Formation of a new receptor-operated channel by heteromeric assembly of TRPP2 and TRPC1 subunits

Chang-Xi Bai^{1*}, Aurélie Giamarchi^{2*}, Lise Rodat-Despoix², Françoise Padilla², Tamyra Downs¹, Leonidas Tsiokas¹⁺ & Patrick Delmas²⁺⁺

¹University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA, and ²CNRS, UMR 6231, Université de la Méditerranée, Marseille, France

Although several protein–protein interactions have been reported between transient receptor potential (TRP) channels, they are all known to occur exclusively between members of the same group. The only intergroup interaction described so far is that of TRPP2 and TRPC1; however, the significance of this interaction is unknown. Here, we show that TRPP2 and TRPC1 assemble to form a channel with a unique constellation of new and TRPP2/TRPC1-specific properties. TRPP2/TRPC1 is activated in response to G-protein-coupled receptor activation and shows a pattern of single-channel conductance, amiloride sensitivity and ion permeability distinct from that of TRPP2 or TRPC1 alone. Native TRPP2/TRPC1 activity is shown in kidney cells by complementary gain-of-function and loss-of-function experiments, and its existence under physiological conditions is supported by colocalization at the primary cilium and by co-immunoprecipitation from kidney membranes. Identification of the heteromultimeric TRPP2/TRPC1 channel has implications in mechanosensation and cilium-based Ca²⁺ signalling.

Keywords: TRP channel; polycystic kidney disease; mechanosensation; primary cilium; polycystin 2; polycystin 1
EMBO reports (2008) 9, 472–479. doi:10.1038/embor.2008.29

INTRODUCTION

TRPP2, also called polycystin 2, belongs to the polycystin subgroup of transient receptor potential (TRP) superfamily (Delmas, 2004, 2005; Montell, 2005; Nilius & Voets, 2005; Giamarchi *et al*, 2006; Köttgen, 2007) and was originally cloned because of its involvement in autosomal dominant polycystic kidney disease (ADPKD; Mochizuki *et al*, 1996). Naturally occurring mutations in TRPP2 are responsible for approximately

15% of all cases of ADPKD, which is one of the most common genetic diseases primarily affecting the kidney. TRPP2 forms a highly conductive cation channel (Gonzalez-Perrett *et al*, 2001) requiring polycystin 1 (PKD1) for its activity (Hanaoka *et al*, 2000; Delmas *et al*, 2004). The channel is highly versatile and is involved in epidermal growth factor-mediated currents (Ma *et al*, 2005; Tsiokas *et al*, 2007), intracellular Ca²⁺ release (Koulen *et al*, 2002) and mechanodetection (Nauli *et al*, 2003) in kidney cells.

One of the most exciting properties of TRP channels is their ability to heteromultimerize. The first example, to our knowledge, of a functional interaction between two TRP channels was reported for *Drosophila* homologues TRP and TRPL (Xu *et al*, 1997). Subsequently, protein–protein interactions have been shown for various members of TRPC and TRPV subfamilies (Nilius *et al*, 2007). Interestingly, all these interactions occurred exclusively between members of the same group, implicating primary sequence homology as the underlying factor in protein–protein associations.

Long before the functional characterization of TRPP2 or TRPC1, Tsiokas *et al* (1999) described a protein–protein interaction between them. On the basis of biochemical data, the interaction was mediated by two distinct domains in TRPP2: one involving the carboxy-terminal cytosolic tail, and the other involving a larger domain containing transmembrane segments 2–6 and intervening loops. There is also evidence for the role of TRPC1 in store- and receptor-operated Ca²⁺ entries (Beech, 2005; Ambudkar, 2007; Yuan *et al*, 2007). Recently, two further roles of TRPC1 in mechanotransduction (Maroto *et al*, 2005; Dietrich *et al*, 2007; Gottlieb *et al*, 2008) and chemoattraction (Shim *et al*, 2005; Wang & Poo, 2005) have been reported.

