Published in final edited form as:
Heart Rhythm. 2010 May ; 7(5): . doi:10.1016/j.hrthm.2009.12.017.

Structural basis for $K_V7.1$–$KCNE_x$ interactions in the I_{Ks} channel complex

Alicia Lundby, PhD[*,†], **Gea-Ny Tseng, PhD**[‡], and **Nicole Schmitt, PhD**[*]

[*]Danish National Research Foundation Centre for Cardiac Arrhythmia, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

[†]Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen, Denmark

[‡]Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond, Virginia

Abstract

The cardiac I_{Ks} current is involved in action potential repolarization, where its primary function is to limit action potential prolongation during sympathetic stimulation. The I_{Ks} channel is mainly composed of $K_V7.1$ ion channels associated with KCNE1 auxiliary subunits. The availability of KCNE1 solution structure by nuclear magnetic resonance spectroscopy in conjunction with biochemical assays addressing $K_V7.1$–KCNE1 residue interactions has provided new insights into the structural basis for $K_V7.1$ modulation by KCNE1. Recent evidence further suggests that KCNE2 may associate with the $K_V7.1$–KCNE1 channel complex and modulate its current amplitude. Here we review recent studies in this area and discuss potential roles for multiple $KCNE_x$ subunits in I_{Ks} generation and modulation as well as the clinical relevance of the new information.

Keywords

I_{Ks}; KCNE1; KCNE2; KCNQ1; $K_V7.1$

Introduction

In human heart, the cardiac delayed rectifier current comprising I_{Ks} (cardiac slow delayed rectifier current) and I_{Kr} (cardiac rapid delayed rectifier current) is an important determinant of action potential duration. With its slow rate of activation, I_{Ks} primarily contributes to action potential repolarization during β-adrenergic stimulation, when its current amplitude is increased and rate of activation accelerated via the protein kinase A pathway. A number of studies have identified different signaling molecules, such as calmodulin and phosphatidylinositol 4,5-bisphosphate (PIP_2), which contribute to regulation of I_{Ks} and I_{Kr} in the heart (for an excellent review on these topics, see Charpen-tier et al[1]). The α-subunit that mediates I_{Ks} is $K_V7.1$ (also known as KCNQ1 or KvLQT1; see "The I_{Ks} Babylon" in the Online Supplemental Data). $K_V7.1$ channels are tetramers, with each subunit containing

Address reprint requests and correspondence: Dr. Alicia Lundby, NNF Center for Protein Research, Blegdamsvej 3B 6.2.42, DK-2200 Copenhagen N, Denmark. alicia.lundby@cpr.ku.dk..

Appendix Supplementary data
Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.hrthm.2009.12.017.

six transmembrane segments forming peripheral voltage-sensing domains (S1–S4) and a central pore domain (S5–S6) (Figure 1A). The significance of $K_V7.1$ in normal heart function is highlighted by more than 240 identified *KCNQ1* mutations associated with arrhythmias such as long QT syndrome, short QT syndrome, and atrial fibrillation (http://www.fsm.it/cardmoc/). In the majority of cases, $K_V7.1$ mutations associated with loss of function of the I_{Ks} current appear to result in long QT syndrome, whereas gain-of-function mutations lead to short QT syndrome or atrial fibrillation. However, $K_V7.1$ mutations simultaneously linked to long QT syndrome and atrial fibrillation have been reported.[2] As $K_V7.1$ properties are differentially modulated by the KCNE accessory subunits (Figure 1), this complexity may be at least partially due to a heterogeneous pattern of $K_V7.1$ association with different KCNE subunits in the heart.

Compelling evidence has established that KCNE1 is the major accessory subunit of the I_{Ks} channel. KCNE1 increases $K_V7.1$ channel conductance, shifts its activation to a more positive voltage range, and, importantly, confers the unique slow activation rate of I_{Ks}. Computational work suggests that KCNE1 resides in a cleft between voltage-sensing domains in the $K_V7.1$ channel structure. In support of this model, three $K_V7.1$ mutations associated with cardiac arrhythmia that reveal their phenotype only upon co-expression with KCNE1 all localize to a voltage-sensing domain–pore domain interface that is part of the open-state cleft where KCNE1 resides.[3] Since 1999, other members of the KCNE family have been cloned and characterized[4] (note that KCNE1 is equivalent to minK, and KCNE2–KCNE5 corresponds to minK-related peptides or MiRP1-4 in previous nomenclature; see "The I_{Ks} Babylon" in the Online Supplemental Data). All KCNE genes are reportedly transcribed into mRNA in the human heart,[5] and expression of KCNE1–KCNE4 proteins has been detected. Emerging evidence suggests a role for KCNE2 in regulating I_{Ks} as well. KCNE2 reduces the $K_V7.1$ current amplitude and confers a constitutively active current component (Figures 1B–1D).[6] If I_{Ks} in some cardiac myocytes is mediated by a $K_V7.1$ channel complex encompassing KCNE2, it would have major functional consequences. Understanding the structural requirements for KCNE modulation of $K_V7.1$ is important for delineating the role of KCNE subunits in I_{Ks} generation and regulation. Here we review current knowledge of the structural basis for $K_V7.1$–KCNE1 interactions (with focus on the membrane-spanning regions), describe evidence for the presence of additional KCNE proteins in the channel complex (with focus on KCNE2), and discuss the clinical relevance of these recent findings.

