Published in final edited form as:

Physiol Rev. 2025 July 01; 105(3): 1553–1607. doi:10.1152/physrev.00018.2024.

Physiologic mechanisms underlying polycystic kidney disease

Alessandra Boletta[1], **Michael J. Caplan**[2]

[1.] IRCCS San Raffaele Scientific Institute, Milan, Italy

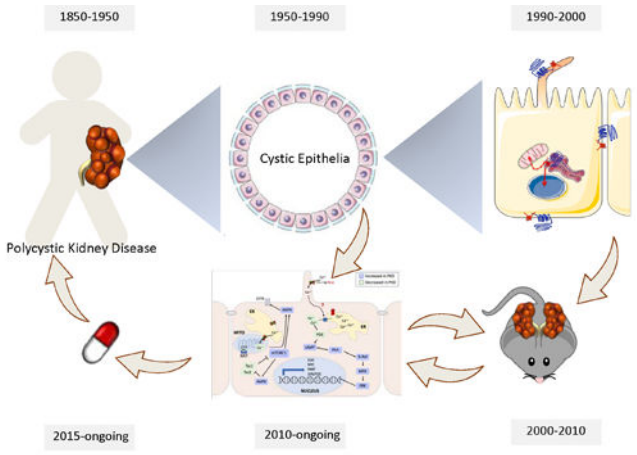
[2.] Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT USA

Abstract

Polycystic Kidney Disease (PKD) encompasses a class of disorders presenting with bilateral cyst formation in the kidney. PKD can be inherited as a dominant (ADPKD) or a recessive (ARPKD) trait, due to mutations into multiple genes, the most frequent being *PKD1*, *PKD2* and *PKHD1*. The protein products of these genes (polycystin-1, polycystin-2 and Fibrocystin, respectively) have been shown to reside within the primary cilium or to be important for the maturation and trafficking of proteins to the primary cilium. The primary cilium is an organelle protruding from the apical surfaces of renal epithelial cells that functions to sense extracellular signals and translate them into intracellular biochemical information. PKD represents the most common monogenic disorder affecting the kidney and the most common manifestation of human ciliopathies. The precise functions of the polycystin and Fibrocystin proteins have not yet been fully elucidated, nor have the molecular basis underlying the renal tubule cyst formation that occurs in the absence of sufficient functional expression of these proteins. The genes that are muted in PKD were cloned three decades ago and since their identification a wealth of information regarding their structure, cell biology and physiological properties has been developed. Here, we provide a broad review of the relevant literature and summarize a large body of experimental evidence, while focusing particularly on more recent findings that are poised to change our understanding of the field.

Graphical Abstract

Contact information: **Alessandra Boletta**: boletta.alessandra@hsr.it, **Michael Caplan**: Michael.caplan@yale.edu.



Overview of the progress on PKD research from the discovery of the disease to the initiation of molecular and pharmacological studies

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most prevalent potentially lethal genetic disease. It affects on the order of 1:1,000 people worldwide and roughly half of these individuals will develop end stage kidney disease, requiring renal replacement therapy in the form of dialysis or kidney transplantation. The are no racial, ethnic, geographic or gender differences in the prevalence of ADPKD. The physical appearance of a kidney affected by late stage ADPKD is quite dramatic. A human kidney is normally between 100 and 200 ml and contains ~1.2 million nephrons. Late stage ADPKD kidneys can exhibit volumes of several liters. This massive size expansion is due to the formation within a few hundred to a few thousand nephrons of large fluid-filled cysts. As these cysts expand, they impinge upon the surrounding normal renal parenchyma, compromising its function. ADPKD is a slowly developing disease process. A person who inherits a mutated form of one of the disease-related genes from one or the other parent may begin to acquire a small number of cysts in early adolescence, and cysts continue to develop and expand over the course of decades. Renal function is usually preserved until patients reach middle age. Patients with ADPKD can accumulate cysts in non-renal tissues, including in the bile ducts of the liver and the secretory ducts of the exocrine pancreas. In an important subset of patients ADPKD is associated with significant non-cystic pathologies, including the formation of vascular aneurysms and cardiac abnormalities. In addition to progressive renal insufficiency, ADPKD patients can develop hypertension and are susceptible to painful cyst rupture, infection and nephrolithiasis. These associated conditions can substantially impact a patient's quality of life.

The identities of the genes whose mutation causes ADPKD have been known for more than two decades. While much has been learned about the structures, localizations and molecular properties of the polycystin proteins that are encoded by these genes, we still lack a consensus on their physiological functions and we do not understand clearly why cysts form in their absence. This review will summarize the extensive and complex literature that reports on the functions of the polycystin proteins and the signaling pathways upon

which they exert profound impacts. Insights into these pathways are yielding new potential therapeutic targets whose development have the potential to alter dramatically the clinical course of this common and often devastating disease.

A. Genetics of PKD

As mentioned above, PKD presents with two distinct modalities of transmission: either autosomal dominant (ADPKD) or autosomal recessive (ARPKD). Besides sharing the key manifestation of developing large and polycystic kidneys, the two disorders are very different (Figure 1). They are caused by inherited mutations in different genes, manifest at different time points and in different regions of the renal tubule, and they show quite different extrarenal manifestations. However, they both belong to the family of genetic diseases known as “ciliopapthies” and share common pathogenetic pathways that likely intersect at multiple levels.

i. PKD1 and 2 genes/polycystins 1 and 2: ADPKD presents with a number of clinical features that allow a diagnosis to be made without performing any molecular testing. The most typical forms of ADPKD are caused by mutations in two genes, *PKD1* and *PKD2,* localized on Chromosome 16p13.3 and on chromosome 4q13 respectively. The two genes were cloned in 1994 and 1996, respectively, through classical positional cloning efforts. Patients inheriting mutations in the *PKD1* gene tend to have a more aggressive form of the disease, while patients with inherited *PKD2* mutations tend to have on average a somewhat milder disease progression. Nevertheless, the two pathologies are indistinguishable based solely on their clinical manifestations. The two genes encode for the two large membrane proteins Polycystin-1 and Polycystin-2 (see below), that interact to form a complex at the plasma membrane, including in primary cilia (Figure 2). This property explains from a molecular point of view the remarkable overlap in their manifestations. Mutations in *PKD1* account for approximately 85% of cases of typical ADPKD, while mutations in *PKD2* account for approximately 15% of typical ADPKD cases (see below minor genes).

a. Two hit model/Loss of heterozygosity: Although ADPKD is inherited in a dominant manner, the disease was described to be recessive at the molecular level. Microdissection studies performed in the mid-1970s on ADPKD kidneys in early stages of disease development had shown a quite remarkable feature of renal cysts in ADPKD, i.e. they appeared as “outpouchings” of the renal tubule forming ballooning structures originating from, and attached to, the renal tubule throughout all tubular segments (Figure 1). This observation had been proposed to be a possible indication of the fact that renal cysts in ADPKD originated from a single cell. Indeed, a subsequent study demonstrated that the cystic epithelia in ADPKD cysts is clonal, i.e. all cells derive from a single cell [Qian 96]. Indeed, the same study had reported that somatic mutations could be detected in the genomic DNA isolated from individual cysts, and that the mutations identified were different from the germline inherited one. This observation led investigators to propose that a “second hit” in the normally inherited allele of either *PKD1* or *PKD2* leading to a loss-of-heterozygosity could constitute the initiating event of cystogenesis, explaining at once both the clonality and the focal nature of cysts. Similar mechanisms were subsequently shown in the liver (1). It should be noted that at the time in which these studies were

first reported, there were major technical problems in sequencing of the *PKD1* gene, due to the fact that approximately two thirds of the gene is present in duplicated regions on the same chromosome. Therefore, only about one third of the gene (the 3' end) could be appropriately sequenced to detect mutations. Consistent with this technical limitation, the original study only reported mutations in approximately 30% of cysts. This observation prompted investigators to challenge the model and to question whether the mechanism of cyst formation was indeed that of a LOH. As the technology was evolving, however, it became clear that more and more cysts manifested with somatic mutations. In fact, a recent report that used whole genome sequencing found that 93% of cysts present with a second hit somatic mutation (2).

b. Haploinsufficiency/third hit model: Immediately after the *PKD1* gene had been cloned, one of the hypotheses formulated was that perhaps the disease could be due to haploinsufficiency, i.e. the heterozygosity was leading to a reduced expression/function of the polycystins to half of the required dosage for maintenance of a normal renal tubule and prevention of cyst formation. Extensive studies from animal models have instead demonstrated that half dosage of the polycystins is sufficient to maintain their function in the renal tubule and thus to prevent cyst formation (while haploinsufficiency could be the cause of some of the extrarenal manifestations of the disease, as evidenced by some animal studies). Elegant studies have provided support for two important concepts: i) haploinsufficiency is not sufficient to initiate cyst formation; ii) reduced expression of Polycystin-1 as low as to 11% of its normal level is no longer able to prevent cyst formation (3). This led to the demonstration that haploinsufficiency is not sufficient to cause initiation of cyst formation, but complete loss of polycystins is not necessary for cysts to initiate. Thus, one could speculate that in the 7% of renal cysts in which the second hit could not be identified, there could be epigenetic, or other types of alterations, that cause downregulation of the polycystins' function below a critical threshold. In strong support of this notion is genetic evidence from humans. Indeed, a rare case has been described of a family presenting with a particularly mild PKD manifestation, with recessive inheritance that was caused by a homozygous inheritance of a hypomorphic *PKD1* mutation (p.R3277C) (4). This study represents landmark evidence of the potential for multiple mechanisms of cyst formation in response to mutations in the *PKD1* gene. Indeed, up to this report, prior evidence had all supported the supposition that homozygous mutations in the *PKD1* gene are not compatible with life. It should be noted that this specific family suffered from a disease that, based on symptoms, should not have been classified as ADPKD, particularly because the cysts in these kidneys appeared as a continuous dilatation of the renal tubule, more similar to the manifestation of recessive PKD. While this case was the only one reported to date and therefore this type of patient appears to be extremely rare, the fact that the parents presented with completely normal kidneys and no evidence of PKD does raise a question regarding the two-hit model of the disease. Indeed, if the combination of two extremely mild mutations in homozygosity is sufficient to cause cyst formation, why do the parents that were carrying the same variant in heterozygosity not present with cyst formation if the somatic mutations in the *PKD1* gene occur stochastically and very frequently? This question remains unanswered to this day. However, additional studies in the mouse have provided evidence of the possible existence of what has been defined as a "third hit".

Indeed, work in the mouse has demonstrated that inactivation of both *PKD* alleles at the somatic level is sufficient to induce cystogenesis, but with a varying degree of delay from the time of inactivation to the time of manifestation. In this context, it has been shown that ischemic injury (5), or other types of insult (6), can favor cyst formation after the second somatic mutation has been acquired by the cell by possibly favoring proliferation. Thus, we could summarize our current knowledge about the mechanism of cyst formation as follows: i) somatic mutations are observed in the vast majority of cysts and are likely the events initiating cyst formation; ii) the time that passes from acquisition of a somatic mutation to a cyst manifestation can be long; iii) external events that provide a tissue insult could contribute to accelerating either the time to cyst manifestation after the second hit has occurred or the actual acquisition of somatic mutation; iv) cyst formation does not require a complete loss of function of the polycystins, thus the second hit could be a mild somatic mutation or even a non-somatic mutation causing a drop in expression of function of the polycystins.

It should be noted that a few caveats remain. One: what if somatic mutations occur after the cells have initiated cyst formation and have started to proliferate? If this were the case, however, one would expect cysts not to be clonal, but rather a mixture of mutants, and this would make it technically impossible to clearly detect somatic mutations, which are instead quite clearly detected. In theory, one such mutant that has acquired a somatic mutation could take over the entire population of cells comprising a cyst. Even in this case, however, one would expect that the percentage of cysts in which a clear mutation can be identified would be much lower than it is (93%). Perhaps the most robust argument that persists against the otherwise widely accepted theory of the second hit as causative in most cyst initiation is the phenotype of the human carriers of the p.R3277C mutation (4). The reason that these particular patients should be protected from acquiring second hits remains quite mysterious.

c. Over-expression: While today there is a general consensus for ADPKD being a loss of function disease, initial studies carried out immediately after the cloning of the *PKD1* and *PKD2* genes led to the proposal that ADPKD might be a gain of function disease. This initial model was based on the evidence that immunostaining of tissues from ADPKD patients had highlighted a strong upregulation of immunostaining using antibodies directed against polycystin-1. Multiple studies in the following years have demonstrated that the specificities of these antibodies were worrisome and thus they could not be considered as providing reliable assessments of polycystin expression in the absence of perfectly matching negative controls. Interestingly, however, two distinct groups had developed transgenic mice carrying over-expression of human polycystin-1 and had reported the development of a PKD-like phenotype (7, 8). Subsequent work has demonstrated that overexpression of up to three copies of the *PKD1* gene do not result in an appreciable renal cystic phenotype (9), and there are no documented cases of human ADPKD that are associated with over-expression of PC1 or PC2. While these discrepancies have yet to be resolved, the general consensus in the field is that loss or reduced function of the polycystins lies at the base of polycystic kidney disease manifestation in humans. However, we can conclude that, unless additional recombination events could be observed in the transgenic animals or additional mechanistic

explanations could be provided, a strong overexpression of the *PKD1* gene could in principle produce PKD.

ii. ARPKD gene/Fibrocystin: The genetics of recessive PKD appears to be much simpler than that of Dominant PKD. Patients inherit two mutated copies of the gene *PKHD1*, which sits on chromosome 6p12. The gene was discovered at about the same time by two groups independently. The first group was able to identify the gene by mapping and cloning the gene responsible for the PKD phenotype in the cpk rat, which had been known for many years and had been utilized as a model of dominant PKD due to its slowly progressive phenotype. The second group identified the gene by classical positional cloning. Both described *PKHD1* as a very large gene encoding for a large protein containing a single transmembrane domain, which was named Fibrocystin (now accepted the name) or Polyductin. The presence of various splicing variants makes the protein a bit more complicated to study than initially appreciated, with possible variants containing extracellular domains lacking the transmembrane domain and thus likely to be released. The intracellular C-tail of the protein can be cleaved in multiple sequential sites through a mechanism that was described to resemble that of the Notch Pathway. Importantly, the *PKD1/2* and *PKHD1* gene can cross talk to each other both genetically and at the protein level. Fibrocystin, like the Polycystins, is localized at cilia where it is believed to exert its most important function.

iii. Minor PKD genes (e.g. GANAB): Finally, as suggested above, more recent clinical and genetic investigations have described additional minor genes involved in Autosomal Dominant Polycystic Kidney Disease. Indeed, today it is estimated that about 5% of ADPKD cases are not caused by mutations in either *PKD1* or *PKD2* (10). For clarity, it should be stressed here that all these cases are described to be atypical cases of ADPKD, with an unusual clinical presentation that can include the formation of multiple very large cysts that are limited in number, with an echogenicity that is different from that of typical ADPKD kidneys. This explains why for two decades almost the totality of patients that were diagnosed with ADPKD based on clinical criteria had been ultimately associated with mutations in either the PKD1 or PKD2 genes, with only very few exceptions. More recent analysis, however, has discovered that some of the “atypical” cases of ADPKD are due to mutations in a list of genes that include several components of the glycosylation machineries important for maturation of membrane proteins in the secretory pathway. It is interesting to note that several of such genes, such as GANAB, were shown to impair the proper trafficking of the polycystins. This, once again, brought the function of the polycystins to the center of the picture and led to the proposal that whenever the function of the polycystins is decreased below a given threshold in a renal epithelium, a cyst ensues.

A similar consideration can apply to a very different type of inherited cystic disorder called Autosomal Dominant Polycystic Liver Disease (ADPLD). In this case, patients inherit one mutant copy of one of a set of genes that are also involved in the maturation and quality control of proteins going through the secretory pathway. For unclear reasons, these individuals acquire a second somatic mutation in the second allele of the same gene, i.e. they undergo a somatic mutation, which occurs only in the cholangiocytes. This, again,

causes trafficking or function of the polycystin proteins to drop below a given threshold of activity ultimately leading to cyst initiation. Of great interest, when these ADPLD genes are forcedly inactivated in the renal epithelium in the mouse, invariably a PKD phenotype can be observed. Indeed, some of these patients do present with the formation of a few renal cysts, though these events are much rarer. Why different genes acquire somatic mutations preferentially in different cell types remains a mystery. But from a clinical point of view, it is interesting to note that ADPKD and ADPLD appear to be a continuum, whereby patients acquire cyst formation in the bile ducts or in the renal tubule at different rates. But the underlying mechanism of cyst formation appears to be due in all of these disorders to the reduced activity of the Polycystins. Finally, atypical cases of ADPKD were also reported to be associated with suspected or proven cases of mosaicism, whereby the *PKD1* gene (in the majority of cases), and the *PKD2* (in a minority of cases), could manifest a germline or a somatic mosaicism, in which case an individual could carry a mixture of cells with wild-type alleles, and homozygote mutant alleles (11, 12).

iv. Genes associated with recessive syndromic cystic diseases (ciliopathies): Finally, a separate mention should be given to the class of disorders collectively called the renal ciliopathies. These disorders can be rare to very rare, tend to be inherited in a recessive manner (with the only exception being medullary cystic kidney disease which is inherited in a dominant manner) and manifest with different degrees of cystogenesis. These disorders are typically syndromic and renal cyst formation is just one of the manifestations. These types of disorders can be very diverse and include pathologies such as Bardet-Biedl Syndrome (BBS), Nephronophthisis (NPHP), and oral-facial-digital syndrome (OFD) among others. In several of these syndromic diseases formation of multiple renal cysts can be appreciated. They are all classified as ciliopathies because they are most often caused by mutations in genes whose function is essential for the normal function of primary cilia, and the resulting cyst formation is likely due to dysfunction of this organelle. If and how these disorders are functionally linked to Polycystic Kidney Disease and to the *PKD1/2* or *PKHD1* genes and their products remains unclear and will likely be the subject of studies in the years to come.

I. Physiology and cell biology of the polycystin proteins

A. Structures of the polycystin proteins and the polycystin complex

i. Primary structures, proteins motifs, homologies: Polycystin-1 (PC-1) is a large transmembrane protein of 4302 amino acids, with a predicted molecular weight of a 462kDa and an apparent molecular weight of ~520 kDa, most likely due to its abundant glycosylation (13, 14). The large N-terminal region (3072 aa) of the protein is structured in multiple domains that include leucine-rich repeats (LRR), a cell wall integrity and stress response component (WSC), a C-type lectin domain (CL), a low density lipoprotein-like domain (LDL-A domain), 16 copies of immunoglobulin (Ig)-like polycystic kidney disease (PKD) domain (called the PKD repeats) and 1000 aa long Receptor for Egg Jelly (REJ) region that takes its name from its high degree of homology with the receptor for the Jelly in the Sea Urchin. This last region was shown to contain a FNIII repeats domain and a less structured region (14, 15) (Figure 2).

Based on these studies it is conceivable that PC-1, by virtue of its extendible N-terminal domains (both the PKD and the FNIII repeats) acts as a mechanosensor (16) (17). A cryoEM structure of PC-1 containing its ectodomain is unfortunately not available yet and further studies will be required to understand the function of the N-terminal domain in the context of the PC-1/PC-2 native complex(18).

Immediately C-terminal to the extracellular ectodomain, the protein possesses a proteolytic site which shares homology with a G-protein coupled receptor proteolytic site (GPS), an autoproteolytic site shown to release the entire N-terminal domain (19). Following the GPS site the protein has 11 transmembrane (TM) domains, alternated by five intracellular and five extracellular loops. The first intracellular loop hosts a PLAT domain (polycystin-1, lipoxygenase, and alpha toxin) previously shown to bind lipids and the third extracellular loop contains a large domain called the TOP domain, sharing homology with the PKD2 protein, PC-2, and described to have important functions in channel gating. Finally, the short intracellular C-terminal domain (C-terminal tail, CTT) of approximately 198aa contains a coiled-coil domain, a putative G-protein binding domain, two putative polyproline motifs, one of which was shown to actively bind SH3 domains, a nuclear localization signal and a mitochondrial localization signal(20–23). Of interest, some of these domains overlap in identical regions, possibly suggesting the mutual exclusion in the localization and functional properties of this multitasking receptor (20–23).

PC-1 interacts with PC-2, a TRP-channel family member sharing homology with voltage-activating calcium channels and which has been reported to function as a cation channel with different cation permeability preferences depending on whether it is assembled as a homotetramer or as a heterotetramer associated with PC-1 subunits (see below, channel properties of PC-1) (24) (25).

ii. Cryo EM 3D structures of polycystin 2 homotetramer and polycystin 1 and 2 heterotetramer: Recent advancements in the technology for performing CryoEM studies have enabled progress in our understanding of multiple complex protein structures formed by the assembly of several subunits. The ADPKD field particularly benefitted from these technological advancements. Shortly after the cloning of their two encoding genes the PC1 and PC2 proteins had been described to form a protein-protein interacting complex (26, 27). Homology to previously identified proteins led to the classification of PC-1 as a membrane receptor capable of interaction with multiple protein-protein interactions likely relevant for cell adhesion, while the PKD2 protein was immediately postulated to be a channel based on its homology to voltage-dependent calcium channels (28). Further to this, both proteins were found to contain C-terminally located coiled-coil domains that were demonstrated to mediate an interaction between the two proteins (26). Subsequent models had therefore been hypothesized whereby the PC1 functioning as an adhesion or mechanosensory receptor could modulate the activity of the PC2 channel (29). These models, however, did not take into consideration an important structural feature of PC1 described above and that is the strong homology identified between the last six transmembrane domains of PC1 with the six transmembrane domains of PC2 (28) (Figures 2 and 3). This homology played an important role in the identification and cloning of the *PKD2* gene two years after the *PKD1* gene had been identified (28). When the first

channel activity of the PC1/PC2 complex was identified in CHO cells overexpressing both proteins, it was hypothesized that the PC-1 protein was essential for the trafficking of the PC2 protein to the plasma membrane where the novel channel activity could be recorded (30). These results were subsequently validated by some investigators, while they could not be reproduced by other investigators (31). The first cryoEM structure of the PKD2 protein in lipid nanodiscs had identified the presence of the expected homotetrameric structure of the PKD2 protein (32). The data had shown that the structure of this homotetramer was consistent with the channel having a pore domain that is too small to conduct calcium was seen as corroborating evidence for the previously reported preference for conducting small cations such as Na^+ and K^+ over the conductance of large cations such as Ca^{++} (32). Subsequent work has determined the cryo-EM structure of the PC1/PC2 proteins in a complex (18) (Figure 3). In this case, the structure showed that both PC-1 and PC-2 can assemble into a pore-forming channel with a stoichiometry of 1:3 (18, 24, 25), supporting previous biochemical evidence of this stoichiometric ratio(33). This study employed a PC1 construct that lacked the NTF and C terminal tail domains, and thus it does not provide insight into these critical components of the PC1 protein. The information that this work has provided on the organization of PC1 transmembrane segments in association with those of PC2, however, has been wonderfully illuminating. The fact that the last six transmembrane domains of PC1 could assemble with three subunits of PC2 to constitute the pore-forming structure of a PC1/PC2 heterotetramer provided a biological rationale for the strong homology between the last six transmembrane domains of the two proteins (32) (Figure 3). Evidence collected from these studies also highlighted that the interaction between PC-1 and PC-2 does not only occur via the intracellular c-terminal tail containing the coiled-coil domains, but also via the transmembrane domains, further corroborating the formation of a channel complex (18). Electrophysiological studies using patch clamp analysis of cells has provided supportive functional evidence for this conclusion (see the section entitled “Ion channel capabilities of the polycystin proteins”).

B. Localizations of the polycystin proteins

i. Tissue localizations: Immediately after the cloning of the *PKD1* gene(34–37), numerous and extensive efforts were undertaken to immunolocalize PC-1 (and subsequently PC-2), by generating antibodies raised against different epitopes of these proteins (38) (39). While at the time these studies were initiated there was no accepted positive (over-expression of the proteins) or negative (reduced-expression of the proteins by knock-down or knock-out) controls that could be used to validate the reagents and provide solid support to the results, a plethora of different efforts delineated a quite detailed similar picture of the tissue distribution of the proteins (38, 40) (39). With the advent of subsequent technologies and the capability to accurately characterize the antibody specificity several studies were published to revise or reinforce the original findings (13, 41). In particular, while PC-2 antibodies were readily available and have been validated in multiple conditions by multiple groups, the anti-PC-1 antisera appeared to be more problematic, with positive staining being observed also in knock-out tissues. Nevertheless, while caution should be used when interpreting some of the earlier studies, today a consensus picture of the results can be gathered. Both PC-1 and PC-2 appear to have a quite wide distribution in multiple tissues, besides the kidney and liver that represent the primary sites for disease manifestations (21,

38) (39, 42). In the kidney, both PC-1 and PC-2 transcript, protein and posttranslational modifications are modulated during development with maximal protein expression being observed during pre-natal development and expression levels (at least of PC-1) dropping below detectability in the adult tissue, unless specific enrichment strategies such as immunoprecipitation are used to enhance the signal (21) (43, 44). Microarrays and RNA sequencing data have confirmed that the expression of the *PKD1* and *PKD2* genes is almost ubiquitous, with reasonable expression levels being detected in endothelial cells from the vasculature in both blood vessels and lymphatics and in vascular smooth muscles (45–47). These data are not surprising given the cardiovascular manifestations of the disease which include aneurysm formation and hypertension. Additional sites of expression are the liver, where both hepatocytes and bile ducts epithelia are found exhibit the expression of both transcript and proteins, in line with some of the hepatic manifestations of the disease(41). Similarly, pancreatic ductal cells were found to be positive for expression of the *PKD1* and 2 genes and proteins, again in line with the clinical manifestations of the disease that include a minority of patients presenting with the formation of pancreatic cysts(38–40). Additional sites for expression of the polycystins are the testis, bone, muscle (both skeletal and cardiac muscle), skin and nervous system including olfactory epithelia, mesenchymal cells and adipocytes. Very informative studies on the multiple tissue distributions of PC-1 came from studies describing the expression of tagged, endogenous PC-1. Tagging the C-terminus of endogenous PC-1 with either a 3xHA or a 5xMyc tag resulted in a fully functional protein that was able to rescue lethality in knock-out mice(43). Analysis of the different tissues confirmed the broad distribution of PC-1 in multiple tissues and unexpectedly revealed that the lung is the tissue with the highest expression levels of PC-1 in adult animals (43). Of interest, while PC1 was found to be developmentally regulated, with a drastic drop of expression in the kidney after the first few days of life, the expression levels in the lung were found not to drop after birth nor in the adult (43). Additional sites of expression were the central nervous system, where the *Pkd1* transcript and protein were found in multiple regions of the CNS. Of interest, cells of the ependyma and of the choroid plexus were found to express reasonably high levels of PC-1 and in line with these findings, inactivation of the *Pkd1* gene in these sub-compartments resulted in hydrocephalus, consistent with the presence of the protein in the cilia of these tissues (see below) (43). Finally, of interest embryonic stem cells (ES) of the mouse also express reasonable levels of the *Pkd1* gene (Boletta and colleagues unpublished).

The quite wide distribution of the polycystins is of great interest and raises the question as to why the disease manifestations appear to be so narrowly limited to certain tissues if the expression of the Polycystin proteins is much wider. One possibility is that the mechanism of second hit mutagenesis (48), proposed to serve as the basis for disease initiation in most cases (48), at least for focal manifestations such as cysts, only occurs in specific tissues. A second possibility is that the inactivation of the PKD genes provides a growth advantage to cells in some tissues, but not in others. An additional possible explanation is that the function of the PKD genes is essential in certain organs, but not in others. Indeed, inactivation of *Pkd1* gene in multiple tissues including vessels, bone and CNS were reported to generate quite severe phenotypes in the mouse (43). However, inactivation of the *Pkd1* gene in the skeletal muscle or in Schwann cells in the peripheral nervous system (both

tissues that express the Polycystins) did not result in any overt manifestation (Boletta and team unpublished).

ii. Subcellular localizations: The subcellular distribution of PC-1 and PC-2 has also been subject to the same difficulties that were encountered in establishing their tissue distributions, i.e. the difficulty to identify antibodies that would be sufficiently specific and sensitive to enable their use for precise subcellular localizations. PC-1 was initially described as being present at cell-cell junctions, either adherens junctions or desmosomes, where functional assays also indicated that it could play a role, and at cell-matrix interfaces such as focal adhesions (41, 49, 50). PC-2 was reported to be predominantly and abundantly in the endoplasmic reticulum, where it was described to have a possible specific function in calcium release from this compartment. When heterologous overexpression systems became available it was shown that over-expression of PC-1 could drive PC-2 localization to the plasma membrane and that PC-2 overexpression can drive membrane localization of PC-1, although this inter-dependence was not always observed (30).

The one subcellular localization that today has been validated by multiple scientists and is universally accepted for the polycystins is that in primary cilia (see also the chapter on cilia) (51, 52). Both PC-1 and PC-2 were reported to be present in primary and motile cilia in multiple different cell types, organs and tissues. Trafficking of the polycystins to primary cilia is not only demonstrated to be an essential process of the functional maturation of the polycystin complex (with specific calcium-channel activity being recorded in individually patch-clamped cilia), but also their ciliary trafficking has been demonstrated to be essential for preventing cyst formation in the kidney (see also section on trafficking) (Figure 4)

Additional membrane-associated subcellular localizations, for PC-1 in particular, were also reported. Indeed PC-1 undergoes multiple alternative cleavages releasing different fragments of the protein (see also the chapter on cleavage). The most commonly observed cleavage product, CTF which retains all 11 transmembrane domains, has been shown to be present at mitochondrial associated membranes of the ER (MAMs, also called mitochondria-ER contact sites or MERCs) where it possibly regulates mitochondrial calcium uptake (53). A second, alternative, cleavage product called p100 (see section entitled p100) which most likely retains the last six transmembrane domains of the protein, was reported to localize within the ER and to mediate Calcium uptake from the cytosol in conjunction with the protein STIM1 (54). Additionally, two smaller fragments released from cleavages that occur after the last transmembrane domain create soluble portions of the PC-1 C-terminal domain: one of approximately 25–30 kDa that should comprise the whole c-terminal tail of PC-1 was reported to either translocate to the nucleus where it can modulate transcription by direct interaction with co-factors mediating the DNA binding activity of specific transcription factors (55) (56), or else to the mitochondrial matrix where the protein can interact with multiple partners (23). Finally, a smaller, 17 kDa membrane-free fragment, likely released from the last portion of the C-terminal tail, was also shown to translocate to the nucleus where it modulates the function of the STAT family of transcription factors (57). All of these fragments have been reported to have different functions and localizations, which may be regulated by the cell's metabolic or redox state (58). Whether these multiple cleavages occur sequentially, releasing progressively smaller portions starting from the same longer C-

terminal fragment or whether each is generating starting from the same Full-length protein is currently unknown. Likewise, if and how a corresponding N-terminal portion for each of these cleavage products is released remains unknown (Figure 2).

iii. Exosomes: In addition to the above-mentioned subcellular localizations, the polycystins have also been localized extracellularly. In particular, the large ectodomain of PC-1, following cleavage at the GPS site, can be shed into the medium as assessed in PC-1 overexpression systems (19). Furthermore, several isoforms of both PC-1 and PC-2 have been reported to be contained in extracellular vesicles such as exosomes (59). The first evidence that this was the case derived from scanning electron microscopy studies that detected numerous extracellular vesicles associated with primary cilia on the apical side of renal epithelia (59). This prompted the investigators to analyze their content and to discover that PC-1, PC-2 and also FPC (Fibrocystin, Polyductin the protein encoded by the gene mutated in recessive PKD, *PKHD1*) were found associated with these extracellular structures (60). Notably, ever since this first finding, multiple investigators have confirmed the association of the Polycystins with exosomes. Exosomes are membrane-bounded structures that can be released by cells into the extracellular milieu. These findings were also confirmed in lower organisms such as the worm C. Elegans. In addition, it was discovered that exosomes and small vesicles can be released directly from cilia, a process subsequently confirmed and described in multiple cell types of different tissues of origin (61). These studies have triggered active research in the field of cell-to-cell communication that could be mediated by ectosomes released and sensed at primary cilia (61, 62).

In addition, it has been suggested that exosomes might contain important information regarding their cells of origin Their analysis performed on the urine of healthy and affected individuals seem to capture important signatures that are specific for the PKD disease state(63). Thus, exosomes have been proposed as a good source for analysis and identification of specific biomarkers of disease progression. Notably, more recent work has highlighted the fact that the expression levels of the polycystins themselves on exosomes might serve as good indicators of disease progression, and/or could possibly be utilized as evidence of target engagement in therapeutic approaches aimed at developing therapeutics that could enhance expression of PC-1 as a way to retard disease progression, such as treatment with anti-miR-17 that are currently being developed as a possible therapy for the disease(64). Indeed, this approach has been shown in animal models to profoundly change the expression of a number of genes that are detrimental for PKD progression, and among these genes investigators have shown that the *PKD1* transcript and protein are themselves targets for this anti-miR molecules(64). Based on this, the expression levels of PC-1 on exosomes seems to be a quite specific way to test whether the compound has reached the kidney and that it is effective in engaging its target, using a non-invasive readout.

C. Evidence for a role of the cilium

In the late 1990s and early 2000s key evidence was collected demonstrating that primary cilia are fundamentally related to polycystic kidney disease. The first study described the identification of a novel gene involved in mating behavior in the nematode C. Elegans, that shared homology with the *PKD1* gene product Polycystin-1. The gene, named lov-1, was

found to play a fundamental role in ciliated sensory cells in the worm (65). Furthermore, this very same study reported evidence that GFP-fused lov-1 and GFP-fused PKD2 localized in the cilia of multi-ciliated sensory cells in the worm (65). A second study shortly after reported that the gene Tg737, long before identified in a mouse screening as a mutant causing PKD (66), encodes the protein IFT88, an intra-flagellar transport protein sharing high homology with the corresponding protein in the algae *Chlamydomonas,* and that their disruption resulted in defective flagella/cilia (67). The IFT proteins had long been studied and known to regulate critical properties of the *Chlamydomonas* flagelli, thus connecting the PKD field to decades of accumulated knowledge on these fundamental organelles. Two additional studies published shortly later deserve further highlight here. The first one was the report that mechanical bending of primary cilia in cultured renal epithelial cells (MDCK) was sufficient to regulate a specific ER-calcium release into the cytosol, initiated by a membrane-bound calcium channel (68). At about the same time it was demonstrated that the Polycystins assemble into a complex that could generate a calcium flux on the membranes of cells, confirming the prediction based on the homology between Polycystin-2 and voltage-gated calcium channels, described when the *PKD2* gene had been cloned (30). These several lines of evidence, collectively, resulted in the initial formulation of the hypothesis that sensory cilia might perhaps play an important role in renal cystogenesis (14), a concept that has since been firmly proven (see below). The evidence of the localization of the mammalian polycystins in primary cilia came shortly thereafter (51, 52) while tagging of the endogenous murine Polycystin-1 subsequently confirmed its localization both in monociliated and multiciliated cells (43). These studies officially opened the doors for two decades of intense investigations.

i. Structure of the cilium: The primary cilium is a cellular antenna that protrudes from most cell types in the human body. It has the appearance of a string-like structure extending from the membrane of the cell for a few micrometers and with a cross section of less than one micron (69, 70). In polarized epithelia (such as renal tubular cells) it extends from the apical side of the membrane, and it protrudes well beyond the microvilli of cells that are endowed with brush borders. Major structural components of the primary cilium are the basal body, the axoneme and the transition zone (69, 70). Despite its apparent simplicity, the building, maintenance and functioning of this organelle is extremely complex (69, 70). Indeed, the process of building a primary cilium, called ciliogenesis, initiates when the mother centriole of a non-dividing cell migrates towards the apical side to become the basal body of the nascent cilium, and starts extending a microtubule-based structure (71). The membrane surrounding the primary cilium is in continuity with that of the apical side of the cell, yet both the protein and lipid composition of the cilium is highly specialized (69–72). This compartmentalization is achieved by a structure located above the basal body, called the transition zone. The transition is characterized by Y-shaped assemblies called the transition fibers, which are constructed to create a gating filter for proteins and compounds that can enter the axoneme, with a cut-off of ≈70kDa (69–72). The axoneme is composed of a central microtubule core (typically 9+0) that serves as a rail along which “trains” composed of intraflagellar transport proteins (IFT) move up (towards the tip) or down (towards the base) using kinesin-2 or dynein-2 as motor proteins, respectively, to transport key proteins and receptors whose expression, retrieval and function needs to be precisely regulated (69–72).

ii. Functions of the cilium: The function of the primary cilium seems to be very diverse and highly dependent on the cell type and tissue context (69–72). As a general overarching function, primary non-motile cilia are sensory organelles that perceive signals from the extracellular environment ranging from mechanical stimuli to chemical or physical cues, and translate them into intracellular biochemical responses dictating important downstream cellular functions such as cell proliferation, apoptosis, migration and cell fate decisions (69–72). In keeping with the broad distributions of these tiny and apparently simple organelles, their sensory properties can be extremely varied, and are determined by the types of receptors they express on their membrane in the different tissues at different time-points (69–72).

i. Role of the cilium in flow sensing/mechanotransduction: One of the important functions of the primary cilium is that of perceiving extracellular mechanical forces and translating them into intracellular biochemical pathways (73). In general terms mechanical stimuli are perceived by receptors referred to as mechanosensors that can be positioned strategically at cell-cell interaction sites, at cell-matrix interaction sites, on the apical membranes of polarized cells (epithelial or endothelial) and also on primary cilia in those cell types that possess them. The first evidence that primary cilia might activate intracellular pathways upon mechanical bending came from landmark studies that demonstrated that bending of primary cilia either by applying an apical membrane flow on renal epithelial cells (MDCK) grown polarized, or by pulling on primary cilia using a micropipette, was sufficient to elicit an intracellular calcium release signal from intracellular ER storage sites mediated by IP3 receptors (68). Subsequent work has been performed on a variety of different cell types including primary renal epithelial cells isolated from the murine kidney, or on primary endothelial cells. In this case, applying an apical flow was able to elicit a different pattern of intracellular calcium release mediated by IP3 receptors (74). Of interest, this type of signal was almost completely lost when measured in primary epithelial or endothelial cells isolated from *Pkd1* mutant mice(75). This finding birthed a new hypothesis that posited that primary cilia on the apical side of renal epithelia perceiving changes in the nascent urine flow through a mechanism that was largely mediated by the polycystins (74). Subsequent work from multiple labs has questioned the validity of the proposed model based on various considerations, including the fact that a small calcium influx that could be observed into primary cilia upon bending did not appear to be mediated by the polycystins. Furthermore, analysis of the calcium influx into the ciliary axoneme at timepoints within milliseconds demonstrated that a sharp peak of intra-axonemal calcium could be measured, but this did not result in a broader calcium release from the ER. Conversely, inducing a strong calcium release from ER intracellular stores did result in elevated calcium levels being detected in the axoneme, leading to a reversal of the proposed model, i.e. that the intraciliary calcium elevation could be produced as a consequence of calcium release from the ER, but that the opposite, i.e. calcium influx into cilia driving release of a larger calcium pool from the ER, did not occur. It is important to note, however, that with regard to the capability of the polycystins (and in particular of polycystin-2) to mediate a calcium influx into the ciliary cytosol, the initial controversial findings have been validated. Indeed, many different laboratories were able to measure a polycystin-mediated ciliary calcium channel, at least in renal epithelial cells and in nodal monocilia.

ii. Role of the cilium in ligand-mediated signaling pathways (e.g. Hedgehog): In addition to their role as a mechanosensors, primary cilia also host a variety of receptors on their membrane whose activation occurs specifically on primary cilia. Indeed, multiple signaling pathways are activated in primary cilia including Receptor Tyrosine Kinases (RTKs), members of the TGF β family of receptors and G-Protein Coupled Receptors (GPCRs) whose expression on the membrane of cilia varies between different tissues and cell types (70–72).

