Review Article

# Role of ATP binding and hydrolysis in the gating of the cystic fibrosis transmembrane conductance regulator

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**Abstract:**

The CFTR gene is unique within the ATP-binding cassette (ABC) protein family, predominantly of transporters, by coding a chloride channel. The gating mechanism of ABC proteins has been characterized by the ATP Switch model in terms cycles of dimer formation and dissociation linked to ATP binding and hydrolysis, respectively. It would be of interest to assess the extent that Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), a functional channel, fits the ATP Switch model for ABC transporters. Additional transporter mechanisms, namely those of Pgp and HlyB, are discussed for perspective. Literature search of databases selected key references in comparing and contrasting the gating mechanism. CFTR is a functional chloride channel facilitating transmembrane anion flow down electrochemical gradients. A dysfunctional CFTR protein results in cystic fibrosis, a fatal pleiotropic disease currently managed symptomatically. Understanding the gating mechanism will help target drug development aimed at alleviating and curing the disease.

**Key words:**

ATP-binding cassette proteins, cystic fibrosis transmembrane conductance regulator, cystic fibrosis, gating mechanism

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## Methodology

Literature search of medical and scientific databases up to August 2011 was performed to select key references in comparing and contrasting the gating mechanism. The publication titles and abstracts were assessed for their quality and relevance in synthesizing information for the topics covered.

## Background

### ATP-binding cassette proteins

The ATP-binding cassette (ABC) protein superfamily contains mainly transporters responsible for a range of functions from nutrient uptake to toxin export and these proteins are found in all organisms ranging from bacteria to mammals. The first ABC transporter was identified more than two decades ago and currently in the human genome 48 ABC transporters have been identified and grouped into seven families. These proteins are highly conserved among vertebrates, especially in their structure and function of their nucleotide-binding domains (NBD), in contrast to the varied transmembrane domains (TMD) giving rise to functional diversity [Figure 1].

### Evolutionary perspective

ABC superfamily has evolved to include numerous transporters as well as other functions, including DNA repair by MutS and Rad50, $K^+$ channel regulation by Sur1, and a functional chloride channel by Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). NBDs being the fundamental biological engines driving the ABC proteins are highly conserved.[1] TMDs on the other hand are biological machinery resulting in varied function and therefore structural diversity. Evolutionary conservation of NBD structure and function allows the CFTR gating cycle to be studied directly and by indirect comparisons.

### Characterizing cystic fibrosis

The understanding of CF pathophysiology and its treatment has greatly advanced over the last 70 years, since it was first comprehensively reported by Dorothy H. Andersen in 1938 as a discrete disease.[2] She characterized the disease in terms of its symptoms that included the destruction of the pancreas together with airway damage caused by infections. The New York heat wave of 1953 helped Dr. Paul di Sant'Agnese to publish on the excessive salt loss of CF patients and this led to the development of the "Sweat Test."[3] Since its establishment in the 1950s, the sweat test remains to this day the main and simplest diagnostic tool for cystic fibrosis with chloride concentrations: >60 mEq/l indicating clinical cystic fibrosis, 40-60 mEq/l is intermediate, and <40 mEq/l is normal.[4] In 1989, the CFTR gene was cloned and sequenced using reverse genetics that opened the door to the exciting field of gene therapy.[5,6]

## Cystic fibrosis transmembrane conductance regulator protein

Cystic fibrosis is a Mendelian autosomal recessive disease of the young, due to its lethal nature in the early years of life, and affects roughly one in 2 500 newborns with a total of 70 000 people worldwide.[7] The CFTR protein is widely expressed in the body including the lungs, gastrointestinal tract, liver, pancreas, sweat glands, and the reproductive tract. The domain structure of CFTR resembles the other ABC proteins containing two TMDs, each made up of six membrane spanning α helices, and two NBDs found within the cytoplasm [Figure 1]. The unique CFTR modification to this basic structural outline is an additional fifth regulatory (R) domain linking the two homologous heterodimers [Figure 2]. This regulatory domain needs to be phosphorylated by PKA to facilitate the gating cycle.[8]

