StarCellBio

StarCellBio Exercise 1 Answer Key – Protein localization

Goal

In this exercise, you will use StarCellBio, a cell and molecular experiment simulator, to examine the subcellular localization of different proteins using fluorescence microscopy.

Learning Objectives

After completing this exercise, you will be able to:

1. Use StarCellBio to perform simulated fluorescence microscopy experiments.
2. Design and implement an experiment in StarCellBio using the appropriate negative and/or positive controls.
3. Analyze fluorescence microscopy images to identify the subcellular localization of proteins.
4. Propose a hypothesis that could explain how mutations in a given gene may result in a change in its protein's subcellular localization.

Accessing StarCellBio

To begin:

1. Using **Google Chrome**, navigate to: http://starcellbio.mit.edu.
2. Sign in to your StarCellBio student account. If you need to set up a student account, use the course code **SCB_SampleExercises**. Note: while you can complete these exercises as a guest by clicking on **Try an Experiment** on the right side of the StarCellBio homepage, your work will not be saved.
3. Select **"Exercise 1"** from the **Assignments** window.

Introduction

You are completing a summer research project in a lab that studies various signal transduction pathways involved in human diseases. Your advisor has asked you to determine the subcellular localization of a newly discovered set of proteins, called Protein A, B, C, and D within human cells. These proteins are components of a signal transduction pathway that regulates cell proliferation. Mutations in other known components of this pathway result in increased proliferation and tumorigenesis. To characterize the roles of Proteins A-D in this signal transduction pathway, you first investigate their subcellular localization. To do this, you generate human cell lines, each stably expressing a fusion protein in which Green Fluorescent Protein (GFP) is fused in frame to one of the five proteins. These fusion cell lines are called **GFP-ProA, GFP-ProB**, etc. You then look at the localization of these fusion proteins using fluorescence microscopy.

Your collaborator in a clinical cancer lab has identified two cancer patients with mutations in this pathway; one with a mutation in the gene encoding Protein A and one with a mutation in the gene encoding Protein B. These mutations do not affect the production of Protein A and B. To understand how these mutations affect the function of Protein A and B, you clone the Mutant A and Mutant B genes and create cell lines stably expressing each of the mutant proteins fused to GFP. These cell lines are called **GFP-Mut ProA** and **GFP-Mut ProB**. Your research project is to determine if these mutations affect the localization of Protein A and B using fluorescence microscopy.

Background Information

Cell Lines

You are provided with the following cell lines:

Strain	Description
No GFP	A human cell line without expression of any GFP or GFP fusion proteins.
GFP	A human cell line stably expressing GFP, which localizes to both the **nucleus** and **cytoplasm**.
GFP-ProA	A human cell line stably expressing Protein A fused to GFP.
GFP-ProB	A human cell line stably expressing Protein B fused to GFP.
GFP-ProC	A human cell line stably expressing Protein C fused to GFP.
GFP-ProD	A human cell line stably expressing Protein D fused to GFP.
GFP-Mut ProA	A human cell line stably expressing the mutant Protein A fused to GFP.
GFP-Mut ProB	A human cell line stably expressing the mutant Protein B fused to GFP.
GFP-Nuc	A human cell line stably expressing Histone H2B, a protein that localizes to the **nucleus** (nuc), fused to GFP.
GFP–Cyto	A human cell line stably expressing RPS20, a protein that localizes to the **cytoplasm** (cyto), fused to GFP.
GFP-PM	A human cell line stably expressing LCK, a protein that localizes to the **plasma membrane** (PM), fused to GFP.
GFP-ER	A human cell line stably expressing CALNEXIN, a protein that localizes to the **endoplasmic reticulum** (ER), fused to GFP.
GFP-NM	A human cell line stably expressing LAMIN B1, a protein that localizes to the **nuclear membrane (NM),** fused to GFP.

Microscopy

You are provided with the following conditions for fluorescence microscopy experiments:

Condition	Description
GFP (green)	Fluorescence microscopy image captured using the green channel [1,2].

Notes:

[1]When GFP is excited with a laser emitting the appropriate wavelength of light, it fluoresces, emitting green light. A scientist can view the green light emitted by GFP molecules in the cells through a microscope's viewfinder when the appropriate emission filter is used on the microscope.

