Unique ATP-inhibitable K⁺ channels (K_ATP) in the kidney determine the rate of urinary K⁺ excretion and play an essential role in extracellular K⁺ membrane conductance regulator (CFTR) genes. Co-injection of CFTR and Kir1.1a cRNA into Xenopus oocytes lead to the expression of K⁺ selective channels that retained the high open probability behavior of Kir1.1a but acquired sulfonylurea sensitivity and ATP-dependent gating properties. Similar to the K_ATP channels in the kidney but different from K_ATP channels in excitable tissues, the Kir1.1a/CFTR channel was inhibited by glibenclamide with micromolar affinity. Since the expression of Kir1.1a and CFTR overlap at sites in the kidney where the low sulfonylurea affinity K_ATP are expressed, our study offers evidence that these native K_ATP channels are comprised of Kir1.1a and CFTR. The implication that Kir subunits can interact with ABC proteins beyond the subfamily of sulfonylurea receptors provides an intriguing explanation for functional diversity in K_ATP channels.

Extracellular potassium homeostasis, maintained through the regulation of renal potassium excretion (1), is dependent on unique epithelial ATP-sensitive K⁺ channels (K_ATP) (2). Providing the major, if not exclusive, route for potassium transport into the distal nephron lumen, these channels constitute the final regulated component of kidney's potassium secretory apparatus. Physiological changes in secretory K_ATP channel activity subsequently determine the extent of urinary K⁺ excretion in accord with the demands of potassium balance. While the molecular basis of these physiologically important channels has remained unresolved, elucidation of their biophysical nature by the patch-clamp technique has provided important insights. Most telling are the important similarities and differences exhibited between the secretory channel and the ATP-sensitive K⁺ channels identified in the cardiac myocyte (3) and the islet beta cell (4). Characterized by the ability of cytoplasmic ATP to induce channel closure (5), susceptibility to antidiabetic sulfonylurea inhibition (6) and weak inward rectification (7), the distal nephron secretory channel exhibits common functional features of all K_ATP channels (8). However, the renal epithelial K_ATP channels are readily distinguished from other ATP-sensitive K⁺ channels by their relatively low affinity for sulfonylurea agents (6, 9) and cytoplasmic ATP (5), suggesting the secretory K_ATP channel might be encoded by particularly unique members of the multimeric family of K_ATP proteins.

Several recent breakthroughs in the field offered tangible clues for testing this hypothesis. Defining a new class of K⁺ channel proteins, characterized by their inward rectifying properties (Kir) (10) and a unique two-transmembrane structural motif, Ho et al. (11) isolated a novel K⁺ channel cDNA, called ROMK1 or Kir1.1a, from rat kidney. As required for a secretory K⁺ channel gene, Kir 1.1a is specially expressed in the distal nephron (12) on the apical membrane (13). While Kir1.1a shares many functional features of the secretory channel, the notable absence of ATP (11) or sulfonylurea sensitivity suggested that the native channel was more complex than Kir 1.1a alone. Certainly, the recent discovery of Aguilar-Bryan et al. (14) gave credence to this notion. These investigators showed that the high affinity sulfonylurea receptor expressed in the pancreatic islet beta cell is encoded by a unique ATP-binding cassette protein (ABC protein or traffic ATPase), SUR1. The sulfonylurea binding protein does not exhibit any channel activity itself. Instead, SUR1 interacts with a pancreatic inward rectifying K⁺ channel subunit, Kir 6.2, to form a K_ATP channel like those observed in beta-islet cells (15). With the subsequent discovery that the cardiac K_ATP channel is comprised of a related ATP-binding cassette protein, SUR2A, and the inward-rectifying K⁺ channel isofrom, Kir 6.2, a molecular paradigm for other K_ATP channels began to be established (16).

