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REVIEW

Kv Channel Ancillary Subunits: Where Do We Go from Here?

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Voltage-gated potassium (Kv) channels each comprise four pore-forming α-subunits that orchestrate essential duties such as voltage sensing and K^+ selectivity and conductance. In vivo, however, Kv channels also incorporate regulatory subunits—some Kv channel specific, others more general modifiers of protein folding, trafficking, and function. Understanding all the above is essential for a complete picture of the role of Kv channels in physiology and disease.

cardiac arrhythmia; KCNE; KCNQ; MinK-related peptide; potassium channel

Introduction

Voltage-gated potassium (Kv) channels are miracles of evolution. They possess charged domains that sense electrical changes across the cell membrane and respond to them by inducing conformational shifts that alter the conductivity of the pore (1). Most Kv channels work primarily to regulate cellular excitability by opening in response to plasma membrane depolarization (2, 3). This can involve repolarizing a cell to end an action potential, maintaining the resting membrane potential to disfavor action potential firing, or shaping action potential morphology during the action potential (4, 5). The pore itself is able to distinguish K^+ from the smaller, similarly charged Na^+, an essential property since without this capability there would be no action potential. The Kv channel pore does this by mimicking the K^+ hydration shell with carbonyl oxygens, so that K^+ ions can pass through at incredible rates (as high as 60,000 ions per channel protein per second) by binding and unbinding to the pore as they pass through. The smaller Na^+ ion is not coordinated in this way in the K^+ channel pore, and so its passage is relatively disfavored (6–10). Each Kv channel has four pore-forming (α) subunits that must come together in a tetrameric pseudosymmetric assembly to form a functional pore (11). Each α-subunit possesses a voltage-sensing domain, but, depending on the channel, not all four sensors must necessarily be moved for channel opening (12).

Once one moves beyond the essential functions described above, the devil is very much in the details. Of the 40 Kv channel α-subunits in the human genome (13), some can form heterotetrameric channels with different α-subunits [usually, but not always (14, 15), within the same subfamily, e.g., KCNQ2 with KCNQ3 (16) or KCNQ5 (17)] that exhibit altered properties compared with homomers formed from their constituent α-subunits and are considered essential complexes in vivo (18). Many cells are highly polarized in terms of their architecture, and Kv channels must be delivered to the correct location to efficiently serve their role (19–21). Kv channels are also subject to multiple forms of posttranslational modifications that alter their function or even location (22, 23). Kv channels are also constantly modulated by endogenous and exogenous small molecules that can bind to functionally influential domains to alter channel behavior (24–27).

In addition, contributing heavily to the richness of Kv channel biology are the non-pore-forming ancillary or β-subunits (28). Ancillary subunits influence all the properties described above. Many of the ancillary subunits can interact with and differentially modulate multiple α-subunits (29–31), and vice versa (32), and some are essential components for specific native currents, e.g., KCNE2 (MiRP1) in gastric parietal cell complexes with KCNQ1, forming the constitutive K^+ current required to support gastric H^+-K^+-ATPase activity (33–35). Others may form part of a toolkit for variable regulation of channels in a given tissue, as is postulated to be the case for the entire KCNE β-subunit family in the heart (32, 36). In this review, outstanding questions such as this are pondered, in addition to a consideration of Kv channel ancillary subunit controversies and the future of the field.

Current State of the Field

> *Now, in order to answer the question, "Where do we go from here?" which is our theme, we must first honestly recognize where we are now.*
>
> —Dr. Martin Luther King, Jr. (37)

The two broad classes of Kv channel ancillary subunits are the cytoplasmic isoforms that interact primarily with the intracellular portion of the α-subunits and the

transmembrane (TM) β-subunits that also heavily interact with the α-subunit transmembrane segments (28) (FIGURE 1A). According to searches in PubMed, within the cytosolic ancillary subunits the Kv β-subunits (38, 39) are the most studied, with the potassium channel interacting proteins (KChIPs) (40) a distant second, whereas among the TM ancillary subunits the single TM-spanning (1TM) KCNE subunits (41, 42) are the most studied, followed by the 1TM dipeptidyl peptidase-like proteins (DPPs) (43, 44).

Other notable Kv channel ancillary subunits include the "silent" Kv α (KvS) subunits that do not form functional channels alone but can modulate functional Kv2 subfamily α-subunits in heteromeric complexes with them (45, 46), and regulation of Kv2 channels is a fascinating topic in and of itself. The electrically silent KvS subunits number 10 in the human genome and have historically been given various classifications but more recently have been designated as Kv5.1, Kv6.1–Kv6.4, Kv8.1, Kv8.2, and Kv9.1–Kv9.3 (47, 48). Briefly, all KvS subunits bear the canonical GYG K^+ selectivity sequence and Kv α-subunit 6TM topology yet cannot form functional homomeric channels because they are retained in the endoplasmic reticulum (ER) unless rescued by coexpression with Kv2 α-subunits. They functionally regulate Kv2 α-subunits but not those from the Kv1, 3, or 4 subfamilies, a selectivity mediated by intersubunit interactions (or lack thereof) between the soluble domains (47). KvS subunits are thought to regulate hippocampal physiology, and their disruption has been associated with human epilepsy (49, 50) and with impaired spermiogenesis in mice (51). By the same token, some Kv α-subunits also exhibit nonconducting functions. Kv2.1, for example, in addition to functioning as a bona fide Kv channel, also creates stable membrane contact sites between the ER and plasma membrane by virtue of interactions with ER-resident proteins. Kv2.1 channels in this context are electrically silent, probably because of the high expression density they exhibit when fulfilling this role; this density-dependent silencing was also recently found for Kv2.2, Kv1.4, and Kv1.5 (52). Kv2 subfamily channels also form complexes with the neuronal adhesion proteins amphotericin-induced gene and open reading frame 1, 2, and 3 (AMIGO1–3), and it was recently discovered that AMIGOs and Kv2 channels reciprocally regulate one another, with the details depending on the specific isoform combination (53). Kv2.1 and heteromeric Kv2.1/Kv6.4 channels are also regulated by multiple members of the KCNE subunit family (54–57). In addition, SUMO was long thought to be solely a nuclear protein but has since been shown to participate in plasma membrane complexes with Kv channels, including Kv2.1 and KCNQ1 (58–61). Finally, Kv2.1 coimmunoprecipitates in cardiac tissue with K^+ channel-associated protein (KChAP), a chaperone that enhances current and protein levels of Kv2.1, and also Kv1.3, Kv2.2, and Kv4.3 (62–64).

There are also various regulatory proteins that can modulate the function of nonchannel proteins but are also essential in some cases for channel function, including calmodulin, which is required for KCNQ channel formation and function (65–71), and a host of kinases and phosphatases (72–77). Some proteins may modulate Kv channels only in disease states, or the modulation may only become important during a disease state; this may be the case for regulation of Kv channels by the amyloid precursor protein C99 fragment (78). Other classes of protein that serve other functional roles in their own right form in some cases reciprocally regulating complexes with Kv channels, e.g., sodium-coupled solute transporters (79–81). Finally, ancillary subunits may coordinate coassembly of tertiary regulators into Kv channel complexes (82) or modify the way in which other secondary regulators interact with the Kv α-subunits (70).

