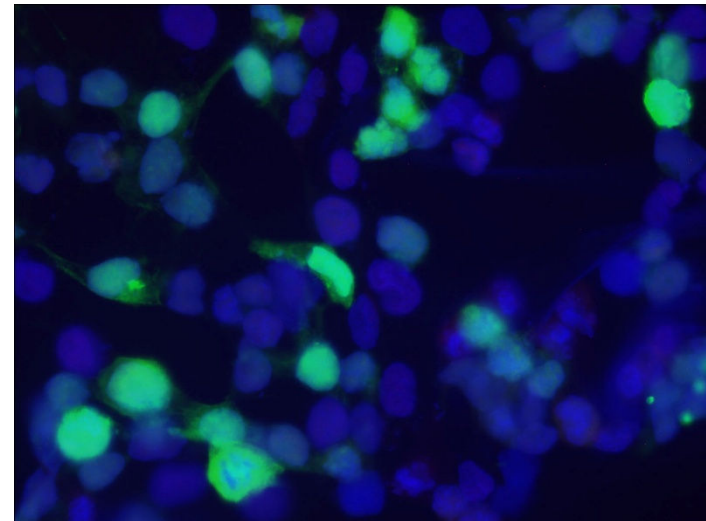


Project: Protein Localization in Mammalian Cells

- 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

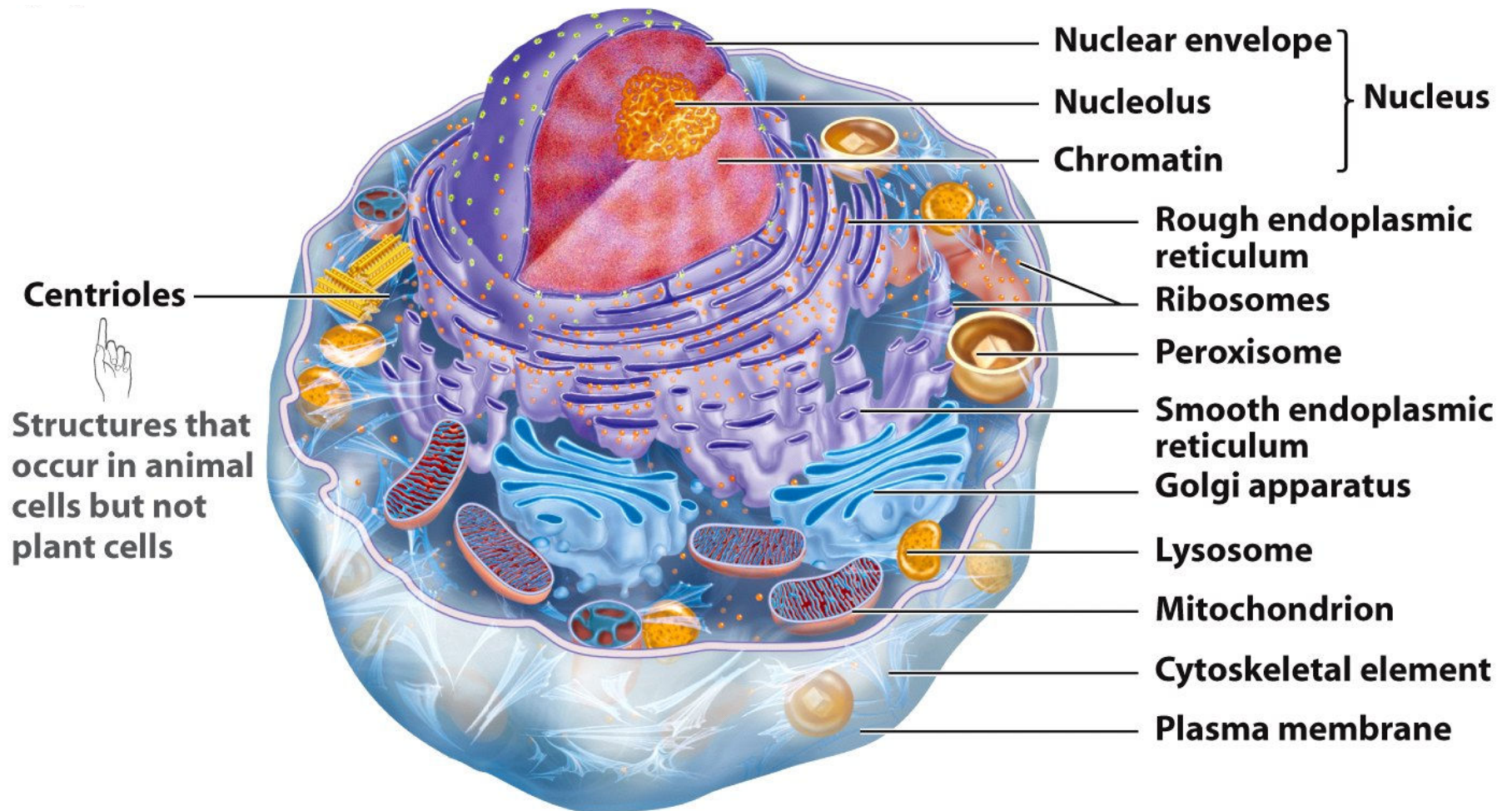


Figure 7-6a Biological Science, 2/e

Diseases associated with defects in protein transport

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

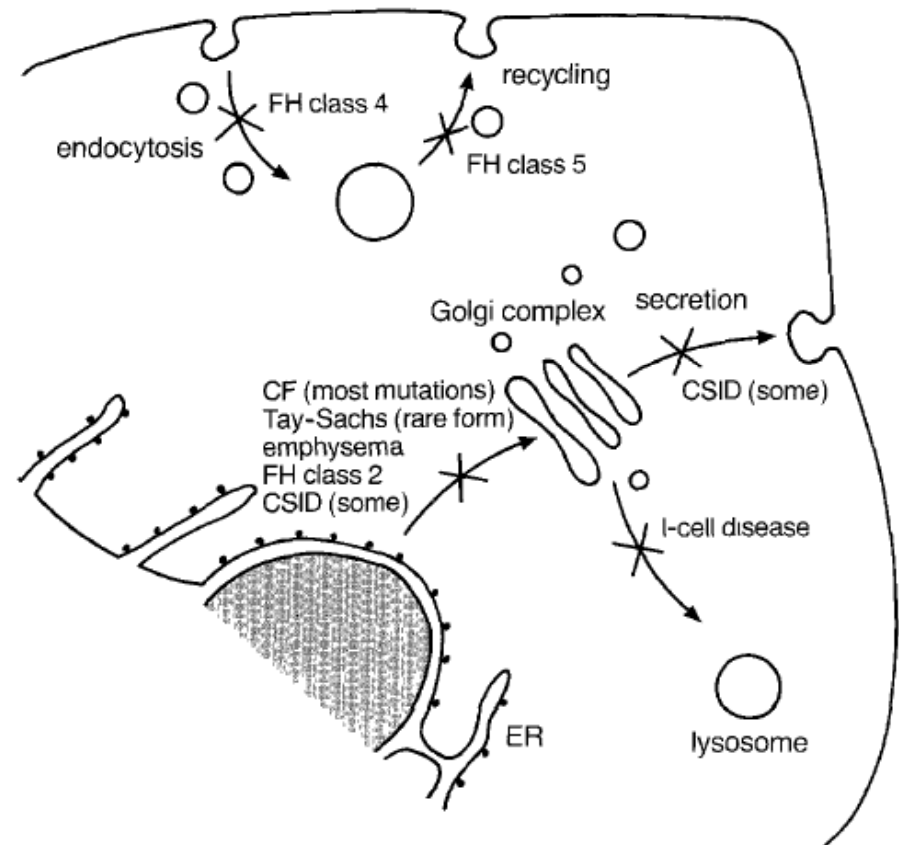


FIGURE 1

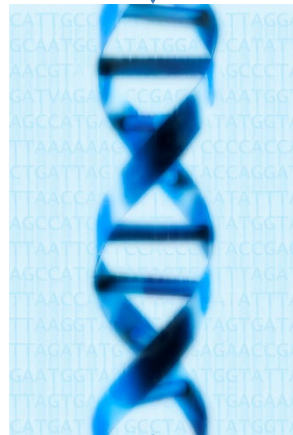
