Course Materials for Week 4: Vesicle Trafficking

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Assignment Questions

1. The two *primary* components of vesicles are (select all that apply):

    a. plasma membrane
    b. lipid bilayer
    c. interior content
    d. none of the above

2. The steps leading to a vesicle releasing its content include (select all that apply):

    a. recycling of vesicle interior content
    b. docking at the plasma membrane
    c. recycling of membrane
    d. fusing/merging vesicle membrane with the plasma membrane
    e. none of the above

3. Which of the following statements are true? (select all that apply):

    a. yeast is a single cell organism
    b. yeast does not have a cell wall
    c. yeast uses similar vesicle transport system as neurons
    d. yeast uses vesicles to transport membrane proteins
    e. none of the above

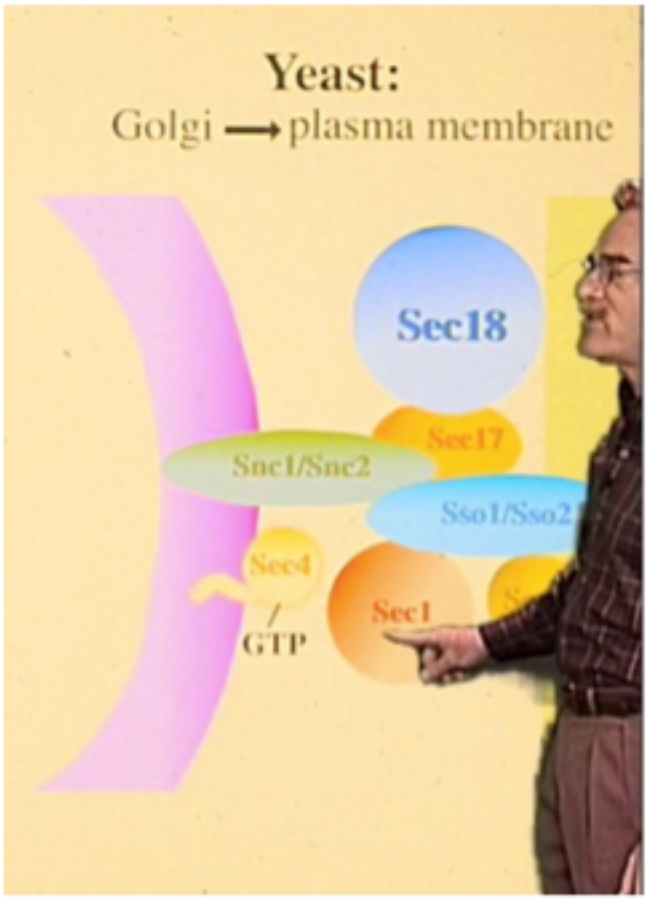
4. Temperature sensitive mutations are useful because (select all that apply):

    a. scientists can study mutations in essential proteins
    b. the mutation only destabilizes the protein at higher temperature
    c. the mutation only destabilizes the protein at lower temperature
    d. scientists can use random genetic mutagenesis to create mutants
    e. none of the above

5. Which of the following is true about Sec1 mutant yeast cells: (select all that apply)

    a. the vesicle size is larger on average than wild-type vesicles
    b. vesicles fill the entire cell and bud
    c. the type of molecules contained in the vesicles are the same as in the wild-type vesicles
    d. the vesicle cannot fuse with the plasma membrane
    e. None of the above

6.

    a. In his talk, Dr. Schekman presents the following image:
        i. Figure 3:
        ii. 

        iii. About the model presented here, check all that apply:
            ❏ Such a model can be created solely using electron microscopy data

- ❏ Such a model can be created solely using mutant yeast cells
- ❏ Electron microscopy, combined with yeast cell mutagenesis can provide enough information to build such models
- ❏ None of the above

