



Published in final edited form as:

Science. 2018 March 02; 359(6379): 1047–1050. doi:10.1126/science.aao3264.

An Evolutionarily Conserved Gene Family Encodes Proton-Selective Ion Channels

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Abstract

Ion channels form the basis for cellular electrical signaling. Despite the scores of genetically identified ion channels selective for other monatomic ions, only one type of proton-selective ion channel has been found in eukaryotic cells. By comparative transcriptome analysis of mouse taste receptor cells, we identified Otopetrin1 (OTOP1), a protein required for development of gravity-sensing otoconia in the vestibular system, as forming a proton-selective ion channel. We found that murine OTOP1 is enriched in acid-detecting taste receptor cells and is required for their Zn²⁺-sensitive proton conductance. Two related murine genes, *Otop2* and *Otop3*, and a *Drosophila* ortholog also encode proton channels. Evolutionary conservation of the gene family and its widespread tissue distribution suggest a broad role for proton channels in physiology and pathophysiology.

Ion channels include a large and diverse group of membrane proteins that rapidly, and with great selectivity, move ions across the cell membrane, performing crucial roles in cell signaling and homeostasis (1). Ion channels selective for each of the physiologically relevant ions, Na⁺, K⁺, Ca²⁺ and Cl⁻, have been described at the molecular and structural levels (2, 3), but only a few types of proton-selective ion channels (proton channels) have been described (4). One is the 96-amino acid M2 protein of influenza A, which conducts protons

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into the virion interior, an essential step in the replication of the virus (5). The only one identified in eukaryotes is the voltage-gated Hv1 (6-8), which is present in immune cells, where it extrudes protons into the phagosome to inactivate infectious agents (9). Functional evidence indicates that ion channels that selectively transport protons into eukaryotic cells must also exist. For example, in acid-sensing taste receptor cells (TRCs), an inward-conducting Zn^{2+} -sensitive proton current that is biophysically distinct from currents carried by Hv1 has been described (10, 11).

To identify candidates encoding such a proton channel, we compared the transcriptome of mouse TRCs positive for the inward-conducting Zn^{2+} -sensitive proton current (PKD2L1 cells) with that of TRCs that lack the current (TRPM5 cells; Fig 1A). We selected genes that were enriched in PKD2L1 cells and that encoded poorly or uncharacterized transmembrane proteins (Fig. 1A and Table S1) (see methods). We expressed the candidates in human embryonic kidney (HEK-293) cells or *Xenopus* oocytes and measured ionic currents in response to lowering the extracellular pH (pH_o) in the absence of extracellular Na^+ . Of the forty-one cDNAs tested, only Otopetrin1 (*Otop1*), which encodes a protein with 12 predicted transmembrane domains (12), generated large Zn^{2+} -sensitive inward currents in response to extracellular acidification (Fig 1B).

We characterized functional properties of OTOP1 expressed in *Xenopus* oocytes. Unless otherwise noted, the extracellular solution used in recordings was Na^+ -free [N-methyl-D-glucamine (NMDG^+)-based]. OTOP1 currents increased monotonically as pH_o was lowered (Fig 1, C through E) and the reversal potential (E_{rev}) shifted toward more positive voltages (Fig. S1A). The currents showed a small time-dependent change in amplitude in response to hyperpolarizing voltage steps, indicating that gating of OTOP1 is mildly voltage-sensitive (Fig. S1, B and C).

