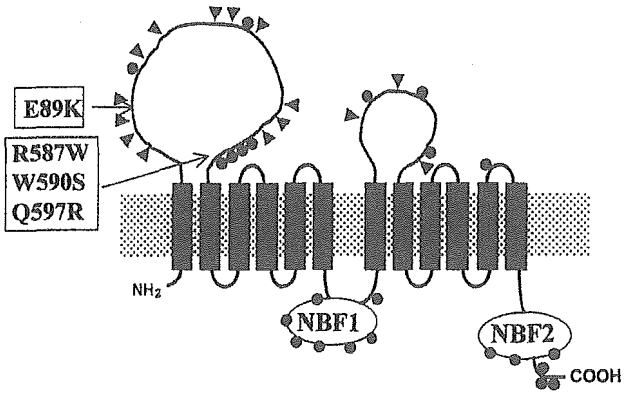


Fig. 2. Putative secondary structure of human ABCA1 and mutations that cause familial hypoalphalipoproteinemia and Tangier disease. ●, mutation site; ▲, putative glycosylation site

transported as low-density lipoprotein to peripheral cells. Cholesterol is not catabolized in the peripheral cells and therefore is mostly released and transported to the liver for conversion to bile acids to maintain cholesterol homeostasis. The assembly of HDL particles by helical apolipoproteins with cellular lipid is the major mechanism of cellular cholesterol release.[52,53] The importance of this active cholesterol-releasing pathway became apparent with the finding that it is impaired in cells from patients with Tangier disease.[7,8]

Tangier Island is located in Chesapeake Bay in Virginia, USA. Dr. Frederickson of the NIH discovered that a 5-year-boy living on the island had unusually large orange tonsils and virtually no HDL in the plasma in 1964.[54] More than 30 years after the discovery, mutations were identified in *ABCA1* of Tangier disease (TD) patients.[55–57] Mutations mapped to the *ABCA1* gene in patients with familial hypoalphalipoproteinemia (FHA) and TD seem to be clustered in several regions of the ABCA1 protein, such as the first extracellular domain (ECD1), the first nucleotide-binding fold (NBF1), the linker region, the second extracellular domain, and the C-terminal region (Fig. 2).

The primary substrates and mechanism of action of ABCA1 have not been precisely defined. The current model of ABCA1 functionality is based on the following.

1. Topological models. The current consensus topological model predicts two large extracellular loops between TM1 (amino acids 23–44) and TM2 (amino acids 642–660) and TM7 (amino acids 1346–1368) and TM8 (amino acids 1655–1677) (Fig. 2). It is supported by computer modeling,[58] tag insertion,[58] antibody reactivity,[58] and glycosylation analysis.[58–60] These large extracellular domains are unique to the ABCA subfamily, and other ABC proteins, such as MDR1, do not have them. We also predicted that the first hydrophobic segment serves as a signal peptide.[58] This idea is based on an estimation with the algorithm SignalP[61] and on the inability to detect an HA epitope fused to the N-terminus of ABCA1 by Western blotting.[58] However, the EGFP–ABCA1 fusion construct functioned without being processed by signal peptidase.[59] It is not clear yet if the segment serves as a signal peptide or a signal anchor sequence.
2. Subcellular localization of ABCA1–GFP. The subcellular distribution of ABCA1 was studied using a GFP-fused protein.[48,62] ABCA1–GFP was localized not only to the plasma membrane but also to the intracellular compartments. It was colocalized partly with a marker for the Golgi apparatus, with a marker for early endosomes, and with lysotracker, a marker for acidic compartments. When the cells were treated with monensin, which prevents delivery of protein from endosomes to the cell surface, after the blocking of new protein synthesis, the ABCA1–GFP on the cell surface decreased and the vesicular distribution increased. These results suggested that newly synthesized ABCA1–GFP was first delivered to the plasma membrane through the endoplasmic reticulum (ER) and Golgi and then shuttled rapidly between the plasma membrane and intracellular vesicles. Fluorescent apoA-I was accumulated into ABCA1–GFP-containing endosomes[63] that shuttled between late endosomes and the cell surface.[64] In late endocytic vesicles of cells from TD patients, both cholesterol and sphingomyelin were accumulated and massive amounts of NPC1 were retained. These late endocytic trafficking defects were corrected by adenovirally mediated ABCA1–GFP expression.[64]

   In polarized intestine[65] and liver cells,[66] ABCA1 is mainly expressed on the basolateral surface, suggesting an important role for ABCA1 in the absorption of dietary cholesterol from intestine[67] and for hepatocyte ABCA1 in the regulation of the levels of plasma HDL.[66]
3. ApoA-I dependence of ABCA1-mediated HDL formation. HEK293 cells stably expressing human ABCA1 show apoA-I-mediated release of cholesterol and choline phospholipids.[58,59,62] Without the addition of apoA-I, virtually no release of lipids is observed compared to the nontransfected HEK293 cells. ApoA-I is supposed to interact with ABCA1 directly, because ApoA-I retards calpain-mediated degradation of ABCA1 at the cell surface[68,69] and because apoA-I can be cross-linked with ABCA1.[46,47,70] Interaction with apoA-I could be required to allow ABCA1 to internalize from the plasma membrane to endocytic compartments. Takahashi and Smith[71] first reported that cellular cholesterol efflux involves endocytosis and resecretion of apoA-I. However, it is not yet clear that ABCA1 and apoA-I are internalized obligatorily together to early and late endocytic compartments after interaction on the cell surface.
4. Functional analysis of TD mutants. Many mutations in patients with TD and FHA have been identified in ECD1 of ABCA1, and three mutations (R587W, W590S, Q597R) cluster amino acids 587 to 597[46,48,72] (see Fig. 2). When these three TD mutations were introduced into ECD1 of ABCA1-GFP and the mutants were transiently or stably expressed in HEK293, R587W and Q597R appeared to be distributed mainly in the ER and not the plasma membrane. In contrast, the W590S mu-

tant was localized to the plasma membrane to the same extent as the wild type. Immunostaining with the antibody against ECD1 confirmed the proper orientation of W590S. However, the apoA-I-mediated release of cholesterol and choline phospholipids from cells expressing W590S was as low as that of the other two mutants and less than 10% of that in cells expressing the wild-type ABCA1. The W590S mutation does not have any apparent effect on ATP hydrolysis or the interaction between apoA-I and ABCA1. Analyses of W590S should facilitate our understanding of the mechanism of HDL formation.

5. ATP hydrolysis by ABCA1. We previously showed that ABCA1 is able to bind ATP and hydrolyze it using crude membrane of HEK293 cells stably expressing human ABCA1.[48] Recently, we expressed human ABCA1 in insect Sf9 cells and succeeded in purifying it (Takahashi et al., in preparation). The purified detergent-soluble ABCA1 showed significant ATPase activity, and the activity was stimulated by the addition of phospholipids. The Walker A lysine mutation K939M in the first nucleotide-binding fold, resulting in a defect in HDL formation, impaired ATPase activity. ATP hydrolysis is suggested to be involved in the apoA-I-dependent secretion of phospholipids and cholesterol.

Model for ABCA1-mediated HDL formation

Figure 3 is our working model of the action of ABCA1. The lipid-free apoA-I interacts with the extracellular domain of ABCA1. ABCA1–apoA-I complexes are internalized into early endosomes and can either recycle back to the plasma membrane or move to late endocytic compartments. ABCA1 likely loads phospholipid and cholesterol onto apoA-I in a manner dependent on ATP hydrolysis. However, it is not clear yet where this "lipidation of apoA-I" occurs. As described later, when human ABCA3, predominantly expressed in alveolar type II cells, was expressed in HEK293 cells, it localized to the intracellular vesicle membrane, and ABCA3 is involved in accumulating phospholipids and cholesterol in the vesicles.[73] ABCA1 may function as ABCA3 to accumulate phospholipids and cholesterol in intracellular compartments, in which apoA-I–lipid complexes are generated. A specific environment could be also required for the generation of the nascent HDL. ABCA1 in late endosomes may make it possible to remove the excess endocytosed LDL-derived cholesterol from the cell and likely plays a critical role in the macrophage, where unregulated uptake of oxidized LDL triggers foam cell formation. The "lipidated" apoA-I in intracellular compartments moves to the cell surface and is released as the nascent HDL particle. Alternatively, the nascent HDL particles may be generated either on the cell surface or in early or late endocytic compartments. Each nascent HDL particle could be generated from different pools of lipids. In any case, we speculate that ABCA1 functions as a transporter of lipids as well as a receptor for apoA-I.

Transcriptional and posttranscriptional regulation of ABCA1

The hyperaccumulation of cholesterol is harmful to cells, but at the same time cholesterol is indispensable for cells. Therefore, the expression of ABCA1 is highly regulated at both the transcriptional and posttranscriptional level. The transcription of ABCA1 is regulated by the intracellular oxysterol concentration via the LXR/RXR nuclear receptor,[74] and the turnover of ABCA1 protein occurs rapidly with a half-life of 1–2 h[68,69,75] to avoid depletion of cholesterol.

Some TD mutations are clustered at the C-terminal region of ABCA1,[76] and one mutation, which was identified in a TD patient and was predicted to delete the last 46 amino acids, impaired the function of ABCA1.[77] Furthermore, the C-terminal region contains amino acid sequences well conserved among ABCA subfamily proteins. Therefore, this region was expected to be functionally important. Proteins that interact with this region were screened using the yeast two-hybrid method and three PDZ-binding proteins, α_1- and β_2-syntrophin and Lin7, were isolated as candidate proteins interacting with ABCA1.[78,79] α_1-Syntrophin is mainly expressed in brain, skeletal muscle, and heart in mice, and indeed mouse ABCA1 was coimmunoprecipitated with α_1-syntrophin from mouse brain.[79] This interaction was confirmed to be specific, because ABCA1 was not precipitated from the brain of α_1-*Syn*$^{-/-}$ mice.

Coexpression of α_1-syntrophin in HEK293 cells retarded degradation of ABCA1 and made the half-life of ABCA1 five times longer than in the cells not expressing α_1-syntrophin. This effect is not common among PDZ-containing proteins interacting with ABCA1, because Lin7 did not have a significant effect on the half-life of ABCA1. Coexpression of α_1-syntrophin significantly increased the apoA-I-mediated release of cholesterol.[79] These results suggest that cellular proteins, including α_1-syntrophin, may be involved in intracellular signaling, which determines the stability of ABCA1 and modulates cellular cholesterol release.

There may be other motifs in the C-terminal region of ABCA1 that are involved in the regulation. A novel, highly conserved motif (VFVNFA) was reported to be required for lipid efflux.[80] Alteration of this motif, which is present in some but not all members of the ABCA family, did not prevent trafficking of the transporter to the plasma membrane but did eliminate its binding of apoA-I. When a peptide containing the VFVNFA sequence was introduced into ABCA1-expressing cells, ABCA1-mediated lipid efflux was markedly inhibited. It is not clear yet that this motif participates in novel protein–protein interactions.

Phosphorylation seems to play a role in controlling ABCA1 protein levels and activity. Interaction with lipid-free apoA-I stabilizes ABCA1 against degradation by calpain and increases its protein level in cells. Two protein kinases, protein kinase C (PKC) and protein kinase A (PKA), are reported to play an important role in the apoA-I-induced stabilization of ABCA1. It is suggested that the removal of sphingomyelin by lipid-free apoA-I in generat-

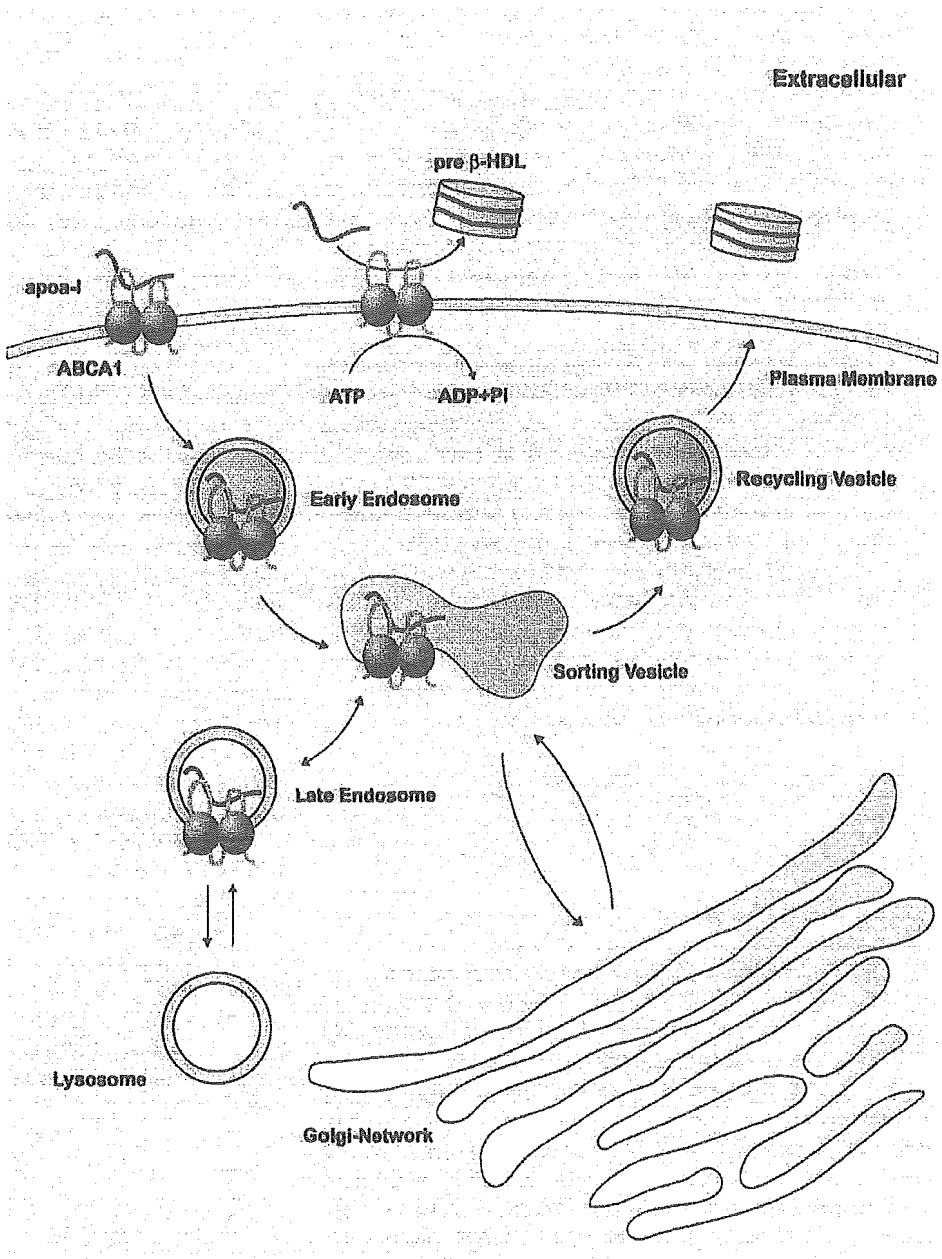


Fig. 3. Model for ABCA1-mediated high-density lipoprotein (HDL) formation

ing new HDL generates diacylglycerol by a replenishment reaction, and diacylglycerol activates PKC, which in turn phosphorylates and stabilizes ABCA1.[81] Peptides with an amphipathic helical structure that are competent in promoting lipid efflux from cells also promote the phosphorylation and stabilization of ABCA1.[82] ABCA1 contains a cytosolic PEST sequence that, when phosphorylated, directs calpain-mediated proteolysis of ABCA1.[83] The interaction of apolipoproteins with cells prevents this phosphorylation of PEST and thus stabilizes ABCA1. ApoA-I binding is also suggested to induce intracellular cAMP release, which leads to the phosphorylation of ABCA1 via a PKA-dependent mechanism,[84] and PKA-mediated phosphorylation of ABCA1 may be essential for optimal lipid transport activity.[85] It is also reported that apoA-I stimulates the autophosphorylation of protein-tyrosine kinase Janus kinase 2 (JAK2), which in turn activates a process that enhances apolipoprotein interactions with ABCA1 and lipid removal from cells.[86] However, it is not clear if JAK2 phosphorylates ABCA1 directly.

