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INVITED REVIEW

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# Intracellular regulation of inward rectifier K+ channels

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**Abstract** Inward rectifier potassium (Kir) channels comprise a relatively young gene family of ion channels whose first member was isolated in 1993. A common property its members share is a strong dependence on intracellular regulators such as polyamines, nucleotides, phospholipids, kinases, pH and guanosine-triphosphate-binding proteins (G-proteins). The physiological role of Kir channels is to modulate the excitability and secretion of potassium ($K^+$) to maintain $K^+$ homeostasis, under the control of various intracellular second messengers. Structurally, Kir channels are assembled from four alpha-subunits each carrying the prototypic $K^+$-channel pore region lined by two transmembrane segments with intracellular N- and C-termini. The exact molecular mechanism of Kir channel gating by intracellular second messengers is of considerable biophysical interest. Recent studies have gained significant insight into the molecular mechanism of intracellular regulation by pH. This review illustrates the various modes of regulation of this class of ion channel and the present knowledge of the underlying molecular mechanisms.

**Keywords** Potassium channels · GIRK · Kir · $K_{ATP}$ · pH-regulation

## Introduction

The molecular nature of inward rectifier $K^+$ (Kir) channels was discovered in 1993, when the first two subunits (ROMK1/Kir1.1 and IRK1/Kir2.1) were cloned [32, 43]. Since then a large number of Kir proteins have been identified, and grouped in a $K^+$ channel gene family, sharing a common feature of only two transmembrane segments in each of the four $K^+$ channel subunits [14, 20]. This class of ion channel plays a key role in cell physiology: Kir channels maintain the resting potential near the $K^+$ reversal potential ($E_K$), set a threshold for excitation that depends on both membrane voltage ($E$) and the regulatory state of the channels [31], and they control $K^+$ homeostasis and $K^+$ secretion [68]. The biophysical properties behind these physiological functions are the channels' characteristic inward rectification [18] and their strong modulation by intracellular factors and second messengers [50, 53, 54]. The best known examples of the physiological importance of Kir regulation are as follows: the ATP dependence of Kir6.X ($K_{ATP}$) channels in the control of insulin secretion [2, 3] and the determination of myocardial resistance to hypoxia [7, 9]; the regulation of Kir3.X (GIRK) $K^+$ channels by G-proteins to account for the vagal control of heart rate [41, 70]; the regulation of Kir1.X (ROMK) channels by $[K^+]$ and pH, which controls $K^+$ secretion in kidney [67]; and the regulation of Kir4.1 channels in glial cells [37] and the inner ear [29], controlling $K^+$ homeostasis and excitability. In this review, I give an overview of the present data available on the intracellular regulation of Kir channels and provide some insight into the mechanisms and present understanding of the underlying molecular processes.

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## Intracellular regulators of Kir channels

### Regulation by polyamines

The inward rectification of Kir channels is caused by a rapid and highly voltage-dependent block by intracellular polyamines [17, 46] and also $Mg^{2+}$ ions [48]. Spermine (SPM), which is fourfold positively charged, is the most potent member of this class of molecule [18]. Spermidine, which is threefold positively charged, is the second most potent member. Figure 1 shows the differential effect of SPM on so-called "weak" and "strong" inward rectifier channel subtypes, Kir1.1 and Kir2.1.

Strong inward rectifiers are highly sensitive to blockade by polyamines in a highly voltage-dependent manner [18]. Heterotetrameric Kir channels composed of both

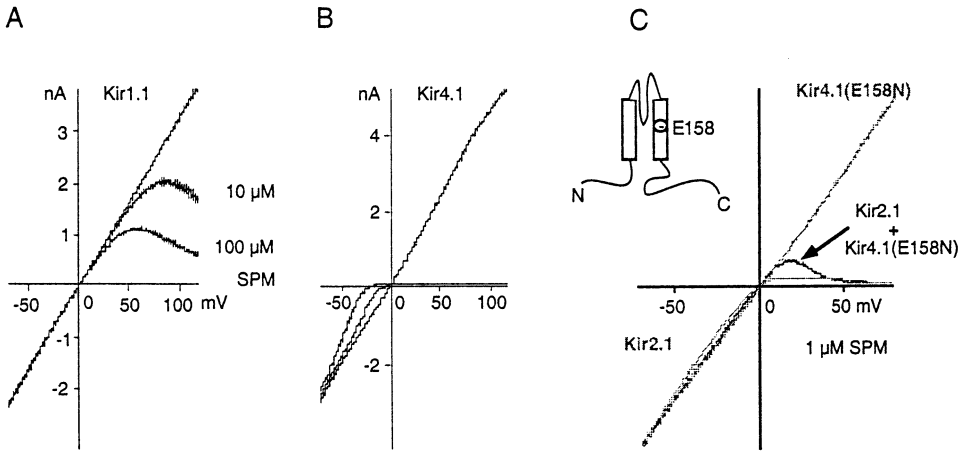



**Fig. 1A–C** The differential effects of the polyamine spermine (*SPM*) on strong (Kir2.1, Kir4.1) and weak [Kir1.1, Kir4.1(E158N)] rectifiers. **A** Effect of 10 and 100 µM SPM applied to the intracellular side of Kir1.1 channels in giant inside-out patches from *Xenopus* oocytes expressing Kir1.1 after cRNA injection. SPM voltage-dependently but incompletely inhibits the outward current. **B** Same experiment as in **A** but with Kir4.1 channels, showing much stronger inhibition of outward current. **C** Similar to Kir4.1 channels, Kir 2.1 channels (*lower grey trace*) are strongly inhibited by SPM (1 µM). Kir4.1 channels are no longer inhibited if the negatively charged glutamate at position 158 in the second transmembrane segment is mutated to glutamine (E158N; *upper grey trace*). Mixing Kir2.1 and Kir4.1(E158N) cRNAs results in channels with intermediate SPM sensitivity, indicating that heteromultimeric channels have formed. Graphs modified from [16, 24]

strong and weak rectifier subunits have intermediate sensitivity to polyamines [24]. This differential sensitivity can be used to identify and isolate heteromeric channel populations and to estimate the subunit stoichiometry of this class of $K^+$ channel [20, 24] (Fig. 1). If co-expressed, Kir2.1 and Kir4.1 [16], Kir3.1 and Kir3.4 [41] as well as Kir4.1 and Kir5.1 (BIR9) [52] form preferential heteromultimers. For these pairs of subunits, co-expression leads to a homogeneous population of channels, whereas most other possible combinations, for example Kir2.1 and Kir1.1, either do not form heteromultimers at all or the resulting heteromultimeric populations represent only a small percentage of the channels formed upon co-expression. For example, Kir1.1 and Kir4.1 form heteromultimers, but the probability of this occurrence is only about 10% that of the corresponding homomers [24]. It seems likely that heteromer formation is a mechanism for controlling the strength of rectification and polyamine sensitivity by controlling the amount of gene expression.

The extracellular concentrations of ions also determine the sensitivity to intracellular blockade by SPM. This is caused by an interaction between $K^+$ ions located in the external part of the $K^+$ channel pore and the blocking polyamine molecules [51]. Through this interaction, the extracellular $K^+$ concentration regulates the rate at which the blocking SPM molecule is removed (the "off-rate"). This mechanism accounts for the correlation between the threshold voltage for the SPM-induced block and $E_K$. At physiological SPM concentrations (around 10 µM free SPM; up to 97% of SPM is bound to intracellular nucleic acids and nucleotides), strong inward rectifiers are barely blocked at $E_K$ but are completely blocked at around 30 mV positive to $E_K$. Thus, the functional role of SPM in strong rectifiers is to switch off Kir channels when the membrane is significantly depolarized, and to stabilize the resting potential at membrane potentials below the threshold of SPM-induced block. This is an important way for the cell's "electrochemical battery" to save energy. Thus, strongly rectifying Kir channels do not shunt an action potential with a substantial $K^+$ current, such as would be mediated by a permanently open $K^+$ channel.

