Observing Trafficking Behaviors in Mutated Cystic Fibrosis Transmembrane Conductance Regulator Protein (CFTR)

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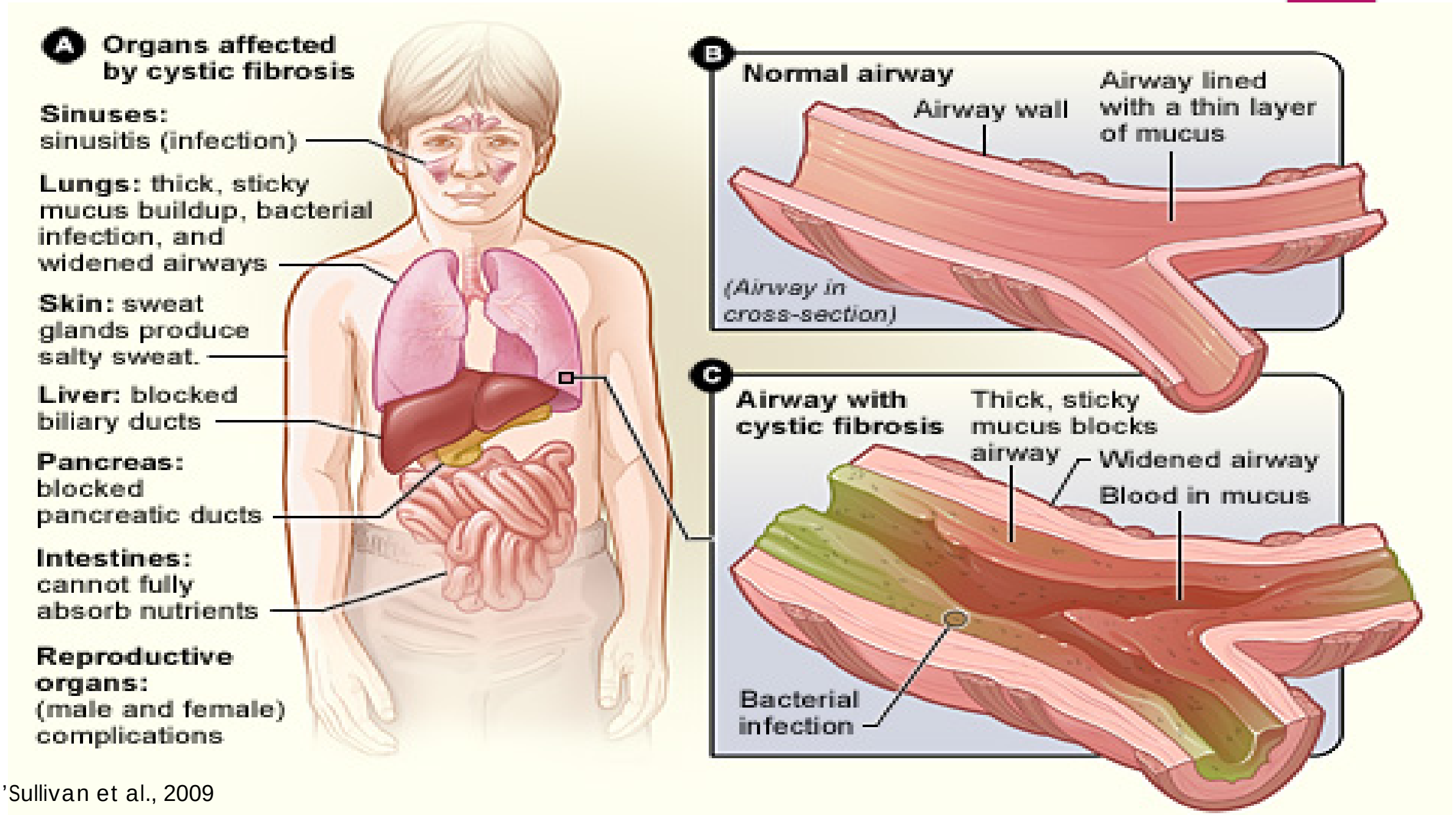
Introduction

