I added 6 points to everyone's score (or 7 points if you wrote your name on every page) to account for several locations in the exam where most of you were not clear on how specific you had to be (Q7), how to describe your experiment (Q8) or what type of control you should provide (Q 8). Please look over the key so that you are clear on these points for the final exam. After comparing your answers to the key, if you think there was an error in grading your test, please follow the re-grade procedure described in the syllabus.

Median of adjusted scores = 45
Average of the top 3 adjusted scores on the exam was: 59.

MIDTERM EXAM KEY (60 pts)

You will receive 1 point for writing your name on EVERY page. You should have 5 pages. You will have 50 minutes to complete the exam. You may begin working as soon as you receive the exam.

Multiple Choice (Circle the letter corresponding to the correct answer)

NOTE: the RED words after each question refer to the Bloom's level of thinking required to answer that question. There is a list of study activities on the web page targeted to the different levels of Bloom's (Study Activities). The majority of questions on the exams in this class will be application, analysis, evaluation and synthesis level, with a smaller proportion being knowledge & comprehension

____1. (2 pt.) You have cloned a protein-coding gene from *C. elegans* that you think is involved in neuron development. You perform a sequence comparison analysis using BLAST to identify related genes in other eukaryotes. You find a related mouse gene that is known to be essential for neuron development and compare the DNA sequence, predicted mRNA sequence and predicted amino acid sequence corresponding to the coding region of the two genes. Which of the following sequences would you expect to show the greatest similarity between the two organisms?

COMPREHENSION

A. The amino acid sequences
B. The mRNA sequences
C. The DNA sequences
D. All three types of sequences are likely to show the same degree of similarity

___ 2. (2 pt.) Which of the following pieces of information can you learn from a SDS-polyacrylamide gel?

COMPREHENSION

A. Size of an individual protein
B. Size of a protein complex (multiple proteins interacting with each other)
C. Amount of a protein
D. All of the above
E. A and C only

____3. (2 pts.) Which of the following techniques would best allow you to test whether a newly identified channel protein can be post-translationally inserted into the mitochondrial membrane?

APPLICATION

A. Subcellular fractionation and immunoprecipitation with an antibody to the channel protein
B. In vitro mitochondrial transport assay with digitonin-treated cells and fluorescent-tagged channel protein
C. Fluorescence microscopy of cells expressing a channel protein/GFP fusion protein under the control of a constitutive promoter
D. Fluorescence microscopy of cells expressing a channel protein/GFP fusion protein under the control of the channel protein's promote

____ 4. (6 pts). You want to test the hypothesis that a new importin protein you have discovered is sufficient to target NLS-containing proteins to the nucleus. You perform an in vitro nuclear transport assay on digitonin treated HeLa cells. You add your newly identified importin protein and a fluorescently-tagged NLS-containing protein to the cells and then view the cells in a fluorescent microscope. Assuming the importin protein is stable, will this approach allow you to determine if the importin protein is sufficient to localize a NLS-containing protein to the nucleus?

Circle one: **YES** NO

If YES, explain **why this approach will work** and describe a **negative** and **positive control** you would need to perform.

If NO, explain **why this approach will not work** and briefly describe **what experimental approach would allow you to test your hypothesis**.

ANALYSIS

YES (+1) , this approach will test sufficiency because you are asking whether the importin protein, on its own (in the absence of other soluble proteins), is enough to target the NLS-protein to the nucleus (+1)

Controls:

(+2) Negative control: NLS protein on its own to show that the nuclear protein doesn't move to the nucleus on its own without soluble factors OR could include a mutated NLS-containing protein control.

Not accepted: No digitonin treatment/untreated cell (this will not show you anything since no reagents are able to get into this cell)

(+2) Positive control: Adding back entire cytoplasm to show that it is possible to recreate transport of a nuclear protein if all the components of the system are added back in to the digitonin-treated cell (this is the key positive control for an in vitro assay).

Other positive controls: Adding a different importin protein

___ 5. (12 pts.).

a. (2 pts. each) For each of the following statements, decide whether the stated evidence would or would not support the hypothesis that nuclear positioning plays a role in gene regulation (Circle Yes for support or No for would not support)

COMPREHENSION

i. Both gene-dense and gene-poor chromosomes are located at the nuclear periphery	YES **NO**
ii. Two IgH alleles which are bi-allelically expressed co-localize in human lymphocytes	**YES** NO
iii. RNA polymerase II activity is concentrated in a few locations in the nucleus	**YES** NO
iv. The Myosin Heavy Chain (MyHC) gene, a marker for mature muscle cells, is found in the same 3D nuclear location in myoblasts and myotubes	YES **NO**

b. (4 pts.) Explain your reasoning for why you think statement iv above does or does not support the hypothesis:

COMPREHENSION

If nuclear positioning plays a role in gene regulation, you would expect the MyHC gene to shift positions (+2) in the nucleus when it is expressed in myotubes (+1), compared to its position when it is not expressed in myoblasts (+1).

