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Polycystin 2: a calcium channel, channel partner, and regulator of calcium homeostasis in ADPKD

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Abstract

Polycystin 2 (PC2) is one of two main protein types responsible for the underlying etiology of autosomal dominant polycystic kidney disease (ADPKD), the most prevalent monogenic renal disease in the world. This debilitating and currently incurable condition is caused by loss-of-function mutations in *PKD2* and *PKD1*, the genes encoding for PC2 and Polycystin 1 (PC1), respectively. Two-hit mutation events in these genes lead to renal cyst formation and eventual kidney failure, the main hallmarks of ADPKD. Though much is known concerning the physiological consequences and dysfunctional signaling mechanisms resulting from ADPKD development, to best understand the requirement of PC2 in maintaining organ homeostasis, it is important to recognize how PC2 acts under normal conditions. As such, an array of work has been performed characterizing the endogenous function of PC2, revealing it to be a member of the transient receptor potential (TRP) channel family of proteins. As a TRP protein, PC2 is a nonselective, cation-permeant, calcium-sensitive channel expressed in all tissue types, where it localizes primarily on the endoplasmic reticulum (ER), primary cilia, and plasma membrane. In addition to its channel function, PC2 interacts with and acts as a regulator of a number of other channels, ultimately further affecting intracellular signaling and leading to dysfunction in its absence. In this review, we describe the biophysical and physiological properties of PC2 as a cation channel and modulator of intracellular calcium channels, along with how these properties are altered in ADPKD.

1. Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic renal disease worldwide, presenting with a prevalence of 1:400 – 1000¹. Mutations in *PKD2*, the gene encoding for Polycystin 2^{2,3}, account for approximately 15–20% of ADPKD cases, with mutations in *PKD1*, the gene encoding for Polycystin 1 (PC1)⁴, being responsible for the remaining 80–85%⁵. Although ADPKD is inherited in a dominant pattern, the

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homozygosity of mutations in *PKD2* or *PKD1* are embryonic lethal⁶, suggesting a role for the polycystins as essential cellular proteins. As a result, the development of cysts requires a second-hit mutation after birth, and as such, the progression to end stage renal disease (ESRD) typically occurs later in life for these patients. Approximately 50% of patients with mutations in *PKD1* develop ESRD by 54 years of age, and those with mutations in *PKD2* approximately 20 years later⁷. The development of renal cysts occurs long before ESRD, and patients can now be diagnosed with ADPKD through genetic testing; thus, patients may be diagnosed decades before renal failure develops. Despite the prevalence of ADPKD, there unfortunately exists only one approved therapy to slow ADPKD progression, and this treatment presents with severe aquaretic side effects⁸. This drug, Tolvaptan, is a selective vasopressin V2 receptor antagonist only recently approved for treatment in several countries including the U.S., Japan, Canada, Australia, and Europe, but often physicians depend upon medications to merely manage the painful symptoms of ADPKD until eventual ESRD necessitates renal replacement therapy⁹. The lack of effective therapies, especially treatments without severe side effects, makes clear the need for further research on ADPKD pathophysiology. A solid understanding of the mechanism of disease, along with identification of novel pathways and targets for its treatment is urgently needed.

Polycystin 2 (PC2 or TRPP1, formerly TRPP2) is a ubiquitously-expressed^{10–12} member of the transient receptor potential (TRP) channel family of proteins, where it is located on the endoplasmic reticulum (ER), primary cilia, and plasma membrane. It is most well-characterized in renal epithelial cells due to its role in ADPKD, for which it was named^{1,13}. However, unlike PC1, PC2 is ubiquitously expressed throughout the body, and evidence suggests that PC2 plays an important role in organ physiology beyond the kidney^{10,14–17}. As a member of the TRP channel family, PC2 forms a calcium (Ca^{2+})-permeant cation channel where it can function as a homomeric tetramer or in complex with PC1 and other TRP channels^{18–20}. Specifically, PC2 plays a critical role in maintaining normal Ca^{2+} signaling, a process that, when disrupted, plays a key role in the progression of ADPKD. Thus, PC2-mediated dysregulation of Ca^{2+} signaling is thought to play a major role in cystogenesis^{21,22}.

Beyond its functional capacity to conduct cations, PC2 acts as a physical modulator of numerous other ion channels, including fellow TRP family members, along with the critical intracellular Ca^{2+} release channels Inositol 1,4,5-trisphosphate Receptor (InsP3R) and Ryanodine Receptor (RyR). The ability of PC2 to regulate intracellular Ca^{2+} via its function as a cation channel and through its interactions with other proteins makes it an interesting target to help understand how improper Ca^{2+} levels lead to pathophysiology. In this review, we discuss 1) the function of PC2 as a Ca^{2+} -permeant cation channel in the ER, 2) the biophysical and regulatory interactions between PC2 and other channels, and 3) how Ca^{2+} dysregulation contributes to the development of ADPKD.