ABCA7 is also involved in HDL formation

ABCA7 is highly homologous to ABCA1. This gene product was first reported as a human sterol-sensitive ABC transporter.[87] While investigating the functional importance of ECD1 of ABCA1, we discovered that the amino acid sequence of autoantigen SS-N, an epitope of Sjögren's syndrome, is highly homologous to a part of the ECD1 of ABCA1, and isolated a ABC protein gene coding for autoantigen SS-N,[58] which was *ABCA7*.

ABCA7 induces an apolipoprotein-mediated assembly of cholesterol-containing HDL similar to that induced by ABCA1.[51,88] ABCA7 protein was detected with a polyclonal antiserum when macrophages were incubated with AcLDL[87] as ABCA1. However, ABCA7 is expressed in a rather tissue-specific manner compared to ABCA1, and mRNA of ABCA7 is predominantly found in myelolymphatic tissues (bone marrow, lymph node, spleen, thymus, and peripheral blood cells) and in brain and trachea.[87,88] Alternative splicing could be involved in the posttranscriptional regulation of the expression and function of human ABCA7.[88] The human *ABCA7* gene produces at least two types of splicing variants in a tissue-specific manner, and type II mRNA, which does not produce a functional ABCA7, was detected rather abundantly in many tissues.[88] We have failed to detect ABCA7 protein in human peripheral blood cells, although poly A^+ RNA was detected by Northern blot hybridization. There was a report that human ABCA7 is highly expressed in keratinocytes and upregulated during differentiation.[89] Mouse ABCA7 protein is expressed in spleen, lung, adrenal, and brain[87] whereas rat ABCA7 protein is expressed specifically in platelets and red blood cells.[90] Mouse ABCA7 was reported to promote the apoA-I-dependent release of phospholipids, but not cholesterol,[91] although human ABCA7 mediates the apoA-I-dependent release of cholesterol and phospholipids.[51,88] It is worth examining the difference in physiological function between human and mouse ABCA7.

ABCA3 and pulmonary surfactant secretion

ABCA3 is predominantly expressed in lung[92,93] and localized to the limiting membrane of lamellar bodies in alveolar type II cells in humans and rats.[94,95] Recently it was revealed that *ABCA3* gene mutations cause a fatal deficiency of surfactant in newborns.[96] The lamellar body is a member of lysosome-related organelles, in which pulmonary surfactant is stored. Lamellar bodies are secreted into the alveolar space by exocytosis, and secreted pulmonary surfactant coats the lumen of alveoli, where it reduces the surface tension at the alveolar air–liquid interface, thus preventing alveoli from collapsing and reducing the workload of breathing. Pulmonary surfactant is composed of lipids (90%) and surfactant proteins (SP-A, SP-B, SP-C, and SP-D), which are densely packed into multilamellar structures. The most abundant lipid in pulmonary surfactant is phosphatidylcholine, especially dipalmitolyphosphatidylcholine. The mechanism by which lipids are packed into lamellar bodies is unknown.

When expressed in HEK293 cells, human ABCA3 localized to the intracellular vesicle membrane.[73] The diameters of the vesicles observed in HEK293/hABCA3, where ABCA3 was expressed, were about 1 μm, corresponding to those of lamellar bodies. ABCA3 is efficiently labeled by 8-azido-[α-^{32}P]ATP, but not by 8-azido-[γ-^{32}P]ATP, when the membrane fraction is incubated in the presence of orthovanadate, indicating that ABCA3 shows strong ATPase activity in the isolated membrane. Photoaffinity labeling was largely reduced by membrane pretreatment with 5% methyl-β-cyclodextrin (MβCD), which depletes cholesterol. Therefore, cholesterol would be the first candidate of a transport substrate for ABCA3. Indeed, a filipin–

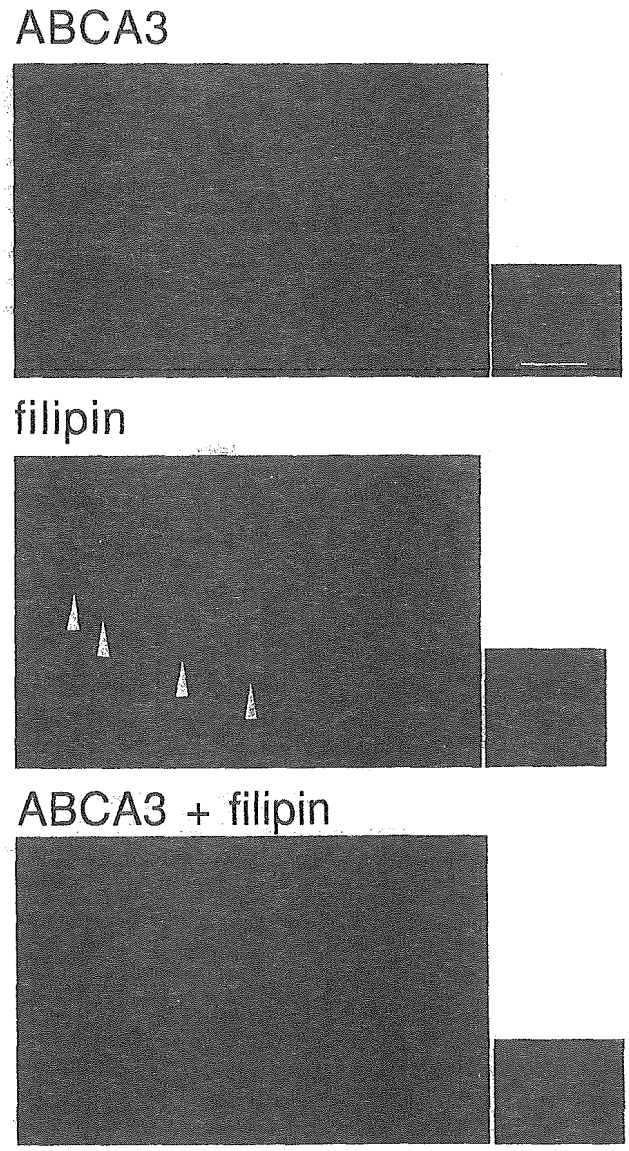


Fig. 4. *Filipin* staining of HEK293/hABCA3 cells. *A3 + filipin*, overlaid image of *ABCA3* fluorescence and *filipin* staining. Bar 1 μm

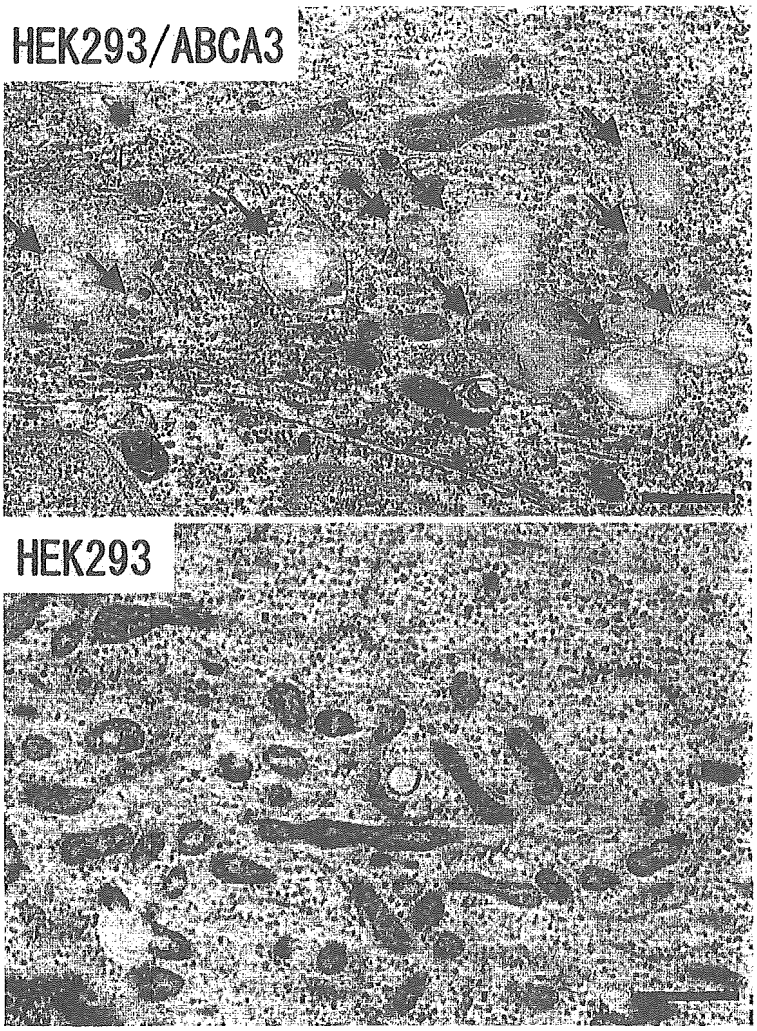


Fig. 5. Electron micrograph of *HEK293/hABCA3* cells (*top*) and *HEK293* host cells (*bottom*). Lamellar body-like structures are indicated by *arrows*. Modified from Nagata et al.[73] Bar 1 μm

cholesterol complex was preferentially detected in the vesicular structures, in which ABCA3 is located, when HEK293/hABCA3 cells were stained with filipin (Fig. 4). However, MβCD might deplete phospholipids together with cholesterol. Because dipalmitolyphosphatidylcholine, the most abundant lipid in pulmonary surfactant, has high affinity for cholesterol, it is possible that phospholipids with saturated fatty acid chains are depleted from the HEK293 cell membrane together with cholesterol. This might cause the suppression of vanadate-induced nucleotide trapping in ABCA3.

Electron micrographs show that HEK293/hABCA3 cells contain multivesicular, lamellar body-like structures, which do not exist in HEK293 host cells (Fig. 5). Some fuzzy components such as lipids accumulate in the vesicles.[73] These results suggest that ABCA3 shows ATPase activity, which is induced by lipids, and may be involved in accumulating phospholipids and cholesterol in lamellar bodies and in generating multivesicular structures.

Conclusion

Among 49 human ABC proteins, there still exist many whose physiological functions or endogenous substrates are unclear. Even though we know the human disease phenotype with which the dysfunction of ABC proteins is associated, it is difficult to define the primary substrates and mechanism of action of ABC proteins, because most of their substrates are lipophilic and their binding affinity for ABC proteins is quite low, and because the substrate specificity is often quite low also. The regulation of their expression and functions is sophisticated and thus very complicated. However, ABC proteins play critical roles in the homeostasis of lipids and other metabolites, making them important therapeutic targets. Basic biochemical studies as well as cellular level and animal model studies will improve our understanding of the physiological roles of ABC proteins.

