RNA Interference Reveals That Endogenous Xenopus MinK-related Peptides Govern Mammalian K⁺ Channel Function in Oocyte Expression Studies*

Received for publication, December 16, 2002, and in revised form, January 14, 2003
Published, JBC Papers in Press, January 15, 2003, DOI 10.1074/jbc.M212751200

Arun Anantharam‡§§, Anthony Lewis‡§§, Gianina Panaghie§§, Earl Gordon§§, Zoe A. McCrossan§, Daniel J. Lerner¶¶, and Geoffrey W. Abbott§§§

From the §Division of Cardiology, Departments of Medicine and Pharmacology, ¶Graduate Program of Neuroscience, and ¶¶Graduate Program of Pharmacology, Weill Medical College of Cornell University, New York, New York 10021

The physiological properties of most ion channels are defined experimentally by functional expression of their pore-forming α subunits in Xenopus laevis oocytes. Here, we cloned a family of Xenopus KCNE genes that encode MinK-related peptide K⁺ channel β subunits (xMiRPs) and demonstrated their constitutive expression in oocytes. Electrophysiological analysis of xMiRP2 revealed that when overexpressed this gene modulates human cardiac K⁺ channel α subunits HERG (human ether-a-go-go-related gene) and KCNQ1 by suppressing HERG currents and removing the voltage dependence of KCNQ1 activation. The ability of endogenous levels of xMiRP2 to contribute to the biophysical attributes of overexpressed mammalian K⁺ channels in oocyte studies was assessed next. Injection of an xMiRP2 sequence-specific short interfering RNA (siRNA) oligo reduced endogenous xMiRP2 expression 5-fold, whereas a control siRNA oligo had no effect, indicating the effectiveness of the RNA interference technique in Xenopus oocytes. The functional effects of endogenous xMiRP2 silencing were tested using electrophysiological analysis of heterologously expressed HERG channels. The RNA interference-mediated reduction of endogenous xMiRP2 expression increased macroscopic HERG current as much as 10-fold depending on HERG cRNA concentration. The functional effects of human MiRP1 (hMiRP1)/HERG interaction were also affected by endogenous xMiRP2. At high HERG channel density, at which the effects of endogenous xMiRP2 are minimal, hMiRP1 reduced HERG current. At low HERG current density, hMiRP1 paradoxically up-regulated HERG current, a result consistent with hMiRP1 rescuing HERG from suppression by endogenous xMiRP2. Thus, endogenous Xenopus MiRP subunits contribute to the baseline properties of K⁺ channels like HERG in oocyte expression studies, which could explain expression level- and expression system-dependent variation in K⁺ channel function.

Voltage-gated potassium (Kᵥ) channels form an aqueous pore through the plasma membrane by the assembly of four α subunits, each with six transmembrane helices and a pore loop (1). The favored method for evaluating potassium channel function is by microinjection of channel cRNA into Xenopus laevis oocytes to facilitate two-electrode voltage clamp (TEVC) or patch clamp studies. However, observed differences in the behavior of potassium channel genes when expressed in oocytes compared with mammalian cells suggest that endogenous factors in either or both systems dictate channel properties to some extent (2–5). Studies of cardiac Kᵥ channels HERG and KCNQ1 involve a further level of complexity because they co-assemble with transmembrane β subunits, designated KCN channels, or MiRP-related peptides (MiRPs), to generate native outward current; RT, reverse transcriptase; EST, expressed sequence tag; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid.

† These authors contributed equally to this work.

‡ These authors contributed equally to this work.

* This work was supported by grants from the Greenberg Atrial Fibrillation Fund and the American Heart Association (to G. W. A. and Z. A. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF545500, AF545501, and AF545502, for X. laevis MinK, MiRP2, and MiRP4.1, respectively.

The abbreviations used are: Kᵥ, channel; voltage-gated potassium channel; MiRP, MinK-related peptide; hMiRP, human MiRP; CHO, Chinese hamster ovary; RNAi, RNA interference; siRNA, short interfering RNA; TEVC, two-electrode voltage clamp; Iᵥ,Kᵣ, slowly activating potassium current; Iᵥ,Kᵣ, rapidly activating potassium current; Iᵥ,s, transient outward current; RT, reverse transcriptase; EST, expressed sequence tag; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid.

