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Characterization of an early-diverging KCNE potassium-channel auxiliary subunit in the jawless vertebrate lamprey

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eLife Assessment

The authors describe a new member of the KCNE auxiliary subunits of potassium channels from a lamprey. This new subunit represents an early evolutionary member which confers new properties when expressed along with KCNQ channels. In the revised version of the manuscript, the authors present **convincing** evidence from several experimental approaches. The contents of this manuscript are **important** and should be relevant to understanding both the mechanism of modulation of KCNQ channels by KCNE subunits and the evolutionary history of these subunits, which this manuscript now extends to the divergence of early vertebrates.

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Abstract

The KCNE (KCNE1–6) proteins are single-pass transmembrane auxiliary subunits of the voltage-gated K⁺ channel KCNQ1. KCNQ1–KCNE complexes have been well studied in jawed vertebrates ranging from zebrafish to humans, but KCNE subunits from earlier-diverging vertebrates remain poorly characterized. Here, we functionally characterize a single KCNE-like gene in lamprey, a jawless vertebrate, and designate it *kcne0* as an early-diverging member of the KCNE family. KCNE0 shows moderate amino acid sequence similarity to KCNE1–6 but is not particularly similar to any single isoform. Both *kcnq1* and *kcne0* transcripts were detected in multiple lamprey organs. When co-expressed with lamprey KCNQ1, KCNE0 produced a constitutively active current, similar to KCNE3. By contrast, KCNE0 modulated KCNQ1 from other species less effectively, suggesting species-specific tuning of KCNQ1–KCNE compatibility. Introducing into KCNE0 an intracellular tetra-leucine motif analogous to that in KCNE4 markedly reduced KCNQ1 current amplitude, conferring a KCNE4-like inhibitory effect. Overall, this work provides a functional reference for comparing KCNE-dependent modulation of KCNQ1 across vertebrates and suggests an underlying compatibility mechanism.

Introduction

Modulation of ion channels by auxiliary subunits is essential for generating diverse physiological functions^{1,2}. KCNE proteins are single-pass transmembrane auxiliary subunits of the voltage-gated K⁺ channel KCNQ1 (Kv7.1). In jawed vertebrates, six members (KCNE1–6) have been identified^{3–10}. Among them, KCNE1 and KCNE3 are the best studied because they produce opposite effects on KCNQ1 gating with clear physiological roles. KCNE1 shifts the voltage dependence of KCNQ1 to more positive potentials to generate the slow delayed-rectifier K⁺ current (I_{Ks}), which is essential for ventricular repolarization and inner-ear K⁺ homeostasis^{11–14}. By contrast, KCNE3 shifts the voltage dependence to more negative potentials to produce a constitutively active current that

supports epithelial K⁺ recycling^{1,2,7}. Other KCNE subunits also modulate KCNQ1 gating, but their physiological roles are not well understood. For example, KCNE2 produces a constitutively active current, whereas KCNE5 and KCNE6 shift it to more positive potentials. KCNE4 suppresses KCNQ1 current^{2,10,15,16}.

Since the first KCNE member was cloned from rat kidney (originally described as minK; rat *Kcne1*)³, KCNE subunits have been identified and characterized not only in humans but also in a variety of non-human jawed vertebrates, including mice^{7,17}, horses¹⁸, frogs¹⁹, and zebrafish^{20,21}. Comparative genomic analyses have not identified canonical KCNE genes in invertebrates²², although *Caenorhabditis elegans* encodes *mip1–4*, nematode-specific single-pass membrane proteins that are sometimes described as MiRP/KCNE-like^{23,24}. Together, these observations suggest that canonical KCNE genes expanded and became widespread after the rise of jawed vertebrates²². However, KCNE subunits from early-diverging vertebrates remain functionally uncharacterized, limiting our understanding of how KCNE-dependent KCNQ1 modulation originated and diversified.

To address this gap, we searched for KCNE-like sequences outside jawed vertebrates and found a single *KCNE*-like gene in lamprey, a cyclostome (jawless vertebrate) that diverged early in the vertebrate lineage^{25,26}. RT-PCR and re-analysis of deposited RNA-seq data²⁷ detected transcripts of *kcnq1* and the KCNE-like gene in multiple lamprey organs. When co-expressed with the lamprey KCNQ1, this lamprey KCNE-like subunit produced constitutive activity, similar to KCNE3, and modestly reduced current amplitude. By contrast, its effect on KCNQ1 from other species was weaker, suggesting species-specific compatibility between KCNQ1 and KCNE. Introducing into the lamprey subunit a short intracellular tetra-leucine motif homologous to that in human KCNE4 markedly reduced KCNQ1 current, conferring a KCNE4-like inhibitory effect. Based on these sequences and functional data, we designate this subunit KCNE0 and propose that it represents an early-diverging member of the KCNE family.

Results

Characterization of lamprey KCNE0

Guided by previous comparative analyses that have not identified canonical KCNE family genes in invertebrates²², we surveyed cyclostomes, the extant jawless vertebrates including lampreys and hagfishes, as an early-diverging vertebrate group^{25,26}. Public genome databases at NCBI and Ensembl revealed no annotated *kcne*-like sequences in hagfish assemblies, whereas a single *kcne*-like gene was present in two lamprey species, sea lamprey (*Petromyzon marinus*, Pm) and Far Eastern brook lamprey (*Lethenteron reissneri*, Lr), together with *kcnq1*. We additionally obtained Arctic lamprey (*Lethenteron camtschaticum*, Lc) and cloned its *kcne* and *kcnq1* cDNAs using the sea lamprey and Far Eastern brook lamprey sequences as templates (Supplementary Figs. 1 [↗](#) and 2 [↗](#)). The three lamprey KCNE proteins share ~95% amino-acid identity (Fig. 1A–C [↗](#)). In the Ensembl genome browser, the sea lamprey *kcne* locus lies near the *rab20* locus (Fig. 1D [↗](#)). Pairwise alignments against human KCNE1–5 and zebrafish KCNE6 showed moderate identity but did not unambiguously match a single isoform (Fig. 1A–C [↗](#)). Therefore, we refer to the lamprey subunit as KCNE0 throughout for clarity.

To explore whether *kcne0* is co-expressed with *kcnq1* at the transcript level across lamprey tissues, we next examined the organ distribution of *kcnq1* and *kcne0* transcripts in lamprey. We analyzed deposited RNA-seq data from Far Eastern brook lamprey (*Lethenteron reissneri*) (BioProject PRJNA558325; SRX6711574–SRX6711583) covering 10 organs²⁷ and quantified transcript levels. Transcripts of *kcnq1* and *kcne0* were detected in most tissues at low levels (approximately 1–1.5 counts per million, CPM), whereas testis showed lower *kcnq1* levels and *kcne0* was not detected under our analysis threshold (Fig. 1E [↗](#)). We next performed RT-PCR on cDNA synthesized from total RNA isolated from six organs of Arctic lamprey (*Lethenteron camtschaticum*) and detected amplicons for both *kcnq1* and *kcne0* (Fig. 1F [↗](#) and Supplementary Fig. 3 [↗](#)). Although these data do not demonstrate protein expression or native complex formation, they indicate that *kcnq1* and *kcne0* transcripts show a broadly overlapping organ distribution in at least two lamprey species.

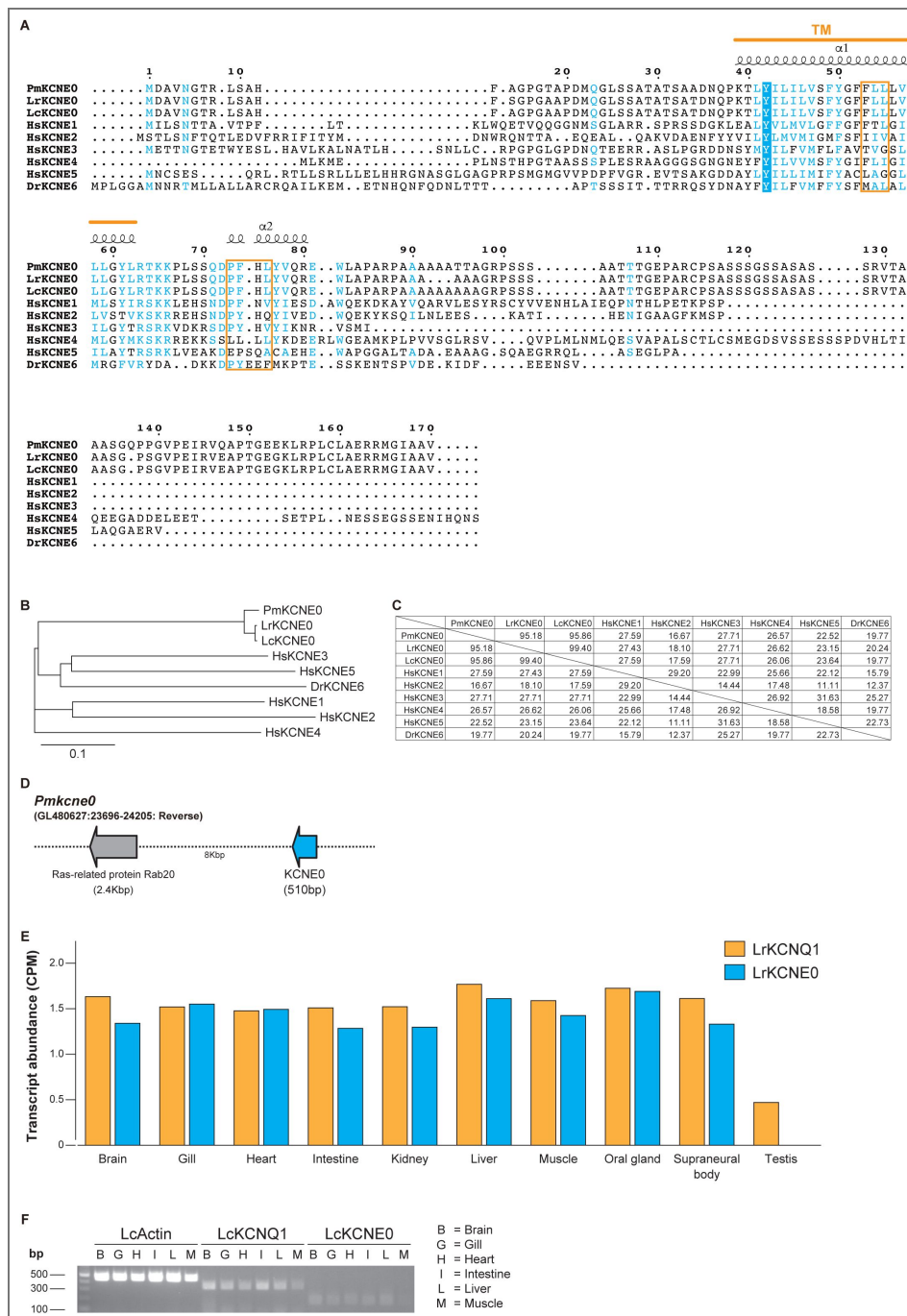


Figure 1. Sequence, genomic context, and transcript distribution of lamprey KCNE0.