References

1. Chen C, Chin JE, Ueda K, Clark DP, Pastan I, Gottesman MM, Roninson IB (1986) Internal duplication and homology with bacterial transport proteins in the *mdr1* (P-glycoprotein) gene from multidrug-resistant human cells. Cell 47:381–389
2. Gros P, Niriah YB, Croop JM, Housman DE (1986) Isolation and expression of a complementary DNA that confers multidrug resistance. Nature (Lond) 323:728–731
3. Ueda K, Cardarelli C, Gottesman MM, Pastan I (1987) Expression of full-length cDNA for the human "MDR1" gene confers resistance to colchicine, doxorubicin, and vinblastine. Proc Natl Acad Sci USA 84:3004–3008
4. Riordan JR, Deuchars K, Kartner N, Alon N, Trent J, Ling V (1985) Amplification of P-glycoprotein genes in multidrug-resistant mammalian cell lines. Nature (Lond) 316:817–819
5. Cole SP, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AM, Deeley RG (1992) Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science 258:1650–1654
6. Smit JJ, Schinkel AH, Oude Elferink RP, Groen AK, Wagenaar E, van Deemter L, Mol CA, Ottenhoff R, van der Lugt NM, van Roon MA, van der Valk MA, Offerhaus GJA, Berns AJM, Borst P (1993) Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. Cell 75:451–462
7. Francis GA, Knopp RH, Oram JF (1995) Defective removal of cellular cholesterol and phospholipids by apolipoprotein A-I in Tangier disease. J Clin Invest 96:78–87
8. Remaley AT, Schumacher UK, Stonik JA, Farsi BD, Nazih H, Brewer HB Jr (1997) Decreased reverse cholesterol transport from Tangier disease fibroblasts. Acceptor specificity and effect of brefeldin on lipid efflux. Arterioscler Thromb Vasc Biol 17: 1813–1821
9. Welsh MJ, Anderson MP, Rich DP, Berger HA, Denning GM, Ostedgaard LS, Sheppard DN, Cheng SH, Gregory RJ, Smith AE (1992) Cystic fibrosis transmembrane conductance regulator: a chloride channel with novel regulation. Neuron 8:821–829
10. Riordan JR (1993) The cystic fibrosis transmembrane conductance regulator. Annu Rev Physiol 55:609–630
11. Gadsby DC, Nagel G, Hwang TC (1995) The CFTR chloride channel of mammalian heart. Annu Rev Physiol 57:387–416
12. Tsui LC, Buchwald (1991) Biochemical and molecular genetics of cystic fibrosis. Adv Hum Genet 20:153–266, 311–312
13. Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JPI, Boyd AEI, Gonzalez G, Herrera-Sosa H, Nguy K, Bryan J, Nelson DA (1995) Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. Science 268:423–426
14. Ueda K, Matsuo M, Tanabe K, Kioka N, Amachi T (1999) Comparative aspects of the function and mechanism of SUR1 and MDR1 proteins. Biochim Biophys Acta 1461:305–313
15. Matsuo M, Tanabe K, Kioka N, Amachi T, Ueda K (2000) Different binding properties and affinities for ATP and ADP among sulfonylurea receptor subtypes, SUR1, SUR2A, and SUR2B. J Biol Chem 275:28757–28763
16. Shitan N, Bazin I, Dan K, Obata K, Kigawa K, Ueda K, Sato F, Forestier C, Yazaki K (2003) Involvement of CjMDR1, a plant MDR-type ABC protein, in alkaloid transport in *Coptis japonica*. Proc Natl Acad Sci USA 100:751–756
17. Hyde SC, Emsley P, Hartshorn MJ, Mimmack MM, Gileadi U, Pearce SR, Gallagher MP, Gill DR, Hubbard RE, Higgins CF (1990) Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport. Nature (Lond) 346:362–365
18. Smith CA, Rayment I (1996) Active site comparisons highlight structural similarities between myosin and other P-loop proteins. Biophys J 70:1590–1602
19. Hrycyna CA, Ramachandra M, Germann UA, Cheng PW, Pastan I, Gottesman MM (1999) Both ATP sites of human P-glycoprotein are essential but not symmetric. Biochemistry 38: 13887–13899
20. Takada Y, Yamada K, Taguchi Y, Kino K, Matsuo M, Tucker SJ, Komano T, Amachi T, Ueda K (1998) Non-equivalent cooperation between the two nucleotide-binding folds of P-glycoprotein. Biochim Biophys Acta 1373:131–136
21. Urbatsch IL, Beaudet L, Carrier I, Gros P (1998) Mutations in either nucleotide-binding sites of P-gycoptotein (Mdr3) prevent vanadate trapping of nucleotide at both sites. Biochemistry 37: 4592–4602
22. Sauna ZE, Muller M, Peng XH, Ambudkar SV (2002) Importance of the conserved Walker B glutamate residues, 556 and 1201, for the completion of the catalytic cycle of ATP hydrolysis by human P-glycoprotein (ABCB1). Biochemistry 41:13989–14000
23. Payen LF, Gao M, Westlake CJ, Cole SP, Deeley RG (2003) Role of carboxylate residues adjacent to the conserved core Walker B motifs in the catalytic cycle of multidrug resistance protein 1 (ABCC1). J Biol Chem 278:38537–38547
24. Locher KP, Lee AT, Rees DC (2002) The *E. coli* BtuCD structure: a framework for ABC transporter architecture and mechanism. Science 296:1091–1098
25. Jones PM, George AM (1999) Subunit interactions in ABC transporters: towards a functional architecture. FEMS Microbiol Lett 179:187–202
26. Smith PC, Karpowich N, Millen L, Moody JE, Rosen J, Thomas PJ, Hunt JF (2002) ATP binding to the motor domain from an ABC transporter drives formation of a nucleotide sandwich dimer. Mol Cell 10:139–149
27. Chen J, Lu G, Lin J, Davidson AL, Quiocho FA (2003) A tweezers-like motion of the ATP-binding cassette dimer in an ABC transport cycle. Mol Cell 12:651–661
28. Dork T, Mekus F, Schmidt K, Bosshammer J, Fislage R, Heuer T, Dziadek V, Neumann T, Kalin N, Wulbrand U, et al. (1994) Detection of more than 50 different CFTR mutations in a large group of German cystic fibrosis patients. Hum Genet 94:533–542
29. Hashimoto K, Uchiumi T, Konno T, Ebihara T, Nakamura T, Wada M, Sakisaka S, Maniwa F, Amachi T, Ueda K, Kuwano M (2002) Trafficking and functional defects by mutations of the ATP-binding domains in MRP2 in patients with Dubin-Johnson syndrome. Hepatology 36:1236–1245
30. Rosenberg MF, Velarde G, Ford RC, Martin C, Berridge G, Kerr ID, Callaghan R, Schmidlin A, Wooding C, Linton KJ, Higgins CF (2001) Repacking of the transmembrane domains of P-glycoprotein during the transport ATPase cycle. EMBO J 20: 5615–2625
31. Rosenberg MF, Kamis AB, Callaghan R, Higgins CF, Ford RC (2003) Three-dimensional structures of the mammalian multidrug resistance P-glycoprotein demonstrate major conformational changes in the transmembrane domains upon nucleotide binding. J Biol Chem 278:8294–8299
32. Higgins CF, Gottesman MM (1992) Is the multidrug transporter a flippase? Trends Biochem Sci 17:18–21
33. Ruetz S, Gros P (1994) Functional expression of P-glycoproteins in secretory vesicles. J Biol Chem 269:12277–12284
34. Smit JJM, Schinkel AH, Oude Elferink RPJ, Groen AK, Wagenaar E, van Deemter L, Mol CAM, Ottenhoff R, van der Lugt NMT, van Roon MA, van der Valk MA, Offerhaus GJA, Berns AJM, Borst P (1993) Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. Cell 75:451–462
35. De Vree JM, Ottenhoff R, Bosma PJ, Smith AJ, Aten J, Oude Elferink RP (2000) Correction of liver disease by hepatocyte transplantation in a mouse model of progressive familial intrahepatic cholestasis. Gastroenterology 119:1720–1730
36. Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH (1999) Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. Cell 98:13–23
37. Allikmets R, Shroyer N, Singh N, Seddon J, Lewis R, Bernstein P, Peiffer A, Zabriskie N, Li Y, Hutchinson A, Dean M, Lupski J, Leppert M (1997) Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. Science 277: 1805–1807
38. Imanaka T, Aihara K, Takano T, Yamashita A, Sato R, Suzuki Y, Yokota S, Osumi T (1999) Characterization of the 70-kDa peroxisomal membrane protein, an ATP binding cassette transporter. J Biol Chem 274:11968–11976

39. Tanaka AR, Tanabe K, Morita M, Kurisu M, Kasiwayama Y, Matsuo M, Kioka N, Amachi T, Imanaka T, Ueda K (2002) ATP binding/hydrolysis by and phosphorylation of peroxisomal ABC proteins PMP70 (ABCD3) and ALDP (ABCD1). J Biol Chem 277:40142–40147
40. Gerloff T, Stieger B, Hagenbuch B, Madon J, Landmann L, Roth J, Hofmann AF, Meier PJ (1998) The sister of P-glycoprotein represents the canalicular bile salt export pump of mammalian liver. J Biol Chem 273:10046–10050
41. Bodzioch M, Orso E, Klucken J, Langmann T, Bottcher A, Diederich W, Drobnik W, Barlage S, Buchler C, Porsch-Ozcurumez M, Kaminski WE, Hahmann HW, Oette K, Rothe G, Aslanidis C, Lackner KJ, Schmitz G (1999) The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat Gen 22:347–351
42. Brooks-Wilson A, Marcil M, Clee SM, Zhang L-H, Roomp K, van Dam M, Yu L, Brewer C, Collins JA, Molhuizen HOF, Loubser O, Ouelette BFF, Fichter K, Ashbourne-Excoffon KJD, Sensen CW, Scherer S, Mott S, Denis M, Martindale D, Frohlich J, Morgan K, Koop B, Pimstone S, Kastelein JJP, Genest J Jr, Hayden MR (1999) Mutations in ABC 1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet 22:336–345
43. Marcil M, Brooks-Wilson A, Clee SM, Roomp K, Zhang LH, Yu L, Collins JA, van Dam M, Molhuizen HO, Loubster O, Ouellette BF, Sensen CW, Fichter K, Mott S, Denis M, Boucher B, Pimstone S, Genest J Jr, Kastelein JJ, Hayden MR (1999) Mutations in the ABC1 gene in familial HDL deficiency with defective cholesterol efflux. Lancet 354:1341–1346
44. Remaley AT, Rust S, Rosier M, Knapper C, Naudin L, Broccardo C, Peterson KM, Koch C, Arnould I, Prades C, Duverger N, Funke H, Assman G, Dinger M, Dean M, Chimini G, Santamarina-Fojo S, Fredrickson DS, Denefle P, Brewer HB Jr (1999) Human ATP-binding cassette transporter 1 (ABC1): genomic organization and identification of the genetic defect in the original Tangier disease kindred. Proc Natl Acad Sci USA 96:12685–12690
45. Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette J-C, Deleuze J-F, Brewer HB, Duverger N, Denefle P, Assmann G (1999) Tangier disease is caused by mutations in the gene encoding ATP binding-cassette transporter 1. Nat Genet 22:352–355
46. Fitzgerald ML, Morris AL, Rhee JS, Andersson LP, Mendez AJ, Freeman MW (2002) Naturally occurring mutations in ABCA1's largest extracellular loops can disrupt its direct interaction with apolipoprotein A-I. J Biol Chem 277:33178–33187
47. Rigot V, Hamon Y, Chambenoit O, Alibert M, Duverger N, Chimini G (2002) Distinct sites on ABCA1 control distinct steps required for cellular release of phospholipids. J Lipid Res 43: 2077–2086
48. Tanaka AR, Abe-Dohmae S, Ohnishi T, Aoki R, Morinaga G, Okuhira KI, Ikeda Y, Kano F, Matsuo M, Kioka N, Amachi T, Murata M, Yokoyama S, Ueda K (2003) Effects of mutations of ABCA1 in the first extracellular domain on subcellular trafficking and ATP binding/hydrolysis. J Biol Chem 278:8815–8819
49. Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, Kwiterovich P, Shan B, Barnes R, Hobbs HH (2000) Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 290:1771–1775
50. Ikeda Y, Abe-Dohmae S, Munehira Y, Aoki R, Kawamoto S, Furuya A, Shitara K, Amachi T, Kioka N, Matsuo M, Yokoyama S, Ueda K (2003) Post-transcriptional regulation of human ABCA7 and its function for the apoA-I-dependent lipid release. Biochem Biophys Res Commun 311:313–318
51. Abe-Dohmae S, Ikeda Y, Matsuo M, Hayashi M, Okuhira K, Ueda K, Yokoyama S (2004) Human ABCA7 supports apolipoprotein-mediated release of cellular cholesterol and phospholipid to generate high density lipoprotein. J Biol Chem 279:604–611
52. Hara H, Yokoyama S (1991) Interaction of free apolipoproteins with macrophages. Formation of high density lipoprotein-like lipoproteins and reduction of cellular cholesterol. J Biol Chem 266: 3080–3086
53. Yokoyama S (2000) Release of cellular cholesterol: molecular mechanism for cholesterol homeostasis in cells and in the body. Biochim Biophys Acta 1529:231–244
54. Fredrickson DS (1964) The inheritance of high density lipoprotein deficiency (Tangier disease). J Clin Invest 43:228–236
55. Brooks-Wilson A, Marcil M, Clee S, Zhang L, Roomp K, van Dam M, Yu L, Brewer C, Collins J, Molhuizen H, Loubser O, Ouelette B, Fichter K, Ashbourne-Excoffon K, Sensen C, Scherer S, Mott S, Denis M, Martindale D, Frohlich J, Morgan K, Koop B, Pimstone S, Kastelein J, Hayden M (1999) Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet 22:336–345
56. Bodzioch M, Orso E, Klucken J, Langmann T, Bottcher A, Diederich W, Drobnik W, Barlage S, Buchler C, Porsch-Ozcurumez M, Kaminski W, Hahmann H, Oette K, Rothe G, Aslanidis C, Lackner K, Schmitz G (1999) The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat Genet 22:347–351
57. Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette J, Deleuze J, Brewer H, Duverger N, Denefle P, Assmann G (1999) Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat Genet 22:352–355
58. Tanaka AR, Ikeda Y, Abe-Dohmae S, Arakawa R, Sadanami K, Kidera A, Nakagawa S, Nagase T, Aoki R, Kioka N, Amachi T, Yokoyama S, Ueda K (2001) Human ABCA1 contains a large amino-terminal extracellular domain homologous to an epitope of Sjogren's syndrome. Biochem Biophys Res Commun 283: 1019–1025
59. Fitzgerald ML, Mendez AJ, Moore KJ, Andersson LP, Panjeton HA, Freeman MW (2001) ABCA1 contains an N-terminal signal-anchor sequence that translocates the protein's first hydrophilic domain to the exoplasmic space. J Biol Chem 276:15137–15145
60. Bungert S, Molday LL, Molday RS (2001) Membrane topology of the ATP binding cassette transporter ABCR and its relationship to ABC1 and related ABCA transporters: identification of N-linked glycosylation sites. J Biol Chem 276:23539–23546
61. Nielsen H, Engelbrecht J, Brunak S, von Heijne G (1997) Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng 10:1–6
62. Neufeld EB, Remaley AT, Demosky SJ, Stonik JA, Cooney AM, Comly M, Dwyer NK, Zhang M, Blanchette-Mackie J, Santamarina-Fojo S, Brewer HB Jr (2001) Cellular localization and trafficking of the human ABCA1 transporter. J Biol Chem 276:27584–27590
63. Smith JD, Waelde C, Horwitz A, Zheng P (2002) Evaluation of the role of phosphatidylserine translocase activity in ABCA1-mediated lipid efflux. J Biol Chem 277:17797–17803
64. Neufeld EB, Stonik JA, Demosky SJ, Knapper CA, Combs CA, Cooney A, Comly M, Dwyer N, Blanchette-Mackie J, Remaley AT, Santamarina-Fojo S, Brewer HB Jr (2004) The ABCA1 transporter modulates late endocytic trafficking: insights from the correction of the genetic defect in Tangier disease. J Biol Chem 279:15571–15578
65. Ohama T, Hirano K, Zhang Z, Aoki R, Tsujii K, Nakagawa-Toyama Y, Tsukamoto K, Ikegami C, Matsuyama A, Ishigami M, Sakai N, Hiraoka H, Ueda K, Yamashita S, Matsuzawa Y (2002) Dominant expression of ATP-binding cassette transporter-1 on basolateral surface of Caco-2 cells stimulated by LXR/RXR ligands. Biochem Biophys Res Commun 296:625–630
66. Neufeld EB, Demosky SJ Jr, Stonik JA, Combs C, Remaley AT, Duverger N, Santamarina-Fojo S, Brewer HB Jr (2002) The ABCA1 transporter functions on the basolateral surface of hepatocytes. Biochem Biophys Res Commun 297:974–979
67. Mulligan JD, Flowers MT, Tebon A, Bitgood JJ, Wellington C, Hayden MR, Attie AD (2003) ABCA1 is essential for efficient basolateral cholesterol efflux during the absorption of dietary cholesterol in chickens. J Biol Chem 278:13356–13366
68. Arakawa R, Yokoyama S (2002) Helical apolipoproteins stabilize ATP-binding cassette transporter A1 by protecting it from thiol protease-mediated degradation. J Biol Chem 277:22426–22429
69. Wang N, Chen W, Linsel-Nitschke P, Martinez LO, Agerholm-Larsen B, Silver DL, Tall AR (2003) A PEST sequence in ABCA1 regulates degradation by calpain protease and stabilization of ABCA1 by apoA-I. J Clin Invest 111:99–107
70. Wang N, Silver D, Costet P, Tall A (2000) Specific binding of ApoA-I, enhanced cholesterol efflux, and altered plasma membrane morphology in cells expressing ABC1. J Biol Chem 275: 33053–33058

