UNIVERSITÀ DEGLI STUDI DI MILANO
FACOLTÀ DI MEDICINA E CHIRURGIA

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PBL: STUDY CASE on CF

Case

Mr. and Mrs. Gottleib took their nine-month-old infant, Jeremy, to the emergency room because he had been suffering from a cough and diarrhea for almost a week. They told the doctor that Jeremy would sometimes "*wheeze*" a lot more than they thought was normal for a child with "*just a cold*" which is what their pediatrician said the problem was last week.

Upon arriving at the emergency room, the attending pediatrician noted that salt crystals were present on Jeremy's skin, and chest auscultation revealed abnormal sounds. The attending pediatrician ordered a chest x-ray and asked Jeremy's parents to sit with him in his office to discuss the situation. The pediatrician told the Gottleibs that Jeremy was small for his age, something he called "failure to thrive". He also mentioned the salt crystals on the baby's skin, at which time Mr. Gottleib said "*Jeremy has always had salty skin, I call him my 'Little Pretzel Stick' because his skin tastes salty when I kiss him*." He said Jeremy's symptoms all point toward a specific disease, and a sweat test would be needed to confirm his suspicions.

To do a sweat test, a small patch of skin on the child's forearm is first cleaned. A gauze pad saturated with pilocarpine, a chemical that makes the skin sweat, is then applied to the area. Electrodes are hooked up, and a mild electric current is turned on for five minutes, which drives the pilocarpine into the skin. A sweaty area appears on the skin where the gauze had been placed, and a piece of dry filter paper is taped over it to absorb the sweat for about a half hour. A technician then measures the concentration of chloride in the pad.

The following day - "*I'll never forget that day*" - Jeremy's mother says, the pediatrician called with the results of the sweat test. The doctor told Mrs. Gottleib that Jeremy's chloride level was much higher than normal. This meant that the test was positive and suggests that Jeremy could be affected by cystic fibrosis.

Learning Objectives

1. Learn to work in small groups effectively to solve a clinical problem from the bench side.

2. Describe the how cystic fibrosis could be diagnosed.

3. Describe the normal versus pathologic transport mechanisms of the CFTR channel.

4. Discuss the effect of an obstructive lung disorder on pulmonary mechanics.

5. Describe how an SDS-PAGE gel works.

6. Describe how to select a possible personalized therapy based on the use of the CFTR modulators

Learning Objectives

2. Describe the how cystic fibrosis could be diagnosed.

5. Describe how an SDS-PAGE gel works.

Learning Objective 2

Next Generation
Sequencing machines
(NGS)

VS

CFTR-dependent diseases

CFTR loss of function: Cystic Fibrosis

- **The most common life-limiting autosomal recessive disease among Caucasians, affecting approximately 1 every 2,500-4,000 newborns**
- **More than 2,000 mutations are found associated with CFTR gene so far and among these 200 have been proved to be disease-causing. 70% of patients carry the mutation F508del. On the basis of the effect on CFTR, the mutations are divided into 6 major classes**

-Class I: mutations preventing the production of a full-length CFTR protein

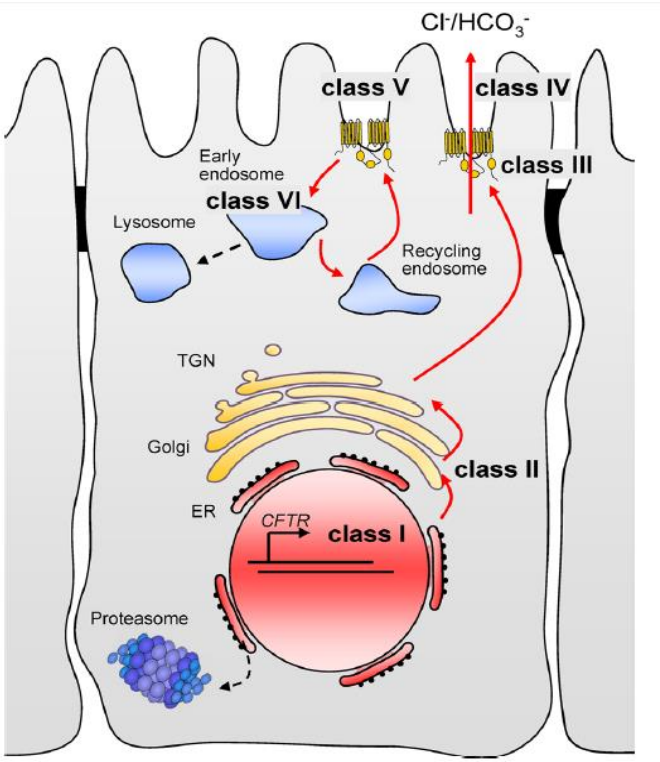
-Class II: mutations altering the cellular processing of the protein

-Class III: mutations disturbing the regulation of the Cl^- channel

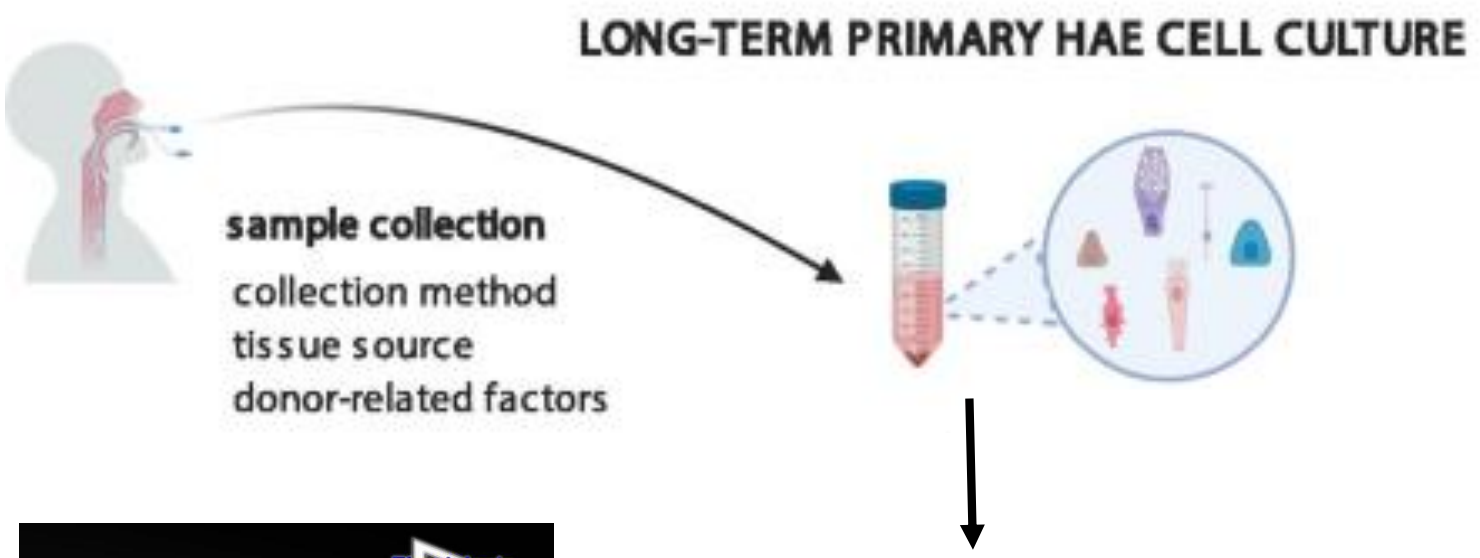
-Class IV: mutations altering the conduction of the Cl^- channel

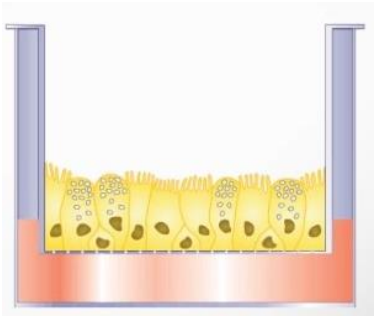
-Class V: mutations reducing the amount of functional CFTR protein

-Class VI: mutations destabilize the channel in post-ER compartments and/or at the PM



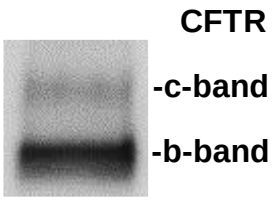
Learning Objective 2





Human primary nasal epithelial cells differentiated at the air liquid interface

Biochemical evaluation of the efficacy of the treatment in term of maturation of the mutated CFTR



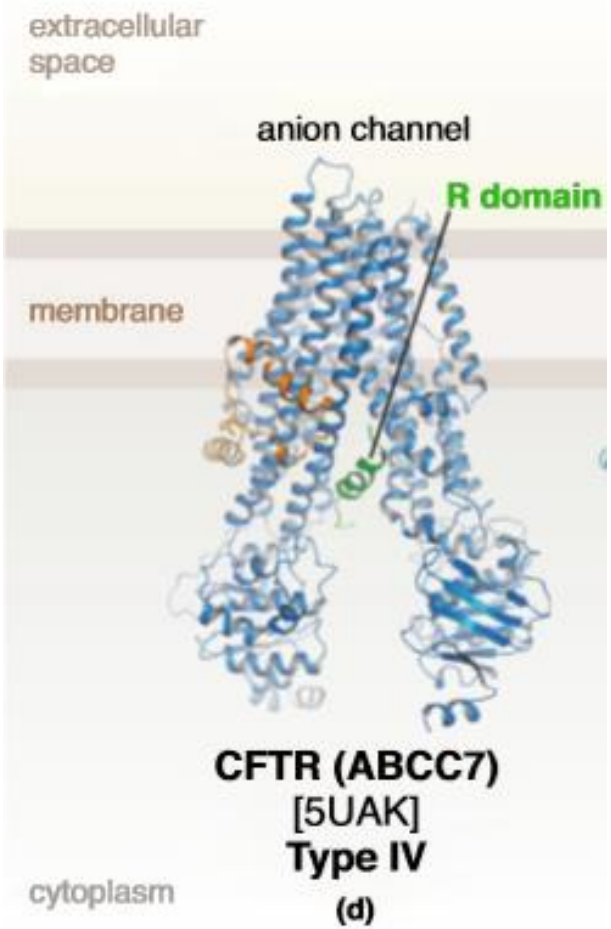
Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)

