Brief Review
Gating Mechanism of $K_{\text{ATP}}$ Channels: Function Fits Form

D. Enkvetchakul and C.G. Nichols
Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110

Based initially on their prominence in the squid giant axon, their useful pharmacology, and subsequently on being the first cloned K channels, voltage-gated K (Kv) channels have received the lion’s share of attention to their biophysical properties. Kv and Ca-activated K channel kinetics and gating mechanisms have been analyzed, and modeled, in exquisite detail (Hille, 2001). Inward rectifier K (Kir) channels have received less attention, and perusal of the literature indicates that there is still no common understanding of kinetic mechanisms in Kir channels. It is our contention, however, that one is in reach. Amongst Kir channels, ATP-sensitive ($K_{\text{ATP}}$) channels are uniquely regulated by cytoplasmic nucleotides and specific pharmacological agents. As such, they play a critical role in coupling cellular metabolism to electrical activity, and are major drug targets in pancreatic, vascular smooth muscle and cardiac muscle (Ashcroft, 1988; Nichols and Lederer, 1991). We would argue that these unique properties also permit elucidation of important features of channel gating that are relevant to the whole class of Kir channels. The molecular mechanisms of $K_{\text{ATP}}$ channel regulation have occupied many groups for the last twenty years. Kinetic measurements have led to mathematical models of gating, mutagenesis has indicated relevant molecular elements, and crystallization of various K channel subunits and domains now provides templates for the channel structure. Distilling a consistent model of channel activity and regulation from this broth of data is the challenge for the field, and the topic of this Brief Review.

The $K_{\text{ATP}}$ channel is formed from four Kir6.2 pore-forming subunits, and four regulatory sulfonylurea receptor (SUR) subunits (Clement et al., 1997; Inagaki et al., 1997; Shyng and Nichols, 1997) (Fig. 1). Activity is modulated by voltage and by multiple ligands, including ATP and PIP$_2$, which act on the Kir6.2 subunits themselves, as well as sulfonylureas, potassium channel openers, and Mg-nucleotides, which act on the SUR subunit. Inhibitory ATP binds to the Kir6.2 subunit, while MgATP- and ADP-activation results from interaction with the SUR subunits (Matsuo et al., 1999, 2000; Tanabe et al., 1999; Ueda et al., 1999; MacGregor et al., 2002; Vanoye et al., 2002). $K_{\text{ATP}}$ channel behavior is undoubtedly complex, and at present a complete kinetic model of channel activity, including pharmacological regulation through the SUR subunits is impossible. However, we will argue that a consistent model of Kir6.2 channel activity does arise, and that from this model, the additional complexity of heteromeric complexes will ultimately emerge. We will first consider how thermodynamic and kinetic measurements lead to a model that can explain gating, then consider the structural basis of this behavior.

A Consistent Kinetic Model for Channel Gating

Gating of the $K_{\text{ATP}}$ channel: a tetrameric model for Kir channel gating in the absence of ATP. Even in the absence of ATP, single $K_{\text{ATP}}$ channel kinetics are complex, and different

Figure 1. The complex $K_{\text{ATP}}$ channel. (A) The channel is formed from two dissimilar subunits: Kir6.2 subunits generate the channel pore, SUR subunits generate the regulatory subunit. (B) Each channel is a functional octamer of four Kir6.2 subunits, each associated with four SUR subunits. (C) Likely locations of Kir6.2 channel gating are at the selectivity filter (1) or at the lower end of the inner cavity formed by the M2 helices (2).
laboratories report quantitatively widely differing lifetimes. Nevertheless, certain kinetic components are clearly distinguishable. Single-channel analyses consistently reveal a single-exponential open lifetime distribution, and a multiexponential closed lifetime distribution (Alekseev et al., 1998; Drain et al., 1998; Fan and Makielski, 1999; Enkvetchakul et al., 2000, 2001; Proks et al., 2001). There is invariably a predominant short closed time and one open time (Alekseev et al., 1998; Drain et al., 1998). These short open and closed times, frequently analyzed as the “intraburst” events, are voltage dependent, and are affected by mutations of residues in or near the selectivity filter of the channel (Proks et al., 2001). In addition, there are always several longer closed times that comprise “interburst” closures. As considered below, similar kinetic properties are replicated in other Kir channel family members (e.g., Choe et al., 1999; Bard et al., 2000), consistent with a common underlying gating mechanism.

