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Review

Comparative aspects of the function and mechanism of SUR1 and MDR1 proteins

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Abstract

ATP-binding cassette (ABC) superfamily proteins have divergent functions and can be classified as transporters, channels, and receptors, although their predicted secondary structures are very much alike. Prominent members include the sulfonylurea receptor (SUR1) and the multidrug transporter (MDR1). SUR1 is a subunit of the pancreatic β-cell K_{ATP} channel and plays a key role in the regulation of glucose-induced insulin secretion. SUR1 binds ATP at NBF1, and ADP at NBF2 and the two NBFs work cooperatively. The pore-forming subunit of the pancreatic β-cell K_{ATP} channel, Kir6.2, is a member of the inwardly rectifying K^+ channel family, and also binds ATP. In this article, we present a model in which the activity of the K_{ATP} channel is determined by the balance of the action of ADP, which activates the channel through SUR1, and the action of ATP, which stabilizes the long closed state by binding to Kir6.2. The concentration of ATP could also affect the channel activity through binding to NBF1 of SUR1. MDR1, on the other hand, is an ATP-dependent efflux pump which extrudes cytotoxic drugs from cells before they can reach their intracellular targets, and in this way confers multidrug resistance to cancer cells. Both NBFs of MDR1 can hydrolyze nucleotides, and their ATPase activity is necessary for drug transport. The interaction of SUR1 with nucleotides is quite different from that of MDR1. Variations in the interactions with nucleotides of ABC proteins may account for the differences in their functions.

Keywords: ABC protein; Sulfonylurea receptor SUR1; Multidrug transporter MDR; Transporter; K channel; ATP

Contents

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1. Introduction

Every cell is surrounded by a plasma membrane, which separates it from the outside world. To take up essential nutrients and to excrete metabolic waste products and environmental toxic substances, cells have developed ways of transferring materials across their membranes. To regulate intracellular ion concentrations, they have developed ways of transferring ions across their membranes. These transfer activities are handled by special membrane proteins, transporters and channels. Cells also have a family of receptor membrane proteins for transmitting signals from outside to inside for intercellular communication and for responding to the external environment.

The functions of these three types of membrane proteins, transporters, channels, and receptors, are quite different (Fig. 1). Transporters bind tightly to their specific substrates and transfer the bound substrates across the membrane coupled with conformational changes. Channel proteins, on the other hand, do not bind tightly to the transport ions. They undergo conformational changes to enter the conducting state, but ion transport is not coupled to these changes. Receptors transmit the information of ligand binding, but do not necessarily transport the ligands themselves. The most striking and interesting feature of the ATP-binding cassette (ABC) protein superfamily is that its members exhibit three different types of functions despite being very much alike in their predicted secondary structures.

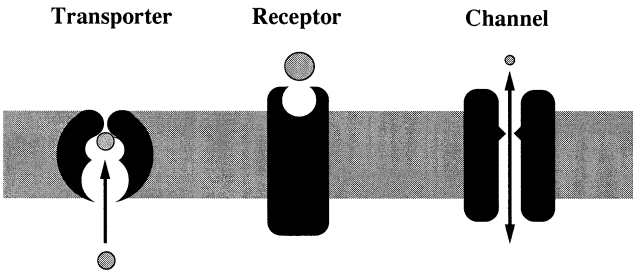

Fig. 1. Three types of functional membrane proteins.

The images of Fig. 2 represent transporters, channels, and receptors to show how these three groups of membrane proteins differ in their functions. MDR1/P-glycoprotein is an active transporter, in which ATP hydrolysis provides the free energy necessary to continue extruding chemotherapeutic drugs from cells like a water pump. Cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel, is like a pipe with a gate. ATP hydrolysis may allow the gate to open or close, but is not coupled to ion flow. Sulfonylurea receptor (SUR1) may function like a switch which regulates the K^+ channel. We have previously studied how ATP is used by functionally different ABC proteins SUR1 and MDR1. In this article, we describe working models of SUR1 and MDR1, with a brief description of techniques used to analyze them, to clarify their similarities and differences.

