Chapter 11

The Role of G-protein Coupled Receptor Proteolytic Site (GPS) Cleavage in Polycystin-1 Biogenesis, Trafficking and Function

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Abstract

Polycystin-1 (PC1) is encoded by *PKD1*, the principal gene mutated in autosomal dominant polycystic kidney disease (ADPKD). The protein regulates terminal differentiation of tubular structures in the kidney and is required to maintain their structural integrity. A fundamental property of PC1 is post-translational modification by cis-autoproteolytic cleavage at the G-protein coupled receptor proteolytic site (GPS) motif located at the base

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of the extracellular ectodomain. Defective cleavage likely plays a significant role in the pathogenesis of ADPKD. In mouse models, GPS cleavage of PC1 is essential for the integrity of the distal nephron segments during the postnatal period. While the exact cellular and biochemical functions of PC1 have yet to be fully elucidated, its trafficking and function must be precisely regulated at the subcellular level to ensure proper structure and function of the kidney. Recent evidence shows that GPS cleavage plays a central role in PC1 biogenesis, trafficking and function *in vivo*. GPS cleavage results in the N-terminal fragment ($PC1_{NTF}$) and C-terminal fragment ($PC1_{CTF}$), which remain non-covalently associated to form a heterodimeric PC1 molecule. Cleavage is required for ciliary trafficking of PC1, which occurs in a two-step mechanism. First, PC1 interacts with polycystin-2 (PC2) in the ER and binds Rabep1. PC1/2 complex formation is required for transition of PC1 to the trans-Golgi compartment. Second, once arriving at the trans-Golgi, PC1/2-bound Rabep1 recruits GGA1 and the small GTPase Arl3 sequentially for ciliary targeting. In the absence of cleavage, the PC1/2 complex cannot reach the trans-Golgi. This article will discuss the roles of GPS cleavage for PC1 structure, trafficking and function that are relevant for normal activity of polycystin-1 and in cystogenesis of ADPKD.

Key words: Autosomal dominant polycystic kidney disease; Ciliary trafficking; Cystogenesis; GPS cleavage; Polycystin-1

Introduction

Polycystin-1 (PC1) is encoded by *PKD1* (1), the principal gene mutated in autosomal-dominant polycystic kidney disease (ADPKD) (2). Prior work indicates that PC1 regulates signaling pathways essential for proper tubular structures in kidney and liver (3-7) and suggests that a threshold level might be required to prevent cyst formation (8, 9). Cystogenesis will begin when the level of functional PC1 is below the critical threshold (10, 11). This is thought to occur through a "two-hit" mechanism once the cells with inherited heterozygous germline mutations acquire a second, somatic mutation to inactivate the remaining normal allele (12-17). Moreover, the degree to which PC1 activity falls below the threshold will determine disease severity, with lower levels leading to earlier and faster cyst growth. In addition, the level of PC1 is the central determinant of cyst formation in other types of cystic diseases such as autosomal recessive polycystic kidney disease (ARPKD) and autosomal dominant polycystic liver diseases (ADPLD) (18).

PC1 is a 4302-amino acid (aa) 11-transmembrane (TM) receptor-like glycoprotein with a large N-terminal extracellular region (ectodomain) of 3072 residues and a short cytoplasmic C-terminus of ~200 residues (1) (Figure 1). The ectodomain contains a combination of functional domains involved in protein-protein interactions and the ~1000 residue receptor for egg jelly (REJ) module that harbors four bona fide fibronectin III domains (19, 20). Situated at the base of the ectodomain is the ~50-aa G-protein coupled receptor proteolytic site (GPS) motif (21). The cytoplasmic C-terminus is responsible for activating a number of intracellular signaling pathways including the Ca2+, cAMP, JAK2/STAT, PI3kinase and mTOR pathways (6, 22, 23). This region contains a coiled-coil domain that binds polycystin-2 (PC2) (24, 25), the *PKD2* gene product (26), which belongs to the transient receptor potential channel family and acts as an ER calcium release channel (27). PC1 is found in a range of subcellular compartments, notably at the plasma membrane and the primary cilium, an organelle that appears most relevant to the pathogenesis of ADPKD (28, 29). PC1 and PC2 are thought to form a receptor-channel complex in cilia (29, 30), with PC1 acting as a sensor of extracellular signals and PC2 as a regulated cation channel. The ciliary polycystin complex is proposed to mediate Ca^{2+}-dependent signaling pathways in response to either mechanical or chemical signals through an unknown mechanism (29, 31). While the exact cellular and biochemical functions of PC1 have yet to be fully elucidated, its trafficking and function must be precisely regulated at the subcellular level to ensure proper structure and function of the kidney and other organs.

A fundamental property of PC1 is post-translational modification by cleavage at the juxtamembrane GPS motif (5, 32, 33) (Figure 1). GPS cleavage of PC1 is frequently disrupted in ADPKD (33-36). Disease-associated *PKD1* mutations that disrupt cleavage result in loss of the functional properties of PC1 to activate the JAK2-STAT pathway and induce *in vitro* tubulogenesis of MDCK cells in three-dimensional culture (33). Therefore, defective GPS cleavage of PC1 likely has a significant contribution in the pathogenesis of ADPKD. Prior evidence indicates that GPS cleavage plays a central role in PC1 biogenesis, trafficking and function *in vivo*. This article will discuss the roles of GPS cleavage in these processes, which are central to the normal functional activity of PC1 and to cystogenesis in ADPKD.