What kind of model can accommodate these kinetic properties? Numerous schemes have been proposed, based on time-homogeneous Markov models, to describe the open-closed behavior of $K_{\text{ATP}}$ channels (Qin et al., 1989; Nichols et al., 1991; Shyng et al., 1997; Alekseev et al., 1998; Enkvetchakul et al., 2000, 2001; Proks et al., 2001; Li et al., 2002). Unfortunately, many models focus on describing limited aspects of channel behavior and do not account for other critical features, which severely limits their predictive utility. Kir channels are tetramers (Glowatzki et al., 1995; Shyng and Nichols, 1997; Doyle et al., 1998; Nishida and MacKinnon, 2002) (see below) and we would strongly argue that relatively simple, tetrameric kinetic models not only replicate all essential features of $K_{\text{ATP}}$ channel gating but also have important predictive properties. As illustrated in Fig. 2 A, 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 (Enkvetchakul et al., 2000). Assuming that the open channel can close as a concerted event (i.e., “fast” gating), or by individual subunit closure, such a model (Scheme 0, see Fig. 2 A) will produce one short and five (or four disregarding order of the subunits) “long” closed states, but only one open state, consistent with observed lifetime distributions (Enkvetchakul et al., 2000). This simple tetrameric model will produce bursts of openings, with the intraburst events dominated by the “fast” gating transitions, and interburst closures dominated by subunit closures (Enkvetchakul et al., 2000). It is important to note that even if the subunit open- and closed-durations overlap with the “fast” events, there will still be “bursting” (since there will still be long multi-subunit closures), although a significant number of subunit closures will now be included within bursts in any burst-discriminator analysis.

**ATP Interactions with the $K_{\text{ATP}}$ Channel—Implicating ATP Interaction with Each Subunit**

The major consistently reported kinetic feature of ATP gating is that “long” closed lifetime distributions get progressively longer in the presence of inhibitory ATP
Tetrameric models (Enkvetchakul et al., 2000, 2001; Markworth et al., 2000) not only replicate these qualitative features of channel gating, but provide quantitative agreement with additional critical features. While multiple closed lifetimes that all lengthen as ATP increases would require multiple unbound closed states in a linear model, a tetrameric model with only a single ATP-bound subunit conformation (e.g., Scheme I) automatically generates multiple overlapping lifetimes that progressively lengthen as [ATP] (and hence occupancy of C state in Scheme I, Fig. 2 A) increases (Enkvetchakul et al., 2000, 2001). Second, steady-state dose–response curves for ATP inhibition of K<sub>ATP</sub> channels are not well fitted by a symmetrical Hill relationship, since they are considerably steeper at higher [ATP] than they are at lower [ATP] (Fig. 2 B) (Ashcroft and Gribble, 1998; Enkvetchakul et al., 2000; Nichols et al., 1991). Such an asymmetric dose–response curve is automatically generated by a tetrameric subunit model, since only a single subunit transition into a C state is necessary to close the channel (and allow access to a long-lived ATP-bound state) but, at saturating [ATP] concentrations (i.e., with each of the four subunits in the C state), four ATP molecules must dissociate for the channel to open (Ashcroft and Gribble, 1998; Enkvetchakul et al., 2000; Markworth et al., 2000).

Antagonistic Behavior of PIP<sub>2</sub> and Open State Stability Mutants on ATP Inhibition: Implicating an Allosteric Four Subunit Model