2. Regulation of β-cell K_{ATP} channels by adenine nucleotides and pharmacological agents

ATP-sensitive potassium (K_{ATP}) channels play many important roles in various tissues by linking the cell metabolic state to the membrane potential [1–3]. The K_{ATP} channels in pancreatic β-cells, for example, are critical in the regulation of glucose-induced insulin secretion [4–8]. Electrophysiological studies have provided clues to the complex control of K_{ATP} channels by ATP and ADP. In the current model (Fig. 3) of glucose-induced insulin secretion, glucose is transported through the glucose transporter and the subsequent metabolism of glucose in-

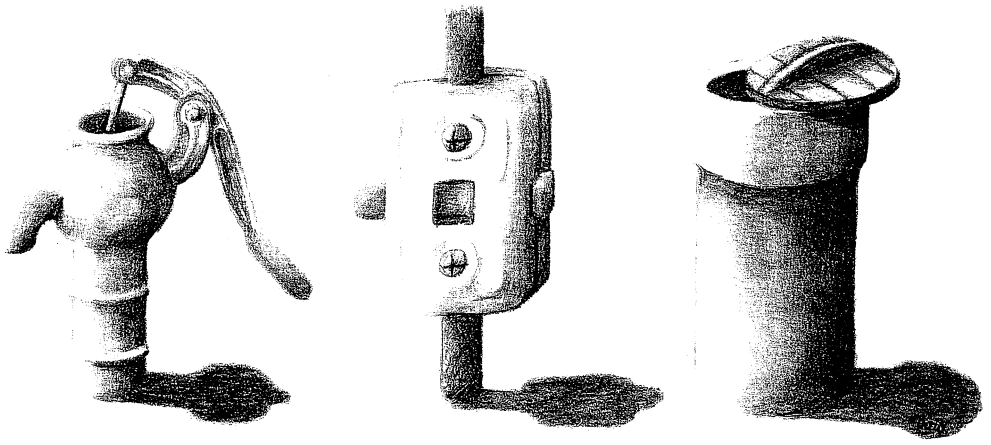

Fig. 2. Drawings representing the three types of ABC proteins.

creases ATP and concomitantly decreases ADP in pancreatic β-cells. The increase in the ATP/ADP ratio closes the K_{ATP} channels, which depolarizes the β-cell membrane, leads to the opening of voltage-dependent Ca^{2+} channels, and allows a Ca^{2+} influx into the β-cells. The resultant rise in intracellular Ca^{2+} concentration triggers insulin release. The pancreatic β-cell K_{ATP} channels are also regulated by important therapeutic pharmacological agents, such as sulfonylureas and K^+-channel openers. Sulfonylureas, widely used in the treatment of non-insulin dependent diabetes mellitus, stimulate insulin secretion by closing the K_{ATP} channels, while K^+-channel openers inhibit insulin secretion by opening the K_{ATP} channels [9].

The pancreatic β-cell K_{ATP} channel is a complex of two subunits [10,11]: SUR1 subunits of the ABC superfamily with two nucleotide binding folds (NBF1 and NBF2) and Kir6.2 subunits, of the inwardly rectifying K^+ channel family. SUR1 and Kir6.2 coassemble in a 4:4 stoichiometry to form K_{ATP} channels [12–14], and this fully assembled octameric stoichiometry is necessary for proper K_{ATP} channel gating [15]. Because mutation in either of the NBFs of SUR1 abolish MgADP-induced channel activation [14,16,17], SUR1 is thought to mediate the stimulatory effect of MgADP. SUR1 is also the primary target for the pharmacological agents, sulfonylureas, such as glibenclamide and K^+-channel openers, such as diazoxide [18,19]. The primary site of ATP inhibition of K_{ATP} channel activity appears to be in Kir6.2 [20,21]. However, the regulation of the β-cell K_{ATP} channels by adenine nucleotides and pharmacological agents is quite complex, even paradoxical. First, when membrane patches are excised into a nucleotide-free solution, the β-cell K_{ATP} channels can be observed. However, the current magnitude of the β-cell K_{ATP} channels slowly declines (run-down) after patch excision. This 'run-down' can be restored following exposure to MgATP [22], although ATP inhibits the β-cell K_{ATP} channels. MgATP actually activates K_{ATP} channels containing a mutation in the Kir6.2 subunit that impairs ATP inhibition (R50G) [23]. Second, although MgADP stimulates K_{ATP} channels, ADP shows inhibitory effects at high concentrations [24,25]. Third, although

