StarCellBio Exercise 1 – 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:

1When 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.

2The 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.

ii.

Protein B

i.

ii.

Protein C

i.

ii.

Protein D

i.

ii.

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.

**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).

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.

**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).