CYSTIC FIBROSIS
AND THE
CFTR

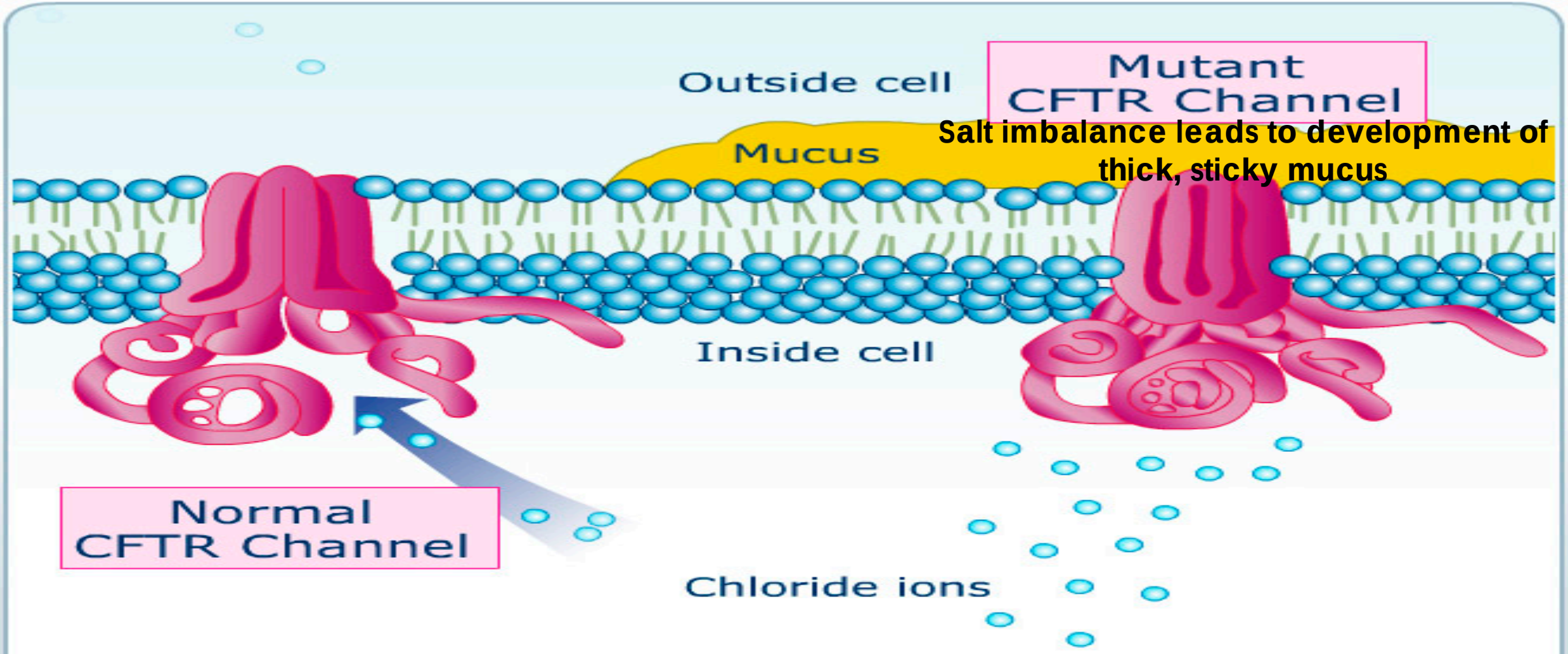
Cystic Fibrosis (CF)

- Genetic autosomal recessive disease
- Results in CF from mutations in the Cystic Fibrosis Transmembrane Conductance Regulator Gene
- At least 2,000 mutations that result in CF
- About 1,000 patients are diagnosed every year
- Most prevalent lethal disease in Caucasians (1/3,200 births)
- Affects respiratory, digestive and reproductive systems
- Symptoms include chronic lung infections and deterioration, bronchiectasis, nutritional deficiencies, and possible sterility

