BCDB Qualifying Exam Part I: Day 1
2021

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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).

Anita Corbett: 404-421-9061
Mike Koval: 610-659-5381
Tracey Wright: 310-406-6194 (OneDrive or logistical questions)

Question 1 (Day 1)

You are performing research on a human neuromuscular syndrome, lateral muscle dystonia (LMD). Your lab previously set up an *in vitro* culturing system where cultured neurons can be grown on a porous mesh. Dendritic arbors penetrate the mesh and can be isolated through mechanical shearing. Using this approach, dendritic compartment extracts were compared to somatic extracts derived from the cell soma/cell body. Using RNA-seq, you identified several mRNAs significantly enriched in the dendritic fraction compared to the fraction prepared from the soma. Through bioinformatics analysis, you identified a 6-nucleotide motif (6-mer) common to the 3'UTR of these mRNAs. Your calculations suggest this motif is significantly overrepresented in the dendritic dataset relative to the somatic RNAs or random chance.

1) Propose ONE hypothesis that would explain how these specific mRNAs are targeted to dendrites? (2 points)

2) Describe ONE detailed experimental approach to test your hypothesis. Include appropriate positive and negative controls. (8 points)

3) Microtubules are important for localization of many subcellular cargoes to dendrites. Describe ONE strategy that would test whether microtubules are necessary for localization of the RNAs you have identified to dendrites? Include appropriate controls (2 points)

4) You select one mRNA for in depth analysis, *LMD1* mRNA. Describe ONE detailed experimental approach to determine whether the 6-mer *cis*-element directs *LMD1* mRNA localization to dendrites. Include appropriate controls. (4 points)

5) Describe ONE strategy to test the hypothesis that *LMD1* mRNA is locally translated within dendrites. Describe the technique and necessary reagents/tools you will use. Include appropriate controls. (4 points)

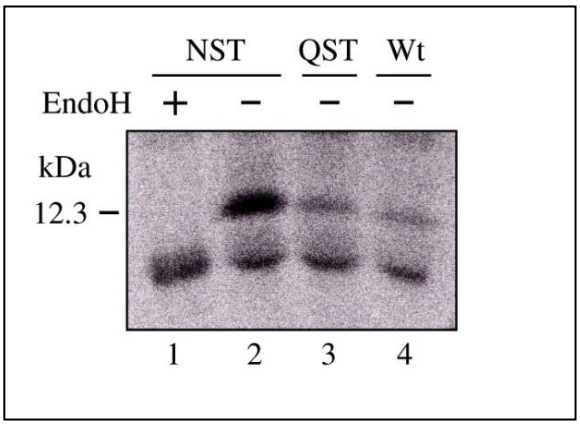
Question 2 (Day 1)

The membrane-bound protein X (MBPX) of the BARF-E (Biologically Appalling Raw Food - Enterovirus) has been proposed to form an ion channel in the lipid bilayer and is essential for pathogenicity. MBPX is similar to the M2 proton channel of the influenza virus. In the case of influenza virus, Amantadine inhibits M2 by binding to the channel gate formed by an Asn residue within its transmembrane domain. Similar to the M2 protein, you hypothesize that MBPX could be a target to develop therapeutics for BARF-E disease. Multiple topologies have been reported for related proteins from other enteroviruses. Thus, it is necessary to experimentally determine the topology of MBPX before you can consider developing therapeutics.

```
        10         20         30         40         50
MYSFVSEETG TLIVNSVLLF LAFVVFLLVT LAILTALRLC AYCCNIVNVS
        60         70
LVKPSFYVYS RVKNLNSSRV PDLLV
```

1) In the figure above that shows the amino acid sequence of MBPX, underline the transmembrane domain. (2 points)

