The cAMP Signaling Pathway and Direct Protein Kinase A Phosphorylation Regulate Polycystin-2 (TRPP2) Channel Function*

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Background: TRP channels are targets for kinase phosphorylation.
Results: PKA phosphorylates and thus controls Polycystin-2 (TRPP2, PC2) channel function.
Conclusion: Phosphorylation of Ser-829 in the carboxy terminus of PC2 is a gating mechanism of channel activity.
Significance: Learning how cAMP/PKA local pathways participate in the regulation of PC2 channel function is crucial for understanding renal physiology and the pathogenesis of ADPKD.

Polycystin-2 (PC2) is a TRP-type, Ca\(^{2+}\)-permeable non-selective cation channel that plays an important role in Ca\(^{2+}\) signaling in renal and non-renal cells. The effect(s) of the cAMP pathway and kinase mediated phosphorylation of PC2 seem to be relevant to PC2 trafficking and its interaction with polycystin-1. However, the role of PC2 phosphorylation in channel function is still poorly defined. Here we reconstituted apical membranes of human syncytiotrophoblast (hST), containing endogenous PC2 (PC2\(_{hst}\)), and in vitro translated channel protein (PC2\(_{iv}\)). Addition of the catalytic subunit of PKA increased by 566% the spontaneous PC2\(_{hst}\) channel activity in the presence of ATP. Interestingly, 8-Br-cAMP also stimulated spontaneous PC2\(_{hst}\) channel activity in the absence of the exogenous kinase. Either stimulation was inhibited by addition of alkaline phosphatase, which in turn, was reversed by the phosphatase inhibitor vanadate. Neither maneuver modified the single channel conductance but instead increased channel mean open time. PKA directly phosphorylated PC2, which increased the mean open time but not the single channel conductance of the channel. PKA phosphorylation did not modify either R742X truncated or S829A-mutant PC2\(_{iv}\) channel function. The data indicate that the cAMP pathway regulates PC2-mediated cation transport in the hST. The relevant PKA site for PC2 channel regulation centers on a single residue serine 829, in the carboxy terminus.

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited human kidney disease and is caused by mutations in either PKD1 (85%) or PKD2 (15%) (1). The ADPKD gene products, polycystin-1 (PC1, TRPP1) and polycystin-2 (PC2, TRPP2) interact to form a heteromeric complex that regulates key signaling pathways in the kidney and other target organs (2, 3). The PC1/PC2 complex requires a physical interaction between the two proteins, via carboxy-terminal coiled-coil domains (4–6). This physical interaction has an important regulatory role in PC2 channel function (7). Nonetheless, both polycystins have different cell and developmental patterns of expression, and thus either protein has functional properties independent of the other (8). PC2 has intrinsic TRP type channel properties, which are regulated by a number of different metabolic and regulatory pathways. Contrary to the primary location of PC1 in the plasma membrane, PC2 has been found at the ER, the plasma membrane and the primary cilium (9–13).

Protein phosphorylation is a common post-translational modification that affects protein trafficking, subcellular location, protein-protein interactions, and ultimately their function. Kinase phosphorylation of PC2 has been implicated in the selectivity of the channel to Ca\(^{2+}\) signals (14), and regulates trafficking events that target the channel protein to various cellular domains (15). A stretch of acidic amino acids in the carboxyl terminus of PC2 mediates the interaction with PACS-1 and PACS-2 (16). The association between PC2 and the PACS proteins is regulated through the phosphorylation of a serine residue at position S812 of PC2 (16) but there is conflicting evidence as to whether phosphorylation is important for the retention of PC2 in the ER (14, 16). Mutant proteins lacking most of the carboxy terminus have been shown to escape from the ER (9, 17). Thus, a postulated mechanism of PC2 trafficking includes phosphorylation by casein kinase 2 (CK2) at Ser-812, "crossmark"}

