Regulation of calcium signaling by polycystin-2

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Cantiello, Horacio F. Regulation of calcium signaling by polycystin-2. Am J Physiol Renal Physiol 286: F1012–F1029, 2004; 10.1152/ajprenal.00181.2003.—Autosomal dominant PKD (ADPKD) is a common lethal genetic disorder characterized by progressive development of fluid-filled cysts in the kidney and other target organs. ADPKD is caused by mutations in the PKD1 and PKD2 genes, encoding the transmembrane proteins polycystin-1 (PC1) and polycystin-2 (PC2), respectively. Although the function and putative interacting ligands of PC1 are largely unknown, recent evidence indicates that PC2 behaves as a TRP-type Ca\(^{2+}\)-permeable nonselective cation channel. The PC2 channel is implicated in the transient increase in cytosolic Ca\(^{2+}\) in renal epithelial cells and may be linked to the activation of subsequent signaling pathways. Recent studies also indicate that PC1 functionally interacts with PC2 such that the PC1-PC2 channel complex is an obligatory novel signaling pathway implicated in the transduction of environmental signals into cellular events. The present review purposely avoids issues of regulation of PC2 expression and trafficking and focuses instead on the evidence for the TRP-type cation channel function of PC2. How its role as a cation channel may unmask mechanisms that trigger Ca\(^{2+}\) transport and regulation is the focus of attention. PC2 channel function may be essential in renal cell function and kidney development. Nonrenal-targeted expression of PC2 and related proteins, including the cardiovascular system, also suggests previously unforeseeable roles in signal transduction.

THIS REVIEW PURPOSELY AVOIDS detailing regulatory aspects of polycystin-2 (PC2) trafficking and developmental expression and cellular location of the channel protein, including cell biological issues of autosomal dominant polycystic kidney disease (ADPKD), for which highly comprehensive reviews can be found (49, 84, 137). This review focuses instead on the description of PC2 as a channel, its structural and functional similarities with other related transient receptor potential (TRP) channels, to which family it is a recent addition, and potential roles of PC2 in Ca\(^{2+}\) signaling. Educated guesses can be drawn insofar as PC2 function, regulation, and/or interaction with other proteins are concerned, in particular new information on the molecular interaction with PC1 and expected regulation found in other TRP channels.

PKD1 AND PKD2 GENE PRODUCTS AND POLYCYSTIC KIDNEY DISEASE-RELATED GENES

Most cases of ADPKD (>80%) are accounted for by mutations in the PKD1 gene, encoding a large glycosylated, transmembrane protein, which has a core molecular mass of 460 kDa (8, 48) (Fig. 1). The extracellular domain of PC1 contains an assorted variety of protein motifs, which are potential targets to mostly unknown ligands (70). These include leucine-rich repeats flanked by cysteine-rich domains, a C-type lectin and WSC domains, and several so-called polycystic kidney disease (PKD) repeats with homology to immunoglobulins. The function of these motifs is yet to be clearly known. However, the specific extracellular domains of PC1 interact with extracellular matrix proteins such as fibronectin, and laminin (70), an interaction that raises mechanistic possibilities for a role of PC1 in cell-matrix interactions and cell proliferation. Invoking the potential ability of PC1 to act as a putative membrane receptor, interactions with highly relevant regulatory mechanisms, such as G protein activation, lipid metabolism regulation, and cytoskeletal interactions, are expected. Conversely, the cytoplasmic domain of PC1 may be a target for regulation by phosphorylation events (31, 92). A G protein-coupled receptor proteolytic site is also present in PC1, which is situated between the last PKD repeat and the first transmembrane (TM) domain of the protein (103) (Fig. 1). This region follows downstream of the receptor for the egg jelly (REJ) domain (103). Although of unknown function, the sea urchin receptor for egg jelly (suREJ), a PC1 homolog, has recently been implicated in the Ca\(^{2+}\) influx acrosomal reaction (72). Thus physiological cleavage of this domain, if confirmed, may represent a novel effector site for normal PC1 function (103). Although PC1’s role in cell function is still unknown, its massive NH\(_2\)-terminal extracellular tail suggests cell-to-cell and cell-matrix interactions (reviewed in Refs. 2 and 49) and the ability to sense environmental signals (85) (Fig. 1). The most intriguing part of PC1, however, is its putative 11 TM
domains. The TM region of PC1 may interact and convey specific protein conformations to PC2, with which it interacts and shares partial homology (77). The TM domain of PC1 may also have distinct cellular functions yet to be determined. Further evidence for potential functions, target location, and interacting proteins has arisen from the demonstration of PKD1 homologous genes in other organisms. The Caenorhabditis elegans Pkd1 gene ortholog (Lov-1) was originally described based on its ability to center on the location of vulva (LOV) behavioral pattern in this organism (3). Lov-1 is not only found in sensory neurons, but it was found to associate with the Pkd2 ortholog in C. elegans, from which a sensory role of this complex may be expected. Several PKD1 gene homologs have also been found, including PKDREJ (126), PKD1L1 (148), and, more recently, PKD1L2 and PKD1L3 in mouse and human genomes (61). The putative proteins encoded by these newly discovered genes range from 1,732 to 2,843 amino acids long, all containing putative large extracellular domains and topological features that suggest functional similarities to PC1 and suREJ. Expression of PKD1 homologs not associated with ADPKD may be a reflection of their widespread involvement in cell signaling events yet to be identified.

PC1 has been linked to a number of signaling events and regulatory effects in various cell functions (Fig. 1). PC1 was shown to regulate the cell cycle through the JAK-STAT signal transducing transcription pathway (6). Madin-Darby canine kidney (MDCK) cells stably expressing human PC1 have been assessed. Protein overexpression is still controversial as to its validity to assess normal cell function. Nonetheless, the evidence may provide a primer for further studies targeting direct regulatory pathways. See text for details and references.
decreased proliferation rate and growth arrest in G0/G1, an effect that may be correlated with the cell substratum surface onto which the cells grow (6). This phenomenon, which apparently requires the presence of PC2 (6), was associated with the PC1-induced inhibition of CD2-type kinases and activation of STAT transcription factors (6), thus regulating cell signaling mechanisms. The expression of the COOH-terminal end of PC1, for example, is also associated with the regulation of renal tubulogenesis, by stimulating TCF-dependent gene transcription and the Wnt signaling pathway in embryonic kidney cells (53). This may be partially associated with PC1 interactions with intermediate filaments (144) and activation of the PKC signaling pathway (86) (Fig. 5). Another potentially relevant signaling pathway associated with PC1 function entails G protein regulation (24). The COOH-terminal tail of PC1 activates Gs/Gi-type G proteins in brain lysates in vitro (93) (Fig. 1). PC1 activation of G proteins may be regulated by PC2 (24), although the molecular steps of this interaction will require further understanding. Overexpression of the PC1 COOH-terminal tail has also been linked to activation of endogenous cation channels in Xenopus oocytes (124) and purinergic receptor regulation of Ca2+ transport (1). Of particular relevance to an understanding of the onset and potential pathophysiological implications in ADPKD is demonstrated by the structural/functional interactions between PC1 and PC2 (123, 143). The coiled-coil domain of PC1 interacts with the COOH-terminal end of PC2 (Fig. 1) in a process that implies functional changes in the PC2 protein. This evidence may suggest, however, that when interacting, both proteins are required as a novel signaling pathway to work properly (85).

THE PC2 PROTEIN

In 1996, Mochizuki et al. (77) discovered the second locus responsible for ADPKD (Fig. 1). Positional cloning determined the presence of the PKD2 gene. This predicted a 968-amino acid peptide, PC2, for the gene product containing the paradigmatic six putative transmembrane (6TM) domains of TRP channels (Fig. 2), with homology with Na+ and Ca2+ voltage-gated channels (77). Homology between PC2 and other TRP channels centers in a 270-amino acid stretch from TM2 through TM6 and intervening loops of PC2 particularly similar to the voltage-activated α-subunit of Ca2+ channels. It is expected, but yet to be proven, that PC2 as do other TRP channels (66) functionally behaves as a homo- or heterotetramer (Fig. 2). The α-subunit of Ca2+-permeable cation channels of excitable tissues is topologically assembled as four domains each with a 6TM topology (16). The similarity also extended to the COOH-terminal end of PC2 (Fig. 2), including the EF-hand domain present in some TRP channels, and the α-subunit of the L-type Ca2+ channel (77). This is particularly relevant in light of the fact that the EF-hand provides a regulatory region of voltage-gated channel inactivation (107). The above homology led to the original hypothesis that PC2 may behave as a cation channel (77). PC2 has been identified in several renal and nonrenal tissues (12, 18, 28), including pancreas, liver, lung, bowel, brain, thymus, reproductive organs, and placenta (77, 91). In the kidney, PC2 was originally found in the plasma membrane of thick ascending limb of Henle and the distal convoluted tubule. Weaker labeling has been found in the proximal tubule and collecting ducts (71).
Conflicting results have been reported, however, for the presence of PC2 in the proximal tubule (28). PC2 has also been detected in vascular tissues and the mammalian heart, where it may play an important role in nonrenal manifestations of ADPKD. Contrary to PC1, whose expression seems highest during fetal development, expression of PC2 is similar in both fetal and adult tissues. This suggests that the two proteins may not always be associated with and/or functional in the same complex (18). Early evidence whereby PC2 location was assessed in cells heterologously expressing the protein (12) would indicate that PC2-overexpressing cells retain the protein in the endoplasmic reticulum (ER). While confirming PC2 targeting to the ER, a recent study showed that inner medullary collecting duct (IMCD) and 293T cells indeed express endogenous amounts of functional plasma membrane PC2 (69). However, LLC-PK1 cells have endogenous intracellular PC2 (69). This would be in agreement with a predicted ER-retention motif in the PC2 protein sequence (amino acids 787–820). This region also contains two putative phosphorylation sites with consensus motifs for PKA and PKC (77) (Fig. 2). However, the role of PC2 phosphorylation in the trafficking and regulation of the protein is still unknown. Truncated PC2 at, or before Arg742, thus missing most of the COOH tail, can more easily reach the plasma membrane (19, 58). The COOH-terminal tail of PC2 is also potentially relevant for putative interactions with cytoskeletal proteins that bind to the channel, including tropomin I (65) and tropomysin-1 (62).