2) Residue Cys40 is required for the stability of MBPX. Cys43 and Cys44 have been postulated to form disulfide bonds with Bike, the membrane-bound surface glycoprotein of BARF-E that is involved in viral entry into the cell. Thus, it is not advisable to employ Cys-mutagenesis to determine the topology of MBPX. Instead, the canonical N-glycosylation sequence motif (Asn-Ser-Thr or NST) was used as a topological reporter and inserted near the N-terminus of MBPX (closer to the N-terminal residue than the transmembrane domain). Three radiolabeled versions of MBPX were prepared by *in vitro* transcription/translation experiments in the presence of ER-derived microsomes and [^{35}S]-labelled amino acids: 1. MBPX-NST (or NST) that can be glycosylated, the control MBPX-QST (Gln-Ser-Thr or QST) that cannot be glycosylated, and the wild-type MBPX (Wt). The resulting microsomes that had either of the three MBPXs imbedded in them, were isolated, treated with or without endoglycosidase H (Endo H), and visualized in the shown gel by autoradiography. Describe the results, including all of the bands shown, and explain your conclusion about the topology of the MBPX. (10 points)

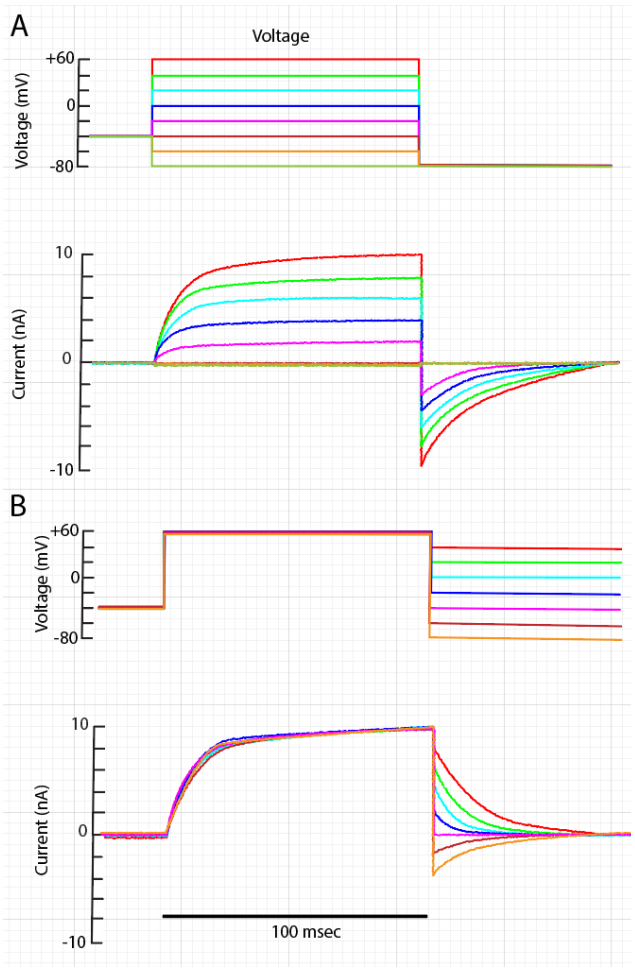


3) Due to sequence similarity to previously characterized proteins, a hypothesis has emerged that a short sequence (4-5 residues) outside the transmembrane domain can bind to the virion membrane and modulate the activity of MBPX. Please (a) identify this short sequence by the residue numbers within the sequence presented, (b) explain why you think it is the most likely candidate to bind virion membrane and modulate the activity of MBPX, and (c) design an experiment to test the binding of this short sequence to the membrane bilayer. (8 points)

Question 3 (Day 1)

You have submitted a manuscript to *Nature* that shows depolarization of hippocampal neurons by addition of high potassium to the bathing solution causes the transcription factor NFAT to translocate to the nucleus. Two reviewers loved the paper, but a third reviewer has asked for new experiments. One experiment the reviewer requested was to record ionic currents using whole-cell patch clamp of the hippocampal neurons. When you ask your graduate students to learn patch clamp, they are excited! Two days later, they come back with these data and want help interpreting them. Answer the following questions they have about the data.

