NATURE|Vol 440|23 March 2006|doi:10.1038/nature04711

K_{ATP} channels as molecular sensors of cellular metabolism

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In responding to cytoplasmic nucleotide levels, ATP-sensitive potassium (K_{ATP}) channel activity provides a unique link between cellular energetics and electrical excitability. Over the past ten years, a steady drumbeat of crystallographic and electrophysiological studies has led to detailed structural and kinetic models that define the molecular basis of channel activity. In parallel, the uncovering of disease-causing mutations of K_{ATP} has led to an explanation of the molecular basis of disease and, in turn, to a better understanding of the structural basis of channel function.

Action potentials, which define electrical excitability, depend on the activity of voltage-gated Na^+, Ca^{2+} and K^+ (Kv) channels (see the review in this issue by Sanguinetti and Tristani-Firouzi, p. 463). But electrical activity without modulation would be like an automobile with one speed, no steering and no brakes. In addition to the channels that play the leading parts, bio-electric signalling involves many crucial bit players (see the review in this issue by Miller, p. 484) that provide essential gears, steering and brakes. These include a family of K^+ channels, the so-called inward rectifier (Kir) channels, which lack steep voltage-gating. By stabilizing the resting membrane potential, these channels act as a brake on excitability. One member, the K_{ATP} channel, modulates electrical activity in multiple tissues[1–4]. In a complex interplay of mechanisms, the K_{ATP} channel is inhibited by the non-hydrolytic binding of ATP, but activated by interactions with Mg^{2+}-bound nucleotides (Mg-nucleotides) at separate sites. The inhibitory effect dominates, and channels are closed, when cellular phosphorylation potential is high, but as metabolism decreases, the activating effect wins out and channels open. In this way, the channel provides a unique electrical transducer of the metabolic state of the cell.

What mechanisms of channel regulation allow this transduction? Many questions remain to be addressed, but as discussed below, the cloning of constituent subunits of the K_{ATP} channel and the crystallization of structurally related proteins have provided the raw materials to gain a detailed understanding of how nucleotides interact with the K_{ATP} channel and an explanation of the molecular basis of channel activity and of channel-dependent human disease.

Protein architecture of K_{ATP} channels

K_{ATP} channels are formed by the unique combination of two dissimilar proteins[2] (Fig. 1a). One, Kir6, is a member of the Kir channel family; the other, SUR (for sulphonylurea receptor), is a member of the ATP-binding cassette (ABC) protein family, which includes CFTR (cystic fibrosis transmembrane conductance regulator; see the review in this issue by Gadsby, p. 477). As discussed in detail below, ATP inhibition results from interaction with the Kir6 subunits, whereas Mg-nucleotide activation reflects interaction with SUR subunits. In the vertebrate genome, there are two Kir6 genes (*KCNJ8*, *KIR6.1* and *KCNJ11*, *KIR6.2*) and two SUR genes (*ABCC.8*, *SUR1* and *ABCC.9*, *SUR2*), and co-expression of combinations of the two genes replicates all of the key features of classical K_{ATP} channel activity[5–7]. Biophysical and biochemical experiments established that four Kir6.2 subunits generate the channel pore, each Kir6.2 being associated with one SUR1 protein[8,9] (Fig. 1b). Stoichiometry has not been tested for other Kir6/SUR combinations, and it is conceivable that Kir6 complexes occur naturally without an associated SUR subunit, or with different stoichiometry. In recombinant cells, Kir6.2 can form channels without SUR1 when truncated at the carboxyl terminus[10]. In this case, truncation removes a trafficking sequence (Arg-Lys-Arg) that otherwise causes Kir6.2 retention in the endoplasmic reticulum (ER). Similarly, SUR1, which also contains an ER-retention sequence, is not efficiently trafficked to the surface membrane in the absence of Kir6.2, which indicates that the subunits normally act to mutually shield these trafficking signals[10].

