

# Cystic Fibrosis

- lethal autosomal recessive disease
- incidence: 1 in 2000-3000; predominantly Caucasian populations  
(carrier frequency 1 in 22-28)
- disease gene CFTR (cystic fibrosis transmembrane conductance regulator) is a regulated epithelial Cl<sup>-</sup> channel; influences other ion channels

“Woe to the child which when kissed on the forehead tastes salty. He is bewitched and soon will die” - old proverb

## Clinical Features of CF

### **affects epithelia in multiple organs**

- chronic lung infections and inflammatory destruction of lungs
  - nutritional abnormalities due to gastrointestinal obstruction and lack of fluid secretion by intestinal cells
  - pancreatic insufficiency
  - males infertile due to congenital malformation of vas deferens
  - excessive salt loss from the sweat glands
  - ion imbalance, dehydration, cardiac arrhythmias
- thick, viscous mucus*

### Relationship of clinical features to residual CFTR function

% normal CFTR function	clinical features
< 1	pancreatic insufficiency and below
< 4.5	pulmonary infection and below
< 5	positive sweat test and symptoms below
< 10	congenital absence of vas deferens
10-49	none
50-100	none

- 34% of patients reach adulthood
- 10% live past age of 30

ave. life expectancy - male: 31  
female: 28

### Lung Physiology

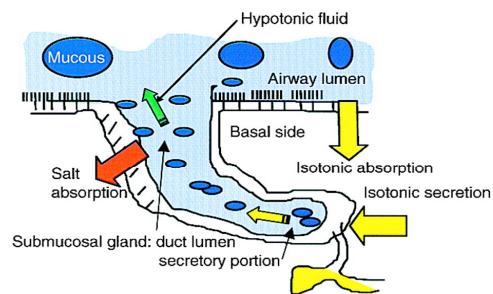
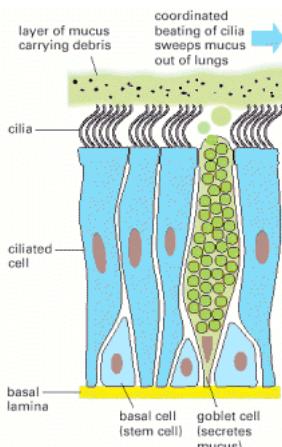
- the lung and other airways (nasal, tracheal) contain several different cell types (all play a role in innate immunity against pathogens)

*Epithelia:* polarized cells that form the lining of the airway tissues; they contain cilia that beat in a single, coordinated direction

*Goblet cells:* mucus producing cells; principal component in mucus is mucins, highly glycosylated and negatively charged molecules that help trap pathogens

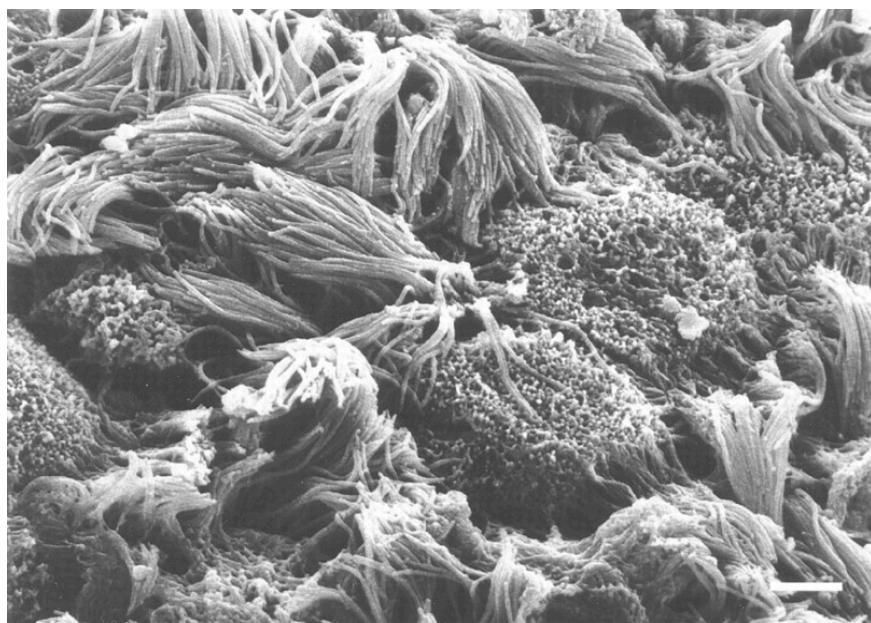
*Submucosal glands:* contains both mucus (mucoid) and fluid (serous)-producing cells and ducts that mix and carry the two components to the surface epithelial layer

