An evolutionarily conserved gene family encodes proton-selective ion channels

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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 zinc-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.

To identify candidates encoding such a proton channel, we compared the transcriptome of mouse TRCs positive for the inward-conducting Zn2+-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 characterized or uncharacterized transmembrane proteins (Fig. 1A and table S1) (see methods). We expressed the candidates in human embryonic kidney 293 (HEK-293) cells or Xenopus oocytes and measured ionic currents in response to lowering the extracellular pH (pH4) in the absence of extracellular Na+. Of the 41 CDNAs tested, only Otopetrin1 (OTOP1), which encodes a protein (OTOP1) with 12 predicted transmembrane domains (12), generated large Zn2+-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 was lowered (Fig. 1, C to E) and the reversal potential (E<sub>rev</sub>) shifted toward more positive voltages (Fig. 1D and 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 to H). An N-terminal YFP (yellow fluorescent protein)-tagged protein confirmed the presence of OTOP1 at the cell surface (fig. S2A). Lowering pH<sub>e</sub> elicited large inward currents in OTOP1-expressing cells and, as in oocytes, the current magnitude increased monotonically with pH<sub>e</sub> (Fig. 1, F to 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 μm<sup>3</sup> volume), a H<sup>+</sup> current of 1000 pA flowing for 1 s will increase the total (bound + free) intracellular concentration of H<sup>+</sup> 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-transfected 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. 2A, 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<sub>e</sub> from 7.4 to 5.5 and measured the effect of changing NMDG<sup>+</sup> in the extracellular solution for equimolar concentrations of Na<sup>+</sup>, Li<sup>+</sup>, or Cs<sup>+</sup> (360 mM each) or Ca<sup>2+</sup> (40 mM) replacing NMDG<sup>+</sup> in the extracellular solution as indicated (Fig. 1B). Percentage change in currents was 0.4 ± 0.7 (n = 8), 2.7 ± 0.7 (n = 8), 2.4 ± 0.5 (n = 8), and 3.6 ± 1.7 (n = 7) for each ion replacement, respectively. (D) Isolated OTOP1 currents in response to voltage ramps (1 V/s) at varying extracellular pH (pHi = 6.0; Zn<sup>2+</sup>-sensitive component is shown; see fig. S5 and methods). (E) E<sub>rev</sub> as a function of ΔpH (pHi - pH<sub>e</sub>) from experiments as in (D). The red line is the equilibrium potential for H<sup>+</sup>, E<sub>H</sub>. The data were fit by linear regression with a slope of 53 mV/ΔpH and a y intercept of 3.6 mV (correlation coefficient R<sup>2</sup> = 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 multisequence alignment of 13 otopetin domain proteins. Scale bar indicates amino acid substitutions per site. dm, Drosophila melanogaster; ce, Caenorhabditis elegans. (B) Distribution of Otop genes in selected murine tissues from microarray data (16). Scale represents expression level in arbitrary units (mean ± SEM, n = 2). 
(C, F, I) Representative traces (V_m = −80 mV) showing currents evoked in Xenopus oocytes expressing mOTOP2, Otop3, or dmOTOP LC in response to varying pHm 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), and (I). 
(E, H, K) The average current induced at V_m = −80 mV (±) as a function of pH for oocytes expressing each of the channels (black circles; mean ± SEM, n = 3 to 7) and for uninjected oocytes (gray triangles, mean ± SEM, n = 3).

providing evidence that, like OTOPI, they form proton channels.

There are three genes in the genome of Drosophila melanogaster that encode proteins that appear to be evolutionarily related to mOTOP1 (22, 44) (Fig. 3A). The transcript CG42265 encodes dmOTOP LC, a protein of 1576 amino acids that over the region of similarity bears 14.1% amino acid identity with OTOPI. Despite the modest level of conservation, when expressed in Xenopus oocytes, dmOTOP LC conducted large currents in response to decreasing extracellular pH, indicating that it too forms a proton channel (Fig. 3, I to 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 otocoria, calcium carbonate–based structures that sense gravity and acceleration in the vestibular system. Two mutations of Otop 1, tilted (ttl) and mergulhador (mlh; fig. S11A), lead to vestibular dysfunction in mice (44). 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 functional properties, such as sensitivity to Zn2+ (fig. S11, B and C), similar to those of wild-type OTOPI. This reduction in current magnitude may contribute to the vestibular dysfunction.

Finally, we sought to determine if OTOPI contributes to the proton current in acid-sensing taste receptor cells (10, 41). We confirmed that in single-cell transcriptome data, Otop 1 was expressed in PKD2LI cells (19 out of 19) implicated in sour transduction (48), whereas Otop 2 and Otop 3 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 OTOPI was present in taste cells in mouse circumvallate papillae that express Pkd2LI (Fig. 4B). To directly determine if OTOPI 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 ttl mutation of Otop 1. Mutation of Otop 1 resulted in significantly smaller proton currents than those measured in taste cells from wild-type mice (Fig. 4, C and D), over a range of H+ concentrations (Fig. 4, E and F), indicating that OTOPI is a component of the proton channel in taste cells. Although the contribution of proton currents to acid-sensing or sour taste behavior by mice is still speculative and complicated by contributions from multiple sensory organs and sensory receptors (19), the identification of OTOPI as forming a proton channel provides a tool with which to start dissecting this system.

Our data show that the otopetrin genes encode a family of ion channels that are unrelated structurally to previously identified ion channels and are highly selective for protons. Unlike Hv1, OTOPI is only weakly sensitive to voltage. Whether, like the viral proton channel M2 (15), low pH gates OTOPI is not clear. OTOPI 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 that OTOPI 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 otocoria (44), OTOPI has been shown to protect mice from obesity-induced metabolic dysfunction (15), and it is up-regulated 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.
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-sequencing 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), OTOPI (magenta), and TRPM5 (cyan). Scale bar, 10 μm. Arrow indicates taste pore. (C) Current in response to a pH 5.0 stimulus in isolated PKD2L1 TRCs from tlt mutant or wild-type (WT) 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). (E) Voltage-gated Na⁺ currents in TRCs from tlt and wild-type mice were indistinguishable (P > 0.05, two-tailed t test).

REFERENCES AND NOTES

SUPPLEMENTARY MATERIALS
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Materials and Methods
Figs. S1 to S11
Table S1
References (21–34)

13 July 2017; accepted 8 January 2018
Published online 25 January 2018
10.1126/science.aaq3264
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Science 359 (6379), 1047-1050.
DOI: 10.1126/science.aao3264 originally published online January 25, 2018

The proton channel behind sour taste
Although many proteins that form ion channels in cell membranes have been described, none that selectively conduct protons into eukaryotic cells have been identified. Tu et al. used a genetic screen to pinpoint candidate genes that might encode such a protein from mouse taste receptor cells (see the Perspective by Montell). They identified the known protein otopetrin and showed that it conferred proton conductance when expressed in cultured human cells. Their results indicate that otopetrin may function in sensory recognition of sour (acidic) taste in humans and other organisms. Science, this issue p. 1047; see also p. 991

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