This paper is available on line at http://www.jbc.org

Printed in U.S.A.
unit currents vary with the expression system or with the concentration of MiRP cRNA, even at levels where α-subunit saturation would be expected (6, 16, 17). This finding suggests that these interactions depend on factors intrinsic to the expression system used. Clearly, resolving the true heteromultimeric identity of native ion channels and currents recorded in heterologous expression studies is important for our understanding of channel structure-function and physiology. Here, we cloned a family of xMRPs endogenously expressed in Xenopus oocytes and demonstrated that xMRP2 interacts with overexpressed HERG to shape the functional attributes of HERG during oocyte expression studies.

EXPERIMENTAL PROCEDURES

Molecular Biology—xMinK, xMRP2, xMRP1.4, and xMRP4.2 were cloned from cDNA isolated by RT-PCR from stages V and VI X. laevis oocyte mRNA using primer sequences as follows: xMinK, 5′-ATGGCCAGGTTTACACCACCTGCC-3′ (forward) and 5′-CTACTTCTGGCAGAAGGGGATTATA-3′ (reverse); xMRP2, 5′-CAGTTTGGAGTTGAGTGTTGATC-3′ (forward) and 5′-TAGATGCTGGAGCTGCTG-3′ (reverse); xMRP4.1, 5′-ATGAAAAATTGATTTAATACCTTCGT-3′ (forward) and 5′-CTAAGATTGAGTTATCCTCCATAT-3′ (reverse); xMRP4.2, 5′-CGGGAATCAGACTTCTGTCC-3′ (forward) and 5′-CATCAAGATGCGACTGTCG-3′ (reverse) and 5′-GCTTGATATAAGCACTAG-3′ (reverse) Genes were identified by a BLAST search of EST data bases with mammalian KCNE gene sequences using the blastn algorithm. All genes showed a canonical initiation ATG site with G at −3, A at +3, and no intervening methionines 3′ of the nearest 5′ stop codon. For functional expression, xMRP2 was ligated into pBA1 and linearized with SacI for transcription of cRNA for injection into oocytes. For studies in oocytes, cRNA transcripts for xMRP2, human MiRP1 (hMiRP1) (in pGA1), KCNKQ1 (in a pBluescript-based vector), and HERG (in pSP64, Promega) were co-transfected with green fluorescent protein (in pBOB) using Superfect transfection reagent (Qiagen), and currents were recorded 24–36 h later.

For RNAi, 500 pg of double-stranded siRNA 21-mer oligos (Dharmacon) corresponding to bases 104–124 of xMRP2 (top strand 5′-AAGGGACACGCACAGGCCA-3′) was injected into oocytes immediately after the injection of α subunit cRNA. siRNA oligo for xMRP2 was co-transfected into CHO cells with the appropriate channel cDNA. For the assessment of RNAi gene silencing at the mRNA level, an equal amount of oocytes was injected with either xMRP2 cRNA or scrambled control siRNA, and RNA was extracted using Trizol (Invitrogen) on the same day that functional experiments were carried out and day 1 of oocyte mRNA (Fig. 1). Homologues of mammalian MiRP1 and MiRP3 were not detected in Xenopus EST data bases. Mammalian MiRP2 co-assembles with several diverse K+ channel α subunits, removing the voltage dependence of KCNQ1 (9), suppressing HERG current without altering gating kinetics (9, 11), and converting Kv3.4 to a subthreshold-activating delayed rectifier (7). Here we investigated the effects of the interaction between xMRP2 and the cardiac-delayed rectifiers HERG and KCNQ1. Effects of human MiRP2 on Kv3.4 are pronounced in mammalian cells but not in Xenopus oocytes (7). This interaction was not covered here but is currently under study.

RESULTS

A Xenopus MiRP Family Is Constitutively Expressed in Oocytes—Xenopus EST data bases were searched with mammalian KCNE gene sequences, leading to the identification of four Xenopus clones designated here as xMinK, xMRP2, xMRP4.1, and xMRP4.2 on the basis of their closest mammalian KCNE relatives. The four Xenopus genes were subsequently cloned from cDNA isolated by RT-PCR from stages V and VI Xenopus oocyte mRNA (Fig. 1). Homologues of mammalian MiRP1 and MiRP3 were not detected in Xenopus EST data bases. Mammalian MiRP2 co-assembles with several diverse K+ channel α subunits, removing the voltage dependence of KCNQ1 (9), suppressing HERG current without altering gating kinetics (9, 11), and converting Kv3.4 to a subthreshold-activating delayed rectifier (7). Here we investigated the effects of the interaction between xMRP2 and the cardiac-delayed rectifiers HERG and KCNQ1. Effects of human MiRP2 on Kv3.4 are pronounced in mammalian cells but not in Xenopus oocytes (7). This interaction was not covered here but is currently under study.