### *Overview of the organization of lung cells and their functions*



\* the epithelial lining of tissues in the digestive system share a similar physiology

### **Electron Micrograph of Lung Epithelial Surface**



## Lung Defenses

human airway lining contains two, distinct aqueous layers:

1. mucus layer - traps inhaled bacteria and foreign particles
2. airway surface liquid (ASL) - provides microenvironment for beating cilia to clear mucus layer with assistance of coughing

*(ASL: rich broth of proteases/antiproteases, antibiotics, antibodies, and oxidant/antioxidants backed by cellular immune mechanisms)*

Defensins: small peptide molecules (12-50 amino acids, contain positively charged and hydrophobic residues) that kill microbes in a salt-sensitive manner (mechanism unclear; might involve membrane disruption or neutralization of cytosolic factors in pathogens)

Non-specific action of defensins makes it difficult for microbes to acquire resistance

***high salt normally inactivates defensins***

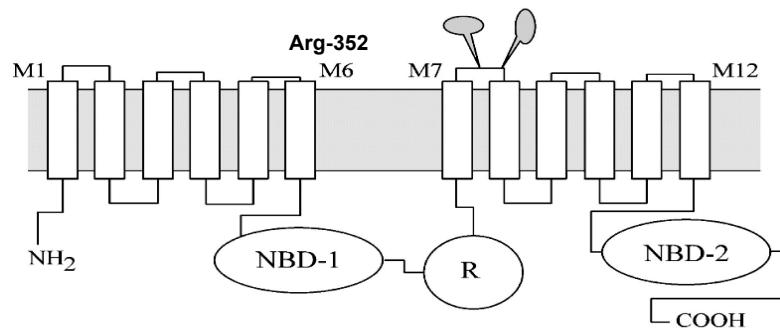
## CF lung disease

- lungs are plagued by persistent bacterial infections

main culprits: *P. aeruginosa*, *B. cepacia*, *S. aureus*

- colonization occurs early and is nearly impossible to eradicate; lung tissue eventually destroyed by onslaught of immune cells (i.e. neutrophils) that respond to the infections

## Transmembrane Topology of CFTR and Charge Selectivity



positive charge of Arg = electrostatic barrier

Cl<sup>-</sup> to Na<sup>+</sup> permeability ratio = 150 (without Arg352 = 15)

## Domains in CFTR protein

- membrane-spanning domains (MSD) - two set of six membrane-spanning segments that anchor protein to the plasma membrane and form the ion channel (represent 19% of the total protein)
- nucleotide-binding domains (NBD-1, NBD-2) - responsible for binding and hydrolyzing ATP, control ion channel gating (opening and closing)
- regulatory domain (R) - responsible for activation of CFTR

## Classes of CFTR mutations

Class I - premature termination codons (truncated proteins) and splicing abnormalities (unstable mRNAs) leads to severe reduction in CFTR production; roughly 5% of total mutations fall into Class I

Class II - these mutations including the common DF508, deletion of a Phe codon, results in CFTRs that are not folded properly and are degraded in the endoplasmic reticulum; represent large majority of total mutations (75%)

Class III - mutations in the nucleotide-binding regions and regulatory domain lead to defective regulation and gating (protein reaches surface of cell but channel remains closed); severe disease seen in patients with these mutations

Class IV - mutations in the membrane-spanning regions result in CFTR molecules that are correctly processed but exhibit altered channel functions (unable to move chloride ions through efficiently); result in less severe disease with no pancreatic dysfunction

*Only 50% of the newly-synthesized CFTR is correctly folded and trafficked to the cell surface (large size, etc.)*

nature of mutations in CFTR correlate well with severity of pancreatic disease and degree of sweat Cl- abnormality  
(genotype <--> phenotype)

relationship between genotype and pulmonary phenotype less robust

- genetic modifiers (stronger immunity, etc)
- environmental factors

## Why is there such a high frequency of CFTR heterozygotes?

genetic defects leading to reduced CFTR protein expression or chloride transport capacity lead to cystic fibrosis

*conversely...*

overstimulation of CFTR in intestinal epithelial cells by bacterial toxins leads to secretory diarrhea (having less CFTR may provide a selective advantage)