Polycystin-1 cleavage at the GPS motif via a cis-autoproteolytic mechanism

PC1 cleavage occurs at the His-Leu-*Thr^{3049} (* indicates scissile bond, with the amino acid number based on human PC1) tripeptide sequence within the GPS motif (37) (Figure 1). PS cleavage takes place in the early stages of the secretory pathway,

presumably within the ER shortly after PC1 synthesis. The cleavage reaction most likely occurs through a *cis*-autoproteolytic mechanism, which is due to a self-catalyzed chemical rearrangement and does not require the intervention of exogenous proteases. The chemical rearrangement is based on the ability of the nucleophilic Thr residue of the tripeptide His-Leu-*Thr to initiate a proximal N–O acyl rearrangement, which converts the peptide (amide) bond to a more reactive ester intermediate (37). The attack of the ester by a second nucleophile, such as a water molecule, leads to the irreversible cleavage of the scissile bond. An analogous mechanism was described for a group of cis-autoproteolytic proteins including hedgehog, nucleoporin and glycosylasparaginase, an Ntn hydrolase (38).

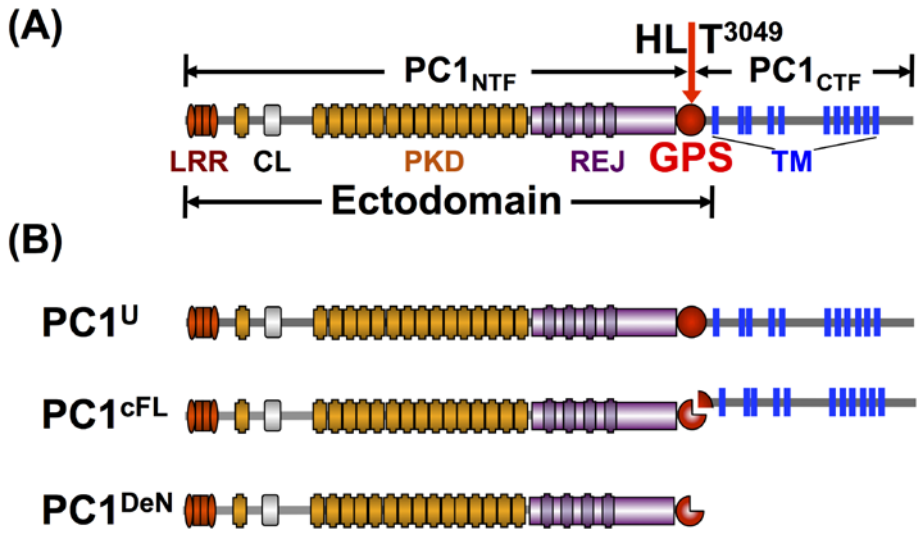

Figure 1. Cleavage of polycystin-1 at the G-protein coupled receptor proteolytic site (GPS) motif. (A) Schematic diagram of the structure of polycystin-1. LRR, leucine-rich repeat; PKD, PKD repeats; CL, C-type lectin; REJ, receptor for egg jelly module with its four fibronectin III domains; GPS, G-protein coupled receptor proteolytic site domain; TM, transmembrane domain. PC1 cleavage occurs at the HL*T3049 tripeptide (amino acid numbering based on human PC1) within the GPS motif at the base of the ectodomain, resulting in PC1NTF and PC1CTF fragments as indicated. (B) Polycystin-1 products generated by GPS cleavage. PC1U, uncleaved full-length PC1; PC1cFL, cleaved full-length PC1 in which PC1NTF and PC1CTF remain non-covalently associated at the GPS; PC1DeN, a separate and stable form of the PC1NTF molecule derived from PC1 cFL once it has dissociated from PC1CTF.

GPS cleavage and polycystin-1 molecular complexity

GPS cleavage of PC1 results in formation of an N-terminal fragment ($PC1_{NTF}$) and a C-terminal fragment ($PC1_{CTF}$) (33, 37) (Figure 1). A unique outcome of this cleavage is that the two fragments remain tightly and non-covalently associated to form a stable but dissociable heterodimer termed $PC1^{cFL}$ (33, 39). GPS cleavage is generally very extensive in most tissues, with a very small proportion of uncleaved PC1 ($PC1^{U}$) molecules detected. In the kidney, cleavage appears to be developmentally regulated. Castelli *et al* (40) recently found that $PC1^{U}$ is the predominate molecular form in early embryonic kidneys but decreases over time, with the relative amount of cleaved PC1 increasing in a complementary manner. After birth, the proportion of GPS-cleaved PC1 differs between proximal and distal nephron segments (5). While $PC1^{U}$ remains at a significant level (>50%) in cells of the proximal nephron, most of PC1 is present as the GPS-cleaved molecules (>90%) in distal nephrons.