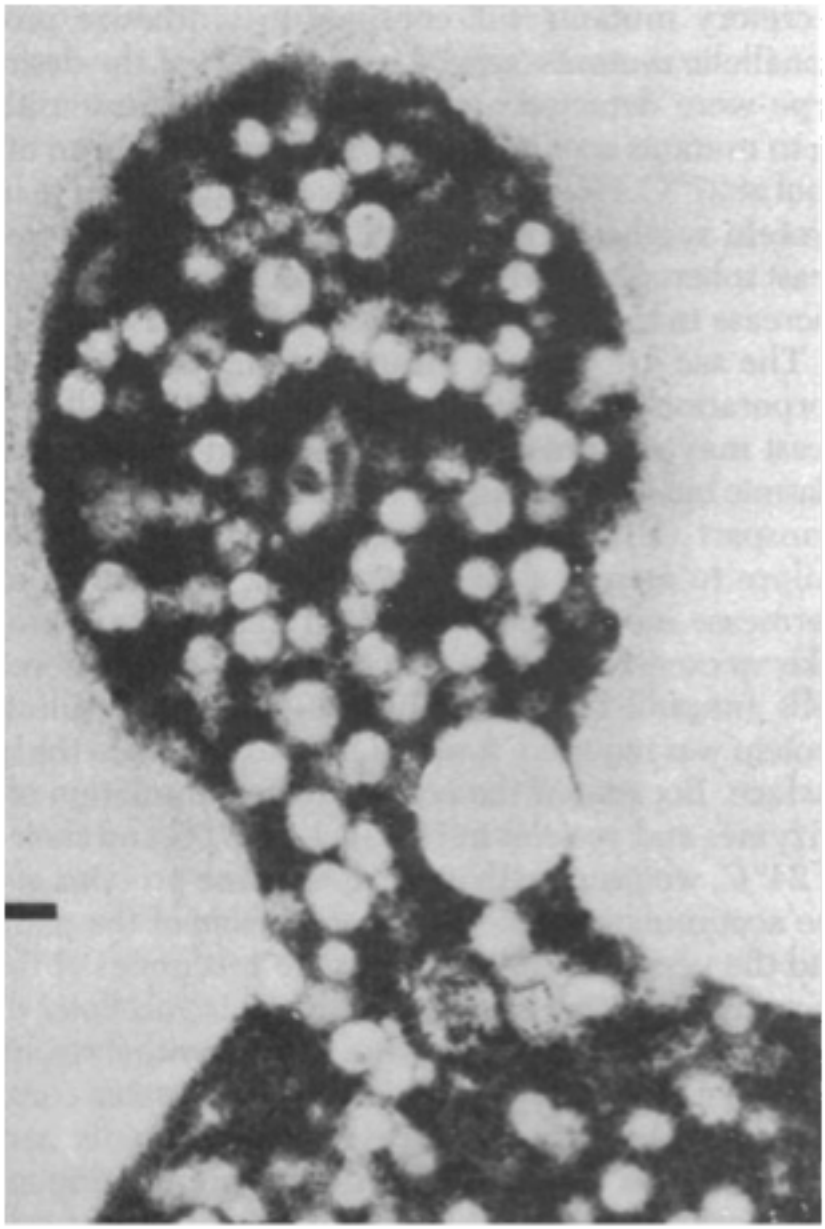
b. Use the space below to explain your answer to the previous question in a few sentences:

7. Which of the following steps of the secretion pathway may be altered in mutants displaying an arrested budding phenotype?

    a. Vesicle trafficking to the plasma membrane
    b. Production of vesicles inside the cell
    c. Docking of vesicles to the plasma membrane
    d. Fusion of vesicles with the plasma membrane
    e. None of the above

8. In the image below is an electron micrograph of a budding *Saccharomyces cerevisiae* cell. In the following list, check all the statements that apply about the mechanism observed in this image:

    a. The phenotype of this cell is indicative of an arrested budding phenotype
    b. There is an accumulation of vacuoles inside the cell
    c. There is likely a defect in the secretory pathway of this cell
    d. None of the above



9. Regarding the image above, would you say that: (check all that apply)

    a. These cells have a similar phenotype to the temperature-sensitive Sec-7 mutants incubated at 37 degrees Celsius
    b. These cells have a similar phenotype to the temperature-sensitive Sec-1 mutants incubated at 20 degrees Celsius
    c. None of the above

10. The table below describes the results obtained from a secretion experiment used to screen conditional mutants in *Saccharomyces cerevisiae*. It includes data from a Wild-Type strain (WT) and two mutants (M1 and M2). The cells were first incubated in regular growth medium (non-stimulating conditions) and acid phosphatase concentration in the medium was used as a measure of secretion levels. Then, cells were incubated in a secretion-inducing medium, either at 37°C or at 25°C, and secretion was measured again using the same method.