Among the signaling pathways that are affected by primary cilia the one that has been most robustly linked to the essential role of cilia is that of Sonic Hedgehog, which strictly depends on the presence of cilia for its activation during development. Indeed, among the multiple signaling pathways modulated by cilia, the Shh is the only one that appears to be highly specific for primary cilia and not activated in other cellular sub-compartments. In this pathway the main receptor is called PTCH1 and it is normally localized in the ciliary membrane from where it inhibits the receptor smoothened (SMO) (71, 72). Under these circumstances, the signal transducer Gli is cleaved to generate a repressed form which inhibits hedgehog-dependent transcription. In the presence of the ligand hedgehog, PITCH is removed from the membrane of cilia, its inhibitory activity on SMO is released and in turn SMO is able to induce activation of the Gli transcription factor thus inducing transcription of hedgehog-dependent transcript (71, 72). This complicated regulatory circuit ensures a precise and timely activation of the pathway particularly relevant during development in which it plays a fundamental role. Despite almost two decades of studies on the HH pathway, the only pathway firmly linked to cilia, many molecular details are still missing on the regulation of the pathway, including how the activating or repressing forms of Gli are generated (72).

The second most represented type of signaling cascade activated in cilia is the one that lies downstream of G-protein-coupled receptors A GPCR-mediated ciliary-specific cAMP signaling output has been described using optogenetic tools (69). These studies show that this cAMP signal substantially differs from non-ciliary activated cAMP pathways. As mentioned above, several additional cascades can be regulated by primary cilia, including Receptor Tyrosine Kinases (RTK) such as PDGF receptor and the TGF-β/BMP signaling pathway. The last two might be particularly relevant on the context of kidney disease where the TGF-β/BMP pathway is the most important signaling cascade resulting in fibrosis when hyperactivated (70, 71).

iii. Dual Role of Cilia in Renal Cystogenesis: the CDCA pathway: When considering the role that cilia or the polycystins localized at cilia play in the prevention of renal cystogenesis, it is important to mention here that several studies have pointed to a quite complicated interdependence (76) (Figure 5). First of all, while deletion of cilia in the renal tubules by ablation of key ciliary genes such as Kif3b or Ift88 invariably manifests with a robust cystogenesis, it should also be considered that the phenotypes of these mutants is substantially less severe than the phenotypes observed when the polycystins are deleted from the same cell types. Furthermore, and quite unexpectedly, co-deletion of the same ciliary genes *Kif3b* or *Ift88* along with the polycystins causes a quite robust amelioration of the polycystin-depletion induced cystogenesis (76). This conundrum has been explained

by the hypothesis that the polycystins in cilia negatively regulate a specific signal which is in itself pro-cystogenic and depends on primary cilia to be activated (76). This pathway has been defined as cilia-dependent cyst-activating pathway (CDCA) (Figure 5). In this scenario one would imagine that removing the polycystins without interfering with primary cilia structure could cause a massive and continuous activation of a signal that by itself and by default would induce cystogenesis. Hence, when the polycystins are removed from cilia the cystogenic pathway is strongly activated. Based on the assumption that this still elusive signal would require primary cilia for activation, if primary cilia are removed in the absence of the polycystins one would indeed expect a rescue of the cystogenic phenotype, which has been observed (Figure 5). While such a mechanism might appear to be convoluted, a similar regulatory mechanism governs the activation and inhibition of the most emblematic pathway mediated by cilia, i.e. that of sonic hedgehog (70). Thus, in principle, this mechanism is in line with previously identified counter-regulatory processes occurring at cilia. The CDCA pathway, however, does not really explain why removal of cilia is sufficient to induce cystogenesis. Indeed, if the pro-cystogenic signal requires cilia, their removal should by default shut off the activity of this pathway. This seems especially logical because the polycystins should still be expressed in cells that lack only cilia(76). A recent step forward has been made in identification of the molecular components regulating this pathway. Using translating ribosome affinity purification followed by RNA sequencing in murine models carrying mutations in the Pkd genes or in the Pkd-cilia composite mutants, investigators have identified a translatome signature that appears to belong to possible CDCA components. Following up on this observation, investigators have identified the transcription factor Glis2 as a candidate responder of the pro-cystic pathway. Indeed, *Glis2* overexpression in vitro mirrors the effect of Pkd gene inactivation and its deletion *in vivo*, or targeting with an Antisense oligonucleotide (ASO), retards disease progression. Multiple pathways might converge on regulating or being part of the defined CDCA pathway (77). Recent work suggests that one of the key components might be represented by key regulators of cellular metabolism, such as glycolysis and glutaminolysis, which were found to be dysegulated in an opposite manner when the Polycystins are removed (78, 79), or when cilia are removed (see also below) (80).

Assembly and trafficking of the polycystin proteins

Any discussion of the trafficking of the polycystin proteins needs to begin with the disclaimer that we do not yet have a complete picture of their functionally relevant subcellular distributions. It is clear that both of the polycystin proteins localize to the primary cilium and furthermore that this localization appears to be critically related to at least some of these proteins' physiological functions (51, 52, 76, 81). It is also clear, however, that the cilium is not the only sight of polycystin accumulation and activity. Recent studies demonstrate, for example, that pools of both PC1 and PC2 that are present in the endoplasmic reticulum participate in processes that are relevant both to their normal physiological functions and to the suppression of cystic disease (53, 82, 83). Similarly, the polycystins have been detected in the plasma membranes of renal epithelial cells, where they may contribute to cell-cell and cell matrix adhesion (49, 84–88).

Biosynthesis, membrane insertion, glycosylation and quality control

The trafficking of newly synthesized membrane proteins begins at their site of synthesis (89, 90). The mRNAs that encode multi-spanning transmembrane proteins such as PC1 and PC2 are translated on polysomes that become physically bound to the membrane of the rough endoplasmic reticulum (91, 92). The protein products of this translation are co-translationally inserted into and woven across the membrane of the endoplasmic reticulum (93). The targeting of polysomes engaged in translating membrane proteins to the surface of the rough endoplasmic reticulum is mediated by linear sequences embedded within the proteins themselves. Many membrane proteins, including PC1, are initially directed to the rough endoplasmic reticulum by virtue of an N terminal signal sequence that is recognized as it emerges from the ribosome by a cytosolic complex of protein and RNA called the Signal Recognition Particle (SRP) (94). This interaction with the SRP guides a polysome that is engaged in synthesizing a nascent membrane protein to docking sites on the rough endoplasmic reticulum and ultimately to the translocon, a transmembrane complex of proteins that serve as a portal through which the nascent protein is threaded into and across the membrane of the endoplasmic reticulum (95). In the case of multi-spanning proteins such as PC1, specialized set of translocon components acts as a tunnel that can open in two modes in order to facilitate the insertion of proteins across and into the membrane with the correct topology (96–98). Hydrophilic portions of the nascent chain that are destined to be exposed at the non-cytoplasmic surface of the membrane traverse the pore of the translocon and are delivered into the lumen of the endoplasmic reticulum. Hydrophobic sequences that are destined to serve as transmembrane domains exit the translocon laterally to enter the lipid bilayer membrane of the endoplasmic reticulum. For many but not all transmembrane proteins that possess an N-terminal signal sequence, this motif is cleaved by the signal peptidase enzyme, which resides in close proximity to the translocon, during or immediately after the nascent protein's membrane insertion (99). Presumably, this is the case for PC-1, although it does not appear to have been determined directly whether its signal sequence is, in fact, removed. It is important to note that many transmembrane proteins, including PC-2, do not possess N-terminal signal sequences. For these proteins, the first transmembrane domain to emerge from the ribosome as the polypeptide chain elongates serves as the motif that targets the nascent protein and the polysome engaged in its synthesis to the rough endoplasmic reticulum (93).

As the hydrophilic domains of secretory and membrane proteins emerge from the translocon tunnel into the lumen of the endoplasmic reticulum they become substrates for a variety of post-translational modifications and maturation processes, including glycosylation, disulfide bond formation and chaperone-assisted folding. Both PC1 and PC2 are modified through N-linked glycosylation (100). The covalent addition of N-linked sugars can occur on a subset of exposed asparagine (N) residues. The canonical motif that is recognized by the enzymatic machinery that mediates this N-linked glycosylation is the tri-peptide sequence Asn-X-Ser/Thr (101). Addition of N-linked sugars involves transfer of a pre-assembled branched polysaccharide chain that is covalently linked to dolichol phosphate, a transmembrane lipid molecule. The N-linked sugar chains that are added in the endoplasmic reticulum have a stereotypical composition and structure that includes two N-acetylglucosamine residues, nine mannose residues and three glucose residues. This "high mannose" sugar assemblage

undergoes modifications that include trimming of all but three of the mannose residues and subsequent addition of N-acetyl glucosamine, galactose and sialic acid residues as the newly synthesized protein travels along the secretory pathway from the endoplasmic reticulum through the successive cis, medial and trans cisternae of the Golgi complex (101, 102).

Prior to embarking on its journey along the secretory pathway, a newly synthesized protein must undergo an inspection of the state of its folding and assembly by the quality control machinery that resides in the lumen of the endoplasmic reticulum. Mis-folded proteins or incompletely assembled multi-protein complexes are recognized and prevented from departing the endoplasmic reticulum. This recognition and retention process involves a complex and elegant interplay that employs enzymes that modify the protein's sugar structure as well as sugar binding lectins that are associated with the luminal surface of membrane of the endoplasmic reticulum (103, 104). The oligosaccharide that is transferred from the dolichol phosphate lipid to a newly synthesized secretory or membrane protein includes three glucose residues at the terminus of one of its branched chains. The first two of these glucose residues are trimmed in succession by the endoplasmic reticulum resident glucosidase I and glucosidase II. The remaining glucose residue can be recognized and bound by calnexin or calreticulin, two lectin proteins that are resident in the endoplasmic reticulum. Binding of the monoglucosylated protein to calnexin or calreticulin can lead to retention of the newly synthesized protein in the endoplasmic reticulum and can facilitate chaperone interactions that contribute to the protein's attaining its fully folded conformation. Enzymatic trimming of the remaining glucose residue eliminates the capacity of calnexin or calreticulin to bind and retain the protein in the endoplasmic reticulum. If, however, the protein has not yet completed its folding or has assumed a mis-folded conformation then the proteins is recognized by UDP-glucose:glycoprotein glucosyltransferase 1, which detects incompletely folded proteins and adds a single glucose residue back to its oligosaccharide chain (105, 106). The re-glucosylated protein is thus once again a substrate for calnexin or calreticulin-mediated retention in the endoplasmic reticulum. Thus, the surveillance function conducted by UDP-glucose:glycoprotein glucosyltransferase 1 ensures that only fully and correctly folded proteins are permitted to exit the endoplasmic reticulum. The mis-folded proteins are retained in the endoplasmic reticulum until either they are folded sufficiently well to avoid detection by UDP-glucose:glycoprotein glucosyltransferase 1 or until they are marked for degradation.

The preceding rather detailed discussion of the initial stages in the synthesis and maturation of transmembrane proteins is occasioned by the fact that these processes appear to be especially complex or critical in the case of the polycystin proteins. Five of the genes whose mutation leads to autosomal dominant polycystic liver disease encode proteins that are components of the endoplasmic reticulum protein complexes that mediate the co-translational transmembrane translocation and sugar processing of newly synthesized membrane proteins (Figure 4). These include SEC63 (107), GANAB (108), PRKCSH (109), ALG8 and SEC61B (110). SEC61B encodes one of the three subunits that together form the primary pore of the translocon tunnel (111). The sec63 protein encoded by the SEC63 gene is a component of the translocon that appears to modulate the open state of the translocon's lateral gate (112). In the absence of sec63 the lateral gate may be unable to open, thus preventing newly synthesized membrane proteins from being able to access the bilayer. Both

GANAB and PRKCSH are components of the glucosidase II. The GANAB gene encodes the catalytic α-subunit of glucosidase II, while PRKCSH encodes this enzyme's non-catalytic β-subunit. Both subunits are necessary to achieve the levels of glucose trimming activity that is required for a newly synthesized protein's sugar chains to undergo appropriate maturation and thus to permit the protein's departure from the endoplasmic reticulum. The protein encoded by ALG8, alpha-3-glucosyltransferase, is required to add the second glucose residue to the sugar tree as it is synthesized in association with the dolichol phosphate intermediate (113).

It is quite surprising that mutations in genes encoding several components of the machinery that is required for the general membrane insertion, post-translational processing and quality control of all newly synthesized membrane proteins leads to polycystic liver disease and not to more profound, multi-organ pleiomorphic phenotypes. These findings suggest that the initial stages of the biosynthesis of the polycystin proteins are likely to be especially complex and dependent upon the participation of these participants in the endoplasmic reticulum's translocation and quality control apparatus. Strong evidence in support of this interpretation is provided by the observation that in cell lines harboring these mutations the levels of mature polycystin-1 protein are substantially reduced (110). Furthermore, in mice that carry mutations in PRKCSH or SEC63 the severity of the renal cystic disease that is observed is directly related to the amount of PC-1 protein that is expressed (9). Genetic reduction in the levels of the PC-1 or 2 proteins exacerbates cyst development, whereas modest over-expression of polycystin-1 or 2 from BAC transgenes can suppress the disease phenotype. Similar observations have been made in the context of the renal cystic disease that is caused by the mutational loss of the ER chaperone protein DNAJB11 (114). Taken together these data suggest that the key perturbation that leads to cystic disease for each of these mutations is a reduction in the levels of functional polycystin proteins. Thus, the post-synthetic maturation of the polycystin proteins appears to be uniquely sensitive to perturbations in the cellular pathways that participate in this maturation processing.

Assembly of the PC1-PC2 complex

The determination of the structure of a heterotetrameric complex that includes three molecules of PC2 and one molecule of PC1 provides a clear demonstration that these two proteins can assemble with one another (18). PC1 is elaborately intertwined with PC2 in this complex, through an organizational scheme in which each of its four protein components shares two of its transmembrane helices with its neighbor to the right (when viewed from the top) (115) (Figure 3). The protein constructs that were employed in this landmark study were designed with goal of minimizing the inclusion of sequence domains that might be disorganized or unfolded and thus complicate the interpretation and structural interpretation of the cryo-electron microscopic images. Thus, substantial portions of cytoplasmic and extracellular domains are missing and their contributions to the interaction between PC1 and PC2 cannot be directly ascertained from the structure (18). This is especially relevant since several papers suggest that interactions between the coiled-coil domain in the C terminal tail of PC1 and its counterpart the C terminal tail of PC2 are important contributors to complex formation (116–118). Interactions among extracellular loops of both proteins have also been shown to be important components of the inter-molecular interactions that stabilize the

heterotetramer (119). As discussed in detail below, it appears that initial assembly of PC1 and PC2 occurs early in the course of the biosynthetic pathway, while both proteins still reside in the endoplasmic reticulum. It remains to be determined whether this assembly is functionally irreversible, or whether regulated or spontaneous disassembly of the complex could release the polycystin proteins, which would then be free to participate in other interactions or pathways (120, 121).

G-protein Coupled Receptor Proteolytic Site (GPS) Cleavage

Most newly synthesized proteins are largely passive participants in the various steps of their post-biosynthetic processing. They are generally substrates for, rather than drivers of, the shaping and styling that is performed upon them by the many chaperones and enzymes that operate along the length of the secretory pathway. In at least one important sense PC1 constitutes a fascinating exception to this pattern of behavior. As previously noted, the primary sequence of PC1 includes a G-protein Coupled Receptor Proteolytic Site (GPS), which is homologous to the sequence domains at which a sub-class of G protein coupled receptors undergo an autocatalytic cleavage that severs the covalent connection between their extracellular N-termini and their transmembrane domains (122, 123). In a seminal study published in 2002, it was demonstrated that PC1 is cleaved at its GPS site (Figure 2). These investigators further showed that pathogenic mutations known to be causative of ADPKD can interfere with this cleavage (19). They used a classical pulse-chase protocol combined with immunoprecipitation and analysis by SDS-PAGE to ascertain that the apparent molecular weight of newly synthesized PC1 protein is ~520 kDa. Within 10 minutes of its synthesis and while it thus still resided in the endoplasmic reticulum, PC1 underwent a cleavage that resulted in the production of a ~370 kDa N terminal fragment and a ~150 kDa C terminal fragment. The C terminal fragment includes all of the PC1 protein's eleven transmembrane spans and its cytoplasmic C terminal tail, while the N terminal fragment is comprised of the ~3,000 amino acid sequence that extends from the protein's N terminus to the beginning of the first transmembrane segment. The soluble N terminal fragment remains non-covalently attached to the C terminal fragment. The nature of the non-covalent bonds that tether the N terminal fragment to the membrane-associated C terminal fragment has not been fully elucidated. It is likely, however, that ionic bonds play an important role since exposure to alkaline pH can effectively disrupt this association, leading to the shedding of the N terminal fragment (124).

A series of very elegant biochemical studies established the molecular mechanism through which this cleavage occurs (125). Using chimeric constructs that connected the sequence of the human PC1 N terminal fragment to the IgG protein Fc domain, Edmund degradation-based protein sequencing revealed that cleavage between Leucine 3048 and Threonine 3049. They further showed that Histidine 3047 initiates the cleavage process by deprotonating Threonine 3049, leading to a nucleophilic attack on the carboxyl group of Leucine 3048. This leads to the creation of a cyclic intermediate that spontaneously opens, forming an ester linkage. The spontaneous severing of this ester linkage by hydrolysis completes the cleavage process, resulting in the conversion of the full length PC1 precursor into a non-covalent assembly of its N and C terminal fragments.

Although residues within PC1 are necessary and sufficient to catalyze this protein's cleavage at the GPS site, it is clear that the presence of PC2 is an important facilitator of this process. Co-expression of PC2 with PC1 in transfected cultured cell systems dramatically increases the fraction of PC1 precursor protein that successfully undergoes GPS site cleavage (126). It is unclear whether this stimulatory effect of PC2 requires its assembly with PC1 into the heterotetrameric complex whose structure has been determined (18). Were this the case, it might suggest that assembly of the PC1-PC2 complex influences the three-dimensional structure of PC1 to create a confirmation that favors one or more steps of the autocatalytic cleavage reaction. Alternatively, it is also possible that the influence of PC2 on the cleavage process could occur at a distance through effects that PC2 might exert on the environment of the lumen of the endoplasmic reticulum. It is interesting to note in this regard that a pathogenic PC2 mutation that disrupts its channel activity (D511V) substantially reduces the ability of PC2 to enhance the extent of PC-1 cleavage (126). This observation suggests the interesting possibility that PC2-dependent influences on the ionic environment in the lumen of the endoplasmic reticulum could modulate the chemical states and inter-relationships among the critical PC1 residues that mediate GPS cleavage without the need for direct physical interaction between PC1 and PC2.

The mechanism through which PC2 influences PC1 GPS cleavage remains unresolved. It is clear, however, that its effects are exerted early in the life of the newly synthesized PC1 protein, while it still resides in the endoplasmic reticulum. A number of studies that have examined the time course of GPS cleavage support the conclusion that this event occurs while the PC1 protein still carries the Endoglycosidase H-sensitive high mannose glycosylation structure (127) that is indicative of residency in the endoplasmic reticulum. Furthermore, cleavage appears to be a prerequisite for departure of the PC1 protein from the endoplasmic reticulum (126, 128–131), although at least one study suggests that it is not required for ciliary localization (132). Mutant forms of PC1 that are unable to undergo GPS cleavage appear to be retained in the endoplasmic reticulum (129, 130), strongly suggesting that GPS cleavage is an obligate event that must be completed in order for PC1 to be deemed acceptable by the endoplasmic reticulum's quality control apparatus and thus permitted to continue its itinerary to its sites of functional residence.

To assess whether cleavage at the GPS site is required in order for the PC1 protein to mediate its full suite of physiological functions *in vivo*, a gene targeting approach was used to generate a mouse model that was homozygous for a mutant form of PC1 in which the catalytic threonine residue at the GPS cleavage site is mutated to valine and which is consequently unable to perform the autocatalytic proteolytic reaction (133). These animals express only the V/V mutant form of the PC1 protein throughout their embryonic development. Lack of expression of functional PC1 during embryonic development leads to embryonic lethality that is notable for the presence of massively enlarged kidneys and profound pulmonary, pancreatic and vascular abnormalities (46, 134). It is surprising, therefore, that none of these developmental defects are detected in the V/V mice. Their appearance at birth is normal. These animals develop rapidly progressing cystic disease that involves distal segments of the nephron but that leaves proximal tubules unaffected. In contrast, the cysts that form in the absence of functional PC1 expression are not limited to distal segments but affect proximal tubules with equal penetrance. It remains to be

determined how and why cells of the distal tubule are more dependent on the consummation of the PC1 GPS cleavage than are their counterparts in the proximal nephron. The fact that expression of the V/V mutant form of PC1 is sufficient to prevent the embryonic phenotypes that are observed in multiple organs is similarly mysterious. Taken together, however, these observations suggest the very interesting possibility that cleavage at the GPS is required for some, but not all, of the roles that PC1 protein is called upon to play. This behavior is consistent with an emerging picture of polycystin biology that recognizes that both PC1 and PC2 may participate in a number of cellular processes, each of which may manifest distinct requirements for parameters such as PC1 cleavage, assembly of a PC1/PC2 complex or subcellular localization of the polycystin proteins.

C-Terminal Tail Cleavage

The C terminal tail of the PC1 protein is 200 amino acid residues in length, comprising residues 4,102–4,302 and extending into the cytoplasm from the end of the eleventh and final transmembrane span. In spite of its relatively short length in comparison to the gargantuan size of the full length PC1 protein, this segment appears to play an out-sized role in several aspects of PC1 function. As will be discussed below, the C terminal tail of PC1 hosts a number of interactions with protein partners that may govern aspects of PC1 signaling, localization and stability. In addition, the PC1 C terminal tail appears to participate in several processes that require it to wander far from the bulk of its parent protein (131). In 2004 a cleavage fragment corresponding to the PC1 C terminal tail was detected both in mouse kidneys *in vivo* and in cultured cell lines transfected to express the full length PC1 protein (55) (Figures 2 and 6). This fragment appeared to embody the bulk of the predicted mass of the PC1 C terminal tail, manifesting an apparent molecular weight of ~35 kDa as determined by SDS-PAGE. Immunolocalization analyses performed on mouse kidneys and on PC1-transfected cultured cells suggested that the C terminal tail fragment could accumulate in cell nuclei. This study identified a 21 amino acid sequence, residing between residues 4,134 and 4,154, that appeared to be responsible for driving the fragment's accumulation in the nucleus. The precise cleavage site involved generating the C terminal tail fragment has not yet been determined. One study suggested that the intramembranous protease γ-secretase participates at some stage in whatever proteolytic cascade leads ultimately to the production of the PC1 C terminal tail fragment (135, 136), although a subsequent study did not confirm a role for this enzyme (23). Though the mechanism responsible for the release of the C terminal tail fragment was not determined, it appeared that cessation of renal tubular flow stimulated the cleavage, since ureteral ligation led to increased detection of the PC1 C terminal tail in distal segments of the renal tubule. Similarly, genetic disruption of cilia formation also led to detection of the PC1 CTT terminal protein in the nuclei of mouse renal epithelial cells. Cleavage and nuclear localization of the PC1 C terminal tail appears to require the presence of PC2 protein with intact channel activity, although it appears to be insensitive to cytosolic calcium levels (137). Though the polycystin proteins have been implicated in a variety of ways in aspects of ciliary mechano-sensation (75, 81, 120, 121, 138–150), it remains to be determined whether PC1 or PC2 are direct participants in or respondents to whatever flow sensing mechanism may contribute to the regulation of the cleavage of the PC1 C terminal tail.

The nuclear population of the liberated PC1 C terminal tail appears to interact with a number of transcription factors and proteins that modulate transcription factor function. These include β-catenin and TCF, which are key modulators and effectors of the Wnt pathway, as well CHOP, which induces an apoptosis response to ER stress (135, 151). The PC1 CTT reduces the capacity of TCF and CHOP to interact with the transcriptional co-activator p300/CBP and thus it reduces the expression of downstream targets of the TCF and CHOP pathways. The PC1 CTT also interacts with the transcriptional co-activator TAZ (152, 153). TAZ is a versatile signaling protein that participates in a number of signaling processes, including the Hippo pathway, which regulates cell growth (154). TAZ stimulates the activity of RunX2, a transcription factor that plays a critical role in osteoblast differentiation and bone formation. The PC1 CTT appears to act through TAZ to stimulate osteoblast cell function (152, 153) and bone deposition is profoundly perturbed in the absence of PC1 expression (155–157).

A smaller fragment of the PC1 C terminus is also released by cleavage and enters the nucleus where it influences transcriptional pathways (Figure 2 and 6). This ~17 kDa fragment appears to be cleaved from PC1 in response to reduced tubule fluid flow (57, 138, 158). It has been reported to accumulate in cyst epithelial cells and to stimulate the activity of the STAT3 and STAT6 components of the JAK/STAT signaling pathway. This influence has been suggested to stimulate proliferation and enhance cyst formation. The protease responsible for producing this fragment has not been identified, and it remains to be determined whether or how the production of this fragment is hierarchically related to the larger PC1 CTT fragment discussed in the preceding paragraphs.

To this point the discussion of the post-cleavage fates of PC1 CTT fragments has focused on the capacity of these fragments to enter the nucleus and to influence the activities of transcription processes. Recently it was shown that the destinations available to the liberated PC1 CTT are more numerous and varied. The PC1 CTT can enter the inner mitochondrial matrix (159) (Figure 6). This result was observed in studies that employed both the full length PC1 protein and constructs that encoded the sequence of the presumed PC1 CTT fragment. Remarkably, the sequence that appears to be required to target the PC1 CTT to mitochondria significantly overlaps with or is identical to the motif that mediates it nuclear localization. Expression of the PC1 CTT partially corrected mitochondrial morphological changes that were observed in cells that lack PC1 expression. Furthermore, transgenic expression of the PC1 CTT decreased exercise capacity and viability while also increasing CO_2 production. Another study has reported that the PC1 CTT can undergo cleavage to create sub-fragments, and that modulation of a cell's redox state influences whether the cleaved PC1 CTT and its fragments are targeted to the nucleus or to mitochondria (58). Taken together, these data provided strong support for the concept that the PC1 CTT is able to enter mitochondria and to exert a direct influence on mitochondrial metabolism.

Further support for a mitochondrial role for the PC1 CTT was provided in a study of the effects of transgenic expression of this protein fragment in the renal epithelial cells of a conditional knockout mouse model of ADPKD. A stop-flox approach (160) was employed to generate a BAC-transgenic mouse model in which inducible expression of Cre led to homozygous disruption of the *Pkd1* genes as well as to activation of

expression of the transgenic sequence encoding the PC1 CTT (161). These investigators found that expression of the PC1 CTT in the C57BL/6N strain of mice substantially suppressed the development of cystic disease, as evidenced by reduced kidney weight/body weight ratio, serum BUN levels and serum creatinine levels as compared to littermates that lacked PC1 CTT expression. Previous studies indicated that the polycystin proteins can accumulate at mitochondria-associated membranes (MAMs) (also known as mitochondria-ER contact sites, or MERCs), which are areas of close apposition between the endoplasmic reticulum and mitochondria (53). Mass spectrometric analysis detected nicotinamide nucleotide transhydrogenase (NNT) as the only major interaction partner that co-immunoprecipitated with PC1 from a MAM fraction prepared from mouse kidney. NNT is a transmembrane protein embedded in the inner mitochondrial membrane, where it exploits the transmembrane proton gradient to drive the exchange of hydrides between NAD(H) and NADP(H). In its “forward mode”, NNT catalyzes transfer of a reducing equivalent from NADH to NADP to create NADPH, which in turn participates in the production of reduced glutathione and hence the suppression of oxidative damage. Another study designed to identify PC1 protein partners has confirmed this interaction (159). The C57BL/6J strain of mice is closely related to C57BL/6N and is widely used in studies focused on ADPKD pathogenesis and therapeutic development. Interestingly, a spontaneous mutation in the C57BL/6J line disrupted the gene encoding NNT, and these animals completely lack production of NNT protein and enzymatic activity. Conditional expression of the PC1 CTT in association with homozygous disruption of *Pkd1* in C57BL/6J mice does produce any suppressive effect on the progression of cystic pathology, strongly suggesting that the availability of the interaction between the PC1 CTT and NNT is a critical prerequisite in order for the PC1 to slow disease development. Biochemical assays showed that the PC1 CTT stimulates the rate of NNT activity and this effect was not observed with an NNT construct that lacked its putative nuclear localization sequence. Taken together with the work that demonstrated the mitochondrial translocation of the PC1 CTT, these observations demonstrate that entry of the PC1 CTT into mitochondria can exert a profound effect on ADPKD pathogenesis. The mechanisms involved in this effect and the relationship between NNT activity and cystogenic pathways remain to be determined.

Interestingly, a similar behavior has been observed with Fibrocystin/Polyductin (FPC), the protein encoded by the *Pkhd1* gene that is mutated in Autosomal Recessive Polycystic Kidney Disease (162). FPC is a large protein that spans the membrane once. Its cleaved C-terminal tail can enter mitochondria, where it enhances oxidative phosphorylation. Loss of the FPC C-terminal tail increases the severity of the cystic disease that develops in mice that are homozygous for the expression of a hypomorphic allele of *Pkd1* that encodes a version of the PC1 protein that cannot undergo cleavage at the GPS site. This study strongly suggests that PC1 and FPC exert at least some of their physiological effects through a common pathway, and further that this common pathway involves the effects of these protein’s C terminal tail fragments on aspects of mitochondrial function.

p100

The preceding discussions have focused on cleavages that sever the covalent attachments between the 12 transmembrane PC1 C terminal fragment and that extracellular PC1 N

terminal fragment and the intracellular PC1 C terminal tail. A third variety of PC1 cleavage takes place within the PC1 CTF itself. This cleavage may occur in a cytoplasmic loop between transmembrane domains 5 and 6 of the PC1 CTF, creating a fragment that is referred to as p100 and that corresponds to the final 6 transmembrane domains of the PC1 CTF (54) (Figure 2). This fragment has been detected both in mouse kidneys *in vivo* as well as in numerous cultured cell lines. The final 6 transmembrane domains of PC1 manifest homology to PC2 (28), and in the 3 dimensional structure of the PC1/PC2 heterotetramer, these last 6 transmembrane domains for extensive contacts with corresponding portions of the neighboring PC2 components of the complex (18). The enzyme responsible for the creation of p100 and the precise cleavage site have not been determined. Because p100 appears to localize to the endoplasmic reticulum it is has been suggested that this cleavage occurs to a population of PC1 that resides in the endoplasmic reticulum. This population could represent newly synthesized PC1 that has not yet departed the endoplasmic reticulum, or it instead could derive from a stable endoplasmic reticulum-resident pool of PC1. It is not clear whether or how this cleavage might be regulated, or whether it instead occurs constitutively. The p100 fragment interacts with STIM1, an endoplasmic reticulum resident protein that measures the calcium content within this organelle's luminal compartment. Release of calcium from the endoplasmic reticulum results in the formation of close contacts between STIM1-containing portions of the endoplasmic reticulum and calcium permeable ion channels in the plasma membrane, including Orai (163, 164), leading to the activation of store-operated calcium entry. Expression of p100 interferes with STIM1 function and suppresses store-operated calcium entry. The relationship between these effects and the multitude of signaling pathways that are influenced by the polycystin proteins remains to be determined.

Trafficking of the Polycystin Proteins to Cilia

The secretory pathway, first elucidated more than half a century ago (165–168), constitutes the route taken by newly synthesized secretory and membrane proteins as they travel from their site of synthesis at the rough endoplasmic reticulum to their sites of ultimate functional residence. Upon departing the endoplasmic reticulum, most proteins are transported by vesicular carriers to the Golgi complex, arriving at the cis surface of this organelle and progressing through its medial stacks until they arrive at the trans Golgi network (169). As they traverse the cisternal stations of the Golgi complex, proteins undergo successive steps in the posttranslational modification of their N-linked sugar structures and in some cases receive additional embellishments, such as o-linked sugars (170). In the case of newly synthesized membrane proteins *en route* to the apical or basolateral plasma membrane domains of polarized epithelial cells, the trans Golgi network constitutes an important sorting station at which they will be sorted into cargo vesicles that will transport them directly to one or the other surface domain or to a variety of endosomal structures that serve as intermediate waystations as they pursue this polarized delivery process (Figure 4).

Primary cilia emerge from the apical surfaces of polarized epithelial cells, and their membranes are continuous with those of the surrounding apical plasma membranes. In spite of this intimate connection, however, the ciliary membrane constitutes a biochemically distinct entity whose protein and lipid compositions are quite distinct from those of the

apical membrane from which it arises. To sustain its compositional integrity, the cilium must possess a selective gate that can prevent the diffusive intermixing of cilium and apical membrane components and rigorously control the entry and exit of delivered and recycling proteins (171). This gate, referred to as the transition zone, is an intricate multi-protein complex. Many of its protein components are encoded by members of gene families, such as NPHP, mutation of which result in ciliopathy syndromes that can include renal cysts among their prominent symptoms. Vesicles cannot enter the cilium. Instead, carrier vesicles deliver their cargoes of newly synthesized proteins to the base of the primary cilium. It appears that the base of the primary cilium can act as a hot spot for the delivery of cargo vesicles carrying either proteins destined for the apical membrane or for the cilium, and that the selectivity imposed by the ciliary transition zone determines which of these protein cargoes can gain access to the cilium versus diffusing laterally into the apical membrane surface (172). A variety of protein motifs are thought to act as targeting signals that direct the trafficking of newly synthesized membrane proteins to the base of the primary cilium and that permit them to pass through the selective filter at the transition zone. Once membrane proteins arrive at the base of the primary cilium and are granted access to cross the transition zone, they travel towards the tip of the cilium in association with intraflagellar transport protein complexes, which are molecular motors that drive the anterograde and retrograde transport of their soluble or intramembranous client molecules along the extent of the cilium. The details of ciliary transition zone structure and protein trafficking are beyond the scope of the present discussion, and a number of comprehensive reviews on these subjects have been published (173–177). In addition, an excellent recent review focuses on the trafficking of the PC proteins to cilia (100). It is worthwhile to discuss briefly, however, the numerous motifs, partners and pathways that have been implicated in the trafficking of PC1 and PC2 to cilia.