2. Polycystin 2 is a transient receptor potential channel

The categorization of PC2 as a TRP channel family member was first predicted from its amino acid sequence²³, which encompasses 6 transmembrane regions, a pore-forming loop, N-glycosylation and phosphorylation sites, a polycystin-specific ‘tetragonal opening for

polycystins' (TOP) domain²⁴, and a C-terminal tail that includes a Ca^{2+} -binding EF hand, coiled-coil domain, and ER retention tag^{25,26}. Notably, PC2 contains high sequence similarity to fellow TRP genes that include a characteristic voltage-sensing domain (VSD), which in PC2 spans transmembrane helices 1–4 (Fig. 1)²⁷. Single channel studies confirmed that PC2 acts as a Ca^{2+} -activated channel that is non-selectively permeable to both monovalent and divalent cations. Recently, the structure of PC2's membrane-spanning domain predicted from cryo-EM studies has allowed for further insights into the biophysical properties of PC2 as a Ca^{2+} -activated tetrameric channel, garnering indispensable information on the unique properties of PC2 as well as characteristic similarities to its fellow TRP channel family members^{24,27–29}.

2.1 Cation permeability

The first studies characterizing the channel properties of PC2 were performed using single channel experiments through reconstitution into planar lipid bilayers. Using this technique, our lab and others demonstrated that PC2 forms a cation-permeable channel^{30,31}. Because full-length PC2 contains a C-terminal ER retention sequence (Glu787-Ser820), the majority of wild-type PC2 is retained in the ER, whereas mutated forms of PC2 missing this region become mis-localized in kidney epithelial cells²⁶, contributing to disease pathology. Because of its subcellular localization, the channel activity of PC2 can be studied by collecting ER microsomes and reconstituting them in planar lipid bilayers. Experiments testing the channel properties of PC2 identified it as a carrier of divalent cations (Ca^{2+} , Ba^{2+} , and Mg^{2+})^{30,31}. This finding is distinctive as, in contrast to the voltage gated calcium channel (VGCC) which is both impermeable to Mg^{2+} and noticeably more permeable to Ba^{2+} , PC2 shows no preference for which divalent cation it conducts^{32–34}. Additionally, the permeability of PC2 to monovalent cations (Cs^+ , K^+ , and Na^+) is high (permeability ratio $P_{\text{Ca}}/P_{\text{Na}} \approx 0.25$), whereas the VGCC is highly specific as a Ca^{2+} channel (permeability ratio $P_{\text{Ca}}/P_{\text{Na}} \approx 1000$)^{31,35,36}. However, though biophysically nonselective, the localization of PC2 as an ER-resident channel is of important physiological relevance, as the Ca^{2+} gradient between the ER lumen and cytoplasm is so prominent that PC2 would preferentially conduct Ca^{2+} in native systems. The levels of Ba^{2+} , Mg^{2+} , Cs^+ , K^+ , and Na^+ do not significantly differ across the ER membrane, thus making these cations irrelevant to the endogenous function of PC2, and essentially making PC2 a Ca^{2+} -specific cation channel when located on the ER. Interestingly, further studies have measured a change in the Ca^{2+} permeability of PC2 depending on whether it is measured in the ER or primary cilia ($P_{\text{Ca}}/P_{\text{Na}} \approx 0.06$ when measured in whole cilia)^{36,37}. These results raise the possibility that PC2 associates with different proteins in distinct locations within the cell, ultimately tailoring its channel activity and permeability contingent upon its subcellular localization. In support of this, the transient receptor potential cation channel subfamily M member 3 (TRPM3) was shown to be required to measure Ca^{2+} currents in PC2-dependent channels of primary cilia³⁶. Although PC2 forms a Ca^{2+} -permeable channel in homotetrameric form (Fig. 1), accounting for where and with what monomers it is potentially interacting to form Ca^{2+} -permeable heterotetramers must be considered when considering the channel properties of PC2. In addition to permeability measurements, computational modeling of PC2's transmembrane core estimates a pore diameter of $\sim 1 \text{ \AA}$ in its closed state^{27,29}, $\sim 1.7 \text{ \AA}$ in its open state^{28,29}, and $\sim 1.4 \text{ \AA}$ in its inhibited (multiple ion) state²⁸. However, functional studies performed by

our group indicate that the minimum pore diameter of the open PC2 channel is approximately 11 Å based upon its ability to conduct cations of increasing size, including tetrapentylammonium (TPeA⁺), whose diameter is 11.1–13.2 Å³⁸. The discrepancy between these pore sizes is likely due to the lack of N- and C-termini in the structures of PC2, as both termini contain multiple important functional domains, including the N- and C-terminal phosphorylation sites, and C-terminal coiled-coil domain, EF-hand, and ER retention tag, all of which may alter channel activity and other protein-protein interactions.

2.2 Calcium-dependency of Polycystin 2

PC2 not only conducts Ca²⁺ from ER stores, but is also intimately related to cytosolic Ca²⁺, as it relies on cytosolic Ca²⁺ levels to regulate its open probability. Low levels of Ca²⁺ (up to ~1 μM) increase the open probability of PC2, whereas high levels (> 1 μM) are inhibitory, ultimately giving PC2 a bell-shaped Ca²⁺ response³⁹. The EF hand located on the cytosolic C-terminal tail of PC2 (Fig. 1A) is responsible for sensing intracellular Ca²⁺ levels⁴⁰, and Ca²⁺ binding to the EF hands of PC2 in its tetrameric form is required for the opening of full-length PC2⁴¹. Mutants lacking or mutated in this region, such as PC2-L703X, a pathological mutant found in human ADPKD patients, show no change in open probability with differential Ca²⁺ levels³⁰. In comparing the structures of PC2 in its single-ion (open) versus multiple-ion (inhibited) state, it appears that the pore of PC2 is blocked by Ca²⁺ binding at the entrance of the selectivity filter in conditions of high Ca²⁺. This comparison provides a structural explanation for the mechanism of PC2 inhibition due to high Ca²⁺ levels²⁸.