There are hundreds of published studies on the cellular electrophysiological effects and other modes of modulation of the various ancillary subunits on their Kv α-subunit channel partners, ranging from heterologous expression system studies using *Xenopus laevis* oocytes or immortal mammalian cell lines to native cells including cardiac myocytes, neurons, and epithelial cells, using techniques including two-electrode voltage-clamp, whole cell patch-clamp, macro-patch, single-channel, and noise variance analysis. Over the decades, effects on channel function have been

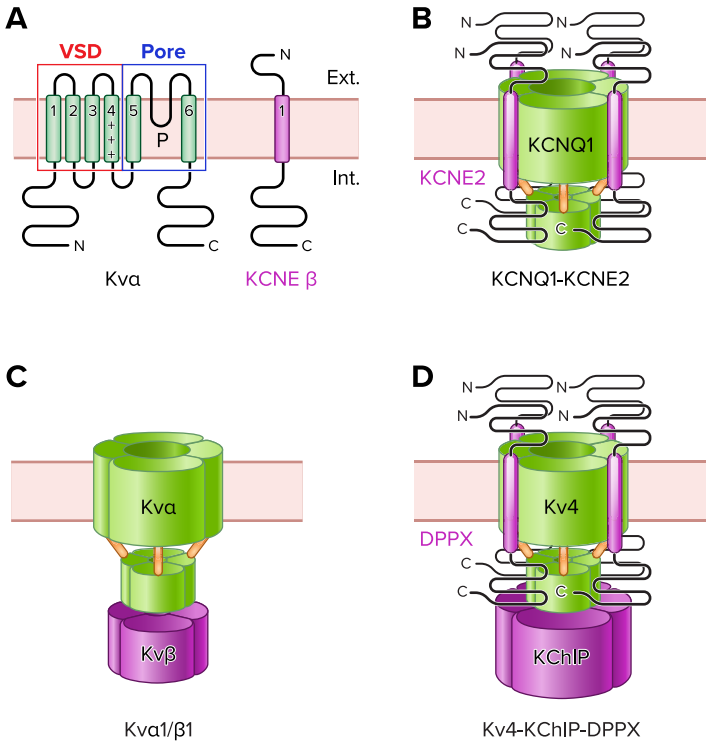

FIGURE 1. **Topology and stoichiometry of Kv channel α and ancillary subunits**
A: membrane span topology of Kv α and KCNE subunits. *B*: representation of a KCNQ1-KCNE2 complex depicting 4 KCNQ1 and 4 KCNE2 subunits. *C*: representation of a Kvα-Kvβ complex depicting 4 Kvα and 4 Kvβ subunits. *D*: representation of a Kv4-KChIP-DPPX complex depicting 4 of each subunit type. VSD, voltage sensing domain.

TABLE 1. ***Summary of known interactions between members of electrically active mammalian Kv α-subunit subfamilies and main Kv channel ancillary subunit classes***

Kv α Subfamily	Kvβ	KCNE	DPP	KChIP	KChAP	KvS	AMIGO	SUMO
Kv1 (KCNA)	x	x		x	x			
Kv2 (KCNB)		x			x	x	x	x
Kv3 (KCNC)		x						
Kv4 (KCND)		x	x	x	x			
Kv7 (KCNQ)		x						x
Kv11 (ERG)		x						

Summary of known interactions between members of electrically active mammalian Kv α-subunit subfamilies and main Kv channel ancillary subunit classes, based on in vitro and/or in vivo evidence. For details of specific subunit-subunit interactions, see main text and prior in-depth reviews (47, 115, 116). x, Interaction; blank, no reported interaction.

determined at the single-channel level through to population effects.

Of the 12 subfamilies that constitute the 40-gene human Kv α-subunit family, members of several participate in interactions with the main classes of true Kv channel ancillary subunits. KCNQ1, hERG, and α-subunits from the Kv1, Kv2, Kv3, and Kv4 subfamilies are particularly highly studied for their interactions with ancillary subunits (40, 62, 63, 83–90). KCNQ1 (originally named KvLQT1) was originally discovered by positional cloning via its disease linkage with the ventricular arrhythmia long QT syndrome (LQTS) (91, 92) and is well known to generate the ventricular myocyte slow-activating Kv current (I_{Ks}), for which it requires coassembly with KCNE1 (93, 94). However, KCNQ1 is more highly expressed in the thyroid and gastric epithelia than it is in the heart (95, 96), both locations in which it forms constitutively activating, largely voltage-independent (across the physiological voltage range) K^+ channels with KCNE2 (34, 95, 97–99). KCNQ1 also forms complexes with KCNE3, 4, and 5, discussed in more detail below (32). Kv1 α-subunits are regulated by Kvβ subunits (38, 100, 101) and also by KCNEs (21, 82, 102–108); Kv2 subunits are regulated by KCNEs (54, 55, 109); and Kv4 subunits form complexes with KCNEs, KChIPs, and DPP subunits (110–114). The main classes of Kv α-ancillary interactions are summarized in Table 1.

Animals including mice, rats, zebrafish, *Caenorhabditis elegans*, rabbits, dogs, and pigs have been electrophysiologically, genetically, and biochemically studied at the subcellular and cellular levels through to the tissue and whole animal levels to determine the native roles and necessity of Kv channel regulatory subunits in specific cells and tissues and their potassium currents and physiological functions—often with the use of transgenic approaches, transient gene knockdown/up-regulation, and/or pharmacological strategies. *KCNE* genes, for example, have been found in the genomes of some model invertebrates, e.g., the roundworm *C. elegans* (117, 118), but not others; *KCNE*s appear to be absent from flies, including *Drosophila melanogaster*, from which Kv channels were first cloned (*Shaker*) (119, 120), demonstrating that KCNEs are not always required for an organism to generate its repertoire of Kv currents. The *Drosophila* genome does, however, contain a single Kvβ subunit gene (*Hyperkinetic*) (121), the protein product of which regulates *Drosophila* Kv α-subunits including *Shaker* (121) and *Ether-a-go-go* (122), and also couples mitochondrial electron transport to sleep via its oxidoreductase domain (123). In contrast, there are six Kvβ genes in the human genome (115, 124,125); both *Drosophila* and human Kvβ subunits function as redox sensors (126); in mammals this enables a role in oxygen-dependent regulation of vascular tone (127). The author could not find a description of *C. elegans* Kvβ subunits in the literature, but a BLAST search using the human Kvβ1 protein sequence identified three predicted Aldo-Keto Reductase domain-containing proteins with ≤30% identity with human Kvβ1 (sequence IDs: NP_509242.1, NP 498580.2, NP 506323.1).

The *C. elegans* KCNE subunits are highly interesting, and they illustrate the complexity of Kv channel regulation even in a relatively simple organism. The four *C. elegans* KCNE orthologs (MPS-1, 2, 3, and 4) include one, MPS-1, that is both a Kv channel ancillary subunit and a serine/threonine kinase (74). MPS-1 forms complexes with KHT-1 (a Kv α-subunit with 73% homology to human Kv3.1). Phosphorylation of KHT-1 by MPS-1 permits neuronal adaptation to mechanical stimulation (74). KCNEs are also found in model organisms zebrafish (*Danio rerio*) and African clawed frog (*Xenopus laevis*). KCNQ1 is, however, much more highly expressed than KCNE1 in zebrafish cardiomyocytes, and it is suggested that KCNQ1 channels lacking KCNE1 contribute significantly to generating cardiac I_{Ks} in zebrafish heart (128). KCNE subunits are endogenously expressed in *X. laevis* oocytes, a major expression system for heterologous expression and cellular electrophysiology studies of ion channels, and their presence can influence the functional characteristics of mammalian Kv channels heterologously expressed in oocytes, especially at lower expression levels (109, 129). The mammalian genomes studied thus far each contain five KCNE

genes, although there are differences in expression and gene structure. For example, human hearts express KCNE3, unlike mouse hearts (109, 129). Also, primates express a longer splice variant of KCNE3 (KCNE3L, 147 residues) in addition to a shorter variant (KCNE3, a.k.a. KCNE3S, 103 residues), whereas mice express only the short variant. Similarly, mice express the 170-residue-long KCNE4S variant of KCNE4, yet lack the 221-residue-long KCNE4L, whereas other mammals and also reptiles express both KCNE4L and KCNE4S (106, 130). Some Kv channel ancillary subunits have been discovered to be essential for one or more native currents and their associated physiological processes. Human genetics studies have enabled discovery of ancillary subunit-gene-disease associations and predispositions, although true disease linkages in large pedigrees are rare.

