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Identification of domains that control the heteromeric assembly of Kir5.1/Kir4.0 potassium channels

Angelos-Aristeidis Konstas, Christoph Korbmacher, and Stephen J. Tucker
University Laboratory of Physiology, Oxford, OX1 3PT, United Kingdom

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Konstas, Angelos-Aristeidis, Christoph Korbmacher, and Stephen J. Tucker. Identification of domains that control the heteromeric assembly of Kir5.1/Kir4.0 potassium channels. *Am J Physiol Cell Physiol* 284: C910–C917, 2003. First published November 27, 2002; 10.1152/ajpcell.00479.2002.—Heteromultimerization between different inwardly rectifying (Kir) potassium channel subunits is an important mechanism for the generation of functional diversity. However, little is known about the mechanisms that control this process and that prevent promiscuous interactions in cells that express many different Kir subunits. In this study, we have examined the heteromeric assembly of Kir5.1 with other Kir subunits and have shown that this subunit exhibits a highly selective interaction with members of the Kir4.0 subfamily and does not physically associate with other Kir subunits such as Kir1.1, Kir2.1, and Kir6.2. Furthermore, we have identified regions within the Kir4.1 subunit that appear to govern the specificity of this interaction. These results help us to understand the mechanisms that control Kir subunit recognition and assembly and how cells can express many different Kir channels while maintaining distinct subpopulations of homo- and heteromeric channels within the cell.

inwardly rectifying potassium channel

ION CHANNEL DIVERSITY CAN be enhanced by a variety of different mechanisms. Many ion channels physically associate with accessory "β"-subunits that modulate channel activity, while other channels employ complex processes such as alternative splicing or even mRNA editing to generate functional diversity from a limited number of gene products. However, for potassium channels where the conductive pore consists of four subunits, it is often found that different combinations of subunits coassemble to generate heteromeric channels with novel functional properties (8). However, K^+ channel subunits are not completely promiscuous, and the specificity of coassembly is carefully controlled to prevent unwanted or potentially damaging combinations from disrupting cellular activity.

Recent studies have begun to elucidate the molecular mechanisms that control channel assembly and in the case of the voltage-gated (Kv) channels, a specific NH_2-terminal intracellular "tetramerization" domain is responsible for both the ability of the channel to form tetramers and for the specificity of subunit interactions (14), whereas BK channels use a domain within the proximal COOH terminus to control heteromeric subunit assembly (20). However, the mechanisms that control the heteromeric interaction of the inwardly rectifying potassium (Kir) channels remain unclear. These channels contain a large intracellular COOH terminus that physically associates with the intracellular NH_2 terminus. Roles for both the NH_2 terminus, transmembrane (TM) domains, and the COOH terminus have been proposed in controlling subunit assembly (6, 12, 27), whereas two 80 amino acid domains within the second TM domain and proximal COOH terminus have been implicated in controlling the heteromeric assembly of members of the Kir2.0 channel family (26).

Heteromeric interactions have now been demonstrated between many different Kir channels (5, 7, 19, 21). In some cases, these interactions produce nonfunctional channels that are degraded, while in other cases channels with novel functional properties are generated. One example in vivo is the cardiac muscarine-sensitive channel, which comprises Kir3.1 and Kir3.4 (13), whereas another is found in the kidney and involves Kir5.1. This subunit only forms functional homomeric channels when coexpressed with PSD-95 (24). However, it has been shown to form functional heterotetramers with both Kir4.1 (19) and Kir4.2 (17, 18). These heteromeric Kir5.1/Kir4.0 channels exhibit novel biophysical properties and an enhanced sensitivity to inhibition by intracellular pH (pH_i) within the physiological range (25, 29, 31). Recent evidence now suggests that these Kir5.1/Kir4.0 channels underlie the basolateral small-conductance K^+ channel in the distal nephron (15), which is thought to play a key role in the transport and recycling of K^+ across the basolateral membrane. However, the expression of these subunits is not restricted to renal epithelia, and so heteromeric Kir5.1/Kir4.0 channels are likely to be found in other cell types.

In addition to Kir5.1, renal epithelial cells have been shown to express members of the Kir1.0, Kir2.0, Kir4.0, Kir6.0, and Kir7.0 subfamilies (1–3, 9, 15, 16). Each of these channels has a defined role, and in polarized renal epithelia their expression is often restricted to apical or basolateral membranes. Therefore, it is essential to control heteromultimerization be-

Address for reprint requests and other correspondence: S. J. Tucker, Univ. Laboratory of Physiology, Parks Rd., Oxford, OX1 3PT, United Kingdom (E-mail: stephen.tucker@physiol.ox.ac.uk).