Injected HERG and KCNQ1 are Modulated by Overexpressed xMRP2 in Oocytes—Injection of HERG cRNA into oocytes resulted in the expression of rapidly inactivating delayed rectifier K+ currents that passed large tail currents during a −30-mV tail pulse because of rapid recovery from inactivation and slow deactivation. Co-injection of xMRP2 cRNA with HERG in oocytes resulted in the complete suppression of HERG current (Fig. 2, A and B). The results demonstrate that xMRP2 functions like hMiRP2 to strongly inhibit HERG current when it is overexpressed in Xenopus oocytes. Overexpression of xMRP2 with KCNQ1 in oocytes resulted in an increase in the time- and voltage-independent component of KCNQ1 current as observed previously for human MiRP2 (Fig. 2, C and D). Xenopus MiRP2 can thus modulate human K+ channels when overexpressed in oocytes.

Endogenous Oocyte xMRP2 Is Suppressed by the Injection of siRNA Oligos—To evaluate whether endogenous levels of KCNE subunits are sufficient to modulate cloned mammalian K+ channel α subunits expressed in oocytes, we tested the applicability in oocytes of the RNAi sequence-specific posttranscriptional silencing technique that has been shown to suppress gene expression in a variety of other cell types (30). A double-stranded xMRP2-specific, siRNA oligo was injected into Xenopus oocytes, and the effects were compared against those of a scrambled control siRNA oligo. To ensure that xMRP2 siRNA was causing a loss of xMRP2 mRNA, RNA was isolated from oocytes injected with siRNA for xMRP2, scrambled siRNA, or cRNA for xMRP2. RT-PCR to produce cDNA was followed by normalization of samples to Xenopus β-actin cDNA concentration (Fig. 3A, top panel). Next, normalized cDNA samples were probed for xMRP2 cDNA by PCR using sequence-specific primers (Fig. 3A, middle panel). Compared with samples of oocytes injected with scrambled control siRNA, the band intensity of amplified xMRP2 cDNA from batches of oocytes injected with xMRP2 siRNA was reduced 8-fold as assessed by densitometry (Fig. 3A, bottom panel). Injection of 3 ng of xMRP2 cRNA, in contrast, increased the band intensity of amplified xMRP2 cRNA 30-fold (n = two batches of 8–12 oocytes). To assess the effects of xMRP2 siRNA at the protein
level, antibodies raised to full-length human KCNE3 and 80% similar to xMiRP2 in the transmembrane and C-terminal domains were used to probe injected oocyte lysates (Fig. 3B, upper panel). Probing the oocyte lysates with anti-MiRP2 antibody gave a band at 15–18 kDa, in the range expected for xMiRP2. Although MiRP2 has one or more predicted N-glycosylation sites, depending on the prediction method used, only one band was visible here. We concluded therefore that the large majority of MiRP2 protein present in the oocyte at baseline is in one particular glycosylation state, probably the mature form, as observed previously for mouse MiRP2 in the murine C2C12 skeletal muscle cell line (7). Similar levels of xMiRP2 protein were detected in water-injected oocytes and oocytes injected with scrambled control siRNA, whereas oocytes injected with xMiRP2 siRNA showed a 5-fold reduction of endogenous xMiRP2 protein as assessed by densitometry (Fig. 3B, lower panel). Thus, RNAi can be used in Xenopus oocytes to specifically suppress endogenous genes, in this case xMiRP2.