Pulse-chase experiments showed that GPS cleavage of newly synthesized PC1 can be detected after 15 min of chase, but only about half of the population was cleaved within 2 h, whereas the other half remained uncleaved for a prolonged period of time before finally being degraded (33). Therefore, a significant portion of nascent PC1 molecules appears to be in a cleavage-resistant or blocked state. This notion has led to a model in which newly synthesized PC1 can proceed through two competing pathways: the 'cleavage' pathway, which leads to irreversible cis-autoproteolytic cleavage; and the 'non-cleavage' pathway, which traps $PC1^{U}$ in the blocked state (37). These data suggested the possibility that cellular factors, including ligand binding, may affect the extent to which PC1 molecules proceed to the cleavage versus the blocked state. It remains to be determined how the differential patterns of GPS-cleaved PC1 observed during kidney development or between different nephron segments are regulated. As discussed in later sections, $PC1^{U}$ and $PC1^{cFL}$ may have non-redundant functions in different biological processes.

$PC1_{NTF}$ can also be dissociated from $PC1_{CTF}$, and present as a separate and stable molecule, termed $PC1^{deN}$ (39). In fact, $PC1^{deN}$ exists in significant amounts and can be as much as 10-times the level of $PC1^{cFL}$ in the kidney. The molecular mechanism by which $PC1^{deN}$ is generated is unknown. One possible scenario may involve the differential degradation of $PC1_{CTF}$ due to the PEST sequence (a signal sequence for protein degradation) within the C-terminal tail. Together, GPS cleavage generates a considerable level of complexity of PC1 molecules *in vivo*.

Structural basis of GPS cleavage and subunit association of adhesion GPCRs and the implications for polycystin-1

GPS cleavage is the defining feature of the class of adhesion G-protein coupled receptors (aGPCRs), the second largest subgroup of GPCRs, which are also characterized by long N-termini with multiple functional domains (41, 42). aGPCRs and PC1 have no structural or functional relationship outside of the GPS motif. However, a recent crystallographic study of aGPCRs by Arac et al (34) provided critical insights into both the structural basis of GPS cleavage and the association of cleaved subunits, which have important implications for PC1. The GPS motif forms five β-strands that are tightly integrated into a larger ~320-residue domain termed the GPCR-Autoproteolysis INducing (GAIN) domain that is also present in PC1 (Figure 2).

In the structure of the uncleaved GAIN domain, the scissile bond within the His-Leu-*Thr tripeptide of the GPS motif is positioned at a sharply-kinked loop between the last two β-strands. This distorted and strained geometry favors the equilibrium toward an N–O rearrangement to facilitate ester formation, and thereby provides the necessary driving force for the cis-autoproteolytic reaction. Three structural elements are responsible for keeping the sharp β-turn in place: (1) two disulphide bonds between neighboring β-strands (PC1 has only one disulphide bond, C3015-C3043); (2) an extensive network of hydrophobic interactions between the last β-strand and other residues within the GPS motif; and (3) the trapping of the Leu side chain of the His-Leu-*Thr tripeptide within a conserved hydrophobic pocket. The analogous structural elements are schematically depicted for PC1 in Figure 2.

Following cleavage, the last β-strand remains tightly bound to the remainder of the GPS motif by an extensive network of hydrophobic interactions mediated between side chains. This finding provided the structural basis for the heterodimeric association of GPS cleavage fragments. The overall structural composition of the aGPCR heterodimers is proposed to allow them to participate in different types of cell guidance (34, 43, 44). By analogy, the heterodimeric structure of PC1cFL is likely to be important in enabling the unique biological functions of PC1 within the kidney.

GPS cleavage and polycystin-1 ciliary trafficking

The primary cilium is the key organelle for the control of tubule diameter (45, 46). Defects of cilia lead to ciliopathies, diseases that include cystogenesis in kidneys (47, 48). The ciliary membrane is separated from the plasma membrane by a periciliary diffusion barrier (49).

Ciliary membrane proteins must therefore be transported to the cilium from their site of synthesis in the rough endoplasmic reticulum (ER) for proper ciliary function (50). Experimental evidence favors a targeted delivery model, whereby ciliary membrane proteins are sorted in the Golgi and are then targeted to the cilium by the vesicular pathway via a number of protein complexes such as the BBSome (50) and intraflagellar transport complexes (51).