High-resolution structures have been solved for Kv channels formed by α-subunits in complexes, including Kv1.1 (KCNA1) cytosolic T1 domains in complex with Kvβ2 subunits (131) (FIGURE 2A) and the Kv1.2 (KCNA2) channel in complex with Kvβ2 subunits (132) (FIGURE 2B), Kv4.3 (KCND3) with KChIP1 or KChIP4a (133–135), Kv4.2 with DPP6S and/or KChIP1 (136) (FIGURE 3A), and KCNQ1 with KCNE3 and/or calmodulin (71, 137) (FIGURE 3B). As is often the case, the structures have resolved some controversies but not all (see below). Despite thousands of articles having been published since the late 1980s on Kv channel ancillary subunits, some substantial questions remain unanswered. Below, those questions are discussed together with the reasons for their resistance to study and suggestions to remedy this.

Accessory Versus Necessity

Nothing has more strength than dire necessity.
—Euripides (138)

Few if any Kv channel ancillary subunits are essential for channel function per se. That is, when heterologously expressed in *Xenopus* oocytes or immortal cell lines, Kv channel α-subunits can get to the plasma membrane as tetramers, sense voltage, and pass current just fine in the absence of any ancillary subunits, even those known to be essential for recapitulating native currents. There is at least one notable exception: KCNQ channels require calmodulin for channel folding and assembly (and also for Ca^{2+} sensitivity) (65–67, 139). Furthermore, electrically silent KvS subunits do not form channels alone, but they are "rescued" by complex formation with other compatible α-subunits, not β-subunits (45, 140).

Despite Kv channel α-subunits being able to generate currents without ancillary subunit assistance in most cases, α-subunit-only channels are often not sufficient for specific physiological roles and this is reflected by the effects of ancillary subunit gene disruption identified in human genetics and transgenic mouse studies. However, recent comprehensive evaluations of the human genetics of long QT syndrome (LQTS) have questioned the majority of the previously reported associations of ion channel regulatory subunits with congenital LQTS (and of most ion channel α-subunits, leaving only KCNQ1, KCNH2, and SCN5A as clearly LQTS linked) (141). KCNE1, CAV3, and the inward-rectified α-subunit KCNJ2 are now

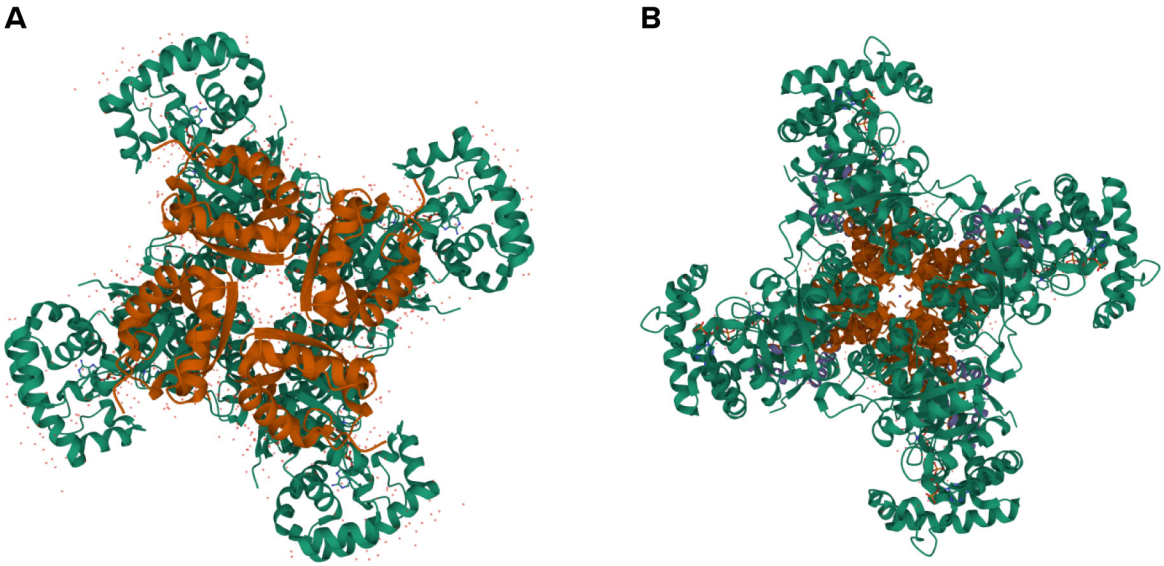

FIGURE 2. **High-resolution structures of bipartite Kv channel complexes: KCNA channel regulation by Kvβ subunits**
A: X-ray crystallographic structure of Kv1.1 (KCNA1) cytosolic T1 domains (brown) in complex with Kvβ2 subunits (green). Image from the RCSB PDB (rcsb.org) of PDB ID 1EXB (Ref. 131; PDB: https://www.rcsb.org/structure/1EXB). *B*: X-ray crystallographic structure of Kv1.2 (KCNA2) channel in complex with Kvβ2 subunits (green). Image from the RCSB PDB (rcsb.org) of PDB ID 2A79 (Ref. 132; PDB: https://www.rcsb.org/structure/2A79).

described as having "limited" evidence for LQTS association, whereas KCNE2, ANK2, AKAP9, SCN4B, SNTA1, and KCNJ5 are listed as "disputed," considered the lowest level of evidence for LQTS association (141). Many of these associations were originally deduced by candidate gene approaches, but gene variants in ANK2, a regulator of various ion channel types, were reported to cause LQTS based on the evidence from a linkage analysis, considered a more rigorous approach less open to bias (142). For the recent reappraisal, blinded teams of gene curators were trained in application of a standard operating procedure for gene curation within the ClinGen Gene curation framework. The framework is intended to constitute a systematic and evidence-based approach to assessing previously reported gene-disease associations, based on clinical validity classifications. The classifications depend on the sum of genetic and experimental data, e.g., linkage analysis scores better than associations identified by a candidate gene approach (141).

Assuming we give credence to this reappraisal, where does it leave our understanding of the cardiac roles of the two Kv channel ancillary subunits in the downgraded group, KCNE1 and KCNE2? KCNQ1-KCNE1 complexes are considered essential for formation of the canonical I_{Ks} current, which at room temperature takes many seconds to saturate at depolarized voltages and cannot be generated by KCNQ1 alone (or by association with any other known regulatory subunit) (93, 94). In turn, the I_{Ks} current is considered an important component of ventricular repolarization in the human myocardium, no current that looks like homomeric KCNQ1 has been found to play an important role in cardiac myocytes (of any species to my knowledge), and indeed KCNQ1 is one of the few genes that survived the overhaul of congenital LQTS assignments (141). Should we now throw out decades of studies of KCNE1 as an essential cardiac Kv channel regulatory subunit because of the recent revelations? The answer of course is no, and much of the explanation is that biology is complex and no matter how much scientists and journals prefer clear-cut, declarative statements on functions, physiological roles, and disease associations, the full story is often extremely nuanced.

