

Course Materials for Week 2: Organization of Cytoplasm

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Assignment Questions

1. What are non-membrane-bound compartments (choose all that apply)?
 - a. Organelles that are not bound to the cell membrane
 - b. Areas in the cell where protein complexes are organized together without a membrane separation
 - c. None of the above

2. Choose examples of the notion of “emergent properties for collections of individuals” from the list below (choose all that apply):
 - a. The activity of a protein that is lost from joining a protein complex
 - b. New properties that can emerge from protein complexes, but that were not found in isolated proteins making up this complex
 - c. The new function of a compartment that could not be executed by the protein complexes that make it up
 - d. None of the above

3. How can double-stranded RNA be introduced into *C. elegans* in an RNA interference experiment (choose all that apply)?
 - a. It can be injected into the nematode
 - b. It can be fed to the nematode
 - c. None of the above

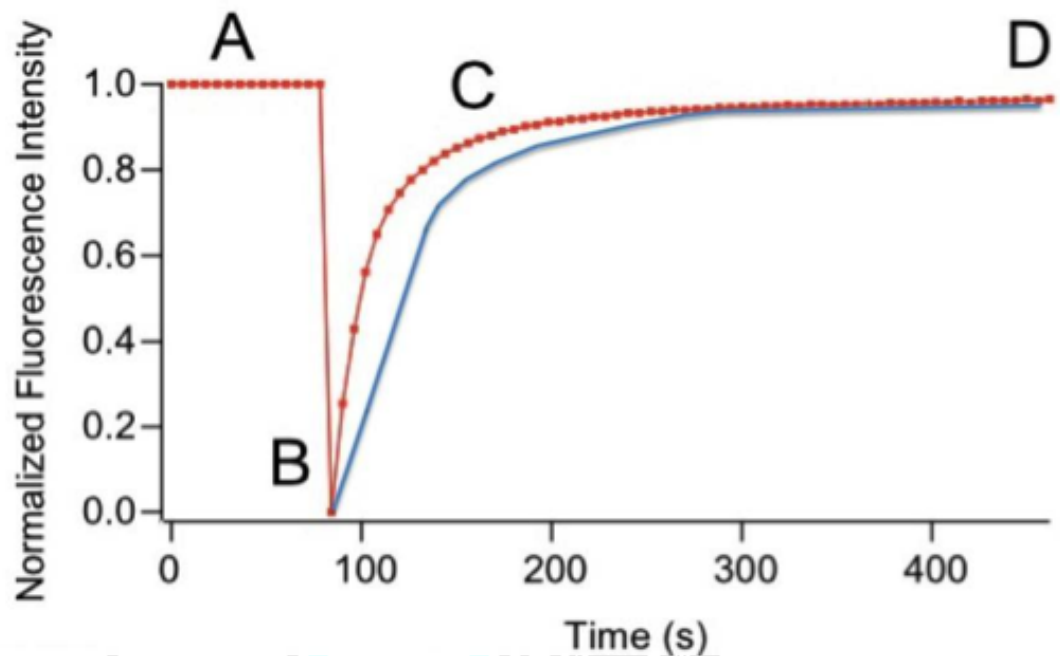
4. You decide to conduct an RNA interference experiment to identify the function of a gene in cultured cells. Shortly after adding the trigger dsRNA, you decide to analyze the phenotype of your cells and you find that it is just like the negative control's.

From the list below, choose all the statements that may apply to this situation:

- a. The negative result could be attributed to the fact that the protein run-down was insufficient for you to see the full effect of RNA interference

- b. The negative result could be attributed to the fact that the siRNA molecules could not bind to the trigger dsRNA like they were supposed to
 - c. None of the above
- 5. Regarding the genome wide-scan described in the talk, choose all the statements that are correct:
 - a. It used photobleaching experiments
 - b. It used RNA interference
 - c. It used Mass Spectroscopy
 - d. None of the above
- 6. What is a "run-down" in RNA interference?
 - a. The process of lowering the concentration of RNA inside the cell
 - b. The process of running out of siRNA after a while
 - c. The process of slowly running out of proteins inside the cell
 - d. None of the above
- 7. The results from the genome-wide scan produced the following type of information:
 - a. A list of proteins corresponding to each of the genes involved in cell division
 - b. A catalog of genes required for the first cell division
 - c. A definition of the specific functional activity of each of the proteins involved in cell division
 - d. None of the above
- 8. What is a Dicer?
 - a. An enzyme that catalyzes the separation of two nucleic acid strands
 - b. A protein that chops up double stranded RNA molecules into smaller pieces
 - c. An siRNA that binds to enzymes
 - d. None of the above

