

Adenylate Kinase Activity in ABC Transporters*

Published, JBC Papers in Press, August 12, 2005, DOI 10.1074/jbc.R500009200

Christoph O. Randak¹ and Michael J. Welsh²

From the Howard Hughes Medical Institute, Departments of Internal Medicine and Physiology and Biophysics, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, Iowa 52242

ABC transporters³ are remarkably versatile machines that move a wide array of substances, including both hydrophilic and hydrophobic molecules across membranes (1–4). They are constructed with a modular design; each transporter has two nucleotide-binding domains (NBDs) and two membrane-spanning domains (often comprising six transmembrane helices). The NBDs are highly conserved modules that interact with ATP to control transport. The membrane-spanning domains show little sequence conservation, consistent with highly divergent functions within the ABC transporter family. In some cases, additional modules (solute-binding and/or regulatory domains) participate in the ABC transporter complex to regulate function. The design and adaptability of ABC transporters have apparently served life well throughout evolution, because they form one of the largest families of proteins in eubacteria, archaea, and eukarya. Not surprisingly, loss of ABC transporter function is implicated in many diseases, and they are therefore important targets for therapeutics (5).

Some ABC transporters are pumps; their NBDs function as ATPases ($Mg^{2+}\cdot ATP + H_2O \rightarrow Mg^{2+}\cdot ADP + P_i$), and they spend the energy of ATP hydrolysis to move substances across a membrane against a gradient (6–8). However, the mechanisms coupling enzymatic activity to substrate transfer remain controversial, and the stoichiometry of transport, the relationship between the number of ATP molecules hydrolyzed and the number of substrate molecules transported, remains uncertain (1).

Uncertainty about the stoichiometry of transport is highlighted in the ABC transporter CFTR. CFTR forms a channel in which anions flow passively, moving across membranes down an electrochemical gradient. Thus, there is no fixed stoichiometry between ion movement and ATP utilization. Once the regulatory (R) domain has been phosphorylated, ATP interacts with the NBDs to gate the channel (for reviews, see Refs. 9–11). Earlier work showed that both NBD1 and NBD2 bind ATP and that NBD1 has little if any enzymatic activity, whereas NBD2 can function as an ATPase. When ATP is the only nucleotide present, normal activity requires ATP hydrolysis. This has long seemed puzzling, because ATP hydrolysis does not fuel ion movement, and the number of ions flowing through the channel bears no fixed stoichiometric relationship to ATP hydrolysis. Moreover, no other ion channel is known to require the large energy of ATP hydrolysis ($\Delta_r G^\circ \sim -32$ kJ/mol; Ref. 12).

Recent data show that CFTR can function as an adenylate kinase ($Mg^{2+}\cdot ATP + AMP \rightleftharpoons Mg^{2+}\cdot ADP + ADP$), a readily reversible reaction that releases no free energy under physiologic conditions (12–14). This finding begins to resolve the apparent puzzle of whether an ion channel requires a large energy input. This discovery may also give us clues about the transport energetics of other ABC transporters. Here we briefly review recent findings of CFTR adenylate kinase activity, and we discuss the implications for other ABC transporters.

* This minireview will be reprinted in the 2005 Minireview Compendium, which will be available in January, 2006. This work was supported in part by NHLBI/National Institutes of Health Grants HL29851-21 and HL1234-05.

¹ Supported by Cystic Fibrosis Foundation Grant RANDAK05F0.

² An Investigator of the Howard Hughes Medical Institute. To whom correspondence should be addressed: 500 EMRB, Howard Hughes Medical Inst., Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA 52242. Tel.: 319-335-7619, Fax: 319-335-7623, E-mail: michael-welsh@uiowa.edu.

³ The abbreviations used are: ABC transporter, ATP-binding cassette transporter; ADP-NH₂, adenylyl 5'-phosphoramidate; AMP-NH₂, adenosine 5'-monophosphoramidate; AMPK, AMP-activated protein kinase; Ap₅A, P₁,P₅-di(adenosine-5') pentaphosphate; CFTR, cystic fibrosis transmembrane conductance regulator; NBD, nucleotide-binding domain; $\Delta_r G^\circ$, standard transformed Gibbs energy of reaction.

