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Homework 6

Due May 22th at the beginning of lecture

Instructions:

You are welcome to discuss concepts with your classmates but must compose your own answers. If you are unsure of the honor code for this course, please ask or look at the course website. http://www.its.caltech.edu/~bi1/Bi1__Micro-_to_Macro-Biology/Policies.html
The goal of this assignment is to help you understand a dense research paper. Many of the questions do not have a single correct answer. You will be given full credit as long as your answer is reasonable. The point is for this to help train you to pull out the main point from a paper, even if you don't get every last detail.
The answers must be legible and should not extend past the allotted space. Keep in mind that a few well-written sentences can give a higher score than a whole page of text.
Remember to write your full name on each page.

Read Hamamura, Y, et. Al (2011) Live-cell imaging reveals the dynamics of two sperm cells during double fertilization in *Arabidopsis thaliana*. *Curr Biol* 21: 497-502 **carefully and answer the following questions.**

1) What are the main goal and significance of the paper? (4 points)
The goal of the paper is to observe sperm cell behaviors during double fertilization by live-cell imaging. The fluorescent and improved imaging techniques allow the authors to see and identify the previous unknown process.

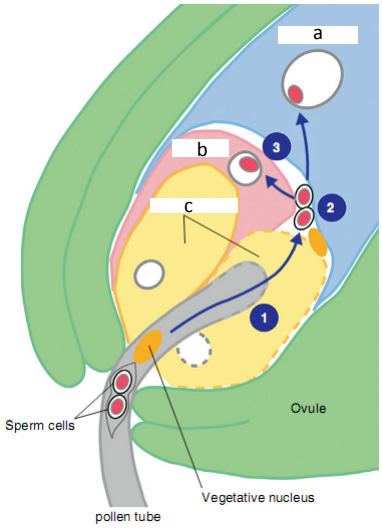

Figure 1:
Steps of double fertilization in *Arabidopsis thaliana*

2) Refer to Figure 1
i. Write down the name of cell a, b and c. (2 points)

a: Central cell
b: Egg cell
c: Synergid cells

ii. In your own words, describe the three steps (1, 2 and 3) of sperm cell behavior observed by the authors (5 points)

Step 1: pollen tube discharge
Step 2: immobility
Step 3: double-fertilization

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iii. The chromosome number of haploid cells is n, diploid cells is 2n and triploid cells 3n. Write down the chromosome numbers of cells a, b, and c before and after fertilization in the following table. (4 points)

	Before Fertilization	After
Cell a	2n	3n
Cell b	n	2n
Cell c	n	n

3) One conclusion of the paper is that there is no preferential fertilization in *Arabidopsis thaliana*. Explain what preferential fertilization is. From which experimental result(s) could the author make the conclusion? (4 points)

When double fertilization happens, one sperm unites with the central cell and the other sperm fertilizes the egg. Preferential fertilization describes the sperm is more likely to fertilize either central or egg cell
Using photo-switchable fluorescent tags, the authors were able to not only distinguish the two sperms but also track their targets. They found the probabilities for each sperm to fertilize the egg and central cell are similar, demonstrating no preferential fertilization in the plant.

4) Some plants can be fertilized by their own pollens, called self-fertilization, but the majority of angiosperms have developed mechanisms to prevent it from happening.

i. What might be the benefit of self-fertilization? (3 points)

The parental character can be preserved
It has greater fertilization possibility

ii. Under what circumstance would self-fertilization be detrimental? What might be the evolutionary advantage of preventing self-fertilization? What strategies can plants use to prevent self-fertilization? (Name 2)(7 points)

Self-fertilization produces genetically identical offspring. When a new environment is introduced, the lack of genetic variations might be harmful for their adaptation or survival. Preventing self-fertilization may help plants obtain useful and eliminate undesirable characters.

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Strategies: timing (female and male mature at different times), morphology (stigma taller than anther, one flower with only one set of reproductive organs), biochemistry (chemical prevents pollen tube germinate on the same flower)

Fluorescent Microscopy

5) Give a short description of how fluorescent tags function and how are they visualized in fluorescent microscopy? Explain the difference between the excitation spectrum and emission spectrum of a fluorescent tag and how this affects fluorescent microscopy. (5 points)
   - Discussion of how the fluorescent protein tag changes conformation when it absorbs energy from light in the excitation spectrum of the protein, and then relaxes and emits light in the emission spectrum. For fluorescent microscopy, to visualize the protein you need illuminate the sample with light in the excitation spectrum and then filter out the excitation spectrum from the acquired image and instead focus on light in the emission spectrum of the fluorescent tag.

6) With a few sentences, describe how fluorescent tags can be attached genetically to a protein of interest and how this fluorescent tag can be useful for cellular and sub-cellular localization? (Hint: look back at earlier recitation sections and papers!) (5 points)
   - Any reasonable description of how to genetically attach a fluorescent tag to a protein, such as using restriction enzymes to make complementary sticky ends followed by ligation of the gene for the fluorescent to the gene encoding the desired protein.
   - The fluorescent tag is useful for cellular localization because you can attach fluorescent tags to cell membranes or other structural features of a cell. It is also useful for sub-cellular localizations because you can observe the sub-cellular localization of particular molecules fused to fluorescent tags.

7) What do you think are some possible problems that could occur? (5 points)
   - The attachment of the fluorescent tag can cause the protein to fold incorrectly and result in a non-functional protein.
   - The fluorescent tag can cause the tagged protein to not be localized correctly
   - The fluorescent tag could block the active site of the protein it is attached to, resulting in a nonfunctional protein.

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8) Design an experiment to better visualize any pathway discussed in class to this date with the use of one or more fluorescent tags and describe what the results will tell you. What pathway interests you? What would you label? What would the expected results be? (6 points)

- This is a very open ended question and any answer that is reasonable and discusses the pathway of interest, how the tags are attached, and what the experimental results will tell us will get full credit. There is specific answer to this question.