71. Takahashi Y, Smith JD (1999) Cholesterol efflux to apolipoprotein AI involves endocytosis and resecretion in a calcium-dependent pathway. Proc Natl Acad Sci U S A 96:11358–11363
72. Schmitz G, Kaminski WE, Orso E (2000) ABC transporters in cellular lipid trafficking. Curr Opin Lipidol 11:493–501
73. Nagata K, Yamamoto A, Ban N, Tanaka AR, Matsuo M, Kioka N, Inagaki N, Ueda K (2004) Human ABCA3, a product of a responsible gene for abca3 for fatal surfactant deficiency in newborns, exhibits unique ATP hydrolysis activity and generates intracellular multilamellar vesicles. Biochem Biophys Res Commun 324:262–268
74. Venkateswaran A, Laffitte BA, Joseph SB, Mak PA, Wilpitz DC, Edwards PA, Tontonoz P (2000) Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXR alpha. Proc Natl Acad Sci U S A 97:12097–12102
75. Wang Y, Oram JF (2002) Unsaturated fatty acids inhibit cholesterol efflux from macrophages by increasing degradation of ATP-binding cassette transporter A1. J Biol Chem 277:5692–5697
76. Singaraja RR, Brunham LR, Visscher H, Kastelein JJ, Hayden MR (2003) Efflux and atherosclerosis: the clinical and biochemical impact of variations in the ABCA1 gene. Arterioscler Thromb Vasc Biol 23:1322–1332
77. Brousseau ME, Schaefer EJ, Dupuis J, Eustace B, Van Eerdewegh P, Goldkamp AL, Thurston LM, FitzGerald MG, Yasek-McKenna D, O'Neill G, Eberhart GP, Weiffenbach B, Ordovas JM, Freeman MW, Brown RH Jr, Gu JZ (2000) Novel mutations in the gene encoding ATP-binding cassette 1 in four Tangier disease kindreds. J Lipid Res 41:433–441
78. Buechler C, Boettcher A, Bared SM, Probst MC, Schmitz G (2002) The carboxyterminus of the ATP-binding cassette transporter A1 interacts with a beta2-syntrophin/utrophin complex. Biochem Biophys Res Commun 293:759–765
79. Munehira Y, Ohnishi T, Kawamoto S, Furuya A, Shitara K, Imamura M, Yokota T, Takeda S, Amachi T, Matsuo M, Kioka N, Ueda K (2004) α_1-Snntrophin modulates turnover of ABCA1. J Biol Chem 279:15091–15095
80. Fitzgerald ML, Okuhira KI, Short GF III, Manning JJ, Bell SA, Freeman MW (2004) ABCA1 contains a novel C-terminal VFVNFA motif that is required for its cholesterol efflux and apoA-I binding activities. J Biol Chem 279:48477–48485
81. Yamauchi Y, Hayashi M, Abe-Dohmae S, Yokoyama S (2003) Apolipoprotein A-I activates protein kinase C alpha signaling to phosphorylate and stabilize ATP binding cassette transporter A1 for the high density lipoprotein assembly. J Biol Chem 278: 47890–47897
82. Arakawa R, Hayashi M, Remaley AT, Brewer BH, Yamauchi Y, Yokoyama S (2004) Phosphorylation and stabilization of ATP binding cassette transporter A1 by synthetic amphiphilic helical peptides. J Biol Chem 279:6217–6220
83. Martinez LO, Agerholm-Larsen B, Wang N, Chen W, Tall AR (2003) Phosphorylation of a pest sequence in ABCA1 promotes calpain degradation and is reversed by ApoA-I. J Biol Chem 278: 37368–37374
84. Haidar B, Denis M, Marcil M, Krimbou L, Genest J Jr (2004) Apolipoprotein A-I activates cellular cAMP signaling through the ABCA1 transporter. J Biol Chem 279:9963–9969
85. See RH, Caday-Malcolm RA, Singaraja RR, Zhou S, Silverston A, Huber MT, Moran J, James ER, Janoo R, Savill JM, Rigot V, Zhang LH, Wang M, Chimini G, Wellington CL, Tafuri SR, Hayden MR (2002) Protein kinase A site-specific phosphorylation regulates ATP-binding cassette A1 (ABCA1)-mediated phospholipid efflux. J Biol Chem 277:41835–41842
86. Tang C, Vaughan AM, Oram JF (2004) Janus kinase 2 modulates the apolipoprotein interactions with ABCA1 required for removing cellular cholesterol. J Biol Chem 279:7622–7628
87. Kaminski W, Orso E, Diederich W, Klucken J, Drobnik W, Schmitz G (2000) Identification of a novel human sterol-sensitive ATP-binding cassette transporter (ABCA7). Biochem Biophys Res Commun 2000:532–538
88. Ikeda Y, Abe-Dohmae S, Munehira Y, Aoki R, Kawamoto S, Furuya A, Shitara K, Amachi T, Kioka N, Matsuo M (2003) Post-transcriptional regulation of human ABCA7 and its function for the apoA-I-dependent lipid release. Biochem Biophys Res Commun 311:313–318
89. Kielar D, Kaminski WE, Liebisch G, Piehler A, Wenzel JJ, Mohle C, Heimerl S, Langmann T, Friedrich SO, Bottcher A, Barlage S, Drobnik W, Schmitz G (2003) Adenosine triphosphate binding cassette (ABC) transporters are expressed and regulated during terminal keratinocyte differentiation: a potential role for ABCA7 in epidermal lipid reorganization. J Invest Dermatol 121:465–474
90. Sasaki M, Shoji A, Kubo Y, Nada S, Yamaguchi A (2003) Cloning of rat ABCA7 and its preferential expression in platelets. Biochem Biophys Res Commun 304:777–782
91. Wang N, Lan D, Gerbod-Giannone M, Linsel-Nitschke P, Jehle AW, Chen W, Martinez LO, Tall AR (2003) ATP-binding cassette transporter A7 (ABCA7) binds apolipoprotein A-I and mediates cellular phospholipid but not cholesterol efflux. J Biol Chem 278: 42906–42912
92. Connors TD, Van Raay TJ, Petry LR, Klinger KW, Landes GM, Burn TC (1997) The cloning of a human ABC gene (ABC3) mapping to chromosome 16p13.3. Genomics 39:231–234
93. Klugbauer N, Hofmann F (1996) Primary structure of a novel ABC transporter with a chromosomal localization on the band encoding the multidrug resistance-associated protein. FEBS Lett 391: 61–65
94. Yamano G, Funahashi H, Kawanami O, Zhao LX, Ban N, Uchida Y, Morohoshi T, Ogawa J, Shioda S, Inagaki N (2001) ABCA3 is a lamellar body membrane protein in human lung alveolar type II cells. FEBS Lett 508:221–225
95. Mulugeta S, Gray JM, Notarfrancesco KL, Gonzales LW, Koval M, Feinstein SI, Ballard PL, Fisher AB, Shuman H (2002) Identification of LBM180, a lamellar body limiting membrane protein of alveolar type II cells, as the ABC transporter protein ABCA3. J Biol Chem 277:22147–22155
96. Shulenin S, Nogee LM, Annilo T, Wert SE, Whitsett JA, Dean M (2004) ABCA3 gene mutations in newborns with fatal surfactant deficiency. N Engl J Med 350:1296–1303

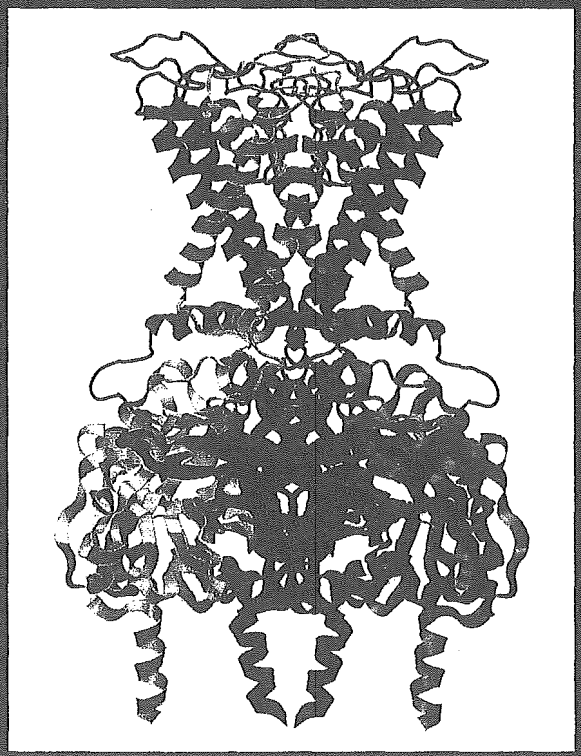
Volume 38, issue 6, June 2005 ISSN 0022-2828

ELSEVIER

Potassium Ion Channels:
From Biogenesis to Disease

Journal of Molecular and Cellular Cardiology

Celebrating 35th anniversary



ISHR
International Society for Heart Research

Published for the International Society for Heart Research

Journal of Molecular and Cellular Cardiology
Official Organ of the International Society for Heart Research

Aims and Scope

The *Journal of Molecular and Cellular Cardiology,* the official organ of the International Society for Heart Research, provides a forum for research papers dealing with the molecular biology, physiology, pharmacology, and pathophysiology of the heart and cardiovascular system. Intended mainly for reports of original research in molecular and cellular cardiology, the journal also publishes editorials, special articles, and book reviews that highlight important developments in these areas. By presenting a broad range of research reports, the journal provides investigators in the many subject areas of experimental cardiology with an overview of the latest developments in molecular and cellular cardiology. Recognizing the growing impact of these subjects on the care of the cardiac patient, the journal is also directed to clinical cardiologists and other health professionals who whish to follow the raidly advancing frontiers of our basic knowledge of the heart and circulation.

Research areas include: Molecular and genetic aspects of the heart and circulatory system, Cardiac metabolism and metabolic regulation, Biochemistry and biophysics of cardiac performance, Cardiovascular morphology, pathology, and pathophysiology, Cardiac electrophysiology, Smooth muscle physiology, Basic and clinical aspects of cardiovascular pharmacology, and Experimental and clinical aspects of heart disease that reflect altered molecular and cellular properties of the heart and cardiovascular system.

Submission of manuscripts

Manuscripts must be submitted online at http://jmcc.editorialmanager.com. Step-by-step online submission instructions are available at this website through the Instructions to Authors link. See also the Journal's website: http://www.elsevier.com//locate/issn/0022-2828 and click on Author Gateway (under Authors) on the right side.

The following items are required for submission and should be uploaded as individual files: abstract, cover letter and manuscript. Authors should also upload a completed manuscript submission form and any potentially overlapping work, either in preparation, already published, or in press. Acceptable file formats include Word, WordPerfect, RTF, LaTeX2e, TIFF, GIF, JPEG, EPS, Postscript, PICT, and PDF.

Revised manuscripts must include a file containing the point-by-point response to reviewer comments. Changes within the text of revised manuscripts must be indicated with underlining or italics. the text of revised manuscripts must be uploaded as Word or WordPerfect file so word processible documents are available to the publishers upon acceptance.

Questions regarding submission should be directed to the editorial office:

Journal of Molecular and Cellular Cardiology
Department of Medicine
525 B St., Suite 1900
San Diego, CA 92101
Phone: (619) 699-6342
Fax: (619) 699-6700
Email: jmcc@elsevier.com

See for a detailed Instructions to Authors the backmatter pages of each issue of the journal.

Abstracted/Indexed in:

Index Medicus, EMBASE and Current Contents.

Subscription Information 2005

Volumes 38-39 are scheduled for publication in 12 issues. Publication frequency: monthly. Subscription prices are available upon request from the Publisher or from the Regional Sales Office nearest you or from this journal's website (http://www.elsevier.com/locate/issn/0022-2828). Subscriptions are accepted on a prepaid basis only and are entered on a calendar year basis. Issues are sent by standard mail (surface within Europe, air delivery outside Europe). Priority rates are available upon request. Claims for missing issues should be made within six months of the date of dispatch.

Orders, claims, and journal enquiries: please contact the Customer Service Department at the Regional Sales Office nearest you:

Orlando: Elsevier, Customer Service Department, 6277 Sea Harbor Drive, Orlando, FL 32887-4800, USA; phone: (+1) (877) 8397126 [toll free number for US customers], or (+1) (407) 3454020 [customers outside US]; fax: (+1) (407) 3631354; e-mail: usjcs@elsevier.com

Amsterdam: Elsevier, Customer Service Department, PO Box 211, 1000 AE Amsterdam, The Netherlands; phone: (+31) (20) 4853757; fax: (+31) (20) 4853432; e-mail: nlinfo-f@elsevier.com

Tokyo: Elsevier, Customer Service Department, 4F Higashi-Azabu, 1-Chome Bldg, 1-9-15 Higashi-Azabu, Minato-ku, Tokyo 106-0044, Japan; phone: (+81) (3) 5561 5037; fax: (+81) (3) 5561 5047; e-mail: jp.info@elsevier.com

Singapore: Elsevier, Customer Service Department, 3 Killiney Road, #08-01 Winsland House I, Singapore 239519; phone: (+65) 63490222; fax: (+65) 67331510; e-mail: asiainfo@elsevier.com

Sponsored supplements and/or commercial reprints: For more information please contact Elsevier Life Sciences Commercial Sales, Radarweg 29, 1043 NX Amsterdam, The Netherlands; phone: (+31) (20) 485 2939 / 2059; e-mail: LSCS@elsevier.com.

Advertising information

Europe, USA, Canada and ROW: Advertising orders and enquiries can be sent to: e-mail: commercialsales@elsevier.com. Miss Katrina Barton, phone: (+44) (0) 20 7611 4117; fax: (+44) (0) 20 7611 4463. **South America:** Advertising orders and enquiries can be sent to: Mr Tino De Carlo, The Advertising Department, Elsevier Inc., 360 Park Avenue South, New York, NY 10010-1710, USA; phone (+1) (212) 633 3815; fax: (+1) (212) 633 3820; e-mail: t.decarlo@elsevier.com.

Us mailing notice – The Journal of Molecular and Cellular Cardiology (ISSN 0022-2828) is published monthly by Elsevier B.V., P.O. Box 211, 1000 AE Amsterdam, The Netherlands. The annual institutional subscription price in the USA is US$ 2670 (valid in North, Central and South America), including air speed delivery. Periodical postage rate paid at Jamaica, NY 11431. USA Postmaster: send address changes to Nuclear Physics B, Publications Expediting Inc., 200 Meacham Avenue, Elmont, NY 11003. Airfreight and mailing in the USA by Publications Expediting Inc., 200 Meacham Avenue, Elmont, NY 11003.

