Molecular mechanism of the assembly of an acid-sensing receptor ion channel complex

Yong Yu1,†, Maximilian H. Ulbrich2,3,4, Ming-hui Li1, Scott Dobbins1, Wei K. Zhang1, Liang Tong1, Ehud Y. Isacoff2,5 & Jian Yang1

Polycystic kidney disease (PKD) family proteins associate with transient receptor potential (TRP) channel family proteins to form functionally important complexes. PKD proteins differ from known ion channel-forming proteins and are generally thought to act as membrane receptors. Here we find that PKD1L3, a PKD protein, functions as a channel-forming subunit in an acid-sensing heteromeric complex formed by PKD1L3 and TRPP3, a TRP channel protein. Single amino-acid mutations in the putative pore region of both proteins alter the channel’s ion selectivity. The PKD1L3/TRPP3 complex in the plasma membrane of live cells contains one PKD1L3 and three TRPP3. A TRPP3 C-terminal coiled-coil domain forms a trimer in solution and in crystal, and has a crucial role in the assembly and surface expression of the PKD1L3/TRPP3 complex. These results demonstrate that PKD subunits constitute a new class of channel-forming proteins, enriching our understanding of the function of PKD proteins and PKD/TRPP complexes.
**Results**

**PKD1L3 and TRPP3 form an acid-sensing ion channel.** With two-electrode voltage clamp (TEVC), we recorded robust acid-induced currents from *Xenopus* oocytes expressing PKD1L3 and TRPP3 (Fig. 1a). Currents were not detected when TRPP3 or PKD1L3 was expressed alone (Fig. 1a,b), or when TRPP3 or TRPP2 was coexpressed with PKD1 or PKD1L3, respectively (Fig. 1b). As reported in HEK 293T cells, the PKD1L3/TRPP3 current was observed only when oocytes were neutralized after a brief low pH treatment (citric acid, pH 2.8), and this current was quickly and reversibly inhibited by reaplication of H⁺ (Fig. 1a). This current could be an off-response or could be due to the removal of proton blockage of open channels. As discussed in a previous study, such an off-response correlates well with the off-response associated with the perception of sour taste in mammals. The current–voltage (I–V) relationship of the acid-induced current showed a reversal potential of -9.6 ± 2.8 mV (n = 148) in a bath solution containing 100 mM NaCl (Fig. 1c,d), suggesting that the PKD1L3/TRPP3 channel has a similar permeability to Na⁺ and K⁺.

**PKD1L3 is an ion channel pore-forming subunit.** We next investigated whether PKD1L3, as well as TRPP3, contribute to form the channel pore. A widely held and extensively tested prediction of channel-forming proteins is that mutating residues lining the ion conduction pathway significantly alters the ion selectivity (not simply current amplitude) of the channel. Based on amino-acid sequence alignment (Fig. 2a), a pore-forming loop has been proposed to exist between the last two transmembrane segments in both TRPP3 and PKD1L3 (ref. 28). To test whether these proteins are channel-forming subunits, we carried out extensive mutagenesis in this region of both proteins and examined the effect of the mutations on the ion selectivity of PKD1L3/TRPP3 channels. Dimethylamine⁺, trimethylamine⁺ and Mg₂⁺ were chosen as charge carriers for their size and/or charge and compared to Na⁺. Figure 2b–d shows the I–V relationships of currents recorded from wild-type (WT) PKD1L3/TRPP3 channels in bath solutions containing 100 mM dimethylamine⁺, 100 mM trimethylamine⁺, 70 mM Mg²⁺ or 100 mM Na⁺, with which each of the other ions was compared in the same oocyte. The reversal potential in dimethylamine⁺ and trimethylamine⁺ was -34.0 ± 5.7 mV (n = 55) and -57.0 ± 7.9 mV (n = 54), respectively (Fig. 2b,c), indicating that PKD1L3/TRPP3 channels are much less permeable to these ions than to Na⁺ (P_{Na⁺}/P_{dimethylamine⁺} = 1.03±0.16). The reversal potential in Mg²⁺ was -13.7 ± 4.2 mV (n = 49), close to that in Na⁺ (Fig. 2d), indicating that Mg²⁺ permeates well through PKD1L3/TRPP3 channels (P_{Na⁺}/P_{Mg²⁺} = 1.065).

Our examination of the sequences suggested several residues that could line the pore in both PKD1L3 and PKD1L1, including D523 and D525 in TRPP3 and D2067, K2069 and E2072 in PKD1L3. Mutations of these residues not only changed the amplitude of acid-induced currents in most cases (Supplementary Fig. S1) but also changed the ion selectivity of PKD1L3/TRPP3 channels (Fig. 2e–j, Table 1, Supplementary Figs S2, S3 and Supplementary Table S1). For example, mutating D523 of TRPP3 to glutamine (D523Q) or D525 of TRPP3 to lysine (D525K) increased the relative permeability of dimethylamine⁺ and trimethylamine⁺ and decreased the relative permeability of Mg²⁺ (Fig. 2e,f, Table 1 and Supplementary Fig. S2a,b). D523 of TRPP3 has also been shown to be important to Ca²⁺ permeation.