Weak rectifiers are also modulated by intracellular SPM, which may decrease their conductance up to tenfold (Fig. 1). These Kir channels are important for controlling $K^+$ secretion in the distal part of the nephron (Kir1.X; ROMK) and for regulating slow action potential bursts in secreting cells (Kir6.2; $K_{ATP}$). The sensitivity of Kir6.2 channels to spermine-induced block is specifically regulated by intracellular pH [6]. At an alkaline pH of around 8, the channels are relatively sensitive to SPM, while at an acidic pH of around 6, SPM has almost no blocking effect. As well as controlling polyamine sensitivity through subunit combination, extracellular ion concentrations and intracellular pH, it is assumed that cellular levels of SPM are regulated by the enzyme ornithine decarboxylase. The latter controls the rate-limiting step in the polyamine synthesis pathway, which may control cellular polyamine concentrations for the purpose of regulating electrical excitability [8].

## Regulation by protein kinases

Protein kinase A and protein kinase C as well as tyrosine kinases, such as src kinase, regulate almost all kinds of ion channel. For the Kir channels, the effects of such kinases on $K^+$ current amplitude have been observed by many authors [11, 12, 19, 27, 49, 67, 68, 71]. Some of these effects, such as current stimulation by activation of

PKA, are common to all members of the Kir family and are not correlated to a particular phosphorylation site in the primary sequences. This may be because of the influence of kinases on other regulatory pathways, such as the phosphatidylinositolphosphate pathway (see below).

Other effects are highly subunit specific, such as the effect of PKC on Kir2.3 [27]. It is not known, however, whether the phosphorylation site is on the Kir2.3 protein. Defined sites for phosphorylation in the C-terminal end of Kir2.1 and Kir2.3 [11] have been confirmed biochemically. The effects of PKA-dependent phosphorylation are not completely clear: PKA either has an inhibitory effect (in Kir2.1) or it modulates pH sensitivity (in Kir1.1). The interaction of these channels with PDZ-domain-carrying proteins, such as PSD95, is thought to be regulated by this C-terminal phosphorylation site [11]. Kir1.1 channels are down-regulated by src kinase [69] on a slow time scale. The latter might be involved in the adaptation of $K^+$ secretion in the kidney to the organism's $K^+$ intake.

## Regulation by intracellular pH

In contrast to indirect regulation by protein kinases, the effect of intracellular pH on Kir1.1 and Kir4.1 channels is mediated by the channel protein itself [21, 54, 57]. Intracellular acidification closes Kir1.1 and Kir4.1 channels rapidly and with a steep pH dependence (Fig. 2).

Kir1.1 (expressed in the nephron) is half-maximally active around pH 7.0 and fully blocked at pH 6.7 while Kir4.1, a channel that is found for example in Müller glia cells in the eye, closes in a pH-dependent manner only below a pH of 6.0. This sensitivity to pH is allosterically linked to a sensitivity to extracellular ion concentrations [13]. At low extracellular $K^+$ and $NH_4$ concentrations, the channels do not open even at very alkaline pH values, while at low intracellular pH values around 6.5 even 120 mM external $K^+$ is not enough to keep the channel in the open state. This interaction may represent an important regulatory link between the luminal $K^+$ concentration and pH in the distal part of the nephron.

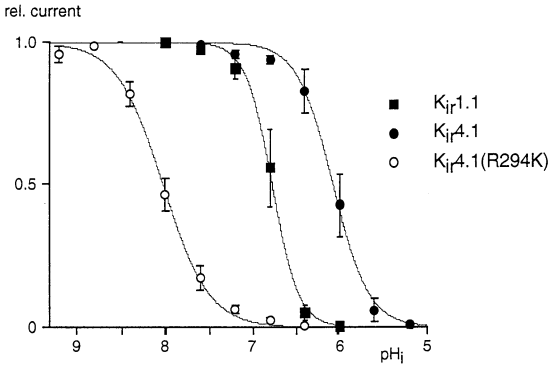



**Fig. 2** The dependence of Kir1.1 and Kir4.1 channels on intracellular pH ($pH_i$) differs. A slight modification of the C-terminal positively charged residue at position 294 in Kir4.1 shifts the pH dependence of Kir4.1 by two orders of magnitude (from [57])

## Regulation by phospholipids

Among the various phospholipids, phosphatidylinositolbisphosphate ($PIP_2$) has the most important role in receptor-controlled second messenger processes. This phospholipid is produced from phosphatidylinositol (PI) by phosphorylation via PI-kinase and PIP-kinase [23]. Moreover, $PIP_2$ is the substrate for PLC which cleaves $PIP_2$ into the other important second messenger molecules inositol trisphosphate ($IP_3$) and diacylglycerol (DAG). Based on the recent observation that $PIP_2$ itself has a significant effect on ion channels [33], it was proposed that some of the phenomena ascribed to the PLC pathway are caused by a breakdown of $PIP_2$ rather than by the effects of $IP_3$ and DAG. One of the major downstream targets of the breakdown of $PIP_2$ might be the Kir channels. Kir channels, including kidney K-secretion channels (Kir1.X), strong inward rectifiers (Kir2.X), G-protein-regulated (Kir3.X) and $K_{ATP}$ (Kir6.X) channels, react in the presence of $PIP_2$ in the following way: their open probability increases, they do not run-down in excised patches and they are insensitive to inhibitory stimuli [5, 33, 60]. For example, $K_{ATP}$ channels may react to stimulation of P2Y-receptors, which activate PLC, via the breakdown of $PIP_2$ [5] (Fig. 3).

On the other hand, $PIP_2$ couples Kir channels to other second messenger mechanisms through regulation of phosphoinositide kinases and phosphatases such as PI-kinase, which produces PIP from PI and requires Mg-ATP as a cosubstrate. The activity of these enzymes is regulated by heteromeric G-proteins and protein kinases [23, 38, 39]. Furthermore, it is downregulated by $Ca^{2+}$ [36]. This is one possible explanation for the numerous observations showing that Kir channels depend on almost all second messenger pathways [19, 53]: GTP[$\gamma$-*S*] plus $Mg^{2+}$ activates PLC, which downregulates Kir channels by breaking down $PIP_2$, while ATP and PKA may upregulate them by increasing $PIP_2$ production via PI-kinase. $Ca^{2+}$ may downregulate by inhibiting PI-kinase, while oxidation may up- or downregulate them, by inactivating protein phosphatase or PI-kinase. Inversely DTT may do both (up- and downregulation), by reactivating these enzymes. These pathways are still hypothetical; however, they may become important soon.

## Regulation by nucleotides

Before the cloning of Kir subunits, the blocking of $K_{ATP}$ channels by ATP was a major topic in $K^+$-channel research [2, 3]. The $K_{ATP}$ channel remains the best established example of the importance of intracellular regulation of a $K^+$ channel, and of the pharmacological significance of drugs that influence $K^+$ channel regulation (for example, $K_{ATP}$ blockers such as glibenclamide and $K^+$