Endogenous Oocyte xMiRP2 Inhibits Heterologously Expressed HERG Currents—The effects of the suppression of endogenous xMiRP2 expression on injected HERG currents were assessed next. HERG is an especially suitable indicator of endogenous xMiRP2 contribution to heterologously expressed K+ currents, because the unique gating attributes of HERG channels (i.e. rapid inactivation at positive voltages and rapid recovery from inactivation at negative voltages (31)) allow the assessment of small HERG tail currents at −30 mV, a voltage at which contamination from other oocyte currents and leaks is negligible. Varying amounts of HERG cRNA were injected into oocytes in the absence or presence of xMiRP2-specific siRNA to silence endogenous xMiRP2, and HERG tail currents were measured by TEVC after 3 days. At levels of HERG cRNA that normally expressed virtually zero HERG tail current (as much as 0.7 ng), co-injection with xMiRP2 siRNA rescued HERG currents to 500 nA as anticipated when an endogenous protein that suppresses HERG is silenced (Fig. 4A and B). Indeed, xMiRP2 silencing increased HERG currents at all concentrations tested below 5 ng of HERG (Fig. 4C). This effect was specific because scrambled control siRNA had no effect (Fig. 4D). To eliminate the possibility that xMiRP2 siRNA oligos increased HERG current because of an artifact via direct interaction with HERG protein rather than by post-transcriptional xMiRP2 gene silencing, we switched expression systems and assessed the effects of xMiRP2 siRNA on HERG currents in CHO cells using a whole-cell voltage clamp. xMiRP2 siRNA should have no effect on HERG current density in CHO cells, because even if CHO cells express endogenous hamster MiRP2, xMiRP2 siRNA is sequence-specific for Xenopus MiRP2. Transfection of cells with xMiRP2, siRNA, and HERG cDNA produced a mean current density not significantly different from cells transfected with HERG cDNA alone. This result argues against our previous oocyte results being an artifact caused by direct interaction of xMiRP2 siRNA with HERG protein (Fig. 4E).

Variability in Effects of hMiRP1 on HERG Suggests Rescue From Inhibition by Endogenous xMiRP2—In contrast to human and Xenopus MiRP2, which completely suppress HERG currents expressed in oocytes (9) (presumably by inhibiting functional HERG channel formation), hMiRP1 partially reduces HERG currents by the formation of lower conductance fast deactivating functional channel complexes (6). The variability of hMiRP1 effects on HERG currents depending on expression system or HERG expression level has been reported previously. Although hMiRP1 mutations have been linked to inherited and acquired arrhythmia in three studies, and the effects of hMiRP1 on HERG have been noted by several groups, the reported effects differ (6, 11, 13–18). We hypothesized that some of the variability of the effects of hMiRP1 on HERG results from the displacement of xMiRP2 and that addition of hMiRP1 cRNA would rescue HERG currents at lower HERG levels (at which HERG is normally inhibited by endogenous
xMiRP2) by forming functional hMiRP1/HERG complexes in favor of nonfunctional xMiRP2/HERG complexes.

We first recapitulated previous reports of hMiRP1 modulation of HERG in both Xenopus oocytes and CHO cells. Using 10 ng of HERG cRNA/oocyte to achieve a high HERG current density, we compared current attributes with and without the

Fig. 2. Overexpressed xMiRP2 modulates HERG and KCNQ1 in oocytes. A, exemplar raw current traces generated from TEVC of oocytes injected with 5 ng of HERG or co-injected with 3 ng of xMiRP2 and 5 ng of HERG cRNA. Oocytes were held at −80 mV and stepped to voltages between −120 and +60 mV, with a −30-mV tail pulse (protocol inset). Dashed lines indicate zero current level. B, mean tail current-voltage relationships from traces as in panel A for HERG (solid squares) or xMiRP2/HERG (open squares) channels in oocytes, n = 8. Error bars indicate S.E. C, exemplar raw current traces generated from TEVC of oocytes injected with 10 ng of hKCNQ1 or co-injected with 3 ng of xMiRP2 and 10 ng of hKCNQ1 cRNA. Dashed lines indicate zero current level. Oocytes were held at −80 mV and stepped to voltages between −120 and +60 mV, with a −30-mV tail pulse (protocol inset). D, mean current-voltage relationships from traces as in panel C, for hKCNQ1 (solid squares) or xMiRP2/hKCNQ1 (open squares) channels in oocytes (n = 9–12). Error bars (S.E.) are smaller than point size.