CFTR-NBD2 Has ATPase and Adenylate Kinase Activity

When ATP is the only nucleotide present, a recombinant CFTR-NBD2 polypeptide functioned as an ATPase (Fig. 1, A and B). However, when AMP was added with ATP, it functioned as an adenylate kinase (15). The reaction was reversible; with ADP applied alone, the NBD2 generated ATP (Fig. 1C) (16). Moreover, both AMP and ADP suppressed the ATPase activity (15). Ap₅A, which specifically inhibits adenylate kinases by binding simultaneously to the ATP and AMP-binding sites (17, 18), inhibited both enzymatic activities (Fig. 1D) (15). These and additional data established that an isolated NBD2 could function either as an ATPase or as an adenylate kinase. The two enzymatic activities shared an ATP-binding site, and there was an additional AMP-binding site (15, 16, 19). Fig. 1E shows a model. When we disrupted the ATP-binding site by mutating the conserved Walker A Lys or the conserved Walker B Asp that coordinates Mg²⁺, we abolished both enzymatic activities. In contrast, a mutation that probably lies outside the ATP site (N1303K) (20, 21) eliminated adenylate kinase activity but had minimal effects on ATPase activity (16). Thus, NBD2 harbored distinct ATP- and AMP-binding sites.

CFTR Has Intrinsic Adenylate Kinase Activity in NBD2 That Gates the Channel

While earlier work demonstrated that the NBD2 enzymatic activity required for normal channel gating could be an ATPase, more recent data indicate that it also can be an adenylate kinase (16).

First, when ATP is the only nucleotide present, the relationship between ATP concentration and current (or channel opening rate) shows simple Michaelis-Menten behavior with a Hill coefficient of 1 (Fig. 2A) (16, 20, 22, 23). These results suggest that interaction of a single ATP molecule (or interactions of ATP with equivalent sites) opens the channel. Adding AMP alone evoked no current on its own, but when added with ATP, AMP switched the relationship between ATP concentration and current to one with positive two-site cooperativity for ATP with a Hill coefficient of 1.6 (Fig. 2A). These data indicate that at least two ATP molecules interact with CFTR and that interaction of one ATP enhances interaction of the other. Thus, AMP induced a different gating mechanism.

Second, transfer of a phosphate between ATP and AMP (the adenylate kinase reaction) was required for normal current generation and positive cooperativity. Supporting this conclusion, AMP-NH₂, which cannot serve as a phosphate acceptor, interacted with the AMP site (16) but inhibited current non-competitively with ATP and did not induce positive cooperativity.

Third, a specific inhibitor of adenylate kinases, Ap₅A, reduced the rate of channel opening and induced a pattern of negative cooperativity for ATP. The data indicated that Ap₅A inhibited CFTR by simultaneously interacting with two sites, an ATP-binding site and an AMP-binding site (Figs. 1E and 2A).

Fourth, mutating the ATP-binding site in NBD2 disrupted both ATPase-dependent and adenylate kinase-dependent gating. In contrast, mutating Asn¹³⁰³ (which appears to lie outside the ATP-site based on the structure of other NBDs (21)) to Lys (N1303K) abolished the adenylate kinase-dependent gating without reducing the potency of ATP in ATPase-dependent gating. Thus, N1303K selectively interfered with adenylate kinase activity, just as it did in a recombinant NBD2.

Fifth, ADP inhibits current (24–26), but in behavior that is very unusual for an inhibitor, it also generated a Hill coefficient for ATP of >1 (Fig. 2B). This result suggests cooperativity with interaction of one ATP enhancing the interaction of a second ATP. These data are consistent with AMP formation from ADP via phosphotransfer.