Hinzpeter et al., 2014; Cant et al., 2014; O'Sullivan et al., 2009



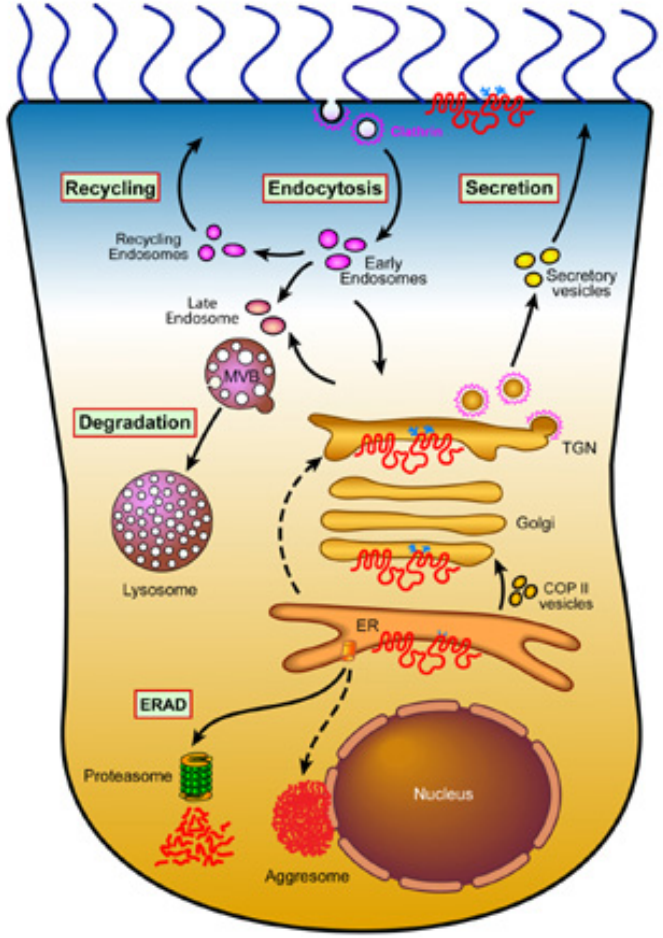
O'Sullivan et al., 2009



A normal-functioning CFTR channel moves chloride ions to the outside of the cell while a mutant CFTR channel does not, causing sticky mucus to build up on the outside of the cell.

Clancy et al., 2013

CFTR Trafficking

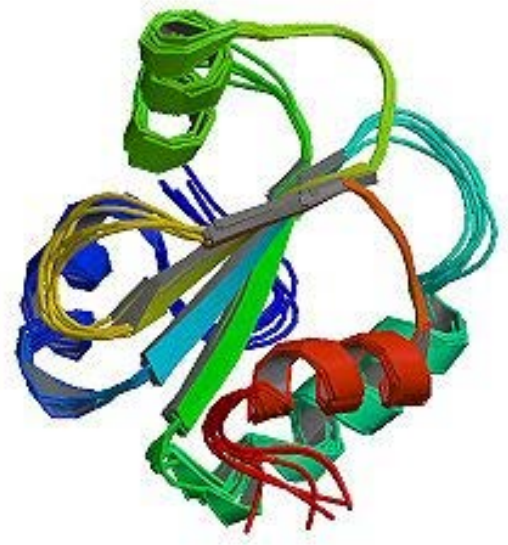


- Trafficking: movement from ribosome in endoplasmic reticulum at protein synthesis site to the apical membrane of the epithelial cells
- Cystic Fibrosis resulting mutations occur when the trafficking process is not completed or not started at all
- Molecular chaperone: protein that helps other proteins fold or traffick

