Potassium channel regulation

Structural insights into the function of the nucleotide-binding domains of the human sulphonylurea receptor

Jeff D. Campbell^{1,2}, Mark S.P. Sansom² & Frances M. Ashcroft^{1*}

¹University Laboratory of Physiology, and ²Laboratory of Molecular Biophysics, Department of Biochemistry, University of Oxford, Oxford, UK

The sulphonylurea receptor (SUR) is a member of the ATP-binding cassette (ABC) family of membrane proteins. It functions as the regulatory subunit of the ATP-sensitive potassium (K_{ATP}) channel, which comprises SUR and Kir6.x proteins. Here, we review data demonstrating functional differences between the two nucleotide binding domains (NBDs) of SUR1. In addition, to explain the structural basis of these functional differences, we have constructed a molecular model of the NBD dimer of human SUR1. We discuss the experimental data in the context of this model, and show how the model can be used to design experiments aimed at elucidating the relationship between the structure and function of the K_{ATP} channel.

EMBO reports 4, 1038–1042 (2003)

doi:10.1038/sj.embor.7400003

INTRODUCTION

During recent years, it has become clear that the ATP-sensitive potassium (K_{ATP}) channel has a key role in the physiology of many cells, and that defects either in the channel itself or in its regulation cause human and animal disease (Seino & Miki, 2003). The function of this channel is best understood in the pancreatic β -cell, where it couples changes in plasma glucose concentration to electrical excitability and insulin release (Ashcroft & Gribble, 1999). Studies of genetically modified mice have revealed that K_{ATP} channels are also involved in protection against neuronal seizures and ischaemic stress in heart and brain, in the regulation of vascular smooth muscle tone and in glucose uptake in skeletal muscle (Seino & Miki, 2003). These properties derive from the ability of the K_{ATP} channel to couple cell metabolism to electrical activity, by sensing changes in the cytosolic levels of ATP and MgADP. These nucleotides have antagonistic actions on K_{ATP} channels, with ATP acting as a channel blocker, and MgADP as a channel opener.

The opposing effects of ATP and MgADP on K_{ATP} channel activity are attributable to the presence of two distinct proteins in the octameric channel complex (Seino & Miki, 2003). The pore of the channel is formed from four inwardly rectifying K^+ channel subunits (Kir6.2 or Kir6.1). Each of these is associated with a sulphonylurea receptor subunit (SUR1, SUR2A or SUR2B, depending on the tissue), which regulates the opening and closing of Kir6.x (Matsuo *et al.*, 2002a). Studies on cloned K_{ATP} channels have shown that ATP inhibits channel activity by binding to Kir6.2, whereas channel activation is mediated by the interaction of Mg-nucleotides with SUR (Tucker *et al.*, 1997).

The importance of SUR in metabolic regulation of the K_{ATP} channel was revealed by the discovery that mutations in the β -cell isoform (SUR1) cause congenital hyperinsulinism (CHI) (Sharma *et al.*, 2000). More than 40 disease-causing mutations in SUR1 have been described, and their functional characterization has shown that they fall into two groups: those that prevent correct targeting of the channel to the plasma membrane and those that cause loss of sensitivity to the endogenous activator, MgADP. In both cases, K_{ATP} channels fail to open when metabolism falls during hypoglycaemia, which produces the hyperinsulinaemia that characterizes CHI.

SUR1 is an ABC protein

SUR is a member of the ATP-binding cassette (ABC) family of proteins, which function as transporters, ion channels and channel regulators in both prokaryotes and eukaryotes (Higgins, 2001). Sequence similarities place SUR1 (ABCC8) in the ABCC subfamily, which includes the cystic fibrosis transport regulator (CFTR/ABCC7) and the multidrug-resistance-related protein 1 (MRP1/ABCC1, to which SUR1 is most closely related).