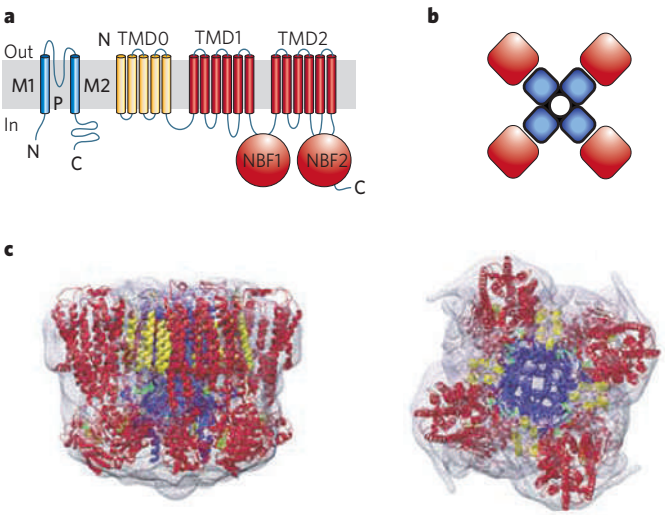

Figure 1 | The K_{ATP} channel is formed from two dissimilar subunits. **a**, Inward rectifier K^+ channel Kir6 subunits generate the channel pore and sulphonylurea receptor (SUR) subunits generate the regulatory subunit. TMD, transmembrane domain; NBF, nucleotide-binding fold; M1, M2, transmembrane helices; P, pore. **b**, The channel is a functional octamer of four Kir6 subunits, and each subunit is associated with four SUR subunits. **c**, Images at 18 Å resolution of the entire K_{ATP} complex viewed in the plane of the membrane (left) or from above the membrane (right) require tight packing of subunit models. Reproduced, with permission, from ref. 33.

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A high-resolution model of Kir6.2

Crystallization of the bacterial KcsA K^+ channel[11] heralded a revolution in our understanding of channel structure. The overall architecture of the KcsA transmembrane domain, consisting of two transmembrane helices (M1 and M2) bridged by an extracellular loop that generates the narrow portion of the pore and controls ion selectivity, is likely to be present in all major cation channel family members, including Kir6.2 (Fig. 2a). Crystallization of linked amino- and carboxy-terminal domains of Kir3.1 (ref. 12) and Kir2.1 (ref. 13), and of full-length prokaryotic Kir channel homologues KirBac1.1 (ref. 14) and KirBac3.1 (ref. 15) (Protein Data Bank accession numbers 1XL4 and 1XL6), revealed an extended cytoplasmic pore and the likely location of ligand-binding sites. A consensus model of Kir6.2, based on elements of both the eukaryotic and the prokaryotic Kir structures[16,17], provides satisfying agreement with mutational studies of Kir6.2, particularly with respect to the inhibitory ATP-binding site (Fig. 2b; see below). At the membrane interface, the verity of the model, which relies heavily on the KirBac1.1 structure, is less clear. KirBac1.1 and KirBac3.1 are part of a distinct subfamily of prokaryotic Kir proteins, with potentially important primary sequence differences and functional differences from eukaryotic Kir channels[18,19] that need to be understood for the correct interpretation of molecular models. The lesson from CLC chloride channels (see p. 484), that homologous structures can encode both ion channels and ion exchangers, is a sobering one. Importantly, although all eukaryotic Kir channels are strongly activated by interaction with phosphatidylinositol-4,5-bisphosphate (PIP_2), KirBac1.1 is inhibited by PIP_2 (ref. 19). The linkers between the cytoplasmic domain and the transmembrane domain are shorter in KirBac1.1 than in euk-Kir channels[19] and the missing residues in the C terminus include two key residues (Arg 176 and Arg 177 in Kir6.2) that are predicted to interact directly with PIP_2 (refs 20–22). It is an intriguing hypothesis that KirBac channels are normally active in bacterial membranes (which typically contain no phosphoinositides) and that eukaryotic Kir channels acquired these additional residues specifically to restore function in the face of otherwise inhibitory PIP_2.

Templates for SUR

As with other members of ABC subfamily C (see p. 477), SUR1 (ABCC8) and SUR2 (ABCC9) each contain two six-helix transmembrane domains (TMD1 and TMD2). In addition, SURs have an N-terminal TMD0 domain that consists of five transmembrane helices (Fig. 1a). There is evidence[23] that TMD0 is crucial for trafficking Kir6.2 subunits to the surface membrane, and its role in controlling the gating of Kir6.2 (refs 24, 25) suggests an intimate relationship with the pore-forming subunit. Low homology between the transmembrane domains of crystallized ABC proteins and SURs makes molecular modelling risky, although provocative insights and hypotheses have been gleaned from the exercise[2,26], including speculations about locations of the binding sites for the channel opener diazoxide and the inhibitory sulphonylurea glibenclamide[2].

SUR also contains one nucleotide-binding fold (NBF1) between TMD1 and TMD2, and a second (NBF2) after TMD2 (Fig. 1a). Several crystal structures are now available for isolated NBFs from bacterial ABC proteins[27–29]; in almost every case, the NBFs crystallize as 'head-to-tail' dimers. Rather than each NBF having a self-contained nucleotide-binding site (ABS), each ABS is formed at the dimer interface and contains elements from both NBFs. CFTR mutant cycle analysis provides evidence for NBF1–NBF2 interactions in the full-length protein (see p. 477), and modelling of the SUR NBFs as heterodimers[26] (Fig. 3b) seems reasonable given the high degree of conservation among NBF sequences. Experimentally, azido-ATP labelling experiments have shown that nucleotide binding to NBF2 stabilizes ATP binding to NBF1 (ref. 30). In addition, NBF1 and NBF2 can be co-immunoprecipitated[31], and a soluble NBF1–GFP (green fluorescent protein) construct is recruited to the membrane by a TMD2–NBF2 construct when co-expressed in insect cells[32].