- toxins (such as cholera) activate the protein kinases responsible for “priming” the CFTR channel

- much larger world health problem: 3 million deaths per year of children under the age of 5

## Function of ion channels in epithelium

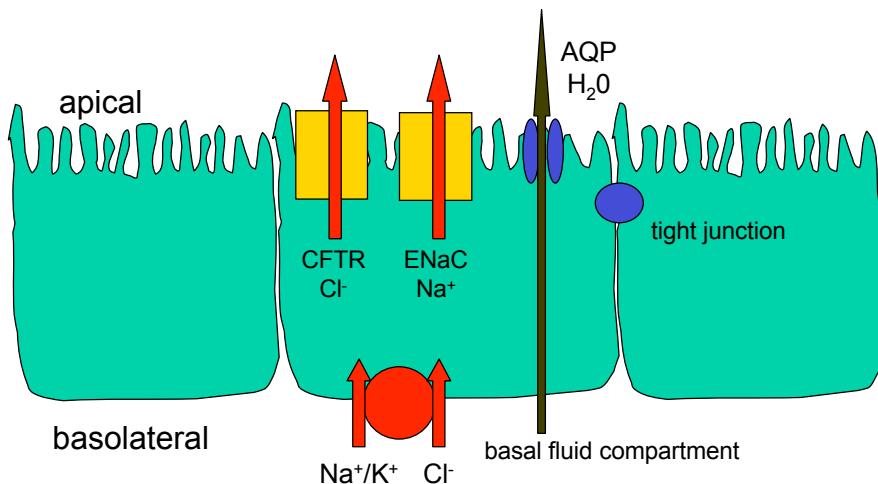
- besides the general function of regulating osmolarity, ion channels such as CFTR provide more specific functions

1) by pumping Cl<sup>-</sup> ions out of the cell, CFTR can regulate water secretion in some epithelial cells (pancreas and intestine)

2) by pumping Cl<sup>-</sup> ions into the cell, CFTR can regulate absorption of ions in excess of water, thereby creating hypotonic water outside the cell (lung)

CFTR is the main chloride channel in epithelia of various tissues

- ENaC - epithelial sodium channel
- CFTR - chloride channel
- AQP - aquaporin (water channel)



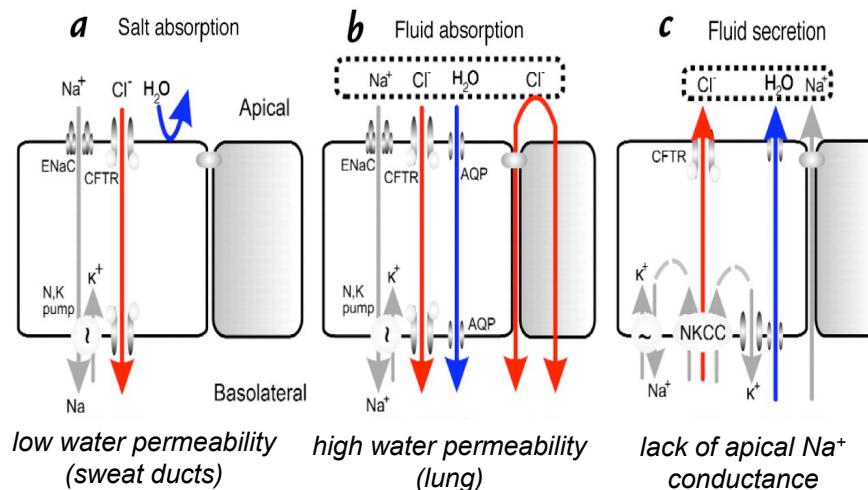
CFTR is the main chloride channel in epithelia of various tissues

*epithelia perform diverse functions:*

- 1) water or volume-absorbing (airways and intestinal tract)
- 2) salt-absorbing (sweat duct, lung)
- 3) water or volume-secretory (pancreas, lung)