All ABC proteins contain four structural domains: two transmembrane domains (TMDs) containing 6–8 transmembrane helices and two cytosolic nucleotide-binding domains (NBDs) that are involved in nucleotide binding and hydrolysis (Higgins, 2001). In prokaryotes, these domains are often separate subunits that co-assemble to produce a functional ABC protein, whereas in eukaryotes, a single gene usually encodes both NBDs and TMDs. Both the sequence and the structure of the NBDs are highly conserved across all eukaryotic

¹University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, UK

²Laboratory of Molecular Biophysics, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK

*Corresponding author. Tel: +44 1865 272 478; Fax: +44 1865 272 469; E-mail: frances.ashcroft@physiol.ox.ac.uk

Submitted 5 May 2003; accepted 5 August 2003

and prokaryotic ABC proteins. Each contains a conserved Walker A (W_A) motif, a Walker B (W_B) motif, an intervening linker motif (LSGGQ), known as the ABC signature sequence, and a histidine (H-loop) and glutamine (Q-loop) residue (Fig. 1). Mutagenesis studies have implicated all of these in ATP binding and hydrolysis, and/or in coupling nucleotide binding and hydrolysis to changes in protein activity. Like MRP1, SUR1 has three sets of TMDs: the TMD1 and TMD2 shared with all ABC proteins and an additional carboxy-terminal set of five transmembrane helices (TMD0, Fig. 1A).

Many CHI mutations alter residues that are located within the NBDs of SUR1 (Sharma *et al.*, 2000), with which Mg-nucleotides also interact. Thus, our understanding of how Mg-nucleotides and CHI mutations exert their functional effects would be greatly facilitated by a three-dimensional (3D) structure of the NBDs at atomic resolution. Although no such structure exists for SUR1, the NBD

structures of several prokaryotic ABC proteins have recently been solved (Diederichs *et al.*, 2000; Gaudet & Wiley, 2001; Hung *et al.*, 1998; Karpowich *et al.*, 2001; Yuan *et al.*, 2001). In this review, we construct a homology-based model of the NBDs of SUR1, which provides some structural understanding of the functional effects of both site-directed and CHI mutations.

Functional studies reveal that the NBDs interact

Functional studies of both eukaryotic and bacterial ABC proteins have provided evidence that the two NBDs of ABC proteins interact and operate as a functional unit (for a review, see Kerr, 2002). These studies support a model in which the NBDs dimerize in a ‘nucleotide-sandwich’ conformation, with the signature sequence (linker) of one NBD monomer located close to the W_A motif of the other. Experimental evidence in favour of this conformation comes from both functional and crystallographic studies of ABC proteins (Fetsch & Davidson, 2002; Locher *et al.*, 2002; Smith *et al.*, 2002). Because of the high degree of structural and sequence conservation among the NBDs of the different ABC transporters, it is expected that they will also share a common dimer architecture. We therefore constructed a molecular model of the SUR1–NBD dimer on the basis of the known structure of MJ0796 (for details, see legend of Fig. 1), as this protein was co-crystallized with Na-ATP and diffracts to the highest resolution of any NBD nucleotide-sandwich dimer crystal (Smith *et al.*, 2002). Figure 1B gives the sequence alignment on which the model was based.

A model of the NBD dimer of SUR1

Figure 1C shows a model of the SUR1–NBD dimer with bound ATP. As expected from the 3D structure of the MJ0796 template, ATP lies in a binding pocket formed by the W_A and W_B motifs of one NBD and the linker motif of the other. The crucial histidine and glutamine residues are in close proximity to the phosphate tail of ATP.

A consequence of using the nucleotide-sandwich structure as a template is that there is now a difference between the NBD encoded in the primary sequence, and the nucleotide-binding pocket observed in the 3D structure. This is because the W_A and W_B of NBD1 associate with the linker sequence of NBD2, and vice versa. For this reason, we refer to the two nucleotide-binding pockets as