2.3 Posttranslational regulation of Polycystin 2

In addition to modulation by cytosolic Ca²⁺ levels, multiple posttranslational modifications help to regulate PC2's activity. Numerous experimentally validated (Ser76, Ser801, Ser812, and Ser829 in mammals, and Ser534 in *C. elegans*) phosphorylation sites have been described within PC2's N- and C-termini. These modifications have been shown to be important for multiple levels of protein regulation, including trafficking, Ca²⁺ dependence, and channel activity of PC2. The phosphorylation of Ser76 is carried out by glycogen synthase kinase-3 (GSK-3), and loss of this modification interferes with the subcellular localization of PC2 in vitro and zebrafish embryonic development in vivo⁴². Ser801 phosphorylation by protein kinase D (PrKD) is required for proper cell proliferation and inositol trisphosphate (InsP3)-mediated ER Ca²⁺ release⁴³. Constitutive Ser812 phosphorylation by casein kinase 2 (CK2) is critical to maintain proper Ca²⁺ dependence of PC2 along with its retrograde transport³⁹. Phosphorylation of Ser829 is regulated both by protein kinase A (PKA) and PC2's interaction with PC1⁴⁴. Similar to Ser801 phosphorylation, the Ser829 phosphoserine site is also thought to enhance InsP3-mediated Ca²⁺ transients. Phosphorylation and dephosphorylation of Ser534 by CK2 and calcineurin (CaN), respectively, mediates PC2 ciliary localization in *C. elegans*⁴⁵, but its importance requires validation in human cells, and further extensive studies are required to determine the role, if any, that phosphorylation of this residue plays in mammalian kidney homeostasis.

Posttranslational protein modifications to PC2 also include the N-glycosylation of five residues within the expansive TOP domain of PC2 (amino acids: Asn299, 305, 328, 362, and

375)²⁸. When these asparagine residues are mutated to disallow their N-glycosylation, the stability of PC2 decreases dramatically, leading to its engulfment by lysosomes⁴⁶. Insights from cryo-EM structures of PC2 indicate that these N-glycosylation sites are differentially modified depending on the open state of PC2²⁸. In its multiple-ion (inhibited) state, Asn299, 328, 362 and 375 are N-glycosylated, and are important for maintaining well-ordered loop interactions. In contrast, the single-ion open state exhibits a more disordered TOP domain, in part due to the N-glycosylation of Asn305, 362, and 375, but not Asn375²⁸. The N-glycosylation of Asn305 is predicted to prevent the intra-tetramer interactions that occur in the multiple-ion state, thus leaving the single-ion state less ordered. However, the exact mechanisms by which, and if, these sugar modifications directly affect channel activity remain to be explored.

2.4 ADPKD-linked mutations in Polycystin 2

The critical protein domains described above that contribute to the activity, stability, and trafficking of PC2 as an ER-resident channel are natural hotspots for mutations leading to PC2 dysfunction and ADPKD. Interestingly, a large number of pathogenic *PKD2* mutations are clustered within the TOP domain⁴⁷, highlighting the currently understudied importance of this region for proper PC2 function. Multiple other mutations are found within the N- and C-termini and membrane-spanning regions. The importance of the N-terminus, beyond containing the site for Ser76 phosphorylation and consequent PC2 localization, includes its role in the trafficking of PC2 to primary cilia, likely through its association with retromer⁴⁸. In addition, the N-terminus contains a crucial oligomerization domain required for the quaternary structure formation of functional PC2⁴⁹, and therefore mutations in this region are more likely to affect these processes. The C-terminus of PC2 also contains multiple important structural domains that, when mutated, lead to the development of ADPKD. This includes the Ca²⁺-sensing EF hand, a coiled-coil domain, and three linker regions. Pathogenic mutations found in the EF hand (i.e. Frameshift 720–736X) are unsurprisingly responsible for the altered Ca²⁺-dependency regulating PC2 channel function⁵⁰. The coiled-coil domain of PC2 appears to be equally important for proper PC2 function, as multiple mutations in this region are responsible for ADPKD pathogenesis (i.e. E837X and Frameshift 837–843X). This domain has been shown to be required for self-oligomerization of PC2's C-terminal coiled-coil domains and consequent heteromeric polycystin complex formation⁵¹. Notably, the C-terminal tail of PC2 has likewise been described as a critical site of interaction between PC2 and other proteins, some of which are described below, including channels affecting intracellular Ca²⁺ homeostasis and cell signaling in ADPKD. Overall, the fact that pathogenic mutations can arise in essentially all regions of *PKD2* tells us that, while all components of PC2 may not be critical for ion conductance of PC2, all aspects (activity, stability, regulation, and trafficking) must be intact to ensure proper cell function.