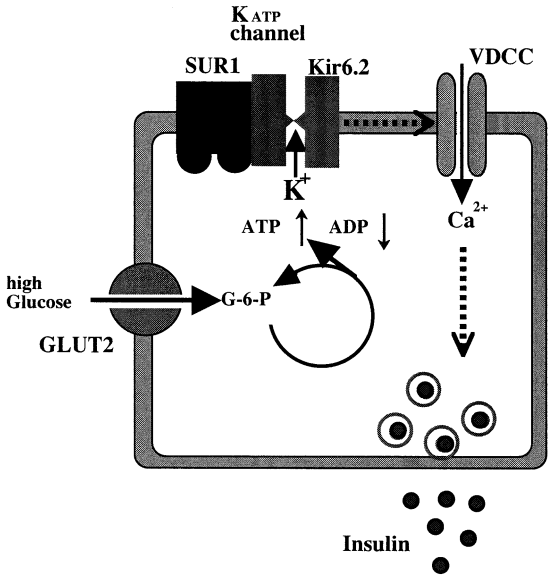

Fig. 3. A model for glucose-induced insulin secretion via the pancreatic β-cell K_{ATP} channel. GLUT2, glucose transporter 2; VDCC, voltage-dependent calcium channel.

the interaction of sulfonylureas with SUR1 abolishes the stimulatory effect of MgADP on K_{ATP} channels [20], the sensitivity of the K_{ATP} channel to sulfonylureas increases more in the presence of MgADP than in its absence [26–28].

3. Interaction of SUR1 with adenine nucleotides

SUR1 was transiently expressed in Cos-7 cells and examined to see if it could be photolabeled with 8-azido-[α-^{32}P]ATP. Surprisingly, SUR1 was efficiently photolabeled even in the absence of Mg^{2+} or vanadate [29]. This feature of ATP binding of SUR1 was quite different from that of MDR1 and MRP [30,31], because both vanadate and Mg^{2+} were required for the photoaffinity labeling of MDR1 and MRP. Binding of 8-azido-[α-^{32}P]ATP or 8-azido-[γ-^{32}P]ATP to SUR1 was apparently saturated at around 10 μM and then increased with increasing concentrations of 8-azido ATP. These results suggest that the bound nucleotide in SUR1 is ATP, and that SUR1 may have two ATP binding sites, one of high affinity and the other of low affinity. Analysis of ATP binding of mutants suggested that NBF1 is the high-affinity ATP binding site. 8-Azido-ATP was found to continue to bind to SUR1 stably in the presence of Mg^{2+} for more than 15 min at 0°C [32]. We expected that this strong and stable ATP binding to NBF1 might make it possible to investigate the interaction between the two NBFs of SUR1.

Two procedures, a 'preincubation procedure' and a 'postincubation procedure' were used, to analyze the interactions of SUR1 with adenine nucleotides (Fig. 4). First, the membrane proteins were *preincubated* with ADP at 0°C, and then allowed to react with 8-azido-[α-^{32}P]ATP in the presence of ADP ('preincubation procedure') [29]. With the preincubation procedure, ADP, in the presence of Mg^{2+}, strongly antagonized 8-azido-ATP binding. This inhibitory effect of MgADP was reduced by mutations in NBF2. ADP weakly antagonized 8-azido-ATP binding in the absence of Mg^{2+}. MgADP bound at NBF2 was assumed to facilitate MgADP binding at NBF1, thereby preventing 8-azido-ATP binding to NBF1 and two NBFs of SUR1 were suggested to work cooperatively. To further analyze this cooperative interaction between the two NBFs of SUR1, we used the 'postincubation procedure' (Fig. 4) [32]. In this procedure, membrane proteins were first incubated with 8-azido-[α-^{32}P]ATP at 37°C for 3 min, and free ligand was removed. Next, the membrane proteins were *postincubated* for 15 min in the presence or absence of unlabeled nucleotide, and irradiated with UV. With this procedure, we found that MgADP and MgATP stabilize prebound 8-azido-ATP binding, but it dissociates gradually at 37°C in the presence of Mg^{2+} alone. The effects of MgADP and MgATP on the stabilization of prebound 8-azido-ATP binding to SUR1 were concentration-dependent from 10 μM, and maximal effects were at 0.5 mM for both. The slowly hydrolysable ATP analog, ATP-γS, had no greater stabilizing effect than Mg^{2+} alone. Mutations in the Walker A and B motifs of NBF2 had almost no effect on the first 8-azido-ATP binding, but abolished the stabilizing effects of MgADP on 8-azido-ATP binding. These results suggest that MgADP, either by direct binding to NBF2 or hydrolysis of bound MgATP, very likely induces a conformational change at NBF2 which transduces another conformational change in NBF1 to stabilize ATP binding at NBF1. When SUR1, which binds with 8-azido-ATP at NBF1, was incubated with sulfonylurea glibenclamide in the presence of 0.5 mM MgADP or MgATP, photoaffinity labeling was reduced in a concentration-dependent manner [32], suggesting that glibenclamide modulates the cooperative interaction of the two NBFs of SUR1.