◀ **Fig. 1 | Modelling SUR1.** (A) The transmembrane topology of the sulphonylurea receptor SUR1, showing the transmembrane domains (TMDs) and the nucleotide-binding domains (NBDs). (B) Multiple sequence alignment used to generate the homology model, generated using ClustalW. Green shading indicates sequence homology and blue indicates sequence identity. Walker A (W_A), Walker B (W_B) and linker motifs are indicated. The asterisks indicate the conserved glutamine (in the Q-loop) and histidine (H-loop) residues. (C) Model of the NBDs of SUR1, showing the nucleotide-sandwich dimer and the conserved motifs. The W_A , W_B motifs and signature sequence are yellow, orange and green, respectively. NBD1 is blue and NBD2 is purple. Site 1 and site 2 are indicated. The SUR1 sequence was aligned with that of MJ0796 using ClustalW (Thompson, 1994) and the model generated using MODELLER 6v2 (Sali & Blundell, 1993) with the MJ0796 dimer crystal structure (PDB code 1L2T; Smith *et al.*, 2002) input as the template. The MgATP molecule (shown in red) was added to each SUR model by least-squares fitting to the backbone atoms in the W_A motif of MJ0796. The Na-ATP coordinates were extracted from the MJ0796 PDB and Mg^{2+} was assumed to occupy the same position as Na^+ .

'site 1' (Walker motifs of NBD1, linker NBD2) and 'site 2' (Walker motifs of NBD2, linker NBD1). This will also be the case for other ABC proteins in which the sequences of the NBDs differ, such as CFTR and MRP1 (although this has not been widely appreciated). The sandwich dimer structure provides an explanation for the functional cooperativity observed between the NBDs, because nucleotide binding and the subsequent conformational change in one site is likely to induce an allosteric change in the other. For example, MgADP binding to NBD2 stabilizes ATP binding to NBD1 in both SUR1 and MRP1 (Hou *et al.*, 2003; Ueda *et al.*, 1997, 1999), and ATP binding to NBD1 stabilizes nucleotide binding to NBD2 in MRP1 (Hou *et al.*, 2003).

Nucleotide-binding studies indicate that ATP and ADP bind with high affinity to NBD1 of SUR1 in a Mg-independent fashion, whereas both nucleotides bind with lower affinity to NBD2 in a Mg-dependent fashion (Matsuo *et al.*, 2000a). Furthermore, ATP hydrolysis at NBD1, if it occurs at all, is much less frequent than at NBD2. These properties have been deduced by photo-affinity labelling SUR1 with 8-azido-[³²P]-ATP, mildly digesting the protein with trypsin, and immunoprecipitating tryptic fragments containing either NBD1 or NBD2. To determine if the properties of site 1 correspond to those measured experimentally for NBD1 or for NBD2, it is therefore necessary to know whether ATP remains bound to the W_A motif, or to the signature sequence, following trypsin digestion. Serendipitously, the ability of N-ethylmaleimide (NEM) to prevent ATP binding at NBD1 is abolished by mutation of C717 (Matsuo *et al.*, 1999), which lies in site 1 of the dimer model. Therefore, site 1 binds ATP with higher affinity than site 2 (IC₅₀s of 4 µM and 60 µM, respectively). This is also the case for ADP (IC₅₀s of 26 µM and 100 µM in NBDs 1 and 2, respectively). Similarly, ATP binds preferentially to site 1 of MRP1 (Gao *et al.*, 2000). Thus, in both MRP1 and SUR1, ATP is preferentially bound to site 1 and preferentially hydrolysed at site 2.

Functional differences relate to structural differences

Our model suggests some reasons for the functional differences observed between site 1 and site 2 in both SUR1 and MRP1. In all the NBD crystal structures that have been resolved so far, adenosine forms a π -stacking interaction with a conserved aromatic residue in the active site. There are two possible sequence alignments in this region, but in both cases there are differences in the putative residues that interact with adenosine, which may contribute to the different nucleotide affinities of the two sites of SUR1. In addition, there is a highly conserved glutamate immediately preceding the linker sequence in both NBD1 (E824, site 2) and NBD2 (E1476, site 1). In both sites, this residue is in a position to form a hydrogen bond with the hydroxyl group of the ribose moiety of ATP. In site 2, however, an adjacent arginine residue (R825) could disrupt this interaction and contribute to the lower affinity of this site (Fig. 2B). Similarly, SUR2 and MRP1, which also show differences in ATP binding between the NBDs, have an arginine at the equivalent position in site 2, but not site 1.