9. Which of the following experiments can be used to identify the components of a given protein complex:
- Photobleaching
 - Mass spectrometry
 - None of the above
10. How can one describe the differences in stability of the components of protein complexes and compartments (choose all that apply)? [in the future, simplify the options below]
- The fact that protein complexes make up compartments explains the fact that their turnover is faster than the compartment itself.
 - Compartments have faster turnover rates than protein complexes because they are the basic components of these protein complexes.
 - Protein complexes have slower turnover rates than compartments because they are exchanged for other protein complexes in compartments.
 - None of the above.
11. To measure the turnover of proteins in a protein complex or in a compartment, scientists can use a method described by Dr. Hyman in his talk called Fluorescence Recovery After [Photobleaching](#) (FRAP), where a bright, targeted, laser is used to bleach fluorescently-labeled proteins. The intensity of the fluorescence emitted by labeled proteins before and after bleaching is represented below, where A is the intensity of fluorescence before photobleaching, B is the intensity of fluorescence immediately following photobleaching and D is the maximum possible recovery from photobleaching (source: Wikimedia Commons, Author: MDougM, 2008).



Comparing the data obtained with the red sample and with the blue sample, check all statements that are true (choose all that apply):

- a. Recovery of fluorescence after photobleaching is faster in the blue sample than in the red sample
- b. Turnover of labeled proteins is higher in the red sample than in the blue sample
- c. Exchange of photobleached proteins for non-photobleached proteins is faster in the blue sample than in the red sample
- d. Proteins in the blue sample are more stable than proteins in the red sample
- e. None of the above

12. This week in class, we will be discussing the following question:

In this talk, Hyman discussed two methods; RNA interference and mass spectrometry following immunoprecipitation. Each of these methods produces different information for analysing a protein's function or its contribution to a particular complex. To assess which method provides which information answer the following questions.

- i. How does RNA interference work? What type of data will it produce?
- ii. How do IP/Mass Spectrometry work together? What type of data will it produce?
- iii. Which one should you use to study a protein's function vs. its contribution to a protein complex.
 1. To prepare for this question, answer the following sub-questions:
 2. How does RNA interference work? What type of data will it produce?
 3. How do IP/Mass Spectrometry work together? What type of data will it produce?
 4. Which one should you use to study a protein's function vs. its contribution to a protein complex.

Assignment Answers

1. b
2. b and c
3. a and b
4. a
5. b
6. c
7. b
8. b
9. b
10. c
11. b and d
12. i. RNA interference will allow the investigator to deplete a targeted mRNA from the cell and therefore decrease the concentration of the targeted protein. By analyzing the resulting phenotype, the investigator can determine the effect of the protein on specific cellular processes. A genome-wide scan using RNAi can yield information on groups of proteins that involved in similar processes, and this data can be used to search for protein complexes.
- ii. IP/Mass Spectrometry: The target protein can be used to immunoprecipitate the protein complex in which it is included. Mass Spectrometry can be then used to

analyze the other components of the protein complex, and determine which proteins interact with each other.

- iii. Therefore, RNA interference is used mainly to analyze protein function, whereas IP/Mass Spectrometry would be used to study protein-protein interaction in a protein complex]

Rubric

Assignment (12pts)	Pass (points)	No Pass (no point)
For each subquestion: Length and clarity (2 pts per question, 6 pts total)	The student provided an answer to each question, using full sentences or illustrations with descriptions.	The student did not answer the question or used the information provided was incomplete.
For each question: Content of answer (2 pts per question, 6 pts total)	The answer relates directly to the question and is based on valid scientific data, models or principles.	The answer is off topic and it is not based on any scientific data, models or principles.