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5 The abbreviations used are: ADPKD, autosomal dominant polycystic kidney disease; PC, polycystin; hST, human syncytiotrophoblast; TRP, transient receptor potential; POPC, 2-oleoyl-palmytoylethanolamine; POPE, 2-oleoyl-palmytoylethanolamine; PKA, protein kinase A.
which is retrieved at the ER via binding to PACS-2/COPI. Conversely, dephosphorylation of PC2 by protein phosphatase 2α (PP2α) may facilitate its translocation to the Golgi compartment (18). Interestingly, the serine/threonine kinases Neks (Never in Mitosis A-related kinases) have been implicated in cystic diseases (19). Nek8, mutated in the jck (juvenile cystic kidneys) mouse, a model of autosomal recessive polycystic kidney disease (ARPKD) (20, 21), was recently found to interact with PC2, but not PC1. The jck mutation leads to abnormal phosphorylation of PC2, longer cilia and ciliary accumulation of PC1 and PC2. Thus, PC2 phosphorylation events seem to play a central role in its trafficking, localization and function.

At least two evolutionarily conserved phosphorylation sites in PC2 were suggested to control its subcellular localization: serine residue 76 (Ser-76)/Ser-80 and Ser-812, which are phosphorylated by glycogen synthase kinase 3 (GSK3) (15) and casein kinase 2 (CK-2) (14), respectively. It has also been shown that PKC-dependent phosphorylation at Ser-801 is essential for the normal function of PC2 as an ER Ca2+ release channel (22). Additionally Nek8, mutated in some cases of nephronopthisis (NPHP), induced abnormal PC2 phosphorylation and its accumulation in primary cilia (21). We recently reported that PKA-catalyzed phosphorylation of PC2 at Ser-829 is dynamically regulated by the specific binding of PC1 to recruit and localize PP1α (18).

Despite the role(s) of PC2 phosphorylation on its trafficking and subcellular location, little is known regarding the role phosphorylation plays in PC2 channel function. Cai et al. (14) provided the first indication that constitutive phosphorylation at Ser812, a putative CK2 site in PC2, could regulate its channel function. Interestingly in that study, it was observed that wild type and S812A mutant PC2 functioned as divergent cation channels with similar current amplitudes. In contrast, a T721A mutant PC2 channel was non-functional. Channel open probabilities for the wild type and S812A mutated PC2 showed a bell-shaped dependence on cytoplasmic Ca2+ with a rightward shift in the Ca2+ dependence such that S812A PC2 was 10-fold less sensitive to Ca2+ activation/inactivation than the wild type PC2 channel. Previous immunochemical and electrophysiological studies on primary cilia of LLC-PK1 renal epithelial cells have shown the expression of ciliary vasopressin 2 (V2R) and local cAMP production (23), which regulates ciliary ion channels, including PC2. No information is currently available on the role PKA and its activating patterns play on PC2 channel properties.

Here we explored the effect of direct PKA phosphorylation on PC2 function by reconstitution of the endogenous channel from apical membranes of hST (PC2het) and the in vitro translated channel protein (PC2iv). PC2iv but not PC2het was regulated by maneuvers that control a local cAMP/PKA pathway present in the apical membrane of the hST, which may at least contain adenyl cyclases, PKA and phosphatases. Our data also indicate that PC2 is readily phosphorylated by PKA in either preparation, which in turn prolongs its mean open time but not the single channel conductance. The relevant phosphorylating site was identified at Ser-829, in the carboxyl terminus of the channel protein.

### Experimental Procedures

**Human Placenta Membrane Preparation**—Apical hST plasma membranes from term human placenta were obtained as previously described (24). Briefly, normal placenta from vaginal deliveries were obtained and immediately processed. The villous tissue was fragmented, washed with ice-cold unbuffered NaCl saline (150 mM), and minced into small pieces. The fragmented tissue was stirred for 1 h in a solution containing a protease inhibitor mixture adjusted to pH 7.4. The tissue preparation was filtered and centrifuged, as reported. The final pellet was resuspended in a buffer solution containing (in mM): HEPES 10, sucrose 250, and KCl 20, adjusted to pH 7.4. Apical hST enrichment was usually higher than 20-fold. Membrane fractions containing abundant endogenous PC2 (PC2het) were aliquoted and stored frozen until the time of the experiment.