Our model also suggests a structural basis for the different hydrolytic properties of sites 1 and 2. There is only one possible alignment of the W_B motif residues involved in ATP binding and hydrolysis. This reveals that, in SUR1, SUR2 and MRP1, the W_B motif has the consensus sequence DD in NBD1 (D853, D854) but DE in NBD2 (D1505, E1506; Fig. 1B). It has been suggested that a glutamate (E) acts as a key hydrolytic residue in the NBD of MJ0796

Fig. 2 | The sulphonylurea receptor SUR1 model. Expanded view of (A) site 1 and (B) site 2 of the sulphonylurea receptor SUR1 model. Nucleotide-binding domains (NBDs) 1 and 2 are shown in yellow and purple, respectively. The ATP molecule is shown in green and Mg²⁺ in brown. Residues that lie within 4 Å of the nucleotide are shown in ball-and-stick configuration.

(Moody *et al.*, 2002). This may explain why ATP is preferentially hydrolysed at site 2 of both SUR1 and MRP1, which also contain a W_B glutamate. In NBD1, the semi-conserved glutamate is replaced by an aspartate (D854), and although this residue can perform the same hydrolytic steps as glutamate, it is ~1.5 Å further away from the γ -phosphate. Our model suggests that mutation of E1506 in NBD2 to aspartate might reduce ATP hydrolysis, whereas the D854E mutation in site 1 might enhance hydrolytic activity. It is worth noting that in P-glycoprotein (the multidrug-resistance protein, MDR1), both sites have glutamate at the W_B hydrolytic position, and both sites hydrolyse ATP (Urbatsch *et al.*, 1995). However, mutation of the glutamate to either glutamine or alanine did not abolish 8-azido-[³²P]-ADP trapping in Pgp (Sauna *et al.*, 2002). Studies of nucleotide binding and hydrolysis at the individual NBDs of SUR1 after mutation of the W_B glutamate residues would therefore be of interest.

The model also reveals that the backbone carbonyls of A858 in NBD1 and S1510 in NBD2, which lie immediately downstream of the W_B motifs, are ~ 3 Å away from the γ -phosphate of ATP in site 2 and site 1, respectively (Fig. 2). Potentially, these residues could form a water-mediated hydrogen bond that might be involved in catalysing nucleotide hydrolysis or stabilizing the hydrolytic transition state. In site 1, S1510 also interacts with Q1483 in the linker motif of NBD2, which might physically disrupt any interaction with ATP and contribute to the low hydrolytic capacity of NBD1 (Fig. 2B). A glycine (G831) is found at the equivalent position in NBD1, which would not be expected to disrupt the interaction. Interestingly, although the linker sequence of most ABC proteins is a highly conserved LSGGQ, that of NBD2 in both SUR2 and MRP1 (which also preferentially hydrolyse ATP at site 2) is FSVFQ, which resembles the FSQFQ of SUR1 (Fig. 1B).

The NBD dimer model was built without recourse to functional data on SUR1. It is therefore encouraging that mutation of residues in close proximity to the nucleotide-binding sites (Fig. 2) has functional effects. In our model, the W_A motif interacts with the β -phosphate of ATP in both NBDs. High-affinity ATP binding to site 1 of SUR1 or MRP1 is abolished by mutation of the W_A lysine in NBD1, and mutation of the W_A lysine in NBD2 prevents the ability of MgADP to stabilize ATP binding at NBD1 (Ueda *et al.*, 1997). Mutation (independently or together) of the lysine residues in the W_A motif of NBD1 and NBD2 of SUR1 (K719, K1384) abolished channel activation by both MgATP and MgADP (Gribble *et al.*, 1997; Shyng *et al.*, 1997). The loss of MgADP binding and activation may be explained by the fact that the W_A lysine interacts with the β -phosphate of the nucleotide (Fig. 2). The data also suggest that ATP binding at site 1 is not influenced by mutations in site 2, and that both ATP binding at NBD1 and MgADP binding at site 2 are essential for nucleotide activation of SUR1. In SUR2A, binding of ATP at NBD1 is proposed to stabilize the MgADP-bound state at site 2 (Zingman *et al.*, 2002).

The effect of mutating the invariant W_B aspartate is similar to the results reported for the W_A lysine, in that mutation of the W_B aspartate at either NBD (D853, D1505) abolishes channel activation by MgADP (Gribble *et al.*, 1997; Shyng *et al.*, 1997), and mutations in NBD1 abolish ATP binding (Ueda *et al.*, 1997). The structural effects of these mutations may be to disrupt the electrostatic coordination of the ATP molecule. However, the exact role of the W_B aspartate is unclear as it lies 6 Å away from the ATP molecule.