(A–C) Amino-acid sequence alignment (A), phylogenetic tree (B), and percent identity (C) of lamprey and human KCNE subunits. Alignments were generated with Clustal Omega⁶¹ and displayed using ESPrnt3⁶². Residues corresponding to the “triplet”^{42,43} motif are highlighted with an orange square. (D) Genomic region containing the *kcne* locus in sea lamprey, shown from the Ensembl genome browser⁶³. For sequence comparison, KCNE0 from lamprey species (sea lamprey, PmKCNE0; NCBI Accession Number XP_032831116.1; Far Eastern brook lamprey, LrKCNE0; XP_061431545.1 and Arctic lamprey, LcKCNE0; see Supplementary Fig. 2⁴), the five human KCNE subunits (HsKCNE1, NP_000210.2; HsKCNE2, NP_751951.1; HsKCNE3, NP_005463.1; HsKCNE4, NP_542402.4; and HsKCNE5, NP_036414.1), and zebrafish KCNE6 (DrKCNE6)¹⁰ were used. (E) RNA-seq-based transcript levels of KCNQ1 (XM_061564454.1) and KCNE0 (XM_061575561.1) from 10 organs of Far Eastern brook lamprey quantified using a decoy-aware Salmon index. Values are shown as counts per million of the library (CPM). (F) RT-PCR detection of KCNQ1 and KCNE0 transcripts using cDNA synthesized from total RNA isolated from six organs of Arctic lamprey.

To investigate how KCNE0 modulates KCNQ1 gating in lamprey, we used two-electrode voltage clamp (TEVC) in *Xenopus laevis* oocytes. When expressed alone, all three lamprey KCNQ1 channels (PmKCNQ1 WT, LrKCNQ1 WT, and LcKCNQ1 WT) showed voltage-dependent activation with a sigmoidal conductance–voltage (G–V) relationship, comparable to KCNQ1 from other species. Their $V_{1/2}$ values differed modestly but significantly, with PmKCNQ1 activating at slightly more negative voltages than LrKCNQ1 and LcKCNQ1 (PmKCNQ1 WT, -35.8 ± 0.9 mV; LrKCNQ1 WT, -32.9 ± 0.6 mV; LcKCNQ1 WT, -32.1 ± 0.6 mV; $n = 5$ each; Supplementary Table 1). By contrast, co-expression with their corresponding KCNE0 WT subunits produced a constitutively active current, resembling the mammalian KCNQ1–KCNE3 complex (Fig. 2A–G; Supplementary Table 1).

To examine whether the KCNE0-dependent G–V changes arise from altered voltage-sensor movements, we performed voltage-clamp fluorometry (VCF). VCF monitors fluorescence from a dye attached near the fourth transmembrane segment (S4), the principal voltage sensor of KCNQ1, so changes in the fluorescence–voltage (F–V) relationship report conformational movements of the voltage-sensor domain (VSD) and allow direct comparison of F–V and G–V relationships in the same construct²⁸. Because the PmKCNQ1/PmKCNE0 pair produced the most consistent recordings, we focused on this pair for VCF and subsequent biophysical analyses. We generated a VCF construct (PmKCNQ1 C205A/G210C; PmKCNQ1_{vcf} WT) by introducing a cysteine mutation at position G210 in the extracellular S3–S4 loop, aligned with the site commonly used for VCF in human KCNQ1 (G219)^{29,30} (Supplementary Fig. 4). Previous VCF studies of human KCNQ1 have shown that KCNQ1 expressed alone often exhibits an F–V relationship that is well described by a double-Boltzmann function, consistent with stepwise VSD activation. Within the established six-state gating framework of KCNQ1, channel gating is governed by three VSD positions (down, intermediate, and up/activated) and two pore domain (PD) conformations (closed and open). Accordingly, KCNQ1 can open in an intermediate-open (IO) and an activated-open (AO) state during VSD activation^{31–33}. In such recordings, the dominant fluorescence component (F_1), corresponding to the transition from down to intermediate VSD positions, typically overlaps with the G–V relationship.

In our analysis, PmKCNQ1_{vcf} WT expressed alone showed overall features similar to human KCNQ1. Its G–V relationship was fit with a single-Boltzmann function, whereas the F–V relationship was fit with a double-Boltzmann function. The dominant F_1 overlapped with the G–V relationship, consistent with channel opening within the established IO/AO framework^{31–33} (Fig. 2H–J; Supplementary Table 1). By contrast, co-expression of PmKCNE0 altered the fluorescence behavior of PmKCNQ1_{vcf} WT. The G–V relationship remained close to 1 over the tested voltage range, whereas the F–V relationship was best described by an apparent single-Boltzmann function. This suggests that the down-to-intermediate VSD transition occurs at voltages more negative than those accessible in our recordings, such that the corresponding fluorescence component was not resolved. Accordingly, the observed F–V relationship indicates stabilization of an intermediate-like VSD conformation (Fig. 2H–J; Supplementary Table 1). Consistent with this interpretation, similar KCNE-dependent changes in KCNQ1 VSD motion have been widely reported, in which KCNE subunits alter VSD-PD coupling and stabilize distinct VSD positions. For example, KCNE1 stabilizes an intermediate closed (IC) state, whereas KCNE3 stabilizes an intermediate open (IO) state at most physiological voltage ranges^{31,33–39}. Taken together, these results indicate that lamprey KCNE0 modulates KCNQ1 gating, at least in part, by altering VSD movement, consistent with mechanisms described for the mammalian KCNQ1–KCNE3 complex.

Next, to identify which regions of KCNE0 are required for modulating KCNQ1 gating, we generated a series of PmKCNE0 truncations at the N and C termini and co-expressed them with PmKCNQ1 WT (Fig. 3A). For N-terminal truncations, removing as few as six residues (PmKCNE0ΔN6) largely abolished modulation of PmKCNQ1 (Fig. 3B–F; Supplementary Table 1). For C-terminal truncations, the modulatory effect decreased progressively as the C terminus was shortened and was nearly lost in the construct lacking 105 residues (PmKCNE0ΔC105) (Fig. 3G–N; Supplementary Table 1). Confocal imaging indicated that neither N- nor C-terminal truncations

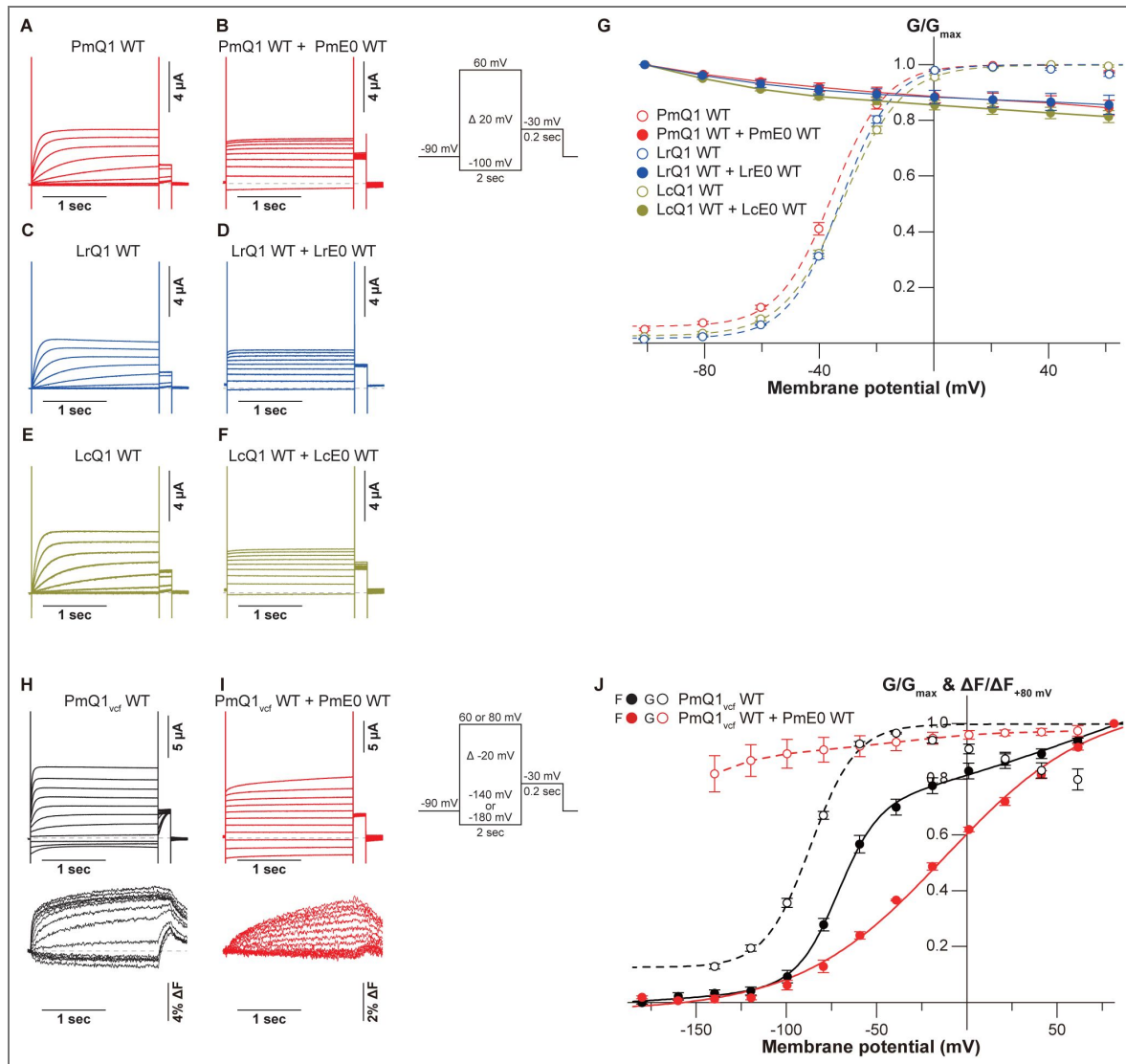


Figure 2. Biophysical properties of lamprey KCNQ1 and modulation by KCNE0.