In the absence of ATP, application of negatively charged phospholipids (in particular PIP<sub>2</sub>) to K<sub>ATP</sub> channels results in an increased open probability time course of PIP<sub>2</sub> (PIP<sub>2</sub> = 20 + 30,000*[1 - exp(-time/tau)]<sup>1.5</sup>, tau = 5 min), and the predicted current in 0, 0.1, and 1 mM ATP. Equilibrium constants used to simulate the model in this and subsequent figures are given in Table I (slightly modified from those calculated using the rate constants given in Enkvetchakul et al., 2001). L defines the intrinsic open-closed equilibrium in the unliganded subunit, p and 1/a define the relative stabilization of open/closed state ratios, when the subunit is PIP<sub>2</sub> bound, or ATP bound, respectively. (B). Relationship between K<sub>1/2,ATP</sub> (the ATP concentration causing half-maximal inhibition) and Po,zero for wild-type K<sub>ATP</sub> channels after application of PIP<sub>2</sub> from patches like that shown in A (data from Enkvetchakul et al., 2000). The red dashed and solid lines are the predictions of the model, the limits of the solid line again corresponding to PIP<sub>2</sub> = 4 and 8,192 arbitrary units, points indicate the predictions for each doubling of PIP<sub>2</sub>. Also shown are measured data and the predicted relationship between K<sub>1/2,ATP</sub> and Po for truncated Kir6.2<sub>C36</sub> channels expressed without SUR1 (see Table I) after application of PIP<sub>2</sub> (from Enkvetchakul et al., 2000). The blue dashed and solid lines are the predictions of the model, the limits of the solid line again corresponding to PIP<sub>2</sub> = 4 and 8,192 a.u., points indicating the predictions for each doubling of PIP<sub>2</sub>.
(Po,zero) (Hilgemann and Ball, 1996; Fan and Makielski, 1997). Since these first reports on KATP channels, a similar action has been demonstrated on all Kir channels (Liou et al., 1999; Rohacs et al., 1999, 2003; Zhang et al., 1999; Lopes et al., 2002). Importantly, PIP2 has also been shown to be synergistic to activating ligands (e.g., G-proteins on Kir channels) and antagonistic to inhibitory ATP on Kir6.2 channels (Baukrowitz et al., 1998; Shyng and Nichols, 1998; Fan and Makielski, 1999; Kobrinsky et al., 2000; Okamura et al., 2001; Sadja et al., 2001). It has been suggested that the two effects on KATP channels (i.e., to increase Po,zero and to reduce ATP-sensitivity) may reflect distinct processes (Fan and Makielski, 1999; Okamura et al., 2001), based primarily on the argument that increase of Po,zero is detectable before loss of ATP sensitivity is. However, such a temporal disparity also follows directly from the tetrameric model (Fig. 3A, for modeling details see figure legends and original publications; Enkvetchakul et al., 2000, 2001). To explicitly incorporate PIP2 binding steps, Scheme I must be extended, e.g., to Scheme II (Enkvetchakul et al., 2001), which is a restricted case of the fully allosteric model (Scheme III, Fig. 2). The essence of such models is that each subunit can exist in open or closed states whether or not any particular ligand is bound. The intrinsic “open state stability” is a useful descriptor of the intrinsic open/closed equilibrium of the unliganded subunit (denoted by the equilibrium constant L in the modeling below). The open/closed equilibrium is then weighted by some factor (a or p in the modeling), when ATP or PIP2 is bound (so that although L describes the unliganded open/closed equilibrium, it will reflect the equilibrium distribution at any given [PIP2]). The restricted scheme II may be a reasonable approximation, consistent with the likely structural basis of ligand sensitivity, since PIP2 and ATP binding may be mutually exclusive (MacGregor et al., 2002; Vanoye et al., 2002).

Fig. 3 A shows the time course of change in Po,zero and ATP sensitivity from a wild-type Kir6.2 tetrameric model (Fig. 3A, for modeling details see figure legends and original publications; Enkvetchakul et al., 2000, 2001). To explicitly incorporate PIP2 binding steps, Scheme I must be extended, e.g., to Scheme II (Enkvetchakul et al., 2001), which is a restricted case of the fully allosteric model (Scheme III, Fig. 2). The essence of such models is that each subunit can exist in open or closed states whether or not any particular ligand is bound. The intrinsic “open state stability” is a useful descriptor of the intrinsic open/closed equilibrium of the unliganded subunit (denoted by the equilibrium constant L in the modeling below). The open/closed equilibrium is then weighted by some factor (a or p in the modeling), when ATP or PIP2 is bound (so that although L describes the unliganded open/closed equilibrium, it will reflect the equilibrium distribution at any given [PIP2]). The restricted scheme II may be a reasonable approximation, consistent with the likely structural basis of ligand sensitivity, since PIP2 and ATP binding may be mutually exclusive (MacGregor et al., 2002; Vanoye et al., 2002).