3. Polycystin 2 and its interactions with other channels

Beyond its role as a cation-permeable channel, PC2 also modulates Ca²⁺ signaling via associations with a number of other channels, both on the ER and plasma membrane. In this section we describe these interactions along with how they are implicated in ADPKD.

3.1 Polycystin 1

PC1 is a large (460 kDa) membrane-bound protein consisting of 11 transmembrane passes, a cytosolic C-terminal tail containing a coiled-coil domain and multiple protein cleavage sites, and an extensive extracellular N-terminal domain with a number of structural regions that allow for wide-ranging protein modifications and signaling events. As the appreciably larger of the polycystins, it is unsurprising that PC1 accounts for the majority of ADPKD-causing mutations, as there are 46 exons encoding for 4303 amino acids compared to the 15 exons and 968 amino acids of PC2 that could carry potential mutations. PC1 has been characterized as a channel by some groups, but it currently remains controversial as to whether PC1 acts as a channel in the absence of PC2. Ca^{2+} -permeable nonselective cation currents have been reported in cells expressing PC1 alone⁵², whereas others have described a necessity for PC2 to conduct Ca^{2+} current^{19,37} and propose that PC1 acts as a chemically- and mechanically-sensitive protein that works in partner with PC2 to transduce Ca^{2+} signals across the cell membrane in response to flow and pressure^{18,53–55}. It has been suggested that PC1 interacts with PC2 via each protein's respective C-terminal coiled-coil domain, based upon the observations that the disruption of these domains abolishes the PC2/PC1 interaction^{39,56–58}. However, other reports suggest that the N-terminal domain of PC1 is required for its interaction with PC2^{27,40,49,52}. Interestingly, the cryo-EM structure of the PC2/PC1 complex did not include the N- or C-termini of either PC2 or PC1⁵⁴, suggesting that these two proteins may assemble independent of these regions at a site currently unidentified. It is also currently unclear as to whether PC2 translocation to primary cilia requires an association with PC1^{58–62}. Along with this, the widely-studied theory that primary cilia are Ca^{2+} -responsive mechanosensors has been recently challenged in a study that failed to observe ciliary Ca^{2+} signaling in response to flow stimulus⁶³. The considerable lack of consensus regarding the functional capabilities of PC1, PC2, and primary cilia demonstrates the need for further studies to better understand these interactions and their roles in regulating intracellular Ca^{2+} . Nonetheless, it is ubiquitously agreed that PC1 and PC2 are capable of interacting to affect cell signaling.

Findings of PC2/PC1-mediated dysregulated signaling in ADPKD are extensive, all of which confirm that both PC2 and PC1 must function properly to maintain cellular health. Structural mapping of human ADPKD mutations support the claim that disruptions of the PC2/PC1 complex are disease causing⁵⁴. Interestingly, PC2 and PC1 depend on each other not only for mechanically-or chemically-induced Ca^{2+} influx, but PC2 also appears to be required for PC1 processing and trafficking to the ciliary membrane⁶⁴. As such, intact versions of each polycystin is mutually crucial for the PC2/PC1 complex to function properly. Notably, overall ablation of primary cilia⁶⁵, PC1⁶⁶, or PC2⁶⁷ all lead to the development of ADPKD. Because the PC2/PC1 complex localizes to primary cilia, ADPKD has historically been characterized as a ciliopathy⁶⁸. However, recent work has identified ADPKD as a metabolic disease independent of cilia function, showing direct effects on mitochondrial function through PC2 and PC2/PC1 complex signaling at the ER-mitochondrial junction^{69–71}. Together, these findings help us appreciate the importance of the PC2/PC1 complex not only in cilia, but in maintaining proper cell signaling throughout the cell, thus explaining the requirement for both polycystins in maintaining homeostasis and how mutations in either polycystin can distinctly affect cell function.

3.2 Inositol 1,4,5-trisphosphate receptor

The localization of PC2 as an ER-resident channel allows for ample opportunity to interact with the InsP3R, another ER-localized Ca^{2+} channel. The InsP3R is a cation channel activated by InsP3 that, like PC2, does not show high preference toward Ca^{2+} over monovalent cations, but is widely known as a Ca^{2+} channel due to its location on the ER membrane where Ca^{2+} is the only relevant cation with an electrochemical gradient⁷². The InsP3R is a large (314 kDa per subunit), ubiquitously-expressed homotetrameric complex with a diverse number of regulators, including phosphorylation, ubiquitination, oxidation, Ca^{2+} , and adenosine triphosphate (ATP)^{73–77}. Co-immunoprecipitation experiments demonstrate that the C-terminal tail of PC2 competes with InsP3⁷⁸ to bind the ligand-binding domain (LBD) of the InsP3R. This interaction is mediated through a positively charged cluster (KKFR⁵⁴) in the suppressor domain of the InsP3R LBD that interacts with an acidic cluster (aa810–818 SEEEDEDS) within PC2's C-terminal tail⁷⁹. Notably, this acidic cluster contains Ser812, one of the phosphorylation sites of PC2 shown to affect its channel function³⁹. Through this interaction, over-expression of PC2 enhances both the maximal Ca^{2+} response approximately two-fold and the half-decay time of InsP3-induced Ca^{2+} transients approximately ten-fold. Conversely, expression of the PC2-L742X mutant, which does not interact with the InsP3R, does not change the InsP3-dependent ER Ca^{2+} release, and the channel-dead mutant PC2-D511V actually leads to decreased amplitude of Ca^{2+} responses upon InsP3 stimulation. The PC2/InsP3R interaction is a reciprocally symbiotic relationship, as not only does PC2 binding enhance InsP3-mediated Ca^{2+} release, but activation of PC2 occurs as a result of its direct association with the InsP3R⁸⁰. Following InsP3-stimulated ER Ca^{2+} release, this interaction creates a signaling microdomain containing high levels of Ca^{2+} , eliciting Ca^{2+} -induced Ca^{2+} release through PC2. This complementary PC2/InsP3R interaction helps to explain the unchanged Ca^{2+} signaling observed with PC2-L742X expression. Because the loss of the acidic cluster within PC2's C-terminal tail abolishes the PC2/InsP3R interaction, therefore prohibiting the formation of the Ca^{2+} -rich microdomain, Ca^{2+} -induced Ca^{2+} release through PC2 is not possible. Additionally, whereas PC2-D511V can physically interact with the InsP3R, PC2 itself is unable to conduct Ca^{2+} across the ER membrane, and thus there is a loss of total Ca^{2+} flow into the cytoplasm.

