Molecular Template for a Voltage Sensor in a Novel K⁺ Channel. II. Conservation of a Eukaryotic Sensor Fold in a Prokaryotic K⁺ Channel

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KvLm, a novel bacterial depolarization-activated K⁺ (Kv) channel isolated from the genome of *Listeria monocytogenes*, contains a voltage sensor module whose sequence deviates considerably from the consensus sequence of a Kv channel sensor in that only three out of eight conserved charged positions are present. Surprisingly, KvLm exhibits the steep dependence of the open channel probability on membrane potential that is characteristic of eukaryotic Kv channels whose sensor sequence approximates the consensus. Here we asked if the KvLm sensor shared a similar fold to that of *Shaker*, the archetypal eukaryotic Kv channel, by examining if interactions between conserved residues in *Shaker* known to mediate sensor biogenesis and function were conserved in KvLm. To this end, each of the five non-conserved residues in the KvLm sensor were mutated to their *Shaker*-like charged residues, and the impact of these mutations on the voltage dependence of activation was assayed by current recordings from excised membrane patches of *Escherichia coli* spheroplasts expressing the KvLm mutants. Conservation of pairwise interactions was investigated by comparison of the effect of single mutations to the impact of double mutations presumed to restore wild-type fold and voltage sensitivity. We observed significant functional coupling between sites known to interact in *Shaker* Kv channels, supporting the notion that the KvLm sensor largely retains the fold of its eukaryotic homologue.
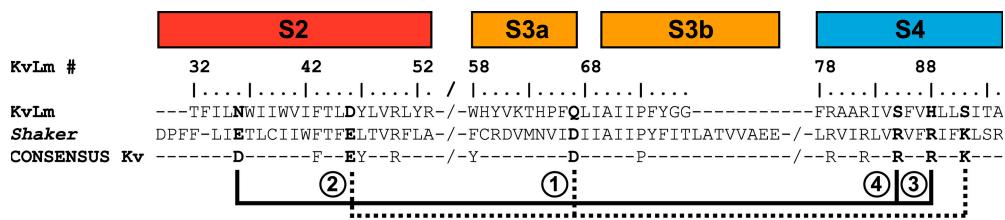
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INTRODUCTION

Eukaryotic and prokaryotic voltage-gated K⁺ (Kv) channels exhibit significant sequence homology and functional similarity and are therefore presumed to also share a common structural fold. The new model for a Kv channel that emerged from the structures of the rodent Kv1.2 (Long et al., 2005a) and the bacterial depolarization-activated KvAP (Jiang et al., 2003a) channels argues strongly for conservation of the sensor fold in prokaryotes and eukaryotes. The salient difference between the two structures is a long continuous kinked S3 helix in Kv1.2 that in KvAP is shorter (by two helical turns) and broken into two helical segments (S3a and S3b). The structural differences are surmised to exert little impact on how voltage is sensed by both channels. The distinct conformations (kinked versus broken) for S3 may be ascribed to different conformations of the voltage sensor captured in the crystal. The increase in length of the transmembrane segments occurs at a position in the sequence that exhibits low conservation (S3b, S3-S4 loop, and N terminus of S4) and may, therefore, not be essential for voltage sensing.

This model predicts pairwise interactions between conserved charged residues in the voltage sensor of the eukaryotic Kv channel *Shaker* to be conserved in prokaryotic Kv channels. In the voltage sensor of *Shaker*, the three conserved acidic residues on S2 and S3 interact with three C-terminal basic residues in S4 (Papazian et al., 1995, 2002; Planells-Cases et al., 1995; Seoh et al., 1996; Tiwari-Woodruff et al., 1997, 2000). In contrast, in the prokaryotic KvLm, all but one of these positions has been replaced by polar residues: asparagine (N36) and glutamine (Q67) in S2 and S3 and serine (S85 and S91) and histidine (H88) in S4 (Fig. 1). These replacements suggest that if prokaryotic and eukaryotic sensors share a fold, the underlying interactions must differ: electrostatic for *Shaker* and hydrogen bond for KvLm. This notion was tested by exchanging each hydrogen bond interaction in KvLm by the electrostatic interaction in *Shaker* and characterizing the impact of each mutation on the voltage dependence of the activation of KvLm on excised membrane patches of enlarged bacteria (spheroplasts) expressing the mutant. This was accomplished by mutation of polar residues N36 in S2, Q67 in S3, and S85, H88, and S91 in S4 of the KvLm sensor to aspartate (N36D and Q67D) and arginine (S85R, H88R, and S91R), one at a time and in pairs, to generate the *Shaker*-like interactions (Fig. 1). If the interactions were conserved, the imposition of a fold stabilized predominantly by electrostatic interactions on the inherently

