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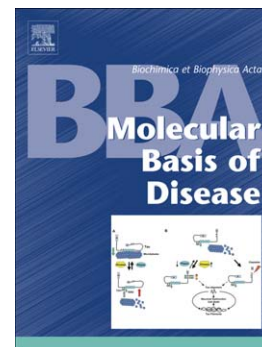
TRPP2 and autosomal dominant polycystic kidney disease

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**TRPP2 and Autosomal Dominant Polycystic  
Kidney Disease**

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**Abstract**

Mutations in TRPP2 (polycystin-2) cause autosomal dominant polycystic kidney disease (ADPKD), a common genetic disorder characterized by progressive development of fluid-filled cysts in the kidney and other organs. TRPP2 is a  $\text{Ca}^{2+}$ -permeable nonselective cation channel that displays an amazing functional versatility at the cellular level. It has been implicated in the regulation of diverse physiological functions including mechanosensation, cell proliferation, polarity, and apoptosis. TRPP2 localizes to different subcellular compartments, such as the endoplasmic reticulum (ER), the plasma membrane and the primary cilium. The channel appears to have distinct functions in different subcellular compartments. This functional compartmentalization is thought to contribute to the observed versatility and specificity of TRPP2-mediated  $\text{Ca}^{2+}$  signaling. In the primary cilium, TRPP2 has been suggested to function as a mechanosensitive channel that detects fluid flow in the renal tubule lumen, supporting the proposed role of the primary cilium as the unifying pathogenic concept for cystic kidney disease. This review summarizes the known and emerging functions of TRPP2, focusing on the question of how channel function translates into complex morphogenetic programs regulating tubular structure.

**Keywords:** Polycystic kidney disease, PKD2, polycystin-2, cilia,  $\text{Ca}^{2+}$ , ADPKD.

## 1. Introduction

Many genetic diseases of ion channels, so called channelopathies, have been identified in the past decade. Mutations in over 60 ion channel genes are now known to cause human disease [1]. Most channelopathies are rare disorders, but some, like autosomal dominant polycystic kidney disease (ADPKD), are among the most common monogenetic diseases known [2, 3]. Mutations in the transient receptor potential channel TRPP2, formerly known as polycystin-2, cause autosomal polycystic kidney disease (ADPKD), a disease that is characterized by progressive replacement of functional renal tissue with fluid-filled cystic structures [4]. The function of the kidney critically depends on the proper structure of its tubule system. Yet regulation of tubular morphogenesis and diameter are poorly understood phenomena. Cystic diseases of the kidney offer unique opportunities to study these fundamental biological processes.

Transient receptor potential (TRP) channels were first described in *Drosophila*, where mutations in the *trp* gene caused a transient response of photoreceptors to continuous light [5, 6]. Unlike many other ion channels, most TRP channel family members were identified by their homology rather than by specific ligands or selectivity. Owing to this, their physiological functions and activation mechanisms are disparate and often unknown [7]. The members of the TRP superfamily of ion channels share several common features including 6 transmembrane segments and permeability to cations (Fig. 2A) [7, 8]. Nevertheless, the TRP family displays an amazing degree of diversity in activation mechanisms, in selectivity and in biological functions. Remarkably, a single TRP

channel can be activated through seemingly disparate mechanisms and integrate multiple signals, as the response to one input is modified by another. A common feature of most members of the TRP channel family is that they have a role in sensory physiology, but not just in the usual organismal definition, that is the five Aristotelian senses. TRP channels are intrinsic sensors of the cellular environment [8].

The TRP channel superfamily is divided into six subfamilies on the basis of amino acid sequence homology (TRPC, TRPM, TRPV, TRPA, TRPP and TRPML) [7, 8]. Unlike other TRP channels, the founding members of the TRPP and TRPML subfamilies, TRPP2 and TRPML1, were identified in searches for disease genes (ADPKD and Mucopolysaccharidosis type IV, respectively) [4, 9]. One of the most striking features of TRPP2 is its functional versatility. It has been implicated in cellular processes as diverse as mechanosensation, proliferation, apoptosis, and polarity [10-12]. TRPP2 functions as a  $\text{Ca}^{2+}$ -permeable nonselective cation channel and assembles into a receptor-channel complex with polycystin-1 [13, 14]. This protein complex forms the core of a signaling pathway that uses  $\text{Ca}^{2+}$  as a second messenger. A fascinating question that arises from the functional versatility of TRPP2 is how this channel is able to fulfil its diverse physiological roles using a promiscuous second messenger like  $\text{Ca}^{2+}$  and, at the same time, ensure signaling specificity. To address these complex problems, TRPP2 research needs to take place at the interface of ion channel physiology,  $\text{Ca}^{2+}$  signaling, cell biology, developmental biology, genetics, and clinical medicine, making this endeavour equally challenging and exciting.

This review is not intended to provide a comprehensive coverage of the extensive body of work on all aspects of the genetics, biophysical properties, and trafficking of TRPP2, which has been the topic of many excellent reviews [10, 11, 15-28]. Rather, it is attempted to integrate known and emerging functions of TRPP2 at all levels of complexity, from single channel, to cell, to the whole organism. Hence this review gravitates around the central question of how TRPP2 channel activity translates into the complex biological program that regulates tubule morphogenesis and maintenance.

## **2. Autosomal Dominant Polycystic Kidney Disease**

Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenetic disease in humans. It affects more than 1 in 1000 live births and is a common cause for end stage renal disease (accounting for 7-10% of hemodialysis patients, 500000 persons in the United States and 4-6 million worldwide) [2, 3]. Cysts arise as a focal outpouch from any segment of the nephron, which eventually separate from the parental nephron, and increase in size and number throughout the patient's life [2, 29]. The continuous increase of fluid-filled cystic structures gradually replaces the normal tissue resulting in end stage renal disease in ~50% of the patients by the sixth decade [2]. Although ADPKD is characterized by kidney cysts and renal failure, it should be regarded as a systemic disorder with cysts occurring in other organs (liver, pancreas) and a range of cardiovascular abnormalities (reviewed in [2]).

ADPKD results from mutations in *PKD1* or *PKD2* [30, 31]. Mutations in either gene cause virtually indistinguishable clinical presentations. The near identity of disease symptoms, irrespective of the causative gene, suggests that the gene products polycystin-1 and polycystin-2 (now TRPP2) function in a common signaling pathway. This hypothesis is supported by a variety of observations, including the similarity of phenotypes between mouse knock-outs of either gene, the discovery that the two proteins interact, and the identity of the phenotypes caused by mutations in the homologous genes in *C. elegans*.