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tween these channels to maintain these distinct subpopulations of K^+ channels. In this study, we set out to understand why Kir5.1 only forms functional heteromeric channels with members of the Kir4.0 family and the molecular mechanisms that underlie this specificity. We found that the specificity of these interactions reflects an inability of most Kir channels to physically interact with Kir5.1 and that a small region within the proximal COOH terminus of Kir4.1 appears to play a key role in the control of this process.

MATERIALS AND METHODS

Molecular Biology

Rat Kir1.1a, mouse Kir2.1, rat Kir4.1, mouse Kir4.2, rat Kir5.1, and mouse Kir6.2 were subcloned in the oocyte expression vector pBF. Chimeras and mutants were also constructed in this vector. The COOH-terminal 14-amino acid endoplasmic reticulum (ER) retention sequence of the human α_2C adrenergic receptor (KHILFRRRRRGFRQ) were fused in frame to the COOH terminus of Kir5.1 by standard PCR protocols. Chimeras between Kir4.1 and Kir1.1a were created by using extension overlap PCR. The splice junctions of each chimera are shown in Table 1. Site-directed mutagenesis was performed using the QuickChange XL protocol (Stratagene). Capped mRNAs were synthesized in vitro by using the T7 or SP6 mMESSAGE mMACHINE kit (Ambion).

Isolation of Oocytes and Injection of cRNA

Xenopus laevis oocytes were prepared and injected as described (10). Defolliculated oocytes were injected with various cRNA combinations. Unless otherwise stated, 1 ng of test cRNA was coinjected with 10 ng of Kir5.1R cRNA. For a 50:1 ratio of Kir5.1R-Kir2.1, 0.2 ng of Kir2.1 RNA was used. Injected oocytes were kept in modified Barth's saline [in mM: 88 NaCl, 1 KCl, 2.4 $NaHCO_3$, 0.3 $Ca(NO_3)_2$, 0.41 $CaCl_2$, 0.82 $MgSO_4$, and 15 HEPES, adjusted to pH 7.6 with Tris].

Electrophysiology

Two-electrode voltage clamp. Oocytes were studied 24–48 h after injection using the two-electrode voltage-clamp technique, as previously described (10). Oocytes were routinely clamped at a holding potential of −60 mV. To obtain whole cell currents, voltage pulse protocols were performed using consecutive 400-ms step changes of the clamp potential from −60 to −120 mV up to +60 in 20-mV increments. The Ba^{2+}-sensitive current was determined at −120 mV by subtracting the whole cell current trace (using the last 100 ms) recorded in the presence of 1 mM Ba^{2+} from that recorded before the addition of Ba^{2+} (in mM: 95 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES, adjusted to pH 7.4 with Tris). Data are given as mean values ± SE; *n* indicates the number of oocytes. Significance was evaluated by the appropriate version of Student's *t*-test. Results were reproducible in three different batches of oocytes.

Table 1. *Chimeric junctions used to contruct the chimeras used in this study*

Chimera	Kir1.1	Kir4.1
30A	^{74}D-M^{391}	^{1}M-V^{60}
30C	^{1}M-H^{197}	^{185}A-V^{379}
30E	^{1}M-V^{73}; ^{198}A-M^{391}	^{61}D-H^{184}
30F	^{198}A-M^{391}	^{1}M-H^{184}
30H	^{1}M-F^{173}; ^{198}A-M^{391}	^{161}I-H^{184}
30I	^{1}M-H^{197}; ^{221}L-M^{391}	^{185}A-L^{217}
30K	^{1}M-H^{197}; ^{260}L-M^{391}	^{185}A-D^{245}
30L	^{1}M-V^{105}	^{93}H-V^{379}
30R	^{1}M-F^{173}; ^{221}L-M^{391}	^{161}I-L^{217}
30T	^{1}M-N^{259}	^{245}D-V^{379}

Kir, inwardly rectifying potassium channel. Nos. describe the amino acids for Kir1.1 (Kir1.1a) and Kir4.1.

Surface labeling of oocytes. Experiments were essentially performed as recently described (10, 23, 32) using 1 μg/ml rat monoclonal anti-HA antibody (clone 3F10; Boehringer) as primary antibody and 2 μg/ml peroxidase-conjugated affinity-purified $F(ab)_2$ fragment goat anti-rat IgG antibody (Jackson ImmunoResearch) as secondary antibody. Results were reproducible in three different batches of oocytes.

RESULTS

Physical Association of Kir5.1 With Kir4.1 But Not Kir1.1

To measure the physical association of Kir5.1 with Kir4.1, we engineered an ER retention sequence from the COOH terminus of the α_2C adrenergic receptor on the COOH terminus of Kir5.1 (Kir5.1R). This "ER-trap" approach has been used previously by Schwappach et al. (23, 32) to monitor the interaction between the sulfonylurea receptor and Kir6.2 coexpressed in *Xenopus* oocytes. The tetrameric assembly of K^+ channel monomers occurs within the ER (4), and so this assay relies upon the ability of the tagged ER-trap protein to prevent surface expression of any coexpressed subunits that it coassembles with. A reduction in the level of surface expression of the coexpressed protein therefore reflects physical association with the tagged protein. In the case of Kir4.1, surface expression can be monitored by measuring both whole cell current levels and surface expression of Kir4.1.