$K_V7.1$–KCNE1 channel stoichiometry

To resolve the structural basis for $K_V7.1$–$KCNE_x$ interactions, it is essential to know the stoichiometry of the channel complex. The number of KCNE subunits in the I_{Ks} complex has been a matter of debate. Recently, an elegant approach was used: iterative rounds of channel blocking/modification by a chemically releasable channel inhibitor were used to bind the channel pore and simultaneously covalently modify a cysteine-bearing KCNE1 subunit. The study convincingly showed that $K_V7.1$ and KCNE1 associate in a 4:2 stoichiometry.[7] There are at least two possible mechanisms for such a stoichiometry in $K_V7.1$–KCNE1 channel complexes. First, K_V channel assembly may involve first dimerization of *α*-subunits, followed by dimerization of dimers to form a tetramer. For $K_V7.1$–KCNE1 channels, KCNE1 has been speculated to be involved in such a dimeric assembly process.[7] Recent reports support this mechanism. The carboxy end of KCNE1 (aa 109–129) binds to a double-helix bundle (helices C) in the cytoplasmic carboxy-terminus of $K_V7.1$,[8] and these helix C bundles may be involved in dimerization of $K_V7.1$ dimers during channel biogenesis.[9] Second, the KCNE1 solution nuclear magnetic resonance (NMR) structure implies that binding of additional KCNE1 subunits in the $K_V7.1$–KCNE1 channel complex may face steric hindrance due to helical secondary structures present in the KCNE1

extracellular and intracellular domains, which form an interface large enough to span multiple $K_V7.1$ subunits.[3]

KCNE1 location in $K_V7.1$ channels

Experimental observations suggest that KCNE1 is located in close proximity to the $K_V7.1$ pore domain so that the transmembrane segment of KCNE1 interacts directly with S6 of $K_V7.1$.[10–12] For example, it has been shown that KCNE1 position 42 can come close to $K_V7.1$ position 324 in S6 in the open state so that cysteine side chains engineered into these two positions can form a disulfide bond and lock the channel in the open state.[13] In addition to interacting with the pore domain, KCNE1 interacts with the $K_V7.1$ voltage-sensing domain. For example, $K_V7.1$ position 226 in S4 can come close to KCNE1 position 44 in the open state so that cysteine side chains engineered into these positions can form a disulfide bond and stabilize S4 in its activated state.[14] These interactions may be related to the ability of KCNE1 to right-shift the voltage-dependency of $K_V7.1$ channel activation. Although the association of KCNE1 with the $K_V7.1$ channel is mediated mainly by the transmembrane regions, secondary association between the carboxyl end of KCNE1 and bundles of helices C of $K_V7.1$ also contribute.[8]

Based on the $K_V1.2$ crystal structure and the KCNE1 solution NMR structure, models for the $K_V7.1$–KCNE1 complex in the open and the closed states have been proposed (Figure 2).[3] Computational analysis of the channel complex localizes KCNE1 to a cleft between pore and voltage-sensing domains but with different interaction interfaces in the two states.[3] In the closed state, the extracellular end of KCNE1 transmembrane segment forms contacts with S3 of one subunit and with S5 and S6 of another subunit, whereas in the open state, it forms an interface with S1, S5, and S6 from three different subunits.[3] In general, these predictions are in agreement with functional studies using cysteine scanning mutagenesis and disulfide trapping approaches.[12,13] Hence, although still not completely resolved, a picture emerges that KCNE proteins are in contact with several helices from distinct subunits of the $K_V7.1$ channel.