The cyst epithelium is less differentiated than normal tubular epithelial cells. Increased proliferation and fluid secretion are thought to drive cyst growth [32, 33]. Abnormalities in apoptosis, cell polarity, gene expression, and extracellular matrix have been implicated in the pathogenesis of ADPKD, but the mechanism of cyst formation remains incompletely understood [12, 32, 34-38]. Cyst development is a focal process and it has been estimated that only around 1% of tubular cells give rise to cysts [29]. A two-hit mechanism of cyst formation has been proposed which explains the focal nature of cyst development and the variability of the cystic phenotype [39, 40]. According to this model, tubular epithelial cells that have inherited a germline mutation in one allele of *PKD1* or *PKD2* acquire somatic mutations (second hits) of the normal allele, which initiates cyst formation (Fig. 1). Most cases of ADPKD (80-85%) are caused by mutations in the *PKD1* gene, which encodes polycystin-1 [31, 41]. Human polycystin-1 is a huge glycosylated protein that consists of 4302 amino acids and was shown to localize to the plasma membrane and other subcellular locations [14, 42, 43]. It is predicted to have an



extracellular N-terminal segment of 3000 amino acids, 11 transmembrane domains and an intracellular C-terminus of ~200 amino acids (Fig. 2B). The extracellular portion includes motifs commonly involved in protein-protein and protein-carbohydrate interactions and a G-protein-coupled receptor proteolytic site (GPS domain) [44, 45]. The intracellular C-terminus includes a coiled-coil domain that mediates binding to TRPP2 [14, 46, 47], several predicted phosphorylation sites [41], and a putative binding sequence for heterotrimeric G proteins [48]. Although the *in vivo* function of polycystin-1 is still unclear, it has been suggested to be a cell-cell or cell-matrix adhesion receptor, and it might function as an atypical G protein coupled receptor [48-50]. More recently, polycystin-1 has also been proposed to function as a mechanosensor [51, 52]. While polycystin-2 has been recognized as a bona fide TRP family member, polycystin-1 has been proposed to belong to this family as well and the name TRPP1 was suggested [7]. This name is probably misleading: although the last 6 transmembrane domains display some sequence homology with TRPP2, polycystin-1 does neither display structural nor functional features of a TRP channel. The molecular genetics and function of polycystin-1 have been extensively reviewed elsewhere [12, 34, 53, 54], and will therefore only be addressed here when required for the understanding of TRPP2 function. Although there has been considerable progress in the understanding of some of the cellular functions of the polycystins, the physiological functions of TRPP2 and polycystin-1 *in vivo* are only now beginning to be unraveled.

### 3. TRPP2: identification and structural features

In 1996, Mochizuki et al. discovered the second locus responsible for ADPKD on chromosome 4, mutated in ~15 % of the patients [30]. The *PKD2* gene was identified by positional cloning and encodes TRPP2 [30], a cation channel that is evolutionarily conserved in vertebrates and invertebrates. In addition to TRPP2, the TRPP family includes two other mammalian proteins referred to as TRPP3 (PKD2L1, polycystin-L, PCL) [55, 56] and TRPP5 (PKD2L2) [57, 58]. TRPP2 homologs have been found in yeast [59], sea urchins [60] *C. elegans* [61, 62], *Drosophila* [63, 64], zebrafish [65-67] and mice [68]. TRPP2 is composed of 968 amino acids and contains 6 predicted transmembrane domains (Fig. 2A). TRPP proteins share only moderate amino acid identity to the most closely related TRPC proteins, TRPC3 and TRPC6 [7]. Mammalian TRPP2 contains a Ca<sup>2+</sup>-binding motif (EF-hand) and an endoplasmic reticulum (ER) retention motif near the C-terminus, but does not include any ankyrin repeats or a TRP domain like other TRP channels. One structural feature that distinguishes TRPP2 from most other TRP channel family members is a long extracellular loop between the first and second predicted transmembrane segments (Fig. 2A). TRPP2 is widely expressed in many tissues, including kidney, heart, vascular smooth muscle, pancreas, intestine, bile ducts and placenta. In the kidney, TRPP2 is expressed in all nephron segments, but has not been detected in glomeruli [12]. TRPP2 has been shown to localize to different subcellular compartments, such as the ER [69, 70], plasma membrane [71-73], primary cilium [74], centrosome [75] and mitotic spindles in dividing cells

[76]. The functional implications of compartment-specific TRPP2 channel activity will be discussed below (6.2).

#### 4. TRPP2 channel function

The predicted topology of TRPP2 suggested that it might function as an ion channel [30]. However, it took a surprisingly long time until the first report on the channel function of TRPP2 was published [14]. The reason for the difficulties in measuring channel activity in the plasma membrane was unraveled by Cai et al., who found that overexpressed TRPP2 is retained in the ER, due to an ER retention motif in the C-terminal domain [69]. Truncation mutants in which this ER retention motif was deleted, translocated to the cell surface where they could be detected by immunofluorescence and electrophysiological methods [69, 77]. While the majority of studies detected no channel activity of overexpressed full length TRPP2 in the plasma membrane, two studies reported TRPP2 channel function either upon treatment with chemical chaperones or proteasome inhibitors [78], or upon massive overexpression that may eventually override the ER retention machinery [79]. Even though it is now generally accepted that TRPP2 functions as a  $\text{Ca}^{2+}$ -permeable nonselective cation channel, there is still uncertainty regarding several important issues. One key question is whether TRPP2 requires the presence of polycystin-1 to form a functional channel. The first report on TRPP2 channel activity by Hanaoka et al. provided evidence that polycystin-1 and TRPP2 interact to form a functional heteromeric complex [14]. In this study, it was shown that

TRPP2 alone was unable to form functional channels in Chinese hamster ovary cells. Co-expression of polycystin-1 and TRPP2, however, promoted the translocation of TRPP2 to the plasma membrane and gave rise to channel activity, with biophysical properties similar to TRPP3 [14, 80]. This finding was later confirmed in neurons where overexpressed polycystin-1 and TRPP2 formed a functional receptor-channel complex [13]. Channel activity was not observed when the C-terminal interaction between polycystin-1 and TRPP2 was abrogated by polycystin-1 truncation mutants, implying that co-assembly of both proteins is required for TRPP2 channel function in the plasma membrane [14]. Yet, whether polycystin-1 is a pore-forming component of the channel complex or rather acts as a "chaperone" is still unclear. Hanaoka et al. reported that a TRPP2 truncation mutant (R742X), which does not bind polycystin-1, translocates to the plasma membrane but displays no channel activity, suggesting that TRPP2 channel function requires polycystin-1 [14]. However, Delmas et al. reported that the same truncation mutant gave rise to channel activity, with a selectivity profile that was identical to one of the polycystin-1-TRPP2 complex [13]. Meanwhile several other studies reported TRPP2 channel activity at the plasma membrane without co-expression of polycystin-1 [77, 78, 81]. The differential cellular and subcellular localization of polycystin-1 and TRPP2 also supports the idea that both proteins might have independent functions in addition to their joint function [71]. Remarkably, a recent study reported that overexpression of polycystin-1 gave rise to nonselective cation channel activity that was independent of TRPP2, suggesting that polycystin-1 itself might function as a channel [82]. Although the last six

transmembrane domains of polycystin-1 share a moderate degree of sequence homology with TRPP2, which might constitute the structural basis for channel function, it cannot be excluded that the currents induced by expression of polycystin-1 were due to indirect effects on endogenous channels other than TRPP2.