Here, we show that TRPP2 and TRPC1 assemble to form a heteromeric channel with biophysical properties and modes of activation distinct from that of the individual channels. One of the physiological roles of this new channel is to mediate G-protein-coupled receptor (GPCR)-induced conductance in native kidney epithelial cells.

RESULTS AND DISCUSSION

Formation of a new GPCR-activated TRP channel

We initiated our studies by testing whether TRPP2 and TRPC1 could form a heteromeric channel in cultured rat sympathetic

¹University of Oklahoma Health Sciences Center, 975 NE 10th Street, Oklahoma City, Oklahoma, 73104, USA

²CNRS, UMR 6231, IFR Jean Roche, Université de la Méditerranée, Boulevard Pierre Dramard, 13916, Marseille Cedex 20, France

*These authors contributed equally to this work

+Corresponding author. Tel: +1 405 271 8001 ext 46211; Fax: +1 405 271 3758; E-mail: leonidas-tsiokas@ouhsc.edu

++Corresponding author. Tel: +33 4 91 69 89 70; Fax: +33 4 91 69 89 77; E-mail: patrick.delmas@univmed.fr

Fig 1 | Differential activation mechanisms of transient receptor potential ion channel complexes. (A) Schematic diagram of an outside-out perforated microvesicle obtained with amphotericin-B (Ampho-B) in the patch pipette solution. (B–E) Single-channel activities from cells expressing (B) TRPP2, (C) PKD1/TRPP2, (D) TRPC1 and (E) TRPP2/TRPC1. Each microvesicle was exposed to the MR3 antibody (1/100 for 2 min) or BK (500 nM for 30 s). Traces are shown at different timescales. $V_h = -40$ mV. The cell in (E) was microinjected with 300 ng/ μ l and 100 ng/ μ l of TRPP2 and TRPC1 complementary DNAs, respectively. (F) Amplitude histograms of cation channel currents for the cells shown in (C–E). Bottom-right panel: superimposed Gaussian curves for PKD1/TRPP2, TRPC1 and TRPP2/TRPC1 channels showing open-state amplitude levels of -5.55 , -2.01 and -0.79 pA, respectively. Vertical bars indicate the number of events. BK, bradykinin; PKD, polycystin; TRP, transient receptor potential.

neurons, using perforated microvesicles (Fig 1A). Fig 1B–E shows single-channel activities observed in microvesicles excised from cells expressing TRPP2, PKD1/TRPP2, TRPC1 and TRPP2/TRPC1. Neither application of the PKD1-specific MR3 antibody ($n = 9/9$), which functions as an activating stimulus (Delmas *et al*, 2004), nor stimulation of endogenous B_2 bradykinin receptors (BK, 500 nM; $n = 12/12$) resulted in channel activation (Fig 1B). However, TRPP2-like cation channels were activated by MR3 in 21 out of 71 microvesicles isolated from cells expressing PKD1 and TRPP2 (Fig 1C,F). Mean amplitude at -40 mV was -6.15 ± 0.4 pA ($n = 20$; Fig 2A). PKD1/TRPP2 channels did not open in response to exposure to BK ($n = 12/12$; Fig 1C). By contrast, BK—but not

MR3—activated single-channel events of -0.9 ± 0.1 pA in microvesicles expressing TRPC1 (Figs 1D,F,2A). TRPP2 and TRPC1 complementary DNAs microinjected at a ratio of 1:1 resulted in BK-induced cation channels with a mean unitary amplitude at -40 mV of -1.7 ± 0.2 pA ($n = 23/38$; Fig 2A). Channels with a mean unitary amplitude of -2.14 ± 0.1 pA ($n = 20$) were detected in microvesicles derived from cells microinjected with an excess of TRPP2 plasmid compared with TRPC1 (3:1, respectively; Fig 2A).