KCNE Disease Associations and the Complexities of Biological Systems

> *Out of intense complexities, intense simplicities emerge.*
>
> —Winston Churchill (143)

Even though there are gene variants (such as D76N) in KCNE1 that act as dominant-negative mutants in I_{Ks} channels (144–146), it appears that there is not sufficient evidence to define them as causal in purely congenital LQTS, i.e., disease causing in the absence of other agents reducing ventricular repolarization reserve (141). Reasons for this could include that there is typically sufficient redundancy to compensate for this loss of K^+ current and that there might be quality-control mechanisms in real myocytes in vivo that recognize and disfavor the presence of mutant channels. KCNQ1 channels are now known to also form complexes with hERG (KCNH2) channels (14, 15); it may be that disruption of these channels contributes to KCNQ1-linked LQTS and that KCNE1 mutations have less of an effect on this. Furthermore, I_{Ks} channels are formed not just by KCNQ1 and KCNE1 but by an entire signaling platform that involves Yotiao (AKAP9), protein kinase A (PKA), and protein phosphatase 1 (PP1)

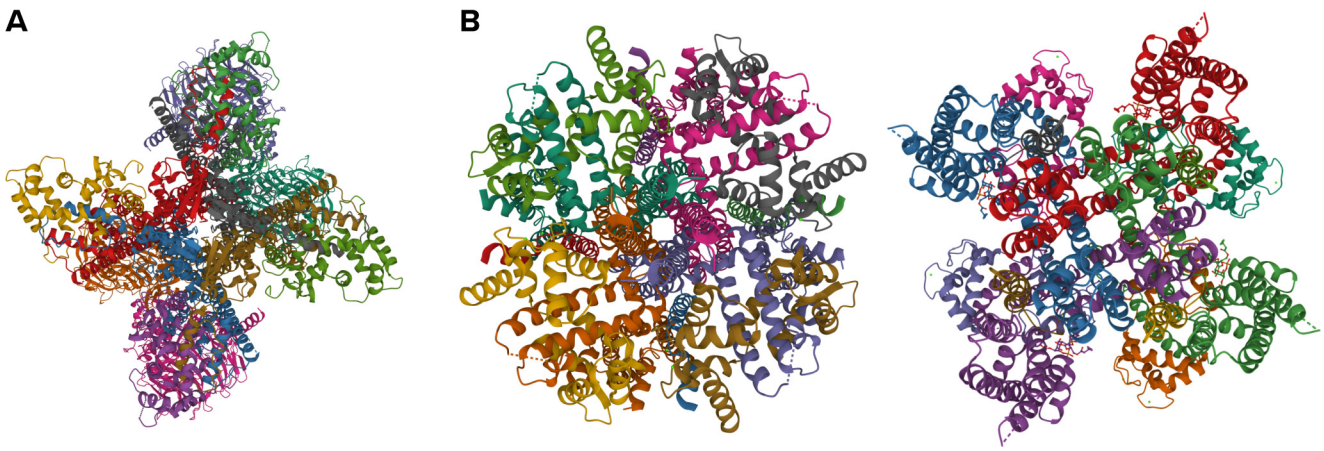

FIGURE 3. **High-resolution structures of tripartite potassium channel complexes**
A: cryo-electron microscopy (cryo-EM) structure of Kv4.2 in complex with DPP6S and KChIP1 (red/blue, orange and gold, respectively, in *top left* grouping). Image from the RCSB PDB (rcsb.org) of PDB ID 7E8H (Ref. 136; https://www.rcsb.org/structure/7E8H). *B*: cryo-EM structure of KCNQ1 in complex with KCNE3 and calmodulin (dark green, purple, and light green, respectively, in *top left* grouping of *left* panel; cobalt, gray, and pink, respectively, in *top left* grouping of *right* panel) in the absence (*left*) and presence (*right*) of phosphatidylinositol 4,5-bisphosphate (PIP_2). Images from the RCSB PDB (rcsb.org) of PDB ID 6V00 (*left*) and 6V01 (*right*) [Ref. 137; PDB: https://www.rcsb.org/structure/6V00 (*left*), https://www.rcsb.org/structure/6V01 (*right*)].

(147–149). The large majority of heterologous expression investigations into the functional effects of KCNQ1 or KCNE1 mutations fail to incorporate these proteins, which could influence the functional outcome of the mutations. In addition, we do not yet know the true molecular heterogeneity of I_{Ks} complexes within the functioning human heart in vivo, at least partly because assumptions have been made about I_{Ks} channels being rigidly formed by KCNQ1-KCNE1, calmodulin, and the Yotiao-coordinated signaling platform. Given that all five KCNE isoforms are expressed in human ventricles and that all five are able to functionally regulate KCNQ1 and in some cases might form mixed-KCNE isoform complexes with KCNQ1, it is fair to say that the majority of heterologous expression studies of the effects of KCNE1 mutations on KCNQ1, while being fine explorations of the biophysical and other functional effects on KCNQ1-KCNE1 complexes, do not touch upon the complexities of what the mutation will experience and the effects it will have when in a true native complex. In addition, there are of course likely to be many different versions of what a native I_{Ks} complex looks like in vivo, depending on location, age, physiological state, etc., further complicating the issue (30–32, 150).

The conclusion of the recent reappraisal of LQTS genes is that *KCNE1* mutations may nevertheless underlie some cases of Jervell and Lange-Nielsen syndrome (JLNS), an autosomal recessive disease involving both LQTS and sensorineural deafness affecting I_{Ks} channels in both the heart and the inner ear (141). In JLNS, two functionally severe mutant *KCNE1* alleles are considered likely to be causal (as is considered the case for most *KCNQ1*-linked JLNS) (151–154). In addition, it is still thought that *KCNE1* gene variants can predispose to acquired LQTS (aLQTS) (141), which describes cases in which an environmental perturbation (drug block, electrolyte imbalance, structural heart disease) contributes to the loss of repolarization reserve (155) and can combine with loss-of-function gene variants to induce LQTS in cases where the gene variant itself was not sufficient (41). aLQTS can almost certainly also occur without any genetic component, the most famous and a relatively common example being drug block of the hERG potassium channel, which generates the rapidly activating ventricular myocyte potassium current (I_{Kr}) (26). As hERG is very sensitive to inhibition by a wide range of small molecules (156–159), and it also generates the primary repolarizing current in human ventricles (26), aLQTS is very much in the minds of cardiac electrophysiologists and drug companies alike, and the latter must demonstrate lack of hERG-induced cardiotoxicity during drug development (160–162).

The discussion of hERG and aLQTS brings us to KCNE2, which we originally named MinK-related Peptide 1 when we cloned it and discovered together with human geneticists its association with aLQTS (41). KCNE2 is the poster child for physiological and pathophysiological complexity among Kv channel ancillary subunits (150). In our original study we discovered that KCNE2 forms complexes with hERG that display altered gating, unitary conductance, and voltage dependence compared with hERG alone, and using a candidate gene approach our geneticist collaborators found cases of aLQTS in which KCNE2 gene variants were present and other known LQTS gene variants were not. Strikingly, in one case in that original study we found that the KCNE2-Q9E gene variant both causes baseline loss of function in hERG-KCNE2 channels and increases hERG-KCNE2 channel sensitivity to block by the macrolide antibiotic (clarithromycin) that prolonged the QT interval in the woman in which KCNE2-Q9E was first identified (41). In a follow-up study we described how KCNE2-T8A, which is a KCNE2 polymorphism present in 1.6% of United States Caucasians, has no baseline effect on hERG-KCNE2 channel function but increases sensitivity to block by the antibiotic sulfamethoxazole, which was found to prolong QT in a man carrying the KCNE2-T8A polymorphism (163). A subsequent study demonstrated a highly novel disease etiology: KCNE2-T8A disrupted N-glycosylation of KCNE2 by removing the glycosylation site; loss of the protective sugar shield rendered hERG-KCNE2-T8A channels susceptible to pore block by the antibiotic sulfamethoxazole (164).