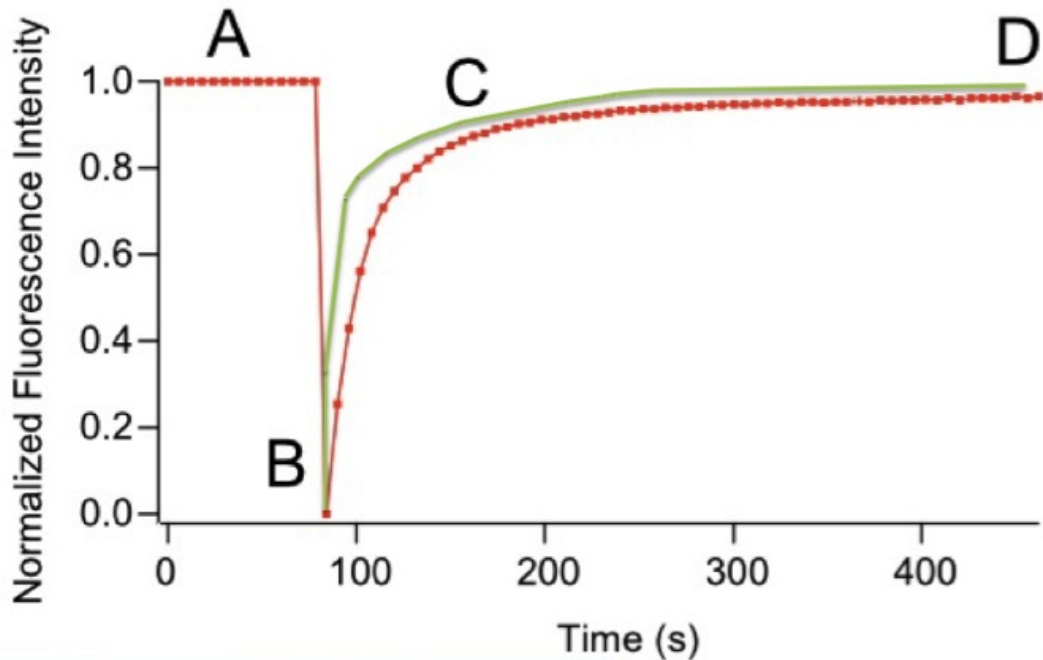
In-Class Quiz Questions

1. You decide to conduct an RNA interference experiment to identify the function of a gene in cultured cells. Shortly after adding the trigger dsRNA, you decide to

analyze the phenotype of your cells and find that it is just like the negative control.

From the list below, choose some of the experiments that could help you determine what may have happened in this experiment:

- a. Measure number of DNA copies
 - b. Measure endogenous mRNA levels
 - c. Measure protein run-down
 - d. None of the above
2. To measure the turnover of proteins in a protein complex or in a compartment, scientists can use a method described by Dr. Hyman in his talk called Fluorescence Recovery After [Photobleaching](#) (FRAP), where a bright, targeted, laser is used to bleach fluorescently-labeled proteins. The intensity of the fluorescence emitted by labeled proteins before and after bleaching is represented below, where A is the intensity of fluorescence before photobleaching, B is the intensity of fluorescence immediately following photobleaching and D is the maximum possible recovery from photobleaching (source: Wikimedia Commons, Author: MDougM, 2008).



Comparing the data obtained with the red sample and with the green sample, check all statements that are true (choose all that apply):

- a. Turnover of fluorescently labeled proteins is higher in the red sample than in the green sample
 - b. Exchange of photobleached proteins for non-photobleached proteins is faster in the green sample than in the red sample
 - c. Proteins in the green sample are more stable than proteins in the red sample
 - d. Recovery of fluorescence after photobleaching is faster in the green sample than in the red sample
 - e. None of the above
3. A new protein, Hymanein, is thought to be involved cell division, although it is not clear what its function would be. Describe a method you would use to confirm Hymanein's involvement in cell division, briefly explain how this method works and what type of information it can yield.

In-Class Quiz Answers

1. b and c
2. b and d
3. The method of choice is RNA interference. RNAi will allow the investigator to deplete Hymaein mRNA from the cell and therefore decrease the concentration of the Hymaein protein. By analyzing the resulting phenotype the investigator can determine the effect of the protein on cell division. See Hyman's talk for mechanism of RNAi.

2: Mechanism of RNA interference (see Hyman talk for details)

Rubric

In Class (10pts)	Pass (points)	No Pass (no point)
Completeness and clarity (5 pts total)	The student described the method and provided a clear (brief) explanation of the mechanism. Optional: described the types of results obtained.	The student did not describe the method and did not provide a clear explanation of the mechanism.
Content of answer (5 pts total)	The answer relates directly to the discussion question and includes one of the accurate answers provided in the key, the course notes or the talk or is based on valid scientific data and models.	The answer is off topic and it is inaccurate and cannot be justified scientifically.