KCNE1 interaction with $K_V7.1$ voltage sensor

Computational model simulations of $K_V7.1$–KCNE1 interactions suggest that the intracellular end of the KCNE1 transmembrane segment is close to the S5 end of the S4–S5 linker in $K_V7.1$. This may be the mechanism by which KCNE1 opposes S4–S5 linker movement and accordingly slows $K_V7.1$ channel opening.[3,15] This scenario is supported by functional studies reporting differential effects of S4–S5 linker mutations dependent on channel assembly with KCNE1.[16,17] Furthermore, KCNE1 is proposed to impede salt bridge formation between charged residues in S2 and S4 of the $K_V7.1$ voltage sensor.[18] The pattern of salt bridge formation appears to differ between the channel's open and closed states, which correlates with axial rotation of KCNE1 transmembrane segments during I_{Ks} gating transitions between the two states.[13] Compelling evidence thus supports a strategic location of KCNE1 between the $K_V7.1$ voltage sensor and pore domains, which allows KCNE1 to effectively modulate the $K_V7.1$ gating transition between the closed and open states.

How does KCNE1 slow $K_V7.1$ activation?

KCNE1 can transform $K_V7.1$-mediated currents into the characteristic slowly activating I_{Ks} by two possible mechanisms. KCNE1 either slows voltage sensor movement or delays coupling of voltage sensor movement to the activation gate. A careful study combining cysteine scanning mutagenesis and varying voltage-clamp pulse durations suggests that $K_V7.1$–KCNE1 voltage sensors reach their equilibrium position in less than 100 ms.[19] Together with KCNE1's restriction of S4–S5 linker movement, it seems likely that the rate-limiting step in $K_V7.1$–KCNE1 channel activation is in the opening of the activation gate. In

K^+ channels, conformational changes allowing gating are attributed to a so-called gating hinge located deep within the membrane. In most K^+ channels, the hinge is a conserved glycine residue, but $K_V7.1$ channels carry an alanine at the homologous position (336). $K_V7.1$ comprises a Pro-Ala-Gly sequence further downstream on S6, which corresponds to the K_V channel Pro-Val-Pro motif that adds to the flexibility of the region. In the absence of KCNE1, the ability of $K_V7.1$ to kink at Ala 336 facilitates gating, whereas the integrity of the Pro-Ala-Gly motif is required for channel activation.[20] Thus, it can be speculated that an additional mechanism by which KCNE1 slows $K_V7.1$ activation is by restraining the tendency of S6 to bend at either of these regions.

Structural determinants for diverse KCNE effects on $K_V7.1$ channels

Additional members of the KCNE family may contribute to I_{Ks} generation. The five KCNE proteins all have diverse effects on $K_V7.1$ conduction properties in heterologous systems, and their importance for cardiac function is evidenced by co-segregation of mutations in each of them and cardiac disorders (for reviews on these subjects, see Charpentier et al[1] and McCrossan and Abbott[4]). A cysteine scanning mutagenesis study suggested a similar position and orientation of the KCNE2 transmembrane segment with respect to $K_V7.1$ as found for KCNE1, with one face of KCNE2 transmembrane segment making intimate contact with the $K_V7.1$ pore domain and another face making more dynamic contacts with the voltage-sensing domain.[6] However, it appears that minor structural differences determine the specific modulatory effects of KCNEs on $K_V7.1$. For instance, the drastic effect of KCNE3 in converting $K_V7.1$ into a constitutively active channel by shifting the equilibrium position of its voltage sensors to the activated state at all potentials[19,21] can be altered into that of KCNE1 by swapping just one amino acid within the transmembrane segment.[22] Likewise, exchange of a single amino acid within the KCNE2 transmembrane segment converts the $K_V7.1$ modulatory properties of KCNE2 to those of KCNE1.[6] It seems that discrete contact points between $K_V7.1$ and the KCNEs are determinants of the distinct modulatory effects of the different KCNE subunits on the $K_V7.1$ channel.

KCNE2 as a dynamic I_{Ks} regulator

As KCNE2–KCNE4 protein expression has been confirmed in human heart,[23–25] I_{Ks} may not be a simple channel complex encompassing four $K_V7.1$ subunits and two KCNE1 subunits. Experiments using heterologous expression systems have shown that different members of the KCNE family can associate with the same $K_V7.1$ channel simultaneously,[26–29] and exchange of KCNE1–KCNE2 subunits can occur even during the lifespan of $K_V7.1$–$KCNE_x$ channel complexes in cells.[29] Because only KCNE1 can confer the unique slow activation rate of I_{Ks}, it is an obligate part of the I_{Ks} channel complex. Given the constraint that $K_V7.1$ and KCNE proteins associate in a 4:2 stoichiometry, it is conceivable that a portion of I_{Ks} channels in human myocytes may be mediated by a channel complex consisting of four $K_V7.1$ subunits, one KCNE1 subunit, and one KCNE subunit other than KCNE1. Thus, spatial heterogeneity in the KCNE expression profile is a potential mechanism for cellular regulation of I_{Ks} density.