Fig. 3. Sequence-specific RNAi efficiently suppresses endogenous xMiRP2 in oocytes. A, semiquantitative PCR of cDNA generated by RT-PCR of mRNA isolated from Xenopus oocytes. Upper panel, Xenopus mRNA normalized to endogenous β-actin gene. Left to right, oocytes injected with 500 pg of scrambled control siRNA, 500 pg of xMiRP2 siRNA, and 3 ng of positive control xMiRP2 cRNA. Bands were generated by PCR using primers specific for Xenopus β-actin and indicate equal amounts of mRNA-generated cDNA loaded in each lane. Center panel, PCR from cDNA generated from Xenopus oocyte mRNA by RT-PCR and normalized to Xenopus β-actin (see above). Left to right, oocytes injected with 500 pg of scrambled control siRNA (con siRNA), 500 pg of xMiRP2 siRNA (xM2 siRNA), or 3 ng of positive control xMiRP2 cRNA (xM2 cRNA). Bands were generated by PCR using primers specific for Xenopus MiRP2 and indicate specific post-transcriptional gene silencing of xMiRP2 by xMiRP2 siRNA. Lower panel, densitometry of xMiRP2 bands indicating an 8-fold reduction of xMiRP2 cDNA band intensity using xMiRP2 siRNA and a 30-fold increase using MiRP2 cRNA. B, upper panel, Western blot using KCNE3-specific antibodies from lysates of Xenopus oocytes injected with (left to right) water, 500 pg of xMiRP2 siRNA (xM2 siRNA), or 500 pg of scrambled control siRNA (con siRNA). Lines indicate 18 kDa (upper) and 6.4 kDa (lower). Lower panel, densitometry of xMiRP2 bands indicating a 5-fold reduction of xMiRP2 protein concentration using xMiRP2 siRNA and no significant reduction with scrambled control siRNA.
injection of hMiRP1 cRNA. Co-injection of either 10 or 20 ng of hMiRP1 cRNA/oocyte resulted in similar ~60% reductions in HERG tail currents (Fig. 5A, left panel). hMiRP1 also reduced HERG currents in CHO cells, producing an ~40% reduction in tail current density compared with HERG co-transfected with blank plasmid (Fig. 5A, right panel). Next, similar experiments were conducted using lower concentrations of HERG cRNA in oocyte expression studies. In contrast to the high current density HERG experiments, co-injection of hMiRP1 cRNA (2 ng/oocyte) with lower levels of HERG cRNA (0.5 ng/oocyte) actually up-regulated HERG ~30-mV tail currents ~3-fold after 2 and 3 days of expression (Fig. 5B). Similar effects were observed with xMiRP2 siRNA or a combination of hMiRP1 cRNA and xMiRP2 siRNA in the same batch of oocytes (Fig. 5B). The results suggest that, paradoxically, because hMiRP1 is able to couple with HERG and prevent suppression by endogenous levels of xMiRP2, at low HERG levels hMiRP1 increases HERG currents despite the formation of channels with lower unitary conductance.

**DISCUSSION**

Previously, the finding that the KCNQ1 α subunit is expressed endogenously in *X. laevis* oocytes and up-regulated by the heterologous expression of mammalian MinK led to the discovery that human cardiac *Ik*₅ is formed by MinK/KCNQ1 complexes in *vivo* (10). Here we demonstrate that *Xenopus* oocytes also express relatives of mammalian MiRPs and that these endogenous xMiRPs can interact with injected mammalian K⁺ channel α subunits. HERG, like KCNQ1, is a human cardiac K⁺ channel widely studied because of the role it plays in cardiac repolarization and the propensity of HERG to interact nonspecifically with a wide range of therapeutic agents, a propensity that contributes to acquired cardiac arrhythmia (21, 32). The functional properties of HERG are modified by mammalian MinK, MiRP1, and MiRP2 (6, 9, 11, 14, 18, 29). We have demonstrated that endogenous levels of xMiRP2 are sufficient to modify overexpressed HERG in oocytes. HERG currents recorded after the injection of HERG cRNA into oocytes are thus the sum product of “pure” HERG channels, xMiRP2-suppressed HERG, and/or channels formed by HERG with other xMiRP subunits. This fact can dramatically affect the interpretation of previous and future studies on HERG gating, pharmacology, and trafficking. We have yet to elucidate the mechanism by which xMiRP2 inhibits HERG, but this complete suppression suggests the formation of a non-functional channel, perhaps one that fails even to reach the plasma membrane. A question then remains to be answered. What happens to MiRP2-sequestered HERG, and can it be rescued by stimulating regulatory pathways or with use of drugs that bind to HERG and that rescue HERG channels containing trafficking mutations associated with long QT syndrome (33, 34)? If so, the association of endogenous xMiRP2 with HERG may impact previous and future oocyte-based studies of HERG regulation and pharmacological rescue. The hMiRP1 versus xMiRP2 data presented here suggest that endogenous xMiRP2 forms the
molecular basis for some of the previously reported variability in hMiRP1/HERG oocyte studies. The hMiRP1 versus xMiRP2 findings also highlight the importance of understanding the molecular background of expression systems used in α-β subunit interaction studies. We anticipate that this phenomenon (i.e., the functional effects of an introduced mammalian β subunit resulting partially from the displacement of an endogenous expression system β subunit) will prove to be a recurring theme once the full range of Kₐ channel interactions with *Xenopus* and mammalian MiRPs is explored in oocytes and in other cell types.