Mutation of the conserved serine in the signature (linker) sequence of either site 1 (S1482) or site 2 (S830) did not affect nucleotide binding (Matsuo *et al.*, 2002b). However, mutation of the linker serine in NBD1 reduced, and in NBD2 abolished, K_{ATP} channel activation by MgADP, indicating that the serine is involved in transduction, and showing once again the functional asymmetry of the NBDs. In both NBDs, the linker serine interacts with the β -phosphate of ATP and the backbone of the W_A glycine, but it is not clear why its mutation to arginine should have these functional effects.

The invariant glutamine (Q1426), and histidine (H1537) in NBD2, which markedly decrease channel activation by MgADP (Matsuo *et al.*, 2002b), are both at positions within site 2 at which they may influence nucleotide hydrolysis and/or transduction.

Disease-causing mutations

Several mutations in the NBDs of SUR1 are associated with CHI. Although some of these mutations prevent targeting of the protein to the plasma membrane, others, such as G1381S, R1420C, E1506K and L1551V (Fig. 3) cause CHI by impairing K_{ATP} channel activation in response to metabolic inhibition or MgADP (Huopio *et al.*, 2000). Our model offers new insights into the mechanism by which these mutations produce their functional effects. For example, E1506 is close to the γ -phosphate and associated Mg^{2+} atom. Therefore, it is possible that substitution of a positively charged lysine for glutamate will reduce Mg-nucleotide binding by electrostatically preventing the coordination of Mg^{2+} .

In the absence of structural information, it was difficult to envisage why the L1551V mutation abolished K_{ATP} channel activation by MgATP and MgADP (Reimann *et al.*, 2003), because in the primary sequence, L1551 is not positioned close to any functionally important conserved motifs. In the 3D model, however, L1551 lies within 3 Å of the conserved W_A serine in NBD2 (S1382) and 3.5 Å of the W_A lysine (K1385), and is therefore in a position to influence nucleotide binding. A similar argument can be made for the CHI mutation G1381S, which lies within the W_A motif of NBD2, and might therefore influence nucleotide binding at site 2 and so prevent channel activation by MgADP (Shyng *et al.*, 1998).

The main effect of the CHI mutation R1420C is to prevent cooperative nucleotide binding (Matsuo *et al.*, 2000b). This can be explained by the fact that in our model, R1420 is proximal to the α -helical subdomain in NBD2 that communicates between site 2 and site 1. Binding of MgADP at site 2 may therefore be expected to influence ATP binding at site 1.

Fig. 3 | Location of congenital hyperinsulinism mutations in the nucleotide-binding domains of sulphonylurea receptor SUR1. Nucleotide-binding domains (NBDs) 1 and 2 are shown in blue and purple, respectively. The ATP molecule is shown in yellow and Mg^{2+} in blue. Residues mutated in congenital hyperinsulinism are shown in green and in ball-and-stick configuration.

Conclusions

Our homology model of the NBDs of SUR1 is able to explain the functional effects of some site-directed and natural mutations and reveals interactions that are not evident from the primary sequence. This suggests that the model may provide a valuable tool for structure–function studies of the K_{ATP} channel and that it can be used to guide future experiments. Combining theory and experimental data will help to refine the model and further clarify the functional roles of the NBDs of SUR1.

ACKNOWLEDGEMENTS

We thank the Wellcome Trust for support. J.D.C. is a Wellcome Trust structural biology student and F.M.A. is the Royal Society GlaxoSmithKline Research Professor.