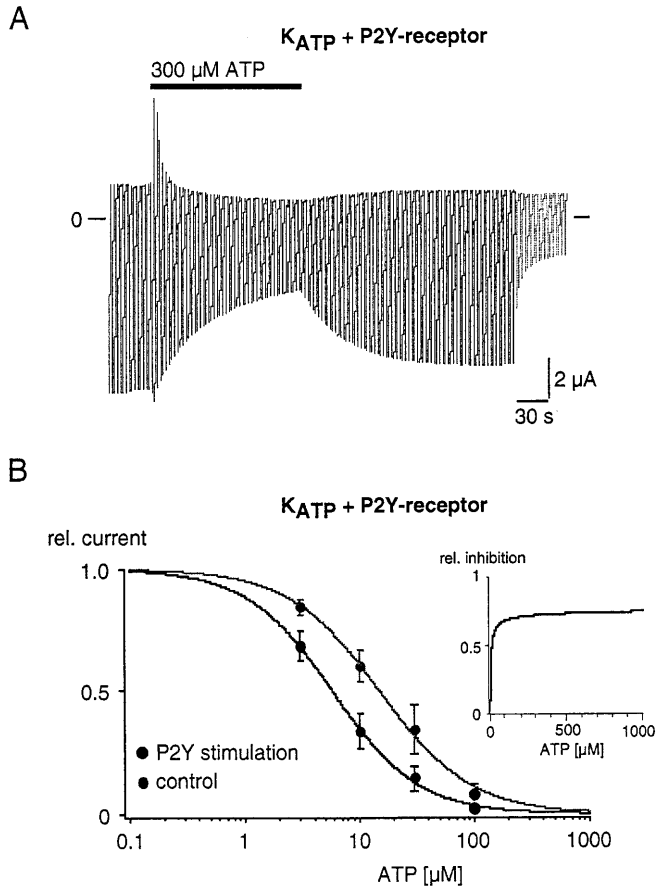



**Fig. 3A,B** Stimulation of coexpressed P2Y-receptors decreases $K_{ATP}$-mediated currents by increasing ATP sensitivity. **A** Responses to voltage ramps from –120 mV to 50 mV measured in whole *Xenopus* oocytes [protein kinase C (*PKC*) inhibited by 100 nM staurosporine]. Application of 300 µM ATP activates P2Y-receptors. The activation evokes a transient increase in outward current, caused by $Ca^{2+}$-dependent activation of $Cl^-$ channels followed by a reversible decrease in $K_{ATP}$-mediated current. **B** Dose–response of ATP-induced inhibition of $K_{ATP}$ channels measured in oocyte patches prior to (*control*) and after stimulation of P2Y-receptors (*P2Y stimulation*). P2Y stimulation shifts the dose–response curves for ATP by breaking down $PIP_2$. *Inset*: relative inhibition (calculated from the ratio of the two dose-evoked responses) of $K_{ATP}$ currents by P2Y-receptor stimulation is independent of intracellular ATP at concentrations >100 µM (from [5])

channel openers such as diazoxide). The control of insulin-producing pancreatic β-cells by $K_{ATP}$, which links insulin secretion to the metabolic state and thereby to the blood glucose level, is a prototype of a molecularly defined physiological regulation mechanism [3]. $K_{ATP}$ channels, their regulation and molecular structure have been reviewed many times before, and will not be a focus here. Nevertheless, it seems important to discriminate between three different actions of nucleotides on Kir channels. The first is inhibition (e.g. by ATP) exclusively described in $K_{ATP}$ channels. The regulatory target site is probably located on the Kir6.X subunit, since $K_{ATP}$ channels expressed in the absence of the SUR subunit (SUR, the sulphonylurea receptor subunit of the channel, is an ATP-binding-cassette protein that mediates the effects of sulphonylureas such as glibenclamide and $K^+$ channel openers) remain dependent on ATP [64]. This regulatory effect is strongly dependent on regulation by $PIP_2$ [5, 60]. The second is upregulation by ADP, which is also a property of $K_{ATP}$ channels, but is mediated by SUR [25, 59]. The third is upregulation of Kir channels by intracellular Mg-ATP (called refreshment), a phenomenon that is shared by most of the Kir subtypes. This effect requires the presence of $Mg^{2+}$ in a complex with ATP, and does not occur with non-hydrolysable ATP analogues. It is assumed that Mg-ATP refreshment effects can be ascribed to a patch-adherent PI- and PIP-kinase, which needs hydrolysable ATP to maintain the concentration of $PIP_2$ in the patch [30].

## Regulation by other proteins

β-Subunits that co-assemble with the pore-forming α-subunit have been identified in almost all classes of ion channel. Among Kir channels, only $K_{ATP}$ (Kir6.X) seems to have such an additional subunit, the sulphonylurea receptor: SUR1a, b and SUR2 [4]. It is presently assumed that the stimulatory effects of dinucleotides such as ADP are mediated by the SUR [25]. Apart from their sensitivity to $K_{ATP}$ blockers and openers, SUR also have a significant effect on the ATP sensitivity of the Kir subunits [64].

A further protein-based regulation of Kir channels is the interaction of Kir3.X channels with heterotrimeric GTP-binding proteins. The main stimulatory effect on Kir3.1 channels is caused by the $G_{\beta\gamma}$ subunit of the heterotrimeric G-protein that binds to Kir3.X channels and activates them [35, 61, 70]. Inhibition of Kir3.X channels by $G_\alpha$ has also been described [61] but seems to be less potent than activation by $G_{\beta\gamma}$ molecules. In *Xenopus* oocytes, a high density of Kir3.X channels leads to $K^+$ channels being activated to an increased resting level even in the absence of a receptor molecule [28]. A possible explanation for this is that the channels may absorb so many $G_\alpha$ molecules that an increase in the concentration of free $G_{\beta\gamma}$ molecules is generated. In other words, by binding $G_\alpha$, Kir3.X channels compete with the active receptors which are meant to bind the whole G-protein trimer complex, and thereby induce receptor-independent auto-activation.

Recently, a family of GTPase-activating proteins known to regulate G-protein signalling (RGS) were shown to accelerate the deactivation kinetics of GIRK channels in native cells [28, 73]. RGS proteins probably accelerate the deactivation kinetics of GIRK channels by speeding up the GTP hydrolysis rate without compromising the peak amplitude of receptor-mediated currents. It is not yet clear whether they interact directly with the Kir channel proteins or only influence the G-protein subunits.

## Mechanisms underlying intracellular regulation of Kir channels

### Protein interactions

Little is known about how interacting proteins, such as β-subunits or G-protein subunits, regulate Kir channels. Therefore, the published mechanistic models discussed here are rather speculative. Figure 4 shows current cartoons of the conformations and possible interactions.

It is presently assumed that $G_{\beta\gamma}$ causes the opening of Kir3.X channels primarily by binding to a C-terminal domain of the channel protein [35, 42], and that a leucine residue at position 339 in Kir3.4 may play a key role in this process [26]. An interaction with the N-terminus has also been discussed [34, 42]. $G_\alpha$ probably associates with the N-terminus of the Kir channel subunit, perhaps while it is bound to the seven-segment receptor [61]. Micro-clusters in which Kir channels, $G_\alpha$ subunits and receptors are bound to each other to form complexes may explain why, in several types of cell, Kir3.X channels are opened only by particular receptors (e.g. M2-receptors but not $\beta_1$-receptors regulate Kir in atrial myocytes), even though Kir channels show no specificity for particular $G_{\beta\gamma}$ subunits of certain G-protein subtypes. Another component of such micro-clusters might be RGS proteins, which have been found to compete with the $G_\alpha$ binding of the Kir channel [10, 28, 55, 56] and probably also have a direct activating effect on Kir3.X channels [10]. The established function of RGS proteins is to react with $G_\alpha$ in its active state where it has bound GTP and to accelerate the breakdown of GTP into GDP and inorganic phosphate.

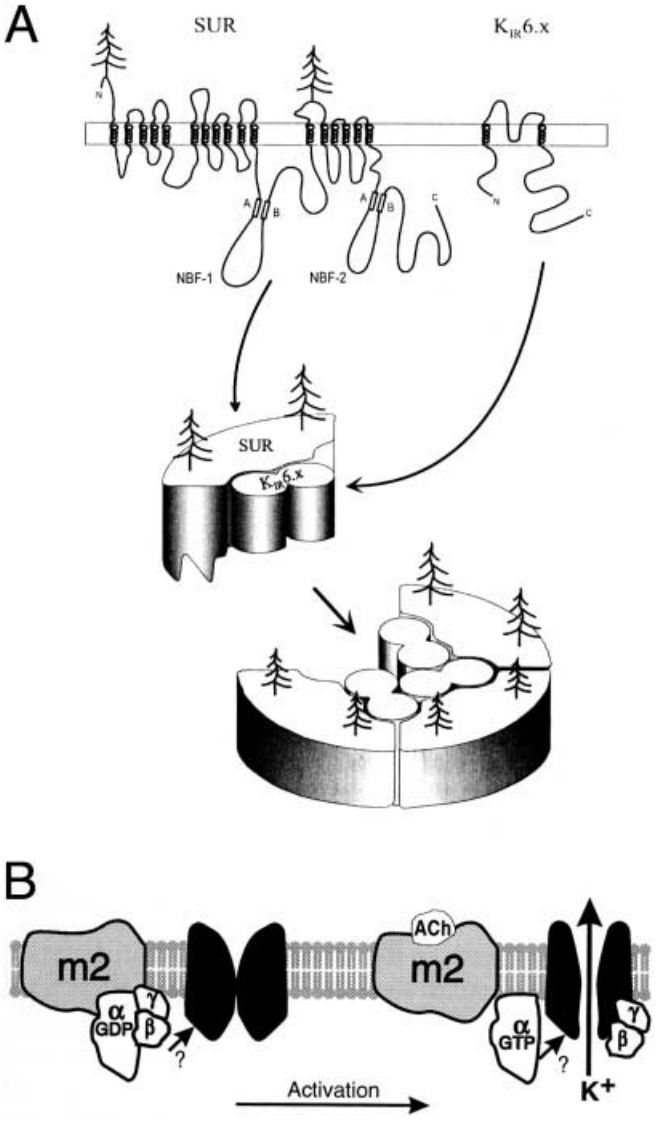



**Fig. 4 A** Coordination of Kir6.2 subunits and SUR subunits in $K_{ATP}$ channels [4]. (*SUR* The sulphonylurea receptor subunit of the channel is an ATP-binding-cassette protein that mediates the effects of sulphonyureas such as glibenclamide and $K^+$ channel openers.) **B** Interactions of Kir3.X channels with $G_\alpha$ and $G_{\beta\gamma}$ at N- and C-termini respectively and the G-protein coupling receptor [61] (*m2 receptor* muscarinic acetylcholine receptor; *NBF* nucleotide binding fold)