Rich et al., 1990; Rubenstein et al., 2013

ERp29: CFTR Molecular Chaperone

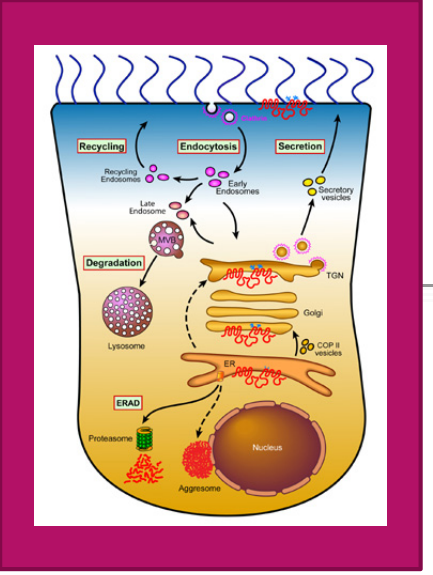
- ER luminal protein of 29 kDa
- Prominent in brain and lungs; ubiquitously expressed
- Thought by Das to play a role in CFTR trafficking
- Previous Suaud studies suggest:
  - 4- phenylbutyrate increased ERp29 mRNA and protein expression
  - Overexpression of ERp29 in Xenopus oocytes and mammalian cells increased functional and surface expression of wild type and mutation F508-CFTR