1) Why do the currents in panel A increase slowly with time during depolarization and then reverse direction and decay with time after stepping the voltage to -80 mV? (4 points)
2) Plot the steady-state IV (current-voltage) curve (from panel A) and the IV curve of tail currents (from panel B). Label the graphs as you would for publication. What is the value of the reversal potential (E_{rev})? Indicate the reversal potential on your IV plot and label inward and outward currents. What is the conductance of this current? (4 points)
3) Your students tell you that they used a pipet solution containing 150 mM KCl, 15 mM NMDG-Cl, 100 nM free $CaCl_2$ (buffered with EGTA), 10 mM HEPES pH 7.4 and a bath solution containing 150 mM NMDG-Cl, 4 mM KCl, 10 mM $CaCl_2$, 10 mM HEPES pH 7.4. For this experiment, NMDG is considered to be impermeant to all channels. Do you think this channel is highly selective for only one ion? If yes, which ion? If no, what ion(s) could be permeable through this channel given the data? In answering this question, make a table listing all ions in the experiment, their calculated Nernst potentials, and a brief explanation why each ion could be permeant or not. If you think the ion is permeant, what direction would the ion move at +60 mV. (4 points)
4) How would you experimentally test your answer in *Part 3* of this question? (4 points)



Whole-cell currents recorded from a hippocampal neuron using the solutions described in Part 3. In each panel, the colors of the current and voltage traces correspond.

5) You know that NFAT translocation into the nucleus is regulated by intracellular free calcium concentration. Why would addition of potassium to the bathing solution cause cells to depolarize and how would this likely result in increased intracellular Ca^{2+} levels. Is it possible that this channel plays a role in NFAT translocation that you have observed? Why or why not? (4 points)

Question 4 (Day 1)

TGR5 is a bile acid-activated G-protein-coupled receptor (GPCR) that is expressed by cells in the liver, gastrointestinal tracts, and other tissues in the body. For many GPCRs, their signaling is subject to negative feedback that prevents GPCR overstimulation. This is broadly called "desensitization" and refers to the decrease in the response of the GPCR after repeated or continuous stimulation.

However, certain cells that express TGR5 are exposed repeatedly to high concentrations of bile acids, and these cells need TGR5 to keep responding to bile acids. An unusual property of TGR5 is that the receptor does not undergo desensitization after repeated activation by bile acids. Your project in the lab is to understand how/why TGR5 does not undergo desensitization after binding bile acids.

1) Propose two mechanistically distinct hypotheses for why TGR5, unlike most GPCRs, does not undergo desensitization. For each hypothesis, also describe the experimentally testable predictions that this hypothesis makes. (6 points).

2) Describe a detailed experimental approach to test one of the two hypotheses you proposed above, including relevant controls needed to interpret the results. Assume that you have a fully stocked lab with all the basic reagents and equipment needed for your experiments (8 points).

3) In addition to TGR5, bile acids can also activate the G-protein-coupled receptor called sphingosine-1-phosphate receptor 2 (S1PR2). An important difference between the two receptors is that TGR5 is coupled to heterotrimeric G-proteins in the Gs family whereas S1PR2 is coupled to heterotrimeric G-proteins in the Gi/o family.

   In the lab, you are studying bile acid signaling in brain using mouse brain tissue explants and brain organoids. For your studies, you add vehicle or bile acids to your cultures. Then after a short incubation period, you prepare protein extracts from the cells for immunoblotting to measure the levels of phosphorylation of different proteins that are targets of important kinases such as Protein kinase A (PKA), Protein Kinase B (PKB/AKT) and members of the MAPK/ERK family.

   You find that there is a specific decrease in phosphorylation of Protein Kinase A target proteins after addition of bile acids. You hypothesize that extracellular bile acids are acting through the bile acid receptor S1PR2 to reduce PKA activity in your brain cultures. However, the cells in your explants and organoid cultures also express low levels of TGR5 mRNA and it is possible that extracellular bile acids could be acting through TGR5.

   Describe a <u>detailed experimental approach</u> to test your hypothesis that bile acids are signaling to reduce PKA activity through S1PR2 and not TGR5. Be sure to include appropriate controls and describe the result you would obtain that would support or refute your hypothesis (6 points)

Question 5 (Day 1)

Your laboratory started studies on a complex of two proteins, X and Y. The nucleic acid sequences encoding these two proteins recently became available. Based on bioinformatic analysis, protein X has a predicted molecular weight (M.W.) of 150 kDa and protein Y has a predicted molecular weight of 65 kDa. Preliminary data suggest that the proteins form a heterodimer with one copy of each protein. Protein X has 4 potential glycosylation sites and 5 cysteines. Proteins Y and X each have 2 phosphorylation sites. Your data suggest that post-translational modifications are critical for the X + Y complex to form. There are currently no structures available for the individual proteins or the complex of X and Y. However, there are available solved structures of proteins with relatively high sequence homology (>60%) to both X and Y.