Having this multimeric Kir/ABC protein motif in mind, we explored the possibility that the distal nephron K_ATP channel is encoded by unique Kir and ATP-binding cassette proteins as a basis for its distinct functional properties. While Kir 1.1a appeared to be an excellent candidate for the inward rectifying K⁺ channel subunit, the identity of the ABC subunit remained less certain. As might be surmised from the low sulfonylurea sensitivity of renal K_ATP channels, neither the pancreatic islet beta cell (SUR1) nor the cardiac myocyte (SUR2A) ABC proteins are expressed in the kidney (14, 16). Another ABC protein, the cystic fibrosis transmembrane conductance regulator (CFTR), does standout as a plausible candidate. First, CFTR is expressed abundantly in the kidney and is localized along the entire nephron (17) on the apical membrane (18). Subsequently, the cellular and subcellular expression overlap with Kir1.1a in the cortical collecting duct. Second, the cystic fibrosis gene product is the only other member of the ABC superfamily that is known to bind sulfonylurea agonists (19). Interestingly, the sulfonylurea affinity of CFTR is similar to the
renal epithelial K\textsubscript{ATP} channels. Finally, besides acting as a chloride channel itself, there is a growing consensus that CFTR may also regulate other channels (20).

In agreement with this general hypothesis, McNicholas et al. (21) have recently reported that CFTR confers glibenclamide sensitivity on a closely related Kir1.1a isoform, ROMK2 (Kir1.1b). However, two critical questions remained unanswered. First, does CFTR interact with other members of the ROMK family? Second, and more importantly, does coexpression of CFTR with any ROMK (Kir1.1) isoform reconstitute ATP-sensitive K\textsuperscript{+} channels? In the present study, we have specifically addressed these issues by measuring the functional consequences of CFTR/Kir1.1a interaction in Xenopus oocytes injected with CFTR and Kir1.1a cRNA. Our results demonstrate that CFTR associates with Kir 1.1 to modify single channel conductance and to confer both ATP and sulfonlurea sensitivity. The data are compatible with the notion that CFTR and Kir1.1 physically associate to form a hybrid channel with properties that are reminiscent of the distal nephron K\textsubscript{ATP} channel. The implication of this study that Kir subunits can interact with ATP-binding cassette proteins beyond the subfamily of sulfonlurea receptors provides an intriguing explanation for functional diversity in K\textsubscript{ATP} channels.

**EXPERIMENTAL PROCEDURES**

**cRNA Synthesis**—Complementary RNA was transcribed in vitro in the presence of capping analogue (G\textsubscript{5}ppp\textsubscript{5}G\textsubscript{5} from 1)\textsubscript{1},\textsubscript{1}O\textsubscript{1}linearized Kir1.1p-SPEPORT (ROMK1 (11) and 2)\textsubscript{2} Smal linearized CFTR-pBS vector (Genzyme). TT or SP6 RNA polymerase were used in the two reactions, respectively (Ambion, mMESSAGE mMACHINE \textsuperscript{TM}). Following DNase treatment, cRNA was purified by phenol-chloroform extraction and ammonium-acetate/ethanol precipitation. The yield and concentration were quantified spectrophotometrically.

**Oocyte Injection**—Female *Xenopus laevis* frogs were obtained from NASCO (Fort Atkinson, WI). Standard protocols were followed for the isolation and care of *X. laevis* oocytes. Briefly, frogs were anesthetized by immersion in 0.2% tricaine and a partial oophorectomy was performed through an abdominal incision. Oocyte aggregates were manually dissected from the ovarian lobes and then were incubated in a calcium-free ORII medium (82.5 mM NaCl, 2 mM KCl, 1 mM MgCl\textsubscript{2}, 5 mM HEPES, pH 7.4 (Sigma type 1A, Worthington collagenase type 3, 2 mg/ml) for 12–24 h at room temperature to remove the follicular layer. After washing the oocytes extensively with collagenase-free ORII, they were placed in a modified L15 medium (50% Leibovitzi’s medium, 10 mM HEPES, pH 7.5) and stored at 19 °C. 12–24 h following isolation, healthy looking Dumont stage V–VI oocytes were anaerobically injected with 50 nl of water containing cRNA and then stored at 19 °C. Oocytes were either injected with Kir1.1a cRNA alone (5 ng) or Kir1.1a (5 ng) and a 5-fold molar excess of CFTR cRNA to force the potential interaction by mass action.