Cover illustration: Model of the Kir6.2 tetramer viewed from the side.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular and Cellular Cardiology 38 (2005) 907–916
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Focused issue on K_{ATP} channels

K_{ATP} channel interaction with adenine nucleotides

Michinori Matsuo, Yasuhisa Kimura, Kazumitsu Ueda *

Laboratory of Cellular Biochemistry, Division of Applied Life Sciences, Kyoto University Graduate School of Agriculture, Kyoto 606-8502, Japan

Received 17 May 2004; received in revised form 18 June 2004; accepted 12 November 2004

Available online 05 February 2005

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular and Cellular Cardiology 38 (2005) 907–916
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Focused issue on K_{ATP} channels

K_{ATP} channel interaction with adenine nucleotides

Michinori Matsuo, Yasuhisa Kimura, Kazumitsu Ueda *

Laboratory of Cellular Biochemistry, Division of Applied Life Sciences, Kyoto University Graduate School of Agriculture, Kyoto 606-8502, Japan

Received 17 May 2004; received in revised form 18 June 2004; accepted 12 November 2004

Available online 05 February 2005

Abstract

ATP-sensitive potassium (K_{ATP}) channels are regulated by adenine nucleotides to convert changes in cellular metabolic levels into membrane excitability. Hence, elucidation of interaction of SUR and Kir6.x with adenine nucleotides is an important issue to understand the molecular mechanisms underlying the metabolic regulation of the K_{ATP} channels. We analyzed direct interactions with adenine nucleotides of each subunit of K_{ATP} channels. Kir6.2 binds adenine nucleotides in a Mg^{2+}-independent manner. SUR has two NBFs which are not equivalent: NBF1 is a Mg^{2+}-independent high affinity nucleotide binding site, whereas NBF2 is a Mg-dependent low affinity site. Although SUR has ATPase activity at NBF2, it is not used to transport substrates against the concentration gradient unlike other ABC proteins. The ATPase cycle at NBF2 serves as a sensor of cellular metabolism. This may explain the low ATP hydrolysis rate compared to other ABC proteins. Based on studies of photoaffinity labeling, a model of K_{ATP} channel regulation is proposed, in which K_{ATP} channel activity is regulated by SUR via monitoring the intracellular MgADP concentration. K_{ATP} channel activation is expected to be induced by the cooperative interaction of ATP binding at NBF1 and MgADP binding at NBF2.

Keywords: K_{ATP} channels; Adenine nucleotides ATP; Photoaffinity labeling; ABC proteins

1. Introduction

ATP-sensitive potassium (K_{ATP}) channels link the metabolism of the cell to the membrane potential [1–4]. K_{ATP} channels are expressed in various tissues including pancreatic β-cells, neurons, cardiac muscle, skeletal muscle and smooth muscle, and play important physiological roles in those tissues (Table 1). Pancreatic K_{ATP} channels play a key role in glucose-stimulated insulin secretion. Cardiac K_{ATP} channels are regulatory components in the general adaptation syndrome, and protect the myocardium from lethal injury during ischemia. K_{ATP} channels protect neuron cells against neuronal damage during metabolic stress in brain. Vascular smooth muscle K_{ATP} channels are thought to play a role in regulation of vascular tone. Electrophysiological studies have suggested that an increase in the ATP/ADP ratio inhibits K_{ATP} channel activity, while a decrease in the ratio stimulates activity. However, regulation by adenine nucleotides and pharmacological agents was very complex and even paradoxical. We analyzed direct interactions with adenine nucleotides of each subunit of K_{ATP} channels to unveil the regulatory mechanism of K_{ATP} channels.

2. SUR as an ABC protein

K_{ATP} channels are hetero-octamers composed of sulfonylurea receptor (SUR) and Kir6.x subunits in a 4:4 stoichiometry [5–7] (Table 1). SUR is a member of the ABCC subfamily of ATP binding cassette (ABC) proteins and have three subtypes: SUR1, SUR2A, and SUR2B, where SUR2A and SUR2B are splicing variants [8–10]. The ABC proteins are characterized by well-conserved nucleotide binding folds (NBFs) and multispanning transmembrane domains. Like other members of the eukaryote ABC proteins, SUR has two

Table 1
Subunit compositions of K_{ATP} channel subtypes

K_{ATP} channel subtype	Composition
Pancreatic type	SUR1 + Kir6.2
Cardiac type	SUR2A + Kir6.2
Vascular smooth muscle type	SUR2B + Kir6.1
Smooth muscle type	SUR2B + Kir6.2

* Corresponding author. Tel.: +81-75-753-6124; fax: +81-75-753-6104.
E-mail address: uedak@kais.kyoto-u.ac.jp (K. Ueda).

0022-2828/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.yjmcc.2004.11.021

large intracellular loops, containing NBFs, and multispanning transmembrane domains. NBFs of SUR contain highly conserved motifs among ABC proteins: Walker A motif, Walker B motif, ABC signature motif (also called as linker sequence or LSGGQ motif), and an invariant glutamine and histidine residue (also called as the Q-loop and H-loop, respectively). Walker A and Walker B motifs are directly involved in nucleotide binding. ABC signature motif characterizes ABC proteins, but the role of the motif is not fully understood. SUR has 17 transmembrane segments (TMs), arranged in three groups of five, six and six TMs [11], while MDR1 (also termed as ABCB1), the first ABC protein gene isolated in human, has 12 TMs, arranged in two groups of six and six TMs [12,13]. MDR1 is associated with multidrug resistance of tumor cells [14]. In humans, there exist 49 ABC protein genes, many of which were identified as key genes responsible for hereditary diseases, such as cystic fibrosis [15], Dubin–Johnson syndrome [16,17], recessive Stargardt macular dystrophy [18], and Tangier disease [19–21]. However, there still remain many ABC proteins whose functions are unclear.

ABC proteins are found in most cells of all species from prokaryotes to humans, and hence make up one of the largest protein superfamilies [22]. ABC proteins can be divided into several subfamilies based on the type and direction of substrate transport. Although most of the eukaryotic ABC proteins are supposed to be functioning as transporters, there are some exceptions. Cystic fibrosis transmembrane conductance regulator (CFTR also termed as ABCC7) is clearly proven to function as a channel [23,24]. CFTR is a voltage-independent Cl^- channel found in the epithelial cells of many tissues, and plays a major role in regulating Cl^- fluxes. Mutations in CFTR cause cystic fibrosis, one of the most common serious diseases, which affects one in 2000–2500 people in northern Europe and the United States. In addition to its Cl^- channel activity, CFTR is also suggested to act as a regulator of both an outwardly-rectifying Cl^- channel (ORCC) [25] and an epithelial Na^+ channel [26]. Numerous abnormalities of cystic fibrosis are believed to be related to the multifunctionality of CFTR [27]. Another ABC protein with a function other than transport is SUR as described in this review.

3. Interaction of Kir6.2 with adenine nucleotides

Kir6.x, which forms a pore of the K_{ATP} channels as a tetramer, is a member of the inwardly rectifying potassium channel family and has two subtypes: Kir6.1 and Kir6.2 [28,29]. Although only fully functional octameric complexes reach the plasma membrane, truncation of the last 26–36 amino acids from Kir6.2 (Kir6.2ΔC) allows this subunit to reach the surface membrane in the absence of SUR, and to form K_{ATP} channels [30]. This suggested that ATP-induced inhibition of the K_{ATP} channels is via Kir6.2 subunit. However, it was not clear that nucleotides interact directly with Kir6.2, because Kir6.2 does not have any known nucleotide binding motif. We and others examined whether Kir6.2 could be specifically photoaffinity-labeled with ATP analogs, 8-azido-ATP, ATP-4-azidoanilido, 2-azidoadenosine 5′-triphosphate-biotin (2-N_3-ATP bio) and adenosine 5′-triphosphate azidoanilidebiotin (ATP-[γ]azidoanilide-bio), and demonstrated that ATP binds directly to Kir6.2 [31–33]. Because each Kir6.2 monomer in the K_{ATP} channel complex has its own ATP-binding site, one channel would contain four ATP-binding sites [34]. However, binding of ATP to just one subunit is reported to be sufficient to induce channel closure [35].

The exact location of the ATP-binding site on Kir6.2 is not clear. The C terminal domain of Kir6.2, expressed in *Escherichia coli*, binds the ATP analog, 2′,3′-*O*-(2,4,6-trinitrophenylcyclo-hexadienylidene) [36]. Mutations in R50, K185, I182, R201 and G334 reduce the channel ATP sensitivity [30,37–39], and direct interaction of the N and C terminal domains of Kir6.2 has also been demonstrated [40,41]. These suggest that both the N and C terminal domains are involved in the ATP-binding pocket. Indeed, both R50G and K185Q mutations reduced photoaffinity labeling of Ki6.2 by 8-azido-ATP [31]. The cysteine substitution and thiol modification study also suggested that R50 and K185 interact with the γ-phosphate and β-phosphate, respectively [42]. These data, altogether, suggest that R50 and K185 are involved in ATP binding.

4. Interaction of SUR with adenine nucleotides

Because ATP-induced inhibition of the K_{ATP} channels is via Kir6.2 subunit, the nucleotide interaction with SUR was expected to modulate the ability of ATP to keep pore impermeable for potassium ions. There was a possibility that SUR functioned as a transporter of some endogenous substrates, which regulated Kir6.x channels from outside of the cells in an autocrine manner. Another possibility was that SUR functioned as a direct regulator of Kir6.x channel. MgADP stimulates Kir6.2/SUR1 channel activity, although it blocks Kir6.2ΔC26 [30]. Mutations within the Walker A (K719A and K1385M) or Walker B (D853N, D1506A and D1506N) motifs of both NBFs of SUR1 abolished the activation of K_{ATP} channels by MgADP [43–45]. These results suggested that MgADP stimulates K_{ATP} channel activity via the SUR subunit. Gribble et al. [46] also reported that MgATP activated SUR1/Kir6.2 channels by interaction with NBFs of SUR1 when SUR1 was coexpressed with Kir6.2-R50G, an ATP-insensitive mutant. The reconstituted channels, composed of Kir6.2-R50G and a Walker A lysine mutant of SUR1, was not activated by MgATP. Therefore, both MgATP and MgADP can stimulate K_{ATP} channel activity via NBFs of SUR subunit. If SUR functions as an active transporter like other ABC proteins, it would expend the energy of ATP hydrolysis to transport compounds. Stimulation of the channel activity by MgADP made us expect SUR functioning as a regulator of Kir6.x channel rather than an active transporter.

To elucidate the molecular basis of the channel regulation by SUR, the nucleotide-binding properties of two NBFs of

SUR1 were examined using the photoaffinity analog 8-azido-[^{32}P]ATP [47]. SUR1, expressed in COS-7 cells, was photoaffinity-labeled with 8-azido-ATP even in the absence of Mg^{2+} or vanadate. Labeling of 8-azido-[α-^{32}P]ATP or 8-azido-[γ-^{32}P]ATP to SUR1 was apparently biphasic and suggested that SUR1 has two ATP-binding sites, one high-affinity site and the other low affinity site [47]. Analysis of ATP binding of mutants suggested NBF1 to be the high-affinity ATP-binding site. The high-affinity 8-azido-ATP binding was very stable at 4 °C, because the bound 8-azido-ATP did not dissociate from NBF1 when membrane fractions were washed with excess cold buffer. This differs from MDR1 and MRP1 (ABCC1), in which photoaffinity labeling requires Mg^{2+} and is stable only in the presence of vanadate [48,49]. Mutations of either the Walker A or Walker B motifs of NBF1, K719M and D854N abolished the high-affinity 8-azido-ATP labeling of SUR1, whereas the equivalent mutations in NBF2 did not affect it [47]. A thiol-modifying agent *N*-ethylmaleimide (NEM) inhibited the high-affinity 8-azido-ATP labeling by interacting with a C717 in the Walker A motif of NBF1 [50]. These indicated that NBF1 of SUR1 is the high-affinity 8-azido-ATP binding site.

To examine the nucleotide binding of two NBFs in more detail, SUR photoaffinity labeled with 8-azido-ATP was digested mildly with trypsin [51,52]. Tryptic fragments were immunoprecipitated with antibodies against NBF1 or NBF2. The ATP binding properties of the tryptic fragments indicated that NBF1 of SUR binds 8-azido-ATP in a Mg^{2+}-independent manner, and that NBF2 binds 8-azido-ATP in a Mg^{2+}-dependent manner, suggesting that NBF2 is responsible for the channel activation by MgADP, because K_{ATP} channels were activated by ADP only in the presence of Mg^{2+} [44].

Reimann et al. [53] reported that the Walker A mutation of NBF2 of SUR2A (K1348A) abolished the channel activation by MgADP, whereas the corresponding mutation of NBF1 (K707A) did not. This also supports the idea that NBF2 of SUR is essential for MgADP activation by SUR. Gribble et al. [44] reported that mutations in the Walker A motif of both NBFs of SUR1 (K719A and K1385A) abolished the channel activation by MgADP. We found that the K719M mutation of SUR1 affects 8-azido-ATP binding not only to NBF1 but also to NBF2 (Matsuo M, et al., unpublished data). Nucleotide binding at NBF1 could be also important for MgADP activation by affecting nucleotide binding at NBF2.

NBF2 of SUR was photoaffinity-labeled with 8-azido-[α-^{32}P]ATP but not with 8-azido-[γ-^{32}P]ATP at 37 °C, whereas NBF1 was photoaffinity-labeled with both 8-azido-[α-^{32}P]ATP and 8-azido-[γ-^{32}P]ATP [51,52]. This suggests that NBF2 has ATPase activity and bound MgATP is hydrolyzed to MgADP as other ABC proteins, and that NBF1 has little, if any activity. In the photoaffinity labeling method, it is assumed that one ATP hydrolysis cycle can be detected, but it is not possible to quantify the ATPase activity. It has been shown that when NBF2 of SUR2 fused to the maltose-binding protein (MBP), it had higher ATPase activity than that of a similar fusion protein containing NBF1 [54,55]. Bienengraeber et al. [54] showed that SUR2A/Kir6.2 channels, immunoprecipitated from cardiac cell membranes, have ATPase activity (31 nmol per min per mg). It was reported that MDR1 (320–3900 nmol per min per mg) [56–61] and MRP1 (460 nmol per min per mg) [62] have relatively high ATPase activity, whereas CFTR has low ATPase activity (50–70 nmol per min per mg) [63–65]. Recently, we overproduced SUR1 in insect Sf9 cells and purified it (Kimura Y, et al., in preparation). The purified SUR1 showed quite low ATPase activity compared to MDR1 purified by the same procedure. While SUR operates in a complex with Kir6.x and may possess altered characteristics when purified without Kir6.2, the lower ATPase activity of SUR could be related to its role as a channel regulator rather than as a transporter.

