

R356 Dispatch

Human disease: Calcium signaling in polycystic kidney disease

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Polycystic kidney disease results from loss of function of either of two novel proteins, polycystin-1 or polycystin-2. Recent studies show that intracellular calcium signaling is important in kidney development, and define defects in this signaling pathway as the basis of cyst formation in polycystic kidney disease.

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The inherited human disorder known as autosomal dominant polycystic kidney disease, ADPKD for short, affects more than 1 in 1000 live births and is the most common monogenic cause of kidney failure in man [1]. In the mid 1990s, two genes, *PKD1* and *PKD2*, were identified as the sites of mutations responsible for ADPKD, with virtually indistinguishable pathologies. The molecular characterization of these genes was achieved by positional cloning [2,3], facilitated by early phases of the human genome project. The products of these two genes, dubbed polycystin-1 and polycystin-2, were both novel proteins, though they did show some sequence similarity to each other [3]. The dominance of *PKD1* and *PKD2* mutations was subsequently found to be caused by somatic mutation of the wild-type allele [4,5]. This is reminiscent of the way tumor suppressor mutations cause cancer predisposition syndromes; in both cases, a ‘second hit’ mutation leads to homozygous loss of function.

The near identity of the diseases caused by *PDK1* or *PDK2* mutation, either in humans or mice mutant for homologs of these genes [6,7], together with the discovery that polycystin-1 and polycystin-2 can physically interact [8,9], led to the suggestion that two proteins exhibit functional cooperativity. From their sequences, polycystin-1 and polycystin-2 were predicted to be components of a receptor/channel complex. Indirect evidence to support this hypothesis came from studies of structurally related proteins in other organisms [10–12]. Now, Gonzalez-Perrett *et al.* [13] have reported the first direct evidence that polycystin-2 is indeed a cation channel, while Hanaoka *et al.* [14] have found that co-expression and co-assembly of the two polycystins induces a novel cell-surface cation channel activity. The elusive *in vivo* functions of the ADPKD proteins are slowly coming to light, and with this should come improved understanding of both human disease and fundamental cellular processes.

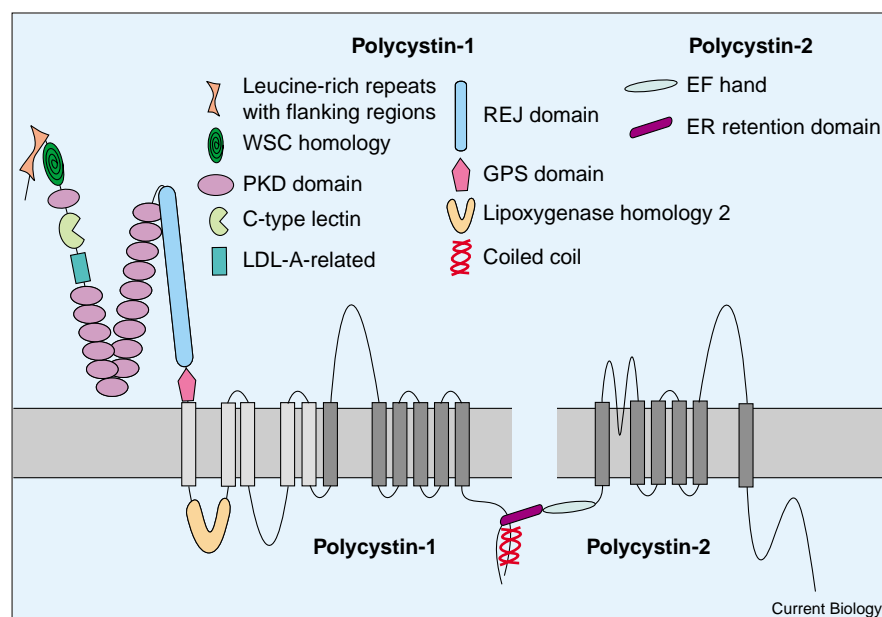
Polycystin-1 is an integral membrane glycoprotein of about 4300 amino acids, predicted to have an extracellular domain of about 3000 amino acids with a multitude of putative interaction domains, eleven membrane-spanning regions and a small cytoplasmic tail (Figure 1) [15]. Polycystin-2 is predicted to have six membrane-spanning regions, and is the prototypical member of a sub-family of the ‘TRP’ superfamily of Ca²⁺ channel proteins (Figure 1). Polycystin-2 also exhibits sequence similarity with the last six membrane-spanning regions of polycystin-1 [3], indicating that polycystin-1 might also participate in channel function. The sequencing of diverse genomes from prokaryotes to man has shown that proteins related to polycystin-1 and polycystin-2 are highly conserved throughout metazoan evolution, but are absent from unicellular organisms.