You are assigned a project to investigate the atomic structure of the complex (X+Y) and to explore the interactions between the complex components.

1) Discuss the selection of an appropriate expression system to produce X and Y. Justify your choice and briefly discuss TWO purification methods and describe how you would validate protein quality. (6 points)

2) Describe two different structural biology strategies that could be employed to obtain high-resolution structures of the X + Y complex. Discuss TWO specific advantages and two disadvantages of each approach. (10 points)

3) In case you are not able to obtain high-resolution data on the X + Y complex that you successfully purified, what would be TWO alternative structural approaches that may help you obtain low-resolution structural information? Discuss ONE advantage and ONE disadvantage of each approach. (4 points)

Question 6 (Day 1)

To investigate the mechanism of actin-based motility which is powered by actin turnover (i.e. assembly and disassembly), an *in vitro* system to mimic actin-based movement was reconstituted (Fig. 1). Polystyrene beads (white spots in Fig. 1) were coated with N-WASP (an activator of Arp2/3 complex) and incubated in a solution containing a minimum set of proteins including globular (G-) actin, Arp2/3 complex (a nucleator of actin polymerization), cofilin (actin filament severing and depolymerizing protein), and capping protein (a protein that blocks polymerization and depolymerization from the plus (barbed) ends of actin filaments). An ATP regeneration system was also included to prevent ATP depletion over time. Under appropriate conditions, the beads started moving with formation of comet tails comprising of actin filaments (visible as dark tails behind the beads in Fig. 1A).

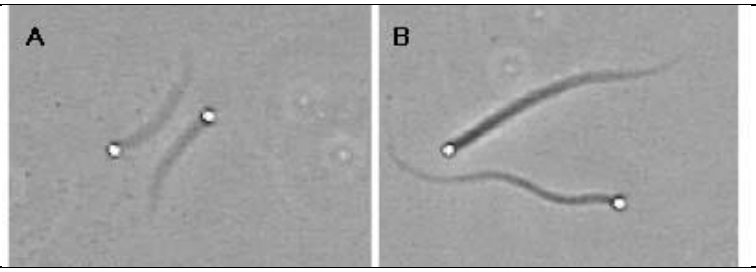


Fig. 1. *In vitro* reconstitution of actin-based motility in different protein compositions. Both (A) and (B) were performed with N-WASP-coated polystyrene beads, Arp2/3 complex, capping protein, and ATP regeneration system. However, (A) was done with cofilin, while (B) was done without cofilin.

1) In this system, actin is expected to undergo treadmilling within the actin tails. State your hypothesis for how actin monomer subunits turn over within the actin tails. Be sure to include a specific statement on the direction of flow of actin monomers in the tail as well as sites of actin polymerization and depolymerization on individual actin filament (2 points), and state how Arp2/3 complex and cofilin contribute to this process (4 points). There is no need to explain the function of capping protein.

2) Design an experiment to test your above hypothesis of actin turnover within actin tails. The experiment needs to be able to determine the direction of flow of actin dynamics within the tails, i.e. where are the monomers polymerizing at and depolymerizing from? (5 points)

3) One day, you set up this experiment with all the components including ATP but forgot to include the ATP regeneration system. Describe and explain how and why the absence of the ATP regeneration system affects the velocity and morphology of the actin tails. (4 points)

4) The experiments shown above were performed under different conditions. In Fig. 1A, the experiment was done with all the stated components. However, in Fig. 1B, the experiment was done without cofilin, and the actin tails became longer than those in Fig. 1A. State your prediction on the velocity of the polystyrene beads in Fig. 1B as compared with that in Fig. 1A (slower, faster, or the same) and explain the basis of your prediction. (5 points)

BCDB Qualifying Exam Part I: Day 2
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Question 7 (Day 2)

Lipid transfer proteins such as the Aster proteins that we discussed in Foundations play a critical role in maintaining cellular lipid homeostasis. In the case of the Aster proteins, these proteins function to move high density lipoprotein (HDL)-derived cholesterol from the plasma membrane to the endoplasmic reticulum. To further study the functions of Aster proteins, your project in the lab is to develop additional tools such as chemical inhibitors of Aster protein function.