**Preparation of in Vitro Translated PC2**—The in vitro translated PC2 gene products (PC2iv) were generated as follows. Generation of a human polycystin-2-pkTag plasmid has been previously reported (8). A Ser-829 > Ala mutation was generated by site-directed mutagenesis (Stratagene) confirmed by sequencing. PC2-pkTag and PC2 Ser829A-pkTag protein was generated using a reticulocyte lysate system TNT T7 (Promega). Briefly, the plasmid DNA (1 μg) and reaction mixture (50 μl) were incubated at 30 °C for 90 min. A sample (5 μl) was separated by SDS-PAGE and Western blotting with a pkTag antibody confirmed the presence of full length PC2-pkTag and PC2 Ser829A-pkTag. The different in vitro translated channel products were introduced by dialysis into liposomes formed by a mixture of the lipids 2-oleoyl-palmytoylcholine (POPC): 2-oleoyl-palmytoylethanolamine (POPE) in 7:3 ratio.

**Ion Channel Reconstitution**—Vesicles containing PC2 were incorporated into lipid bilayers built in a reconstitution system as previously reported (24). The lipid mixture was made of a 7:3 ratio of POPC and POPE (20–25 mg/ml, Avanti Polar Lipids, Birmingham, AL) in n-decane, and painted in the opening of a bilayer chamber cuvette (Warner Instruments Corp.) to seal a membrane. Once the membrane was formed, it was broken and again painted with the vesicle preparation with lysins. Unless otherwise stated, the cis chamber was bathed with a solution containing: KCl 150 mM, CaCl2 10 μM, HEPES 10 mM, at pH 7.40, and the trans side contained a similar solution with lower KCl (15 mM), to create a KCl chemical gradient. PC2het was identified as previously reported (24), by a large conductance (~170 pS), K+-conducting channel in a KCl chemical gradient, which was inhibited by trans (external) amiloride, and cis (cytoplasmic side of PC2) anti-PC2 antibody, properties that also ensured the identification of its orientation in the reconstituted membrane.

**Reagents**—All reagents were analytical grade unless otherwise stated. The reagents sodium ortho-vanadate (#450243), the catalytic subunit of PKA (P2645), and alkaline phosphatase (P6774) were obtained from Sigma-Aldrich and prepared as stock solutions as recommended by the manufacturer and added at final concentrations indicated in the respective experiments. The PKA specific blockers H89 (100 μM) and PKI (5 μg/ml) were obtained from Tocris Chemicals, Bristol, UK. The anti-PC2 rabbit polyclonal antibody raised against amino acids
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689–969 of the human protein (sc-25749, Santa Cruz Biotechnology Inc.), was added at a dilution 1:1000 to the cis reconstitution hemi-chamber.

Electrophysiological Data Acquisition and Analysis—PC2 channel currents were obtained with a PC501A patch-clamp amplifier (Warner Instruments, Hamden, CT) with a 10 GΩ feedback resistor. Output signals were acquired from the patch-clamp amplifier without internal filtering (5 kHz), and were low-pass-filtered at 700 Hz (3 dB) with an analog eight-pole, Bessel-type filter (Frequency Devices, Haverhill, MA). Signals were then digitized with a TL-1 with DMA A/D converter at 80 kHz (Axon Instruments, Foster City, CA). Digital signal acquisition with the pClamp 5.5 suite was set to 100 µs. BLM current tracings were further filtered for display purposes only. Unless otherwise stated, the software pClamp 10.2 (Axon Instruments), was used for data analysis and the software Sigmaplot 11.0 (Jandel Scientific, Corte Madera, CA), was used for statistical analysis and graphics. Unless otherwise stated, all tracings were obtained at holding potentials between 40 and 60 mV.

Signal Analysis and Statistics—PC2 channel currents were expressed as follows: single channel BLM currents were integrated for single sweep 12-s episodes. The data expressed as $N_{Po}$ were analyzed as follows, the mean membrane current for each tracing was determined prior to averaging data from each condition separately. Data represented $I = N \times i \times P_{o}$, where $N$ is the total number of active channels in the reconstituted membrane, $i$ is single channel current, and $P_{o}$ the open probability of the channel at a given holding potential expressed as the mean ± S.E. (n) under each condition, where "n" represents the total number of experiments analyzed. A channel’s open probability was calculated such that $P_o = t_o/NT$, where $t_o$ was the total time a channel was found in the open state, and $T$ is the total observation time. Statistical significance was obtained by paired Student’s t test comparison of sample groups of same size, and accepted at $p < 0.05$ (25).