The SUR subunit of $K_{ATP}$ channels is thought to associate in a stoichiometry of one to one with the Kir6.2 subunit, forming a channel complex that consists of eight subunits in total [1, 4]. Both subunits have strong functional interactions. The SUR subunit mediates the sensitivity of $K_{ATP}$ channels to stimulation by intracellular MgADP [25, 59]. This allows the channel to react to the ATP/ADP ratio rather than just the ATP concentration, which makes the channels more sensitive to metabolic changes in which a decrease in ATP is associated with an increase in ADP. This effect of MgADP on the SUR subunit occurs when it interacts with the second nucleotide-binding fold of SUR. As well as its physiological actions, the SUR subunit also mediates the pharmacological regulatory effects of $K^+$ channel openers such as diazoxide and cromakaline. These drugs bind to the intracellular loop between the 13th and 14th transmembrane domains as well as to the C-terminus and the two C-terminal transmembrane domains 16 and 17 [65]. A certain sequence motif (arginine-lysine-arginine; RKR) in the C-termini of both SUR and Kir6.2 inhibits the transport of these $K_{ATP}$ channel subunits to the surface membrane as long as they are not associated with each other [74].

### Phospholipid interactions

The molecular mechanism of $PIP_2$-dependent regulation of Kirs is hypothesized as follows: the negatively charged phosphate groups of the lipid interact with positively charged amino acids in the Kir protein. Since $PIP_2$ influences the open probability of probably all Kir channels [5, 22, 33, 40, 44, 45, 60, 62, 72], it seems likely that these positively charged residues are somehow conserved. There are several conserved arginine and lysine residues in the C-terminus of Kir channels. Mutation of a conserved arginine to glutamine at position 188 reduces

the ability of ROMK1 to bind $PIP_2$ and increases its sensitivity to inhibition by $PIP_2$ antibodies [33]. Accordingly in Kir6.2 the mutation R176A significantly influences the effect of $PIP_2$ [5, 22]. Besides its general effect on open probability, $PIP_2$ may have other specific effects on most of the Kir subtypes, such as decreasing the ATP sensitivity of Kir6.2 [5].

## Electrostatic effects

In Kir channels the voltage dependence of rectification is either strong or weak. It is well established that two sites, one in TM2 and one in the C-terminus (the latter exists exclusively in Kir2.X channels), interact with positively charged intracellular blockers such as magnesium ions and polyamines [17, 47, 63]. Recently it was shown that in cloned $K_{ATP}$ channels (Kir6.2 + SUR1) polyamine-mediated rectification is not constant but is modulated by intracellular pH in the physiological range. Inward rectification was found to be prominent at basic pH, while at acidic pH it was only weak. Such pH dependence of polyamine-induced block is specific for $K_{ATP}$, as shown in experiments with other Kir channels. Systematic mutagenesis uncovered a titratable C-terminal histidine residue (H216) in Kir6.2 as the structural determinant, and electrostatic interaction between this residue and polyamines as the molecular mechanism underlying the pH-dependent rectification [6]. An electrostatic mechanism has also been suggested to account for the interaction between $PIP_2$, which is negatively charged, and the positively charged residues in the C-terminus of Kir channels (Figs. 5B, 6) [22].

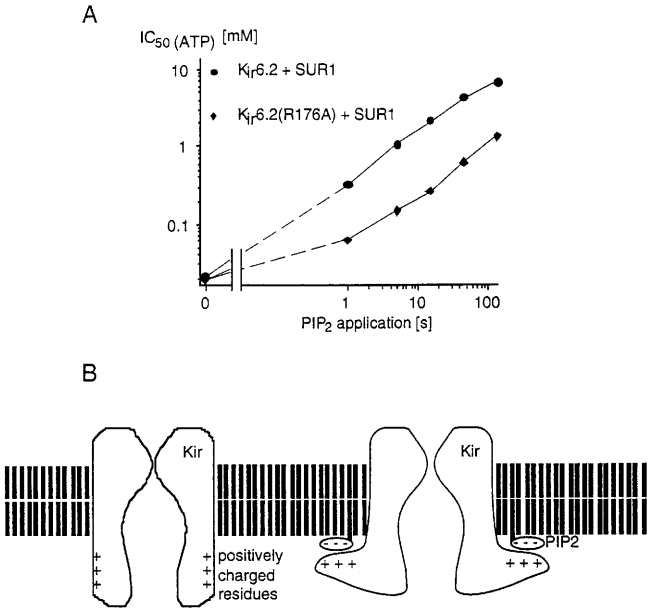



**Fig. 5A,B** Simple model of the activation of Kir channel open probability by $PIP_2$. **A** $PIP_2$ stabilizes the open state of Kir6.2/SUR1 channels against ATP-dependent closure. Saturation of patches with $PIP_2$ continuously shifts the $IC_{50}$ for ATP-dependent block to higher values. This stabilizing effect of $PIP_2$ is weaker in the mutant whose arginine at position 176 in the C-terminus of Kir6.2 has been neutralized (modified from [5].) **B** Simple model of the interaction of $PIP_2$ with the Kir protein. By electrostatic interaction the negatively charged phospholipid induces a conformational change that stabilizes the open state