Assume that you have access to purified recombinant Aster protein, radiolabeled [^{3}H]cholesterol, a fluorescent cholesterol analog (22-NBD-cholesterol) as well as a fully stocked lab with all the basic reagents and equipment that you would need. You can use any of those reagents for your assay.

1) Design an assay you will employ to screen a pre-existing library of compounds to identify novel compounds that inhibit Aster-cholesterol binding. Describe the assay in detail, including controls required to interpret the data you obtain (8 points)

2) Draw what the dose-response plots for each of the following types of compounds would look like:

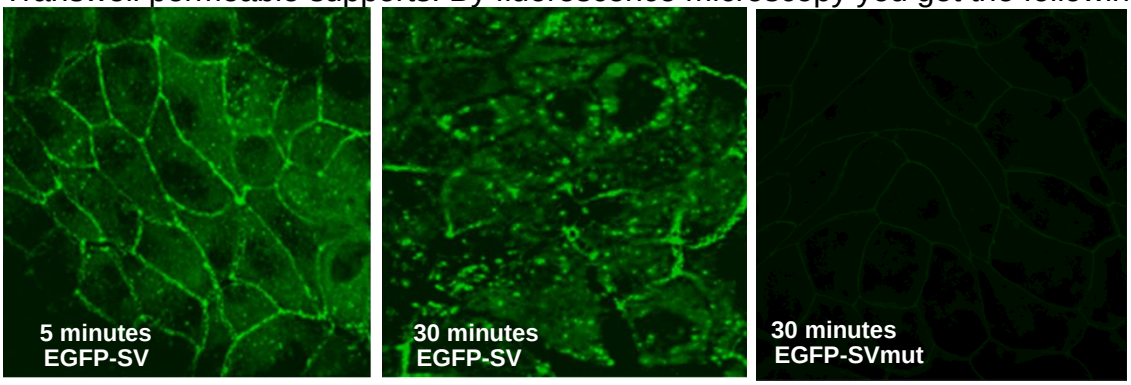
   i. a compound with potent Aster cholesterol binding-inhibitory activity (4 points)

   ii. a compound with weak Aster cholesterol binding-inhibitory activity (4 points)

   iii. a compound that had no Aster cholesterol binding-inhibitory activity (4 points)

Question 8 (Day 2)

A collaborator has given you a fluorescently tagged Sharona Virus (EGFP-SV) where the Spork protein is fused to GFP as well as a version of the virus that has a mutation in the gene encoding the Spork surface protein (EGFP-SVmut). You use these viruses to infect Caco2 cells cultured on Transwell permeable supports. By fluorescence microscopy you get the following results:



You also measure transepithelial resistance (TER) prior to SV incubation (0 minutes), at the 5 minute and 30 minute time points and obtain the following values:

Virus	0 min	5 min	30 min
EGFP-SV	1528 $\pm$ 125 Ohm x cm^2	1134 $\pm$ 205 Ohm x cm^2	282 $\pm$ 185 Ohm x cm^2
EGFP-SVmut	1378 $\pm$ 163 Ohm x cm^2	1255 $\pm$ 312 Ohm x cm^2	1298 $\pm$ 215 Ohm x cm^2

1) What are you measuring when you measure TER? How does this reflect epithelial barrier function? What would you expect if you were to use dye flux instead of TER as a measure of barrier function? (3 points)

2) Describe a method to identify the green punctate structures at the 30 minute time point for cells incubated with EGFP-SV. (5 points)

3) Propose a hypothesis explaining a role for the Spork protein in causing the obvious changes in the pattern of green fluorescence as well as different results for the TER measurements. (4 points)