We subsequently found that KCNE2-T10M was present in several members of a family, most of whom had no cardiac electrophysiological abnormalities. One woman in the family, however, who carried the KCNE2-T10M gene variant and had experienced prior episodes of auditory-induced syncope, presented with LQTS and ventricular fibrillation (VF) with hypomagnesemia and hypocalcemia after running a marathon and subsequent VF with hypokalemia. hERG-KCNE2-T10M channels exhibit impaired function at baseline and no additional sensitivity to perturbed electrolytes than wild-type hERG-KCNE2 channels, but the combination of impaired baseline capabilities and perturbed electrolytes including hypokalemia (which reduces I_{Kr} density) is thought to have caused the LQTS and VF (165).

In all the cases described above, we emphasized that KCNE2 predisposes to aLQTS rather than being a sole cause of pure LQTS; therefore, the latest reevaluation of LQTS genes that places KCNE2 in the aLQTS but not LQTS gene category is highly consistent with our prior conclusions. Of course, the question arises, when do LQTS cases become aLQTS? If all LQTS cases are purely genetic, why do individuals carrying LQTS variants not succumb to LQTS-associated VF early in life? As with most other aspects of biology and disease, it is a sliding scale and there must of course be environmental components that determine when an LQTS gene variant carrier has a cardiac event. It is well known that auditory stimuli provoke KCNH2-

associated LQTS cases (166), while emotional arousal is a reported trigger in KCNQ1, and even more so KCNH2-associated LQTS and torsades de pointes (TdP) (167) associate with stressful life events and mental stress (168). If an external trigger is frequently identified, could these not also be classified as aLQTS with a genetic component?

Beyond the discussion of LQTS versus aLQTS, the story of KCNE2 has additional complexities. KCNE2 is a highly promiscuous, ubiquitously expressed subunit. In addition to regulating hERG (41), it also converts KCNQ1 into a constitutively activating K^+ channel (169). The possible contribution of KCNQ1-KCNE2 to human cardiac potassium channels is not known, but KCNQ1-KCNE2 channels are essential for normal gastric, thyroid, and choroid plexus epithelial function, where their ability to remain open and not inactivated at moderately negative membrane potentials permits K^+ flux to support the activity of transporters—the sodium-iodide symporter (NIS) in thyroid epithelial cells, the H^+-K^+-ATPase in gastric parietal cells, and the sodium-coupled *myo*-inositol transporter (SMIT1) in choroid plexus (34, 95, 170). *KCNQ1* or *KCNE2* mutations that perturb functions of KCNQ1-KCNE2 channels in these extracardiac tissues not only have the potential to alter thyroid or gastric function but, because of the effects of, e.g., thyroid activity on the heart, could indirectly alter cardiac function (95, 171–173). However, if those effects included structural heart disease, the affected patients might have been excluded from studies of "pure" congenital LQTS with the assumption that the structural heart disease was a confounding factor rather than part of a wider syndrome. Interestingly, anemia, hypergastrinemia, and gastric hyperplasia, which result from achlorhydria such as that caused by KCNQ1-KCNE2 channel dysfunction, have been correlated with LQTS in a pedigree carrying a KCNQ1 loss-of-function gene variant (174).

KCNE2 also regulates Kv channels from the Kv1, Kv2, Kv3, and Kv4 subfamilies, including those that are cardiac expressed, further complicating extrapolation of effects of KCNE2 variants on a given channel to the much more complex situation when a KCNE2 variant might alter function of multiple channel subunit partners in vivo (150). Human *KCNE2* gene variants have been associated in large-scale studies with altered lung function and risk of coronary artery disease, both of which factors influence, and are influenced by, cardiac function (175, 176). This further illustrates the complexity of disease associations of multifunctional, ubiquitously expressed proteins such as KCNE2 and highlights the futility of approaches in which only a single-channel type, single-tissue approach is pursued when investigating the physiology and pathobiology of proteins with this profile. Unfortunately, much of the scientific review process, be it with respect to funding or publication (which are of course also tightly linked, as one does not occur without the other for very long) is partitioned into tissue-specific siloes and ill designed for multiorgan studies of physiology and disease pathogenesis even when focused on a single gene. The upshot of this is that such studies are often selected against in favor of cleaner, more straightforward studies that fit more neatly into a silo, such as "cardiovascular" or "arrhythmias." Cleaner often does not equate to being a truer picture of the complex scenario in vivo. More flexibility and open-mindedness in this respect would facilitate multiorgan studies that often better reflect the true nature of biology and disease. The National Institute of General Medical Science (NIGMS) has achieved one solution with its R35 MIRA funding mechanism, which funds a principal investigator's research program rather than a specific project and does not permit specific aims. To achieve this, MIRA study sections are highly multidisciplinary in their makeup.

For manuscript review, this approach is more difficult than it seems. It requires recruitment of reviewers from each of the relevant disciplines if, for example, a manuscript is to be assessed that includes cardiovascular, endocrine, and pulmonary functional studies in addition to transcriptomic and ion channel data sets. Furthermore, recruiting just a single reviewer in each area, which is often the case in review of this type of manuscript, leads to a high likelihood that one person's view on, e.g., endocrine physiology will be the sole and final word on that section of the manuscript. With the current and almost universally adopted approaches employed by journals to review manuscripts, the above situation is unlikely to change, and in many ways this is understandable. Future improvements one might imagine involve artificial intelligence-directed selection of reviewers, crowd reviewing, specific journals that specialize and are set up for a novel approach to complex, multiorgan, multitechnique data sets, or assignment of subject matter expert reviewers tasked only with specialized review of a particular aspect of a manuscript. None of these solutions is perfect by any means. Anything that increases the number of reviewers expected to provide a full review of a given manuscript is to be avoided; scientists are already overworked, overcommitted, and expected to work for free in most review assignments.

The Pros and Cons of Model Organisms

> *Striving to better, oft we mar what's well.*
>
> —Shakespeare (177)
>
> *Le mieux est l'ennemi du bien (the best is the enemy of the good).*
>
> —Voltaire (178)

Even human disease-gene associations, as discussed above (141), can be misleading. Over time, however,

with sufficient studies from a range of groups studying a range of ethnicities and populations, one can arrive at an answer as to whether variants in a specific gene are truly causal for a given disease, whether there is no association at all, or whether there is a genetic predisposition that typically requires an environmental, acquired component. Of course, this eventual arrival at the correct answer assumes no sampling biases such as might occur if one excluded individuals from a study because of a confounding factor, not knowing that this factor is actually a component of the same single gene-associated syndrome (173) one was investigating (because of the tendency toward a one-tissue, one-molecular correlate mindset).

Furthermore, just because a gene is found not to be causal for a purely congenital human disease, for example LQTS, this does not mean that the protein product is unimportant in electrical function in the heart. Perhaps there is redundancy built into the system such that there is a wide tolerance for dysfunction, or compensatory effects via altered expression of other proteins, or perhaps the gene disruption shortens life span arising from multifactorial but subtle perturbations yet causes undetectable effects on a year-by-year basis. Perhaps the gene disruption causes other perturbations that mask the effects one was searching for, or paradoxically improves function of the heart or other interacting tissues in other ways, such as we have discovered in *Kcne2*-deficient mice (179, 180).