Experiments performed on rat cardiomyocytes suggest a role for KCNE2 in I_{Ks}.[27,28] As KCNE2 decreases the amplitude of $K_V7.1$–KCNE1 currents (Figure 1C),[2,28] it is tempting to speculate that KCNE2 functions as a down-regulator of $K_V7.1$–KCNE1-mediated I_{Ks} (Figure 3).[27–29] The feasibility of this proposition is supported by experiments on adult guinea pig ventricular myocytes, where expression of KCNE2 protein by adenovirus-mediated gene transfer reduces I_{Ks} current density but not its gating kinetics or $K_V7.1$ protein level.[29]

Clinical relevance

The clinical aspects of the information presented in this review are twofold. First, recent studies suggest that the subunit composition of I_{Ks} channels in cardiac myocytes likely is more complex and dynamic than previously envisioned. Not only KCNE1 but also KCNE2–KCNE4 may be involved in I_{Ks} generation. Because these other KCNE subunits can confer distinct phenotypes to $K_V7.1$ channels, changes in the subunit composition can have profound impact on I_{Ks} current amplitude and/or gating kinetics and thus action potential shape and duration. Concurrent atrial fibrillation and QT prolongation have been reported for individuals carrying mutations in $K_V7.1$. How $K_V7.1$ can form the molecular substrate for both conditions is perplexing, as QT prolongation is caused by a reduced repolarization reserve, whereas atrial fibrillation can be caused by increased K^+ currents that shorten the repolarization phase and increase the risk of multiple reentrant wavelets. The complexity conveyed by the KCNE subunits may help explain some of the paradoxical phenotypes of $K_V7.1$ mutations. As a case in point, a carrier of the $K_V7.1$ mutation Q147R presented with concomitant atrial fibrillation and prolonged QT interval.[2] Functional studies revealed that the mutation causes a gain of function for channels encompassing KCNE2 and a loss of function for channels encompassing KCNE1. That functional consequences of the mutation are manifested only in presence of KCNE subunits underscores the importance of KCNE subunits for I_{Ks} generation. However, perhaps more importantly, it offers a possible molecular explanation for the patient's phenotype. Assuming a heterogenous distribution of KCNE1 and KCNE2 subunits in the heart, where KCNE1 plays a dominant role in ventricular I_{Ks} generation and KCNE2 is important for atrial I_{Ks} generation, the Q147R mutation may simultaneously lead to loss of function for I_{Ks} in the ventricles (QT prolongation) and gain of function for I_{Ks} in the atria (atrial fibrillation).

The second clinical implication is that the dynamic nature of I_{Ks} channel subunit composition can influence the channel's sensitivity to antiarrhythmic agents. It has long been recognized that KCNE1 association with $K_V7.1$ can dramatically increase its sensitivity to experimental I_{Ks} channel blocker (azimilide) or activator (mefenamic acid). On the other hand, KCNE2 association with $K_V7.1$ does not affect its sensitivity to either azimilide (unpublished observation) or mefenamic acid.[27]

Conclusion

Reasoning that specific current densities may be regulated by dynamically altering the composition of accessory subunits in a particular ion channel complex rather than by regulating plasma membrane expression of the entire complex correlates with the finding that most protein complexes contain entities that are both periodically and constitutively expressed.[30] The task of delineating in detail how changes in I_{Ks} channel subunit composition influence its sensitivity to clinically used antiarrhythmic agents is important and, just like resolving the heterogeneity in cardiac KCNE protein expression profiles, requires future investigations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Danish National Research Foundation to Drs. Lundby and Schmitt and by the National Heart, Lung, and Blood Institute of the National Institutes of Health Grant RO1-HL67840 to Dr. Tseng.