Rod outer segments are the specialized cilia of retinal photoreceptor cells. Studies of the trafficking of membrane proteins including rhodopsin and retinol dehydrogenase have suggested that the motif VxPx is sufficient to direct these proteins to this ciliary structure (178–180). The PC2 protein includes a RVxP sequence in its cytoplasmic N terminal tail that has been shown to be capable of mediating this protein’s ciliary targeting in studies employing heterologous expression in cultured cell models (181). The PC1 protein possesses a sequence at its C terminus (KVHPSST) that includes the VxPx motif, and it has been proposed that this sequence is required for the ciliary targeting of PC1 (182). This sequence has been suggested to interact with a complex including several small GTPases, including Arf4, Rab6 and Rab11, all of which have been implicated in membrane protein trafficking. A subsequent study suggested that VxPx sequences are not required for PC1 or PC2 ciliary localization and further reported that the interactions that are hosted by the PC1 KVHPSST sequence are similarly not involved in determining PC1’s ciliary distribution.(132).

It has been reported that at least some membrane proteins may pursue a non-canonical path *en route* to the cell surface or to the primary cilium. While the classical secretory pathway includes an obligate transit of the cis, medial and trans cisternae of the Golgi complex, there is evidence indicating that a number of membrane proteins travel directly in vesicular carriers from their sites of synthesis in the rough endoplasmic reticulum or from the cis cisternae of the Golgi complex to the plasma membrane or the cilium, thus bypassing some or all of the Golgi cisternae (183, 184). Intriguingly, experiments that employed metabolic

labeling and imaging modalities to follow the trafficking of newly synthesized PC2 to the cell surface and cilium in the ciliated LLC-PK1 line of cultured pig kidney epithelial cells indicated that the pool of PC2 that reaches the non-ciliary domains of the plasma membrane transit the entire Golgi complex, whereas the pool of PC2 that is destined to reside in the cilium is transported there directly from the cis cisterna of the Golgi (185, 186). Similarly, another study employing LLC-PK1 cells transfected to express PC1 and PC2 employed a pulse-chase imaging approach to examine the delivery of PC1 to the cilium and to the plasma membrane reported that treatments that perturb trafficking of newly synthesized proteins through the Golgi complex, including brefeldin A (187, 188) or by incubation at 20° C (189, 190), prevent the delivery of PC1 to the plasma membrane but do not interfere with its delivery to the cilium (124). A very different result was obtained by investigators employing the MDCK line of canine kidney epithelial cells transfected to express PC1 and PC2. In these studies cilia were harvested from cells by treatment with ammonium sulfate (191). The ciliary populations of PC1 and PC2 were subjected to digestion with Endoglycosidase H and analyzed by SDS-PAGE and western blotting. This approach found that all of the PC1 and PC2 protein present in the membranes of the cilia derived from these cells was resistant to Endoglycosidase H digestion, demonstrating that these proteins had undergone at least some steps of the processing of their N-linked sugar structures that take place in the Golgi complex (192). This study went on to show that the post-Golgi delivery of PC1 and PC2 to the cilium requires the involvement of a protein complex that assembles at the trans-Golgi network and that includes the Golgi and ciliary trafficking proteins GGA1, Rabep1 and Arl1. It has also been found that components of BBSome, a protein complex that is involved in targeting proteins to cilia and whose loss leads to the cystic disease-associated ciliopathy Bardet-Biedl Syndrome, are necessary for the ciliary delivery of PC1 but not for this protein’s delivery to the non-ciliary domains of the plasma membrane (193).Taken together, the results of these three studies raise the possibility that the newly synthesized polycystin proteins may pursue multiple trafficking itineraries as they travel from the endoplasmic reticulum to the cell surface and the cilium. Further studies will be required to determine whether the details of these itineraries vary in different cell types or in response to physiological or environmental stimuli.

Functions of the polycystin proteins

It can be argued that our understanding of the primary physiological functions of the polycystin proteins is perhaps undermined, somewhat paradoxically, by the enormously rich literature detailing the wealth of cellular processes in which they have been implicated. The number of candidate pathways that are influenced by the polycystin proteins has proliferated dramatically in the nearly three decades since the genes encoding them were first identified (194). In spite of, or perhaps because of, their influence on numerous cellular functions, it has been challenging to define the collection of molecular mechanisms that connect these proteins directly to the most proximal of the many processes that they impact. While it has been difficult to ascertain precisely what these proteins are supposed to do, however, an analysis of their primary and tertiary structures provides clear and valuable insight into two types of things that they are designed to be capable of doing. The polycystin complexes share features of both ion channels and of receptors.

Ion channel capabilities of the polycystin proteins

The sequences of the PC1 and PC2 proteins (28, 195), together with the three-dimensional structures of the PC2 homotetramer and of the PC2/PC1 heterotetramer (18, 32, 196), demonstrate that these complexes are built like ion channels. A very large body of structural and functional evidence supports the widely accepted conclusion that the polycystins participate in the formation of ion channel complexes. Since the biophysical data in support of this contention has been recently and extensively reviewed (197), the present discussion will focus on aspects of these proteins' channel functions that may be of greatest relevance to these proteins' physiological functions and their roles in suppressing cystic disease.

In discussing the channel properties of the polycystin proteins it is important to keep in mind that these parameters may be influenced by the experimental setting in which they are evaluated. Electrophysiological studies using a variety of native and reconstituted preparations have produced a fairly wide range of observations regarding the gating properties, single channel conductance and selectivity behaviors of channels formed by native polycystins and by those studied in heterologous expression systems (81, 197). Perhaps the most physiologically relevant measurements, therefore, derive from studies that used patch clamp analysis and knockout cell and animal models to interrogate the characteristics of native polycystin channels expressed in the cilia of renal epithelial cells *in situ* and in culture (81, 141, 142). These efforts revealed that the polycystins in the membranes of cilia participate in the formation of a relatively high conductance (~100 pS) cation permeable channels that are activated by depolarized voltages and by intra-ciliary calcium in the low micromolar range. Examination of these channels' selectivity indicates that they favor monovalent over divalent cations by a factor of ten. Interestingly, while disruption of PC2 expression abrogated these currents, disruption of PC1 expression did not appear to reduce or change them (81). Previous studies have identified proteins that appeared to be capable of stimulating the polycystin channel to open, including mDia1 and Wnt ligands (198, 199). Under the conditions employed in the cilia patch clamp experiments these factors did not appear to increase the channel's open probability (81). A subsequent patch clamp study found that extracellular application of polypeptides whose sequence correspond to portions of the PC1 NTF was sufficient to increase the open probability of a channel comprised of PC1 and PC2 (200). This fascinating finding suggests that NTF may itself act as a channel-activating ligand. It remains to be determined whether the NTF activates ciliary polycystin channels in renal epithelial cells *in vivo* and, if so, whether this activation is mediated by interactions among neighboring polycystin complexes or if NTF shed into the tubule fluid can act as a paracrine activator of "downstream" polycystins.

Structure-function studies of the polycystin channels have been complicated by the lack of a consensus understanding of the physiological cues or ligands that induce channel opening. This difficulty has been addressed in part through the identification of a gain of function mutation that produces constitutive channel activity (33). Employing a strategy used successfully in a previous study performed on the TRP channel family member TRPML1 (201), scanning mutagenesis carried out on the fifth transmembrane segment of PC2 and on the sequence linking the fourth and fifth transmembrane spans revealed that changing residue 604 from phenylalanine to proline (F604P) traps the channel in an

open confirmation. This channel activity was detected with PC2 expressed in Xenopus oocytes in the absence of exogenous PC1, suggesting that it is produced by homotetramers of PC2. Structural studies reveal that this conductance change is produced through an alteration the confirmation of the PC2 channel's lower pore gate (202). Interestingly, the monovalent cation conductance of the PC2 F604P channel is blocked by extracellular calcium, suggesting that homotetrameric channels comprised of PC2 alone do not facilitate the entry of extracellular calcium. Small molecule modulators of the channel activity of the PC-2 related TRP channel TRPML have been shown to both increase and inhibit the PC-2 channel activity, depending upon the concentrations at which they are applied (203). Co-expression of PC1 with PC2 F604P substantially reduces the constitutive channel activity. Further mutagenesis efforts performed on PC2 identified two additional gain of function mutations, L677A and N681A. Co-expression with PC1 in Xenopus oocytes does not suppress the channel activity produced by PC2 carrying both of these mutations (PC2 AA). Furthermore, the channel complexes produced by PC2 AA together with PC1 manifest a degree of permeability to extracellular calcium (24). These results suggest the exciting possibility that the permeability properties of homotetrameric PC2 polycystin channels differ in important ways from those of heterotetrameric PC1/PC2 channels. It further supports the hypothesis that the subunit compositions of the channels produced by the polycystins may both influence and determine their physiological roles. The extent to which native polycystin channels *in vivo* manifest heterogenous subunit compositions and permeability properties remains to be determined.

Cilia are mechanosensitive organelles that can modulate ionic currents in response to the application of shear stress (68, 204) and chemical stimuli. The discovery that polycystin proteins localize to the primary cilium (51, 52) in renal epithelial cells has focused attention on the possibility that the ciliary polycystins constitute the cell's most physiologically important locale for polycystin channel function (75, 81). In keeping with this interpretation, it has been shown that the cilium-enriched oxysterol molecule 7β,27-DHC can activate the conductance of the wild type PC2 channel that does not carry any gain of function activating mutations (205). This intriguing result both identifies a potential biologically relevant channel-activating ligand for the polycystin channel complex and offers a possible explanation for the observation that the wild type channel exhibits little channel activity when expressed heterologously in non-ciliated cells. It remains to be determined whether this oxysterol compound constitutes the principle physiological activator of the polycystin channel *in vivo* and, if so, what stimuli regulate its presence in cilia.

It is important to note, however, that polycystin channel activity is also present in the endoplasmic reticulum, and this activity appears to play important roles in regulating calcium release from the endoplasmic reticulum (31). While direct calcium permeation through endoplasmic reticulum polycystin channels may participate to some extent in endoplasmic reticulum calcium release, it has also been shown that polycystins can interact with and modulate the activities of the IP3 receptor and the ryanodine receptor, which serve as the endoplasmic reticulum's major calcium release channels (206–208). It has also been shown that monovalent cation conductance through polycystin channels can play a major role in endoplasmic reticulum calcium release. The movement of large quantities of calcium across the membrane of the endoplasmic reticulum membrane is only possible

if the associated charge movement is offset by the movement of cations in the opposite direction (or the movement of anions in the same direction) to maintain electroneutrality. The role of the polycystins in endoplasmic reticulum calcium release may be to serve as a monovalent cation electrical “shunt” to avoid the creation of a large membrane potential that would oppose the release of biochemically meaningful quantities of calcium. In support of this concept is the fact that the reduction in endoplasmic reticulum calcium release that is associated with loss of polycystin expression can be corrected through the expression of TRIC-B, which is a potassium channel that is localized to the endoplasmic reticulum (82). TRIC-B expression also was found to suppress cystogenesis in the kidneys of mice that lack PC2 expression. Taken together, these data suggest the very interesting possibility that a major function of the polycystin ion channels is not related to their residence in the cilium or to their capacity to mediate calcium flux. Instead, the role of the polycystin channels in indirectly facilitating endoplasmic reticulum calcium release may constitute a major aspect of their physiological functions.

The endoplasmic reticulum and mitochondria form functional contact sites with one-another at specialized interaction structures originally known as “mitochondria associated membranes” or MAMs, and which are now referred to as “mitochondrial-ER contact sites (MERCs)(209–211). (Figure 4). MERCs are created and stabilized through interactions between mitochondrial outer membrane proteins and endoplasmic reticulum proteins, and they serve as hot spots for exchange of substances between these two organellar compartments, including membrane lipids and calcium (210, 211). Delivery of calcium from the endoplasmic reticulum into mitochondria at MERCs can dramatically influence the rate of oxidative phosphorylation by controlling the activity of calcium-dependent enzymes that participate in the tricarboxylic acid cycle (212). The endoplasmic reticulum population of polycystin proteins appears to regulate calcium entry into mitochondria from the endoplasmic reticulum (53) and to modulate the stability of MERCs connections (83). The availability of oxygen appears to regulate the polycystin-associated component of calcium delivery from the endoplasmic reticulum to mitochondria through the oxygen-dependent activity of prolyl hydroxylase, which hydroxylates proline residues on polycystin proteins, which in turn modulates the subcellular localization of the polycystin proteins as well as magnitude of secretagogue-induced calcium release from the endoplasmic reticulum (53). These findings fit well with the observations, noted above, that PC2 functions as a monovalent cation channel that supports calcium release from the endoplasmic reticulum through the IP3 receptor. Taken together, these findings suggest the intriguing possibility that the suppression of oxidative phosphorylation and the adoption of a Warburg-like metabolism (213) that is observed in cells that lack polycystin expression (79, 214) may be due to reductions in polycystin-dependent calcium flux from the endoplasmic reticulum to the mitochondria (215).

Receptor properties of polycystins

A large body of evidence has developed over the past 25 years that provides strong biochemical and genetic support for the surprising conclusion that PC1 mediates G protein-coupled receptor (GPCR)-like signal transduction. As discussed in the previous section, the channel properties of the polycystin proteins can be readily inferred from their primary

and tertiary structures. The same cannot be said of PC1's GPCR-like function. All of the members of the GPCR family adhere rather rigidly to a shared structural design, of which the presence of seven transmembrane domains is an invariant feature (216). In contrast, PC1 has 11 transmembrane domains and its cytosolic loops do not resemble those cytoplasm-facing domains of GPCRs that interact directly with trimeric G proteins (18). In defiance of structural orthodoxy, however, there is strong biochemical evidence that PC1 can interact through its cytoplasm-facing C terminal tail with several different trimeric G protein complexes (217). This interaction involves a sequence in the PC1 C-terminal tail that overlaps entirely with the residues that have also been found to be required for the nuclear localization (55) and the mitochondrial (23) localization of the cleaved C-terminal tail.

Several trimeric G proteins have been suggested to serve as obligate participants in aspects of polycystin signaling, including $G\alpha_q$, $G\alpha_i$, $G\alpha_{12}$, $G\alpha_{13}$, $G\alpha_{14}$ and $G\alpha_s$ (20, 218–223). Assembly with PC2 appears to suppress the involvement of PC1 in G protein-related signaling, suggesting the interesting possibility that a distinct pool of PC1 protein that is not involved in a PC1/PC2 complex mediates the PC1-related receptor functions (224). Studies suggest that at least one G protein, $G\alpha_{12}$, acts as a driver of cyst formation whose influence is suppressed by PC1-mediated signaling (222). Studies performed in Xenopus embryos support the intriguing hypothesis that the interaction of PC1 with trimeric G proteins does not serve to activate signaling through G protein α-subunits, but instead serves to sequester G protein β and γ-subunits whose unfettered availability somehow contributes to cyst formation (223).

Perhaps the most compelling evidence for the physiological significance of the interaction between PC1 and trimeric G proteins derives from experiments that evaluated *in vivo* the effects of a mutation in the PC1 sequence that interferes with this interaction (225). The L4132Δ mutant form of PC1 lacks a single leucine residue that resides within the G protein binding site on the PC1 C-terminal tail. This mutation was originally discovered in ADPKD patients and is considered to be pathogenic. Analysis of a mouse model that carries the L4132Δ allele demonstrates that this protein is non-functional, and its expression does not prevent the development of severe cystic disease. Experiments performed in cells transfected to express PC1 L4132Δ demonstrated that the protein successfully completed its post-synthetic processing, including cleavage at the GPS site and surface delivery but it was unable to activate PC1-associated G-protein coupled signaling. In addition, cells co-expressing the PC1 L4132Δ protein and PC2 did not exhibit the channel activity that was observed when wild type PC1 and PC2 were co-expressed, suggesting that the capacity to modulate G protein activity may influence the channel capability of the PC1/PC2 complex. Finally, it is worth noting in this context that loss of the G-protein signaling modulator 1 protein, which regulates the level of G protein signaling, produces a phenotype that is synthetic with, and increases the severity of, that attributable to the homozygous expression of a hypomorphic PC1 allele (226). Taken together these data provide very convincing evidence that the G protein signaling role of PC1 is an obligate component of its physiological function.

Like PC1, the adhesion GPCR subfamily of GPCRs (aGPCR) have large extracellular N-terminal domains that undergo autocatalytic cleavage, which severs the covalent bond

that connects the N-terminal domain to the transmembrane segments. This cleavage occurs at a GPS site in a GAIN domain that is shared among aGPCR family members and with PC1 (227, 228). Activation of aGPCRs is a two-step process. The binding of a ligand leads to the displacement or shedding of the N-terminal domain, which in turn exposes the short extracellular N terminus, or stalk, that was created on the receptor's transmembrane component by the GPS cleavage. This stalk peptide acts as a tethered agonist that interacts with the extracellular loops of the transmembrane component and, by so doing, initiates G protein-dependent signal transduction (229–231). Based upon the presence of the GAIN domain and sequence similarities between the stalk peptides of aGPCRs and the corresponding region of PC1, it is been proposed that PC1may function as a structurally "atypical" aGPCR (228). Substantial evidence in support of this proposition has emerged, which provides strong support for the likelihood that the PC1 protein's stalk peptide-like sequence can activate signaling through trimeric G proteins (131, 232, 233). The downstream targets of this aGPCR-like activity remain to be defined, as does the identity of the ligand or ligands that might activate it. Interesting candidate ligands include Wnt9b (199) and the N-terminal domain of the PC1 protein itself (200), both of which have been shown to bind to the PC1 protein and to initiate signaling processes.

Polycystin interactomes

A sophisticated understanding of the nature of the interactomes of PC1 and PC2, their subcellular and tissue localizations, the factors that regulate their assembly and disassembly, and the processes that polycystin macromolecular complexes are capable of influencing, would provide deep insight into the physiological functions of the polycystins and into the pathophysiology of cystic disease. Fortunately or unfortunately in this regard, many hundreds of proteins have been reliably shown to interact with the polycystins and this census is growing steadily. The first PC1 interactome that made use of tagged PC1 protein expressed at native levels by generation of a knock-in mouse model, followed by quantitative SILAC Mass-Spectrometry studies on cells derived from these mice, demonstrated that PC1 interacts with a number of cytoskeletal proteins and participates in pathways that are involved in sensing and responding to the stiffness of the extracellular matrix (234). These data suggest a role for PC1 as a sensor that detects and respond to the biomechanical properties of the extracellular environment. Perturbations in this sensing function could contribute to the dysregulation of cell growth and morphogenesis that is associated with cyst formation (17). Another thoughtfully-designed and carefully interpreted study used mass spectrometry to analyze the proteins that co-immunoprecipitate with an epitope tagged form of PC1 expressed by genetic knock-in in mice (159). These immunoprecipitations were performed on lysates prepared from mouse heads, since this tissue was found to express high levels of PC1. In addition to PC1 and PC2, the list of proteins that met the threshold criteria established in this study ran to 821 entries. The principal challenge created by this embarrassment of riches clearly lies not in identifying putative interactors but in validating them individually and in elucidating their roles in the activities mediated by polycystin protein complexes. Such validation studies can take the form of *in vitro* biochemical analyses and/or *in vivo* demonstrations of genetic collaboration. In either case, building a convincing case for the importance of any single interaction identified through proteomic studies can require months to years of experimentation.

A recent review does an excellent job of enumerating numerous well-studied interactors and places them in the context of biological pathways (235). Thus, the large collection of interactors identified to date will not be discussed in extensive detail here. Not surprisingly, many of these interactors can be assigned to a few broad classes of proteins that share common structural and functional properties. These include: ion channels and regulators of ion channel function; components of several signal transduction pathways including G-protein signaling, cAMP signaling, Wnt signaling, and receptor tyrosine phosphatase pathways; components of intercellular junctions and components of the extracellular matrix; cytoskeletal proteins and components of the vesicular and ciliary trafficking machineries; transcription factors and regulators of transcription; protein degradation machinery; and proteins that mediate or regulate mitochondrial function (235).

It is important to note that the repertoire of proteins that interact with the polycystins is likely to be strongly influenced by the tissue and cell types in which it is interrogated and by the methods that are employed to detect the interactors. Similarly, distinct pools of the polycystin proteins that reside within different subcellular localizations almost certainly associate with non-identical subsets of proteins partners. In this regard, it is especially interesting to think about the cleavage fragments of the PC1 C-terminal tail, which can gain access to the nucleus and the inner mitochondrial matrix, compartments from which the full length polycystin proteins are excluded (131). Within the nucleus the cleaved C-terminal tail of PC1 has been shown to interact with several transcription factors and regulators of transcription, including WWTR1 (also known as TAZ) (56, 135, 152) and participants in the STAT pathway (57, 138, 158). Within the inner mitochondrial matrix, the PC1 C-terminal tail interacts with and modulates the activity of NNT and thus appears to be able to influence cellular redox state and energy metabolism (159, 161). Data from genetic experiments demonstrate that this interaction is required in order for the PC1 C-terminal tail to suppress cyst formation in mouse models of ADPKD (161). Clearly, developing a thorough understanding of polycystin function will require the field to continue its arduous efforts to identify from among the richly populated list of putative polycystin interactors those that are the most physically proximate, biologically important and of pathophysiological significance.

Dysregulated signaling pathways and cyst formation

While we do not yet know the primary functions of the polycystin proteins, there can be no doubt that they influence a bewilderingly large number of signaling pathways. The polycystin proteins appear to reside at the center of a web whose tendrils extend into a richly populated array of regulatory processes (Figure 8). The evidence for alterations in these many pathways in the absence of polycystin expression is, in most cases, quite strong. Frustratingly, however, the molecular mechanisms through which the polycystins exert their influence on these pathways, and the relationships between these pathways and the proliferative, secretory and inflammatory processes that drive cyst formation remain largely unknown. A large body of evidence is consistent with the hypothesis that polycystins govern injury repair processes, and that cyst formation constitutes a dysregulated repair response (5, 236–241) (Figure 8).

A thorough discussion of the literature surrounding each signaling pathway that has been implicated in ADPKD would rival in length the Oxford English Dictionary and would likely be less interesting to read in its entirety than that weighty tome. In addition, a number of excellent review articles have explored in depth the evidence supporting the best documented of these many pathways (194, 236). Thus, it is most useful to provide here to list some of the best characterized and most relevant signaling pathways along with references to key papers that explored the mechanistic connections of these pathways to polycystin function and cystic disease. Among the most thoroughly explored of these pathways are cAMP, mTOR, calcium signaling, planar cell polarity, cytoskeletal assembly, tyrosine kinase activity, the Wnt pathway, the STAT pathway, Hippo signaling, cMyc regulation, and regulation of the cell cycle (Figure 8).

cAMP and calcium: Arguably the first and perhaps the most extensively studied of the signaling pathways that have been found to be perturbed in ADPKD relates to the second messenger cAMP. Over thirty years ago Dr. Jared Grantham, together with his many collaborators, demonstrated that cAMP levels are elevated in cells that lack expression of the polycystin proteins (242–245). These elevated cAMP levels are thought to stimulate the accumulation of cyst fluid as a consequence of the protein kinase A-mediated activation of the CFTR chloride channel, which in turn is thought to drive the secretion of fluid into cyst lumens (246–249) (Figure 9). In addition, cAMP has been suggested to stimulate the high level of cell proliferation that characterizes renal cyst epithelial cells. This effect has been connected to increased B-Raf and ERK activity that may be induced by cAMP in the context of the reduced levels of cytosolic calcium that may characterize renal cyst epithelial cells (250–256) (Figure 8).

The mechanism through which loss of polycystin protein expression leads to elevated cAMP levels remains to be fully explicated. It has been suggested, however, that polycystin expression negatively regulates the activity of GSK3β, a kinase that governs a number of signaling pathways. In the absence of this polycystin-associated suppressive influence, GSK3β expression may be induced through a “feed forward” mechanisms through which cAMP activates the transcription factor CREB to increase GSK3β expression, which in turn increases the activity of adenylate cyclase, leading to increased levels of cAMP (257). In addition, cytosolic calcium levels appear to be reduced in a variety of experimental systems in which polycystin protein expression has been reduced (258–260). It remains to be determined whether any such reductions in cytosolic calcium levels are due to perturbations in calcium entry across the ciliary or somatic plasma membrane or are instead attributable to reductions in the release of calcium from endoplasmic reticulum stores or in the size of those stores (31, 75, 82, 142, 208). Independent of their etiology, however, reduced cytoplasmic levels have the potential to elevate cytosolic cAMP levels because at least some of the enzymes that govern cAMP levels are regulated by calcium. The AC5 and AC6 isoforms of adenylate cyclase are inhibited by calcium (261). In addition, the PDE4 isoform of the cAMP-degrading enzyme phosphodiesterase is activated by calcium (262). Thus, reduced cytosolic calcium levels could increase cAMP levels both by increasing its synthesis and suppressing its degradation. Alterations in the expression levels of these and other enzymes involved in determining cAMP levels have been detected in cells that

lack polycystin expression (263). Furthermore, genetic manipulation of the expression of calcium-sensitive adenylate cyclases and phosphodiesterases exert profound effects on the development and progression of cystic disease in mouse models (262, 264, 265) (Figure 8). Finally, it is worth noting that compartmentalized alterations in cAMP levels may also participate in the pathogenesis of renal cystic disease. This may be especially true in the primary cilium, where alterations in the local concentrations of cAMP may be able to alter cellular metabolism and initiation cyst formation (266).

mTOR: The mechanistic target of rapamycin (mTOR) pathways are mediated by the TORC1 and TORC2 kinases. TORC1 integrates metabolic signals to regulate protein synthesis, cell growth and proliferation (267–269). TORC1 activity is stimulated by the GTP-bound form of the small GTP binding protein Rheb. The protein complex formed by hamartin and tuberin (Tsc1 and Tsc2) can serve as a GTPase-activating protein (GAP) that stimulates the GTPase activity of Rheb, leading to its conversion to the GDP bound form which is unable to stimulate TORC1. Genetic loss of the Tsc1 or Tsc2 is the cause of Tuberous Sclerosis Complex, a multiorgan syndrome that includes renal cysts and is associated with elevated levels of mTOR activity (270). A number of studies have shown that TORC1 activity is elevated in several different cell and animal models of polycystic kidney disease (271–274). Furthermore, pharmacological inhibition of mTOR activity slows the progression of cystic disease in orthologous and non-orthologous models of ADPKD (273, 275–281). Taken together, these observations support the conclusions that excessive mTOR activity results from the loss of polycystin expression and contributes substantially to the consequent development of cystic disease (Figure 8).

Several mechanisms have been suggested to account for the increase in mTOR activity in the context of reduced polycystin expression. The gene encoding the Tsc2 protein resides very close to the *Pkd1* gene, and deletions that affect both genes are associated with early onset and rapidly progressing renal cystic disease (282). It has been suggested that the Tsc2 protein interacts with PC1 and that this interaction is required for the proper post-synthetic trafficking of PC1 (273, 283). Loss of PC1 is associated with elevated activity of the ERK kinase, which phosphorylates and inhibits Tsc2, thus permitting excessive mTOR activation (284). In the absence of PC1 there appears to be excessive activity of the kinase associated with the c-met receptor, which has also been suggested to be a source of excessive mTOR activation (285).

The GAP activity of Tsc1-Tsc2, which is required for its negative influence on mTOR, is stimulated by phosphorylation of the Tsc2 protein by the adenosine monophosphate-stimulated protein kinase (AMPK) (286–290). AMPK is a heterotrimeric protein complex composed of α, β and γ subunits. Conditions of cellular energy depletion lead to elevated levels of AMP. AMP binds to the γ-subunit of AMPK, producing a conformational change that causes the α-subunit to become a substrate for regulatory phosphorylation by upstream kinases including Lkb1 and CAMKK. Once activated by this phosphorylation, AMPK phosphorylates downstream substrates to both turn off energy utilization and to initiate pathways of energy production. In the present context, AMPK suppresses the energy utilization inherent in protein synthesis, cell growth and cell proliferation through its phosphorylation of Tsc2, activating its intrinsic GAP activity, leading to reduced GTP-bound

Rheb and hence to reduced mTOR activity. As a consequence, perhaps, of the metabolic alterations associated with polycystic kidney disease, AMPK activity levels are suppressed in cyst epithelial cells (78, 214, 215, 291–293). This, in turn, could reduce Tsc-mediated GAP activity and result in elevated mTOR activation. In addition, activation of ciliary flow sensing can also modulate AMPK and hence mTOR activity (Figure 8). A subpopulation of the Lkb1 kinase that appears to be localized to the primary cilium is activated by ciliary bending, resulting in activation of AMPK and consequently in suppression of mTOR (121, 294, 295). Thus, perturbations in ciliary function could also result in inappropriately high levels of mTOR tone. Finally, the lysosome serves as a hub of cellular nutrient sensing pathways and protein complexes associated with the lysosome regulate the activity both of mTOR (296) and of the TFEB and TFE3 transcription factors, which govern lysosome biogenesis and autophagy (297–299) (Figure 8). Perturbations in the conversation between mTOR, TFEB and TFE3 can result in excessively high levels of TFEB and TFE3 activity, which can in turn result in the development of cystic disease in mouse models (300, 301). It remains to be determined whether and how each of these potential mechanisms might account for the elevated mTOR that appears to constitute an important driver of the development of renal cystic disease.

Cell cycle regulation, apoptosis and injury: Cell proliferation is a widely accepted hallmark of cyst-lining epithelial cells which, along with fluid secretion, plays a major role in cyst development and expansion (302). Under normal circumstances renal tubule epithelial cells are only minimally mitotically active. Injury to the renal tubule can induce epithelial cell proliferation as a component of the repair mechanisms that reestablish tubule integrity. It has been suggested that the proliferation associated with polycystic kidney disease arises as the product of a dysregulated repair response (239). Direct roles for the polycystin proteins in the response to injury remain to be established. In keeping with a recurrent theme in this review, the mechanisms that connect the polycystin proteins to the processes that impact cell proliferation are largely unknown. It is abundantly clear, however, that loss of polycystin protein expression alters the activities of a large number of signaling pathways and cellular factors that impact cell number by influencing proliferation and cell death. A selected list of these (together with a necessarily incomplete subset of related literature references) include oncogene and tumor suppressor-related cascades such as c-Myc, p53, Ras and B-Raf (253, 255, 303–307), proliferation-related kinases such as MAPKK/ERK/MEK/JNK and Auroroa Kinase A (20, 219, 308–315), transcription regulators such as JAK/STAT/p21 (57, 158, 240, 316), Wnt/β-catenin (135, 151, 199, 317–320) and Hippo/YAP/TAZ (152, 153, 303, 321–324), cyclin-dependent cell cycle regulating kinases (325, 326), and modulators of apoptotic cell death (219, 220, 304, 327–330) (Figure 8). In the context of the putative role of the polycystin proteins in responding to or overseeing injury repair it is interesting to note that pathways designed to address a variety of cell stresses also respond to the absence of polycystin protein expression and may participate in cyst-related processes. This list includes hypoxic and oxidative stress as well endoplasmic reticulum protein folding-associated associated stress (58, 331–342). The relationships connecting these stress-related pathways to the polycystins, to one another, and to the proximate mechanisms responsible for cyst formation remain to be defined.

Macrophages and the immune system: Thus far this discussion of polycystin-related signaling perturbations linked to cyst formation has focused exclusively on connections observed between the polycystin proteins and processes intrinsic to renal epithelial cells. While reduced levels of functional polycystin proteins in renal epithelial cells appears to be a prerequisite for the initiation of polycystic kidney disease, a number of important studies demonstrate that non-cell autonomous factors can influence profoundly the rate and extent of disease progression. Among the best documented of these are cells of the innate immune system and elements of the adaptive immune system (343). Renal epithelial cells that lack polycystin expression secrete cytokines, including MIF-1 and MCP-1, that profoundly influence macrophage accumulation and activity (343–347). There is no evidence that macrophages are attracted to cystic tissue in response to tissue damage and the consequent release of damage associated molecular patterns (DAMPs). Macrophages accumulate in cystic kidneys and preventing this accumulation, either with chemical treatments that lead to macrophage depletion or by eliminating MCP-1 expression, dramatically slows the progression of renal cystic disease in mouse models (345, 348, 349). It remains to be determined how and why loss of polycystin expression induces the secretion of macrophage attractants. The mechanisms through which macrophages that respond to these cytokines stimulate cyst growth are also mysterious. In this context it is interesting to consider the possible contributions of the metabolic perturbations that characterize renal cyst epithelial cells (214) to the initiation of the pro-cystic macrophage phenotype. Secretion of lactic acid by tumors leads to polarization of macrophages into the M2 phenotype that can in turn contribute to tumor cell proliferation (350). It remains to be seen whether macrophages and cyst epithelial cells conduct a similar dialog.

While macrophages can send messages that promote cyst development, cells of the adaptive immune system appear to be capable of exerting a suppressive influence (343). $CD8^+$ T cells accumulate in polycystic kidneys, and depleting these cells exacerbates disease progression (351). ADPKD cyst epithelial cells manifest a number of features that are reminiscent of tumor cells, including their propensity for rapid proliferation and their “Warburg-like” shift to glycolytic ATP production even in the presence of oxygen (214, 306). Like tumor cells, cyst epithelial cells appear to be capable of blocking the capacity of cells of the adaptive immune system to interfere with cyst growth, and the administration of therapeutic agents that allow immune cells to overcome this block slows the rate of cystic disease progression to some extent (352). Finally, it appears that cyst epithelial cells produce compounds, including the tryptophan metabolites kynurenines (353), that can suppress immune cell function. Knocking out the expression of indoleamine 2,3-dioxygenase 1, the enzyme responsible for the production of these metabolites, enhances T cell accumulation and slows the progression of cystic disease in an orthologous mouse model of ADPKD.

Cilium-dependent cystogenic activity and cilium-derived extracellular vesicles: As perhaps befits an organelle that manifests at least a passing resemblance to a rapier, the primary cilium can be described as behaving as “a double-edged sword” in its relationship to the processes that drive cystogenesis. As has been noted in a previous section, while lose of polycystin expression is sufficient to drive renal cyst formation, loss of both polycystin expression and cilium formation profoundly suppresses cyst formation

in adult onset mouse models of ADPKD (76). This seminal observation led to the formulation of the intriguing hypothesis that signals deriving from cilia that lack polycystin expression contribute to the development of cystic disease. According to this model, this "cilium-dependent cystogenic activity" (CDCA, see also above) sends stimulatory signals to pathways that drive cyst formation, including perhaps some or all of the pathways noted in the sections above. The nature of these signals, and the molecules responsible for their generation and transmission, remain to be determined. In light of their capacity to contribute to cyst formation, it is quite perplexing that loss of cilia early in embryonic development is also sufficient to produce forms of renal cystic disease (354–356). The cyst formation associated with cilium absence has been interpreted to suggest that cilia produced a cilia-localized cyst-inhibition activity (CLCI) (357). Once again, the molecular nature of the CLCI remains mysterious. Interestingly, however, the genesis of both CDCA and CLCI appears to depend upon the involvement of a protein called TULP3 (358, 359). TULP3 is an adaptor protein that participates in connecting proteins that are destined for ciliary localization to the intraflagellar transport machinery that controls the delivery of cargo proteins into the cilium (360). Loss of TULP3 suppresses cyst formation in the absence of the polycystins (358, 359), suggesting that TULP3 participates in the delivery of a critical CDCA pathway component to the cilium. Defining the cohort of TULP3 client proteins may help to shed light upon the nature of the CDCA signal as well as the means through which CDCA is suppressed by polycystin protein function (357).