(A–G) Representative current traces (A–F) and G–V relationships of lamprey KCNQ1 WT expressed alone or co-expressed with the corresponding KCNE0 WT. (H–J) Representative ionic-current and fluorescence traces (H,I) and F–V relationship (J) of PmKCNQ1_{vcf} WT expressed alone or co-expressed with PmKCNE0 WT. Error bars denote mean $\pm$ s.e.m. for $n = 5$ in (G,J). Statistical comparisons of G–V $V_{1/2}$ values for lamprey KCNQ1 WT expressed alone are provided in Supplementary Table 1. $V_{1/2}$ values were not determined for KCNQ1–KCNE0 complexes because the G–V relationships were nearly saturated over the tested voltage range.

detectably altered membrane localization under our conditions (Fig. 3O–T). Overall, the KCNE0 N-terminus is highly sensitive to truncation for proper modulation of KCNQ1, while the C-terminus is more tolerant and loses most of its effect only after deleting 105 residues (Δ C105).

Species-specific tuning of KCNQ1–KCNE compatibility

Species-specific tuning of KCNQ1–KCNE interactions has been observed across chordates. For example, we previously reported that KCNQ1 from the vase tunicate *Ciona intestinalis* (CiKCNQ1) is not effectively modulated by mammalian KCNE subunits, including KCNE1 and KCNE3⁴⁰. These findings suggest that functional compatibility between KCNQ1 and KCNE subunits is tuned in a species-specific manner. To examine whether KCNE0 modulation also exhibits such species-specific tuning, we performed cross-pairing experiments. We co-expressed PmKCNE0 WT with KCNQ1 WTs from human (*Homo sapiens*, Hs), zebrafish (*Danio rerio*, Dr), and vase tunicate (*Ciona intestinalis*, Ci). PmKCNE0 modulated HsKCNQ1 and DrKCNQ1, but the effects were weaker and less consistent than those observed with PmKCNQ1. In HsKCNQ1, PmKCNE0 produced a mixed effect, with partial constitutive activity at negative voltages and a positive shift of the half-activation voltage ($V_{1/2}$; HsKCNQ1 WT, -26.5 ± 0.8 mV; HsKCNQ1 WT–PmKCNE0 WT, 14.6 ± 1.5 mV; $n = 5$ each) (Fig. 4A–C; Supplementary Table 1). In DrKCNQ1, PmKCNE0 produced partial constitutive activity at negative voltages with only a mild change in $V_{1/2}$ (DrKCNQ1 WT, -44.2 ± 1.1 mV; DrKCNQ1 WT–PmKCNE0 WT, -40.4 ± 1.3 mV; $n = 5$ each) (Fig. 4D–F; Supplementary Table 1). By contrast, CiKCNQ1 was not detectably modulated by PmKCNE0 under our conditions (Fig. 4G–I; Supplementary Table 1).

Reciprocally, we tested whether lamprey KCNQ1 is efficiently modulated by human KCNE subunits. Co-expression of PmKCNQ1 with HsKCNE1 produced only a small positive shift of $V_{1/2}$ (PmKCNQ1 WT, -35.8 ± 0.9 mV; PmKCNQ1 WT–HsKCNE1 WT, -19.3 ± 1.5 mV; $n = 5$ each) (Fig. 4J, L; Supplementary Table 1), which is markedly smaller than the strong positive shift typically observed for HsKCNQ1 with HsKCNE1 (about +50 mV positive shift)^{1,3}. Likewise, pairing PmKCNQ1 with HsKCNE3 produced only a small negative shift of $V_{1/2}$ (PmKCNQ1 WT, same as above; PmKCNQ1 WT–HsKCNE3 WT, -41.3 ± 1.2 mV; $n = 5$) and partial constitutive activity at negative voltages (Fig. 4K, L; Supplementary Table 1), weaker than the robust constitutive effect typically observed for HsKCNQ1 with HsKCNE3^{1,7}. Together, these cross-species results support the idea that KCNQ1–KCNE functional compatibility is tuned in a species-specific manner.

Intracellular leucine substitutions shift KCNE0 toward a KCNE4-like inhibitory effect

Because KCNE subunits can show diverse effects on KCNQ1 gating^{3–10}, we asked whether the early-diverging KCNE0 phenotype can be shifted by a small number of mutations. We first tested a KCNE4-related intracellular leucine motif that has been linked to KCNQ1 inhibition. KCNE4 contains a tetra-leucine sequence in a juxtamembrane intracellular region that is required for interaction with calmodulin and functional suppression of KCNQ1⁴¹ (Fig. 5A, B). Because PmKCNE0 already contains one leucine in the corresponding region (L76), we first added one leucine just before L76 by introducing H75L. This single substitution strongly reduced PmKCNQ1 current amplitude compared with PmKCNE0 WT, while the remaining current still showed constitutive activity across the tested voltages (Fig. 5C–F). We next introduced P73L, F74L, and H75L to create a tetra-leucine stretch from L73 to L76 together with the native L76. This change further attenuated KCNE0-dependent activation and shifted the G–V relationship toward that of PmKCNQ1 expressed alone (Fig. 5C–F; Supplementary Table 1). These results show that KCNE0 can be shifted toward a KCNE4-like inhibitory effect by introducing leucine substitutions in a small intracellular region. By contrast, we tested whether KCNE0 could be shifted toward a KCNE1-like effect. A previous study³⁷ showed that the KCNE3 effect can be partially shifted toward a KCNE1-like effect by mutations in the middle of the KCNE transmembrane segment (the “triplet” motif)^{42,43} (Supplementary Fig. 5A, B; Supplementary Table 1). We therefore introduced an HsKCNE1-like change into the corresponding region of PmKCNE0 (PmKCNE0 L54T). However, this

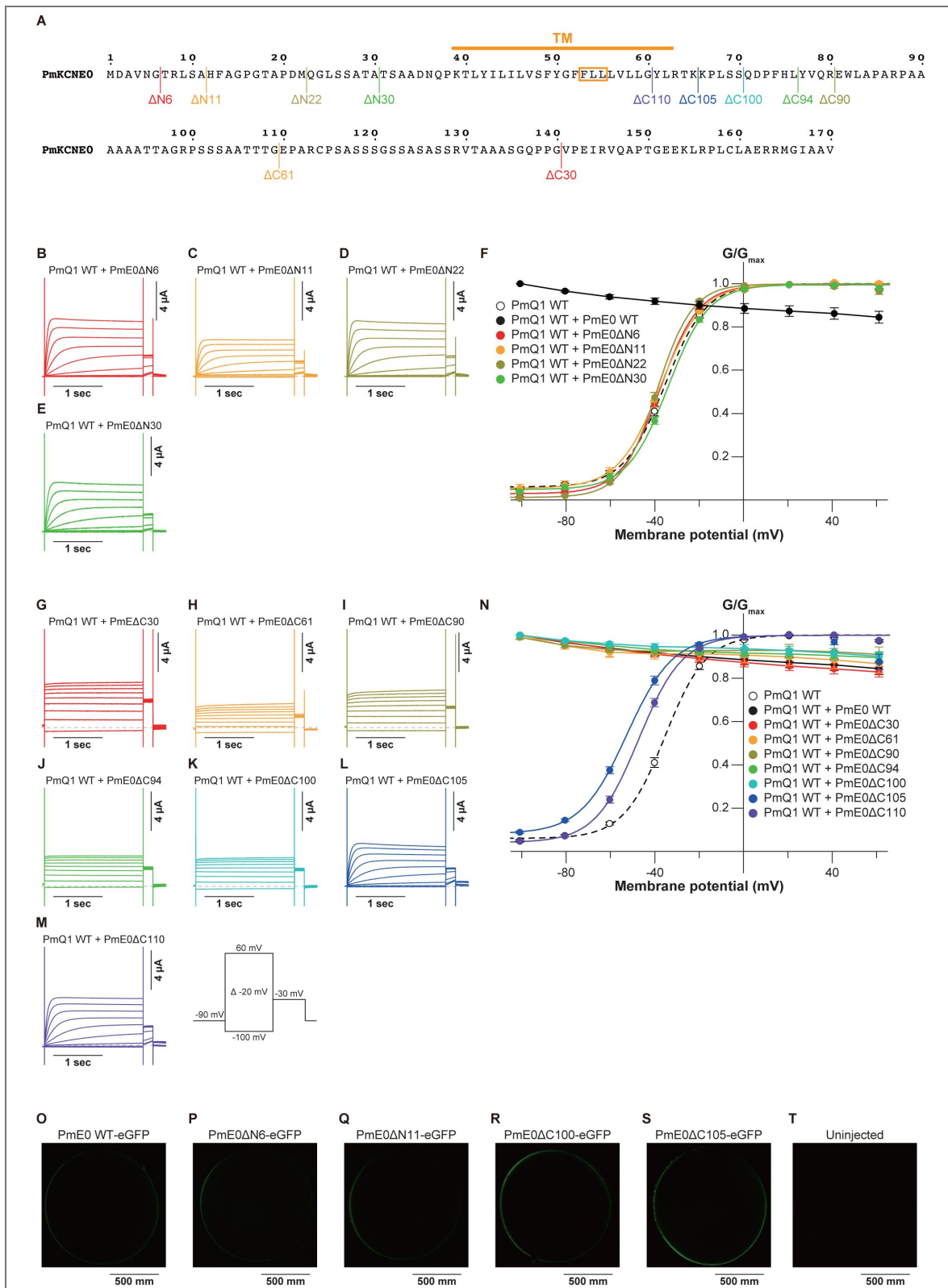


Figure 3. Regions of KCNE0 important for KCNQ1 modulation.

(A) Schematic diagram of N- and C-terminal truncation constructs of PmKCNE0. (B-F) Representative current traces (B-E) and G-V relationship (F) of PmKCNQ1 WT expressed alone or co-expressed with each of the N-terminally truncated PmKCNE0 constructs. (G-N) Representative current traces (G-M) and G-V relationship (N) of PmKCNQ1 WT expressed alone or co-expressed with each of the C-terminally truncated PmKCNE0 constructs. Error bars denote mean $\pm$ s.e.m. for $n = 5$ in (F,N). (O-T) Confocal images of oocytes expressing C-terminally eGFP-tagged PmKCNE0 WT or truncation constructs, and an uninjected oocyte (control).