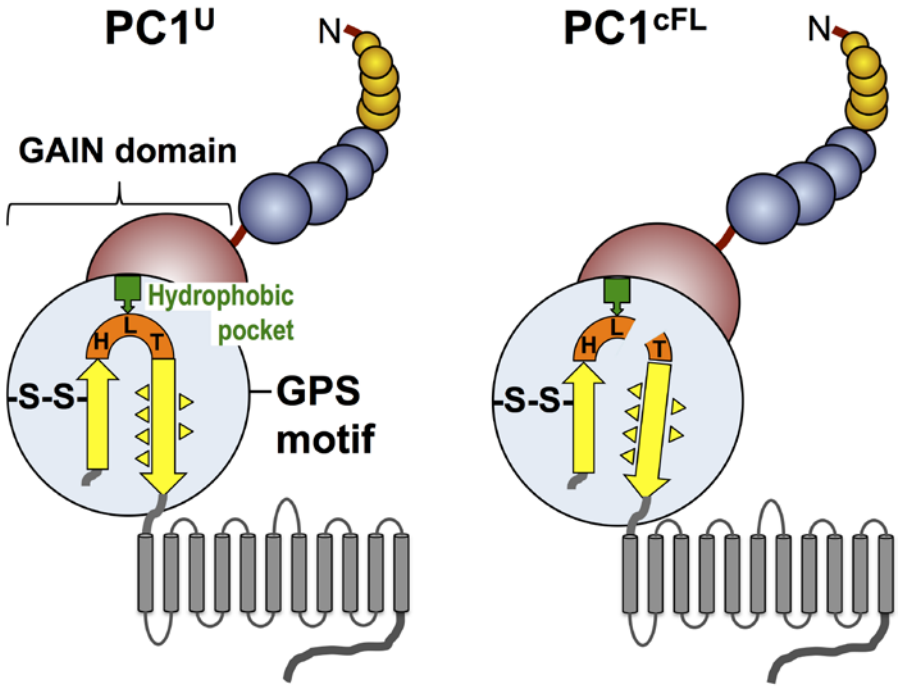

Figure 2. Structural model of the GPS/GAIN domain and heterodimeric association in polycystin-1. The GPS motif in polycystin-1 is schematically depicted based on analogy with the structure of the GAIN domain of the aGPCRs by Arac *et al* (34). In the uncleaved GPS, the scissile bond of the His-Leu-*Thr (HL*T) tripeptide is positioned within a sharply-kinked β-turn between the last two β-strands (the other three β-strands are not shown). This distorted and strained geometry provides the necessary driving force for cis-autoproteolytic cleavage. The structural elements that maintain the sharp β-turn are depicted. In the cleaved GPS, the last β-strand remains tightly bound to the rest of the GPS by the extensive network of hydrophobic interactions as indicated by the triangles. See text for details.

Previous efforts to understand ciliary trafficking of the large transmembrane PC1 and PC2 proteins have focused on the identification of their cilia-targeting motifs and the binding proteins that may mediate their trafficking. For PC1, the ciliary targeting sequence is at its extreme C-terminus (52), while for PC2, the targeting sequence is within the first 15 amino acids at the N-terminus (53). In some studies, PC2 was able to localize to cilia independently of PC1 (53, 54), while other studies show that this requires PC1 (29, 55, 56). In addition, different trafficking routes have been reported for PC1 and PC2 to reach the cilium. PC1 is trafficked to cilia from the trans-Golgi network (TGN) via post-Golgi vesicles in an Arf4-dependent manner (52), whereas PC2 is trafficked to the cilia directly from the cis-Golgi compartment without traversing the Golgi apparatus (54).

Kim *et al* (57) have recently shown that GPS cleavage is required for ciliary trafficking of PC1, which occurs via a two-step mechanism (Figure 3). First, the PC1/2 complex is formed in the ER and binds Rabep1. PC1/2 complex formation is required for transition of PC1 to the trans-Golgi compartment. Second, once arrived at the TGN, PC1/2-bound Rabep1 recruits GGA1 and the small GTPase Arl3 sequentially to enable subsequent ciliary targeting. The following three sections describe these two ciliary trafficking steps for PC1 and discuss the potential roles for GPS cleavage.

Trafficking of polycystin complex to the trans-Golgi network

Kim *et al* (57) have shown that endogenous PC1 and PC2 are mutually required for their ciliary localization in cells and kidney tissues (57). Therefore, the ciliary-targeting signal in each protein appears not to be sufficient for ciliary trafficking in renal epithelial cells. Ectopic expression of PC1 could induce the ER-resident endogenous PC2 to translocate to the cilium. However, amino acid substitutions within the coiled-coil domain of PC1, which disrupt interaction with PC2, abolished the ability of PC1 to traffic, and to induce translocation of PC2, to the primary cilium. PC1 and PC2 must therefore interact to form a molecular complex in order to traffic to cilia. *N*-glycosylation analyses of native PC1/2 protein complex provided further insights into the role of polycystin complex formation in ciliary trafficking. In the kidney, a significant fraction of the PC1/2 complex was found to be Endo H resistant and characterized by PC2 with a higher molecular weight of ~130 kDa, rather than the predominant cellular PC2 that is Endo H sensitive and has a molecular weight of ~120 kDa. Depletion of PC2 abolished the ability of PC1 to acquire Endo H resistance and to traffic to cilia. These data indicate that PC1-PC2 interaction is required for ciliary trafficking by enabling the polycystin complex to move to the TGN. Given that PC1 is far less abundant than PC2, PC1 is likely the rate-limiting factor for ciliary trafficking of the PC1/2 complex in renal epithelial cells.