Several PKD2 homologous genes have been identified to date (reviewed in Refs. 49 and 112). Polycystin-like (PCL), encoded by Pkdl2L (89), was the first member of the “polycystin” TRPP subfamily of TRP channels to be identified as a cation channel (20). Not itself a cause of ADPKD, PCL is highly expressed in fetal kidney and liver and is deleted in Krd mice expressing renal and eye defects (89). A functional role of PCL in these defects still requires experimental proof. PCL shares significant sequence homology (50% identity and 71% similarity) and similar topology and domain organization with PC2. Chen et al. (20) demonstrated that expression of PCL in Xenopus oocytes was associated with a novel large-conductance (137 pS) Ca2+-permeable nonselective cation channel. PKD2 homologs can now be considered a subfamily of transmembrane proteins with potential channel-like properties. Both PCL and PC2 have been found to display highly similar channel properties (20, 34). Other highly homologous PKD2 gene homologs have been identified, for which there is no apparent link between their expression and ADPKD (89, 126). However, it is possible that their homology to the PKD2 and PKDL genes reflects sensory functions based on their intrinsic channel properties, yet to be determined. Mutations in either the PKD1 or the PKD2 genes present rather similar clinical manifestations of ADPKD. Similar pathophysiological features are also manifested in animal models carrying mutations in either gene (10, 68, 141). Thus the current evidence suggests that PC1 and PC2 are both an integral part of an obligatory functional complex implicated in signaling events linked to one or more cell functions. Considering the systemic nature of the disease, potential roles for the PC1-PC2 partnership include the regulation of ion transport, activation of kinase cascades, and/or other regulatory cell functions. Recent evidence shows that PC2 and homologs act as Ca2+-permeable cation channels (20, 34, 125). Thus PC1-PC2 interactions may regulate Ca2+ transport and cell signaling (85) (Fig. 3), renal epithelial cell growth, and normal tubular morphogenesis (86). This suggests that mutations in either gene likely result in a common dysfunctional Ca2+ signaling mechanism, which controls various cell functions. Several of the functional features and potential physiological roles of PC2 arise from structural/functional homology with TRP channels.

**PC2 and the TRP Channel Superfamily**

The TRP paradigm was initially observed in spontaneous Drosophila mutants with a unique photoreceptor phenotype (76). The TRP mutant has a transient rather than a prolonged response during light stimulation. This basic defect implied the reduced activity of a Ca2+-permeable light-sensitive conductance, which is required for a sustained response (75). Interestingly, addition of micromolar concentrations of La3+ to wild-type, but not TRP mutant, photoreceptors mimicked the TRP phenotype (75, 118). The above findings gave rise to the original hypothesis that the TRP mutant implicates a novel Ca2+-permeable cation channel. The cloning of TRP and mammalian gene homologs (80, 97, 134, 150) has led to rapid progress in the identification and characterization of a number of TRP channel family members (7, 23, 75, 79). The TRP superfamily of cation channels includes >20 proteins with variable homologies (reviewed in Refs. 75 and 78). TRP channel activity may represent ubiquitous mechanisms for Ca2+ entry and subsequent contribution to the rise in intracellular Ca2+. TRP proteins are found in a variety of organs, including the brain, heart, kidney, testis, lung, liver, spleen, ovaries, intestine, prostate, placenta, uterus, and vascular tissues, and numerous cell types (reviewed in Ref. 75).

TRP proteins contain 6TM, S1–S6, with a characteristic pore region between transmembrane segments S5 and S6, which is typical of voltage-gated channels (7, 97). This sequence is the most conserved region in all members of the TRP superfamily. TRP proteins display homology with the extended family of voltage- and cyclic nucleotide-gated channels, in particular the transmembrane domains of the pore region (97). However, the voltage sensor present in the S4 domain of voltage-gated cation channels is missing in TRP channels (reviewed in Refs. 75 and 78). A 25-amino acid “TRP domain” of unknown function is also present in the COOH terminus of most, but not all, TRP channels. Variations in the either short or long cytoplasmic tails may provide further specificity. The NH2-terminal cytoplasmic domain of TRPC and TRPV channels contains several ankyrin repeats, whereas the TRPC and TRPM COOH termini contain proline-rich motifs. Interestingly, TRP channels also display distinct sequences for putative interactions with calmodulin (CaM), dystrophin, and the PDZ-scaffolding protein ENAD in their COOH-terminal ends (reviewed in Ref. 75). These sequences may be relevant in the regulation of channel function by such mechanisms as Ca2+-induced channel inactivation and cytoskeletal control of channel function. Evidence is mounting to suggest that TRP channels oligomerize with homolog or heterologous partners, enabling channel complexes with distinct functional features (7, 67, 122, 145). The suggestion that a tetramer of TRP subunits underlies the prototypical channel structure (7) may also be in reasonable agreement with the partial homology of TRP channels with voltage-gated cation channels of excitable tissues. The α-subunit of the
L-type Ca\(^{2+}\) channel indeed has four consecutive 6TM-containing structural domains (16).

Based on their functional properties, TRP proteins are nonselective cation channels with diverse cation permeability properties, including a high Ca\(^{2+}\) selectivity in some, but not all, TRP channels (75). The two light-sensitive *Drosophila* TRP channels were originally identified, based on their high \(P_{\text{Ca}}/P_{\text{Na}} > 100:1\) or low \(P_{\text{Ca}}/P_{\text{Na}} \sim 4:1\) Ca\(^{2+}\) permeability. The low-Ca\(^{2+}\)-selective TRP conductance is encoded at least in part by the TRP-like (TRPL) gene, \(~40\%\) identical to TRP (87, 97). TRP channels are linked to sensory stimuli, including cold and heat, osmotic challenges, and other receptor stimulatory responses (78, 130). The TRP family was initially classified (41) into short (TRPC), osm-9-related (TRPV, vanilloid), and long (melastatin-related, TRPM) channels, including the sensory channels involved in phototransduction, and cold and hot sensations (21). TRP channels are now classified in a newly encompassing and comprehensive nomenclature that has divided this family into at least six subgroups (78, 79) linked to activating and/or regulatory mechanisms. The TRPC (canonical) subfamily is the closest to *Drosophila* TRP. The other two groups include TRP channels associated with other sensory responses, including TRPV and TRPM receptors. Thus three original subfamilies of TRP channels were designated, namely, as short, long, and osm-9-like, which respond to a nomenclature based on the length, and thus potential regulation of their cytoplasmic tails (41). The osm-9-like gene encodes a TRP protein associated with osmotic responses in the *C. elegans* (113). The TRP superfamily has now been extended to 6TM proteins with weaker homology but potentially similar topological features and regulatory roles in cell function. New members of the TRP channel superfamily include the recently discovered epithelial Ca\(^{2+}\) channels CaT1 and ECaC, now renamed TRPV6 and TRPV5, respectively (45, 96, 149). CaT1 and ECaC are highly homologous (\(~75\%\) sequence identity) (95) and may represent a major contributing factor to the apical Ca\(^{2+}\) absorption step in transporting epithelia. CaT1 is preferentially expressed in intestinal tissues, whereas ECaC is expressed in the apical membrane of epithelial cells lining the rabbit, mouse, and rat distal nephron (44). Both CaT1 and ECaC channels are permeable to Ca\(^{2+}\) (45, 95). However, channel activity is inversely proportional to intracellular Ca\(^{2+}\) concentrations, and single-channel conductance in the presence of external physiological concentrations of the ion deems the conductance undetectable (95, 128). More recently, the Ca\(^{2+}\)-permeable nonselective cation channels PC2 and PCL (20, 77) have been incorporated in the TRPP subfamily of TRP proteins (79). Further information on the growing number and physiological properties of these channels is the basis of this review. The PC2 topologically similar protein mucolipin-1 (TRPML subfamily), which is genetically linked to mucolipidosis type IV, is also speculated to preserve the cation channel structure of PC2 (117). Mucolipin homologs have been recently implicated in sensory functions that also imply potential ion channel activity (25). At least one recent study reports that ML1 expression in *Xenopus* oocytes is associated with a novel