---

**Five human polycystic kidney disease (PKD) proteins are known to exist, with wide tissue distribution and limited knowledge of their biological functions.** Members of the PKD protein family associate with members of the transient receptor potential channel P subfamily (TRPP) to form heteromeric protein complexes. TRPP proteins belong to the large transient receptor potential (TRP) cation channel family that expresses in diverse species and cell types and has critical roles in sensory physiology. The founding members of PKD and TRPP families, PKD1 (also known as polycystin-1) and TRPP2 (also known as polycystin-2 or PKD2), coassemble to form a receptor/ion channel complex. Mutations in these proteins cause autosomal dominant PKD, one of the most common inherited human diseases. PKD1L3, another PKD family member, interacts with another TRPP family member, TRPP3 (also known as polycystin-L or PKD2L1). These proteins colocalize in a subset of acid-sensing taste cells; these taste cells and HEK 293 cells heterologously expressing both proteins respond to extracellular acid, making the PKD1L3/TRPP3 complex a good candidate for a sour taste receptor. Several lines of evidence from studies on animals and humans further support the hypothesis that the PKD1L3/TRPP3 complex is a candidate sour taste receptor. (i) Selective ablation of TRPP3-expressing taste cells abolishes all gustatory response to acidic stimuli. (ii) Two patients with sour ageusia are reported to have no expression of TRPP3, PKD1L3 and several acid-sensing ion channel proteins in their taste cells. (iii) TRPP3-expressing taste cells exhibit acid responses similar to those of exogenously expressed PKD1L3/TRPP3 complexes. (iv) TRPP3 knockout mice show clear sour taste deficit. Although no significant effect on sour taste was observed in PKD1L3 knockout mice and demonstrate that PKD1L3, a close relative of PKD1, is a channel-forming subunit. We also find that this complex contains one PKD1L3 and three TRPP3 subunits, and a TRPP3 C-terminal coiled-coil domain has a crucial role in determining this stoichiometry.

---
of PKD1L3/TRPP3 channels. When the two corresponding positions in PKD1L3, T2067 and K2069, were mutated to aspartic acid, all three ions became more permeant (Fig. 2g,i, Table 1 and Supplementary Fig. S2f). In both cases, the reversal potential of dimethylamine (Fig. 2g,i, Table 1 and Supplementary Fig. S2d). The E2072A mutation slightly decreased the relative permeability of dimethylamine (Fig. 2g,i, Table 1 and Supplementary Fig. S2f). However, it significantly decreased Mg$^{2+}$ permeability (Fig. 2g,i, Table 1 and Supplementary Fig. S2d). In both cases, the reversal potential of dimethylamine (Fig. 2g,i, Table 1 and Supplementary Fig. S2d), indicating that Mg$^{2+}$ even became positive (Fig. 2g,i and Supplementary Fig. S2c,e), indicating that Mg$^{2+}$ became more permeant than K$^+$ and Na$^+$ (Table 1). In contrast, when T2067 was mutated to proline, the mutant channels became less permeable to Mg$^{2+}$, albeit they still exhibited increased relative permeability to dimethylamine$^+$ and trimethylamine$^+$ (Fig. 1h, Table 1 and Supplementary Fig. S2d). The E2072A mutation slightly decreased the relative permeability of dimethylamine$^+$ but did not affect that of trimethylamine$^+$ (Fig. 1j, Table 1 and Supplementary Fig. S2f). However, it significantly decreased Mg$^{2+}$ permeability (Fig. 1j, Table 1 and Supplementary Fig. S2f).

We also examined the relative permeability of WT channel and two mutant channels to N-methyl-D-glucamine$^+$ (NMDG$^+$) and K$^+$. NMDG$^+$ was only slightly permeant through WT channels, whereas K$^+$ was as permeant as Na$^+$ (Supplementary Fig. S3 and Supplementary Table S1). The TRPP3_D523Q mutation altered the relative permeability of NMDG$^+$ (Supplementary Fig. S3a and Supplementary Table S1), as it did for other ions (Table 1). This mutation also affected K$^+$ permeation, as indicated by the more negative reversal potential in K$^+$ (Supplementary Fig. S3a). On the other hand, despite its strong effect on the relative permeability of dimethylamine$^+$, trimethylamine$^+$ and Mg$^{2+}$ (Table 1), the PKD1L3_K2069D mutation did not affect the relative permeability of NMDG$^+$ and K$^+$ (Supplementary Fig. S3b and Supplementary Table S1). This is perhaps because the size of NMDG$^+$ and K$^+$ are either too big or too close to that of Na$^+$ such that their permeation is insensitive to this single mutation.

Taken together, these results provide strong evidence that PKD1L3 and TRPP3 are both pore-forming subunits.

**The TRPP3 homomeric complex contains three TRPP3 subunits.** TRP channels are tetramers. We previously showed that the PKD1/TRPP2 complex expressed in the oocyte plasma membrane contains one PKD1 and three TRPP2. (ref. 29) What is the subunit stoichiometry of heteromeric PKD1L3/TRPP3 channels? To address this question, we used a single-molecule photobleaching method that allows subunit counting of membrane proteins in live cells. In this approach, enhanced green fluorescent protein (EGFP)-tagged proteins are expressed in the plasma membrane of *Xenopus* oocytes at low levels and visualized with total internal reflection fluorescence (TIRF) microscopy. EGFP bleaches with continuous excitation. The number of EGFP bleaching steps represents the number of tagged subunits in a complex. When PKD1L3-EGFP was expressed alone, few EGFP spots were seen on the oocyte surface (Supplementary Fig. S4), suggesting that coexpression of TRPP3 is required to bring PKD1L3 to the surface. On the other hand, when TRPP3-EGFP was expressed alone, numerous fluorescent spots were seen on the plasma membrane (Fig. 3a), indicating that TRPP3 can reach the surface membrane either on its own or,
unpaired two-tailed Student’s t-test, except the middle panel in j. Representative I–V curve for each condition is shown in Supplementary Fig. S2. Results are shown as mean and s.d. n = number of measurements.