Note: If you answered YES to part iv. and provided consistent reasoning (e.g. similar cell types have chromosomes in similar positions), you should have received at least partial credit.

___ 6. (4 pts) Unlike the ER signal sequence, nuclear localization signals are not removed when proteins are transported into the nucleus. List two reasons why a cell would not want to remove nuclear localization signals.

ANALYSIS

1. Some proteins (e.g transcription factors, importins) cycle in and out of the nucleus, so they need to keep their nuclear localization signal (+2)

2. When the nuclear envelope breaks down during cell division, all nuclear proteins are released into the cytosol. Nuclear proteins must be re-targeted to the nucleus in the daughter cells (+2)

Also accepted:

-nucleus is leaky, so proteins may diffuse out and need to be re-targeted (but this answer needed to be distinct from the 2nd reason given)

-to keep proteins in the nucleus (but needed to be clear that these had an export signal on them)

-signal sequence could be in the middle of the protein or some other critical domain, so removing it would disrupt the function of the protein

___ 7. (12 pts) You are studying nuclear transport in yeast cells. You identify a conditional mutant which has a defect in nuclear transport at 37°C, but behaves normally at room temperature (22°C). To study this further, you decide to look at transport of a steroid receptor in your mutant cells. You create a plasmid that encodes an Estrogen Receptor Ligand Binding Domain/GFP fusion protein. You transfect the mutant yeast cells with this construct and get the results shown in Fig. 1.

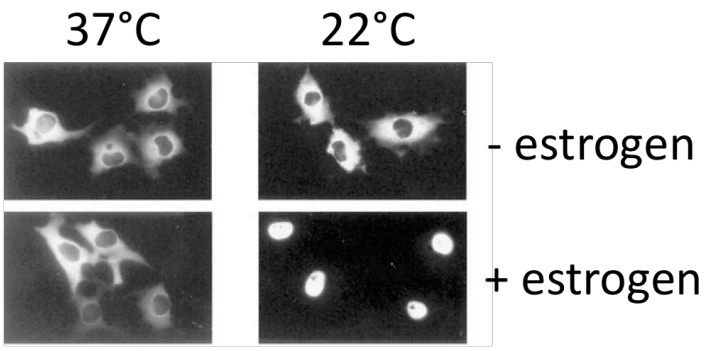

Fig. 1. Transport of the Estrogen Receptor in mutant yeast cells grown at room temperature (22°C) or at 37°C Cells were treated with or without estrogen hormone, as indicated, and then analyzed by fluorescence microscopy. **The white color indicates fluorescence.** Grey or black color indicates absence of fluorescence.

___ A. (4 pts) What can you conclude from the **room-temperature data** about estrogen receptor transport? (Be as specific as possible, using the words necessary and/or sufficient when appropriate)

ANALYSIS

The ligand binding domain is sufficient (+2) to mediate transport of the estrogen receptor (+1) from the cytosol to the nucleus in the presence of hormone (+1).

Remember that steroid hormone receptors have multiple domains (Ligand binding, transcription activation, DNA binding) so it is important to state which domain is sufficient in this experiment).

Partial credit: Hormone is necessary for transport of the estrogen receptor

___ B. (4 pts).Based on the data, explain how a mutation in Importin could result in the phenotype observed at 37°C.

ANALYSIS

The Importin mutation could disrupt the ability of importin to bind NLS of Estrogen receptor (+2), (or can bind NLS, but cannot bind FG repeats on nucleoporins), so estrogen receptor is not localized to the nucleus even in the presence of hormone (+2).
Also accepted: estrogen itself may be unable to bind and promote transport
Partial credit given for stating that temperature disrupted the folding of importin, but not stating how that would disrupt transport
It is important to be specific with your conclusions. Many of you left off the statement "even in the presence of hormone" or did not describe how the mutation would disrupt transport.

___ C. (4 pts.). Based on the data, explain how a mutation in Exportin could result in the phenotype observed at 37°C.

ANALYSIS

Exportin mutant binds NES even in the presence of estrogen (+2) causing the receptor to be exported to the cytoplasm as soon as it is imported to the nucleus by importin (+2).
Other possible answers accepted as long as consistent with data.
It is important to be specific with your conclusions. Many of you left off the statement "even in the presence of hormone" or did not describe how the mutation would disrupt transport.