4) Describe a detailed experimental approach to test your hypothesis. Be sure to include negative and positive controls required to interpret your data. (8 points)

Question 9 (Day 2)

You are studying a DNA Repair Protein, DRP, in cancer cells. DRP is an N-glycosylase that uses a catalytic lysine residue in a reaction that cleaves the damaged base in DNA to facilitate subsequent steps of DNA repair. In your studies, you find that when you compare a control cell line (Control) to a cell line derived from a breast cancer tissue (Cancer), there is an increase in the steady-state level of the DRP protein. You examine a number of other breast cancer cell lines and find a strong correlation between the level of DRP and the aggressiveness of the cancer (more aggressive cancer cells have more DRP).

Assume you have access to all tools required including an antibody directed against DRP, a construct to express DRP in cultured mammalian cells, a clone to express recombinant DRP in bacteria and anything else that you might need for the proposed studies.

1) You decide to define the mechanism(s) that regulate(s) DRP protein levels in the cell lines you are studying. Describe the first experiment you would perform to address the mechanism(s) regulating DRP in breast cancer cell lines. Be sure to include appropriate controls and describe the result you obtain- multiple results are possible- pick one. (5 points)

2) Given the results that you obtained in (A), what is the next experiment you would perform to understand at the mechanistic level how DRP levels are regulated in breast cancer cell lines? (6 points)

3) Based on the observation that DRP protein levels are increased in more aggressive cancer cell lines, you set out to test the hypothesis that an increase in DRP catalytic function is necessary to drive tumor phenotypes such as anchorage-independent growth. (6 points)

4) You complete your studies and submit your work for publication. However, reviewers raise a concern that all the work employs cell lines. How could you address this concern? (3 points)

Question 10 **(Be sure to continue to the next page for Parts 2-4 of this Question) (Day 2)**

Cytosine methylation at the C_5 position is the most important covalent modification in DNA, occurring predominantly at CpG sites. This modification within the promoter region of genes plays a key role in genomic imprinting and X-chromosome inactivation and its dysfunction is highly associated with various human diseases including cancer. CpG methylation also plays crucial roles in evolution. Once methylated, 5mC can be deaminated to thymidine, which occurs 10-50 times faster than the equivalent process on an unmodified cytosine. Inefficient DNA repair in vertebrates leads to the formation of a TpG dinucleotide after DNA replication. Genomes from more primitive organisms are more abundant in CpG sites which, through the process of methylation, deamination, and subsequent mutation to TpG sites, can produce new transcription factor binding sites. Thus, the paradigm is that CpG is methylated to 5mCpG which can deaminated and mutated to TpG (i.e., CpG → 5mCpG → TpG).

You suspect that this process of 5mCpG methylation may create a transcription factor binding site for a steroid receptor (SR1) that contains a TpG as part of their preferred DNA binding sequence. You also suspect that binding to a 5mCpG-containing DNA site might have put selective pressure on these sites favoring their conservation, enrichment and eventual mutation to TpG-containing DNA sites in higher mammals.

Characterizing the SR1-DNA interaction in vitro

1) Design an experiment to determine and compare the binding of purified Steroid Receptor 1 (SR1) to the different DNA sequences highlighted in Figure 1. Propose two complementary approaches and describe the benefits and limits of each approach you choose (e.g., equilibrium binding?, Kd or apparent affinity?, thermodynamic information?). You may assume that working with just the SR1 DNA binding domain (which has a mass of 26 kDa) is sufficient for these experiments. You may also assume you have access to purified protein and can order your required DNA oligomers. (10 points)