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**Fig. 3. Effect of PC1 COOH tail on wild-type PC2 channel activity.** *A*, in vitro translated PC2 was reconstituted in a lipid bilayer system, where single-channel activity was observed at the beginning of the experiment (top trace, left). Inactivation occurred either spontaneously (shown at the beginning of the trace) or after holding of the membrane to negative potentials (data not shown). Addition of PC1 COOH-terminal tail (P1CC) restored PC2 channel activity (top trace, right). Representative expanded traces are shown below for each condition, including spontaneous channel activity (*a*), spontaneous inactivation (*b*), and 2 subsequent channel reactivation levels after the addition of P1CC (*c* and *d*). Data are reproduced from Ref. 143 with permission. *B*: effect of various PC1 tail constructs and negative controls on wild-type PC2. Addition of the GST prepared as a control for the glutathione S-transferase (GST)-PC1 chimeras was without effect, as expected. Neither addition of the NH\(_{2}\)-terminal portion of the COOH tail of PC1 (P1CN) missing the coiled-coil domain nor the coiled-coil containing tail mutated in Q421SP had an effect. Addition of P1CC, the wild-type tail, reestablished wild-type PC2 channel activity. *C*: model of the regulation by the PC1 tail of PC2. Neither mutated PC1 nor truncated PC2 interacts with each other such that a functional channel complex is elicited.
Ca$^{2+}$-permeable cation conductance (60). TRP proteins, of which PC2 and homologs are newly joined members, are ubiquitous channel proteins likely involved in transient Ca$^{2+}$ influx and subsequent transduction signals. The conserved TM domain topology among members of the TRP superfamily of channels contrasts with the wide diversity of tissue and cell expression and the variety of signaling mechanisms in which they are involved. It is likely that TRP channels are regulated by a number of specific intracellular ligands, most associated with their cytoplasmic domains. These include putative PKC (TrpC, TrpV) and other kinases PKA [TrpP1-poly cystin-2, TrpV1–2, 5–6, CaT1, endothelial Ca$^{2+}$ channel (ECaC1)], and phosphatidylinositol 3-kinase (TrpC2–3, 5–7, TrpV1, 4, 6), inositol 1,4,5-trisphosphate (IP3) receptor (IP3R) and calmodulin interactions (all TrpC), and PDZ domains (Trp, TrpC4, 5), including INAD and NINAC myosin III (reviewed in Refs. 66 and 78). Other putative ligands for intracellular regulation include cytoskeletal proteins, such as dystrophin-like motifs (TrpC1), and interactions with troponin I and tropomyosin-I (PC2 or TrpP1). Other expected ligands for intracellular regulation may directly or indirectly involve trimeric and small G proteins, ATP, IP3, and diacylglycerol (DAG), and arachidonic acid (AA) ligands such as AA itself. AA byproducts of potential DAG kinases and lipases reactions include anandamides (TrpV1) and HPETE (TrpV), which are linked to regulation of the vanilloid receptors (reviewed in Ref. 4).

**PCL and PC2 as Cation Channels**

The gene product of *Pkd2L* (PCL) was the first PC2-like protein to be determined as a functional cation channel (20). PCL permeates a number of mono- and divalent cations, including Na$^+$, K$^+$, Ba$^{2+}$, and Cu$^{2+}$, respectively. PCL has a 4:1 divalent-monovalent permselectivity ratio, and the channel displays slight outward rectification in the presence of asymmetrical Na$^+$/K$^+$ (20). Channel activity by PCL is regulated by Ca$^{2+}$, whose channel activity can be substantially increased when either extracellular or intracellular Ca$^{2+}$ is raised (20). PCL-expressing oocytes preincubated in a Ca$^{2+}$-free solution evoke large transient (<30 s) currents after addition of external Ca$^{2+}$ (5 mM) (20). A second addition of Ca$^{2+}$, however, inhibits the currents. Thus external Ca$^{2+}$ induces both activation and subsequent inhibition of PCL. This suggests a Ca$^{2+}$-induced inactivation process somewhat reminiscent of that observed in voltage-gated cation channels (11, 152) and some members of the TRP family (88). This may be in contrast with TRP, TRPL, and TRPV6, which are blocked by varying concentrations of Mg$^{2+}$ (39, 129), but whose function has little dependence on extracellular Ca$^{2+}$. The Mg$^{2+}$ block in some TRP channels, however, is modulated by extracellular Ca$^{2+}$ (75). Recently, Li et al. (64) entertained the possibility that the putative Ca$^{2+}$ binding EF-hand region in the COOH-terminal tail of PCL may be implicated in its Ca$^{2+}$ regulation. Spliced versions of PCL from liver and testis, containing and lacking this region, respectively, showed the same extent of channel inactivation by external Ca$^{2+}$, indicating functional differences for Ca$^{2+}$ regulation between PCL and voltage-gated cation channels. The two PCL splice variants have different activating currents, however (64), suggesting that the role of this region will require further study. PC2, which contains the EF-hand domain, may also be regulated by Ca$^{2+}$ by a yet unknown mechanism.

Evidence for the cation channel properties of PC2 was obtained as an attempt to identify the molecular nature of the most abundant nonselective cation channel in the outer membrane of term human placenta, the syncytiotrophoblast (hST) (34). Interestingly, the placenta is one of the three most important organs responsible for Ca$^{2+}$ homeostasis, the other two being the kidney and intestine (95). The intrinsic properties of the PC2 cation channel activity were later confirmed by expression of human protein in Sf9 insect cells (34) and by reconstitution of the in vitro translated gene product (34). The spontaneously active cation-selective channel ascribed to PC2 functions in hST has a single 177-pS channel conductance in a K$^+$ gradient. A 134-pS nonrectifying single-channel conductance is observed in symmetrical K$^+$ (34). Channel activity shows at least four subconductance states of ~30 pS each (34). Placental PC2 function has a 1:1 Na$^+$/K$^+$ permselectivity with a slightly increased permeability to Ca$^{2+}$ (1:3:1) compared with monovalent cations. Similar results were found for reconstituted human PC2 (34). Interestingly, Ca$^{2+}$ permeation through the PC2 channel inhibited its own channel activity (34), suggesting a Ca$^{2+}$-induced Ca$^{2+}$ inactivation effect. This inhibition takes two forms, including fast flickering of the large-conductance state and a slow decrease in subconductance states of the channel (34). Inhibition by Ca$^{2+}$ has only been observed by exposure of the channel to high extracellular Ca$^{2+}$. Thus it is unlikely that physiological extracellular Ca$^{2+}$ may resemble the inhibition observed in other Ca$^{2+}$-permeable TRP channels such as CaT1 (TrpV6) and ECaC (TrpV5) (46, 88). Whether the EF-hand present in the COOH terminus of the protein, PC2 regulatory proteins, or the pore conductance itself plays a role in this inhibition is yet to be determined. Reported data by Vassilev et al. (125) showed different permselectivity ratios and rectification patterns in *Xenopus*-expressed PC2. Nevertheless, a large single-channel conductance, multiple substates, Cu$^{2+}$ regulation, and a slight divalent cation preference all seem to be intrinsic properties of the PC2 cation channel. While PC2 cation channel activity is not intrinsically outwardly rectifying, its open probability displays evident voltage dependence (33). This may be an intrinsically relevant mechanism of PC2 channel regulation. Voltage dependence of PC2’s open probability has been confirmed in the ER-localized PC2 (58) and more recently in IMCD and MDCK cells expressing the endogenous protein (69). Like other nonselective cation channels and in particular some TRP family members, PC2 channel function is inhibited by the nonselective cation channel blockers La$^{3+}$, Gd$^{3+}$, and the diuretic amiloride, also a known inhibitor of epithelial Na$^+$-permeable channels (34).

Channel activity by PC2 has been determined in various cells and expression systems. A nonselective cation channel was recently observed in the plasma membrane of IMCD and MDCK renal epithelial cells, which was consistent with the presence of endogenous PC2 (69). This was actually suggested by immunocytochemical and Western blot analyses, where plasma membrane PC2 was found in cells expressing endogenous, but not the overexpressed protein (69). The 170-pS single-channel conductance found in K$^+$ is similar to that previously observed. However, the high (7:1) K$^+$/Na$^+$ permselectivity ratio differs from reported data in Sf9 cells and the endogenous hST reconstituted channel (34). Channel activity...
was increased in epithelial cells overexpressing PC2, suggesting that both endogenous and heterologously expressed PC2 behave similarly (69). Confirmation that this channel activity is indeed mediated by PC2 alone is still lacking. Nevertheless, Vassilev et al. (125) had previously reported that PKD2-cRNA injection in Xenopus oocytes is associated with the expression of a highly similar cation-selective, Ca\(^{2+}\)-permeable ion channel. In another study, Hanaoka et al. (37) showed that heterologous coexpression of PC2 and PC1 in Chinese hamster ovary (CHO) cells, but neither one alone produced a novel, Ca\(^{2+}\)-permeable cation conductance not previously observed in untransfected cells. In that report, the cation conductance induced by PC1-PC2 coexpression was prevented by removing the COOH-terminal end of either protein. However, this is at odds with the fact that the truncated PC2 should traffic more freely and reach the plasma membrane (19). Interestingly, the coexpressed PC1-PC2 whole cell data in CHO cells showed outward rectification and a sixfold higher permeability to Ca\(^{2+}\) compared with monovalent cations (37). The available information albeit with minor discrepancies is overall consistent with PC2’s being a nonselective cation channel with weak preferential cation permeability for Ca\(^{2+}\), over Na\(^{+}\) and K\(^{+}\) (compared with canonical TRP channels). PC2 likely represents a relevant molecular pathway for Ca\(^{2+}\) entry into epithelial tissues.