3.2.1 Stromal interaction molecule 1—The interaction between InsP3R and PC2 is dependent on both intracellular Ca^{2+} levels as well as the cellular abundance of PC1^{81,82}. PC2 appears to compete with Stromal Interaction Molecule 1 (STIM1) to bind the InsP3R, and increased levels of PC1 cause a decrease in the association between PC2 and the InsP3R by enhancing InsP3R/STIM1 binding. Because PC2 positively promotes InsP3-mediated ER Ca^{2+} release, the presence of PC1 leads to a decrease in ATP-stimulated Ca^{2+} signaling by disrupting the interaction between PC2 and the InsP3R and instead promoting the interaction between STIM1 and the InsP3R. This PC1-mediated blunting of Ca^{2+} signaling can be reversed through inhibiting the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway, which is activated by increased levels of cyclic adenosine monophosphate (cAMP), a characteristic phenotype of cystic cells⁸³. Alternately, depletion of ER Ca^{2+} stores increases the PC2/InsP3R interaction by decreasing the interaction between InsP3R and STIM1, leaving STIM1 free to interact with calcium release-activated calcium channel

protein 1 (Orai1) and promote store operated calcium entry (SOCE). Work from our group demonstrated that while kidney cells with PC2 knocked down develop cysts in 3D cell culture, as anticipated, knockdown of InsP3R types 1 and 3 led to cyst development characterized by greater size, increased cell death, increased apoptosis, and deciliation compared to PC2 knockdown cells⁸⁴. Thus, modulation of both PC2 and InsP3R appear to play a role in ADPKD development through the disruption of normal Ca^{2+} signaling.

3.3 Ryanodine receptor

The cardiac ryanodine receptor (RyR2), like the InsP3R, is an essential intracellular Ca^{2+} release channel located on the sarcoplasmic reticulum (SR) that, when activated, releases Ca^{2+} from intracellular stores through Ca^{2+} -induced Ca^{2+} release⁸⁵. RyR2 is highly expressed within muscle tissues and is required for proper excitation-contraction coupling via Ca^{2+} release following plasma membrane voltage changes. Because ADPKD patients often present with cardiac complications along with renal cyst development, the interaction between PC2 and RyR2 helps to inform how Ca^{2+} signaling in the hearts of *PKD2*^{+/-} patients is altered to affect cardiac function. RyR2 contains high sequence homology to the InsP3R in its fifth and sixth transmembrane domains. However, unlike the InsP3R, studies mapping the PC2/RyR2 interaction demonstrated that both the N- and C-termini of PC2 associate with the RyR2, and that the nature of this interaction is dependent on the open state of RyR2⁸⁶. In its closed state, RyR2 interacts with amino acids 130–220 in the N-terminus of PC2 but does not interact with the PC2 C-terminus. However, when maintained in the open state, RyR2 associates with both the N- and C-termini of PC2. The PC2 N-terminus appears to be necessary for the physical PC2/RyR association, whereas the C-terminus acts to decrease the open probability of RyR2 in a Ca^{2+} -dependent manner. These findings support experiments showing that *Pkd2*^{+/-} cardiomyocytes exhibit increased spontaneous Ca^{2+} oscillations, decreased caffeine-stimulated SR Ca^{2+} release, and a concomitant depletion of SR Ca^{2+} stores⁸⁶. Functional studies testing the requirement of PC2 to regulate RyR2 in cardiomyocytes demonstrated that abolishing PC2 function in zebrafish and mice caused aberrant Ca^{2+} handling, leading to impaired cardiac output and calcium-contraction coupling, lowered heart rate and stroke volume, shortened ventricular action potential, and increased the incidence of atrioventricular block^{16,17}. The absence of PC2 in these cardiomyocytes seemingly abrogates the negative regulation of RyR2 Ca^{2+} release, leading to Ca^{2+} overload and cardiac dysfunction. It remains to be seen whether PC2 interacts with the RyR isoforms expressed outside of the heart, RyR1 and RyR3. Nevertheless, the interaction of PC2 with RyR2 supports an essential role for PC2 to regulate the mobilization of intracellular Ca^{2+} stores in extrarenal tissues reliant on tightly regulated Ca^{2+} for proper function.

