Function and regulation of TRPP2 at the plasma membrane

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Running head: Activation modes of TRPP2

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Abstract

The vast majority (~99%) of all known cases of autosomal dominant polycystic kidney disease (ADPKD) are caused by naturally occurring mutations in two separate, but genetically interacting loci, \textit{pkd1} and \textit{pkd2}. \textit{Pkd1} encodes a large multispansing membrane protein (PKD1) of an unknown function, while \textit{pkd2} encodes a protein (TRPP2, Polycystin-2, or PKD2) of the transient receptor potential (TRP) superfamily of ion channels. Biochemical, functional, and genetic studies support a model whereby PKD1 physically interacts with TRPP2 to form an ion channel complex that conveys extracellular stimuli to ionic currents. However, the molecular identity of these extracellular stimuli remains elusive. Functional studies in cell culture show that TRPP2 can be activated in response to mechanical cues (fluid shear stress) and/or receptor tyrosine kinase (RTK) and G protein couple receptor (GPCR) activation at the cell surface. Recent genetic studies in \textit{Chlamydomonas reinhardtii} show that CrPKD2 functions in a pathway linking cell-cell adhesion and Ca$^{2+}$ signaling. The mode of activation depends on protein-protein interactions with other channel subunits and auxiliary proteins. Therefore, understanding the mechanisms underlying the molecular make-up of TRPP2 containing complexes is critical in delineating the mechanisms of TRPP2 activation, and most importantly, the mechanisms by which naturally occurring mutations in \textit{pkd1} or \textit{pkd2} lead not only to ADPKD, but also to other defects reported in model organisms lacking functional TRPP2. This review will focus on the molecular assembly, function, and regulation of TRPP2 as a cell surface cation channel and discuss its potential role in Ca$^{2+}$ signaling and ADPKD pathophysiology.
Keywords: TRP channels, ADPKD, Ca$^{2+}$ signaling
**Introduction**

TRPP2 (Polycystin-2, PC2, or PKD2) is the protein encoded for by the *pkd2* gene originally identified as one of the genes responsible for autosomal dominant polycystic kidney disease (ADPKD) (50). ADPKD is one of the most common genetic diseases affecting 1:400-1,000 individuals (24, 28) with the development of large fluid-filled kidney, liver, and, pancreatic cysts. In addition to defects associated with polycystic kidney disease (91, 92), homozygous deletion of *pkd2* in mice results in randomization of embryonic turning manifested as abnormal left-right axis patterning and abnormal heart looping morphogenesis (67). These data highlight the indispensable role of TRPP2 in vertebrate development and health.

Structurally, TRPP2 belongs to the TRP superfamily of channel proteins (87). Mammalian TRP channels have been grouped into seven categories (TRPC, TRPM, TRPP, TRPV, TRPML, TRPN, and TRPA1) based on primary sequence homology (54). All TRP channels span the plasma membrane six times with their N- and C-termini located in the cytoplasm. The region responsible for forming the ionic pore is believed to be between transmembrane segments five and six. With the exception of TRPV5 and TRPV6, which are highly Ca\(^{2+}\) selective and TRPM4 which is practically impermeable to Ca\(^{2+}\), all other members of the TRP superfamily form non-selective cation channels, which permeate divalent cations to various degrees (61). One of the most exciting properties of TRP channels is their ability to heteromultimerize resulting in the formation
of channels with new biophysical properties, modes of activation, or simply more efficient trafficking of the ion conducting subunit to the plasma membrane (87).

TRPP2 has been shown to physically interact with PKD1 (75, 85) resulting in the formation of a functional ion channel complex (29). The interaction between the two proteins is mediated through their C-termini and involves amino acid residues 822 to 895 of human TRPP2 (84). PKD1, the gene product of \textit{pkd1}, is a large protein of \~4300 amino acids with an N-terminal extracellular domain of \~2500 residues (32). The extracellular portion of PKD1 contains several domains present in known cell adhesion molecules (Figure 1). Of particular interest is a domain similar to the receptor of egg jelly (REJ) in sea urchin (55). This domain is believed to mediate egg-sperm interactions in this organism. Just before the first transmembrane segment, there is a G protein couple receptor proteolytic site (GPS) (69), which is responsible for the proteolytic cleavage of full length PKD1 into two pieces (49, 90). The N-terminal fragment encompasses almost the entire extracellular domain (90), while the C-terminal fragment contains the remaining molecule which is predicted to span the plasma membrane eleven times with the C-terminus residing in the cytoplasm. The two fragments have been suggested to be held together via non-covalent protein-protein interactions (90). In this context, it has been proposed that PKD1 acts as a receptor activated by a yet to be identified ligand, in a signal transduction scheme which is vital for normal tubulogenesis.