The issue of MiRP/α-subunit interaction is particularly contentious when cardiac K channels are considered. Both HERG and KCNQ1, the α subunits that generate the two principal repolarization currents, Iₚₕ and Iₚₛ, respectively, in human ventricular myocardium, are modulated by MinK, MiRP1, and MiRP2 in at least one expression system (35). KCNQ1 is also strongly suppressed by MiRP3 (KCN4E) (36) and forms with MiRP4 a slowly activating channel that resembles MinK/KCNQ1 channels but with a much more positive voltage dependence of activation (37). Little doubt exists that MinK/KCNQ1 complexes form human cardiac Iₚₛ. Compared with currents formed by overexpression of KCNQ1 alone, MinK/KCNQ1 channels exhibit a 4-fold higher conductance and a greatly slowed activation rate, generating a current that strongly resembles Iₚₛ in terms of biophysical attributes, regulation, and pharmacology. Further, mutations in both subunits that diminish channel function are associated with long QT syndrome in humans (19, 23, 24, 38–40). More debate exists concerning the precise molecular correlate of human Iₚₛ. Although the introduction of HERG α subunits into oocytes or immortal mammalian cell lines creates a potassium current with many of the attributes of cardiac Iₚₛ (21, 32), coexpression with human MiRP1 alters the single channel properties of HERG to produce a current more closely resembling previous in vivo recordings of Iₚₛ (6). Mutations in hMiRP1 diminish flux through hMiRP1/HERG channels and are associated with inherited long QT syndrome (6, 17). Further, some hMiRP1 mutations increase sensitivity to the blockade of hMiRP1/HERG channels in vitro by drugs known to block Iₚₛ (e.g., clarithromycin). Importantly, in each case the same drug (e.g., sulfamethoxazole) had precipitated drug-induced torsades de pointes and a prolonged QTc interval, specifically in patients from whom each mutation was isolated (6, 15). However, largely as a result of the reported wide variability in effects of MiRP1/HERG coexpression, the assignment of this combination of subunits as the molecular basis of human Iₚₛ is still questioned. Here we have noted one cause of the reported variability in hMiRP1/HERG behavior: the dependence of hMiRP1 effects on HERG expression level because of interference from endogenous MiRPs such as xMiRP2. At low levels of HERG cRNA, HERG current is completely suppressed by endogenous xMiRP2. The fact that we were able to subsequently reconstitute HERG current by either silencing the expression of xMiRP2 or introducing an ostensibly preferred partner illustrates just how promiscuous the association between an α subunit and the MiRPs can be. Can we infer from this that HERG will favor in every cell type an interaction with MiRP1 over other β subunits? Enough evidence does not exist at this time to support such a claim. However, our findings in conjunction with those from other studies highlight the important idea that native currents such as Iₚₛ are probably formed from a spectrum of molecular components with temporal and spatial dependence, and thus the success of direct native-cloned current comparisons is governed not only by the choice of expression system but also by the inclusion of possible β subunit partners and by the precise choice of native tissue source. Increasingly, for example, evidence suggests that Iₚₛ is regulated by MinK in some cardiac myocytes: MinK up-regulates HERG in some mammalian cell lines, knockdown of MinK suppresses HERG in murine myocytes, and immunoprecipitation of MinK from horse heart pulls down HERG, and KCNQ1 (25, 29, 41, 42).