Results

Effect of PKA on PC2_hst-mediated $K^+$ Currents—The role of the cAMP signaling pathway on PC2_hst channel function was tested by addition of its endogenous substrate, the catalytic subunit of PKA (Fig. 1, a–c) to reconstituted hST apical membranes in the presence of a KCl chemical gradient, as previously reported (24). The activated PKA (100 nm) was added in the presence of MgATP (1 mM) to the cis compartment of the reconstitution chamber. PKA stimulated PC2_hst, mediated K+ currents by 566% ($N_{Po} = 0.12 ± 0.07$, versus $0.80 ± 0.16$, n = 10, $p < 0.01$, Fig. 1c). Channel currents were largely (87%) inhibited by addition of amiloride (100 µM) to the trans compartment (Fig. 1c), and by addition of an anti-PC2 antibody as previously reported (24, data not shown). The single channel conductance and reversal potential (173 ± 15 pS, and −56.7 mV, respectively) were identical to previous reports (24) (Fig. 1b).

Effect of Activation of the cAMP Pathway on PC2_hst $K^+$ Currents—To further explore the presence of a local cAMP regulatory pathway effecting PC2_hst channel regulation, apical hST vesicles were reconstituted in the presence of a KCl chemical gradient as for the previous experiments. To determine the effect of local cAMP production on PC2_hst function, the non-hydrolyzable cAMP analog, 8-Br-cAMP (500 µM) was added to the cis chamber, also containing MgATP (1 mM). The cAMP analog stimulated PC2_hst mediated K+ currents by 260% ($N_{Po} = 0.10 ± 0.035$ versus $0.26 ± 0.15$, n = 7, $p < 0.05$, Fig. 2, a and c). In contrast, addition of either H89 (100 µM) or the PKA inhibitor PKI (5 µg/ml), prevented the PKA stimulation of PC2_hst (Fig. 2, b and f).

Effect of Protein Dephosphorylation on the PC2_hst Function—To further test that the stimulatory effect of PKA-activation on PC2_hst K+ currents was indeed mediated by direct phosphorylation, alkaline phosphatase (160 µg/ml) was added to the cis reconstitution chamber containing PKA-ATP-activated PC2_hst channel activity. Addition of the enzyme completely inhibited PC2_hst channel function ($0.80 ± 0.16$, $n = 6$ versus $0.02 ± 0.01$, $n = 3$, $p < 0.01$, Fig. 2, d and h). Conversely, hST vesicles pre-incubated with the phosphatase inhibitor vanadate (25 µM) prior to reconstitution showed a 400% channel stimulation ($N_{Po} = 0.12 ± 0.07$, versus $0.60 ± 0.40$, $n = 7$, $p < 0.05$, Fig. 2, c and g), suggesting a role of endogenous phosphatases in the inhibiting, or otherwise decreasing the stimulatory effect of the phosphorylating event, thus decreasing PC2-mediated K+ channels in hST.

Effect of PKA and ATP on Purified PC2iv—To confirm that the cAMP-PKA stimulatory pathway targetted PC2, and not any other regulatory protein, wild type PC2iv (see “Experimental
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Procedures”) was tested instead. Addition of PKA (100 nm) and MgATP (1 mM) activated PC2
interpreted as the PKA inhibitor PKI (5 mg/ml) to the cis compartment, prevented
PKA activation of the PC2hst currents. c, representative single channel tracings of
PC2hst (n = 4) were activated by incubation with vanadate (25 mg/ml), d, rep-
resentative single channel tracings of PC2hst (n = 10) activated by PKA-acti-
vated were inhibited by addition of alkaline phosphatase (160 mg/ml) to the
cis chamber. e, bar graph representing mean NPo data for control, after ATP
addition, and further addition of 8-Br-cAMP (n = 7). f, bar graph representing
mean NPo data for control condition, after stimulation with PKA/ATP and
subsequent inhibition with either PKI (5 mg/ml, n = 10), or H89 (100 mg/ml, n =
doubled the cis compartment. g, bar graph representing mean NPo data for
control condition and after incubation with the phosphatase inhibitor
vanadate (25 mg/ml) prior to reconstitution (n = 7). h, bar graph showing mean
NPo data for control condition and after addition with alkaline phosphatase
(n = 3).