Strain	Basal Levels (Acid Phosphatase U/mL)	Secretion at 37°C (Acid Phosphatase U/mL)	Secretion at 25°C (Acid Phosphatase U/mL)
WT	27	193	174
M1	27	28	147
M2	30	27	31

Regarding this data, choose all that apply:

a. M1 and M2 are both conditional mutants
b. Secretion is inhibited in both mutants at body temperature, compared to wild-type
c. Secretion is inhibited in both mutants at room temperature, compared to wild-type

d. The mutations in M1 and M2 must affect vesicle docking and membrane fusion
e. None of the above

11. The table below represents the cell density measured by centrifugation of the Wild-Type (WT) and mutant cells (M1 and M2) incubated in similar conditions as described above. It also includes the cell density of the Sec-1 conditional mutant described in Dr. Schekman's talk.

Strain	Cell density (g/mL) after secretion experiment at 25°C	Cell density (g/mL) after secretion experiment at 37°C
WT	1.110	1.122
Sec-1 conditional mutant	1.104	1.157
M1	1.113	1.161
M2	1.103	1.111

Regarding this data, choose all that apply:

a. The M1 mutation likely results in accumulation of vesicles inside the cell
b. The M2 mutation likely results in accumulation of vesicles inside the cell
c. None of the above

12. The investigators then obtained the following electron micrographs in their experiment when incubating cells at 37 degrees Celsius in (to add) Question 11.

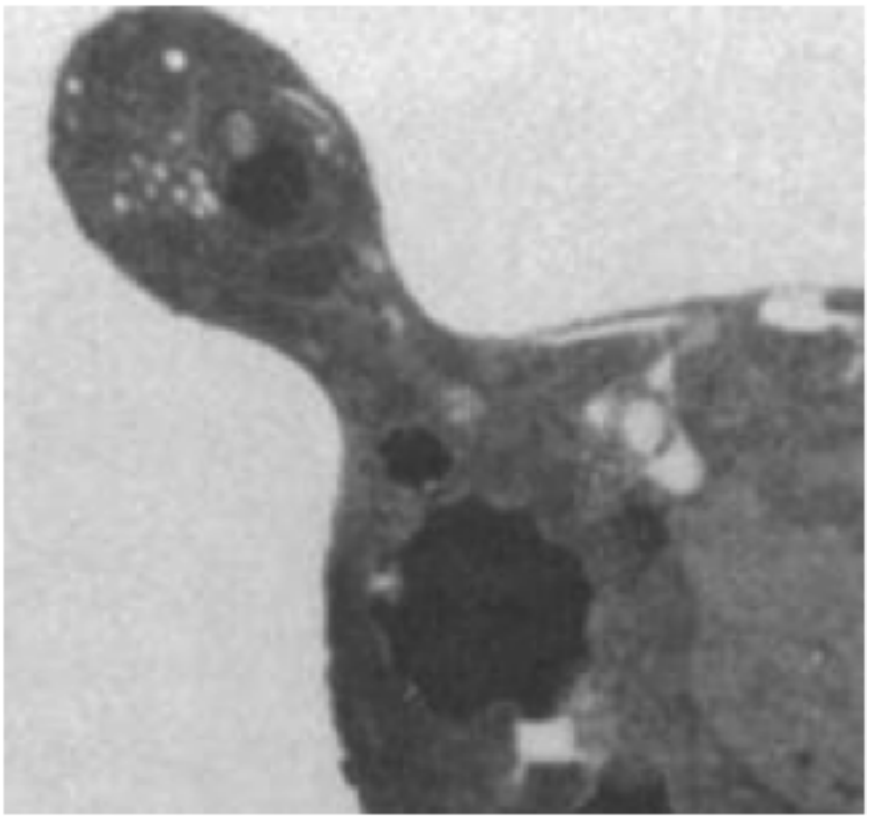
Figure 1: 

Figure 2: 

In these images, figure 1 could represent _____ or _____ cells. Figure 2 could represent _____ or _____ cells.

a. Sec-1 mutant, M1 mutant, Wild-type, M2 mutant
b. Wild-type, M1 mutant, Sec-1 mutant, M2 mutant
c. Sec-1 mutant, M2 mutant, Wild-type, M1 mutant
d. Wild-type, M2 mutant, Sec-1 mutant, M1 mutant
e. None of the above

13. Discussion Question:

In this talk, Randy Schekman describes a genetic screen in yeast and then follow-up studies *in vitro* (in the test tube).