3.4 Transient receptor potential channels

The characterization of PC2 as a TRP channel validates its structural homology to other TRP superfamily members and supports the suggestion that there are inter-TRP interactions to form heteromeric channels. In this section, we describe the interactions of other TRP channels with PC2.

3.4.1 Transient Receptor Potential Cation Channel Subfamily V Member 4—

As a member of the TRP superfamily, the transient receptor potential cation channel subfamily V member 4 (TRPV4; 98 kDa) is a nonselective, thermo and mechanosensitive Ca^{2+} -permeant cation channel. Located on the plasma membrane and in primary cilia, TRPV4 contains the characteristic VSD located in transmembrane regions 1–4 of PC2 (Fig. 1)^{87–89}. The mechanosensitive nature of TRPV4 is notable in its interaction with PC2, as PC2 has been shown to be critical for mechanical-dependent ciliary Ca^{2+} influx¹⁸, but, as described in Section 2, PC2 itself does not contain any mechanosensitive properties. It has been suggested that PC2 accomplishes its mechanosensitive signaling by interacting with PC1 or TRPV4 in primary cilia, where the PC2/TRPV4 heterocomplex forms with a 2:2 alternating stoichiometry via the C-termini of both channels^{90,91}. It is thought that PC2 enhances mechanically-induced TRPV4 currents by altering its channel properties⁹², giving the PC2/TRPV4 heterocomplex properties distinct from either PC2 or TRPV4 alone⁹³. This heterocomplex has been shown to be required for flow-mediated Ca^{2+} responses and, interestingly, shows increased expression following deciliation of renal epithelial cells⁹⁴. TRPV4-deficient zebrafish embryos develop hydrocephalus, which is a canonical phenotype of PC2-deficient ADPKD zebrafish.⁹⁰ However, double deletion of both PC2 and TRPV4 does not show synergism for the development of ADPKD, indicating that the pathogenesis of this disease in relation to TRPV4 is dependent upon its interaction with PC2.

3.4.2 Transient Receptor Potential Cation Channel Subfamily C Member 1—

The interaction between PC2 and the transient receptor potential cation channel subfamily C member 1 (TRPC1; 91 kDa) was first hypothesized based upon the similarity between these proteins' topology and homology, both of which contain channel pore-forming, TRP-like, and helical regions.⁹⁵ TRPC1, another TRP channel family member, forms a tetrameric nonselective cation channel and has been described as having localization both on the plasma membrane⁹⁶ and within the ER⁹⁷. It is thought to facilitate Ca^{2+} influx in response to ER Ca^{2+} store depletion, phospholipase C-mediated intracellular signaling, and protein kinase C-dependent phosphorylation.⁹⁸ Similar to the PC2/TRPV4 interaction, PC2 and TRPC1 form a heterotetramer on the plasma membrane with a 2:2 stoichiometry and an alternating subunit arrangement,⁹⁹ an interaction mediated by aa679–743 and aa379–643 within the C-terminal tail of PC2 and by aa639–750 in TRPC1⁹⁵. Also similar to PC2/TRPV4, the formation of the PC2/TRPC1 heterotetramer has distinct channel properties separate from PC2 or TRPC1 homomers alone; the conductance of this hetero-complex is an intermediate between the large and small conductances of PC2 and TRPC1 homomers, respectively, and whereas PC2 homomers are sensitive to channel blockage by pH changes, the PC2/TRPC1 hetero-complex shows no functional difference with altered pH. Additionally, although PC2 shows reduced current in the presence of amiloride, with the TRPC1 contribution to the heterotetramer, the channel is no longer affected by amiloride addition^{100,101}. Interestingly, beyond the more well-characterized PC2/TRPV4 and PC2/TRPC1 hetero-complexes, it has also been described that, due to the cross-subfamily assembly capabilities of the TRP channels, all three channels can assemble to form a PC2/TRPV4/TRPC1 flow-sensitive heteromeric channel that requires the channel activity of each TRP to function properly.¹⁰² Notably, though most research has focused on the biophysical properties of PC2 in complex with TRPC1 and TRPC1/V4 channels, the physiological

consequences of these interactions in the progression of human ADPKD still remain unknown, and further studies must be done to determine the requirement of these hetero-complexes in maintaining renal homeostasis in vivo.

4. Calcium signaling in polycystic kidney disease

Given the extensive effect that PC2 has on maintaining Ca^{2+} homeostasis both through its action as a channel and a regulator of Ca^{2+} -permeant ion channels, it is unsurprising that Ca^{2+} dysregulation is widely accepted as a major player in the pathogenesis of ADPKD. Ca^{2+} signaling is an extremely diverse and conserved process involved in a multitude of pathways, including cell proliferation, survival, and death, all of which are highly implicated in cystogenesis and ADPKD development.