The question arises, in this time when translational and biomedically relevant research are the primary emphasis of many reviewers, funding institutions, and journals, if perturbation of a gene does not unequivocally cause the human disease syndrome that we thought it did should we no longer prioritize, fund, or publish its study? There are at least five reasons why we should still care about proteins that fall into this category. First, the obvious: it might be associated with a completely different and unexpected syndrome. Second, if we are to understand physiological systems in their entirety, which is one of the stated or unstated end points for the biological/biomedical research community as a whole, we must understand every component of the system. Third, we should also strive to understand every component and complexity of biological systems if we are to design precision medicine-based interventions, diets, therapies, etc. for integrated and holistic treatments of patients. Fourth, some genes are so important that we never find disease linkages because disruption of the gene is incompatible with life. Fifth, a protein can still be an important therapeutic drug target even if its disruption is not unequivocally linked to a purely congenital human disease.

Between human disease linkage and cellular electrophysiology studies of ion channel function and dysfunction lies the middle ground populated primarily now by animal studies. Among animal studies, gene knockout and other transgenic approaches such as CRISPR/Cas9 gene editing have afforded investigators the potent tool of being able to remove, mutate, or overexpress a gene or genes in a model organism to see exactly what happens to physiological processes in that organism, without the confounding variety of genetics, other disease states, diet, and other lifestyle differences associated with human population studies. Within transgenic animal studies, those of mice are by far the dominant variety—for obvious reasons including cost, tractability, cage sizes, reproductive rates, and ethical and optics considerations.

In the cardiovascular field, there is a significant problem with studying effects of ion channel gene disruption: the relatively small size of the mouse heart and its relatively high heart rate (10 times that of the human heart) make it a highly imperfect system in which to model the human heart and even generate the types of arrhythmias that can propagate in the much larger human heart (181). For potassium channel enthusiasts, the imperfections are confounded by the fact that, unlike voltage-gated sodium channels, for example, the cadre of cardiac Kv channels is very different between *Mus musculus* and *Homo sapiens* (26, 93, 182–184). Human heart relies primarily on I_{Ks} and I_{Kr} (generated by KCNQ1 and KCNH2, respectively, in complexes with 1 or more KCNE subunit isoforms and other regulatory assembles as discussed above) for ventricular repolarization, in addition to the transient outward Kv current, I_{to}, generated by Kv4 (KCND) in complexes with KChIP, KCNE, and DPP subunits (4, 106, 185). The human heart reliance on I_{Kr} and I_{Ks} reflects the relatively long ventricular myocyte action potential that requires repolarizing channels to open slowly or in the case of I_{Kr}, open rapidly, inactivate rapidly, and then recover from inactivation once conditions permit to provide a robust repolarizing force in phase 3 of the action potential (186–188). In mouse heart, the much faster heartbeat requires a much shorter action potential, and therefore I_{Ks} and I_{Kr} are absent. In mouse ventricles, I_{to} is more important for ventricular repolarization than in human ventricles and is composed of two kinetically and genetically distinct currents. $I_{to,f}$ (fast component) is generated by Kv4 subunits in complexes with KChIPs, KCNE, and/or DPP subunits; $I_{to,s}$ (slow component) is generated by Kv1.4 (KCNA4). Mouse ventricles also require the $I_{K,slow}$ current for timely repolarization (40, 182, 183). $I_{K,slow}$ is divided into $I_{K,slow1}$, generated by Kv1.5 (KCNA5) in complexes with KCNE2, KCNE4, and KCNE5, and $I_{K,slow2,}$ generated by Kv2.1 (KCNB1) and KCNE5 (21, 54, 189).

All model organisms are imperfect, but they permit study of physiological systems in ways that in vitro studies do not, and in a manner not possible in the human population. Mice offer the unique advantage of

relatively low cost, relatively short breeding times, and genetic tractability while providing access to a real and whole physiological system. Especially with the advent of CRISPR, other organisms are moving into this realm, but mice are still uniquely cheap mammals to house and maintain and quick to breed. Yet studies of voltage-gated potassium currents in the hearts of mice have suffered from the all-too-easy criticism that the molecular correlates differ from those of people. I contend that we have such a deep baseline knowledge of the roles of Kv α-subunits in generating mouse ventricular Kv currents, and a building knowledge of the type of ancillary subunit regulation that occurs in this type of system, that it is foolish to dismiss this system. Once one accepts the imperfections of the mouse model, one can learn a great deal about how Kv channel function and regulation occurs in a real cardiac system and how specific perturbation of a gene or genes alters function in the absence of substantial uncontrolled genetic and environmental variation (190, 191).

As discussed above, consider this: of 17 gene associations with LQTS that were previously deduced and to a great extent accepted over the past quarter century, less than half are now considered reliable (141). All these associations were derived from studies of human populations; many were reinforced by in vitro analysis of the channel mutants to investigate the putative damaging effects of the gene variants in a controlled system using cellular electrophysiology techniques. Does this mean we should stop studying potential arrhythmia-gene variant associations in human populations or investigating the mechanistic effects of the gene variants upon channel activity, using reductionist, immortal cell lines or *Xenopus* oocytes in the channels? Or course, we should continue those studies. They are imperfect but still part of the overall picture—as are the mouse studies.

Understanding the complex physiology and disease processes that occur within the mammalian heart takes a variety of approaches and model systems in addition to human genetics studies. Diversity both in the approaches and in the research groups aiming to understand the system is crucial; when cliques develop, so do bias and refusal to consider the value of opinions and data from outside that group. Knowledge is built over time, and mistakes made along the way very often augment that knowledge and teach us about both the physiological system and disease processes, and about the investigative approaches themselves. We currently lack an in-depth understanding of how Kv channel regulatory subunits dynamically shape electrical activity in the human heart, let alone other tissues. As organoid, stem cell, single-cell omics and big data genetics approaches continue to revolutionize capabilities to understand cardiovascular processes in human systems, it will also be crucial to consider what has been learned from animal models, including those from mice.

Mouse Studies of KCNE Subunits Teach Us to Expect the Unexpected

> *He who does not expect will not find out the unexpected, for it is trackless and unexplored.*
>
> —Heraclitus (192)

To give some examples of the types of unique information that can be gleaned by studying a whole organism with relatively tight control over general genetic variability and firm control over a specific gene perturbation, I give a brief overview of work describing genetic deletion from mice of each of the five members of the *Kcne* gene family in turn. Disruption of *Kcne1* in mice causes deafness, urinary and fecal salt wasting, hypokalemia, and hyperaldosteronism, with only mild electrocardiographic abnormalities, reflecting roles in the inner ear with KCNQ1 and in the kidneys, adrenal gland, and pancreas with KCNQ1 and/or other ion channels (193–200). Strikingly, the Sandoz group recently discovered that KCNE1 forms native complexes with TMEM16A, a Ca^{2+}-activated Cl^- channel, conferring voltage dependence to the chloride channel and forming a complex essential for dynamic renin-angiotensin regulation of proximal tubule cells, explaining some of the above defects of *Kcne1*$^{-/-}$ mice (201).

The author's laboratory, in collaboration with others, generated and studied *Kcne2*, *3*, *4*, and *5* knockout ($^{-/-}$) mice; our work built on the approaches used in earlier studies of *Kcne1* and also on seminal work investigating the roles in mouse heart of the Kv channel α-subunits themselves (40, 182, 183), which laid the groundwork for our cardiac electrophysiology experiments. Using transgenic approaches combined with whole animal, tissue, and cellular electrophysiology, molecular biology, transcriptomics, and biochemistry, we found that knockout of *Kcne2*, *Kcne3*, or *Kcne4* genes delayed ventricular myocyte repolarization, causing QT prolongation in an age-, sex-, and/or location-specific manner (21, 173, 179, 189, 202, 203). Interestingly, deletion of the X-linked *Kcne5* gene speeded repolarization in our mouse studies (54), paralleling its association with human Brugada syndrome (204). We found that in mouse ventricles Kcne2, Kcne4, and Kcne5 proteins regulate Kv1.5; Kcne5 regulates Kv2.1; and Kcne2, Kcne4, and Kcne5 regulate Kv4.2 (21, 54, 189).