possibly, in complex with an unknown endogenous oocyte protein. Regardless, the TRPP3 complex that lacks PKD1L3 does not respond to acid (Fig. 1a,b). Green fluorescence spots observed with TIRF that are immobile were selected for further analysis, each spot representing a single TRPP3 homomeric complex (Fig. 3a, green circles). In the photobleaching experiments, most selected spots showed 1, 2 or 3 EGFP bleaching steps, whereas a small number of spots bleached in 4 steps (Fig. 3b,c). The distribution of 1-to-3 bleaching steps was well fit by a binomial distribution, in which each spot contains three EGFP molecules and the probability of EGFP being fluorescent is 70% (Fig. 3c), a probability of EGFP fluorescence similar to what has been seen in fusions to other ion channels29,31,32. The presence of the small number of spots bleaching in 4 steps (Fig. 3b,c).
Table 1 | Permeability ratios of WT PKD1L3/TRPP3 and the listed mutant channels for dimethylamine $^\dagger$, trimethylamine $^\dagger$ and Mg$^{2+}$.

| Channel type   | Dimethylamine $^\dagger$ | Trimethylamine $^\dagger$ | Mg$^{2+}$ |
|----------------|--------------------------|---------------------------|-----------|
|                | $\Delta E_{\text{rev}} \pm \text{s.d. (n)}$ | $P_{\text{dimethylamine}}/P_{\text{Na}}$ | $\Delta E_{\text{rev}} \pm \text{s.d. (n)}$ | $P_{\text{trimethylamine}}/P_{\text{Na}}$ | $\Delta E_{\text{rev}} \pm \text{s.d. (n)}$ | $P_{\text{Mg}}/P_{\text{Na}}$ |
| Wild type      | $-23.6 \pm 5.3$ (55)     | 0.39                      | $-46.6 \pm 8.4$ (54)   | 0.16                     | $-5.4 \pm 3.7$ (49)   | 0.65                          |
| TRPP3_D523Q    | $-8.1 \pm 4.1$ (8)       | 0.73                      | $-16.6 \pm 8.9$ (9)    | 0.52                     | $-23.1 \pm 9.1$ (8)    | 0.27                          |
| TRPP3_D525K    | $-12.2 \pm 6.9$ (11)     | 0.62                      | $-13.4 \pm 4.7$ (10)   | 0.60                     | $-12.6 \pm 9.1$ (9)    | 0.45                          |
| PKD1L3_T2067D  | $-4.8 \pm 1.2$ (10)      | 0.83                      | $-16.3 \pm 4.6$ (8)    | 0.53                     | $13.2 \pm 5.4$ (11)    | 1.91                          |
| PKD1L3_T2067P  | $-2.2 \pm 0.8$ (7)       | 0.92                      | $-9.7 \pm 2.9$ (7)     | 0.68                     | $-25.2 \pm 4.8$ (10)   | 0.24                          |
| PKD1L3_K2069D  | $-4 \pm 0.7$ (7)         | 0.85                      | $-13.8 \pm 2.2$ (7)    | 0.58                     | $22.6 \pm 0.8$ (6)     | 3.46                          |
| PKD1L3_E2072A  | $-26.8 \pm 2.7$ (7)      | 0.35                      | $-51.4 \pm 9$ (6)      | 0.13                     | $-15.5 \pm 1.2$ (7)    | 0.39                          |

$\Delta E_{\text{rev}}$ is the change of reversal potential upon switching the bath solution from Na$^+$ to the test ions ($\Delta E_{\text{rev}} = E_{\text{rev,x}} - E_{\text{rev,Na}}$). Statistical comparisons are given in Fig. 1. Results are shown as mean and s.d. $n$ = number of measurements.

Figure 3 | Homomeric TRPP3 complexes on the plasma membrane of Xenopus oocytes contain three TRPP3 subunits. (a) TIRF image from an oocyte expressing TRPP3-EGFP. Immobile spots were selected (circles) for photobleaching analysis. Scale bar, 2 μm. (b) Time course of photobleaching of two representative spots in a, showing three EGFP bleaching steps (arrows). Green bar indicates EGFP excitation. (c) Distribution of observed and calculated bleaching steps for TRPP3-EGFP fluorescence spots. The 1–3 step distribution (green bars) is well fit by a binomial distribution, in which each spot contains three EGFP molecules and the probability of EGFP being fluorescent is 78% (Fig. 4c). The distribution of 1–to-3 bleaching steps was well fit by a binomial distribution, in which each spot contains three EGFP molecules and the probability of EGFP being fluorescent is 78% (Fig. 4c). The distribution of 1–to-3 bleaching steps was well fit by a binomial distribution, in which each spot contains three EGFP molecules and the probability of EGFP being fluorescent is 78% (Fig. 4c). The distribution of 1–to-3 bleaching steps was well fit by a binomial distribution, in which each spot contains three EGFP molecules and the probability of EGFP being fluorescent is 78% (Fig. 4c). The distribution of 1–to-3 bleaching steps was well fit by a binomial distribution, in which each spot contains three EGFP molecules and the probability of EGFP being fluorescent is 78% (Fig. 4c).

