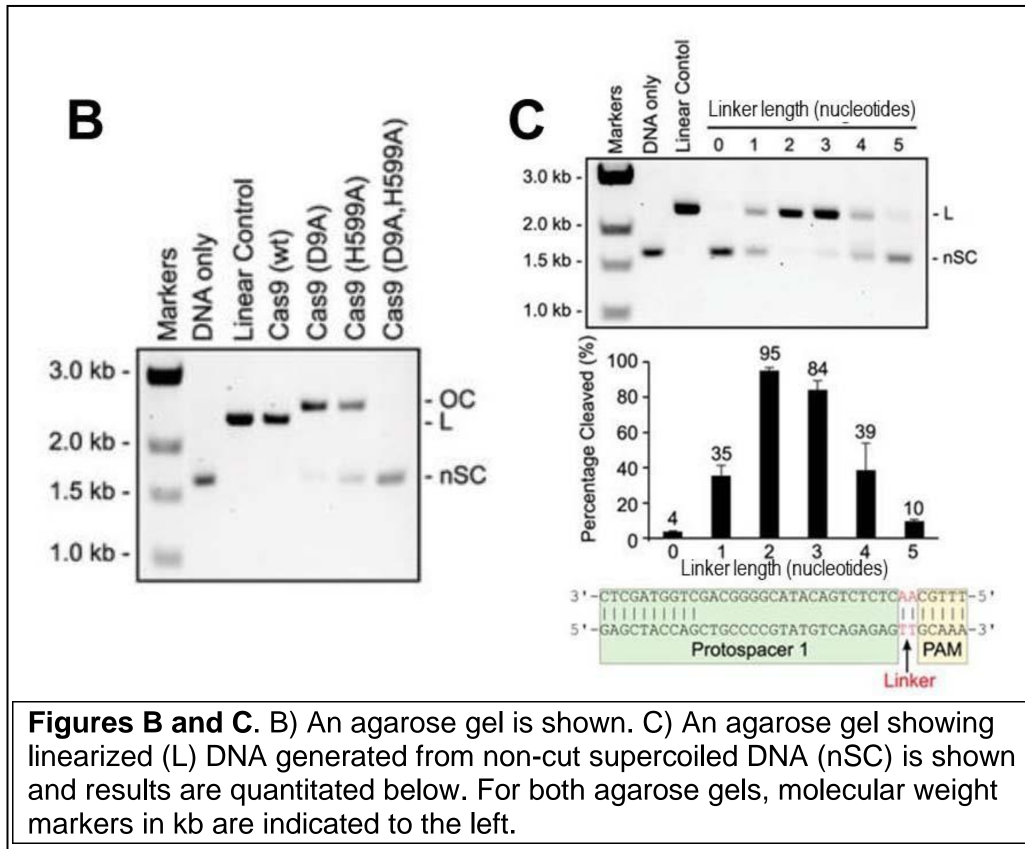
(B) Select **ONE** of your hypotheses and propose **ONE** experimental strategy that could test the model proposed. **(8 points)**

(C) Describe **ONE** possible mechanism that could account for the enlarged volume and **ONE** possible mechanism that could account for the decreased volumes in the brain of these mutant animals. **(6 points)**

Question 7



The discovery of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system has revolutionized biology both by facilitating genome editing in the laboratory and by opening new possibilities for therapeutic approaches. As shown in **Figure A**, the *Streptococcus thermophiles* Cas9 nuclease functions with the aid of two RNAs (crRNA and tracrRNA) that can be ligated to form a single guide RNA. The CRISPR/Cas9 system generates double-stranded breaks in the DNA backbone by creating two single-stranded nicks in the DNA (as indicated by the two scissors).



Figures B and C. B) An agarose gel is shown. C) An agarose gel showing linearized (L) DNA generated from non-cut supercoiled DNA (nSC) is shown and results are quantitated below. For both agarose gels, molecular weight markers in kb are indicated to the left.

The CRISPR/Cas9 system has been studied *in vitro* by reconstituting the components. Investigators can incubate a circular plasmid containing a target site with the appropriate components and monitor cleavage of the non-cut super-coiled (nSC) plasmid to nicked, open circular DNA (OC) or fully cleaved (with two close nicks) linear (L) DNA product (**Figure B**). The Cas9 nuclease contains two putative nuclease domains, termed RuvC and HNH (**Figure A**). The investigators used different active site mutants to investigate the mechanism of Cas9 (**Figure B**). The D9A amino acid substitution occurs in the RuvC-like domain while the H599A amino acid substitution occurs in the HNH domain. **(Continue on next page)**

Question 7 (continued from the previous page)

(A) What do the results of the experiments shown in **Figure B** tell you about the mechanism of cleavage by Cas9? (**5 points**)

(B) As shown in the **Figure A** in Page 1, Cas9-mediated cleavage occurs between the protospacer sequence (green) and a target site termed the PAM sequence (red). To investigate the impact of the nucleotide length of the linker between these sequences, the authors took advantage of the *in vitro* cleavage assay as shown in **Figure C**.