While there are multiple lines of independent evidence that TRPP2 and PKD1 form a heteromultimeric ion channel complex with an indispensable role in kidney development,
there are still outstanding questions on the activation mechanisms of TRPP2/PKD1 channel, their exact cellular functions, and their specific roles in ADPKD pathophysiology. Data accumulated over the last five years support three major modes of TRPP2 activation: activation by fluid shear stress (mechanosensitive TRPP2), activation by cell surface receptor stimulation (receptor-operated TRPP2), or activation by cell adhesion (cell adhesion-mediated activation of TRPP2). This review summarizes data of current modes of TRPP2 activation at the plasma membrane and discusses the relevance of each one of these modes in the pathophysiology of ADPKD. For further reading on the function of TRPP2, the reader is directed to many excellent reviews (1, 11, 15, 16, 35, 38, 86).
Mechanosensitive TRPP2 The identification of the primary cilium, as perhaps the most relevant organelle in ADPKD pathophysiology (65, 94), along with the expression of TRPP2 and PKD1 there (66, 95), prompted investigations on whether these proteins have a ciliary function. Cilium bending has been shown to be associated with increased Ca\(^{2+}\) entry into the cell (70, 71, 73). Initially, Nauli et al. (56) showed that fluid shear stress applied to ciliated cells resulted in Ca\(^{2+}\) entry into the cell body, which was dependent on TRPP2 or PKD1. Kidney epithelial cells derived from mice lacking \textit{pkd1} did not respond to fluid flow, when they were allowed to form cilia. Similarly, a TRPP2-specific antibody raised against the first extracellular loop of TRPP2 blocked fluid flow-induced Ca\(^{2+}\) entry in ciliated cells. Nauli et al. (56) proposed that TRPP2 and PKD1 play a mechanosensitive role in kidney cells and loss of fluid-flow-induced PKD1/TRPP2-mediated Ca\(^{2+}\) entry through the cilium may be the primary defect in ADPKD.

Consistent with the notion that TRPP2 could function as a mechanosensitive channel, the Cantiello and Chen groups have shown that the actin cytoskeleton can directly modulate TRPP2 activity in several in vitro systems. TRPP2 activity was identified and characterized in membrane vesicles derived from apical membranes of human syncytiotrophoblasts (hST) (26). Lipid bilayer reconstituted TRPP2 displayed eight-fold increased activity in response to cytochalasin D, an F-actin depolymerizing agent, added to the \textit{cis} (intracellular) compartment of the recording chamber (26). Moreover, gelsolin, an actin cytoskeleton severing protein also increased TRPP2 activity in the same experimental setting (52). Because gelsolin was effective only in the presence of intracellular Ca\(^{2+}\), the authors proposed an interesting mechanism by which, Ca\(^{2+}\) entry
through TRPP2 could activate endogenous gelsolin to induce actin cytoskeleton remodeling that could also affect TRPP2 by a positive feedback mechanism (52). The implication of these data is that TRPP2 activity can modulate the actin cytoskeleton and this modulation may account for downstream effects of TRPP2 signaling. This intriguing hypothesis awaits further support from whole cell-based systems. In agreement with a role of actin cytoskeleton in modulating TRPP2 activity, Li et al (44) identified α-actinin-2 as an interacting partner with an N-terminal domain (M1-K215) of human TRPP2 using a yeast two-hybrid screen. Directed protein-protein interaction studies identified a second interaction domain within the C-terminal cytosolic region of TRPP2 encompassing residues 821 to 878 (44). The interaction of TRPP2 and actinin was confirmed by multiple in vitro and in vivo binding and yeast two-hybrid assays. Most importantly, Li et al (44) showed that α-actinin modulated TRPP2 single channel activity. Using lipid bilayer reconstitution of TRPP2 from hST membranes, these investigators showed that purified α-actinin added to the cis compartment increased TRPP2 single channel activity by almost 15-fold. Using the same approach, Montalbetti et al (51) showed that changes in osmotic pressure or hydrostatic pressure affected TRPP2 activity and this modulation was again dependent on an intact actin cytoskeleton. In sum, these studies provided evidence for the direct coupling of TRPP2 to the actin cytoskeleton and set forth the hypothesis that TRPP2 activity could be modulated by mechanical changes transferred to the channel through the actin network. However, this type of regulation is expected to occur mainly at the plasma membrane and not at the microtubule-based cilium, which lacks actin. In regard to regulation of TRPP2 by microtubules, it was recently shown that taxol, a microtubule stabilizing agent increased, while colchicine, a microtubule
destabilizing agent decreased TRPP2 activity (53). Purified tubulin and GTP increased reconstituted TRPP2 activity from apical hST membrane, but they were without effect on purified TRPP2, suggesting that the regulation by tubulin was indirect. KIF3B, a kinesin motor, was found to be present in the apical membrane of hSTs and to also physically interact with TRPP2 (53, 93). TRPP2 interacted with KIF3B through its C-terminal cytosolic domain encompassing amino acid residues 682 to 968 of human TRPP2 (93). Therefore, a model was proposed whereby, microtubular structures could regulate TRPP2 activity via KIF3B/KIF3A (53). While this type of regulation was described in preparation derived from the apical surface of hSTs, it could be also extrapolated to the primary cilium which is rich in tubulin, KIF3A, and TRPP2. Overall, a great deal of effort has been employed to test whether TRPP2 is a mechanosensitive channel. Supporting evidence comes from studies using measurements of intracellular Ca^{2+} concentration in response to fluid flow (47, 56, 57, 89) and single channel currents in response to osmotic and hydrostatic pressure (51). Physical interaction of TRPP2 and actin cytoskeleton and microtubules are also supportive (53). However, whether TRPP2 is a *bona fide* mechanosensitive channel, according to certain criteria recently set for mechanosensitive channels (13), it still remains an outstanding question. Furthermore, it is also unknown whether the mechanosensitive mode of TRPP2 is relevant to ADPKD.