Why is the PC1–PC2 interaction required for the complex to move to the TGN? It was previously reported that PC2 is continuously released from the ER to the Golgi in a COPII-dependent manner but immediately returned to the ER via an ER retention/retrieval signal present in its C-terminus (54, 58, 59). The PC1–PC2 interaction may be required to counteract and overcome the retrograde transport of PC2 to the ER, perhaps by masking the ER retention/retrieval signals or alternatively by allowing the complex to pass quality-control checkpoints in the ER as found for many GPCRs (60).

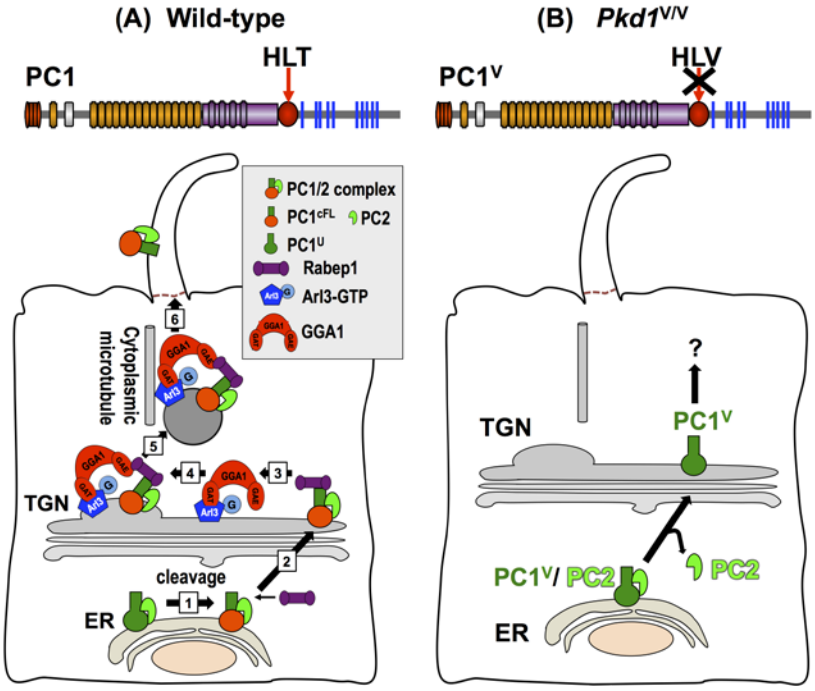

Figure 3. Model for ciliary trafficking of the polycystin complex and the role of GPS cleavage. (A) In wild-type cells, PC1 is cleaved at GPS and forms a complex with PC2 in the ER (1). Rabep1 binds PC1's cytoplasmic C-terminal tail at a pre-Golgi compartment (2), and this complex traffics to the trans-Golgi network (TGN). At the TGN, Rabep1 couples the polycystin complex to a GGA1/Arl3 module (3), which is formed by Arl3-GTP binding to GGA1. GGA1 then assembles the clathrin coat (4) to form the vesicle carrier (5). The resulting polycystin complex-bearing vesicle traffics along the cytoplasmic microtubules to the base of cilia (6) and enters the cilium by an unknown mechanism. (B) In the absence of GPS cleavage as in Pkd1V/V mice, PC1V is able to interact with PC2 to form a PC1V/PC2 complex in the ER. However, PC1V cannot maintain a stable association with PC2 at the trans-Golgi, nor traffic to cilia. The final subcellular location of PC1V remains to be determined. The figure is adapted from "Ciliary membrane proteins traffic through the Golgi via a Rabep1/GGA1/Arl3-dependent mechanism" by Kim et al., Nat Commun. 2014 Nov 18;5:5482. doi: 10.1038/ncomms6482 (57).

Rabep1/GGA1/Arl3-dependent ciliary trafficking of polycystin complex

N-glycosylation analyses of intact, isolated ciliary preparations showed that cilia contain only Endo H-resistant forms of PC1 and PC2 (57). This result provides direct biochemical evidence that the ciliary polycystins are derived from the TGN. How is the polycystin complex at the trans-Golgi directed to cilia?

We recently published data demonstrating that a novel protein complex composed of Rabep1, GGA1 and Arl3 is critically involved in mediating the sorting and targeting of the polycystin complex from the TGN to the cilium (57) (Figure 3). Using the yeast two-hybrid system, we identified Rabep1 as a binding partner of PC1 at its C-terminus. Rabep1 is an effector of multiple Rab GTPases involved in various steps of intracellular vesicular trafficking but not previously known for ciliary trafficking (61). Rabep1 knockdown in collecting duct cells abolished the ciliary localization of PC1 and PC2. Rabep1 binds the polycystin complex and thereby accompanies it to the Golgi. Once arrived at the TGN, PC1/2-bound Rabep1 binds Golgi-localized GGA1 (Golgi-localized, gamma adaptin ear-containing, ARF-binding), which is known to mediate the ARF-dependent recruitment of clathrin to the TGN (62-64). Rabep1-GGA1 interaction was previously described in the context of the fusion of TGN-derived vesicles with endosomes (65). GGA1 knockdown in collecting duct cells abolished the ciliary localization of PC1 and PC2. Arf4 was previously proposed to the involved for ciliary trafficking of a PC1 C-terminal fragment (52). However, Arf4 was not detected in the native polycystin complex and therefore does not appear to play a significant role in its ciliary trafficking (57). Instead we found association of a closely related member of the Arf family, Arl3, with the polycystin complex. Moreover, Arl3 knockdown in collecting duct cells abolished the ciliary localization of PC1 and PC2, indicating a critical role for Arl3 in the ciliary targeting of the polycystin complex. In mice, Arl3 inactivation was previously shown to result in renal cystogenesis (66) that is similar to the *Pkd1*$^{V/V}$ knockin mutant mice (5) (see following sections). Collectively, our data support a model in which Rabep1 recruits the polycystin complex to GGA1/Arl3 at TGN for ciliary trafficking.