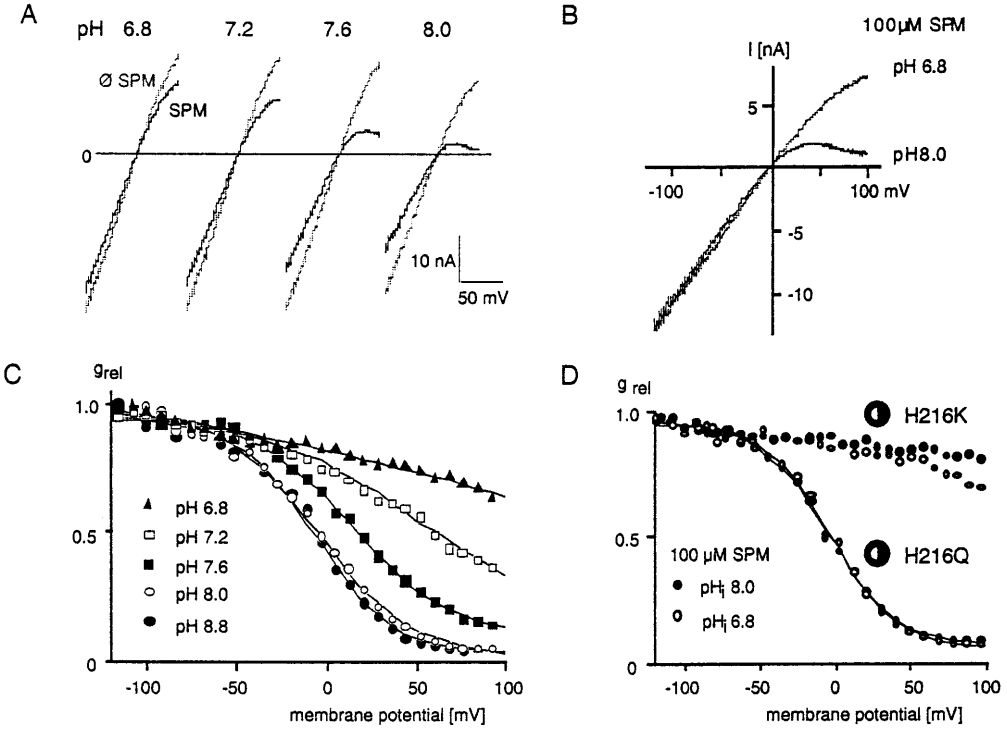
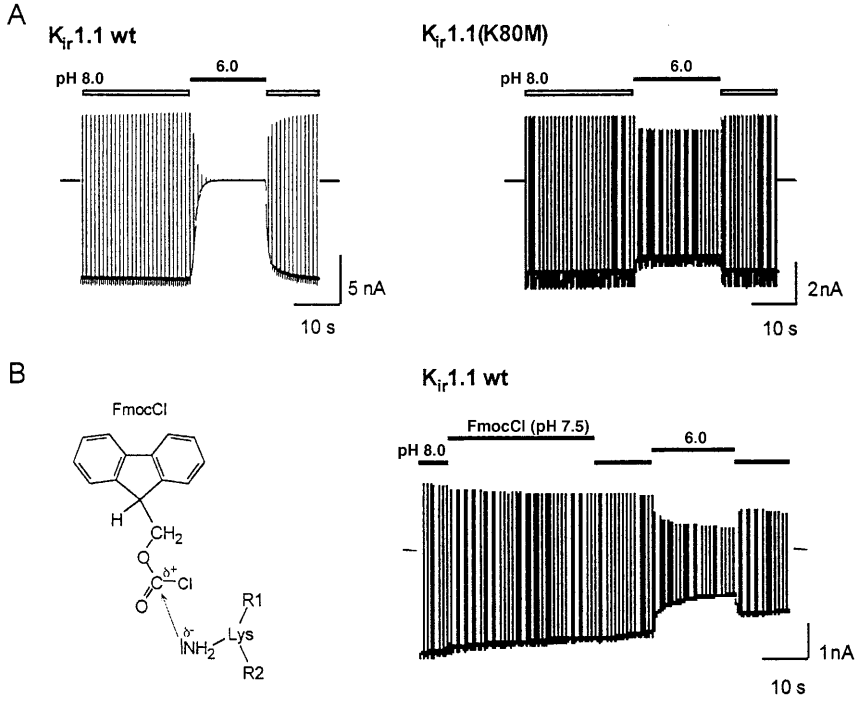



**Fig. 6A–D** Spermine block of Kir6.2 is regulated by intracellular pH. **A** The effect of 100 µM SPM on the voltage dependence of Kir6.2/SUR channels strongly depends on pH. **B** Comparison between the current–voltage dependencies measured at pH 6.8 and at pH 8.0. **C** Conductance–voltage curves at different pH values in the presence of 100 µM SPM for the wild-type channels. The curves vary strongly between pH 6.8 and 8.0. **D** No pH-dependent variation of the rectification is observed after mutation of H216. Mutation to the non-charged residue glutamine (*Q*) results in a curve as observed in the wild-type at pH 8.8, while mutation to the permanently positively charged lysine (*K*) results in reduced SPM sensitivity as for pH 6.8 in the wild-type. Modified from [6]

Intracellular conformational changes

The pH-gating of Kir1.1 and Kir4.1 channels is presently the best understood gating mechanism in Kir channels. It is driven by protonation/deprotonation of the amino group of a lysine residue at position 80 in Kir1.1 (position 76 in Kir4.1) [21, 54]. In contrast to the general chemical behaviour of lysine residues, this particular lysine residue is not protonated at neutral pH values. This has been shown by modification of the non-charged amino group by the amino-reagent 9-fluorenylmethoxy-carbonylchloride (FmocCl, Fig. 7) in inside-out patches, which destroys pH-dependent regulation of these channels. Protonation of the K80 residues in the four subunits of Kir1.1 occurs at pH values below 7.0 and induces the channels to close in a highly cooperative manner.

Titration of K80 is shifted by more than 3 pH units in the acidic direction compared to the free amino acid ($pK_A$ of free lysine is 10.53). This anomalous titration is due to electrostatic interactions within an intrasubunit arginine-lysine-arginine triad formed by lysine 80, arginine 41 in the N-terminus and arginine 311 in the C-terminus of the Kir1.1 protein. Disruption of this triad results in defective ROMK function (e.g. in some of the mutations underlying antenatal Bartter syndrome) [57] (Fig. 8).



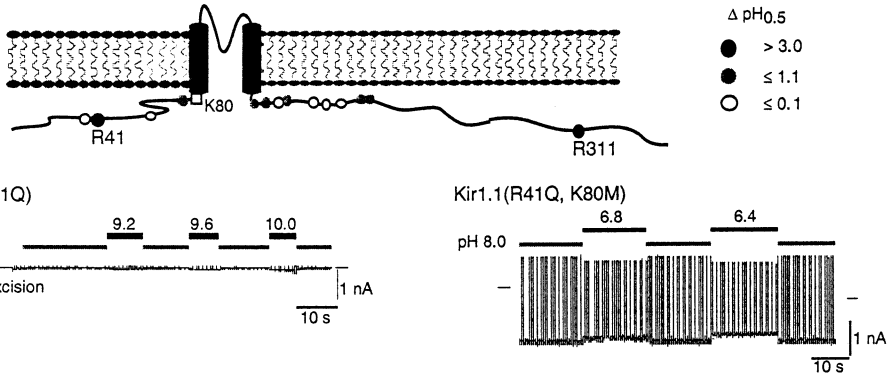




**Fig. 7A,B** Titration of K80 mediates the regulation of Kir1.1 channels by intracellular pH. **A** pH-dependent closure of Kir1.1 channels in giant inside-out patches is removed in Kir1.1(K80M) channels, where pH 6.0 induces only a small current decrease. **B** Similarly the pH sensitivity of Kir1.1 wild-type channels can be removed by a reaction of the neutral lysine residue with the amino-reagent FmocCl at pH 7.6. This demonstrates that K80 is neutral at pH 7.5 and that protonation of K80 is necessary for the pH-dependent regulation of Kir1.1 channels [57]

**Fig. 8** Mutagenesis reveals two arginine residues that interact with K80. The cartoon shows all positively charged residues of Kir1.1 and displays their influence on the pH dependence of the current. Mutagenesis of the residues marked in *white* does not influence pH-dependent regulation. The *grey circles* reflect those arginine residues that, if mutated to glutamine, shift the pH dependence significantly in the alkaline direction but by 1 pH unit or less. The residues R41 and R311 marked in black shift the pH sensitivity by more than 3 pH units. Almost no current recovers at pH 10. This inhibitory effect is fully rescued, however, by co-mutating lysine 80 to methionine in order to remove the critical positive charge at this position. The double mutant Kir1.1(*R41Q, K80M*) is fully active and pH insensitive. The same is true for R311Q in Kir1.1. Modified from [57]

Patch-clamp experiments revealed that at an acidic intracellular pH recovery from pH-dependent inactivation of the channels was only fully reversible in the presence of antioxidant compounds such as DTT. This suggests state-dependent oxidation (only occurring in the inactive state) of cysteine residues. This effect was pronounced in an irreversible way when the channels were treated with the oxidizing reagent 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) from the intracellular side. Mutagenesis of all intracellular cysteine residues (C), either alone or in combination, revealed that two residues are targeted by DTNB, one in the N-terminus (C49) and one in the C-terminus (C308) of the channel protein [58]. Both sites react with the thiol reagents, but only in the closed state and not in the open state. Both residues are located close to the arginine residues at positions 41 and 311, which are part of the channel's pH sensor. These results indicate that the pH-dependent gating of Kir1.1 channels involves the movement of protein domains in both the N- and the C-termini of the Kir1.1 protein.