Kcne2 deletion impaired Kv1.5 trafficking to the intercalated disks, a finding that could not have been uncovered in vitro in standard cell lines (21). *Kcne2* deletion only prolonged the QT interval when combined with an environmental factor, e.g., the QT-prolonging anesthetic sevoflurane, aging, a Western diet, or

female sex (21, 173, 205). This is remarkably reminiscent of the human *KCNE2* association with acquired LQTS (41, 163). *Kcne2* deletion also caused atherosclerosis, a novel finding for deletion of an ion channel subunit, and this too was exacerbated by a Western diet (unsurprisingly) and by female sex—so much so that female *Kcne2*$^{-/-}$ mice fed a Western diet exhibited premature ventricular complexes and sudden cardiac death (>50% dead by 15 wk) (205), again reminiscent of findings that human *KCNE2* gene variance is associated with predisposition to coronary artery disease (175, 206, 207). Despite a different α-subunit repertoire between human and mouse heart, there are clear parallels between the cardiovascular effects of *Kcne2* disruption in either species.

Kcne2 deletion in mice also causes achlorhydria, gastric cancer, hypothyroidism, seizures due to a choroid plexus *myo*-inositol transport defect, lung dysfunction, splenomegaly, anemia, type 2 diabetes mellitus, differential responses to ischemia-reperfusion injury, and heart failure, this latter condition being surprisingly more severe and causing earlier death in cardiac-specific *Kcne2*$^{-/-}$ mice than in global *Kcne2*$^{-/-}$ mice, a paradox that appears to arise from gut microbiome changes linked to the achlorhydria in global *Kcne2*$^{-/-}$ mice (20, 34, 35, 95, 173, 179, 180, 205, 208–212). Deletion of a ubiquitously expressed ancillary subunit therefore causes a complex syndrome characterized and complicated by interconnectedness of the affected organ systems and impossible to predict without the use of transgenic organisms and the willingness to explore multiple organ systems and accept the complicated realities of biology and pathobiology.

Kcne3$^{-/-}$, *Kcne4*$^{-/-}$, and *Kcne5*$^{-/-}$ mice are perhaps not so complicated, but they have incredibly interesting eccentricities. The importance of searching outside the heart for factors contributing to arrhythmia was underscored by study of *Kcne3*$^{-/-}$ mice. KCNE3/Kcne3 is expressed in human heart but not mouse heart, yet *Kcne3* deletion nevertheless causes LQTS in mice. Remarkably, the mechanism is via elevated serum aldosterone arising from an adrenal-targeted autoimmune response (despite Kcne3 also not being expressed in mouse adrenal glands) (202). This mechanism seems highly unlikely yet was borne out by a different group who subsequently observed similar effects in a *Kcne3*$^{-/-}$ mouse line that was independently derived and generated in a different genetic background (107).

Kcne3 deletion, as with human *KCNE3* mutation, causes skeletal muscle dysfunction. In KCNE3-R83H *Homo sapiens* this manifests as periodic paralysis (22, 83); in *Mus musculus*, in which species Kcne3 is highly expressed in the skeletal muscles, *Kcne3* deletion manifests as abnormal hindlimb clasping when tail suspended and loss of the typical decline in contractile force observed when the hindlimb muscle is repetitively stimulated (213).

Kcne4 is positive regulated by testosterone in mouse heart, and consequently *Kcne4* deletion of young adult mice only impairs ventricular repolarization in males. However, in postmenopausal female mice, which exhibit higher testosterone levels than those of younger female mice and therefore have higher baseline levels of cardiac *Kcne4*, deletion of the latter also delays ventricular repolarization (189). KCNE4 is something of a mystery in human heart—it is highly expressed there, but its function in human heart is not clear. KCNE4 strongly inhibits KCNQ1 in vitro, but no functional role in vivo has been assigned to this property (214–216). *Kcne4* deletion also sex-specifically impairs vascular reactivity, providing the first known sex-specific molecular basis for this phenomenon (217), and sex-dependently alters the effects of cardiac and hepatic ischemia-reperfusion injury (203, 218).

Kcne5, in contrast, is X-linked, and its deletion in mice upregulated cardiac Kv currents, abbreviated the ventricular refractory period, caused ventricular premature beats, and increased susceptibility to induction of polymorphic ventricular tachycardia (54); human *KCNE5* mutations are suggested to associate with Brugada syndrome (204) and atrial fibrillation (219, 220), both of which are conditions that can arise from increased myocyte Kv currents. Thus, strong parallels are apparent once more between the effects of human and mouse KCNE5/Kcne5 disruption, despite the differences in the Kv channel α-subunits expressed in the hearts of either species. In all, the findings suggest that KCNEs can be viewed as master regulators with parallel roles across species, the puppet masters for an array of subservient Kv α-subunits, the identity and gating characteristics of which determine species-specific features such as cardiac myocyte action potential morphology and duration (29).

How Many Ancillary Subunits in a Complex?

> *In all things which have a plurality of parts, and which are not a total aggregate but a whole of some sort distinct from the parts, there is some cause.*
>
> —Aristotle (221)

Although the tetrameric organization of α subunits appears set in stone for Kv α-subunits, controversies surround the β-subunit stoichiometry in some cases. A recent cryo-electron microscopy (cryo-EM) study very nicely demonstrated that Kv4.2 forms dodecameric complexes with KChIP1 and DPP6S, giving a stoichiometry of 4:4:4, and can form octameric (4:4) complexes with either subunit type alone (136) (FIGURE 2) as also demonstrated by prior functional and bio-

chemical studies of channels formed by Kv4.2 with KChIP2 (222) and Kv4.2 with DPP6 (114). Similarly, Kvβ subunits form octameric (4:4) complexes with Kv channels, such as Kv1.1-Kvβ2 (131) (FIGURE 3). For KCNEs, the picture is controversial and less clear. In a recent high-resolution structure, KCNQ1-KCNE3-calmodulin were found to assemble in a dodecameric complex (4:4:4) (137). A range of previous studies based on photobleaching of fluorescent protein-tagged subunits, dominant-negative mutant-based subunit counting, and toxin counting came to varying conclusions for KCNQ1-KCNE1. Some groups contend that variable stoichiometries (4:1 through 4:4, respectively) are possible depending on the relative KCNQ1:KCNE1 expression levels (223–226), whereas the Goldstein group, regardless of which counting system they adopt, also sees 4:2 (227–229). We recently counted subunits in KCNQ1-KCNE2 complexes using fluorescence fluctuation spectroscopy and total internal reflection fluorescence (TIRF) microscopy and found only 4:4 complexes (230). Others examined, using photobleaching/single-molecule counting, complexes formed between KCNE2 and the monovalent cation nonselective cardiac pacemaker channel (HCN1–4) α-subunits and found that HCN:KCNE2 stoichiometry varied between 4:1 and 4:4 depending on both relative expression levels and mutations in KCNE2 (231). Variable stoichiometry can in turn lead to variable function and pharmacology, and therefore may be a physiologically impactful phenomenon if verified in a native system. The emerging pattern is that there is a tendency for unity with respect to each subunit in a mixed-subunit Kv channel complex (4 of each type), but questions remain about whether this can be flexible based on relative expression levels (or other factors) and whether KCNQ1-KCNE1 is an exception (4:2).