5. Cooperative nucleotide binding of two NBFs of SUR

As described above, 8-azido-ATP continues to bind to NBF1 stably in the presence of Mg^{2+} for more than 15 min at 4 °C [66]. We expected that the strong and stable ATP-binding to NBF1 would make it possible to investigate the functional interaction between the two NBFs of SUR1. Two procedures, a "pre-incubation procedure" and a "post-incubation procedure" were used, to analyze the interactions of SUR1 with adenine nucleotides (Fig. 1). First, the mem-

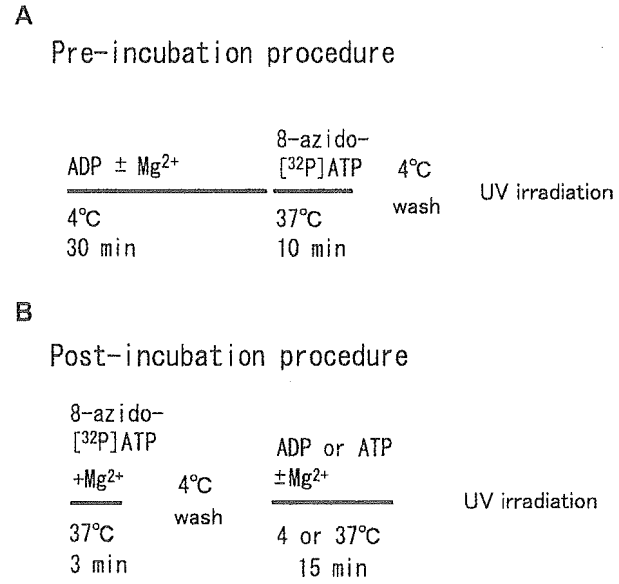


Fig. 1. Schematic diagrams of reactions of SUR1 with adenine nucleotides. A. Pre-incubation procedure. Membrane proteins were first incubated with 10–500 μM ADP for 30 min at 4 °C. 8-Azido-[α-^{32}P] ATP was then added to the mixture to a final concentration of 5 μM, and the mixture was incubated for 10 min at 37 °C. The reactions were stopped by the addition of 500 μl of ice-cold buffer, free 8-azido-[^{32}P]ATP was removed after centrifugation, and proteins were irradiated on ice. B. Post-incubation procedure. Membrane proteins were incubated with 10 μM 8-azido-[α-^{32}P]ATP or 8-azido-[γ-^{32}P]ATP for 3 min at 37 °C. The reactions were stopped, and free 8-azido-[^{32}P]ATP was removed. Membrane proteins were then mixed with buffer containing 10–500 μM ADP or ATP. The mixture was incubated for 15 min at 4 or 37 °C, and irradiated on ice.

brane proteins were pre-incubated with ADP at 4 °C, then allowed to react with 8-azido-[α-^{32}P]ATP in the presence of ADP ("pre-incubation procedure") [66]. With the pre-incubation procedure, ADP, in the presence of Mg^{2+}, strongly antagonized 8-azido-ATP binding. The 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 "post-incubation procedure" (Fig. 1) [66]. 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 post-incubated for 15 min in the presence or absence of unlabeled nucleotide, and irradiated with UV. With this procedure, we found that MgADP and MgATP stabilized prebound 8-azido-ATP binding, but it dissociated 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-hydrolyzable ATP analog, ATP-γS, had no greater stabilizing effect than Mg^{2+} alone. Mutations in the Walker A and Walker 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 induced a conformational change in NBF2 which transduced another conformational change in NBF1 to stabilize ATP-binding at NBF1. Similar cooperativity was found for nucleotide binding to the two NBFs of SUR2A and SUR2B [52]. Zingman et al. [67] report that cooperative nucleotide binding is essential for K_{ATP} channel opening, suggesting that the cooperativity is functionally important.

The crystal structures of two bacterial ABC proteins, MsbA from *Vibrio cholera* [68] and BtuCD from *E. coli* [69], have been reported. Two NBFs interact with each other, and two ATP molecules lie at the dimer interface formed by the Walker A and Walker B motif of one NBF and the ABC signature motif of the other NBF. It is demonstrated that NBFs of ABC proteins form dimers in the presence of ATP [70,71]. Because the two NBFs of SUR1 expressed in *E. coli* formed a dimer [72], the cooperativity of two NBFs may be mediated by direct interaction and communication of NBFs.

Sulfonylurea drugs, which close K_{ATP} channels to stimulate and release insulin, are widely used for treatment of non-insulin-dependent diabetes mellitus (NIDDM) patients. Reconstituted SUR1/Kir6.2 channels are inhibited by glibenclamide with IC_{50} of <10 nM, whereas SUR2A/Kir6.2 channels are inhibited with IC_{50} of 1.2 μM [9]. SUR1 is photoaffinity-labeled with iodide-glibenclamide with K_d of <10 nM [10], indicating that channel blockers bind to SUR. It is suggested that the transmembrane domain 12–17 is the sulfonyl group binding site and the intracellular loop between TM5 and TM6 is the benzamide group binding site [73–75]. However, the molecular mechanism of closure of K_{ATP} channels by the binding of sulfonylurea to SUR is not clear. Glibenclamide did not affect the affinity of two NBFs of SUR1 for MgATP or MgADP in nanomolar order (Matsuo M, et al. unpublished result). 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 by glibenclamide in a concentration-dependent manner [66], suggesting that glibenclamide modulates the cooperative interaction of the two NBFs of SUR1. Thus, glibenclamide may convert the active state of SUR1 directly to the inactive state by dissociating ATP from NBF1.

6. Interaction of disease related mutants of SUR1 and Kir6.2 with adenine nucleotides

Altered function of K_{ATP} channels is responsible for human diseases, because K_{ATP} channels play important physiological roles. It has been reported that Kir6.2 polymorphism (E23K) is associated with type 2 diabetes [76–78]. This mutation lies within N terminal cytosolic region of Kir6.2, and reduced ATP sensitivity of reconstituted K_{ATP} channels. Heterozygous missense mutations were identified in patients with permanent neonatal diabetes [79]. Among them, R201H mutation, which lies in the C terminal cytosolic region, reduced ATP sensitivity when Kir6.2-R201H was coexpressed with SUR1 in *Xenopus* oocyte. Because changes in ATP sensitivity caused diabetes on genetically engineered mice [80], mutations of Kir6.2, which affect interaction of Kir6.2 with adenine nucleotides, may decrease the threshold ATP concentration of insulin release and cause diabetes.

A frameshift mutation (frameshift at L1524) and a missense mutation (A1513T) of SUR2A were identified to be responsible for dilated cardiomyopathy [81]. Structural modeling of NBF2 of SUR2A suggested that both mutations, located within C terminal region of SUR2A, disrupt folding of the C terminal β-strand and affect the structure of nucleotide binding pocket of NBF2. Purified mutant NBF2 showed reduced ATPase activity without altering ATP-binding compared with wild-type. Reconstituted SUR2A/Kir6.2 channels showed that the mutations reduced both ATP sensitivity and response to MgADP, suggesting that defective catalysis-mediated pore regulation is a mechanism for channel dysfunction and susceptibility to dilated cardiomyopathy.

Persistent hyperinsulinemic hypoglycemia of infancy (PHHI) is a genetic disorder characterized by inappropriate insulin secretion despite severe hypoglycemia [82,83]. Loss of K_{ATP} channel function by mutations in SUR1 or Kir6.2 in pancreatic β-cells leads to a phenotype of PHHI. Many missense mutations are found within the NBFs of SUR1 and impair K_{ATP} channel activity by affecting MgADP activa-

tion. Nichols et al. [84] reported that the activation by MgADP was reduced when SUR1-G1479R, in which a G1479 within NBF2 was mutated into an arginine residue, was coexpressed with Kir6.2 in COSm6 cells. Shyng et al. [85] analyzed the function of several PHHI missense mutations and reported that most mutations reduced the response to stimulation by MgADP. In these mutant channels, loss of MgADP activation underlies the inability of the channel to respond to metabolic inhibition. In some cases, the mutant channel retains sensitivity to diazoxide and MgADP activation is not completely lost.

A missense SUR1 mutation (R1420C) was identified in Japanese PHHI patients [86]. They were siblings from a consanguineous family and homozygous for the mutation, and their clinical characteristics consisted of a mild form of PHHI. Verkarre et al. [87] also reported this mutation in a patient with focal adenomatous hyperplasia of pancreatic islets. R1420 is located between the Walker A motif and the ABC signature motif of NBF2. An $^{86}Rb^{+}$ efflux study revealed that K_{ATP} channels composed of SUR1-R1420C and Kir6.2 are not activated by metabolic inhibition as much as wild-type channels. It also decreases the expression of functional K_{ATP} channels. This may be related to the fact that the expression level of SUR1-R1420C was only about half that of the wild-type channel when it was transiently expressed in COS-7 cells [86]. Photoaffinity labeling experiments revealed that the R1420C PHHI mutation does not affect the affinity of NBF1 for nucleotides, but lowers the affinity of NBF2 for ATP and ADP [88]. The R1420C mutation impairs the cooperative nucleotide binding of two NBFs, although it does not show direct effects on the high-affinity 8-azido-ATP binding to NBF1. Increases in the EC_{50} for MgADP activation of channel activity were observed only when measured by coexpression with Kir6.2-R50G. These suggest that some PHHI missense mutations of SUR1, which show a mild phenotype, might directly or indirectly affect the interaction with nucleotides, even though those mutations apparently alter channel properties only minimally when measured by coexpression with the wild-type Kir6.2. Structural modeling of NBFs of SUR1 showed that R1420 is proximal to the α-helical subdomain in NBF2, suggesting that the R1420C mutation affects the interaction of NBFs with nucleotides indirectly [89].

7. Differences between SUR1, SUR2A and SUR2B on the interaction with adenine nucleotides

Pancreatic, cardiac, and vascular smooth muscle K_{ATP} channels, which consist of different subtypes of SUR, differ in their responses to cellular metabolic state (Fig. 2). Under normal conditions, pancreatic β-cell K_{ATP} channels stay open to maintain membrane potential, and close when elevation of blood glucose concentration results in increased intracellular concentration of ATP to trigger insulin secretion [1,2,84,90]. On the other hand, cardiac muscle K_{ATP} channels remain closed under normal conditions, and they open when the intracellular ATP concentration decreases under ischemic stress to shorten the action potential duration and protect the myocardium from lethal injury [3,90,91]. Cardiac K_{ATP} channels open to maintain cardiac cellular homeostasis in the adaptive reaction to stress [92]. To explore the possibility that different interactions of SUR with nucleotides cause differential regulation of K_{ATP} channels, we analyzed the properties of NBFs of SUR1, SUR2A, and SUR2B [52].

Several nucleotide-binding properties have been found to be similar among all of the SUR subtypes: (1) NBF1 is a Mg^{2+}-independent nucleotide binding site; (2) NBF2 is a Mg^{2+}-dependent nucleotide binding site; (3) 8-azido-ATP binds to NBF1 stably and does not dissociate at 4 °C; (4) MgATP or MgADP binding to NBF2 stabilizes 8-azido-ATP binding at NBF1; (5) NBF2 has ATPase activity, whereas NBF1 shows little or no ATPase activity. However, the affinities of NBFs of SUR for MgATP and MgADP differ among SUR subtypes (Table 2). The affinities of NBF1 of SUR1 for ATP and ADP, especially ATP, are significantly higher than those of SUR2A and SUR2B. The affinity of NBF1 of SUR2B

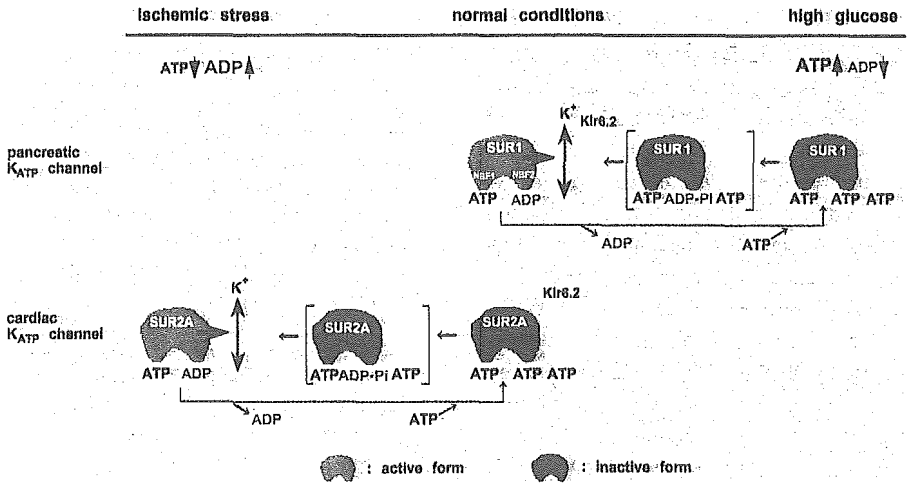


Fig. 2. The model of regulation of K_{ATP} channels, composed of SUR and Kir6.2, by ATP and ADP. Under normal conditions, pancreatic K_{ATP} channels stay open, but cardiac K_{ATP} channels remain closed. Differences in nucleotide binding affinities and ATPase kinetics between SUR1 and SUR2A may explain the higher ability of SUR1 to open the K_{ATP} channels than SUR2A.

Table 2
Affinity of SUR for adenine nucleotides

SUR subtype	NBF	Ki (μM) ATP	Ki (μM) ADP
SUR1	1	4.4 ± 3.7	26 ± 8.6
	2	60 ± 26	100 ± 26
SUR2A	1	110 ± 41	86 ± 23
	2	120 ± 39	170 ± 70
SUR2B	1	51 ± 13	66 ± 7.5
	2	38 ± 26	67 ± 40

for ATP is relatively higher than that of SUR2A, and the affinities of NBF2 of SUR2B for ATP and ADP are significantly higher than those of SUR2A. These differences in nucleotide binding affinities of SURs are possibly related to the differential regulation of K_{ATP} channels (Fig. 2). Furthermore, the recent finding of SUR2A mutations, identified in human dilated cardiomyopathy, underscored the essential role of the intrinsic ATP hydrolysis reaction in cardiac channel pore regulation [81]. Differences in the activities and properties of ATP hydrolysis by SUR1, SUR2A and SUR2B may lead to different channel regulations of SUR1/Kir6.2, SUR2A/Kir6.2 or SUR2B/Kir6.1 channels and account for their different physiological roles (Fig. 2).