Primary cilia are thought to function primarily as sensory organelles that receive and transduce signals from the environment (361). It is also clear, however, that cilia can serve as the source of signals that are able to influence the behaviors of distant cells. Through a process of self-sacrificial shedding, cilia produce membrane-encapsulated extracellular vesicles that carry away portions of the ciliary membrane and its internal contents (362–364) and this phenomenon has been observed in renal epithelial cells (365). Cilia-derived extracellular vesicles can carry polycystin proteins (59, 366) or polycystin protein fragments (367). In the kidney cilia-derived vesicles may be capable of transmitting messages from upstream to more down-stream nephron segments. The compositions of the extracellular vesicles produced in kidneys that lack polycystin expression differ from those produced by wild type kidney cells (368). Furthermore, it appears that these extracellular vesicles can participate in exacerbating cystic disease (62, 369). The molecular natures of the messages carried by these extracellular vesicles remain to be determined, and the means through which renal epithelial cells detect, receive and respond to these messages are similarly mysterious. These observations, underscore, however, the concept that cilia both generate and transduce signals that can both stimulate and suppress the development of renal cysts. Interpreting the dialogue between cilia and polycystin proteins will go a long way towards illuminating both the as yet poorly understood physiological functions of the polycystin proteins and the pathological processes unleashed in their absence.

Metabolism

In addition to the multiple signaling pathways de-regulated in ADPKD, several lines of evidence in the past decade have delineated a central role for metabolic reprogramming in the initiation and progression of the disease (Figure 10). Indeed, all cells in the human

body face the need for energy production to function normally (78, 215, 370). The primary source of energy for cells derives from the consumption of carbon molecules that are catabolized in the metabolic process described as “central carbon metabolism”. Briefly, the three major sources of carbon in cells derive from glucose, glutamine, or fatty acids (213). All three sources are catabolized initially in the cytosol and their products are funneled into the TCA cycle taking place into mitochondria, where, in the presence of oxygen, the optimized ATP-producing activity of oxidative phosphorylation (OXPHOS, via the electron transport chain complexes) takes place. Whenever oxygen is not available, or when cells proliferate, there is an obligate switch to an anabolic mode to cope with the need to produce “building blocks” required to duplicate DNA, RNA, proteins and lipids at each cell division. This continuous requirement for shifting the balance between the different sources of carbon and their relative use for energy production or for the neo-synthesis of cellular components provides to cellular metabolism a uniquely flexible character (213). Perhaps due to this flexibility under physiological conditions, metabolic derangement is a relatively common feature in disease, particularly in those disorders characterized by increased proliferation such as cancer and, to a lesser extent, PKD (78). Interestingly, however, very often disease-related metabolic reprogramming also leads to an increased dependency on specific sources of energy, and thus to reduced cellular flexibility, exposing important vulnerabilities of the system that provide unique opportunities for therapy (78). Indeed, some of these vulnerabilities have been identified and shown to be targetable leading to effective retardation of disease progression in animal models of ADPKD (78).

i. Glycolysis: The first evidence of the importance of metabolic reprogramming in Polycystic Kidney Disease came from the demonstration that mouse embryonic fibroblasts (MEFs) lacking the *Pkd1* gene showed increased extracellular acidification and were dependent on glucose for their ATP production (214). Furthermore, animal models of PKD and human-derived cystic epithelial cells showed increased expression of key glycolytic enzymes and this was mirrored, in the mouse kidney carrying *Pkd1* deletion, by increased uptake of glucose and its conversion into lactate (214). Furthermore, and in line with this evidence, the administration of a glucose analogue, 2-deoxy-D-glucose (2-DG), previously demonstrated to interfere with glucose utilization in tissues, proved quite effective in inhibiting glycolysis *in vivo* and concomitantly improving disease progression in PKD murine models of increasing severity (214). These multiple lines of evidence prompted investigators to propose that ADPKD cystic epithelia were characterized by a Warburg effect similar to the one observed in cancer (213). These studies also prompted investigators to revisit a few studies that had previously described an altered expression of metabolic enzymes in human or murine PKD tissues and to propose that metabolic reprogramming might be a broad and quite strong feature of PKD (371). Oxidative phosphorylation (OXPHOS) is the process of phosphorylating ADP to ATP by extracting energy from electrons released in the breaking of carbon bonds, and for which oxygen acts as a final acceptor in the process that is mediated by the electron transport chain in mitochondria (372). Notably, the carbon bonds that are broken provide electron reducing equivalents to the tricarboxylic acid cycle (TCA, also known as the Krebs cycle after its discoverer). This mitochondrial oxygen-consuming process is dampened in all PKD models investigated to date, including fibroblasts and renal epithelial cells mutant for the *Pkd1* gene, as well

as epithelia derived from mice or human samples carrying mutations in the *PKD1* gene (78, 215, 371, 372). While there is broad consensus on the downregulation of OXPHOS in the absence of functional polycystins, more controversial has been the demonstration that glycolysis, the only biochemical reaction discovered to date to be able to generate energy in proliferating cells that cannot rely on mitochondria for their ATP production (214, 370) accounts for the bulk of ATP production in cells that lack polycystin expression. The most likely explanation for this initial controversy relates to issues that prevented some investigators from detecting either by transcriptional profiling or by the use of more sophisticated methods the presence of an increased utilization of glucose and lactate production either in cells or in tissues. However, the most elegant demonstration of the strong increase in glycolysis observed in PKD (in a process similar to the Warburg effect observed in cancer cells) was provided by investigators employing metabolic tracing using 13C-labelled molecules both in vitro and in vivo to demonstrate the high uptake of glucose and the production of lactate in cells and tissues (78, 79, 214, 373). These elegant studies have provided the formal proof that this process occurs in PKD. Further support for this model comes from the multiple demonstrations in the literature of the strong suppression of disease progression achieved by administration of glucose analogues such as 2-deoxy-D-glucose (2-DG) in all animal models tested to date (214, 373), as well as the strong improvement achieved upon fasting or exposure to ketogenic diets (293, 374) in animal models. Thus, increased glucose utilization is a major feature of PKD and a good target for therapy.

ii. miR-17: Among the first evidence that cellular metabolism could be dysregulated in PKD came from a set of microarray studies performed on the epithelia lining the cysts derived from patients carrying mutations in the *PKD1* gene (375). Multiple signaling pathways had been identified as transcriptionally dysregulated, as might be expected when analyzing samples from relatively late stage disease (376). Among the de-regulated pathways reduced OXPHOS was identified as a very prominent feature. Additional metabolic pathways were also described to be dysregulated (371, 372), however, little emphasis was placed on these data initially(376). When it was reported that increased glycolysis (214) and later that reduced mitochondrial activity (53) were prominent features of PKD, these data acquired greater relevance. Additional evidence in support of the metabolic dysregulation in PKD came from the interesting observation that PKD animal models displayed an upregulation of the miR-17~92 cluster and that, in line with this, genetic deletion of the cluster in mice, or treatment with anti- miR-17~92 anti-sense oligonucleotides resulted in a drastic improvement of polycystic kidney disease (377). Of great interest, the authors had attributed the effect of miR-17~92 in PKD to a c-myc dependent downregulation of oxidative phosphorylation and of metabolic pathways (377). Indeed, the authors demonstrated that c-Myc drives the upregulation of miR-17~92 in cystic kidneys, and that the downstream effects of these mIRs are based in part on inhibition of oxidative phosphorylation on the one hand and repression of Pparα on the other hand (376) Thus, given the strong effect of the cluster miR-17~92 on the mitochondrial metabolism and the very strong and promising therapeutic efficacy of anti-miR-17, the data seem to be supportive of a strong miR17-dependent dysregulation in metabolic processes.

iii. Calorie restriction: The fact that mTOR has been implicated as a kinase important for disease progression, along with the emergent role of glucose as a primary carbon source of energy in ADPKD tissues (due to the Warburg effect, see above), has prompted investigators to propose and test the hypothesis that inducing a ketogenic state in animals, and possibly in patients, could provide some benefit to the progression of PKD (214, 273). Indeed, inducing a ketogenic state in patients has long been used as a way to lower circulating glucose levels in those diseases, such as diabetes, for which induction of hypoglycemia would lead to a beneficial outcome (378, 379). Ketogenesis can be induced by ketogenic diets, by calorie restriction, or by intermittent fasting (378, 379). All of these diets have been tested in humans for multiple disease conditions. In PKD, it was first demonstrated that reducing calorie intake in mice would provide a robust retardation of disease progression (293). Subsequent work has demonstrated that a ketogenic diet as well as intermittent fasting also provides strong improvement in disease progression in animal models of the disease and, interestingly, in *Pkd1* mutant cats (374) In this last study, it was also shown that the increased production and release of ketone bodies such as hydroxybutyrate from the liver was part of the mechanism and, in line with this finding, administration of Beta-hydroxybutyrate (BHB) in the absence of any added dietary intervention was sufficient to improve disease progression (374). Subsequent studies have reported controversial findings on the efficacy of calorie restriction versus intermitted fasting in animal models (380). Recent work has also provided evidence that a reduction in calorie intake produces very beneficial results leading to improved kidney function when it was applied to individuals with high BMI that would lose weight through the dietary intervention, while it had no renal function-preserving beneficial effect when applied to individuals with a normal BMI and in the absence of weight loss (380). These data seem to indicate that an elevated BMI impacts negatively on the functional decline of renal function in ADPKD, but that intermittent fasting per se would not provide any benefit. These initial studies have prompted multiple investigators and centers to test whether an induction of a ketogenic state by diets that would include intermittent fasting provide a beneficial effect on ADPKD patients (380). Multiple clinical trials have been initiated and are ongoing as we are writing this review article. The first data reported from a clinical trial in humans suggests the feasibility of this approach with indications of a positive outcome, though much more data will be required to demonstrate possible efficacy and safety in humans (381).

iv. Glutamine-dependence and other amino acids: In addition to the dramatic alteration in glucose metabolism, cells lacking the *Pkd1* gene were found to have more profound and broad metabolic reprogramming features (79). In particular, it was shown using metabolic tracing studies by feeding Pkd1 mutant cells with either $^{13}C_6$-glucose or with $^{13}C_5$-glutamine that mutant cells divert most of the glucose-derived carbons into the pyruvate-lactate axis, and that they compensate for the reduced utilization of the glucose-derived pyruvate in the TCA cycle by importing excessive amounts of glutamine (79). Glutamine-derived carbons were found to provide a modest amount of fuel to the TCA cycle and, in particular, to the oxidative branch of the TCA cycle (79). This flux helps to compensate for the reduced utilization of glucose-derived carbons and thus to maintain minimal levels of OXPHOS activity, which is essential to maintain the mitochondrial membrane potential and to avoid collapse of mitochondria and apoptosis (79). Surprisingly,

the data also showed that cells lacking the *Pkd1* gene have tremendously high levels of reductive carboxylation which results in synthesis of citrate, which is exported into the cytosol and broken down in oxalacetate and AcetylCoA, which is extensively utilized for lipid de novo biosynthesis (Figure 10) (79).

Notably, glutamine is a non-essential amino acid, which becomes essential under nutrient stress conditions. Its essential role is based upon its capability to be oxidized in the TCA cycle to sustain minimal levels of OXPHOS to maintain the mitochondrial membrane potential under conditions of reduced utilization of glucose or lipids as energy sources. In this context, it was shown that the enhanced levels of glutamine utilization in PKD ensure this minimal OXPHOS generation (79). Further to this, glutamine is also a fundamental component of glutathione biosynthesis. A previous study had shown that polycystic kidney disease is characterized by a severe dependency on glutamine, because its utilization is essential for glutathione biosynthesis which helps to buffer an oxidative unbalance in PKD (382). In this case, it was shown that blocking the enzyme glutaminase, which converts glutamine into glutamate and releases an ammonia group, was able to ameliorate disease progression in pre-birth treatment (382). A second study confirmed that the Gls1 inhibitor had a beneficial effect on retarding disease progression, but only in one and not in another animal model tested in this case (383). Finally, it should be noted that the first evidence of a possible involvement of glutamine rewiring in PKD came from the demonstration that in the cpk ARPKD rat model there could be an increased utilization of glutamine, associated with an increased production of the oncometabolite 2-Hydroxy-Glutarate (2-HG) (384). While this is not an orthologous model of ADPKD, it is an orthologous model of ARPKD. These data thus suggest that alterations similar to the one observed in dominant PKD could be likely identified in recessive PKD, though more work will be needed to refine the metabolic pathways involved (384). This study showed for the first time that glutamine dependence could be a general feature of PKD (384). Notably, a recent study also showed that cells lacking the Pkhd1 gene present with reduced OXPHOS activity that very much resembles the observations in ADPKD, providing additional evidence for the existence of metabolic similarities among these conditions (162).

Finally, additional amino acids have been shown to exhibit deranged metabolism in ADPKD animal models (385). Of special note in this context is methionine, whose altered metabolism was shown to be critical because of its capability to participate in RNA methylation pathways that influence mRNA translation and stability. Indeed, Ramalingam et al have shown that elevated levels of methionine and its derivative S-adenosylmethionine in PKD murine kidneys correlates with an upregulation of expression of the methyltransferase Mettl3, which in turn regulates expression of a number of transcripts by controlling m6A levels (385). Overexpression of Mettl3 is sufficient to induce tubular dilatation and mild cystogenesis, while deletion of the enzyme in PKD models greatly ameliorates disease progression (385). Importantly, the investigators have shown that dietary methionine restriction improves disease progression in PKD, adding a new opportunity for nutritional intervention in PKD.

v. Metabolic profiling and the identification of biomarkers: Finally, a number of studies have explored the metabolic alterations present in ADPKD by profiling different

types of tissues using various metabolomic platforms (353, 386–390). While all of these studies invariably showed alterations in multiple metabolic pathways, including central carbon metabolism, it is worth noting here that while developing a profile of metabolites might be a great strategy for identifying possible biomarkers, such profiling alone is unlikely to provide a mechanistic view of what is happening in a given tissue. Indeed, metabolic profiling only offers a snapshot of metabolites that can be identified at a given time in a given tissue, but it does not provide any information as to whether the flow through a particular metabolic pathway is increased or decreased. Indeed, to study in detail the metabolic alterations observed in a given sample, one needs to be able to manipulate the system so as to follow alterations in time. Typically, isotopologues of carbon or nitrogen are utilized to follow the fate of individual metabolites once they have been uptaken by cells or tissues. For instance, the use of $^{13}C_6$-Glucose was used *in vivo* to show that in PKD kidneys an enhanced production of lactate from glucose could be identified, therefore defining, and quantifying the degree of Warburg effect (214, 373). These studies *in vivo* can be very demanding, time consuming and expensive. Recently, investigators have been able to set up organotypic renal cultures fed with isotopologue-labelled metabolites to study the fate of glucose, glutamine and lipids, the three main sources of carbons in cells, in the individual cells of the kidney in a first successful utilization of spatial metabolomics (391, 392). Future studies in PKD should likely move in this direction to specify which cells are affected by which metabolic alterations by following the fate of energy sources in time (391, 392). While the multiple metabolic profiles generated in the past several years provide important information for possible alterations, the use of more sophisticated systems will likely unveil important mechanistic information.

IV. Models and Extrarenal Activities: A number of animal models have been created and are used to study the pathophysiology of ADPKD and the function of the Polycystins. These range from the use of Drosophila Melanogaster (393) to the use of C. Elegans (65), the fish Danio rerio (394) and includes a number of mammalian models (395). Here, we will concentrate on the mammalian animal systems, with the intent to describe the models that, in our view, better recapitulate the human condition.

Organoid models of ADPKD

Recent advances in the methods for creating renal organoids have made it possible to interrogate the pathways and processes related to polycystin function and cyst formation in nephron-like structures comprised of human renal epithelial cells. A number of renal organoid systems have been developed that employ protocols for differentiating renal tubule structures from induced pluripotent stem cells (iPSCs)(396–401), fetal embryonic stem cells (402, 403) and nephron progenitor cells (404, 405). Each of these approaches can produce three dimensional cultures of human renal epithelial cells that manifest gene expression patterns and cellular morphologies that resemble those of nephron epithelial cells in human kidneys, although the cultures produced through these approaches often resemble immature embryonic renal epithelial cells. Gene editing techniques have been used to create renal organoids that manifest disrupted expression of polycystin proteins. Gratifyingly, these models can be induced to form cysts whose morphological and gene expression properties resemble those of the cysts found in human ADPKD patients and in rodent models of

ADPKD (395, 397, 398, 401, 402, 404–410). “Organoid on a chip” models that incorporate the possibility of fluid flow through the cultured nephron structures have also been created to model ARPKD (411). The mechanisms through which cysts form in organoid models of PKD is a subject of very active research (407, 412). Interestingly, in a number of these systems the cysts form “inside-out”, with the basolateral surfaces of the cyst epithelial cells facing the cyst lumen and the apical surfaces facing the culture medium. It will be interesting to understand how and why this apparent inversion of cyst topology occurs, and to explore what it tells us about the extent to which these model systems can be used to model faithfully the pathogenesis of cystic disease. It is becoming clear, however, that organoid systems have the potential to serve as versatile and accessible systems in which to perform the early stages of preclinical testing of novel therapeutic strategies for PKD (395, 401, 402, 412, 413).

Rodent models of ADPKD

Over the space of two decades several animal models have been generated to study the function of the polycystins. While prior of the cloning of the PKD1 and PKD2 genes, the preferred animal models employed in the field were either rats or mice that showed a spontaneous PKD phenotype (414). After the two genes mutated in humans had been identified, however, it became clear that the animal models that better reflect the development of PKD are those carrying mutations or deletions in the genes orthologous to the human PKD genes (134, 415). Indeed, multiple animal models have been developed over the years and extensively studied. It was immediately clear from the very early analysis of animal models of the Pkd1 or 2 genes that heterozygosity was not sufficient to recapitulate the human phenotype, most likely due to a lack of somatic second hits able to that recapitulate the focal reduction in the presence of functional polycystins complexes (134, 415). Indeed, the only murine model that nicely recapitulates the human condition is a *Pkd2* mutant mouse model (the so called WS25) that carries an unstable allele of the *Pkd2* gene, and this indeed develops a disease characterized by focal formation of cysts along over the life course of the mouse. This model is certainly the best developed to date in mice (416). As a downside, this murine PKD model, like the human condition, is characterized by a slowly progressive and hugely variable phenotype. Additional attempts at generating animal models that would both recapitulate the human condition and be more reliably usable for functional analysis or therapeutic testing have led to the generation of floxable *Pkd1* or 2 alleles that when intercrossed with either constitutively active or inducible Cre recombinase murine lines, results in a cystic phenotype caused by loss-of-function of the *Pkd* alleles and initiation/evolution of a renal cystic phenotype (417). Further analysis also led to the exciting and surprising discovery that time of inactivation can make a great difference in the phenotype observed, with early activity of the Cre recombinase in young, newborn mice results resulting in a quite aggressive PKD phenotype, whereas inactivation at later timepoints in juvenile or adult mice results resulting in increasingly mild phenotypes (418). These observations, besides helping to establish better animal models, might also suggest that the great variability observed in humans might reflect a degree of stochasticity whereby the time when the second hit is initiated in humans might determine a more or less aggressive disease progression. In this scenario, the time of acquisition of the second hit might therefore by itself be a strong determinant of phenotypic variability (417, 418).

Finally, one additional approach that has been taken to generate better murine models to study disease progression and therapeutic interventions came from the unexpected observation that in extremely rare cases in humans, patients were found to inherit two mild hypomorphic mutations in the PKD1 gene in homozygosity (418). These rare cases of “recessively inherited dominant PKD” have been shown to progress in a particularly mild manner and to be characterized by the formation of homogeneously sized small cysts progressing during the course of the life lives of these patients. Of great interest, introduction into the murine allele of the corresponding human mis-sense mutation (to generate a Pkd1 murine mutant allele called Pkd1RC) also showed that in homozygosity this presumably partially functional hypomorphic mutant develops an extremely slowly progressive disease. When intercrossed with a null allele, as well, the Pkd1RC allele results in a slowly progressive disease model developing over the course of months (418). While this animal model reproduces an extremely rare human condition and not the most common cases of ADPKD, the fact that animals develop a slowly progressive disease caused by mutations in the Pkd1 gene, have made this model extremely useful to study in long-term studies of the disease progression as well as assessments of possible therapeutic interventions (418).

One final note that deserves mention is the fact that among mammals, two species seem to develop PKD that is similar to the human condition: cats and minipigs (374, 419). Indeed, an inbred Persian cat species develops spontaneous dominant Polycystic Kidney Disease due to heterozygous inheritance of a missense mutation in the *PKD1* gene (420). Likewise, a mini pig model engineered to carry deletion in one *PKD1* allele develops a spontaneous disease manifestation in a relatively slowly progressive manner with an apparent full penetrance (419). These animals would in principle be the best possible experimental model(s) as they: i) are orthologous models caused by mutations in the *PKD1* gene; ii) develop a spontaneous PKD similar to the one observed in humans (likely due to second hits); iii) in the case of the minipig, they develop over the course of three years making the disease slowly progressive and the model usable for testing therapeutic compounds; iv) minipigs appear to have a renal physiology which is quite close to the one in humans. For these models, however, time, costs and ethical issues might limit the usability in extensive experimental studies.

Extra renal expression/effects

One important aspect to consider is that ADPKD is not only a renal disorder, but rather a systemic one as described above (421). Thus, several efforts have been made to try to recapitulate all aspects of the disease, at least in vertebrate systems. In particular, rodent systems have been extensively utilized to study extra-renal manifestations with a special focus on cardiovascular and hepatic manifestations (422). Cardiovascular manifestations have been extensively described in Pkd mouse models and data seem to support the idea that haploinsufficiency might drive some of the cardiovascular manifestations such as hypertension and possibly aneurism formation (46, 423, 424). Likewise, multiple lines of evidence have provided important information about the generation of hepatic cysts upon biallelic inactivation of the Pkd1 or Pkd2 genes specifically in the cholangiocytes of the bile duct. Likewise, inactivation of the Pkhd1 gene in these cell types does results in gross liver

cystic disease, even if patients affected by ARPKD do not manifest with cysts in the liver, but only with a severe hepatic fibrosis (425).

Rodent models of ARPKD and other recessive ciliopathies

Recessive PKD, unlike dominant PKD, has not benefited as yet from the generation of faithful animal models of the disease. One of the strategies that was employed to identify the PKHD1 gene that is mutated in ARPKD was identified involved work with an animal model (426), the cpk rat, which had been used extensively as a model of ADPKD because it manifests very massive polycystic kidney disease in adult rats. Molecular analysis revealed retrospectively that this rat carries truncating mutations in the Pkhd1 gene. Why a rat carrying a mutation in the Pkhd1 gene develops a slowly progressive disease remains unclear, but at least rats do develop a PKD phenotype. Conversely, multiple different murine mouse models carrying various types of PKHD1 mutations were generated, but with the exception of few that manifest a mild cystic phenotype in the kidney, most animal models do not develop a kidney phenotype that could serve the purpose of supporting studies of the disease pathogenesis or of defining the effectiveness of possible therapies (427). Of interest, several of these murine models generated over the years do develop a polycystic liver disease, which is not a feature of ARPKD in patients(425, 428). Conversely, these animals do not develop the typical hepatic fibrosis phenotype observed in patients (428).

Therapeutic targets

If we knew exactly what the polycystin proteins are supposed to be doing then we would have a clear idea of what needs to be fixed to prevent or reverse the cystic disease that develops in their absence. It is both paradoxical and predictable that, in the absence of a single unified understanding of polycystin function, the list of potential therapeutic targets is at least as large as the inventory of signaling pathways outlined above that are dysregulated in these proteins' absence. A thorough discussion of each of these targets and of the many creative efforts that have been made to exploit is well beyond the scope of the present discussion. It is worthwhile, however, to explore briefly the broad categories of new and developing therapeutic avenues, as they are all based in large measure upon the types of polycystin-related effects and perturbations that are reviewed here.

Signaling pathways

Perturbations of the cAMP signaling pathway were the first ADPKD-related cellular abnormalities to be explored in depth and, fittingly, are the first to be addressed by an approved therapy. Stimulation of the V2R vasopressin receptor by anti-diuretic hormone induces cAMP production in the principal cells of the renal collecting duct (429). Administration of Tolvaptan, a small molecule inhibitor of the V2R, reduces cAMP accumulation in V2R-expressing cells and slows cyst development in numerous animal models of ADPKD (430). More importantly, administration of this compound slows cyst growth and preserves renal function in ADPKD patients (431). Consequently, Tolvaptan has obtained international regulatory approval as a therapeutic for ADPKD. It is worth noting that other approaches to reducing renal epithelial cell cAMP production, including among others by activating the somatostatin receptor or by inhibiting the β3 adrenergic receptor, have also been explored (432) and may be indicative of alternatives that have the potential

to reduce cAMP levels in a broader cross-section of renal epithelial cell types than can be targeted by Tolvaptan, whose molecular target is expressed primarily in collecting duct principal cells.

Small molecules that target additional perturbed signaling pathways have also shown substantial promise in preclinical studies employing animal models. An incomplete list of these approaches includes efforts to use small molecules to inhibit the mTOR pathway, aberrant ceramide production, the cell cycle, Wnt signaling, protein kinase activities and epigenetic perturbations. In particular, rapamycin was employed in multiple non-orthologous and orthologous animal models of PKD and produced a very robust response in these preclinical systems (273, 276). Subsequent studies in humans, however, showed much less promising responses to mTOR inhibition (433, 434), which may be attirbutable at least in part to severe side effects that did not allow to the high doses of inhibitors that had been used in the rodent models to be achieved. Although some of these directions, including ceramide-directed interventions (435), have been evaluated in human clinical trials, none of these approaches have yet been shown to be safe and effective in slowing disease progression in ADPKD patients.

The successful development of Tolvaptan was clearly predicated upon the preclinical studies that identified aberrant cAMP signaling as a driver of ADPKD cyst formation. Equally importantly, however, the effort that led to its successful development owes at least as much to the enormous effort invested by a large number of clinical and translational scientists in developing the metrics that were required to assess therapeutic efficacy and who designed and conducted the trials that employed these metrics. The approval of Tolvaptan offers the ADPKD patient community with a long-awaited therapeutic option that can slow disease progression and prolong renal function. Its value to the ADPKD community also derives from the fact that the remarkable community of investigators who moved Tolvaptan from the laboratory to the clinic pioneered a pathway for the testing and approval of future therapies that may offer better efficacy and side effect profiles.

Metabolism

The metabolic alterations that characterize cells that lack polycystin expression suggest attractive therapeutic vulnerabilities. Dietary interventions, including calorie restriction and imposition of ketosis-inducing diets, have been shown to slow disease progression in animal models of ADPKD (293, 374, 436). Starving glycolysis-dependent cyst cells for glycolytic substrates by administering 2-deoxyglucose shows great potential promise (373, 419, 437). Similarly, ADPKD cells manifest a need for glutamine as an energy source and interrupting the availability of glutamine by inhibiting the activities of the asparagine metabolizing enzymes that feed this dependence constitutes a new avenue for therapeutic development (78, 79). The metabolic perturbations experience by epithelial cells affected by ADPKD lead to additional amino acid-related perturbations, including the accumulation of methionine, which can in turn influence pathways that regulate mRNA translation and contribute to cyst formation (385). Directly or indirectly modulating the pathways that consume or produce a variety of bioactive metabolites thus constitutes a very active area of research into ADPKD therapeutic directions. Finally, it is worth noting that a number of existing drugs that

target important modulators of metabolism have shown efficacy in preclinical models of ADPKD. These include indirect and direct AMPK activators such as metformin (438), thiazolidinediones(439), bempadoic acid (440), and salsalate (441) (291). While in small clinical trials these compounds have not exhibited significant efficacy (442–444), larger ongoing trials may reveal whether or not these well-tolerated compounds have the potential to be repurposed as ADPKD therapeutics.

Finally, inhibitors of mTOR were also tested in preclinical and clinical trials (see above) providing only limited benefit in the patients. This was partly due to the low doses of rapalogues that can be employed in patients, as opposed to the high doses that had been used in preclinical studies. Additionally, it should be considered that, while several of the metabolic alterations observed in PKD were shown to be secondary to mTOR upregulation and AMPK deregulation (214), no study has specifically looked at whether at least some of the extensive metabolic alterations observed in PKD do not depend on the mTORC1 branch of the mTOR pathway, potentially explaining the limited effect of its inhibition.

Inflammation and Immunity

Inflammatory stimuli, such as renal stone formation, have been shown to exacerbate cyst formation in ADPKD animal models and may do so as well in patients (6). Macrophages and other components of the innate immune system make important contributions to cystic disease progression (344–349, 445–450). Cells of the adaptive immune system appear to be capable of slowing cystic disease, and cyst cells produce chemical signals that may suppress the capacity of the adaptive immune system to control cystic disease (343, 351–353). A human clinical trial testing the anti-inflammatory agent bardoxalone as an agent to possibly slow ADPKD was discontinued. There exists, however, an enormous and varied trove of pharmacological agents that target aspects of inflammation and adaptive immune function. This pharmacopeia is likely to constitute a promising source of clinically tolerable compounds that may be capable of suppressing or activating the relevant components of the innate and adaptive immune systems in order to control the course of ADPKD.

Genetics

Even in the absence of a clear understanding of the functions of the polycystin proteins, it can be stated with confidence that ADPKD is a genetic disease that develops as a direct consequence of insufficient expression levels of functional polycystin proteins. Thus, interventions designed to increase functional polycystin expression are likely to be effective. This statement derives support from experiments that employed a clever approach to engineer the conditional re-expression of PC1 or PC2 in a mouse model that had developed advanced cystic disease as a consequence of prior disruption of the Pkd1 or Pkd2 gene. Remarkably, re-expression of the relevant polycystin protein produced a dramatic reversal of cystic pathology and recovery of renal function in animals that had reached the end stages of cystic disease (451). Thus, strategies that increase functional expression of polycystin proteins have the potential not only to slow disease progression but also to ameliorate extant cystic pathology. In the case of disease attributable to loss of PC2, the size of the Pkd2 gene is sufficiently small to permit its packaging in standard viral gene therapy systems. While the Pkd1 gene is far to large to be delivered in its entirety via viral gene therapy,

the observation that the expression of the C-terminal tail of the PC1 protein suppresses disease progression in Pkd1 conditional knockout mice (161) suggests the possibility that gene therapy designed to provide a small but functionally important piece of PC1 might offer a pathway for the development of Pkd1-directed gene therapy approaches.

Most ADPKD-causing mutations lead to complete loss of PC1 or PC2 protein expression. In as many as ~30% of patients, however, a pathogenic missense mutation may result in the production of a polycystin protein that does not fold, traffic or function at levels sufficient to prevent disease development (4, 128). It is possible that a subset of patients carrying such hypomorphic alleles may be candidates for “molecular chaperone” therapies that increase the size of the functionally active pool of polycystin protein (452). This approach has proved to be wonderfully successful in the context of cystic fibrosis, in which the folding and functional deficiencies of a subset of CFTR proteins encoded by a subset of pathogenic alleles can be rescued to levels sufficient to produce remarkable clinical benefit. In the case of cystic fibrosis, ~90% of patients carry at least one copy of the pathogenic ΔF508 CFTR allele. Consequently, therapies that enhance the functional expression of the ΔF508 CFTR protein are of potential value to a large majority of patients. In the case of ADPKD, there are no predominant hypomorphic alleles. Hence, it remains to be determined whether any chemical chaperone approach will enhance the functional expression of a broad cross section of mis-folded or dysfunctional polycystin proteins encoded by the large and diverse array of pathogenic hypomorphic alleles. It is important to note in this context that reduced polycystin protein expression leads to upregulation of the expression of a micro-RNA, miR17, which suppresses the translation of the mRNAs encoding PC1 and PC2 (64, 376). Thus, interfering with miR17-mediated actions could increase polycystin expression and suppress disease development in settings in which increasing the expression of a pathogenic hypomorphic allele could result in levels of polycystin protein function sufficient to slow disease progression.

Conclusions

If nothing else, this review makes a strong case for the premise that ADPKD is complicated. When examined at every level of resolution, from molecular to cellular to organ to organismal, ADPKD reveals complex webs of pathways and processes. In spite of two decades of intense and creative effort, we have to admit that we still do not know the physiological functions of the polycystin proteins or the identities of their most proximate downstream effectors. Similarly, we do not have a deep understanding of the processes that drive the formation of cysts by renal epithelial cells that lack functional expression of the polycystin proteins. It is safe to say, however, that studies of the proteins and pathways involved in ADPKD have generated an enormous quantity of fundamental and multidisciplinary biological insight. Furthermore, these insights have led to the identification of a number of potential therapeutic targets and strategies. While many of these novel approaches are likely to not survive translation into actual clinical tools, their pursuit is providing a wealth of knowledge about the pathogenesis and possible vulnerabilities of ADPKD. Furthermore, the rapidly expanding number and variety of these nascent therapeutic avenues reflects a tremendous amount of creativity and enthusiastic effort that is being focused on this problem by a large international community of focused and devoted

scientists and clinicians. The rapid and accelerating pace of discovery in the field is cause for optimism that the next several years will see the creation of a number of effective therapies ADPKD.

Acknowledgements

The authors wish to extend their heartfelt gratitude to all of the members of their laboratory groups, present and past, whose efforts and insights have been instrumental in shaping the authors' understanding of the field. The authors are also extremely grateful to their many colleagues and collaborators whose perspectives and contributions have helped the authors tremendously in their efforts to present a balanced perspective view of a complex and evolving discipline. Work in the authors' laboratories is supported by grants from the US Department of Defense (AB and MJC), European Union (AB), Italian association for research on PKD (AB), Italian Association for Research on Cancer (AB), the NIH (MJC), and the Polycystic Kidney Disease Foundation (MJC).