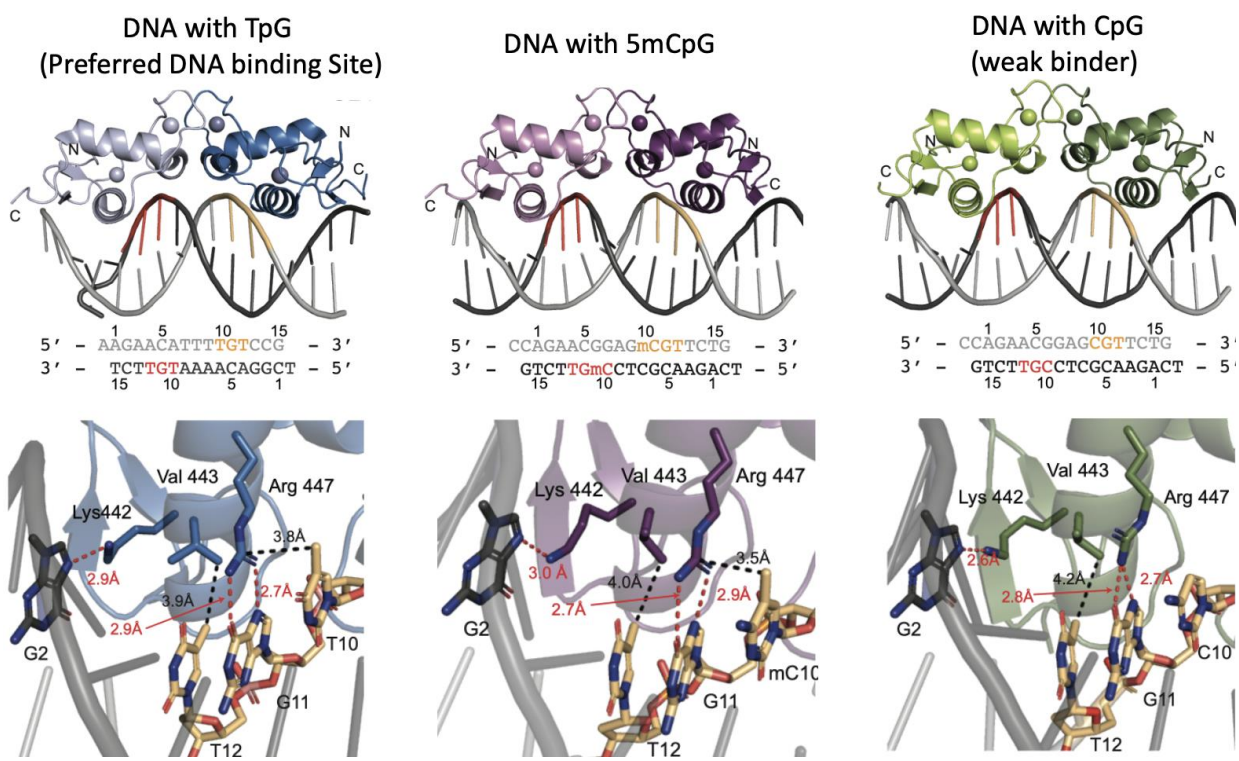


Figure 1. (Left) SR1 binds to its canonical DNA response element (or DNA binding site) as a head-to-head dimer. A close-up view of the SR1 DNA structure is shown on the bottom left highlighting the protein-DNA contacts important for DNA sequence specificity. (Middle) SR1 structure in complex with a 5mC-containing DNA sequence where 5mC is located in both the sense and antisense DNA strands. (Right) SR1 structure in complex with a non-ideal DNA sequence where the CpG has not been methylated.

You have determined the X-ray crystal structures SR1 in complex with three DNA sequences containing: The canonical DNA binding site, which contains a T at position 10; the methylated DNA binding site containing a 5mC at position 10; and a non-methylated DNA sequence containing a C at position 10.

2) What are the protein-DNA interactions that permit specific recognition of DNA bases G2, T12 and G11? (3 points)

3) Propose amino acid substitutions that would test your hypothesis regarding interactions with G2, T12 and G11. Try to be more exciting that suggesting alanine which can often create additional (favorable) hydrophobic contact at a protein-DNA interface. (3 points)

4) What molecular interactions explain how methylation of the DNA sequence in Figure 1 may enhance binding affinity for SR1? Highlight two differences between the middle (5mC) and right (CpG-containing) structures. (4 points)

Question 11 (Day 2)

While studying the recently sequenced genome of a new species of fly, you identify a gene that appears to encode a novel, single copy histone H3 variant, which you name H3.XX. Although the amino acid sequence of this new histone variant is 93% identical to canonical H3, a number of amino acid changes are evident, including Lys4→Ala4. This new fly species has all of the genetic and molecular tools found in other model organisms, and can easily be grown and propagated in the lab. You wish to examine the role of H3.XX, if any, in gene regulation in this fly species.