The PKD1L3/TRPP3 complex contains one PKD1L3 and three TRPP3. To determine the subunit stoichiometry of the PKD1L3/TRPP3 complex, we coexpressed EGFP-tagged TRPP3 with mCherry-tagged PKD1L3. Under these conditions, we observed numerous red fluorescent spots on the cell surface (Fig. 4a), supporting the notion that coexpression with TRPP3 helps transport PKD1L3 to the plasma membrane. Fluorescent spots showing green and red dual fluorescence, which are expected to be PKD1L3/TRPP3 complexes, were selected for further analysis. Again, most spots that had both green and red fluorescence showed 1, 2 or 3 EGFP bleaching steps, while a small number of spots bleached in 4 steps (Fig. 4b,c). The distribution of 1-to-3 bleaching steps was well fit by a binomial distribution, in which each spot contains three EGFP molecules and the probability of EGFP being fluorescent is 78% (Fig. 4c). The distribution of 1-to-3 bleaching steps was well fit by a binomial distribution, in which each spot contains three EGFP molecules and the probability of EGFP being fluorescent is 78% (Fig. 4c). The distribution of 1-to-3 bleaching steps was well fit by a binomial distribution, in which each spot contains three EGFP molecules and the probability of EGFP being fluorescent is 78% (Fig. 4c). The distribution of 1-to-3 bleaching steps was well fit by a binomial distribution, in which each spot contains three EGFP molecules and the probability of EGFP being fluorescent is 78% (Fig. 4c). The distribution of 1-to-3 bleaching steps was well fit by a binomial distribution, in which each spot contains three EGFP molecules and the probability of EGFP being fluorescent is 78% (Fig. 4c). The distribution of 1-to-3 bleaching steps was well fit by a binomial distribution, in which each spot contains three EGFP molecules and the probability of EGFP being fluorescent is 78% (Fig. 4c). The distribution of 1-to-3 bleaching steps was well fit by a binomial distribution, in which each spot contains three EGFP molecules and the probability of EGFP being fluorescent is 78% (Fig. 4c). The distribution of 1-to-3 bleaching steps was well fit by a binomial distribution, in which each spot contains three EGFP molecules and the probability of EGFP being fluorescent is 78% (Fig. 4c). The distribution of 1-to-3 bleaching steps was well fit by a binomial distribution, in which each spot contains three EGFP molecules and the probability of EGFP being fluorescent is 78% (Fig. 4c).
Coiled-coil trimer is critical for TRPP3 homomeric assembly.

To test the effect of the TRPP3 coiled-coil trimer interaction on the assembly of the full-length TRPP3 homomeric complex, two TRPP3 mutants were made to disrupt the coiled-coil trimer: one was TRPP3 with mut6 (named TRPP3_mut6) and the other was TRPP3 with its coiled-coil domain (G704-K741) replaced with eight glycines (named TRPP3_CCto8G). FLAG-tagged TRPP3 carrying each mutation was coexpressed with HA-tagged WT TRPP3 with its coiled-coil trimer interaction on the assembly of the full-length TRPP3 homomeric complex, two TRPP3 mutants were made to disrupt the coiled-coil trimer: one was TRPP3 with mut6 (named TRPP3_mut6) and the other was TRPP3 with its coiled-coil domain (G704-K741) replaced with eight glycines (named TRPP3_CCto8G). FLAG-tagged TRPP3 carrying each mutation was coexpressed with HA-tagged WT TRPP3 in *Xenopus* oocytes. Coimmunoprecipitation showed that homomeric TRPP3 association was markedly weakened by both mutations (Fig. 6a). TIRF imaging further showed that mut6 greatly decreased the surface expression of TRPP3 homomeric complexes in oocytes (Fig. 6b). These results indicate that the integrity of the TRPP3 C-terminal trimer is critical for the assembly of the homomeric TRPP3 complex and its surface expression.

**TRPP3 trimer is crucial for PKD1L3/TRPP3 surface expression.** When PKD1L3 was coexpressed in oocytes with either TRPP3_mut6 or TRPP3_CCto8G, acid-induced currents were greatly diminished (Fig. 7a). This loss of current was primarily due to a diminished surface expression of the mutant PKD1L3/TRPP3 complexes. TIRF imaging showed that mut6 greatly decreased the surface expression of PKD1L3/TRPP3 complexes in oocytes (Fig. 7b). This result was confirmed by detecting surface protein with surface biotinylation (Fig. 7c). While FLAG-tagged WT TRPP3 (FLAG–TRPP3) showed detectable expression on the plasma membrane (Fig. 7c, lane 2 in upper gels), FLAG–TRPP3_mut6 showed little surface expression (Fig. 7c, lane 5). Likewise, while coexpressing WT PKD1L3 and WT TRPP3 produced measurable surface expression of both proteins (Fig. 7c, lane 4), coexpressing WT PKD1L3 with either of the two aforementioned TRPP3 mutants produced greatly reduced surface expression of either protein (Fig. 7c, lanes 6 and 7 and bar graph). Consistent with TIRF imaging of PKD1L3 alone (Supplementary Fig. S4), surface biotinylation showed that PKD1L3 expressed poorly on the plasma membrane on its own.
were obtained assuming that the protein is a monomer (for mut6), a trimer (for WT) or a tetramer (for WT).