We therefore coexpressed Kir4.1 with and without Kir5.1R in *Xenopus* oocytes and measured the resulting Ba^{2+}-sensitive whole cell currents by two-electrode voltage clamp. Figure 1*A* shows representative currents from these two groups of oocytes and demonstrates that, when a 10-fold excess of Kir5.1R mRNA was coexpressed with Kir4.1, there was a dramatic decrease in Kir4.1 currents. The results are summarized in Fig. 1*C*. Coexpression with Kir5.1R causes a 95% reduction in Kir4.1 currents (4.6 ± 2.9% of control, $n = 63$) compared with Kir4.1 expressed alone. To test whether this inhibition of Kir4.1 currents is the result of a specific interaction with Kir4.1, we also coexpressed Kir5.1R with Kir1.1. It has been shown previously that Kir5.1 does not functionally interact with Kir1.1, and Fig. 1, *B* and *C*, shows that Kir5.1R had no effect on the Ba^{2+}-sensitive whole cell currents expressed by Kir1.1 (102 ± 9.2% of control, $n = 56$), indicating that Kir5.1R does not physically associate with Kir1.1.

We next examined the levels of surface expression of Kir4.1 in the presence and absence of Kir5.1R. We have previously monitored surface expression of Kir1.1 and Kir4.1 by engineering hemagglutinin (HA) epitopes in the first extracellular loop of these channels (11). Both