4.1 Calcium-mediated cell proliferation in ADPKD

Renal cystogenesis involves the energetically demanding process of enhanced proliferation in polycystin-deficient epithelial cells. As a hallmark of cyst formation, a number of studies have focused on the altered signaling pathways involved in highly proliferative cystic cells, many of which implicate dysregulated Ca^{2+} signaling as a source of pathologically enhanced proliferation and survival¹⁰³. A multitude of these signaling pathways are regulated upstream by the ubiquitous second messenger molecule, cAMP. cAMP activates many downstream targets that promote cell growth and proliferation (Fig. 2), including B-Raf and extracellular signal-regulated kinase (ERK)¹⁰⁴, cAMP response element binding protein (CREB)¹⁰⁵, Hippo signaling¹⁰⁶, Ras homolog enriched in brain (RHEB)¹⁰⁷, and mammalian/mechanistic target of rapamycin (mTOR)¹⁰⁸, many of these being activated primarily through cAMP activation of protein kinase A (PKA). cAMP is generated by the conversion of ATP into cAMP by adenylyl cyclase (AC), a plasma membrane-bound enzyme with multiple Ca^{2+} -sensitive subtypes (AC5/6) whose enzymatic activity is inhibited in the presence of Ca^{2+} . Under conditions of low cytosolic Ca^{2+} , such as in PC2-deficient cells, Ca^{2+} inhibition of AC5/6 is relieved, leading to increased levels of cAMP and consequent activation of PKA¹⁰⁹. It is hypothesized that the imbalance between Ca^{2+} and cAMP in cystic cells promotes their transition from a normal absorptive, quiescent state to a pathological secretory, proliferative states¹⁰³. In support of this, cystic phenotypes can be rescued through the restoration of normal cytosolic Ca^{2+} or by decreasing cAMP levels¹¹⁰, making the intracellular Ca^{2+} /cAMP balance a promising target to help counteract cystogenesis.

4.2 Calcium-mediated cell death in ADPKD

In addition to cell proliferation, a concomitant hallmark of cyst development in ADPKD is the dramatic increase of apoptosis in cyst-lining cells. The combination of enhanced cell proliferation and death contributes to the filling of renal cysts with excess fluid and dead cells, leading to the slow formation of cysts and eventual ESRD. Because the process of apoptosis is directly regulated by Ca^{2+} signaling, it is unsurprising that apoptosis is intimately correlated to polycystin function. Indeed, mice lacking the anti-apoptotic factor B-cell lymphoma 2 (BCL-2) develop polycystic kidneys, and over-expression of BCL-2 prevents cyst cavitation in Madin-Darby canine kidney (MDCK) cells, validating the role

that apoptosis plays in cystogenesis^{111–113}. It is hypothesized that the Ca^{2+} -mediated increase in cAMP levels in cystic cells contributes to apoptosis. In support of this, treatment of vascular smooth muscle cells (VSCMs) with an intracellular Ca^{2+} chelator increases levels of both cAMP and apoptosis¹¹⁴, and treatment of mice with a vasopressin V2 receptor antagonist slows the progression of the renal apoptotic index in a rodent model of polycystic kidney disease¹¹⁵. In addition, our lab showed that PC2 acts as a “molecular brake” of Ca^{2+} transmission at the mitochondria-associated ER membrane (MAM)⁶⁹, the inter-organellar region responsible for transmitting Ca^{2+} stores into the mitochondria (Fig. 2). Thus, the combination of dysregulated cAMP and loss of ER-to-mitochondria Ca^{2+} inhibition efficiently primes PC2-deficient cells for mitochondrial Ca^{2+} overload and apoptosis initiation following cell stress and apoptotic stimuli.

5. Future Directions

Since the identification and characterization of PC2 as the product of the “second” gene responsible for autosomal dominant polycystic kidney disease, *PKD2*, many strides have been made toward understanding the function of this protein, particularly in the context of the kidney. However, there still remain numerous questions surrounding not only ADPKD, but the general role of PC2 in the cell. Because it is expressed in all tissue types, one of the most important remaining questions regarding PC2 is its function in the normal cell, and the reason for its requirement in all tissues. Though PC2 is named after its causative role in kidney disease, ADPKD can also present with many extrarenal pathologies, including cerebral aneurisms¹¹⁶, hernia^{117,118}, colonic diverticula^{119,120}, and the development of hepatic¹²¹, pancreatic¹²², and seminal vesicle^{123,124} cysts, suggesting that the polycystins are important for homeostatic maintenance in all tissues. In support of this, work from our lab showed that loss of PC2 results in cardiac dysfunction independent of renal abnormalities^{15,125}, demonstrating the need for further studies to help elucidate the requirement of PC2 in extrarenal tissues, and how its loss affects signaling and health in all cells.

In addition to the study of PC2 in ADPKD, the characterization of PC2’s function as a channel has likewise continued to develop since first being identified as a TRP family member. Much of this can be attributed to work establishing the channel properties of PC2, including its selectivity, ion sensitivity, and voltage dependency. Recently, structural solving of the membrane-spanning regions of PC2 have also provided important insights into its channel characteristics. However, a major limitation of these studies is the lack of N- and C-termini, which contain many important functional domains that have a significant effect on channel function and quaternary structure formation. Because these domains are important for PC2-protein interactions, structures including N- and C-termini would provide valuable information regarding the tetrameric assembly of PC2-containing channels, which likewise remains incompletely understood. Along these lines, the question remains as to whether PC2 natively forms a channel that fluxes ions in vitro, as there is still a lack of evidence that PC2 functions as a homomeric channel in the ER of cells. Unfortunately, the lack of a specific agonist or antagonist for PC2 contributes to the difficulty in measuring PC2 currents in vitro, and future work studying the discovery or development of PC2 agonists or antagonists will be important for furthering our understanding of PC2 channel activity.