Conductance similar to that of wild type PC2hst (Fig. 3b). Addition
of PKA and ATP, however, were entirely without effect on the
channel function of mutant S829A-PC2 (n = 9). The single
channel conductance of PC2hst obtained in a KCl chemical gradient was 145 ± 15 pS (n = 16), while that of PC2S829A under identical conditions was, 154 ± 11 (n = 9, Fig. 3b), almost identical to each other (p < 0.1). Under control conditions single
channel currents of both wild type- and S829A-PC2 were
completely inhibited by addition of the anti-PC2 antibody
(no plasmid).

Discussion

Cyclic AMP and PKA Channel Regulation—The second mes-
senger cAMP has important role(s) in the stimulation of tran-
sepithelial fluid movement as well as cell proliferation. In the
mammalian nephron, chiefly in principal cells of the collecting
duct, for example, cAMP-mediated signals are quintessentially
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**a.** Single channel tracings are representative of wild type PC2 \(_{iv}\) (top tracings), and PC2\(_{S829A}\) (bottom tracings), respectively. Channels were reconstituted in the presence of a KCl chemical gradient. Addition of PKA (100 nM) and anti-PC2 antibody (1:1000 dilution) completely inhibited PC2 channel function. Both PC2\(_{iv}\) and PC2\(_{S829A}\) single channel currents were completely inhibited by addition of anti-PC2 antibody (1:1000 dilution iv/v) to the cis chamber. b, bar graph representing mean \(N_p\) data for control, after ATP/PKA addition for PC2 of different origins as indicated, and further exposure for 1 min to the anti-PC2 antibody. Single asterisk indicates \(p < 0.05\) (\(n = 5\)) for the PKA/ATP-treated wild type PC2\(_{iv}\) respect to control value. Double asterisks indicate \(p < 0.001\) (\(n = 19\) and 12, respectively) for the difference between before and after anti-PC2 antibody inhibition.

**FIGURE 4. Effect of anti-PC2 antibody on in vitro translated PC2.** a, single channel tracings are representative of wild type PC2\(_{iv}\) (\(n = 19\), top tracings), and PC2\(_{S829A}\) (\(n = 12\), bottom tracings), respectively. Channels were reconstituted in the presence of a KCl chemical gradient. Addition of PKA (100 nM) and MgATP (1 mM) only activated PC2\(_{iv}\) single channel currents. Both PC2\(_{iv}\) and PC2\(_{S829A}\) single channel currents were completely inhibited by addition of anti-PC2 antibody (1:1000 dilution iv/v) to the cis chamber.

**FIGURE 5. Schematics of cAMP/ PKA regulation of polycystin-2.** Hypothetical model of PKA regulation of PC2, based on the present findings in hST and the isolated protein, our previous results on PC1 dephosphorylation events on Ser-829 of PC2 (18), and a local cAMP signaling pathway in primary cilia (23). The CAMP production by local adenylyl cyclases (AC) activates PKA, which phosphorylates Ser-829 in the carboxyl terminus of PC2. This event in turn, increases channel open time, but not the conductive properties of the pore. Basal PC2 channel function in the hST is tightly coupled with phosphorylation/dephosphorylation events since alkaline phosphatase (AP) inhibits PC2 channel function and conversely, ortho-phanacetin activates it. Possible feedback mechanisms include the triggering of Ca\(^{2+}\)-dependent cAMP decreasing events elicited by its influx through the channel. Further, whenever present, PC1 facilitates the dephosphorylation of Ser-829 of PC2, by recruiting PP1 to the site (18). The interaction between cAMP/PC2 in hST further suggests possible receptors and/or yet to be identified AKAPs in this preparation.

while drugs that reduce cAMP levels inhibit cyst growth in experimental animals and retard kidney enlargement in humans with the disease (30). Although the reason for higher cytosolic cAMP concentrations in cystic cells is still not well understood, one possible scenario may be that the polycystin proteins alter the activity of G-protein-coupled receptors e.g. V2R, that signal via cAMP (30, 31). In the present study, we explored the role that the cAMP signaling pathway plays in PC2 channel function (Fig. 5). Our findings indicate that stimulation of either a local cAMP pathway, or direct addition of PKA, induced or stimulated PC2 channel activity from apical hST membranes. These findings also confirmed direct phosphorylation by PKA of both PC2\(_{iv}\) and PC2\(_{hst}\). This is in agreement with previous findings showing that AVP stimulation of a local cAMP pathway activates PC2 in primary cilia of LLC-PK1 renal epithelial cells (23).