Qualitatively similar behavior is observed for the gating of Kir6.2 in both the presence and absence of SUR subunits (Tucker et al., 1997). In each case, the channels are inhibited by ATP, and activated by PIP2 (which causes both an increase in Po,zero and decreased sensitivity to inhibitory ATP; Baukrowitz et al., 1998; Enkvetchakul et al., 2000). However, there are significant quantitative differences: the Po,zero is considerably lower, and the stimulatory effect of PIP2 is weaker, on Kir6.2ΔC36 channels expressed without SUR1 (Fig. 3B, closed symbols, from Enkvetchakul et al., 2000). Are these features predicted in any straightforward way by the tetrameric-allosteric model? Steady-state relationships are well modeled quantitatively by simply assuming that the effect of removing SUR is to stabilize the ligand-unbound state (Enkvetchakul et al., 2000). As shown in Fig. 3B, stabilizing the unliganded channel (by increasing Kα and Kβ by 50-fold and 1,000-fold, respectively), quantitatively predicts both the steady-state and the time-dependent changes of K1/2ATP,Po,zero relationships after application of PIP2 for Kir6.2ΔC36 channels expressed without SUR1. Predicting the behavior of the channel in the absence or presence of SUR1 thus requires no change in L, i.e., no change in the properties of the subunit gate itself.

Mutations which change the binding affinity of ATP per se (i.e., which alter Kα), will not affect current in

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<th>Table I</th>
<th>Equilibrium Constants Used in Simulating 6-state Allosteric Tetramer Model (Scheme II)</th>
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<td>Wild-type Kir6.2 + SUR1</td>
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<th>Adjustments for mutant channels</th>
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<td>Δ36 (-SUR1)</td>
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<td>R176A</td>
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<tr>
<td>Kp</td>
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<td>0.0333 au</td>
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*aArbitrary units.

*bParameter adjustments are combined for double mutant (Kir6.2 [L164C,R176A] etc.) channels in Fig. 4 C.

p and 1/a are the relative stabilization of open/closed state ratios, when the subunit is PIP2 bound, or ATP bound, respectively.

measured parameters (Po,zero and K1/2ATP) change with a time course that varies considerably from experiment to experiment, they remain closely correlated to one another, as predicted by Scheme II (Enkvetchakul et al., 2000).
the absence of ATP. However, many point mutations of Kir6.2 have been shown to cause correlated increase of 
Po,zero and decrease of ATP sensitivity (Shyng et al., 1997; Drain et al., 1998; Trapp et al., 1998; Tucker et al., 1998; Enkvetchakul et al., 2000). How are these effects incorporated into the gating scheme? Such mutations can change ATP sensitivity and Po,zero by changing the intrinsic equilibrium between the open and closed states of the unliganded subunit (i.e., the intrinsic “open state stability,” L) (Enkvetchakul et al., 2001). An important prediction of the model is that in addition to reducing the efficacy of ATP inhibition, a plateau of inhibition will be observed at saturating [ATP] if L is increased sufficiently (i.e., so that L/a becomes large enough, such that the O, occupancy becomes reasonable at saturating [ATP]). This important prediction is actually met by certain mutations in the lower part of M2 of Kir6.2 (Enkvetchakul et al., 2001). In particular, Kir6.2[L164C]+SUR1 channels are extremely insensitive to ATP, and show very little inhibition by even 10 mM ATP (Fig. 4). Scheme II predicts that combining this mutation with a mutation to decrease PIP2 binding (i.e., to decrease Kp) will increase the apparent sensitivity to ATP, and reveal a plateau of inhibition above ~1 mM ATP (Fig. 4). Strikingly, this is indeed observed experimentally for Kir6.2[L164C, R176A] mutations (Fig. 4) (Enkvetchakul et al., 2001).