OTOP1 also generated an ionic current in HEK-293 cells (Fig 1, F through H). An N-terminal YFP-tagged protein confirmed the presence of OTOP1 at the cell surface (Fig. S2A). Lowering pH_o elicited large inward currents in OTOP1-expressing HEK-293 cells and, as in oocytes, the current magnitude increased monotonically with pH_o (Fig. 1, F through H). OTOP1 currents in HEK-293 cells decayed within seconds, with faster kinetics observed in response to more acidic stimuli (Fig. 1F). The decay of the currents is likely to be due, in part, to a reduction in the driving force as protons accumulate in the cytosol. For a 15- μm diameter cell (1767 fL volume), a H^+ current of 1000 pA flowing for 1 second will increase the total (bound + free) intracellular concentration of H^+ by ~6 mM (4). We confirmed that OTOP1 mediated flux of protons into the cell cytosol with the membrane-permeant pH indicator pHrodo Red. In OTOP1-expressing HEK-293 cells, but not in mock-transfected cells, lowering extracellular pH from 7.4 to 5.0 caused a large increase in emission of pHrodo Red (Fig 2, A and B), corresponding to a large change in intracellular pH (Fig. S2, B and C).

Hv1 and M2 are highly selective for protons, present in high nanomolar concentrations, over other cations whose concentrations are a million times higher (4, 13). To determine if OTOP1 is similarly proton-selective, we evoked OTOP1 currents by lowering pH_o from 7.4 to 5.5 and measured the effect of exchanging NMDG^+ in the extracellular solution for

K). The shallow relation between the current amplitude and pH may endow the channel with a broader dynamic range.

OTOP1 is required for the development of otoconia, calcium carbonate-based structures that sense gravity and acceleration in the vestibular system. Two mutations of OTOP1, tilted (*tl*) and mergulhador (*mlh*; Fig. S11A), lead to vestibular dysfunction in mice (14). These mutations affect trafficking of the protein to the cell surface in vestibular supporting cells (17). Mutant channels expressed in *Xenopus* oocytes, produced smaller currents but otherwise had similar functional properties, such as sensitivity to Zn^{2+} (Fig. S11, B and C), to wild-type OTOP1. This reduction in current magnitude may contribute to the vestibular dysfunction.

Finally, we sought to determine if OTOP1 constitutes the proton current in acid-sensing taste receptor cells (10, 11). We confirmed that in single cell transcriptome data, *Otop1* was expressed in all PKD2L1 cells (19/19) implicated in sour transduction (18), whereas *Otop2* and *Otop3* were expressed in much lower amounts, and none of the three *Otop* transcripts were detected in TRPM5 cells, which lack proton currents (Fig. 4A). By immunocytochemistry, we confirmed that OTOP1 was present in taste cells in mouse circumvallate papillae that express *Pkd21l1* (Fig. 4B). To directly determine if OTOP1 contributes to the proton current in taste cells, we measured currents in taste cells from either wild-type mice or mice that were homozygous for the *tl* mutation of OTOP1. Mutation of OTOP1 resulted in significantly smaller currents than those measured in taste cells from wildtype mice (Fig 4C and D), over a range of H^+ concentrations (Fig 4E and F), indicating that OTOP1 is a component of the proton channel in taste cells. While the contribution of proton currents to acid-sensing/sour taste behavior by mice is still speculative and complicated by contributions from multiple sensory organs and sensory receptors (19), the identification of OTOP1 provides a tool with which to start dissecting this system.

Our data show that the *Otop* gene family encodes a family of ion channels that are unrelated structurally to previously identified ion channels and are highly selective for protons. Unlike Hv1, OTOP1 is only weakly sensitive to voltage. Whether, like the viral proton channel M2 (13), low pH gates OTOP1 is not clear. OTOP channels conduct protons at normal resting potentials and can mediate the entry of protons into cells. Most cells guard against proton entry, which is generally cytotoxic. Thus, we expect OTOP channels are restricted to cell types that use changes in intracellular pH for cell signaling or to regulate biochemical or developmental processes. Along with a role in formation of vestibular otoconia (14), OTOP1 has been shown to protect mice from obesity-induced metabolic dysfunction (15), and it is upregulated in dorsal root ganglion cells in response to cell damage (20). The knowledge that this gene family encodes proton channels can be used to understand its contribution to physiology and disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

E.L. and USC have filed a provisional patent application no. 62/537,900 that claims methods of screening molecules that modulate Otopetrin-dependent ion channel activities. We thank S. Rao, L. Goggins and A. Bernanke for technical assistance, J. Bushman for assistance with electrophysiology and D. Arnold, B. Bean, B. Herring and R. Kramer for careful reading of the manuscript. Funding was provided by the National Institutes of Health grants R01DC013741 and R21DC012747 to E.R.L. and R01HG006015 to A.D.S.