List of Abbreviations

ADPKD	autosomal dominant polycystic kidney disease
ADPLD	autosomal dominant polycystic liver disease
aGPCR	adhesion G protein-coupled receptor
AMPK	adenosine monophosphate-stimulated protein kinase
ARPKD	autosomal recessive polycystic kidney disease
BBS	Bardet-Biedl Syndrome
BMP	bone morphogenetic protein
cAMP	cyclic adenosine monophosphate
CDCA	cilia-dependent cyst activating activity
CFTR	cystic fibrosis transmembrane conductance regulator
CL	C-type lectin domain
CLCI	cilia-localized cyst-inhibition activity
CREB	cyclic AMP response element binding protein
CryoEM	cryo-electron microscopy
CTF	C-terminal fragment of PC1
CTT	C-terminal tail of PC1
DAMP	Damage-associated molecular pattern
EM	electron microscopy
ER	endoplasmic reticulum
ERK	extracellular regulated kinases

FPC	Fibrocystin, polyductin
GAIN	G-protein-coupled receptor autoproteolysis-inducing domain
GANAB	gene encoding Glucosidase II Alpha Subunit
GAP	GTPase activating protein
GFP	green fluorescent protein
GPCR	G protein-coupled receptor
GPS	G-protein coupled receptor proteolytic site
GSK3β	glycogen synthase kinase 3β
IFT	intraflagellar transport
IP3	inositol triphosphate
LRR	leucine rich repeat
MAM	mitochondria-associated membrane
MDCK	Madin Darby canine kidney cell
MEF	mouse embryonic fibroblast
MERC	mitochondria-ER contact site
mTOR	mechanistic target of rapamycin
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NNT	nicotinamide nucleotide transhydrogenase
NTF	N-terminal fragment of PC1
OFD	oral-facial-digital syndrome
OXPHOS	oxidative phosphorylation
PC1	polycystin 1
PC2	polycystin 2
PDE4	phosphodiesterase 4
PDGF	platelet-derived growth factor
PKD	polycystic kidney disease
PLAT	Polycystin-1, Lipoxygenase, Alpha-Toxin domain

PTCH1	patched receptor
REJ	receptor for egg jelly
RTK	receptor tyrosine kinase
SDS-PAGE	sodium dodecyl-sulfate polyacrylamide gel electrophoresis
SMO	smoothened receptor
SRP	signal recognition particle
STAT	“signal transducer and activator of transcription” transcription factor
STIM1	stromal interaction molecule 1
TAZ	transcriptional activator with a PDZ domain
TCA	tricarboxylic acid cycle
TGFβ	tissue growth factor β
TM	transmembrane
TOP domain	polycystin domain
TRP	transient receptor potential
V2R	V2 vasopressin receptor
WSC	wall stress component

References

1. Watnick TJ, Torres VE, Gandolph MA, Qian F, Onuchic LF, Klinger KW, et al. Somatic mutation in individual liver cysts supports a two-hit model of cystogenesis in autosomal dominant polycystic kidney disease. Mol Cell. 1998;2(2):247–51. [PubMed: 9734362]
2. Zhang Z, Bai H, Blumenfeld J, Ramnauth AB, Barash I, Prince M, et al. Detection of PKD1 and PKD2 Somatic Variants in Autosomal Dominant Polycystic Kidney Cyst Epithelial Cells by Whole-Genome Sequencing. J Am Soc Nephrol. 2021.
3. Lantinga-van Leeuwen IS, Dauwerse JG, Baelde HJ, Leonhard WN, van de Wal A, Ward CJ, et al. Lowering of Pkd1 expression is sufficient to cause polycystic kidney disease. Hum Mol Genet. 2004;13(24):3069–77. [PubMed: 15496422]
4. Hopp K, Ward CJ, Hommerding CJ, Nasr SH, Tuan HF, Gainullin VG, et al. Functional polycystin-1 dosage governs autosomal dominant polycystic kidney disease severity. J Clin Invest. 2012;122(11):4257–73. [PubMed: 23064367]
5. Takakura A, Contrino L, Zhou X, Bonventre JV, Sun Y, Humphreys BD, et al. Renal injury is a third hit promoting rapid development of adult polycystic kidney disease. Hum Mol Genet. 2009;18(14):2523–31. [PubMed: 19342421]
6. Torres JA, Rezaei M, Broderick C, Lin L, Wang X, Hoppe B, et al. Crystal deposition triggers tubule dilation that accelerates cystogenesis in polycystic kidney disease. J Clin Invest. 2019;129(10):4506–22. [PubMed: 31361604]

7. Pritchard L, Sloane-Stanley JA, Sharpe JA, Aspinwall R, Lu W, Buckle V, et al. A human PKD1 transgene generates functional polycystin-1 in mice and is associated with a cystic phenotype. Hum Mol Genet. 2000;9(18):2617–27. [PubMed: 11063721]
8. Thivierge C, Kurbegovic A, Couillard M, Guillaume R, Cote O, Trudel M. Overexpression of PKD1 causes polycystic kidney disease. Mol Cell Biol. 2006;26(4):1538–48. [PubMed: 16449663]
9. Fedeles SV, Tian X, Gallagher AR, Mitobe M, Nishio S, Lee SH, et al. A genetic interaction network of five genes for human polycystic kidney and liver diseases defines polycystin-1 as the central determinant of cyst formation. Nat Genet. 2011;43(7):639–47. [PubMed: 21685914]
10. Bergmann C, Guay-Woodford LM, Harris PC, Horie S, Peters DJM, Torres VE. Polycystic kidney disease. Nat Rev Dis Primers. 2018;4(1):50. [PubMed: 30523303]
11. Iliuta IA, Kalatharan V, Wang K, Cornec-Le Gall E, Conklin J, Pourafkari M, et al. Polycystic Kidney Disease without an Apparent Family History. J Am Soc Nephrol. 2017;28(9):2768–76. [PubMed: 28522688]
12. Hopp K, Cornec-Le Gall E, Senum SR, Te Paske I, Raj S, Lavu S, et al. Detection and characterization of mosaicism in autosomal dominant polycystic kidney disease. Kidney Int. 2020;97(2):370–82. [PubMed: 31874800]
13. Boletta A, Qian F, Onuchic LF, Bragonzi A, Cortese M, Deen PM, et al. Biochemical characterization of bona fide polycystin-1 in vitro and in vivo. Am J Kidney Dis. 2001;38(6):1421–9. [PubMed: 11728985]
14. Boletta A, Germino GG. Role of polycystins in renal tubulogenesis. Trends Cell Biol. 2003;13(9):484–92. [PubMed: 12946628]
15. Chapin HC, Caplan MJ. The cell biology of polycystic kidney disease. J Cell Biol. 2010;191(4):701–10. [PubMed: 21079243]
16. Schroder S, Fraternali F, Quan X, Scott D, Qian F, Pfuhl M. When a module is not a domain: the case of the REJ module and the redefinition of the architecture of polycystin-1. Biochem J. 2011;435(3):651–60. [PubMed: 21314639]
17. Nigro EA, Boletta A. Role of the polycystins as mechanosensors of extracellular stiffness. Am J Physiol Renal Physiol. 2021;320(5):F693–F705. [PubMed: 33615892]
18. Su Q, Hu F, Ge X, Lei J, Yu S, Wang T, et al. Structure of the human PKD1-PKD2 complex. Science. 2018;361(6406).
19. Qian F, Boletta A, Bhunia AK, Xu H, Liu L, Ahrabi AK, et al. Cleavage of polycystin-1 requires the receptor for egg jelly domain and is disrupted by human autosomal-dominant polycystic kidney disease 1-associated mutations. Proc Natl Acad Sci U S A. 2002;99(26):16981–6. [PubMed: 12482949]
20. Parnell SC, Magenheimer BS, Maser RL, Zien CA, Frischauf AM, Calvet JP. Polycystin-1 activation of c-Jun N-terminal kinase and AP-1 is mediated by heterotrimeric G proteins. J Biol Chem. 2002;277(22):19566–72. [PubMed: 11912216]
21. Chauvet V, Qian F, Boute N, Cai Y, Phakdeekitacharoen B, Onuchic LF, et al. Expression of PKD1 and PKD2 transcripts and proteins in human embryo and during normal kidney development. Am J Pathol. 2002;160(3):973–83. [PubMed: 11891195]
22. Wodarczyk C, Distefano G, Rowe I, Gaetani M, Bricoli B, Muorah M, et al. Nephrocystin-1 forms a complex with polycystin-1 via a polyproline motif/SH3 domain interaction and regulates the apoptotic response in mammals. PLoS One. 2010;5(9):e12719. [PubMed: 20856870]
23. Lin CC, Kurashige M, Liu Y, Terabayashi T, Ishimoto Y, Wang T, et al. A cleavage product of Polycystin-1 is a mitochondrial matrix protein that affects mitochondria morphology and function when heterologously expressed. Sci Rep. 2018;8(1):2743. [PubMed: 29426897]
24. Wang Z, Ng C, Liu X, Wang Y, Li B, Kashyap P, et al. The ion channel function of polycystin-1 in the polycystin-1/polycystin-2 complex. EMBO Rep. 2019;20(11):e48336. [PubMed: 31441214]
25. Caplan MJ. Holding open the door reveals a new view of polycystin channel function. EMBO Rep. 2019;20(11):e49156. [PubMed: 31556469]
26. Qian F, Germino FJ, Cai Y, Zhang X, Somlo S, Germino GG. PKD1 interacts with PKD2 through a probable coiled-coil domain. Nat Genet. 1997;16(2):179–83. [PubMed: 9171830]

27. Tsiokas L, Kim E, Arnould T, Sukhatme VP, Walz G. Homo- and heterodimeric interactions between the gene products of PKD1 and PKD2. Proc Natl Acad Sci U S A. 1997;94(13):6965–70. [PubMed: 9192675]

28. Mochizuki T, Wu G, Hayashi T, Xenophontos SL, Veldhuisen B, Saris JJ, et al. PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. Science. 1996;272(5266):1339–42. [PubMed: 8650545]

29. Douguet D, Patel A, Honore E. Structure and function of polycystins: insights into polycystic kidney disease. Nat Rev Nephrol. 2019;15(7):412–22. [PubMed: 30948841]

30. Hanaoka K, Qian F, Boletta A, Bhunia AK, Piontek K, Tsiokas L, et al. Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. Nature. 2000;408(6815):990–4. [PubMed: 11140688]

31. Koulen P, Cai Y, Geng L, Maeda Y, Nishimura S, Witzgall R, et al. Polycystin-2 is an intracellular calcium release channel. Nat Cell Biol. 2002;4(3):191–7. [PubMed: 11854751]

32. Shen PS, Yang X, DeCaen PG, Liu X, Bulkley D, Clapham DE, et al. The Structure of the Polycystic Kidney Disease Channel PKD2 in Lipid Nanodiscs. Cell. 2016;167(3):763–73 e11. [PubMed: 27768895]

33. Arif Pavel M, Lv C, Ng C, Yang L, Kashyap P, Lam C, et al. Function and regulation of TRPP2 ion channel revealed by a gain-of-function mutant. Proc Natl Acad Sci U S A. 2016;113(17):E2363–72. [PubMed: 27071085]

34. The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. The European Polycystic Kidney Disease Consortium. Cell. 1994;78(4):725.

35. The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. The European Polycystic Kidney Disease Consortium. Cell. 1994;77(6):881–94. [PubMed: 8004675]

36. Polycystic kidney disease: the complete structure of the PKD1 gene and its protein. The International Polycystic Kidney Disease Consortium. Cell. 1995;81(2):289–98. [PubMed: 7736581]

37. Burn TC, Connors TD, Dackowski WR, Petry LR, Van Raay TJ, Millholland JM, et al. Analysis of the genomic sequence for the autosomal dominant polycystic kidney disease (PKD1) gene predicts the presence of a leucine-rich repeat. The American PKD1 Consortium (APKD1 Consortium). Hum Mol Genet. 1995;4(4):575–82. [PubMed: 7633406]

38. Griffin MD, Torres VE, Grande JP, Kumar R. Immunolocalization of polycystin in human tissues and cultured cells. Proc Assoc Am Physicians. 1996;108(3):185–97. [PubMed: 8774052]

39. Palsson R, Sharma CP, Kim K, McLaughlin M, Brown D, Arnaout MA. Characterization and cell distribution of polycystin, the product of autosomal dominant polycystic kidney disease gene 1. Mol Med. 1996;2(6):702–11. [PubMed: 8972485]

40. Geng L, Segal Y, Peissel B, Deng N, Pei Y, Carone F, et al. Identification and localization of polycystin, the PKD1 gene product. J Clin Invest. 1996;98(12):2674–82. [PubMed: 8981910]

41. Ibraghimov-Beskrovnaya O, Dackowski WR, Foggensteiner L, Coleman N, Thiru S, Petry LR, et al. Polycystin: in vitro synthesis, in vivo tissue expression, and subcellular localization identifies a large membrane-associated protein. Proc Natl Acad Sci U S A. 1997;94(12):6397–402. [PubMed: 9177229]

42. Peters DJ, van de Wal A, Spruit L, Saris JJ, Breuning MH, Bruijn JA, et al. Cellular localization and tissue distribution of polycystin-1. J Pathol. 1999;188(4):439–46. [PubMed: 10440756]

43. Wodarczyk C, Rowe I, Chiaravalli M, Pema M, Qian F, Boletta A. A novel mouse model reveals that polycystin-1 deficiency in ependyma and choroid plexus results in dysfunctional cilia and hydrocephalus. PLoS One. 2009;4(9):e7137. [PubMed: 19774080]

44. Castelli M, Boca M, Chiaravalli M, Ramalingam H, Rowe I, Distefano G, et al. Polycystin-1 binds Par3/aPKC and controls convergent extension during renal tubular morphogenesis. Nat Commun. 2013;4:2658. [PubMed: 24153433]

45. Qian Q, Li M, Cai Y, Ward CJ, Somlo S, Harris PC, et al. Analysis of the polycystins in aortic vascular smooth muscle cells. J Am Soc Nephrol. 2003;14(9):2280–7. [PubMed: 12937304]

46. Outeda P, Huso DL, Fisher SA, Halushka MK, Kim H, Qian F, et al. Polycystin signaling is required for directed endothelial cell migration and lymphatic development. Cell reports. 2014;7(3):634–44. [PubMed: 24767998]
47. Coxam B, Sabine A, Bower NI, Smith KA, Pichol-Thievend C, Skoczylas R, et al. Pkd1 regulates lymphatic vascular morphogenesis during development. Cell reports. 2014;7(3):623–33. [PubMed: 24767999]
48. Qian F, Watnick TJ, Onuchic LF, Germino GG. The molecular basis of focal cyst formation in human autosomal dominant polycystic kidney disease type I. Cell. 1996;87(6):979–87. [PubMed: 8978603]
49. Huan Y, van Adelsberg J. Polycystin-1, the PKD1 gene product, is in a complex containing E-cadherin and the catenins. J Clin Invest. 1999;104(10):1459–68. [PubMed: 10562308]
50. Drummond IA. Polycystins, focal adhesions and extracellular matrix interactions. Biochim Biophys Acta. 2011;1812(10):1322–6. [PubMed: 21396443]
51. Pazour GJ, San Agustin JT, Follit JA, Rosenbaum JL, Witman GB. Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in orpk mice with polycystic kidney disease. Curr Biol. 2002;12(11):R378–80. [PubMed: 12062067]
52. Yoder BK, Hou X, Guay-Woodford LM. The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. J Am Soc Nephrol. 2002;13(10):2508–16. [PubMed: 12239239]
53. Padovano V, Kuo IY, Stavola LK, Aerni HR, Flaherty BJ, Chapin HC, et al. The polycystins are modulated by cellular oxygen-sensing pathways and regulate mitochondrial function. Mol Biol Cell. 2017;28(2):261–9. [PubMed: 27881662]
54. Woodward OM, Li Y, Yu S, Greenwell P, Wodarczyk C, Boletta A, et al. Identification of a polycystin-1 cleavage product, P100, that regulates store operated Ca entry through interactions with STIM1. PLoS One. 2010;5(8):e12305. [PubMed: 20808796]
55. Chauvet V, Tian X, Husson H, Grimm DH, Wang T, Hiesberger T, et al. Mechanical stimuli induce cleavage and nuclear translocation of the polycystin-1 C terminus. J Clin Invest. 2004;114(10):1433–43. [PubMed: 15545994]
56. Merrick D, Bertuccio CA, Chapin HC, Lal M, Chauvet V, Caplan MJ. Polycystin-1 cleavage and the regulation of transcriptional pathways. Pediatr Nephrol. 2014;29(4):505–11. [PubMed: 23824180]
57. Talbot JJ, Shillingford JM, Vasanth S, Doerr N, Mukherjee S, Kinter MT, et al. Polycystin-1 regulates STAT activity by a dual mechanism. Proc Natl Acad Sci U S A. 2011;108(19):7985–90. [PubMed: 21518865]
58. Pellegrini H, Sharpe EH, Liu G, Nishiuchi E, Doerr N, Kipp KR, et al. Cleavage fragments of the C-terminal tail of polycystin-1 are regulated by oxidative stress and induce mitochondrial dysfunction. J Biol Chem. 2023;299(9):105158. [PubMed: 37579949]
59. Hogan MC, Manganelli L, Woollard JR, Masyuk AI, Masyuk TV, Tammachote R, et al. Characterization of PKD protein-positive exosome-like vesicles. J Am Soc Nephrol. 2009;20(2):278–88. [PubMed: 19158352]
60. Lea WA, McGreal K, Sharma M, Parnell SC, Zelenchuk L, Charlesworth MC, et al. Analysis of the polycystin complex (PCC) in human urinary exosome-like vesicles (ELVs). Sci Rep. 2020;10(1):1500. [PubMed: 32001768]
61. Wang J, Nikonorova IA, Silva M, Walsh JD, Tilton PE, Gu A, et al. Sensory cilia act as a specialized venue for regulated extracellular vesicle biogenesis and signaling. Curr Biol. 2021;31(17):3943–51 e3. [PubMed: 34270950]
62. Ding H, Li LX, Harris PC, Yang J, Li X. Extracellular vesicles and exosomes generated from cystic renal epithelial cells promote cyst growth in autosomal dominant polycystic kidney disease. Nature communications. 2021;12(1):4548.
63. Hogan MC, Bakeberg JL, Gainullin VG, Irazabal MV, Harmon AJ, Lieske JC, et al. Identification of Biomarkers for PKD1 Using Urinary Exosomes. J Am Soc Nephrol. 2015;26(7):1661–70. [PubMed: 25475747]

64. Lakhia R, Ramalingam H, Chang CM, Cobo-Stark P, Biggers L, Flaten A, et al. PKD1 and PKD2 mRNA cis-inhibition drives polycystic kidney disease progression. Nat Commun. 2022;13(1):4765. [PubMed: 35965273]

65. Barr MM, Sternberg PW. A polycystic kidney-disease gene homologue required for male mating behaviour in C. elegans. Nature. 1999;401(6751):386–9. [PubMed: 10517638]

66. Yoder BK, Richards WG, Sommardahl C, Sweeney WE, Michaud EJ, Wilkinson JE, et al. Functional correction of renal defects in a mouse model for ARPKD through expression of the cloned wild-type Tg737 cDNA. Kidney Int. 1996;50(4):1240–8. [PubMed: 8887283]

67. Pazour GJ, Dickert BL, Vucica Y, Seeley ES, Rosenbaum JL, Witman GB, et al. Chlamydomonas IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. J Cell Biol. 2000;151(3):709–18. [PubMed: 11062270]

68. Praetorius HA, Spring KR. Bending the MDCK cell primary cilium increases intracellular calcium. J Membr Biol. 2001;184(1):71–9. [PubMed: 11687880]

69. Reiter JF, Leroux MR. Genes and molecular pathways underpinning ciliopathies. Nat Rev Mol Cell Biol. 2017;18(9):533–47. [PubMed: 28698599]

70. Anvarian Z, Mykytyn K, Mukhopadhyay S, Pedersen LB, Christensen ST. Cellular signalling by primary cilia in development, organ function and disease. Nat Rev Nephrol. 2019;15(4):199–219. [PubMed: 30733609]

71. Mill P, Christensen ST, Pedersen LB. Primary cilia as dynamic and diverse signalling hubs in development and disease. Nat Rev Genet. 2023;24(7):421–41. [PubMed: 37072495]

72. Derderian C, Canales GI, Reiter JF. Seriously cilia: A tiny organelle illuminates evolution, disease, and intercellular communication. Dev Cell. 2023.

73. Ishikawa T, Ueno H, Omori T, Kikuchi K. Cilia and centrosomes: Ultrastructural and mechanical perspectives. Semin Cell Dev Biol. 2021;110:61–9. [PubMed: 32307225]

74. Praetorius HA, Spring KR. The renal cell primary cilium functions as a flow sensor. Curr Opin Nephrol Hypertens. 2003;12(5):517–20. [PubMed: 12920399]

75. Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, et al. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nat Genet. 2003;33(2):129–37. [PubMed: 12514735]

76. Ma M, Tian X, Igarashi P, Pazour GJ, Somlo S. Loss of cilia suppresses cyst growth in genetic models of autosomal dominant polycystic kidney disease. Nat Genet. 2013;45(9):1004–12. [PubMed: 23892607]

77. Zhang C, Rehman M, Tian X, Pei SLC, Gu J, Bell TA 3rd, et al. Glis2 is an early effector of polycystin signaling and a target for therapy in polycystic kidney disease. Nature communications. 2024;15(1):3698.

78. Podrini C, Cassina L, Boletta A. Metabolic reprogramming and the role of mitochondria in polycystic kidney disease. Cell Signal. 2020;67:109495. [PubMed: 31816397]

79. Podrini C, Rowe I, Pagliarini R, Costa ASH, Chiaravalli M, Di Meo I, et al. Dissection of metabolic reprogramming in polycystic kidney disease reveals coordinated rewiring of bioenergetic pathways. Commun Biol. 2018;1:194. [PubMed: 30480096]

80. Steidl ME, Nigro EA, Nielsen AK, Pagliarini R, Cassina L, Lampis M, et al. Primary cilia sense glutamine availability and respond via asparagine synthetase. Nat Metab. 2023;5(3):385–97. [PubMed: 36879119]

81. Liu X, Vien T, Duan J, Sheu SH, DeCaen PG, Clapham DE. Polycystin-2 is an essential ion channel subunit in the primary cilium of the renal collecting duct epithelium. Elife. 2018;7.

82. Padhy B, Xie J, Wang R, Lin F, Huang CL. Channel Function of Polycystin-2 in the Endoplasmic Reticulum Protects against Autosomal Dominant Polycystic Kidney Disease. J Am Soc Nephrol. 2022;33(8):1501–16. [PubMed: 35835458]

83. Kuo IY, Brill AL, Lemos FO, Jiang JY, Falcone JL, Kimmerling EP, et al. Polycystin 2 regulates mitochondrial Ca(2+) signaling, bioenergetics, and dynamics through mitofusin 2. Sci Signal. 2019;12(580).

84. Newby LJ, Streets AJ, Zhao Y, Harris PC, Ward CJ, Ong AC. Identification, characterization, and localization of a novel kidney polycystin-1-polycystin-2 complex. J Biol Chem. 2002;277(23):20763–73. [PubMed: 11901144]

85. Roitbak T, Ward CJ, Harris PC, Bacallao R, Ness SA, Wandinger-Ness A. A polycystin-1 multiprotein complex is disrupted in polycystic kidney disease cells. Mol Biol Cell. 2004;15(3):1334–46. [PubMed: 14718571]

86. Scheffers MS, van der Bent P, Prins F, Spruit L, Breuning MH, Litvinov SV, et al. Polycystin-1, the product of the polycystic kidney disease 1 gene, co-localizes with desmosomes in MDCK cells. Hum Mol Genet. 2000;9(18):2743–50. [PubMed: 11063733]

87. Streets AJ, Wagner BE, Harris PC, Ward CJ, Ong AC. Homophilic and heterophilic polycystin 1 interactions regulate E-cadherin recruitment and junction assembly in MDCK cells. J Cell Sci. 2009;122(Pt 9):1410–7. [PubMed: 19351715]

88. Roitbak T, Surviladze Z, Tikkanen R, Wandinger-Ness A. A polycystin multiprotein complex constitutes a cholesterol-containing signalling microdomain in human kidney epithelia. Biochem J. 2005;392(Pt 1):29–38. [PubMed: 16038619]

89. Muth TR, Caplan MJ. Transport protein trafficking in polarized cells. Annual review of cell and developmental biology. 2003;19:333–66.

90. Stoops EH, Caplan MJ. Trafficking to the apical and basolateral membranes in polarized epithelial cells. J Am Soc Nephrol. 2014;25(7):1375–86. [PubMed: 24652803]

91. Blobel G, Dobberstein B. Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. J Cell Biol. 1975;67(3):835–51. [PubMed: 811671]

92. Blobel G, Dobberstein B. Transfer of proteins across membranes. II. Reconstitution of functional rough microsomes from heterologous components. J Cell Biol. 1975;67(3):852–62. [PubMed: 811672]

93. Blobel G Intracellular protein topogenesis. Proc Natl Acad Sci U S A. 1980;77(3):1496–500. [PubMed: 6929499]

94. Elvekrog MM, Walter P. Dynamics of co-translational protein targeting. Curr Opin Chem Biol. 2015;29:79–86. [PubMed: 26517565]

95. Rapoport TA, Li L, Park E. Structural and Mechanistic Insights into Protein Translocation. Annual review of cell and developmental biology. 2017;33:369–90.

96. McGilvray PT, Anghel SA, Sundaram A, Zhong F, Trnka MJ, Fuller JR, et al. An ER translocon for multi-pass membrane protein biogenesis. Elife. 2020;9.

97. Smalinskaite L, Kim MK, Lewis AJO, Keenan RJ, Hegde RS. Mechanism of an intramembrane chaperone for multipass membrane proteins. Nature. 2022;611(7934):161–6. [PubMed: 36261528]

98. Sundaram A, Yamsek M, Zhong F, Hooda Y, Hegde RS, Keenan RJ. Substrate-driven assembly of a translocon for multipass membrane proteins. Nature. 2022;611(7934):167–72. [PubMed: 36261522]

99. Stroud RM, Walter P. Signal sequence recognition and protein targeting. Curr Opin Struct Biol. 1999;9(6):754–9. [PubMed: 10607673]

100. Hu J, Harris PC. Regulation of polycystin expression, maturation and trafficking. Cell Signal. 2020;72:109630. [PubMed: 32275942]

101. Aebi M N-linked protein glycosylation in the ER. Biochim Biophys Acta. 2013;1833(11):2430–7. [PubMed: 23583305]

102. Helenius A, Aebi M. Intracellular functions of N-linked glycans. Science. 2001;291(5512):2364–9. [PubMed: 11269317]

103. Helenius A How N-linked oligosaccharides affect glycoprotein folding in the endoplasmic reticulum. Mol Biol Cell. 1994;5(3):253–65. [PubMed: 8049518]

104. Ellgaard L, Helenius A. Quality control in the endoplasmic reticulum. Nat Rev Mol Cell Biol. 2003;4(3):181–91. [PubMed: 12612637]

105. Caramelo JJ, Parodi AJ. A sweet code for glycoprotein folding. FEBS Lett. 2015;589(22):3379–87. [PubMed: 26226420]

106. Maattanen P, Gehring K, Bergeron JJ, Thomas DY. Protein quality control in the ER: the recognition of misfolded proteins. Semin Cell Dev Biol. 2010;21(5):500–11. [PubMed: 20347046]

107. Davila S, Furu L, Gharavi AG, Tian X, Onoe T, Qian Q, et al. Mutations in SEC63 cause autosomal dominant polycystic liver disease. Nat Genet. 2004;36(6):575–7. [PubMed: 15133510]
108. Porath B, Gainullin VG, Cornec-Le Gall E, Dillinger EK, Heyer CM, Hopp K, et al. Mutations in GANAB, Encoding the Glucosidase IIalpha Subunit, Cause Autosomal-Dominant Polycystic Kidney and Liver Disease. Am J Hum Genet. 2016;98(6):1193–207. [PubMed: 27259053]
109. Li A, Davila S, Furu L, Qian Q, Tian X, Kamath PS, et al. Mutations in PRKCSH cause isolated autosomal dominant polycystic liver disease. Am J Hum Genet. 2003;72(3):691–703. [PubMed: 12529853]
110. Besse W, Dong K, Choi J, Punia S, Fedeles SV, Choi M, et al. Isolated polycystic liver disease genes define effectors of polycystin-1 function. J Clin Invest. 2017;127(5):1772–85. [PubMed: 28375157]
111. Hartmann E, Sommer T, Prehn S, Gorlich D, Jentsch S, Rapoport TA. Evolutionary conservation of components of the protein translocation complex. Nature. 1994;367(6464):654–7. [PubMed: 8107851]
112. Itskanov S, Kuo KM, Gumbart JC, Park E. Stepwise gating of the Sec61 protein-conducting channel by Sec63 and Sec62. Nat Struct Mol Biol. 2021;28(2):162–72. [PubMed: 33398175]
113. Stagljar I, te Heesen S, Aebi M. New phenotype of mutations deficient in glucosylation of the lipid-linked oligosaccharide: cloning of the ALG8 locus. Proc Natl Acad Sci U S A. 1994;91(13):5977–81. [PubMed: 8016100]
114. Ghosh Roy S, Li Z, Guo Z, Tran Long K, Rehrl S, Tian X, et al. Dnajb11 -Kidney Disease Develops from Reduced Polycystin-1 Dosage but not Unfolded Protein Response in Mice. J Am Soc Nephrol. 2023.
115. Padovano V, Caplan MJ. The Polycystin Complex Reveals Its Complexity. Biochemistry. 2018;57(51):6917–8. [PubMed: 30540438]
116. Yu Y, Ulbrich MH, Li MH, Buraei Z, Chen XZ, Ong AC, et al. Structural and molecular basis of the assembly of the TRPP2/PKD1 complex. Proc Natl Acad Sci U S A. 2009;106(28):11558–63. [PubMed: 19556541]
117. Giamarchi A, Feng S, Rodat-Despoix L, Xu Y, Bubenshchikova E, Newby LJ, et al. A polycystin-2 (TRPP2) dimerization domain essential for the function of heteromeric polycystin complexes. EMBO J. 2010;29(7):1176–91. [PubMed: 20168298]
118. Zhu J, Yu Y, Ulbrich MH, Li MH, Isacoff EY, Honig B, et al. Structural model of the TRPP2/PKD1 C-terminal coiled-coil complex produced by a combined computational and experimental approach. Proc Natl Acad Sci U S A. 2011;108(25):10133–8. [PubMed: 21642537]
119. Salehi-Najafabadi Z, Li B, Valentino V, Ng C, Martin H, Yu Y, et al. Extracellular Loops Are Essential for the Assembly and Function of Polycystin Receptor-Ion Channel Complexes. J Biol Chem. 2017;292(10):4210–21. [PubMed: 28154010]
120. Sharif-Naeini R, Folgering JH, Bichet D, Duprat F, Lauritzen I, Arhatte M, et al. Polycystin-1 and -2 dosage regulates pressure sensing. Cell. 2009;139(3):587–96. [PubMed: 19879844]
121. Viau A, Kotsis F, Boehlke C, Braeg S, Klein M, Nitschke R, et al. Divergent function of polycystin 1 and polycystin 2 in cell size regulation. Biochem Biophys Res Commun. 2020;521(2):290–5. [PubMed: 31668373]
122. Qian F The Role of G-protein Coupled Receptor Proteolytic Site (GPS) Cleavage in Polycystin-1 Biogenesis, Trafficking and Function. In: Li X, editor. Polycystic Kidney Disease. Brisbane (AU)2015.
123. Trudel M, Yao Q, Qian F. The Role of G-Protein-Coupled Receptor Proteolysis Site Cleavage of Polycystin-1 in Renal Physiology and Polycystic Kidney Disease. Cells. 2016;5(1).
124. Gilder AL, Chapin HC, Padovano V, Hueschen CL, Rajendran V, Caplan MJ. Newly synthesized polycystin-1 takes different trafficking pathways to the apical and ciliary membranes. Traffic. 2018;19(12):933–45. [PubMed: 30125442]
125. Wei W, Hackmann K, Xu H, Germino G, Qian F. Characterization of cis-autoproteolysis of polycystin-1, the product of human polycystic kidney disease 1 gene. J Biol Chem. 2007;282(30):21729–37. [PubMed: 17525154]

126. Chapin HC, Rajendran V, Caplan MJ. Polycystin-1 Surface Localization Is Stimulated by Polycystin-2 and Cleavage at the G Protein-coupled Receptor Proteolytic Site. Mol Biol Cell. 2010;21(24):4338–48. [PubMed: 20980620]

127. Robbins PW, Hubbard SC, Turco SJ, Wirth DF. Proposal for a common oligosaccharide intermediate in the synthesis of membrane glycoproteins. Cell. 1977;12(4):893–900. [PubMed: 202393]

128. Gainullin VG, Hopp K, Ward CJ, Hommerding CJ, Harris PC. Polycystin-1 maturation requires polycystin-2 in a dose-dependent manner. J Clin Invest. 2015;125(2):607–20. [PubMed: 25574838]

129. Cai Y, Fedeles SV, Dong K, Anyatonwu G, Onoe T, Mitobe M, et al. Altered trafficking and stability of polycystins underlie polycystic kidney disease. J Clin Invest. 2014.

130. Kurbegovic A, Kim H, Xu H, Yu S, Cruanes J, Maser RL, et al. Novel functional complexity of polycystin-1 by GPS cleavage in vivo: role in polycystic kidney disease. Mol Cell Biol. 2014;34(17):3341–53. [PubMed: 24958103]

131. Padovano V, Mistry K, Merrick D, Gresko N, Caplan MJ. A cut above (and below): Protein cleavage in the regulation of polycystin trafficking and signaling. Cell Signal. 2020;72:109634. [PubMed: 32283256]

132. Su X, Wu M, Yao G, El-Jouni W, Luo C, Tabari A, et al. Regulation of polycystin-1 ciliary trafficking by motifs at its C-terminus and polycystin-2 but not by cleavage at the GPS site. J Cell Sci. 2015;128(22):4063–73. [PubMed: 26430213]

133. Yu S, Hackmann K, Gao J, He X, Piontek K, Garcia-Gonzalez MA, et al. Essential role of cleavage of Polycystin-1 at G protein-coupled receptor proteolytic site for kidney tubular structure. Proc Natl Acad Sci U S A. 2007;104(47):18688–93. [PubMed: 18003909]

134. Lu W, Peissel B, Babakhanlou H, Pavlova A, Geng L, Fan X, et al. Perinatal lethality with kidney and pancreas defects in mice with a targetted Pkd1 mutation. Nat Genet. 1997;17(2):179–81. [PubMed: 9326937]

135. Merrick D, Chapin H, Baggs JE, Yu Z, Somlo S, Sun Z, et al. The gamma-Secretase Cleavage Product of Polycystin-1 Regulates TCF and CHOP-Mediated Transcriptional Activation through a p300-Dependent Mechanism. Dev Cell. 2012;22(1):197–210. [PubMed: 22178500]

136. Lal M, Caplan M. Regulated intramembrane proteolysis: signaling pathways and biological functions. Physiology (Bethesda). 2011;26(1):34–44. [PubMed: 21357901]

137. Bertuccio CA, Chapin HC, Cai Y, Mistry K, Chauvet V, Somlo S, et al. Polycystin-1 C-terminal cleavage is modulated by polycystin-2 expression. J Biol Chem. 2009;284(31):21011–26. [PubMed: 19491093]

138. Low SH, Vasanth S, Larson CH, Mukherjee S, Sharma N, Kinter MT, et al. Polycystin-1, STAT6, and P100 function in a pathway that transduces ciliary mechanosensation and is activated in polycystic kidney disease. Dev Cell. 2006;10(1):57–69. [PubMed: 16399078]

139. Xia S, Li XG, Johnson T, Seidel C, Wallace DP, Li R. Polycystin-dependent fluid flow sensing targets histone deacetylase 5 to prevent the development of renal cysts. Development. 2010;137(7):1075–84. [PubMed: 20181743]

140. Yoshiba S, Shiratori H, Kuo IY, Kawasumi A, Shinohara K, Nonaka S, et al. Cilia at the node of mouse embryos sense fluid flow for left-right determination via Pkd2. Science. 2012;338(6104):226–31. [PubMed: 22983710]

141. Kleene SJ, Kleene NK. The native TRPP2-dependent channel of murine renal primary cilia. Am J Physiol Renal Physiol. 2017;312(1):F96–F108. [PubMed: 27760766]

142. Kleene SJ, Kleene NK. Inward Ca(2+) current through the polycystin-2-dependent channels of renal primary cilia. Am J Physiol Renal Physiol. 2021;320(6):F1165–F73. [PubMed: 33969696]

143. MacKay CE, Leo MD, Fernandez-Pena C, Hasan R, Yin W, Mata-Daboin A, et al. Intravascular flow stimulates PKD2 (polycystin-2) channels in endothelial cells to reduce blood pressure. Elife. 2020;9.

144. Patel A, Honore E. Polycystins and renovascular mechanosensory transduction. Nat Rev Nephrol. 2010.

145. Peyronnet R, Martins JR, Duprat F, Demolombe S, Arhatte M, Jodar M, et al. Piezo1-dependent stretch-activated channels are inhibited by Polycystin-2 in renal tubular epithelial cells. EMBO Rep. 2013;14(12):1143–8. [PubMed: 24157948]

146. AbouAlaiwi WA, Takahashi M, Mell BR, Jones TJ, Ratnam S, Kolb RJ, et al. Ciliary polycystin-2 is a mechanosensitive calcium channel involved in nitric oxide signaling cascades. Circ Res. 2009;104(7):860–9. [PubMed: 19265036]

147. Kottgen M, Buchholz B, Garcia-Gonzalez MA, Kotsis F, Fu X, Doerken M, et al. TRPP2 and TRPV4 form a polymodal sensory channel complex. J Cell Biol. 2008;182(3):437–47. [PubMed: 18695040]

148. Xu C, Rossetti S, Jiang L, Harris PC, Brown-Glaberman U, Wandinger-Ness A, et al. Human ADPKD primary cyst epithelial cells with a novel, single codon deletion in the PKD1 gene exhibit defective ciliary polycystin localization and loss of flow-induced Ca2+ signaling. Am J Physiol Renal Physiol. 2007;292(3):F930–45. [PubMed: 17090781]

149. Xu C, Shmukler BE, Nishimura K, Kaczmarek E, Rossetti S, Harris PC, et al. Attenuated, flow-induced ATP release contributes to absence of flow-sensitive, purinergic Cai2+ signaling in human ADPKD cyst epithelial cells. Am J Physiol Renal Physiol. 2009;296(6):F1464–76. [PubMed: 19244404]

150. Nauli SM, Rossetti S, Kolb RJ, Alenghat FJ, Consugar MB, Harris PC, et al. Loss of polycystin-1 in human cyst-lining epithelia leads to ciliary dysfunction. J Am Soc Nephrol. 2006;17(4):1015–25. [PubMed: 16565258]

151. Lal M, Song X, Pluznick J, Di Giovanni V, Merrick D, Rosenblum N, et al. Polycystin-1 C-terminal tail associates with beta-catenin and inhibits canonical Wnt signaling. Hum Mol Genet. 2008;17(20):3105–17. [PubMed: 18632682]

152. Merrick D, Mistry K, Wu J, Gresko N, Baggs JE, Hogenesch JB, et al. Polycystin-1 regulates bone development through an interaction with the transcriptional coactivator TAZ. Hum Mol Genet. 2019;28(1):16–30. [PubMed: 30215740]

153. Xiao Z, Baudry J, Cao L, Huang J, Chen H, Yates CR, et al. Polycystin-1 interacts with TAZ to stimulate osteoblastogenesis and inhibit adipogenesis. J Clin Invest. 2018;128(1):157–74. [PubMed: 29202470]

154. Zhao B, Lei QY, Guan KL. The Hippo-YAP pathway: new connections between regulation of organ size and cancer. Curr Opin Cell Biol. 2008;20(6):638–46. [PubMed: 18955139]

155. Xiao Z, Zhang S, Cao L, Qiu N, David V, Quarles LD. Conditional disruption of Pkd1 in osteoblasts results in osteopenia due to direct impairment of bone formation. J Biol Chem. 2010;285(2):1177–87. [PubMed: 19887454]

156. Xiao Z, Zhang S, Magenheimer BS, Luo J, Quarles LD. Polycystin-1 regulates skeletogenesis through stimulation of the osteoblast-specific transcription factor RUNX2-II. J Biol Chem. 2008;283(18):12624–34. [PubMed: 18321855]

157. Xiao Z, Zhang S, Mahlios J, Zhou G, Magenheimer BS, Guo D, et al. Cilia-like structures and polycystin-1 in osteoblasts/osteocytes and associated abnormalities in skeletogenesis and Runx2 expression. J Biol Chem. 2006;281(41):30884–95. [PubMed: 16905538]

158. Talbot JJ, Song X, Wang X, Rinschen MM, Doerr N, LaRiviere WB, et al. The cleaved cytoplasmic tail of polycystin-1 regulates Src-dependent STAT3 activation. J Am Soc Nephrol. 2014;25(8):1737–48. [PubMed: 24578126]

159. Lin CC, Menezes LF, Qiu J, Pearson E, Zhou F, Ishimoto Y, et al. In vivo Polycystin-1 interactome using a novel Pkd1 knock-in mouse model. PLoS One. 2023;18(8):e0289778. [PubMed: 37540694]

160. Soriano P Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat Genet. 1999;21(1):70–1. [PubMed: 9916792]

161. Onuchic L, Padovano V, Schena G, Rajendran V, Dong K, Shi X, et al. The C-terminal tail of polycystin-1 suppresses cystic disease in a mitochondrial enzyme-dependent fashion. Nature communications. 2023;14(1):1790.