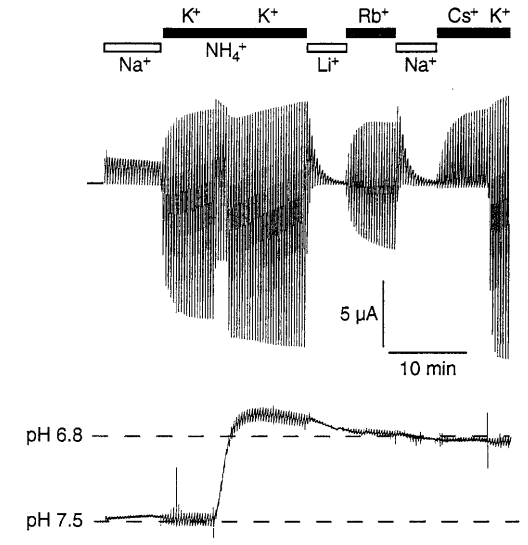



**Fig. 9** Regulation of Kir1.1 channels by extracellular ions measured in whole *Xenopus* oocytes with the two-microelectrode voltage-clamp technique. Simultaneous recording of the intracellular pH is performed with a third pH-sensitive microelectrode. In the absence of extracellular $K^+$, currents mediated by Kir1.1 channels slowly decline. Sudden exposure to a $K^+$-rich solution (90 mM) results in a rapid current increase developing within 2 min. Next the oocyte is exposed to 90 mM ammonium ($NH_4^+$). This yields an increase in the internal hydrogen ion concentration, because of the influx of $NH_4^+$ and the subsequent $NH_3$ outflux through the cell membrane, leaving a hydrogen ion in the cytoplasm. Accordingly the pH-sensitive microelectrode measures an intracellular pH shift from 7.5 to about 6.8. Although this is not enough to decrease the current at high $K^+$, a subsequent exposure to zero-$K^+$ high-$Na^+$ solution generates a very rapid current decline. The increased speed of current decline in zero $K^+$ demonstrates that the internal pH allosterically couples to the external $K^+$ binding site. As well as $K^+$, $Rb^+$ and $Cs^+$ are also able to upregulate Kir1.1 channels, while $Li^+$ downregulates them similar to $Na^+$. Modified from [13]

## Allosteric regulation

Kir1.1 channels expressed in the apical membrane of renal tubular cells control $K^+$ homeostasis by mediating $K^+$ secretion. Native apical $K^+$ channels are indirectly controlled by the $K^+$ concentration at the basolateral membrane through a cascade of intracellular second messengers [66]. Moreover it has been found that Kir1.1channels are regulated directly by the extracellular $K^+$ concentration [13]. As shown in Fig. 9 this extracellular $K^+$ regulation is allosterically coupled to the intracellular pH.

The molecular determinants of external $K^+$ sensitivity are in the central membrane-spanning domain of the channel protein, whereas the pH sensitivity is mainly determined by the N- and C-termini. This supports the allosteric nature of the interaction between extracellular $K^+$ and intracellular pH [13]. Moreover in the continuous absence of extracellular $K^+$, the closing reaction induced by intracellular acidification can no longer be reversed by subsequent alkalization (unpublished observation), demonstrating that the interaction of the channel with extracellular $K^+$ is necessary for the opening reaction. Extracellular $K^+$ regulation and its coupling with pH is specific to Kir1.1 channels, but is equally present whether Kir1.1 channels are expressed in human embryonic kidney (HEK293) cells or *Xenopus* oocytes after cRNA injection (unpublished results). The dependence on extracellular $K^+$ may serve as a way for the tubular $K^+$ concentration to feedback positively on $K^+$ secretion. It may be physiologically important in mammalian renal tubular cells.

## A possible model of Kir channel gating

The gating transition of Kir1.1 involves intracellular pH and the extracellular $K^+$ concentration; it depends on the pore region and the N- and C-termini of the $K^+$ channel subunit. From the cysteine modification experiments, it may be additionally concluded that the corresponding conformational change must be so pronounced that the two cysteine residues (49 and 308) in the N- and C-termini are suddenly exposed to the intracellular fluid after the closing reaction. This suggests that the triad of R41, K80 and R311 opens up because of electrostatic repulsion in response to protonation of lysine 80, which appears logical since a positively charged lysine must repulse the two arginine residues. The opening of the triad structure formed by the N- and C-termini then may lead to an allosteric closing of the channel pore, squeezing out the external $K^+$ ion to account for the channel closure and the interaction of intracellular pH with the extracellular $K^+$ concentration. Summing up these assumptions to an integral hypothesis of Kir1.1 gating leads to the model in Fig. 10. Repulsion between the N- and C-termini caused by the lysine titration leads to the opening of the inner side of the $K^+$ channel structure, characterized by the accessibility of the two cysteine residues. This leads to a tilt in the transmembrane helices TM1 and TM2, causing the channel to close by narrowing the upper half

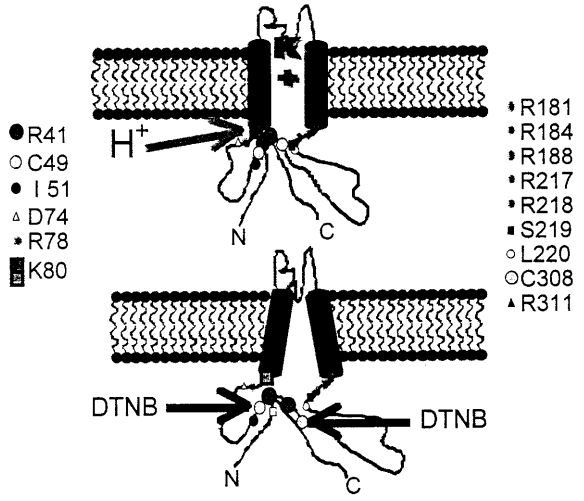



**Fig. 10** Model of pH-dependent gating of Kir1.1. At pH values above 7.0, lysine 80 (*blue square*) is deprotonated because of the vicinity of the arginine residue at position 41 and 311 (*orange spots*). The two cysteine residues at position 49 and 308 (*yellow spots*) are not accessible to 5,5′-dithiobis-(2-nitrobenzoic acid) (*DTNB*) because of the narrow structural packing. Other residues that have been found to contribute to this structure by influencing pH dependence are marked *green* or *black*. Protonation of K80 at pH values below 6.8 (*orange square*) results in the disruption of the narrowly packed structure by electrostatic repulsion. This has two consequences: the pore closes and $K^+$ escapes from its external binding site. The two cysteine residues are accessible to DTNB from the inside. Modification with DTNB may freeze the channels in the closed state

of the pore-lining structure. This may lead to an escape of the $K^+$ ion bound to the extracellular $K^+$ binding site. Then the channel fully collapses and cannot be recovered by an alkaline intracellular pH. The tilting of the transmembrane segments may also lead to channel closure without the release of the $K^+$ ion from the external $K^+$ binding site. On the other hand, the tilting of the transmembrane segments is facilitated by the dissociation of the external $K^+$ ion.

## Discussion

Intracellular regulation of Kir channels is complex. There are various types of regulators, namely $H^+$ ions, nucleotides, polyamines and phospholipids and larger proteins such as $G_{\beta\gamma}$. The sites of interaction are also diverse. Some are located in the N-terminus, some in the C-terminus, and some are mediated through the SUR subunit. This complexity is surprising, since the Kir subunits have a rather simple tertiary and quaternary structure, with two transmembrane segments lining the selectivity filter in each of the four channel subunits [15]. This raises the question of whether all the regulators converge on a common gating transition for Kir channels that underlies their opening and closure. This has yet to be clarified; however, there are some data in the literature that hint in this direction. Upregulation by $PIP_2$ interaction may antagonize ATP-dependent closure in $K_{ATP}$ channels [5]. At the same time, mutated Kir2.1 channels can recover from pH-dependent closure in the presence of Mg-ATP, which is thought to act via $PIP_2$ kinase [54]. Thus, it seems likely that the stimulating effect of $PIP_2$ counteracts the inactivated states induced by ATP in Kir6.2 channels and by $H^+$ in mutated Kir2.1 channels. Moreover, it has been shown that $PIP_2$ interacts positively with the effect of $G_{\beta\gamma}$ on Kir3 channels [33]. These multiple interactions of the various regulators suggest that there is a common principle of allosteric interaction between the different regulators, indicative of a common gating mechanism. One possible conformational change underlying such a gating mechanism is the model shown in Fig. 10. On the other hand, there are also some counterarguments against a common principle of Kir channel gating. Application of $PIP_2$ to Kir1.1 did not change the steady-state parameters of pH gating [57]. Moreover, the interaction between PIP2 and $G_{\beta\gamma}$ has recently been interpreted differently: $PIP_2$ may also simply modulate the opening of Kir3 channels without directly interacting with the mechanism underlying $G_{\beta\gamma}$-dependent opening [40]. Thus, it may well be that the various members of the Kir channel family use different conformational changes in their protein structure during the various processes of regulation by intracellular second messengers. Future work analysing the interactions of Kir regulators in combination with future studies of structure–function relationships will probably provide further insight into the gating mechanism of this class of $K^+$ channel.