Activation of TRPP2/TRPC1 channels by BK was secondary to phospholipase C activation, as pretreatment with U73122 (10 μ M for 10 min), but not its inactive analogue, abolished BK-induced channel activity ($n = 7/7$; data not shown). None

Fig 2 | Single-channel conductance of TRPP2 and TRPC1 channel complexes. (A) Scatter graph showing single-channel amplitude in microvesicles from cells expressing TRPP2, PKD1/TRPP2, TRPC1, TRPC1/TRPP2, TRPC1/TRPP2-D511V and PKD1/TRPP2-D511V. Cells were intranuclearly microinjected with 100 ng/ μ l (1) or 300 ng/ μ l (3) complementary DNA. The mean $\pm$ SEM is shown. $V_h = -40$ mV. The grey bar indicates the limit of resolution at the filter frequencies used. (B,C) Mean pooled I - V relationships of (B) PKD1/TRPP2 and PKD1/TRPP2-D511V channels and (C) TRPC1 and TRPC1/TRPP2 channels. Each data point represents the mean $\pm$ SEM of 10–17 microvesicles. Mean unitary conductances of PKD1/TRPP2, TRPC1 and TRPC1/TRPP2 channels were 142 ± 5 , 16 ± 1 and 40 ± 5 pS, respectively. Dashed lines represent 95% confidence limits. BK, bradykinin; MR3, PKD1 specific antibody; PKD, polycystin; TRP, transient receptor potential; TRPP2-D511V, channel-dead mutant.

of the TRPP2/TRPC1-expressing microvesicles responded to MR3 challenge (Fig 1E). Single-channel conductance of PKD1/TRPP2, TRPC1 and TRPC1/TRPP2 channels determined under similar recording conditions were 142 ± 2 , 16 ± 0.5 and 40 ± 2 pS, respectively (Fig 2B,C).

To provide further evidence for a functional interaction between TRPP2 and TRPC1, single-channel activities were analysed in microvesicles expressing TRPC1 and the channel-dead mutant TRPP2-D511V (Fig 2A). Out of 17 microvesicles challenged with BK, only 4 showed a BK-induced cation current with a unitary amplitude at -40 mV of approximately -1 pA, which was close to that of TRPC1 expressed alone, indicating a dominant-negative effect of TRPP2-D511V on TRPC1. The lack of TRPP2-D511V activity was confirmed by coexpression of PKD1, in which no channel activity was seen in response to MR3 ($n = 14/14$; Fig 2A,B).

Pharmacological properties of TRPP2/TRPC1 channels

We compared the effects of lanthanoides on single-channel activities (supplementary Fig 1 online). When $100 \mu\text{M}$ La^{3+} was applied to PKD1/TRPP2-expressing cells stimulated with MR3, rapid inhibition of PKD1/TRPP2 channel activity was observed

(supplementary Fig 1A online). Similar results were obtained for both TRPC1- and TRPP2/TRPC1-mediated ion channel currents during exposure to BK (supplementary Fig 1B,C online), indicating that PKD1/TRPP2, TRPC1 and TRPP2/TRPC1 channel complexes cannot be discriminated by using lanthanoides (supplementary Fig 2 online).

We and others have shown that the PKD1/TRPP2 channel is inhibited by bath application of amiloride (Gonzalez-Perrett *et al*, 2001, 2002; Delmas *et al*, 2004). Therefore, we tested whether amiloride could also inhibit TRPC1 and TRPP2/TRPC1 channels. Consistent with previous results, treatment of PKD1/TRPP2 channels with amiloride ($100 \mu\text{M}$) increased the number of short flicker closings and reduced single-channel amplitude at -40 mV by approximately $69 \pm 2.5\%$ (Fig 3A,D). Higher concentrations also reduced p_o (data not shown). By contrast, amiloride had little effect on TRPC1 current, but reduced TRPP2/TRPC1 single-channel current by $40.7 \pm 2\%$ (Fig 3B–D).