Sixth, in a recombinant CFTR-NBD2 (Fig. 1C), two ADP molecules interacted cooperatively. Moreover in CFTR, current inhibition showed positive cooperativity for ADP (27). These and other data suggest that the reverse adenylate kinase reaction contributes to ADP-dependent inhibition.

These results indicate that NBD2 harbors intrinsic adenylate kinase activity that gates CFTR. Because this enzymatic reaction releases little if any energy under physiologic conditions, these findings bring the energetics of CFTR ion transfer and gating in line with that of other ion channels, none of which are known to require the energy of ATP hydrolysis for gating. They also demonstrate a previously unrecognized enzymatic activity for an ABC transporter.

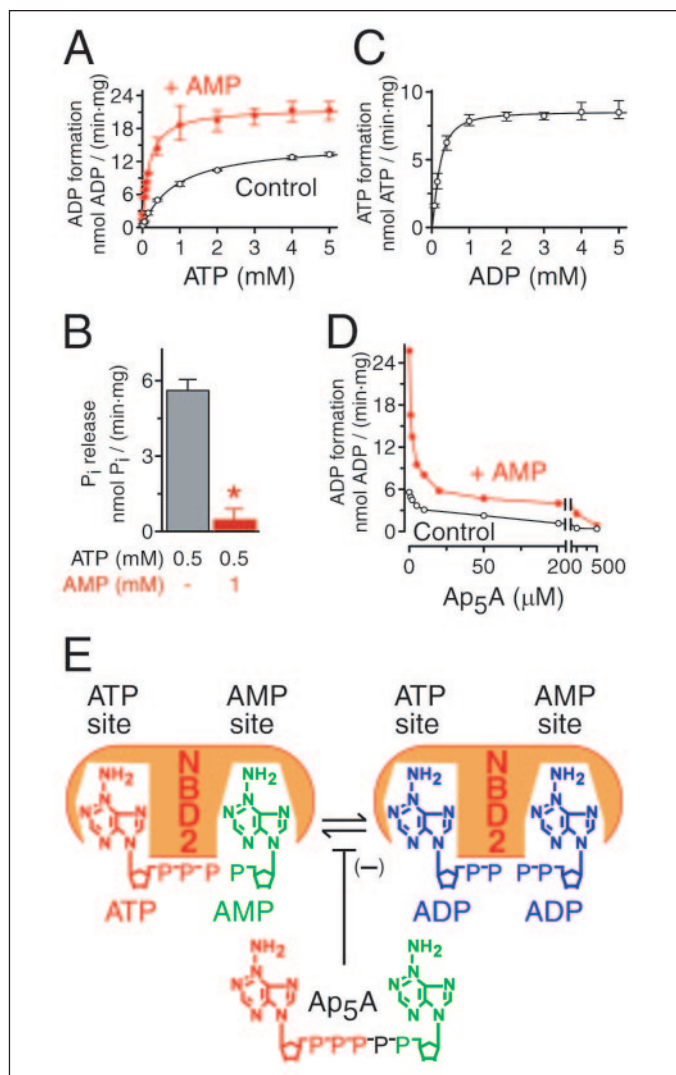


FIGURE 1. **ATPase and adenylate kinase activities of a recombinant NBD2.** *A*, enzymatic activity measured as ADP release from ATP. "Control" is in the presence of ATP alone. *B*, ATPase activity measured as P_i release. *C*, phosphotransfer measured as ATP formation; the line is the fit to Hill equation with a Hill coefficient of 1.49 ± 0.14 . *D*, inhibition of enzymatic activity by Ap₅A in the presence of 1 mM ATP alone (Control) or 1 mM ATP plus 0.1 mM AMP. *E*, model of CFTR-NBD2 with ATP- and AMP-binding sites. Data are from Refs. 15 and 16 with permission.

Does ATPase or Adenylate Kinase Activity Gate CFTR *in Vivo*?