**Electrophysiology**—Channel activity was assessed 2–6 days post-injection by patch-clamp under conditions where K\textsuperscript{+} channels could be discerned from chloride channels by differences in reversal potential. For most studies, the pipette contained 140 mM KCl, 1 mM CaCl\textsubscript{2}, 5.0 mM HEPES, pH 7.4, while the bath solution was comprised of 120 mM sodium gluconate, 20 mM KCl, 1 mM CaCl\textsubscript{2}, 5.0 mM HEPES, pH 7.4 (E\textsubscript{K} = +50, E\textsubscript{Na} = −50 mV). For ion selectivity studies, bath sodium gluconate was replaced with equimolar KCl. In all studies, we purposely excluded protein kinase A to prevent CFTR activation in the excised cell-attached patch (22). Glibenclamide was added from a freshly prepared alkaline stock as before (9). The magnesium salt of ATP (vanadate-free, Sigma) was used in the ATP studies. All ATP containing solutions were used immediately (<30 min) after preparation. After the addition of ATP or glibenclamide the pH was titrated to 7.4. Solution exchange was accomplished by an oil gate apparatus as described previously (23). All ATP and glibenclamide studies were performed at E\textsubscript{Cl} (<50 mV cell relative to patch), so inward K\textsuperscript{+} currents could be isolated.

In these studies, the vitelline membrane was removed from oocytes following hypersonomotric shrinking (24). Patch-clamp electrodes, pulled from filaments borsilicate glass, had resistances of 0.5–5 megohms. Single channel currents were measured with Axopatch-1C patch clamp amplifier and recorded on video tape via Neuro-corder (model DR-886). Data were replayed and filtered by an eight-pole Bessel filter (Frequen-

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**RESULTS**

**Co-expression of CFTR Reduces the Single Channel Conductance of Kir 1.1a**—Since Kir 1.1a exhibits a larger conductance (35 pS) than the values initially reported for the native secretory K\textsubscript{ATP} channel measured at the same temperature (22–25 pS) (25), we first asked whether the potential interaction between Kir 1.1a and CFTR might be revealed by a change in the unitary conductance of Kir 1.1a. As shown by hypothesis was borne out from co-expression studies. Co-injection of Kir1.1a and a 5-fold molar excess of CFTR cRNA into oocytes led to the expression of unique small conductance K\textsuperscript{+} channels. The Kir1.1a/CFTR channel exhibited similar high open probability kinetic properties as Kir1.1a (P\textsubscript{o} = 0.77 ± 0.04 Kir1.1a/ CFTR; P\textsubscript{o} = 0.95 ± 0.02 Kir1.1a at −50 mV, Fig. 1A). Kir1.1a/ CFTR was, however, easily distinguished from Kir1.1a by a significantly smaller, albeit variable, unitary conductance (Fig. 1B). Compared with the inward slope conductance of Kir 1.1a (51.5 ± 2.15 pS, as reported by others) (11), the Kir1.1a/CFTR channel (19.89 ± 6.0 pS (P > 0.01)) was significantly smaller (Fig. 1C). Consistent with a variable interaction stoichiometry, the smaller average unitary conductance of Kir 1.1a/CFTR reflects the expression of a least four distinct channel types with conductances ranging from 24 pS to 12 pS (24 ± 2 pS (13.8%), 20 ± 2 pS (23.1%), 16 ± 2 pS (36.92%) and 12 ± 2 pS (9.23%) n = 65 channels/52 patches) (Fig. 2). K\textsuperscript{+} channels of this smaller conductance fingerprint were never observed in unjected oocytes or in oocytes injected with either Kir 1.1a or CFTR cRNA alone. The occasional detection a channel with the larger conductance signature of the Kir 1.1a channel in coinjected oocytes (17% of patches contained channels with a conductance of 35 ± 5 pS) is consistent with unmodified Kir1.1a channels.