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                  S2                    S3a          S3b                   S4
KvLm #       32      42      52    58      68                      78      88
             |....|....|....|....|. / |....|....|....|....           |....|....|....|.
KvLm         ---TFILNWIIWVIFTLDYLVRLYR-/-WHYVKTHPFQLIAIIPFYGG----------FRAARIVSFVHLLSITA
Shaker       DPFF-LIETLCIIWFTFELTVRFLA-/-FCRDVMNVIDIIAIIPYFITLATVVAEE-/-LRVIRLVRVFRIFKLSR
CONSENSUS Kv -------D------F--EY--R----/-Y--------D-----P------------/--R--R--R--R--K---
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hydrogen bond–dominated architecture of the KvLm sensor is presumed to generate mutants whose voltage of activation deviates little from wild type. Our results show this to be the case for two of the four interactions tested. For the others, we surmise that the residues tested are only part of the native *Shaker*-like interaction. The dramatic effects of neutralization of the three charged conserved positions in the KvLm sensor (D46 in S2, and R79 and R81 in S4) are consistent with the view that all eight voltage-sensing positions in *Shaker* are conserved in KvLm, and that six of these form a network of hydrogen bond interactions that sustains a fold homologous to that of *Shaker*. These findings provide further functional constraints on the mechanism of voltage sensing of prokaryotic Kv channels in accord with the new structural model proposed for Kv channels.

MATERIALS AND METHODS

All mutations were performed using the QuikChange site-directed mutagenesis kit (Stratagene). Unless otherwise indicated, all procedures were done as described in the accompanying paper (see Santos et al. on p. 283 of this issue).

RESULTS

Site-specific Replacements at Conserved S4 Positions Considered Key for Sensing

Exhaustive mutational studies of the S4 basic residues on numerous Kv channels demonstrated that these are involved in voltage gating and, depending on their position along S4, they impact sensing to a different extent (Papazian et al., 1991; Aggarwal and MacKinnon, 1996; Seoh et al., 1996; Bezanilla, 2000; Mannikko et al., 2002; Latorre et al., 2003; Sesti et al., 2003; Ahern and Horn,

TABLE I
Conductance and Gating of KvLm and Single and Double KvLm Mutant Channels

Mutant	γ	$V_{1/2}$	z	n	N
	pS	*mV*	*e*		
Wild type	18.4 ± 0.3	154 ± 1	2.0 ± 0.1	12	10,560
N36D	17.6 ± 0.5	172 ± 1	2.8 ± 0.2	5	3,676
D46N	17.6 ± 0.7	–	–	8	111
Q67D[a,b]	17.9 ± 0.1	175 ± 2	1.8 ± 0.2	5	676
R79A[a]	18.2 ± 0.1	>200	–	3	687
R82A[a]	18.7 ± 0.9	>200	–	4	573
S85R[a]	18.1 ± 0.2	170 ± 3	1.6 ± 0.2	3	1,051
H88R	17.4 ± 0.4	153 ± 1	2.0 ± 0.1	9	8,489
S91R	19.1 ± 0.3	120 ± 1	2.7 ± 0.2	5	5,760
N36D/S85R[a,b] (4)	19.2 ± 0.7	175 ± 1	1.8 ± 0.1	6	1,222
N36D/H88R (3)	17.3 ± 0.6	158 ± 2	2.3 ± 0.3	6	7,974
D46N/S91R[a] (2)	17.2 ± 0.9	>200	–	7	2,732
Q67D/S91R (1)	17.1 ± 0.3	153 ± 2	1.7 ± 0.1	6	7,579
R79A/R82A[a]	17.6 ± 0.5	>200	–	3	62
S85R/H88R/S91R (ΣS4)	17.7 ± 0.5	120 ± 2	1.3 ± 0.1	5	3,083

(1), (2), (3), and (4) in the "mutant" column refer to interactions defined in Fig. 1.

[a]Open probability did not saturate within the voltage range tested.