Because SUR2A and SUR2B share the same amino acid sequence except for their C-terminal 42 amino acids [8–10], the C-terminal region may change the nucleotide-binding properties of both NBFs, affecting the physiological roles of SUR2A and SUR2B. It has been reported that much higher concentrations of MgADP are needed to activate SUR2A/Kir6.2 channels than SUR1/Kir6.2 or SUR2B/Kir6.2 channels [93], and that seven residues at the central portion of the 42 amino acids localize near the Walker A motif of NBF2 in structural modeling of NBF2 of SUR2 [94]. Mutations within the C terminal region of SUR2A affected ATP hydrolysis kinetics, reducing ATP sensitivity and response to MgADP of the channels [81]. Babenko et al. [95] reported that the C-terminal 42 amino acids of SUR specify the effect of ATP on gating. The C-terminal regions may alter the sensitivity of K_{ATP} channels to adenine nucleotides by affecting the affinity and/or ATP hydrolysis of NBFs of SUR.

Mutations within the ABC signature motif of NBF2 of SUR1 (S1482R) and SUR2B (S1446R) abolished the channel activation by MgADP, whereas the corresponding mutation of NBF1 of SUR1 (S830R) and SUR2B (S809R) did not [96]. These mutations within the ABC signature motif did not show strong effects on ATP-binding, nor did they abolish ATP hydrolysis of SUR1, suggesting the ABC signature motif is involved in transducing nucleotide binding into channel activation rather than adenine nucleotide binding itself. This idea is supported by the study that the serine mutation of the ABC signature motif did not affect the binding of substrate peptide or ATP by TAP1 (ABCB2) and TAP2 (ABCB3) [97]. Interestingly, the serine mutation of NBF1 (S809R) and NBF2 (S1446R) of SUR2A did not affect the channel activation by MgADP [96]. The C-terminal 42 amino acids of SUR2 may interact with the ABC signature motif of NBF2 in the transduction process, and this interaction differs between SUR2A and SUR2B.

8. SUR as an intracellular ADP sensor and the role of ATP hydrolysis

Based on the studies analyzing nucleotide-binding properties, we propose a model for the open and closed states of K_{ATP} channels (Fig. 3). Because MgADP binding at NBF2 seems to be essential for channel activation, we can assume that SUR binding MgADP at NBF2 activates the channel. Zingman et al. [55] have demonstrated that the K_{ATP} channel closes when the ATPase cycle of SUR2A is trapped by beryllium fluoride in a prehydrolytic state, which mimics the MgATP binding form, and that K_{ATP} channel open when ATPase cycle is trapped by orthovanadate in a posthydrolytic state, which mimics the MgADP binding form. This supports the former assumption and suggests that SUR binding MgATP at NBF2 does not activate the channel. The intracellular ATP concentration is thought to be several mM, which is more than 10 times higher than the intracellular ADP concentration [43,98–100]. This suggests that most NBF1 of SUR bind MgATP rather than MgADP. Because NBF1 of SUR has low or no ATPase activity, NBF1 of SUR binds MgATP irrespective of channel open and closed states. Therefore, the state of SUR binding MgATP (or ATP) at NBF1 and MgADP at NBF2 is the active state to open the pore of the Kir6.2 sub-

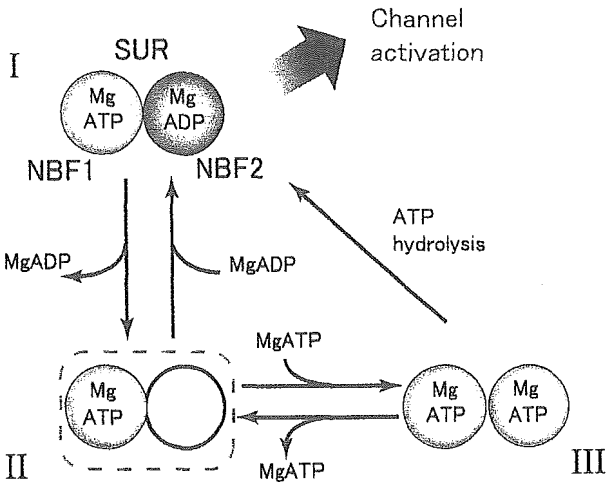


Fig. 3. Model for nucleotide activation of the K_{ATP} channels by SUR subunits. State I is the 'active state' in which SUR stimulates channel activity. State II is transient, and states II and III are 'inactive states' in which SUR cannot stimulate channel activity. Channel activation is induced when SUR binds MgATP at NBF1 and MgADP at NBF2 (state I). When the cellular metabolism is stimulated and intracellular MgATP concentration increases and MgADP concentration concomitantly decreases, MgADP dissociates from NBF2 and MgATP binds to NBF2 instead (state I–III through state II). MgADP interacts with NBF2 either by direct binding (state II–I) or hydrolysis of bound MgATP (state III–I by hydrolysis). MgADP might slow the off-rate of MgADP binding at NBF2. States I and II are of equilibrium, and most of the SUR subunits will be in state I at high MgADP concentrations and in state II or III at low MgADP concentrations. MgADP dissociation from NBF2 leads to instability of MgATP binding at NBF1, allowing the release of MgATP from NBF1. Because the intracellular concentrations of nucleotides are high enough under physiological conditions, state II would be transient and nucleotides (MgATP or MgADP) would bind to NBF2 to stabilize MgATP binding at NBF1 before the release of MgATP from NBF1. Consequently, SUR is in either state I, II, or III under physiological conditions.

unit. Because NBF2 of SUR has ATPase activity, bound MgATP is hydrolyzed to MgADP at NBF2. When the MgADP dissociates from NBF2 followed by binding of MgATP, the three states of SUR form a cycle.

In low metabolic states, such as low blood glucose levels or ischemic conditions, cellular ATP and ADP concentrations decrease and increase, respectively, in pancreatic β-cells or cardiomyocytes. It is unlikely that MgADP directly replaces with MgATP at NBF2 of SUR, because the cytosolic ADP concentration changes within the 100 μM range and is still about 10 times lower than ATP concentration even in low metabolic states [101]. Application of creatin kinase system, which scavenge ADP and accelerate the ATPase cycle of SUR2A, inhibits the channel, and application of MgADP, on the contrary decreases the rate of ATP hydrolysis with IC_{50} at about 100 μM [55]. Therefore, an increase of cytosolic MgADP concentration decelerates the ATP hydrolysis rate and increases duration of MgADP binding at NBF2, which is the active state. In this way, SUR work as intracellular ADP sensors.

These assumptions and the recent finding by Bienengraeber et al. [81] underline the importance of the ATPase activities of NBF2 of each SUR subtype. A comparison of ATPase activity of SUR subtypes would be of great help to understand the molecular mechanism of K_{ATP} channels, because different kinetics of ATPase activity may lead to tissue-specific channel regulation of K_{ATP} channel subtypes. The regulatory pattern of K_{ATP} channel may depend on intracellular local nucleotide concentrations regulated by intracellular phosphotransfer systems [55,102,103]. Recently, syntaxin 1A was shown to inhibit K_{ATP} channel by interaction with NBF1 of SUR1 [104]. It is also possible that other cellular systems or ligands modify the nucleotide binding and/or ATP hydrolysis of each SUR subtype in a different way.

9. Conclusion

K_{ATP} channels are regulated by adenine nucleotides to convert changes in cellular metabolic levels into membrane excitability. Hence, elucidation of interaction of SUR and Kir6.x with adenine nucleotides is an important issue to understand the molecular mechanisms underlying the metabolic regulation of the K_{ATP} channels. Kir6.2 binds adenine nucleotides in a Mg^{2+}-independent manner. SUR has two NBFs, which are not equivalent: NBF1 is a Mg^{2+}-independent high-affinity nucleotide binding site, whereas NBF2 is a Mg-dependent low affinity site. Although SUR has ATPase activity at NBF2, it is not used to transport substrates against the concentration gradient unlike other ABC proteins. The ATPase cycle at NBF2 serves as a sensor of cellular metabolism. This may explain the low ATP hydrolysis rate compared to other ABC proteins. Based on studies of photoaffinity labeling, a model of K_{ATP} channel regulation is proposed, in which K_{ATP} channel activity is regulated by SUR via monitoring the intracellular MgADP concentration. K_{ATP} channel activation is induced by the cooperative interaction of ATP-binding at NBF1 and MgADP binding at NBF2.

Acknowledgement

We thank Dr. Andre Terzic for reading the manuscript.

References

[1] Aguilar-Bryan L, Clement IV JP, Gonzalez G, Kunjilwar K, Babenko A, Bryan J. Toward understanding the assembly and structure of K_{ATP} channels. Physiol Rev 1998;78:227–45.
[2] Ashcroft FM, Gribble FM. Correlating structure and function in ATP-sensitive K^+ channels. Trends Neurosci 1998;21:288–94.
[3] Babenko AP, Aguilar-Bryan L, Bryan J. A view of SUR/Kir6.X, K_{ATP} channels. Annu Rev Physiol 1998;60:667–87.
[4] Seino S, Miki T. Physiological and pathophysiological roles of ATP-sensitive K+ channels. Prog Biophys Mol Biol 2003;81(2):133–76.
[5] Inagaki N, Gonoi T, Seino S. Subunit stoichiometry of the pancreatic b-cell ATP-sensitive K^+ channel. FEBS Lett 1997;409:232–6.
[6] Shyng S-L, Nichols CG. Octameric stoichiometry of the K_{ATP} channel complex. J Gen Physiol 1997;110:655–64.
[7] Clement IV JP, Kunjilwar K, Gonzalez G, Schwanstecher M, Panten U, Aguliar-Bryan L, et al. Association and stoichiometry of K_{ATP} channel subunits. Neuron 1997;18:827–38.
[8] Yamada M, Isomoto S, Matsumoto S, Kondo C, Shindo T, Horio Y, et al. Sulfonylurea receptor 2B and Kir6.1 form a sulfonylurea-sensitive but ATP-insensitive K^+ channel. J Physiol 1997;499:715–20.
[9] Inagaki N, Gonoi T, Clement IV JP, Wang C-Z, Aguilar-Bryan L, Bryan J, et al. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. Neuron 1996;16:1011–7.
[10] Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement IV JP, Boyd III AE, Gonzalez G, et al. Cloning of the β cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. Science 1995; 268:423–6.
[11] Raab-Graham KF, Cirilo LJ, Boettcher AA, Radeke CM, Vandenbergs CA. Membrane topology of the amino-terminal region of the sulfonylurea receptor. J Biol Chem 1999;274:29122–9.
[12] Ueda K, Cardarelli C, Gottesman MM, Pastan I. Expression of a full-length cDNA for the human "MDR1" gene confers resistance to colchicine, doxorubicinm and vinblastine. Proc Natl Acad Sci USA 1987;84:3004–8.
[13] Chen C-J, Chin JE, Ueda K, Gottesman DM, Roninson IB. Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoptotein) gene from multidrug-resistant human cells. Cell 1986;47:381–9.
[14] Fojo AT, Ueda K, Slamon DJ, Poplack DG, Gottesman MM, Pastan I. Expression of a multidrug-resistance gene in human tumors. Proc Natl Acad Sci USA 1987;84:265–9.
[15] Riordan JR, Rommens JM, Kerem B-S, Alon N, Rozmahel R, Grzelczak Z, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 1989;245:1066–73.
[16] Ito K, Suzuki H, Hirohashi T, Kume K, Shimizu T, Sugiyama Y. Molecular cloning of canalicular multispecific organic anion transporter defective in EHBR. Am J Physiol 1997;272(1 Pt 1):G16–G22.
[17] Paulusma CC, Bosma PJ, Zaman GJR, Bakker CTM, Otter M, Scheffer GL, et al. Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene. Science 1996;271:1126–8.
[18] Allikmets R, Shroyer NF, Singh N, Seddon JM, Lewis RA, Bernstein PS, et al. Mutation of the stargardt disease gene (ABCR) in age-related macular degeneration. Science 1997;277:1805–7.