**Phosphorylation of TRP Channels—**Both phosphorylation and dephosphorylation cycles are reversible post-translational modifications that regulate the structure and function of a variety of TRP channels (32), affecting both trafficking to the plasma membrane and regulation of channel function. Protein channel phosphorylation has been associated with both TRP channel activation and inhibition. Because PKA signaling has a positive or sensitizing effect on a large number of mechanosensitive TRP channels, including V1 (33), V4 (34), M7 (35), and TRPC subtypes (36) our findings are consistent with the fact that PKA phosphorylation of PC2 may control, as for other TRP channels, Ca\(^{2+}\) entry, and thus the feedback of Ca\(^{2+}\) signals. PC1 and PC2 are also phosphorylated. PC1 is phosphorylated by tyrosine kinase (TK) and PKA (37). TK elicits cell matrix interaction with E-cadherin, while inhibiting the PC1 interaction with focal adhesion kinase (pp125FAK) (38). The elevated phosphorylation of PC1 in ADPKD patients may interfere with its interaction with PC2/E-cadherin/β-catenin, causing the
depletion of both PC1 and E-cadherin from the plasma membrane (39). CK2 phosphorylation of PC2 at Ser-812 (14) and dephosphorylation by PP2α, affect its trafficking to distinct subcellular compartments (18). The proteins PACS-1 (phosphofurin acidic cluster sorting protein-1) and PACS-2 bind to phosphorylated PC2, causing its retrieval to the Golgi and endoplasmic compartments, respectively. Only CK2 phosphorylation of Ser-812 in PC2 has been shown to significantly modify channel function by increasing its Ca\textsuperscript{2+} sensitivity (14). Although PC2 can be phosphorylated at multiple sites in its carboxyl-terminal domain, further understanding of the pathogenesis of ADPKD may implicate a functional balance between the phosphorylation and dephosphorylation events at Ser-829 in PC2 involving, respectively PKA and PP1. Recently, we demonstrated that the PKA-mediated phosphorylation status of Ser-829 is dynamically regulated by the binding of PC1 through the recruitment of PP1 (18). Ser-829 hyperphosphorylation of PC2 may not just be a marker of PC1 inactivation, but may have direct functional cellular consequences for the development of the cystic phenotype in PKD1 patients. Truncating mutations in PKD1 resulted in the constitutive phosphorylation of this residue, in part due to the loss of binding between PC1 and PC2 and the concomitant mislocalization of PP1 dephosphorylation activity (18). PK1 activity would help terminate the phosphorylating activation, which requires the binding of PC1 to PC2 (18). The PC1 truncation mutant (R4227X) which cannot bind to PC2 but still binds PP1 was defective in dephosphorylating Ser-829. Although the functional consequences of these features remain to be further explored, the data in the present study suggest that PC2 phosphorylation is an important feature in both, its basal channel function and regulation by PC1, adding new levels of complexity to the functional sensory and transport capabilities of the PC1/PC2 complex. Our results with apical hST vesicles are consistent with a local cAMP signaling pathway adjacent to PC2inh. Direct addition of either the AC stimulator forskolin (data not shown), or the cAMP analog 8-Br-cAMP, stimulated PC2inh channel activity. Phosphatase inhibition with ortho-vanadate was also stimulatory. Thus, data are consistent with spontaneous channel activity from constitutively phosphorylated PC2inh. This finding was further supported by the inhibitory effect of alkaline phosphatase on spontaneous PC2inh channel activity. Addition of activated PKA in the presence of ATP stimulated PC2 in both the hST apical membrane preparation and the wild type in vitro translation product, demonstrating that PC2 is itself a target for PKA phosphorylation. This was confirmed by two negative experimental maneuvers. Truncation of the carboxyl terminus of PC2 with an ADPKD-causing mutation (R742X) eliminated PKA stimulation of the channel, while maintaining its basal channel activity. Secondly, mutation of Ser-829, a key target site of PKA phosphorylation of PC2, rendered channel function completely insensitive to PKA. The location of this relevant PKA phosphorylation site suggests regulation of possible assembly and/or gating properties of the channel complex, but not single channel conductance.