The molecular mechanism by which Arl3 regulates ciliary trafficking of polycystin complex is unclear. Arl3 is exclusively found in ciliated organisms (67), and localizes to a number of microtubule-dense structures including the centrosome and cilia, as well as on the Golgi (68). It has microtubule-binding activity and is suggested to function in microtubule-dependent processes in mammalian cells (68). In fact, Arl3 is described to regulate vesicle transport from the Golgi to pericentrioles in photoreceptors (69). One possibility is therefore that Arl3 might direct polycystin-bearing vesicles to cytoplasmic microtubules for cytoplasmic dynein-driven transport to the cilium. Cytoplasmic dynein (distinct from ciliary dynein) is a multi-subunit molecular motor that drives microtubule-based

retrograde vesicle transport toward minus-ends (70). It is reported to drive transport of rhodopsin-bearing vesicles from Golgi to the connecting cilium in photoreceptors (71). Recent evidence also indicated a role for Arl3 in releasing lipid-modified proteins from their soluble transport proteins inside cilia (72, 73). It remains to be determined whether Arl3 has a similar role for the polycystin complex once arrived inside the cilium.

Role of GPS cleavage in ciliary trafficking of polycystin complex

Analyses of intact ciliary preparations isolated from renal epithelial monolayers expressing recombinant PC1 showed that cilia contain only cleaved PC1 (57). The uncleaved $PC1^{U}$ was absent from the cilia, although it was present at levels similar to cleaved PC1 within the cell body. Two possible mechanisms could be responsible for the lack of $PC1^{U}$ in cilia. First, PC1 has to be cleaved in order to traffic to cilia. Second, $PC1^{U}$ can traffic to cilia but becomes rapidly cleaved before reaching the cilia. To differentiate between these two possibilities, a non-cleavable PC1 mutant termed $PC1^{V}$, with a Thr-to-Val substitution at the His-Leu-*Thr cleavage site was engineered and analyzed (37, 39) (Figure 3). Thr and Val differ solely by one functional group at the very terminus of the side chain (-OH vs. -CH3). While effectively preventing cleavage by preventing the nucleophile attack, the critical initial step of cis-autoproteolysis (37), this smallest possible change seems less likely to significantly alter the conformation surrounding the cleavage site. The equivalent mutation did not cause conformational changes in the cis-autoproteolytic protein as shown by structural analysis (74). It is reasonable to assume that this mutation only blocks cleavage, without major unintended "side effects" that may confound the interpretation of the results. In fact, $PC1^{V}$ did not traffic to cilia, nor did it induce the ciliary translocation of endogenous PC2. These findings indicate a critical role for GPS cleavage in ciliary trafficking of polycystin complex, rather than reflect efficient cleavage of PC1 prior reaching the cilia.

N-glycosylation analyses with *Pkd1*$^{V/V}$ knockin mice, which harbor the same Thr-to-Val substitution and thus express mutant $PC1^{V}$, provided critical insights into the role of GPS cleavage in ciliary trafficking of the polycystin complex (39, 57). We found that $PC1^{V}$ acquires Endo H resistance to a significant degree (~50%) in *Pkd1*$^{V/V}$ tissues and cells. This finding indicates that GPS cleavage is not a prerequisite for trafficking of PC1 to the Golgi compartment. $PC1^{V}$ retains its ability to interact with PC2 and forms a $PC1^{V}$/PC2 complex in the ER (Figure 3). However, PC2 in this complex remains entirely sensitive to Endo H. These results indicate that GPS cleavage is not necessary for the formation of the PC1/2 complex, but is required to enable the complex to traffic to the trans-Golgi compartment. In the absence of cleavage, the $PC1^{V}$/PC2 complex cannot reach the trans-Golgi; instead, $PC1^{V}$ arrives at the Golgi without the association of PC2. Therefore, GPS cleavage may be required to prevent premature dissociation of the PC1/2 complex. One possibility is that

cleavage increases the affinity of PC1-PC2 binding and thereby ensures stable association of the complex at the Golgi. An alternative, but not mutually exclusive, idea is that GPS cleavage may increase the ER-to-Golgi transportation rate of the polycystin complex, thereby outpacing PC2 dissociation during the transition. It would be of interest to determine whether GPS cleavage may also play a critical role in recruiting Rabep1, GGA1 or Arl3, and/or whether PC2 is important for this process.