Oocytes remain an expression system with numerous advantages for the assessment of ion channel function. Here we have described the first reported use (to our knowledge) of RNAi post-transcriptional gene silencing in the oocyte system. The use of RNAi to control endogenous modifying subunits in oocytes, mammalian cell expression systems (which may also contain endogenous MiRPs), and native studies will improve our understanding of the heteromultimeric molecular basis of human K currents and also allow us to accurately define the properties of “pure” α subunit complexes. This knowledge will facilitate the linkage of channel α and β subunit genes to inherited and acquired disease, the rational design of channel-directed therapeutic drugs, and screening for avoidance of unwanted channel-drug interactions. Consideration of endoge-
Oocyte xMiRPs Govern Mammalian K⁺ Channel Function

Acknowledgment—We are grateful to D. Christini for critical reading of the manuscript.

REFERENCES

1. Yellen, G. (2002) Nature 419, 35–42
2. Seebohm, G., Lerche, C., Busch, A. E., and Bachmann, A. (2001) Pflugers Arch. Eur. J. Physiol. 442, 891–895
3. Weber, W. M. (1999) J. Membr. Biol. 170, 1–12
4. Weber, W. M. (1999) Biochim. Biophys. Acta 1421, 213–233
5. Weber, W. M., Liebold, K. M., Reifarth, F. W., and Claus, W. (1995) J. Membr. Biol. 148, 265–275
6. Abbott, G. W., Sesti, F., Splawski, I., Buck, M. E., Lehmann, M. H., Timothy, K. W., Keating, M. T., and Goldstein, S. A. (1999) Cell 97, 175–187
7. Abbott, G. W., Butler, M. H., Bendahhou, S., Dalakas, M. C., Piacse, L. J., and Goldstein, S. A. (2001) Cell 104, 217–231
8. Barhanin, J., Lesage, F., Guillermare, E., Fink, M., Lazdunski, M., and Romey, G. (1996) Nature 384, 78–80
9. Schroeder, B. C., Waldegger, S., Fehr, S., Bleich, M., Warth, R., Greger, R., and Jentsch, T. J. (2000) Nature 403, 196–199
10. Sanguinetti, M. C., Curran, M. E., Zou, A., Shen, J., Spector, P. S., Atkinson, D. L., and Keating, M. T. (1996) Nature 384, 80–83
11. Abbott, G. W., and Goldstein, S. A. (2002) FASEB J. 16, 390–400
12. Takumi, T., Ohkubo, H., and Nakashima, S. (1988) Science 242, 1042–1045
13. Weerapura, M., Nettel, S., Chartier, D., Caballero, R., and Hebert, T. E. (2002) J. Physiol. 540, 15–27
14. Cui, J., Melman, Y., Palma, E., Fishman, G. I., and McDonald, T. V. (2000) Curr. Biol. 10, 671–674
15. Sesti, F., Abbott, G. W., Wei, J., Murray, K. T., Sakena, S., Schwartz, P. J., Priori, S. G., Roden, D. M., George, A. L., Jr., and Goldstein, S. A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 10613–10618
16. Zhang, M., Jiang, M., and Tseng, G. N. (2001) Circ. Res. 88, 1012–1019
17. Isbrandt, D., Friederich, P., Solth, A., Haverkamp, W., Ebneth, A., Berggren, M., Funke, H., Sauter, K., Breithardt, G., Pongs, O., and Schulze-Bahns, E. (2002) J. Mol. Med. 80, 524–532
18. Mazhari, R., Greenstein, J. L., Winslow, R. L., Marban, E., and Nuss, H. B. (2001) Circ. Res. 89, 33–38
19. Splawski, I., Tristani-Firouzi, M., Lehmann, M. H., Sanguinetti, M. C., and Keating, M. T. (1997) Nat. Genet. 17, 338–340
20. Splawski, I., Shen, J., Timothy, K. W., Lehmann, M. H., Priori, S., Robinson, J. L., Moss, A. J., Schwartz, P. J., Towbin, J. A., Vincent, G. M., and Keating, M. T. (2000) Circulation 102, 1178–1185