[b]Values for $V_{1/2}$ and z were estimated by normalizing the mutant channel P_o to the wild-type P_{max}.
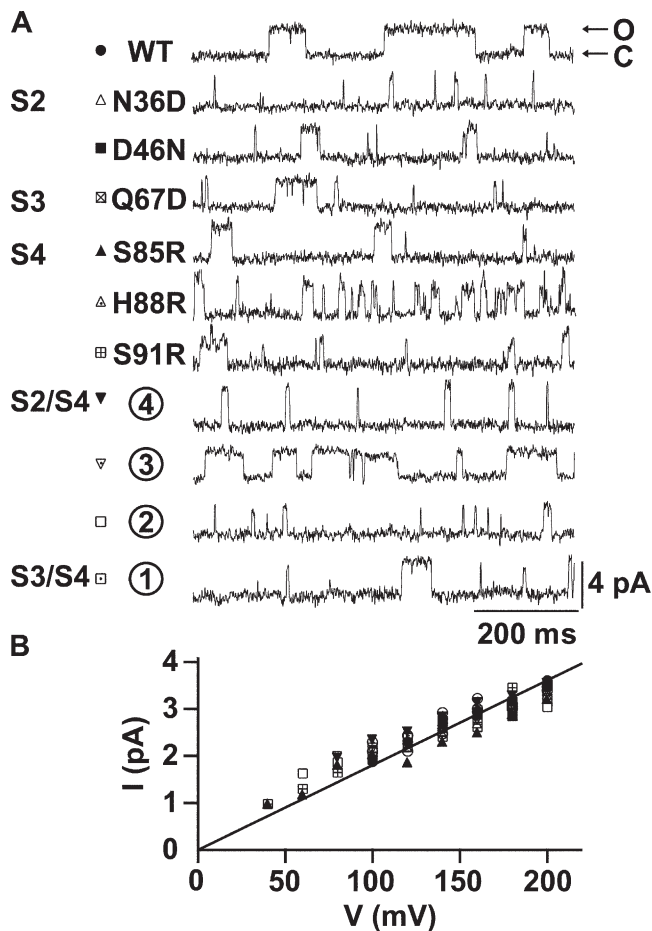
Figure 2. Voltage sensor mutations on KvLm do not alter the permeation pathway. (A) Single channel currents from wild-type and mutant channels were evoked from a holding potential of 0 to 180 mV, except for the S91R mutant, which was tested at 120 mV. (B) Single channel I-V relationships for the wild-type channel and each of the mutant channels. The wild-type channel has a slope conductance $\gamma = 18.4 \pm 0.3$ pS (mean $\pm$ SD); average γ of mutant channels = 17.9 ± 0.6 pS (mean $\pm$ SEM; solid line). Single channel currents of double mutants are identified according to interaction number defined in Fig. 1. Other conditions as in Fig. 4 of accompanying paper (Santos et al., 2006).

2004a; Starace and Bezanilla, 2004; Tombola et al., 2005b). Thus, we systematically replaced the residues of KvLm at the conserved positions on S4 of *Shaker* considered critical for sensing and examined the consequences on voltage gating (see Figs. 3 and 4; Table I). First, we confirmed that the mutant proteins largely retain a native fold and do not alter the conduction pathway, as judged by the unmodified single channel conductance (Fig. 2, A and B).

Remarkably, replacing the S4 C-terminal serine by arginine (S91R) evokes channel activity at lower voltages than required to open the wild-type channel, affecting a left shift of the P_o-V curve by $\sim$35 mV. Concurrently, the addition of one charge at position 91 significantly increases the apparent gating charge (z) moved in the

activation process, affecting an increase in the steepness of the slope of the P_o-V curve (Fig. 4, A and B; Fig. 5; Table I). Conversely, mutation of the N-terminal arginines to alanines (R79A and R82A) produces a right shift of the P_o-V curve of $\geq$45 mV (Fig. 3; Table I). Thus, the nature of the residue at these flanking ends of S4 sharply modulates voltage sensing. Position matters as mutation of serine 85 (S85R), located roughly at the middle of S4, produces a surprising right shift of $\sim$15 mV and decrease in z ($V_{1/2} = 170 \pm 3$ mV, $z = 1.6 \pm 0.2$ e; Fig. 4 D and Fig. 5). Replacing H88, which conceivably could be charged within the sensor core, with arginine (H88R) does not affect the voltage dependence of channel activation ($V_{1/2} = 153 \pm 1$ mV, $z = 2.0 \pm 0.1$ e) (Fig. 4 C and Fig. 5). Does the number of S4 arginines fine tune the sensor? The triple mutant containing only arginines in all key positions (S85R/H88R/S91R) produced a left shift commensurate with that affected by the single mutant S91R ($V_{1/2} = 120 \pm 2$ mV, $z = 1.3 \pm 0.1$ e) (Figs. 3; Fig. 4, A and B; Fig. 5). This implies that, given only S4, an endpoint in the dynamic range of the P_o-V curve is at $V_{1/2} = 120$ mV, which is reached with only three arginines on S4 at positions 79, 82, and 91. And it raises the following question: what is the minimum number of S4 arginines that can support sensing? The double mutant (R79A/R82A), which contains no arginines at any key position, produced a right shift of at least 45 mV (Fig. 3; Table I). Therefore, it is plausible to infer that, fundamentally, no positively charged residues on S4 are required for voltage sensing. We hypothesize that the vestigial voltage sensitivity of this double mutant arises at least in part from a charge on S2. These results define the presence on KvLm of an evolutionary conserved voltage-sensing S4 in which, in keeping with a *Shaker* Kv channel similarity, two out of five charged residues on key positions are uncharged.