Competition between TRPC1 and PKD1 for TRPP2

As TRPP2 interacted with PKD1 and TRPC1 through the same region in its C-terminal cytoplasmic tail and formed heteromeric channels with different biophysical properties, we tested whether

Fig 3 | Differential effects of amiloride on TRPP and TRPC1 channels. Inhibition of (A) PKD1/TRPP2, (B) TRPC1 and (C) TRPP2/TRPC1 cation channels by 100 μ M amiloride (Amil.). Sample traces obtained from the same recordings are given at two different time resolutions. Amplitude histograms in the absence and presence of amiloride are also given. $V_h = -40$ mV. (D) Conductances of PKD1/TRPP2, TRPC1 and TRPP2/TRPC1 channels were plotted against the decrease in single-channel amplitude induced by amiloride. Each data point is the mean $\pm$ SEM of 4–6 microvesicles. BK, bradykinin; Ctr, control; PKD, polycystin; TRP, transient receptor potential.

PKD1 competed functionally with TRPC1 for binding to TRPP2. Supplementary Fig 3 online shows that expression of PKD1 along with TRPP2 and TRPC1 reduced whole-cell TRPP2/TRPC1 or TRPP2/PKD1 currents activated by BK or MR3, respectively, indicating competition between TRPC1 and PKD1 for TRPP2. These data have implications for the role of TRPC1 in ADPKD and might explain why deletion of TRPC1 did not result in a PKD phenotype. Our data predict that pathological conditions resulting in TRPC1 upregulation could produce PKD-type pathologies.

GPCR activation of TRPP2/TRPC1 in mIMCD3 wells

Next, we studied whether TRPP2/TRPC1 channel complexes exist in mIMCD3 (murine inner medullary collecting duct) kidney cells, which are known to express TRPP2 (Luo *et al*, 2003) and TRPC1 (supplementary Fig 4B online, lane 2). Perforated patch-clamp recordings showed that the amplitude of basal currents increased by 3.8 ± 0.7 pA/pF at -80 mV in response to oxotremorine-M (Oxo-M), an M1-muscarinic cholinergic receptor agonist, and was

suppressed by $48.3 \pm 1.9\%$ by amiloride in native mIMCD3 cells (Fig 4A,E,I,K). Transfection of TRPP2 alone caused a net increase in the basal activity by -18.3 ± 1.5 pA/pF, no significant activation by Oxo-M (1.4 ± 0.4 pA/pF) and $65.3 \pm 1.5\%$ inhibition by amiloride (Fig 4B,F,I–K). Transfection of TRPC1 alone increased the basal activity by -22.9 ± 1.4 pA/pF, showed significant activation by Oxo-M (7.8 ± 0.3 pA/pF) and little inhibition by amiloride ($12.8 \pm 0.9\%$; Fig 4C,G,I–K). Co-transfection of TRPP2 and TRPC1 yielded an increase in the basal activity by -63.3 ± 2.1 pA/pF, which was higher than the additive effect (-41.2 pA/pF) of TRPP2 and TRPC1 (new property; Fig 4D,H,I), higher activation by Oxo-M compared with TRPC1 alone (11.8 ± 0.7 pA/pF; TRPC1-specific property; Fig 4J) and inhibition by amiloride ($63.4 \pm 2.4\%$; TRPP2-specific property; Fig 4K). In addition, co-expression of TRPP2 and TRPC1 resulted in a positive shift of approximately 8 mV in the reversal potential (E_{rev}), which was not observed with TRPP2 or TRPC1 alone (Fig 4E–H). Extracellular cation substitution experiments showed that

Fig 4 | Functional expression of transfected TRPP2/TRPC1 in mIMCD3 kidney cells. (A–D) Oxo-M-induced normalized currents in (A) untransfected cells and in cells expressing (B) m1AChR/TRPP2, (C) m1AChR/TRPC1 and (D) m1AChR/TRPP2/TRPC1. Currents were recorded using a voltage clamp ramp protocol. Oxo-M, 10 μ M; amiloride, 500 μ M. Each panel represents the mean $\pm$ SEM of 5–7 cells. (E–H) Mean I–V relationships for basal currents (black), Oxo-M-induced current (green) and post-amiloride current (red) in (E) untransfected cells and in cells expressing (F) m1AChR/TRPP2, (G) TRPC1 and (H) TRPP2/TRPC1. Black, green and red arrows in (A–D) indicate the time points at which I–V curves were taken. (I–K) Summary data of normalized currents before the application of (I) 10 μ M Oxo-M (basal), (J) net Oxo-M-induced inward currents and (K) percentage block of maximum current (after application of Oxo-M) by amiloride (500 μ M) obtained from untransfected cells (1), TRPP2 (2), TRPC1 (3) or TRPP2/TRPC1 (4) expressing mIMCD3 cells ($n=5-7$). The red lines indicate current densities of untransfected cells. * $P<0.05$. (L,M) I–V curves derived from cells co-transfected with TRPP2 ($n=7$), TRPC1 ($n=7$) or TRPP2/TRPC1 ($n=7$) in the presence of (L) 135 mM extracellular Na^+ or (M) 10 mM CaCl_2 . (N) Na^+ and Ca^{2+} permeability profiles of TRPP2, TRPC1 and TRPP2/TRPC1 in mIMCD3 cells. mIMCD, murine inner medullary collecting duct; Oxo-M, oxotremorine-M; TRP, transient receptor potential.