## Cystic fibrosis as a disease

With over one thousand recorded allelic mutations of the cystic fibrosis gene in addition to environmental factors results in a huge variety of disease pathophysiology and progression patterns, there are some common symptoms mainly of the airways and the digestive tract. Chronic lung infections predominantly by *Pseudomonas aeruginosa* occur in trapped mucous in the airways and progressively destroy lung tissue. Pancreatic ducts occluded by mucous prevent essential digestive enzymes reaching the gastrointestinal tract, which occurs in two-thirds of the patients. Small bile ducts are clogged up by mucous that leads to liver damage and also impedes digestion. The reproductive system of male patients is severely disrupted with the absence of fine ducts, including the vas deferens, accounting for the 95% infertility rates. Women patients may also be infertile due to a viscous mucous plug blocking sperm entry to the uterus. Finally, dysfunctional CFTR in the sweat glands of the skin result in excessively salty sweat and provides a simple, yet effective, method of diagnosis.[4]

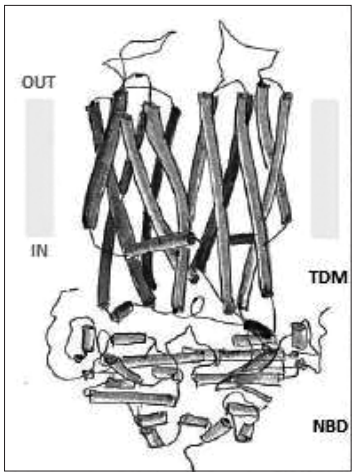


**Figure 1:** Schematic of a general ABC protein. The diagram is based on P-glycoprotein in its closed dimer conformation. The nucleotide-binding domains (NBDs) are the biological engines of ABCs and are located in the cytoplasm. The ATP at the dimer interface functions as the "molecular glue" in holding the dimer in its closed head-to-tail conformation. The transmembrane domains (TMDs) span the lipid bilayer and provide the translocation pathway. In normal ABC transporters, ligand transport entails conformational changes transmitted through TMDs to translocate the ligand across the membrane and subsequent lowering of affinity at the ligand-binding site releases the ligand on the other side of the membrane. CFTR on the other hand acts as a channel and the role of the TMDs is simply to form the pore for chloride ion transport. Nevertheless, all ABCs have the basic domain structure together with the highly conserved NBDs and this gives rise to the notion that perhaps there is a general mechanism, such as the ATP Switch model, that can be uniquely adapted for each ABC protein.

In the last century, cystic fibrosis was characterized using these symptoms into a discrete disease;[2] however, with the genetic revolution, variations in pathophysiology and progression can now be related back to a vast array of distinct genotype mutations, with their subsequent phenotypes. In the therapeutic section, an exciting novel small-molecule targeting ΔF508-CFTR mutant to potentiate channel gating will be mentioned.[9]

## Functional overview

ATP catalysis occurs at the NBDs, the biological engines of ABC proteins, to power the gating mechanism and over the years, there has been much progress in assigning the individual components of ATP binding, ATP hydrolysis, and finally ADP and $P_i$ release to the mechanistic steps. The traditional alternating catalytic site model proposed by Alan Senior was based on research into P-glycoprotein (Pgp) and put down the foundations for further mechanistic developments. The model held the long-established view that ATP hydrolysis was the major source of power for conformational changes and ligand transport.[10] The more recent ATP Switch model, proposed by Higgins and Linton,[11] is based on the innovative notion that ATP binding drives ligand transport and ATP hydrolysis resets the transporter. The ATP Switch model is based on ATP binding and hydrolysis driving the formation and dissociation of an NBD dimer, respectively [Figure 3]. Hence the NBD dimer is the fundamental feature of this model as it links ATP catalysis at the NBD to ligand transport at the TMD via conformational changes transmitted between these domains.

It would be interesting to compare and contrast the ATP Switch model designed for ABC transporters with the CFTR gating cycle model for a chloride channel. The 'power stroke' in ABC transporters is seen as the transport of the ligand across the membrane via the TMDs and the subsequent release on the other side associated with a lowered ligand binding affinity [Figure 4]. It is difficult to apply this concept to CFTR channels that allow chloride ion flow over a prolonged period, although opening of the TMD pore may be seen as an analogous process [Figures 5 and 6]. This functional diversity can be simplified to the "power stroke" driving ligand transport in ABC transporters *vs* pore opening in CFTR.