Is adenylate kinase activity only a laboratory phenomenon or does it actually occur in cells? Cellular ATP concentrations lie well above the K_m for both reactions (TABLE ONE), so ATP concentrations will not determine which enzymatic activity occurs in cells. Importantly, intracellular AMP concentrations range from 10 to 700 μM (28–32), and the AMP K_m value of CFTR is $\sim 70 \mu\text{M}$ (16). With these values, cellular AMP would switch CFTR enzymatic activity from ATPase to adenylate kinase. Therefore, we conclude that CFTR will function predominantly as an adenylate kinase *in vivo*. This conclusion is further supported by the fact that other adenylate kinases have AMP K_m values similar to CFTR (33, 34). We therefore expect that like them, the CFTR adenylate kinase function will be active under physiologic conditions.

Implications for Structure

How is it that one protein is capable of functioning either as an ATPase or as an adenylate kinase? The crystal structures of ABC transporter NBDs (4) and adenylate kinases (14, 35) provide some clues. ATPases possess an ATP-binding site, and adenylate kinases contain an ATP-binding site plus an AMP-binding site. Importantly, the core ATP-binding region containing a Walker A motif is very similar for both protein families (36, 37). This structure most

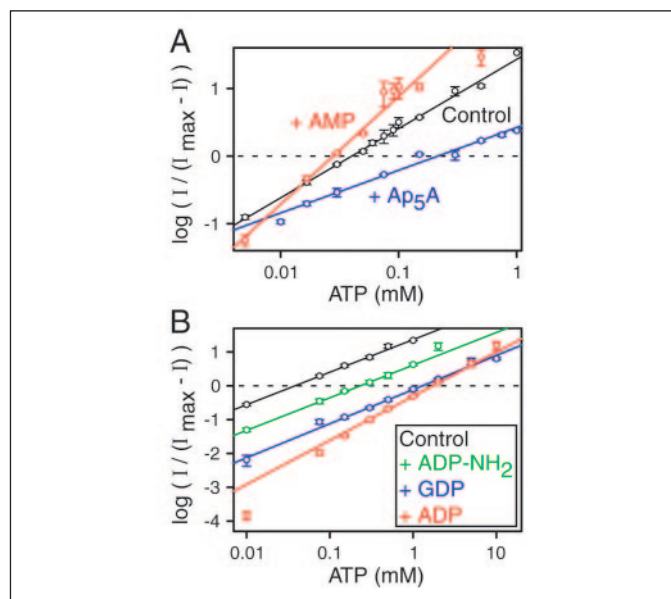


FIGURE 2. **Relationship between ATP concentration and Cl^- current (I).** Data are Hill plots in the presence of ATP alone (Control) or with a 1 mM concentration of the indicated nucleotides. The Hill coefficients are as follows: control, 1.02 ± 0.04 (A) and 0.96 ± 0.04 (B); AMP, 1.60 ± 0.07 ; Ap₅A, 0.63 ± 0.04 ; ADP, 1.30 ± 0.08 ; ADP-NH₂, 0.97 ± 0.08 ; and GDP, 1.01 ± 0.07 . Data were derived from Ref. 16.

likely comprises the ATP site that we found was common to the ATPase and adenylate kinase activities of CFTR.

Where is the AMP-binding site? In adenylate kinases, the AMP site can display considerable structural diversity (38) and can show little sequence conservation between kingdoms (39, 40). In CFTR, the AMP site is unknown, but we speculate that it may lie near the NBD1:NBD2 dimer interface. Positioning the AMP site there would place it near the ATP site where it is available for phosphotransfer.

Implications for NBD Dimerization

Biochemical and structural evidence suggest that the NBDs of ABC transporters can form dimers (Fig. 3) (21, 41–44). In the dimer structure, the NBDs sit in a head-to-tail configuration forming two ATP-binding sites at their interface. Both NBDs contribute to each ATP-binding site, with the Walker A motif of one NBD and the "Signature" LSGGQ motif of the other NBD sandwiching an ATP between them. Interestingly, dimerization is a mechanism that generates positive cooperativity. Thus, finding that AMP altered CFTR gating so that two ATP molecules cooperated in opening CFTR suggests that adenylate kinase activity induces ATP-dependent NBD1:NBD2 dimerization. These data indicate that NBD dimerization in an ABC transporter has a functional consequence; in the case of CFTR that consequence is channel opening.