> **B.1** Based on the data presented, what do you conclude is the optimal nucleotide spacer length and why? (**5 points**)
> **B.2** How would you test whether the sequence of the spacer region matters? Describe the experimental approach and how you would interpret the data you obtain. (**6 points**)

(C) Studies have been performed suggesting that CRISPR/Cas9 can be employed to treat many types of diseases including cancer, muscular dystrophy, blood disorders and neurological disease. If you were to choose one type of disease to be treated with a CRISPR/Cas9-based therapy, what type of disease would you choose? Describe the target gene including the nature of the disease-causing change and the tissue/cell type you would need to target. Provide a rationale for your choice. (**4 points**)

Question 8

(A) You want to test if a certain transcription factor (TF) interacts with RNA polymerase in live *Escherichia coli* (*E. coli*) cells. Describe **ONE** experimental strategy to do this. Explain the principles of your technique of choice and appropriate controls. **(5 points)**

(B) If they do interact, you want to characterize the dynamics of such interaction in live *E. coli* cells. Could you use the same technique as you described in **(A)**, or do you need a different technique? Explain your rationale, the second technique if necessary, and describe what parameter you would measure as a function of time. **(5 points)**

(C) Describe **ONE** strategy to characterize the diffusion of TF and of RNA polymerase in live *E. coli* cells. What parameter could be calculated which characterizes diffusion of these two proteins? **(5 points)**

(D) You are able to purify TF and RNA polymerase for *in vitro* experiments. Describe **ONE** strategy to visualize the interaction between TF and RNA polymerase *in vitro.* Explain your rationale for the selection of a technique, and be sure to include appropriate controls. Feel free to draw an illustration. **(5 points)**

Question 9

A previous student in your lab found that treating mice for 4 weeks with a non-absorbable drug that acts only in the intestine somehow reduces the development of fatty liver. Your PI hypothesizes that the drug is increasing production of an intestinal or gut microbiome-derived substance that is carried directly from the intestine in the portal blood to the liver, its site of action.

To test this hypothesis, serum was isolated from portal blood of drug-treated or control (vehicle-treated) mice, and then added to the media of mouse hepatocytes grown in culture. After incubating for 24 h at 37°C, the media was removed and the cells were harvested to prepare a lipid extract. The amount of triglyceride in the lipid extract was then measured using an enzymatic assay that uses an enzyme (lipase) specific for triglycerides to cleave off the fatty acids from the glycerol backbone. The amount of glycerol produced is measured by a coupled colorimetric assay as a measure of total triglycerides. The amount of triglyceride is normalized to the number of cells or total cellular protein. Remarkably, incubation with serum from the drug-treated mice (but not control mice) reduced the amount of triglyceride that was stored in the mouse hepatocytes in culture.

Your project is to identify the substance responsible for this effect and understand its mechanism of action. It is unclear if the active substance in serum is a lipid, alternatively is a protein, small peptide, nucleic acid, or other small metabolite. However you have plenty of portal blood serum from the drug-treated and vehicle treated mice, and you can use the hepatocyte assay described above to help you in your search for that substance.

(A) Describe a biochemical experiment to determine if the active substance is a lipid. **(5 points)**

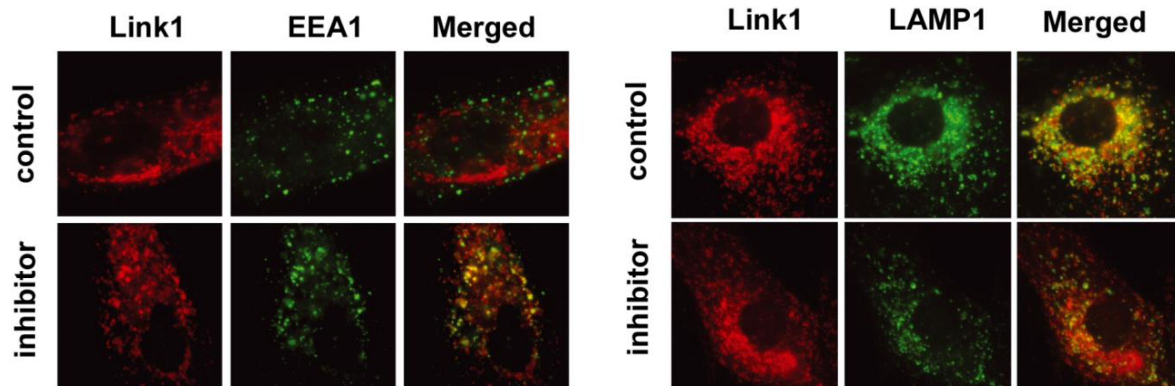
(B) The results from part **(A)** suggest that the active substance is a lipid.

