Post-translational modifications of the polycystin proteins

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited cause of kidney failure and affects up to 12 million people worldwide. Germline mutations in two genes, *PKD1* or *PKD2*, account for almost all patients with ADPKD. The ADPKD proteins, polycystin-1 (PC1) and polycystin-2 (PC2), are regulated by post-translational modifications (PTM), with phosphorylation, glycosylation and proteolytic cleavage being the best described changes. A few PTMs have been shown to regulate polycystin trafficking, signalling, localisation or stability and thus their physiological function. A key challenge for the future will be to elucidate the functional significance of all the individual PTMs reported to date. Finally, it is possible that site-specific mutations that disrupt PTM could contribute to cystogenesis although in the majority of cases, confirmatory evidence is awaited.

Introduction

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common inherited renal disorder, affecting over 12 million people worldwide, with a prevalence of ca. 1/1,000 [1]. It is caused by germline mutations in one of two genes: *PKD1* (80%) and *PKD2* (15%) encoding the proteins PC1 and PC2 respectively. As ~7% of cases are genetically unresolved, the existence of a third locus (PKD3) has been postulated. Whole exosome sequencing of these families have identified mutations in two additional genes, *GANAB* and *DNAJB11*, which typically result in a late-onset atypical phenotype. *GANAB*, encodes the glucosidase II alpha subunit, and may account for ≈0.3 % of ADPKD [2]. *DNAJB11* is responsible for the regulation of normal protein folding and assembly in the ER [3]. Mutations in both genes result in a defect in the maturation and trafficking of PC1.

Disease is believed to occur by a loss of function mechanism i.e. by 'two hit' or haploinsufficiency models [4]. In the 'two hit' model, cystogenesis occurs in cells carrying a germline mutation on one allele; however a somatic mutation to the other allele is required to trigger cyst formation. The alternative hypothesis of cyst formation is haploinsufficiency, which supposes that mutations in one allele may be sufficient to initiate cyst formation and other non-cystic features in ADPKD. This is a gene-dosage-dependent mechanism in which gene expression level may be more variable due to the presence of only one functional allele. Lowering the level of PC1 or/and PC2, below a tissue specific threshold could predispose renal epithelial cells to stochastic events which trigger cyst initiation [5]. Human *PKD1* may be particularly sensitive to a dosage reduction as abnormal splicing across intron 21 and 22 typically results in a significant number of mRNA transcripts (28.8-61.5%) terminating early [6].

In typical adult-onset disease, thousands of renal cysts are found in most ADPKD patients by the fifth decade, while only a few are evident before 20 years of age. However, in rare very-early onset cases (under 2 years of age), the phenotype can be severe and is associated with a high prevalence of biallelic or compound heterozygous mutations in *PKD1* [7, 8]. A high rate of somatic mutation in *PKD1* has been reported in end-stage human ADPKD kidney cysts indicating that this could contribute to cyst progression in typical late-onset disease [9].

The polycystin proteins

Polycystin-1

PC1 is a 4,303 amino acid protein with a predicted unglycosylated molecular weight of 462kDa. Its topology is that of a Type 1 receptor consisting of a large N-terminal extracellular region, a transmembrane region (11 predicted domains) and a short C-terminus [10]. The extracellular N-terminus (3000 aa) has a highly modular structure and contains various domains (LRR, WSC, C-lectin, LDL-A, PKD, REJ, GAIN) which give it the ability to perform multiple functions including cell recognition, cell-cell and cell-matrix adhesion [11-14]. Cis-proteolytic cleavage mediated by the GAIN domain at the conserved G-protein coupled receptor proteolytic cleavage site (GPS) generates N- and C-terminal fragments (NTF, CTF) which remain tethered by non-covalent bonds [15]. Although PC1 has been localised to the primary cilium where it is proposed to act as a mechanoreceptor [16], a significant body of work has also reported its localisation to the lateral plasma membrane where a role in mediating and stabilising cell adhesion has been proposed [12, 17]. In this context, the presence of the GAIN domain typical of adhesion-GPCRs (ADGR) is suggestive that the latter could be its main site of action [18]. Overall PC1 is still considered an orphan receptor though experimental evidence that it could act as a co-receptor for Wnt and signal through PC2 in a non-canonical Wnt pathway (Wnt/Ca^{2+}) has been demonstrated in some systems [19, 20]. Isolated PC1 domains (C-type lectin, LRR) have been shown to bind several extracellular matrix proteins *in vitro* [21, 22].

The polycystin-1 lipoxygenase A-toxin (PLAT) domain is evolutionarily conserved in all orthologues and paralogues of PC1 [23]. Located in the first intracellular loop, it may act as a protein scaffold regulating the intracellular signalling, trafficking and endocytosis of PC1 [24]. The PC1 intracellular C-terminus (CT1) comprising the last 200 amino acids is the most extensively studied region and contains several key motifs including a coil-coiled region which mediates interaction between PC1 and PC2 [17, 25].

Polycystin-2

PC2 is a Type 2 968 amino acid protein (109 kDa) with six transmembrane domains and intracellular N- and C-termini [26]. PC2 (TRPP2) has been adopted into the Transient Receptor Potential (TRP) channel superfamily as the founding member of the TRP(P) subfamily [27]. Although there is good evidence that PC1 and PC2 form a functional heterodimeric complex, its main location and site of action relevant to cyst formation remain controversial [5]. Similar

to PC1, PC2 has been localised to primary cilia and the lateral plasma membranes [16, 17, 28, 29]. However, PC2 is predominantly located in the ER as well as at the centrosomes, mitotic spindles and sorting endosomes [17, 20, 30-32].