These observations and speculations raise additional questions. (a) If dimerization is key for channel opening and ATP applied alone opens the channel, then why do we not see ATP-dependent positive cooperativity in the absence of AMP? When ATP is the only nucleotide present, it produces similar gating, and it generates the same net activity as when AMP is also present. Thus, although we expect dimerization to occur with ATP alone, there is no correlate for it in the ATP dose-response curve (16, 20, 22, 23). Perhaps relatively stable ATP binding (and the lack of enzymatic activity) at the NBD1 Walker A:NBD2 Signature interface leads to opening when ATP binds to the NBD2 Walker A:NBD1 "LSGGQ" site (45–47). This scenario could account for the lack of observable cooperativity as evidenced by the Hill coefficient of 1 (Fig. 2A). (b) What conformational changes do ATP:AMP or ADP:ADP phosphotransfer induce? How does it facilitate dimerization? (c) Is the dimer state the open state? If yes, it may explain why AMP influences the opening rate (by facilitating dimer formation) but not the closing rate.

Might ABC Transporters Alter Nucleotide Concentrations in Membrane Microenvironments?

Adenylate kinase activity links AMP with the ATP/ADP equilibrium. This raises the question of whether ABC transporters could alter ATP, ADP, and/or AMP concentrations in restricted membrane microenvironments. Such

TABLE ONE

Cellular nucleotide concentrations and K_m values for CFTR

Data for CFTR are approximate values from Ref. 16. Values of K_{ADP} are the K_i value for CFTR in the presence of 1 mM ATP (27) and the K_m value for an isolated NBD2 (Fig. 1C). Estimates of cellular nucleotide concentrations are derived from Refs. 28–32.

Protein	Enzymatic activity	K_m		K_{ADP}
		ATP	AMP	
CFTR	ATP \rightarrow ADP + P _i	40 μ M		430 μ M
CFTR	ATP + AMP \rightleftharpoons ADP + ADP	28 μ M	70 μ M	200 μ M
		[ATP] _c = 1–11.7 mM	[AMP] _c = 10–700 μ M	[ADP] _c = 0.1–3 mM

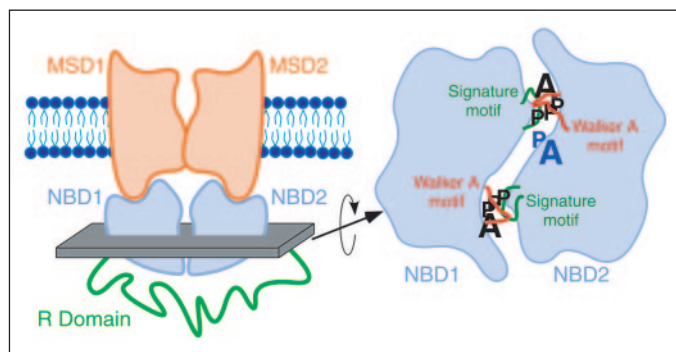


FIGURE 3. Simplified model of dimeric structure of NBD1 and NBD2 of CFTR. ATP is in black and AMP in blue. The AMP-binding site is unknown.

effects might become apparent during states of heavy energy consumption, and an ABC transporter might serve to maintain ATP concentrations. Might this activity affect other proteins or CFTR function? Several ABC transporters regulate the activity of other membrane transport proteins (48), and we wonder whether alterations in local nucleotide concentrations could be responsible in some cases. Moreover, by altering cellular AMP:ATP ratios CFTR might affect the AMP-activated protein kinase (AMPK) (49), which, once activated, switches on catabolic pathways generating ATP and switches off ATP-consuming processes. AMPK-mediated phosphorylation of CFTR also reduces its channel activity (50) suggesting the possibility of a physiologic feedback loop.