After the initial report on TRPP2 whole cell currents, several single channel studies in placental preparations [79], *Xenopus* oocytes [78], HEK 293 cells [83], and in cell-free lipid bilayer systems [79] characterized the channel properties in more detail. Despite some discrepancies between these studies [17], it was concluded that TRPP2 can form a functional channel in the plasma membrane with constitutive activity upon overexpression. The detailed biophysical properties of the channel are reviewed elsewhere [17, 18]. In brief, TRPP2 showed a slightly higher selectivity of  $\text{Ca}^{2+}$  over  $\text{Na}^{+}$  and  $\text{K}^{+}$ , a high single channel conductance (100-200 pS), and a relatively high spontaneous open probability (20-40%) [17, 18]. Koulen et al. studied the single channel properties of ER-resident TRPP2 using reconstitution of microsomes in lipid bilayers [70]. This study demonstrated that microsomal TRPP2 also functions as a  $\text{Ca}^{2+}$ -permeable nonselective channel. However, some of the biophysical properties clearly differed from those reported for TRPP2 in other studies [17, 70, 79]. While some of these discrepant results (e.g. single channel conductance) may be explained by technical differences in the experimental conditions, others are more difficult to reconcile. These include the very low spontaneous open probability of <1% in ER-microsome preparations compared to ~20-40% in the plasma membrane, the strong voltage dependence of

TRPP2 which was not observed consistently in other studies, and the variable effects of C-terminal truncations on the single channel conductance of TRPP2 [17, 70]. Some of these differences might be explained by interaction of TRPP2 with other proteins that modulate its function. Since these interactions may depend on factors like cell type, subcellular compartment, and differentiation state, they can potentially account for the discrepant results. However, it cannot be excluded to date that some of the currents reported in the literature did not flow through TRPP2, but through the pore of another channel that is up-regulated or modulated by expression of TRPP2. The notion that TRPP2 participates in pore formation, though very likely, still awaits final proof by demonstration that the ion selectivity of TRPP2 is altered by mutations in the putative pore-forming region.

It is evident that future studies on TRPP2 channel function will be required to learn more about potential regulatory mechanisms, including the functional role of TRPP2 association with other channels or regulatory proteins. Furthermore, the identification of the physiological activation mechanisms and specific inhibitors of TRPP2 will be critical to study its channel function under more physiological conditions, i.e. in the native tissue.

## **5. TRPP2-mediated signal transduction: versatile yet specific**

In the face of TRPP2's functional versatility how can TRPP2-mediated signal transduction be specific? One of the fundamental challenges of modern biology is to understand how cells within a developing organism generate, integrate, and

respond to dynamic cues. Over the last decades, many pathways of the responsible signal transduction networks have been identified and functionally characterized. From this work, it has become evident that a complicated interplay between signaling pathways, involving extensive feedback regulation and multiple levels of cross-talk, underlies even the “simplest” developmental decision. Thus, a signaling pathway can no longer be thought of as a rigid linear process, but rather must be considered a dynamic and self-adjusting network.

Significant progress has been made in the identification of signaling pathways by which TRPP2 and polycystin-1 exert their versatile functions [12, 18, 34, 84]. Polycystin-1 has been reported to participate in various signaling pathways, including the PI3 kinase pathway [85], the JAK/STAT pathway [86], as well as Wnt- [87], mTOR- [88], NFAT- [89] and PKC pathways [90]. Some of these signaling functions of polycystin-1 appear to require TRPP2, but it is not known how the different pathways are integrated *in vivo* to regulate tubular morphogenesis and maintenance.

As mentioned above, multiple lines of evidence indicate that TRPP2 and polycystin-1 function in a common signaling pathway; in this review  $\text{Ca}^{2+}$  signaling shall be the focus. Although it was predicted early on that the protein-protein interaction between TRPP2 and polycystin-1 would promote  $\text{Ca}^{2+}$ -mediated signaling, this was formally demonstrated only much later [13, 14]. Meanwhile it is generally accepted that polycystin-1 and TRPP2 form the core of a signaling pathway that utilizes  $\text{Ca}^{2+}$  as a second messenger. However, neither the activation

mechanisms nor the effectors downstream of the  $\text{Ca}^{2+}$  signal have yet been identified (Fig. 3A).  $\text{Ca}^{2+}$  ions are ubiquitous and versatile signaling molecules, capable of decoding and integrating a variety of extracellular stimuli. These stimuli are translated into diverse cellular actions ranging from contraction, secretion, fertilization and proliferation to cell death (reviewed in [91, 92]). Multiple upstream and downstream players in  $\text{Ca}^{2+}$  signal transduction are expressed within a single cell (Fig. 3B). Thus, a fundamental question is how a given cell is able to translate the wide array of extracellular signals into specific cellular responses using the same second messenger. The key to input-output specificity of this pleiotropic signaling molecule is the complex spatiotemporal organization of  $\text{Ca}^{2+}$  signals, which allows specific effectors to be recruited [91, 92]. Subcellular heterogeneity in  $\text{Ca}^{2+}$  concentration at rest and upon stimulation, so-called cytosolic  $\text{Ca}^{2+}$  microdomains, are critical for the selective activation of  $\text{Ca}^{2+}$ -dependent effectors that determine specific cellular responses (Fig. 3C) [93].

In the context of ADPKD this means that we need to understand why loss of function of the  $\text{Ca}^{2+}$ -permeable channel TRPP2 causes such profound morphogenetic changes, whereas related channels (e.g. TRPV5), permitting  $\text{Ca}^{2+}$  entry in the close proximity (within a few micrometers), give rise to completely different phenotypes when mutated, such as renal  $\text{Ca}^{2+}$  wasting and reduced bone thickness in mice lacking TRPV5 [94]. In order to understand TRPP2 signaling specificity, we need to identify the activation mechanisms and downstream effectors of TRPP2 that execute specific cellular functions. The multifunctional properties of TRPP2 are likely to be mediated in part by differential interactions



with other proteins. In fact, TRPP2 has been shown to interact with different proteins including, cytoskeletal proteins [95-97], adapter proteins [81, 98, 99], ion channels [100], and others. Yet, the physiological relevance of most of these protein-protein interactions with TRPP2 remains to be clarified. Moreover, in order to accomplish input-output specificity in TRPP2-mediated signaling, there is probably crosstalk between  $\text{Ca}^{2+}$  signals and other "polycystin-signaling" pathways mentioned above (Fig. 3B). Current and potential future experimental approaches to address these complex problems will be discussed below. As a first step to elucidate the spatial regulation of TRPP2-mediated  $\text{Ca}^{2+}$  signals many laboratories have begun to study the compartment-specific functions of TRPP2.