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One Sentence Summary

A conserved gene family encodes ion channels that selectively transport H⁺ across cellular membranes and regulate pH.

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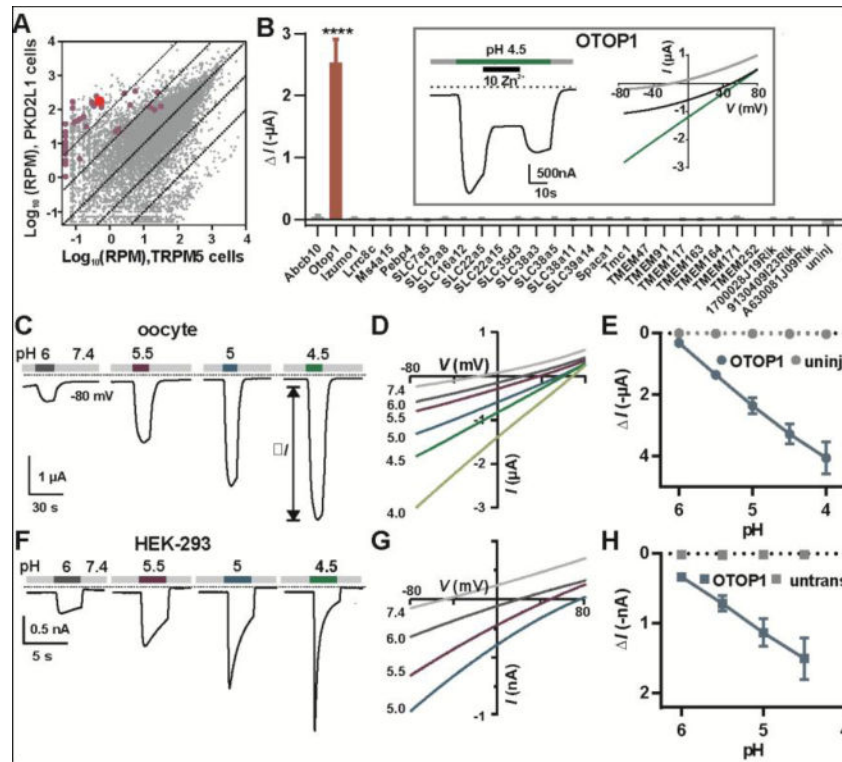


Fig. 1.

Expression analysis of taste-cell enriched genes identifies OTOPI as a novel proton channel. (A) Transcriptome profiling of PKD2L1 and TRPM5 taste receptor cells (Each data point represents the average of 5 replicates). Genes tested by electrophysiology are highlighted in magenta or red (OTOPI). RPM, reads per million. (B) Magnitude of currents evoked in response to pH 4.5 Na⁺-free solution in *Xenopus* oocytes expressing the genes indicated ($V_m = -80$ mV; data are mean \pm SEM, $n = 3 - 37$; for OTOPI, $n = 5$). ****, $P < 0.0001$ compared to uninjected oocytes ($n = 3$). One-way ANOVA with Bonferroni correction. Inset: currents evoked in an OTOPI-expressing oocyte to the acid stimulus at $V_m = -80$ mV (left) and the current-voltage relationship before (gray), during acid application (green) and during Zn²⁺ application (black). (C) Current measured by two-electrode voltage clamp in a *Xenopus* oocyte expressing OTOPI in response to Na⁺-free extracellular solutions with pH_o as indicated ($V_m = -80$ mV). (D) I-V relation of the current in (A) from voltage ramps (1V/s). (E) Evoked current (I ; mean \pm SEM) as a function of pH in *Xenopus* oocytes expressing OTOPI (blue circle; $n = 4$) and uninjected oocytes (grey circles; $n = 4$). (F) Currents measured by whole-cell patch clamp recording in a HEK-293 cell expressing OTOPI in Na⁺-free extracellular solutions (pH_i = 7.3, $V_m = -80$ mV). (G) I-V relation of currents in OTOPI-expressing HEK-293 cell from experiments as in (F) with voltage ramps (1V/s). (H) Evoked currents (I ; mean \pm SEM) as a function of pH in HEK-293 cells expressing OTOPI (blue squares; $n = 5$) and untransfected cells (grey squares; $n = 3$).