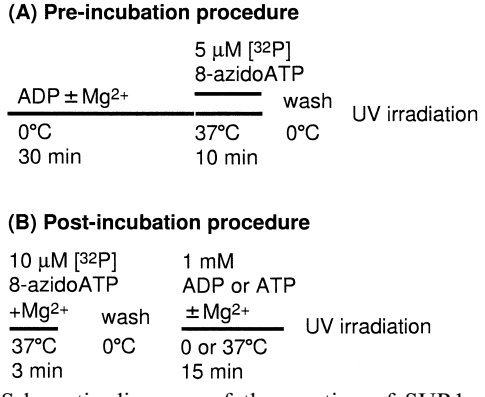

Fig. 4. Schematic diagrams of the reaction of SUR1 with nucleotides. (A) Preincubation procedure [29]. (B) Postincubation procedure [32].

4. Cooperative ATP binding/hydrolysis cycle model of SUR1

Based on these biochemical results, a model of the cooperative ATP binding/hydrolysis cycle of SUR1 was proposed (Fig. 5) [32]. In this model, SUR1 changes its conformation by binding ATP in NBF1 and ADP in NBF2. This state 1 is proposed to be the active form of SUR1. When the intracellular ADP concentration decreases, ADP dissociates from NBF2 (state 2). ADP interacts with NBF2 either by direct binding or hydrolysis of bound ATP (state 3). States 1 and 2 are states of equilibrium, and most of the SUR1 subunits will be in state 1 at high ADP concentration and in state 2 at low ADP concentrations. ADP dissociation from NBF2 leads to instability of ATP binding at NBF1, allowing the release of ATP (state 4). This dissociation of ATP from NBF1 may be involved in channel inactivation. Under physiological conditions, the intracellular ATP concentration is high enough, and ATP bound to NBF2 is readily hydrolyzed to ADP. Consequently, SUR1 is in either state 1, 2, or 3. According to this model, ATP binding at NBF1 is required for the active state of SUR1, and the intracellular concentration of ADP is the primary factor determining the activity of SUR1. Importantly, glibenclamide may convert an active state of SUR1 directly to the inactive state by dissociating ATP from NBF1. Glibenclamide causes release of ATP from NBF1 in cooperation with ADP at NBF2.