## **6. Compartment-specific functions of TRPP2**

The long-standing debate whether TRPP2 resides and functions in the plasma membrane or in the ER has been reconciled by the recent demonstration that the subcellular localization and transport of TRPP2 are controlled by phosphorylation and multiple interactions with adapter proteins [23, 98, 101-103]. Polycystin-1 [14], glycogen synthase kinase 3 (GSK3) [103] and Golgi- and ER-associated protein 14 (PIGEA-14) [98] have been shown to facilitate forward transport of TRPP2 in the secretory pathway, whereas PACS-2 and PACS-1 have been shown to mediate phosphorylation-dependent retention or retrieval in the ER and Golgi apparatus, respectively [81]. TRPP2 appears to serve distinct functions in different subcellular compartments and its localization is probably dynamically regulated (Fig. 4).

### 6.1. TRPP2 function in the plasma membrane

Functional expression of TRPP2 in the plasma membrane was first reported by Hanaoka et al. and subsequently confirmed by others using different overexpression systems [13, 14, 72, 79, 81]. Furthermore, the activity of endogenous ion channels with biophysical properties similar to overexpressed TRPP2 has been detected in several cell types [72, 83, 104]. Based on the finding that TRPP2 and polycystin-1 interact *in vitro* and *in vivo*, it was suggested that both proteins could form a receptor-ion channel complex that senses extracellular cues and translates them into intracellular  $\text{Ca}^{2+}$  signals. In this model, binding of hitherto unidentified ligands to polycystin-1 would regulate the gating of TRPP2. Delmas et al. provided evidence supporting this concept by showing that polycystin-1-TRPP2 complexes reconstituted in neurons can be activated by applying antibodies directed against the receptor for egg jelly (REJ) domain of polycystin-1 [13]. This elegant study revealed striking mechanistic parallels with the acrosome reaction in sea-urchin spermatozoa [105]. The acrosome reaction requires activation of REJ1/REJ3, two polycystin-1 orthologs which bind components of egg jelly [106]. Antibodies directed against the REJ domain of sea urchin REJ proteins induce the acrosome reaction by opening sperm  $\text{Ca}^{2+}$ -permeable channels of unknown identity [107]. Delmas et al. suggested that polycystin-1 activates TRPP2 via conformational coupling, since the REJ antibodies increased whole cell currents independent of G protein and phospholipase C signaling [13]. However, it is also possible that TRPP2 can be activated indirectly by polycystin-1 under certain

conditions. Recently, it has been reported that TRPP2 channel activity can be enhanced in response to epidermal growth factor (EGF) receptor stimulation [108], in a process that is mediated via phospholipase C ( $\gamma$ 2) and phosphoinositide 3-kinase (PI3-kinase). Since polycystin-1 was shown to increase PI3-kinase activity in another study [85] one might hypothesize that polycystin-1-induced PI3-kinase activity may regulate TRPP2, providing an indirect activation mechanism that does not require physical interaction between polycystin-1 and TRPP2. In tubular epithelial cells TRPP2 has been shown to localize to the basolateral membrane [71, 73] and it has been suggested that polycystin-1-TRPP2 complexes cell-matrix or at cell-cell junctions in the basolateral membrane may regulate processes like adhesion, differentiation and proliferation (Fig. 4B). However, experimental evidence supporting the functional relevance of this model is still sparse.

### *6.2. TRPP2 function in the ER*

After it was reported that native and transfected TRPP2 accumulated in the ER [69], an obvious question was whether the channel also functions in this subcellular compartment. Ion channel function in the ER is well established for the IP<sub>3</sub> receptor and the ryanodine receptor. For most ion channels, however, intracellular compartments, including ER and Golgi apparatus, have been viewed as "whistle stops" on the way to their final functional destination, the plasma membrane. Recent studies have revealed that members of other ion channel families, like ClC chloride channels, which had been thought to function in the plasma membrane exclusively, have important intracellular functions [109]. Koulen et al. reported that

TRPP2 may function as a  $\text{Ca}^{2+}$  release channel in the ER, which amplifies  $\text{Ca}^{2+}$  transients triggered by  $\text{IP}_3$  [70] (Fig. 4A). TRPP2, reconstituted in lipid bilayers, appears to be directly activated by  $\text{Ca}^{2+}$  and the open probability displays a bell-shaped  $\text{Ca}^{2+}$  dependence [70]. The sensitivity of microsomal TRPP2 to  $\text{Ca}^{2+}$  stimulation was shown to be significantly increased by phosphorylation of S812, a putative CK2 phosphorylation site [110]. Recently, it has been reported that TRPP2 interacts physically and functionally with the  $\text{IP}_3$  receptor in an *Xenopus* oocyte expression system and it has been proposed that this interaction is critical for TRPP2 function in the ER [111]. Furthermore, vascular smooth muscle cells of TRPP2<sup>+/-</sup> mice were reported to display altered intracellular  $\text{Ca}^{2+}$  homeostasis, which may potentially be linked to TRPP2 channel function in the ER [112]. Although the physiological relevance of TRPP2 channel function in the ER remains to be clarified, the existence of other ER-resident "cystoproteins" like bcl-2 [113], PRKCSH (glucosidase II  $\beta$  subunit) [114] or SEC63 [115] points to a potential role of the ER in cystogenesis. Unpublished results from our laboratory indicate that TRPP2 may regulate the  $\text{Ca}^{2+}$  concentration in the ER and thereby control apoptosis sensitivity, a critical cellular function for tubule morphogenesis and tissue homeostasis (see also 7.1).