In regard to the role of mechanosensitive TRPP2 in ADPKD, Kottgen et al (37) recently showed that TRPP2 formed a heteromultimeric channel with TRPV4, which was required for fluid flow-induced Ca^{2+} entry in MDCK cells. Interestingly, TRPP2 alone or in association with endogenous PKD1 could not support fluid-flow induced Ca^{2+} entry, as
knockdown of TRPV4 eliminated Ca\textsuperscript{2+} entry in response to fluid flow. Heterologous expression of TRPP2 and TRPV4 in *Xenopus laevis* oocytes resulted in the formation of a mechano- and thermosensitive channel complex. However, kidney cysts were not formed in mice or zebrafish lacking TRPV4. Kottgen et al (37) concluded that while TRPP2 can form a mechanosensitive channel in association with TRPV4, loss of this activity cannot account for cyst formation.

**Receptor-operated TRPP2** G protein coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs) comprise a very large group of cell surface receptors that elicit their physiological responses through the production of inositol (1, 4, 5) trisphosphate (IP\textsubscript{3}) (6) (Figure 2). Upon receptor-activation, newly synthesized IP\textsubscript{3} acts on IP\textsubscript{3} receptors (IP\textsubscript{3}Rs) to trigger a rapid increase in the intracellular Ca\textsuperscript{2+} concentration by releasing free Ca\textsuperscript{2+} from intracellular stores (9). Intracellular Ca\textsuperscript{2+} concentration returns to normal levels by extrusion of cytoplasmic Ca\textsuperscript{2+} to the extracellular space by plasma membrane Ca\textsuperscript{2+}-ATPase and Na\textsuperscript{+}-Ca\textsuperscript{2+} exchangers, re-admission of Ca\textsuperscript{2+} into the endoplasmic reticulum (ER) by the SERCA pump, and Ca\textsuperscript{2+} entry via the store- and receptor-operated Ca\textsuperscript{2+} channels (20, 74).

While there is general agreement that store-operated channels are defined by their ability to open directly in response to the depletion of the internal stores, the term receptor-operated channels is loosely defined (also discussed in (63)). To avoid confusion, receptor-operated channels will be considered as the channels activated in response to GPCR/RTK-induced activation of second messengers, but not store depletion. Therefore,
cell surface receptor stimulation will result in the activation of both store- and receptor-operated channels, whereas pharmacological inhibition of the SERCA pump by thapsigargin (TG) or passive depletion of internal stores by cell dialysis using Ca\(^{2+}\) chelators will result in the activation of only the store-operated channels.