- CFTR is a CL^- and HCO_3^- channel expressed at the apical surface of many epithelia and codified by the gene CFTR located in the long arm of chromosome 7

- CFTR is a single polypeptide chain formed by 1480 aa and it is belonging to the ABC family C7

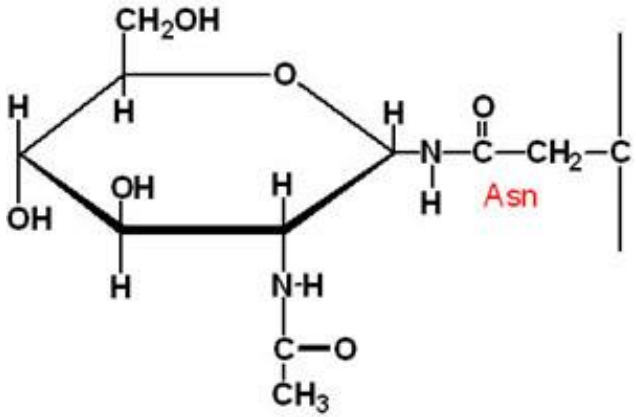
- CFTR synthesis starts in the ER but because of the complex structure of the protein the folding have a low yield, indeed only the 20% of the neobiosynthesized proteins reach the Golgi apparatus for the final maturation and trafficking to plasma membrane.

- The complexity of CFTR is also due to the important and massive post-translational modifications



N-glycoproteins

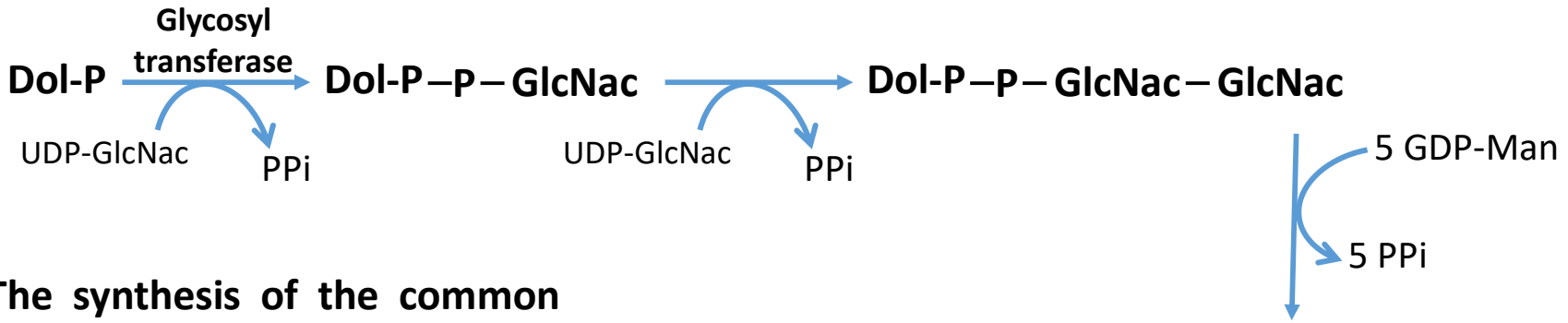
N-glycoproteins are proteins characterized by the presence of a polysaccaride chain, which is linked to an asparagine residues of the protein with an N-acetyl-glucosamine (N-GlcNac)



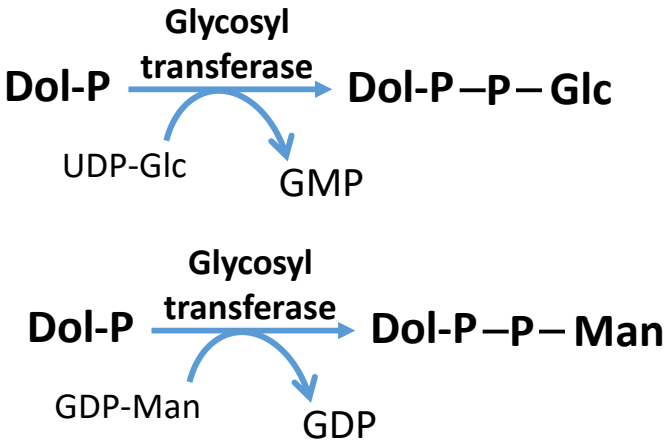
***N*-GlcNAc**

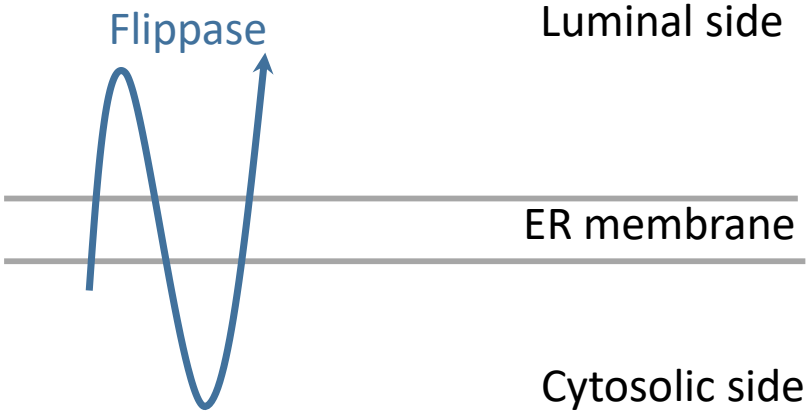
Post-translational modifications of CFTR: N-glycosylation

ER: cytosolic side



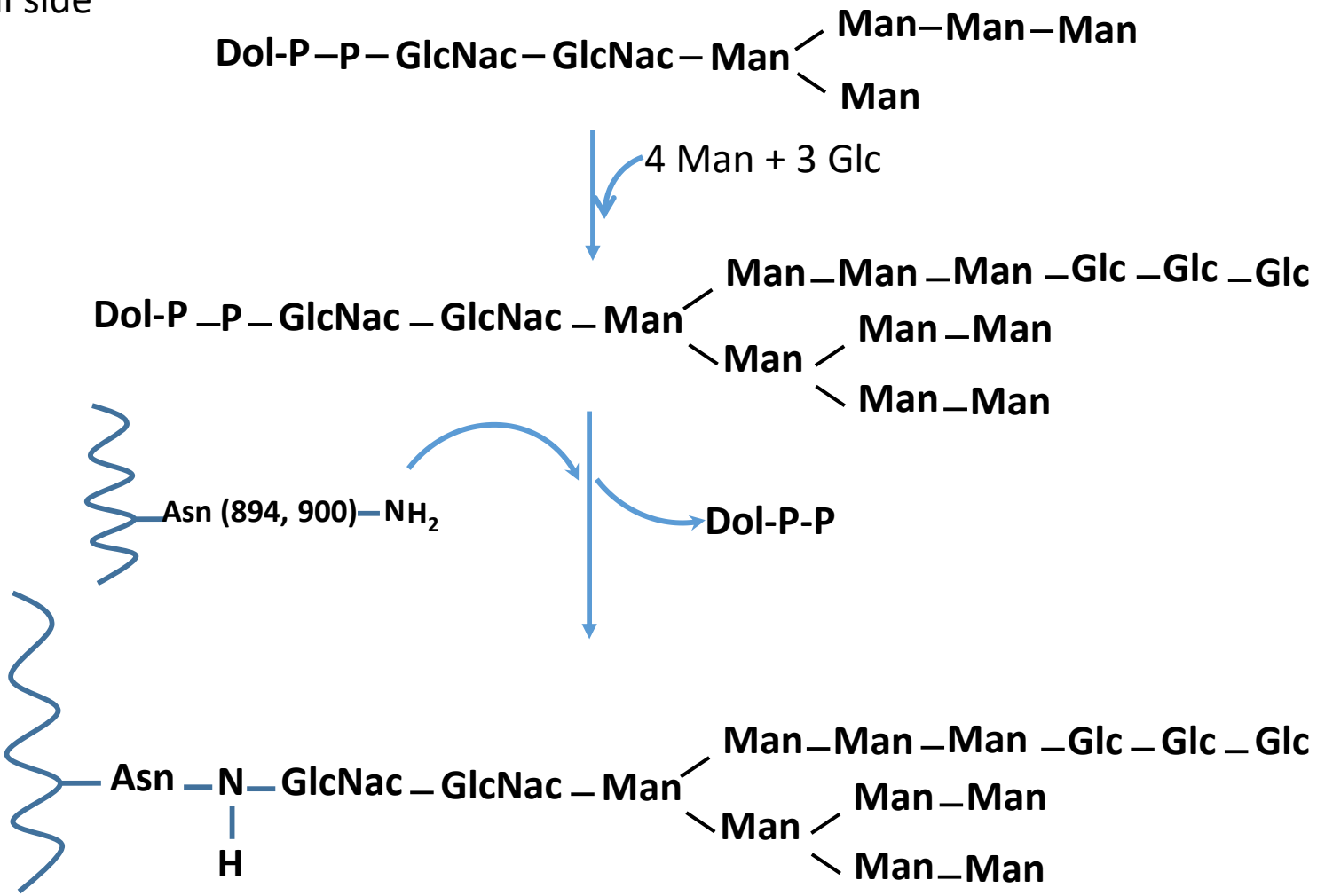
The synthesis of the common ologosaccaridic portion shared by all the N-glycoproteins occur in the ER and is in parallel with the protein biosynthesis.