### 6.3. TRPP2 function in the primary cilium

An amazing convergence of findings from several species, including algae, nematodes, fruit flies and mice implicate defects in structure or function of primary cilia as a possible common mechanism in the development of polycystic kidney

disease [116-118]. The primary cilium is an antenna-like structure that emanates from the surface of virtually every cell in the mammalian body and functions as a sensory organelle [119, 120]. Ultrastructurally, cilia consist of a ciliary membrane that is continuous with the cell membrane and a central axoneme that is composed of microtubules. The cilia axoneme originates from the basal body, a centriole-derived microtubule-organizing center [117, 119]. The role of cilia in sensing the extracellular environment is best understood in the context of olfaction and photoreception. In addition to sensing chemicals and light, cilia can sense mechanical cues. These functions have been well characterized in model organisms. Ciliary TRPV channels in *Drosophila* and *C. elegans* may function as mechanosensors. In *Drosophila*, two TRPV channels (Inactive and Nanchung) on the cilia of auditory neurons mediate reception of sound vibrations at the antenna [121, 122], and the TRPN channel NOMPC is required for touch sensation in mechanosensory bristles [123]. Similarly, two *C. elegans* TRP channels (OSM-9 and OCR-2) localize to cilia of sensory neurons, where they respond to nose touch, chemicals and high osmolarity [124]. The functional importance of renal cilia has been largely ignored until a series of recent studies suggested that structural or functional ciliary defects are associated with cystic kidney disease [62, 125, 126]. The first hint that cilia might be involved in cystogenesis in the kidney came from the *orpk* mouse model of PKD. The *orpk* phenotype is caused by a hypomorphic allele of the *Tg737* gene, and is characterized by multiple abnormalities including cystic kidneys [127]. The discovery that *Tg737* is the mouse homolog of IFT88, a protein required for the assembly of flagella in the green algae, *Chlamydomonas*

*reinhardtii*, was a breakthrough linking structural abnormality of cilia to the development of PKD [125]. In addition, around the same time it was shown that the *C. elegans* homologs of TRPP2 (*pkd-2*) and polycystin-1 (*lov-1*) localize to cilia in sensory neurons and function in a common pathway, regulating male mating behavior [61, 62]. Remarkably, it was shown in several studies that the majority of mammalian cystoproteins including TRPP2 and polycystin-1 are localized to primary cilia in the kidney [74, 128]. None of the human PKD genes appears to be associated with assembly of cilia. Rather PKD patients form cilia that are normal in appearance, but are missing proteins required for sensory transduction. In the kidney the primary cilium would be ideally positioned to act as a sensor extending from the apical cell surface into the tubule lumen. But what is it that the cilium is sensing? Based on elegant studies by Praetorius and Spring it was proposed that renal cilia function as mechanosensors that detect fluid flow in the tubule lumen. The bending of cilia on MDCK cells by laminar fluid flow or a micropipette led to an increase in the cytosolic  $Ca^{2+}$  concentration that was strictly dependent on the presence of cilia [129, 130]. This calcium influx through an unknown ciliary ion channel triggered  $Ca^{2+}$ -induced  $Ca^{2+}$  release from the ER which amplified the signal [129]. Subsequently it was shown by Liu et al. that flow-mediated  $Ca^{2+}$  signals in isolated microperfused collecting ducts of *orpk* mice (which have stunted cilia) were decreased compared to wild type animals [131], supporting the flow-hypothesis in a more physiological experimental setting. The  $Ca^{2+}$  permeability and localization of TRPP2 make it an attractive candidate for a ciliary  $Ca^{2+}$  entry pathway (Fig. 4C). Nauli et al. presented the first evidence that

polycystin-1 and TRPP2 might be involved in flow sensing, using cells derived from mice lacking polycystin-1 [51]. In this study polycystin-1-deficient cells did not show an increase in the cytosolic  $\text{Ca}^{2+}$  concentration in response to fluid flow. Furthermore, antibodies directed against the first extracellular loop of TRPP2 (between transmembrane segments 1 and 2) abolished the flow response in wild type mice [51]. Interestingly, an antibody directed against an intracellular epitope that served as a negative control also blocked about 50% of the flow-induced  $\text{Ca}^{2+}$  signal. As an independent indication for the involvement of TRPP2 in the flow response the authors reported that TRPP2 is not detectable in cilia of polycystin-1-deficient cells. This observation, however, is at odds with a recent study showing that an N-terminal motif in TRPP2 is responsible for its targeting to the primary cilium in a process that did not require polycystin-1 [101]. The notion that TRPP2 is part of a flow-mediated  $\text{Ca}^{2+}$  entry pathway in the primary cilium of tubular epithelial cells would be strengthened by the demonstration that the flow-induced  $\text{Ca}^{2+}$  signal is reduced or abolished in TRPP2-deficient cells. A recent study was the first to provide evidence for single channel cation currents from isolated primary cilia [132]. However, the molecular identity and mechanosensitivity of these ciliary channels remains to be established. Another hint for a potential role of TRPP2 in ciliary mechanosensation was revealed by a recent unexpected observation. Pennekamp et al. found that targeted disruption of TRPP2 in mice causes *situs inversus*, in addition to the known abnormalities in the kidney and the cardiovascular system [133]. The establishment of left-right asymmetry of visceral organs is known to require directed fluid-flow in an embryonic structure called the

node [134]. Consistent with a potential mechanosensory role of TRPP2 it was subsequently reported that the channel is expressed in cilia of the embryonic node, where it appears to be required for the transduction of leftward nodal fluid flow into perinodal  $\text{Ca}^{2+}$  signals [135]. Interestingly, none of the polycystin-1-deficient mouse strains have been reported to exhibit laterality defects, which correlates with the absence of polycystin-1 in nodal cilia. This supports the view that, at least in the embryonic node, TRPP2 may be involved in mechanosensation in the absence of polycystin-1. However, recent evidence suggests that nodal flow results in morphogen transport via nodal vesicular particles, which is supposedly critical for the generation of laterality [136]. In this model asymmetric elevation of intracellular  $\text{Ca}^{2+}$  in the node is caused by chemical rather than mechanical cues [134]. Overall, there is some evidence to support the model that the polycystin-1-TRPP2 complex may function as a mechanosensor that transduces mechanical stimuli into intracellular  $\text{Ca}^{2+}$  signals. However, direct experimental evidence demonstrating that the polycystin-1-TRPP2 complex is mechanosensitive is still missing.

The mechanism whereby mechanical force is transduced to regulate the activity of TRPP2 is currently unknown. Since there is no experimental evidence indicating that homomultimeric TRPP2 channels are mechanosensitive, one may hypothesize that TRPP2 assembles with other TRP channels to form a heteromeric mechanosensitive channel complex. This would be consistent with observations in *C. elegans*, whereby differential assembly of heteromeric TRP channel complexes specifies distinct sensory modalities including mechanosensation [124]. Unpublished results from the Delmas group and from ourselves indicate that



TRPP2 might in fact assemble with two candidate TRP channels, TRPC1 and TRPV4, to form a mechanosensory complex (Fig. 4C) (reviewed in [20]).