The right shift of the latter indicates a decrease in molecular mass. (f) Gel filtration profile of MBP-tagged WT TRPP3 C-terminal fragment (TRPP3_CT) and MBP-TRPP3_CT_mut6. Measured molecular masses were obtained by static light scattering. Calculated molecular masses of MBP–TRPP3_CT and MBP–TRPP3_CT_mut6. The underline amino acids were mutated to alanine to generate mut6. (g) Superposition of the structures of TRPP3–G699–W743 fragment, showing that the TRPP3 coiled-coil domain forms a trimer. (d) Side chains, shown in sticks, of hydrophobic residues involved in the formation of the coiled-coil domain trimer. The underlined amino acids were mutated to alanine to generate mut6. (c) Crystal structure of TRPP3-G699–W743 fragment, showing that the TRPP3 coiled-coil domain forms a trimer. (b) Amino-acid sequence alignment of TRPP2 and TRPP3 coiled-coil domains. Red: identical; green: conserved. Asterisks indicate hydrophobic residues at the 1st and 4th positions in characteristic heptad repeats of canonical coiled-coil domains. (a) Putative transmembrane topology of TRPP2 and TRPP3. The C terminus of both proteins contains a coiled-coil domain (red bar). (e) Superposition of the structures of TRPP3 and TRPP2 coiled-coil domains (PDB: 3HRN).

Discussion

Mutations of residues that line the ion conduction pathway of an ion channel usually significantly alter its ion selectivity. This concept has often been applied to test whether a protein is an ion channel-forming subunit. On the other hand, mutations of non-pore-lining regulatory proteins that even interact directly with ion channels rarely affect the channels’ ion selectivity. To our knowledge, the only reported non-pore lining protein whose mutations affect a channel’s ion selectivity is mink (KCNE1), a K^+ channel regulatory subunit. In that case, however, only one residue was found that, when mutated, was able to increase the relative permeability of one ion (Na^+) by sevenfold (ref. 39).

In this study, we found that single amino-acid mutations at three positions in PKD1L3 greatly changed the permeability of the PKD1L3/TRPP3 complex to three different cations (Fig. 2, Table 1 and Supplementary Fig. S2). These results strongly support the notion that PKD1L3 regulates the TRPP3 channel directly by coassembling with it to form the channel pore, showing that a member of the PKD family functions as a channel-forming subunit in addition to its possible role as a cell surface receptor. Whether other PKD proteins also function as channel-forming subunits remains to be determined. It is interesting to note that although PKD1 has been postulated to function solely as a regulator of the TRPP2 channel, its putative pore-lining region also exists in PKD1 and the PKD1/TRPP2 complex also has a 1:3 stoichiometry. It is possible that PKD protein serving as a pore-forming subunit may be a common feature of PKD/TRPP complexes.

The TRPP2 coiled-coil domain forms a trimer and mediate the homomeric trimer assembly of the TRPP2 C terminus. In this study, we found that single amino-acid mutations at three positions in PKD1L3 greatly changed the permeability of the PKD1L3/TRPP3 complex to three different cations (Fig. 2, Table 1 and Supplementary Fig. S2). These results strongly support the notion that PKD1L3 regulates the TRPP3 channel directly by coassembling with it to form the channel pore, showing that a member of the PKD family functions as a channel-forming subunit in addition to its possible role as a cell surface receptor. Whether other PKD proteins also function as channel-forming subunits remains to be determined. It is interesting to note that although PKD1 has been postulated to function solely as a regulator of the TRPP2 channel, its putative pore-lining region also exists in PKD1 and the PKD1/TRPP2 complex also has a 1:3 stoichiometry. It is possible that PKD protein serving as a pore-forming subunit may be a common feature of PKD/TRPP complexes.
of full-length TRPP2. In this study we found that a similar coiled-coil domain is present in TRPP3, forms a trimer, and is critical for the trimeric assembly of the TRPP3 C terminus and of full-length TRPP3. Amino-acid sequence alignment suggests that a coiled-coil domain also exists in the C terminus of TRPP5 (also known as polycystin-L2 or PKD2L2), the third TRPP family protein. This conservation suggests that homomeric trimer assembly may be a common property of the TRPP subfamily. A possible utility of forming a stable homotrimer rather than a stable homotetramer is that it allows the TRPP trimer to associate with either a fourth TRPP subunit to form a homomeric complex or other channel-forming subunits like PKD proteins to form a heteromeric complex. A similar heteromeric assembly mechanism is seen in the cyclic nucleotide-gated channels of rod photoreceptors. This channel contains three A1 and one B1 subunits, and that this 3:1 stoichiometry is determined by a trimer-forming coiled-coil domain in the C terminus of the A1 subunit.

The C-terminal coiled-coil domain is critical for the direct interaction between TRPP2 and PKD1 and the assembly of the TRPP2/PKD1 complex. The molecular determinants of TRPP3 and PKD1L3 association remain to be determined. The transmembrane domains have been reported to be important. Disruption of the TRPP3 coiled-coil trimer does not eliminate direct association of TRPP3 and PKD1L3 (Fig. 7d), but it greatly reduces the surface expression of the PKD1L3/TRPP3 complex (Figs 7b,c). Thus, the C-terminal coiled-coil domain is essential for the proper assembly of both TRPP2/PKD1 and PKD1L3/TRPP3 complexes.

In conclusion, we find that PKD1L3 functions as a channel-forming subunit and associates with TRPP3 in a 1:3 stoichiometry to form an acid-activated heteromeric channel. As a candidate sour taste receptor, PKD1L3 and TRPP3 colocalize in sour taste cells. Besides, both TRPP3 and PKD1L3 are widely expressed and their expression overlaps in many tissues, suggesting that the PKD1L3/TRPP3 complex may exist in tissues other than the tongue. The physiological role of PKD1L3/TRPP3 complexes in those tissues remains to be explored.