6. Conclusion

The pathogenesis of ADPKD is extensive and extremely complex, especially considering the multitude of mutations that can lead to disease development present both in *PKD2* and *PKD1*. Although the process of cystogenesis undoubtedly results from a combination of many dysregulated signaling pathways, the functional characterization of PC2 has helped identify Ca^{2+} signaling as a major contributor to ADPKD development. Despite the challenge that all mechanisms through which PC2 interacts with other proteins to regulate Ca^{2+} signaling remain incompletely understood, especially in the context of disease pathology, the success of restoring Ca^{2+} and Ca^{2+} -regulated pathways in ameliorating cystogenesis and disease progression presents promising mechanistic targets for ADPKD drug development and therapies. We have focused this review on the role that PC2 plays as a Ca^{2+} modulator on the ER membrane, but it is possible that PC2 may carry different functions depending on its subcellular localization and with which proteins it interacts. For example, it may exclusively interact with PC1 or TRPV4 in cilia to form a mechanosensory channel, whereas in the ER/SR with InsP3R or RyR2 to modulate ER Ca^{2+} release to regulate cell health. In addition, emerging evidence shows that PC2 function is critical in whole-body physiology, and thus PC2 may play specific roles in different tissue and cell types. This possibility only increases the complexity of disease, and thus further studies must be performed to elucidate the role of PC2 in particular subcellular locations and cell types if effective therapeutics are to be designed to help treat this incurable disease.

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HIGHLIGHTS

- Polycystin 2, encoded by *PKD2*, resides on the ER, primary cilia, and plasma membrane
- Polycystin 2 is a nonselective tetrameric cation channel, of the TRP channel family
- Polycystin 2 interacts with many ion channels to modulate intracellular Ca^{2+} signaling
- Mutated polycystin 2 has altered function and leads to polycystic kidney disease

Figure 1: Polycystin 2 is a membrane-bound transient receptor potential channel

A) Schematic depicting the topology of a full-length human PC2 monomer. PC2 contains six transmembrane helices and cytosolic N- and C-termini. Serine phosphorylation sites can be found in both termini; from N- to C-terminus: Ser76, 801, 812, and 829. The unique ‘tetragonal opening for polycystins’ (TOP, pink) domain between transmembrane regions 1–2 is rich in N-glycosylation sites: from N- to C-terminus: Asn299, 305, 328, 362, and 375. The characteristic voltage-sensing domain (VSD, yellow) of PC2 spans transmembrane helices 1–4. PC2’s pore-forming loop containing pore helices 1 and 2 (P, green) sits between

transmembrane helices 5 and 6 (blue) and allows the transfer of Ca^{2+} from the ER lumen to the cytosol. The C-terminal tail of PC2 contains a Ca^{2+} -binding EF hand, ER retention tag, and coiled-coil domain. Numbers depict amino acid locations. B) Structure of a truncated PC2 monomer (PDB: 5MKF) containing amino acids K215-K695, without the cytosolic N- or C-termini. Yellow highlights the VSD; pink highlights the TOP domain; green highlights the pore helices; blue highlights transmembrane helices 5–6. N-glycosylated residues within the TOP domain are labeled and highlighted in teal. C) Top-down (luminal) view of truncated PC2 (PDB: 5K47) highlighting the location of these same domains within a homotetrameric quaternary structure. Opaque coloring highlights a single monomer.

Figure 2: Polycystin 2 interacts with ion channels to modulate intracellular Ca^{2+} signaling

Diagram depicting PC2/channel interactions in a healthy cell, and how normal Ca^{2+} signaling prevents the hyper-proliferation and -survival observed in PC2-deficient cells. PC2 acts to release ER Ca^{2+} through its intrinsic channel activity, or through its interactions with the InsP3R or RyR. The PC2/InsP3R interaction at the ER-mitochondrial interface serves to reduce the levels of mitochondrial Ca^{2+} uptake, preventing overload and apoptotic initiation. The PC2/PC1 and PC2/TRPV4 interactions on the primary cilium allow for mechanically stimulated Ca^{2+} influx. PC2/TRPC1 and PC2/TRPV4/TRPC1 heterocomplexes on the plasma membrane facilitate cation transport into the cytoplasm. The proper maintenance of cytosolic Ca^{2+} levels acts to limit the activity of AC5/6, thus regulating the cellular levels of cAMP. Without proper Ca^{2+} signaling, cAMP levels are enhanced, leading to the activation of PKA and numerous downstream pathways to promote cell proliferation and survival. Polycystin 2 (PC2); polycystin 1 (PC1); transient receptor potential cation channel subfamily V member 4 (TRPV4); transient receptor potential cation channel subfamily C member 1 (TRPC1); ryanodine receptor (RyR2); inositol 1,4,5-trisphosphate receptor (InsP3R); endoplasmic reticulum (ER); mitochondrion (Mito); adenylyl cyclase 5/6 (AC5/6); adenosine triphosphate (ATP); cyclic adenosine monophosphate (cAMP); protein kinase A (PKA).