ABBREVIATIONS

I_{Kr}	cardiac rapid delayed rectifier current
I_{Ks}	cardiac slow delayed rectifier current
K_V	voltage-gated potassium channel
NMR	nuclear magnetic resonance
PIP_2	phosphatidylinositol 4,5-bisphosphate

References

1. Charpentier F, Merot J, Loussouarn G, Baro I. Delayed rectifier K(+) currents and cardiac repolarization. J Mol Cell Cardiol. 2010; 48:37–44. [PubMed: 19683534]
2. Lundby A, Ravn LS, Svendsen JH, Olesen SP, Schmitt N. KCNQ1 mutation Q147R is associated with atrial fibrillation and prolonged QT interval. Heart Rhythm. 2007; 4:1532–1541. [PubMed: 17997361]
3. Kang C, Tian C, Sonnichsen FD, et al. Structure of KCNE1 and implications for how it modulates the KCNQ1 potassium channel. Biochemistry. 2008; 47:7999–8006. [PubMed: 18611041]
4. McCrossan ZA, Abbott GW. The MinK-related peptides. Neuropharmacology. 2004; 47:787–821. [PubMed: 15527815]
5. Gaborit N, Le BS, Szuts V, et al. Regional and tissue specific transcript signatures of ion channel genes in the non-diseased human heart. J Physiol. 2007; 582:675–693. [PubMed: 17478540]
6. Liu XS, Zhang M, Jiang M, Wu DM, Tseng GN. Probing the interaction between KCNE2 and KCNQ1 in their transmembrane regions. J Membr Biol. 2007; 216:117–127. [PubMed: 17676362]
7. Morin TJ, Kobertz WR. Counting membrane-embedded KCNE beta-subunits in functioning K^+ channel complexes. Proc Natl Acad Sci U S A. 2008; 105:1478–1482. [PubMed: 18223154]
8. Haitin Y, Wiener R, Shaham D, et al. Intracellular domains interactions and gated motions of I(KS) potassium channel subunits. EMBO J. 2009; 28:1994–2005. [PubMed: 19521339]
9. Wiener R, Haitin Y, Shamgar L, et al. The KCNQ1 (Kv7.1) COOH terminus, a multitiered scaffold for subunit assembly and protein interaction. J Biol Chem. 2008; 283:5815–5830. [PubMed: 18165683]
10. Melman YF, Um SY, Krumerman A, Kagan A, McDonald TV. KCNE1 binds to the KCNQ1 pore to regulate potassium channel activity. Neuron. 2004; 42:927–937. [PubMed: 15207237]
11. Panaghie G, Tai KK, Abbott GW. Interaction of KCNE subunits with the KCNQ1 K^+ channel pore. J Physiol. 2006; 570:455–467. [PubMed: 16308347]
12. Xu X, Jiang M, Hsu KL, Zhang M, Tseng GN. KCNQ1 and KCNE1 in the IKs channel complex make state-dependent contacts in their extracellular domains. J Gen Physiol. 2008; 131:589–603. [PubMed: 18504315]
13. Chung DY, Chan PJ, Bankston JR, et al. Location of KCNE1 relative to KCNQ1 in the I(KS) potassium channel by disulfide cross-linking of substituted cysteines. Proc Natl Acad Sci U S A. 2009; 106:743–748. [PubMed: 19131515]
14. Nakajo K, Kubo Y. KCNE1 and KCNE3 stabilize and/or slow voltage sensing S4 segment of KCNQ1 channel. J Gen Physiol. 2007; 130:269–281. [PubMed: 17698596]
15. Shamgar L, Haitin Y, Yisharel I, et al. KCNE1 constrains the voltage sensor of Kv7.1 K^+ channels. PLoS ONE. 2008; 3:e1943. [PubMed: 18398469]
16. Chouabe C, Neyroud N, Richard P, et al. Novel mutations in KvLQT1 that affect Iks activation through interactions with Isk. Cardiovasc Res. 2000; 45:971–980. [PubMed: 10728423]
17. Franqueza L, Lin M, Splawski I, Keating MT, Sanguinetti MC. Long QT syndrome-associated mutations in the S4-S5 linker of KvLQT1 potassium chan nels modify gating and interaction with minK subunits. J Biol Chem. 1999; 274:21063–21070. [PubMed: 10409658]

18. Restier L, Cheng L, Sanguinetti MC. Mechanisms by which atrial fibrillation-associated mutations in the S1 domain of KCNQ1 slow deactivation of IKs channels. J Physiol. 2008; 586:4179–4191. [PubMed: 18599533]

19. Rocheleau JM, Kobertz WR. KCNE peptides differently affect voltage sensor equilibrium and equilibration rates in KCNQ1 K^+ channels. J Gen Physiol. 2008; 131:59–68. [PubMed: 18079560]

20. Seebohm G, Strutz-Seebohm N, Ureche ON, et al. Differential roles of S6 domain hinges in the gating of KCNQ potassium channels. Biophys J. 2006; 90:2235–2244. [PubMed: 16326905]

21. Panaghie G, Abbott GW. The role of S4 charges in voltage-dependent and voltage-independent KCNQ1 potassium channel complexes. J Gen Physiol. 2007; 129:121–133. [PubMed: 17227916]

22. Melman YF, Domenech A, de la LS, McDonald TV. Structural determinants of KvLQT1 control by the KCNE family of proteins. J Biol Chem. 2001; 276:6439–6444. [PubMed: 11104781]

23. Delpon E, Cordeiro JM, Nunez L, et al. Functional effects of KCNE3 mutation and its role in the development of Brugada syndrome. Circ Arrhythm Electro-physiol. 2008; 1:209–218.