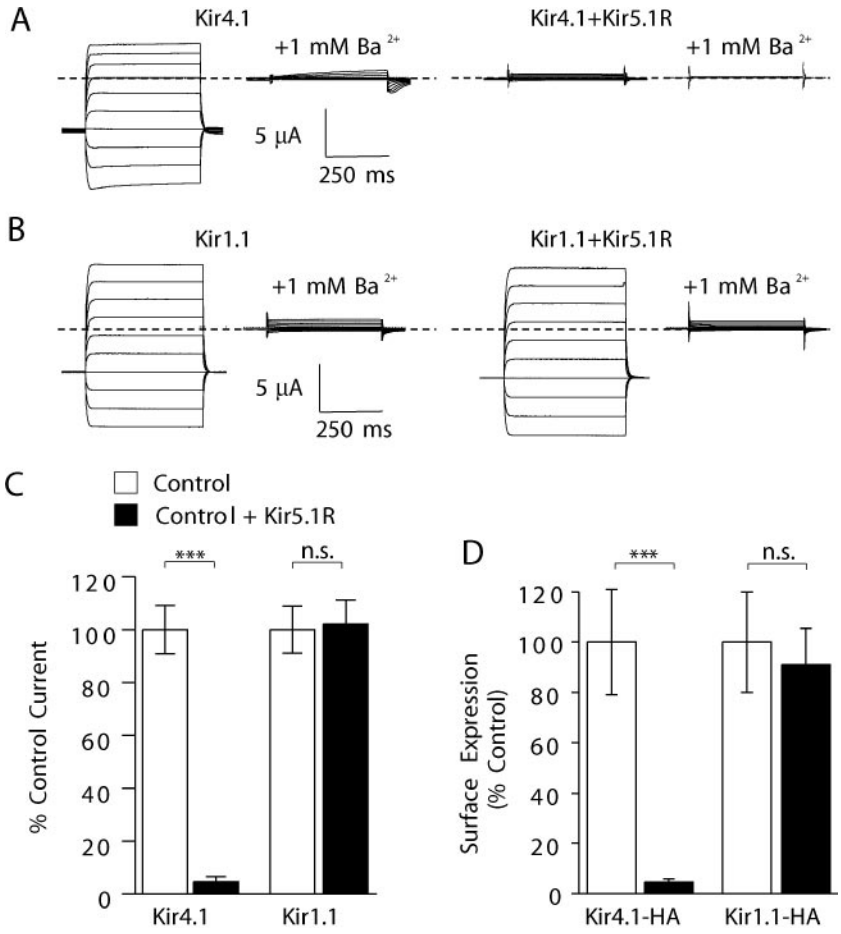


Fig. 1. Inwardly rectifying potassium (Kir) channel 5.1 interacts with Kir4.1 but not the closely related Kir1.1. *A*: representative whole cell current traces obtained from voltage-step protocols of oocytes expressing Kir4.1 or Kir4.1 with a 10-fold excess of Kir5.1R (see MATERIALS AND METHODS). Kir currents were almost abolished after the addition of 1 mM Ba^{2+}. Dotted line represents zero current level. *B*: representative whole cell current traces obtained from oocytes expressing Kir1.1 or Kir1.1 + Kir5.1R. No effect of Kir5.1R was observed. *C*: Ba^{2+}-sensitive currents in control oocytes expressing either Kir4.1 or Kir1.1 (open bars) or coinjected with Kir5.1 containing an endoplasmic reticulum (ER) retention sequence (Kir5.1R; filled bars). Currents were expressed as a percentage of the Ba^{2+}-sensitive control current. *D*: chemiluminescence antibody detection assay was used to measure surface expression of Kir4.1-hemagglutinin (HA) and Kir1.1a-HA in oocytes expressing either Kir4.1-HA or Kir1.1-HA alone (open bars) or coexpressing Kir5.1R with Kir4.1-HA or Kir1.1-HA (filled bars). Surface expression was normalized for each group according to the values obtained for Kir4.1-HA or Kir1.1-HA control oocytes. ***$P < 0.005$. NS, not significant.

Kir4.1-HA and Kir1.1-HA are functional, and surface expression is measured by chemiluminescent detection of antibody binding to the exposed HA epitope. Figure 1*D* shows that coexpression with Kir5.1R caused a reduction in the surface expression of Kir4.1-HA (4.7 ± 1.1% of control, $n = 10$), similar to the reduction in current levels seen in Fig. 1*C*. Likewise, there was no significant reduction in the surface expression of Kir1.1-HA (91.3 ± 9.4% of control, $n = 10$), consistent with the lack of effect of Kir5.1R on Kir1.1 current levels. Thus the mechanism of inhibition of Kir4.1 currents by Kir5.1R is the result of a reduction in the surface expression of Kir4.1. This indicates that Kir5.1R physically associates with Kir4.1 to trap it within the cell and prevents surface expression but it does not associate with Kir1.1.

Highly Selective Association of Kir5.1 With Members of the Kir4.0 Family

We next examined the selectivity of Kir5.1 association with other Kir channels by coexpressing Kir4.2, Kir2.1, and Kir6.2 with and without Kir5.1R. For Kir6.2, we used a COOH-terminal truncation (Kir6.2ΔC26) that removes an intrinsic ER-retention motif and permits surface expression of Kir6.2 (28). Figure 2 shows that coexpression of Kir5.1R caused complete suppression of Kir4.2 Ba^{2+}-sensitive currents (4.3 ± 2.1% of control, $n = 10$). By contrast, it had no effect on Kir2.1 or Kir6.2ΔC26 currents (97.6 ± 10.1 and 91 ± 16.4% of control, respectively, $n = 14$ for each group).

Identification of Domains in Kir4.1 That Permit Association with Kir5.1

We therefore set out to exploit the clear difference in behavior between Kir4.1 and Kir1.1 to examine which regions of Kir4.1 were capable of physical association with Kir5.1. We made chimeras between Kir4.1 and Kir1.1 and coexpressed these with and without Kir5.1R. Figure 3 summarizes the results obtained. Chimeras 30C and 30F were both inhibited by coexpression with Kir5.1R (3.2 ± 2.4 and 6.1 ± 2.3% of control, respectively, $n = 21$ for each group), indicating that multiple regions are involved in this process.

To further narrow down these regions, smaller domains were exchanged between Kir4.1 and Kir1.1. Chimeras 30A and 30E demonstrate that exchanging the NH_2 terminus has no effect on retention; therefore, it is unlikely to play a major role. Chimera 30L contained only the second TM and P-loop of Kir4.1 and exhibited partial retention (37 ± 8.1% of control, $n = 14$), suggesting a consistent but weaker interaction with Kir5.1R. Further attempts to narrow down the regions in TM2 were complicated by the fact that none