A protein related to polycystin-1 has been shown to play a part in fertilization: this is the receptor protein REJ, a component of the sea urchin sperm membrane which mediates the ‘acrosome reaction’ — a calcium-regulated exocytic membrane fusion process that is an essential part of fertilization [10]. A variant of REJ, REJ3, has been shown to be proteolytically cleaved at a so-called ‘GPS’ domain into roughly equal-sized polypeptides [16]. Antibodies against the carboxy-terminal half of REJ3 are sufficient to induce calcium influx and the acrosome reaction, although it is uncertain whether REJ3 itself forms part of the channel [16]. The GPS domain is conserved in polycystin-1, raising the possibility that human polycystin-1 is also cleaved *in vivo* [15]. Sea urchin sperm also contains a homolog of polycystin-2 [17].

A homolog of *PKD1* has also been identified in the nematode worm *Caenorhabditis elegans* — *lov-1*, a gene essential for male mating function [11]. Unlike REJ3, LOV-1 has an unrelated extracellular domain but membrane-spanning segments similar to those of the polycystins. *C. elegans* also has a homolog of *PKD2*, which is expressed in the same subset of ciliated sensory neurons as *lov-1*, supporting the view that polycystin-related proteins have acted in common cellular pathways throughout evolution [11]. Most recently, mutations in a human protein related to polycystin-2 have been found to cause mucopolysaccharidosis, type IV disease [18]. This storage disorder results from defects in membrane sorting and the late stages of endocytosis. Taken together, the phenotypes in these pathways involving polycystin-related proteins suggest a general role in chemosensory or mechanosensory signal transduction affecting intracellular transport of proteins and lipids.

Figure 1

Schematic representation of the domain structure, topology and association of polycystin-1 and polycystin-2 (not to scale). Polycystin-1 is predicted to have an ~3,000 amino acid extracellular region with a complex domain structure, followed by 11 membrane spans and a cytoplasmic tail. The predicted domain structure is based on reference [15]. Polycystin-2 has ~1,000 amino acids with six membrane spans and intracellular amino and carboxyl termini. The region of similarity between the proteins resides in the like-shaded membrane spans. Polycystin-1 and polycystin-2 require intact carboxyl termini and the coiled-coil domain to interact with each other [8,9,14]. This association is essential for their normal function. The GPS domain is a potential proteolytic cleavage site [15,16].



Following the discovery of *PKD2* [3], several homologous genes have been identified from expressed sequence tag (EST) and genome databases of a variety of organisms. One of the human proteins, polycystin-like (PCL) was found, when its gene was expressed in *Xenopus* oocytes, to behave as a calcium-modulated, calcium-permeable, non-selective cation channel [12]. This was the first demonstration of channel activity for a member of the polycystin family. Formal demonstration that polycystin-2 is a channel, however, remained elusive, at least in part because polycystin-2 does not translocate to the plasma membrane in most cell-based systems [14,19]. This limitation was overcome in the recent work of Gonzalez-Perrett *et al.* [13], who made single channel recordings in reconstituted planar lipid bilayers. They obtained very similar results using polycystin-2 from a variety of sources, including apical membrane vesicles from human placental syncytiotrophoblasts, epitope-tagged affinity-purified protein and *in vitro* translated protein.

Gonzalez-Perrett *et al.* [13] found that polycystin-2 behaves as a large conductance, non-selective cation channel with similar relative ion selectivities for Ca^{2+} , Na^{+} and K^{+} . This differs from PCL, which exhibits four-fold higher selectivity for Ca^{2+} than for monovalent cations [12]. The measured single channel conductance for polycystin-2 was approximately 140 pS [13], similar to that observed for PCL [12], although the polycystin-2 channel displayed a range of subconductance states not seen in the latter homolog. The polycystin-2 conductance was found to be inhibited by Ca^{2+} , La^{3+} , Gd^{3+} , lower pH and amiloride