Observations that the predominant expression of the smaller conductance K\textsuperscript{+} channel in co-injected oocytes was unaffected by patch excision from the cell, imply that the interaction between CFTR and Kir1.1a is membrane delimited. While we can not rule out the role of unidentified linker proteins, such as cytoskeletal elements, these observations suggest that the functional interaction between CFTR and Kir1.1a may be direct and physical. The *Kir 1.1a/CFTR Channel Is K\textsuperscript{+} Selective*—As predicted from our multimeric Kir1.1a/CFTR model of an epithelial K\textsubscript{ATP} the small conductance Kir1.1a/CFTR channel is K\textsuperscript{+} selective (Fig. 3). In the excised inside-out configuration with 140 mM KCl in the pipette, replacement of cytoplasmatic KCl with equal molar sodium gluconate (KCl + sodium gluconate = 140 mM) changed the reversal potential of the small conductance channel from 1.4 ± 2.4 mV in 140 mM KCl to 22.6 ± 2.05 mV in 50 mM KCl (90 mM sodium gluconate) to 49.3 ± 4 mV in 20 mM KCl (120 mM sodium gluconate) (n = 6). The shift of 51 mV per decade change in KCl is in close agreement for the change in the equilibrium potential for K\textsuperscript{+}, indicating the small conductance Kir1.1a/CFTR channel is highly K\textsuperscript{+} selective over sodium and chloride.
CFTR-dependent Acquisition of Sulfonylurea Sensitivity—Having shown that CFTR interacts with Kir 1.1a to form a K⁺ channel with conduction properties similar to the secretory channel, we asked whether this channel also acquires the characteristic features of KATP, such as sulfonylurea sensitivity. An oil gate was employed in these studies to ensure immediate and complete solution exchange. This experimental design allowed an obvious distinction between the specific inhibitory effects of glibenclamide and spontaneous channel rundown, a property of Kir1.1 (11, 26), the native secretory channel (5) and Kir1.1/CFTR. The high open probability behavior of the Kir 1.1a was not affected by exposure up to 100 μM glibenclamide (1.5 ± 1.6% decrease in channel activity, n = 4), incompatible with properties of the native secretory Kₐₐₜp channel (Fig. 4A). In dramatic contrast to Kir 1.1a and as expected for Kₐₐₜp to the subconductance level observed with Kir 1.1a is consistent with the stabilization of the smaller conductance state. C, current voltage relationships of the Kir 1.1a and the Kir1.1a/CFTR channels confirm a bona fide reduction in the inward slope conductance upon CFTR coexpression. inhibitory effects of glibenclamide occurred with low affinity, almost identical to the effects on CFTR alone (19) and similar to the native secretory kidney Kₐₐₜp channel. Indeed dose-dependent glibenclamide inhibition of the Kir 1.1a/CFTR channel reveal a Kᵢ of 33 μM (n = 6), compared with the nanomolar sulfonylurea-sensitivity of the Kₐₐₜp channels expressed in the pancreatic beta cell (Kir6.2/SUR1, Kᵢ = 8.6 nM) (16) and cardiac myocytes (Kir6.2/SUR2A, Kᵢ = 350 nM) (16).