/16 pts

8. (19 pts.). Your friend Amalia has identified a new mouse protein which she named Nup 333 because she thinks this protein is a component of the nuclear pore complex. To test this hypothesis, she created a plasmid containing a strong promoter driving expression of a Nup 333/GFP fusion protein. She transfected a mouse cell line with this plasmid and then monitored the location of the GFP fusion protein using fluorescence microscopy. She saw fluorescent rings around the mouse nuclei and concluded that Nup133 is located at nuclear pores.

SYNTHESIS

___ A. (2 pts.) What is **one** other possible interpretation of where Nup 333 is located? Be as specific as possible with your terminology.

Nup 333 could be associated with the nuclear envelope, outer nuclear membrane, inner nuclear membrane, nuclear lamina etc.

Based on several other experiments, you learn that:

1) Nup 333 is indeed a part of the nuclear pore complex and
2) Nup 333 is only expressed in B-cells (lymphocytes).

You develop a hypothesis about the function of Nup333 in B-cells that includes the word "necessary" (any idea is fine, as long as it leads to a testable prediction and is consistent with what you know about Nup 333).

___ B. (3 pts.) Hypothesis:

Nup 333 is necessary for transport of B-cell specific proteins
Nup 333 is necessary for expression of B-cell specific genes
Nup 333 is necessary for positioning of chromosomes in B-cells

Note: also accepted that Nup 333 is necessary for transport of all proteins in B cells – however, if this is the case, then creating a knock-out/knock-down of Nup 333 would be lethal and so not feasible unless you created a conditional knock-out.

___ C. (2 pts.). You want to test your hypothesis. List **one prediction** that you can make from your hypothesis.

Possibilities:
B-cell specific genes will not be expressed in Nup 333 mutants
Chromosomes will not be correctly positioned in Nup 333 mutants/RNAi knockdown B-cells
B-cell specific proteins will not be transported to the nucleus in Nup 333 mutants

___ D. (6 pts.) You design an experiment to test your prediction in part C. You can assume that you have any antibody, cells etc. available to you for your experiment.

List the experimental method(s) you would use to test your prediction (your experiment may involve one or more methods) AND the purpose of that method for **your** experiment (be specific about what you would be doing/measuring in this experiment but do not describe predicted results).

	Name of Method State the name not a description	Purpose of Method in **your** proposed experiment
1.	RNAi (or other mutant/knockdown method) (this method was needed to test Necessity)	Decrease/eliminate production of Nup 333 protein
2.	Many possibilities: FISH RT-PCR	Many possibilities: FISH: Determine location of chromosomes in cells lacking Nup 333

	In vitro nuclear transport assay GFP-tagging/fluorescence microscopy	RT-PCR: Determine expression of B-cell specific genes in cells lacking Nup 333 In vitro nuclear transport: Determine ability of cells lacking Nup 333 to transport B-cell specific proteins GFP-tagging: Visualize transport of a GFP-tagged nuclear protein in Nup333 deficient cells

+2 correct terminology of method/purpose of method
+2: methods can be used to test prediction
+2 specific for this experiment /13 pts

___ E. (3 pt.) Briefly describe a **specific control** for **one** of your methods listed in part D and the purpose of that control. This should not be an experimental control group e.g. wild-type cells compared to mutant cells. Do not just write "negative" or "positive" control.

Control:
Many possibilities – needed to be appropriate for one of the methods in part D.
Many of you described the wild-type control group (e.g. cells not treated with RNAi). This is considered part of your actual experiment treated vs. untreated. I was looking for a control for the actual method. How do you know the results are not due to some artifact of the treatment itself.
Remember to fill out a Methods rubric (on web site) to help you with thinking of good controls for each method we learn
Possible answers:
GFP tagging: use GFP alone– to show that GFP is normally localized to the cytosol, so any observed nuclear transport is due to the tagged protein itself)
RNAi: Add a non-specific RNA or a different RNA that you do not expect to disrupt Nup 333 expression
RT-PCR: Measure levels of a gene that you do not expect to change in the presence of absence of Nup 333

Purpose of Control:
COMPREHENSION
+2 describing a control appropriate to the method
+1 for explaining the purpose of the control
e.g. RT-PCR control: Use Actin as a positive control for a gene that is expected to be expressed in both B-cells and other cells or FISH – use a probe to a gene whose expression doesn't change e.g. actin.

___ F. (3 pts). Briefly describe **what your expected results would be for the experiment you described in part D** based on your hypothesis.
1 pt. stating what will be observed (i.e. what the output is for the experiment)
2 pt. for correctly predicting what will be observed if hypothesis is supported
Need to state what you will observe, not what you will conclude here