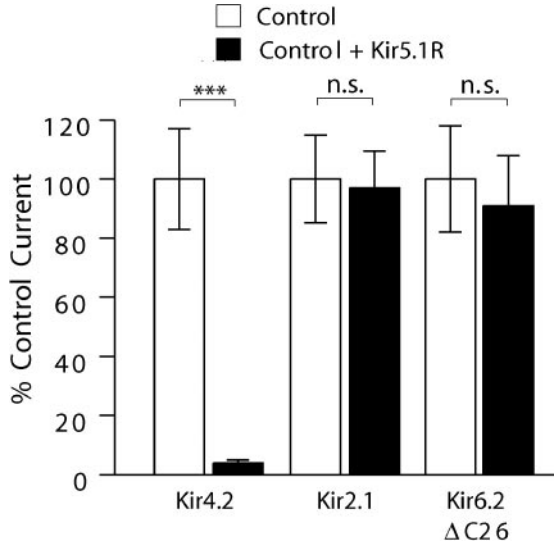
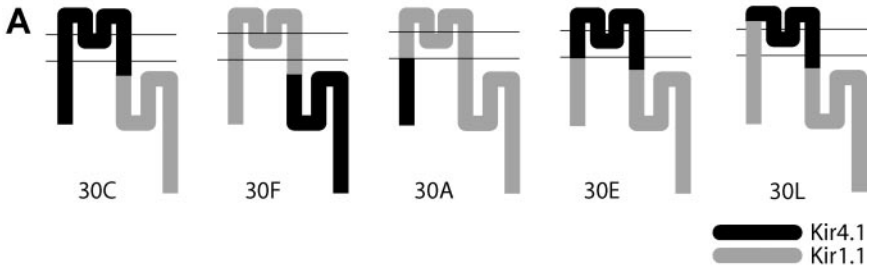


Fig. 2. Kir5.1 only interacts with members of the Kir4.0 family. Ba^{2+}-sensitive currents in control oocytes expressing either Kir4.2, Kir2.1, or Kir6.2ΔC26 (open bars) or coinjected with Kir5.1 containing an ER retention sequence (Kir5.1R; filled bars). Currents were expressed as a percentage of the control current. $^{***}P < 0.005$.

of the chimeras expressed functional K^+ channels (data not shown).

Analysis of the COOH terminus revealed that the distal domains of Kir4.1 do not contribute to coassembly with Kir5.1. Figure 4*A* shows that surface expression of chimeras 30K-HA and 30T-HA, which contained the distal regions of Kir4.1, were not suppressed by Kir5.1R (96.3 ± 9.1 and 95.1 ± 8.4% of control, respectively, $n = 10$ for each group). However, the chimera 30R was able to interact with Kir5.1R and showed significant suppression of surface expression when coexpressed with Kir5.1R (14.6 ± 4% of control, $n = 42$). The region of Kir4.1 exchanged in the chimera was a 44-amino acid stretch covering the very end of the second TM and the proximal COOH terminus. This region was then subdivided to form two further chimeras (30H and 30I). However, Fig. 5 shows that neither of these regions was sufficient by themselves to permit interaction with Kir5.1R.

Sequence comparison of the regions exchanged in the 30R chimera shows that this region is highly conserved between Kir4.1 and Kir1.1 (Fig. 6). We therefore examined the role of key residues within this domain. Several point mutations were made within the 30R chimera at the positions that differed between the two Kir subunits. In each case, the Kir4.1 residue was exchanged for the corresponding Kir1.1 residue. The A183S, R193T, Q196K, and N204G mutations in 30R all resulted in nonfunctional channels. However, the E177K mutation resulted in functional K^+ channels. Figure 6, *B* and *C*, shows that these 30R(E177K) currents were not suppressed by coexpression with Kir5.1R (99.7 ± 3.1% of control, $n = 14$), and neither was surface expression of 30R-HA(E177K) affected (95.9 ± 9.9% of control, $n = 10$). These results indicate that both regions contained within chimeras 30I and 30H are necessary but not sufficient for this interaction



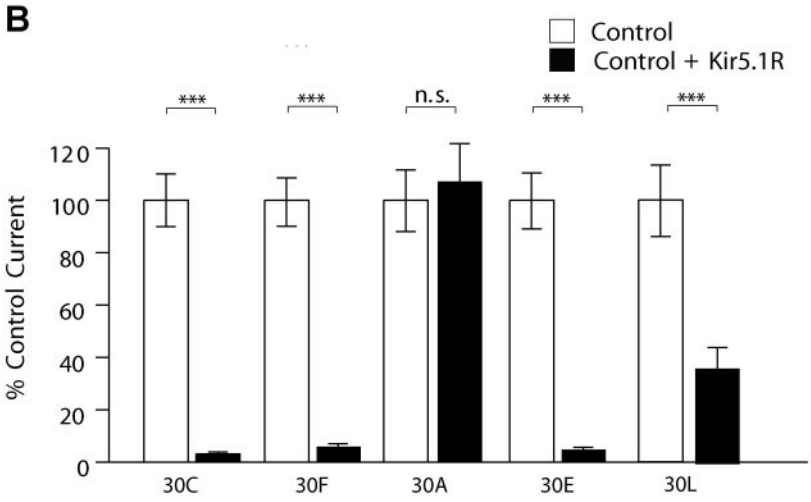


Fig. 3. Chimeric analysis of Kir4.1 and Kir1.1. *A*: schematic representation of the chimeras used (see Table 1). *B*: currents expressed by each chimera expressed alone (open bars) or coexpressed with Kir5.1R (filled bars). Currents were normalized for each group according to the values obtained for control oocytes. $^{***}P < 0.005$.