Site-specific Replacements at Conserved S2 and S3 Positions Considered Key for Sensing

What about the determinants on the S2 and S3 components of the sensor? For KvLm, the residues on S2 and S3 at positions corresponding to conserved acidic residues of Kv channels, thought of as countercharges for the positively charged residues on S4, are N36 and D46 on S2, and Q67 on S3 (Fig. 1). Their influence on KvLm voltage sensing was assessed by mutational analysis aiming to preserve side chain size while changing charge (Figs. 4 and 5; Table I). Whenever possible, residues were mutated to the residue that occurred with the highest frequency at that position (e.g., N36D and Q67D) in an alignment of 73 Kv channel sequences (see Fig. S1 in Santos et al., 2006). However, the contribution of residue D46 could not be evaluated using this approach and so the residue was mutated to asparagine, one of the only two nonacidic residues found at this position in the alignment (N in *Soneid*, gi: 24375262, and
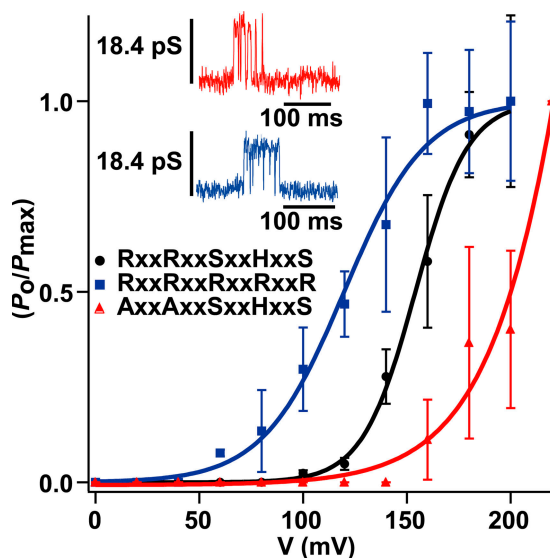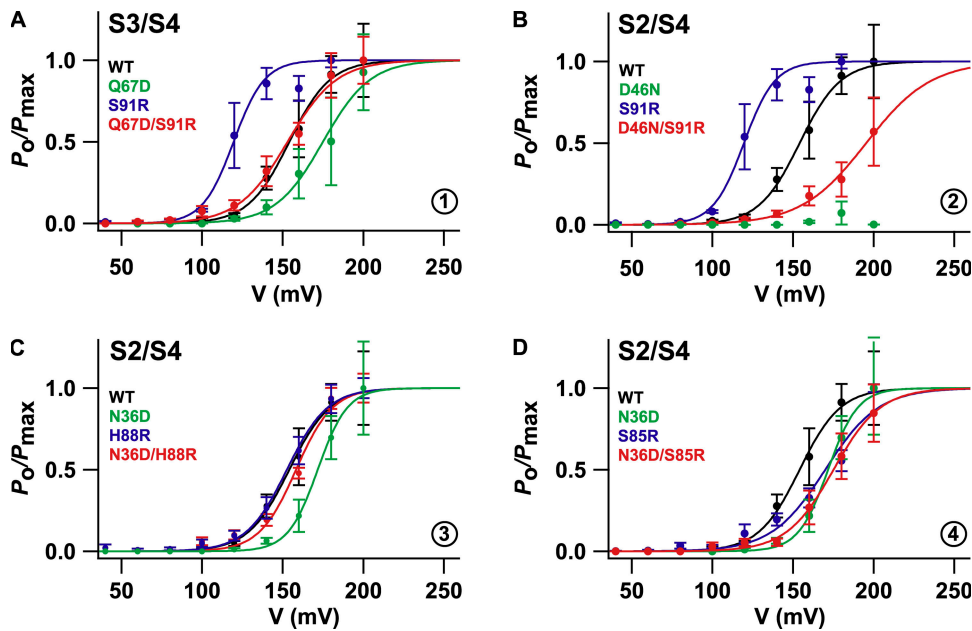
Figure 3. The rudimentary S4 motif of KvLm is a component of the voltage sensor. Representative single channel records and the voltage dependence of the open probability for wild-type KvLm (RxxRxxSxxHxxS) and KvLm channels with an uncharged S4 (AxxAxxSxxHxxS, red), and a fully charged, *Shaker*-like S4 (RxxRxxRxxRxxR, blue). A Boltzmann distribution $(P_o/P_{max}) = (1 + \exp[-zF(V - V_{1/2})/RT])^{-1}$ was fitted to the data (solid line), where $V_{1/2}$ is the voltage at which half of the channels are open $(P_o/P_{max} = 0.5)$, and z is the apparent gating charge. Fit values are shown in Table I. Other conditions are as in Fig. 2.