Do Other ABC Transporters Function as Adenylate Kinases?

The apparent disconnect between a protein that forms a passive pore and yet utilizes the high energy ATPase activity drove the discovery of endogenous adenylate kinase activity in CFTR. The power of the patch clamp technique for studying single CFTR molecules facilitated that work. We now wonder if other ABC transporters also function as adenylate kinases. Certainly for ABC transporters that actively pump substrate against electrochemical gradients, energy from an ATPase will be required, and adenylate kinase activity would not be expected.

However, it is not likely that all other ABC transporters actively pump substrate, and in fact, in many cases it has been difficult to determine whether or not an ABC transporter performs work (51). For example, some ABC transporters move hydrophobic substrates; because such substrates associate with membranes, it has been difficult to learn if transport occurs against a concentration gradient. In addition, following their translocation, some substrates are rapidly modified so that the concentration of unmodified substrate remains low on the side of the membrane where they are released. As a result, it is unknown whether substrate movement occurs uphill against an electrochemical gradient or downhill. Finally, some ABC transporters, such as the sulfonyleurea receptor (SUR) (52), are not known to perform any transmembrane transport; instead, they regulate other processes.

If substrate flow is energetically neutral or downhill, then adenylate kinase activity could suffice to control transport. When the ABC multidrug transporter LmrA of *Lactococcus lactis* was studied in the presence of ADP, downhill substrate transport and ATP formation were demonstrated (53). It would be interesting to determine whether ATP was synthesized from ADP and P_i or from two ADP molecules via adenylate kinase activity. We also speculate that the adenylate kinase reaction could provide a way to couple transport to the cellular metabolic state. For example, SUR1 is an ABC transporter that senses the cellular metabolic state and controls inwardly rectifying K⁺ channels (Kir6.2) (54). High ATP concentrations close the SUR1-Kir6.2 complex, depo-

larizing pancreatic β cells so they secrete insulin. When ATP concentrations fall or in the presence of ADP or ATP plus AMP, channels open, thereby suppressing insulin secretion (55, 56). Adenylate kinase activity could regulate this process if a cytosolic adenylate kinase associated with the SUR-Kir complex or if an NBD had intrinsic adenylate kinase activity. We speculate that adenylate kinase activity intrinsic to other ABC transporters might provide a physiologic mechanism to couple function with the metabolic state of a cell by sensing the relative amounts of ATP, ADP, and AMP.

The existence of two different but related enzymatic activities in other ABC transporters may help explain the current uncertainty about how enzymatic activity couples to substrate transfer, as well as the difficulty in establishing the relationship between the number of ATP molecules utilized and the number of substrate molecules transported (1, 51). For example, specific experimental conditions could be critical in determining whether adenylate kinase, ATPase, or some combination of both activities control function. Some previous interpretations might have been confounded if only one of the reactions was considered, and relating *in vitro* data to function in cells, which have ATP, ADP, and AMP, may be complicated. Finally, these new findings raise the possibility that under physiologic conditions ABC transporters might switch back and forth between the two enzymatic reactions. Perhaps substrate and/or energy availability could determine their enzymatic mechanism. Further investigations of adenylate kinase activity may help illuminate these persistent uncertainties. They may also suggest novel targets for agonists and antagonists of this medically important family of proteins.