Post-translational modifications of CFTR: N-glycosylation

ER: luminal side



Post-translational modifications of CFTR: N-glycosylation

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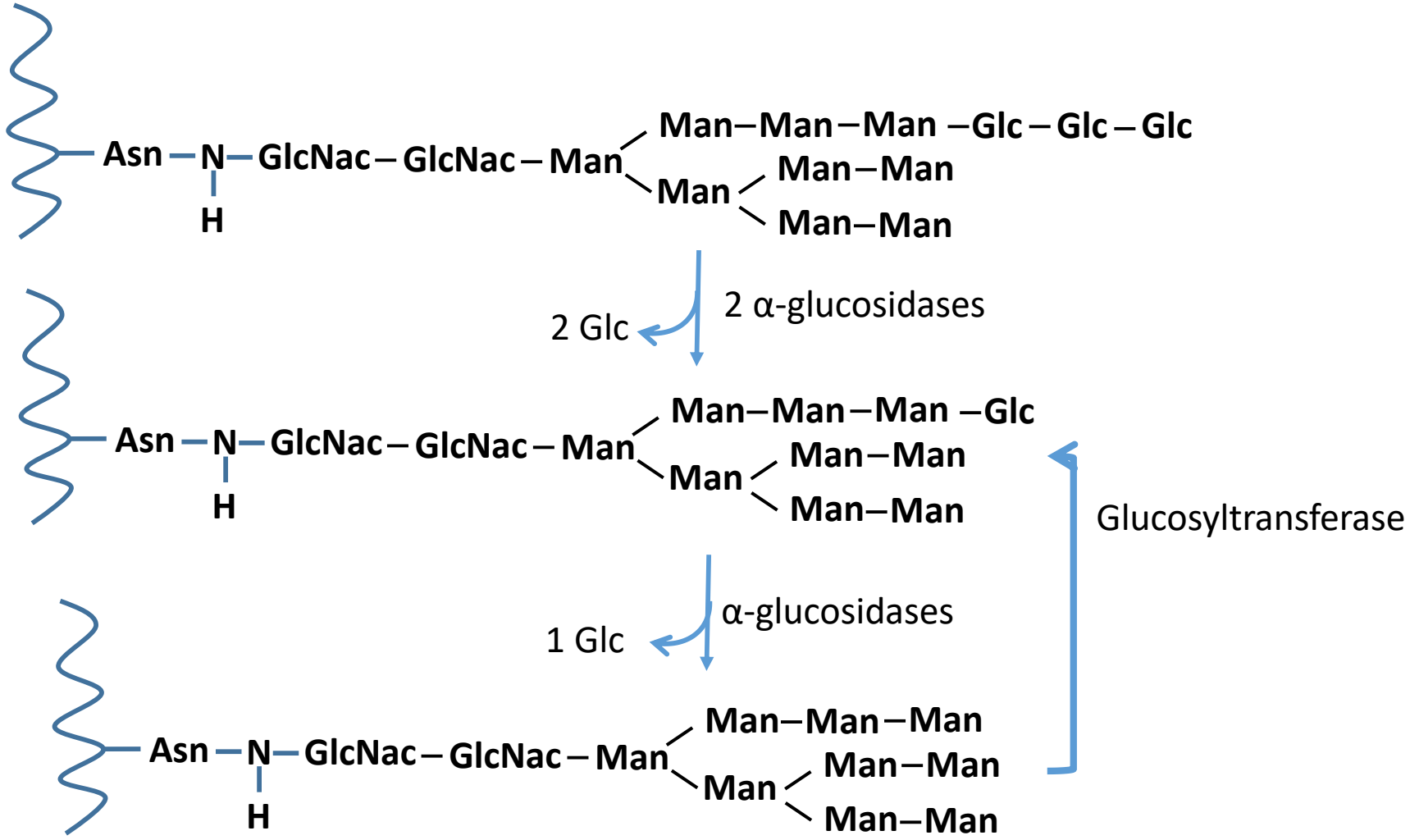
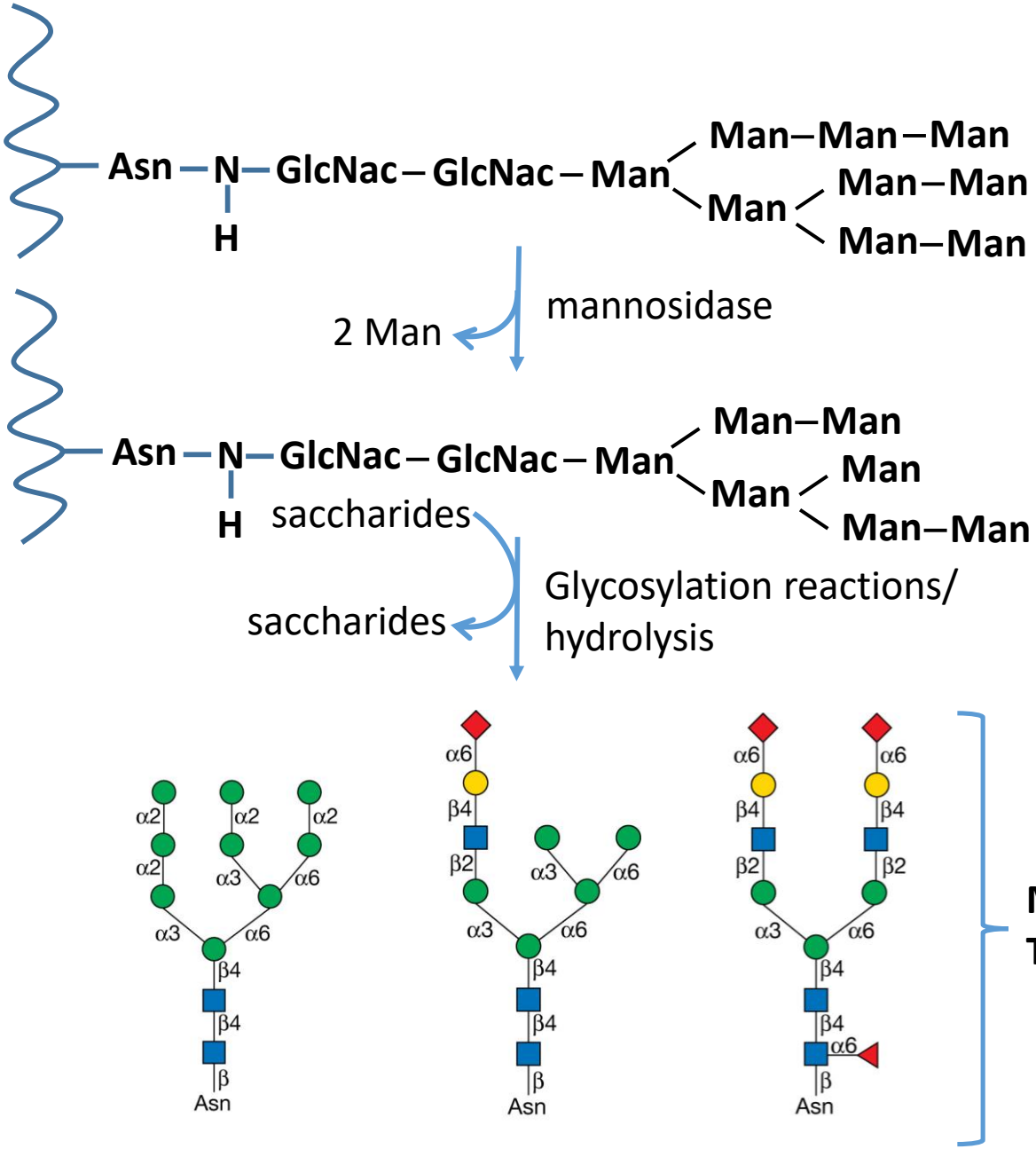