Q in *Vvulni-2*, gi: 27364075). We characterized the single site mutant channels. The N36D mutant on S2 produced a right shift of 18 mV and an increase in z ($V_{1/2} = 172 \pm 1$ mV, $z = 2.8 \pm 0.2$ e) (Fig. 4, C and D; Fig. 5). Interestingly, the only mutation of the KvLm voltage sensor that yields a non–voltage-gated channel phenotype is the D46N mutant on S2 (Fig. 4 B and Fig. 5). This is consistent with the observations that D46 is one of the five most conserved sites on the voltage sensor of the Kv channel superfamily (Fig. 1; and see Fig. S1 in Santos et al., 2006), and it contributes significantly to the gating current and structural fold of *Shaker*, which indicates that this is a critical residue for sensing and structural stability (Papazian et al., 1995; Planells-Cases et al., 1995; Aggarwal and MacKinnon, 1996; Seoh et al., 1996; Tiwari-Woodruff et al., 1997; Tiwari-Woodruff et al., 2000). The observed phenotype for D46N supports the hypothesis that this charged residue is in part responsible for the voltage sensitivity of the S4 mutant, R79A/R82A. For S3, replacing Q67 with an acidic residue (Q67D) (Fig. 4 A and Fig. 5) produced a right shift of ~20 mV and a small decrease in z ($V_{1/2} = 175 \pm 2$ mV, $z = 1.8 \pm 0.2$ e). Together, these findings indicate that S2 and S3 are crucial components of the KvLm minimal sensor, in accord with findings on other Kv channels (Papazian et al., 1995; Planells-Cases et al., 1995; Seoh et al., 1996; Tiwari-Woodruff et al., 1997, 2000; Papazian et al., 2002).

Conservation of Pairwise Interactions between S2, S3, and S4 Components of the Sensor

Given the significant sequence divergence between the sensor on KvLm and on other eukaryotic Kv channels, the question arises of whether Kv channels share a common sensor architecture. We approached this issue by designing double mutants between both S2 and S4, and S3 and S4 to examine if specific pairs of interacting residues required for folding and function in *Shaker* Kv channels are conserved on KvLm. Specifically we mutated each of the residues in the proposed interacting pair in KvLm to its *Shaker* sequence homologue and looked for the ability of a mutation in the second interacting site to attenuate the effects produced by a mutation on the first site. Because the choice of mutations aims to recapitulate the *Shaker*-like interaction, the assay provides information not only on whether the sites are interacting but also on whether the KvLm shares a fold with *Shaker*. We focus first on the interplay between S3 and S4 (interaction one in Fig. 1). *Shaker* Kv channel residues corresponding to KvLm Q67 on S3 and S91 on S4 are known to interact (Papazian et al., 1995; Planells-Cases et al., 1995; Seoh et al., 1996; Tiwari-Woodruff et al., 1997, 2000), as they do in KvLm (Fig. 4 A; Fig. 5; Table I); whereas the S91R mutation effects a left shift of the P_o-V curve and the Q67D produces a right shift, the double mutant Q67D/S91R restores the wild-type phenotype ($V_{1/2} = 153 \pm 2$ mV, $z = 1.7 \pm 0.1$ e). This indicates that the interplay between the partner residues Q67 and S91 causes the same functional effect irrespective of whether the interaction is established by an ionic or an H bond.