REFERENCES

- Ashcroft, F.M. & Gribble, F.M. (1999) ATP-sensitive K^+ channels and insulin secretion: their role in health and disease. *Diabetologia*, **42**, 903–919.
- Diederichs, K., Diez, J., Grell, G., Muller, C., Breed, J., Schnell, C., Vonrhein, C., Boos, W. & Welte, W. (2000) Crystal structure of MalK, the ATPase subunit of the trehalose/maltose ABC transporter of the archaeon *Thermococcus litoralis*. *EMBO J.*, **19**, 5951–5961.
- Fetsch, E.E. & Davidson, A.L. (2002) Vanadate-catalyzed photocleavage of the signature motif of an ATP-binding cassette (ABC) transporter. *Proc. Natl Acad. Sci. USA*, **99**, 9685–9690.
- Gao, M., Cui, H.R., Loe, D.W., Grant, C.E., Almquist, K.C., Cole, S.P.C. & Deeley, R.G. (2000) Comparison of the functional characteristics of the nucleotide binding domains of multidrug resistance protein 1. *J. Biol. Chem.*, **275**, 13098–13108.
- Gaudet, R. & Wiley, D.C. (2001) Structure of the ABC ATPase domain of human TAP1, the transporter associated with antigen processing. *EMBO J.*, **20**, 4964–4972.
- Gribble, F.M., Tucker, S.J. & Ashcroft, F.M. (1997) The essential role of the Walker A motifs of SUR1 in K-ATP channel activation by Mg-ADP and diazoxide. *EMBO J.*, **16**, 1145–1152.
- Higgins, C.F. (2001) ABC transporters: physiology, structure and mechanism—an overview. *Res. Microbiol.*, **152**, 205–210.
- Hou, Y.X., Riordan, J.R. & Chang, X.B. (2003) ATP binding, not hydrolysis, at the first nucleotide-binding domain of multidrug resistance-associated protein MRP1 enhances ADP-Vi trapping at the second domain. *J. Biol. Chem.*, **278**, 3599–3605.
- Hung, L.W., Wang, I.X.Y., Nikaido, K., Liu, P.Q., Ames, G.F.L. & Kim, S.H. (1998) Crystal structure of the ATP-binding subunit of an ABC transporter. *Nature*, **396**, 703–707.
- Huopio, H., Reimann, F., Ashfield, R., Komulainen, J., Lenko, H.L., Rahier, J., Vauhkonen, I., Kere, J., Laakso, M., Ashcroft, F. & Otonkoski, T. (2000) Dominantly inherited hyperinsulinism caused by a mutation in the sulfonylurea receptor type 1. *J. Clin. Invest.*, **106**, 897–906.
- Karpowich, N., Martsinkevich, O., Millen, L., Yuan, Y.R., Dai, P.L., MacVey, K., Thomas, P.J. & Hunt, J.F. (2001) Crystal structures of the MJ1267 ATP binding cassette reveal an induced-fit effect at the ATPase active site of an ABC transporter. *Structure*, **9**, 571–586.
- Kerr, I.D. (2002) Structure and association of ATP-binding cassette transporter nucleotide-binding domains. *Biochim. Biophys. Acta*, **1561**, 47–64.
- Locher, K.P., Lee, A.T. & Rees, D.C. (2002) The *E. coli* BtuCD structure: a framework for ABC transporter architecture and mechanism. *Science*, **296**, 1091–1098.
- Matsuo, M., Tucker, S.J., Ashcroft, F.M., Amachi, T. & Ueda, K. (1999) NEM modification prevents high-affinity ATP binding to the first nucleotide binding fold of the sulphonylurea receptor, SUR1. *FEBS Lett.*, **458**, 292–294.
- Matsuo, M., Tanabe, K., Kioka, N., Amachi, T. & Ueda, K. (2000a) Different binding properties and affinities for ATP and ADP among sulfonylurea receptor subtypes, SUR1, SUR2A, and SUR2B. *J. Biol. Chem.*, **275**, 28757–28763.
- Matsuo, M., Trapp, S., Tanizawa, Y., Kioka, N., Amachi, T., Oka, Y., Ashcroft, F.M. & Ueda, K. (2000b) Functional analysis of a mutant sulfonylurea receptor, SUR1-R1420C, that is responsible for persistent hyperinsulinemic hypoglycemia of infancy. *J. Biol. Chem.*, **275**, 41184–41191.