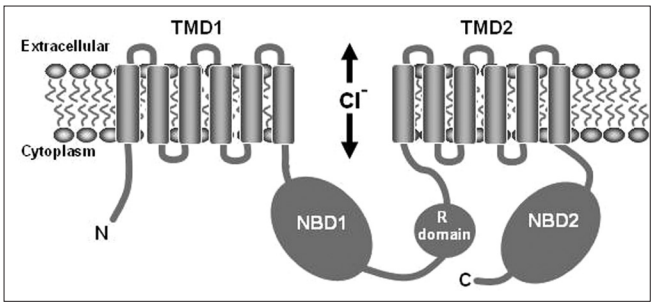


**Figure 2:** Domain structure of CFTR. The unique R domain is essential to the regulation of the channel and needs to be phosphorylated by PKA to facilitate the gating cycle. TMD, transmembrane domain; NBD, nucleotide-binding domain; R, regulatory domain.

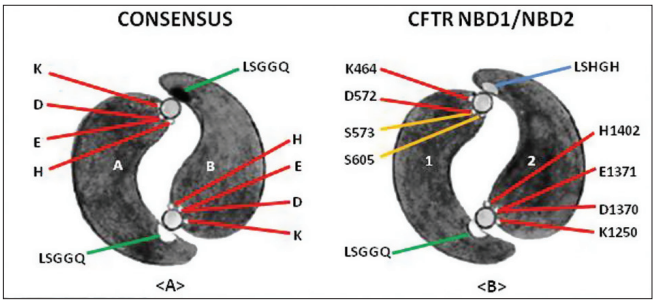


**Figure 3:** Head-to-tail arrangement of NBD dimer. (a) The standard NBD homodimer found in most ABC transporters with the full consensus of catalytic residues at each NBD. The interfacial composite site binding an ATP is made up of a major "head" region containing the Walker A motif, Walker B motif, and the D-loop together with the minor "tail" containing the LSGGQ signature motif. (b) This contrasts with the NBD heterodimer arrangement of CFTR that contains only a single consensus catalytic site at NBD2. These structural findings are an indication of the function of CFTR, with studies showing ATP catalysis at the catalytically active NBD2 to be sufficient to drive the gating cycle.

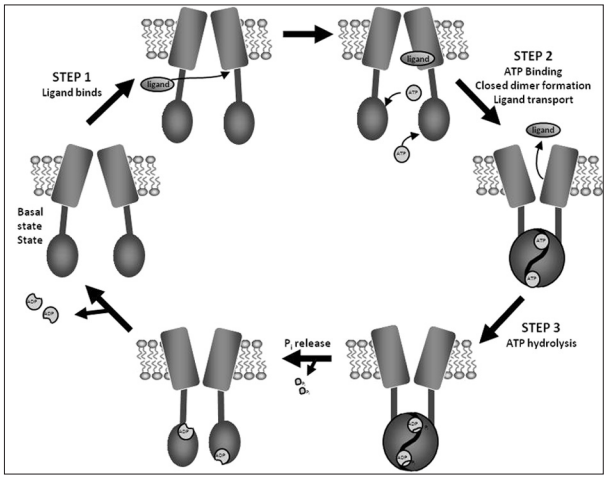


**Figure 4:** ATP Switch model. Step 1: Cycle initiated by ligand-binding TMDs. Conformational changes transmitted to NBDs enable ATP binding. Step 2: Bound ATP acts as molecular glue in maintaining closed "head-to-tail" NBD dimer, which transmits conformational changes back to TMDs. Resulting in ligand translocation, seen as the power stroke in the transport mechanism. Step 3: ATP hydrolysis enables the conformational changes of NBD dimer dissociation to be transmitted to the TMDs. Step 4: Basal state restored after sequential release of $P_i$ and ADP.

Additional transporter mechanisms, namely those of Pgp and HlyB, are reviewed for perspective. Studies of Pgp by Alan Senior led to the traditional alternative catalytic sites model and further research has provided a rich source of structural and biochemical data.[10] The HlyB transporter is also well reported in ABC literature and provides a second benchmark for assessing the points raised when modeling CFTR on the ATP Switch mechanism.

