Molecular Basis of CF

Learning Outcomes

- Be aware of discovery of the gene (CFTR) involved in causing cystic fibrosis
- Explain the molecular basis of cystic fibrosis – the CFTR protein and its structure
- Explain the role of CFTR in epithelial cells which mediate signs and symptoms
- Distinguish the classes of CFTR gene variants
- Describe the most common variant: F508del

Historical Background

Cystic fibrosis (CF) has been recognized for centuries, primarily due to the characteristic saltiness of sweat in affected infants and children. The condition was first described in 1938, focusing on its impact on the pancreas, where infants would "waste away" despite adequate nutrition. Initially termed "cystic fibrosis of the pancreas," the understanding of CF has evolved significantly over time.

Clinical Manifestations

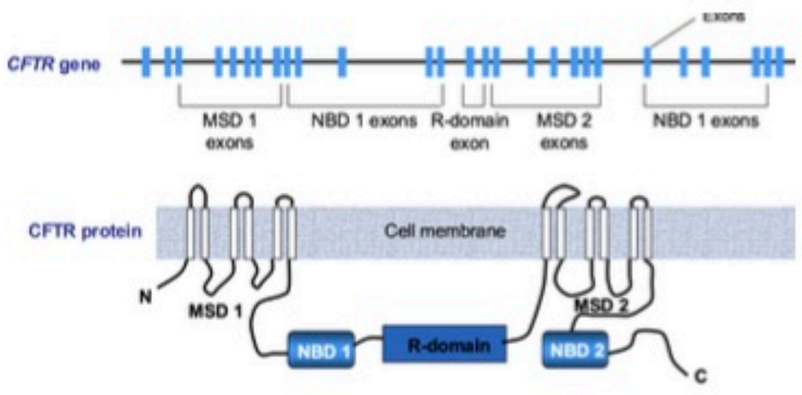
CF is characterized by several key features:

- Malabsorption of nutrients
- Poor growth and development
- Lung disease
- Abnormal electrolyte composition in sweat, leading to its salty taste

Advancements in treatment have dramatically improved life expectancy, which now ranges from 40 to 55 years.

Genetic Basis

The gene associated with cystic fibrosis was identified in 1989 through positional cloning. This process involved mapping the gene's location on chromosome 7 by studying families with CF and identifying common genetic markers. The CF gene encodes for the cystic fibrosis transmembrane conductance regulator (CFTR), a protein that plays a crucial role in ion transport.



CFTR Protein Structure

The CFTR protein consists of:

- 27 exons coding for a large membrane-spanning protein
- 1480 amino acids
- Four canonical domains, including two membrane-spanning domains and two nucleotide-binding domains

CFTR regulates anion transport, primarily chloride and bicarbonate ions, which are essential for mucociliary clearance, immunity, and inflammation. Expressed in apical (lumen-facing) epithelial cells in a wide variety of tissues.

Mechanism of Action

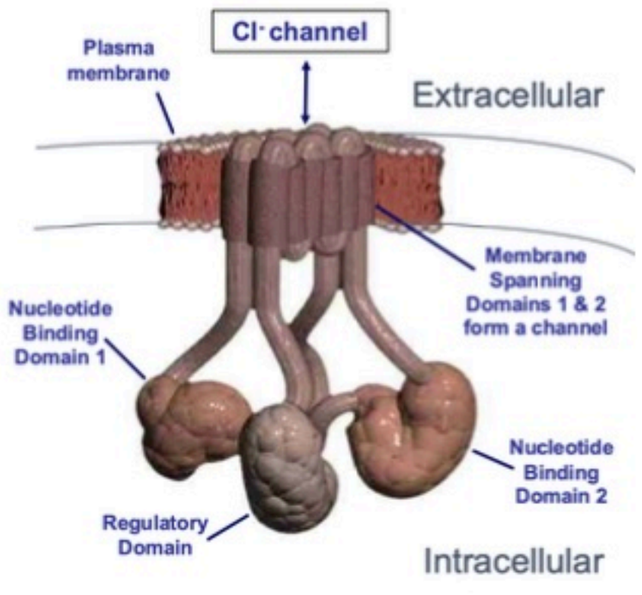
The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is a member of the ABCC superfamily of membrane transporters. It is characterized by a conserved structure comprising four canonical domains, all encoded by a single gene that forms a polypeptide chain. Unlike most ABC transporters that function through active transport, CFTR operates via diffusion down a concentration gradient, although ATP binding is still involved in its function.