162. Walker RV, Yao Q, Xu H, Maranto A, Swaney KF, Ramachandran S, et al. Fibrocystin/Polyductin releases a C-terminal fragment that translocates into mitochondria and suppresses cystogenesis. Nature communications. 2023;14(1):6513.

163. Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. Orai1 is an essential pore subunit of the CRAC channel. Nature. 2006;443(7108):230–3. [PubMed: 16921383]
164. Yeromin AV, Zhang SL, Jiang W, Yu Y, Safrina O, Cahalan MD. Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. Nature. 2006;443(7108):226–9. [PubMed: 16921385]
165. Jamieson JD, Palade GE. Intracellular transport of secretory proteins in the pancreatic exocrine cell. IV. Metabolic requirements. J Cell Biol. 1968;39(3):589–603. [PubMed: 5699933]
166. Jamieson JD, Palade GE. Intracellular transport of secretory proteins in the pancreatic exocrine cell. 3. Dissociation of intracellular transport from protein synthesis. J Cell Biol. 1968;39(3):580–8. [PubMed: 5699932]
167. Jamieson JD, Palade GE. Intracellular transport of secretory proteins in the pancreatic exocrine cell. II. Transport to condensing vacuoles and zymogen granules. J Cell Biol. 1967;34(2):597–615. [PubMed: 6035648]
168. Jamieson JD, Palade GE. Intracellular transport of secretory proteins in the pancreatic exocrine cell. I. Role of the peripheral elements of the Golgi complex. J Cell Biol. 1967;34(2):577–96. [PubMed: 6035647]
169. Dunphy WG, Rothman JE. Compartmentation of asparagine-linked oligosaccharide processing in the Golgi apparatus. J Cell Biol. 1983;97(1):270–5. [PubMed: 6223041]
170. Moremen KW, Tiemeyer M, Nairn AV. Vertebrate protein glycosylation: diversity, synthesis and function. Nat Rev Mol Cell Biol. 2012;13(7):448–62. [PubMed: 22722607]
171. Reiter JF, Blacque OE, Leroux MR. The base of the cilium: roles for transition fibres and the transition zone in ciliary formation, maintenance and compartmentalization. EMBO Rep. 2012;13(7):608–18. [PubMed: 22653444]
172. Stoops EH, Hull M, Olesen C, Mistry K, Harder JL, Rivera-Molina F, et al. The periciliary ring in polarized epithelial cells is a hot spot for delivery of the apical protein gp135. J Cell Biol. 2015;211(2):287–94. [PubMed: 26504168]
173. Park K, Leroux MR. Composition, organization and mechanisms of the transition zone, a gate for the cilium. EMBO Rep. 2022;23(12):e55420. [PubMed: 36408840]
174. Nachury MV, Mick DU. Establishing and regulating the composition of cilia for signal transduction. Nat Rev Mol Cell Biol. 2019;20(7):389–405. [PubMed: 30948801]
175. Webb S, Mukhopadhyay AG, Roberts AJ. Intraflagellar transport trains and motors: Insights from structure. Semin Cell Dev Biol. 2020;107:82–90. [PubMed: 32684327]
176. Nachury MV. The molecular machines that traffic signaling receptors into and out of cilia. Curr Opin Cell Biol. 2018;51:124–31. [PubMed: 29579578]
177. Garcia-Gonzalo FR, Reiter JF. Open Sesame: How Transition Fibers and the Transition Zone Control Ciliary Composition. Cold Spring Harbor perspectives in biology. 2017;9(2).
178. Deretic D, Williams AH, Ransom N, Morel V, Hargrave PA, Arendt A. Rhodopsin C terminus, the site of mutations causing retinal disease, regulates trafficking by binding to ADP-ribosylation factor 4 (ARF4). Proc Natl Acad Sci U S A. 2005;102(9):3301–6. [PubMed: 15728366]
179. Mazelova J, Astuto-Gribble L, Inoue H, Tam BM, Schonteich E, Prekeris R, et al. Ciliary targeting motif VxPx directs assembly of a trafficking module through Arf4. EMBO J. 2009;28(3):183–92. [PubMed: 19153612]
180. Luo W, Marsh-Armstrong N, Rattner A, Nathans J. An outer segment localization signal at the C terminus of the photoreceptor-specific retinol dehydrogenase. J Neurosci. 2004;24(11):2623–32. [PubMed: 15028754]
181. Geng L, Okuhara D, Yu Z, Tian X, Cai Y, Shibazaki S, et al. Polycystin-2 traffics to cilia independently of polycystin-1 by using an N-terminal RVxP motif. J Cell Sci. 2006;119(Pt 7):1383–95. [PubMed: 16537653]
182. Ward HH, Brown-Glaberman U, Wang J, Morita Y, Alper SL, Bedrick EJ, et al. A conserved signal and GTPase complex are required for the ciliary transport of polycystin-1. Mol Biol Cell. 2011;22(18):3289–305. [PubMed: 21775626]
183. Grieve AG, Rabouille C. Golgi bypass: skirting around the heart of classical secretion. Cold Spring Harbor perspectives in biology. 2011;3(4).

184. Marie M, Sannerud R, Avsnes Dale H, Saraste J. Take the 'A' train: on fast tracks to the cell surface. Cell Mol Life Sci. 2008;65(18):2859–74. [PubMed: 18726174]

185. Witzgall R Golgi bypass of ciliary proteins. Semin Cell Dev Biol. 2018.

186. Hoffmeister H, Babinger K, Gurster S, Cedzich A, Meese C, Schadendorf K, et al. Polycystin-2 takes different routes to the somatic and ciliary plasma membrane. J Cell Biol. 2011;192(4):631–45. [PubMed: 21321097]

187. Donaldson JG, Finazzi D, Klausner RD. Brefeldin A inhibits Golgi membrane-catalysed exchange of guanine nucleotide onto ARF protein. Nature. 1992;360(6402):350–2. [PubMed: 1448151]

188. Lippincott-Schwartz J, Yuan L, Tipper C, Amherdt M, Orci L, Klausner RD. Brefeldin A's effects on endosomes, lysosomes, and the TGN suggest a general mechanism for regulating organelle structure and membrane traffic. Cell. 1991;67(3):601–16. [PubMed: 1682055]

189. Saraste J, Kuismanen E. Pre- and post-Golgi vacuoles operate in the transport of Semliki Forest virus membrane glycoproteins to the cell surface. Cell. 1984;38(2):535–49. [PubMed: 6432345]

190. Matlin KS, Simons K. Reduced temperature prevents transfer of a membrane glycoprotein to the cell surface but does not prevent terminal glycosylation. Cell. 1983;34(1):233–43. [PubMed: 6883510]

191. Kim H, Xu H, Yao Q, Li W, Huang Q, Outeda P, et al. Ciliary membrane proteins traffic through the Golgi via a Rabep1/GGA1/Arl3-dependent mechanism. Nature communications. 2014;5:5482.

192. Zilberstein A, Snider MD, Porter M, Lodish HF. Mutants of vesicular stomatitis virus blocked at different stages in maturation of the viral glycoprotein. Cell. 1980;21(2):417–27. [PubMed: 6250721]

193. Su X, Driscoll K, Yao G, Raed A, Wu M, Beales PL, et al. Bardet-Biedl syndrome proteins 1 and 3 regulate the ciliary trafficking of polycystic kidney disease 1 protein. Hum Mol Genet. 2014;23(20):5441–51. [PubMed: 24939912]

194. Harris PC, Torres VE. Genetic mechanisms and signaling pathways in autosomal dominant polycystic kidney disease. J Clin Invest. 2014;124(6):2315–24. [PubMed: 24892705]

195. Hughes J, Ward CJ, Peral B, Aspinwall R, Clark K, San Millan JL, et al. The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell recognition domains. Nat Genet. 1995;10(2):151–60. [PubMed: 7663510]

196. Grieben M, Pike AC, Shintre CA, Venturi E, El-Ajouz S, Tessitore A, et al. Structure of the polycystic kidney disease TRP channel Polycystin-2 (PC2). Nat Struct Mol Biol. 2017;24(2):114–22. [PubMed: 27991905]

197. Esarte Palomero O, Larmore M, DeCaen PG. Polycystin Channel Complexes. Annu Rev Physiol. 2023;85:425–48. [PubMed: 36763973]

198. Bai CX, Kim S, Li WP, Streets AJ, Ong AC, Tsiokas L. Activation of TRPP2 through mDia1-dependent voltage gating. EMBO J. 2008;27(9):1345–56. [PubMed: 18388856]

199. Kim S, Nie H, Nesin V, Tran U, Outeda P, Bai CX, et al. The polycystin complex mediates Wnt/Ca(2+) signalling. Nat Cell Biol. 2016;18(7):752–64. [PubMed: 27214281]

200. Ha K, Nobuhara M, Wang Q, Walker RV, Qian F, Schartner C, et al. The heteromeric PC-1/PC-2 polycystin complex is activated by the PC-1 N-terminus. Elife. 2020;9.

201. Dong XP, Wang X, Shen D, Chen S, Liu M, Wang Y, et al. Activating mutations of the TRPML1 channel revealed by proline-scanning mutagenesis. J Biol Chem. 2009;284(46):32040–52. [PubMed: 19638346]

202. Zheng W, Yang X, Hu R, Cai R, Hofmann L, Wang Z, et al. Hydrophobic pore gates regulate ion permeation in polycystic kidney disease 2 and 2L1 channels. Nature communications. 2018;9(1):2302.

203. Wang Z, Chen M, Su Q, Morais TDC, Wang Y, Nazginov E, et al. Molecular and structural basis of the dual regulation of the polycystin-2 ion channel by small-molecule ligands. Proc Natl Acad Sci U S A. 2024;121(12):e2316230121. [PubMed: 38483987]

204. Praetorius HA, Frokiaer J, Nielsen S, Spring KR. Bending the primary cilium opens Ca2+-sensitive intermediate-conductance K+ channels in MDCK cells. J Membr Biol. 2003;191(3):193–200. [PubMed: 12571753]

205. Ha K, Mundt-Machado N, Bisignano P, Pinedo A, Raleigh DR, Loeb G, et al. Cilia-enriched oxysterol 7beta,27-DHC is required for polycystin ion channel activation. Nature communications. 2024;15(1):6468.

206. Anyatonwu GI, Estrada M, Tian X, Somlo S, Ehrlich BE. Regulation of ryanodine receptor-dependent calcium signaling by polycystin-2. Proc Natl Acad Sci U S A. 2007;104(15):6454–9. [PubMed: 17404231]

207. Li Y, Wright JM, Qian F, Germino GG, Guggino WB. Polycystin 2 interacts with type I inositol 1,4,5-trisphosphate receptor to modulate intracellular Ca2+ signaling. J Biol Chem. 2005;280(50):41298–306. [PubMed: 16223735]

208. Mekahli D, Sammels E, Luyten T, Welkenhuyzen K, van den Heuvel LP, Levtchenko EN, et al. Polycystin-1 and polycystin-2 are both required to amplify inositol-trisphosphate-induced Ca2+ release. Cell Calcium. 2012;51(6):452–8. [PubMed: 22456092]

209. de Brito OM, Scorrano L. Mitofusin 2 tethers endoplasmic reticulum to mitochondria. Nature. 2008;456(7222):605–10. [PubMed: 19052620]

210. Filadi R, Theurey P, Pizzo P. The endoplasmic reticulum-mitochondria coupling in health and disease: Molecules, functions and significance. Cell Calcium. 2017;62:1–15. [PubMed: 28108029]

211. Raturi A, Simmen T. Where the endoplasmic reticulum and the mitochondrion tie the knot: the mitochondria-associated membrane (MAM). Biochim Biophys Acta. 2013;1833(1):213–24. [PubMed: 22575682]

212. Cardenas C, Miller RA, Smith I, Bui T, Molgo J, Muller M, et al. Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca2+ transfer to mitochondria. Cell. 2010;142(2):270–83. [PubMed: 20655468]

213. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 2009;324(5930):1029–33. [PubMed: 19460998]

214. Rowe I, Chiaravalli M, Mannella V, Ulisse V, Quilici G, Pema M, et al. Defective glucose metabolism in polycystic kidney disease identifies a new therapeutic strategy. Nat Med. 2013;19(4):488–93. [PubMed: 23524344]

215. Padovano V, Podrini C, Boletta A, Caplan MJ. Metabolism and mitochondria in polycystic kidney disease research and therapy. Nat Rev Nephrol. 2018;14(11):678–87. [PubMed: 30120380]

216. Hilger D, Masureel M, Kobilka BK. Structure and dynamics of GPCR signaling complexes. Nat Struct Mol Biol. 2018;25(1):4–12. [PubMed: 29323277]

217. Parnell SC, Magenheimer BS, Maser RL, Rankin CA, Smine A, Okamoto T, et al. The polycystic kidney disease-1 protein, polycystin-1, binds and activates heterotrimeric G-proteins in vitro. Biochem Biophys Res Commun. 1998;251(2):625–31. [PubMed: 9792824]

218. Puri S, Magenheimer BS, Maser RL, Ryan EM, Zien CA, Walker DD, et al. Polycystin-1 activates the calcineurin/NFAT (nuclear factor of activated T-cells) signaling pathway. J Biol Chem. 2004;279(53):55455–64. [PubMed: 15466861]

219. Yu W, Kong T, Beaudry S, Tran M, Negoro H, Yanamadala V, et al. Polycystin-1 protein level determines activity of the Galpha12/JNK apoptosis pathway. J Biol Chem. 2010;285(14):10243–51. [PubMed: 20106977]

220. Yu W, Ritchie BJ, Su X, Zhou J, Meigs TE, Denker BM. Identification of polycystin-1 and Galpha12 binding regions necessary for regulation of apoptosis. Cell Signal. 2011;23(1):213–21. [PubMed: 20837139]

221. Xu JX, Lu TS, Li S, Wu Y, Ding L, Denker BM, et al. Polycystin-1 and Galpha12 regulate the cleavage of E-cadherin in kidney epithelial cells. Physiol Genomics. 2015;47(2):24–32. [PubMed: 25492927]

222. Wu Y, Xu JX, El-Jouni W, Lu T, Li S, Wang Q, et al. Galpha12 is required for renal cystogenesis induced by Pkd1 inactivation. J Cell Sci. 2016;129(19):3675–84. [PubMed: 27505895]

223. Zhang B, Tran U, Wessely O. Polycystin 1 loss of function is directly linked to an imbalance in G-protein signaling in the kidney. Development. 2018;145(6).

224. Delmas P, Nomura H, Li X, Lakkis M, Luo Y, Segal Y, et al. Constitutive activation of G-proteins by polycystin-1 is antagonized by polycystin-2. J Biol Chem. 2002;277(13):11276–83. [PubMed: 11786542]

225. Parnell SC, Magenheimer BS, Maser RL, Pavlov TS, Havens MA, Hastings ML, et al. A mutation affecting polycystin-1 mediated heterotrimeric G-protein signaling causes PKD. Hum Mol Genet. 2018;27(19):3313–24. [PubMed: 29931260]

226. Kwon M, Pavlov TS, Nozu K, Rasmussen SA, Ilatovskaya DV, Lerch-Gaggl A, et al. G-protein signaling modulator 1 deficiency accelerates cystic disease in an orthologous mouse model of autosomal dominant polycystic kidney disease. Proc Natl Acad Sci U S A. 2012;109(52):21462–7. [PubMed: 23236168]

227. Arac D, Boucard AA, Bolliger MF, Nguyen J, Soltis SM, Sudhof TC, et al. A novel evolutionarily conserved domain of cell-adhesion GPCRs mediates autoproteolysis. EMBO J. 2012;31(6):1364–78. [PubMed: 22333914]

228. Promel S, Langenhan T, Arac D. Matching structure with function: the GAIN domain of adhesion-GPCR and PKD1-like proteins. Trends Pharmacol Sci. 2013;34(8):470–8. [PubMed: 23850273]

229. Mathiasen S, Palmisano T, Perry NA, Stoveken HM, Vizurraga A, McEwen DP, et al. G12/13 is activated by acute tethered agonist exposure in the adhesion GPCR ADGRL3. Nat Chem Biol. 2020;16(12):1343–50. [PubMed: 32778842]

230. Nieberler M, Kittel RJ, Petrenko AG, Lin HH, Langenhan T. Control of Adhesion GPCR Function Through Proteolytic Processing. Handb Exp Pharmacol. 2016;234:83–109. [PubMed: 27832485]

231. Beliu G, Altrichter S, Guixa-Gonzalez R, Hemberger M, Brauer I, Dahse AK, et al. Tethered agonist exposure in intact adhesion/class B2 GPCRs through intrinsic structural flexibility of the GAIN domain. Mol Cell. 2021;81(5):905–21 e5. [PubMed: 33497605]

232. Pawnikar S, Magenheimer BS, Munoz EN, Maser RL, Miao Y. Mechanism of tethered agonist-mediated signaling by polycystin-1. Proc Natl Acad Sci U S A. 2022;119(19):e2113786119. [PubMed: 35522707]

233. Maser RL, Calvet JP. Adhesion GPCRs as a paradigm for understanding polycystin-1G protein regulation. Cell Signal. 2020:109637. [PubMed: 32305667]

234. Nigro EA, Distefano G, Chiaravalli M, Matafora V, Castelli M, Pesenti Gritti A, et al. Polycystin-1 Regulates Actomyosin Contraction and the Cellular Response to Extracellular Stiffness. Sci Rep. 2019;9(1):16640. [PubMed: 31719603]

235. Hardy E, Tsiokas L. Polycystins as components of large multiprotein complexes of polycystin interactors. Cell Signal. 2020;72:109640. [PubMed: 32305669]

236. Formica C, Peters DJM. Molecular pathways involved in injury-repair and ADPKD progression. Cell Signal. 2020;72:109648. [PubMed: 32320858]

237. Happe H, Leonhard WN, van der Wal A, van de Water B, Lantinga-van Leeuwen IS, Breuning MH, et al. Toxic tubular injury in kidneys from Pkd1-deletion mice accelerates cystogenesis accompanied by dysregulated planar cell polarity and canonical Wnt signaling pathways. Human Molecular Genetics. 2009;18(14):2532–42. [PubMed: 19401297]

238. Patel V, Li L, Cobo-Stark P, Shao X, Somlo S, Lin F, et al. Acute kidney injury and aberrant planar cell polarity induce cyst formation in mice lacking renal cilia. Hum Mol Genet. 2008;17(11):1578–90. [PubMed: 18263895]

239. Weimbs T Polycystic kidney disease and renal injury repair: common pathways, fluid flow, and the function of polycystin-1. Am J Physiol Renal Physiol. 2007;293(5):F1423–32. [PubMed: 17715262]

240. Weimbs T, Olsan EE, Talbot JJ. Regulation of STATs by polycystin-1 and their role in polycystic kidney disease. Jak-Stat. 2013;2(2):e23650. [PubMed: 24058808]

241. Zhou J, Ouyang X, Schoeb TR, Bolisetty S, Cui X, Mrug S, et al. Kidney injury accelerates cystogenesis via pathways modulated by heme oxygenase and complement. J Am Soc Nephrol. 2012;23(7):1161–71. [PubMed: 22518005]

242. Mangoo-Karim R, Uchic M, Lechene C, Grantham JJ. Renal epithelial cyst formation and enlargement in vitro: dependence on cAMP. Proc Natl Acad Sci U S A. 1989;86(15):6007–11. [PubMed: 2474825]

243. Mangoo-Karim R, Uchic ME, Grant M, Shumate WA, Calvet JP, Park CH, et al. Renal epithelial fluid secretion and cyst growth: the role of cyclic AMP. FASEB J. 1989;3(14):2629–32. [PubMed: 2480260]

244. Calvet JP, Grantham JJ. The genetics and physiology of polycystic kidney disease. Seminars in nephrology. 2001;21(2):107–23. [PubMed: 11245774]

245. Yamaguchi T, Nagao S, Kasahara M, Takahashi H, Grantham JJ. Renal accumulation and excretion of cyclic adenosine monophosphate in a murine model of slowly progressive polycystic kidney disease. Am J Kidney Dis. 1997;30(5):703–9. [PubMed: 9370187]

246. Brill SR, Ross KE, Davidow CJ, Ye M, Grantham JJ, Caplan MJ. Immunolocalization of ion transport proteins in human autosomal dominant polycystic kidney epithelial cells. Proc Natl Acad Sci U S A. 1996;93(19):10206–11. [PubMed: 8816777]

247. Davidow CJ, Maser RL, Rome LA, Calvet JP, Grantham JJ. The cystic fibrosis transmembrane conductance regulator mediates transepithelial fluid secretion by human autosomal dominant polycystic kidney disease epithelium in vitro. Kidney Int. 1996;50(1):208–18. [PubMed: 8807590]

248. Sullivan LP, Wallace DP, Grantham JJ. Epithelial transport in polycystic kidney disease. Physiol Rev. 1998;78(4):1165–91. [PubMed: 9790573]

249. Yang B, Sonawane ND, Zhao D, Somlo S, Verkman AS. Small-molecule CFTR inhibitors slow cyst growth in polycystic kidney disease. J Am Soc Nephrol. 2008;19(7):1300–10. [PubMed: 18385427]

250. Sutters M, Yamaguchi T, Maser RL, Magenheimer BS, St John PL, Abrahamson DR, et al. Polycystin-1 transforms the cAMP growth-responsive phenotype of M-1 cells. Kidney Int. 2001;60(2):484–94. [PubMed: 11473631]

251. Yamaguchi T, Wallace DP, Magenheimer BS, Hempson SJ, Grantham JJ, Calvet JP. Calcium restriction allows cAMP activation of the B-Raf/ERK pathway, switching cells to a cAMP-dependent growth-stimulated phenotype. J Biol Chem. 2004;279(39):40419–30. [PubMed: 15263001]

252. Yamaguchi T, Pelling JC, Ramaswamy NT, Eppler JW, Wallace DP, Nagao S, et al. cAMP stimulates the in vitro proliferation of renal cyst epithelial cells by activating the extracellular signal-regulated kinase pathway. Kidney Int. 2000;57(4):1460–71. [PubMed: 10760082]

253. Yamaguchi T, Nagao S, Wallace DP, Belibi FA, Cowley BD, Pelling JC, et al. Cyclic AMP activates B-Raf and ERK in cyst epithelial cells from autosomal-dominant polycystic kidneys. Kidney Int. 2003;63(6):1983–94. [PubMed: 12753285]

254. Belibi FA, Reif G, Wallace DP, Yamaguchi T, Olsen L, Li H, et al. Cyclic AMP promotes growth and secretion in human polycystic kidney epithelial cells. Kidney Int. 2004;66(3):964–73. [PubMed: 15327388]

255. Parnell SC, Raman A, Zhang Y, Daniel EA, Dai Y, Khanna A, et al. Expression of active B-Raf proto-oncogene in kidney collecting ducts induces cyst formation in normal mice and accelerates cyst growth in mice with polycystic kidney disease. Kidney Int. 2022;102(5):1103–14. [PubMed: 35760151]

256. Yamaguchi T, Hempson SJ, Reif GA, Hedge AM, Wallace DP. Calcium restores a normal proliferation phenotype in human polycystic kidney disease epithelial cells. J Am Soc Nephrol. 2006;17(1):178–87. [PubMed: 16319189]

257. Kakade VR, Tao S, Rajagopal M, Zhou X, Li X, Yu AS, et al. A cAMP and CREB-mediated feed-forward mechanism regulates GSK3beta in polycystic kidney disease. J Mol Cell Biol. 2016;8(6):464–76. [PubMed: 27190311]

258. Marquez-Nogueras KM, Vuchkovska V, Kuo IY. Calcium signaling in polycystic kidney disease-cell death and survival. Cell Calcium. 2023;112:102733. [PubMed: 37023534]

259. Brill AL, Ehrlich BE. Polycystin 2: A calcium channel, channel partner, and regulator of calcium homeostasis in ADPKD. Cell Signal. 2020;66:109490. [PubMed: 31805375]

260. Kuo IY, DesRochers TM, Kimmerling EP, Nguyen L, Ehrlich BE, Kaplan DL. Cyst formation following disruption of intracellular calcium signaling. Proc Natl Acad Sci U S A. 2014;111(39):14283–8. [PubMed: 25228769]

261. Pinto CS, Reif GA, Nivens E, White C, Wallace DP. Calmodulin-sensitive adenylyl cyclases mediate AVP-dependent cAMP production and Cl- secretion by human autosomal dominant polycystic kidney cells. Am J Physiol Renal Physiol. 2012;303(10):F1412–24. [PubMed: 22952279]

262. Pinto CS, Raman A, Reif GA, Magenheimer BS, White C, Calvet JP, et al. Phosphodiesterase Isoform Regulation of Cell Proliferation and Fluid Secretion in Autosomal Dominant Polycystic Kidney Disease. J Am Soc Nephrol. 2016;27(4):1124–34. [PubMed: 26289612]

263. Mehta YR, Lewis SA, Leo KT, Chen L, Park E, Raghuram V, et al. "ADPKD-omics": determinants of cyclic AMP levels in renal epithelial cells. Kidney Int. 2022;101(1):47–62. [PubMed: 34757121]

264. Wang Q, Cobo-Stark P, Patel V, Somlo S, Han PL, Igarashi P. Adenylyl cyclase 5 deficiency reduces renal cyclic AMP and cyst growth in an orthologous mouse model of polycystic kidney disease. Kidney Int. 2018;93(2):403–15. [PubMed: 29042084]

265. Ye H, Wang X, Sussman CR, Hopp K, Irazabal MV, Bakeberg JL, et al. Modulation of Polycystic Kidney Disease Severity by Phosphodiesterase 1 and 3 Subfamilies. J Am Soc Nephrol. 2016;27(5):1312–20. [PubMed: 26374610]

266. Hansen JN, Kaiser F, Leyendecker P, Stuven B, Krause JH, Derakhshandeh F, et al. A cAMP signalosome in primary cilia drives gene expression and kidney cyst formation. EMBO Rep. 2022;23(8):e54315. [PubMed: 35695071]

267. Dowling RJ, Topisirovic I, Alain T, Bidinosti M, Fonseca BD, Petroulakis E, et al. mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. Science. 2010;328(5982):1172–6. [PubMed: 20508131]

268. Fingar DC, Richardson CJ, Tee AR, Cheatham L, Tsou C, Blenis J. mTOR controls cell cycle progression through its cell growth effectors S6K1 and 4E-BP1/eukaryotic translation initiation factor 4E. Mol Cell Biol. 2004;24(1):200–16. [PubMed: 14673156]

269. Sarbassov DD, Ali SM, Sabatini DM. Growing roles for the mTOR pathway. Curr Opin Cell Biol. 2005;17(6):596–603. [PubMed: 16226444]

270. Astrinidis A, Henske EP. Tuberous sclerosis complex: linking growth and energy signaling pathways with human disease. Oncogene. 2005;24(50):7475–81. [PubMed: 16288294]

271. Belibi F, Ravichandran K, Zafar I, He Z, Edelstein CL. mTORC1/2 and rapamycin in female Han:SPRD rats with polycystic kidney disease. Am J Physiol Renal Physiol. 2011;300(1):F236–44. [PubMed: 20943770]

272. Ibraghimov-Beskrovnaya O, Natoli TA. mTOR signaling in polycystic kidney disease. Trends Mol Med. 2011;17(11):625–33. [PubMed: 21775207]

273. Shillingford JM, Murcia NS, Larson CH, Low SH, Hedgepeth R, Brown N, et al. The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. Proc Natl Acad Sci U S A. 2006;103(14):5466–71. [PubMed: 16567633]

274. Zhou J, Brugarolas J, Parada LF. Loss of Tsc1, but not Pten, in renal tubular cells causes polycystic kidney disease by activating mTORC1. Hum Mol Genet. 2009;18(22):4428–41. [PubMed: 19692352]

275. Novalic Z, van der Wal AM, Leonhard WN, Koehl G, Breuning MH, Geissler EK, et al. Dose-dependent effects of sirolimus on mTOR signaling and polycystic kidney disease. J Am Soc Nephrol. 2012;23(5):842–53. [PubMed: 22343118]

276. Shillingford JM, Piontek KB, Germino GG, Weimbs T. Rapamycin ameliorates PKD resulting from conditional inactivation of Pkd1. J Am Soc Nephrol. 2010;21(3):489–97. [PubMed: 20075061]

277. Zafar I, Ravichandran K, Belibi FA, Doctor RB, Edelstein CL. Sirolimus attenuates disease progression in an orthologous mouse model of human autosomal dominant polycystic kidney disease. Kidney Int. 2010;78(8):754–61. [PubMed: 20686448]

278. Wu M, Wahl PR, Le Hir M, Wackerle-Men Y, Wuthrich RP, Serra AL. Everolimus retards cyst growth and preserves kidney function in a rodent model for polycystic kidney disease. Kidney Blood Press Res. 2007;30(4):253–9. [PubMed: 17596700]

279. Zafar I, Belibi FA, He Z, Edelstein CL. Long-term rapamycin therapy in the Han:SPRD rat model of polycystic kidney disease (PKD). Nephrol Dial Transplant. 2009;24(8):2349–53. [PubMed: 19321761]

280. Wahl PR, Serra AL, Le Hir M, Molle KD, Hall MN, Wuthrich RP. Inhibition of mTOR with sirolimus slows disease progression in Han:SPRD rats with autosomal dominant polycystic kidney disease (ADPKD). Nephrol Dial Transplant. 2006;21(3):598–604. [PubMed: 16221708]

281. Mekahli D, Decuypere JP, Sammels E, Welkenhuyzen K, Schoeber J, Audrezet MP, et al. Polycystin-1 but not polycystin-2 deficiency causes upregulation of the mTOR pathway and can be synergistically targeted with rapamycin and metformin. Pflugers Arch. 2014;466(8):1591–604. [PubMed: 24193408]

282. Brook-Carter PT, Peral B, Ward CJ, Thompson P, Hughes J, Maheshwar MM, et al. Deletion of the TSC2 and PKD1 genes associated with severe infantile polycystic kidney disease--a contiguous gene syndrome. Nat Genet. 1994;8(4):328–32. [PubMed: 7894481]

283. Kleymenova E, Ibraghimov-Beskrovnaya O, Kugoh H, Everitt J, Xu H, Kiguchi K, et al. Tuberin-dependent membrane localization of polycystin-1: a functional link between polycystic kidney disease and the TSC2 tumor suppressor gene. Mol Cell. 2001;7(4):823–32. [PubMed: 11336705]

284. Distefano G, Boca M, Rowe I, Wodarczyk C, Ma L, Piontek KB, et al. Polycystin-1 regulates extracellular signal-regulated kinase-dependent phosphorylation of tuberin to control cell size through mTOR and its downstream effectors S6K and 4EBP1. Mol Cell Biol. 2009;29(9):2359–71. [PubMed: 19255143]

285. Qin S, Taglienti M, Nauli SM, Contrino L, Takakura A, Zhou J, et al. Failure to ubiquitinate c-Met leads to hyperactivation of mTOR signaling in a mouse model of autosomal dominant polycystic kidney disease. J Clin Invest. 2010;120(10):3617–28. [PubMed: 20852388]

286. Corradetti MN, Inoki K, Bardeesy N, DePinho RA, Guan KL. Regulation of the TSC pathway by LKB1: evidence of a molecular link between tuberous sclerosis complex and Peutz-Jeghers syndrome. Genes Dev. 2004;18(13):1533–8. [PubMed: 15231735]

287. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol Cell. 2008;30(2):214–26. [PubMed: 18439900]

288. Inoki K, Ouyang H, Zhu T, Lindvall C, Wang Y, Zhang X, et al. TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. Cell. 2006;126(5):955–68. [PubMed: 16959574]

289. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. Cell. 2003;115(5):577–90. [PubMed: 14651849]

290. Shaw RJ, Bardeesy N, Manning BD, Lopez L, Kosmatka M, DePinho RA, et al. The LKB1 tumor suppressor negatively regulates mTOR signaling. Cancer Cell. 2004;6(1):91–9. [PubMed: 15261145]

291. Caplan MJ. AMPK and Polycystic Kidney Disease Drug Development: An Interesting Off-Target Target. Front Med (Lausanne). 2022;9:753418. [PubMed: 35174190]

292. Lian X, Zhao J, Wu X, Zhang Y, Li Q, Lin S, et al. The changes in glucose metabolism and cell proliferation in the kidneys of polycystic kidney disease mini-pig models. Biochem Biophys Res Commun. 2017;488(2):374–81. [PubMed: 28501615]

293. Warner G, Hein KZ, Nin V, Edwards M, Chini CC, Hopp K, et al. Food Restriction Ameliorates the Development of Polycystic Kidney Disease. J Am Soc Nephrol. 2016;27(5):1437–47. [PubMed: 26538633]

294. Boehlke C, Kotsis F, Patel V, Braeg S, Voelker H, Bredt S, et al. Primary cilia regulate mTORC1 activity and cell size through Lkb1. Nat Cell Biol. 2010;12(11):1115–22. [PubMed: 20972424]

295. Orhon I, Dupont N, Zaidan M, Boitez V, Burtin M, Schmitt A, et al. Primary-cilium-dependent autophagy controls epithelial cell volume in response to fluid flow. Nat Cell Biol. 2016;18(6):657–67. [PubMed: 27214279]

296. Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y, Sabatini DM. mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H(+)-ATPase. Science. 2011;334(6056):678–83. [PubMed: 22053050]

297. Sardiello M, Palmieri M, di Ronza A, Medina DL, Valenza M, Gennarino VA, et al. A gene network regulating lysosomal biogenesis and function. Science. 2009;325(5939):473–7. [PubMed: 19556463]

298. Settembre C, Di Malta C, Polito VA, Garcia Arencibia M, Vetrini F, Erdin S, et al. TFEB links autophagy to lysosomal biogenesis. Science. 2011;332(6036):1429–33. [PubMed: 21617040]

299. Settembre C, Zoncu R, Medina DL, Vetrini F, Erdin S, Erdin S, et al. A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB. EMBO J. 2012;31(5):1095–108. [PubMed: 22343943]

300. Di Malta C, Zampelli A, Granieri L, Vilardo C, De Cegli R, Cinque L, et al. TFEB and TFE3 drive kidney cystogenesis and tumorigenesis. EMBO Mol Med. 2023;15(5):e16877. [PubMed: 36987696]

301. Napolitano G, Di Malta C, Esposito A, de Araujo MEG, Pece S, Bertalot G, et al. A substrate-specific mTORC1 pathway underlies Birt-Hogg-Dube syndrome. Nature. 2020;585(7826):597–602. [PubMed: 32612235]

302. Lee K, Gusella GL, He JC. Epithelial proliferation and cell cycle dysregulation in kidney injury and disease. Kidney Int. 2021;100(1):67–78. [PubMed: 33831367]

303. Cai J, Song X, Wang W, Watnick T, Pei Y, Qian F, et al. A RhoA-YAP-c-Myc signaling axis promotes the development of polycystic kidney disease. Genes Dev. 2018;32(11–12):781–93. [PubMed: 29891559]

304. Kurbegovic A, Trudel M. The master regulators Myc and p53 cellular signaling and functions in polycystic kidney disease. Cell Signal. 2020;71:109594. [PubMed: 32145315]

305. Park EY, Sung YH, Yang MH, Noh JY, Park SY, Lee TY, et al. Cyst formation in kidney via B-Raf signaling in the PKD2 transgenic mice. J Biol Chem. 2009;284(11):7214–22. [PubMed: 19098310]

306. Parker E, Newby LJ, Sharpe CC, Rossetti S, Streets AJ, Harris PC, et al. Hyperproliferation of PKD1 cystic cells is induced by insulin-like growth factor-1 activation of the Ras/Raf signalling system. Kidney Int. 2007.