Consistent with the definition of receptor-operated Ca\(^{2+}\) channels, TRPP2 can be activated in response to epidermal growth factor (EGF) in the kidney epithelial cell line, LLC-PK1 (45). In these cells, addition of EGF in the extracellular solution caused a rise in cytosolic Ca\(^{2+}\) concentration that was entirely dependent on extracellular Ca\(^{2+}\) and did not involve depletion of intracellular stores and activation of store-operated Ca\(^{2+}\) channels (Figure 3). The physiological relevance of EGF-induced activation of TRPP2 in LLC-PK1 cells was supported by whole animal studies, whereby homozygous deletion of the egfr gene resulted in cystic dilatation of collecting ducts (83), an area that was also predominantly affected by pkd2 mutations (92). Mechanistically, TRPP2 overexpression increased EGF-induced conductance in LLC-PK1 kidney epithelial cells, while knock down of endogenous TRPP2 by RNAi or expression of the pathogenic, missense variant, TRPP2-D511V, blunted the EGF-induced response. Pharmacological experiments indicated that the EGF-induced activation of TRPP2 occurred independently of store depletion, but required the activity of phospholipase C (PLC) and phosphoinositide 3-kinase (PI3K). Pipette infusion of purified phosphatidylinositol-4, 5-bisphosphate (PIP\(_2\)) suppressed the TRPP2-mediated effect on EGF-induced conductance, while pipette infusion of phosphatidylinositol-3, 4, 5-trisphosphate (PIP\(_3\)) had no detectable effect on this conductance. Overexpression of type I\(\alpha\) phosphatidylinositol-4-phosphate 5-kinase
(PIP(5)Kα), which catalyzes the formation of PIP₂, suppressed EGF-induced TRPP2 currents. Biochemically, TRPP2 interacted with PLC-γ2 and co-localized the primary cilium with EGFR and PIP₂. Overall, TRPP2 functioned as a bona-fide receptor-operated ion channel downstream of EGFR activation in the plasma membrane of LLC-PK1 cells. These studies identified a cellular, physiological signal transduction pathway of which TRPP2 was an important component and had implications of RTK-mediated Ca²⁺ signaling in ADPKD.

Following up on the activation of TRPP2 by EGF, Bai et al (4) recently showed that native TRPP2 was gated by an intracellular protein, the mammalian homolog of the Diaphanous related formin 1 (mDia1). mDia1 exists in an autoinhibited or “closed” state whereby the C-terminus loops around to bind the N-terminus (30, 88). In response to RhoA-C activation, activated GTP-bound forms of RhoA-C proteins bind to the N-terminus of mDia1 resulting in mDia1 unwinding by disrupting the intramolecular interaction between its N- and C-termini. The unwinding of mDia1 results in the exposure of internal formin homology domains 1 and 2 (FH1 and 2), which form docking sites for multiple effector molecules containing SH3 and WW domains. mDia1 was previously shown to physically associate with the very C-terminal tail of TRPP2 (amino acid residues 872 to 968 of human TRPP2) and to mediate anchoring of TRPP2 to the mitotic spindles during metaphase (78). In the recent study (4), mDia1 was shown to function as an intracellular voltage-dependent gate for TRPP2 at the plasma membrane in cells in interphase. Specifically, at resting physiological potentials, mDia1 was shown to bind to and block TRPP2. Membrane depolarization or EGF stimulation resulted in the
sequential activation of RhoA and mDia1. It was then hypothesized that activated mDia1 dissociated or “swung away” from TRPP2 releasing its block on the channel. This model of TRPP2 activation implies a “ball-and-chain” mechanism for TRPP2 activation (Figure 4), which has never been proposed for any of the known 28 mammalian TRP channels. However, it still remains to be investigated how the PLC-γ2/PIP2 pathway functionally interacts with the RhoA/mDia1 pathway to regulate TRPP2 activation by EGF and possibly other ligands acting through receptor tyrosine kinases.

A new study reported that TRPP2 could function downstream of PLC activation, in a GPCR-activated mode. Bai et al (3) showed that TRPP2 complexed with TRPC1, but not PKD1, was able to be activated in response to bradykinin (BK) stimulation, acting through PLC-coupled BK receptor subtypes. This study followed up an earlier report that transfected TRPP2 could physically interact with TRPC1 (84). The interaction involved both their C-termini and transmembrane segments. Detailed structure-function analysis identified residue D886 in the cytosolic tail of TRPP2 responsible for the interaction with TRPC1, but not PKD1 (84). Subsequently, a completely independent study identified an affected family with a missense mutation at the very same residue (76). Tsiokas et al (84) proposed that the interaction through the soluble cytosolic domains may have a regulatory role, while the interaction through the transmembarne segments may have a more structural role in forming the ionic pore of the heteromultimeric channel.