The localisation of PC2 and PC1 to primary cilia has led to ADPKD as a disease being adopted into the group of diseases known as 'ciliopathies'. Whether loss of cilia-localised PC2 in isolation is sufficient to lead to cyst formation remains uncertain. In addition, PC2 may function as an ER Ca^{2+} -regulated Ca^{2+}-release channel, in association with IP3R and RyR in different cell types [33, 34]. There is also biochemical and functional evidence that PC2/TRPP2 can with other TRP channel subunits such as TRPC1, TRPV4, TRPM3 to form channels with unique properties [35-37]. The relevance of these TRPP combinations to the pathogenesis of cystic disease remains undefined.

The interacting motifs and stoichiometry for the formation of a PC1 and PC2 complex have been defined. PC2 has been shown to oligomerise via a coiled-coil domain in its C-terminus (CT2) to form trimers or dimers and this step appears to be critical for PC1 binding via a coil-coiled domain in CT1 [38, 39]. PC2 is essential for PC1 maturation and its surface (and cilia) localisation and vice-versa [40]. However, the trafficking of PC2 into cilia does not always depend on PC1 expression or binding [41]. Co-regulation of PC2 by PC1 and vice-versa have been reported in some experimental systems [42-44]. The proposed role of PC1 as a flow-activated mechanoreceptor regulating PC2 channel opening in primary cilia has been disputed by recent studies [16, 45, 46]. Finally, although there is agreement that PC2 is a Ca^{2+} permeable channel, there is disagreement about its ion selectivity (monovalent v divalent ions) and mode of activation or inactivation by Ca^{2+} depending on the model systems used [47]. One possibility is that these may differ between different subpopulations of PC2/TRPP2 or in different locations. For instance, a recent study has proposed a role for ER PC2 in protection from cellular stress [48, 49].

Several PC2 structures including one complexed with PC1 have been recently published. Shen et al. were the first to report a 'polycystin domain' in the large first extracellular loop, covalently linked to the first and second helices, and proposed a role for this domain in channel assembly and modulation [50]. Grieben et al. recently performed the first crystallisation of the full closed version of the protein, giving more insights into its structure and function [51]. They confirmed the existence of the polycystin domain, which they renamed the TOP domain (standing for Tetragonal Opening for Polycystins). Wilkes et al. recently reported the presence of two PC2 conformational states; an 'open' conformation where the lipid mediated interaction between the TOP and C-terminal domain lead to a larger opening of the funnel and a more 'closed' state which restricts the passage of ions such as Ca^{2+}, Na^{+} and K^{+} [52]. Ca^{2+} entry was thought to be mediated through a pore formed between the fifth and sixth transmembrane domains of the protein [51]. Of interest, a cryo-EM structure of truncated human PC1 (3049-4169aa) and PC2 (185-723aa) proteins demonstrated a 1:3 stoichiometry of the PC1/PC2 complex [53]. In this study, PC1 was found to interact with PC2 via an extracellular voltage gated ion channel (VGIC) fold providing a second level of interaction besides the C-terminal interactions previously reported.

The function of several motifs in the N- and C-termini were however not clarified in these structural studies. For instance, the EF-hand motif of PC2 forms a pocket-like structure that binds to Ca^{2+} ions and could modify the conformation of the C-terminus: how this regulates channel function is not known [54]. Similarly, the regulatory functions of the phosphorylation residues reported in both N- and C-termini (see below) for channel opening or function remain unclear.

Post-translational modifications

In the past few decades, it has become apparent that the human proteome is vastly more complex than the human genome (**Figure 1**). While the human genome comprises between 20,000 and 25,000 genes, the total number of proteins in the human proteome has been estimated at over 1 million demonstrating that single genes can encode multiple protein isoforms. Genomic recombination, transcription initiation at alternative promoters, differential transcription termination, and alternative splicing of the transcript are mechanisms that can generate different mRNA transcripts from a single gene.

The increase in complexity from the level of the genome to the proteome is amplified by protein post-translational modifications (PTMs). PTMs are chemical modifications that play a key role in protein function regulating its activity, localisation, stability and interaction with other cellular molecules such as proteins, nucleic acids, lipids and cofactors.

Post-translational modifications can occur on the amino acid side chains or at the protein's C- or N-termini **(Table 1)**. They can extend the chemical repertoire of the 20 standard amino acids by modifying an existing functional group or introducing a new one such as phosphate. Phosphorylation is a very common mechanism for regulating the activity of enzymes and is the most common post-translational modification. Many eukaryotic and prokaryotic proteins also have carbohydrate molecules attached to them, a process called glycosylation which can promote protein folding, improve stability as well as serving regulatory functions. Attachment of lipid molecules, known as lipidation, often targets a protein or part of a protein attached to the cell membrane.

Other forms of PTM consist of proteolytic cleavage, as in processing a propeptide to a mature form or removing the initiator methionine residue. The formation of disulfide (covalent) bonds between cysteine residues may also be referred to as a PTM. For instance, the peptide hormone insulin is cut twice after disulfide bonds are formed, and a propeptide is removed from the middle of the chain resulting in a protein consisting of two polypeptide chains connected by disulfide bonds.

Sites that often undergo post-translational modification are those that have a functional group that can serve as a nucleophile in the reaction: the hydroxyl groups of serine, threonine, and tyrosine; the amine forms of lysine, arginine, and histidine; the thiolate anion of cysteine; the carboxylates of aspartate and glutamate; and the N- and C-termini. In addition, although the amide of asparagine is a weak nucleophile, it can serve as an attachment point for glycans. Rarer modifications can occur at oxidized methionines and at some methylenes in side chains.