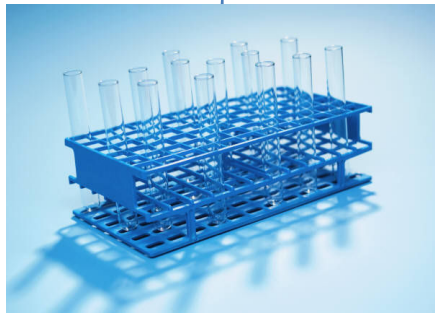
Protein trafficking events disrupted in some genetic diseases. CF, cystic fibrosis; FH, familial hypercholesterolaemia; CSID, congenital sucrase-isomaltase deficiency.

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)



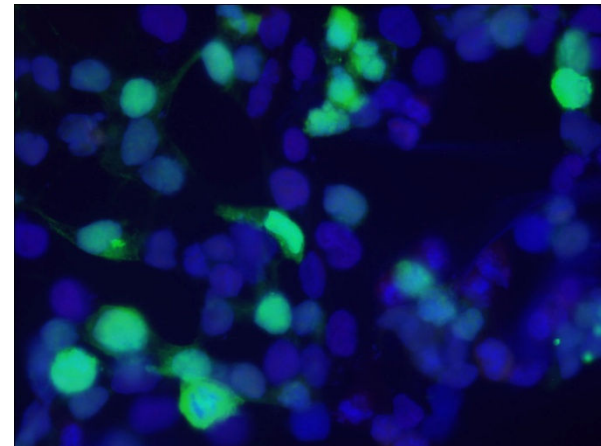
Stain & look at our cells to see
where the protein is (Thurs/Fri)



Transfect to put our DNA in
mammalian cells (Wed)