**Methods**

** Constructs and cloning.** Human TRPP3 (NCBI accession number NM_016112) and mouse PKD1L3 (AY164486) were used. The construct for HA-tagged PKD1L3 was described previously. All mutations were checked by sequencing. TRPP3 constructs used for protein purification for light scattering experiments were cloned into a modified pcDFduet-1 vector (Novagen) containing an N-terminal MBP complementary DNA (cDNA). The TRPP3 C terminus construct used for crystallization was cloned into the pCDFduet-1 vector (Novagen). For constructs used for in vitro RNA synthesis, cDNAs were cloned into a modified pGEMHE vector. Constructs used in TIRF imaging experiments were cloned into a modified pGEMHE vector containing the cDNA for EGFP or mCherry. A flexible linker (SRGTSGGGSSRSGSGLGG) was added between the fluorescent proteins and our constructs.

** Electrophysiology.** cRNA (antisense RNA) was synthesized in vitro and injected into Xenopus oocytes at 15 ng per oocyte. The injected RNA has a molar ratio of TRPP3/PKD1L3 = 1:3 to ensure an excess amount of PKD1L3. Whole-oocyte currents were recorded from oocytes 4 to 5 days after injection using TEVC.

** Solutions.** Standard bath solution contains 100 mM NaCl, 0.5 mM MgCl₂ and 2 mM HEPES (pH 7.5). pH was adjusted with NaOH. Acid solution was generated by adding citric acid into the bath solution until pH reached 2.8. For solutions used in the ion permeability assay, 100 mM NaCl in the standard bath solution and voltage steps were applied to monitor the leak current. Oocytes were then washed with the standard solution while monitoring with voltage steps until the reversal potential shift stopped. Finally, another protocol was reapplied for extracting I–V relationships, 50-ms voltage steps from 80 mV were discarded. Acid solution was applied to qualitied oocytes for 12 s followed by washing with the solution containing dimethylamine + , trimethylamine + or Mg²⁺ until the current reached its peak amplitude (monitored with voltage ramps). Subsequently, the I–V protocol was reapplied for extracting I–V curves and the reversal potential for the test ion. Oocytes were then washed with the standard solution while monitoring with voltage ramps until the reversal potential shift stopped. Finally, another voltage step protocol was applied for extracting the I–V curve and reversal potential in Na⁺.
Relative permeabilities for monovalent cations were calculated based on changes of reversal potential upon switching the bath solution of NaCl to the test solutions. The permeability ratios, $P_N/P_M$, were calculated according to the equation derived from the Goldman-Hodgkin-Katz (GHK) equation:43

$$P_N/P_M = e^{\Delta E_{rev}/RT}$$

where $E_{rev}$ is the reversal potential, $\Delta E_{rev} = E_{rev,Na} - E_{rev,MCl}$ is Faraday’s constant, $R$ is the universal gas constant, and $T$ is the absolute temperature. We considered the effect of Mg$^{2+}$ in the monovalent cation solutions negligible.

$$P_{Na}/P_{Mg} = \frac{[Na^+]_o e^{\Delta E_{rev}/RT}(1 + e^{\Delta E_{rev}/RT})}{4[Mg^{2+}]_o}$$

Activity coefficients for NaCl and MgCl$_2$ were determined from the tables of Robinson and Stokes49, and those for dimethylamine$^+$ and trimethylamine$^+$ were assumed to be equal to that of Na$^+$.
Data were presented as mean ± s.d. Statistical significance was determined by
unpaired two-tailed Student’s t-test.

**TIRF microscopy and EGFP bleaching steps.** The detailed methods have been
described previously29. Oocytes were used at 24–48 h after cRNA injection,
mCherry and EGFP fluorescence was recorded as movie and fluorescent spots that
were immobile during the movie were selected and the number of EGFP bleaching
steps was manually counted.

**Protein fragment expression and purification.** The TRPP3 C-terminal fragment
(His6–TRPP3_K568-S805) used for crystallization was expressed in Rosetta (DE3)
cells (Novagen). Bacteria were lysed with a Branson digital sonifier in a lysis
solution containing 50 mM Tris–HCl, 250 mM NaCl, 2.5% glycerol, and 7 mM
β-mercaptoethanol (pH 7.8). Protein was pulled down with Ni-NTA His-Bind
beads (Novagen) and eluted from the beads with 250 mM imidazole. The His-tag
was removed by incubating overnight with thrombin (Sigma) at 20°C. The protein
was then purified with a Superose 12 column (GE Healthcare) on an Amersham
Pharmacia AKTA Purifier in a solution contained 250 mM NaCl, 1% glycerol,
10 mM HEPES, pH 7.5.

The TRPP3 C-terminal fragment used for light scattering was expressed as an
MBP-tagged protein, purified with an anion exchange resin (New England Biolabs),
and eluted from the beads with 20 mM maltose. The protein was then purified with a
Superose 6 column (GE Healthcare).

Detailed methods for protein purification have been described previously29.

**Light scattering measurements.** Purified protein samples were concentrated
to 10 mg ml–1. Protein (50 µl) was loaded on a gel filtration column
Protein KW–804 (Shodex) with a solution containing 200 mM NaCl and
100 mM Tris–HCl (pH 7.5). Eluates were examined by static light-scattering
(Wyat Technology).