Some types of PTM occur as the consequence of oxidative stress. Carbonylation is one example that targets the modified protein for degradation and can result in the formation of protein aggregates. Specific amino acid modifications can be used as biomarkers indicating oxidative damage.

Post-translational modification of polycystin protein and their functional significance

Since the identification of the polycystin proteins, a great deal of effort has been made to understand their function within the cellular environment. It has become clear that the functions of these proteins could be regulated by distinct post-translational modifications. By far the most studied modification is phosphorylation but glycosylation, proteolytic cleavage, ubiquitination and lipidation have also been described. This section will describe these modifications and evidence for their functional significance.

Polycystin phosphorylation

Polycystin-2

Phosphorylation of PC2 at multiple residues has been detected by mass spectrometry in several large-scale phosphoproteomic studies. The majority of these residues are highly conserved in vertebrates suggesting they may be essential for polycystin function although to date, only a few have been experimentally verified *(https://www.phosphosite.org)* **(Figure 2)**. A summary of how phosphorylation of PC2 may regulate key cellular functions such as its subcellular localisation and/or trafficking, calcium permeability and cell cycle regulation is summarised in **Figure 3**.

Serine 812

The first phosphorylation site within the C-termini of PC2 was identified at Ser^{812} and this remains the most studied residue [55]. Phosphorylation at this site is important for channel function; loss of phosphorylation resulted in reduced sensitivity to PC2 channel activation by intracellular Ca^{2+} in ER-enriched liposomes [55]. A second study demonstrated a role for Ser^{812} phosphorylation in regulating PC2 trafficking [56]. Ser^{812} is located in a cluster of acidic amino acid residues which were found to mediate interaction between polycystin-2 and the phosphofurin acidic cluster sorting (PACS) proteins, PACS-1 and PACS-2. Interaction with these PACS proteins proved essential for the retrograde trafficking of PC2 between the plasma membrane, Golgi and ER. Binding of PACS to PC2 was dependent on CK2 phosphorylation at Ser^{812}; mutation of Ser^{812} to alanine or disruption of the acidic cluster weakened PACS binding resulting in translocation of PC2 to the lateral plasma membrane. These observations were later confirmed in a zebrafish model [57]. In a third study, a phosphomimic Ser^{812D} C-terminal protein (679-968aa) had lower affinity binding to PIGEA14 compared to wild-type PC2, potentially regulating anterograde PC2 transport between the ER and Golgi [58, 59]. Finally, phosphorylation at Ser^{812} was reported to be important for PC2

binding to ID2 (inhibitor of DNA binding 2), a member of the ID family of helix-loop-helix (HLH) transcription factors [60]. Phosphorylated PC2 bound ID2 retaining it in the cytoplasm, preventing its effect on p21 transcription leading to cell-cycle progression.

Other serine residues

Serine 76/80

Although an initial study reported that PC2 was only phosphorylated at a single residue (Ser^{812}) [55], a second GSK3-recognition site was next identified at Ser^{76} with a potential CK1 priming site at Ser^{80} [61]. This phosphorylation event was required for PC2 trafficking to (or retention at) the lateral plasma membrane but not to primary cilia in kidney epithelial cells. The inability of a phosphodeficient Ser^{76} mutant to rescue the cystic phenotype in the zebrafish *Pkd2* pronephric kidney further confirmed its functional significance [61].

Serine 801

A third PC2 phosphorylated residue (Ser^{801}) mediated by protein kinase D (PrKD) was shown to be critical for PC2-mediated ER Ca^{2+} release and in mediating PC2 regulation of proliferation [62]. Interestingly, a reported missense mutation, Ser^{804N}, which lies within the PrKD consensus sequence abolished Ser^{801} phosphorylation [62, 63]. Phosphorylation at Ser^{801} by PrKD thus appears to be essential for PC2 channel function in the ER in response to growth factor stimulation.

Serine 829

A fourth site at Ser^{829} was identified by several groups. In the first study, AurA kinase was identified as the relevant kinase and was shown to reduce the ability of PC2 to limit the amplitude of ER Ca^{2+} release in kidney epithelial cells [64]. A second study reported that Protein kinase G (PKG) could phosphorylate PC2 at Ser^{829}, inhibiting flow induced Ca^{2+} influx in M1-CCD cells; the authors proposed Ser^{721} as a second relevant PKG site [65]. Finally, protein kinase A (PKA) was shown to phosphorylate PC2 at Ser^{829} [44, 66]. In this study, Ser^{829} phosphorylation led to enhanced ATP-dependent ER Ca^{2+} release and loss of growth suppression in cycling cells. Of significance, constitutive Ser^{829} phosphorylation detected with a phosphospecific antibody was observed in PC1 null or mutant cells. The molecular basis of this is the recruitment of protein phosphatase 1 (PP1) by the PC1 C-terminus, a function that is lost when PC1 is mutated [67]. This observation suggested a different paradigm of disease pathogenesis with an inhibitory rather than a cooperative relationship between PC1 and PC2 [44].

Other potential sites

Studies of the worm PC2 homologue has shown that phosphorylation of Ser^{534} by CK2 was important in PC2 ciliary localisation and its function during male mating behaviour [68]. Evolutionary conservation of this CK2 site in human PC2 (Thr^{683}) suggests it's potential importance although this has yet to be shown experimentally.