21. Curran, M. E., Splawski, I., Timothy, K. W., Vincent, G. M., Green, E. D., and Keating, M. T. (1995) Cell 80, 795–803
22. Wang, Q., Curran, M. E., Splawski, I., Burn, T. C., Milholland, J. M., Van Raay, T. J., Shen, J., Timothy, K. W., Vincent, G. M., De-Jager, T., Schwartz, P. J., Towbin, J. A., Moss, A. J., Atkinson, D. L., Landes, G. M., Connors, T. D., and Keating, M. T. (1996) Nat. Genet. 12, 17–23
23. Dugué, P., Vesely, M. R., Watanassartichaigoen, D., Villafane, J., Kaznich, V., and Beggs, A. H. (1998) Circulation 97, 142–146
24. Tyson, J., Tranebaerg, L., Bellman, S., Wrenn, C., Taylor, J. F., Barhanin, J., Aalaksen, B., Sorland, S. J., Lund, O., Malm, C., Pemery, M., Huttunen, S., and Bitter-Glindzicz, M. (1997) Hum. Mol. Genet. 6, 2179–2185
25. Finley, M. R., Li, Y., Hua, F., Lillich, J., Mitchell, K. E., Ganta, S., Gilmour, R. F., Jr., and Freeman, L. C. (2002) Am. J. Physiol. 283, H126–H138
26. Chen, Y. H., Xu, S. J., Bendahhou, S., Wang, X. L., Wang, X. W., Jin, H. W., Sun, H., Su, Y. Y., Zhuang, Q. N., Yang, Y. Q., Li, Y., Liu, Y., Xu, J. H., Li, X. F., Ma, N., Mao, C. F., Chen, Z., Barhanin, J., and Huang, W. (2003) Science 299, 251–254
27. Tinel, N., Dierhot, S., Borsotto, M., Lazzunski, M., and Barhanin, J. (2000) EMBO J. 19, 6326–6336
28. Yu, H., Wu, J., Potapova, L., Wymore, R. T., Holmes, B., Zackerman, J., van Zand, Z., Wang, H., Shi, W., Robinson, R. B., El-Maghrabi, M. R., Benjamin, W., Dixon, J., McKinnon, D., Cohen, I. S., and Wymore, R. (2001) Circ. Res. 88, E84–E87
29. McDonald, T. V., Yu, Z., Ming, Z., Palma, E., Meyers, M. B., Wang, K., Goldstein, S. A., and Fishman, G. I. (1997) Nature 388, 289–292
30. Elbashir, S. M., Martinez, J., Pankaniowka, A., Lendeckel, W., and Tuschl, T. (2001) EMBO J. 20, 6877–6884
31. Smith, P. L., Baukwowitz, T., and Yellen, G. (1996) Nature 379, 833–836
32. Sanguinetti, M. C., Jiang, C., Curran, M. E., and Keating, M. T. (1995) Cell 81, 219–267
33. Rajamani, S., Anderson, C. L., Anson, B. D., and Janz, C. T. (2002) Circulation 105, 2630–2635
34. Pfeffer, R., Oleaga-Paz, C. A., Zhan, S., and Brown, A. M. (2002) J. Biol. Chem. 277, 4899–4998
35. Abbott, G. W., Goldstein, S. A., and Sesti, F. (2001) Circ. Res. 88, 981–983
36. Grunnet, M., Jespersen, T., Rasmussen, H. B., Ljungstrom, T., Jorgensen, N. K., Olsen, S. P., and Klaerke, D. A. (2002) J. Physiol. 542, 119–130
37. Angelo, K., Jespersen, T., Grunnet, M., Nielsen, M. S., Klaerke, D. A., and Olsen, S. P. (2002) J. Physiol. 543, 1997–2006
38. Sesti, F., and Goldstein, S. A. (1998) J. Gen. Physiol. 112, 651–663
39. Sesti, F., Tai, K. K., and Goldstein, S. A. (2000) Biophys. J. 79, 1369–1378
40. Splawski, I., Timothy, K. W., Vincent, G. M., Atkinson, D. L., and Keating, M. T. (1997) J. Biol. Chem. 272, 1562–1567
41. Yang, T., Kupershmidt, S., and Roden, D. M. (1995) Circ. Res. 77, 1246–1253
42. Ohyama, H., Kajita, H., Omori, K., Takumi, T., Hiramoto, N., Iwasaka, T., and Matsuda, H. (2001) Pflugers Arch. Eur. J. Physiol 442, 329–335
43. Chen, J., Seebohm, G., and Sanguinetti, M. C. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 12461–12466
44. Mitcheson, J. S., Chen, J., Lin, M., Colburn, C., and Sanguinetti, M. C. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 12329–12333