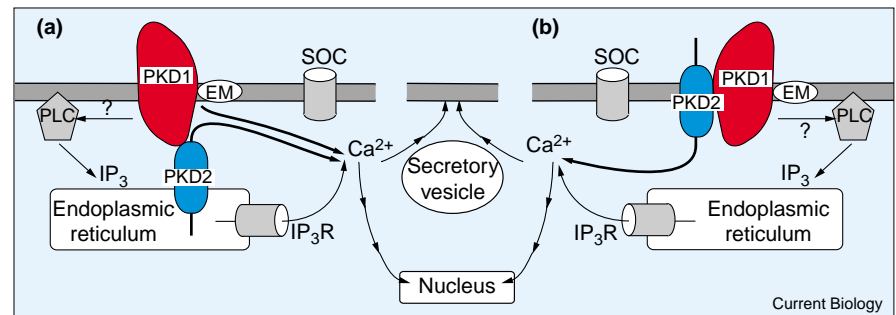
[13], whereas the PCL conductance is activated by Ca^{2+} [12]. It is interesting to note that Gonzalez-Perrett *et al.* [13] detected polycystin-2 channel activity on the surface of Sf9 cells expressing *PKD2* from a baculovirus vector. This is the first time that cell-surface localization of polycystin-2 has been achieved by expression of *PKD2* alone in a cell-based system [19], and may be due to the massive amounts of protein produced in the system overwhelming the endogenous retention machinery.

Hanaoka *et al.* [14] have found a way of expressing polycystin-2 on the surface of mammalian cells in culture that may be somewhat more physiological. By co-immunoprecipitating epitope-tagged versions of polycystin-1 and polycystin-2 from doubly transfected cells, they confirmed that *in vivo* physical association of the two proteins requires both proteins to have intact carboxyl termini. It had previously been shown that full-length polycystin-2 is confined to pre-middle Golgi membranes, and does not appear on the plasma membrane [19]. Hanaoka *et al.* [14] found that full-length polycystin-2 can achieve plasma membrane localization in the presence of heterologously expressed polycystin-1 [14]. Similar findings had previously been reported for transfected cells expressing polycystin-2 and TRPC1, a store-operated Ca^{2+} channel, although no functional cell-surface channel complexes were demonstrated in that study [20].

In the cell-based system used by Hanaoka *et al.* [14], the association between polycystin-1 and polycystin-2, and the consequent chaperoning of polycystin-2 to the cell surface,

Figure 2

Polycystin-1 and polycystin-2 function in intracellular calcium signaling. The diagram illustrates speculative pathways of polycystin signaling based on the understanding of the acrosome reaction [27] in which polycystin-1-related proteins play a role [10,16,17]. Polycystin-1 is a cell surface receptor with possible channel activity, while polycystin-2 is a calcium channel located either in the endoplasmic reticulum (a) [19] or plasma membrane (b) [14]. Localized increases in intracellular calcium may effect secretory vesicle transport unique to tubular epithelia, or regulate gene transcription programs required for the differentiated phenotype of these cells. See text for details. PKD1, polycystin-1;



PKD2, polycystin-2; EM, unknown effector molecules; PLC, phospholipase C; SOC,

store operated channel; IP₃, inositol 1,4,5-trisphosphate; IP₃R, IP₃ receptor.

produces a novel non-selective cation channel that is Ca²⁺ permeable and outwardly rectifying [14]. This channel activity is not observed if the interaction between the polycystins is prevented by removing the carboxyl terminus of either protein [14]. Whole-cell patch-clamp measurements showed that the polycystin channel is six-fold more permeable to Ca²⁺ than to monovalent cations. This relative ion selectivity is similar to that for PCL [12], but differs from that reported for polycystin-2 in the bilayer studies [13]. The polycystin-1/2 channel did, however, show similar inhibition by Ca²⁺, La³⁺ and niflumic acid to that reported for the reconstituted polycystin-2 channel [13]. The polycystin-1/2 and PCL channels have relative Ca²⁺:monovalent cation permeabilities typical of the TRP channel family [14,21]; the single channel conductances reported for polycystin-2 alone are at least two-fold higher than those for any TRP channel reported to date [13,21].