Future of the Field

You really need the whole village.
—Toni Morrison (232)

As high-resolution structural biology of membrane proteins flourishes with improved cryo-EM and more sophisticated understanding and techniques for membrane protein reconstitution for structural studies, the field is starting to enjoy high-resolution structures of multimeric Kv channel complexes, such as KCNQ1-KCNE3-calmodulin (137) and Kv4.2-KChIP1-DPP6S (136). In addition, several structures have been solved for neuronal KCNQ channels with therapeutic drugs bound (233, 234). As we move to a future phase in which high-resolution "structural pharmacology" targets structures of multimeric Kv channels with drugs bound, there is the potential for rational drug design of small-molecule therapeutics that leverage bound ancillary subunits to achieve temporal or spatial specificity of action between closely related Kv channel complexes that differ only by the ancillary subunits they carry. Similarly, high-resolution structural biology of ancillary subunit-containing Kv channel complexes in varying membrane compositions and relative expression levels will help us to understand the full range of possible subunit stoichiometries that are permitted.

Even in the absence of high-resolution structural information, in silico and/or in vitro screening approaches can identify ancillary subunit-dependent pharmacology with potential for therapeutic gain in safety and/or efficacy. To reap the full benefit of this, we must better understand spatial, temporal, sex, and disease state dependence of Kv channel complex composition in whole organisms (not simply focusing on the favorite tissue or cell type). This considerable undertaking may best be achieved by omics approaches in systems ranging from genetically tractable mammals (rodents) through to human beings. The ancillary subunit influence on Kv channel pharmacology can be considerable and applies both to synthetic small molecules and to natural products (24, 27, 235). We recently found several examples of ancillary subunit-specific changes in KCNQ channel responses to medicinal plant extracts

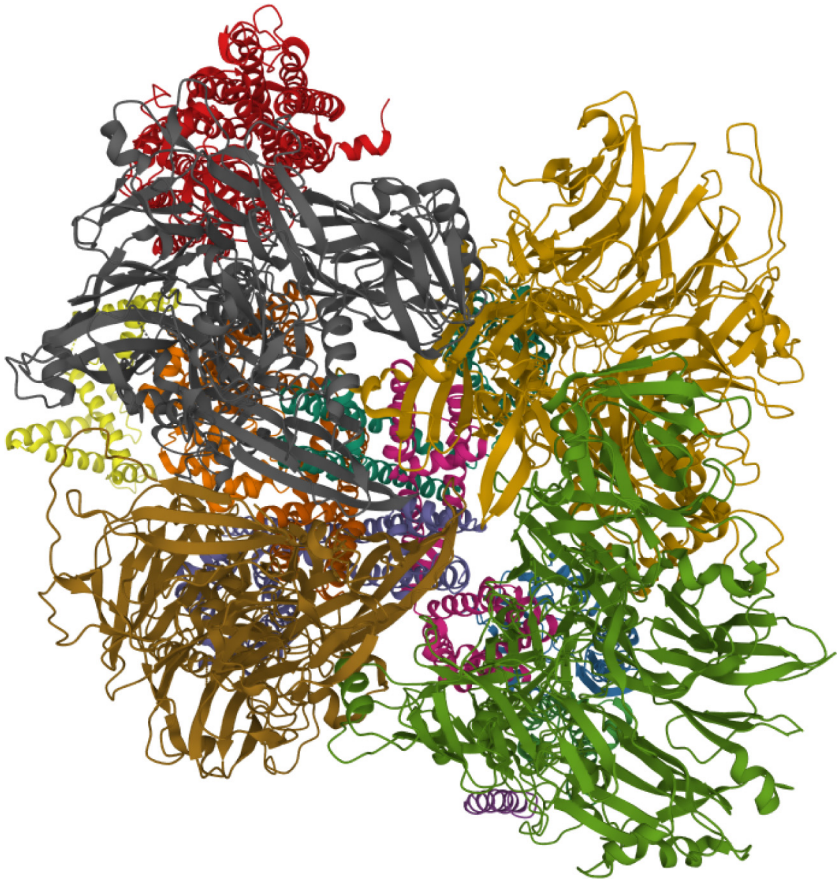

FIGURE 4. **High-resolution structure of a multipartite cation channel-transporter complex**
Cryo-electron microscopy (cryo-EM) structure of a CatSpermasome, a multipartite cation channel-transporter complex consisting of a pore tetramer comprising CatSper1-4 and various ancillary subunits including a single subunit per complex of the organic anion transporter SLCO6C1 (red). Image from the RCSB PDB (rcsb.org) of PDB ID 7EEB (Ref. 248; PDB: https://www.rcsb.org/structure/7EEB).

and their active ingredients. For example, tannic acid increases KCNQ1-KCNE1 activity yet inhibits KCNQ1-KCNE3, at hyperpolarized voltages (236).

As mentioned above, some Kv channels can form complexes with sodium-coupled solute transporters, in vivo and in vitro. We discovered complexes between KCNQ1-KCNE2 and SMIT1 (*SLC5A3*) or SMIT2 (*SLC5A11*), both of which are sodium-coupled *myo*-inositol transporters, which we also later found to interact with KCNQ2 and KCNQ3 (80, 81, 237, 238). Coassembly with SMIT1 alters the pharmacology of all the above KCNQ isoforms (to synthetic small molecules and to the neurotransmitter GABA) and may provide a mechanism to regulate local concentrations of phosphatidylinositol 4,5-bisphosphate (PIP_2), a lipid-derived signaling molecule essential for KCNQ channel activity and for which *myo*-inositol is an important precursor (238–241). Others found that *Drosophila* KCNQ forms complexes with the nontransporting *Drosophila* SMIT2 ortholog *cupcake* (*dSLC5A11*) (242) and that mammalian KCNQ2/3 forms complexes with the sodium-coupled neurotransmitter transporters DAT and GLT1 (243, 244). Also, Kv1.2 (*KCNA2*) forms complexes with LAT1 (*SLC7A5*), a neutral amino acid transporter (245–247).

Clearly, sodium-coupled transporters and Kv channels reciprocally regulate one another, and in that way can be regarded as each other's ancillary subunits. There is much to learn about this type of complex, as they appear to be numerous, can provide a novel approach to therapeutic manipulation of their component subunits, and represent a much-understudied class of biological signaling hub. We do not yet know their subunit stoichiometry, but a recent tour de force in structural biology of membrane proteins may hold clues to this. Lin et al. purified native CatSper cation channel complexes (CatSpermasomes) from mouse sperms and upon determining their structure by cryo-EM discovered that together with a pore tetramer comprising CatSper1-4, a host of ancillary subunits were copurified and visualized. These included a single subunit per complex of the organic anion transporter SLCO6C1, a stochiometric asymmetry perhaps made possible by other inherent asymmetries of the heteromeric CatSpermasome (both the α and the ancillary subunits) (248) (FIGURE 4). High-resolution structural studies directing this type of approach toward Kv channel supercomplexes purified from specific native tissues could answer many of the questions posed above in this review, and it is hoped that there are groups willing to take on this substantial but potentially highly rewarding challenge. Many different groups, working from the atomic level through to whole animal, human, and population genetics, will ultimately be needed to fully elucidate the matrixed and essential roles of ion channel regulators, including the ancillary subunits discussed here that modulate all aspects of Kv channel biology and disease and may hold the key to greater pharmacological efficacy and selectivity. ■

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