The discrepancies among the various reports may fall within different explanations, ranging from simple technical differences to specific cell models and expression systems. However, more complicated scenarios are also plausible, including heterooligomerization of endogenous proteins and PC2-like channels, which display distinct molecular and functional properties. TRP channels can change their phenotypic characteristics depending on whether each protein oligomerizes with itself or other TRP channel isoforms. Xu et al. (145) showed that coexpression of TRP and TRPL in 293T cells led to outwardly rectifying currents distinct from expression of either protein alone. TRPC1 interacts with TRPC5 to enable novel cation channel activity in the brain (115). Most interestingly, however, PC2 has been reported to interact with TRPC1 but not TRPC3 (122). This interaction, which awaits functional proof, was expected by the interacting COOH-terminal ends of either protein. However, TRPC1-PC2 structural interaction was found to be at least partially mediated by their respective S2-S4 interacting TM domains in both proteins. Thus relevant TRP-PC2 topological interactions may render channel complexes of functional properties different from those observed with PC2 alone (i.e., purified reconstituted protein) or by such complexes as the PC1-PC2 partnership (85).

Cells overexpressing PC2 have been found to accumulate the protein in intracellular compartments, in particular the ER (12, 28, 58, 69). In a recent paper, Koulen et al. (58) assessed the role of wild-type and mutated PC2 in intracellular compartments. LLC-PK\(_1\) renal epithelial cells were transfected with wild-type and mutated PC2, including the COOH-terminal truncated L703X-PC2 and a missense mutation of PC2 (D511V-PC2). The protein was almost exclusively found in pre-medial Golgi and the ER, by cell gradient analysis. While full-length and D511V PC2 were confined to pre-medial Golgi membranes, the L703X PC2 partially translocated to the plasma membrane as expected from the loss of the ER retention signal (77). Reconstituted ER membranes from LLC-PK\(_1\) cells overexpressing wild-type PC2 but not naïve membranes displayed inward Ba\(^{2+}\) currents in a Ba\(^{2+}\) chemical gradient (58). This was tantamount to the presence of a functional PC2 channel in the ER. The ER-located PC2 channel had a slope conductance of 114 pS in Ba\(^{2+}\) and slightly smaller conductances in Ca\(^{2+}\) and Mg\(^{2+}\), respectively. The truncated L703X PC2, however, had a reduced single-channel conductance of 28 pS in Ba\(^{2+}\). This is in contrast to data showing that reconstituted L742X PC2 has similar functional properties compared with wild-type PC2 (143). Higher negative potentials increased reconstituted PC2 channel activity, suggesting a voltage-dependent channel activity. This was considered a further suggestion of a functional PC2, as IP\(_3\) receptors present in the ER are not voltage dependent (27, 133). The possibility that the currents of the PC2-overexpressing cells were mediated by either IP\(_3\)R, which activates IP\(_3\)R, or ryanodine (RyR) receptors was further excluded by the absence of IP\(_3\) and addition of the RyR inhibitor ruthenium red, respectively. The L703X mutant PC2 required larger negative membrane potentials for channel activation compared with the wild-type protein. Furthermore, the open probability of wild-type but not L703X PC2 channel increased over a physiological range of Ca\(^{2+}\) concentrations, whereas higher cytoplasmic Ca\(^{2+}\) lowered PC2 channel activity. However, negative potentials that fully activated wild-type PC2 channels had no effect on the L703X PC2. The differences between L703X PC2 and other rather similar truncations (L742X) and wild-type PC2 may suggest a relevant role of this missing domain of PC2 in channel activation. No currents were observed in ER vesicles containing the D511V PC2, suggesting a loss of function in this mutation. Although this evidence does not necessarily make a case for a functional role of intracellular PC2 under physiological conditions, there is no compelling evidence why PC2 may not participate as a Ca\(^{2+}\) transport mechanism in more than one cellular location.

### PC2 Channel Regulation

Regulation of PC2 channel function is essential to our understanding not only of the potential mechanisms of interaction with PC1 and the onset of ADPKD but also its physiological response and functional role in cell function. Two aspects of PC2 channel regulation are herein summarized, including those ascribed to the changes in the intrinsic properties of the channel itself and those potentially linked to putative interactions with associated proteins. This evidence is only emerging from recent studies, and further experimentation will be required to unequivocally ascribe particular roles to the various potential partners of the PC2 channel. It is evident that the COOH-terminal end of PC2, which is rich in putative interacting domains, may play an important role in regulating its channel function. Regulatory target sites include the domain that interacts with the coiled-coil region in the cytoplasmic tail of PC1 (37, 143). Other regulatory sites may be relevant in either trafficking and/or direct interactions with the membrane-associated protein. These are the putative ER-retention site, an EF-hand and calmodulin-binding domain, and putative phosphorylation sites in the COOH-terminal tail of PC2 (77).

#### Effect of pH on PC2 Channel Function

Changes in pH, a byproduct of cell metabolic activity, is known to affect several cellular functions, including ion trans-
port, enzymatic activity, and gene expression. Early studies in excitable tissues indicated that pH changes modify voltage-gated Na\(^+\) channel function. Neutral Na\(^+\) currents are inhibited by a rise in H\(^+\) ions (140). Similar findings were observed for L-type Ca\(^{2+}\) channels. Hess and collaborators (43, 80) originally determined that the L-type Ca\(^{2+}\) channel, which shares homology with PC2 (77), is also sensitive to changes in pH. The voltage dependence and H\(^+\) accessibility suggested that the protonation site of L-type Ca\(^{2+}\) channels is external to the conductance pore (43). Changes in pH have been found to regulate a number of PC2-related TRP channels. The PC2 homolog PCL, for example, is blocked by a reduction in cytoplasmic pH (20), suggesting homologous topologies between PC2 and PCL. However, pH regulation of TRP channel members of the sensory-related TRPV subfamily, such as TrPV1, seems to be stimulatory (15). Thus external acidic pH increases the TrPV1 currents otherwise stimulated by capsaicin and similar ligands. This regulation may take place from the extracellular domain of the protein; however, the channel is also modulated by intracellular PtdIns(4, 5)P_2 (15). Recently, we observed that PC2 channel function is voltage dependent and highly sensitive to changes in pH (33). The protonation site in PC2 with a pK_a of ~6.4 regulates channel activity by controlling the voltage dependence of the single-channel kinetics (33). A reduction in intracellular pH decreased the channel’s open probability but not the single-channel conductance. The data placed the protonation site within the conductance pore of PC2. This was confirmed by the pH effect on the in vitro translated material (33). Furthermore, the R742X PC2, missing most of its cytoplasmic domain and potential interactions with regulatory proteins, preserves the inhibition by changes in intracellular pH (19). The internal pH-sensitive site in PC2 may be a relevant feature of members of the “polycystin” TRPP subfamily of TRP channels and may play an important role in metabolic acidosis. pH regulation has also been determined in the TRP-type CaT1 and ECaC channels, both epithelial Ca\(^{2+}\) channels in renal and intestinal epithelia. However, ECaC is inhibited by extracellular pH (127), a property that seems to be shared by CaT1 (95). Future experiments will be required to assess whether the regulation by pH of the various members of the TRP family of channels is a common functional mechanism ascribed to topologically similar domains of the channel proteins.