[2]The images portrayed in StarCellBio's microscopy experiments are in black and white because they were captured by a black and white camera attached to the microscope. When images are captured by a black and white camera, the areas with the brightest or strongest fluorescence appear as white pixels and the areas with the weakest fluorescence appear as black pixels.

Question 1

Conduct the appropriate fluorescence microscopy experiments to determine the subcellular localization of each protein, Proteins A-D. Ensure you perform any relevant control experiments.

Answer the following two questions for each of Proteins A-D:

i. Where is the protein localized within human cells? For each protein, choose the answer that best describes its subcellular localization: plasma membrane, cytoplasm, endoplasmic reticulum (ER), nucleus, or nuclear membrane.

ii. Explain how you arrived at your answer. Your answer should reference your experimental results and include the relevant controls that you used to arrive at your conclusion.

Answer:

Protein A
i. ER
ii. The fluorescence microscopy image of GFP-ProA cells shows protein expression in a pattern that appears to be a 'meshwork' throughout the cell. This pattern is characteristic of a protein localizing to the ER, and mirrors the GFP fluorescence pattern seen in the GFP-ER positive control strain.

Protein B
i. nucleus
ii. The fluorescence microscopy image of GFP-ProA cells shows protein expression in a small central area of the cell, the nucleus. The remainder of the cell (cytoplasm) is dark, but with the little bit of fluorescent background signal produced by the cell, we can still see the faint outline of the cell. This pattern is characteristic of a protein localizing to the nucleus, and mirrors the GFP fluorescence pattern seen in the GFP-nuc positive control strain.

Protein C
i. plasma membrane
ii. The fluorescence microscopy image of GFP-ProA cells shows protein expression in a pattern that has the most intense protein expression around the exterior of the cell. This pattern is characteristic of a protein localizing to the plasma membrane, and mirrors the GFP fluorescence pattern seen in the GFP-PM positive control strain.

Protein D
i. cytoplasm
ii. The fluorescence microscopy image of GFP-ProA cells shows protein expression throughout the cell excluding the nucleus. This pattern is characteristic of a protein localizing to the cytoplasm, and mirrors the GFP fluorescence pattern seen in the GFP-cyto positive control strain.

Question 2

Perform the appropriate microscopy experiments to determine the subcellular localization of the mutant Protein A in the GFP-Mut ProA cell line. Ensure you perform any relevant controls experiments.

a) Where is the mutant Protein A located in the GFP-Mut ProA cell line**?** Does the mutant Protein A localize to the same place as wild-type Protein A? Explain how you arrived at your answer using your experimental results.

Answer:

Mutant protein A is located in the plasma membrane. This differs from the wild-type localization of Protein A, which is located in the ER.

b) Propose a hypothesis that would explain how a mutation in the gene encoding Protein A would result in the change in subcellular localization, if any, identified in Question 2 (a).

Answer:

One answer is below. Other answers are also possible:

Wild-type Protein A localizes to the ER, but mutant Protein A localizes to the plasma membrane. This change may be explained by a mutation that removes or alters the ER retention signal (KDEL sequence), resulting in mutant Protein A being transported through the secretory pathway from the ER to the Golgi apparatus to the plasma membrane, where it accumulates in the mutant cell line.

Question 3

Conduct the appropriate properly controlled microscopy experiments within StarCellBio to determine the subcellular localization of the mutant Protein B in the GFP-Mut ProB cell line. Ensure you perform any relevant controls experiments.

a) Where is the mutant Protein B located in the GFP-Mut ProB cell line**?** Does the mutant Protein B localize to the same place as wild-type Protein B? Explain how you arrived at your answer using your experimental results.

Answer:

Mutant protein B is located in the cytosol. This differs from the wild-type localization of Protein B, which is in the nucleus.

b) Propose a hypothesis that would explain how a mutation in the gene encoding Protein B would result in the change in subcellular localization, if any, identified in Question 3 (a).

Answer:

One answer is below. Other answers are also possible.
Wild-type Protein B localizes to the nucleus, but mutant Protein B localizes to the cytoplasm. This change may be explained by a mutation that removes or alters its nuclear localization signal, resulting in mutant Protein B remaining in the cytoplasm rather than being transported into the nucleus after translation.