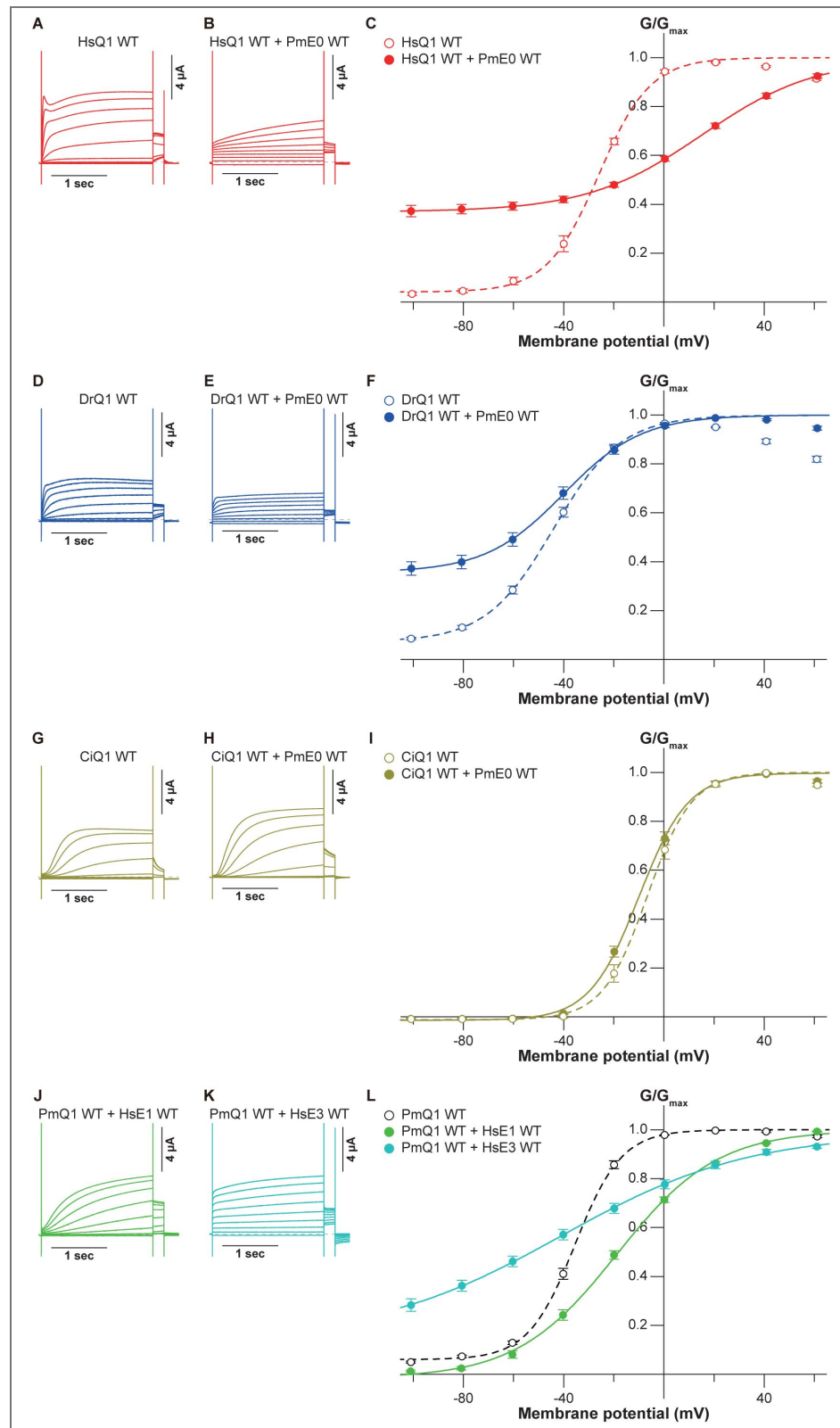


Figure 4. Species dependence of KCNQ1-KCNE compatibility.

(A–C) Representative current traces (A,B) and G–V relationship (C) of HsKCNQ1 WT expressed alone or co-expressed with PmKCNE0 WT. (D–F) Representative current traces (D,E) and G–V relationship (F) of DrKCNQ1 WT expressed alone or co-expressed with PmKCNE0 WT. (G–I) Representative current traces (G,H) and G–V relationship (I) of CiKCNQ1 WT expressed alone or co-expressed with PmKCNE0 WT. (J–L) Representative current traces (J,K) and G–V relationship (L) of PmKCNQ1 WT co-expressed with HsKCNE1 WT or HsKCNE3 WT. Error bars denote mean $\pm$ s.e.m. for $n = 5$ in (C,F,I,L).

mutant produced a constitutively active current when co-expressed with PmKCNQ1, similar to PmKCNE0 WT (Supplementary Fig. 5C, D [↗](#)), indicating that a KCNE1-like modulatory effect cannot be readily recapitulated by introducing a single motif analogous to that of human KCNE1.

Discussion

In this study, we functionally characterized a KCNE subunit from lamprey and designated it KCNE0. Because cyclostomes, the extant jawless vertebrates including lampreys and hagfishes, represent an early-diverging vertebrate lineage^{25,26}, our results provide a useful reference point for understanding how KCNE-dependent modulation of KCNQ1 can operate outside jawed vertebrates. The lamprey KCNE subunit shows moderate amino acid sequence similarity to human KCNE1–5 and zebrafish KCNE6 but is not particularly similar to any single isoform (Fig. 1 [↗](#)). Therefore, this subunit cannot be confidently assigned to a specific KCNE isoform based on sequence alone, and we use “KCNE0” to denote this lamprey KCNE subunit. Importantly, this naming does not imply a strict one-to-one evolutionary relationship with any particular jawed-vertebrate isoform.

Using deposited RNA-seq data from Far Eastern brook lamprey²⁷ and RT-PCR in Arctic lamprey, we detected transcripts of both *kcnq1* and *kcne0* in multiple organs (Fig. 1E, F [↗](#)). Although transcript detection by RNA-seq or RT-PCR does not directly demonstrate protein expression, subcellular localization, or native complex assembly, the broad co-expression of transcripts supports the possibility that KCNQ1–KCNE0 complexes could operate in multiple lamprey tissues *in vivo*. This widespread expression contrasts with the more restricted and isoform-specific functions of KCNE subunits in humans, such as KCNE1, which is essential for ventricular repolarization and inner-ear K⁺ homeostasis, and KCNE3, which supports epithelial K⁺ recycling^{1,2,7}. In this context, it is notable that *KCNE*-like genes can show lineage-specific gains, losses, and pseudogenization. For example, KCNE6 is functional in lower jawed vertebrates, including marsupials, but becomes a pseudogene in eutherians¹⁰, and a KCNE1-related pseudogene (KCNE1P) has been reported in zebra finch⁴⁴. Consistent with these observations, one possible interpretation is that KCNE0 represents a relatively unspecialized KCNE subunit whose function and expression remain less restricted across tissues than those observed in jawed vertebrates, potentially allowing flexible pairing with KCNQ1 in different cellular contexts. Accordingly, in this study, we focus on the biophysical properties of KCNE0, particularly its effects on KCNQ1 in a heterologous expression system, while questions regarding its native physiological roles in lamprey tissues are left for future work.

When co-expressed with lamprey KCNQ1, KCNE0 produced a constitutively active current, resembling the effect typically induced by KCNE3 on mammalian KCNQ1^{1,2,7} (Fig. 2A–G [↗](#); Supplementary Table 1). Our voltage-clamp fluorometry (VCF) experiments further support that KCNE0 modulates lamprey KCNQ1 gating by altering voltage-sensing domain (VSD) movement, consistent with how human KCNE subunits modulate KCNQ1 gating^{31,33,34,36–39} (Fig. 2H–I [↗](#); Supplementary Table 1). An important observation is that KCNQ1–KCNE function is strongly dependent on the pairing of the two partners. In the native lamprey pairing, KCNE0 produced a constitutively active current accompanied by an apparent negative shift of the G–V relationship, whereas cross-species combinations showed weaker, mixed, or no effects on KCNQ1 modulation (Fig. 4 [↗](#); Supplementary Table 1). Previous cryo-electron microscopic structures^{45–47}, together with a subsequent biophysical analysis³⁹ showed extensive contacts between the KCNE transmembrane segment and the first transmembrane segment (S1) of KCNQ1 in the KCNQ1–KCNE complexes. However, the S1 segment is highly conserved among KCNQ1 orthologues across species. In contrast, the KCNQ1 proteins used in this study vary in amino-acid length, largely reflecting differences in the cytoplasmic C-terminal region (HsKCNQ1, 676 amino acids; DrKCNQ1, 575 amino acids; PmKCNQ1, 642 amino acids; LrKCNQ1, 644 amino acids; LcKCNQ1, 644 amino acids; CiKCNQ1, 520 amino acids) (Supplementary Fig. 4 [↗](#)). Therefore, the species-specific effects observed in cross-species pairing experiments are unlikely to be explained by KCNE0 alone. Instead, they probably reflect compatibility between KCNE0 and species-specific features of KCNQ1 α -subunits, including regions beyond the conserved S1 segment, subtle differences within

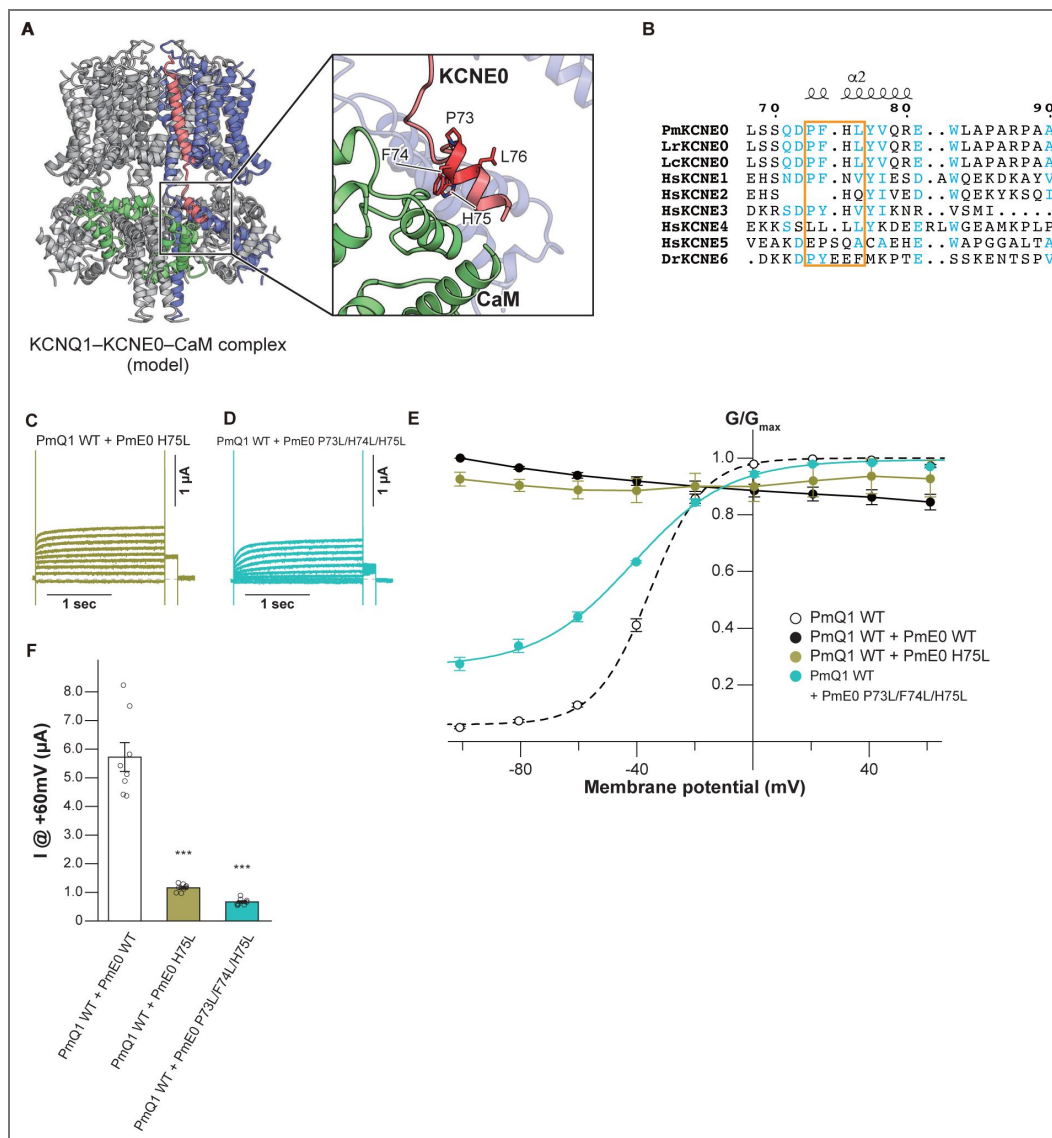


Figure 5. Intracellular leucine substitutions shift KCNE0 modulation toward a KCNE4-like inhibitory effect.