Fig 5 | Functional expression of endogenous TRPP2/TRPC1 in mIMCD3 kidney cells. (A–H) Representative voltage step currents (A–D) and averaged *I*–*V* curves (E–H) of (A,E) untransfected and cells transfected with (B,F) TRPP2-D511V, (C,G) shRNAi^{TRPC1} and (D,H) I-mfa. *I*–*V* curves were constructed from 7 to 8 cells. (I) Effect of amiloride (500 μM) on basal currents (at –100 mV) of mIMCD3 cells transfected with CD8 (mock, *n* = 5), TRPP2-D511V (*n* = 7), shRNAi^{TRPC1} (*n* = 7) or TRPP2-D511V and shRNAi^{TRPC1} (*n* = 7). (J) Summary data of amiloride-sensitive current in cell groups shown in (I). **P* < 0.05. (K) Time course of 10 μM Oxo-M-induced current (at –100 mV) in mIMCD3 cells transfected with CD8 (mock, *n* = 6), TRPP2-D511V (*n* = 7), shRNAi^{TRPC1} (*n* = 7) or TRPP2-D511V and shRNAi^{TRPC1} (*n* = 6). (L) Summary data of Oxo-M-induced current in cell groups shown in (K). **P* < 0.05. mIMCD, murine inner medullary collecting duct; Oxo-M, oxotremorine-M; shRNAi; TRPC1-specific short hairpin RNA interference construct; TRP, transient receptor potential; D511V, construct-dead mutant.

co-transfection of TRPP2 and TRPC1 resulted in the formation of a channel with a higher permeability ratio of Ca²⁺ to Na⁺ compared to transfection of either of the channels alone (Fig 4L–N; new property).

Next, we performed loss-of-function experiments to find a functional interaction between native TRPP2 and TRPC1 in mIMCD3 cells. Cells were transiently transfected with TRPP2-D511V, I-mfa, a known inhibitor of TRPC1-specific current

(Ma *et al*, 2003), or a TRPC1-specific short hairpin RNA interference (RNAi) construct (shRNAi^{TRPC1}). The silencing efficiency of shRNAi^{TRPC1} towards transfected and native mouse TRPC1 was shown in human embryonic kidney (HEK) 293T and mIMCD3 cells, respectively (supplementary Fig 4 online). All these constructs reduced basal currents (Fig 5A–H; supplementary Fig 5 online), consistent with the idea that TRPP2 and TRPC1 contributed to these currents.