Glycoprotein N-GlcNAc: Biosynthesis

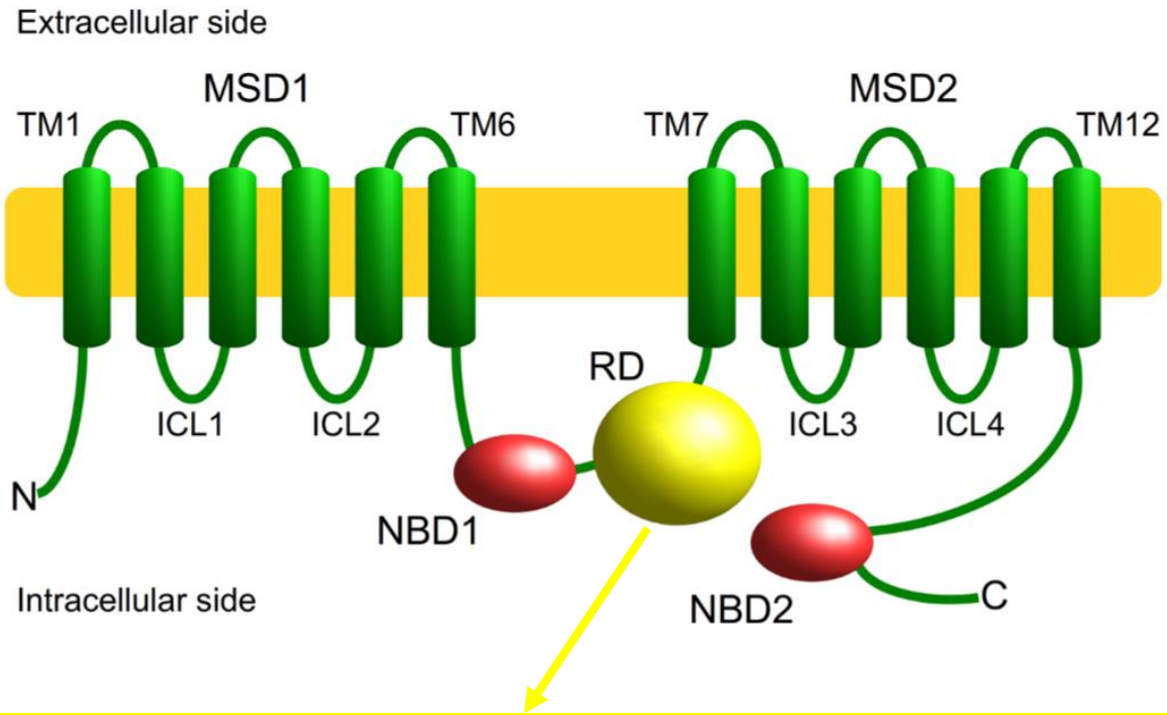


Learning Objectives

3. Describe the normal versus pathologic transport mechanisms of the CFTR channel.

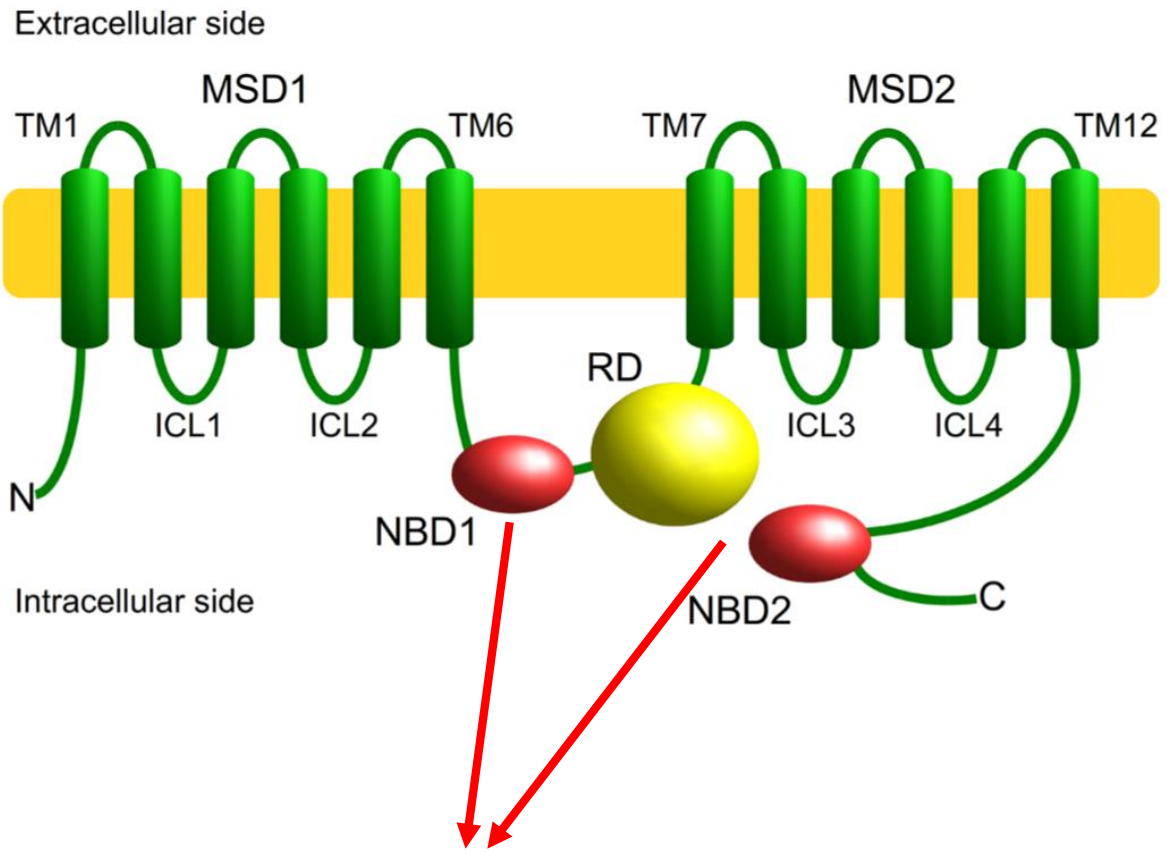
CFTR Structure of R domain

- **CFTR is a channel that allow the ion passage depending on their electrochemical gradient of concentration: ATP hydrolysis is not used to transport chloride**



With respect to the other ABC transporter the unique feature of CFTR is the presence of a lasso and intrinsically disordered portion called regulatory domain linking the two halves of the protein. The Hyper-phosphorylation of RD by PKA and PKC is fundamental for CFTR activation

CFTR Structure of NBDs



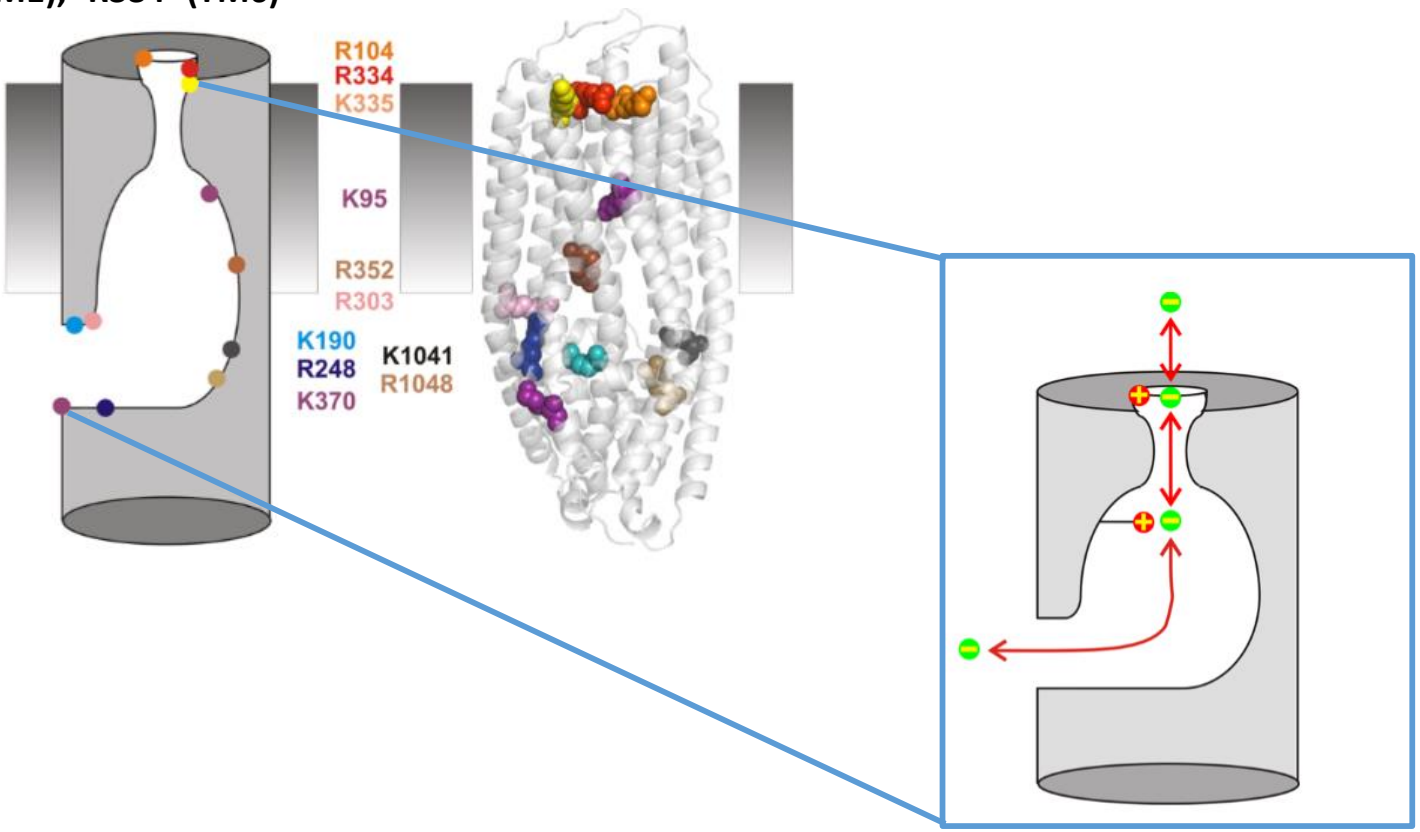
The two domains appear to act independently in the binding and hydrolysis of ATP. NBD1 is a stable ATP-binding site at which hydrolisis occur very slowly (degenerated site). In contrast, at the NBD2 ATP is hydrolysed as rapidly as it is bound and the nucleotide diphosphate hydrolysis product dissociates immediately.

CFTR Structure of the pore

Structure of the pore: a key role is played by positive charged aminoacids found in the outer vestibule, inner vestibule.