Elucidating the exact CFTR gating mechanism should provide a solid foundation for targeted drug development programs in the future. There has been much progress in high-throughput screening (HTS) for small molecule drugs to target trafficking and gating of the ΔF508-CFTR, accounting for two-thirds of cystic fibrosis cases. However, drugs targeting the gating cycle, called potentiators, seem to be more numerous and so may be the more enticing pharmacological agents of study.[9]

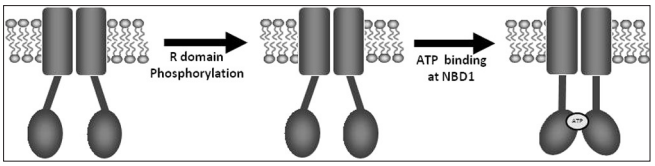


**Figure 5:** Regulation of CFTR activation. R domain phosphorylation followed by ATP binding at the NBD1 site enables the cycling mechanism to proceed [Figure 6]. In the CFTR model, the ATP molecule has adopted functions other than just powering the cycle as it is also required for activating the NBD1 site as well being the hydrolytic ligand in the gating cycle. However, the ATP concentrations within a cell are high enough for constant activation of the channel, thereby making these regulatory steps essential in preventing futile cycling.

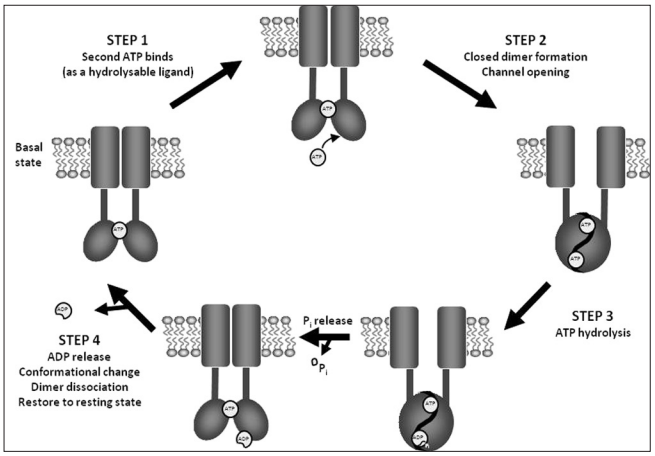


**Figure 6:** Proposed CFTR gating mechanism. CFTR being a functional channel adapts the ATP Switch gating mechanism designed for transporters. Of note, the inbuilt latch mechanism of ABC transporters is replaced by the more complex regulatory steps in CFTR [Figure 5]. Step 1: Initiation by ATP "hydrolysable ligand" binding the NBD2 catalytic site. Step 2: Formation of closed head-to-tail NBD dimer transmits conformational changes to TMDs for pore opening. Step 3: ATP hydrolysis and dimer dissociation initiates restoration. Step 4: $P_i$ and ADP release restores channel to its basal conformation.

## Therapeutics

Even though life expectancy of CF patients has tripled over the last half-century from under 10 years in the 1960s to over 30 years in the new millennium, cystic fibrosis remains a fatal genetic disease of the young. At present, drugs exist only for treating the symptoms, mainly of the airways and digestive tract, but there is still no cure. Even with the most advanced antibiotics (TOBI – "tobramycin solution for inhalation" against *Pseudomonas aeruginosa* infections of the lungs), nutritional regimes, exercise routines, pancreatic enzyme supplements (Creon and Pancrease), and highly complex heart-lungs transplants for the most severe cases, the median life expectancy is still under 37 years.[7]

This indicates that perhaps a new approach is required in developing drugs for CF. However, with full pharmaceutical pipeline costs running into hundreds of millions of pounds, evidence of a reasonable biological rationale, which holds CFTR gating at its epicenter, is needed for drug development programs to proceed. Sequencing of the CFTR gene in 1989 created great potential for gene therapy in the future,[5,6] although barriers to gene transfer vectors at the extracellular and intracellular environments provide many hurdles that

must be overcome.[12] An alternative approach of HTS for "lead generation" has rapidly grown in the 1990s in conjunction with automation advances, improvements in miniturization, and the buildup of vast compound libraries by pharmaceutical companies.[13] Small molecule drugs identified by HTS provide a method to rescue mutated CFTR, while the more ambitious gene therapy aims to replace and/or insert functional CFTR at the epithelia. Importantly, both these approaches have a common goal in targeting the underlying biochemical dysfunction and thereby creating a cure.