Polycystin-1

The functional significance of PC1 phosphorylation is less well defined although several potential sites of interest have been reported. Tyrosine phosphorylation of PC1 was first demonstrated implicating a role for tyrosine phosphorylation in PC1-mediated focal adhesion complexes [69, 70]. C-src, FAK, PKA and a novel kidney cAMP-dependent kinase protein kinase X (PRKX) can phosphorylate CT1 *in vitro*. Site-directed mutagenesis identified Tyr^{4237} as the specific target site for c-src, Ser^{4252} and Ser^{4169} as specific target sites for PKA [71-73] and Ser^{4166} as the specific target site for PRKX [72]. Outside the C-terminus, PC1 can be phosphorylated at Ser^{3164} within the PLAT domain by PKA [24]. This event reduces its binding affinity to PI4P and recruits β-arrestins and the clathrin adaptor AP2 to trigger PC1 internalization. Finally, PC1 in ADPKD cells has been reported to be hyperphosphorylated (by phospholabelling with ^{32}P) and proposed to contribute to the disruption of a PC1/E-cadherin complex [74]. Although the relevant sites were not identified in this paper, an increase in PC1 phosphorylation could result from loss of PP1 binding to CT1, similar to what has been observed for CT2 (see above, **Figure 3**).

Other post-translational modifications of polycystins

Glycosylation

The polycystin proteins are highly glycosylated. Sequence predictions indicate 60 N-linked sites for PC1 and experimentally, several different glycosylated forms of endogenous PC1 have been identified. Treatment with PNGase F (to cleave N-linked sugars) and endoglycosidase H (Endo H) (to differentiate high-mannose sugars) led to the identification of Endo H–resistant and sensitive forms of PC1. An Endo H–sensitive and an Endo H-resistant form of PC1 were found to interact with PC2, with the latter enriched in plasma membrane fractions [17]. It has been estimated that 50% of PC1 is EndoH resistant and localised to the cell surface and that PC2 is critical for this trafficking [40].

Sequence predictions indicate 5 N-linked sites for PC2 (all in the first extracellular loop). Unexpectedly, no EndoH resistant fraction has been consistently found suggesting that the majority of (Endo H-sensitive) PC2 is retained within the ER where it exerts its major functions [17, 30]. An alternative mechanism proposes that a small fraction of PC2 traffics from the cis-Golgi to the cilium without going through the classical secretory pathway [75], possibly mediated by an N-terminal RVxP motif (**Figure 3**) [41]. Nonetheless, PC2 in human urine extracellular vesicles has been reported to be predominantly EndoH-resistant [76]. Similarly, another group reported a minor EndoH-resistant PC2 cilia fraction complexed with PC1, that transited the Golgi via a Rabep1/GGA1/Arl3-dependent ciliary targeting mechanism [77]. N-glycosylation is linked to protein stability and mutation of *PRKSCH* (glucosidase II β subunit) reduces the abundance of PC2 probably through effects on protein folding and/or translation efficiency [78] (see below).

Proteolytic cleavage

GPS cleavage

A key post-translational modification of PC1 is the ability to undergo cleavage via a GPCR proteolysis site (GPS) motif situated distal to the REJ-GAIN domain [79]. GPS cleavage of PC1 has been shown to regulate intracellular trafficking, localisation and function of the protein [80-83]. Cleavage is thought to occur shortly after ER processing and results in the formation of two distinct fragments (NTF, CTF) that are non-covalently associated **(Figure 4)**. The functional importance of this PTM *in vivo* was demonstrated by the characterisation of a knock-in mutant mouse (*Pkd1*$^{V/V}$) with a missense mutation (T3041V) disrupting the GPS cleavage site [84]. Surprisingly, although GPS cleavage was completely prevented, mutant *Pkd1*$^{V/V}$ mice had a hypomorphic phenotype, characterised by delayed onset of cystogenesis, predominant distal tubule involvement and lack of extrarenal cysts indicating a possible role for the uncleaved form in prenatal life, in other tubular segments and extrarenal tissues. GPS cleavage does not however appear to be essential for PC1 maturation [81].

An essential role for GPS cleavage in the cilia trafficking of PC1 has been proposed [77] although cilia localisation can be normal in some mutants [85, 86]. Around 30% of all reported pathogenic PKD1 mutations are located in the REJ-GAIN region with the potential to disrupt GPS cleavage (http://pkdb.mayo.edu) [15].

Other cleavage events

Several groups have reported that CT1 may undergo proteolytic cleavage to release smaller fragments (14, 32, 100kDa) (**Figure 4**) that translocate to the nucleus to regulate gene transcription, in co-operation with other transcription factors such as STAT6, STAT3 and TCF/β-catenin [87-89]. A G-protein activation motif has been shown to be involved in the activation of transcription factor AP-1 mediated signalling [90, 91]: mutation of this motif (L4132Delta) leads to cyst formation [92]. Intriguingly, a CT1 fragment has been identified in mitochondria and could regulate mitochondrial morphology and function [93]. One caveat is that all of these studies were performed using heterologous or recombinant PC1. However, a recent study of native PC1 in human urine extracellular vesicles identified three other proteolytic cleavage sites, two of which could generate PC1 fragments similar in size to those previously reported (11, 94kDa) (**Figure 4**) [76]. Similar to PC1, a low percentage of PC2 (13%) was also found to be cleaved proximal to the coiled-coil domain (aa835-873, CC2) [76]. Unlike GPS cleavage, the functional relevance of these cleavage events is unclear.