Recently, complete K_{ATP} complexes generated from tandem SUR1–

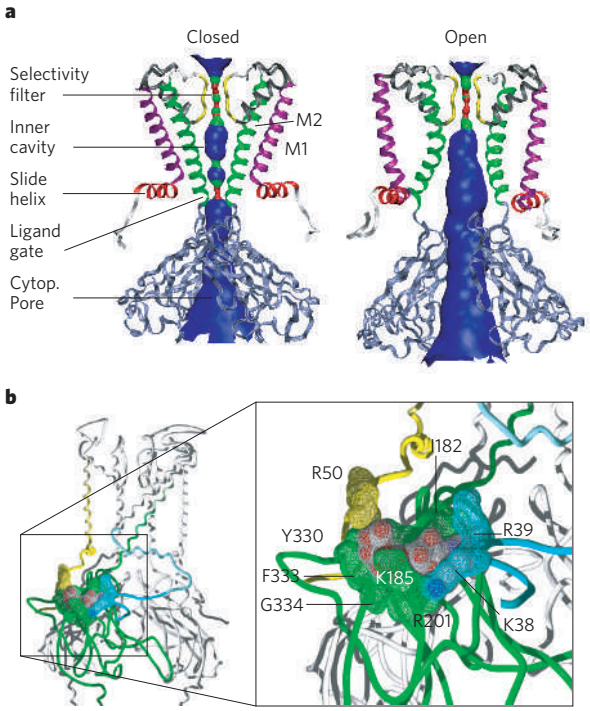


Figure 2 | ATP-dependent gating in high definition. **a**, A model inward rectifier K^+ (Kir) channel showing potential gating states. Closed and open structures modelled on two-dimensional crystals of KirBac3.1 are shown in two different conformational states[15], with critical regions labelled. Transmembrane helix M2 (green) hinges around the glycine in the centre of the segment, opening the entrance to the inner cavity. The blue volume represents free access of water. The channel is closed (left) at the volume coloured in red, where there is insufficient space for a water molecule to pass. Reproduced, with permission, from ref. 15. **b**, Three subunits of a consensus Kir6.2 model[17] are shown with the rear-most subunit removed for clarity. The subunit positioned to the front provides the bulk of one ATP-binding site in its green carboxy-terminal cytoplasmic domain. The amino-terminal domain of this subunit provides the blue 'slide helix' overlying the binding site to the right and then curves back to close the binding pocket around the adenine ring. Beyond the slide helix, at the turn of the N terminus, residue Arg 50 from the subunit to the left (yellow) is predicted to close the binding pocket at the γ-phosphate of ATP. ATP is rendered by atom type, with oxygens in red and nitrogens in blue.

Kir6.2 constructs have been isolated and studied using electron micrography and three-dimensional reconstruction[33] (Fig. 1c). The complex is a compact structure of approximately 18 × 13 nm. It points to the intimate packing of four SUR1 subunits against four Kir6.2 subunits, with the TMD0 domain nestled between TMD1, TMD2 and Kir6.2 (Fig. 1c), and suggests that NBF1 and NBF2 interactions can occur between subunits, a possibility that is yet to be experimentally tested.

Mechanism of nucleotide gating of K_{ATP} channels

Several ligands affect K_{ATP} channel activity. ATP (with or without Mg^{2+}) inhibits, and PIP_2 activates, by direct interaction with Kir6.2 subunits. Sulphonylureas inhibit, and K^+ channel-opener drugs activate, by interaction with the SUR subunit. In addition, in the presence of Mg^{2+}, ATP and ADP can activate the channel through interaction with the NBFs of SUR. Inhibition by ATP binding to Kir6.2 and activation by Mg-nucleotides is almost certainly the primary physiological regulatory mechanism. Figure 4 provides a theoretical model of the biochemical details. How much material do we have to add realistic details to the model?

Where are the gates?

The weight of evidence suggests that K_{ATP} channels contain two independent types of gate in the permeation pathway (Fig. 4). K_{ATP} chan-

nels show fast, ligand-independent gating (Box 1), which indicates the presence of a gate in the selectivity filter[34] (Fig. 2a) — the part of the channel that confers selectivity to particular ions — but here we concern ourselves mainly with the slow, ligand-dependent gates. Crystallization of the bacterial MthK K^+ channel in an apparently open state led to a now widely accepted hypothesis[35] that the main gate in all cation channels involves a hinged motion of M2 (termed S6 in Kv channels; see p. 463), closing the channel at the bottom of the inner cavity, at the narrow collar generated by the crossing of the bundle of M2 helices (Fig. 2a). Consistent with this hypothesis, mutations in M2, particularly near the cytoplasmic end, affect slow gating in multiple Kir channels, including Kir6.2 (refs 36–38). Reduction of single-channel conductance after introduction of positive charge to the inner cavity by methyl thio sulphhydrol ethylamine ($MTSEA^+$) modification of introduced cysteines[39,40] shows that this region can act as a significant barrier in the permeation process. Furthermore, the ability of $MTSEA^+$ and other sulphydryl reagents to access the inner cavity through this region indicates a minimum in the open state diameter. Molecular models of open versus closed states suggest that minimal backbone M2 movement[15,39,41] is required to increase the diameter of the pore through this region considerably (Fig. 2b).