REFERENCES

- Higgins, C. F. (2001) *Res. Microbiol.* **152**, 205–210
- Holland, I. B., and Blight, M. A. (1999) *J. Mol. Biol.* **293**, 381–399
- Ames, G. F. L., Mimura, C. S., Holbrook, S. R., and Shyamala, V. (1992) *Adv. Enzymol. Relat. Areas Mol. Biol.* **65**, 1–47
- Davidson, A. L., and Chen, J. (2004) *Annu. Rev. Biochem.* **73**, 241–268
- Gottesman, M. M., and Ambudkar, S. V. (2001) *J. Bioenerg. Biomembr.* **33**, 453–458
- Bishop, L., Agbayani, R. J., Ambudkar, S. V., Maloney, P. C., and Ames, G. F. L. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 6953–6957
- Davidson, A. L., and Nikaido, H. (1990) *J. Biol. Chem.* **265**, 4254–4260
- Omote, H., and Al-Shawi, M. K. (2002) *J. Biol. Chem.* **277**, 45688–45694
- Sheppard, D. N., and Welsh, M. J. (1999) *Physiol. Rev.* **79**, S23–S45
- Gadsby, D. C., and Nairn, A. C. (1999) *Physiol. Rev.* **79**, S77–S107
- Hanrahan, J. W., Gentzsch, M., and Riordan, J. R. (2003) *ABC Proteins from Bacteria to Man* (Holland, I. B., Cole, S. P. C., Kuchler, K., and Higgins, C. F., eds) pp. 589–618, Academic Press, Amsterdam
- Alberty, R. A., and Goldberg, R. N. (1992) *Biochemistry* **31**, 10610–10615
- Noda, L. H. (1973) in *The Enzymes* (Boyer, P. D., ed) 3rd Ed., Vol. 8, pp. 279–305, Academic Press, New York
- Yan, H., and Tsai, M. D. (1999) *Adv. Enzymol. Relat. Areas Mol. Biol.* **73**, 103–134
- Randak, C., Neth, P., Auerswald, E. A., Eckerskorn, C., Assfalg-Machleidt, I., and Machleidt, W. (1997) *FEBS Lett.* **410**, 180–186
- Randak, C., and Welsh, M. J. (2003) *Cell* **115**, 837–850
- Feldhaus, P., Fröhlich, T., Goody, R. S., Isakov, M., and Schirmer, R. H. (1975) *Eur. J. Biochem.* **57**, 197–204
- Lienhard, G. E., and Secemski, I. I. (1973) *J. Biol. Chem.* **248**, 1121–1123
- Randak, C., Roscher, A. A., Hadorn, H. B., Assfalg-Machleidt, I., Auerswald, E. A., and Machleidt, W. (1995) *FEBS Lett.* **363**, 189–194
- Berger, A. L., Ikuma, M., Hunt, J. F., Thomas, P. J., and Welsh, M. J. (2002) *J. Biol. Chem.* **277**, 2125–2131
- Smith, P. C., Karpowich, N., Millen, L., Moody, J. E., Rosen, J., Thomas, P. J., and Hunt, J. F. (2002) *Mol. Cell* **10**, 139–149
- Szellas, T., and Nagel, G. (2003) *FEBS Lett.* **535**, 141–146
- Csanady, L., Chan, K. W., Seto-Young, D., Kopsco, D. C., Nairn, A. C., and Gadsby, D. C. (2000) *J. Gen. Physiol.* **116**, 477–500
- Anderson, M. P., and Welsh, M. J. (1992) *Science* **257**, 1701–1704
- Schultz, B. D., Venglarik, C. J., Bridges, R. J., and Frizzell, R. A. (1995) *J. Gen. Physiol.* **105**, 329–361
- Weinreich, F., Riordan, J. R., and Nagel, G. (1999) *J. Gen. Physiol.* **114**, 55–70
- Randak, C. O., and Welsh, M. J. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102**, 2216–2220
- Bozzi, A., Martini, F., Leonard, F., and Strom, R. (1994) *Biochem. Mol. Biol. Int.* **32**,