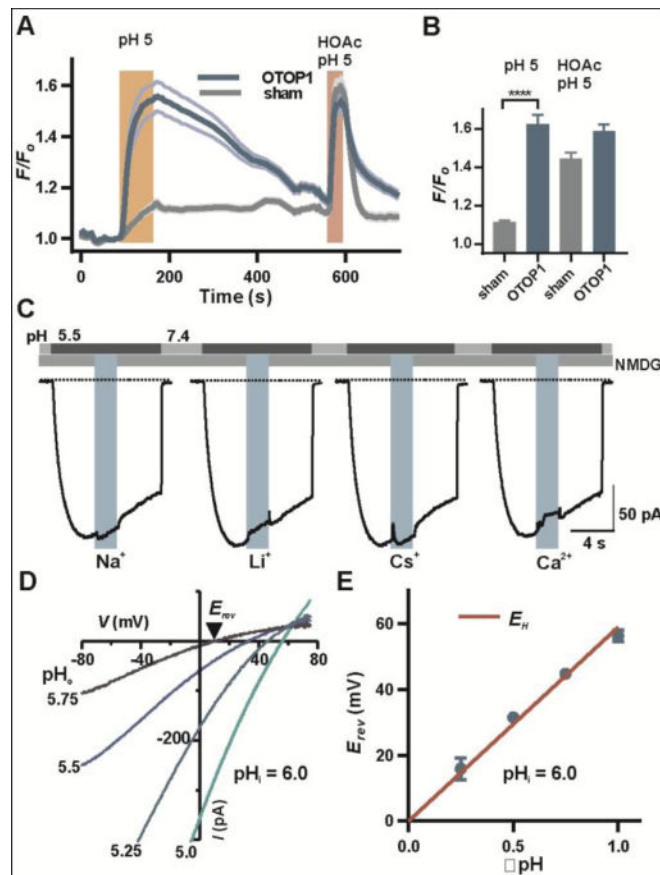


Fig 2.

Selectivity of OTOPI for protons. **(A)** Fluorescence emission of the pH indicator pHrodo Red in HEK-293 cells expressing OTOPI ($n = 9$) and sham-transfected cells ($n = 11$; mean \pm SEM) in response to the stimuli indicated. Similar results were obtained in 3 replicates. **(B)** Average data (mean \pm SEM; $n = 28-29$ cells) were analyzed by 2-tailed t-test. ****: $P < 0.0001$. HOAc, acetic acid, which shuttles protons across membranes (4) served as a positive control. **(C)** OTOPI currents in HEK-293 cells were evoked in response to a pH 5.5 solution with Na^+ , Li^+ or Cs^+ (160 mM each) or Ca^{2+} (40 mM) replacing NMDG^+ in the extracellular solution as indicated ($V_m = -80$ mV). Percentage change in currents was 0.4 ± 0.7 , $n = 8$, 2.7 ± 0.7 , $n = 8$, 2.4 ± 0.5 , $n = 8$, 3.6 ± 1.7 , $n = 7$ for each ion replacement respectively. **(D)** Isolated OTOPI currents in response to voltage ramps (1 V/s) at varying extracellular pH ($\text{pH}_i = 6.0$; Zn^{2+} -sensitive component is shown; see Fig. S5 and methods). **(E)** E_{rev} as a function of pH ($\text{pH}_i - \text{pH}_o$) from experiments as in (D). The red line is E_H . The data were fit by linear regression with a slope of 53 mV/ pH and a Y intercept of 3.6 mV ($R^2 = 0.99$).