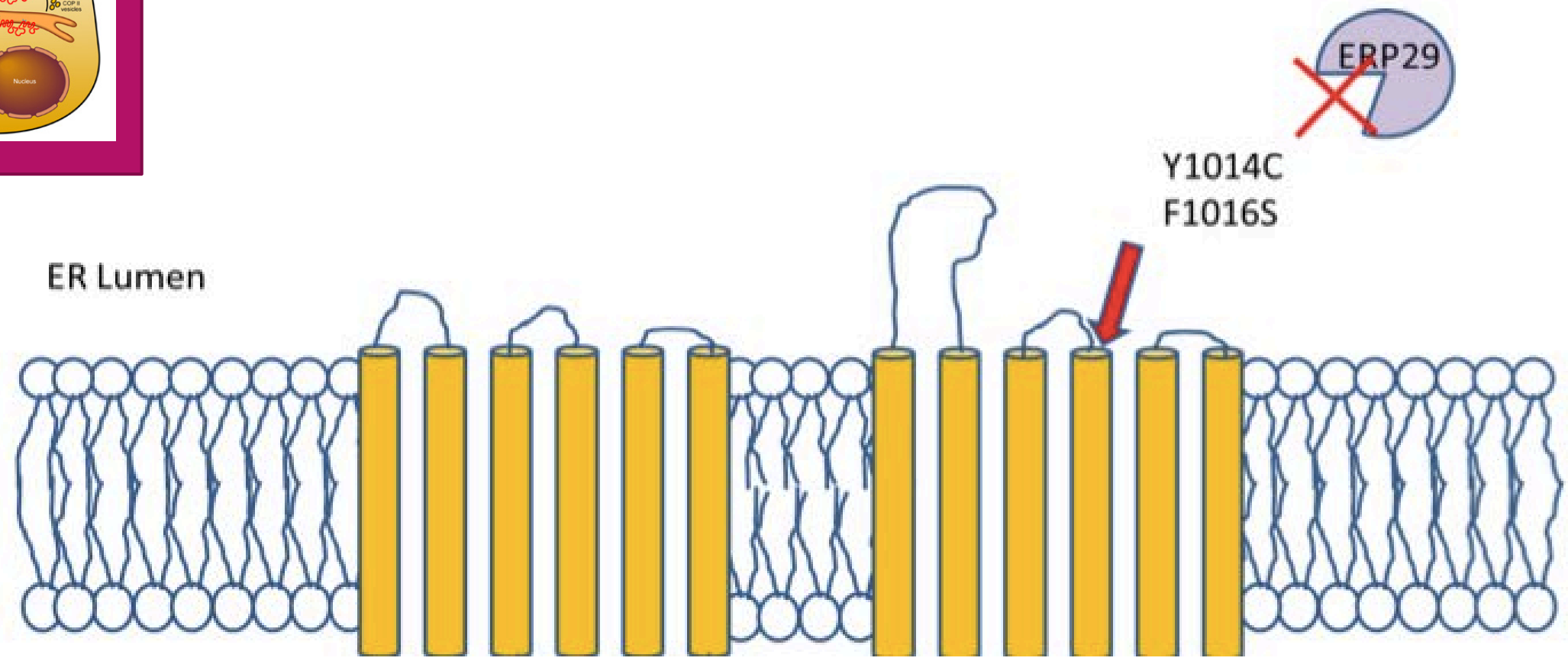


Baryshev et al., 2006; Das et al., 2009; Suaud et al., 2011

Research Question and Hypothesis

- ERp29 is suggested to interact with either –(F,Y)-(F,Y)- or –(F,Y)-X-(F,Y)- motifs
- ERp29 remains outside the ER in the cell
- CFTR has one consistent motif (1014Y-I-F1016), where mutations Y1014C, F1016S and F1016C occur
- **Research Question**: Where do mutations Y1014C, F1016S and F1016C stop trafficking after protein synthesis?
- **Hypothesis 1**: These mutations stop trafficking in between the nucleus and the ER, immediately after protein synthesis.
- **Hypothesis 0**: These mutations stop trafficking between the Golgi and the epithelial apical membrane.


Recycling
Endocytosis
Secretion
Recycling Endosomes
Early Endosomes
Late Endosome
Secretory vesicles
MVB
Degradation
TGN
Golgi
Lysosome
COP II vesicles
ER
ERAD
Proteasome
Nucleus
Aggresome


ERP29
Y1014C
F1016S
ER Lumen

Methods

1. CELL CULTURE

Obtain DNA

2. PCR

Amplify, transform, and sequence

3. MINIPREP

Further amplification

4. BRADFORD ASSAY

Determine proper protein concentrations for immunoblot samples

5. IMMUNOBLOT

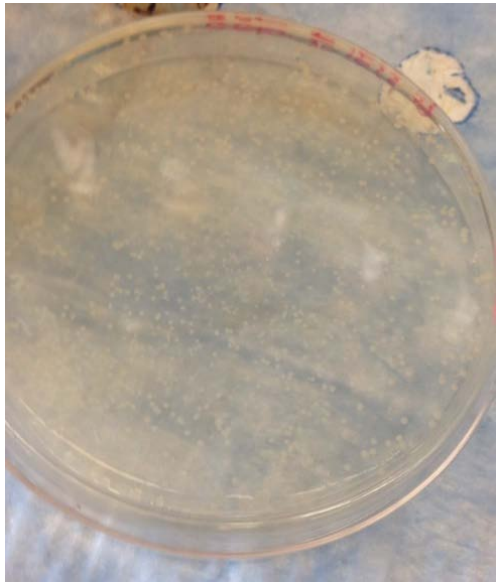
Determine weight of protein, indicating level of processing

6. IMMUNOHISTOCHEMISTRY

Visualize localization of CFTR within the cell

Cell Culture and DNA Amplification

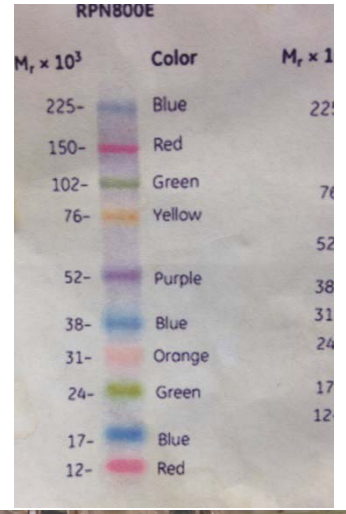
- Cell culture: Immortalized CFBE41o-CF bronchiolar epithelial cells with parental, and CFTR F508 lines were grown in Eagle's minimal essential medium
- PCR: Amplify available DNA
- Products of PCR were then transformed and isolated via Miniprep
- Product of Miniprep was then sequenced to ensure correct mutation or control



Rubenstein et al., 2013

Immunoblot

- CFBE parental cells were mock transfected or transfected with WT, F508del, Y1014C, F1016S and F1016C CFTR
- Proteins were lysed and Bradford assay was performed to determine proper concentrations for immunoblot samples
- Acrylamide gels were created with samples in wells and ran at 100v
- Primary antibody: CFTR mouse
- Secondary antibody: goat anti-mouse
- Gels were transferred onto membranes and washed in PBST
- Membranes were transferred onto film