Ubiquitination

Ubiquitin is conjugated to a lysine residue in the substrate protein by a series of enzymes either singly (mono) or as part of a chain (poly) ubiquitination. Ubiquitination can target a protein for degradation via the proteasome and lysosome, alter their cellular location, affect their activity, and promote or prevent protein interactions. PC2 has been reported to be

ubiquitinated following interaction with Herp and ATPase p97, components of the ER-associated degradation (ERAD) pathway [94]. Of interest, this PTM is inhibited by *PRKCSH*, the β subunit of glucosidase II, which when mutated results in Autosomal Dominant Polycystic Liver Disease (PCLD1) [95].

TAZ, a core component of the Hippo pathway and an adaptor protein in the E3 ubiquitin ligase complex can also target PC2 for degradation [96, 97]. The kinase Nek1 phosphorylates TAZ at a site essential for the ubiquitination and proteosomal degradation of PC2. Loss of TAZ or mutations in Nek1 are associated with renal cysts *in vivo* in the context of increased PC2 expression. *PKD2* transgenic mice, like *PKD1* transgenic mice, develop PKD underscoring the importance of gene dosage in cyst formation [98].

Conversely, PC2 expression has also been shown to be specifically reduced in mice with biliary damage, being modulated post-translationally through the ERAD/proteasome pathway and by ER-stressors and NO-donors through the autophagy pathway via post-translational modification by ubiquitin [99].

Siah1 regulates the degradation of endogenous polycystin-1 via the ubiquitin-proteasome pathway [100]. The binding of Siah1 to CT1 induces ubiquitination and degradation of PC1 in response to cell cycle progression through p21.

Sumoylation

PC2 has recently been shown to be post-translationally modified by SUMO1 (small ubiquitin-like modifier 1) protein in arterial smooth muscle cells (myocytes) [101]. At physiological intravascular pressures, PC2 exists in approximately equal proportions as either non-sumoylated or triple SUMO1-modifed proteins. SUMO-PC2 recycles, whereas unmodified PC2 is surface-resident. Depending on the intravascular pressure and intracellular calcium levels, SUMO-PC2 either recycles to the plasma membrane or undergoes degradation in lysosomes. This post-translational modification allows the physiological regulation of PC2 surface abundance and pressure-mediated activation in myocytes and thus control of arterial contractility.

Palmitoylation

PC1 has been reported to be palmitoylated at its C-terminus, altering its expression levels and surface membrane localisation [102].

Conclusions

Accumulating evidence has revealed that the polycystin proteins undergo a variety of PTMs affecting every aspect of their biology including trafficking, localisation, interaction, channel activity, signalling and stability. The best described changes involve phosphorylation,

glycosylation, proteolytic cleavage and ubiquitination. A key challenge for the future will be to elucidate the functional significance of these modifications and their relevance to disease.

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Figure Legends

Figure 1. Post-translational modifications are key mechanisms that increase proteomic diversity

While the genome comprises 20,000 to 25,000 genes, the proteome is estimated to encompass over 1 million proteins. Changes at the transcriptional and mRNA levels increase the size of the transcriptome relative to the genome, and the myriad of different post-translational modifications exponentially increases the complexity of the proteome relative to both the transcriptome and genome.

Figure 2. Experimentally verified polycystin phosphorylation sites

A total of five phosphorylation sites have been experimentally verified in polycystin-1 and six in polycystin-2. A significantly larger number have been identified in high throughput MS proteomic screening studies but as of yet have not been verified experimentally (https://www.phosphosite.org). Kinases that have been experimentally validated are shown above each residue.

Figure 3. Putative role of phosphorylation in the subcellular functions of the polycystins in kidney tubular cells

Phosphorylation at Ser^{76} is required for PC2 trafficking to or retention at the lateral plasma membrane. Retrograde trafficking of PC2 between plasma membrane, Golgi and ER is dependent on Ser^{812} phosphorylation. PC2 transport to the ciliary membrane independent of Golgi trafficking may occur via an N-terminal RVxP motif; alternatively PC2 complexed to PC1 could traffic to cilia via a Rabep1/GGA1/Arl3 dependent mechanism (dotted arrows). Phosphorylation at Ser^{829}, Ser^{812} and Ser^{801} regulates PC2 channel activity. CT1 phosphorylation can modify GPCR signalling. Phosphorylation of PC1 at Ser^{3164} (PLAT) mediates its internalisation from the plasma membrane. Increased phosphorylation of CT1 and CT2 at specific residues may result from the loss of Protein Phosphatase-1 (PP1) binding to CT1 in *PKD1* truncating mutations.

Figure 4. Domain structure of polycystin-1 indicating sites of proteolytic cleavage

Four putative cleavage sites have been identified in PC1. The GPS site (black arrowhead) has been mapped to $His\text{-}Leu^{3048}$-*Thr^{3049} with *cis*-proteolytic cleavage occurring between Leu^{3048} and Thr^{3049}. Three smaller proteolytic cleavage products of 94-100kDa (P100), 32kDa and 11-14kDa (CTT) respectively have been reported (broken arrows). The blue arrowheads indicate 3 other proteolytic cleavage sites recently reported for PC1 present in human urine extracellular vesicles.