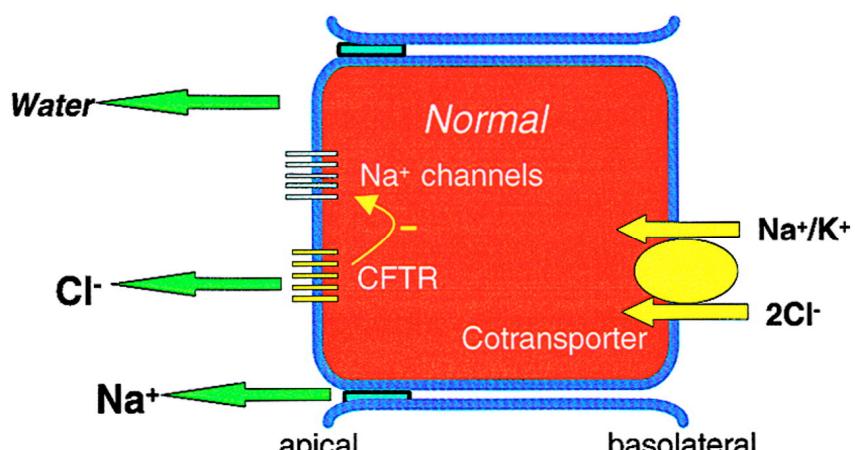
all processes involve chloride ion transport; disruption of this transport in cystic fibrosis leads to multiple effects

### CFTR's multiple roles in fluid and electrolyte transport



### Secretion of NaCl and water in normal epithelial cells

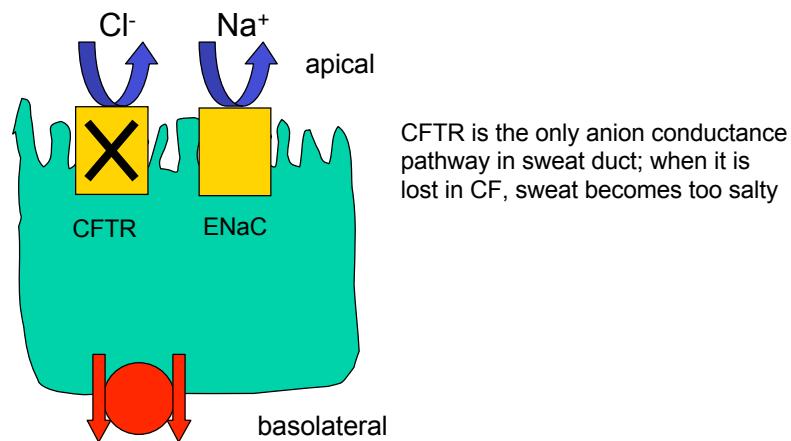
- occurs in pancreas, intestine, and submucosal glands of airways



water “follows the salt” by osmosis

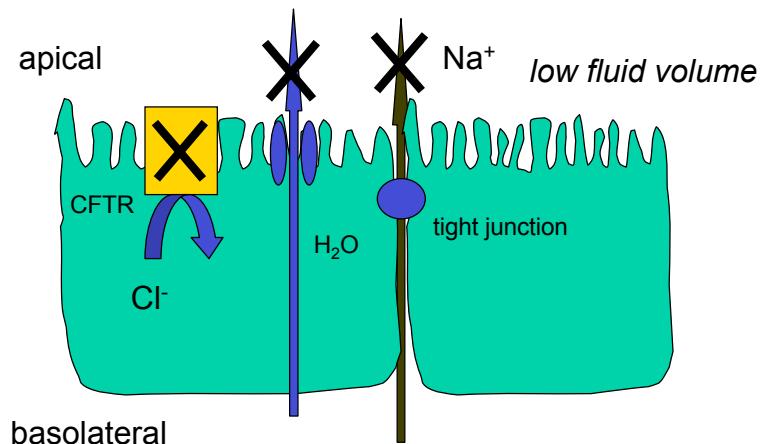
### Organ-specific pathophysiology of CF: sweat ducts

- salt absorption problem - high conductance for  $\text{Na}^+$  and  $\text{Cl}^-$  and relatively low water permeability allows for hypertonic absorption (more salt than water)



### Organ-specific pathophysiology of CF: pancreas, intestine, submucosal gland

- fluid or volume secretion problem - secreting epithelia lack significant  $\text{Na}^+$  conductance; when  $\text{Cl}^-$  transport is blocked in CF,  $\text{Na}^+$  (via passive flow through the tight junctions) and water (following the salt transcellularly) is also eliminated.



### Why is this a problem in the pancreas?

- one of the main function of pancreas is the secretion of digestive enzymes

low fluid secretion leads to formation of thick mucus which prevents enzymes from reaching the intestines:

1) enzymes are retained in the pancreas and eventually destroy all pancreatic tissue

2) lack of digestive enzymes leads to poor nutrient absorption, weight loss, etc.