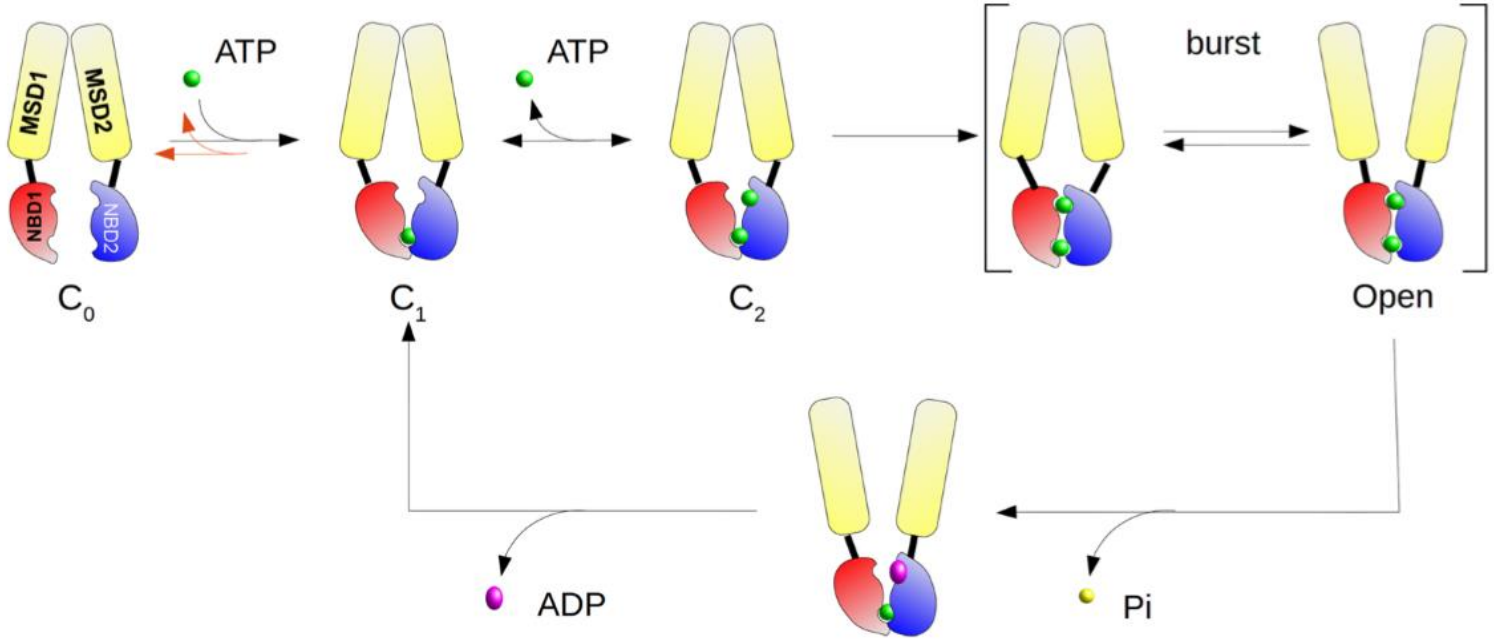
At the outer mouth of the pore, Cl- ions are likely attracted by positively charged amino acid side-chains R104 (TM1), R334 (TM6) and K335 (TM6)

Positively charged side-chains in the intracellular vestibule electrostatically attract cytoplasmic Cl- ions to the internal entrance to the pore



CFTR mechanism of action

First the activation of CFTR by the protein kinase A (PKA)- dependent phosphorylation of multiple sites located at the regulatory domain (RD). The phosphorylation of R domain increase the affinity of NBD1 and NBD2 for ATP. Then binding of ATP promotes the ''dimerization'' of the Nucleotide binding domains, leading to a conformational change at the level of the Multi spanning domains, that in turn leads to channel opening. The hydrolysis of ATP by the enzymatic activity of the Nucleotide binding domains determine channel closure with the release of ADP. In addition, the phosphorylation of R domain seems to be essential to amplify the conformational changes induced by the ATP binding to the MSDs giving a real stroke for the pore opening. Interestingly, as I mentioned before ATP hydrolyses in NBD1 is very slow so the channel could be rapidly activated by the only binding of ATP in NBD2.



Learning Objectives

4. Discuss the effect of an obstructive lung disorder on pulmonary mechanics.

Clinical Feature of cystic fibrosis

The faulty secretion of chloride from epithelial cells alter the electrolytes equilibrium, reduced recall of water and hydration of the mucins present in the lumen of different organs such as lungs, pancreas, gut and testis.

The final results is the production of hyperviscous mucus that alters the organs functionality.

Indeed, even if the pulmonary manifestations are the most severe, CF is considered a multi systemic disease.

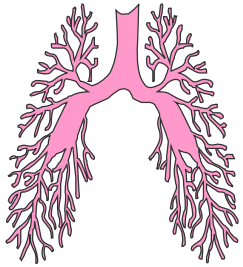
CF lung disease: the main leading cause of death for CF patients

Is caused by the pulmonary hyper-inflammation as a consequence of the formation of a very viscous mucus that does not allow to eliminate bacteria from the lung.

Persistent high-intensity inflammation leads to structural damage of the airways and impaired lung function that may result in respiratory failure and death.

Cystic Fibrosis: therapy

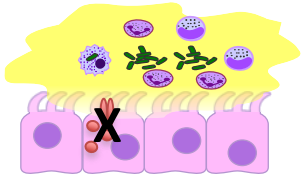
Bronchiestasis & lung insufficiency



Respiratory physiotherapy

Infection & inflammation

Persistent inflammation

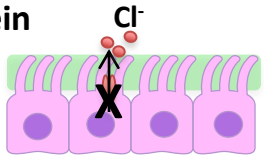


Recurrent infections

CFTR $^{-/-}$

Mucolytics
Antibiotics
Anti-inflammatories

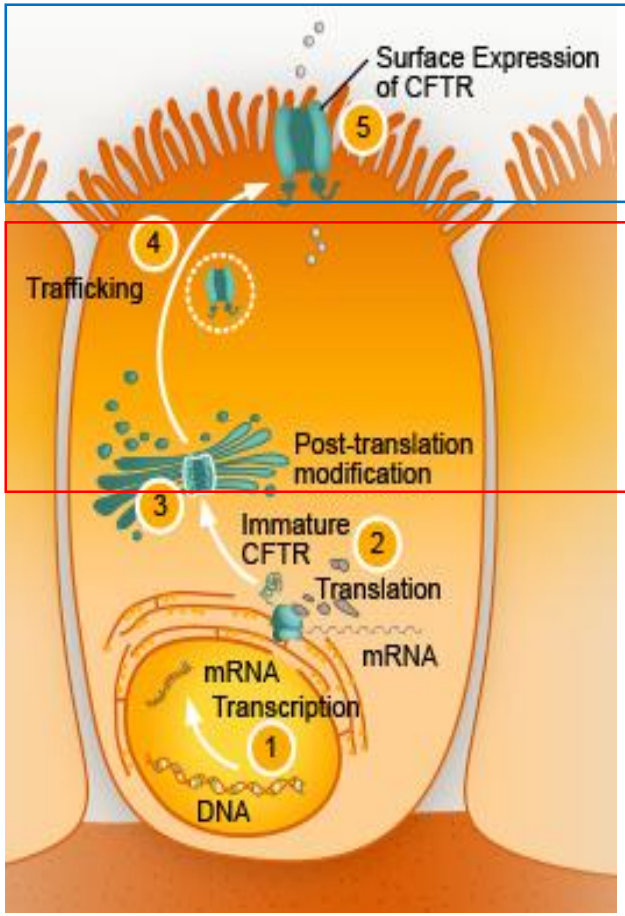
Defective protein



CFTR $^{-/-}$

CFTR-modulators

CFTR-modulators: mechanism of action and outcomes



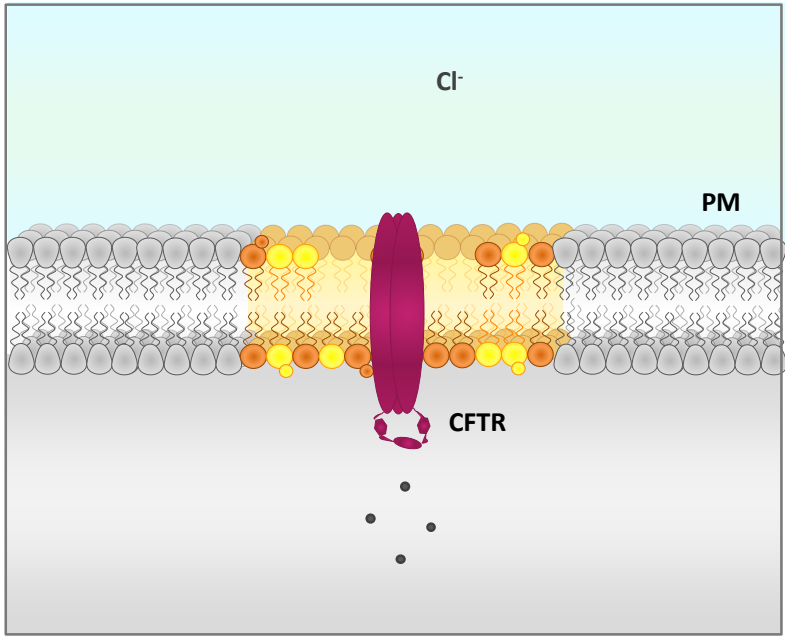
Potentiators
Increase the gating of CFTR, resulting in higher ion flow

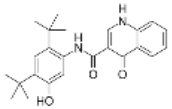
Correctors
Improve the delivery of CFTR to cell plasma membrane

The correctors such as lumacaftor can correct mutated CFTR structure and the potentiators like Ivacaftor improve the channel activity directly at PM. Unfortunately, even if these small molecules work very well for the patients who carry the mutation G551D, for the most common CF-causing mutation their efficacy seems to be time-limited.