(A) Close-up view of the interface between KCNQ1 and KCNE0 within the PmKCNQ1-PmKCNE0-PmCaM complex structure generated by SwissModel server⁶⁴. The model was built with amino acid sequences of PmKCNQ1 (XP_075921450.1), PmKCNE0 (XP_032831116.1), and PmCaM (XP_032811771.1), using the human KCNQ1-KCNE3-CaM structure (PDB: 6V00) as a template. One KCNQ1, KCNE0 and CaM subunit are shown in blue, red, and green. The other regions are shown in gray. Residues used for motif-based mutagenesis are shown as sticks. Molecular graphics were prepared with CueMol (<http://www.cuemol.org/>). (B) Sequence alignment around the intracellular region corresponding to the KCNE4-related juxtamembrane tetra-leucine motif⁴¹. (C-F) Representative current traces (C,D), G-V relationship (E), and current amplitude at +60 mV (F) of PmKCNQ1 WT co-expressed with PmKCNE0 WT, PmKCNE0 H75L, or PmKCNE0 P73L/F74L/H75L. In (E), error bars denote mean $\pm$ s.e.m. for $n = 5$. In (F), error bars denote mean $\pm$ s.e.m. for $n = 8$. Statistical significance among the three constructs was assessed using one-way ANOVA followed by Tukey-Kramer multiple-comparison test. Significant differences are indicated by asterisks (*** $P < 0.001$).

the broader transmembrane interface, and/or differences in intracellular regions that influence channel gating and coupling to KCNE subunits. Thus, sequence similarity around S1 alone is not sufficient to predict functional outcomes across distant lineages, and future structure-guided analyses will be needed to identify the KCNQ1-side determinants of KCNE compatibility.

Another mechanistic insight from this study is that KCNE0 can be shifted toward a KCNE4-like inhibitory effect by changes in a short intracellular region. Guided by prior work⁴¹ showing that KCNE4 contains a juxtamembrane tetra-leucine sequence required for interaction with calmodulin and functional suppression of KCNQ1 (Fig. 5A, B [↗](#)), introducing leucine substitutions that create a local tetra-leucine stretch in the corresponding region of KCNE0 markedly reduced KCNQ1 current amplitude and shifted the G–V relationship toward that of KCNQ1 expressed alone (Fig. 5C–F [↗](#); Supplementary Table 1). One possible explanation for the ability of KCNE0 to acquire a KCNE4-like inhibitory effect is the presence of an extended intracellular region. Among KCNE family members, KCNE4 is distinguished by a long cytoplasmic tail that is essential for its inhibitory action on KCNQ1 (Fig. 1A [↗](#)), including interactions mediated by a juxtamembrane tetra-leucine motif⁴¹. KCNE0 similarly possesses a relatively long intracellular region compared with other KCNE isoforms (Fig. 1A [↗](#)). This shared architectural feature may influence KCNQ1–CaM interaction in a manner similar to KCNE4, thereby enabling KCNE0 to adopt KCNE4-like inhibitory properties. Taken together, these observations suggest that KCNE0 may represent a functionally flexible KCNE subunit that has not yet reached the degree of specialization observed among KCNE isoforms in jawed vertebrates, possibly reflecting an ancestral state preceding lineage-specific subfunctionalization of KCNE genes.

Several limitations of this study define clear next steps. First, we characterized KCNE0 primarily as an auxiliary subunit for KCNQ1 because KCNQ1 is the best-characterized pore-forming α -subunit partner for KCNE proteins. However, previous studies have shown that KCNE subunits can modulate other voltage-gated K^+ channels^{48–53} and a Ca^{2+} -gated Cl^- channel (TMEM16A)⁵⁴ in heterologous expression systems, although at least one study failed to detect modulation of TMEM16A by KCNE1 under comparable conditions⁵⁵. Second, our expression evidence is transcript-based and does not yet demonstrate protein expression, subcellular localization, or *in vivo* complex formation. Therefore, future studies will be required to test whether KCNE0 is functional *in vivo* and to identify its physiological partner(s) and contexts. Nevertheless, the broad tissue distribution of *kcne0* transcripts, together with the ability of KCNE0 to render KCNQ1 constitutively active, similar to the effect of mammalian KCNE3, raises the possibility that KCNQ1–KCNE0 complexes contribute to general ion homeostasis, possibly including K^+ recycling in epithelial tissues, rather than to a highly specialized role like that of KCNQ1–KCNE1 in the mammalian heart and inner ear. Third, while cross-species pairing experiments support species-specific tuning of compatibility, we do not yet know which precise interface features encode compatibility across lineages. Addressing these questions will require additional structure-guided biophysical analyses.

In summary, these findings provide a framework for comparative studies of KCNE-dependent KCNQ1 modulation across vertebrate lineages and suggest that KCNE0 represents a relatively unspecialized KCNE subunit, potentially reflecting an ancestral stage preceding lineage-specific subfunctionalization of KCNE genes in jawed vertebrates.

Materials and methods

Protein expression in *Xenopus laevis* oocytes

The coding regions of KCNQ1 (human, NCBI Accession Number NM_000218.3; zebrafish, NM_001123242.2; sea lamprey, XM_076065335.1; Far Eastern brook lamprey, XM_061564454.1; Arctic lamprey, see Supplementary Fig. 1 [↗](#); vase tunicate, NM_001160065), KCNE0 (sea lamprey, XM_032975225.1; Far Eastern brook lamprey, XM_061575561.1; Arctic lamprey, see Supplementary Fig. 2 [↗](#)), human KCNE1 (NM_000219.6), and human KCNE3 (NM_005472.5) were cloned into the pGEMHE vector⁵⁶. cRNA was transcribed using the HiScribe[®] T7 ARCA mRNA Kit (New England Biolabs, E2065S). Female *Xenopus laevis* frogs were anesthetized in water containing 0.1% tricaine

(Sigma-Aldrich, E10521) for 15–30 min, and oocytes were surgically isolated. Follicle layers were removed by collagenase treatment (Sigma-Aldrich, C0130) for 5–6 h at room temperature. Defolliculated oocytes of similar size at stage V–VI were selected, microinjected with 50 nL of cRNA solution (2–10 ng for KCNQ1 and 1 ng for KCNE) using a Nanoject II (Drummond Scientific), and incubated at 18 °C in Barth's solution (88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO₃, 10 mM HEPES, 0.3 mM Ca(NO₃)₂, 0.41 mM CaCl₂, and 0.82 mM MgSO₄, pH 7.6) supplemented with 0.1% penicillin - streptomycin solution (Sigma-Aldrich, P4333). All procedures involving *Xenopus laevis* were approved by the Animal Care Committee of Jichi Medical University (protocol 21030-04) and complied with institutional guidelines.

Two-electrode voltage clamp (TEVC) recordings

Oocytes were recorded 1–3 days after injection using an OC-725C amplifier (Warner Instruments) at room temperature. The bath was continuously perfused with Ca²⁺-free ND96 (96 mM NaCl, 2 mM KCl, 2.8 mM MgCl₂, 5 mM HEPES, pH 7.6) containing 100 μM LaCl₃ to suppress endogenous hyperpolarization-activated currents^{29,35,39}. Microelectrodes were pulled from borosilicate glass capillaries (Harvard Apparatus, GC150TF-10) using a P-1000 micropipette puller (Sutter Instrument) to 0.2–1.0 MΩ and filled with 3 M KCl. From a holding potential of -90 mV, currents were elicited by voltage steps from -100 to +60 mV in +20 mV increments with 2 s step duration and 10 s intervals. Oocytes with a holding current < -0.4 μA at -90 mV were excluded. Protocol generation and data acquisition were performed using a Digidata 1550 (Molecular Devices) controlled by pCLAMP 10.7. Signals were sampled at 10 kHz and low-pass filtered at 1 kHz.

Voltage dependence analysis (G-V)

G-V relationships were obtained from tail current amplitudes at -30 mV. Fits were performed in pCLAMP 10.7 (Molecular Devices) to a single-Boltzmann function:

$$G = G_{\min} + (G_{\max} - G_{\min}) / (1 + e^{-zF(V-V_{1/2})/RT})$$

where $G_{\max}$ and $G_{\min}$ are the maximal and minimal tail conductances, z is the effective gating charge, $V_{1/2}$ is the half-activation voltage, T is absolute temperature, F is Faraday's constant, and R is the gas constant. Normalized conductance ($G/G_{\max}$) was plotted against voltage for presentation.

Voltage-clamp fluorometry

Oocytes expressing constructs for VCF were incubated for 2–4 days after injection. Labeling was performed for 30 min in KD98 solution (98 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, pH 7.6) with 5 μM Alexa FluorTM 488 C₅ maleimide (Thermo Fisher Scientific, A10254), and unreacted dye was removed by washing with Ca²⁺-free ND96 solution^{29,35,39}. Microelectrodes were pulled from borosilicate glass capillaries (Harvard Apparatus, GC150TF-15), as in TEVC recordings. From a holding potential of -90 mV, currents and fluorescence signals were recorded during voltage steps from +80 to -180 mV, or from +60 mV to -140 mV where indicated, in -20 mV increments with 2 s step duration and 20 s intervals. Oocytes with a holding current < -0.4 μA at -90 mV were excluded, as in TEVC recordings. Protocol generation and data acquisition were performed using a Digidata 1440A (Molecular Devices) controlled by pCLAMP 10.7. Ionic currents were sampled at 10 kHz and low-pass filtered at 1 kHz. Fluorescence signals were digitized at 1 kHz through the Digidata1440A and low-pass filtered at 50 Hz.