Kir 1.1a/CFTR Is a Kₐₐₜp Channel—Cyttoplasmic ATP exposure had no inhibitory effect on Kir1.1a activity (Fig. 5A), confirming initial observations (11). In excised inside-out patches, Kir1.1a exhibited high open probability behavior in the absence (Pₒ = 0.94 ± 0.04) or presence of cytoplasmic 5 mM ATP (Pₒ = 0.88 ± 0.09, n = 6). In dramatic contrast, the same concentration of cytoplasmic ATP inhibited the small conductance Kir1.1a/CFTR channel (12–26 pS) by 84 ± 1.2% (9/11 patches) within 30 s of ATP exposure with recovery after ATP washout (Fig. 5B). Repeated exposures of 5 mM ATP resulted in repeated bouts of channel closure followed by reopening to 34 ± 3.2% of control levels upon return to zero ATP. The response is consistent with an allosteric effect, rather than phosphorylation/dephosphorylation mechanism of action (23). If channel required rephosphorylation to open, the relative constant activity on return to zero ATP would not have been observed.
Dose-response studies (Fig. 5C) revealed that the ATP sensitivity of Kir1.1a/CFTR is more similar to the native kidney $K_{\text{ATP}}$ channel ($K_i = 0.5$ mM) (5) than the higher affinity $K_{\text{ATP}}$ channels in the pancreatic islet beta cell (Kir6.2/SUR1, $EC_{50} \approx 10$ $\mu$M (16), or heart (Kir6.2/SUR2A, $EC_{50} \approx 100$ $\mu$M (16)). Indeed, ATP-dependent channel inhibition of Kir1.1a/CFTR occurred with an $EC_{50}$ of 0.6 mM. Furthermore, at concentrations less than 100 $\mu$M, ATP had the tendency to increase channel activity as has been described for the native channel (5).

Also reminiscent of the native channel, cytoplasmic ADP antagonized the inhibitory effect of ATP (Fig. 6) on Kir1.1a/CFTR. In contrast to the 67.64 ± 2.45% decrease in channel activity observed in 1 mM ATP, cytoplasmic addition of 1 mM ATP + 1 mM ADP caused channel activity to decrease by 45.29 ± 7.3% ($p < 0.005$, $n = 6$). In this regard and as have been and have been shown for the native kidney channel, the ratio of ATP/ADP would appear to be a more important regulator of Kir1.1a/CFTR activity than ATP alone.

In most but not all studies (compare Figs. 5B and 6), the inhibitory effect that was observed in Kir1.1a/CFTR upon elevation of cytoplasmic ATP occurred with a slower time course than would be anticipated from the immediate step increase in cytoplasmic ATP (≈20 ms) (27). Currents relaxed to near zero upon exposure to ATP with a half-time of ≈15 s. While we can not be certain of the mechanism responsible for the time course, in low channel density patches where single channel conductance could be precisely resolved, we frequently observed the transient emergence of the 35–40-pS channel upon exposure to ATP. Such a response may reflect the activation of non interacting ROMK channels or the dissociation of putative CFTR/ROMK1 subunits before ATP-dependent inhibition.

**DISCUSSION**

Unique $K_{\text{ATP}}$ channels in the renal cortical collecting duct, comprising the final regulated component of kidney’s potassium secretory machinery, play an essential role in the regulation of extracellular K+ balance (28). In the present study, we demonstrate that functionally similar low sulfonylurea affinity $K_{\text{ATP}}$ channels are formed by two heterologous molecules, products of Kir1.1a and CFTR genes. Co-injection of CFTR and Kir1.1a cRNA into *Xenopus* oocytes lead to the expression of unique K+ selective channels that retained the high open probability behavior of all Kir1.1a channels but acquired sulfonylurea sensitivity and ATP-dependent gating properties, the *sine qua non* of $K_{\text{ATP}}$. The Kir1.1a/CFTR channel was inhibited by glibenclamide with a much lower affinity than those expressed in excitable tissues (endocrine pancreas, Kir 6.2/SUR1 or heart, Kir6.2/SUR2), similar to the unique $K_{\text{ATP}}$ channels in the kidney (6). With observations that the expression patterns of Kir1.1a (12) and CFTR (18) overlap in the distal nephron along the apical membrane, our study offers compelling evidence that the native potassium secretory $K_{\text{ATP}}$ channels may be comprised of Kir1.1a and CFTR.