Kinetic analyses showing that the inner cavity is only accessible to cytoplasmic $MTSEA^+$ in the open state and that blockers are trapped in the inner cavity in the closed state[42,43] seem to confirm that the ligand-operated gate is located at the bottom of the inner cavity (Fig. 2). At this point, there is debate about whether permeation is stopped by specific side chains blocking ion flow, or is a more diffuse reflection of an unfavourable environment for K^+ dehydration. Whether the final opening transition reflects a concerted motion of all four subunits or whether there are transitional steps involving each subunit is also unresolved[36,38], but available experimental data are well described by models in which each subunit undergoes independent gating transitions (Box 1).

How does ATP close the channel?

The cytoplasmic domains of each Kir6.2 subunit provide the binding sites for ATP and PIP_2, which stabilize closed and open states of the channel, respectively[36] (Box 1). Molecular modelling suggests that there are four ATP-binding sites on the channel[16,17,36], each formed at the Kir6.2 subunit interfaces. Kinetic analyses indicate that unliganded subunits (that is, those not bound to ATP or PIP_2) are relatively unstable and that ATP and PIP_2 binding are mutually excluded[36] (Box 1). Subunits will therefore be predominantly bound to one ligand or the other at any one time. In the absence of ATP, wild-type subunits are bound to membrane PIP_2 (and hence in the permissive, 'open' state) for ~90% of the time. Because all four subunits need to be in the permissive ('open') state for the channel to be active, this results in an observed open probability of ~0.5 (0.9^4). Binding of ATP to the unliganded subunit will trap it in the non-permissive, 'closed', state. With an association constant, K_A, of ~10 μM for wild-type subunits, and with an ATP concentration that is two to three orders of magnitude higher than this in energized cells, each wild-type subunit is probably bound for more than 90% of the time. Trapping of any one subunit in the closed conformation is sufficient to close the channel[44,45], and so for most cells under any but the most extreme conditions, the expected open probability would be $<0.1^4$: that is, negligible without the stimulatory effects of Mg-nucleotides acting at the SUR subunit (see below).

Docking of ATP in the consensus model of Kir6.2 (ref. 17 and Fig. 2b) was guided by the results of numerous mutagenesis studies that have provided candidate residues for the ATP-binding site. Many mutations alter ATP sensitivity independently of ATP-binding affinity, by altering the intrinsic stability of the open state (that is, they control the open–closed equilibrium constant, L; Box 1) and only a few residues (for example, Arg 50 in the N terminus, and Ile 182, Lys 185, Arg 201 and Gly 334 in the C terminus[46–50]) control ATP affinity directly. These residues are well separated in the primary sequence, but are all close to one another at the inter-subunit interface in the model (Fig. 2b). Potential PIP_2-interacting residues (Arg 54, Arg 176, Arg 177 and Arg 206)[20,22,49] are near the ATP site and appropriately located to interact with PIP_2 headgroups (Fig. 3a). Overlapping ATP- and PIP_2-binding sites, and biochemical competition of the two ligands for the non-ligand-bound state[51], are consistent with the proposed 'negative heterotropic' interaction of these two ligands in channel regulation[22,36].

How the binding of ATP or of PIP_2 is coupled to the closing or opening of the gate is still unknown. One interesting feature of the KirBac structures is the existence of a 'slide helix' (Fig. 2) that lies along the plane of the membrane and physically connects the N terminus, which in Kir6.2 forms part of the ATP-binding site, to the cytoplasmic end of M1 (refs 14, 15). It is interesting that naturally occurring disease mutations that affect the channel open-state stability cluster along the slide helix of Kir6.2 (Fig. 5; see below), highlighting a role for this helix in controlling channel gating[14,15].