Critical and restricted functional role of PC1 cleavage at GPS *in vivo*

Important insights into the functional role of GPS cleavage were gained by the characterization of the *Pkd1*$^{V/V}$ knockin mouse (Figure 4), the first *Pkd1* mouse model with a missense mutation. PC1^{V} is present at two to three times the level of wild-type PC1^{U}, with similar stage- and tissue-specific expression patterns (5). The mutant mice exhibit several important phenotypic differences compared to the *Pkd1* knockout mice. While Pkd1 knockout mice develop massive renal and pancreatic cysts during embryonic development and are embryonically lethal (3, 4), *Pkd1*$^{V/V}$ mice are viable with apparently intact kidney and pancreas structure at birth. However, the *Pkd1*$^{V/V}$ mutants develop rapid cystic dilation of collecting ducts (except the papilla tip) and distal convoluted tubules starting at postnatal day 3 (Figure 4).

Unexpectedly, *Pkd1*$^{V/V}$ mice do not display cystogenesis in the proximal tubules, thick ascending limb of loop of Henle, or the pancreas during their lifespan of up to 3 weeks. Therefore, cleavage is dispensable for the embryonic development of various organ systems, including kidney and pancreas, and for proximal nephron segments after birth, but is essential for the integrity of the distal nephron segments during the postnatal period. Collectively, GPS cleavage plays a critical and restricted role in PC1 function *in vivo*.

The importance of GPS cleavage was recently confirmed by the *BAC-Pkd1* transgenic approach using a different non-cleavable mutant, PC1-L3040H, which contains a Leu-to-His substitution at the penultimate position of the His-Leu-*Thr cleavage site (35). Unlike PC1^{V} in the *Pkd1*$^{V/V}$ knockin mice, PC1-L3040H did not rescue the embryonic lethality of *Pkd1*$^{-/-}$ mice, indicating a complete loss-of-function by this mutant. Remarkably, in contrast to PC1^{V}, PC1-L3040H did not acquire Endo H resistance, which is indicative of a trafficking defect. Based on the structural analysis of the aGPCR GPS/GAIN domain (34), Leu3040, which is highly conserved in all GPS motifs, is expected to form part of the hydrophobic pocket at the sharp kink of scissile bond (see Figure 2). Substitution with the bulky and charged His at this position may therefore alter the conformation of the GPS/GAIN domain preventing cleavage and secondarily disrupting exit from the ER, thereby confounding the role of GPS cleavage. These considerations highlight the importance of experimental design for assessing the function of GPS cleavage.