Rubenstein et al., 2013

Immunohistochemistry

- Visualizes localization of CFTR in cells
- Cells were mock transfected or transfected with WT, F508del, Y1014C, F1016C and F1016S
- Cells were treated with antibodies to view landmarks under microscope
  - DAPI: nucleus stain
  - FITC: anti-mouse secondary antibody to CFTR
  - TRITC: anti-rabbit secondary antibody that binds to rabbit antibody that binds to ER protein, calnexin

Rubenstein et al., 2013

Results

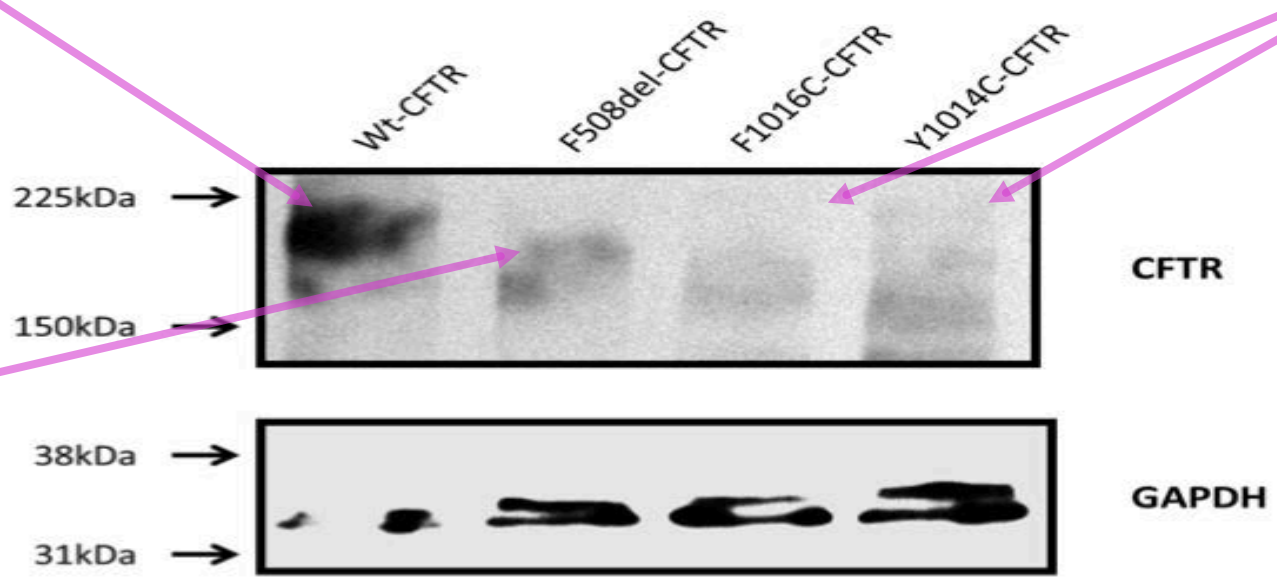
1. IMMUNOBLOT
2. IMMUNOHISTOCHEMISTRY

Immunoblot

Full expression

Bent shape indicates partial processing

Rubenstein et al., 2013

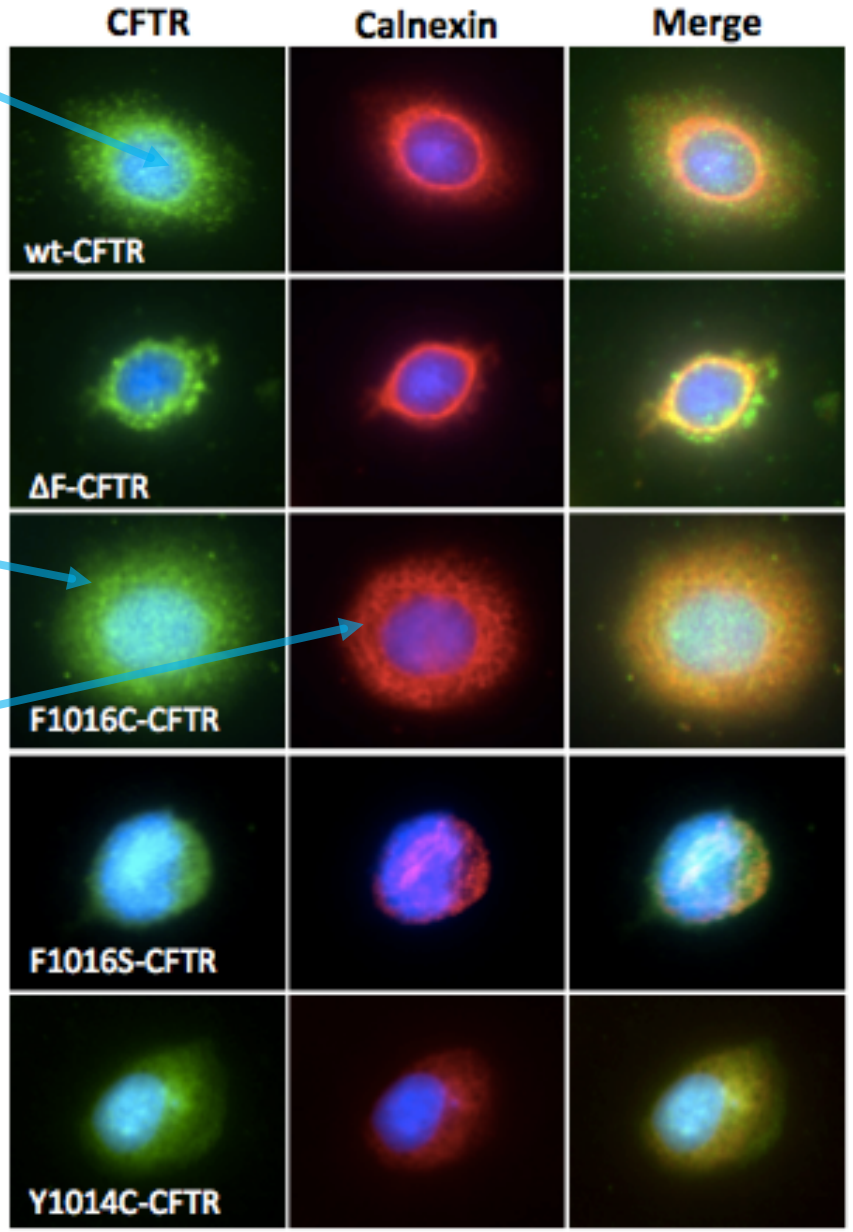


Light expression and weight< 150 kDa indicates little to no processing

DAPI stain= blue
Nucleus

FITC stain= green
CFTR

TRITC stain= red
ER



Proper distribution: CFTR travels beyond processing organelles to membrane

CFTR trafficks just beyond the ER but does not make it to the membrane

Rubenstein et al., 2013

CFTR is centralized outside the nucleus and in the ER

Discussion

WHY DOES THE CFTR STOP TRAFFICKING?

WHY ISN'T THE ERP29 INTERACTING WITH MUTANT CFTR?

HOW MIGHT THIS BE RESOLVED?

Why do the CFTR stop trafficking?

- Results confirm that mutations Y1014C, F1016S and F1016C stop trafficking before traveling through the ER and are therefore not fully processed; **confirms hypothesis** and is a **novel finding**

- ERp29 is in the same location as the proteins stop trafficking

- If ERp29 is helping the CFTR to traffick in wild type forms, we hypothesize that the stopping of trafficking in these mutations is due to the lack of ERp29 interaction.

Why isn't the ERp29 interacting with mutant CFTR?

- We hypothesize that there are some physical boundaries on the surface of the CFTR that do not allow ERp29 interaction, causing the discontinuation of the trafficking mechanism and cystic fibrosis.
- Therapy: drug that could manipulate CFTR to interact properly with ERp29 in order to traffick

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Major Findings

- CFTR mutants Y1014C, F1016S and F1016C stop trafficking between the nucleus and the ER where ERp29 is localized
- ERp29 interaction may be a deciding factor in success of CFTR trafficking and presence of cystic fibrosis
- CFTR mutants present boundaries that block ERp29 interaction and cause CF