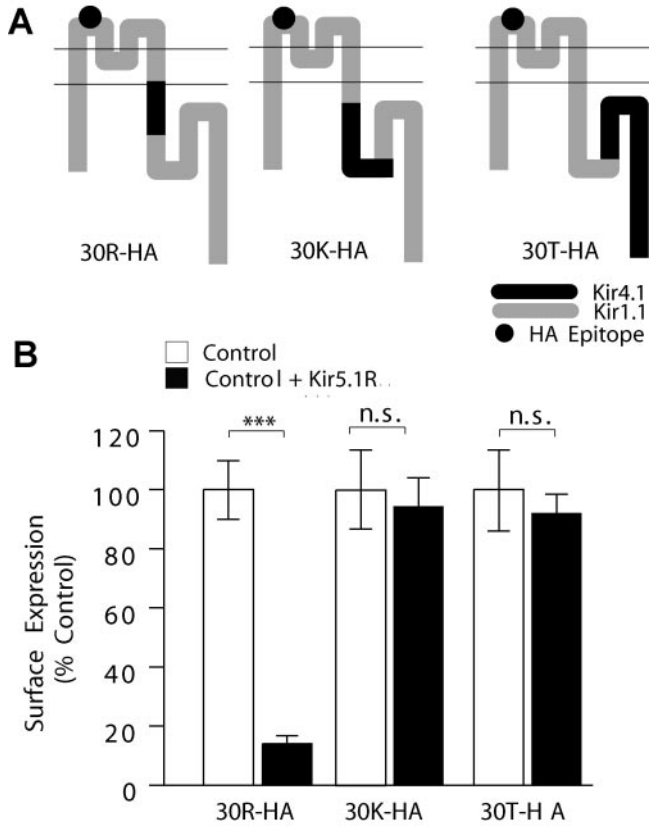


Fig. 4. Role of the COOH terminus in heteromeric specificity. *A*: schematic representation of the chimeras used. In each case, the chimera was tagged with an HA-epitope in the first extracellular loop to detect surface expression. *B*: relative surface expression of each chimera expressed alone (open bars) or coexpressed with Kir5.1R (filled bars). Surface expression was normalized for each group according to the values obtained for control oocytes. $^{***}P < 0.005$.

and that a single point mutation within this "interaction domain" can disrupt this interaction.

DISCUSSION

The main findings of this study are that Kir5.1 exhibits highly selective heteromultimerization with members of the Kir4.0 subfamily and that the lack of functional interaction with other Kir channels is the result of their inability to physically interact. Furthermore, we have identified a small domain within the proximal COOH terminus of Kir4.1 that plays a major role in the interaction between Kir5.1 and Kir4.1. These results help us to understand the mechanisms that enable cells to express many different Kir channels while maintaining distinct subpopulations of homo- and heteromeric channels within the cell.

Physiological Significance of Selective Heteromultimerization

The basolateral K^+ channel in the distal nephron plays an essential role in the recycling of K^+ that has entered the cells via the basolateral Na^+-K^+ pump and thus is essential for Na^+ reabsorption to take place. Recently, Lourdel et al. (15) have identified a Kir5.1/Kir4.0 combination as the likely molecular correlate of these channels. One of the principal features of these heteromeric Kir5.1/Kir4.0 channels is their sensitivity to inhibition by protons within the physiological range (25, 29, 31); when Kir5.1 heteropolymerizes with Kir4.1, a dramatic shift in the pH sensitivity of Kir4.1 is observed, shifting the pKa of Kir4.1 from 6.0 to 7.4 in the heteromeric channel. It has been shown that this shift is the result of the ability of Kir5.1 to "enhance" the intrinsic pH sensitivity of Kir4.1 rather than providing an additional proton sensor per se (18). This dramatic shift in pH sensitivity produces a novel channel suited for pH_i-mediated cross talk with Kir1.1 and the epithelial Na^+ channel, which are both regulated by pH_i in the physiological range. This therefore provides a mechanism for their coordinated regulation, enabling apical Na^+ absorption to be coupled to basolateral K^+ recycling. It is also of considerable importance that Kir5.1 is unable to heteromultimerize with Kir1.1. Given the dramatic shift in pKa when Kir5.1 coassembles with Kir4.1, heteromultimerization of Kir5.1 with Kir1.1 could have the potential to shift the pKa value of Kir1.1 to the alkaline range, which would inhibit Kir1.1 activity at normal pH. Certain Bartter's Syndrome mutations in Kir1.1 have been shown to shift the pKa of the channel to the alkaline range, creating "functional" channels that are inactive at