## References


1. Aguilar-Bryan L, Clement JP IV, Gonzalez G, Kunjilwar K, Babenko A, Bryan J (1998) Toward understanding the assembly and structure of KATP channels. Physiol Rev 78:227–245
2. Ashcroft FM, Harrison D, Ashcroft SJ (1984) Glucose induces closure of single potassium channels in isolated rat pancreatic β-cells. Nature 312:446–448
3. Ashcroft FM, Rorsman P (1989) Electrophysiology of the pancreatic beta-cell. Prog Biophys Mol Biol 54:87–143
4. Babenko AP, Aguilar-Bryan L, Bryan J (1998) a view of SUR/KIR6.X, KATP channels. Annu Rev Physiol 60:667–687
5. Baukrowitz T, Schulte U, Oliver D, Herlitze S, Krauter T, Tucker SJ, Ruppersberg JP, Fakler B (1998) PIP2 and PIP as determinants for ATP inhibition of KATP channels. Science 282:1141–1144
6. Baukrowitz T, Tucker SJ, Schulte U, Benndorf K, Ruppersberg JP, Fakler B (1999) Inward rectification in KATP channels: a pH switch in the pore. EMBO J 18:847–53
7. Benndorf K, Schwalb M, Hirche H (1990) Effects of anoxia on K and Ca currents in isolated guinea pig cardiocytes. Pflügers Arch 416:207–209
8. Bianchi L, Roy M-L, Taglialatela M, Lundgren DW, Brown AM, Ficker E (1996) Regulation by spermine of native inward rectifier $K^+$ channels in RBL-1 cells. J Biol Chem 271: 6114–6121
9. Brown AM (1997) Cardiac potassium channels in health and disease. Trends Cardiovasc Med 7:118–124
10. Bünemann M, Hosey MM (1998) Regulators of G protein signaling (RGS) proteins constitutively activate $G_{\beta\gamma}$-gated potassium channels. J Biol Chem 273:31186–31190
11. Cohen NA, Brenman JE, Snyder SH, Bredt DS (1996) Binding of the inward rectifier $K^+$ channel Kir2.3 to PDS-95 is regulated by protein kinase A phosphorylation. Neuron 17:759–767

12. Di Magno L, Dascal N, Davidson N, Lester HA, Schreibmayer W (1996) Serotonin and protein kinase C modulation of a rat brain inwardly rectifying $K^+$ channel expressed in *Xenopus* oocytes. Pflügers Arch 431:335–40
13. Doi T, Fakler B, Schultz JH, Schulte U, Brändle U, Weidemann S, Zenner H-P, Lang F, Ruppersberg JP (1996) Extracellular $K^+$ and intracellular pH allosterically regulate renal Kir1.1 channels. J Biol Chem 271:17261–17266
14. Doupnik CA, Davidson N, Lester HA (1995) The inward rectifier potassium channel family. Curr Opin Neurobiol 5:268–277
15. Doyle DA, Cabral JM, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998) The structure of the potassium channel: molecular basis of $K^+$ conduction and selectivity. Science 280:69–77
16. Fakler B, Bond CT, Adelman JP, Ruppersberg JP (1996) Heterooligomeric assembly of inward-rectifier $K^+$ channels from subunits of different subfamilies: Kir2.1 (IRK1) and Kir4.1 (BIR10). Pflügers Arch 433:77–83
17. Fakler B, Brändle U, Glowatzki E, König C, Bond C, Adelman JP, Zenner H-P, Ruppersberg JP (1994) A structural determinant of differential sensitivity of cloned inward rectifier channels to intracellular spermine. FEBS Lett 356:199–203
18. Fakler B, Brändle U, Glowatzki E, Weidemann S, Zenner HP, Ruppersberg JP (1995) Strong voltage-dependent inward-rectification of inward rectifier $K^+$ channels is caused by intracellular spermine. Cell 80:149–154
19. Fakler B, Brändle U, Glowatzki E, Zenner H-P, Ruppersberg JP (1994) Kir2.1 inward rectifier $K^+$ channels are regulated independently by protein kinases and ATP hydrolysis. Neuron 13:1413–1420
20. Fakler B, Ruppersberg JP (1996) Functional and molecular diversity classifies the family of inward-rectifier $K^+$ channels. Cell Physiol Biochem 6:195–209
21. Fakler B, Schultz J, Yang J, Schulte U, Brändle U, Zenner HP, Jan LY, Ruppersberg JP (1996) Identification of a titratable lysine residue that determines sensitivity of kidney potassium channels (ROMK) to intracellular pH. EMBO J 15:4093–4099
22. Fan Z, Makielski JC (1997) Anionic phospholipids activate ATP-sensitive potassium channels. J Biol Chem 272:5388–5395
23. Fruman DA, Meyers RE, Cantley LC (1998) Phosphoinositide kinases. Annu Rev Biochem 67:481–507
24. Glowatzki E, Fakler G, Brändle U, Rexhausen U, Zenner HP, Ruppersberg JP, Fakler B (1995) Subunit-dependent assembly of inward-rectifier $K^+$ channels. Proc R Soc Lond [Biol] 261:251–261
25. Gribble FM, Tucker SJ, Ashcroft FM (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
26. He C, Zhang H, Mirshahi T, Logothetis DE (1999) Identification of a potassium channel site that interacts with G protein $\beta\gamma$ subunits to mediate agonist-induced signaling. J Biol Chem 274:12517–12524
27. Henry P, Pearson WL, Nichols CG (1996) Protein kinase C inhibition of cloned inward rectifier (HRK1/KIR2.3) $K^+$ channels expressed in *Xenopus* oocytes. J Physiol (Lond) 495: 681–688
28. Herlitze S, Ruppersberg JP, Mark MD (1999) New roles for RGS2, 5 and 8 on the ratio-dependent modulation of recombinant GIRK channels expressed in *Xenopus* oocytes. J Physiol (Lond) 517:341–352
29. Hibino H, Horio Y, Inanobe A, Doi K, Ito M, Yamada M, Gotow T, Uchiyama Y, Kawamura M, Kubo T, Kurachi Y (1997) An ATP-dependent inwardly rectifying potassium channel, KAB-2 (Kir4.1), in cochlear stria vascularis of inner ear: its specific subcellular localization and correlation with the formation of endocochlear potential. J Neurosci 17: 4711–4721
30. Hilgemann DW, Ball R (1996) Regulation of cardiac $Na^+$,$Ca^{2+}$ exchange and KATP potassium channels by PIP2. Science 273:956–959
31. Hille B (1992) Ionic channels in excitable membranes, 2nd edn Sinauer, Sunderland, Mass., pp 127–130
32. Ho K, Nichols CG, Lederer WJ, Lytton J, Vassilev PM, Kanarzirska MV, Hebert SC (1993) Cloning and expression of an inwardly rectifying ATP-regulated potassium channel. Nature 362:31–38
33. Huang C-L, Feng S, Hilgemann DW (1998) Direct activation of inward rectifier potassium channels by PIP2 and its stabilization by $G\beta\gamma$. Nature 391:803–806
34. Huang C-L, Jan YN, Jan LY (1997) Binding of the G protein $\beta\gamma$ subunit to multiple regions of G protein-gated inward-rectifying $K^+$ channels. FEBS Lett 405:291–298
35. Huang C-L, Slesinger PA, Casey PJ, Jan YN, Jan LY (1995) Evidence that direct binding of $G_{\beta\gamma}$ to the GIRK1 G protein-gated inwardly rectifying $K^+$ channel is important for channel activation. Neuron 15:1133–1143
36. Husebye ES, Flatmark T (1988) Phosphatidylinositol kinase of bovine adrenal chromaffin granules: kinetic properties and inhibition by low concentrations of $Ca^{2+}$. Biochim Biophys Acta 968:261–265
37. Ishii M, Horio Y, Tada Y, Hibino H, Inanobe A, Ito M, Yamada M, Gotow T, Uchiyama Y, Kurachi Y (1997) Expression and clustered distribution of an inwardly rectifying potassium channel, KAB-2/Kir4.1, on mammalian retinal Müller cell membrane: their regulation by insulin and laminin signals. J Neurosci 17:7725–7735
38. Katan M (1998) Families of phosphoinositide-specific phospholipase C: structure and function. Biochim Biophys Acta 1436:5–17
39. Kauffmann-Zeh A, Klinger R, Endemann G, Waterfield MD, Wetzker R, Hsuan JJ (1994) Regulation of human type II phosphatidylinositol kinase activity by epidermal growth factor-dependent phosphorylation and receptor association. J Biol Chem 269:31243–31251
40. Kim D, Bang H (1999) Modulation of rat atrial G protein-coupled $K^+$ channel function by phospholipids. J Physiol (Lond) 517:59–74
41. Krapivinsky G, Gordon EA, Wickman K, Velimirovic B, Krapivinsky L, Clapham DE (1995) The G-protein-gated atrial $K^+$ channel IKACh is a heteromultimer of two inwardly rectifying $K^+$-channel proteins. Nature 374:135–141
42. Krapivinsky G, Kennedy ME, Nemec J, Medina I, Krapivinsky L, Clapham DE (1998) $G_{\beta\gamma}$ binding to GIRK4 subunit is critical for G protein-gated $K^+$ channel activation. J Biol Chem 273:16946–16952
43. Kubo Y, Baldwin TJ, Jan YN, Jan LY (1993) Primary structure and functional expression of a mouse inward rectifier potassium channel. Nature 362:127–133
44. Liou H-H, Zhou S-S, Huang C-L (1999) Regulation of ROMK1 channel by protein kinase A via a phosphatidylinositol 4,5-bisphosphate-dependent mechanism. Proc Natl Acad Sci USA 96:5820–5825
45. Logothetis DE, Zhang H (1999) Gating of G protein-sensitive inwardly rectifying $K^+$ channels through phosphatidyl-inositol 4,5-bisphosphate. J Physiol (Lond) 520:653–660
46. Lopatin AN, Makhina EN, Nichols CG (1994) Potassium channel block by cytoplasmic polyamines as the mechanism of intrinsic rectification. Nature 372:366–369
47. Lu Z, MacKinnon R (1994) Electrostatic tuning of $Mg^{2+}$ affinity in an inward-rectifier $K^+$ channel. Nature 371:243–246
48. Matsuda H (1991) Effects of external and internal $K^+$ ions on magnesium block of inwardly rectifying $K^+$ channels in guinea-pig heart cells. J Physiol (Lond) 435:83–99
49. McNicholas CM, Wang W, Ho K, Hebert SC, Giebisch G (1994) Regulation of ROMK1 $K^+$ channel activity involves phosphorylation processes. Proc Natl Acad Sci USA 91:8077–8081
50. Nichols CG, Lopatin AN (1997) Inward rectifier potassium channels. Annu Rev Physiol 59:171–191
51. Oliver D, Hahn H, Antz C, Ruppersberg JP, Fakler B (1998) Interaction of permeant and blocking ions in cloned inward-rectifier $K^+$ channels. Biophys J 74:2318–2326