Write a paragraph-long answer, keeping in mind Tony Hyman's talk where he explained some of the limitations with the data obtained from the genome-wide RNAi screen.

Why would a scientist reconstruct vesicle budding in vitro when this phenomenon can be readily observed in live cells? What are some of the advantages of using biochemical techniques to study the secretory pathway?

Assignment Answers

1. b and c
2. b, c, and d
3. a, c, and d
4. a, b, and d
5. b, c, and d
6. a. d
7. b. points for providing a clear, scientifically valid answer such as: mutagenesis and/or microscopy will provide information on the cellular processes, either wild-type (without mutagenesis) or the processes that are impacted by genetic modifications. But they will not provide information on molecular interactions.
8. a, b, c, and d
9. a and c
10. c (mutation in Sec-1 at 37 degrees)
11. b (wrong answers: a- M2 is not a conditional mutant - it is constitutive because secretion at 25 degrees is impaired as well; c - only M2 secretion is inhibited at 25 degrees; d - with this data, there is no way to say which point in the secretion pathway is affected)
12. a
13. D

a. Why would a scientist reconstruct vesicle budding *in vitro* when this phenomenon can be readily observed in live cells?
    i. genetics can give you good insight into what proteins might be involved in a process
    ii. a scientist can perform a genome-wide screen to find many of the proteins involved in a pathway
    iii. a scientist can use random mutagenesis to look for mutations that cause a phenotype, therefore finding new genes involved in a pathway

b. However,
    i. genetics do not tell you how the molecules encoded by the genes perform their function (e.g. how a membrane is shaped, how protein molecules are

inserted into the membrane, how the vesicle is delivered, how do vesicles fuse, etc.)

ii. genes involved in the same pathway might produce the same phenotype although the functions are different
iii. the genes might make proteins that are tangentially related to a process. For example, maybe the mutation is in a kinase that regulates the process of vesicle budding, but it is not involved in forming the vesicles
iv. biochemistry can be useful to find the minimal components for a process, for example to make a bud. In Schekman's talk, he ends with a video of vesicle formation which shows what each component does, without biochemistry you would not have this information since the genetics will lead to the same phenotype
v. If you use biochemistry, you can also do IP-Mass Spec to identify more proteins involved in the pathway

Similar to Hyman's talk, Schekman illustrates the power of genetics to help identify proteins that might work together in a pathway, however follow-up studies are necessary to determine what the protein function is and how the proteins interact with one another in the process.

Rubric

Assignment (10pts)	Pass (points)	No Pass (no point)
Length and clarity (5 pts total)	The student provided a paragraph-long answer and used full sentences.	The student's answer was too short (less than a paragraph long) or used short fragments of sentences.
Content of answer (5 pts total)	The answer relates directly to the discussion 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 have isolated a new temperature-sensitive sec mutant which you suspect has a specific defect in secretory vesicle docking and fusion with the plasma membrane (exocytosis). Describe an experiment you could do to test your hypothesis.

2. Design a series of experiments to identify which secretory pathway proteins are present on these secretory vesicles.

3. Among the proteins present in these synaptic vesicles according to mass spec you find a protein which contains a G-domain fold suggesting that it is a G-protein. Briefly outline how, in principle, you would determine which of the other proteins is a Gap for this G-protein?

In-Class Quiz Answers

1. After shifting cells to restrictive temperature, centrifuge on gradient to look for increased buoyant density of cells and look by EM for accumulation of vesicles at the bud site

2. Allow vesicles to accumulate at restrictive temperature, isolate vesicles from lysed cells by centrifugation, trypsinize vesicles and perform mass spectroscopy on isolated vesicles.....compare mass-to-charge ratio with those of predicted tryptic fragments present in yeast genome sequence database to identify proteins present

3. Purify the protein from wild type cells and measure the rate at which it releases gamma phosphate from GTP. Assay proteins in extract for those that stimulate this GTPase activity.