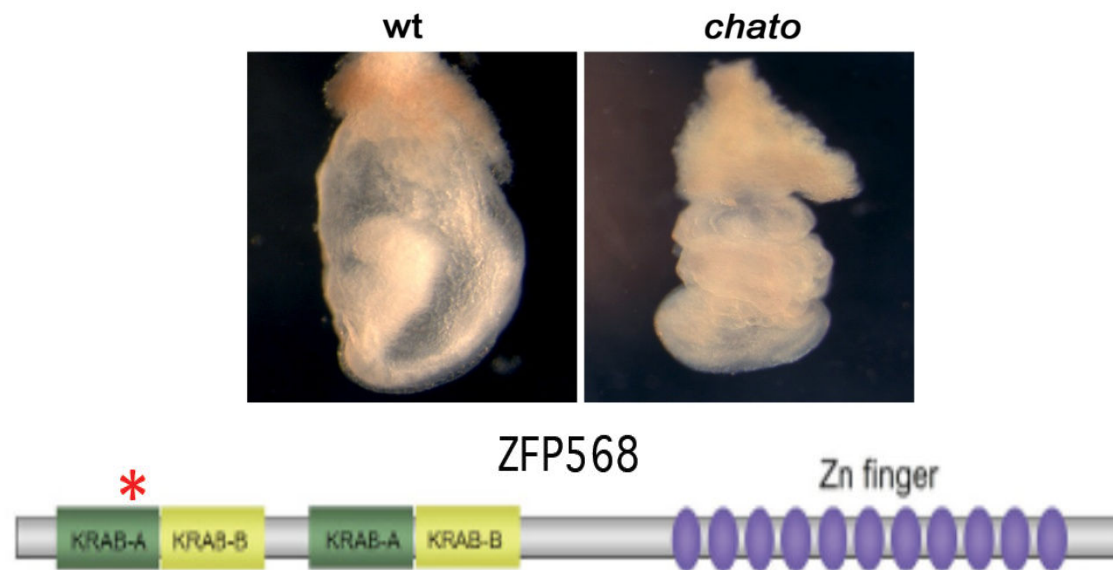
The Two Cell Types We'll Use

- 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

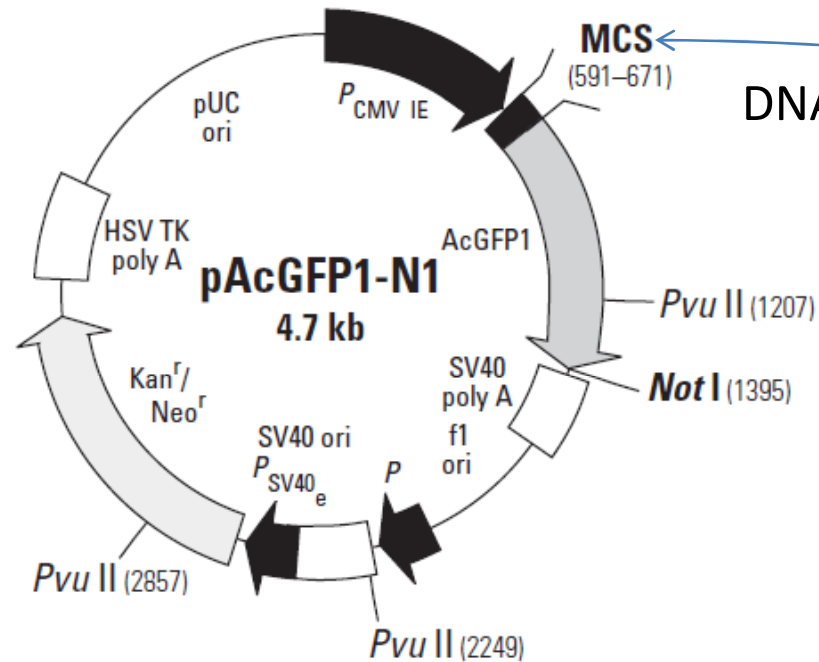
- GFP-ZFP568 (Zinc finger protein 568)
 - Binds to DNA and recruits transcriptional repressor TRIM28
 - Mutation (“chato”) causes embryonic arrest



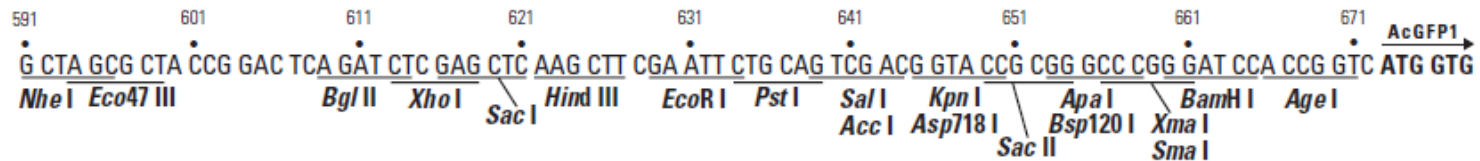
The Second Protein We'll Look At

- 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?

5'...CTCGAG...3'
3'...GAGCTC...5'

XhoI

5'...AAGCTT...3'
3'...TTCGAA...5'

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

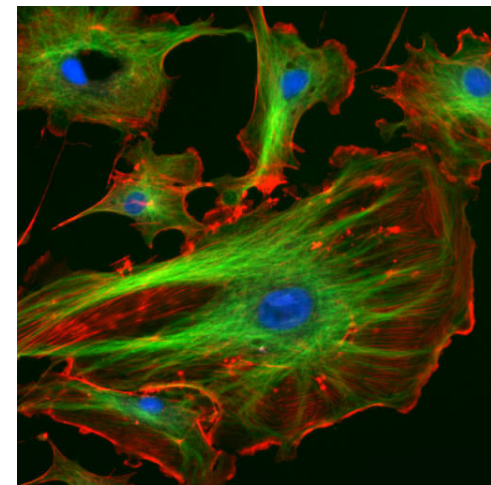
- Purified DNA can be transfected into HEK293T and NIH3T3 cells using Lipofectamine 2000
- Mix DNA in media with the Lipofectamine reagent and then add it to your cells' dish
- Cells will take up DNA and express the proteins (GFP-ZFP568, GFP-GALT, or GFP-alone) within 24hrs



- 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?