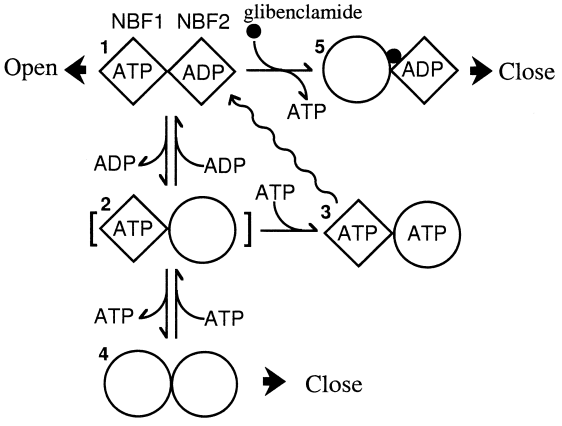

Fig. 5. Model for nucleotide activation of the K_{ATP} channel by SUR1 subunit. Nucleotide binding folds (NBFs) in the inactive state are depicted as circles, those with bound nucleotide and altered conformation as diamonds. State 1 is the 'active state' in which SUR1 potentiates channel activity. States 2 and 3 are transient, and states 4 and 5 are 'inactive states' in which SUR1 inhibits channel activity. The straight arrows indicate binding or unbinding reactions and the wavy arrows indicate a hydrolytic reaction. (Modified from [32].)

5. Working model of the function of the pancreatic β-cell K_{ATP} channel

The pore-forming subunit of pancreatic β-cell K_{ATP} channel, Kir6.2, is a member of the inwardly rectifying K^+ channel family (Fig. 6) [10,11]. Unlike most other Kir channels, expression of Kir6.2 alone does not produce functional channel activity; it requires coexpression with SUR1. Recently, it has been clearly shown that SUR1 and Kir6.2 contain a novel motif for endoplasmic reticulum retention/retrieval, and that this motif is required at multiple stages of the K_{ATP} channel assembly to restrict surface expression to fully assembled and correctly regulated octameric channels [15].

An isoform of Kir6.2, in which the last 26 amino acids including the motif for endoplasmic reticulum retention/retrieval have been removed (Kir6.2ΔC26), is capable of expressing functional K^+ channel activity in the absence of SUR1. Kir6.2ΔC26 retains sensitivity to inhibition by ATP and mutations in this subunit can significantly reduce the inhibitory effect of ATP [20,33]. Recently, the pore-forming subunit Kir6.2 was demonstrated to be directly labeled by 8-azido-[γ-^{32}P]ATP [34], although Kir6.2 has much lower affinity for 8-azido-ATP than SUR1. Mutations in the NH_2-terminus (R50G) and the COOH-

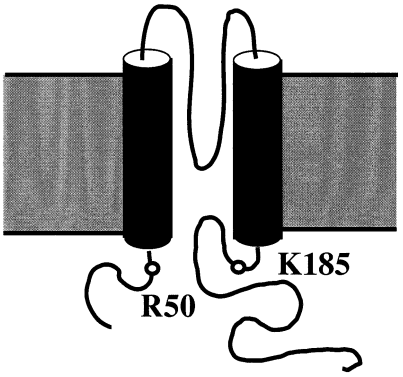

Fig. 6. Schematic showing of Kir6.2. Mutations of arginine-50 and lysine-185 reduce the inhibitory effect of ATP upon Kir6.2 channel activity and ATP binding [34].

terminus (K185Q) reduced photoaffinity labeling of Kir6.2 by more than 50% [34]. These mutations have been shown to reduce the inhibitory effect of ATP upon Kir6.2 channel activity [33]. These results demonstrate that ATP binds directly to Kir6.2 and that both the -NH_2 and -COOH terminal intracellular domains may influence ATP-binding.

Taking these results into consideration, we propose a working model for the function of the K_{ATP} channel (Fig. 7). When the intracellular ADP concentration is high enough, SUR1 cooperatively binds ATP in NBF1 and ADP in NBF2, and activates Kir6.2 to open K^+ channel. After meal consumption, the plasma glucose concentration rises. The increase in glucose metabolism raises the cellular ATP concentration and concomitantly lowers the cellular ADP concentration. The decrease in cellular ADP concentration causes dissociation of ADP from NBF2 of SUR1. ATP may bind to NBF2 after ADP dissociation because of the cellular high ATP concentration, but ATP bound to NBF2 would be readily hydrolyzed to ADP. Then ADP would dissociate from NBF2. ADP dissociation from NBF2 leads to instability of ATP binding at NBF1, allowing the release of ATP. As a result, SUR1 would not remain in the active state, when the cellular ADP concentration is low. With the rise in the cellular ATP concentration, Kir6.2 would remain longer in the ATP binding form. ATP binding to Kir6.2 is speculated to be involved in stabilization of the long closed state of K_{ATP} channel [33]. Therefore, we assume that the activity of the pancreatic β-cell K_{ATP} channel is determined by the balance of the action of ADP, which activates the channel through SUR1, and the action of ATP, which stabilizes the long closed state by binding to Kir6.2. The concentration of ATP could also affect the channel activity by binding to NBF1 of SUR1. SUR1 and Kir6.2 coassemble in a 4:4 stoichiometry to form K_{ATP} channels [12–14], and this fully assembled octameric stoichiometry is necessary for proper K_{ATP} channel gating [15]. Namely, there are four high-affinity ATP binding sites (NBF1), four MgADP binding sites (NBF2), and four low-affinity ATP binding sites (Kir6.2) in a functional K_{ATP} channel. Although it remains an open question how many ATPs and ADPs are necessary for binding to open and close the K_{ATP} channel, we assume that this octameric stoichiometry ensures fine-tuning of the K_{ATP} channel gating regulated by changes in cellular ATP and ADP concentrations.