An alternative function of the primary cilium in the kidney may be in sensing the concentration of specific ligands in the tubule lumen. In such a chemosensory model, binding of ligands to polycystin-1 might regulate the gating of TRPP2, resulting in intracellular  $\text{Ca}^{2+}$  signals. Both models could potentially convey spatial information to the tubular epithelial cell, which is required to form and maintain a polarized epithelial tube with proper diameter [137]. Future studies will have to test whether chemical, mechanical, or both types of stimuli are essential to transduce morphogenetic signals via TRPP2.

Taken together, TRPP2 has been shown to function in at least 3 different subcellular localizations, the plasma membrane, the ER, and the primary cilium (the possibility of TRPP2 function at mitotic spindles is discussed elsewhere [76]). It remains to be determined which pool of TRPP2 is critical for the pathogenesis of ADPKD. For obvious reasons the PKD field has become quite ciliocentric recently. However, one should not exclude the possibility that an interplay between different compartment-specific functions of TRPP2 (and other cystoproteins) is critical for the underlying physiological and pathophysiological processes. The flow-induced  $\text{Ca}^{2+}$  signal is a good example for the interaction between two compartments, the cilium and the ER, since either compartment-specific component of the  $\text{Ca}^{2+}$  signal is critical to transduce the mechanical stimulus [129].

#### 6.4. Activation mechanisms

The different localization-dependent functions described above have provided first insights into the activation mechanisms of TRPP2, which are essential for understanding the physiological function of the channel. In the ER, it has been suggested that  $\text{Ca}^{2+}$  directly activates TRPP2 [70], but the nature of the physiological stimulus that triggers the  $\text{Ca}^{2+}$  increase is not clear in this context. In the primary cilium TRPP2 appears to be activated by mechanical force, probably in a complex with other proteins [51]. In the plasma membrane activation via EGF receptors and polycystin-1 has been reported [13, 108]. One of the most important future challenges will be the identification of polycystin-1 or TRPP2 ligands, which will enable us to study the spatiotemporal aspects of TRPP2 function and  $\text{Ca}^{2+}$  signaling in more physiological settings.

#### 6.5. Downstream effectors

The identity and regulation of  $\text{Ca}^{2+}$ -dependent downstream effectors in the TRPP2 signaling pathway are even less understood. Recently, it was reported that TRPP2 binds the pro-proliferative helix-loop-helix protein Id2 and prevents it from entering the nucleus [138]. Mutations in TRPP2 that disrupt the binding of Id2 were reported to cause nuclear translocation of Id2, resulting in increased proliferation. It is unknown whether this process is regulated by TRPP2-dependent  $\text{Ca}^{2+}$  signals. Furthermore, several polycystin-1-induced downstream signals, including NFAT activation [89], the nuclear translocation of the polycystin-1 C-terminal domain [139], and the JAK/STAT pathway [86] have been shown to depend on TRPP2.

Yet, in none of these examples a direct link to TRPP2-dependent  $\text{Ca}^{2+}$  signals has been established. Consequently, in order to understand TRPP2  $\text{Ca}^{2+}$  signaling specificity, it will be mandatory to identify  $\text{Ca}^{2+}$ -dependent downstream targets in the TRPP2 signaling pathway.

## **7. Cellular consequences of TRPP2 function**

The physiological functions of TRPP2 have been mostly deduced from the loss of function phenotypes observed in cells and tissues of patients and animal models. As mentioned earlier TRPP2 has been implicated in the regulation of cellular differentiation, proliferation, apoptosis, and polarity. Consequently, a fundamental question is how TRPP2 fulfils such diverse physiological functions.

### *7.1. Apoptosis*

Processes that disrupt the delicate balance between the rates of cell proliferation and apoptosis have been reported to result in cyst formation [140, 141]. Apoptosis is increased in polycystic kidney disease and there is ample evidence that apoptosis plays a causal role in cyst formation [142]. Tubular epithelial cell apoptosis is increased early on in most animal models of PKD and in kidneys from humans with ADKPD [143, 144]. Induction of apoptosis results in cyst formation in tubular epithelial cells in 3D collagen culture, and cystogenesis in this system is inhibited by overexpression of the antiapoptotic protein Bcl-2 [145]. Moreover, a recent study demonstrated that caspase inhibition reduces apoptosis and

proliferation in renal epithelia and attenuates cyst formation and kidney failure in PKD animal models [143]. Although the link between apoptosis and PKD is well established, our understanding of the molecular mechanisms of apoptosis regulation by TRPP2 is still sketchy. Recent studies have emphasized the central role of  $\text{Ca}^{2+}$  in the regulation of programmed cell death [146, 147]. Increases in cytosolic  $\text{Ca}^{2+}$  during cell death can arise from several sources, including damage of external membranes, hyper-activation of cation channels or release from  $\text{Ca}^{2+}$  stores in the ER [146, 148]. Apoptotic stimuli such as thapsigargin or ceramide elicit  $\text{Ca}^{2+}$  release from the ER and thereby increase the mitochondrial  $\text{Ca}^{2+}$  concentration [149], which results in induction of apoptosis. Kip et al. reported increased rates of apoptosis in vascular smooth muscle cells of TRPP2<sup>+/-</sup> mice and suggested that this phenotype is caused by a reduction in the cytosolic  $\text{Ca}^{2+}$  concentration in these cells [150]. However, the mechanistic link between the  $\text{Ca}^{2+}$  reduction and increased apoptosis is not clear. Preliminary results from our group indicate that TRPP2 function in the ER protects cells from apoptosis by decreasing the concentration of releasable calcium in the ER [151], a well-established determinant controlling apoptosis sensitivity [149]. This exemplifies that compartmentalized TRPP2 channel function appears to be critical for specific cellular outcomes. Moreover, these results may explain the rather counterintuitive concept that a nonselective cation channel protects cells from apoptosis rather than killing them. Interestingly, polycystin-1 has also been shown to protect cells from apoptosis via the phosphatidylinositol 3-kinase (PI3-kinase)/Akt signaling

pathway [85, 140]. Future studies will have to address whether TRPP2 and polycystin-1 function in a common pathway of apoptosis regulation.