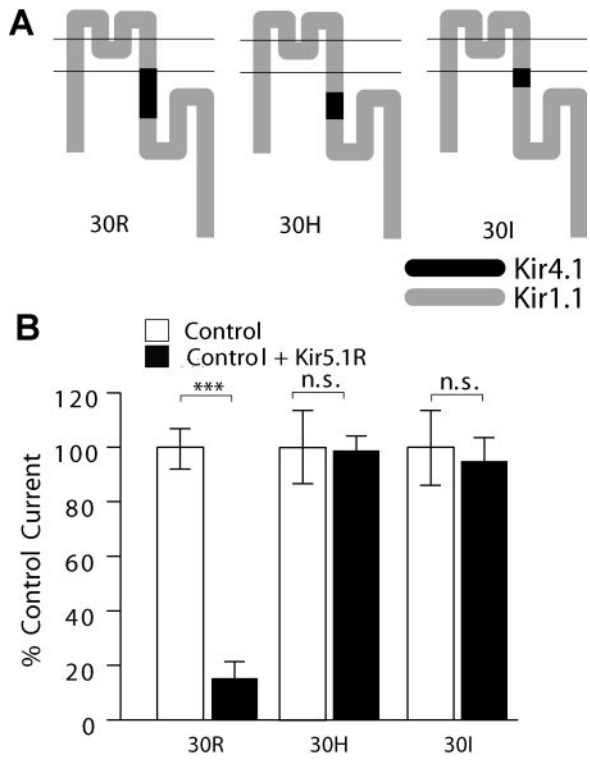


Fig. 5. Role of the proximal COOH terminus in heteromeric specificity. *A*: schematic representation of the chimeras used. The region identified in chimera 30R was further subdivided to form chimeras 30H and 30I (see Table 1). *B*: relative surface expression of each chimera expressed alone (open bars) or coexpressed with Kir5.1R (filled bars). Surface expression was normalized for each group according to the values obtained for control oocytes. $^{***}P < 0.005$.

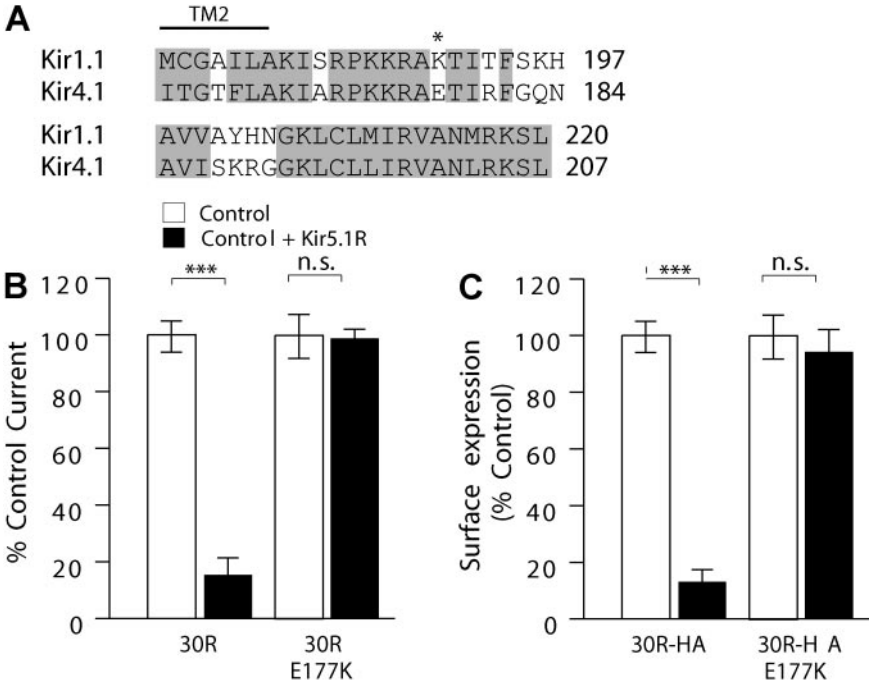


Fig. 6. A single mutation within the proximal COOH-terminus can abolish heteromeric selectivity. *A*: alignment of the region exchanged in the 30R chimera. Identical or functionally equivalent amino acids are shaded. The distal part of transmembrane domain 2 is marked. The asterisk (*) indicates the single amino acid substitution performed in chimera 30R (E177K). *B*: Ba^{2+}-sensitive currents in control oocytes expressing either 30R or 30R-E177K (open bars) or coinjected with Kir5.1R. *C*: relative surface expression of each chimera expressed alone (open bars) or coexpressed with Kir5.1R (filled bars). Surface expression was normalized for each group according to the values obtained for control oocytes. $***P < 0.005$.

physiological pH (22). Thus the similarity in sequence between Kir1.1 and Kir4.1 means that it is essential for their interaction with Kir5.1 to be tightly controlled. Likewise, the inability of Kir5.1 to interact with members of the Kir2.0 and Kir6.0 subfamilies assists in maintaining distinct homomeric subpopulations of these channels within the cell.