Because PC2 channel function largely implicates Ca\textsuperscript{2+} entry into the cell (40), it is likely that channel function is coupled as a negative feedback mechanism to cAMP stimulatory signals. One of the most direct and plausible routes connecting Ca\textsuperscript{2+} to PKA is through Ca\textsuperscript{2+}-mediated regulation of AC activity and thus cAMP production. Specifically, AC1 and AC8 are activated by Ca\textsuperscript{2+} via calmodulin, while other isoforms are inhibited by Ca\textsuperscript{2+} (41). Ca\textsuperscript{2+} may also regulate AKAP function. Ca\textsuperscript{2+}/CaM binding inhibits the interaction of two AKAPs, AKAP79, and gravin/SseCKS, with membrane phospholipids (42), such that changes in local Ca\textsuperscript{2+} may either exclude PKA or alternatively, displace specific AKAP-containing complexes while retaining or enhancing others, an efficient way for Ca\textsuperscript{2+} to regulate the specificity of PKA signaling. It is entirely possible, however, that could be targeted by phosphorylation of other binding partners. PC2 interacts with two other members of the TRP family, chiefly among which are TRPC1 and TRPV4 (43), which have distinct functional properties in renal epithelial cells (44, 45). In this context, PKC activation of TRPV4 significantly increases [Ca\textsuperscript{2+}], in response to flow in isolated split-opened distal nephrons, without affecting its subcellular localization (34). In contrast, forskolin activation of PKA, does not affect the TRPV4-mediated [Ca\textsuperscript{2+}], response to flow but instead markedly shifted its subcellular distribution toward the apical membrane. Thus, while the PKA-dependent cascade promotes TRPV4 trafficking and translocation to the apical membrane, the PKC-dependent pathway increases its channel activity (34). Yet another possible regulatory mechanism of PC2 function, implicating binding partners may involve cytoskeletal structures. We recently demonstrated that Ca\textsuperscript{2+} regulation of PC2 is conveyed by actin-binding proteins (46), including α-actinin and filamin, which in turn, are both PKA targets.

**Cyclic AMP Regulation of Ciliary PC2**—Cystic diseases have been recently linked to the dysfunctional activity of ciliary proteins (47). In this context, it is important to mention second messenger systems which may affect correlates structure-function of the primary cilium. There is an interesting connection between primary cilia, the cAMP and Ca\textsuperscript{2+} pathways, chiefly because our findings of a functional ciliary V2R linked to local cAMP production and activation of PC2 in this organelle. In renal epithelial cells, primary cilia, which are cell cycle-regulated organelles, are implicated in Ca\textsuperscript{2+} signals and cell activation (12, 48). Because cAMP signals also control the rate of renal cell proliferation (31), signals that control ciliary structure may indeed be associated with the initiation-arrest of cell proliferation. In fact, AVP antagonists effectively inhibit renal cystic cell growth (30). We previously demonstrated that AVP activation of the ciliary V2R increases the local production of cAMP and activated PKA (23), which in turn stimulates primary cilium ion channels, including PC2.