Consistently, the TRPP2/TRPPC1 channel complex displayed unique biophysical properties in terms of single channel conductance, ion permeability, and pharmacology (3). TRPP2/TRPC1 showed intermediate single channel conductance from TRPP2/PKD1
and TRPC1 homomultimers, higher permeability to Ca$^{2+}$, and amiloride sensitivity similar to TRPP2/PKD1 but not to TRPC1 alone (3). As the properties of TRPP2/TRPC1 were distinct from that of PKD1/TRPP2, these new data indicated that the TRPP2 mode of activation was conferred through interaction with specific subunits. The identification of TRPP2/TRPC1 was the first example of a functional channel complex containing members of non homologous groups, such TRPP and TRPC, as all known interactions between TRP channels have been shown to occur exclusively between members of the same group. It also extended the functional diversity of TRP channels to an even larger number of heteromultimeric channels as thought before. Importantly, the identification of heteromultimeric TRPP2/TRPC1 has implications in purported roles of TRPP2 in fluid flow-mediated mechanosensation. It was recently shown that endothelial cells sensed fluid shear stress through the activation of bradykinin 2 (BK-2) receptor, a G protein coupled receptor (12). Fluid shear stress activated BK-2 receptor through a conformational change in the receptor, in a ligand-independent manner (12). Mechanical bending of the primary cilium has been shown to induce Ca$^{2+}$ influx through the activation of PLC (72, 73). Therefore, it is quite possible that fluid shear stress or cilium bending could activate a channel complex containing TRPP2 and TRPC1 through GPCR activation. This type of TRPP2-mediated mechanotransduction would be indirect and involve ligand-independent activation of endogenous GPCRs present in the cell rather than activation of true mechanosensitive channels. Therefore, it is conceivable that a pool of TRPP2 associated with TRPC1 could be activated in response to cilium bending through PLC-mediated signaling. In support of the idea that ligand-independent activation of Gq coupled receptors can activate TRP channels, TRPC6 was recently
shown to be activated by the ligand-independent activation of Angiotensin II receptors in vascular smooth muscle cells in response to membrane stretch (48). Interestingly, TRPP2/TRPC4 heteromultimers were shown to form an Angiotensin II-activated plasma membrane channel in glomerular mesangial cells (19).

According to Bai et al (3), GPCR-mediated activation of TRPP2 required TRPC1 and was independent of PKD1. In fact, PKD1 competed with TRPC1 for binding to TRPP2 and activation by BK. An initial analysis of mice lacking TRPC1 did not reveal obvious symptoms of polycystic kidney disease (18). Thus, while TRPP2 could be responsible for Ca\(^{2+}\) entry in response to cilium bending, defective fluid shear stress-and/or cilium bending-induced mechanotransduction is unlikely to be solely responsible for ADPKD, at least based on current data. This idea is supported by Kottgen et al (37), as was discussed earlier, and also by recent data on the temporal inactivation of \(\text{pkd} 1\), \(\text{Ift} 88\), or \(\text{Kif} 3a\) in the mouse (14, 68). \(\text{Ift} 88\), or \(\text{Kif} 3a\) genes encode the proteins IFT88/polaris or Kif3A, which are essential components of the intraflagellar transport (IFT) machinery and required for cilium formation (77). Conditional inactivation of \(\text{Ift} 88\) or \(\text{Kif} 3a\), at different times during postnatal life (newborn versus 16 week-old mice) resulted in complete loss of primary cilia independently of the time of gene inactivation, yet in marked differences in cyst formation (14). Inactivation of these genes in newborn mice resulted in massive cysts, while inactivation in 16 week-old mice resulted in a much weaker cystic phenotype. These data led Davenport et al (14) to conclude that defective mechanosensation cannot simply account for cyst formation. In aggregate, recent results from multiple independent investigations question the loss of cilium-based and possibly, TRPP2-mediated
mechanotransduction/mechanosensation as the initiating event in cystogenesis (3, 14, 37, 68). However, more studies are needed to definitively address the role of mechanosensation in ADPKD.

The identification of the EGFR, TRPP2, and PIP2 in the cilium is worth discussing, as it raises the possibility that receptor-operated TRPP2 could function at the ciliary membrane. The presence of EGFR in the primary cilium and basal body of ovary epithelial cells has been shown earlier (43). It is therefore conceivable that TRPP2 may function as an EGF-activated Ca\textsuperscript{2+} channel at the primary cilium (Figure 5). EGF/EGFR signaling in the kidney has been a mystery since the discovery of EGF. While human EGF was identified in the urine (41, 42) and is known to be secreted from the apical surface of kidney tubular cells (41, 80), EGFR has never been detected in the apical surface of kidney tubular cells. EGFR is almost exclusively present at the basolateral surface of epithelial cells of the thick ascending loop of Henle and distal convulated tubules (25, 79). Therefore, the question of whether EGF present in the urine was capable of signaling has been a long-standing one. The data of Ma et al (45) raise the possibility that EGF could signal through cilium-based EGFR and thus, may provide an answer to this old question. A possible signaling scheme is shown in Figure 4: Prepro-EGF is anchored to the apical surface of the plasma membrane of the cells lining the thick ascending limb of Henle and distal convulated tubules (59) and it is believed to be cleaved by urine proteases and released to the tubular lumen (21, 33, 62). It should be noted that membrane bound prepro-EGF is also biologically active (8). TRPP2 is highly expressed in the thick ascending loop of Henle and distal convulated tubules (22).
Therefore, cilium-resident TRPP2 could be activated by secreted EGF in the urine and/or plasma membrane bound pre-proEGF. If indeed, pre-proEGF is in close proximity to EGFR and TRPP2 channel, it could, in principle, preferentially activate TRPP2 at the base of the cilium. This could generate a gradient of partially activated (sensitized) TRPP2 along the cilium. This type of channel sensitization may have implications in cilium-based mechanotransduction, as it may reduce its threshold of activation by mechanical stimulation and/or other models of TRPP2 activation that work independently of PIP2 breakdown (for example, cell adhesion-mediated activation of TRPP2, see below).