**Effect of Ca\(^{2+}\) on PC2 Channel Function**

The evidence indicates that PC2 is a nonselective cation channel with a weak preferential permeability to divalent cations, including Ca\(^{2+}\). This brings forth the current hypothesis that PC2 functionally behaves as a Ca\(^{2+}\)-permeable channel. Other members of the TRP superfamily are indeed Ca\(^{2+}\)-channels, and the original TRP phenotype was described based on a light-induced Ca\(^{2+}\) signaling event (40). CaT1 and ECaC are TRP-related channels thought to play an important functional role in Ca\(^{2+}\) permeation, despite the fact that their intrinsic divalent currents are often undetectable. Thus Ca\(^{2+}\) itself may be a major contributor to the transient Ca\(^{2+}\) signal as its rise in the intracellular compartment often constitutes a negative feedback mechanism in the regulation of Ca\(^{2+}\) influx. Brehm and Eckert (11) originally observed that while voltage-activated L-type-mediated Ca\(^{2+}\) currents normally inactivate, Ba\(^{2+}\) currents through the same channels did not. Thus it was originally suggested that Ca\(^{2+}\) itself is required for L-type Ca\(^{2+}\) channel inactivation. The Ca\(^{2+}\)-dependent inactivation is present in a number of channels. The nature of the inhibition would be such that the Ca\(^{2+}\) delivered in the cytoplasmic vicinity of the channel is the effector of a self-inhibitory cascade, including potential regulatory sites in the channels themselves or in their surroundings. Ca\(^{2+}\)-induced L-type Ca\(^{2+}\) channel inactivation may be elicited by a COOH-terminal region encompassing at least three distinct domains. This region in L-type Ca\(^{2+}\) channels contains the putative Ca\(^{2+}\)-binding EF-hand motif, two hydrophilic residues (asparagine and glutamic acid) downstream of the EF-hand motif, and a putative IQ CaM binding motif (16, 107, 152). The EF-hand and IQ CaM putative Ca\(^{2+}\) binding motifs have been observed in a number of channels. These include voltage-gated Ca\(^{2+}\) channels (152), cyclic nucleotide gated (CNG) channels (120), TRP family members (97, 111), and NMDA (26) and RyRs (105). The putative Ca\(^{2+}\) binding motifs are usually present in the COOH-terminal end of the channel proteins, where they are most likely implicated in Ca\(^{2+}\) binding and/or the selective Ca\(^{2+}\)-CaM regulation (reviewed in Ref. 107). CaM binding and regulation have been demonstrated in the TRPC1 (111) and TRPL channels (110). Both PC2 (77) and PCL (64) also contain putative Ca\(^{2+}\) binding and CaM-interacting domains, with the expectation that this domain may play an important functional role in their regulation.

Regulation of TRPC1 by Ca\(^{2+}\) and CaM interactions may also bear relevance in potential regulatory mechanisms of PC2 function, as PC2 binds and interacts with various domains of TRPC1 in a TRP homolog-specific manner (107). Extracellular Ca\(^{2+}\)-induced regulation of polycystin channels may thus be an intrinsic mechanism of channel function. Little is known about Ca\(^{2+}\) binding to PC2. However, Ca\(^{2+}\) seems to regulate and inhibit its channel function (34, 125). A recent study by Li et al. (64), however, assessed the role of the EF-hand in the PC2 homolog PCL, where the presence of Ca\(^{2+}\) induced both channel activation and inactivation. PCL splice variants from liver [(PCL-LV) lacking the EF-hand] and testis [(PCL-TS) lacking only 45 amino acids of the COOH tail] were used in that study. Both PCL splice variants exhibited spontaneous cation channel activity and Ca\(^{2+}\)-induced channel activation. However, PCL-LV exhibited threefold increased activation compared with PCL-TS. Ca\(^{2+}\) transport through the PC2-related ECaC and CaT1 (ECaC2) was also shown to present an inhibition of channel activity, consistent with a self-induced inhibition. However, in these two Ca\(^{2+}\)-permeable channels, with different inhibitory kinetics, the domains implicated in the Ca\(^{2+}\)-induced inhibition seem to reside outside of both the NH\(_2\)- and COOH-terminal tails of the proteins (88). The fact that Ca\(^{2+}\) transport through PC2 modifies the residence time for the various subconductance states of the channel (34) may provide a hint as to the presence of a novel Ca\(^{2+}\)-induced inactivating mechanism. At least one recent report indicates that the truncated L703X PC2 has a much higher threshold for voltage activation than the wild-type protein (58). This further suggests a role of the COOH-terminal end of PC2 in the regulation of channel function.
Interactions Between PC1 and PC2

Aside from potential regulatory mechanisms of PC2 channel function that relate to its intrinsic electrophysiological properties, including voltage dependence and charge carrier perm-selectivity properties, little is yet known about how PC2 channel function is regulated. As for other TRP-related channels, however, it is expected that potentially interacting proteins, in particular those that may have an affinity for the COOH-terminal end of the protein, may play a relevant role in channel regulation. In the case of PC2, a potential interaction between both PKD gene products was suspected from the similar clinical manifestations of the disease, regardless of which gene was affected. A structural interaction between PC1 and PC2 was originally described by the cytoplasmic binding of their respective COOH-terminal tails (123). This, in turn, raised the early hypothesis that a PC1-PC2 functional partnership is a potentially relevant physiological regulatory mechanism for what we now know is the affected PC2 channel function. Hanaoka et al. (37) originally observed that in transfected CHO cells overexpressing both proteins, only PC1-PC2 coexpression renders a functional channel complex. Elimination of the cytoplasmic domain of either protein was sufficient to obliterate the PC1-PC2-induced whole cell conductance in CHO cells (37). Truncated R742X PC2, missing the ER retention site, showed no differences from untransfected CHO cells (37). This contrasts with at least one report showing that R742X PC2 reaches the plasma membrane with better ease than its wild-type counterpart (19). Actually, R742X PC2 channel function is remarkably similar to the wild-type channel (19, 143). Thus no auxiliary proteins seem to be required for PC2 to enable channel activity (34). It is likely, however, that while interacting with PC2, PC1 plays a seemingly important regulatory role in its channel function (37, 85).

Colocalization and a structural interaction between PC2 and PC1 would suggest (123) that modulation of PC2 channel activity by PC1 requires the COOH terminus of PC1 and its binding counterpart, the cytoplasmic tail of PC2 (143). To substantiate the importance of PC1-PC2 functional interactions on the channel activity of PC2, Xu et al. (143) reconstituted wild-type PC2 in a bilayer system (Fig. 3). The isolated PC2 channel inactivated both spontaneously and by hyperpolarizing holding potentials. Addition of the coiled-coil-containing domain of the PC1 cytoplasmic tail (P1CC; Fig. 3) reversed the inhibition of wild-type PC2 and increased the activity of already functional channels. The addition of a PC1 tail containing the Q4215P substitution, a mutation expected to disrupt the coiled-coil domain and cause the disease when present in the human gene, however, was unable to elicit a similar activation (143). Thus the COOH tail of PC1 is an agonist that helps stabilize PC2 channel function. Conversely, truncated R742X PC2, itself an active channel, but missing most of the COOH-terminal end of the channel, failed to respond to the normal P1CC tail after channel inactivation (143). Thus modulation of PC2 channel activity by PC1 may be an important component of biological functions ascribed to this molecular complex. This is also potentially relevant as PC1, whose function as a whole protein is yet unknown, may be involved in more than one regulatory function. As a signaling mechanism, physical interactions of the cytoplasmic domain of PC1 with proteins other than PC2 may provide a more flexible regulatory role depending on the effector channels or signaling proteins. Expression of the cytoplasmic tail of PC1 in Xenopus oocytes, for example, was found to activate an endogenous, nonselective cation channel in this cell model (124), representing a structure likely distinct from PC2. This suggests PC1 regulation of more than one target channel protein. Aguiari et al. (1) recently reported that expression of the PC1 tail potentiates purinergic receptor-induced intracellular Ca2+ release in human renal HEK-293 cells. The PC1 cytoplasmic tail has also been found to stabilize endogenous β-catenin and stimulate TCF-dependent gene transcription in HEK-293 cells (53). These disparate findings point to a flexible regulatory role of the cytoplasmic domain of PC1 in diverse signaling pathways, including those effected by changes in channel function.

Polycystins and the Ciliary Connection

Evidence for a physiological interaction between PC1 and the PC2 channel is more clearly established in at least one recent report providing interesting new understanding of the role PC1 plays in transducing environmental signals to PC2. Nauli et al. (85) recently found that wild-type PC1 colocalizes with PC2 and ciliary proteins of the microtubular machinery in the stem and basal body of the primary cilium of mouse embryonic renal epithelial cells. The nonmotile primary cilium of renal epithelial cells, until recently only thought to be a vestigial appendage, has drawn renewed attention because of its potential role in sensory signaling (98, 99, 109). Praetorius and Spring (98) determined that flow-induced stress in MDCK cells is associated with cilium bending and a rise in intracellular Ca2+ elicited by both a transient Ca2+ influx and the subsequent Ca2+ release from intracellular stores. Chemical excision of the primary cilium eliminated this signal (99). Similarly, in highly differentiated embryonic mouse renal epithelial cells with a well-developed primary cilium, Nauli et al. (85) observed that shear flow induced a PC2-mediated Ca2+ influx. In this study, PC1 was localized in the primary cilium of cultured embryonic collecting duct renal epithelial cells from wild-type mice and mice homozygous for the PKD1 exon 34-targeted deletion (Pkd1del34del34). This mutation causes the expression of truncated PC1, which leads to the formation of renal cysts at embryonic days 14–15 (68). PC1 colocalized with PC2 in primary cilia from wild-type but not Pkd1del34del34 renal epithelial cells. PC1-deficient cells did not respond to shear stress with a rapid rise in intracellular Ca2+ as did the wild-type cells (85) (Fig. 4). This change in intracellular Ca2+ was elicited by the PC2-mediated and extracellular Ca2+-dependent Ca2+ influx and the activation of the store-operated intracellular Ca2+ release (Fig. 5). The evidence suggests a functional link between a normal and not a dysfunctional PC1-PC2 channel complex in the transduction of environmental signals. Nevertheless, other compensatory mechanisms such as receptor-induced Ca2+ increase may remain largely intact in the absence of a functional PC1. Inhibition of the PC2-mediated Ca2+ rise in wild-type cells to flow stimulation (85) by added antibodies against either PC1 or PC2 confirmed the requirement of both proteins in the mechanotransduction response of renal epithelial cells. This finding further suggests that functional blockade of either protein obliterated the entire signaling event. It is thus likely that PC1-PC2 interaction is obligatory in that only a normal channel complex between the
two proteins enables a functional response. The response to thrombin was actually higher in the Pkd1 del34/del34 cells compared with those expressing wild-type PC1. Although unknown at present, it is still possible that PC1-independent activation of PC2 may be elicited by receptor-mediated events. Conversely, TRP-related channel events expected from Ca2+-permeable channels such as CaT1 (TrpV6) may also play an either compensatory and/or complementary role in Ca2+ influx steps in renal epithelial cells.

