Project: Protein Localization in Mammalian Cells

- **Idea:** Compare localization of proteins (ZFP568 & GALT) in two types of mammalian cells

- **Significance:** Protein location is essential for proper function, mislocalization is associated with disease

- **Biological Techniques:**
  - DNA extraction & purification
  - Transfection of DNA into HEK293T (human embryonic kidney) & NIH3T3 (mouse embryonic fibroblast) cells
  - Cell staining & fluorescence microscopy to visualize protein location with GFP
Protein Synthesis in the Cell

Nuclear envelope

Nucleolus

Chromatin

Nucleus

Rough endoplasmic reticulum

Ribosomes

Peroxisome

Smooth endoplasmic reticulum

Golgi apparatus

Lysosome

Mitochondrion

Cytoskeletal element

Plasma membrane

Centrioles

Structures that occur in animal cells but not plant cells
Diseases associated with defects in protein transport

- Cystic fibrosis (CF)
- Familial hypercholesterolaemia (FH)
- Congenital sucrase-isomaltase deficiency (CSID)

**FIGURE 1**

Protein trafficking events disrupted in some genetic diseases. CF, cystic fibrosis; FH, familial hypercholesterolaemia; CSID, congenital sucrase–isomaltase deficiency.
Project Goal

• Compare the localization of two proteins (ZFP568 & GALT) in two types of mammalian cells (human embryonic kidney cells & mouse embryonic fibroblasts)

• We’ll do this by
  – Making DNA that codes for our proteins (DNA extraction & purification)
  – Putting that DNA in mammalian cells (transfection)
  – Determining where our proteins are within the cells (cell staining & fluorescent microscopy)
Our Project Plan

Miniprep to extract DNA from bacteria (Tues)

Stain & look at our cells to see where the protein is (Thurs/Fri)

Transfect to put our DNA in mammalian cells (Wed)
The Two Cell Types We’ll Use

- HEK293T cells (human embryonic kidney)
- NIH 3T3 cells (mouse embryonic fibroblast)
- Look at the cell morphology of each: How are they different?
- Which cell type would you want to use for our project?
The First Protein We’ll Look At

- GFP-ZFP568 (Zinc finger protein 568)
  - Binds to DNA and recruits transcriptional repressor TRIM28
  - Mutation ("chato") causes embryonic arrest

The Second Protein We’ll Look At

• GFP-GALT (Galactose-1-phosphate uridylyltransferase)
  – Enzyme important in sugar metabolism: Converts galactose to glucose
  – Mutation in GALT causes galactosemia
    • autosomal recessive mode of inheritance
GFP vector – a plasmid

DNA coding for ZFP568 or GALT can be inserted here

Restriction Map and Multiple Cloning Site (MCS) of pAcGFP1-N1 Vector. Unique restriction sites are shown in bold.
Restriction Enzymes

• XhoI and HindIII
  – Used to cut (digest) DNA coding for ZFP568 so it could be glued (ligated) into GFP-vector

• Digest with these enzymes and DNA coding for ZFP568 should be “cut” from its vector
  – Resulting fragments of DNA will be analyzed using gel electrophoresis
  – What will this gel look like? How will digested GFP-ZFP568 look different from GFP-alone?

\[
\begin{align*}
5' & \ldots CTGAG \ldots 3' \\
3' & \ldots GAGCTC \ldots 5'
\end{align*}
\]
\[
\begin{align*}
5' & \ldots AAGCTT \ldots 3' \\
3' & \ldots TTCGAA \ldots 5'
\end{align*}
\]

XhoI    HindIII
DNA extraction & purification

• GFP-ZFP568, GFP-GALT, and GFP-alone DNA was transformed into bacterial cells → cells were cultured to make more DNA → now DNA can be extracted
• Qiagen MiniPrep Kit
Transfection

• Purified DNA can be transfected into HEK293T and NIH3T3 cells using Lipofectamine 2000
• Mix DNA in media with the Lipofectamine reagent and then add it to your cells’ dish
• Cells will take up DNA and express the proteins (GFP-ZFP568, GFP-GALT, or GFP-alone) within 24hrs

• Why do we transfect the GFP-alone construct?
Cell Staining

• Phalloidin (red)
  – Marks actin filaments, concentrated beneath cell membrane to keep cell shape
  – Actin is part of cytoskeleton

• DAPI (blue)
  – Marks the nuclei of cells

• GFP (green)
  – Tagged to ZFP568 and GALT

• Can take pictures of each using fluorescence microscope and then merge using Photoshop

• Why do we stain with DAPI and Phalloidin?
• Why don’t we have to stain to see ZFP568 or GALT?

Final Questions

• Where is GFP-GALT and GFP-ZFP568 located within the cell? Why?
• What does GFP-alone look like? Why?
• Is protein location different in HEK293T or NIH3T3 cells?
• Why are cells useful for scientists?