MINIREVIEW: Adenylate Kinase Activity in ABC Transporters

95–103

29. Olson, L. K., Schroeder, W., Robertson, R. P., Goldberg, N. D., and Walseth, T. F. (1996) *J. Biol. Chem.* **271**, 16544–16552
30. Pucar, D., Bast, P., Gumina, R. J., Lim, L., Drahl, C., Juranic, N., Macura, S., Janssen, E., Wieringa, B., Terzic, A., and Dzeja, P. S. (2002) *Am. J. Physiol.* **283**, H776–H782
31. Zeleznikar, R. J., Dzeja, P. P., and Goldberg, N. D. (1995) *J. Biol. Chem.* **270**, 7311–7319
32. Malaisse, W. J., and Sener, A. (1987) *Biochim. Biophys. Acta* **927**, 190–195
33. Byeon, L., Shi, Z., and Tsai, M. D. (1995) *Biochemistry* **34**, 3172–3182
34. Okajima, T., Tanizawa, K., and Fukui, T. (1993) *J. Biochem. (Tokyo)* **114**, 627–633
35. Vornrhein, C., Schlauderer, G. J., and Schulz, G. E. (1995) *Structure* **3**, 483–490
36. Higgins, C. F., Hiles, I. D., Salmond, G. P. C., Gill, D. R., Downie, J. A., Evans, I. J., Holland, I. B., Gray, L., Buckel, S. D., Bell, A. W., and Hermodson, M. A. (1986) *Nature* **323**, 448–450
37. Smith, C. A., and Rayment, I. (1996) *Biophys. J.* **70**, 1590–1602
38. Ren, H., Wang, L., Bennett, M., Liang, Y., Zheng, X., Lu, F., Li, L., Nan, J., Luo, M., Eriksson, S., Zhang, C., and Su, X. D. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102**, 303–308
39. Müller, C. W., and Schulz, G. E. (1992) *J. Mol. Biol.* **244**, 159–177
40. Vornrhein, C., Bonisch, H., Schafer, G., and Schulz, G. E. (1998) *J. Mol. Biol.* **282**, 167–179
41. Moody, J. E., Millen, L., Binns, D., Hunt, J. F., and Thomas, P. J. (2002) *J. Biol. Chem.* **277**, 21111–21114
42. Janas, E., Hofacker, M., Gompf, S., van der Does, C., and Tampé, R. (2003) *J. Biol. Chem.* **278**, 26862–26869
43. Chen, J., Lu, G., Lin, J., Davidson, A. L., and Quioco, F. A. (2003) *Mol. Cell* **12**, 651–661
44. Locher, K. P., Lee, A. T., and Rees, D. C. (2002) *Science* **296**, 1091–1098
45. Basso, C., Vergani, P., Nairn, A. C., and Gadsby, D. C. (2003) *J. Gen. Physiol.* **122**, 333–348
46. Aleksandrov, L., Aleksandrov, A. A., Chang, X., and Riordan, J. R. (2002) *J. Biol. Chem.* **277**, 15419–15425
47. Berger, A. L., Ikuma, M., and Welsh, M. J. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102**, 455–460
48. Schwiebert, E. M., Benos, D. J., Egan, M. E., Stutts, J., and Guggino, W. B. (1999) *Physiol. Rev.* **79**, S145–S166
49. Hardie, D. G. (2003) *Endocrinology* **144**, 5179–5183
50. Hallows, K. R., Raghuram, V., Kemp, B. E., Witters, L. A., and Foskett, J. K. (2000) *J. Clin. Invest.* **105**, 1711–1721
51. Higgins, C. F., and Linton, K. J. (2004) *Nat. Struct. Mol. Biol.* **11**, 918–926
52. Matsuo, M., Ueda, K., Ryder, T., and Ashcroft, F. (2003) in *ABC Proteins from Bacteria to Man* (Holland, I. B., Cole, S. P. C., Kuchler, K., and Higgins, C. F., eds) pp. 551–575, Academic Press, Amsterdam
53. Balakrishnan, L., Venter, H., Shilling, R. A., and van Veen, H. W. (2004) *J. Biol. Chem.* **279**, 11273–11280
54. Babenko, A. P., Aguilar-Bryan, L., and Bryan, J. (1998) *Annu. Rev. Physiol.* **60**, 667–687
55. Huopio, H., Shyng, S. L., Otonkoski, T., and Nichols, C. G. (2002) *Am. J. Physiol. Endocrinol. Metab.* **283**, E207–E216
56. Dzeja, P. P., and Terzic, A. (1998) *FASEB J.* **12**, 523–529