Connections between ciliary structures and PKD originally arose from mouse models of autosomal recessive polycystic kidney disease (ARPKD), which are now genetically linked to deficiencies in ciliary proteins and not ADPKD-related genes. The Tg737 gene affected in the orpk Pkd mouse encodes a novel protein, polaris, which localizes to the cilary basal body and axoneme (147). Orpk mice show shortened cilia and left-right symmetry defects (83) in addition to PKD. Orpk mice also show increased ciliary PC2 (94). Homozygous inv mice lacking a functional inversin protein exhibit situs inversus and severe renal and pancreatic cystic disease (82). Inversin has recently been located to primary cilia of renal epithelia (81). Although inversin function is unknown, cytoskeletal interactions and Ca2+ regulation by CaM binding may be expected to regulate this protein (82, 90). The congenital polycystic kidney (cpk) mouse model also provided early clues to a sensory role of ciliary function in PKD. The cpk gene encodes a novel protein, cystin (47), which is expressed and colocalizes with polaris in cilia in renal epithelial cells. Yoder et al. (146) recently reported the colocalization of PC1 and PC2 with cystin, polaris, and ciliary tubulin in cilia of renal epithelial cells. Thus the encompassed evidence suggests novel mechanotransduction signaling mechanisms linking the sensory function mediated by cilia bending with the activation of the PC1-PC2 channel complex (Fig. 4). How this channel complex senses environmental responses and/or couples to ciliary proteins is yet unknown. However, evidence is mounting for all these proteins to comprise novel signaling pathways where all components are required for a normal response. This hypothesis is evidenced by novel TRP channel roles in sensory function in organisms such as C. elegans, where various TRP homologs have been implicated in touch and other sensory functions (3). Worms lacking a truncated PC1 (lov-1), PC2 (Pkd2), and the polaris homolog osm-5 (42) were recently located in ciliated sensory neurons of this organism (3). Worms expressing mutations in any of the genes exhibit similar sensory defects, suggesting a common deficient cilia-mediated sensory pathway (42). As indicated above, PKD-unrelated mutations induce cystic renal disease. It is thus tempting to postulate that novel signaling pathways, involving microtubular and other cytoskeletal proteins, and the channels reviewed in this report may all play a role in transducing environmental information into cellular events. Trp and TrpL, for example,
are distributed along the ciliary appendages of the photoreceptors in adult *Drosophila* (23). This phenomenon, which resembles the one observed with the PC1-PC2 channel complex in renal epithelial cells, raises the interesting question as to their functional connection with the Ca\(^{2+}\)/H\(_{1001}\) storage compartments, with which they are associated (13).

**Cytoskeletal Connection to PC2**

Interestingly, signals associated with shear stress, including cell stretch and anisooosmotic cell volume changes, cell-matrix adhesion, and motility are all linked to, and/or elicited by, changes in cytoskeletal structures. It is thus tempting to postulate cytoskeletal interactions with PC2 (and likely PC1) as potential regulatory pathways of its channel function. This hypothesis requires experimental evidence. Nevertheless, as expected from its putative interaction with the extracellular matrix, potential role in cell-cell interactions, and localization in focal adhesion cell domains, PC1 has been found to interact with several focal adhesion proteins, including pp125FAK, pp60src, p130Cas, and paxillin (31). Focal adhesion complexes are also linked to regulatory actin-binding proteins, including vinculin, talin, and \(\alpha\)-actinin, as well as the cell adherens junction proteins E-cadherin and \(\beta\)- and \(\gamma\)-catenins, which coprecipitate with PC1 (31). Functional correlates for these interactions in the context of PC1-PC2 channel complexes are still missing. However, Geng et al. (31) have shown that PC1-containing multiprotein complexes in cell-cell and cell-matrix regions (see Ref. 137 for details) may still offer another means of regulation based on interactions with cytoskeletal proteins, which may bind to TRP channels, and/or PC2 itself.

Fig. 5. Hypothetical model of Ca\(^{2+}\) entry steps associated with expression of TRP channels and interacting proteins. In this hypothetical cell, combining evidence from epithelial and nonepithelial cells, Ca\(^{2+}\) influx is largely mediated by members of the TRP channel superfamily. TRP channels may coassemble such that the expected channel properties and regulatory pathways regulating their function can be numerous and serve a variety of cell functions. The PC1-PC2 channel elicits an environmentally triggered Ca\(^{2+}\) entry, which, in turn, activates and/or is potentiated by intracellular Ca\(^{2+}\) stores. PC1 can be coupled to G protein receptors by exchanging and/or activating various G proteins, which, in turn, may be regulated by PC2 in certain cells. Other TRP channels may be associated with Ca\(^{2+}\) influx steps in epithelial cells, in particular CaT1 (TrpV6). TRP channels are also regulated by the G protein-induced activation of the PLC pathway and the production of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP\(_3\)). This latter second messenger activates IP\(_3\) receptor (IP\(_3\)R) from endoplasmic reticulum (ER)/sarcoplasmic reticulum (SR) compartments. Other intracellular channels include the Ca\(^{2+}\)-dependent ryonadine receptor (RyR) and PC2 itself. These channels can also regulate, by apposition, plasma membrane TRP channels. This is complementary or in parallel with their expected role in the release of Ca\(^{2+}\) from intracellular stores. Specific TRP channels may have regulatory/activating pathways not shared by other members of the same family. TrpV1 (VR-1), the capsaicin-sensitive vanilloid receptor, is likely regulated by arachidonic acid (AA) and its metabolites such as HPEPE. This may require coupling to receptor activation by DAG lipases and other DAG-related enzymes. The presence of PC1 multiprotein complexes in cell-cell and cell-matrix regions (see Ref. 137 for details) may still offer another means of regulation based on interactions with cytoskeletal proteins, which may bind to TRP channels, and/or PC2 itself.
found that the cytoskeletal proteins troponin I (65) and tropomyosin-I (62) bind directly to PC2, further strengthening a link between cytoskeletal dynamics and the PC2 channel. Recent evidence by the same group provided the first experimental proof for troponin I regulation of PCL channel function (63). Thus a close association between ion channels such as PC2 and environmental signals may be linked through cytoskeletal structures as has been established for other channel proteins (reviewed in Refs. 14 and 52). Interactions between PC2 and homologs with cytoskeletal proteins such as troponin I and tropomyosin-I further stress the relevant role of the actin cytoskeleton in the control of PC2 channel function. This is especially relevant in the regulation of PC2 in extrarenal locations such as the cardiovascular system, also a target of ADPKD.