As discussed above, the major consistently reported kinetic feature of ATP gating is that “long” closed lifetime distributions get progressively longer in the presence of inhibitory ATP, and “burst” durations shorten (Alekseev et al., 1997, 1998; Drain et al., 1998; Enkvetchakul et al., 2000, 2001). The ligand-induced shifts in the distribution of closed state lifetimes are indicative of ATP interaction with the closed state. As discussed above, Scheme II also predicts ATP interaction with the open state. Experimentally this has been more difficult to demonstrate, but in high [ATP] open lifetime distributions clearly shorten in both wild-type (Li et al., 2002) and mutant channels (Enkvetchakul et al., 2001), accounted for in the model by a faster O,A→C,A transition than O,A→O,A or O→C (Enkvetchakul et al., 2001). Thus, ATP can access both open and closed channels, although in wild-type channels, the predominant pathway of gating is predicted to be O,A→C,A. An important caveat of Scheme I (and of this predominant pathway in Scheme II) is that although ATP only (or predominantly) interacts with the closed state (and therefore does not shorten the “real” burst duration, which is determined by subunit closure O→C), experimentally estimated burst durations can still be shortened if subunit opening (C→O) rates are similarly to the C,A→O transition rates, since subunit closures will be falsely included in bursts, but will increasingly be correctly excluded as interburst closures, when ATP increases and C,A→C,A transitions become more frequent. In Scheme II, real “burst” durations will additionally shorten as [ATP] increases, since the O,A→C,A transition is faster than the O→C transition (Enkvetchakul et al., 2001). While it is clearly desirable to know, it is not a trivial procedure to estimate burst durations from Scheme II, and whether the model can adequately account for the experimentally observed shortening of KATP “burst” durations is not yet clear.

What about other Kir Channels? How Generally Applicable Might the ‘Tetrameric-allosteric’ Model Be?

Thus far, we have limited discussion to interaction of ligands (ATP, PIP2) with, and mutations to, the Kir6.2 subunit. In all Kir channels, there are short “intraburst” single closed and open times, modulated by voltage and dependent on the structure of the selectivity filter (Trapp et al., 1998; Choe et al., 1999, 2001; Bard et al., 2000; Lu et al., 2001a,b; Proks et al., 2001; Sadja et al., 2001; Jin et al., 2002). There are also always multiple...
"interburst" closed events, and these are invariably the durations that are predominantly modified by regulatory ligands, including ATP (Alekseev et al., 1998; Fan and Makielski, 1999; Enkvetchakul et al., 2000), G-proteins (Bard et al., 2000), and pH (Wu et al., 2002). It is now clear that all Kir channels are activated by PIP2, involving common structural elements (see below) (Hilgemann and Ball, 1996; Fan and Makielski, 1997; Huang et al., 1998; Kim and Bang, 1999; Rohacs et al., 1999, 2003; Sadja et al., 2001; Lopes et al., 2002; Lu et al., 2002; MacGregor et al., 2002; Vanoye et al., 2002; Schulze et al., 2003) to increase the channel open probability. In so doing, PIP2 always reduces sensitivity to inhibitory ligands and enhances sensitivity to activating ligands, consistent with essentially the same allosteric gating mechanism being involved. Thus, although this has not yet been formally considered, the tetrameric-allosteric model is likely to be generally applicable to the gating of other inward rectifiers.

The subunit gating model we propose is essentially the tetrameric subunit gating model proposed fifty years ago by Hodgkin and Huxley (1952) for voltage-gated K channels, each subunit gating equally and independently. More sophisticated models of voltage gating invoke cooperative interactions between subunits to explain gating of Kv channels (Schoppa and Sigworth, 1998; Zagotta et al., 1994) and Ca-activated K channels (Horrigan et al., 1999; Horrigan and Aldrich, 2002; Magleby, 2003), but the essential tetrameric nature of gating seems established. Conceivably, the fundamental subunit mechanism of voltage gating and ligand gating may be common to all K channels, and the recent demonstration that voltage-gated K channels are also activated by PIP2 (Zhang et al., 2003) brings the possibility closer.