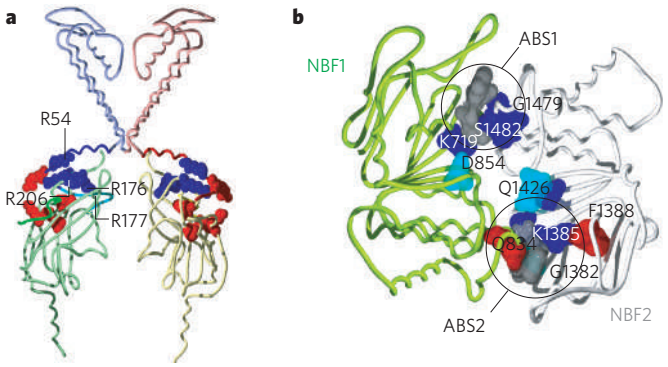


Figure 3 | Binding sites for opening ligands. a, Molecular basis of the 'negative heterotropic' interaction between ATP and PIP_2. Predicted ATP-binding site residues (red) and potential PIP_2-interacting residues (blue) in a consensus Kir6.2 model[92] show how close these groups are likely to be, and how potential PIP_2-binding residues are in the region of phospholipid headgroups. Two diagonally opposed subunits are shown in each case. **b**, Nucleotide-binding fold 1 (NBF1)–NBF2 heterodimeric structure of ATP-binding sites (ABSs) in sulphonylurea receptor SUR1 (modelled on the bacterial ATP-binding protein MsbA using MODELER version 6, verified with PROCHECK). ATP is rendered in grey in ABS1 and ABS2. Mutation of residues in dark and light blue causes reduced or slowed MgADP activation of channel activity, respectively. Mutation of residues in red causes enhanced MgADP activation.

What goes on at the SUR NBFs?

In the face of ATP inhibition at Kir6.2, physiological activation of the K_{ATP} channel arises acutely from the interaction of nucleotides with the NBFs of SUR[52] (Fig. 3b). In bacterial NBFs, dimerization is favoured by the presence of nucleotide, and ATP hydrolysis occurs in a cooperative manner[53,54], leading to the hypothesis that the nucleotide-bound dimer is the catalytically active species. By analogy, it is suggested that Mg^{2+}-dependent ATP hydrolysis at the dimeric SUR NBFs also provides the 'power stroke' that overcomes the inhibitory effect of ATP on Kir6.2 (Fig. 4). The Mg^{2+} dependence of α^{32}P- and γ^{32}P-azido-ATP labelling of SUR NBFs provides indirect evidence that SUR NBFs do hydrolyse ATP[55,56]. Trapping of channels in an activated state by vanadate (a transition-state analogue that mimics a post-hydrolytic, ADP-bound state) and in an inactivated state by beryllium fluoride (a transition-state analogue that mimics the pre-hydrolytic, ATP-bound state)[57] add further consistency to the hypothesis that ATP hydrolysis underlies channel activation.

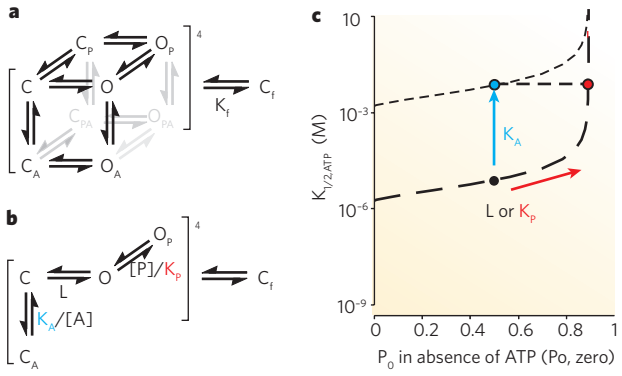
However, direct hydrolysis measurements on SUR NBFs are sparse[33,57–59], and mutations analogous to those that have drastic effects on ATP hydrolysis in bacterial NBFs have only modest effects on SUR NBFs[58]. In patch-clamp experiments, channel activation by exogenous MgADP is much more effective than activation by MgATP, and the

Box 1 | Kinetic mechanism of K_{ATP} channel gating

Even in the absence of ATP, the single-channel kinetics of K_{ATP} are complex, but certain key features are clearly discernible and can be modelled by assuming a single 'fast' gate and ligand-operated 'slow' gates within each subunit[36]. Single-channel analyses consistently reveal one open time and multiple closed times[34,44,50]. The fast gate gives rise to the short open and closed times (the 'intra-burst' events), which are voltage-dependent but ligand-independent, and affected by mutations of residues in or near the selectivity filter[34]. In addition, there are several longer closed times (the 'inter-burst' closures). It is these slow gating events that are modulated by nucleotides and membrane phosphatidylinositol-4,5-bisphosphate (PIP_2)[38,44].

Tetrameric models (shown in panel **a**), in which each subunit undergoes independent gating transitions, not only replicate all essential features of ATP inhibition, but also have important predictive properties[36]. The assumption that each of the four subunits can be in an open or closed conformation (in the absence of ligand) and that the channel conducts only if all subunits are in the open conformation automatically produces multiple closed states[44] and implies that closure of any one subunit will be sufficient to close the channel[45]. Assuming that the open channel can close by such individual subunit closures (O_P to C_P, or O_A to C_A) or, at the selectivity filter, by a concerted event (fast gating, O to C_f controlled by K_f), such a model produces a single short open time and multiple closed times with inter-burst closures dominated by subunit closures[36]. The physical reality is that PIP_2- and ATP-binding sites are probably overlapping (Fig. 3a), consistent with negligible occupancy of C_{PA} and O_{PA} states predicted by the model. Furthermore, O_A and C_P states are rarely sampled in wild-type models.