**PC2 in the Cardiovascular System**

The true systemic nature of ADPKD is perhaps manifested by ever-growing evidence for the importance of PKD1 and PKD2 mutations in extrarenal abnormalities. Extrarenal ADPKD is most commonly associated with premature death and disability by the presence of vascular phenotypes most often associated with ruptured intracranial aneurysms (17, 108), resulting in subarachnoid hemorrhage (108). ADPKD accounts for 9% of familial intracranial aneurysms (106). Common vascular manifestations of ADPKD include saccular intracranial aneurysms, dolichoectasias, aortic root dilatation, dissection of the thoracic aorta and cervicocephalic arteries, and coronary artery aneurysms (29, 108). Both PKD1 and PKD2 mutations have been associated with cranial aneurysms. Moreover, it has been shown that PKD2, PKDL, and PKD2L2 are expressed in the heart (2, 36, 89). Therefore, putative channel function by either one of these proteins may play a yet unrecognized role in the cardiovascular system. This is supported by the expression of polycystins in vascular smooth muscle cells (104) and cardiac myocytes (132). Vascular leakage and multiple focal hemorrhages in mouse embryos homozygous for Pkd1 or Pkd2 null mutations further support a direct role for the polycystins in ADPKD-associated vascular disease (54, 142). A reduced life expectancy has recently been reported in heterozygous Pkd2+/− mice (104). This is accompanied by an early onset hypertension even in the absence of cystic renal disease. Thus haploinsufficiency may play a role in some extrarenal manifestations of ADPKD (104). Qian et al. (104) recently found decreased PC2 expression in Pkd2+/− vascular smooth muscle cells and enhanced intracranial vascular abnormalities in Pkd2+/− mice induced to develop hypertension (104). Freshly dissociated Pkd2+/− vascular smooth muscle cells were found to have lower basal intracellular Ca2+ compared with wild-type cells. Furthermore, caffeine plus thapsigargin-induced Ca2+ release from intracellular stores was also decreased in these cells. Interestingly, this was also associated with decreased store-operated Ca2+ channel activity. Thus inactivation of just one Pkd2 allele is sufficient to significantly reduce PC2 expression and alter intracellular Ca2+ homeostasis in vascular smooth muscle cells. Based on these findings, the authors proposed that the abnormal intracellular Ca2+ regulation linked to Pkd2 haploinsufficiency is directly related to the vascular phenotype. Another report, however, points to the possibility that cardiac expression of Pkd2 homolog genes may have a functional role in cardiac function. Rats left ventricular myocytes display nonselective cation channel activity with characteristics similar to those observed for PC2 and its homologs (132), suggesting that PC2 and/or homologs may underlie this channel function in ventricular myocytes. Addition of extracellular caffeine, by increasing intracellular Ca2+, led to cation channel activation in these cells. Single-channel characterization of these currents showed channel currents with a conductance of ~300 pS, roughly twice as large as that of PC2, and similar permeability for monovalent cations, including Na+, K+, Li+, and Cs+ (132). The channel was also permeable to divalent cations, including Ca2+ and Ba2+. Pharmacological features included inhibition byamiloride with an affinity similar to that reported for PC2, and trivalent cations Gd3+ and La3+, also in a fashion similar to those observed for PC2 in human placenta (34). Single-cell RT-PCR analysis of individual rat left ventricular myocytes revealed the expression of Pkd2 and Pkd2L2 genes but neither PkdL nor Pkd1 (132). Thus a PC2-like channel is present in cardiac ventricular myocytes, whose function is independent of PC1 regulation. Whether this channel phenotype is the functional fingerprint of PC2 alone, and/or in association with the PKD2L2 (or other) gene product, will require future experimentation. A functional PC2 channel may have widespread implications in Ca2+ signaling events.

**Ca2+ Transport and Signal Transduction**

Ca2+ is a ubiquitous intracellular second messenger that controls numerous physiological events. Ca2+ signals are initiated by a sudden, sizable rise in cytoplasmic Ca2+, which is essential for a variety of cellular responses including sensory transduction, cell activation, degranulation and ion secretion, regulation of cell contraction, cell proliferation, and apoptosis. The increase in intracellular Ca2+ can be achieved by one or more consecutive events. These signals include channel-mediated Ca2+ influx and/or receptor-induced Ca2+ release from intracellular stores. This may or may not activate the so-called “capacitative” membrane channel response (reviewed in Refs. 7, 100, 101, and 151). One of the most prevalent pathways implicated in the Ca2+ rise in nonexcitable cells is the triggering of PLC activation and the subsequent production of the ligands DAG and IP3 (7, 38, 74, 75, 100, 101, 138) (Fig. 5). These metabolites target intracellular Ca2+ stores, which elicit the release of Ca2+ in most cells. The release of Ca2+ from intracellular stores is dominated by two different types of Ca2+-permeable channels, RyR, that is itself activated by cytosolic Ca2+, and IP3, which, in turn, is activated by IP3. These channels can be either colococalized in the same ER/sarcoplasmic reticulum (SR) compartments or be in independent pools, providing separate venues for the release of stored Ca2+ to the cytoplasmic compartment. How intracellular Ca2+ release triggers and controls plasma membrane Ca2+-permeable channel activity is still a matter of debate and is beyond the scope of the present review. Nevertheless, the intracellular store-activated response that enables the capacitative Ca2+ influx at the plasma membrane is now being considered as mediated by plasma membrane TRP channels (7, 38, 74, 100, 101).

Stereotypical examples of Ca2+ influx–induced cell activation are the cardiac and smooth muscle cell contractile re-
sponses, where the stimulating Ca\textsuperscript{2+} signal is the combined effort between L-type Ca\textsuperscript{2+} channel activation and the release of intracellular Ca\textsuperscript{2+} from the SR. In cardiomyocytes, for example, the Ca\textsuperscript{2+} entry step is potentiated by an amplifying signal mediated by the Ca\textsuperscript{2+} release from intracellular Ca\textsuperscript{2+} stores (9). In vascular smooth muscle cells, in contrast, the Ca\textsuperscript{2+} entry step is followed by little amplification from intracellular stores (50), which is explained by insufficient recruitment of Ca\textsuperscript{2+} release store sites in the reaction (51). Thus proper apposition of plasma membrane Ca\textsuperscript{2+}-permeable ion channels and store release compartments is required for proper Ca\textsuperscript{2+} signals to ensue. Recently, both store release channels, including IP\textsubscript{3}R (55, 57) and RyR (56), have been associated by direct interactions with plasma membrane-located TRP-related channels. This provides at least one potential mechanism for store channel-initiated Ca\textsuperscript{2+}-permeable channel activation (151). Conversely, an important enzymatic pathway in the regulation of cytosolic Ca\textsuperscript{2+} is the inositol phosphate (IP) lipid cascade (100, 102). This metabolic pathway entails IP-sensitive PLC activation by a variety of G protein-coupled and tyrosine kinase receptors, responding to either hormonal stimuli or growth factors, respectively. PLC activation mediates the generation of both IP\textsubscript{3} and DAG, each branch of which leads to the release of Ca\textsuperscript{2+} from intracellular stores (100, 119). PLC stimulation also regulates Ca\textsuperscript{2+} influx by channels in the plasma membrane and may represent one of the most relevant mechanisms for TRP channel activation (7, 38). The final Ca\textsuperscript{2+} signaling cascade by either mechanism is potentiated by Ca\textsuperscript{2+}-induced feedback signals. These may include cell membrane depolarization, enzyme activation, Ca\textsuperscript{2+} binding to regulatory proteins, and/or vesicle fission (Fig. 5). Ca\textsuperscript{2+} influx is often mediated by activation of the capacitative Ca\textsuperscript{2+} response (5, 9) elicited by store-operated channels (7), which release Ca\textsuperscript{2+} from intracellular compartments. Recent evidence provides an attempt to directly implicate specific TRP channels in Ca\textsuperscript{2+} influx sensory signals (7, 73, 100, 101). The TRP-related CaT1 channel (TrpV6), for example, was recently suggested as the molecular fingerprint of the elusive CRAC conductance when expressed in CHO-K cells (149). CaT1 shares most of the store-activated (Ca\textsuperscript{2+} release-activated) CRAC current. These include inversely correlated activity with intracellular Ca\textsuperscript{2+} concentration, loss of selectivity in the absence of divalent cations, and inhibition by La\textsuperscript{3+} (149). The molecular fingerprint of the Ca\textsuperscript{2+} release-activated current is yet to be unequivocally determined, as other studies have found clear functional discrepancies, most importantly voltage regulation, rectification, and pharmacological differences between endogenous CRAC and expressed CaT1 (131). Unequivocal identification of CRAC is still a difficult task as most cells may have “contaminating” currents with apparently similar physiological roles (22). Endogenous channels may play complementary, compensatory, and/or otherwise misleading roles in assessing intrinsic vs. exogenous Ca\textsuperscript{2+}-permeable channel function. It is highly likely that most cell systems are intrinsically biased by expression of endogenous TRP-like channels, which may coassemble or otherwise interact with the channels under study.