307. van Bodegom D, Roessingh W, Pridjian A, El Dahr SS. Mechanisms of p53-mediated repression of the human polycystic kidney disease-1 promoter. Biochim Biophys Acta. 2010;1799(7):502–9. [PubMed: 20388565]

308. Nagao S, Yamaguchi T, Kusaka M, Maser RL, Takahashi H, Cowley BD, et al. Renal activation of extracellular signal-regulated kinase in rats with autosomal-dominant polycystic kidney disease. Kidney Int. 2003;63(2):427–37. [PubMed: 12631108]

309. Omori S, Hida M, Fujita H, Takahashi H, Tanimura S, Kohno M, et al. Extracellular signal-regulated kinase inhibition slows disease progression in mice with polycystic kidney disease. J Am Soc Nephrol. 2006;17(6):1604–14. [PubMed: 16641154]

310. Joly D, Ishibe S, Nickel C, Yu Z, Somlo S, Cantley LG. The polycystin 1-C-terminal fragment stimulates ERK-dependent spreading of renal epithelial cells. J Biol Chem. 2006;281(36):26329–39. [PubMed: 16790429]

311. Shibazaki S, Yu Z, Nishio S, Tian X, Thomson RB, Mitobe M, et al. Cyst formation and activation of the extracellular regulated kinase pathway after kidney specific inactivation of Pkd1. Hum Mol Genet. 2008;17(11):1505–16. [PubMed: 18263604]

312. Arnould T, Kim E, Tsiokas L, Jochimsen F, Gruning W, Chang JD, et al. The polycystic kidney disease 1 gene product mediates protein kinase C alpha-dependent and c-Jun N-terminal kinase-dependent activation of the transcription factor AP-1. J Biol Chem. 1998;273(11):6013–8. [PubMed: 9497315]

313. Nishio S, Hatano M, Nagata M, Horie S, Koike T, Tokuhisa T, et al. Pkd1 regulates immortalized proliferation of renal tubular epithelial cells through p53 induction and JNK activation. J Clin Invest. 2005;115(4):910–8. [PubMed: 15761494]

314. Islam MR, Jimenez T, Pelham C, Rodova M, Puri S, Magenheimer BS, et al. MAP/ERK kinase kinase 1 (MEKK1) mediates transcriptional repression by interacting with polycystic kidney disease-1 (PKD1) promoter-bound p53 tumor suppressor protein. J Biol Chem. 2010;285(50):38818–31. [PubMed: 20923779]

315. Tham MS, Cottle DL, Zylberberg AK, Short KM, Jones LK, Chan P, et al. Deletion of Aurora kinase A prevents the development of polycystic kidney disease in mice. Nature communications. 2024;15(1):371.

316. Bhunia AK, Piontek K, Boletta A, Liu L, Qian F, Xu PN, et al. PKD1 induces p21(waf1) and regulation of the cell cycle via direct activation of the JAK-STAT signaling pathway in a process requiring PKD2. Cell. 2002;109(2):157–68. [PubMed: 12007403]

317. Kim E, Arnould T, Sellin LK, Benzing T, Fan MJ, Gruning W, et al. The polycystic kidney disease 1 gene product modulates Wnt signaling. J Biol Chem. 1999;274(8):4947–53. [PubMed: 9988738]

318. Lee EJ, Seo E, Kim JW, Nam SA, Lee JY, Jun J, et al. TAZ/Wnt-beta-catenin/c-MYC axis regulates cystogenesis in polycystic kidney disease. Proc Natl Acad Sci U S A. 2020;117(46):29001–12. [PubMed: 33122431]

319. Miller MM, Iglesias DM, Zhang Z, Corsini R, Chu L, Murawski I, et al. T-cell factor/beta-catenin activity is suppressed in two different models of autosomal dominant polycystic kidney disease. Kidney Int. 2011;80(2):146–53. [PubMed: 21389971]

320. Conduit SE, Hakim S, Feeney SJ, Ooms LM, Dyson JM, Abud HE, et al. beta-catenin ablation exacerbates polycystic kidney disease progression. Hum Mol Genet. 2019;28(2):230–44. [PubMed: 30265301]

321. Happe H, van der Wal AM, Leonhard WN, Kunnen SJ, Breuning MH, de Heer E, et al. Altered Hippo signalling in polycystic kidney disease. J Pathol. 2011;224(1):133–42. [PubMed: 21381034]

322. Makita R, Uchijima Y, Nishiyama K, Amano T, Chen Q, Takeuchi T, et al. Multiple renal cysts, urinary concentration defects, and pulmonary emphysematous changes in mice lacking TAZ. Am J Physiol Renal Physiol. 2008;294(3):F542–53. [PubMed: 18172001]

323. Tian Y, Kolb R, Hong JH, Carroll J, Li D, You J, et al. TAZ promotes PC2 degradation through a SCFbeta-Trcp E3 ligase complex. Mol Cell Biol. 2007;27(18):6383–95. [PubMed: 17636028]

324. Yim H, Sung CK, You J, Tian Y, Benjamin T. Nek1 and TAZ interact to maintain normal levels of polycystin 2. J Am Soc Nephrol. 2011;22(5):832–7. [PubMed: 21474562]

325. Bukanov NO, Moreno SE, Natoli TA, Rogers KA, Smith LA, Ledbetter SR, et al. CDK inhibitors R-roscovitine and S-CR8 effectively block renal and hepatic cystogenesis in an orthologous model of ADPKD. Cell Cycle. 2012;11(21):4040–6. [PubMed: 23032260]

326. Li X, Luo Y, Starremans PG, McNamara CA, Pei Y, Zhou J. Polycystin-1 and polycystin-2 regulate the cell cycle through the helix-loop-helix inhibitor Id2. Nat Cell Biol. 2005;7(12):1202–12. [PubMed: 16311606]

327. Peyronnet R, Sharif-Naeini R, Folgering JH, Arhatte M, Jodar M, El Boustany C, et al. Mechanoprotection by polycystins against apoptosis is mediated through the opening of stretch-activated K(2P) channels. Cell reports. 2012;1(3):241–50. [PubMed: 22832196]

328. Goilav B Apoptosis in polycystic kidney disease. Biochim Biophys Acta. 2011;1812(10):1272–80. [PubMed: 21241798]

329. Goilav B, Satlin LM, Wilson PD. Pathways of apoptosis in human autosomal recessive and autosomal dominant polycystic kidney diseases. Pediatr Nephrol. 2008;23(9):1473–82. [PubMed: 18516626]

330. Boletta A, Qian F, Onuchic LF, Bhunia AK, Phakdeekitcharoen B, Hanaoka K, et al. Polycystin-1, the gene product of PKD1, induces resistance to apoptosis and spontaneous tubulogenesis in MDCK cells. Mol Cell. 2000;6(5):1267–73. [PubMed: 11106764]

331. Hasegawa S, Inagi R. Organelle Stress and Crosstalk in Kidney Disease. Kidney360. 2020;1(10):1157–64. [PubMed: 35368784]

332. Ishikawa Y, Fedeles S, Marlier A, Zhang C, Gallagher AR, Lee AH, et al. Spliced XBP1 Rescues Renal Interstitial Inflammation Due to Loss of Sec63 in Collecting Ducts. J Am Soc Nephrol. 2019;30(3):443–59. [PubMed: 30745418]

333. Ishimoto Y, Inagi R, Yoshihara D, Kugita M, Nagao S, Shimizu A, et al. Mitochondrial Abnormality Facilitates Cyst Formation in Autosomal Dominant Polycystic Kidney Disease. Mol Cell Biol. 2017.

334. Lu Y, Sun Y, Liu Z, Lu Y, Zhu X, Lan B, et al. Activation of NRF2 ameliorates oxidative stress and cystogenesis in autosomal dominant polycystic kidney disease. Sci Transl Med. 2020;12(554).

335. Panda DK, Bai X, Zhang Y, Stylianesis NA, Koromilas AE, Lipman ML, et al. SCF-SKP2 E3 ubiquitin ligase links mTORC1/ER stress/ISR with YAP activation in murine renal cystogenesis. J Clin Invest. 2022;132(24).

336. Fedeles SV, So JS, Shrikhande A, Lee SH, Gallagher AR, Barkauskas CE, et al. Sec63 and Xbp1 regulate IRE1alpha activity and polycystic disease severity. J Clin Invest. 2015;125(5):1955–67. [PubMed: 25844898]

337. Belibi F, Zafar I, Ravichandran K, Segvic AB, Jani A, Ljubanovic DG, et al. Hypoxia-inducible factor-1alpha (HIF-1alpha) and autophagy in polycystic kidney disease (PKD). Am J Physiol Renal Physiol. 2011;300(5):F1235–43. [PubMed: 21270095]

338. Bernhardt WM, Wiesener MS, Weidemann A, Schmitt R, Weichert W, Lechler P, et al. Involvement of hypoxia-inducible transcription factors in polycystic kidney disease. Am J Pathol. 2007;170(3):830–42. [PubMed: 17322369]

339. Buchholz B, Eckardt KU. Role of oxygen and the HIF-pathway in polycystic kidney disease. Cell Signal. 2020;69:109524. [PubMed: 31904413]

340. Buchholz B, Schley G, Faria D, Kroening S, Willam C, Schreiber R, et al. Hypoxia-Inducible Factor-1alpha Causes Renal Cyst Expansion through Calcium-Activated Chloride Secretion. J Am Soc Nephrol. 2013.

341. Kahveci AS, Barnatan TT, Kahveci A, Adrian AE, Arroyo J, Eirin A, et al. Oxidative Stress and Mitochondrial Abnormalities Contribute to Decreased Endothelial Nitric Oxide Synthase Expression and Renal Disease Progression in Early Experimental Polycystic Kidney Disease. Int J Mol Sci. 2020;21(6).

342. Maser RL, Vassmer D, Magenheimer BS, Calvet JP. Oxidant stress and reduced antioxidant enzyme protection in polycystic kidney disease. J Am Soc Nephrol. 2002;13(4):991–9. [PubMed: 11912258]

343. Zimmerman KA, Hopp K, Mrug M. Role of chemokines, innate and adaptive immunity. Cell Signal. 2020;73:109647. [PubMed: 32325183]

344. Chen L, Zhou X, Fan LX, Yao Y, Swenson-Fields KI, Gadjeva M, et al. Macrophage migration inhibitory factor promotes cyst growth in polycystic kidney disease. J Clin Invest. 2015;125(6):2399–412. [PubMed: 25961459]

345. Cassini MF, Kakade VR, Kurtz E, Sulkowski P, Glazer P, Torres R, et al. Mcp1 Promotes Macrophage-Dependent Cyst Expansion in Autosomal Dominant Polycystic Kidney Disease. J Am Soc Nephrol. 2018;29(10):2471–81. [PubMed: 30209078]

346. Cowley BD Jr., Ricardo SD, Nagao S, Diamond JR. Increased renal expression of monocyte chemoattractant protein-1 and osteopontin in ADPKD in rats. Kidney Int. 2001;60(6):2087–96. [PubMed: 11737583]

347. Janssens P, Decuypere JP, De Rechter S, Breysem L, Van Giel D, Billen J, et al. Enhanced MCP-1 Release in Early Autosomal Dominant Polycystic Kidney Disease. Kidney Int Rep. 2021;6(6):1687–98. [PubMed: 34169210]

348. Karihaloo A, Koraishy F, Huen SC, Lee Y, Merrick D, Caplan MJ, et al. Macrophages promote cyst growth in polycystic kidney disease. J Am Soc Nephrol. 2011;22(10):1809–14. [PubMed: 21921140]

349. Swenson-Fields KI, Vivian CJ, Salah SM, Peda JD, Davis BM, van Rooijen N, et al. Macrophages promote polycystic kidney disease progression. Kidney Int. 2013;83(5):855–64. [PubMed: 23423256]

350. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, et al. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. Nature. 2014;513(7519):559–63. [PubMed: 25043024]

351. Kleczko EK, Marsh KH, Tyler LC, Furgeson SB, Bullock BL, Altmann CJ, et al. CD8(+) T cells modulate autosomal dominant polycystic kidney disease progression. Kidney Int. 2018;94(6):1127–40. [PubMed: 30249452]

352. Kleczko EK, Nguyen DT, Marsh KH, Bauer CD, Li AS, Monaghan MT, et al. Immune checkpoint activity regulates polycystic kidney disease progression. JCI insight. 2023;8(12).

353. Nguyen DT, Kleczko EK, Dwivedi N, Monaghan MT, Gitomer BY, Chonchol MB, et al. The tryptophan-metabolizing enzyme indoleamine 2,3-dioxygenase 1 regulates polycystic kidney disease progression. JCI insight. 2023;8(1).

354. Lin F, Hiesberger T, Cordes K, Sinclair AM, Goldstein LS, Somlo S, et al. Kidney-specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease. Proc Natl Acad Sci U S A. 2003;100(9):5286–91. [PubMed: 12672950]

355. Jonassen JA, San Agustin J, Follit JA, Pazour GJ. Deletion of IFT20 in the mouse kidney causes misorientation of the mitotic spindle and cystic kidney disease. J Cell Biol. 2008;183(3):377–84. [PubMed: 18981227]

356. Davenport JR, Watts AJ, Roper VC, Croyle MJ, van GT, Wyss JM, et al. Disruption of intraflagellar transport in adult mice leads to obesity and slow-onset cystic kidney disease. CurrBiol. 2007;17(18):1586–94.

357. Walker RV, Maranto A, Palicharla VR, Hwang SH, Mukhopadhyay S, Qian F. Cilia-Localized Counterregulatory Signals as Drivers of Renal Cystogenesis. Front Mol Biosci. 2022;9:936070. [PubMed: 35832738]

358. Hwang SH, Somatilaka BN, Badgandi H, Palicharla VR, Walker R, Shelton JM, et al. Tulp3 Regulates Renal Cystogenesis by Trafficking of Cystoproteins to Cilia. Curr Biol. 2019;29(5):790–802 e5. [PubMed: 30799239]

359. Legue E, Liem KF Jr. Tulp3 Is a Ciliary Trafficking Gene that Regulates Polycystic Kidney Disease. Curr Biol. 2019;29(5):803–12 e5. [PubMed: 30799240]

360. Badgandi HB, Hwang SH, Shimada IS, Loriot E, Mukhopadhyay S. Tubby family proteins are adapters for ciliary trafficking of integral membrane proteins. J Cell Biol. 2017;216(3):743–60. [PubMed: 28154160]

361. Praetorius HA, Spring KR. A physiological view of the primary cilium. Annu Rev Physiol. 2005;67:515–29. [PubMed: 15709968]

362. Wood CR, Huang K, Diener DR, Rosenbaum JL. The cilium secretes bioactive ectosomes. Curr Biol. 2013;23(10):906–11. [PubMed: 23623554]

363. Wang J, Barr MM. Ciliary Extracellular Vesicles: Txt Msg Organelles. Cell Mol Neurobiol. 2016;36(3):449–57. [PubMed: 26983828]

364. Wood CR, Rosenbaum JL. Ciliary ectosomes: transmissions from the cell's antenna. Trends Cell Biol. 2015;25(5):276–85. [PubMed: 25618328]

365. Zuo X, Kwon SH, Janech MG, Dang Y, Lauzon SD, Fogelgren B, et al. Primary cilia and the exocyst are linked to urinary extracellular vesicle production and content. J Biol Chem. 2019;294(50):19099–110. [PubMed: 31694916]

366. Lea WA, Winklhofer T, Zelenchuk L, Sharma M, Rossol-Allison J, Fields TA, et al. Polycystin-1 Interacting Protein-1 (CU062) Interacts with the Ectodomain of Polycystin-1 (PC1). Cells. 2023;12(17).

367. Walsh JD, Wang J, DeHart M, Nikonorova IA, Srinivasan J, Barr MM. Tracking N- and C-termini of C. elegans polycystin-1 reveals their distinct targeting requirements and functions in cilia and extracellular vesicles. PLoS Genet. 2022;18(12):e1010560. [PubMed: 36574451]

368. Pocsfalvi G, Raj DA, Fiume I, Vilasi A, Trepiccione F, Capasso G. Urinary extracellular vesicles as reservoirs of altered proteins during the pathogenesis of polycystic kidney disease. Proteomics Clinical applications. 2015;9(5–6):552–67. [PubMed: 25755179]

369. Carotti V, van Megen WH, Rigalli JP, Barros ER, Sommers V, Rutten L, et al. Extracellular vesicles contribute to early cyst development in autosomal dominant polycystic kidney disease by cell-to-cell communication. FASEB J. 2023;37(7):e23006. [PubMed: 37249915]

370. Menezes LF, Germino GG. The pathobiology of polycystic kidney disease from a metabolic viewpoint. Nat Rev Nephrol. 2019;15(12):735–49. [PubMed: 31488901]

371. Song X, Di Giovanni V, He N, Wang K, Ingram A, Rosenblum ND, et al. Systems biology of autosomal dominant polycystic kidney disease (ADPKD): computational identification of gene expression pathways and integrated regulatory networks. Hum Mol Genet. 2009;18(13):2328–43. [PubMed: 19346236]

372. Menezes LF, Zhou F, Patterson AD, Piontek KB, Krausz KW, Gonzalez FJ, et al. Network analysis of a Pkd1-mouse model of autosomal dominant polycystic kidney disease identifies HNF4alpha as a disease modifier. PLoS Genet. 2012;8(11):e1003053. [PubMed: 23209428]

373. Chiaravalli M, Rowe I, Mannella V, Quilici G, Canu T, Bianchi V, et al. 2-Deoxy-d-Glucose Ameliorates PKD Progression. J Am Soc Nephrol. 2016;27(7):1958–69. [PubMed: 26534924]

374. Torres JA, Kruger SL, Broderick C, Amarlkhagva T, Agrawal S, Dodam JR, et al. Ketosis Ameliorates Renal Cyst Growth in Polycystic Kidney Disease. Cell Metab. 2019;30(6):1007–23 e5. [PubMed: 31631001]

375. Patel V, Williams D, Hajarnis S, Hunter R, Pontoglio M, Somlo S, et al. miR-17~92 miRNA cluster promotes kidney cyst growth in polycystic kidney disease. Proc Natl Acad Sci U S A. 2013;110(26):10765–70. [PubMed: 23759744]

376. Hajarnis S, Lakhia R, Yheskel M, Williams D, Sorourian M, Liu X, et al. microRNA-17 family promotes polycystic kidney disease progression through modulation of mitochondrial metabolism. Nature communications. 2017;8:14395.

377. Lee EC, Valencia T, Allerson C, Schairer A, Flaten A, Yheskel M, et al. Discovery and preclinical evaluation of anti-miR-17 oligonucleotide RGLS4326 for the treatment of polycystic kidney disease. Nature communications. 2019;10(1):4148.

378. Zhu H, Bi D, Zhang Y, Kong C, Du J, Wu X, et al. Ketogenic diet for human diseases: the underlying mechanisms and potential for clinical implementations. Signal Transduct Target Ther. 2022;7(1):11. [PubMed: 35034957]

379. Boletta A Slowing Polycystic Kidney Disease by Fasting. J Am Soc Nephrol. 2016;27(5):1268–70. [PubMed: 26538629]

380. Hopp K, Catenacci VA, Dwivedi N, Kline TL, Wang W, You Z, et al. Weight loss and cystic disease progression in autosomal dominant polycystic kidney disease. iScience. 2022;25(1):103697. [PubMed: 35059607]

381. Cukoski S, Lindemann CH, Arjune S, Todorova P, Brecht T, Kuhn A, et al. Feasibility and impact of ketogenic dietary interventions in polycystic kidney disease: KETO-ADPKD-a randomized controlled trial. Cell Rep Med. 2023;4(11):101283. [PubMed: 37935200]

382. Flowers EM, Sudderth J, Zacharias L, Mernaugh G, Zent R, DeBerardinis RJ, et al. Lkb1 deficiency confers glutamine dependency in polycystic kidney disease. Nature communications. 2018;9(1):814.

383. Soomro I, Sun Y, Li Z, Diggs L, Hatzivassiliou G, Thomas AG, et al. Glutamine metabolism via glutaminase 1 in autosomal-dominant polycystic kidney disease. Nephrol Dial Transplant. 2018;33(8):1343–53. [PubMed: 29420817]

384. Hwang VJ, Kim J, Rand A, Yang C, Sturdivant S, Hammock B, et al. The cpk model of recessive PKD shows glutamine dependence associated with the production of the oncometabolite 2-hydroxyglutarate. Am J Physiol Renal Physiol. 2015;309(6):F492–8. [PubMed: 26155843]

385. Ramalingam H, Kashyap S, Cobo-Stark P, Flaten A, Chang CM, Hajarnis S, et al. A methionine-Mettl3-N(6)-methyladenosine axis promotes polycystic kidney disease. Cell Metab. 2021;33(6):1234–47 e7. [PubMed: 33852874]

386. Baliga MM, Klawitter J, Christians U, Hopp K, Chonchol M, Gitomer BY, et al. Metabolic profiling in children and young adults with autosomal dominant polycystic kidney disease. Sci Rep. 2021;11(1):6629. [PubMed: 33758231]

387. Hopp K, Kleczko EK, Gitomer BY, Chonchol M, Klawitter J, Christians U, et al. Metabolic reprogramming in a slowly developing orthologous model of polycystic kidney disease. Am J Physiol Renal Physiol. 2022;322(3):F258–F67. [PubMed: 35037466]

388. Klawitter J, Jackson MJ, Smith PH, Hopp K, Chonchol M, Gitomer BY, et al. Kynurenines in polycystic kidney disease. J Nephrol. 2023;36(1):83–91. [PubMed: 35867237]

389. Sriwi D, Alabdaljabar MS, Jacob M, Mujamammi AH, Gu X, Sabi EM, et al. Metabolomics Profiling of Cystic Renal Disease towards Biomarker Discovery. Biology (Basel). 2021;10(8).

390. Houske EA, Glimm MG, Bergstrom AR, Slipher SK, Welhaven HD, Greenwood MC, et al. Metabolomic profiling to identify early urinary biomarkers and metabolic pathway alterations in autosomal dominant polycystic kidney disease. Am J Physiol Renal Physiol. 2023;324(6):F590–F602. [PubMed: 37141147]

391. Wang G, Heijs B, Kostidis S, Mahfouz A, Rietjens RGJ, Bijkerk R, et al. Analyzing cell-type-specific dynamics of metabolism in kidney repair. Nat Metab. 2022;4(9):1109–18. [PubMed: 36008550]

392. Wang G, Heijs B, Kostidis S, Rietjens RGJ, Koning M, Yuan L, et al. Spatial dynamic metabolomics identifies metabolic cell fate trajectories in human kidney differentiation. Cell Stem Cell. 2022;29(11):1580–93 e7. [PubMed: 36332571]

393. Watnick TJ, Jin Y, Matunis E, Kernan MJ, Montell C. A flagellar polycystin-2 homolog required for male fertility in Drosophila. Curr Biol. 2003;13(24):2179–84. [PubMed: 14680634]

394. Mangos S, Lam PY, Zhao A, Liu Y, Mudumana S, Vasilyev A, et al. The ADPKD genes pkd1a/b and pkd2 regulate extracellular matrix formation. Dis Model Mech. 2010;3(5–6):354–65. [PubMed: 20335443]

395. Sieben CJ, Harris PC. Experimental Models of Polycystic Kidney Disease: Applications and Therapeutic Testing. Kidney360. 2023;4(8):1155–73. [PubMed: 37418622]

396. Forbes TA, Howden SE, Lawlor K, Phipson B, Maksimovic J, Hale L, et al. Patient-iPSC-Derived Kidney Organoids Show Functional Validation of a Ciliopathic Renal Phenotype and Reveal Underlying Pathogenetic Mechanisms. Am J Hum Genet. 2018;102(5):816–31. [PubMed: 29706353]

397. Freedman BS, Brooks CR, Lam AQ, Fu H, Morizane R, Agrawal V, et al. Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. Nature communications. 2015;6:8715.

398. Kuraoka S, Tanigawa S, Taguchi A, Hotta A, Nakazato H, Osafune K, et al. PKD1-Dependent Renal Cystogenesis in Human Induced Pluripotent Stem Cell-Derived Ureteric Bud/Collecting Duct Organoids. J Am Soc Nephrol. 2020;31(10):2355–71. [PubMed: 32747355]

399. Morizane R, Lam AQ, Freedman BS, Kishi S, Valerius MT, Bonventre JV. Nephron organoids derived from human pluripotent stem cells model kidney development and injury. Nat Biotechnol. 2015;33(11):1193–200. [PubMed: 26458176]

400. Takasato M, Er PX, Chiu HS, Maier B, Baillie GJ, Ferguson C, et al. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. Nature. 2015;526(7574):564–8. [PubMed: 26444236]

401. Vishy CE, Thomas C, Vincent T, Crawford DK, Goddeeris MM, Freedman BS. Genetics of cystogenesis in base-edited human organoids reveal therapeutic strategies for polycystic kidney disease. Cell Stem Cell. 2024;31(4):537–53 e5. [PubMed: 38579684]

402. Tran T, Song CJ, Nguyen T, Cheng SY, McMahon JA, Yang R, et al. A scalable organoid model of human autosomal dominant polycystic kidney disease for disease mechanism and drug discovery. Cell Stem Cell. 2022;29(7):1083–101 e7. [PubMed: 35803227]

403. Takasato M, Er PX, Chiu HS, Little MH. Generation of kidney organoids from human pluripotent stem cells. Nat Protoc. 2016;11(9):1681–92. [PubMed: 27560173]

404. Huang B, Zeng Z, Kim S, Fausto CC, Koppitch K, Li H, et al. Long-term expandable mouse and human-induced nephron progenitor cells enable kidney organoid maturation and modeling of plasticity and disease. Cell Stem Cell. 2024;31(6):921–39 e17. [PubMed: 38692273]

405. Xu Y, Kuppe C, Perales-Paton J, Hayat S, Kranz J, Abdallah AT, et al. Adult human kidney organoids originate from CD24(+) cells and represent an advanced model for adult polycystic kidney disease. Nat Genet. 2022;54(11):1690–701. [PubMed: 36303074]

406. Cruz NM, Reddy R, McFaline-Figueroa JL, Tran C, Fu H, Freedman BS. Modelling ciliopathy phenotypes in human tissues derived from pluripotent stem cells with genetically ablated cilia. Nat Biomed Eng. 2022;6(4):463–75. [PubMed: 35478224]

407. Cruz NM, Song X, Czerniecki SM, Gulieva RE, Churchill AJ, Kim YK, et al. Organoid cystogenesis reveals a critical role of microenvironment in human polycystic kidney disease. Nat Mater. 2017;16(11):1112–9. [PubMed: 28967916]

408. Howden SE, Wilson SB, Groenewegen E, Starks L, Forbes TA, Tan KS, et al. Plasticity of distal nephron epithelia from human kidney organoids enables the induction of ureteric tip and stalk. Cell Stem Cell. 2021;28(4):671–84 e6. [PubMed: 33378647]

409. Mae SI, Hattanda F, Morita H, Nozaki A, Katagiri N, Ogawa H, et al. Human iPSC-derived renal collecting duct organoid model cystogenesis in ADPKD. Cell reports. 2023;42(12):113431. [PubMed: 38039961]

410. Shimizu T, Mae SI, Araoka T, Okita K, Hotta A, Yamagata K, et al. A novel ADPKD model using kidney organoids derived from disease-specific human iPSCs. Biochem Biophys Res Commun. 2020;529(4):1186–94. [PubMed: 32819584]

411. Hiratsuka K, Miyoshi T, Kroll KT, Gupta NR, Valerius MT, Ferrante T, et al. Organoid-on-a-chip model of human ARPKD reveals mechanosensing pathomechanisms for drug discovery. Sci Adv. 2022;8(38):eabq0866. [PubMed: 36129975]

412. Li SR, Gulieva RE, Helms L, Cruz NM, Vincent T, Fu H, et al. Glucose absorption drives cystogenesis in a human organoid-on-chip model of polycystic kidney disease. Nature communications. 2022;13(1):7918.

413. Liu M, Zhang C, Gong X, Zhang T, Lian MM, Chew EGY, et al. Kidney organoid models reveal cilium-autophagy metabolic axis as a therapeutic target for PKD both in vitro and in vivo. Cell Stem Cell. 2024;31(1):52–70 e8. [PubMed: 38181751]

414. Schieren G, Pey R, Bach J, Hafner M, Gretz N. Murine models of polycystic kidney disease. Nephrol Dial Transplant. 1996;11 Suppl 6:38–45.

415. Wu G, Markowitz GS, Li L, D'Agati VD, Factor SM, Geng L, et al. Cardiac defects and renal failure in mice with targeted mutations in Pkd2. Nat Genet. 2000;24(1):75–8. [PubMed: 10615132]

416. Wu G, D'Agati V, Cai Y, Markowitz G, Park JH, Reynolds DM, et al. Somatic inactivation of Pkd2 results in polycystic kidney disease. Cell. 1998;93(2):177–88. [PubMed: 9568711]

417. Menezes LF, Germino GG. Murine Models of Polycystic Kidney Disease. Drug Discov Today Dis Mech. 2013;10(3–4):e153–e8. [PubMed: 25013443]

418. Piontek K, Menezes LF, Garcia-Gonzalez MA, Huso DL, Germino GG. A critical developmental switch defines the kinetics of kidney cyst formation after loss of Pkd1. Nat Med. 2007;13(12):1490–5. [PubMed: 17965720]

419. Lian X, Wu X, Li Z, Zhang Y, Song K, Cai G, et al. The combination of metformin and 2-deoxyglucose significantly inhibits cyst formation in miniature pigs with polycystic kidney disease. Br J Pharmacol. 2019;176(5):711–24. [PubMed: 30515768]

420. Lee YJ, Chen HY, Hsu WL, Ou CM, Wong ML. Diagnosis of feline polycystic kidney disease by a combination of ultrasonographic examination and PKD1 gene analysis. Vet Rec. 2010;167(16):614–8. [PubMed: 21257442]

421. Boulter C, Mulroy S, Webb S, Fleming S, Brindle K, Sandford R. Cardiovascular, skeletal, and renal defects in mice with a targeted disruption of the Pkd1 gene. Proc Natl Acad Sci U S A. 2001;98(21):12174–9. [PubMed: 11593033]

422. Smithline ZB, Nikonova AS, Hensley HH, Cai KQ, Egleston BL, Proia DA, et al. Inhibiting heat shock protein 90 (HSP90) limits the formation of liver cysts induced by conditional deletion of Pkd1 in mice. PLoS One. 2014;9(12):e114403. [PubMed: 25474361]

423. Kurbegovic A, Cote O, Couillard M, Ward CJ, Harris PC, Trudel M. Pkd1 transgenic mice: adult model of polycystic kidney disease with extrarenal and renal phenotypes. Hum Mol Genet. 2010;19(7):1174–89. [PubMed: 20053665]

424. Hamzaoui M, Groussard D, Nezam D, Djerada Z, Lamy G, Tardif V, et al. Endothelium-Specific Deficiency of Polycystin-1 Promotes Hypertension and Cardiovascular Disorders. Hypertension. 2022;79(11):2542–51. [PubMed: 36093769]

425. Besse W, Roosendaal C, Tuccillo L, Roy SG, Gallagher AR, Somlo S. Adult Inactivation of the Recessive Polycystic Kidney Disease Gene Causes Polycystic Liver Disease. Kidney360. 2020;1(10):1068–76. [PubMed: 33554127]

426. Ward CJ, Hogan MC, Rossetti S, Walker D, Sneddon T, Wang X, et al. The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein. Nat Genet. 2002;30(3):259–69. [PubMed: 11919560]

427. Gallagher AR, Esquivel EL, Briere TS, Tian X, Mitobe M, Menezes LF, et al. Biliary and pancreatic dysgenesis in mice harboring a mutation in Pkhd1. Am J Pathol. 2008;172(2):417–29. [PubMed: 18202188]

428. Shan D, Rezonzew G, Mullen S, Roye R, Zhou J, Chumley P, et al. Heterozygous Pkhd1(C642*) mice develop cystic liver disease and proximal tubule ectasia that mimics radiographic signs of medullary sponge kidney. Am J Physiol Renal Physiol. 2019;316(3):F463–F72. [PubMed: 30600684]

429. Olesen ETB, Fenton RA. Aquaporin 2 regulation: implications for water balance and polycystic kidney diseases. Nat Rev Nephrol. 2021;17(11):765–81. [PubMed: 34211154]

430. Gattone VH 2nd, Wang X, Harris PC, Torres VE. Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist. Nat Med. 2003;9(10):1323–6. [PubMed: 14502283]

431. Torres VE, Chapman AB, Devuyst O, Gansevoort RT, Grantham JJ, Higashihara E, et al. Tolvaptan in patients with autosomal dominant polycystic kidney disease. N Engl J Med. 2012;367(25):2407–18. [PubMed: 23121377]

432. Hogan MC, Masyuk TV, Page LJ, Kubly VJ, Bergstralh EJ, Li X, et al. Randomized clinical trial of long-acting somatostatin for autosomal dominant polycystic kidney and liver disease. J Am Soc Nephrol.21(6):1052–61.

433. Serra AL, Poster D, Kistler AD, Krauer F, Raina S, Young J, et al. Sirolimus and kidney growth in autosomal dominant polycystic kidney disease. N Engl J Med. 2010;363(9):820–9. [PubMed: 20581391]

434. Walz G, Budde K, Mannaa M, Nurnberger J, Wanner C, Sommerer C, et al. Everolimus in patients with autosomal dominant polycystic kidney disease. N Engl J Med. 2010;363(9):830–40. [PubMed: 20581392]

435. Gansevoort RT, Hariri A, Minini P, Ahn C, Chapman AB, Horie S, et al. Venglustat, a Novel Glucosylceramide Synthase Inhibitor, in Patients at Risk of Rapidly Progressing ADPKD: Primary Results of a Double-Blind, Placebo-Controlled, Phase 2/3 Randomized Clinical Trial. Am J Kidney Dis. 2023;81(5):517–27 e1. [PubMed: 36535535]

436. Kipp KR, Rezaei M, Lin L, Dewey EC, Weimbs T. A mild reduction of food intake slows disease progression in an orthologous mouse model of polycystic kidney disease. Am J Physiol Renal Physiol. 2016;310(8):F726–F31. [PubMed: 26764208]

437. Kipp KR, Kruger SL, Schimmel MF, Parker N, Shillingford JM, Leamon CP, et al. Comparison of folate-conjugated rapamycin versus unconjugated rapamycin in an orthologous mouse model of polycystic kidney disease. Am J Physiol Renal Physiol. 2018;315(2):F395–F405. [PubMed: 29717938]

438. Takiar V, Nishio S, Seo-Mayer P, King JD Jr., Li H, Zhang L, et al. Activating AMP-activated protein kinase (AMPK) slows renal cystogenesis. Proc Natl Acad Sci U S A. 2011;108(6):2462–7. [PubMed: 21262823]

439. Blazer-Yost BL, Haydon J, Eggleston-Gulyas T, Chen JH, Wang X, Gattone V, et al. Pioglitazone Attenuates Cystic Burden in the PCK Rodent Model of Polycystic Kidney Disease. PPAR Res. 2010;2010:274376. [PubMed: 21052534]

440. Hallows KR, Li H, Saitta B, Sepehr S, Huang P, Pham J, et al. Beneficial effects of bempedoic acid treatment in polycystic kidney disease cells and mice. Front Mol Biosci. 2022;9:1001941. [PubMed: 36504724]

441. Leonhard WN, Song X, Kanhai AA, Iliuta IA, Bozovic A, Steinberg GR, et al. Salsalate, but not metformin or canagliflozin, slows kidney cyst growth in an adult-onset mouse model of polycystic kidney disease. EBioMedicine. 2019;47:436–45. [PubMed: 31473186]

442. Perrone RD, Abebe KZ, Watnick T, Althouse A, Hallows KR, Lalama CM, et al. Primary results of the randomized trial of metformin administration in polycystic kidney disease (TAME PKD). Kidney Int. 2021.