CFTR-modulators: mechanism of action on different mutations

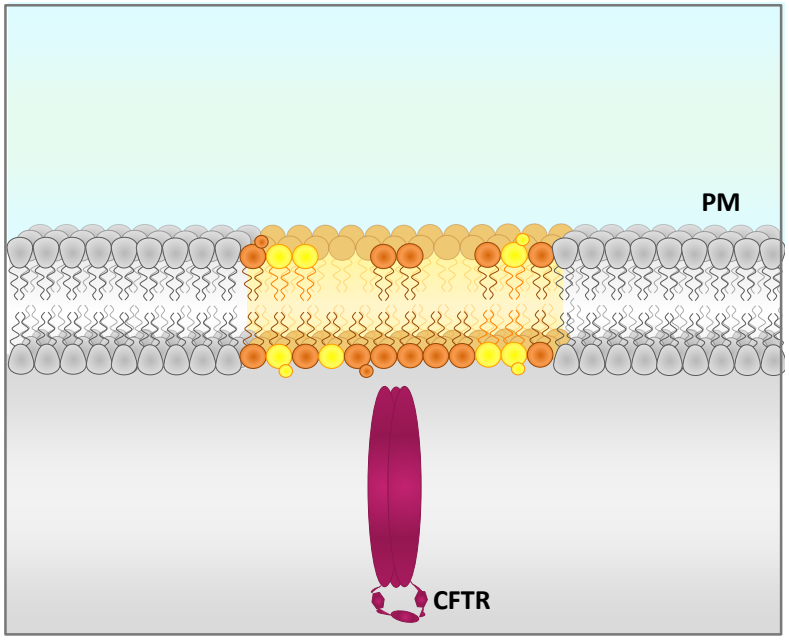
Gating mutation e.g. G551D

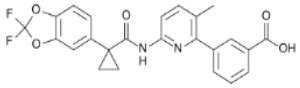




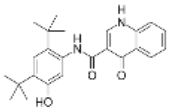
VX-770

Folding mutation e.g. F508del





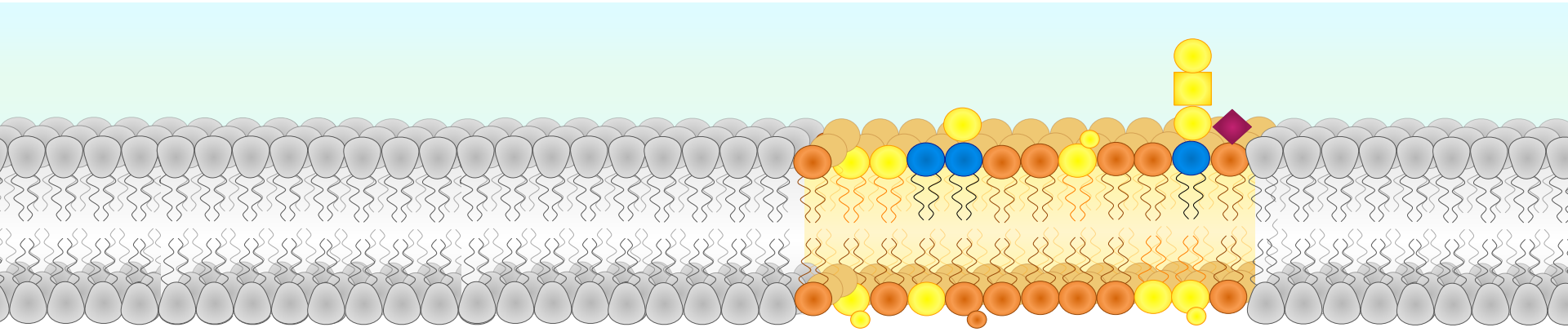
VX-809

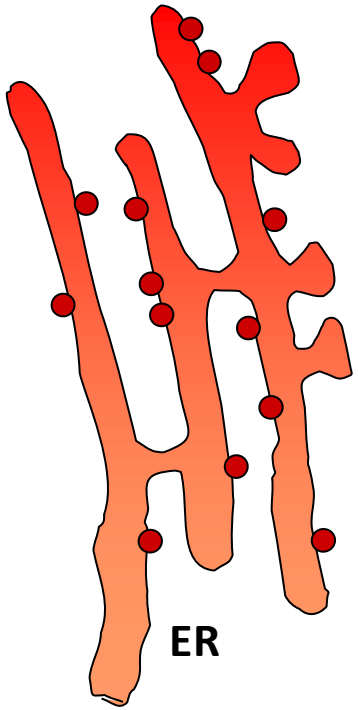


VX-770

In the case of gating mutations, such as G551D where CFTR is in the PM but does not exert its channel function, the therapy with the potentiator VX-770 restore the channel activity. Whereas when the mutations cause the lack of CFTR from the cell surface principally for problem of folding such as the case of F508del, the therapy with corrector VX-809 and potentiator (VX-770) promotes the trafficking of CFTR with the PM but CFTR is not stable and it is immediately internalized and degraded