For wild-type channels, the subunits then exist primarily in only four states (panel **b**) and ATP binds primarily to the closed state. This feature predicts a characteristic nonlinear relationship (panel **c**) between ATP sensitivity (the concentration of ATP causing half-maximal inhibition, $K_{1/2,ATP}$) and open probability (P_O) — one that is clearly adhered to by wild-type and mutant channels under a range of experimental conditions[36]. Changes in intrinsic open-state stability (L) or PIP_2 affinity (K_P) cause the channel to move up or down this nonlinear relationship, whereas changes in ATP affinity (K_A) shift the channel onto a parallel relationship. One consequence of these two effects is that it is possible to reduce apparent ATP sensitivity (as measured by $K_{1/2,ATP}$) to the same value by two mechanistic routes (dashed line), with different consequences for other regulatory features (see main text).



same 'hydrolysis' mutations lead to decreased MgADP stimulation of K_{ATP} channels[58,60–62] (Fig. 3b). Why MgADP should so strongly activate the channel in excised patches if a hydrolytic cycle is required for activation is unclear. To fit this property into the model (Fig. 4) would require that the 'activated' state resulting from hydrolysis persists after MgADP dissociation, at least long enough for rebinding of exogenous MgADP to maintain the state. Just how reasonable this notion is has not been addressed. It remains conceivable that hydrolysis, if it happens at all, is an epiphenomenon and that preferential binding of MgADP at ABS2 is the activator of the channel.

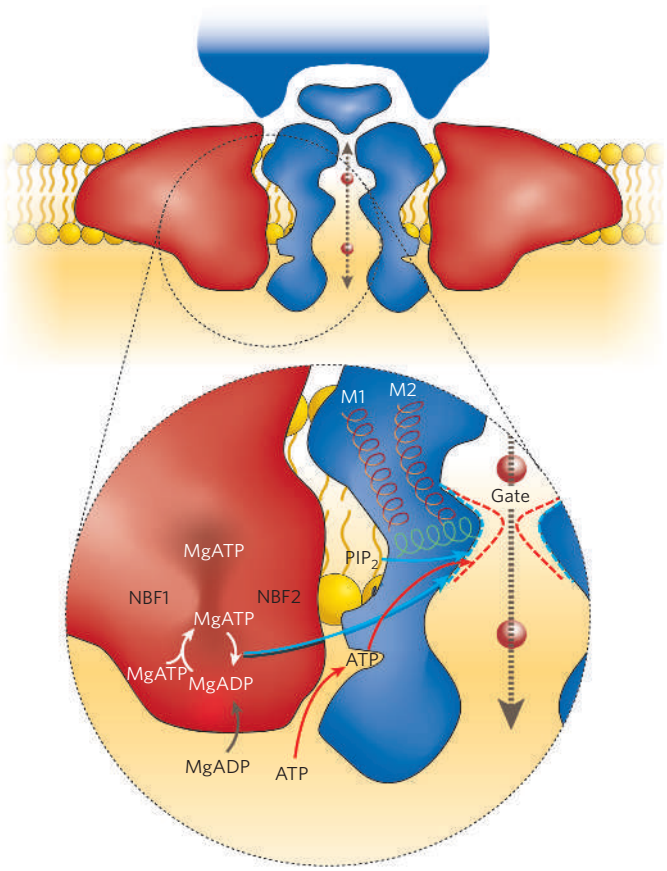


Figure 4 | Nucleotide regulation of K_{ATP} activity. The metabolically controlled gate of the channel is located at the cytoplasmic end of the inner cavity. Phosphatidylinositol-4,5-bisphosphate (PIP_2) interaction provides an energetic pull to open channels, and ATP binding provides the energetic push to close the ligand-operated gate, perhaps through the physical link provided by the 'slide helix' (green). MgATP binds to each of the ATP-binding sites (ABSs) that are formed at the interface between nucleotide-binding folds NBF1 and NBF2 in each sulphonylurea receptor (SUR) subunit. ATP hydrolysis at the second site results in a conformational 'activated' state that is transduced to an 'override' of ATP inhibition at the Kir6.2 subunit. The 'activated' state persists through MgADP dissociation, and can be maintained by MgADP rebinding.

From structure to disease and back

K_{ATP} channels are widely expressed in vertebrate tissues, and genetic manipulation in mice has revealed important consequences of loss of channel subunits in different tissues, highlighting the coordinate nature of the two subunits in generating tissue-specific channel activity[63]. The same tissue-specific phenotype is generated in several cases when one or the other partner is knocked out: both $SUR1^{-/-}$ and $Kir6.2^{-/-}$ reiterate a glucose-insensitive insulin secretory phenotype due to loss of K_{ATP} in the pancreas[64–66]. $SUR2^{-/-}$ and $Kir6.2^{-/-}$ animals both lack muscle K_{ATP}[67,68], and both $SUR2^{-/-}$ and $Kir6.1^{-/-}$ reiterate a phenotype that mimics human Prinzmetal angina[69,70], with spontaneous coronary vasospasm leading to early death, probably due to the vasoconstrictive effects of reduced vascular K_{ATP} current. Overexpression of mutant K_{ATP} subunits in the pancreas also shows how increased or decreased channel activity, respectively, underlies a hypoinsulinaemic, diabetic phenotype, or hyperinsulinaemic phenotype, respectively[71] (Fig. 5a). Alongside these animal models, genetic analyses have demonstrated corollary diseases—neonatal diabetes mellitus (NDM) and hyperinsulinaemia — in humans[1–3,71,72]. Studies of recombinant properties of mutant channels can explain the molecular basis of such diseases, and can also provide important forward genetic evidence for the role of specific structural features of the channel.