Post translational modification	Definition	Frequency
Phosphorylation	The addition of a phosphate group, usually to serine, threonine, and tyrosine	58383
Acetylation	The addition of an acetyl group, either at the N-terminus of the protein or at lysine residues.	6751
N-linked glycosylation	The addition of a glycosyl group to an asparagine residue	5526
Amidation	Formed by oxidative dissociation of a C-terminal Gly residue	2844
Hydroxylation	The addition of an oxygen atom to the side-chain of a Pro or Lys residue	1619
Methylation	The addition of a methyl group, usually at lysine or arginine residues	1523
O-linked glycosylation	The addition of a glycosyl group to serine or threonine residues	1133
Ubiquitylation	The covalent linkage to the protein ubiquitin usually at lysine residues	878
Sulfation	The addition of a sulfate group to a tyrosine	504
Sumoylation	The covalent linkage to the SUMO protein usually at lysine residues	393
Palmiytoylation	Attachment of palmitate, a C_{16} saturated acid usually at cysteine residues	271
Myristoylation	Attachment of myristate, a C_{14} saturated acid usually at glycine residues	140
Farnesylation	The addition of an isoprenoid group (e.g. Farnesol and geranylgeraniol) usually at cysteine residues	77
Deamidation	The conversion of glutamine to glutamic acid or asparagine to aspartic acid	55
S-nitrosylation	Covalent attachment of a nitric oxide group (-NO) to cysteine	53

Table 1. Common post translational modifications and their experimentally observed frequency curated from Swiss-Prot (adapted from Khoury et al. 2011)(46).

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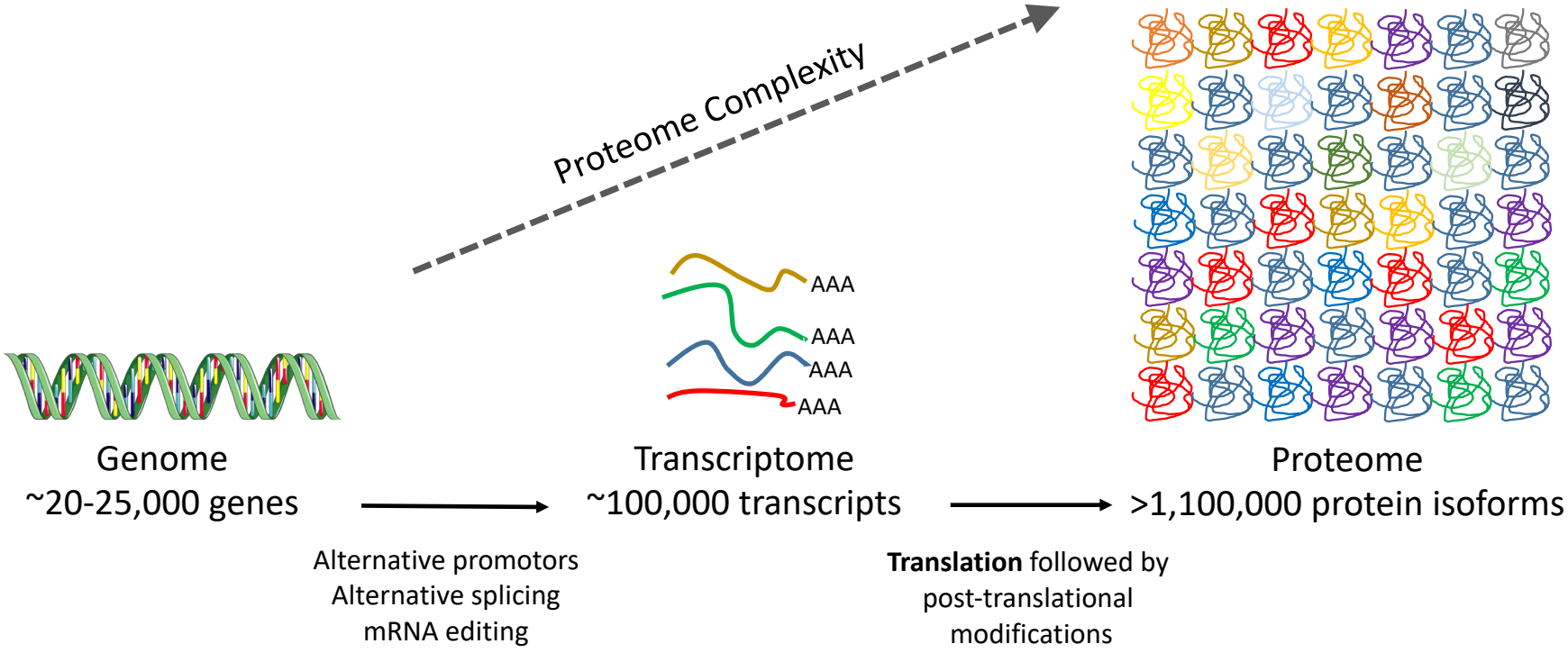
Figure 1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AAA
AAA
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Proteome
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Alternative promotors
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mRNA editing
Translation followed by
post-translational
modifications