The CFTR gene is located on chromosome 7q31.31 and loss of function mutations result in cystic fibrosis. There are currently more than 1000 known mutations of this chloride channel that result in a range of disease states. The most common mutation is the ΔF508 that produces severe form of cystic fibrosis, due to the deletion of phenylalanine at position 508 located in the NBD1, and accounts for two-thirds of all disease cases. The loss of function mutation causes improper folding and trafficking of the channel to the epithelia membrane as well as altered channel gating.[14] There was previously much interest in gene therapy to correct ΔF508 mutation; however, the current drug development approach is to rescue the dysfunctional trafficking, folding, and gating of mutant CFTRs. HTS of compound libraries is used to find small molecule drugs that can target and rescue the various abnormalities of the ΔF508-CFTR function in an additive fashion for maximal clinical benefit.[9]

Understanding the gating mechanism of CFTR is important in many aspects of CF drug development, such as cheminformatics to maximize lead generation by HTS and medicinal chemistry to match drugs with specific gating intermediates. Pharmacogenomics analyzes phenotypes of various CFTR mutation to create tailored drugs, in terms of efficacy and toxicity profiles, on the basis of patient genotype. Work done by Van Goor *et al*. on CF drug development have used HTS to identify a number of small molecules that can rescue the mutant ΔF508-CFTR gating and trafficking processes.[9] They have identified both potentiators (VRT-532), drugs that enhance PKA-regulated Cl- channel gating, as well as correctors (VRT-422 and VRT-325) that improve the folding and trafficking of ΔF508-CFTR in an additive fashion with each other. This up-to-date research offers a promising outlook for drug development based on knowledge of the gating cycle.

## Gating Mechanism

Even though CFTR belongs to the ABC transporter superfamily, it has evolved to function as a channel, which makes it a challenge to compare the similarities and contrast the differences with the general ABC gating mechanism. The ATP Switch model proposed by Higgins and Linton describes how ABC transporters can couple ATP catalysis (ATP binding and hydrolysis) to gating and provides a good starting point for this investigation.[11] As CFTR is a chloride channel, the switch model must be adapted to accommodate the structural and functional differences. Finally, in order to make a balanced analysis of the basic ABC transporter mechanism and how CFTR fits this, it would be useful to refer to other well-studied transporters, including HlyB and Pgp.

### The ATP switch model

The ATP Switch model is based on the conformational changes between an open and closed dimer driving the ABC gating cycle, hence making the NBD pivotal to the mechanism. An energy-converting device, called an NBD, acts as an engine for driving biological processes by using ATP as a source of potential energy. Functional diversity is attributed to the varied TMDs, which are linked to the NBD engine to drive specific transport mechanisms. The general structure and function of NBDs are highly conserved; hence, the NBD dimer-centered ATP Switch mechanism is a good starting point for a general ABC gating mechanism. The most suitable approach to compare and contrast CFTR with the ATP switch model is perhaps by tackling it in a step-by-step fashion [Figures 4 and 6].

#### *Step 1*

Initiation of the transport cycle. ATP Switch model requires ligand binding for the initiating step, which acts as an inbuilt latch mechanism. The cycle is permitted only if there is a ligand to transport, thereby preventing futile catalysis in the presence of high cellular levels of ATP.[11,15,16]

In CFTR, ATP at the NBD2 site functions as both the ligand and the hydrolysable energy source.[8] How can futile catalysis be prevented in CFTR despite the high cellular levels of ATP "hydrolysable ligand"? The answer lies in the preceding regulatory steps, unique to CFTR, that are outside of the gating cycle [Figure 5]. This allows Cl- concentrations to be sensed indirectly and the information transmitted through complex signaling pathways ending in phosphorylation of the R domain and subsequent ATP binding at the NBD1 site.[8]

In the ATP Switch model, the ligand binds to the TMDs, which transmits conformational changes to the NBDs that increase their affinity to ATP. In CFTR model, the ATP "hydrolysable ligand" binds directly to the NBD, therefore skipping the additional step that involves energy-dependent conformational changes. This may in part explain the reduced energy requirements of CFTR gating, which require only a single catalytically active NBD2 site.

The ABCC family of transporters, which include CFTR, have asymmetric NBDs with stably bound ATP at NBD1 and catalytically active NBD2 driving the gating cycle. Structural asymmetry is demonstrated by the 27% homology between NBD1 and NBD2 [Figure 3], compared with almost identical domains of bacterial transporters.[8] Function asymmetry is demonstrated by photolabeled ATP and vanadate trapping experiments.[17] ABC transporters need a change in affinity at the ligand-binding site to facilitate ligand binding and release, which involves energy-consuming conformational changes. CFTR has a stable ionic pore in the open state, which may explain in part the reason for a single catalytically active NBD2 site.