Although not the focus of this review, the identification of TRPP2 as an intracellular Ca\(^{2+}\) release channel (39) may have also implications in receptor-operated Ca\(^{2+}\) signaling in some of the TRPP2 phenotypes reported in model organisms. Knockdown of TRPP2 in zebrafish has been shown to result in three distinct phenotypes: pronephric cysts, defects in body axis curvature, and loss of left-right asymmetry (or laterality defects) (23, 40, 60). Mutation of S812 to A or D in human TRPP2 was shown to result in higher expression in the plasma membrane or trapping in the ER, respectively (36), while Cai et al (10) showed that S812A reduced the sensitivity of TRPP2 to intracellular Ca\(^{2+}\) without affecting trafficking of the mutant channel from the ER to the plasma membrane. Despite the discrepancy in the trafficking properties of TRPP2-S812A between the two studies, both studies are consistent with the fact that mutation of S812 to A resulted in impaired function of TRPP2 at the ER, either by a reduced amount in this organelle (36) or reduced activity of TRPP2 at the ER (10). Introduction of TRPP2-S812A or TRPP2-S812D into zebrafish embryos lacking endogenous TRPP2 resulted in somewhat
surprising results. TRPP2-S812A rescued the kidney cystic phenotype more efficiently than the body axis and laterality phenotypes rescued by the TRPP2-S812D construct (23), suggesting that the reduced Ca\(^{2+}\) sensitivity of this construct was irrelevant in respect to the rescue of cystogenesis. It is more likely that the increased plasma membrane expression of this construct was responsible for rescuing the cystic phenotype. Conversely, TRPP2-S812D rescued more efficiently the body axis and laterality phenotypes than the cystic phenotype compared to TRPP2-S812A (23). A previous study using a TRPP2 mutant lacking an N-terminal GSK3 phosphorylation site which was required for targeting to the plasma membrane showed that while this mutant was efficiently targeted to the cilium did not rescue the cystic phenotype caused by native TRPP2 knockdown (82). These date suggest that proper function of TRPP2 at the plasma membrane is primarily responsible for normal tubulogenesis and prevention of cyst formation.