One recent study has suggested that Kir5.1 acts as a "dominant-negative" regulator of Kir2.1 activity by interacting with Kir2.1 to form electrically silent channels (3). However, our results using the ER-trap assay do not support this conclusion, as we observed no reduction in Kir2.1 currents when coexpressed with a 10-fold excess of Kir5.1R. To confirm these findings, we also attempted to repeat the findings of Derst et al. (3) by coexpressing wild-type Kir5.1 with Kir2.1. Even when coexpressed in a 50-fold excess with Kir2.1, we saw no suppression of Kir2.1 currents (data not shown). The Kir2.1 clone used by Derst et al. was the human clone, which differs from the clone used in this study at a residue within the NH_2 terminus (M84T) and which has been suggested to alter the intracellular trafficking of Kir2.1 (30). We therefore coexpressed Kir5.1 in a 10-, 25-, and 50-fold excess with human Kir2.1 but still saw no effect on current levels (data not shown). We are unable to reconcile these differences, but if Kir5.1 was able to interact with Kir2.1 in vivo then this would have severe functional consequences for the ability of Kir2.1 to form functional homomeric channels.

Molecular Determinants of Heteromeric Specificity

It is now becoming evident that there is a complex series of interactions between the cytoplasmic domains of the Kir channel. These interactions are not only intrasubunit but may also be intersubunit, and, as well as providing a structural framework for channel assembly, they also underlie channel gating and regulation. Previous attempts to isolate the domains responsible for intersubunit interactions have concluded that multiple regions are involved (6, 12, 26, 27). However, one of the most recent studies by Tinker et al. (26) concluded that two regions of Kir2.1, the second TM domain and the proximal COOH terminus (amino acids 156–220 and 220–300, respectively), control the ability of Kir2.1 to interact with other Kir subunits and that the relative importance of these two regions varies depending on which Kir subunit was interacting with Kir2.1.

Our results concur with those of Tinker et al. (26) to suggest that multiple regions contribute to heteromeric assembly and that there is no single assembly domain for Kir channels like there is for the Kv channels. Chimeras 30F and 30E demonstrate that, contrary to previous reports (6), the NH_2 terminus does not play a major role in the compatibility between Kir subunits. Even though chimera 30L showed only partial retention, the effect was consistent and significant. This suggests that the second TM domain also plays a key role in the compatibility between Kir4.1 and Kir5.1. Further attempts to narrow down the region within TM2 were unsuccessful, since none of the resulting chimeras produced functional K^+ channels (data not shown). However, our analysis of the COOH terminus was much more successful. The region isolated in chimera 30R contains a 44-amino acid stretch of Kir4.1 that permits coassembly with Kir5.1, and much of this domain is identical between the two Kir subunits. To date, this is the smallest domain identified that contributes to Kir subunit assembly and con-

sists of the very proximal COOH terminus of Kir4.1. Figure 6 shows that, within this 44-amino acid stretch, 30 amino acids are either identical or functionally equivalent, indicating that only a few key residues are responsible for this difference.

The ability of the E177K mutation to abolish the interaction between the 30R chimera and Kir5.1R also highlights the specificity of this interaction. However, no single amino acid appears to be responsible, since dividing this region into two (chimeras 30H and 30I) produced noninteracting chimeras. Thus it is likely that, within this region, there are multiple points of contact that contribute to this heteromeric interaction. The ability of the E177K mutation to abolish this interaction probably reflects a general allosteric disruption caused by this charge reversal rather than a specific interaction with the glutamate residue in Kir4.1. The significant homology between Kir1.1 and Kir4.1, and indeed with all other Kir subunits, in the proximal COOH terminus region also indicates that very small differences in folding and/or exposure of surfaces are involved in determining the permissiveness of subunit binding and that only a few amino acids may have major effects on subunit interaction. It is also interesting to note that the position of this domain in the 30R chimera correlates with the tetramerization domain identified in the BK channel (20), which is also found in the very proximal COOH terminus. It is therefore possible that channels such as the BK and Kir channels, which both contain large cytoplasmic COOH termini, use similar approaches for subunit recognition and assembly.

In conclusion, we have identified a small domain within Kir4.1 that contributes to the highly selective coassembly of this channel with Kir5.1 and that only a few key residues contribute to this process. Furthermore, the highly selective interaction between Kir5.1 and other Kir channels helps us to understand how cells that express many different Kir subunits are able to maintain distinct subpopulations of homo- and heteromeric channels within the cell.

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Present address of C. Korbmacher: Institut für Zelluläre und Molekulare Physiologie, Universität Erlangen-Nürnberg, Waldstr. 6, D-91054 Erlangen, Germany.

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