As we knew that TRPP2 and heteromeric TRPP2/TRPC1—but not TRPC1—was amiloride sensitive, we used amiloride to test for the existence of native TRPP2/TRPC1 current in mIMCD3 cells. Fig 5I,J shows that knockdown of native TRPC1 reduced whole-cell amiloride-sensitive current by 5.4 pA/pF (from $17.2 \pm 0.6 \text{ pA/pF}$ in mock-transfected cells to $11.8 \pm 0.2 \text{ pA/pF}$ in $\text{shRNAi}^{\text{TRPC1}}$ -transfected cells). We reasoned that this reduction was due to the loss of an amiloride-sensitive pool of TRPC1. This pool is likely to contain TRPC1/TRPP2 because overexpression of TRPP2-D511V suppressed amiloride-sensitive current to levels similar to that in cells transfected with TRPP2-D511V and $\text{shRNAi}^{\text{TRPC1}}$, indicating that TRPP2 and interacting proteins accounted for 76% of all amiloride-sensitive current in mIMCD3 cells (from $17.2 \pm 0.6 \text{ pA/pF}$ in mock-transfected cells to $3.5 \pm 0.4 \text{ pA/pF}$ in TRPP2-D511V-transfected cells). The residual current was carried by proteins unrelated to TRPP2 or TRPC1 ($4.0 \pm 0.3 \text{ pA/pF}$; Fig 5J). Single-channel analysis showed that TRPP2/TRPC1, but not TRPP2 alone or in association with PKD1, was activated secondarily to GPCR activation. Consistently, overexpression of TRPP2-D511V suppressed Oxo-M-induced currents, indicating that native TRPP2 was likely to participate in these currents (Fig 5K,L). It should be noted that TRPP2-D511V did not inhibit total TRPC1 activity but only the pool of TRPC1 associated with TRPP2 (compare basal current density before and after amiloride in cells transfected with TRPP2-D511V alone, and TRPP2-D511V and $\text{shRNAi}^{\text{TRPC1}}$; Fig 5I). Overall, these loss-of-function experiments in mIMCD3 cells were consistent with the existence of a native TRPP2/TRPC1 channel and the involvement of this channel in GPCR-induced currents.

TRPP2 and TRPC1 in kidney cells

We tested whether TRPC1 would be present and colocalize with TRPP2 in primary cilia. Immunofluorescence staining of LLC-PK1 (pig kidney epithelial cells) and mIMCD3 cells showed the expression of TRPC1 in the cilium and its colocalization with TRPP2 (supplementary Fig 6A online). Consistently, supplementary Fig 6B online shows that a mouse polyclonal antibody raised against the amino-terminal cytosolic region of human TRPC1 (anti-TRPC1) was able to immunoprecipitate native TRPP2 from rat kidney membrane lysates.

Here, we have shown that TRPP2 and TRPC1 assemble into a new GPCR-activated channel with biophysical properties distinct from that of individual channels. This conclusion is supported by biochemical, and gain-of-function and loss-of-function experiments using single-channel and whole-cell analysis in two different cell culture systems. TRPP2/TRPC1 mediates native GPCR-activated currents and might explain the role of TRPP2 in mechanosensation and cilium-based Ca^{2+} signalling.

TRPP2 has been postulated to function as a mechanosensitive channel in kidney epithelial cells. Our data indirectly support such a role but cast doubt on the role of mechanosensation in ADPKD. It was recently shown that endothelial cells detect fluid-shear stress through activation of BK (Chachisvilis *et al*, 2006), indicating that GPCR-dependent signalling might actually underlie mechanotransduction. Mechanical bending of the primary cilium has been shown to induce Ca^{2+} influx through the activation of phospholipase C (Praetorius & Spring, 2003). Therefore, it is possible that fluid-shear stress or cilium bending activates TRPP2/TRPC1 either directly (Maroto *et al*, 2005) or

indirectly through the activation of plasma membrane GPCRs. However, an initial analysis of mice lacking TRPC1 did not show obvious symptoms of PKD. Thus, we would like to propose that defective mechanosensation might not be the sole underlying cause of ADPKD. This is now supported by whole animal studies in which delayed inactivation of PKD1 or polaris resulted in mild forms of PKD. It is thus possible that PKD1/TRPP2 might mediate functions other than mechanosensation during kidney development that are crucial for normal tubulogenesis.