In regard to the role of TRPP2 in body axis formation and left-right asymmetry, it is tempting to propose a model (Figure 6), whereby cilia bending could trigger GPCR activation which would then lead to PLC activation and transient release of Ca\(^{2+}\) from the internal stores. The function of TRPP2 as an intracellular Ca\(^{2+}\) release channel could amplify this release and this function could be physiologically relevant in terms of mechanotransduction directly impacting on the left-right asymmetry phenotype. This model is consistent with the independence of ER-resident TRPP2 by PKD1 and lack of a laterality phenotype in \textit{pkd1} knockout mice (34).
Cell adhesion-mediated activation of TRPP2 Because of the presence of multiple domains in vertebrate PKD1 which are predicted to have a role in cell adhesion (32), it has been thought that cell adhesion events could trigger activation of the PKD1/TRPP2 heteromultimer. This idea is also supported by the identification of a heteromultimeric PKD2/PKD1 complex exclusively at the plasma membrane of the acrosomal vesicle in sea urchin sperm (58). While direct evidence for such an activation mechanism is lacking, recent studies on the PKD2 Chlamydomonas reinhardtii homolog (CrPKD2) may lend support to this concept. CrPKD2 was first identified as a component of the Chlamydomonas reinhardtii flagellum using a proteomic approach (64). CrPKD2 was subsequently found to exist in two fragments produced by proteolytic cleavage, occurring at a site close to the C-terminus of the first extracellular loop (31). It was shown that CrPKD2 cleavage occurred at the cell body and the two fragments traveled to the axonemal membrane mostly by the intraflagellar transport (IFT) mechanism. CrPKD2 was essential for gamete mating, a process known to depend on extracellular Ca\(^{2+}\) as an initial step (27). RNAi experiments in this organism demonstrated that loss of CrPKD2 resulted in a block in an earlier step in mating, which could be rescued by restoring downstream components of the mating cascade (31). The proposed model is consistent with several aspects of polycystic kidney disease. Because gamete mating is initiated by cell adhesion through agglutinin molecules present in the flagella of gametes of opposite mating strains (5) and CrPKD2 causes a block in an early stage of mating (31), it is conceivable that CrPKD2 could be activated, directly or indirectly, by cell adhesion. This idea is consistent with the activation of mammalian TRPP2 by cell adhesion mediated perhaps, by one or more of the predicted cell adhesion domains in PKD1. However, it
should be noted that a PKD1 homolog in *Chlamydomonas reinhardtii* has yet to be identified and there are significant differences in the primary amino acid sequence between CrPKD2 and vertebrate TRPP2. Nevertheless, it is possible that in contrast to *C. elegans*, mice, and humans, CrPKD2 may function independently of PKD1 in *Chlamydomonas*. It is conceivable that in *Chlamydomonas*, homophillic interactions between agglutinin molecules, CrPKD2 itself, and/or other yet to be identified molecules sitting on the flagella of gametes of opposing strains could activate CrPKD2. In higher organisms, such as mammals, the initial cell adhesion step maybe mediated through PKD1. While this is a highly hypothetical model, it explains well how CrPKD2-mediated Ca$^{2+}$ signaling could result in an increase in intracellular cAMP concentration, an observation that has been reported in cystic cells in humans (46). In addition, the proposed model does not require Ca$^{2+}$ diffusion from the tip of the flagella to the cell body to initiate cell signaling, which would be inconsistent with the physical properties of the ion (for discussion see (86)). Because of these reasons, these data form an attractive model for the cellular function of CrPKD2 that could also be extrapolated to its mammalian relatives. However, the exact role of Ca$^{2+}$ influx in the mating process of *Chlamydomonas reinhardtii* is not immediately clear, as most of the evidence is indirect and is based on the use of general cation channel blockers, such as lidocaine, La$^{3+}$, Cd$^{2+}$, etc (27, 81). In this regard, while mammalian TRPP2 has been shown to be blocked by trivalents such as La$^{3+}$ (17, 26, 45) or Gd$^{3+}$ (2, 19, 26), it would be interesting to test whether all these inhibitors used to block mating could inhibit CrPKD-2 channel activity in vitro. The most consistent data in terms of Ca$^{2+}$ fluxes during the mating process are data showing Ca$^{2+}$ efflux rather than influx (7, 27). This Ca$^{2+}$ efflux is not associated with

release from the ER, but rather release from cell walls, is downstream of cAMP accumulation, and its physiological relevance to mating is unknown (27). Moreover, mating occurred normally in Ca\textsuperscript{2+}-free extracellular solution and in one report there was at most ~25\% reduction in mating in an extracellular solution containing 5 mM EGTA (7). One of the prevailing models is that Ca\textsuperscript{2+} influx is one of the earliest steps following cell adhesion. Incoming Ca\textsuperscript{2+} binds to Ca\textsuperscript{2+}-binding proteins in the flagella and is released to the mating medium only after a rise in intraflagellar cAMP concentration (27). CrPKD2 may function as the Ca\textsuperscript{2+} channel activated by cell adhesion, although direct demonstration for such a role is currently missing. Nevertheless, the identification of a Ca\textsuperscript{2+}-dependent cellular process requiring CrPKD2 in a whole organism is of great value as it could provide mechanistic insights into its function in mammalian systems.

**Conclusions**

TRPP2 has been proposed to function downstream of mechanical stimulation, cell surface receptor activation, and cell adhesion. These functions can be carried out at the plasma membrane and/or the primary cilium and require physical interactions with other channels subunits such as PKD1, TRPC1, TRPC4, or TRPV4 and auxiliary proteins (i.e., mDia1, actinins, KIF3A/B). Most of these protein-protein interactions are likely to be relevant to ADPKD, as they are eliminated in pathogenic mutants of TRPP2. The mechanosensitivity of TRPP2 has been supported by its involvement in intracellular Ca\textsuperscript{2+} changes associated with fluid flow changes, association and regulation by actin cytoskeleton and microtubules. The receptor-operated mode of TRPP2 activation has been demonstrated in EGF and GPCR signal transduction cascades in native and
overexpressed systems. Activation by cell adhesion is speculated based on its interaction with PKD1 and recent data on the role of CrPKD2 in the process of *Chlamydomonas* mating. However, it still remains to be established how each mode contributes to the biological functions of TRPP2. It is very possible that different modes of TRPP2 activation may serve different biological functions. Closer examination of the phenotypes associated with inactivation of the genes of known interacting partners, mechanisms of functional interaction with PKD1 and other proteins, and role of cilium in the each mode of TRPP2 activation will be critical in establishing a causative role of each mode in ADPKD and other developmental defects resulting by the loss of functional TRPP2.
FIGURE LEGENDS

**Figure 1** Diagram illustrating the membrane topology of PKD1 and TRPP2. LRR-LDL: leucine-rich repeats and low density lipoprotein homology domains, PKD: polycystic kidney disease domain, C-type lectin: C-type lectin homology domain.