#### *Step 2*

Main features of this step are formation of the closed NBD dimer, subsequent transmission of conformational changes to the TMDs, and finally ligand transport.[11] These basic functions are shared by the ATP Switch model and CFTR.

Conformational changes at the TMDs are used for ligand transport in normal ABC transporters or the opening of the TMD

anion pore in CFTR. This functional diversity of TMDs is reflected in their structural and mechanistic variations, although this does not detract from underlying similarity of all ABC proteins being driven by highly conserved biological engines, the NBDs.[1]

The "power stroke" is a defining step in a process and for the ATP Switch model, it is the transport of the ligand across the lipid membrane. Mechanical energy for this step comes from the formation of a closed NBD dimer, which transmits the necessary conformational changes to the TMDs for ligand transport.[18] It appears that CFTR cannot be viewed to have such a distinctive power stroke step as it functions as a channel to facilitate the flow of many chloride ions over a prolonged period. However, opening of the channel can be viewed as the major stroke in the gating cycle and therefore ATP binding, which powers this step, can then be interpreted as the energy input for this "power stroke."

ABC transporters require the ligand-binding site to switch from inside-facing high affinity in the basal state to outside-facing low affinity after the translocation step in order to allow the release of the ligand on the other side of the membrane.[10] CFTR on the other hand does not have a chloride-binding site as such, although positively charged residues throughout the pore attract chloride ions and facilitate rapid flow through the pore.[19] This lack of a chloride-binding site, that would need energy-dependent conformational changes to modify its affinity, may be part of the reason behind CFTR requiring only a single catalytically active NBD2 site for the gating cycle.

The dimer is at the center of the ATP Switch mechanism and it forms by the rigid-body rotation of the main nucleotide-binding site (Walker A and B motifs) on one NBD around the complimentary nucleotide-binding site (LSGGQ signature motif) on the second NBD.[20] Once the two ATPs are bound, they provide the "molecular glue" at the dimer interface for maintaining the head-to-tail conformation.[21] Even though the CFTR protein has an asymmetric dimer, the highly conserved nature of NBDs dictates that dimer formation is fundamental to gating function of all ABC proteins, with CFTR being no exception [Figure 3].

*Step 3*

The main feature of this step is ATP hydrolysis driving the disassociation of the dimer, which initiates the formation of an open dimer state. The associated conformational changes are transmitted reciprocally from NBDs to TMDs and facilitate the return to the basal state.

The ABCC family, in which CFTR is a prominent member, have branched in the tree of ABC protein development to posses only a single catalytically active NBD2 site with the gating cycle driven by the catalysis of a single ATP molecule.[8] Although this is a variation of the standard mechanism, it does not seem to conflict with the formation and dissociation of the dimer, which is essential to the ATP Switch model.

ATP hydrolysis initiates the process leading to the restoration of the protein to its basal state, but it is important to note that this site does not restore the channel directly. Studies with vanadate on Pgp and orthovandate on CFTR have shown that these molecules can replace $P_i$ after the hydrolytic step to form a non-reactive NBD dimer.[17,22] The hydrolyzed ATP molecule is replaced with ADP-vanadate to leave the protein in a transitional active state. This active state has been shown by Basso and colleagues to be particularly apparent when CFTR is treated with orthovanadate as closure of the channel is significantly delayed and this may imply that a subsequent step such as ADP release or even ligand binding is required to fully restore the protein.

*Step 4*

The essential aspect of this step, common to both models, is the restoration of the transporter to its resting state. This process is quite complex, involving many individual steps and is currently not well understood.

The sequential release of the catalysis products, $P_i$ followed by ADP, from the binding pocket facilitates the disassociation of the NBD dimer. This in turn transmits conformational changes to the TMDs that are restored to their resting state. The vanadate experiments mentioned above helped elucidate the order in which the products of ATP catalysis are released,[17,22] and suggests that the release of ADP may be the final step in resetting the transporter.

Repulsion of the two products, ADP and $P_i$, within the binding pocket is one explanation for the release of the catalysis products at the end of the cycle,[20] although this may not be the case for all ABC proteins due to their diversity. Studies of MutS have shown that ADP only dissociates from the NBD and is replaced by ATP upon substrate binding.[23] Further studies are required to elucidate the exact mechanistic steps needed to reset the CFTR channel to its basal state.