Fluorescence recordings were obtained with an MVX10 macrozoom microscope (Olympus) equipped with a 2x objective lens (MVPLAPO 2XC, NA = 0.5, Olympus), a 2x magnification changer (MVX-CA2X, Olympus), a GFP filter cube (U-MGFPHQ/XL, Olympus), and an XLED1 light source with a BDX 450-495 nm LED module (Excelitas Technologies). Fluorescence was detected with a photomultiplier (H10722-110, Hamamatsu Photonics) and recorded in pCLAMP 10.7 simultaneously with ionic currents. The excitation shutter remained open during recordings,

which caused a gradual decrease in fluorescence due to photobleaching. For each trace, the bleaching rate (R) was estimated from the 1.1 s baseline preceding the test pulse and traces were corrected assuming a linear decrease:

$$F_{\text{corrected}} = F_{\text{raw}} \times (1 - R \times t)^{35,39}$$

Corrected traces were then baseline-normalized to 1 at the pre-step level.

VCF analysis

F–V relationships were obtained by plotting the fluorescence change from the baseline (ΔF) against membrane potential. For presentation (Fig. 2), ΔF values were normalized to the response at +80 mV ($\Delta F_{+80\text{mV}}$). Fits were performed in Igor Pro (WaveMetrics). KCNQ1 alone was fit with a double-Boltzmann function, whereas KCNQ1 co-expressed with KCNE0 was fit with a single-Boltzmann function.

Single-Boltzmann function:

$$F = F_{\min} + (F_{\max} - F_{\min}) / (1 + e^{-zF(V - V_{1/2(F)})/RT})$$

Double-Boltzmann function:

$$F = F_{\min} + (F_1 - F_{\min}) / (1 + e^{-z_1 F(V - v_{1/2(F1)})/R1}) + (F_2 - F_1) / (1 + e^{-z_2 F(V - v_{1/2(F2)})}) R^1$$

where $F_{\min}$, F_1 , F_2 , and $F_{\max}$ denote the baseline, intermediate, and maximal fluorescence components, zF is the effective gating charge for the fluorescence component, $V_{1/2(F)}$, $V_{1/2(F1)}$, and $V_{1/2(F2)}$ are the half-activation voltages for the fluorescence components, T is absolute temperature, F is Faraday's constant, and R is the gas constant. Normalized fluorescence change ($\Delta F/\Delta F_{+80\text{mV}}$) was plotted against voltage for presentation.

Isolation of cDNAs, cloning, and RT-PCR from Arctic lamprey

Arctic lampreys (*Lethenteron camtschaticum*) captured in the Ishikari River (Hokkaido, Japan) were commercially obtained and maintained on 12-h light/12-h dark cycles at 4 °C. Total RNA was extracted from the indicated organs using NucleoSpin RNA Plus (MACHEREY-NAGEL, 740984) and reverse-transcribed using PrimeScript II (TaKaRa, 6210) according to the manufacturer's instructions. The resulting cDNA was used as a template for PCR amplification. PCR for cloning was performed using KOD One PCR Master Mix (TOYOBO, KMM-101), and RT-PCR of β -actin, KCNQ1, and KCNE0 was performed using PrimeSTAR Max DNA Polymerase (TaKaRa, R045A), according to the manufacturers' protocols.

For cloning of KCNQ1 and KCNE0, we performed two-step PCR using the following primers

KCNQ1 1st Fw 5'-ATGTCACACGAAAGCGAAGTTCTTCTCACAGAGG-3'

KCNQ1 1st Rv 5'-ACAGCTGTGCTGTTGGCTGAAAGGTATGTGGGCGC-3'

KCNQ1 2nd Fw 5'-AGTGCGGAGCCACCATGTACACGAAAGCGAAG-3'

KCNQ1 2nd Rv 5'-GTCGCGGCCGCTTTAACAGCTGTGCTGTTGGCTGA-3'

KCNE0 1st Fw 5'-GACACGGAGAGAGCGAGCGCCGCGAC-3'

KCNE0 1st Rv 5'-AGGGGCTGGAGGTTAGGAGCTGGGCCC-3'

KCNE0 2nd Fw 5'-AGTGCGGAGCCACCGACGAGAGAGCGAGCGC-3'

KCNE0 2nd Rv 5'-GTCGCGGCCGCTTTAAGGGGCTGGAGGTTAGGAGC-3'

For RT-PCR we used the following primers

β -actin Fw 5'-ACCCAGATCATGTTTGAGACC-3'

β -actin Rv 5'-GACTCCATGCCGATGAATGA-3'

KCNQ1 Fw 5'-CCTGGGTCTCATATTCTCATC-3'

KCNQ1 Rv 5'-TGACATCTCCACAGGCTCTG-3'

KCNE0 Fw 5'-ACATGCAGGGCCTCTCATCG-3'

KCNE0 Rv 5'-TGCACGTAGAGGTGGAACGG-3'

Analysis of deposited RNA-seq data from Far Eastern brook lamprey

The raw paired-end RNA-seq FASTQ files were downloaded from the Sequence Read Archive (SRA). The run identifiers were SRR9964076 (heart), SRR9964077 (gill), SRR9964078 (testis), SRR9964079 (brain), SRR9964080 (liver), SRR9964081 (oral gland), SRR9964082 (kidney), SRR9964083 (intestine), SRR9964084 (supraneural body), and SRR9964085 (muscle). Raw reads were processed for adapter removal, base-quality filtering, and per-read quality evaluation using fastp (v1.1.0)^{57,58}. Quality control reports were generated with FastQC (v0.12.1) and MultiQC (v1.33)⁵⁹ (Supplementary Table 2). A decoy-aware Salmon index was built from two target transcripts (KCNQ1, XM_061564454.1; KCNE0, XM_061575561.1) combined with RefSeq decoy sequences (GCF_015708825.1). Quantification was performed with Salmon (v1.10.3)⁶⁰ (Supplementary Table 3). Transcript-level estimates were summarized at the gene level and reported as counts per million (CPM).

Statistics and reproducibility

Data are presented as mean $\pm$ SEM (n = 5–8). In Figs. 2G⁴, 3F⁴, and 3N⁴, differences in G–V $V_{1/2}$ values among groups were assessed using one-way ANOVA followed by the Tukey–Kramer multiple-comparison test to evaluate pairwise differences. In Fig. 5F⁴, differences in current amplitudes among groups were assessed using one-way ANOVA followed by the Tukey–Kramer multiple-comparison test to evaluate pairwise differences. In Figs. 4C⁴, 4F⁴, 4I⁴, and 4L⁴, differences between two groups were assessed using unpaired two-tailed Welch's t-test. $V_{1/2}$ values were not statistically compared when reliable Boltzmann fits could not be obtained because the G–V relationships were nearly saturated over the tested voltage range. Statistical significance was defined as $P < 0.05$ (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Supplementary figures

LcKCNQ1 orf	
1 ATGTCACACGAAAGCGAAGTTCTTCTCACAGAGGGCCAGCCCGGCCATTACAGAGGCCACCTCCGTGGAGAGACTGCAGGTGCCGGTG	90
1 M S H G K R S S S H R G P A R A I Q E A T S V E R L Q V P V	30
91 TCACCTGTGCGCCTGGAGATGTCGAGACCGCAAGACGCGAGTCGGCGAGGCATCTTGGGACGACATGCTGGGCCGGGCCAGGGCCAG	180
31 S P V R L E M S E T A K T R V G E A S W D D M L G P G Q G Q	60
181 CATGTCGTCGAGACGACCGCAAGTGGCTCTACATGGCCTGCCCCATGACCCCGCAGAGGTCCGCGCTGTCCACGTACAGTCATCTG	270
61 H V V E T T P Q V A L H G L P H D P R R G R R V S T Y S H L	90
271 CTGATGTGCGGACGAACCTCCACCATCCAGTCCCGTGTGTATACTTCTGGAGCGGCCACCGGTGGAATGCTTCCCTTACCACCTTC	360
91 L M C G R T S T I Q S R V Y N F L E R P T G W K C F L Y H F	120
361 TCTGTGTTTCATGATGGTGTCTATCTGCCTTATATTACAGCTGTGTCCACCATCGAGCTGTACACAGACTTTGCCAACAGCACCTCTAT	450
121 S V F M M V L I C L I F S V L S T I E L Y T D F A N S T L Y	150
451 TGGATGGAATAGCACTGGTGGTCTTCTTTGGGGTGGAGTACCTGGTGCCTGTGGTGCAGCCGGCTGCAGGAGCAAGTACGTGGGTGTG	540
151 W M E I A L V V F F G V E Y L V R L W S A G C R S K Y V G V	180
541 ATGGGACGCGTCCGCTTTGGCTACAAGCCCATCTCCATCATCGATCTCGTGGTAGTGGTGGCTTCCATCATGTTCTGTGTGGGGTCC	630
181 M G R V R F A H K P I S I I D L V V V V A S I I V L C V G S	210
631 AACGGGAGGTATTTGCAACCTTGCCATAAGGGGATTCGCTTCTCCAGATCTGCGCATGTTCACGTGGACCGGCAAGGAGGTACC	720
211 N G Q V F A T S A I R G I R F L Q I L R M L H V D R Q G G T	240
721 TGGAGGCTCCTGGGCTCCGTCGCTTTTACCACGGCAGGAGTGATAACGACTCTGTACATTTGGCTTCCTGGGCTCATATTCTCATCG	810
241 W R L L G S V V F I H R Q E L I T T L Y I G F L G L I F S S	270
811 TACTTTGTGTACCTGGCCGAGAAGGATGAAGCCAGGAGAGAAGTGAATTTGCCAGCTATGCTGACGCACCTTTGGTGGGGGGTGGTGACA	900
271 Y F V Y L A E K D E A R E K N E F A S Y A D A L W W G V V T	300
901 GTGACGACGATAGGATATGGCGACAAGGTGCCACAGACCTGGATCGGCAAAACCATCGCATCTTGTCTCTAGTGTTCGCATCTCGCTT	990
301 V T T I G Y G D K V P Q T W I G K T I A S C F S V F A I S F	330
991 TTCGCCCTGCCTGCGGTATACTTGGCTCGGGATTTGCCCTAAAAGTACAACAAAAGCAGAGGCAGAAGCACTTCAACCGGCAATCCCA	1080
331 F A L P A G I L G S G F A L K V Q Q K Q R Q K H F N R Q I P	360
1081 GCGGCAGCAACCCGTGATCCAGAGCCTGTGGAGATGTCACTTGGCCGAGAGTGAGCACCCTCCACAGCCACGTGGATGATGTACGTGAAC	1170
361 A A A T L I Q S L W R C H L A E S E H H S T A T W M M Y V N	390
1171 GCGCATGCACAGCCGGCGGCCCTTTCCGAGACAGCAGTGGATTAGAAAGCAGGAAAAAGAGGACAAGAAGAGGAAAAAGCAGCTGATC	1260
391 A H A Q P G G P F R D S T W I R K Q E K E D K K R K K Q L I	420
1261 AAGCAGGCGAAGGGCGAAGGGCTCGTGAGCCCGGGGCACGGCAGAGTCCAGCACGTCCGAGGGCAACCACGCCAACCTCTGACAAC	1350
421 K Q A K G E G L V S P G A R Q R S S T S E G N H A N T P D N	450
1351 GGCACCAACTCGGCCACAGGACAGATCGAGGCGTGAAGCTCCGAGTGGACGCCCCACCCTCAATAGTAGCGGAGCTTTGACGACGGC	1440
451 G T N S A T G T D R G V E A P S G R P T L N S T A S F D D G	480
1441 GAGGCTCGGAGGACATCTCTTGCCGCGCTGATGCACATCTCAGTTGACAGAATCCCAAGATTGCCATAAGGGTTATTGGAAGA	1530
481 E A S E D I S L P A V M H I S Q L T E S H K I A I R V I R R	510
1531 ATGCAGTATTTGTAGCAAGGAGTAAATTTAGCAAGCCAAGAAACCTACGACGTGAGGGACGTGATAGAGCAGTACTCGCAAGGACAT	1620
511 M Q Y F V A R S K F Q Q A K K P Y D V R D V I E Q Y S Q G H	540
1621 GTCAATATGATGTGCGAATCAAAGAGCTGCAGCGCAGGCTGGATCACTCACTGGGTAAACCAACATGTTTCTGTGAGAGAAGGGGAAA	1710
541 V N M M V R I K E L Q R R L D H S L G K P N M F L S E K G K	570
1711 GATCGAAGCAAAATATACCATTTGGAGCAAGACTGGACAGAGTGAAGAGAAGTCTGACGATGGATCAGAAGCTGGACACCATCTCTGGCA	1800
571 D R S K Y T I G A R L D R V E E K V V R M D Q K L D T I L A	600
1801 TTGCTGAGAGGGCTGAGCGACGAGGGAGGCCACAGCTGCGGCCGGTCCACGTGCAAGTGTGCCCCGAGCCACTGGCACTTGCCTGCTC	1890
601 L L R G L S D E G G P Q L R P V H V Q V S P E P L A L A P S	630
1891 CCCTAAGCGCCACATACCTTTTACGCCAACAGCACAGCTGT	1932
631 P S S A H I P F S Q Q H S C 644	