We turn now to S2 and S4 interactions (interactions two, three, and four in Fig. 1). In KvLm, D46 is proposed to interact with S91. Because the first site in the interaction is already an acidic residue on KvLm as it is in *Shaker* (E293), the single mutation S91R alone effectively reconstructs the ionic interaction present in *Shaker*. The observed disparity with respect to wild type in both parameters characterizing the channel voltage dependence ($V_{1/2} = 120 \pm 1$ mV, $z = 2.7 \pm 0.2$ e) (Fig. 4 B and Fig. 5) indicates that the *Shaker*-like interaction cannot be reproduced within the KvLm architecture. To test for an interaction, we coupled the S91R mutation to D46N, effectively exchanging the position of the charged and polar sites while attempting to preserve the type of interaction (charged residue with polar). Whereas the D46N mutant channel exhibits a non–voltage-gated channel phenotype (Fig. 4 B), the double mutant D46N/S91R restored a measurable, albeit different, voltage sensitivity (Fig. 4 B), arguing for an interacting pair as for *Shaker* Kv channels (Papazian et al., 1995; Planells-Cases et al., 1995; Seoh et al., 1996; Tiwari-Woodruff et al., 1997, 2000). Pair neutralization mutations of *Shaker* Kv channel residues corresponding to KvLm N36 on S2, and S85 and H88 on S4 demonstrate that these interact

Figure 4. Voltage sensor mutations on KvLm reveal specific interactions between highly conserved residues. Circled numbers on bottom right corners of each panel refer to interactions identified in Fig. 1. For each mutant channel, the voltage dependence of the normalized open probability (mean ± SEM) and a fit of a Boltzmann distribution to the data are displayed. Fit values are shown in Table I. (A) The Q67D mutation [green] produces a right shift, whereas the S91R mutation [blue] effects a left shift. The double mutation Q67D/S91R [red] exhibits a phenotype identical to wild-type KvLm [black]. (B) The D46N mutant [green] does not display voltage sensitivity within the voltage range tested, and the channel rarely opens: the open residence time at 200 mV is 0.04 ± 0.009% compared with 25 ± 6% for wild-type KvLm (black). The double mutant D46N/S91R (red) displays a right shift. (C) The H88R (blue) single mutant and the double mutant N36D/H88R (red) express a wild-type phenotype (black). (D) The N36D (green) and S85R (blue) single mutants, and the N36D/S85R (red) double mutant effect right shifts. Other conditions as in Fig. 2.

(Seoh et al., 1996; Tiwari-Woodruff et al., 1997). Indeed, the double mutant N36D/H88R ($V_{1/2} = 158 \pm 2$ mV, $z = 2.3 \pm 0.3$ e) (Fig. 4 C and Fig. 5) restores the wild-type KvLm phenotype. This result suggests that N36 and H88 interact, and implies that the phenotype is comparable regardless of the chemical nature of the bond between the pair. The N36D/S85R double mutant displays gating properties shifted to the right compared with wild-type KvLm ($V_{1/2} = 175 \pm 1$ mV, $z = 1.8 \pm 0.1$ e) (Fig. 4 D), and, significantly, the second site mutation (S85R, $V_{1/2} = 170 \pm 3$ mV, $z = 1.6 \pm 0.2$ e) does not attenuate the effect of a mutation at the first site (N36D, $V_{1/2} = 172 \pm 1$ mV, $z = 2.8 \pm 0.2$ e), which argues that the type of bond between this pair matters, thereby precluding a definite conclusion on whether the residues interact.

Together, these findings indicate that in KvLm, the C-terminal half of S4 (H88 and S91) interacts with N36 on S2 and Q67D on S3 (interactions three and one in Fig. 1), suggesting a conservation of these interactions from *Shaker* to KvLm (Papazian et al., 1995; Planells-Cases et al., 1995; Seoh et al., 1996). For these two interactions, the replacement of a hydrogen bond in KvLm by the salt bridge present in *Shaker* yields a mutant KvLm channel whose voltage activation parameters are comparable to wild-type KvLm (Fig. 5). Therefore, we can also conclude that the *Shaker* fold in these structural regions (N-terminal half of S2 and S3, and C-terminal half of S4) is well recapitulated in the KvLm sensor fold. Functional coupling was also observed for residues D46 and S91 (interaction two in Fig. 1); however in this

structural region, we could not reproduce the *Shaker* fold in KvLm. The result suggests that in this region, either the fold differs in KvLm or there is yet another unidentified partner in the interaction. The proposed interaction between N36 and S85 (interaction four in
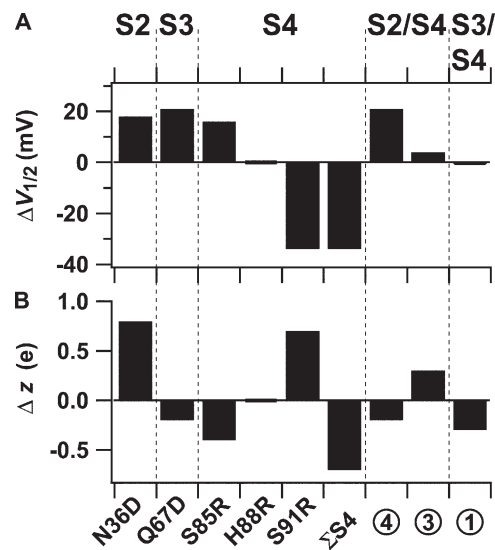