## 7.2. Proliferation

The prevailing model of cystogenesis in ADPKD predicts that the loss of heterozygosity following a somatic mutation of the wild type allele of *PKD1* or *PKD2* causes monoclonal proliferation of cells and thereby initiates cyst formation (Fig.1). Evidence to support this hypothesis has come from microdissection studies of cystic kidneys, which revealed that cyst size is due to an increase in the number of epithelial cells lining the cyst and not to the stretching of the cyst wall [152]. Additionally, cultured epithelial cells from ADPKD cysts display enhanced rates of proliferation, and genes associated with increased proliferation, such as c-Myc, have been found to be overexpressed in cystic epithelium [153, 154]. TRPP2 has also been implicated in regulation of the cell cycle via its calcium channel activity and stimulation of the transcription factor AP-1 [155]. However, there has been little direct evidence tying TRPP2 to this process. Several groups have studied the role of TRPP2 in the regulation of proliferation using both, loss and gain of function approaches. Grimm et al. created a number of cell lines variable in their expression of TRPP2 to demonstrate that that the basal and EGF-stimulated rate of cell proliferation is higher in cells that do not express TRPP2 versus those that do [156]. This indicates that TRPP2 acts as a negative regulator of cell growth. Cells expressing a channel dead mutant of TRPP2 (D511V) proliferated faster than those expressing the wild-type protein, implying that channel activity of TRPP2

may be important in this process [156]. A recent study reported that haploinsufficiency of TRPP2 can result in increased tubular proliferation in the cystic kidney [157]. Since the increase in cell proliferation was prominent in cysts and non-cystic tubules, the authors concluded that it probably represents one of the earliest events in cyst formation. Another study in lymphoblastoid cell lines suggested that loss of TRPP2 in these cells is associated with reduced proliferation, a finding that seems at odds with other studies [158]. The fact that these data were obtained from non-renal cells suggests that TRPP2 may play specific growth regulatory roles in epithelial cells that are not recapitulated in non-epithelial cell types. In addition, the association of TRPP2 with polycystin-1 suggests that TRPP2 may be involved in some of the same growth suppression pathways that have been attributed to polycystin-1 [140]. Work by Bhunia et al. in particular indicates that polycystin-2 aids polycystin-1 in its antiproliferative function via up-regulation of the cell cycle arrest protein p21waf1 [86]. In summary, there is mounting evidence to support an anti-proliferative role for TRPP2 but the mechanistic link to TRPP2 channel function remains to be established.

### *7.3. Polarity*

In addition to apico-basolateral polarity of epithelia there is a second polarity axis in the plane of the epithelium. In contrast to a renal tubule, which possesses clearly distinct longitudinal and circumferential axes, a cyst has no predominant axis. In epithelial tissues it appears that individual cells are able to identify their position and orientation relative to the overall orientation of the epithelial sheet through a

process termed planar cell polarity [159]. The transition from tubule to cyst in ADPKD suggests a profound alteration in this process. The fundamental determinants of planar polarity have been studied in *Drosophila* and the non-canonical Wnt signaling pathway appears to play a central role in this process [159]. More recently, different studies provided first insights that alterations in planar cell polarity might in fact be central to cyst development (reviewed in [160, 161]). Fischer et al. elegantly showed that lengthening of renal tubules is associated with mitotic orientation of cells along the tubule axis, demonstrating intrinsic planar cell polarization [162]. Moreover, they demonstrated that mitotic orientations are significantly distorted in rodent polycystic kidney disease models, suggesting that oriented cell division dictates the maintenance of constant tubule diameter during tubular lengthening and that defects in this process trigger renal tubular enlargement and cyst formation. In addition, Simons et al. provided experimental evidence that links cilia with noncanonical Wnt signaling pathways and planar polarity [163]. This exciting study suggested that the ciliary cystoprotein inversin might act as a flow-regulated molecular switch between canonical and noncanonical Wnt pathways. Notably, loss of inversin function in mice and humans, like TRPP2 disruption, results in left-right axis defects and severe renal cystic disease [164]. The link between flow-mediated  $\text{Ca}^{2+}$  signals and inversin is not clear to date, but it is tempting to speculate that local  $\text{Ca}^{2+}$  signals modulate inversin function. Interestingly, inversin contains an IQ motif and has been shown to bind calmodulin in a  $\text{Ca}^{2+}$ -dependent fashion, supporting this hypothesis [165].

How TRPP2 transduces spatial cues to downstream effectors like inversin to regulate polarity will be an exciting future area of research.

The mechanism by which the diverse cellular defects discussed here ultimately initiate cystogenesis is unclear. The integration of apoptosis, proliferation, and polarity regulation at the tissue level may be critical to understand cyst development. In contrast to the role of increased proliferation in cystogenesis, that of apoptosis appears rather counterintuitive, since kidney size is hugely increased in ADPKD. However, a significant portion of the volume increase is due to cyst fluid rather than increase in cyst tissue. Therefore, a subtle imbalance between cell proliferation and apoptosis is probably sufficient for cyst development and growth. One might speculate that as a result of increased apoptosis, regenerating cells need to orient themselves in the epithelial layer with correct apico-basolateral and planar polarity. Thus, alterations of apoptosis, proliferation and polarity signaling might jointly cause cyst development.

#### **8. TRPP2 function *in vivo*: lessons from animal models**

Homozygous TRPP2 knockout mice are embryonic lethal and develop severely cystic kidneys *in utero* [68]. Besides cysts these animals exhibit a range of nonrenal manifestations that are not seen in human ADPKD, including abnormal vascular permeability, defective cardiac septation and *situs inversus* [68, 133]. Therefore, TRPP2 appears to have functions whose manifestations during embryonic development (revealed in homozygous knockout mice) differ from those



after development (revealed by the delayed, second-hit-mediated loss of TRPP2 function in ADPKD patients). The kidneys of heterozygous TRPP2 knockout mice do not exhibit a prominent cystic phenotype [68]. These results demonstrate that loss of TRPP2 is sufficient to cause renal cysts and support the two-hit model. Further evidence for the two-hit model comes from a unique strain of mice carrying a *Pkd2* allele (WS25) that is prone to genomic rearrangement [68]. The WS25 allele produces wildtype TRPP2 protein. However, during life it can rearrange to produce a null allele, mimicking somatic mutations. Compound heterozygous *Pkd2*<sup>WS25/-</sup> mice represent the most authentic model of human ADPKD to date in that they are viable and develop cysts throughout adult life, resembling the human ADPKD phenotype [68]. Rodent models have proven to be very useful in the analysis of the pathological features of ADPKD, as well as in the development and evaluation of new therapeutic strategies [166]. Conditional inactivation of TRPP2 using the Cre-lox system will take this one step further, since it will allow us to study the tissue-specific functions of TRPP2 in adult animals and the importance of the timing of gene inactivation. However, due to the complexity of these models, studies on the signal transduction networks underlying cystogenesis continue to be very challenging.