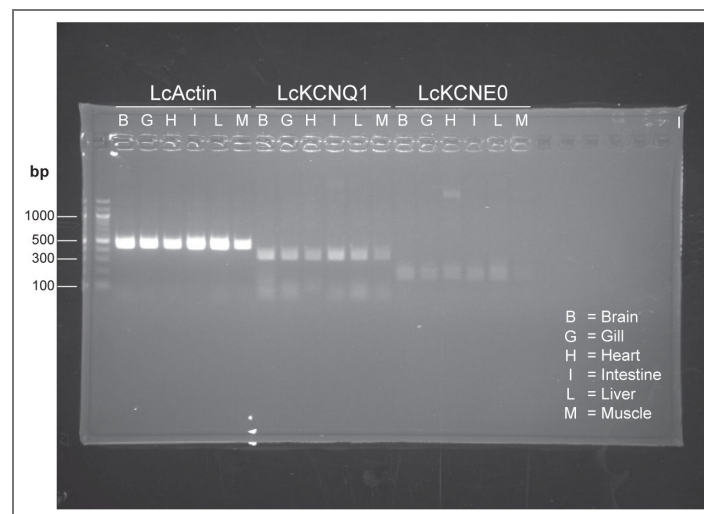
Supplementary Figure 1. ORF nucleotide and corresponding amino-acid sequence of KCNQ1 in Arctic lamprey. The open reading frame (ORF) nucleotide sequence and the translated amino-acid sequence of Arctic lamprey KCNQ1 used for cloning and electrophysiological recordings are shown. The primer-binding sites used for RT-PCR analysis in Fig. 1F are indicated with orange arrows.

Supplementary Figure 2. ORF nucleotide and corresponding amino-acid sequence of KCNE0 in Arctic lamprey.

The open reading frame (ORF) nucleotide sequence and the translated amino-acid sequence of Arctic lamprey KCNE0 used for cloning and electrophysiological recordings are shown. The primer-binding sites used for RT-PCR analysis in Fig. 1F are indicated with orange arrows.

LcKCNE0 orf	
1 ATGGACGCGGTGAACGGAACGCGCCTCTCGGCACACTTCGCCGGGCGGGCGCGCACCGGACATGCAGGGCCTCTCATCGGCCACCGCC 90	
1 M D A V N G T R L S A H F A G P G A A P D M Q G L S S A T A 30	
91 ACCTCCGCCACCGACAACCAGCCCAAGACGCTCTACATCCTCATCTCGTCTCCTTCTACGGCTTCTTCTGCTGCTGCTGCTCGGC 180	
31 T S A T D N Q P K T L Y I L I L V S F Y G F F L L L V L L G 60	
181 TACCTGCGCACCAAGAAGCCGCTGAGCTCCCAGGACCCGTTCCACCTCTACGTGCAGCGGAGTGGCTGGCGCCCGCGCGCCCGCCGCC 270	
61 Y L R T K K P L S S Q D P F H L Y V Q R E W L A P A R P A A 90	
271 GCCGCGGCCCGCGCGGCCCGGGAGGCCGTCGTCGTCGGCGGCCACGACGACGGGGAGCCGGCGCGCTGCCCCAGCGCCTCCTCTTCC 360	
91 A A A A A A A G R P S S S A A T T T G E P A R C P S A S S S 120	
361 GGCTCCTCGGCCTCCGCCTCCTCGAGGGTCACGGCGGCCGCTCGGGGCCGTCAGGGGTGCCCGAGATCCGCGTGGAGGCGCCGACCGGC 450	
121 G S S A S A S S R V T A A A S G P S G V P E I R V E A P T G 150	
451 GAGGGGAAGCTGCGGCCGCTGTGCCTGGCCGAGCGGCGCATGGGCATCGCGGCCGTG 507	
151 E G K L R P L C L A E R R M G I A A V 169	

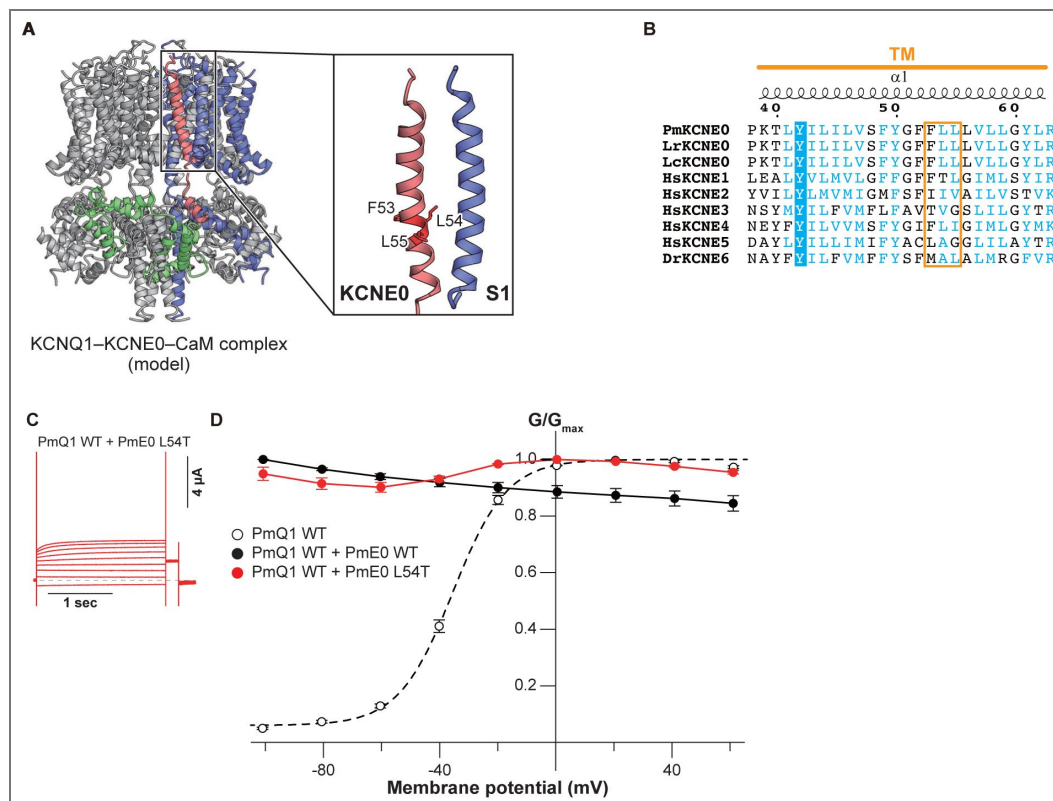
Supplementary Figure 3. Uncropped agarose gel corresponding to the gel image shown in Figure 1F.





Supplementary Figure 4. Amino-acid sequence alignment of KCNQ1 from various species.

Amino acid sequence alignment of KCNQ1 from human, zebrafish, lampreys, and vase tunicate generated with Clustal Omega⁶¹ and displayed with ESPrnt3⁶². The residue corresponding to the labeling site in human KCNQ1 (G219)^{29,30} is highlighted with an orange circle. For sequence alignment, human (HsKCNQ1; NCBI Accession Number NP_000209.2), zebrafish (DrKCNQ1; NP_001116714.1), sea lamprey (PmKCNQ1; XP_075921450.1), Far Eastern brook lamprey (LrKCNQ1; XP_061420438.1), Arctic lamprey, (LcKCNQ1; see Supplementary Fig. 1³⁴), and vase tunicate (CiKCNQ1; NP_001153537.1) KCNQ1 were used.



Supplementary Figure 5. Attempted conversion of KCNE0 toward a KCNE1-like effect.

(A) Close-up view of the interface between KCNE0 and S1 within the PmKCNE0-PmCaM complex corresponding to the structure shown in Fig. 5A. (B) Sequence alignment around the KCNE transmembrane segments highlighting the "triplet" region^{42,43}. (C,D) A representative current trace (C) and G-V relationship (D) of PmKCNE0 WT co-expressed with the HsKCNE1-like triplet mutant of PmKCNE0 (PmKCNE0 L54T). In (D), error bars denote mean $\pm$ s.e.m. for n = 5.