## Final words on cystic fibrosis transmembrane conductance regulator and the ATP switch model

In comparing the ATP Switch and CFTR models, it is obvious that despite the differences, they are fundamentally quite similar. The ATP Switch model attempts to encompass the diversity of transporters by relating back to the highly conserved NBD characteristics of this superfamily, therefore allowing various transporters to be grouped together under its umbrella mechanism.

It seems to me that the ATP Switch model is comparable with the CFTR mechanism in many aspects. CFTR gating is based on ATP binding, driving the opening of the channel followed by hydrolysis to close it, which the associated formation and dissociation of the dimer, respectively.[8] This dimer-based perspective is the foundation of the ATP Switch mechanism, proposed by Higgins and Linton[11], and naturally explains the naming of the model in terms of an on-off dimer "switch."

Considering the view that "*early in evolution an engine called a nucleotide-binding domain* (*NBD*) *was created*" and to this day has a highly conserved sequence makes it likely that the gating mechanism has also been conserved.[1] The ability of the engine to power a diverse range of processes comes from its coupling to various TMDs, which show high sequence variation. In conclusion, it is likely that there is a general gating mechanism applicable to the ABC superfamily and the ATP Switch model fits many of the studies on CFTR and other ABC transporters.

### Evaluating the ATP switch model

The ATP Switch model exemplifies much of the current accepted data on ABC transporters; however, it would be of use to examine a few other well-known transporter mechanisms. First, these further mechanisms should be beneficial for analyzing the points raised in comparing and contrasting the CFTR gating cycle to the ATP Switch model. And second, testing the ATP Switch model, designed to represent all the members of the ABC superfamily, against other well-known mechanisms should bring about constructive assessment of this general model.

Study of HlyB and Pgp has provided the valuable structural and functional insight for CFTR research. This may be exemplified by the generalized schematic diagrams of an ABC protein being based on Pgp [Figure 1] as well as the substrate-assisted catalysis (SAC) models of HlyB emphasizing the importance of a latch mechanisms, respectively. Outlined below are some of the mechanistic advances linked to CFTR research and suggestions of future areas of interest.

### Models of HlyB

The ABC transporter HlyB is an essential *Escherichia coli* protein that exports hemolysin A, a virulence factor that influences metabolism of host cells, across the cell envelope of the bacterium.

The previous model of NBD function in the HlyB transporter was general base catalysis that hypothesized the nucleotide-binding pocket to contain all the necessary amino acids orientated favorably to facilitate ATP hydrolysis, thereby lacking an intrinsic latch mechanism within the gating cycle. The new SAC model, on the other hand, requires ligand binding to form a head-to-tail NBD dimer that creates a microenvironment favorable for ATP hydrolysis. These modifications of physical properties, such as H-bonding and salt bridge formation, occur in specific residues (Asp630/Glu630/H662) that are involved in hydrolysis.[21] So, the SAC provides a theory for how ligand binding and subsequent dimerization can be coupled to ATP hydrolysis without futile catalysis, which emphasizes the importance of the latch mechanism in the ATP Switch model and the need for a further regulatory step in the CFTR mechanism.

The D-loop is a highly conserved and characteristic sequence of ABC proteins located in the major catalytic region of an NBD and it has had little scientific attention over the years [Figure 3]. It has been proposed by Hanekop *et al*. that the D-Loop is part of the molecular machinery allowing the two distant nucleotide-binding sites to interact with each other, which is essential to the cooperativity required in function of the NBD dimer.[21] Further investigation is required to shed light on this motif, especially as the process of dimer formation and dissociation is fundamental to the ATP Switch model.

The mechanochemistry energy model of HlyB looks at two sources of potential energy within the cycle: The first is mechanical energy from dimerization allowing rigid body motion of the helical domains (33 kj/mol) and the second is the chemical energy released from ATP hydrolysis (36 kj/mol). The two potential energies are of comparable magnitude, thereby further supporting the mechanochemical model.[21,24] As a side remark, this model does raise the question of where in the cycle the power stroke is located or even if there are two such strokes, one for mechanical and one for chemical energy release.