### Organ-specific pathophysiology of CF: lungs

- although many theories exist to explain the lung disease of CF, it is generally accepted that improper clearance of mucus and persistent bacterial infection are central to the disease process

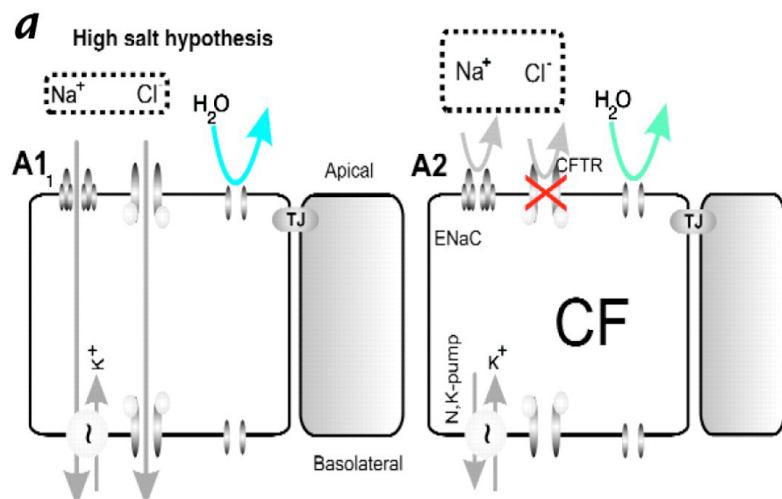
*What are the molecular mechanisms whereby loss of CFTR lead to lung disease?*

### Welsh and colleagues: experimental observations

- grew airway epithelial cells from normal and CF subjects on filters, bacteria were placed on apical surface of cells
  - \* bacteria flourished on CF cultures but were killed on normal cultures; bacteria placed on basolateral surface killed both
  - \* if pure water was added to apical side of CF cultures, they were able to kill the bacteria (evidence of a salt-sensitive factor, defensins)

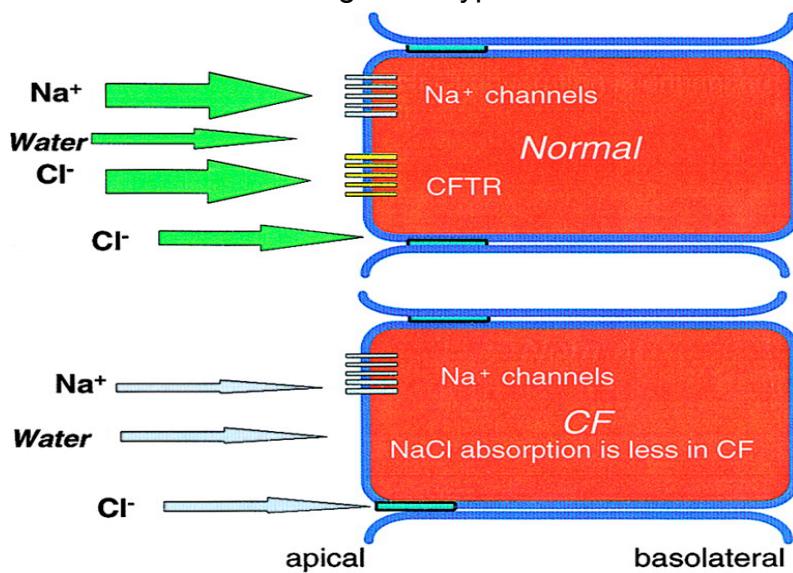
*concluded that both normal and CF airway cells release antibiotics into the ASL but high salt of ASL in CF patients renders them ineffective*

### Welsh and colleagues



ASL volume not changed, higher salt concentration than normal cells

mechanism behind the “high salt hypothesis”



key features: no parallel pathway for Cl<sup>-</sup>, no ENaC inhibition by CFTR

Boucher and colleagues: experimental observations

- grew airway epithelial cells from normal and CF subjects on filters and measured volume and salt

\* found no differences in salt concentration or osmolarity of surface liquid in normal and CF cell layers