> **B.1** Describe **ONE** experimental approach (including controls) to identify this active substance. **(5 points)**
>
> **B.2** You identify several different lipids whose concentration is greatly increased in serum from portal blood of drug-treated versus control (vehicle-treated) mice. Describe **ONE** experimental approach to identify which of the candidate lipids is responsible for the hepatocyte triglyceride-lowering activity. **(5 points)**

(C) The structure of the active lipid (determined in Part **B**) suggests that it could be a ligand for a lipid-activated nuclear receptor. However, you do not know which of the 49 mouse nuclear receptors is involved. Fortunately, both the active lipid and cDNAs for all receptors are commercially available. Describe **ONE** experimental approach to identify the nuclear receptor(s) that are activated (or inhibited) by this active substance. **(5 points)**

Question 10

You are interested in the protein Link1 that is stably localized to an intracellular compartment and want to identify the role of a cytosolic protein co-factor, TRF80, in regulating Link1 trafficking. As a first step in determining how Link1 is targeted, you perform immunofluorescence co-localization experiments using two different markers for intracellular compartments, EEA1 (early endosomes) and LAMP1 (lysosomes). Control panels represent images of an individual unperturbed cell, and inhibitor panels represent images of a cell treated with a pharmacologic TRF80 inhibitor.



(A) Based upon these data, state a hypothesis for how TRF80 regulates Link1 localization. Describe how you would use quantitative image analysis of these data to test this hypothesis. **(6 points)**

(B) Describe **ONE** method to validate the effects of the TRF80 inhibitor on Link1 localization. **(2 points)**

(C) You want to test a second hypothesis that Link1 localization is controlled by a direct interaction with TRF80. Provide **TWO** different experiments (one imaging based and one biochemical method) to test this hypothesis. Be sure to include appropriate controls. **(6 points)**

(D) If the results of the experiment show that Link1 and TRF80 do not interact, propose an alternative hypothesis for how TRF80 regulates Link1. **(2 points)**

(E) Describe **ONE** approach to test this alternative hypothesis as proposed in part (**D**). **(4 points)**

Question 11

(A) Mouse fibroblast cells grown in culture show detectable expression of ~10,000 protein-coding genes, while the remaining 15,000 genes are not transcribed. Many of the silent genes in this cell type are found in facultative heterochromatin. Compare and contrast the chromatin features of an active gene in euchromatin versus a silent gene in facultative heterochromatin. Be sure to invoke the following terms in your answer: nucleosome, histone, post-translational modification, transcription factor, and RNA polymerase. **(4 points)**

(B) During DNA replication, chromatin states can also be replicated to ensure proper transmission of epigenetic information.

B.1 Propose **ONE** model for how a heterochromatin domain in fibroblast cells can be propagated through mitosis. **(4 points)**
B.2 Describe **ONE** experimental approach that would directly test your model. **(4 points)**

(C) Overexpression of pluripotency-associated transcription factors such as OCT4 in a fibroblast cell can cause the cell to revert to a pluripotent state.

C.1 Propose **ONE** model to explain this epigenetic reversion in terms of how silenced genes become activated and vice versa. Be sure to invoke the activity of the transcription factor itself as well as histone modifying and de-modifying enzymes. **(4 points)**
C.2 Following on part **C**, describe **ONE** experimental approach to test whether or not your model about epigenetic reversion is correct. **(4 points)**

Question 12

(A) There are several reasons why specific mRNAs are localized to distinct subcellular compartments.

A.1 Describe **ONE** documented localized mRNA in any type of cells. In your description, state which subcellular compartment(s) this mRNA is localized to. **(1 point)**
A.2 Describe a mechanism for its localization. **(2 points)**
A.3 How is this localization important for the function of this mRNA. Feel free to draw illustrations. **(1 point)**

(B) Assume that you are investigating the role of mRNA that you described in **(A)**.

B.1 Describe **ONE** strategy to determine a cis-element of the mRNA that directs its localization. Include appropriate controls. **(4 points)**
B.2 Describe **ONE** strategy to identify a cellular component that is responsible for localizing the mRNA in your described mechanism in **(A.2)**. Include appropriate controls. **(4 points)**

(C) Describe **ONE** strategy to visualize and quantify the dynamics of mRNA localization in **live** cells. Describe and illustrate how this visualization technique works including necessary components that you will use. Include appropriate controls and describe how you make sure that the observed signals specifically reflect localization of the mRNA. **(8 points)**