**Figure 2** Receptor- and store-operated Ca\(^{2+}\) signaling in non-excitable cells. Activation of G protein coupled receptors (GPCRs) or receptor tyrosine kinases (RTKs) results in the activation of phospholipase C- \(\beta\) (PLC-\(\beta\)) or \(\gamma\) (PLC-\(\gamma\)) isoforms, respectively. Activated PLCs catalyze the breakdown of phosphatidylinositol-4,5-bisphosphate (PIP\(_2\)) to inositol (1, 4, 5) trisphosphate (IP\(_3\)) and diacylglycerol (DAG). IP\(_3\) binds and activates IP\(_3\)Rs in the ER membrane resulting in the release of Ca\(^{2+}\) from the intracellular stores. Depletion of Ca\(^{2+}\) from the ER triggers the translocation of ER-resident STIM1 to the vicinity of the plasma membrane (PM) to activate store-operated Ca\(^{2+}\) (SOC) channels. Receptor-operated channels (ROC) are activated by second messengers other than store depletion (ie, reduction of PIP\(_2\), production of DAG, etc).

**Figure 3** Function of TRPP2 as a receptor-operated channel at the plasma membrane of LLC-PK1 kidney epithelial cells. Activation of EGFR by EGF results in the activation of PLC-\(\gamma2\) and conversion of PIP\(_2\) to IP\(_3\) and DAG. In this cell line, EGF-induced IP\(_3\) is not sufficient to activate intracellular Ca\(^{2+}\) release from the ER and subsequent activation of SOCs. However, EGF-induced activation of PIP\(_2\) breakdown results in the release of PIP\(_2\)-mediated inhibition of TRPP2. In this case, TRPP2 behaves as a *bona fide* ROC.
Figure 4 Activation of TRPP2 by mDia1. At resting membrane potentials, autoinhibited mDia1 (red) binds and blocks TRPP2 activity. In response to EGF or membrane depolarization, mDia1 switches from the autoinhibited state to the activated state releasing the block on TRPP2. Predicted pore-forming region in TRPP2 is shown in green. Formin homology domains 1 and 2 (FH1 and FH2) are shown as red cylinders.

Figure 5 Hypothetical regulation TRPP2 by EGF in the cilium in vivo. TRPP2 co-localizes with PIP₂ and EGFR along the cilium, where it is kept insensitive to flow stimulation due to PIP₂-mediated inhibition. Liberated EGF from prepro-EGF at the apical surface of the plasma membrane through the action of locally acting proteases releases TRPP2 from PIP₂-mediated inhibition generating a gradient in sensitized TRPP2 with higher level of sensitization at the base (shown as yellow) and lower level of sensitization at the tip of the cilium (shown as white). Highly sensitized TRPP2 at the base of the cilium mediates efficient mechanotransduction secondary to fluid flow changes.

Figure 6 Hypothetical role of TRPP2 in mechanotransduction. Cilium bending in response to fluid shear stress may activate GPCR(s) in a ligand-independent fashion probably through mechanical stretching of the plasma membrane and/or associated cytoskeletal structures (arrows). Activation of GPCR would result in intracellular Ca²⁺ release from the ER through the IP₃ pathway and activation of SOCs. ROCs, including plasma membrane TRPP2, could also be activated resulting in a massive and long-lasting
increase in intracellular Ca$^{2+}$ concentration. Broken arrows denote changes in membrane tension in response to cilium bending.

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References


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Figure 1

Tsiokas
ER
PM
ROC
Ca^{2+}
SOC
Ca^{2+}
PIP_{2} \rightarrow IP_{3} + DAG
Ca^{2+}
IP_{3}R
STIM1
PLC(\beta/\gamma)
GPCR
RTK

Figure 2
Tsiokas
Figure 4: mDia1 activation unblocks TRPP2.

TRPP2
extracellular
intracellular

block

extracellular
intracellular

Figure 4
Tsiokas
Figure 5
Tsiokas
Figure 6

Tsiokas