[19] Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, et al. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat Genet 1999;22(4):352–5.
[20] Bodzioch M, Orso E, Klucken J, Langmann T, Bottcher A, Diederich W, et al. The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat Genet 1999;22(4):347–51.
[21] Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, Van Dam M, et al. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet 1999;22(4):336–45.
[22] Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res 2001;11(7):1156–66.
[23] Riordan JR. The cystic fibrosis transmembrane conductance regulator. Annu Rev Physiol 1993;55:609–30.
[24] Gadsby DC, Nagel G, Hwang TC. The CFTR chloride channel of mammalian heart. Annu Rev Physiol 1995;57:387–416.
[25] Julien M, Verrier B, Cerutti M, Chappe V, Gola M, Devauchelle G, et al. Cystic fibrosis transmembrane conductance regulator (CFTR) confers glibenclamide sensitivity to outwardly rectifying chloride channel (ORCC) in Hi-5 insect cells. J Membr Biol 1999;168(3):229–39.
[26] Schreiber R, Hopf A, Mall M, Greger R, Kunzelmann K. The first-nucleotide binding domain of the cystic-fibrosis transmembrane conductance regulator is important for inhibition of the epithelial Na^+ channel. Proc Natl Acad Sci USA 1999;96:5310–5.
[27] Tsui LC, Buchwald M. Biochemical and molecular genetics of cystic fibrosis. Adv Hum Genet 1991;20:153–266.
[28] Inagaki N, Gonoi T, Clement IV JP, Namba N, Inazawa J, Gonzalez G, et al. Reconstitution of IK_{ATP}: an inward rectifier subunit plus the sulfonylurea receptor. Science 1995;270:1166–9.
[29] Inagaki N, Tsuura Y, Namba N, Masuda K, Gonoi T, Horie M, et al. Cloning and functional characterization of a novel ATP-sensitive potassium channel ubiquitously expressed in rat tissues, including pancreatic islets, pituitary, skeletal muscle, and heart. J Biol Chem 1995;270:5691–4.
[30] Tucker SJ, Gribble FM, Zhao C, Trapp S, Ashcroft FM. Truncation of Kir6.2 produces ATP-sensitive K^+ channels in the absence of the sulphonylurea receptor. Nature 1997;387:179–83.
[31] Tanabe K, Tucker SJ, Matsuo M, Proks P, Ashcroft FM, Seino S, et al. Direct photoaffinity labeling of the Kir6.2 subunit of the ATP-sensitive K^+ channel by 8-azido-ATP. J Biol Chem 1999;274:3931–3.
[32] Tanabe K, Tucker SJ, Ashcroft FM, Proks P, Kioka N, Amachi T, et al. Direct photoaffinity labeling of Kir6.2 by [γ-^{32}P]ATP-[γ]4-azidoanilide. Biochem Biophys Res Commun 2000;272(2):316–9.
[33] Wang C, Wang K, Wang W, Cui Y, Fan Z. Compromised ATP binding as a mechanism of phosphoinositide modulation of ATP-sensitive K+ channels. FEBS Lett 2002;532(1–2):177–82.
[34] Markworth E, Schwanstecher C, Schwanstecher M. ATP4– mediates closure of pancreatic beta-cell ATP-sensitive potassium channels by interaction with 1 of 4 identical sites. Diabetes 2000;49(9):1413–8.
[35] Dorschner H, Brekardin E, Uhde I, Schwanstecher C, Schwanstecher M. Stoichiometry of sulfonylurea-induced ATP-sensitive potassium channel closure. Mol Pharmacol 1999;55(6):1060–6.
[36] Vanoye CG, MacGregor GG, Dong K, Tang L, Buschmann AS, Hall AE, et al. The carboxyl termini of KATP channels bind nucleotides. J Biol Chem 2002;277(26):23260–70.
[37] Drain P, Li L, Wang J. K_{ATP} channel inhibition by ATP requires distinct functional domains of the cytoplasmic C terminus of the pore-forming subunit. Proc Natl Acad Sci USA 1998;95:13953–8.
[38] Tucker SJ, Gribble FM, Proks P, Trapp S, Ryder TJ, Haug T, et al. Molecular determinants of K_{ATP} channel inhibition by ATP. EMBO J 1998;17:3290–6.
[39] Shyng SL, Barbieri A, Gumusboga A, Cukras C, Pike L, Davis JN, et al. Modulation of nucleotide sensitivity of ATP-sensitive potassium channels by phosphatidylinositol-4-phosphate 5-kinase. Proc Natl Acad Sci USA 2000;97(2):937–41.
[40] Tucker SJ, Ashcroft FM. Mapping of the physical interaction between the intracellular domains of an inwardly rectifying potassium channel, Kir6.2. J Biol Chem 1999;274:33393–7.
[41] Lippiat JD, Albinson SL, Ashcroft FM. Interaction of the cytosolic domains of the Kir6.2 subunit of the KATP channel is modulated by sulfonylureas. Diabetes 2002;51(3):S377–S380.
[42] Trapp S, Haider S, Jones P, Sansom MSP, Ashcroft FM. Identification of residues contributing to the ATP binding site of Kir6.2. EMBO J 2003;22(12):2903–12.
[43] Gribble FM, Tucker SJ, Ashcroft FM. The interaction of nucleotide with the tolbutamide block of cloned ATP-sensitive K^+channel currents expressed in *Xenopus* oocytes: a reinterpretation. J Physiol 1997;504:35–45.
[44] Gribble FM, Tucker SJ, Ashcroft FM. The essential role of the Walker A motifs of SUR1 in K-ATP channel activation by Mg-ADP and diazoxide. EMBO J 1997;16:1145–52.
[45] Shyng S-L, Ferrigni T, Nichols CG. Regulation of K_{ATP} channel activity by diazoxide and MgADP. J Gen Physiol 1997;110:643–54.
[46] Gribble FM, Tucker SJ, Haug T, Ashcroft FM. MgATP activates the β cell K_{ATP} channel by interaction with its SUR1 subunit. Proc Natl Acad Sci USA 1998;95:7185–90.
[47] Ueda K, Inagaki N, Seino S. MgADP antagonism to Mg^{2+}-independent ATP binding of the sulfonylurea receptor SUR1. J Biol Chem 1997;272:22983–6.
[48] Urbatsch IL, Sankaran B, Weber J, Senior AE. P-glycoprotein is stably inhibited by vanadate-induced trapping of nucleotide at a single catalytic site. J Biol Chem 1995;270:19383–90.
[49] Taguchi Y, Yoshida A, Takada Y, Komano T, Ueda K. Anti-cancer drugs and glutathione stimulate vanadate-induced trapping of nucleotide in multidrug resistance-associated protein (MRP). FEBS Lett 1997;401:11–4.
[50] Matsuo M, Tucker SJ, Ashcroft FM, Amachi T, Ueda K. NEM modification prevents high-affinity ATP binding to the first nucleotide binding fold of the sulphonylurea receptor, SUR1. FEBS Lett 1999; 458:292–4.
[51] Matsuo M, Kioka N, Amachi T, Ueda K. ATP binding properties of the nucleotide binding folds of SUR1. J Biol Chem 1999;274:37479–82.
[52] Matsuo M, Tanabe K, Kioka N, Amachi T, Ueda K. Different binding properties and affinities for ATP and ADP among sulfonylurea receptor subtypes, SUR1, SUR2A, and SUR2B. J Biol Chem 2000;275: 28757–63.
[53] Reimann F, Gribble FM, Ashcroft FM. Differential response of KATP channels containing SUR2A or SUR2B subunits to nucleotides and pinacidil. Mol Pharmacol 2000;58(6):1318–25.
[54] Bienengraeber M, Alekseev AE, Abraham MR, Carrasco AJ, Moreau C, Vivaudou M, et al. ATPase activity of the sulfonylurea receptor: a catalytic function for the KATP channel complex. FASEB J 2000;14(13):1943–52.
[55] Zingman LV, Alekseev AE, Bienengraeber M, Hodgson D, Karger AB, Dzeja PP, et al. Signaling in channel/enzyme multimers: ATPase transitions in SUR module gate ATP-sensitive K+ conductance. Neuron 2001;31(2):233–45.
[56] Shapiro AB, Ling V. ATPase activity of purified and reconstituted P-glycoprotein from Chinese hamster ovary cells. J Biol Chem 1994; 269:3745–54.
[57] Urbatsch IL, Al-Shawi MK, Senior AE. Characterization of the ATPase activity of purified Chinese hamster P-glycoprotein. Biochemistry 1994;33:7069–756.
[58] Urbatsch IL, Senior AE. Effects of lipids on ATPase activity of purified Chinese hamster P-glycoprotein. Arch Biochem Biophys 1995;316:135–40.
[59] Callaghan R, Berridge G, Ferry DR, Higgins CF. The functional purification of P-glycoprotein is dependent on maintenance of a lipid–protein interface. Biochim Biophys Acta (BBA)-. Biomembr 1997; 1328(2):109–24.
[60] Mao Q, Scarborough GA. Purification of functional human P-glycoprotein expressed in *Saccharomyces cerevisiae*. Biochim Biophys Acta (BBA)-. Biomembr 1997;1327(1):107–18.

[61] Ramachandra M, Ambudkar SV, Chen D, Hrycyna CA, Dey S, Gottesman MM, et al. Human P-glycoprotein exhibits reduced affinity for substrates during a catalytic transition state. Biochemistry 1998;37(14):5010–9.

[62] Chang X-B, Hou Y-X, Riordan JR. ATPase activity of purified multidrug resistance-associated protein. J Biol Chem 1997;272:30962–8.

[63] Ramjeesingh M, Li C, Garami E, Huan LJ, Galley K, Wang Y, et al. Walker mutations reveal loose relationship between catalytic and channel-gating activities of purified CFTR (cystic fibrosis transmembrane conductance regulator). Biochemistry 1999;38(5):1463–8.

[64] Ramjeesingh M, Li C, Garami E, Huan L-J, Hewryk M, Wang Y, et al. A novel procedure for the efficient purification of the cystic fibrosis transmembrane conductance regulator (CFTR). Biochem J 1997;327: 17–21.

[65] Li C, Ramjeesingh M, Wang W, Garami E, Hewryk M, Lee D, et al. ATPase activity of the cystic fibrosis transmembrane conductance regulator. J Biol Chem 1996;271:28463–8.

[66] Ueda K, Komine J, Matsuo M, Seino S, Amachi T. Cooperative binding of ATP and MgADP in the sulfonylurea receptor is modulated by glibenclamide. Proc Natl Acad Sci USA 1999;96:1268–72.

[67] Zingman LV, Hodgson DM, Bienengraeber M, Karger AB, Kathmann EC, Alekseev AE, et al. Tandem function of nucleotide binding domains confers competence to sulfonylurea receptor in gating ATP-sensitive K+ channels. J Biol Chem 2002;277(16):14206–10.

[68] Chang G. Structure of MsbA from Vibrio cholera: a multidrug resistance ABC transporter homolog in a closed conformation. J Mol Biol 2003;330(2):419–30.

[69] Locher KP, Lee AT, Rees DC. The E. coli BtuCD structure: a framework for ABC transporter architecture and mechanism. Science 2002; 296(5570):1091–8.

[70] Chen J, Lu G, Lin J, Davidson AL, Quiocho FA. A tweezers-like motion of the ATP-binding cassette dimer in an ABC transport cycle. Mol Cell 2003;12(3):651–61.

[71] Moody JE, Millen L, Binns D, Hunt JF, Thomas PJ. Cooperative, ATP-dependent association of the nucleotide binding cassettes during the catalytic cycle of ATP-binding cassette transporters. J Biol Chem 2002;277(24):21111–4.

[72] Hough E, Mair L, Mackenzie W, Sivaprasadarao A. Expression, purification, and evidence for the interaction of the two nucleotide-binding folds of the sulphonylurea receptor. Biochem Biophys Res Commun 2002;294(1):191–7.

[73] Ashfield R, Gribble FM, Ashcroft SJH, Ashcroft FM. Identification of the high-affinity tolbutamide site on the SUR1 subunit of the K_{ATP} channel. Diabetes 1999;48:1341–7.

[74] Babenko AP, Gonzalez G, Bryan J. The tolbutamide site of SUR1 and a mechanism for its functional coupling to K_{ATP} channel closure. FEBS Lett 1999;459:367–76.

[75] Mikhailov MV, Mikhailova EA, Ashcroft SJ. Molecular structure of the glibenclamide binding site of the beta-cell KATP channel. FEBS Lett 2001;499(1–2):154–60.

[76] Gloyn AL, Weedon MN, Owen KR, Turner MJ, Knight BA, Hitman G, et al. Large-scale association studies of variants in genes encoding the pancreatic β-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. Diabetes 2003;52(2):568–72.

[77] Hani EH, Boutin P, Durand E, Inoue H, Permutt MA, Velho G, et al. Missense mutations in the pancreatic islet beta cell inwardly rectifying K^+ channel gene (Kir6.2/BIR): a meta-analysis suggests a role in the polygenic basis of Type II diabetes mellitus in Caucasians. Diabetologia 1998;41:1511–5.

[78] Schwanstecher C, Meyer U, Schwanstecher. M. KIR6.2 polymorphism predisposes to type 2 diabetes by inducing overactivity of pancreatic β-cell ATP-sensitive K+ channels. Diabetes 2002;51(3): 875–9.

[79] Gloyn AL, Pearson ER, Antcliff JF, Proks P, Bruining GJ, Slingerland AS, et al. Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. New Engl J Med 2004;350(18):1838–49.

[80] Koster JC, Marshall BA, Ensor N, Corbett JA, Nichols CG. Targeted overactivity of beta cell KATP channels induces profound neonatal diabetes. Cell 2000;100(6):645–54.

[81] Bienengraeber M, Olson TM, Selivanov VA, Kathmann EC, O'Cochlain F, Gao F, et al. ABCC9 mutations identified in human dilated cardiomyopathy disrupt catalytic KATP channel gating. Nat Genet 2004;36(4):382–7.

[82] Sharma N, Crane A, Gonzalez G, Bryan J, Aguilar-Bryan L. Familial hyperinsulinism and pancreatic beta-cell ATP-sensitive potassium channels. Kidney Int 2000;57(3):803–8.

[83] Dunne MJ, Cosgrove KE, Shepherd RM, Aynsley-Green A, Lindley KJ. Hyperinsulinism in infancy: from basic science to clinical disease. Physiol Rev 2004;84(1):239–75.

[84] Nichols CG, Shyng S-L, Nestorowicz A, Glaser B, Clement IV JP, Gonzalez G, et al. Adenosine diphosphate as an intracellular regulator of insulin secretion. Science 1996;272:1785–7.

[85] Shyng S-L, Ferrigni T, Shepard JB, Nestorowicz A, Glaser B, Permutt MA, et al. Functional analyses of novel mutations in the sulfonylurea receptor 1 associated with persistent hyperinsulinemic hypoglycemia of infancy. Diabetes 1998;47:1145–51.

[86] Tanizawa Y, Matsuda K, Matsuo M, Ohta Y, Ochi N, Adachi M, et al. Genetic analysis of Japanese patients with persistent hyperinsulinemic hypoglycemia of infancy: nucleotide-binding fold-2 mutation impairs cooperative binding of adenine nucleotides to sulfonylurea receptor 1. Diabetes 2000;49:114–20.

[87] Verkarre V, Fournet JC, de Lonlay P, Gross-Morand MS, Devillers M, Rahier J, et al. Paternal mutation of the sulfonylurea receptor (SUR1) gene and maternal loss of 11p15 imprinted genes lead to persistent hyperinsulinism in focal adenomatous hyperplasia. J Clin Invest 1998;102(7):1286–91.

[88] Matsuo M, Trapp S, Tanizawa Y, Kioka N, Amachi T, Oka Y, et al. Functional analysis of a mutant sulfonylurea receptor, SUR1-R1420C, that is responsible for persistent hyperinsulinemic hypoglycemia of infancy. J Biol Chem 2000;275(52):41184–91.

[89] Campbell JD, Sansom MS, Ashcroft FM. Potassium channel regulation. EMBO Rep 2003;4(11):1038–42.

[90] Seino S. ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. Annu Rev Physiol 1999;61:337–62.

[91] Yokoshiki H, Sunagawa M, Seki T, Sperelakis N. ATP-sensitive K^+ channels in pancreatic, cardiac and vascular smooth muscle cells. Am J Physiol 1998;274:25–37.

[92] Zingman LV, Hodgson DM, Bast PH, Kane GC, Perez-Terzic C, Gumina RJ, et al. Kir6.2 is required for adaptation to stress. Proc Natl Acad Sci USA 2002;99(20):13278–83.

[93] Matsuoka T, Matsushita K, Katayama Y, Fujita A, Inageda K, Tanemoto M, et al. C-terminal tails of sulfonylurea receptors control ADP-induced activation and diazoxide modulation of ATP-sensitive K+ channels. Circ Res 2000;87(10):873–80.

[94] Matsushita K, Kinoshita K, Matsuoka T, Fujita A, Fujikado T, Tano Y, et al. Intramolecular interaction of SUR2 subtypes for intracellular ADP-induced differential control of KATP channels. Circ Res 2002; 90(5):554–61.

[95] Babenko AP, Gonzalez G, Bryan J. Two regions of sulfonylurea receptor specify the spontaneous bursting and ATP inhibition of K_{ATP} channel isoforms. J Biol Chem 1999;274:11587–92.

[96] Matsuo M, Dabrowski M, Ueda K, Ashcroft FM. Mutations in the linker domain of NBD2 of SUR inhibit transduction but not nucleotide binding. EMBO J 2002;21(16):4250–8.

[97] Hewitt EW, Lehner PJ. The ABC-transporter signature motif is required for peptide translocation but not peptide binding by TAP. Eur J Immunol 2003;33(2):422–7.