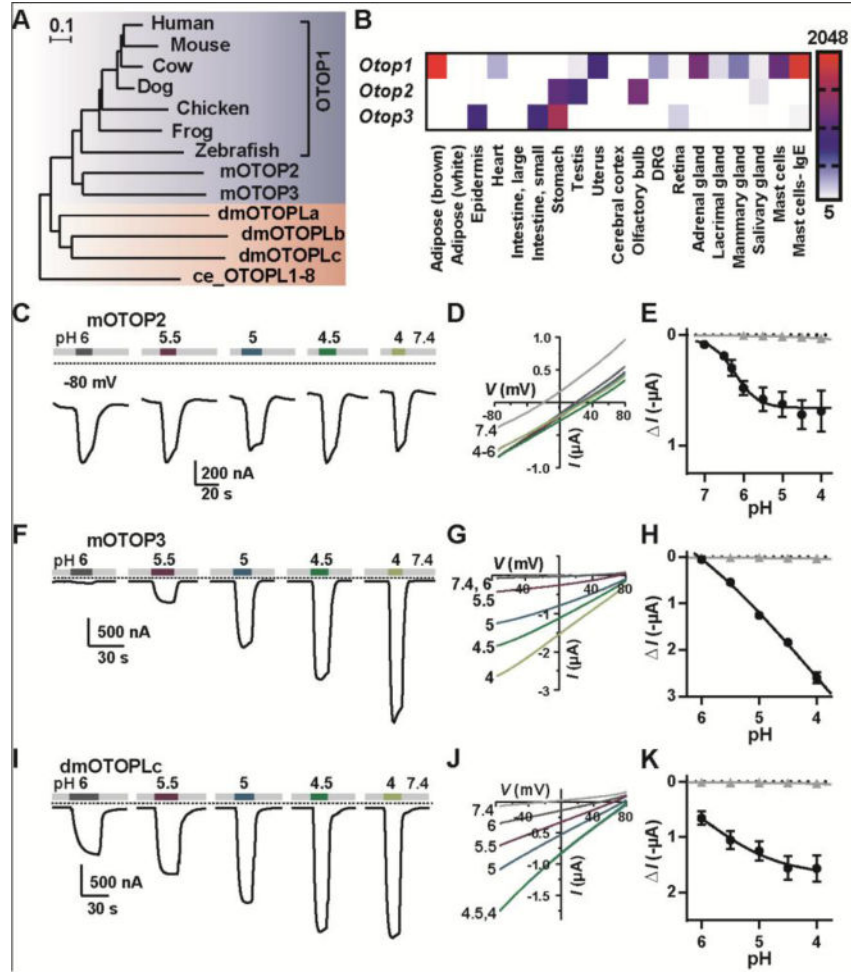


Fig 3. An evolutionarily conserved family of genes, expressed in diverse tissues and encoding proton channels. **(A)** Maximum-likelihood phylogenetic tree from the multi-sequence alignment of 13 otopetrin domain proteins. Scale bar is amino acid substitutions per site. **(B)** Distribution of *Otops* in selected murine tissues from microarray data (16). Scale represents expression level in arbitrary units (mean \pm SEM, $n = 2$). **(C, E, I)** Representative traces ($V_m = -80$ mV) showing currents evoked in *Xenopus* oocytes expressing OTOP2, OTOP3 or dmOTOP1c in response to varying pH_o of the Na^+ -free extracellular solution. **(D, G, J)** I-V relationship (from voltage ramps at 1 V/s) from experiments as in (C, F, I). **(E, H, K)** The average current induced at $V_m = -80$ mV (I) as a function of pH for oocytes expressing each of the channels (black circles; mean \pm SEM, $n = 3-7$) and for uninjected oocytes (gray triangles, mean \pm SEM, $n = 3$).