### Models of P-glycoprotein

Pgp plays an important part in chemotherapeutic resistance of cancer cells and is capable of transporting numerous drugs of variable structures. Due to its pathophysiological significance, it has been widely researched and should help in understanding the general ABC gating mechanism.

Isolated NBD experiments may be inconclusive for studying the gating mechanism as they lack important structural restrictions imposed by TMDs, which apply under physiological conditions. TMDs may be necessary for the formation of important intermediates, such as the occluded Pgp state, and their absence has been proposed to be the reason for symmetrical dimer formation in isolated NBD experiments.[25] The CFTR protein forms an asymmetrical dimer, similar in structure to the occluded Pgp state, although the basis of its formation may be more heavily based on differences in the primary structure of the nucleotide-binding sites than the dynamics of the gating mechanism. However, even if in isolated nucleotide experiments, an asymmetrical CFTR dimer still forms, this does not retract from the importance of TMD interactions with the NBDs throughout gating mechanism and the need for more advanced experimental techniques in the future to study complete proteins.

ATP catalysis powers the gating mechanism; however, the number of ATP molecules required for a single cycle is debatable for different ABC proteins. Most ABC proteins are thought to require the catalysis of two ATP molecules for a complete cycle, with studies on Pgp showing that the energy from a single ATP catalysis and subsequent electrostatic repulsion between the products is insufficient to restore the transporter to its basal state.[25] This contrasts to the ABCC family of transporters that require only a single ATP to drive the complete cycle.[8] However, it seems that the ATP Switch model is sufficiently versatile to accommodate these differences as the model is centered around the dimer that is universal to all ABCs, and so, such differences appear as specifics within a general mechanism.

An interesting way of looking at gating is from a thermodynamic perspective, which analyzes the energy-driven conformational changes between various Pgp intermediates.[26] This kind of model is depicted in terms of numerous transition states and pathways with thermodynamic pressures dictating the actual mechanism, which allows for many potential pathways but a single most likely route that perhaps may also be reflected in the CFTR mechanism.

## Future Prospects

There are many aspects of the CFTR gating mechanism that are currently unknown and present an exciting challenge for scientific advancement. Studies of intermediates, including the Pgp occluded state, have provided a novel take on the mechanism of this transporter as well as potential refinements of the ATP Switch model.[25] Arising from these studies is the difficult question of how reliable isolated NBD experiments really are, considering the absence of TMD interactions that may affect NBD mobility and even change the thermodynamic likelihood of various mechanistic pathways.[21] The ABC

superfamily is an ancient biological machine, with a conserved engine core driving a number of diverse biological tools. The core highly conserved structure is the NBD and the diversification of function is achieved by coupling to a variety of TMDs. It would therefore seem highly likely that there is a general ABC transporter mechanism but under evolutionary pressures has adapted specific characteristics for the different families, such as asymmetrical NBD function of the ABCC family.[8] An interesting question that arises from the asymmetric function of the ABCC family, which is catalytically active only at NBD2, is the need for retaining a redundant ATP-binding site at NBD1. What is the reasoning behind ABC transporters being made up of two NBDs? Perhaps, the formation of an NBD dimer helps amplify small conformational changes during ATP binding and hydrolysis as well as providing a structural base for a sequential process.[1] ATP hydrolysis at one site would drive the first stage of the process, followed by hydrolysis at the second site restoring the protein to its resting state. This alternating catalytic site mechanism was formulated by Alan Senior in his studies of Pgp and opened up the field for further research and led to the proposal of the new ATP Switch model by Higgins and Linton.[10,11] With many further research possibilities such as the overlooked D-Loop, which is thought to be involved in cooperativity between the NBDs, there is still much to discover and refine in the CFTR field.[21]

## Conclusion

There are many intriguing aspects of the CFTR gating mechanism that are as yet unanswered. These problems can be tackled head on by researching the CFTR protein or they can be approached indirectly by examining general mechanistic models of ABC proteins. The ATP Switch model has been one of the most promising approaches for understanding the gating of ABC transporters by looking at the wider picture of dimer formation and dissociation as underlying common factors of the mechanism. It has been useful to refer to other well-studied transporters, mainly HlyB and Pgp, with respect to the ATP Switch model in order to test various aspects of the mechanism. Finally, studying the mechanisms behind CFTR gating can help us better understand this fascinating although potentially lethal channel so as to improve existing and devise novel therapeutic approaches.

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