**Structure determination.** Crystallization of TRPP3_K568–S805 was carried out
with the hanging-drop vapour diffusion method at 20°C. The protein concentra-
tion was 20 mg ml–1 and the reservoir solution contained 18–20% PEG 8000,
100 mM HEPES, pH 7.5. Drops were set up with 1.6
mM HEPES, pH 7.5. Drops were set up with 1.6

**References**

1. Delmas, P. Polycystins: polymodal receptor/ion-channel cellular sensors.
Pflugers Arch. 451, 264–276 (2005).
2. Harris, P.C. & Torres, V.E. Polycystic kidney disease. Annu. Rev. Med. 60,
321–337 (2009).
3. Ong, A.C. & Harris, P.C. Molecular pathogenesis of ADPKD: the polycystin
complex gets complex. Kidney Int. 67, 1234–1247 (2005).
4. Zhou, J. Polycystins and primary cilia: primers for cell cycle progression. Annu.
Rev. Physiol. 71, 83–113 (2009).
5. Damann, N., Voets, T. & Nilius, B. TRPs in our senses. Curr. Biol. 18,
R861–889 (2008).
6. Owsianik, G., D’Hoedt, D., Voets, T. & Nilius, B. Structure-function
relationship of the TRP channel superfamily. Rev. Physiol. Biochem. Pharmacol.
156, 61–90 (2006).
7. Ramsey, I.S., Delling, M. & Clapham, D.E. An introduction to TRP channels.
Annu. Rev. Physiol. 69, 619–647 (2007).
8. Venkatachalam, K. & Montell, C. TRP channels. Annu. Rev. Biochem. 76,
387–417 (2007).
9. Wu, L.J., Sweet, T.B. & Clapham, D.E. Current progress in the mammalian TRP
ion channel family. Pharmacol. Rev. 62, 384–410 (2010).
10. Huang, A.L. et al. The cells and logic for mammalian sour taste detection.
Nature 442, 934–938 (2006).
11. Ishimaru, Y. et al. Transient receptor potential family members PKD1L3 and
PKD2L1 form a candidate sour taste receptor. Proc. Natl Acad. Sci. USA 103,
12569–12574 (2006).
12. Kawaguchi, H. et al. Activation of polycystic kidney disease-2-like 1 (PKD2L1–
PKD1L3) complex by acid in mouse taste cells. J. Biol. Chem. 285, 17277–17281
(2010).
13. LopeiJimenez, N.D. et al. Two members of the TRPP family of ion channels,
PKD1L3 and PKD2L1, are co-expressed in a subset of taste receptor cells.
J. Neurochem. 98, 68–77 (2006).
14. Inada, H. et al. Off-response property of an acid-activated cation channel
complex PKD1L3–PKD2L1. EMBO Rep. 9, 690–697 (2008).
15. Ishii, S. et al. Acidic taste activates PKD1L3–PKD2L1 channel—a candidate sour
taste receptor. Biochem. Biophys. Res. Commun. 385, 346–350 (2009).
16. Ishimaru, Y. & Matsunami, H. Transient receptor potential (TRP) channels and
taste sensation. J. Dent. Res. 88, 212–218 (2009).
17. Huque, T. et al. Sour ageusia in two individuals implicates ion channels of the
ASIC and PKD families in human sour taste perception at the anterior tongue.
PLoS One 4, e7347 (2009).
18. Horio, N. et al. Sour taste responses in mice lacking PKD channel proteins.
PLoS One 6, e20007 (2011).
19. Nelson, T.M. et al. Sour taste function in mice with a targeted mutation of the
T1R3 gene. Proc. Natl Acad. Sci. USA 107, 22320–22325 (2010).
20. Anaytonwu, G.I. & Ehrlich, B.E. Organic cation permeation through the channel
formed by polycystin-2. J. Biol. Chem. 280, 29488–29493 (2005).
21. Chen, X.Z. et al. Polycystin-L is a calcium-regulated cation channel permeable
to calcium ions. Nature 401, 383–386 (1999).
22. Gonzalez-Perrett, S. et al. Polycystin-2, the protein mutated in autosomal
dominant polycystic kidney disease (ADPKD), is a Ca2+-permeable
nonselective cation channel. Proc. Natl Acad. Sci. USA 98, 1182–1187 (2001).
23. Kounel, P. et al. Polycystin-2 is an intracellular calcium release channel. Nat.
Cell Biol. 4, 191–197 (2002).
24. Shimizu, T., Janssens, A., Voets, T. & Nilius, B. Regulation of the murine TRPP3
channel by voltage, pH, and changes in cell volume. Pflugers Arch. 457,
795–807 (2009).
26. Shimizu, T., Higuchi, T., Fujii, T., Nilius, B. & Sakai, H. Bimodal effect of alkalinization on the polycystin transient receptor potential channel, PKD2L1. Pflugers Arch. 461, 507–513 (2011).
27. Mochizuki, T. et al. PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. Science 272, 1339–1342 (1996).
28. Li, A., Tian, X., Sung, S.W. & Somlo, S. Identification of two novel polycystic kidney disease-1-like genes in human and mouse genomes. Genomics 81, 596–608 (2003).
29. Yu, Y. et al. Structural and molecular basis of the assembly of the TRPP2/PKD1 complex. Proc. Natl Acad. Sci. USA 106, 11558–11563 (2009).
30. Fujimoto, C. et al. The single pore residue Asp523 in PKD2L1 determines Ca\(^{2+}\) permeation of the PKD1L3/PKD2L1 complex. Biochim. Biophys. Res. Commun. 404, 946–951 (2011).
31. Ulbrich, M.H. & Isacoff, E.Y. Subunit counting in membrane-bound proteins. Nat. Methods 4, 319–321 (2007).
32. Ulbrich, M.H. & Isacoff, E.Y. Rules of engagement for NMDA receptor subunits. Proc. Natl Acad. Sci. USA 105, 14161–14168 (2008).