24. Jiang M, Zhang M, Tang DG, et al. KCNE2 protein is expressed in ventricles of different species, and changes in its expression contribute to electrical remodeling in diseased hearts. Circulation. 2004; 109:1783–1788. [PubMed: 15066947]

25. Manderfield LJ, George AL Jr. KCNE4 can co-associate with the I(Ks) (KCNQ1-KCNE1) channel complex. FEBS J. 2008; 275:1336–1349. [PubMed: 18279388]

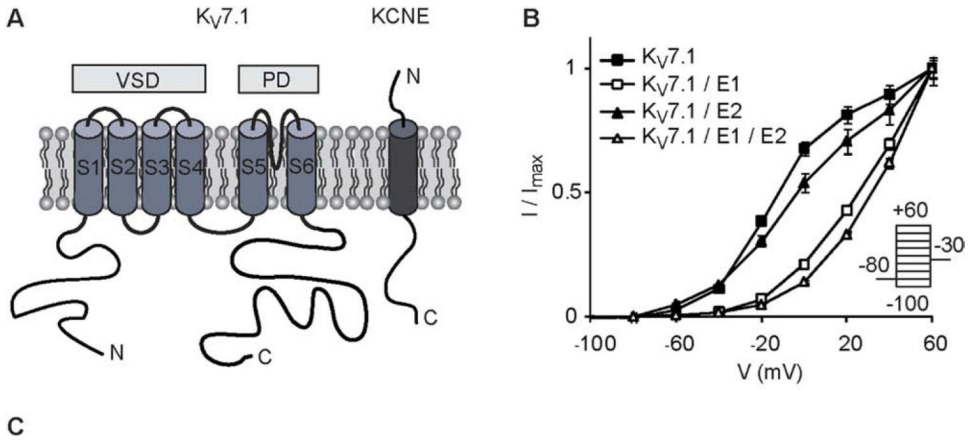
26. Morin TJ, Kobertz WR. A derivatized scorpion toxin reveals the functional output of heteromeric KCNQ1-KCNE K^+ channel complexes. ACS Chem Biol. 2007; 2:469–473. [PubMed: 17602620]

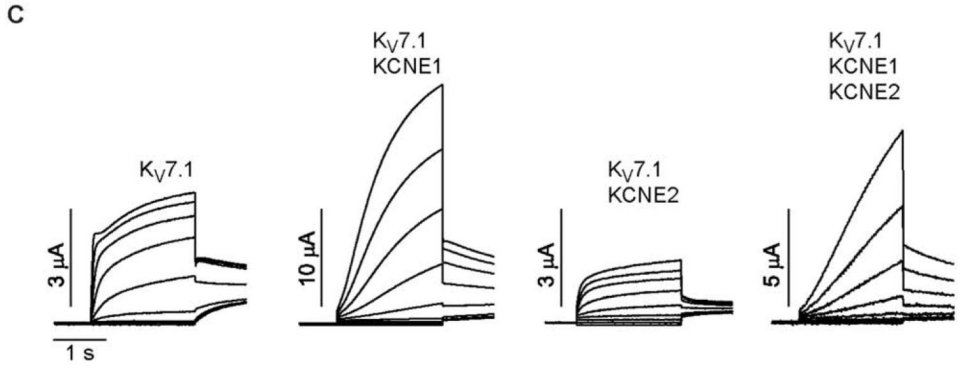
27. Toyoda F, Ueyama H, Ding WG, Matsuura H. Modulation of functional properties of KCNQ1 channel by association of KCNE1 and KCNE2. Biochem Biophys Res Commun. 2006; 344:814–820. [PubMed: 16631607]

28. Wu DM, Jiang M, Zhang M, et al. KCNE2 is colocalized with KCNQ1 and KCNE1 in cardiac myocytes and may function as a negative modulator of I(Ks) current amplitude in the heart. Heart Rhythm. 2006; 3:1469–1480. [PubMed: 17161791]