- Matsuo, M., Ueda, K., Ryder, T. & Ashcroft, F.M. (2002a) in *ABC Proteins: From Bacteria to Man* (eds Holland, B., Kuchler, K., Higgins, C. & Cole, S.). Academic, London, UK.
- Matsuo, M., Dabrowski, M., Ueda, K. & Ashcroft, F.M. (2002b) Mutations in the linker domain of NBD2 of SUR inhibit transduction but not nucleotide binding. *EMBO J.*, **21**, 4250–4258.
- Moody, J.E., Millen, L., Binns, D., Hunt, J.F. & Thomas, P.J. (2002) Cooperative, ATP-dependent association of the nucleotide binding cassettes during the catalytic cycle of ATP-binding cassette transporters. *J. Biol. Chem.*, **277**, 21111–21114.
- Reimann, F., Huopio, H., Dabrowski, M., Proks, P., Laakso, M., Otonkoski, T. & Ashcroft, F.M. (2003) Characterisation of new K_{ATP} -channel mutations associated with congenital hyperinsulinism in the Finnish population. *Diabetologia*, **46**, 241–249.
- Sali, A. & Blundell, T.L. (1993) Comparative protein modelling by satisfaction of spatial restraints. *J. Mol. Biol.*, **234**, 779–815.
- Sauna, Z.E., Muller, M., Peng, X.H. & Ambudkar, S.V. (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.
- Seino, S. & Miki, T. (2003) Physiological and pathophysiological roles of ATP-sensitive K^+ channels. *Prog. Biophys. Mol. Biol.*, **81**, 133–176.
- Sharma, N., Crane, A., Gonzalez, G., Bryan, J. & Aguilar-Bryan, L. (2000) Familial hyperinsulinism and pancreatic β -cell ATP-sensitive potassium channels. *Kidney Int.*, **57**, 803–808.
- Shyng, S.L., Ferrigni, T. & Nichols, C.G. (1997) Regulation of K_{ATP} channel activity by diazoxide and MgADP. *J. Gen. Physiol.*, **110**, 643–654.
- Shyng, S.L., Ferrigni, T., Shepard, J.B., Nestorowicz, A., Glaser, B., Permutt, M.A. & Nichols, C.G. (1998) Functional analyses of novel mutations in the sulfonylurea receptor 1 associated with persistent hyperinsulinemic hypoglycemia of infancy. *Diabetes*, **47**, 1145–1151.
- Smith, P.C., Karpowich, N., Millen, L., Moody, J.E., Rosen, J., Thomas, P.J. & Hunt, J.F. (2002) ATP binding to the motor domain from an ABC transporter drives formation of a nucleotide sandwich dimer. *Mol. Cell*, **10**, 139–149.
- Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, **22**, 4673–4680.
- Tucker, S.J., Gribble, F.M., Zhao, C., Trapp, S. & Ashcroft, F.M. (1997) Truncation of Kir6.2 produces ATP-sensitive K^+ channels in the absence of the sulphonylurea receptor. *Nature*, **387**, 179–183.
- Ueda, K., Inagaki, N. & Seino, S. (1997) MgADP antagonism to Mg^{2+} -independent ATP binding of the sulfonylurea receptor SUR1. *J. Biol. Chem.*, **272**, 22983–22986.
- Ueda, K., Komine, J., Matsuo, M., Seino, S. & Amachi, T. (1999) Cooperative binding of ATP and MgADP in the sulfonylurea receptor is modulated by glibenclamide. *Proc. Natl Acad. Sci. USA*, **96**, 1268–1272.
- Urbatsch, I.L., Sankaran, B., Bhagat, S. & Senior, A.E. (1995) Both P-glycoprotein nucleotide-binding sites are catalytically active. *J. Biol. Chem.*, **270**, 26956–26961.
- Yuan, Y.R., Blecker, S., Martsinkevich, O., Millen, L., Thomas, P.J. & Hunt, J.F. (2001) The crystal structure of the MJ0796 ATP-binding cassette—implications for the structural consequences of ATP hydrolysis in the active site of an ABC transporter. *J. Biol. Chem.*, **276**, 32313–32321.
- Zingman, L.V., Hodgson, D.M., Bienengraeber, M., Karger, A.B., Kathmann, E.C., Alekseev, A.E. & Terzic, A. (2002) Tandem function of nucleotide binding domains confers competence to sulfonylurea receptor in gating ATP-sensitive K^+ channels. *J. Biol. Chem.*, **277**, 14206–14210.