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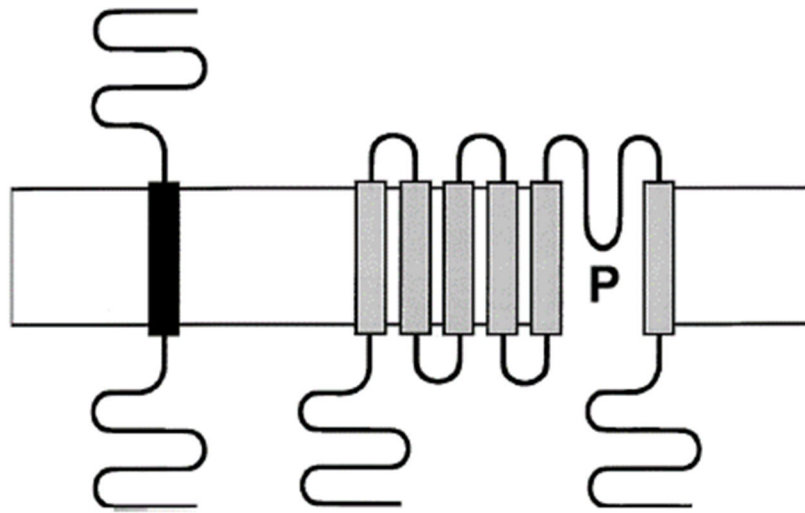
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Do All Voltage-Gated Potassium Channels Use MiRPs?

Geoffrey W. Abbott, Steve A.N. Goldstein, Federico Sesti

Once again, a MinK-related peptide (MiRP) has been implicated in allowing a pore-forming, voltage-gated potassium channel α subunit to achieve its potential. In this issue of *Circulation Research*, Zhang et al¹ show that MiRP1 (encoded by the *KCNE2* gene) can alter the function of Kv4 family subunits (which contribute to I_{to} , transient outward currents in heart and brain) when they are expressed together in *Xenopus* oocytes. After recent reports that MiRP1 affects the behavior of HERG²⁻⁶ and MiRP2 affects the function of KCNQ1, KCNQ4, HERG, and Kv3.4,^{7,8} the MiRP subunits have been accused of widespread promiscuous partnering. Whether this salacious charge is a valid reflection of natural physiology is the critical issue at hand.

MinK and its four recognized relations (MiRP1 through MiRP4 encoded by *KCNE1* through *KCNE5*) are diminutive single-transmembrane subunits that coassemble with α subunits during protein translation to form stable complexes^{9,10} (Figure).



The membrane topology of MiRP subunits (1 transmembrane segment) (left) and pore-forming α subunits (6 transmembrane segments) (right). A central ion-conduction pore (P) is formed by assembly of four α subunits.²³ The subunit stoichiometry of MiRP/ α -subunit complexes and sites of contact between MiRPs and their pore-forming partners remain to be established.^{15,24,25}

In doing so, MinK alters the gating kinetics, permeation attributes, and pharmacology of KCNQ1 α subunits to yield the attributes recorded for native cardiac I_{Ks} channels.^{11,12} So, too, MiRP1 and HERG combine to create complexes with unique functions that recapitulate the properties of cardiac I_{Kr} channels.²⁻⁶ In each of these cases, inherited

mutations in either the MiRP or its associated a subunit partner have been linked to similar pathophysiology. The present work by Zhang et al¹ indicates that MiRP1 can alter the function of Kv4 subunits in experimental cells and demands we consider the possibility that MiRP subunits play a central role in determining cardiovascular excitability through influence over I_{Ks} , I_{Kr} , and I_{to} channels.

In general, three criteria have been used to judge whether a subunit-subunit complex is formed in nature. First, are the subunits expressed in the same native cells at the same time in complexes as assessed at the protein level? Second, when studied in experimental cells, does the complex recapitulate the attributes and pharmacology of native channels? Third, are the subunits both genetically associated with the same physiological or disease processes?

The first criterion is best assessed by coimmunoprecipitation and coimmunolocalization using native cells; the third demands patient recruitment, careful clinical assessment, and the capacity to perform molecular genetic analyses. In this discussion, we consider the second criterion, the place where most studies of accessory subunits begin and culminate. Failure of cloned a subunits expressed in an experimental cell to recapitulate a native current often starts the hunt for a missing subunit, with correlation of new attributes and native currents being the desired outcome. However, studies that seek to correlate clones and native currents are difficult endeavors: recordings of native currents are not always feasible, nonidentical native channels can behave similarly, and identical channels can function differently in different cells and even show altered behavior in the same cell type in health and disease. Similarly, clones expressed in experimental cells will show variable attributes because of altered gene expression, mRNA processing, subunit composition, and channel modulation.