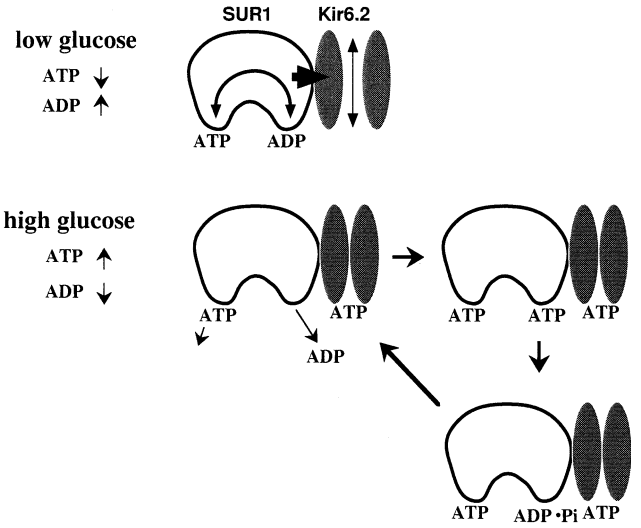

Fig. 7. A working model of the function of the pancreatic β-cell K_{ATP} channel. When intracellular ATP concentration is low and ADP concentration is high (low glucose), SUR1 cooperatively binds ATP in NBF1 and ADP in NBF2, and activates Kir6.2 to open K^+ channel. When intracellular ATP concentration is high and ADP concentration is low (high glucose), ADP dissociates from NBF2. ATP binds to NBF2 after ADP dissociation because of the cellular high ATP concentration, but ATP bound to NBF2 is readily hydrolyzed to ADP. Next, ADP becomes dissociated from NBF2, resulting in instability of the ATP binding at NBF1 and allowing ATP release. Thus, SUR1 does not remain in the active state. ATP binding to Kir6.2 stabilizes the long closed state of the K_{ATP} channel. The activity of the pancreatic β-cell K_{ATP} channel is determined by the balance ADP action, which activates the channel through SUR1, and ATP action, which stabilizes the long closed state by binding to Kir6.2. The concentration of ATP may also affect the channel activity through the binding to NBF1 of SUR1.

6. Interaction of MDR1 with ATP

MDR1 is an ATP-dependent efflux pump that extrudes cytotoxic drugs from cells before the drugs reach their intracellular targets, and in this way confers multidrug resistance to cancer cells [35–37]. It has been suggested that both NBFs of MDR1 can hydrolyze nucleotides, and their ATPase activity is necessary for drug transport [38–40]. Senior et al. have proposed that two NBFs are equivalent in the functioning of MDR1 [41]. Indeed, mutation of the

Walker A lysine residue of either NBF abolishes the ATPase activity of MDR1 and its ability to confer multidrug resistance [42–44]. The covalent modification of the cysteine residue in the Walker A motif of either NBF has also been shown to be sufficient to inactivate the ATPase activity of MDR1 [45–48]. However, some non-equivalent features between the two NBFs have been reported [48].