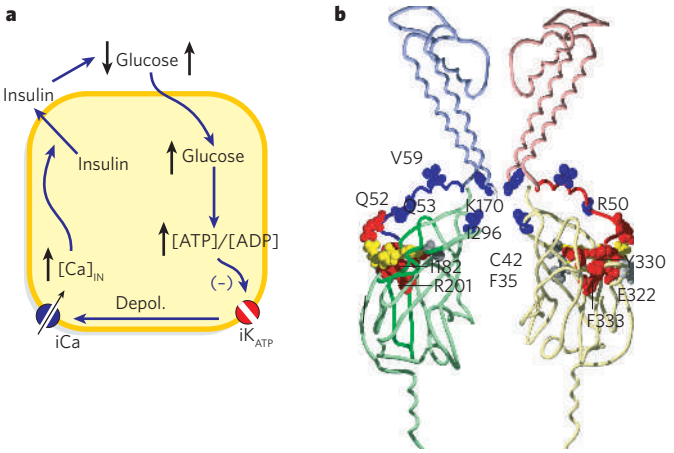


Figure 5 | Molecular defects of neonatal diabetes. **a**, When glucose rises in the pancreatic β-cell, metabolism is stimulated, raising the ratio of ATP to ADP. This closes K_{ATP} channels, which depolarizes the cell, opening Ca^{2+} channels, causing intracellular Ca^{2+} to rise, and triggering insulin secretion. Insensitivity of K_{ATP} to ATP inhibition will cause maintained hyperpolarization and decreased insulin secretion. **b**, Residues of inward rectifier K^+ channel subunit Kir6.2 that are mutated in neonatal diabetes,highlighted in the consensus model[92]. Residues in red contribute to forming the ATP-binding site. Residues in blue, conspicuously clustering at the end of the inner cavity and in the 'slide helix', control intrinsic open-state stability. Residues in grey have not yet been studied in detail. ATP is shown in yellow. Two opposing subunits are shown (subunit *a*,*c* in the transmembrane region; *b*,*d* in the cytoplasmic region).

K_{ATP} defects in hyperinsulinaemia

K_{ATP} channels suppress insulin secretion by hyperpolarizing the β-cell membrane (Fig. 5a), and so loss of K_{ATP} channel activity is expected to cause hypersecretion. Congenital hyperinsulinaemia, a genetic disorder characterized by dysregulated insulin secretion, is the most common cause of hypoglycaemia in infancy, and more than 50 loss-of-function hyperinsulinaemia mutations have now been recognized in K_{ATP} genes[73]. Although some mutations are in Kir6.2, the majority are in SUR1 (refs 74,75) and most cause one of two major defects: biosynthetic or trafficking defects that lead to absent or reduced surface expression of channels; or loss of MgADP activation of expressed channels, which could reflect direct effects on ATP hydrolysis or ADP binding at the NBFs, or defects in the coupling to channel opening. As a result, β-cells remain depolarized and persistently secreting. Hyperinsulinaemia mutations tend to cluster in the SUR1 NBFs, and almost invariably result in reduced MgADP activation[76] (Fig. 3b), highlighting the crucial role of SUR NBFs in the physiological activation of K_{ATP} channels.

One of the most common hyperinsulinaemia mutations (SUR1 [ΔPhe 1388]) causes defective trafficking and lack of surface expression of functional K_{ATP} channels, and altered channel function[77]. The mutant protein appears to be retained in the ER, similar to the CFTR[ΔPhe 508] mutation (see p. 477). Another single amino acid mutation (Arg1394His) also causes defective trafficking in mammalian cells, but rather than being retained in the ER, the protein appears to accumulate in the Golgi[78]. For such mutations, manipulations that allow correction of the trafficking defects might be of therapeutic value[79].

K_{ATP} defects in neonatal diabetes

Whereas loss of K_{ATP} activity causes persistent hypersecretion of insulin, gain of function should cause the opposite: that is, lack of secretion and diabetes. Multiple mutations in Kir6.2 have recently been found to underlie NDM. Random mutations are most likely to cause loss of a specific protein function. In this case, it is loss of ATP sensitivity[71,72,80] resulting from one of two mechanisms (Box 1): decreased apparent affinity of the ATP-binding pocket for the nucleotide; or an increase in the intrinsic stability of the open state[81,82]. Consistent with a direct effect on binding, residues Arg 50, Ile 182 and Arg 201, all identified as mutated in NDM patients, were all previously implicated as likely ATP-binding residues in Kir6.2, and all are located in the ATP-binding site in the model channel (Fig. 5b). Tyr 330 and Phe 333, also found to be mutated in NDM patients, are predicted to lie close to the phosphate tail in the binding pocket[16].