Figure 5. Voltage sensing positions and fold stabilizing interactions in the voltage sensor are conserved. Effect of single and double mutations on the parameters that characterize the voltage dependence of activation ($V_{1/2}$ and z). The changes in $V_{1/2}$ ($\Delta V_{1/2} = (V_{1/2}$ wild type$) - (V_{1/2}$ mutant$)$) and z ($\Delta z = z$ wild type $- z$ mutant) are shown. The "ΣS4" identifies the *Shaker*-like triple mutant (S85R, H88R, and S91R). Circled numbers denote double mutants designed to test for the interactions defined in Fig. 1.

Fig. 1) could not be verified. This is attributed to the ineffective replacement of serine by arginine presumably due to a steric hindrance arising from side chain volume. We defer the presentation of this interpretation to the discussion section.

How do these proximity relationships compare with those observed on the crystal structure of the Kv1.2 sensor? In the *Shaker* Kv family of channels, interacting pairs of residues were unveiled primarily by a technique similar to intragenic suppression; charges in pairs of interacting residues were reversed in double mutants and correct maturation was determined from gel assays (Papazian et al., 1995; Tiwari-Woodruff et al., 1997; Tiwari-Woodruff et al., 2000). The assay assumes that interactions key to channel biogenesis persist in the mature channel and are part of structural intermediates in the activation pathway. From the functional characterization of these mutants, it was surmised that interactions three and four in Fig. 1 occur in a partially activated conformation of the *Shaker* sensor (Tiwari-Woodruff et al., 2000). In the crystal of Kv1.2, the open conformation of the channel appears to have been trapped and therefore a rigorous test for the proximity is possible. For Kv1.2, the distance between the corresponding residues R300 and R303 with E226 is <11 Å measured from C_β to C_β in accord with a proposed short-range interaction, given the caveat that the crystal structure corresponds to the open conformation of the channel (Long et al., 2005a). Therefore, we conclude that in KvLm, H88 comes within a short distance of N36.

It appears, therefore, that the constellation of specific, short-range interactions between highly conserved residues on the S2, S3, and S4 components of the sensor observed in the eukaryotic *Shaker* Kv channel recapitulate those present in the minimal sensor of the bacterial KvLm. This implies that the design of the voltage sensor is conserved among the Kv channel superfamily. Our results support the view that function is the main reason for fold conservation.

DISCUSSION

Admittedly, our knowledge of the KvLm fold is incomplete, and therefore our understanding of the sensing mechanism is speculative. However, a number of interesting inferences can be drawn from our analysis.

Conservation of Sensor Fold: Hydrogen Bond Architecture
The selective replacement of fold stabilizing salt bridges in *Shaker* by a hydrogen bond network of interactions in KvLm argues that sensor diversity arose in part by the sampling of multiple energetic profiles of fold stabilizing interactions within a unique sensor architecture. This function-driven view of sensor evolution suggests a conservation of the voltage-sensing mechanism across all Kv channels only insofar as conservation of fold

stabilization mediated by pairwise interactions between conserved positions on transmembrane segments S2, S3, and S4. Conservation of length and sequence identity of voltage sensor components S2, S3, and S4 are therefore sacrificed in exchange for the conservation of these important interactions. Cases in point are the important sequence deviations harbored in the sensors of KvLm, KvAP, and Kv1.2. Focusing on S3 and S4, KvLm is characterized by a short S3b–S4 (25 residues from A70 to A94) in which S4 has only two arginines. In contrast, in KvAP and Kv1.2 in which S4 harbors five and six charged residues, the length of S3b–S4 increases by 12 residues in KvAP (37 residues from A95 to S132) and by 18 residues in Kv1.2 (48 residues from A262 to H310), in agreement with the observation of an S3b and S4 that is two turns of a helix longer in Kv1.2 (Long et al., 2005a,b). Irrespective of these major changes in length and sequence identity, pairwise interactions between conserved residues in the voltage sensors of Kv1.2 and KvLm are conserved, suggesting that the length and character of S3b and S4 are variables in sensor design. Accordingly, it is predicted that different sensors will exhibit different solvent accessibility profiles owing to different lengths of S3b–S4. In this context, KvLm, KvAP, and Kv1.2 sensors are seen to operate with the same sensor architecture in which KvLm uses the shortest and Kv1.2 the longest paddle to achieve the same function.