Until recently, the study of TRPP2 function within complex signaling networks *in vivo* has been hampered by the lack of genetically tractable model organisms. The discovery of TRPP2 homologs in *C. elegans*, *Drosophila melanogaster* and zebrafish has now greatly facilitated the study of TRPP2 signaling pathways *in vivo* [62-64, 67]. In zebrafish, the expression pattern of the

TRPP2 homolog polycystin-2 was shown to be similar to mammalian TRPP2 [66]. Morpholino-antisense-mediated disruption of zebrafish polycystin-2 gave rise to multiple phenotypes including pronephric cysts and organ laterality defects [66]. Furthermore, large scale mutagenesis in zebrafish has confirmed the role of known cyst genes like HNF-1 $\beta$  as well as identified new "cilia genes" that are involved in cyst formation [67]. The *C. elegans* TRPP2 homolog (*pkd-2*) is expressed in ciliated sensory neurons [62]. Worm *pkd-2* function appears to be essential for stereotyped male mating behavior and it functions in a common pathway with Lov-1, the worm homolog of polycystin-1 [61, 62]. In *Drosophila* the TRPP2 homolog AMO is localized to the sperm tail, a ciliated structure, and is essential for male fertility [63, 64]. Loss of function of AMO does not affect sperm development, or transfer of sperm into the uterus. Rather, there is a defect in the directed movement of sperm from the uterus to sperm storage organs, which is known to be essential for reproductive success in flies [64]. Thus, in worms and in flies TRPP2 appears to have a sensory role in ciliated structures. Whether chemical or mechanical signals activate the channel in these organisms remains to be shown. Although the anthropocentric mind may ask why one should spend time (and money) studying genes involved in kidney disease in organisms that have no kidneys, using these model organisms will likely prove to be very useful in elucidating TRPP2 signaling pathways *in vivo*. Due to the powerful tools for genetic manipulation, many fundamental signaling pathways have been originally identified in *Drosophila* and *C. elegans*. More than half of human disease genes are evolutionary conserved in these organisms. *Drosophila* and *C. elegans* TRP

channels share many basic features with mammalian TRP family members. This suggests that particular classes of *Drosophila* and *C. elegans* TRP channels can provide considerable insight into the function of their mammalian counterparts. Unbiased forward genetic screens in these model organisms are underway to identify new upstream and downstream components in the TRPP2 signaling pathway *in vivo*.

## 9. Conclusions and future perspectives

Since the identification of TRPP2 in the past decade there has been considerable progress in our understanding of the cellular and subcellular functions of this exceptionally versatile ion channel. TRPP2 and polycystin-1 form the core of a signaling pathway that uses  $\text{Ca}^{2+}$  as a second messenger, and it has become evident that TRPP2 exhibits distinct signaling functions in different subcellular compartments. However, the diversity of the processes in which these signaling mechanisms appear to be involved highlights the remaining questions. What are the specific molecular cues that activate these pathways? What are their downstream effectors and what additional factors are responsible for adapting these mechanisms to the unique requirements of each tissue?

A multidisciplinary approach using biochemistry, electrophysiology, cell biology, and animal models ranging from *C. elegans* to mice will be required to address these complex issues. Thus, in order to address the fundamental question why loss of TRPP2 channel function ultimately results in such profound changes in

organ architecture, we will need to integrate information gathered from the analysis of data sets as diverse as single channel recordings, proteomics, TRPP2 signaling networks, worm sex, fly sperm and conditional mouse models. This endeavour would undoubtedly benefit from the continuing collaborative efforts of geneticists, physiologists, cell biologists and developmental biologists. Future results not only promise to improve our understanding of ADPKD pathogenesis, but will also provide insights into the enigmatic mechanisms of  $\text{Ca}^{2+}$  signaling specificity. These are exciting times for TRPP2 research.

It is encouraging that first therapeutic approaches have emerged from basic research studies (reviewed in [167]). Ultimately, improved understanding of ADPKD pathogenesis and the availability of animal models of the human disease will provide an excellent opportunity for the development of rational therapies.

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## Figure legends

**Fig. 1 The two-hit model of cyst formation in ADPKD. (A)** Tubular epithelial cells with a germline mutation in one TRPP2 allele form proper tubules. **(B)** Loss of heterozygosity by a somatic mutation of the normal allele in an individual cell initiates cyst formation (second hit indicated by the star). **(C)** Monoclonal proliferation of the cell in which both alleles are inactivated. **(D)** After disconnecting from its parental nephron, isolated cysts progressively increase in size due to proliferation and secretion of electrolytes and water into the cyst lumen (modified from [168]).

**Fig. 2 Predicted structure of TRPP2 (A) and polycystin-1 (B).** Protein motifs are identified in the figure. Structures are not drawn to scale.

**Fig. 3 Model of TRPP2 function in intracellular  $\text{Ca}^{2+}$  signaling. (A)** TRPP2 and polycystin-1 form a signaling complex that uses  $\text{Ca}^{2+}$  as a second messenger. Neither the activation mechanisms nor the  $\text{Ca}^{2+}$ -regulated downstream effectors are known. **(B)**  $\text{Ca}^{2+}$  is a multifunctional signaling molecule regulating a wide array of downstream effectors, such as calmodulin-dependent kinases, different protein kinase C isoforms, and calcineurin (drawn in blue, effector proteins are schematized by various shapes). Multiple proteins, including G-protein-coupled receptors and ion channels, regulate  $\text{Ca}^{2+}$  dynamics within a single cell (drawn in red). Crosstalk between  $\text{Ca}^{2+}$  signaling pathways and other polycystin-1-regulated

signaling pathways (drawn in green) is likely to occur. **(C)**  $\text{Ca}^{2+}$  signaling microdomains are depicted as a red gradient around the channel pore. These spatially restricted  $\text{Ca}^{2+}$  increases allow for the specific activation of downstream effectors.

Abbreviations: polycystin-1, PC-1; adenosine-trisphosphate, ATP; antidiuretic hormone, ADH; acetylcholine, ACh.

**Fig. 4 Compartment-specific functions of TRPP2.** Various models for the localization-dependent functions of TRPP2 are depicted. **(A)** In the ER, TRPP2 may function as a  $\text{Ca}^{2+}$ -regulated  $\text{Ca}^{2+}$  release channel. TRPP2 channel activity in the ER might be regulated by interaction with polycystin-1 (PC-1) in the plasma membrane or by interaction with the inositol 1,4,5-trisphosphate receptor ( $\text{IP}_3$ -R) in the ER. **(B)** In the basolateral plasma membrane TRPP2 is thought to function in a complex with polycystin-1. This complex resides either at the basal cell-matrix interface or at cell-cell junctions in the lateral membrane. **(C)** In the primary cilium TRPP2 may function as a sensor for mechanical or chemical cues. Flow-mediated bending of the cilium was shown to trigger cytosolic  $\text{Ca}^{2+}$  signals, in a process that was proposed to require the TRPP2- polycystin-1 complex. Other members of the TRP family (TRPV4 and TRPC1) may also be involved in ciliary mechanosensation.