Hanaoka *et al.* [14] argue that, given the similarity of the observed channel properties of the polycystin-1/2 channel to PCL, it is most likely that polycystin-2 functions as the cation channel in the co-assembled complex. The data do not, however, exclude the possibility that polycystin-1 forms the channel and that the absence of activity when either polycystin is truncated is due to a failure to deliver polycystin-1 to the cell surface. The data also do not exclude the possibility that other, as yet undefined, components at the cell surface contribute to the channel [22]. Although it is tempting to speculate that the co-assembled complex in transfected cells is representative of the polycystin-1/2 channel that functions *in vivo*, the final arbiter of the primary site of action of polycystin-2 will be studies based in native kidney, where both polycystins are known to be produced and functional.

Overall, these recent discoveries support the notion that loss of function of either polycystin-1 or polycystin-2

interferes with the proper subcellular localization, assembly, activity and regulation of a novel epithelial channel complex. Loss of the polycystin complex does not, however, manifest as a defect in electrolyte handling by the kidney; rather, it results in dysregulation of Ca²⁺ signaling that controls renal epithelial cell growth and promotes normal tubular morphogenesis and function [14]. Experimentally, polycystin-1 stably over-expressed in MDCK cells exerts anti-proliferative and anti-apoptotic effects, while inducing spontaneous tubule formation in an *ex vivo* model of renal tubular morphogenesis [23]. Furthermore, studies of the human disease and mouse mutant models have shown that polycystin-1 and polycystin-2 are critical for both the establishment and maintenance of the polarized epithelial phenotype — loss of either protein at the level of a single cell results in loss of this specialized phenotype [5–7].

Extensive studies on the origins of epithelial cell polarity have shown that it requires both cell–substratum and cell–cell interactions, very much in keeping with the function predicted for polycystin-1 based on its primary structure. Recent demonstrations that polycystin-1 is localized primarily to the lateral membrane of polarized cells [24,25], and that the extracellular domain is capable of strong homophilic interactions [26], point more to a role in cell–cell interactions. The new studies [13,14] define one of the earliest intracellular steps of the polycystin signaling pathway as being mediated by Ca²⁺. While neither polycystin-1 nor polycystin-2 is likely to participate in the acrosome reaction directly, our understanding of the ligand and channel events underlying sperm capacitation is currently the best available *in vivo* molecular insight we have into the action of proteins structurally related to polycystins.

With the available data [27], it is possible to pose a speculative model for how polycystins may function in polarized

epithelia (Figure 2). In the model, polycystin-1 is targeted to the basolateral membrane where it complexes with polycystin-2, which is either in the plasma membrane or in the endoplasmic reticulum (ER) in close apposition to the plasma membrane (Figure 2). Signals from external stimuli — such as homophilic interactions or as yet unknown ligands — are transduced by polycystin-1 in association with effector molecules, for example, polycystin-2, the ‘regulator of G protein signalling’ RGS7 [28] or other, so far unknown participants. This activation results in increased local $[Ca^{2+}]_i$ (Figure 2). If polycystin-2 is on the cell surface, this increase in $[Ca^{2+}]_i$ results primarily from movement of extracellular Ca^{2+} into the cell, but may be augmented by local release of Ca^{2+} from intracellular stores [27]. If polycystin-2 is in the ER, then cation translocation across the plasma membrane, perhaps through polycystin-1 and associated proteins, could trigger the augmentation by Ca^{2+} release from cytoplasmic stores through opening of the polycystin-2 channel. Whether the receptor for the Ca^{2+} -mobilizing messenger inositol 1,4,5-trisphosphate (IP_3) is involved in intracellular Ca^{2+} release in this pathway is uncertain. In either configuration, store-operated Ca^{2+} channels [20] may be activated in response to intracellular store depletion.

Localized increases in $[Ca^{2+}]_i$ can modulate a variety of subcellular processes. Among these processes are targeted fusion of specialized cytosolic vesicles with the plasma membrane, or activation of kinase cascades leading to cell-type specific gene expression. Either or both of these processes could mediate the function of the polycystin pathway. Involvement of the polycystin pathway in intracellular vesicle transport would be consistent with the inferred functions of polycystin-related molecules in the acrosome reaction, male mating in *C. elegans* and the human disorder mucopolidiosis type IV, as well as with direct observations [29]. The ability to formulate testable hypotheses at the level of discrete cellular pathways clearly demonstrates the substantial progress the field has made since discordant nucleotide sequences were discovered in novel genes of patients with ADPKD. Questions that were posed initially with the aim of furthering our understanding of disease biology should yield answers about normal biology, and the latter, in turn, should yield therapeutic strategies for the disease. Therein will lie one of the many successes of the genome project.

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