Effect of the corrector VX-809

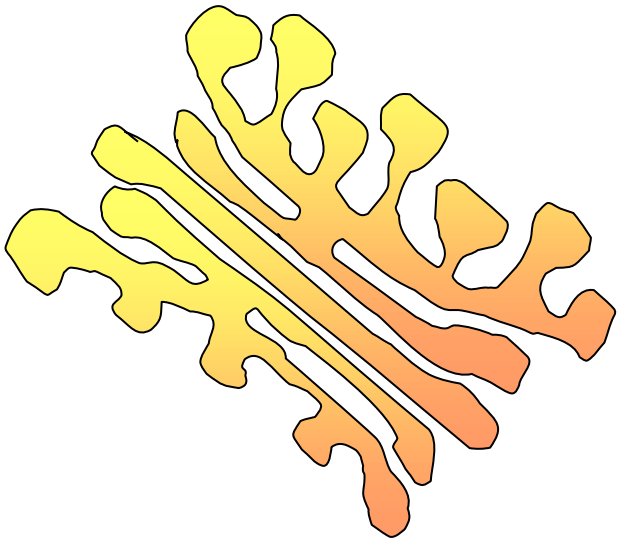




ER

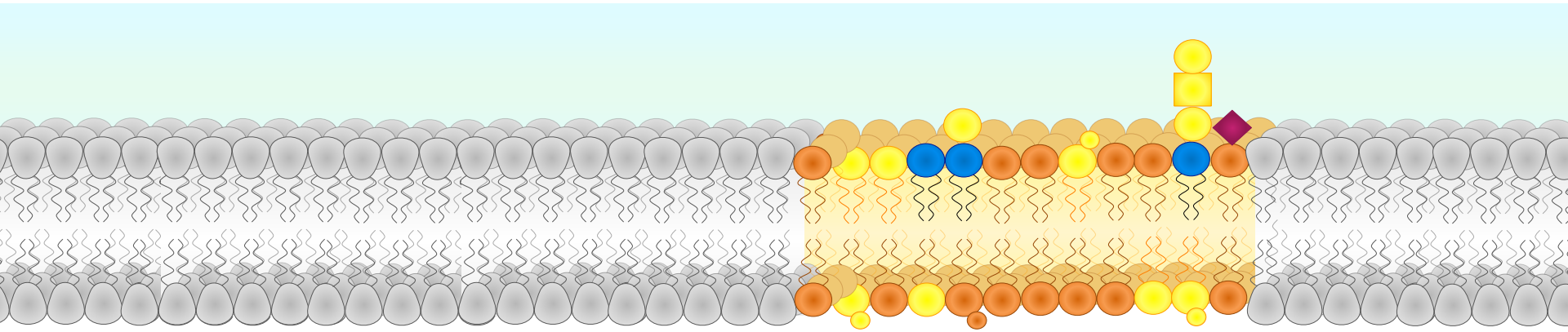
VX-809

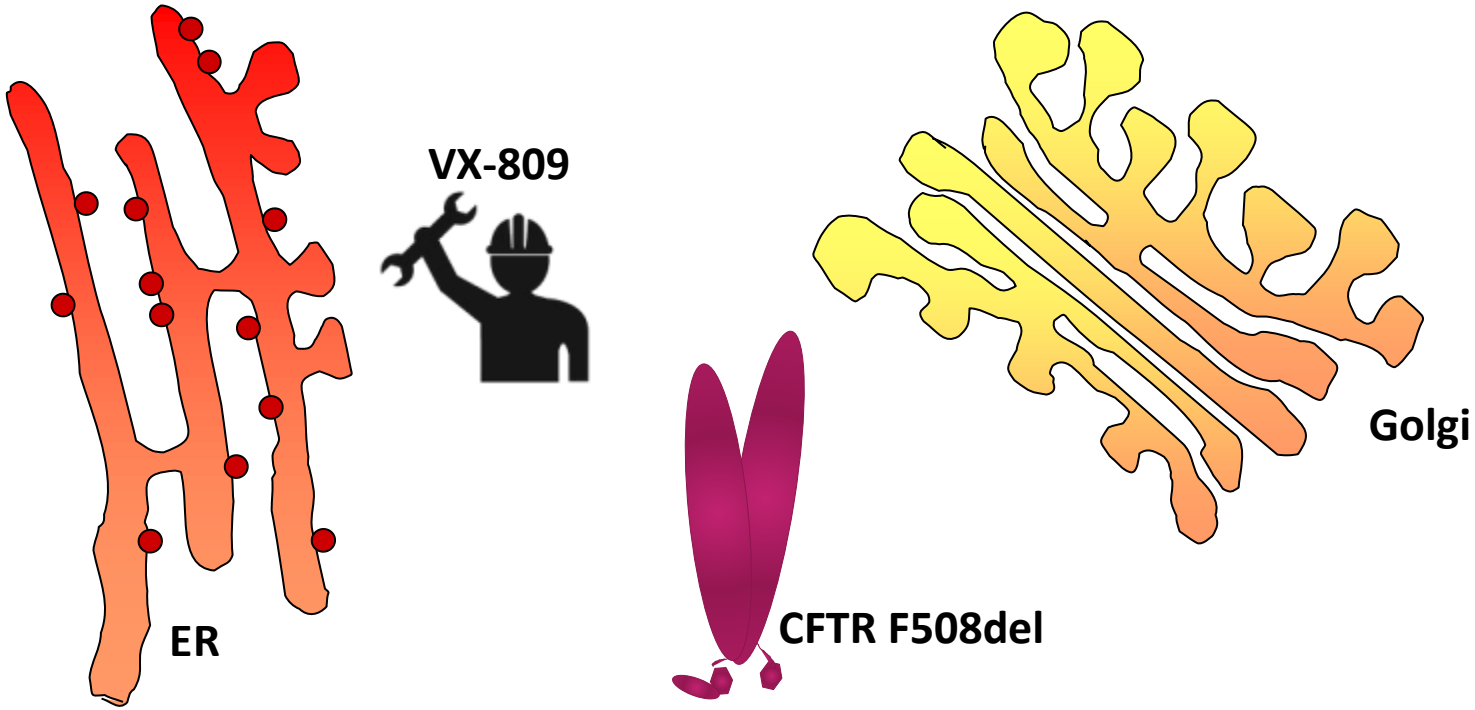
CFTR F508del



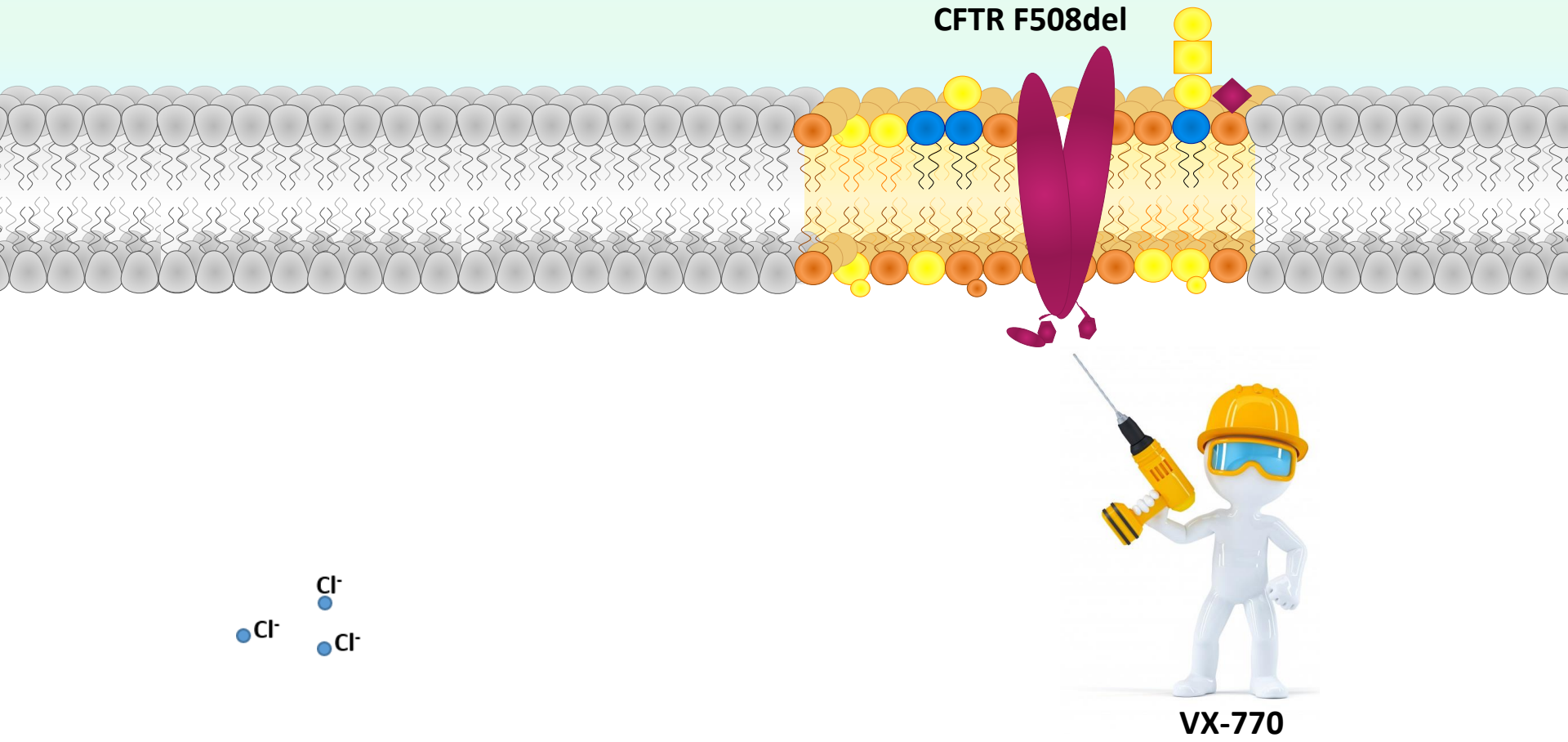
Golgi

Effect of the corrector VX-809

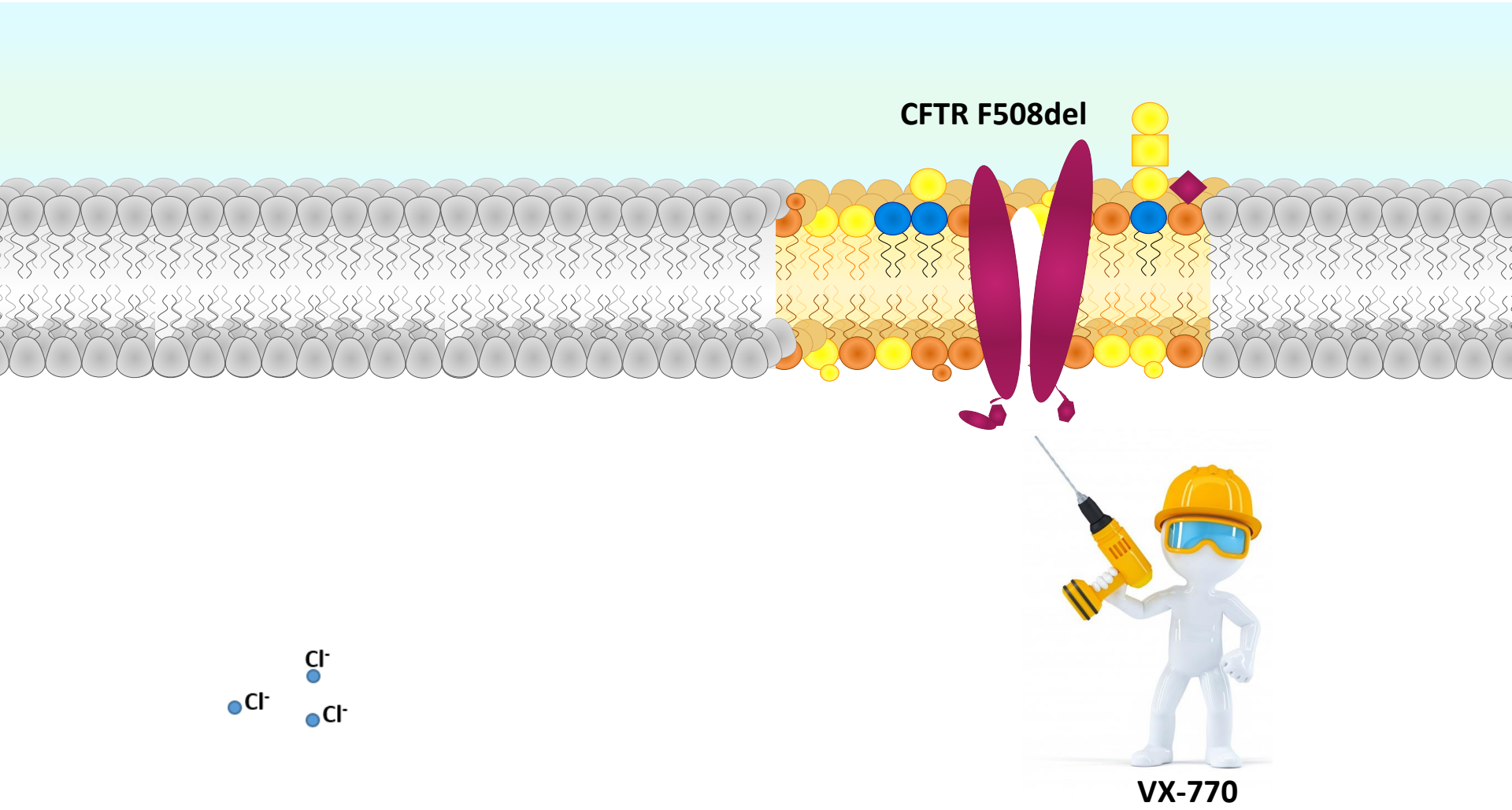


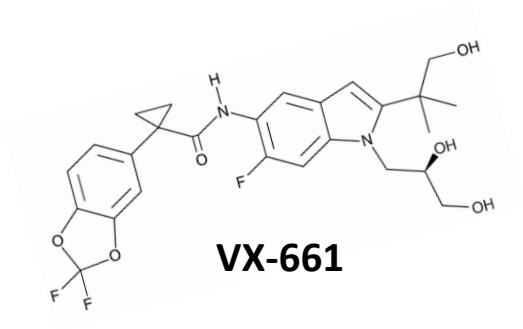


Effect of the potentiator VX-770

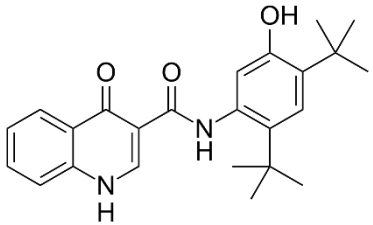


Effect of the potentiator VX-770





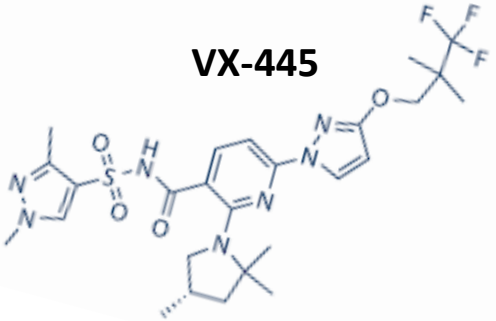
VX-661



VX-770

The era of Trikafta®

VX-445



Learning Objectives

6. Describe how to select a possible personalized therapy based on the use of the CFTR modulators

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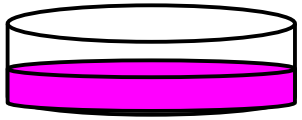


Primary cells from patient

Identification of the Mutation

In case of a new mutation biochemical and physiological characterization of the effect of the channel

(Try to) Found a therapy

Different combinations of corredctors and/or potentiators :