METHODS

DNA constructs. mPKD1, TRPP2-D511 and I-mfa cDNAs have been described previously (Delmas *et al*, 2002a; Ma *et al*, 2003, 2005). TRPP2-D511V is a pathogenic missense variant of TRPP2 lacking channel activity but maintaining hetero- and homo-multimerization (Reynolds *et al*, 1999). mTRPP2 and mTRPC1 were obtained from Open Biosystems (Huntsville, AL, USA) as full-length expressed sequence tags. $\text{shRNAi}^{\text{TRPC1}}$ was obtained by cloning the mTRPC1 sequence 5'-GTGGTGGCTCACAACAAGT-3' into pSUPER-retro (Oligoengine Inc., Seattle, WA, USA) RNAi vector. The human m1AChR cDNA was cloned into pSV-2 neo vector.

Intranuclear cDNA delivery. DNA plasmids were diluted to either 100 or 300 ng/ μl in a KCl-based Ca^{2+} -free internal solution (pH 7.3) containing 0.2% fluorescein isothiocyanate-dextran (70 kDa) and were pressure-injected into the nucleus of neurons, as described previously (Delmas *et al*, 2002a). Cells were maintained in culture for a further 2 days before recording.

Outside-out perforated microvesicles. Microvesicles were obtained as described in the supplementary information online (Delmas *et al*, 2002b).

Whole-cell recordings. Currents in mIMCD3 cells were recorded by using the perforated whole-cell configuration as described in the supplementary information online.

Immunofluorescence and immunoprecipitation. LLC-PK1 or mIMCD3 cells were grown on glass coverslips for 7 days to allow cilia formation and immunostained as described in the supplementary information online. Immunocomplexes from rat kidney tissues were prepared as described previously (Tsiokas *et al*, 1999) and as detailed in the supplementary information online.

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

ACKNOWLEDGEMENTS

We thank I. Bezprozvanny, B. Ceresa, S. Plafker and K. Moore for valuable comments on the manuscript. This work was supported by the PKD Foundation, Oklahoma Center for the Advancement of Science and Technology, and National Institute of Health (to L.T.) and grants from Centre National de la Recherche Scientifique, équipe Fondation pour la Recherche Médicale 2007, Agence Nationale pour la Recherche (ANR)-05-Neur., ANR-05-PCOD, Action Concertée Incitative Jeunes Chercheurs and Fondation Schlumberger and French Ministry (to P.D.).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

- Ambudkar IS (2007) TRPC1: a core component of store-operated calcium channels. *Biochem Soc Trans* **35**: 96–100
- Beech DJ (2005) TRPC1: store-operated channel and more. *Pflügers Arch* **451**: 53–60