Thus, it was an endogenous KCNQ1 subunit in *Xenopus laevis* oocytes that allowed the expression cloning of MinK^{11,13} (and the apparent absence of such in Chinese hamster ovary [CHO] cells that offers electrical silence with MinK until an α subunit partner is provided^{14,15}). The slow-activation kinetics of MinK-induced currents in oocytes suggested their correlation with I_{Ks} currents; however, induction of chloride currents with immoderate amounts of injected MinK cRNA¹⁶ (resulting from nonspecific calcium influx attributable to profound overexpression of these transmembrane subunits^{17,18}) confounded initial attempts at characterization and eventual molecular correlation of MinK with cardiac and auditory I_{Ks} channels.^{11,12}

Another example of the influence of expression environment is offered by MiRP1/HERG complexes.^{2,3} Whereas the subunits coassemble in both CHO cells and oocytes to form channels that resemble cardiac I_{Kr} channels in their gating and permeation properties, the class III agent E-4031 blocks MiRP1/HERG channels in CHO cells (and native cardiac I_{Kr} channels) in the closed state, producing tonic blockade, and additionally demonstrates ready use-dependent inhibition with cyclical stimulation. Conversely, MiRP1/HERG complexes in oocytes show no closed-state blockade and slow relaxation to equilibrium inhibition with \approx 200-fold lower sensitivity.

Considerations such as these support the utility, when allowed by favorable experimental conditions, of side-by-side comparisons of cloned and native channels in both wild-type and mutant form to establish molecular identity. For example, simultaneous characterization of the attributes of MiRP2/Kv3.4 channels in CHO cells and native channels in a skeletal muscle cell line suggested the physiological correlation

of these subthreshold activating currents that set resting membrane potential.⁸ Identity was supported by isolation of an inherited MiRP2 missense mutation associated with periodic paralysis, because expression of the mutant subunit in both CHO and skeletal muscle cells revealed its dominant suppression of both current density and perturbation of resting potential.

Once a native interaction is supported, heterologous expression can provide additional insights that might otherwise be difficult to ascertain. For example, some arrhythmia-associated mutations through KCNQ1 have only mild effects when expressed without MinK but markedly inhibit current in mixed MinK/KCNQ1 complexes.¹⁹ Similarly, arrhythmia-associated HERG mutations that showed only mild abnormalities expressed on their own (making it difficult to rationalize the pathogenesis) were able to act as dominant suppressors in MiRP1/HERG complexes.⁶ Moreover, perturbation of native channel function by adenovirus-mediated overexpression of wildtype and mutant forms of HERG or MinK in cardiac myocytes is now facilitating characterization of genespecific mechanisms of arrhythmogenesis.²⁰

Zhang et al fully recognize these demands of clone/native channel identification, being careful to stress that the effects of MiRP1 on Kv4.2 in oocytes provide a starting point toward addressing the possibility of their native association. (Although we also observe evidence for interaction of the subunits in oocytes,²¹ we suspect these complexes will function primarily in the central nervous system rather than the heart.) Furthermore, some of the conclusions drawn by Zhang et al are questionable because of the unorthodox manner in which the experiments were executed. For example, injection of cRNAs asynchronously and at unusual levels likely explains failure to repeat earlier reports of functional modulation of HERG by MiRP1,²⁻⁶ including one from the same laboratory.²²

Thus far, three MiRP/ α -subunit complexes have garnered considerable support as molecular correlates for native currents: MinK/KCNQ1, MiRP1/HERG, and MiRP2/Kv3.4. Hints that MiRPs may partner more broadly suggest that the array of complexes in native cells will be significantly larger. And yet the questions remain more numerous than the answers. Is MiRP1 central to cardiovascular excitability through interaction with multiple myocardial α subunits? Will other types of pore-forming subunits with similar topology associate with MiRPs (for example, pacemaker and cyclic-nucleotide-gated channel subunits)? Will native complexes contain MiRPs under some conditions and not others? Do native complexes contain more than one MiRP type at the same time? Where do MiRPs contact their pore-forming partners? Do MiRP/ α -subunit complexes show variable subunit stoichiometry (and how many MiRPs are in a complex)?

Do all voltage-gated potassium channels use MiRPs? Although there is no a priori reason to posit a MiRP for every channel complex, this irritating notion remains a viable and exciting possibility.

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