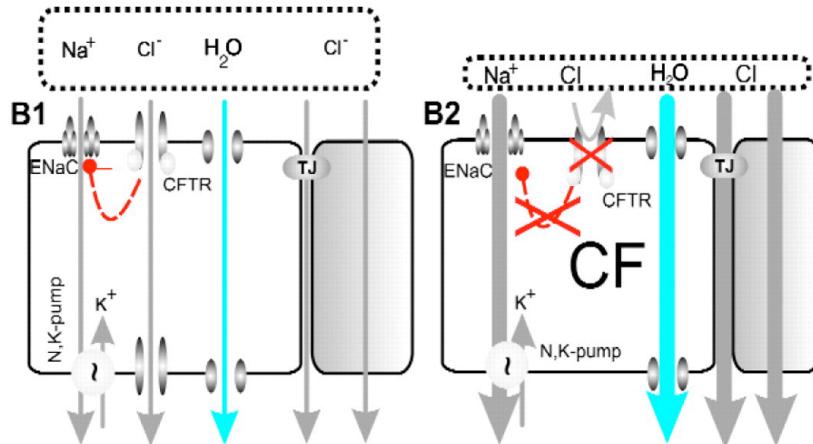
\* found that the liquid layer was reduced from a height of 18μm to 6μm and mucus transport was impaired; effects were reversible

*findings fit with their evidence for Na<sup>+</sup> hyperabsorption in CF epithelia and the long-standing “thick mucus” hypothesis to explain CF lung disease*

Boucher and colleagues

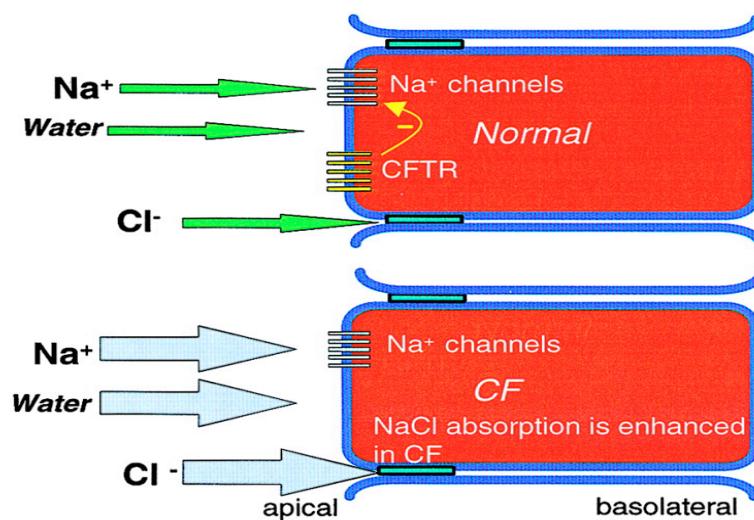
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Low volume hypothesis



ASL volume decreased compared to normal cells, salt concentration unchanged

mechanism behind the “low volume hypothesis”



key features: parallel pathway for Cl<sup>-</sup>, ENaC inhibition by CFTR

## Two sides of the salt controversy...

- high salt hypothesis: CFTR fails to absorb Cl<sup>-</sup> (and Na<sup>+</sup>) in excess of water leading to high salt ASL (volume not changed)

**(high salt inhibits defensin activity)**

- low volume hypothesis: CFTR fails to inhibit ENaC from absorbing Na<sup>+</sup>; water and Cl<sup>-</sup> (via tight junctions) follow leading to a low volume ASL

**(low volume prevents ciliary clearance of mucus)**

## Treatments - pulmonary disease

- antibiotics (tobramycin) - used to control Psuedomonas infections; these can be aerosolized for convenient administration
- smooth muscle relaxants (albuterol) - opens airways for easier mucus clearance; mucus clearance by physical means done regularly to prevent colonization of pathogenic bacteria
- anti-inflammatories (ibuprofen) - used to reduce pulmonary inflammation caused by immune response in lungs
- DNase - DNA from dead cells and bacteria contribute to thickness of mucus; DNA cleaving enzyme helps to thin mucus

## Treatments specific for different class of CF mutations

Class I (no protein synthesis) - gentamicin: an aminoglycoside antibiotic, suppresses premature termination of CFTR mRNA when mutations creating early stop codons are present; allows some “readthrough” and, therefore, full length protein to be made

Class II (folding and trafficking problems) - deoxyspergualin: competes with chaperone molecule Hsp70 for binding to mutant CFTR; prolonged association of mutant CFTR with Hsp70 leads to degradation, deoxyspergualin allows some mutant protein to get to the cell surface  
(the common DF508 mutant has normal chloride channel function)

Class III (activation defects) - CPX: adenosine receptor antagonist that binds and specifically activates CFTR chloride channel; also improves trafficking

Curcumin decreases calcium levels in the ER sparing delta508 CFTR from proteasomal degradation