VX-661

OR

VX-809

AND

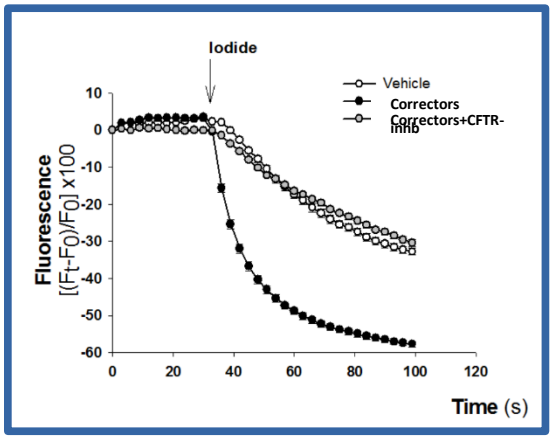
VX-770

CFTR

-band C

-band B

CFTR function



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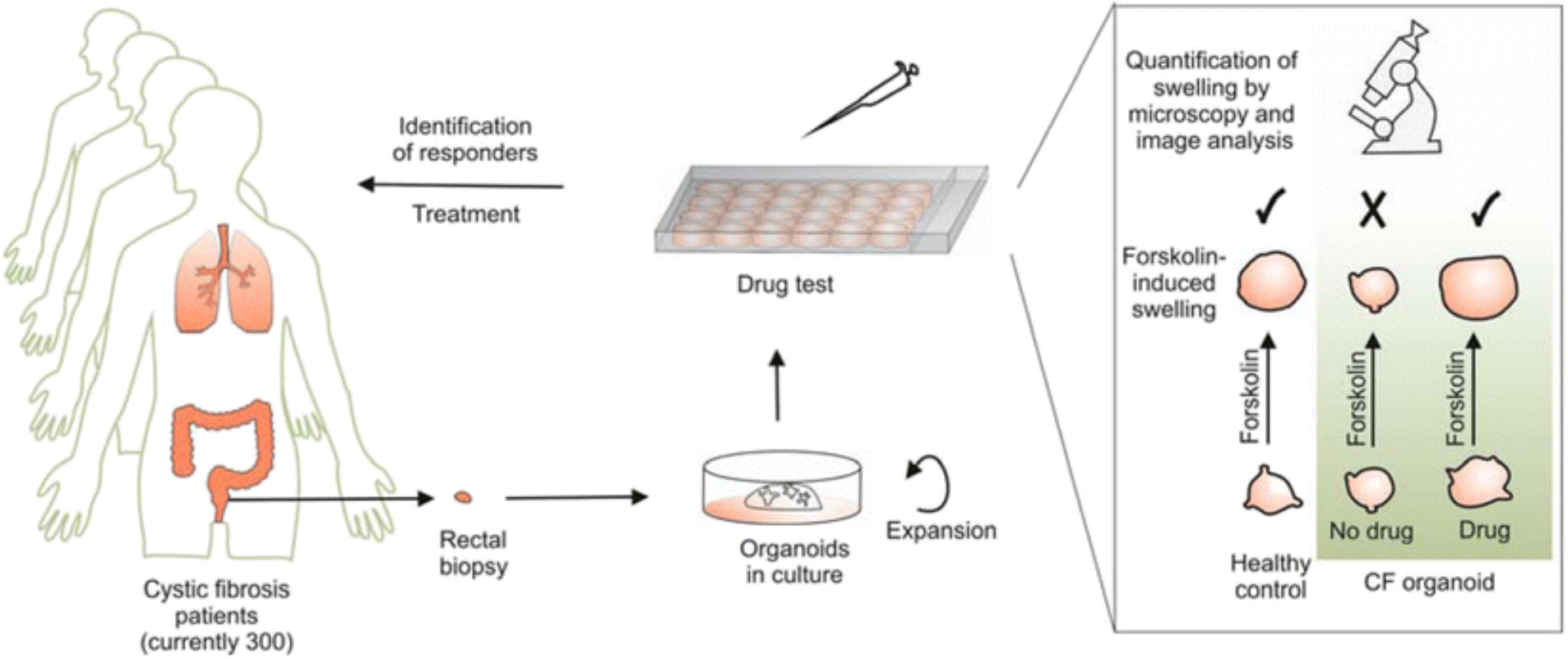


VX-770

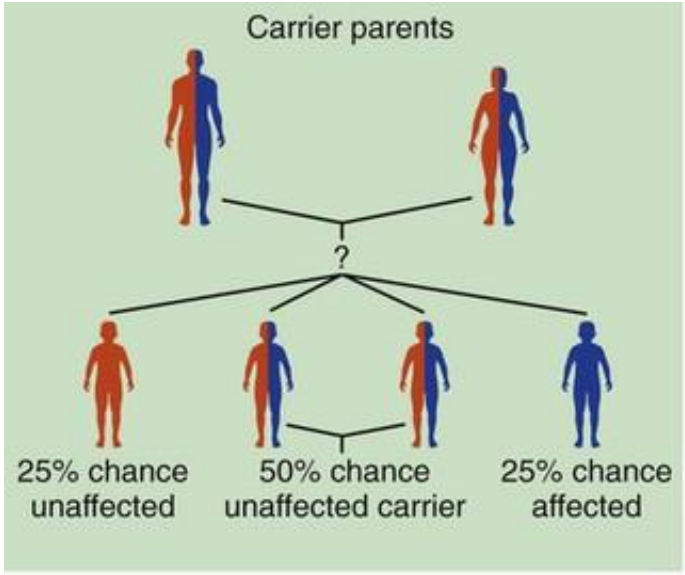
Case

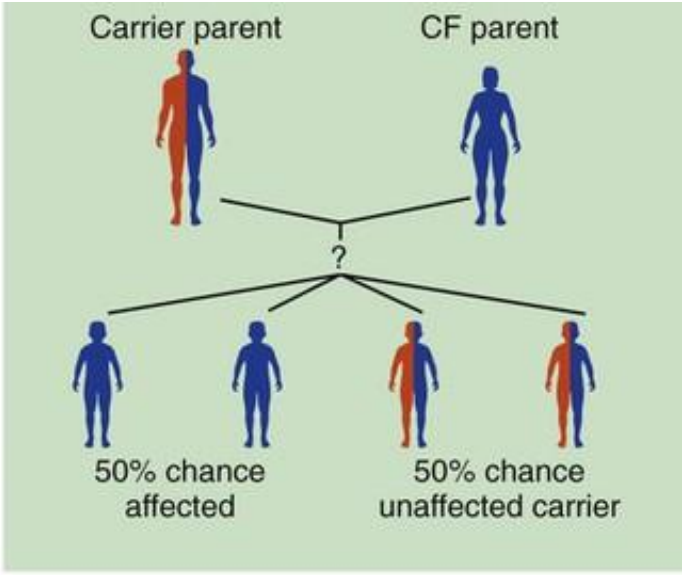
Ten years after Jeremy's diagnosis, the Gottleib's have had a daughter who is also affected with Cystic Fibrosis. Their eldest son who is now 25 years old is interested in marriage and has requested testing to determine if he is a carrier of a cystic fibrosis allele. He has previously tested negative for the same sweat test which Jeremy received. What is the pre-test risk that Jeremy's brother is a heterozygous carrier of a cystic fibrosis allele, given that he does not have the disease?

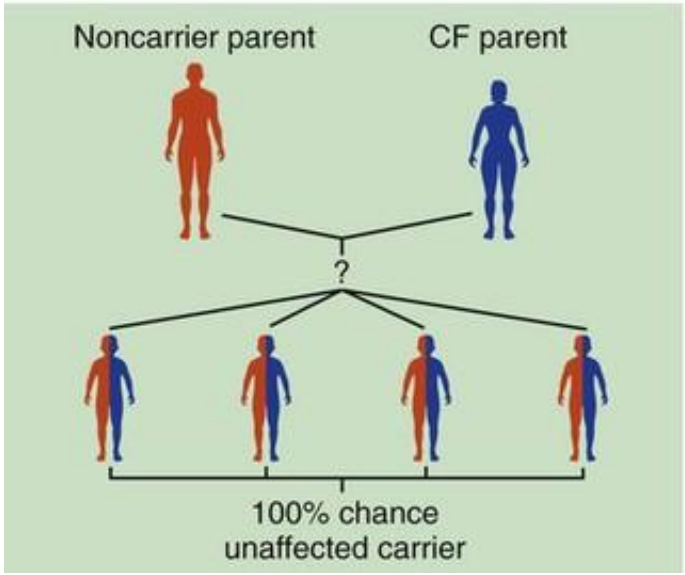
Learning Objectives

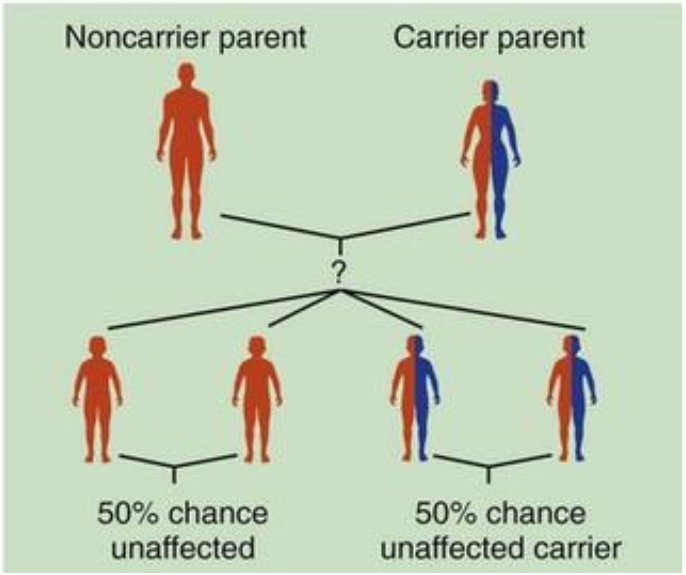
Discuss the inheritance pattern for cystic fibrosis and the likelihood of passing the gene or disease from one generation to another

Standard Mendelian pattern of inheritance of cystic fibrosis









Thank you for your attention