8-Azido-ATP binding with the wild-type MDR1 was inhibited by 100 μM NEM, while 8-azido-ATP binding with the C431A/C1074A mutant form was not, suggesting that the cysteines of Walker A motifs in both NBFs are responsible for the effects of NEM on ATP binding. 8-Azido-ATP binding of the C431A mutant form appeared not to be affected by treatment with 100 μM NEM. However, ATP-binding to the C1074A mutant form was significantly reduced by the same treatment, similar to the wild-type MDR1. These results suggest that NEM modification of NBF1 is responsible for the NEM inhibition of ATP binding in the wild-type protein. NEM modification of NBF1 may have an allosteric effect on ATP binding at NBF2, although that of NBF2 does not affect further ATP binding at NBF1. Alternatively, NBF1 might be the high-affinity ATP binding site in the wild-type MDR1. The effect of mutation of the Walker A lysine in one NBF was also reported to not be equivalent to that in the other NBF of MDR1 [48].

7. Similarities and differences in catalytic cycles of ABC proteins

Cooperative interaction of the two NBFs of ABC proteins has been shown for MDR1 [42–48], SUR1 [29,32], and CFTR [49,50]. In MDR1, if one NBF is non-functional, no ATP hydrolysis occurs even if the other functional NBF contains a bound nucleotide [48]. It is likely that a conformation induced by ATP binding at a regulatory NBF is necessary for ATP hydrolysis at a catalytic NBF. In the alternating catalytic sites cycle model proposed by Senior et al. [41], the two NBFs appear to be functionally equivalent, to alternate in catalysis, and one substrate may be transported by hydrolysis of one ATP. However, because the two NBFs are not necessarily equivalent as described above, the possibility remains that one substrate is transported by hydrolysis of two ATPs.

In CFTR, both of the NBFs appear to hydrolyze ATP and to alternate in catalysis [49,50], as those of MDR1. Cooperativity between the two NBFs is induced by phosphorylation of the regulatory domain by protein kinase A, which is also necessary for channel opening [50]. However, the roles of the two NBFs are predicted to be non-equivalent: NBF1 is involved in channel opening while NBF2 is involved in channel closing [49,50]. Therefore, one complete cycle of opening and closing of a single CFTR channel requires hydrolysis of two ATP molecules, one at each NBF. The catalytic cycle of CFTR appears to be very similar to that of MDR1. However, ATP hydrolysis would not provide the energy necessary for conformational changes of CFTR to transport ions. The tight binding of ATP is predicted to stabilize the channel's open or closed state, until that nucleotide is hydrolyzed, similar to the tight binding of GTP stabilizing the active conformation of a G-protein [51].

In SUR1, the roles of the two NBFs are predicted to be non-equivalent: SUR1 binds ATP strongly at NBF1 and MgADP at NBF2. MgADP interacts with NBF2, either by direct binding or hydrolysis of bound MgATP at NBF2. Similar to CFTR, cooperative nucleotide binding to NBFs would stabilize the active state of SUR1. Interestingly, the predicted active state of SUR1 binds ATP at NBF1 and ADP at NBF2, although, in CFTR, ADP binds at NBF1 and ATP binds at NBF2 [49,50]. We assume that NBF2 of SUR1 hydrolyzes ATP [32] (Matsuo, M. et al., submitted). Because mutations in NBF1 abolish channel activation by adenine nucleotides [14,16,17], NBF1 is also assumed to have ATPase activity [8]. It has also been reported that binding of potassium channel openers to SURs requires a conformational change induced by ATP hydrolysis in both NBFs [52]. However, we have not been able to obtain any data suggesting ATP hydrolysis at NBF1 under the conditions examined (Matsuo, M. et al., submitted). Because the interaction of SUR1 with adenine nucleotides is quite different from MDR1, we assume that SUR1 is not a transporter but a switch, like a G-protein, which regulates the Kir6.2 K^+ channel. Finally, we would like to underline the unique feature of SUR1 functioning

not only as a switch itself, but also monitoring changes in cellular nucleotide concentrations.

In conclusion, although the predicted secondary structures of the ABC superfamily proteins are very much alike, they diverge according to function into transporters, channels, and receptors. The interaction of SUR1 with nucleotides is quite different from that of MDR1. Variations in the interactions with nucleotides of ABC proteins may account for the differences in their functions.

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