Data availability

The numerical data used to generate the figures in this study are provided in LampreyKCNE0_SourceData. All DNA sequence data generated in this study are included in the Supplementary Figures.

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Additional information

Author contributions

G.K. and K.N. conceived and designed the project. G.K. and K.R. performed electrophysiological experiments. G.K. performed confocal microscopy. G.K., B.Z., E.K.Y., and K.N. collected tissue samples and performed RT-PCR. G.K. analyzed deposited RNA-seq data. G.K. and K.N. wrote the original draft, and all authors reviewed and edited the manuscript. G.K. and K.N. supervised the research.

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Additional files

Supplemental Table 1.  Summary of electrophysiological parameters and $V_{1/2}$ statistical comparisons. Summary of electrophysiological parameters for all constructs. For each condition, maximum tail current amplitude (I_{max}) and Boltzmann fit parameters ($V_{1/2}$ and z) are reported. For VCF experiments, parameters from single- or double-Boltzmann fits are reported as appropriate. Values are mean $\pm$ s.e.m.; n indicates the number of oocytes. In the statistical comparisons, P values indicate pairwise comparisons of $G-V$ $V_{1/2}$ values. For datasets containing more than two groups (Figs. 2G, 3F, and 3N), one-way ANOVA was first performed, followed by Tukey–Kramer multiple-comparison tests. For two-group comparisons (Fig. 4C, 4F, 4I, and 4L), an unpaired two-tailed Welch’s t -test was used. $V_{1/2}$ values were not statistically compared when reliable Boltzmann fits could not be obtained because the $G-V$ relationships were nearly saturated over the tested voltage range.

Supplemental Table 2.  Summary of fastp quality control. Quality-control statistics generated by fastp for all RNA-seq runs. For each run, total reads before and after trimming (million reads), percentage of reads retained after trimming (%), mean read length of R1 and R2 before and after

trimming (bp), and the percentage of bases with Phred quality ≥ 30 (Q30) before and after trimming (%) are shown. “Reads” denote paired-end fragments.

Supplemental Table 3. [Quantification summary using Salmon.](#) Summary of transcript detections for Far Eastern brook lamprey KCNQ1 (XM_061564454.1) and KCNE0 (XM_061575561.1) using Salmon. For each run, the number of detected fragments, total processed fragments, and CPM (counts per million of the library) are shown. “Fragments” denote paired-end read pairs (one read pair counted as one fragment).

Source data 1. [Excel file with numerical electrophysiology data acquired in this study.](#)

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Peer reviews

Reviewer #1 (Public review):

[Editors' note: this version has been assessed by the Reviewing Editor without further input from the original reviewers. The authors have addressed the comments raised in the previous round of review.]

Summary:

In this study, the authors describe an early diverging vertebrate KCNE gene present in jawless lampreys that they denote KCNE0.

Three forms of the protein are isolated from different lampreys, which have 95% homology to each other, but only moderate homology to KCNE1-6.

Co-expression with lamprey KCNQ1 produced a non-inactivating current, whereas co-expression with mammalian KCNQ1 resulted in less modulation. Introduction of a tetra-leucine motif from KCNE4 into KCNE0 reduced current on co-expression with KCNQ1, conferring an inhibitory effect.

Strengths:

This is an interesting and uncontroversial report of a new KCNE isoform from lower vertebrates that gives insight into the evolutionary progression of the sequence and functional properties of the accessory protein.

<https://doi.org/10.7554/eLife.111781.2.sa2>

Reviewer #2 (Public review):

Summary:

This study functionally characterizes a single KCNE-like gene, *kcne0*, from a jawless vertebrate. The authors conducted multiple experiments, including TEVC, VCF, RT-PCR, and RNA-seq to show that KCNQ1 and *kcne0* exhibited a broadly overlapping organ distribution in lamprey species, and KCNE0 produced a constitutively active current when co-expressed with lamprey KCNQ1, similar to the effects of human KCNE3 on KCNQ1. This modulation was species-specific, as co-expression of KCNE0 with other species' KCNQ1 was less effective. Moreover, the authors found that truncating the N-terminal had a more significant reduction of the modulatory effects than truncating the C-terminal of KCNE0. Interestingly, the introduction of the tetra-leucine motif from human KCNE4 into KCNE0 conferred KCNE0 with comparable effects of human KCNE4 on KCNQ1.

Strengths:

The authors clearly introduced an early-diverging member of the KCNE family, and convincingly demonstrated the function of this gene, KCNE0. The results are supported by experiments of multiple approaches and are clearly written. The work is significant and will interest readers from the extended research area.

Weaknesses:

No major concerns were identified with the manuscript in general.

<https://doi.org/10.7554/eLife.111781.2.sa1>

Author response:

The following is the authors' response to the original reviews.

Public Reviews:

Reviewer #1 (Public review):

Summary:

In this study, the authors describe an early diverging vertebrate KCNE gene present in jawless lampreys that they denote KCNE0.

Three forms of the protein are isolated from different lampreys, which have 95% homology to each other, but only moderate homology to KCNE1-6.

Co-expression with lamprey KCNQ1 produced a non-inactivating current, whereas co-expression with mammalian KCNQ1 resulted in less modulation. Introduction of a tetra-leucine motif from KCNE4 into KCNE0 reduced current on co-expression with KCNQ1, conferring an inhibitory effect.

Strengths:

This is an interesting and uncontroversial report of a new KCNE isoform from lower vertebrates that gives insight into the evolutionary progression of the sequence and functional properties of the accessory protein.

Thank you for reviewing our manuscript and for your constructive comments. Our point-to-point responses are shown below.

Weaknesses:

(1) No error bars visible for lamprey Q1 isoforms (open symbols) in Figure 2G. No statistical comparison was provided to indicate whether lamprey Q1 isoform V1/2s are significantly different (nor in Supplementary Table 1).

(2) There is the same issue in Figures 3 and 4. No appropriate statistical comparison is made between V1/2s for different truncations of PmKCNE0 (Figure 3), or between KCNQ1 species isoforms with and without PmE0.

We thank you for these helpful comments. Based on your suggestions, we revised the presentation of error bars in Fig. 2G and in other panels showing G–V or F–V relationships (Figs. 2J, 3F, 3N, 4C, 4F, 4I, 4L, and 5E; Supplementary Fig. 5D) to make the SEM bars clearer. We also added statistical comparisons of $V_{1/2}$ values among the three lamprey KCNQ1 orthologs in Fig. 2G and among truncation-series constructs in Figs. 3F and 3N using one-way ANOVA followed by Tukey–Kramer multiple-comparison tests. For Fig. 4, we added statistical comparisons between KCNQ1 species isoforms expressed with or without PmKCNE0 (Figs. 4C, 4F, and 4I), and between PmKCNQ1 expressed alone or with human KCNE1 or KCNE3 (Fig. 4L), using unpaired two-tailed Welch's t-tests. These statistical comparisons are included in Supplementary Table 1.

Reviewer #2 (Public review):

Summary:

*This study functionally characterizes a single KCNE-like gene, *kcne0*, from a jawless vertebrate. The authors conducted multiple experiments, including TEVC, VCF, RT-PCR, and RNA-seq to show that KCNQ1 and *kcne0* exhibited a broadly overlapping organ distribution in lamprey species, and KCNE0 produced a constitutively active current when co-expressed with lamprey KCNQ1, similar to the effects of human KCNE3 on KCNQ1. This modulation was species-specific, as co-expression of KCNE0 with other species' KCNQ1 was less effective. Moreover, the authors found that truncating the N-terminal had a more significant reduction of the modulatory effects than truncating the C-terminal of KCNE0. Interestingly, the introduction of the tetra-leucine motif from human KCNE4 into KCNE0 conferred KCNE0 with comparable effects of human KCNE4 on KCNQ1.*

Strengths:

The authors clearly introduced an early-diverging member of the KCNE family, and convincingly demonstrated the function of this gene, KCNE0. The results are supported by experiments of multiple approaches and are clearly written. The work is significant and will interest readers from the extended research area.

Weaknesses:

No major concerns were identified with the manuscript in general.

We thank you for the positive assessment of our work and for the constructive suggestions. Our point-by-point responses are provided below.

Recommendations for the authors:

Reviewer #2 (Recommendations for the authors):

(1) What is the physiological role of this KCNE0 and lamprey KCNQ1 in the lamprey species? While the authors mention that the physiological roles of KCNE0 are the next focus, it is preferable to discuss some of the potential functional significance of this newly characterised KCNE.

We thank you for this helpful suggestion. We agree that discussing the potential physiological roles of KCNQ1–KCNE0 complexes in lamprey strengthens the manuscript. We have therefore expanded the Discussion to raise the possibility that, given the broad tissue distribution of *kcne0* transcripts and the ability of KCNE0 to render lamprey KCNQ1 constitutively active, KCNQ1–KCNE0 complexes may contribute to general ion homeostasis, potentially analogous to the epithelial K⁺ recycling function of mammalian KCNQ1–KCNE3, rather than to the highly specialized KCNQ1–KCNE1 function in the mammalian heart and inner ear (page 14, lines 263–267).

(2) Human KCNQ1 has 676 amino acids, but LcKCNQ1 contains just 507 amino acids. The species-specific regulatory effects of KCNE0 may not only be attributed to KCNE0 itself but might also be influenced by the species of KCNQ1. Some discussion on this possibility will be helpful.

We thank you for raising this important point. We agree that the species-specific regulatory effects observed in our cross-species pairing experiments are unlikely to be determined by KCNE0 alone and may also be influenced by species-specific features of the KCNQ1 α -subunit. To address this point, we expanded the Discussion to note that the KCNQ1 proteins used in this study vary in amino-acid length, largely reflecting differences in the cytoplasmic C-terminal region, which may affect KCNQ1–KCNE compatibility and thereby influence channel gating and coupling to KCNE subunits (pages 12–13, lines 227–238). We also updated Supplementary Fig. 4 to include LrKCNQ1 and LcKCNQ1, and clarified that the LcKCNQ1 construct used in this study encodes 644 amino acids.

(3) In Supplementary Figure 3, bands corresponding to LcKCNQ1 (507 amino acids, Supplementary Figure 1) were not seen.

We thank you for pointing out this potentially confusing point. Supplementary Fig. 3 shows RT-PCR products amplified from tissue cDNA, not full-length amplification of the LcKCNQ1 ORF. As stated in the Methods section (pages 19–20, lines 374–399), the primers used for RT-PCR in Fig. 1F and Supplementary Fig. 3 were different from those used for cloning the full-length LcKCNQ1 cDNA shown in Supplementary Fig. 1. Therefore, a band corresponding to the full-length LcKCNQ1 coding sequence was not expected in Supplementary Fig. 3.

To clarify this point, we revised the figure legends and indicated the RT-PCR primer-binding sites with orange arrows in Supplementary Figs. 1 and 2.

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