52. Pessia M, Tucker SJ, Lee K, Bond CT, Adelman JP (1996) Subunit positional effects revealed by novel heteromeric inwardly rectifying $K^+$ channels. EMBO J 15:2980–2987
53. Ruppersberg JP, Fakler B (1996) Complexity of the regulation of Kir2.1 $K^+$ channels. Neuropharmacology 35:887–893
54. Ruppersberg JP, Schultz J, Brändle U, Fakler B, Schulte U (1999) Intracellular regulation of inwardly rectifying potassium channels. Curr Topics Membr 46:223–242
55. Saitoh O, Kubo Y, Miyatani Y, Asano T, Nakata H (1997) RGS8 accelerates G-protein-mediated modulation of $K^+$ currents. Nature 390:525–529
56. Saitoh O, Kubo Y, Odagiri M, Ichikawa M, Yamagata K, Sekine T (1999) RGS7 and RGS8 differentially accelerate G protein-mediated modulation of $K^+$ currents. J Biol Chem 274:9899–9904
57. Schulte U, Hahn H, Konrad M, Jeck N, Derst C, Wild K, Weidemann S, Ruppersberg JP, Fakler B, Ludwig J (1999) pH gating of ROMK ($K_{(ir)}1.1$) channels: control by an arg-lys-Arg triad disrupted in antenatal bartter syndrome. Proc Natl Acad Sci USA 96:15298–15303
58. Schulte U, Hahn H, Wiesinger H, Ruppersberg JP, Fakler B (1998) pH-dependent gating of ROMK (Kir1.1) channels involves conformational changes in both N and C termini. J Biol Chem 273:34575–34579
59. Shyng S-L, Ferrigni T, Nichols CG (1997) Regulation of KATP channel activity by diazoxide and MgADP. Distinct functions of the two nucleotide binding folds of the sulfonylurea receptor. J Gen Physiol 110:643–654
60. Shyng S-L, Nichols CG (1998) Membrane phospholipid control of nucleotide sensitivity of KATP channels. Science 282:138–141
61. Slesinger PA, Reuveny E, Jan YN, Jan LY (1995) Identification of structural elements involved in G protein gating of the GIRK1 potassium channel. Neuron 15:1145–1156
62. Sui JL, Petit-Jacques J, Logothetis DE (1998) Activation of the atrial KACh channel by the $\beta\gamma$ subunits of G proteins or intracellular $Na^+$ ions depends on the presence of phosphatidylinositol phosphates. Proc Natl Acad Sci USA 95:1307–1312
63. Taglialatela M, Ficker E, Wible BA, Brown AM (1995) C-terminus determinants for $Mg^{2+}$ and polyamine block of the inward rectifier $K^+$ channel IRK1. EMBO J 14:5532–5541
64. Tucker SJ, Gribble FM, Zhao C, Trapp S, Ashcroft FM (1997) Truncation of Kir6.2 produces ATP-sensitive $K^+$ channels in the absence of the sulphonylurea receptor. Nature 387:179–183
65. Uhde I, Toman A, Gross I, Schwanstecher C, Schwanstecher M (1999) Identification of the potassium channel opener site on sulfonylurea receptors. J Biol Chem 274:28079–28082
66. Wang W, Giebisch G (1991) Dual modulation of renal ATP-sensitive $K^+$ channel by protein kinases A and C. Proc Natl Acad Sci USA 88:9722–9725
67. Wang W, Hebert SC, Giebisch G (1997) Renal $K^+$ channels: Structure and function. Annu Rev Physiol 59:413–436
68. Wang W-H (1995) Regulation of the hyperpolarization-activated $K^+$ channel in the lateral membrane of the cortical collecting duct. J Gen Physiol 106:25–43
69. Wei Y, Ali S, Wang W-H (1999) Protein tyrosine phosphatase (PTP) is involved in regulating the number of apical small-conductance K channels (SK) in the cortical collecting duct (CCD) of the rat kidney. APS conference – biology of potassium channels: from molecules to disease; Abstract 10.7:
70. Wickman K, Clapham DE (1995) Ion channel regulation by G proteins. Physiol Rev 75:865–885
71. Wischmeyer E, Karschin A (1996) Receptor stimulation causes slow inhibition of IRK1 inwardly rectifying $K^+$ channels by direct protein kinase A-mediated phosphorylation. Proc Natl Acad Sci USA 93:5819–5823
72. Xie L-H, Takano M, Kakei M, Okamura M, Noma A (1999) Wortmannin, an inhibitor of phosphatidylinositol kinases, blocks the MgATP-dependent recovery of Kir6.2/SUR2A channels. J Physiol (Lond) 514:655–665
73. Zerangue N, Jan LY (1998) G-protein signaling: fine-tuning signaling kinetics. Curr Biol 8:R313–R316
74. Zerangue N, Schwappach B, Jan YN, Jan LY (1999) A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels. Neuron 22:537–548