29. Jiang M, Xu X, Wang Y, et al. Dynamic partnership between KCNQ1 and KCNE1 and influence on cardiac I_{Ks} current amplitude by KCNE2. J Biol Chem. 2009; 284:16452–16462. [PubMed: 19372218]

30. de Lichtenberg U, Jensen LJ, Brunak S, Bork P. Dynamic complex formation during the yeast cell cycle. Science. 2005; 307:724–727. [PubMed: 15692050]





D

	Current amplitude	$V_{1/2}$ of activation	Constitutive component?
KCNE1	Increase	+ shift	No
KCNE2	Decrease	+ shift	Yes
KCNE3	Increase	?	**Yes**
KCNE4	**Suppressed**	No change	No
KCNE5	?	+ shift	No

Figure 1.

$K_V7.1$–KCNE1–KCNE2 current characteristics. **A:** Topology of $K_V7.1$ and KCNE subunits. $K_V7.1$ subunits encompass six transmembrane segments with intracellular N- and C-termini, where S1–S4 encode the voltage-sensing domain (VSD) and S5–S6 encode the pore domain (PD). The KCNE proteins contain a single transmembrane segment flanked by an extracellular N-terminus and a cytosolic C-terminus. **B:** Normalized amplitudes of currents elicited from *Xenopus* oocytes upon stimulation with the indicated voltage clamp protocol 48 hours after injection of $K_V7.1$ and KCNE1–KCNE2 cRNA as a function of clamp potential reveals that KCNE1 shifts the voltage-dependence of $K_V7.1$ activation in the depolarizing direction to a much greater extent than does KCNE2. **C:** Representative current traces from the experiments analyzed in B illustrate the diverse effects of KCNE proteins on $K_V7.1$ channel properties. Homomeric $K_V7.1$ channels activate relatively rapidly, exhibit sustained currents at maintained depolarization, and slowly deactivate upon repolarization. Co-expression with KCNE1 greatly increases $K_V7.1$ current amplitude and slows activation, in addition to its effect on channel voltage-dependency. Co-expression with KCNE2 reduces current amplitudes and induces a constitutively active current component. Activation of the time-dependent current component is slower than activation in the absence of KCNE2.[28] Co-expression with KCNE1 and KCNE2 generate currents with a mixture of the features, where KCNE1 dictates kinetics, whereas both KCNE1 and KCNE2 contribute to

determination of current amplitude.[2,29] **D:** Effects exerted by KCNE subunits on $K_V7.1$ currents. Strong effects are highlighted in bold.