**Ca\textsuperscript{2+} Transport and Regulation of PC2**—PC2 acts as a Ca\textsuperscript{2+} entry pathway in various epithelial tissues and other organs (24, 10, 49–51). Its abundance in the apical membrane of the hST makes PC2 also relevant to Ca\textsuperscript{2+} transport in human term placenta, where this syncytial epithelium increases the transfer of Ca\textsuperscript{2+} during the last trimester of pregnancy (52, 53). Its contribution to both cell signaling and Ca\textsuperscript{2+} homeostasis is implicated in its reported activity in several cell locations, including the primary cilium, intracellular Ca\textsuperscript{2+} stores, and the plasma membrane (54, 49, 55), thus, playing an important role in Ca\textsuperscript{2+} entry and cell activation. This contribution of PC2 to Ca\textsuperscript{2+}...
transport requires highly tuned feedback mechanisms because of the non-inactivating nature of Ca\(^{2+}\) transport through the channel (40). Despite homology with voltage-gated Na\(^+\), and Ca\(^{2+}\) channels (56), both in the pore region, and in putative Ca\(^{2+}\)-binding domains such as the EF-hand (56, 57), that may contribute to Ca\(^{2+}\)-dependent channel regulation (58, 59), PC2 does not engage in Ca\(^{2+}\) induced self-inhibition present in L-type and similar Ca\(^{2+}\) channels. Cytoplasmic Ca\(^{2+}\) delivered by channel function instead regulates a local pool of cytoplasmic ions (40) that modulate direct interactions with Ca\(^{2+}\)-dependent cytoskeletal proteins (46). Ca\(^{2+}\) transport through PC2 feeds the regulatory sites in the cytoplasmic domain of the channel, which, in turn, modulate its function via direct interaction with actin-binding proteins (ABPs) such as α-actinin, gelsolin, and filamin (46). Thus, the isolated channel protein (PC2\(_{iso}\)) is completely insensitive to Ca\(^{2+}\) (40), requiring Ca\(^{2+}\) binding sites extrinsic to the channel protein for regulation. This regulation of PC2 may be relevant to the pathogenesis of ADPKD. Vassilev et al. (60) reported that the transient activation of wild type PC2 by higher intracellular Ca\(^{2+}\) in Xenopus laevis oocytes overexpressing the protein was absent in the ADPKD-causing R742X truncated PC2. Cai et al. reported that wild-type PC2 shows a Ca\(^{2+}\)-dependent, bell-shaped open probability in response to voltage that shifts in the mutated PC2-S812A, a casein kinase phosphorylation site (14). The above evidence suggests that intracellular Ca\(^{2+}\) signaling regulated by the polycystins could be one of the most proximal events whose dysfunction will lead to cyst formation.

**Downstream Effects of Ca\(^{2+}\) Signaling in ADPKD—**A common finding in different models of ADPKD is an increased cAMP concentration that stimulates cyst fluid and electrolyte secretion (31). The PKA signaling pathway stimulates cell proliferation in ADPKD cysts, but not normal human kidney cells (61–63). Inhibition of the MAPK pathway by PKA-mediated phosphorylation in normal cells is altered in cystic cells, through an effect that has been attributed to lower cytosolic Ca\(^{2+}\) concentration (64, 65). The regulation of cAMP production includes intracellular compartmentalization, where the relation between polycystin dysfunction and cAMP levels in ADPKD may be linked to specific microdomains, either at the plasma and/or intracellular membranes and involving particular AC or PDE isoforms. In this context, there is long standing evidence for relevant connections between the Ca\(^{2+}\) and cAMP/PKA signaling pathways (66, 67). Ca\(^{2+}\) and the cAMP-effector PKA can cross-talk and regulate one another at a number of levels in the pathogenesis of ADPKD, mutations in the genes encoding either PC1 or PC2 lead to the lowering of intracellular Ca\(^{2+}\), with the resulting increase in cAMP due to the released inhibition of Ca\(^{2+}\)-sensitive ACs and/or stimulation of Ca\(^{2+}\)-sensitive phosphodiesterases. Because PC2 mediates a non-inactivating Ca\(^{2+}\) entry (40), its cAMP-dependent activation in such compartments as the primary cilium, would have important implications as a feedback mechanism in the handling of Ca\(^{2+}\) signals, which in turn control cilary structures such as the microtubular axone (68). The most direct route connecting Ca\(^{2+}\) to PKA is the control by Ca\(^{2+}\) of cAMP production by regulating AC activity. Several AC isoforms are regulated by Ca\(^{2+}\), while others are insensitive (41). The isoforms AC1 and AC8 for example, are activated by Ca\(^{2+}\) via calmodulin, while other isoforms are inhibited by Ca\(^{2+}\), either directly or through phospho-regulation by PKC (41).

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