The Structural Basis of Kir Channel Gating

What is the actual gate? Is there a common gate in Kv and Kir channels? What is the subunit “gate” that is stabilized in the closed state by ATP, and in the open state by PIP2? The recent crystal structures of KCsA and MthK demonstrate a plausible gating mechanism for K channels, whereby the M2 helices undergo a rigid body motion to open up the entry to the inner vestibule at the region of the “bundle-crossing” (Fig. 5 B) (Jin et al., 2002; Jiang et al., 2002a,b). Such a model is very consistent with a large body of work demonstrating the ability to trap organic blockers, applied from the cytoplasmic surface, inside Kv channels, when the channels are closed by depolarization, and to block the accessibility of cysteine-reactive molecules to the inner vestibule (Armstrong, 1969; Holmgren et al., 1997; del Camino et al., 2000; del Camino and Yellen, 2001; Shin et al., 2001). Importantly, mutation of M2 residues, particularly residues near the expected bundle crossing (Enkvetchakul et al., 2000, 2001; Sadja et al., 2001; Yi et al., 2001) and moreover at equivalent residues to those that lead to voltage-independent opening of Kv channels (Espinosa et al., 2001; Hackos et al., 2002) can have very profound effects on ligand gating in Kir channels, indicating a clear involvement of M2 in coupling the.
gating sensors to the gates and leading to the implicit supposition that M2 helices themselves act as the subunit gates to pinch off the permeation pathway in the closed state (Enkvetchakul et al., 2000; Loussouarn et al., 2001; Jin et al., 2002).

Significant evidence has been marshaled in support of the idea that a very tight steric closure of Kv and HCN channels does occur at the level of the M2 (S6) “bundle crossing” (Liu et al., 1997; del Camino et al., 2000; del Camino and Yellen, 2001; Shin et al., 2001; Rothberg et al., 2002). Quantitative analysis of the dependence of reagent accessibility on open probability now indicates a critical caveat to interpretation of experiments using accessibility measurements to assess the position of the channel gate in Kir channels (Phillips et al., 2003). With both rapid gating and rapid reagent access, relative to modification rates, the problem of reagent “trapping” can obscure a gated access, unless the degree of closure is very high. For Kir6.2 channels, the data indicate that access of the sulfhydryl reactive MTSEA to the inner vestibule is gated by ATP (Phillips et al., 2003). While we thus favor the interpretation that the “gate” lies at the “bundle crossing” (Jin et al., 2002), the results of reagent accessibility studies in several ligand-gated cation channels, including Kir channels, have been more ambiguous (Flynn and Zaugotta, 2001; Bruening-Wright et al., 2002; Xiao and Yang, 2002; Proks et al., 2003), and there remains considerable controversy on this point.

**The Structure of the K$_{ATP}$ Ligand-binding Domains?**

Many studies have sought to localize the ATP binding site on Kir6.2 by mutagenesis (Shyng et al., 1997, 2000; Tucker et al., 1997, 1998; Shyng and Nichols, 1998; Trapp et al., 1998; Koster et al., 1999; Proks et al., 1999; Reimann et al., 1999; Tanabe et al., 1999; Enkvetchakul et al., 2000; Xu et al., 2001; Cukras et al., 2002a,b). Much evidence has indicated a structurally conserved Kir cytoplasmic domain (Durell and Guy, 2001; Cukras et al., 2002b), containing both NH$_2$ terminus and COOH terminus contributions (Tucker and Ashcroft, 1999; Jones et al., 2001), and this is now confirmed by the recent crystallization of the linked NH$_2$ and COOH termini of Kir3.1 (GIRK-NC) (Nishida and MacKinnon, 2002), which generates a stable tetramer of globular domains (see also Note added in proof). Most mutations that alter apparent ATP sensitivity of K$_{ATP}$ do so by allosterically altering L (i.e., they affect the intrinsic open-closed equilibrium) and very few residues (R50; Proks et al., 1999; Cukras et al., 2002a) in the NH$_2$ terminus, and I182 (Li et al., 2000), K185 (Tucker et al., 1997, 1998; Koster et al., 1999), R201 (Cukras et al., 2002b), and G334 (Drain et al., 1998) in the COOH terminus, actually control ATP affinity itself. Even though these residues are well separated in the primary sequence, the GIRK-NC structure (Nishida and MacKinnon, 2002) shows that the corresponding residues are all located in a single patch on the external surface of each subunit (Fig. 5 A). It is particularly satisfying that two residues, K185 (Tucker et al., 1997; Reimann et al., 1999) and G334 (Drain et al., 1998), which have been most carefully and definitively identified as controlling ATP binding rather than allosteric coupling, are predicted to be extremely close in the 3-D structure (Fig. 5 A). Likely PIP$_2$-interacting residues (R54, R176, R177, R206) (Baukrowitz et al., 1998; Shyng and Nichols, 1998; Shyng et al., 2000; Cukras et al., 2002a,b; Schulze et al., 2003) are also in an overlapping patch, on the top surface of the domain, where they would be expected to be directly opposing lipid headgroups (Fig. 5 A). Since there is an ATP binding site located on each subunit (see also Note added in proof), four separate ATP binding sites per channel are thus established. The overlapping nature of the ATP-binding sites and the PIP$_2$-interacting sites is very consistent with the proposed “negative heterotropic” interaction of these two groups in channel regulation (Shyng and Nichols, 1998). The apparent competition of the two ligands for the same state (i.e., the nonligand bound state) (MacGregor et al., 2002), but without perfect exclusion of one ligand by the other, is also consistent with the proposed gating model (Scheme II above) (Enkvetchakul et al., 2000, 2001). Important details are of course still missing from this structure. The exact orientation of ATP within its binding site needs to be established (see also Note added in proof) and at least part of the potential PIP$_2$ binding site is unresolved. Nevertheless, essential structural features are present and provide additional food for thought regarding the nature of the SUR1–Kir6.2 interaction. Part of the crystallized NH$_2$ terminus is unresolved, a part that likely forms some kind of “lid” over the ATP/PIP$_2$ binding sites (Fig. 5 A). Possibly SUR1 helps to stabilize this fold, such an effect could lead to the destabilization of the ligand-unbound states (relative to either the PIP$_2$, or ATP-bound states; Enkvetchakul et al., 2000).