The Reduced z in all Constructs Bearing the S85R Mutation Suggests a Spatial Constriction Confined to this Region of S4
Conservation of stabilizing interactions in the sensor fold from KvLm to Kv1.2 hints that there are other general structural features of all sensors that are conserved. For example, mutations that evoke a significant right shift in the P_o vs. V curve in the *Shaker* Kv channel are accompanied by a decrease in z. Case in point, in two Kv channels from the *Shaker* family, the largest increase in $V_{1/2}$ elicited by a neutralization mutation in S4 is observed for R368Q (50 mV $\leq \Delta V_{1/2} \leq$ 110 mV) and occurs with a concomitant large decrease in z (-2 e $\leq \Delta z \leq -0.6$ e) (Papazian et al., 1991; Perozo et al., 1994). It follows then that mutations that add a positive charge at a position in S4 presumed to be within the electric field should evoke a left shift of P_o vs. V curve and an increase of z; this was indeed observed for the S91R KvLm mutant (Fig. 4, A and B; Fig. 5; Table I). In that regard, the effect of the single mutant S85R ($\Delta V_{1/2} = 16$ mV, $\Delta z = -0.4$ e) (Fig. 4 D and Fig. 5) is unexpected. A constraint to movement with the electric field imposed by a large increase in side chain volume (1.7x) (Creighton, 1993) effected by replacement of serine with arginine at position 85 would be expected to left shift the voltage of activation by increasing the energy barrier between closed and open states, and decrease z by limiting the distance moved by charges C terminal to S85 in the activation

process. This conjecture is in agreement with the observation that the addition of two charges (S85R and H88R) to the S91R mutant results in the otherwise unexpected decrease of the effective charge moved (Fig. 3; Fig. 4 D; Fig. 5; Table I). These results suggest that in the KvLm sensor architecture, S85 occupies a delicate position in which the proteinaceous environment surrounding it allows only enough space for a small side chain to move, thereby implicating a contribution of nonsensing residues to voltage activation in paving a permissive pathway for the motion of S4. This prediction has been verified for both HERG (Subbiah et al., 2005) and *Shaker* (Lopez et al., 1991) channels in which mutations of hydrophobic residues in S4 shift activation voltages by as much as 80 mV and change z values by $\sim30\%$. A constricted path for the movement of S4 is in accord with the measurement of small ω-currents in mutant *Shaker* sensors with the N-terminal arginines replaced by alanines (Tombola et al., 2005b). We therefore propose that the inability to validate an interaction between residues N36 and S85 in the double mutant (N36D-S85R) can be accounted for by the bulky side chain of arginine that precludes a favorable interaction with aspartate due to steric constrains imposed by the sensor fold.

Toward a Converging Model of Prokaryotic and Eukaryotic Kv Function

The results of decades of exceptional work on the functional characterization of eukaryotic Kv channels have been considered to be incompatible (Cohen et al., 2003; Ahern and Horn, 2004b) with gating models proposed on the basis of structures of two voltage sensors, one from a bacterial source (KvAP) (Jiang et al., 2003a) and the other from rat (Kv1.2) (Long et al., 2005a). At the heart of controversy is the nature of movement of the voltage sensor components: large for structural-based models (Jiang et al., 2003b; Long et al., 2005b) and small for function-derived models (Gandhi et al., 2003; Ahern and Horn, 2005; Chanda et al., 2005; Posson et al., 2005). Determination of amplitude of sensor component motion has relied heavily on accessibility measurements for KvAP (Gandhi et al., 2003; Jiang et al., 2003b; Ruta and MacKinnon, 2004; Ruta et al., 2005) and *Shaker* (Baker et al., 1998; Gandhi et al., 2003; Gonzalez et al., 2005; Phillips et al., 2005). What if a universal gating mechanism were conserved in Kv channels originating from Archaea to *Drosophila* irrespective of conservation of accessibilities? Could the observed differences in accessibility measurements be a reflection of voltage sensor diversity? In support of a converging model for voltage sensing (Tombola et al., 2005a), we favor this view and suggest that much of the known voltage sensor functional diversity may arise from "paddles" of different sizes and permissive paths for S4 motion with different solvent accessible surfaces. The fact that in KvLm, KvAP, and Kv1.2, the size and physico-chemical properties of the presumed moving parts of the sensor (S3–S4) are not conserved and yet all retain significant voltage sensitivity in a sensor fold stabilized by interactions between residues conserved across all Kv channels strongly supports this view.

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