33. Molland, K.L., Narayanan, A., Burgner, J.W. & Yernool, D.A. Identification of the structural motif responsible for trimeric assembly of the C-terminal regulatory domains of polycystin channels PKD2L1 and PKD2. Biochem. J. 429, 171–183 (2010).
34. Molland, K.L., Paul, L.N. & Yernool, D.A. Crystal structure and characterization of coiled-coil domain of the transient receptor potential channel PKD2L1. Biochim. Biophys. Acta 1824, 413–421 (2012).
35. Ishimaru, Y. et al. Interaction between PKD1L3 and PKD2L1 through their transmembrane domains is required for localization of PKD2L1 at taste pores in taste cells of circumvallate and foliate papillae. FASEB J. 24, 4058–4067 (2010).
36. Kang, L., Gao, J., Schafer, W.R., Xie, Z. & Xu, X.Z. C. elegans TRP family protein TRP-4 is a pore-forming subunit of a native mechanotransduction channel. Neuron 67, 381–391 (2010).
37. Prakraiy, M. et al. Oral is an essential pore subunit of the CRAC channel. Nature 443, 230–233 (2006).
38. Yeromin, A.V. et al. Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. Nature 443, 226–229 (2006).
39. Tai, K.K. & Goldstein, S.A. The conduction pore of a cardiac potassium channel. Nature 391, 605–608 (1998).
40. Feng, S., Rodat-Despoix, L., Delmas, P. & Ong, A.C. A single amino acid residue constitutes the third dimerization domain essential for the assembly and function of the tetrameric polycystin-2 (TRPP2) channel. J. Biol. Chem. 286, 18994–19000 (2011).
41. Behn, D. et al. Quantifying the interaction of the C-terminal regions of polycystin-2 and polycystin-1 attached to a lipid bilayer by means of QCM. Biophys. Chem. 150, 47–53 (2010).
42. Guo, L. et al. Identification and characterization of a novel polycystin family member, polycystin-L2, in mouse and human: sequence, expression, alternative splicing, and chromosomal localization. Genomics 64, 241–251 (2000).
43. Zhang, P. et al. The multicentric structure of polycystin-2 (TRPP2): structural-functional correlates of homo- and hetero-multimers with TRPC1. Hum. Mol. Genet. 18, 1238–1251 (2009).
44. Matulef, K. & Zagotta, W.N. Cyclic nucleotide-gated ion channels. Annu. Rev. Cell Dev. Biol. 19, 23–44 (2003).
45. Qian, F. et al. PKD1 interacts with PKD2 through a probable coiled-coil domain. Nat. Genet. 16, 179–183 (1997).
46. Tsikas, L., Kim, E., Arnould, S., Sukhatme, V.P. & Wals, G. Homodimeric and heterodimeric interactions between the gene products of PKD1 and PKD2. Proc. Natl Acad. Sci. USA 94, 6965–6970 (1997).
47. Nomura, H. et al. Identification of PKDL1, a novel polycystic kidney disease 2-like gene whose murine homologue is deleted in mice with kidney and retinal defects. J. Biol. Chem. 273, 25967–25973 (1998).
48. Hille, B. Ionic Channels of Excitable Membranes. (Sinauer Associates Inc., 1992).
49. Robinson, R.A. & Stokes, R.H. Electrolyte Solutions. (Butterworth Scientific Publications 1959).
50. Otwinowski, Z. & Minor, W. Processing of X-ray diffraction data collected in oscillation mode. Methods Enzymol. 276, 307–326 (1997).
51. Storoni, L.C., McCoy, A.J. & Read, R.J. Likelihood-enhanced fast rotation functions. Acta. Crystallogr. D Biol. Crystallogr. 60, 432–438 (2004).
52. Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. Acta. Crystallogr. D Biol. Crystallogr. 60, 2126–2132 (2004).
53. Brunger, A.T. et al. Crystallography & NMR system: a new software suite for macromolecular structure determination. Acta. Crystallogr. D Biol. Crystallogr. 54, 905–921 (1998).

Acknowledgements

We thank Dr H. Matsunami at Duke University for mouse PKD1L3 clone and members of Yang laboratory for commenting on the manuscript. This work was supported by the National Institutes of Health Grants GM085234 and NS045383 (to J.Y.) and NS035549 (to E.Y.I.).

Author contributions

Y.Y. performed molecular biology, biochemistry and electrophysiology experiments as well as protein purification and crystallization. M.H.U. performed single-molecule photobleaching experiments. S.D. tested TRPP3 oligomers in different SDS–PAGE conditions. M.L. solved the crystal structure. W.K.Z. helped with electrophysiology experiments. J.Y. and Y.Y. wrote the manuscript. All authors discussed the results and commented on the manuscript.

Additional information

Accession codes: The coordinates and structural factors for the TRPP3 coiled-coil domain have been deposited in the Protein Data Bank (PDB) under the accession code 4G1F.

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

How to cite this article: Yu Y. et al. Molecular mechanism of the assembly of an acid-sensing receptor ion channel complex. Nat. Commun. 3:1252 doi: 10.1038/ncomms2257 (2012).