Only newly emerging information is available on the possible role of polycystins in eliciting Ca\textsuperscript{2+} signals. A normal PC1-PC2 complex seems to be required for the Ca\textsuperscript{2+} influx step induced by shear flow in embryonic renal epithelial cells (85). This phenomenon is triggered by bending of the primary cilium (98) and seemingly requires normal PC1-PC2 interaction. Cells expressing truncated PC1 (85), or missing the primary cilium (99), lack this response. Although the molecular steps of these important signaling mechanisms require further identification, it is clear that the PC1-PC2 response to volume flow requires external Ca\textsuperscript{2+}, implying Ca\textsuperscript{2+} influx as an initial step in the response. Addition of ryanodine and caffeine, to block RyR and IP\textsubscript{3}R, respectively, but not the IP\textsubscript{3}R inhibitor aminoethoxydiphenyl borate (2-APB), impaired the flow-induced cytosolic Ca\textsuperscript{2+} increase in wild-type renal epithelial cells (85) (Fig. 4). This suggests that although required, Ca\textsuperscript{2+} influx does not account for the massive Ca\textsuperscript{2+} rise detected in embryonic mouse renal epithelial cells (85). Instead, the Ca\textsuperscript{2+} response in these cells may also implicate a store-operated response secondary to the Ca\textsuperscript{2+} influx. Cilia bending in MDCK cells, for example, elicit a Ca\textsuperscript{2+} signal, which also depends on the presence of extracellular Ca\textsuperscript{2+}, but this is abolished by the PLC inhibitor U-73122 (98). Thus after plasma membrane PC1-PC2 channel complex activation takes place, downstream signaling pathways may be either independent or perhaps mutually complementary in different cell types. In embryonic mouse collecting duct cells, addition of GDP\textsubscript{βS}, U-73122, and 2-APB to inhibit G proteins, phospholipase C, and IP\textsubscript{3}R, respectively, failed to impact the flow-induced cytosolic Ca\textsuperscript{2+} signaling event (85). Distinct intraorganellar receptor channel abundance and isotypes may also help elicit different responses. Conversely, the plasma membrane receptor activation may lead to cell-type-specific signals. Thrombin receptor activation in embryonic renal epithelial cells, for example, modified a comparatively similar cytosolic Ca\textsuperscript{2+} increase in both wild-type and Pkd\textsubscript{1}del34/del34 cells lacking normal PC1-PC2 interaction (85) (Fig. 4). This suggests a more typical receptor-mediated, store-activated Ca\textsuperscript{2+} response, which does not require a functional PC1-PC2 complex. Actually, the Ca\textsuperscript{2+} response to thrombin was significantly greater in the Pkd\textsubscript{1}del34/del34 cells compared with wild-type cells (85) (Fig. 4). It is unclear at present as to whether thrombin stimulation is linked to PC1-independent PC2 channel activation. One possibility is that in the absence of functional PC1, PC2 develops partnerships with other regulatory proteins such as receptor-activated responses. Conversely, an impaired PC1-PC2 channel complex may help induce expression of PC2-independent signaling mechanisms of Ca\textsuperscript{2+} entry, including TRP channel isoforms, and, in particular, CaT1 and similar channels in renal epithelial cells. Mutual complementation among various channels in the capacitative entry step may provide flexibility to the regulatory mechanisms for Ca\textsuperscript{2+} rise, thus accommodating a variety of cell environmental signals associated with renal epithelial cell activation.

EMERGING EVIDENCE AND FUTURE DIRECTIONS: ADPKD AND BEYOND

This review summarizes recent evidence for the TRP-type channel properties of PC2, focusing on its electrophysiological characteristics and potential regulatory aspects of its function, particularly those pertaining to Ca\textsuperscript{2+} signals. Foreseeable outcomes in health and disease are still unclear as little is known about the specific role PC2 plays under physiological conditions. It is evident that future studies will be required, in particular concerning potential regulatory mechanisms such as...
PC2 association with other channels and/or regulatory proteins (Fig. 5). New and interesting sensory connections have recently emerged that may help in our understanding what roles the genes implicated in ADPKD have in health and disease. Comparatively, we know more about the role of PC2 in ADPKD than its role under physiological conditions. Furthermore, the new evidence has to eventually help reconcile the body of evidence concerning a number of transport and features in ADPKD epithelia, in particular those associated with the increased cAMP response and fluid secretion (116). The evidence to date strongly contends in favor of PC2 and related TRP proteins having a physiological role in Ca\(^{2+}\)-signaling events (Fig. 5), regardless of putative interactions with other channels or regulatory proteins. In fact, the evidence to date would indicate that the way PC2 is regulated may play an essential role in physiological events, including the eventual onset of disease, such as ADPKD. Interestingly, TRP channel members have been implicated in seemingly unrelated issues. These include, for example, invasiveness of tumorigenic response, suggesting that the putative role of these proteins indeed affect cell growth (32, 135) and malignancy (121, 139). This may be shared by several members of the TRP family, further contending in favor of their role as signal transducers. PC1 overexpression, for example, has been associated with changes in the growth of cultured cells (86). Whether this is a reflection of specific interactions with PC2 is still an open question. It is entirely likely that PC2 may behave as a nonregulated cation channel until such time, or developmental signals ensue, that enable the colocalized PC1-PC2 complexes to elicit the obligatory signaling pathway that triggers Ca\(^{2+}\) influx (Fig. 5). Either protein in the PC1-PC2 channel complex may have otherwise important functional and/or regulatory roles of their own. Conversely, each protein may control cellular pathways otherwise regulated independently in the presence of the PC1-PC2 channel complex. PC1 has been linked to G protein regulation in vivo (24) and activation in vitro (93), which, in turn, may be associated with channel regulation other than that of PC2 (124). Recent evidence suggests yet other regulatory pathways controlled by Pkd gene products. PC1 overexpression induces an increased ATP release in renal epithelial cells (1), which in turn may help elicit a number of cellular responses. Nucleotide release in renal epithelial cells may play a role in regulating ion transport, including activation of ATP-gated P2X cation channels, which elicit sustained Ca\(^{2+}\) influx in target cells. Thus PC2-independent Ca\(^{2+}\) influx pathways may help add diversity to the physiological conditions and conversely serve as alternative Ca\(^{2+}\) signaling mechanisms under PC1-PC2-impaired conditions. Diversity may also be expected under physiological conditions by PC2 association with other channel proteins. PC2 has been shown to interact with the TRP channel TRPC1 (122). This interaction, whose functional correlate awaits experimental proof, suggests that PC2 may heterooligomerize as a TRP channel subunit. A likely scenario may be presented whereby putative heterooligomerizations between TRPs, including PC2 and PCL, in association with, or independent of PC1 (Fig. 5), may enable a wider spectrum of “channel complexes” with diverse cellular functions. Coupling of one or more TRP subunits (monomers?) may provide unexpected gating and regulatory mechanisms not intrinsic to PC2 and homologous channel proteins (homo- and heterotetrameric complexes). An unprecedented diversity in the plasticity of signals that can be expected from TRP channels is based on these previously undetermined TRP heteromeric structures (114). In particular Ca\(^{2+}\) signals may have to be further assessed, depending on which Ca\(^{2+}\)-permeable channels may be expressed in epithelial cells, which may be functional or even overexpressed in the absence of a functional PC2 channel pathway. This, in turn, may provide alternate routes for both Ca\(^{2+}\) transport and/or adaptive signaling mechanisms. CaT1 (TrpV6) and its highly homologous ECaC (TrpV5) are present in renal epithelia, where their role is expected to produce relevant transient Ca\(^{2+}\) currents. The temporal resolution of the CaT1/ECaC Ca\(^{2+}\) transients should be different from those expected from PC2 and related homologs. Contrary to the expected Ca\(^{2+}\)-induced channel inhibition observed in Ca\(^{2+}\)-permeable channels such as the L-type and the CaT-type channels in excitable and nonexcitable, respectively, PC2 is only inhibited by nonphysiological Ca\(^{2+}\) concentrations. Thus it may be speculated that PC2 serves as an important Ca\(^{2+}\) transport mechanism, as opposed to a channel related to Ca\(^{2+}\) signaling events. It would not be surprising that more than one channel structure is implicated in the Ca\(^{2+}\) influx of renal epithelial cells. There is no information to date as to whether either genomic or phenotypic depletion of the Pkd2 gene product renders cellular environments where other putative Ca\(^{2+}\)-permeable channels are up-regulated. The cellular location of endogenous PC2 is still a matter of debate. PC2 may be functional in intracellular compartments such as the ER/SR. Thus membrane apposition with PC1-containing plasma membrane may be required to ensue, a PC2-mediated store-activated response similar to that postulated for L-type Ca\(^{2+}\) channels and RyRs in excitable tissues. Conversely, plasma membrane PC2 may provide a viable Ca\(^{2+}\) entry step in most cells. The most solid evidence for this contention relies on findings that PC2 is located in the primary cilium, clearly a membrane-surrounded organelle.

The fact that TRP-related channels, including PC2, are widely expressed suggests a variety of regulatory roles in cell function. This may be partially dependent on the specific signaling mechanism to which TRP channels are associated (75, 78). Now, sensory function can be extended to cell compartments such as the nonmotile primary cilium to control PC2-mediated Ca\(^{2+}\) transport. Other possibilities for cascading signals, which modify a variety of cell responses, include PC2-induced changes in cell polarization, the regulation of ion transport, vesicle fusion, and cell proliferation. In this regard, it is important to remember that albeit Ca\(^{2+}\) permeable, PC2 is foremost a nonselective cation channel, whose role in Na\(^{+}\) reabsorption, K\(^{+}\) secretion, and changes in membrane polarization still have to be addressed. It is possible that following a rapid and transient Ca\(^{2+}\) response dissipated with the Ca\(^{2+}\) gradient, PC2 may continue to function to control the state of the cell’s resting potential and/or other cellular parameters to control cell function. Future studies will be required to further assess the transport capabilities and/or regulatory signaling pathways ascribed to PC2 channel function in renal tissues and, most interestingly, the role(s) of PC2 in nonrenal tissues. Vascular location of this Ca\(^{2+}\) channel may provide important clues to yet unknown mechanisms of Ca\(^{2+}\) regulation.

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REFERENCES

CALCIUM SIGNALING BY POLYCYSTIN-2