Mutations that alter channel gating in the absence of ATP can occur throughout the Kir6.2 subunit[44,50,83]. By stabilizing the open state of the channel (increasing *L*; Box 1), ATP sensitivity is reduced without a change in ATP-binding affinity[36,84]. Loss of ATP sensitivity coupled to high open probability in Gln52Arg, Cys42Arg, Tyr330Cys, Ile1296Leu and Val59Gly mutations indicates that this mechanism is operating in these NDM mutations[81,82,85]. Gln 52 and Val 59 are located within the slide helix region of Kir6.2 (Fig. 6), lending consistency to the suggestion that this 'linker' serves to physically couple the ATP-binding site to the gating region[14]. An effect on the gate region itself might explain the diabetes-causing effects of mutations at residue Lys 170 (Lys170Asn,Lys170Arg)[86] (Fig. 5b).

The realization that neonatal diabetes results from gain of K_{ATP} channel function has rapidly led to changes in therapy from injected insulin to the painless use of oral sulphonylureas[87,88]. However, understanding of the molecular basis of K_{ATP} function also immediately predicts an underlying caveat: sulphonylureas also stabilize the closed state of the channel, and sulphonylurea sensitivity depends on the open-state stability of the channel, such that mutations that increase open-state stability also reduce sulphonylurea sensitivity[82]. Because different NDM mutations alter ATP sensitivity by two distinct mechanisms (by reducing ATP affinity or by increasing the open-state stability; Box 1), so they show differential sulphonylurea sensitivity[82]—mechanistic information that might help in tailoring pharmacologies for treating the disease.

Future directions

What are the important questions to pursue in future? From a strictly reductionist angle, the first challenge is to generate a high-resolution crystal structure of the K_{ATP} complex. The recent structural study by Mikhailov *et al.*[33] is tantalizing, suggesting that recombinant expression in insect cells will prove to be a useful approach. Beyond this, hints that K_{ATP} can be complexed with enzymes and other proteins[89,90] need to be explored using cellular imaging approaches, as well as with recombinant complexes that could lead to higher-resolution structures. Less ambitiously, short-term gains will be made from crystallizing domains of the channel. Crystallization of the cytoplasmic domains of Kir2.1 (ref. 13) and Kir3.1 (ref. 12), as well as the NBFs of CFTR (see p. 477), indicates that crystallization of the homologous ligand-interacting domains in the K_{ATP} channel could be realized in the near future. Further progress in crystallizing KirBac proteins, combined with biochemical and functional assays, will provide increasing insights into gating mechanisms and the molecular details of channel–ligand interactions.

What are the frontiers in terms of understanding ligand regulation and gating motions in the channel? There is now a consensus that ligand-dependent gating involves motions of M2/S6 that block permeation at the bottom of the inner cavity, but exactly how this is coupled to ligand interactions with cytoplasmic domains is unclear. The conformational changes that result from nucleotide hydrolysis and ADP binding at the NBFs of SUR, or the interaction of sulphonylureas and K^+ channel openers with SUR, and how they ultimately influence the channel gate, are also unknown.

How will future structural insights affect our understanding of pathophysiology or our approach to clinical therapeutics? K_{ATP} could be involved in a whole spectrum of diabetes mellitus[85] and other organ pathologies[63], and we will continue to see effects of K_{ATP} subunit deletion and manipulation affecting the functioning of multiple organs. Because all proteins are evolutionarily tailored to perform specific functions, it follows that disease-causing mutations are most likely to cause loss of the specific function. In the present context, loss of channel activation due to loss of the activating effects of nucleotides on SUR1 underlies net

channel 'loss of function' in most cases of hyperinsulinaemia, whereas loss of ATP sensitivity through mutation of Kir6.2 underlies net 'gain of function' in all NDM mutations identified so far. But it is conceivable that spontaneous mutations also cause increases in an activating function, such as increased MgADP affinity of SUR1 (refs 60, 91). Detection of disease-causing mutations will further inform the molecular basis of channel function, but the sulphonylurea desensitizing effect of open-state stabilizing mutations[82] shows how an understanding of the underlying molecular basis of activity can provide insights into the mechanisms of disease. ■

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Acknowledgements I am grateful to R. Masia, B. Koster, D. Enkvetchakul, H. Kurata and T. Flagg for providing suggestions on the text, and to R. Masia, D. Enkvetchakul, F. Ashcroft and M. Sansom for providing molecular models of SUR1 NBFs and Kir6.2.

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