**Putting it All Together. Does Function Meet Form?**

Beginning with early analyses (Shyng et al., 1997; Nichols et al., 1991; Alekseev et al., 1998), tetrameric allosteric models (Enkvetchakul et al., 2001; Enkvetchakul et al., 2000) can reproduce the essential aspects of K$_{ATP}$ channel gating. Adjusting only one or two parameters in such models allows quantitative prediction of the effects of mutations that alter PIP$_2$ binding, ATP binding, or the intrinsic open state stability on both macro- and microscopic gating. The concepts behind the tetrameric model are not original—both the subunit mechanism and allosteric kinetic modeling...
have been used in other channel fields and protein function fields before (Monod et al., 1965; Zagotta et al., 1994; Sunderman and Zagotta, 1999; Magleby, 2003), but the predictive capacity of such models for KATP channels is quite striking.

How does ligand-binding “gate” Kir channels? The crystallization of open and closed K channel pores and the cytoplasmic domain of a Kir channel now provide dramatic snapshots of the likely structures of both the gating modules and perhaps the gates themselves, in Kir channels. The demonstration of four separated ligand-binding domains per channel (Nishida and MacKinnon, 2002; see Note added in proof) supports a tetrameric subunit model of gating, and provides a plausible physical gating mechanism (Enkvetchakul et al., 2000, 2001; Jiang et al., 2002b) (Fig. 5 B). We propose that ligand-gating of Kir channels occurs by “pinching” of the permeation pathway at the M2 helix bundle crossing (Enkvetchakul et al., 2000; Jiang et al., 2002b; Jin et al., 2002; Phillips et al., 2003). The relatively short distance between the ends of the M1/2 helices and the crystallized GIRK-NC cytoplasmic domain leaves little ambiguity regarding the orientation of the domains relative to one another, and the location of the cytoplasmic domain relative to the membrane. Interaction of residues 54, 176, 177, 206 (in Kir6.2) (Fan and Makielski, 1997; Baukrowitz et al., 1998; Shyng and Nichols, 1998; Shyng et al., 2000; Cukras et al., 2002a; Schulze et al., 2003) with PIP2 is proposed to “pull” the cytoplasmic domain toward the membrane, thereby stabilizing the open state of the channel. Conversely, interaction of an overlapping set of residues (50, 54, 182, 185, 201, 20b, 334) (Tucker et al., 1997; Drain et al., 1998; Baukrowitz et al., 1998; Syling et al., 2000; Li et al., 2000; Shyng et al., 2000) with ATP, will destabilize/exclude the membrane interaction, “pulling” the cytoplasmic domain away from the membrane and thereby stabilizing the closed state. This model achieves the lofty goal of equating specific physical states of each subunit with specific kinetic states. Of course it suffers from being mere conjecture on our behalf. However, it is an explicit model that can be tested—and one that undoubtedly will be in the near future!

We are very grateful to the collaborators who contributed to our own studies, and to the numerous other investigators whose papers continue to guide and stimulate our efforts.

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