443. Brosnahan GM, Wang W, Gitomer B, Struemph T, George D, You Z, et al. Metformin Therapy in Autosomal Dominant Polycystic Kidney Disease: A Feasibility Study. Am J Kidney Dis. 2022;79(4):518–26. [PubMed: 34391872]

444. Ong ACM, Gansevoort RT. TAMEing ADPKD with metformin: safe and effective? Kidney Int. 2021;100(3):513–5. [PubMed: 34420660]

445. Safi W, Kraus A, Grampp S, Schodel J, Buchholz B. Macrophage migration inhibitory factor is regulated by HIF-1alpha and cAMP and promotes renal cyst cell proliferation in a macrophage-independent manner. Journal of molecular medicine. 2020;98(11):1547–59. [PubMed: 32885302]

446. Salah SM, Meisenheimer JD, Rao R, Peda JD, Wallace DP, Foster D, et al. MCP-1 promotes detrimental cardiac physiology, pulmonary edema, and death in the cpk model of polycystic kidney disease. Am J Physiol Renal Physiol. 2019;317(2):F343–F60. [PubMed: 31091126]

447. Ta MH, Harris DC, Rangan GK. Role of interstitial inflammation in the pathogenesis of polycystic kidney disease. Nephrology. 2013;18(5):317–30. [PubMed: 23448509]

448. Viau A, Baaziz M, Aka A, Mazloum M, Nguyen C, Kuehn EW, et al. Tubular STAT3 Limits Renal Inflammation in Autosomal Dominant Polycystic Kidney Disease. J Am Soc Nephrol. 2020;31(5):1035–49. [PubMed: 32238474]

449. Viau A, Bienaime F, Lukas K, Todkar AP, Knoll M, Yakulov TA, et al. Cilia-localized LKB1 regulates chemokine signaling, macrophage recruitment, and tissue homeostasis in the kidney. EMBO J. 2018;37(15).

450. Yang Y, Chen M, Zhou J, Lv J, Song S, Fu L, et al. Interactions between Macrophages and Cyst-Lining Epithelial Cells Promote Kidney Cyst Growth in Pkd1-Deficient Mice. J Am Soc Nephrol. 2018;29(9):2310–25. [PubMed: 30042193]

451. Dong K, Zhang C, Tian X, Coman D, Hyder F, Ma M, et al. Renal plasticity revealed through reversal of polycystic kidney disease in mice. Nat Genet. 2021;53(12):1649–63. [PubMed: 34635846]

452. Ha K, Loeb GB, Park M, Pinedo A, Park CH, Brandes N, et al. ADPKD-Causing Missense Variants in Polycystin-1 Disrupt Cell Surface Localization or Polycystin Channel Function. bioRxiv. 2023.

Clinical Highlights

Autosomal dominant polycystic kidney disease (ADPKD) is the most common potentially lethal genetic disorder, affecting up 1 in 1,000 individuals worldwide and leading to end stage renal disease in ~50% of affected individuals. Mutations in the *PKD1* and *PKD2* genes, encoding the polycystin 1 and polycystin 2 proteins, respectively account for almost all cases of ADPKD. While the identities of these genes and the proteins that they encode have been known for more than 20 years, we still do not understand these proteins' normal cellular roles, nor do we know why renal cysts form in their absence. The polycystin proteins clearly influence, directly and indirectly, a large number of signaling processes. A thorough understanding of the physiological functions in which the polycystin proteins participate is key to elucidating the pathogenesis of cyst formation in ADPKD and to identify promising new therapeutic targets.

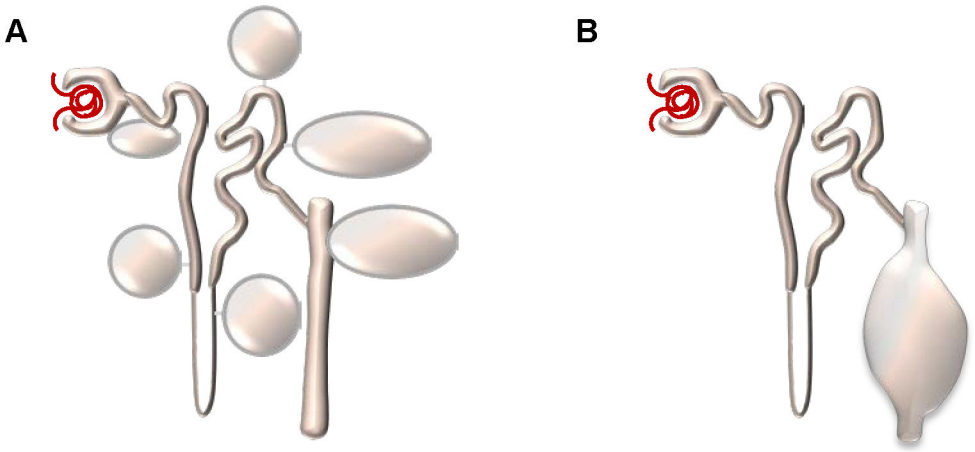


Figure 1:

A. Schematic representation of the focal manifestations of cysts in all nephron segments in ADPKD; **B.** Schematic representation of the fusiform dilatations of the collecting ducts typical of ARPKD.

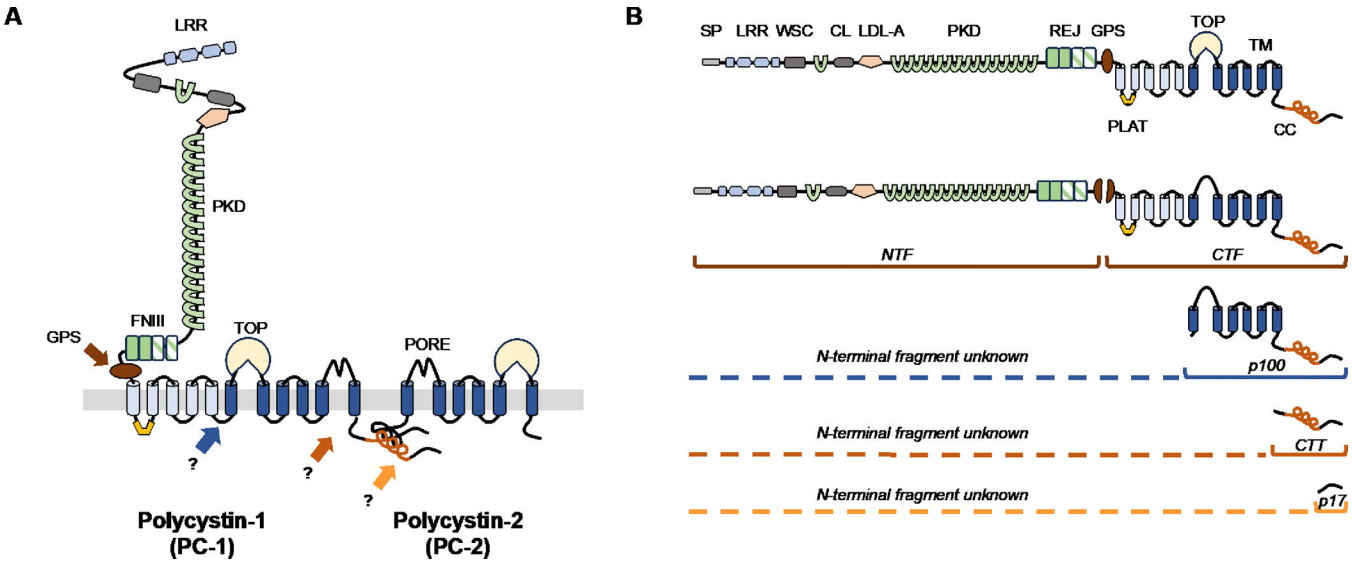


Figure 2:

A. Schematic representation of the Polycystin-1 (PC-1) and Polycystin-2 (PC-2) proteins and their structural domains. LRR, leucine rich repeats; PKD, PKD repeats; FNIII, fibronectin II like domains; GPS, G-protein coupled receptor proteolytic site; TOP, tetragonal opening for polycystins domain. **B.** Structure of the full-length Polycystin-1 protein and its reported cleavage sites.

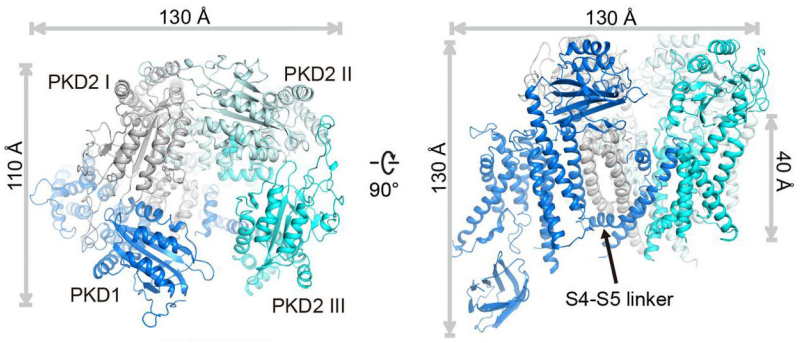


Figure 3:
CryoEM structure of the PC-1/PC-2 complex as published in Su et al., Science 2018.

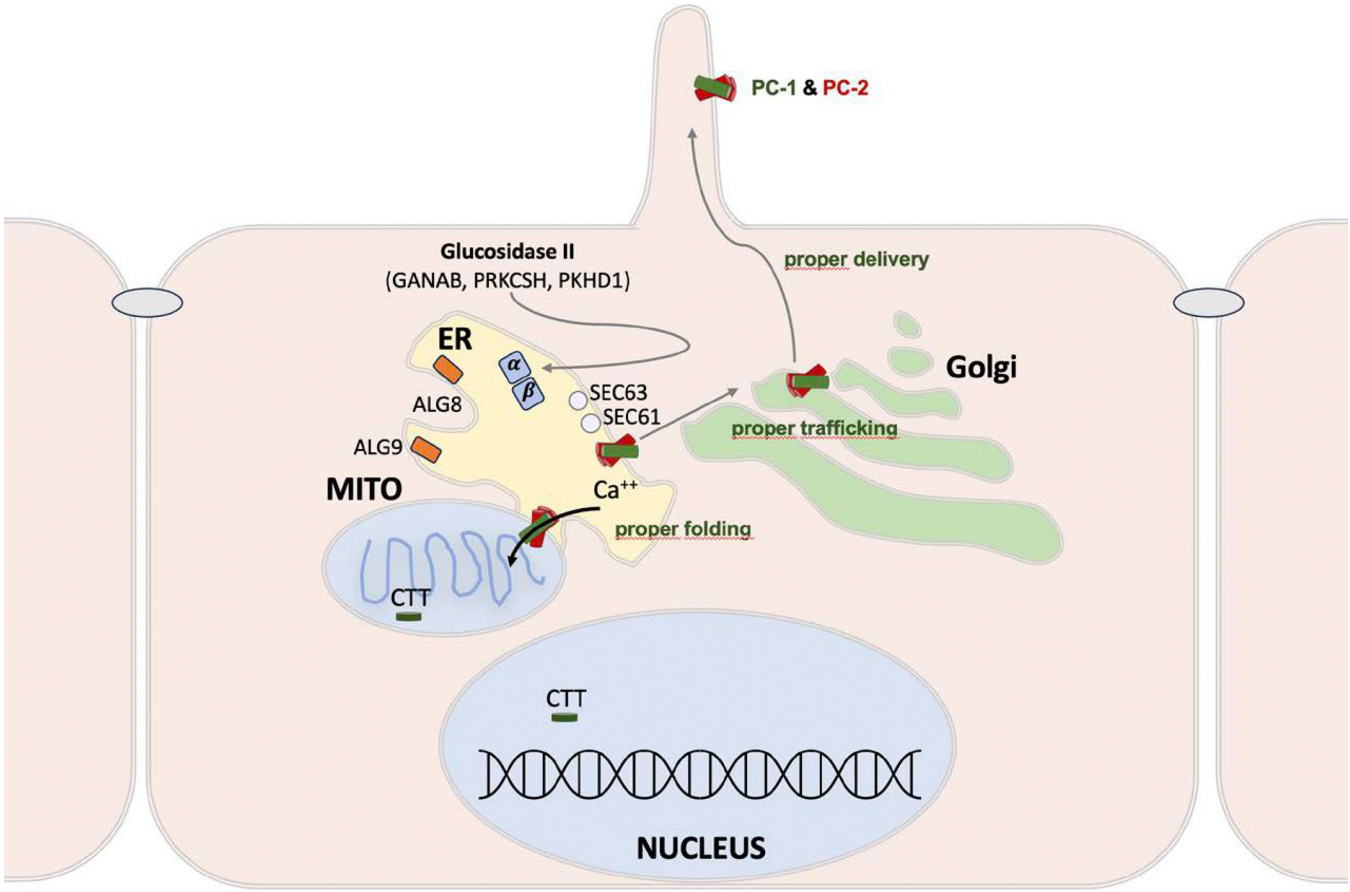


Figure 4:

Cartoon depicting the subcellular sites at which the polycystin proteins are thought to function, including the cilium and Mitochondria-ER contact sites. The diagram also depicts the resident ER proteins that are thought to be involved in the folding and assembly of the newly synthesized polycystin proteins, and whose genetic absence can lead to cystic disease.

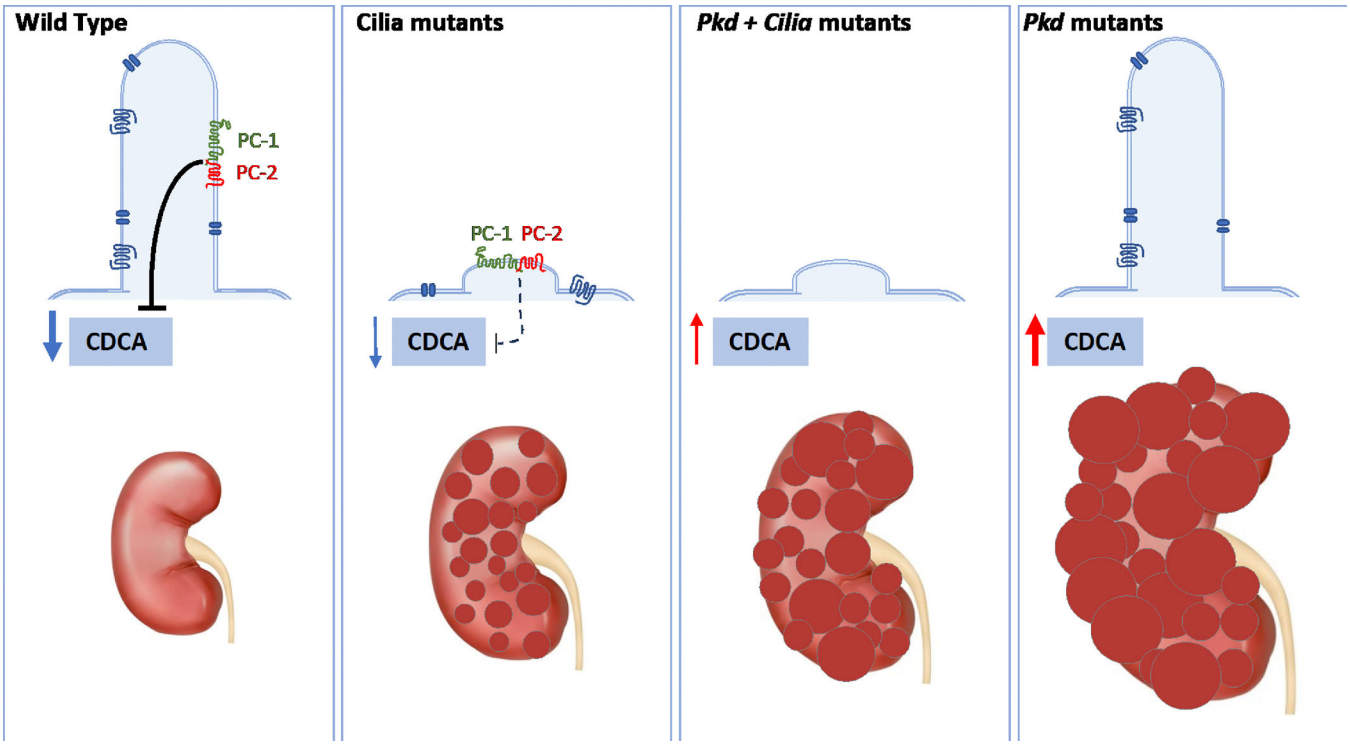


Figure 5:

Cartoon representing the role that the Cilia Dependent Cyst Activating (CDCA) pathway has been suggested to play in PKD. In wild type kidneys the ciliary PC-1/PC-2 complex inhibits a cilia-driven pathway that would otherwise be constitutively active and cause cyst formation. Removal of cilia results in a reduced inhibitory activity by the polycystins, causing a mild cystic disease. Removal of the polycystins in the presence of normal cilia results in a very aggressive PKD phenotype. In this context, removing cilia partially ameliorates the phenotype.

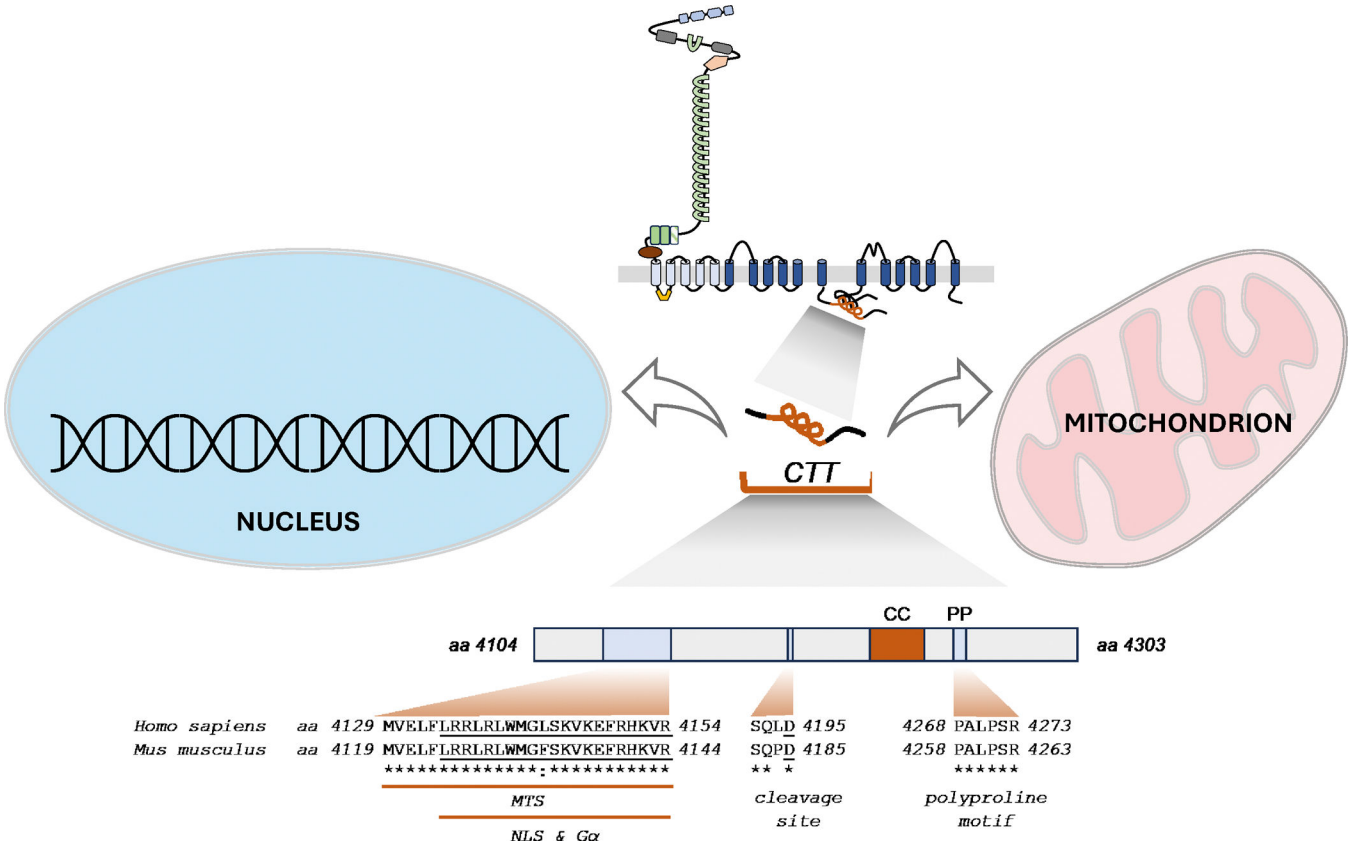


Figure 6:

Structure of PC-1 c-terminal tail. In brown is indicated the coiled-coil (CC) domain, in light blue the mitochondrial targeting sequence (MTS), nuclear localization signal (NLS), Gα binding site (Gα). The caspase cleavage site and the active polyproline motif are also indicated.

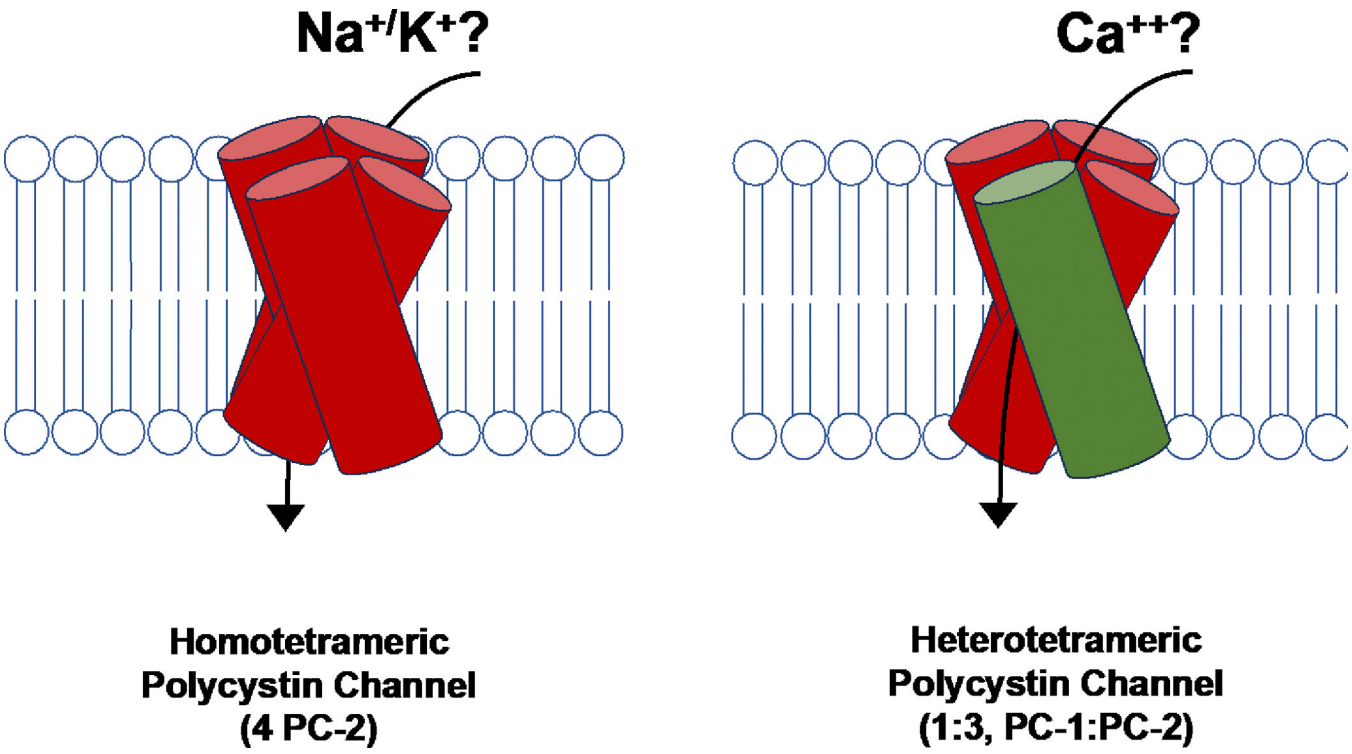


Figure 7:

Schematic representation of the homotetrameric Polycystin-2 channel (left) or heterotetrameric Polycystin-1/Polycystin-2 channel (right) and their reported preferences for monovalent or divalent cations, respectively.

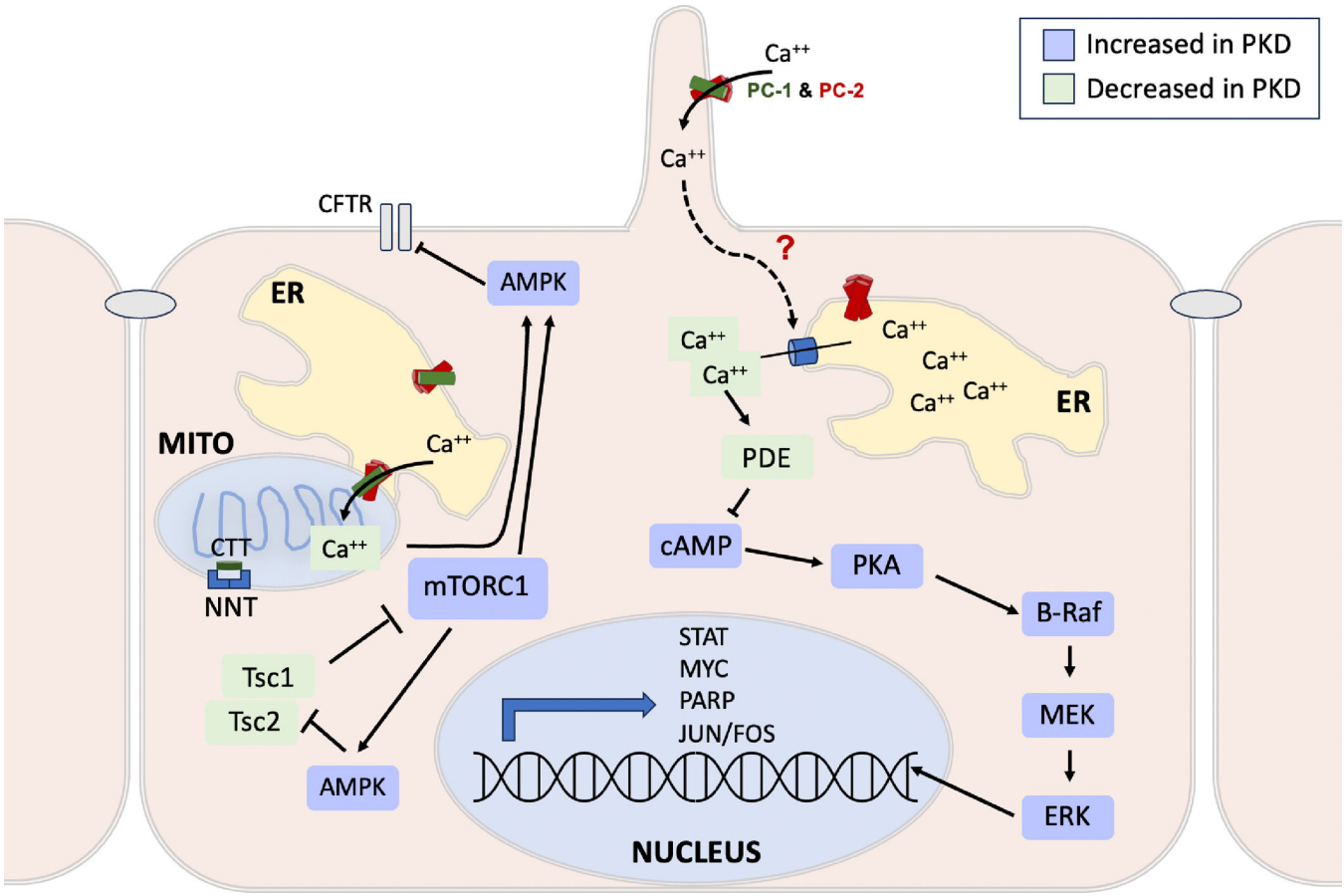


Figure 8:

Overview of selected signaling pathways that are altered in ADPKD cystic epithelia. The polycystins proteins are found in the cilium, the ER and the MERCs. Pathways depicted in green are thought to be downregulated in PKD cyst cells, while those depicted in in violet are thought to be upregulated in PKD.

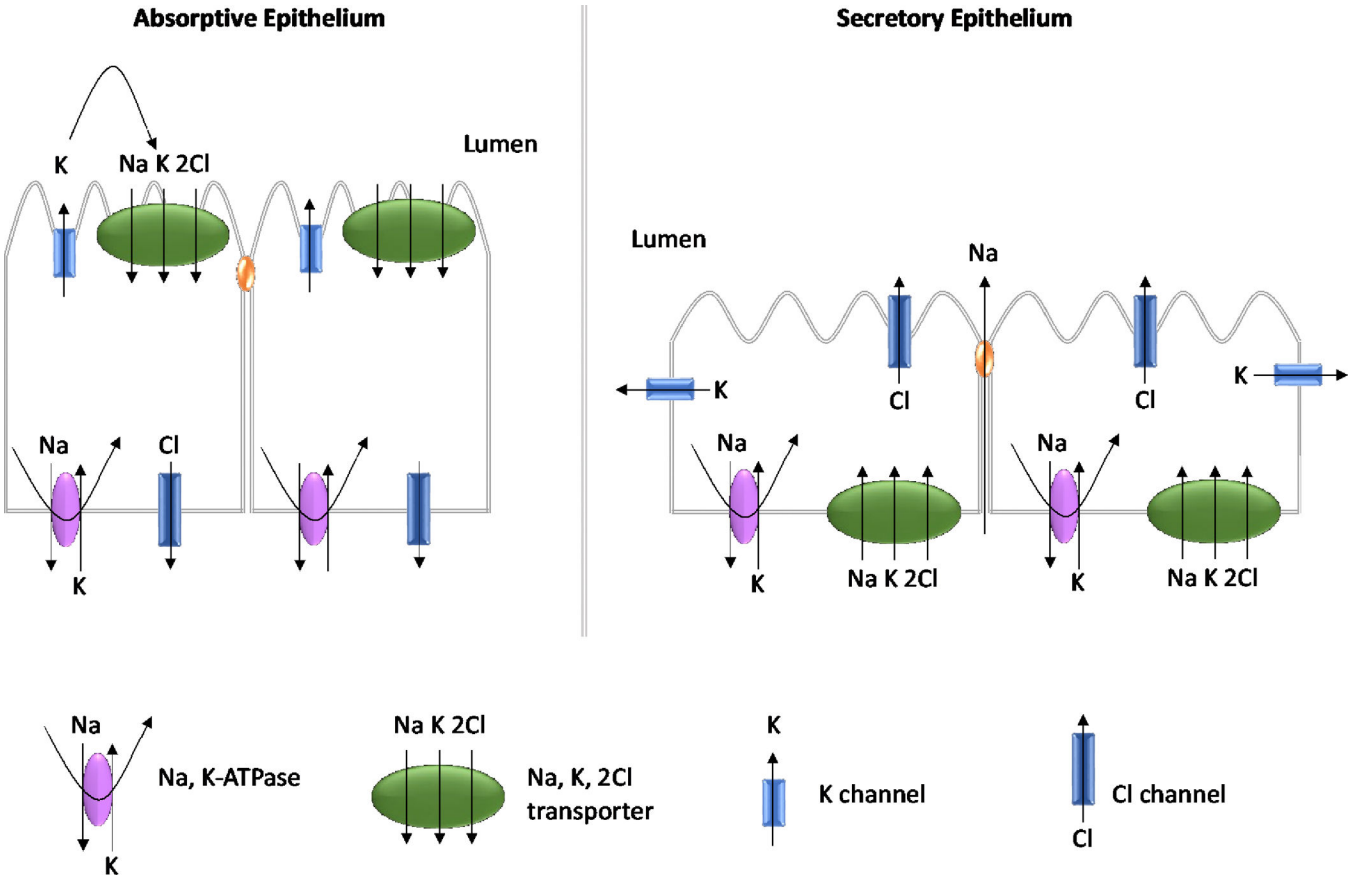


Figure 9:

Both healthy and cystic epithelia are fully polarized, with the Na,K-ATPase pump properly located on the basolateral side. Normal absorptive renal epithelial cells exploit the gradient created by the Na,K-ATPase to drive the apical to basolateral transport of sodium and chloride ions. In the case of cells of the thick ascending limb (right panel), this is accomplished via an apical Na,K,2CL cotransporter (NKCC2) and basolateral chloride and potassium channels. Cystic Epithelia (left panel) secrete fluid and electrolytes by virtue of apical expression of the CFTR chloride transporter in concert with the basolateral NKCC1 isoform of the Na,K,2Cl cotransporter.

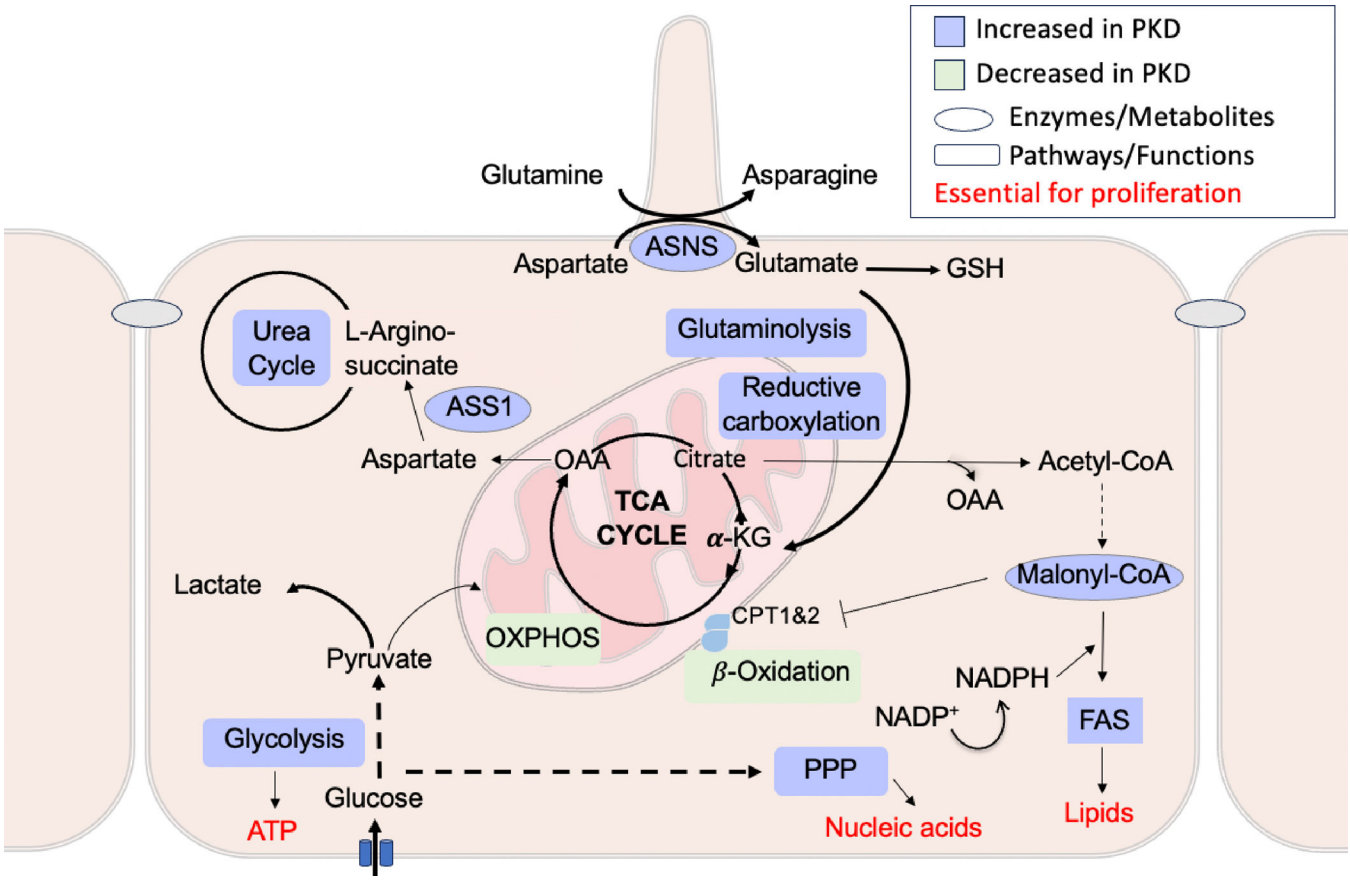


Figure 10:

Overview of the metabolic reprogramming that occurs in PKD. Enhanced glycolysis and reduced Oxidative phosphorylation are observed. A compensatory increased glutaminolysis, which drives reductive carboxylation, enhances citrate export from mitochondria to drive fatty acid biosynthesis. The increased uptake of glucose fuels the pentose phosphate pathway (PPP) which generates reductive power in the form of NADPH to drive fatty acid biosynthesis. The accumulation of Malonyl-CoA blocks the carnitine transporters CPT1 and CPT2, causing reduced beta-oxidation.