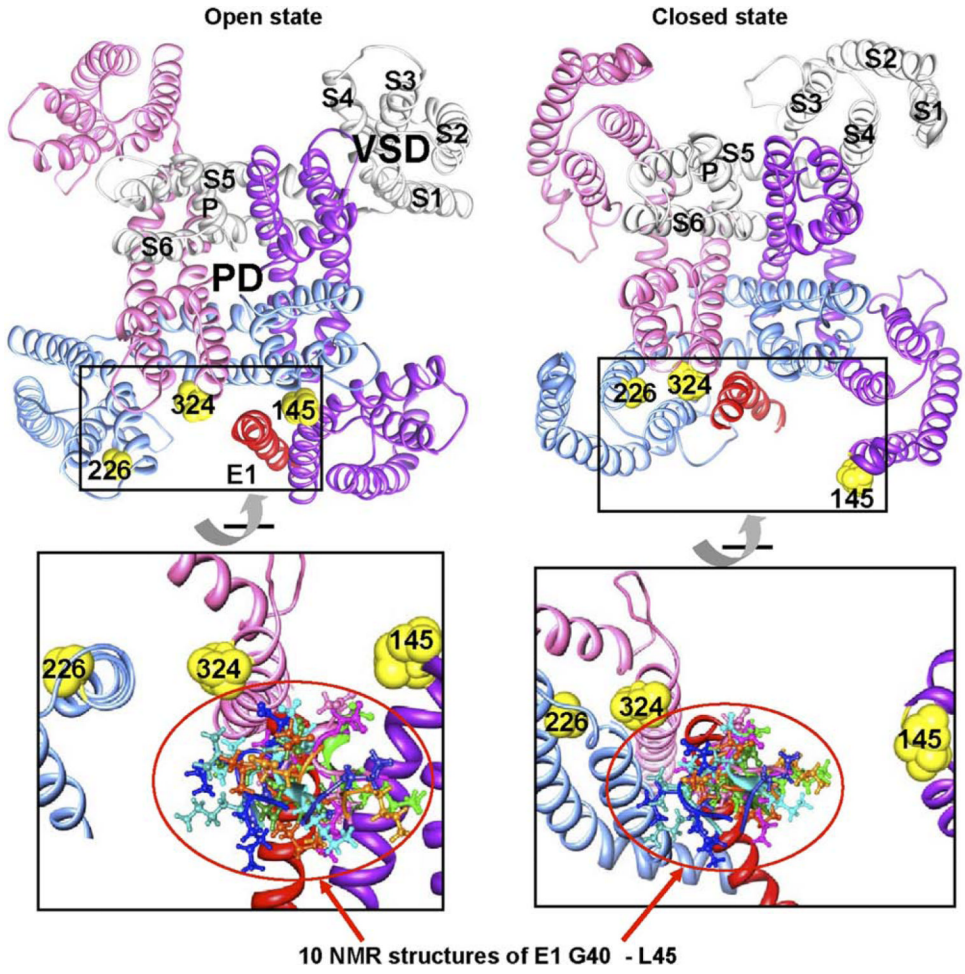


Figure 2.

Model of $K_V7.1$–KCNE1 channel structure. $K_V7.1$–KCNE1 closed- and open-state models as generated by Kang et al[3] and graphed using UCSF-Chimera. **Top:** Docking of the nuclear magnetic resonance (NMR) structure of one KCNE1 transmembrane segment *(red)* onto a homology model of $K_V7.1$ in the open **(left)** and closed **(right)** state, respectively, shows that KCNE1 resides in different clefts in the two states. The channel complex is visualized from the extracellular side. $K_V7.1$ subunits are colored *gray*, *pink*, *blue*, and *purple*, with S1–S6 and P helices labeled for one subunit. The voltage-sensing domain (VSD) and pore domain (PD) are also marked. *Yellow spheres* indicate the extracellular ends of S1 (I145), S4 (A226), and S6 (V324). In the open state, the extracellular end of the KCNE1 transmembrane segment forms an interface with S1, S5, and S6 from three different subunits; in the closed state, it forms contact with S3 of one subunit and S5 and S6 of another subunit. **Bottom:** Enlarged and rotated view of the boxed regions in the top panels, with addition of 10 NMR structures of KCNE1 residues 40–45 (color coded differently for individual NMR structures). This was achieved by aligning the 10 NMR KCNE1 structures (residues 40–70) to the KCNE1 transmembrane segment of the $K_V7.1$–KCNE1 model[6] using the MatchMaker function of UCSF-Chimera (for clarity, only residues 40–45 are shown). Assuming (1) a wide swing of S1–S4 upon transition between closed and open states and (2) changing KCNE1 position and orientation relative to $K_V7.1$ in these states, the model suggests a wide range of motions in the extracellular end of the KCNE1 transmembrane segment (highlighted by the *red circle)* and possibilities of contacts with

K_V7.1. This model is partially supported by the proximity of –S–S– partners as delineated in cysteine mutagenesis, although further experimental confirmation is needed.

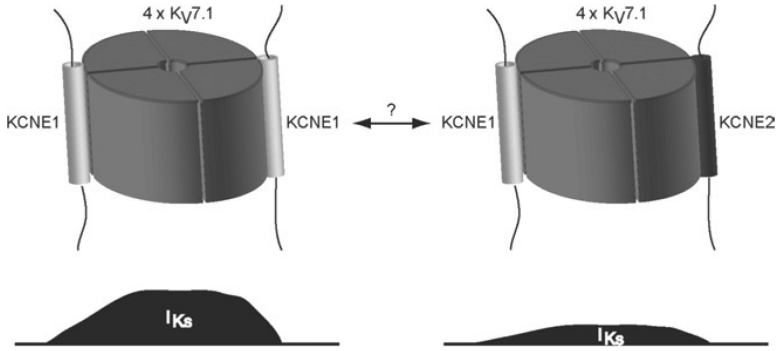


Figure 3.

Possible heterogeneity in I_{Ks} channel components. I_{Ks} density may be dynamically regulated by the composition of KCNE proteins in the channel complex. Expression of $K_V7.1$–KCNE1 channels gives rise to greater current amplitude than do $K_V7.1$–KCNE1–KCNE2 channels. As KCNE2 can modulate current mediated by $K_V7.1$–KCNE1 channels in the plasma membrane, it can be speculated that KCNE2 may function as a dynamical down-regulator of $K_V7.1$–KCNE1 currents, where KCNE2 may be able to exchange position with KCNE1 in the channel complex.[29] Such regulation of I_{Ks} density by KCNE2 could have a temporal and/or a spatial component, but this remains speculative based on the current evidence.