Figure 2

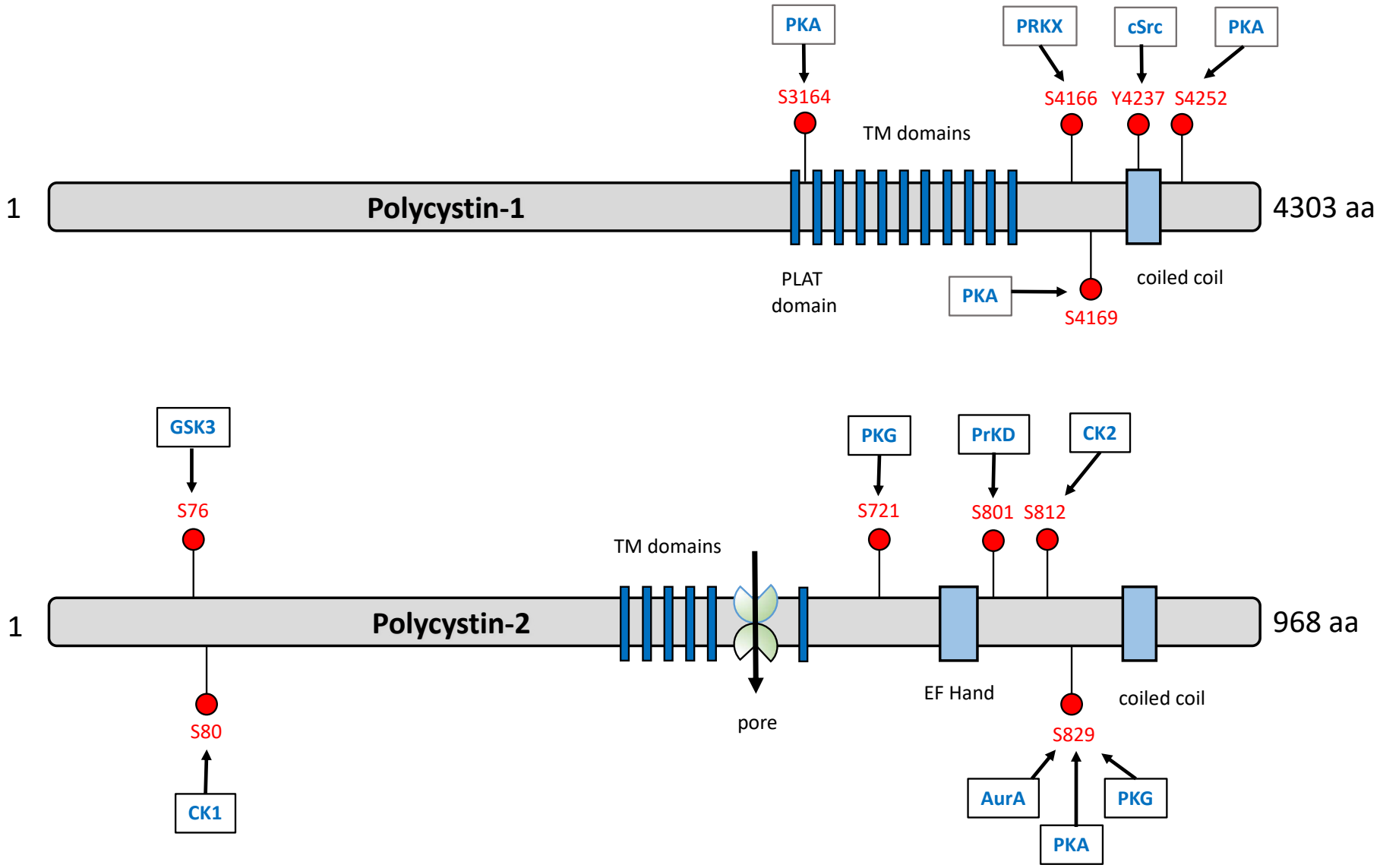
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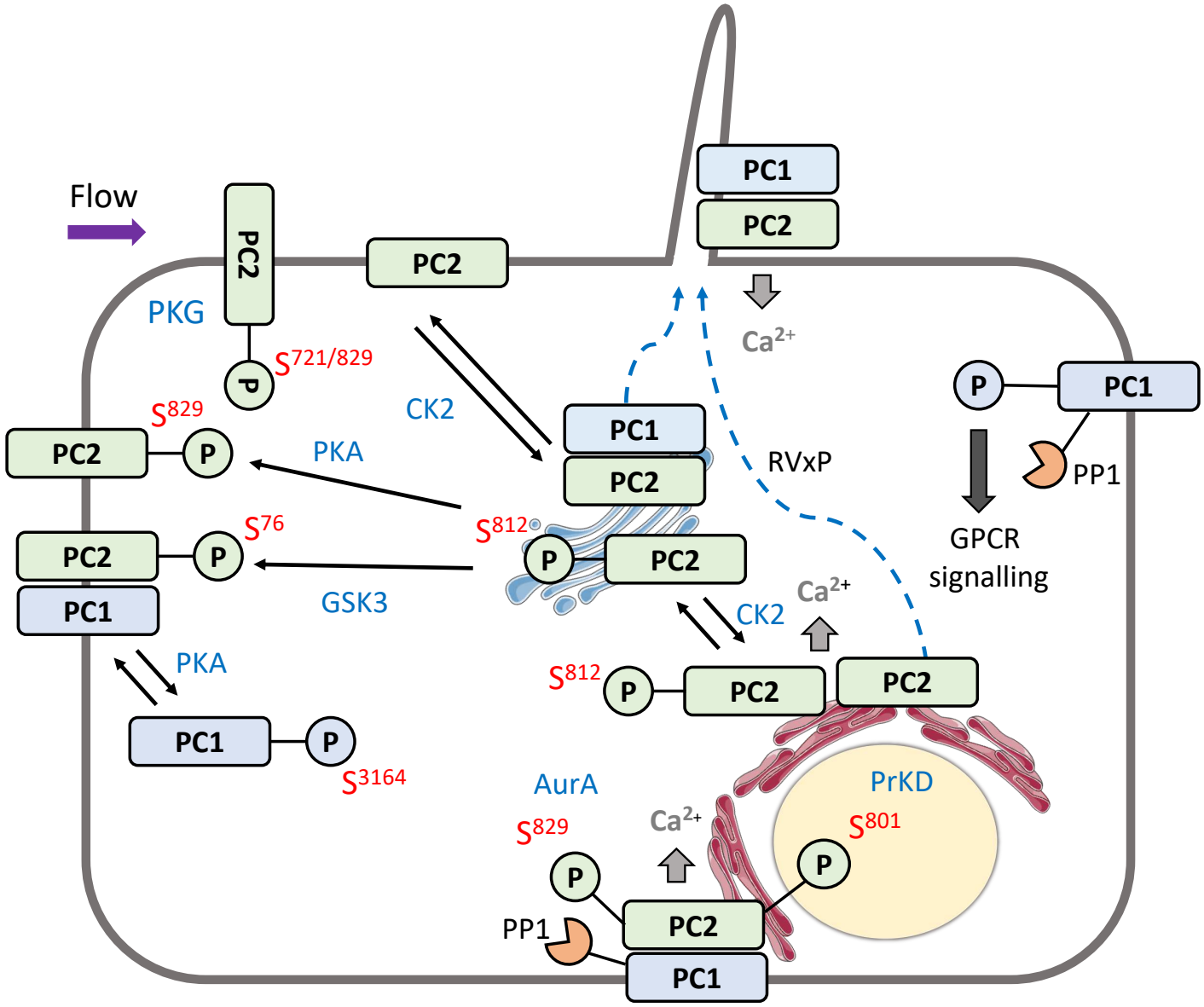
Figure 3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P
PKA
P
PC1
PP1
GPCR
signalling
S812
S76
PC2
P
PC2
P
PC1
GSK3
Ca2+
CK2