Several important aspects of our study differ from a recent report of McNicholas et al. (21) with Kir1.1b(ROMK2) and CFTR. By employing an oil gate for rapid and efficient solution exchange, we demonstrated that glibenclamide directly inhibits its Kir1.1a/CFTR as required for a $K_{\text{ATP}}$ channel. Subsequently it is not necessary to invoke a dephosphorylation-dependent rundown process to explain the glibenclamide effect on Kir1.1a/CFTR as suggested by McNicholas et al. for Kir1.1b/CFTR. In addition to extending and clarifying the mechanism of sulfonylurea sensitivity, we have discovered that co-expression CFTR with Kir1.1a was required to reconstitute ATP-dependent gating properties. In these regards, the renal epithelial $K_{\text{ATP}}$ channel appears to mimic the basic Kir/ATP binding cassette protein paradigm exhibited by other $K_{\text{ATP}}$ channels.
Although the acquisition of ATP and low-affinity sulfonylurea sensitivity are compatible with the reconstitution of the renal secretory $K_{ATP}$ channel, the single channel conductance of Kir1.1a/CFTR deserves some comment. In the present study, we demonstrate a variable downward shift in the single channel conductance of Kir1.1a with co-expression of CFTR, from 36 pS in Kir1.1a alone to an average of 20 pS in Kir1.1a/CFTR. Sulfonylurea sensitivity and ATP-dependent gating properties appeared to be unique characteristic of the smaller conductance channel, making it likely that these particular channels reflect the interaction among CFTR and Kir1.1a subunits. The shift in the mean current amplitude of Kir 1.1a/CFTR to the subconductance level observed with Kir 1.1a is consistent with the CFTR-mediated stabilization of the smaller and more rare conductance state in the Kir 1.1a-only channels. A mutation in the Kir1.1 channel that eliminates a site for protein kinase A phosphorylation also stabilizes the subconductance state (29), suggesting a possible mechanism for the CFTR induced change in the conductive properties.

In any regard, the conductive properties of Kir1.1a/CFTR are more similar to those initially reported for the native secretory channel than Kir1.1a alone. Friedt and Palmer (25) found that the apical membrane $K^+$ channel in the cortical collecting duct exhibited an inward slope conductance of 22–25 pS at room temperature, smaller than Kir1.1a but more similar to Kir1.1a/CFTR. In agreement with these early observations and as predicted by the temperature dependence of aqueous diffusion ($Q_{10} = 1.3–1.8$) (30), latter measurements at 37 °C placed the value at 36 pS (7). Although all these observations are more compatible with Kir1.1a/CFTR than the Kir1.1a channel, it should be pointed out that Palmer et al. (31) have recently come to a different conclusion in a systematic comparison of the native channel to Kir1.1b (ROMK2), a splice variant that lacks the first 19 amino acid residues of Kir1.1a. These investigators now report that the conductive properties of the two channels are more similar (31). At room temperature both channels exhibit an inward slope conductance of 36 pS. At present we can not wholly account for the discordance between these more recent measurements in the native secretory $K_{ATP}$ channel and our own with Kir1.1a/CFTR. However, co-expression of CFTR appears to be absolutely required for the acquisition of ATP and sulfonylurea-sensitivity in Kir1.1a.

The time dependence of ATP inhibition in the native channel has never been systematically studied under ideal conditions (i.e., an oil gate). Nevertheless, based on what can be inferred from work with the native channel, it would appear that inhibition of the Kir1.1a/CFTR channel often occurs at a slower rate than the kidney channel. At present, the mechanism underlying the kinetics of ATP-dependent inhibition in Kir1.1a/CFTR, or any $K_{ATP}$ channel for that matter, are unknown. Based on the variability of the response, however, we speculate that ATP-dependent modulation may be dependent on a particular conformational state of Kir1.1a/CFTR. Obviously, further work to elucidate the mechanism by which CFTR interacts with Kir1.1a to form a $K_{ATP}$ channel will be necessarily before a mechanism is established with any certainty.