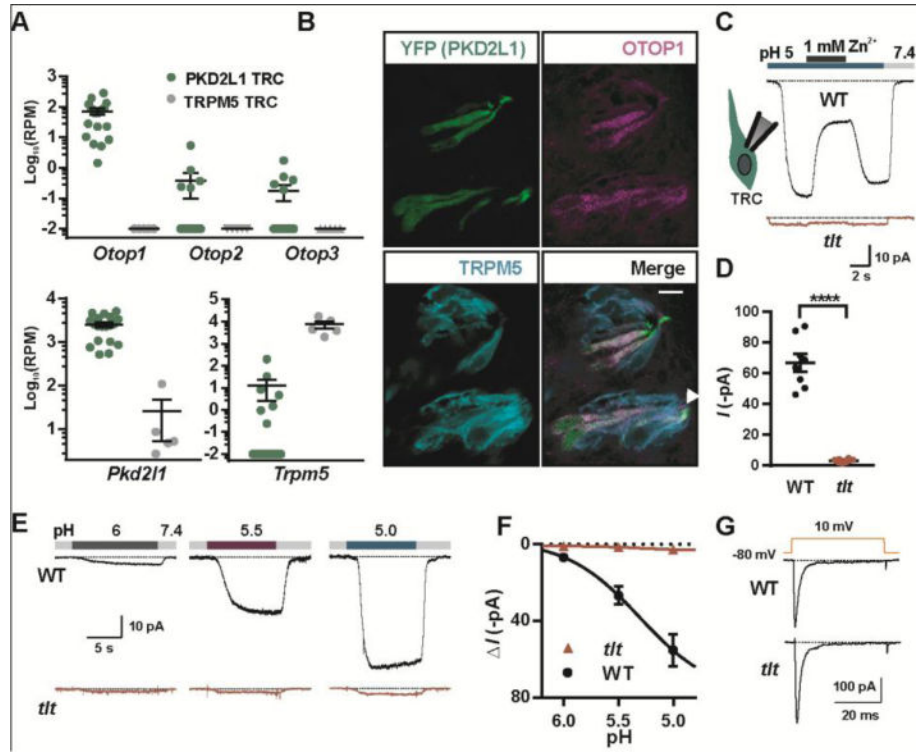


Fig 4. Requirement of OTOP1 for the proton current in taste receptor cells. **(A)** Read counts per million (RPM) for the genes indicated from RNA-seq data obtained from single PKD2L1 ($n = 19$) or TRPM5 taste cells ($n = 5$). 0 RPM was adjusted to 0.01 RPM. **(B)** Confocal images showing taste buds in the circumvallate papillae from a mouse in which *Pkd2l1* drives expression of YFP immunostained with antibodies against YFP (green), OTOP1 (magenta) and TRPM5 (cyan). Scale bar is 10 μ M. Arrow indicates taste pore. **(C)** Current in response to pH 5.0 stimulus in isolated PKD2L1 TRCs from *tlt* mutant or *wildtype* mice in NMDG⁺-based solution ($V_m = -80$ mV). **(D)** Average data from experiments as in (C) (****: $P < 0.0001$ by two tailed t-test, $n = 8$ cells/genotype). **(E)** Response of PKD2L1 TRCs to NMDG⁺-based extracellular solution of varying pH ($V_m = -80$ mV). **(F)** Average data from experiments as in (E). **(G)** Voltage-gated Na⁺ currents in TRCs from *tlt* and *wildtype* mice were indistinguishable ($P > 0.05$, two-tailed t-test).