1) Describe a detailed experimental approach for how you would assess whether H3.XX is expressed in the developing fly and determine whether H3.XX shows tissue-specific expression. (5 points)

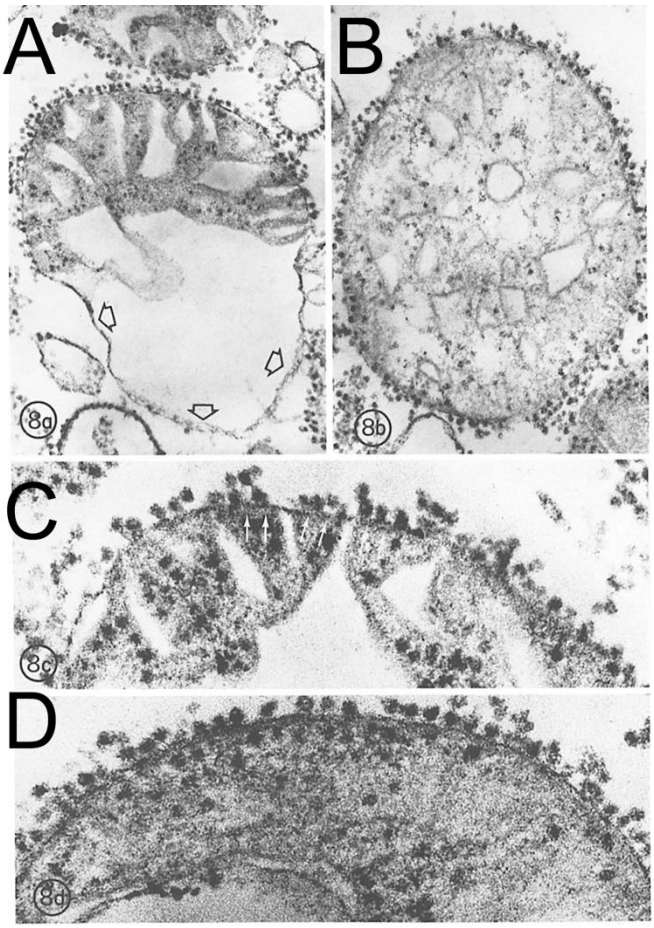
2) You find that H3.XX is only expressed in muscle cells, which can be easily dissected from early developmental stages. Describe an experiment that could determine whether H3.XX binding sites within the genome correlate with gene activation or gene repression during fly muscle development. (6 points)

3) You find that genes that are repressed during muscle development become enriched with H3.XX. You hypothesize that this enrichment of H3.XX is causal for the gene repression you have detected. Describe an experimental approach that would *directly* test your hypothesis. Include both positive and negative controls that would be required to interpret your results (6 points)

4) Which histone post-translational modification(s) would likely correlate with the proposed role of H3.XX role in gene repression? Explain the rationale for your answer. (3 points)

Question 12 (Day 2)

The figure depicts electron microscopic (EM) images of *Saccharomyces cerevisiae* mitochondria isolated by sequential differential and isopycnic sucrose sedimentation. A and B present images of the whole perimeter of a cross-sectioned mitochondrion. Internal structures correspond to cristae. In A, the inner and outer mitochondrial membranes have become detached from one another (see arrows). C and D show high magnification sections where the attachment of inner and outer mitochondrial membranes has been preserved. Note the electron dense particles attached to the outer mitochondrial membrane. These structures correspond to ***ribosomes***.



Based on this information:

1) Design TWO experimental approaches to address whether the binding of these ribosomes to the outer mitochondrial membrane is an artifact of subcellular fractionation or not. (6 points)

2) Describe TWO detailed experimental approaches to determine whether these ribosomes are of cytoplasmic origin or mitochondrial origin. (6 points)

3) Describe THREE experimental strategies to test whether the association of these ribosomes with the mitochondria impacts either the function or the composition of mitochondria. (8 points)