CFTR-dependent modulation of other ion channels, involving either direct or indirect interaction mechanisms (20), provides some clues, however. While still controversial, activation of glibenclamide. The upper tracing illustrates reversible and repeatable inhibition of Kir1.1a + CFTR $K^+$ channel activity upon exposure to 100 μM glibenclamide. At higher gain, unitary events are observed with the small conductance fingerprint of the Kir 1.1a/CFTR channel. Sulfonylurea inhibition occurs with relatively low affinity. Inhibition (1 - $I/I_{max}$) of integrated Kir1.1a + CFTR and Kir1.1a channel activity are shown.
of the outward rectifying chloride channel in respiratory epithelia is perhaps the best characterized example of indirect regulation (32). In this system, CFTR is thought to mediate the efflux of ATP which in turn increases outward rectifying chloride channel activity through a purinergic receptor signaling pathway (33). In contrast to this autocrine mechanism of interaction, our observations point to a membrane delimited pathway. CFTR-dependent modulation of Kir1.1a, revealed by the decrease in single channel conductance, acquisition of sulfonylurea sensitivity and development ATP-dependent gating properties, were observed in the excised patch configuration. Furthermore, the interaction between CFTR and Kir1.1a occurred in the apparent absence of any autocrine regulators, addition of kinases or nucleotides for phosphorylation, making an indirect pathway unlikely. While we can not rule out a role of undefined linker proteins, such as cytoskeletal elements, our observations strongly imply that CFTR modifies Kir1.1a by direct protein-protein interactions, similar to that proposed for CFTR-dependent modulation of the amiloride-sensitive sodium channel (34) and Kir6.2/SUR1 (35). Recently, the pancreatic islet beta cell KATP channel has recently been shown to consist of an (SUR1-Kir6.2)4 octamer, comprised of a Kir 6.2 tetramer, forming the K<sup>1</sup> selective pore, surrounded by four SUR1 subunits (35). Based on homology, it seems plausible that the organization of CFTR and Kir1.1a subunits is similar. While the distribution of several different conductance states in CFTR/Kir1.1a may suggest the CFTR interaction stoichiometry is variable, further experimentation is required before any definitive conclusions can be made.

The domains of the Kir1.1a/CFTR complex that confer ATP and sulfonylurea sensitivity are presently unknown. The mo-
lecular homology of CFTR to sulfonylurea receptors, all members of the ATP-binding cassette family of proteins, suggests that CFTR acts as the sulfonylurea receptor in the Kir1.1a/CFTR channel. Certainly, the close concordance in the affinity of the Kir1.1a/CFTR channel for glibenclamide and that of CFTR alone (19) support this view. The functional domains responsible for ATP-sensitivity remain more obscure. Because CFTR contains two functional nucleotide binding domains (36), similar to the nucleotide binding domains in SUR1 that have been implicated nucleotide-dependent modulation of the islet beta cell $K_{ATP}$ (37), it is possible that these sites play some role. Alternatively, a recent report by Tucker et al. (38) with Kir 6.2 suggests that the critical ATP binding site resides in the Kir subunit. In agreement with this notion with ROMK channels, Kir1.1a contains a functional nucleotide binding domain (39, 40). Subsequently, CFTR may stabilize a more favorable gating conformation in Kir1.1a, allowing direct ATP-binding with Kir1.1a to induce channel closure. Obviously, further study is required to determine the relative importance of each of the nucleotide-binding domains in the two different subunits of the renal epithelial $K_{ATP}$ channel.

In summary, we have shown that co-expression of Kir1.1a (ROMK1) with CFTR in Xenopus oocytes reconstitute unique low-sulfonylurea affinity $K_{ATP}$ similar to those involved in renal $K^+$ excretion and $K^+$ homeostasis. The implication of this study that Kir subunits can interact with ATP binding cassette proteins beyond the subfamily of sulfonylurea receptors provides an intriguing explanation for functional diversity in $K_{ATP}$ channels.

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