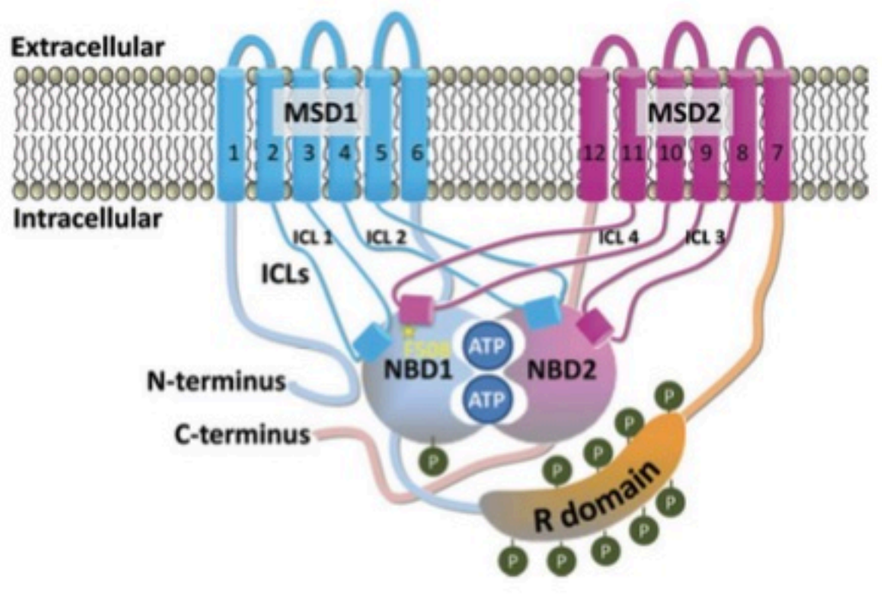
Structure of CFTR

The CFTR protein spans the membrane and consists of:

- Two membrane spanning domains (MSD1 and MSD2) that create the channel for chloride ion passage.
- Two nucleotide binding domains (NBD1 and NBD2) that regulate the channel's opening and closing by binding and hydrolyzing ATP.
- A regulatory domain (R domain) that contains phosphorylation sites influenced by protein kinases.

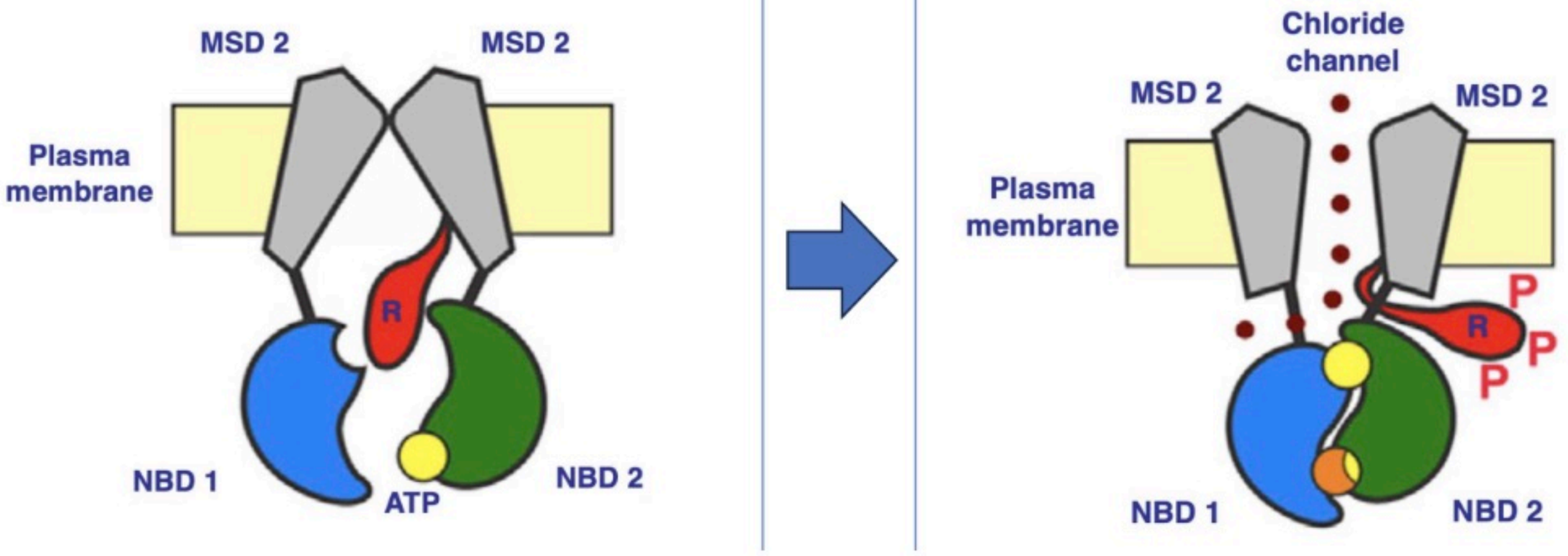
These domains are closely intertwined, allowing for complex interactions that facilitate the protein's function.





Channel Gating Mechanism

The gating of the CFTR channel is regulated by two primary processes: protein kinase phosphorylation and ATP binding. The channel can exist in a closed or open state, which is determined by the conformation of the regulatory domain and the nucleotide binding domains.



Closed Channel State

In the closed state, the membrane spanning domains are depicted in grey, while the nucleotide binding domains are shown in blue and green. The regulatory domain is in an inhibitory position, preventing the nucleotide binding domains from coming together, thus keeping the channel closed. Although one ATP molecule may be bound, it is insufficient to trigger the opening of the channel; both ATP molecules are required.

Open Channel State

When the channel opens, the regulatory domain becomes permissive, allowing the nucleotide binding domains to dimerize. This dimerization occurs when the regulatory domain is phosphorylated, enabling the binding of ATP at both nucleotide binding domains. The channel remains open as long as ATP is bound and hydrolysis occurs at the catalytic site.

Nucleotide Binding Domains

The nucleotide binding domains exhibit structural asymmetry, consisting of a head and a tail. This asymmetry results in two ATP binding sites: one degenerate site that binds ATP but does not hydrolyze it, and one catalytic site that does hydrolyze ATP. The lower catalytic site is crucial for channel function.