- Chachisvilis M, Zhang YL, Frangos JA (2006) G protein-coupled receptors sense fluid shear stress in endothelial cells. *Proc Natl Acad Sci USA* **103**: 15463–15468
- Delmas P (2004) Polycystins: from mechanosensation to gene regulation. *Cell* **118**: 145–148
- Delmas P (2005) Polycystins: polymodal receptor/ion-channel cellular sensors. *Pflügers Arch* **451**: 264–276
- Delmas P et al (2002a) Constitutive activation of G-proteins by polycystin-1 is antagonized by polycystin-2. *J Biol Chem* **277**: 11276–11283
- Delmas P, Wanaverbecq N, Abogadie FC, Mistry M, Brown DA (2002b) Signaling microdomains define the specificity of receptor-mediated InsP(3) pathways in neurons. *Neuron* **34**: 209–220
- Delmas P, Nauli SM, Li X, Coste B, Osorio N, Crest M, Brown DA, Zhou J (2004) Gating of the polycystin ion channel signaling complex in neurons and kidney cells. *FASEB J* **18**: 740–742
- Dietrich A et al (2007) Pressure-induced and store-operated cation influx in vascular smooth muscle cells is independent of TRPC1. *Pflügers Arch* **455**: 465–477
- Giamarchi A, Padilla F, Coste B, Raoux M, Crest M, Honore E, Delmas P (2006) The versatile nature of the calcium-permeable cation channel TRPP2. *EMBO Rep* **7**: 787–793
- Gonzalez-Perrett S, Kim K, Ibarra C, Damiano AE, Zotta E, Batelli M, Harris PC, Reisin IL, Arnaout MA, Cantiello HF (2001) 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
- Gonzalez-Perrett S, Batelli M, Kim K, Essafi M, Timpanaro G, Moltabetti N, Reisin IL, Arnaout MA, Cantiello HF (2002) Voltage dependence and pH regulation of human polycystin-2-mediated cation channel activity. *J Biol Chem* **277**: 24959–24966
- Gottlieb P et al (2008) Revisiting TRPC1 and TRPC6 mechanosensitivity. *Pflügers Arch* **455**: 1097–1103
- Hanaoka K, Qian F, Boletta A, Bhunia AK, Piontek K, Tsiokas L, Sukhatme VP, Guggino WB, Germino GG (2000) Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. *Nature* **408**: 990–994
- Köttgen M (2007) TRPP2 and autosomal dominant polycystic kidney disease. *Biochim Biophys Acta* **1772**: 836–850
- Koulen P, Cai Y, Geng L, Maeda Y, Nishimura S, Witzgall R, Ehrlich BE, Somlo S (2002) Polycystin-2 is an intracellular calcium release channel. *Nat Cell Biol* **4**: 191–197
- Luo Y, Vassilev PM, Li X, Kawanabe Y, Zhou J (2003) Native polycystin 2 functions as a plasma membrane Ca²⁺-permeable cation channel in renal epithelia. *Mol Cell Biol* **23**: 2600–2607
- Ma R, Rundle D, Jacks J, Koch M, Downs T, Tsiokas L (2003) Inhibitor of myogenic family, a novel suppressor of store-operated currents through an interaction with TRPC1. *J Biol Chem* **278**: 52763–52772
- Ma R, Li WP, Rundle D, Kong J, Akbarali HI, Tsiokas L (2005) PKD2 functions as an epidermal growth factor-activated plasma membrane channel. *Mol Cell Biol* **25**: 8285–8298
- Maroto R, Raso A, Wood TG, Kurosky A, Martinac B, Hamill OP (2005) TRPC1 forms the stretch-activated cation channel in vertebrate cells. *Nat Cell Biol* **7**: 179–185
- Mochizuki T et al (1996) PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* **272**: 1339–1342
- Montell C (2005) The TRP superfamily of cation channels. *Sci STKE* **2005**: RE3
- Nauli SM et al (2003) Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet* **33**: 129–137
- Nilius B, Voets T (2005) TRP channels: a TR(1)P through a world of multifunctional cation channels. *Pflügers Arch* **451**: 1–10
- Nilius B, Owsianik G, Voets T, Peters JA (2007) Transient receptor potential cation channels in disease. *Physiol Rev* **87**: 165–217
- Praetorius HA, Spring KR (2003) Removal of the MDCK cell primary cilium abolishes flow sensing. *J Membr Biol* **191**: 69–76
- Reynolds DM et al (1999) Aberrant splicing in the PKD2 gene as a cause of polycystic kidney disease. *J Am Soc Nephrol* **10**: 2342–2351
- Shim S, Goh EL, Ge S, Sailor K, Yuan JP, Roderick HL, Bootman MD, Worley PF, Song H, Ming GL (2005) TRPC1-dependent chemotropic guidance of neuronal growth cones. *Nat Neurosci* **8**: 730–735
- Tsiokas L, Arnould T, Zhu C, Kim E, Walz G, Sukhatme VP (1999) Specific association of the gene product of PKD2 with the TRPC1 channel. *Proc Natl Acad Sci USA* **96**: 3934–3939
- Tsiokas L, Kim S, Ong EC (2007) Cell biology of polycystin-2. *Cell Signal* **19**: 444–453
- Wang GX, Poo MM (2005) Requirement of TRPC channels in netrin-1-induced chemotropic turning of nerve growth cones. *Nature* **434**: 898–904
- Xu XZ, Li HS, Guggino WB, Montell C (1997) Coassembly of TRP and TRPL produces a distinct store-operated conductance. *Cell* **89**: 1155–1164
- Yuan JP, Zeng W, Huang GN, Worley PF, Muallem S (2007) STIM1 heteromultimerizes TRPC channels to determine their function as store-operated channels. *Nat Cell Biol* **9**: 636–645