PKA
S812
P
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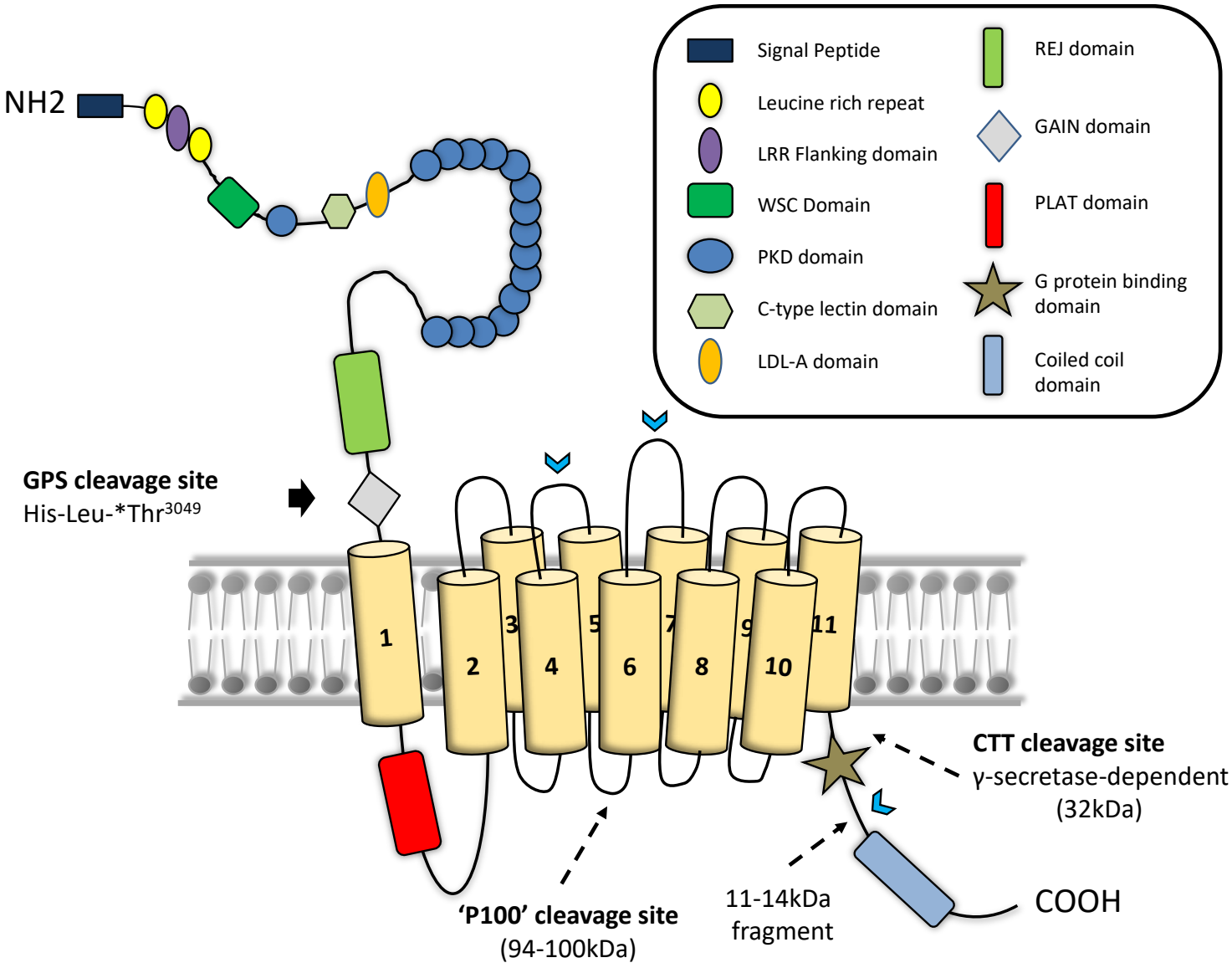
Figure 4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G protein binding domain
Coiled coil domain
GPS cleavage site
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