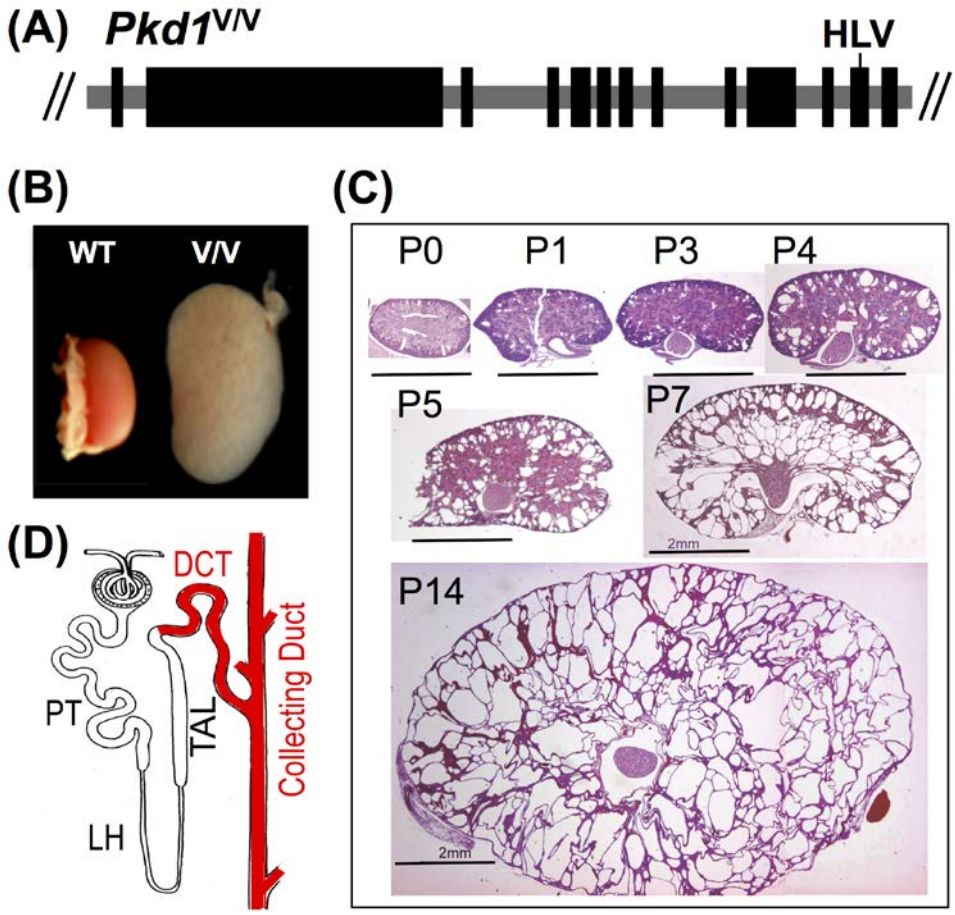

Figure 4. Essential biological function of polycystin-1 cleavage within the GPS motif. (A) Diagram of the *Pkd1*^{V} knock-in allele. Exons are depicted by black boxes. The critical nucleophilic threonine residue at the HLT cleavage site (in exon 25 of the *Pkd1* gene) is mutated to a non-polar valine by homologous recombination. (B) The *Pkd1*$^{V/V}$ kidneys (V/V) are enlarged, pale, and cystic compared with normal kidneys from wild-type (WT) littermates as shown for postnatal day 9. (C) Hematoxylin and eosin-stained sections of *Pkd1*$^{V/V}$ kidneys at various postnatal stages, demonstrate the rapid and progressive cystic dilation of *Pkd1*$^{V/V}$ kidneys during the postnatal period. Scale bars, 2 mm. (D) Diagram of the nephron segments affected by cystogenesis in *Pkd1*$^{V/V}$ kidneys. Cysts are derived from distal convoluted tubule (DCT) and collecting duct (in red). Glomerulus, proximal tubule (PT), loop of Henle (LH), and thick ascending limb (TAL) are not dilated.The figure is adapted from "Essential role of cleavage of Polycystin-1 at G protein-coupled receptor proteolytic site for kidney tubular structure" by Yu *et al*., Proceedings of the National Academy of Sciences of the United States of America 2007, 104:18688-18693 (5).

Do $PC1^{U}$ and $PC1^{cFL}$ have different biological functions?

What is the molecular mechanism by which GPS cleavage affects PC1 function? In principle, GPS cleavage may result in a more active PC1 molecule, for example by the creation of a high-affinity binding pocket for as-yet unidentified ligands, as has been suggested for other receptor molecules, or by more efficient signal transduction after ligand binding (75). Given the distinct patterns of GPS cleavage of PC1 during kidney development and in different nephron segments, and the critical role of cleavage in ciliary trafficking, it is tempting to speculate that $PC1^{U}$ and $PC1^{cFL}$ have non-redundant functions in different biological processes.

In early embryonic stages, PC1 exists largely in $PC1^{U}$ form. Therefore, $PC1^{U}$ might mainly traffic, perhaps apart from PC2, to a non-ciliary location such as cell–cell junctions where it could regulate convergent extension and elongation of developing renal tubules (40, 76). After birth, however, most of the PC1 in distal tubules and collecting ducts is GPS cleaved (5, 39), and $PC1^{cFL}$ traffics to the cilia in the form of the PC1/2 complex to control their proper tubular diameter. On the other hand, GPS cleavage is not required for intact proximal tubules in which $PC1^{U}$ is the more abundant molecule (5). $PC1^{U}$ may play a key role in the integrity of this portion of the nephron. The subcellular trafficking and biological functions of $PC1^{U}$ and $PC1^{cFL}$ in the proximal tubules remain to be examined. $PC1^{deN}$ itself does not have an intracellular region and its function is currently unknown (39). In summary, GPS cleavage may be significant for regulating polycystin trafficking and function in the kidney in a development- or nephron segment-specific manner.

GPS cleavage of polycystin-1 and polycystic kidney disease

GPS cleavage of PC1 appears to be frequently disrupted in human ADPKD. Of the 15 ADPKD-associated missense mutations in the GPS motif and the adjacent REJ module that have been analyzed thus far, all have been shown to disrupt cleavage (33-36). Remarkably, 94 (30%) of the 311 *PKD1* missense mutations classified as pathogenic in the Mayo PKD Mutation Database (http://pkdb.mayo.edu/) are located in the REJ-GPS region. By extrapolation, as much as 30% of the pathogenic missense mutations in *PKD1* have the potential to affect PC1 cleavage. These mutations might affect the critical residues that are involved in establishing the strained geometry critical for the cleavage reaction. Because *cis*-autoproteolysis depends on the correct alignment of critical resides within the GPS/GAIN domain, and thus correct global protein conformation (77), *PKD1* mutations that are distant from the GPS and REJ module might also affect cleavage. On the other hand, genetic modifiers or environmental factors that affect protein folding and maturation

have been reported to affect GPS cleavage and thereby disease progression (18, 78). Therefore, defective GPS cleavage of PC1 may play a significant role in the pathogenesis of ADPKD. Disease-associated *PKD1* mutations that disrupt cleavage may result in defects of intracellular trafficking and loss of the functional properties of PC1 preferentially within distal nephron segments.

Conclusion

GPS cleavage is the central control mechanism of normal PC1 biogenesis, trafficking and function, as well as a significant factor in ADPKD pathogenesis. Future studies will be directed to elucidate molecular mechanisms by which cleavage and a heterodimeric structure allow the PC1 molecule to mediate signal transduction at cilia and possibly other subcellular locations, and to develop strategies to manipulate GPS cleavage or the extent of heterodimeric association of PC1. These studies are expected to result in novel therapies to target ADPKD.

Conflict of interest

The author declares that he has no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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