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Polycystins: From Mechanosensation to Gene Regulation

Minireview

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Polycystin proteins have been suggested to form mechanosensory transduction complexes involved in a variety of biological functions including sperm fertilization, mating behavior, and asymmetric gene expression in different species. Furthermore, their dysfunction is the cause of cyst formation in human kidney disease. This review focuses on the pros and cons of their candidacy as mechanically gated channels and on recent findings that have significantly advanced our physiological insight.

The list of human diseases known to be associated with defects in ion channel functions or channelopathies, has grown considerably over the past decade and is still expanding. Many of these diseases, including epilepsy, ataxia, and cardiac arrhythmia manifest as neuromuscular disorders and result from dysfunctions of voltage-gated ion channels. Defective ion-channel proteins in nonexcitable cells can cause a broad spectrum of disorders including cystic fibrosis, heritable hypertension (Liddle's syndrome), and multiple forms of Bartter syndrome.

Polycystin Proteins and Kidney Disease

The inherited human disorder known as autosomal dominant polycystic kidney disease (ADPKD) is one of such channelopathies. ADPKD is a common nephropathy affecting four to six million of the worldwide population and a leading cause of end-stage kidney failure in man. The disease is typically characterized by defects in the polarized phenotype and function of epithelial kidney cells, leading to abnormal renal tubular cell growth and formation of numerous fluid-filled cysts. Eventually these cysts would overwhelm the kidney and destroy the parenchyma. Though its etiopathology is clearly multifactorial, ADPKD is perhaps the first disease to be linked to loss-of-function mutations in mechanosensitive channels.

In the mid 1990's, mutated genes responsible for ADPKD were identified by positional cloning (Sutters and Germino, 2003 and references therein). ADPKD has been shown to result from loss-of-function mutations of either of two molecules, polycystin-1 (PKD1) and polycystin-2 (PKD2), with PKD1 mutations being the most prevalent cause of the disease. The dominance of PKD1 and PKD2 mutations appears to require both a germ-line mutation of PKD1 or PKD2 and a subsequent somatic mutation of the wild-type allele. This would explain the relatively late development of ADPKD and the focal nature of epithelial cells giving rise to cysts. Consistent with the broad expression of both genes during early organogenesis, mouse models for ADPKD derived from targeted disruption of either PKD genes die in utero or perinatally with cardiac septal defects and severe cystic manifestations in nephrons and pancreatic ducts.

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Polycystin Complexes in Sensory Ciliary Functions

PKD1 and PKD2 have markedly different molecular architecture and represent the two main phylogenetic branches of the polycystin family, which contains multiple members and exist throughout the animal kingdom (Supplemental Table S1 and Supplemental Figure S1 are available at http://www.cell.com/cgi/content/full/118/2/145/DC1). PKD1 is a large multimodular glycoprotein of about 4300 amino acids with 11 transmembrane-spanning domains, an extensive extracellular region encompassing a number of adhesive domains and a short C-terminal cytoplasmic tail harboring a G protein binding site (Supplemental Figure S1 available on *Cell* website). These predicted structural elements, both data- and knowledge-driven, implicate PKD1 in cell-cell and cell-matrix signaling pathways. PKD1 interacts by means of a coiled-coil domain with PKD2, a six transmembrane domain protein that functions as a Ca^{2+}-permeant cation channel and has significant homology with TRP channels (after transient receptor potential, the founding member in *Drosophila* phototransduction) (Supplemental Figure S1 available on *Cell* website). On this basis, PKD1 and PKD2 are thought to be interacting partners within a heteromeric polycystin complex. This has been intuited before from the observation that mutations in PKD1 or PKD2 in humans or in their homologs in lower organisms produce virtually identical phenotypes irrespective of the causative gene (Barr and Sternberg, 1999; Sutters and Germino, 2003).

What is so detrimental in the loss-of-function of polycystin complexes that would cause severe alterations in the functions of cyst-lining epithelial cells? Recent studies have shown that PKD1 and PKD2 colocalize in primary cilia of renal epithelial cells where they function in transducing sensory information, such as shear stress during fluid flow (Nauli et al., 2003). The primary cilium of renal epithelial cells is a solitary nonmotile structure that arises from the apical membrane and extends into the lumen. It serves as a flow sensor because it can reversibly bend in response to flow rates comparable to those observed in renal tubules. Fluid shear-force bending of the cilium is known to cause Ca^{2+} influx through mechanically sensitive channels that reside in the membrane cilium (Praetorius and Spring, 2001). This Ca^{2+} signal is then amplified by Ca^{2+} release from IP3- and/or ryanodine-sensitive stores and spreads to neighboring cells through tight junction communication. Thus, the cilium acts as a mechanosensor that transduces stimulus energy into change in ion permeability, "sensing" locally, and transmitting global information chemically. PKD1 and PKD2 colocalize to the primary cilium and mediate the cellular response to flow (Nauli et al., 2003). Thus, the polycystin complex may "sense" luminal flow rates via ciliary bending, directing

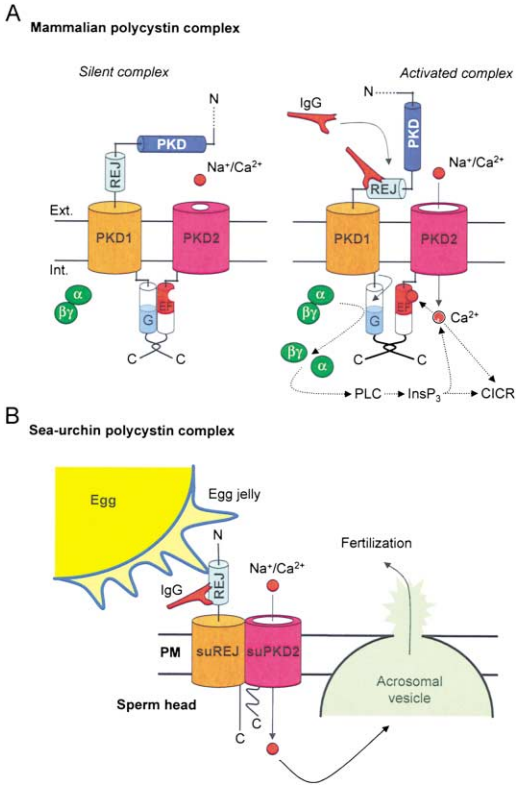

Figure 1. Gating of Polycystin Complexes

(A) Mammalian polycystins: within the complex, PKD1 acts as a cell surface receptor, while PKD2 is the ion-translocating pore. At rest, PKD1/PKD2 complex has little background activity. It activates upon binding of an antibody (IgG) to the REJ domain of PKD1, which results in (1) activation of G protein pathways and (2) opening of PKD2 channels. Localized stimulation of phospholipase C and increase in $InsP_3$ and Ca^{2+} may lead to Ca^{2+}-induced Ca^{2+} release (CICR).

(B) Sea-urchin sperm polycystins: see text for details.

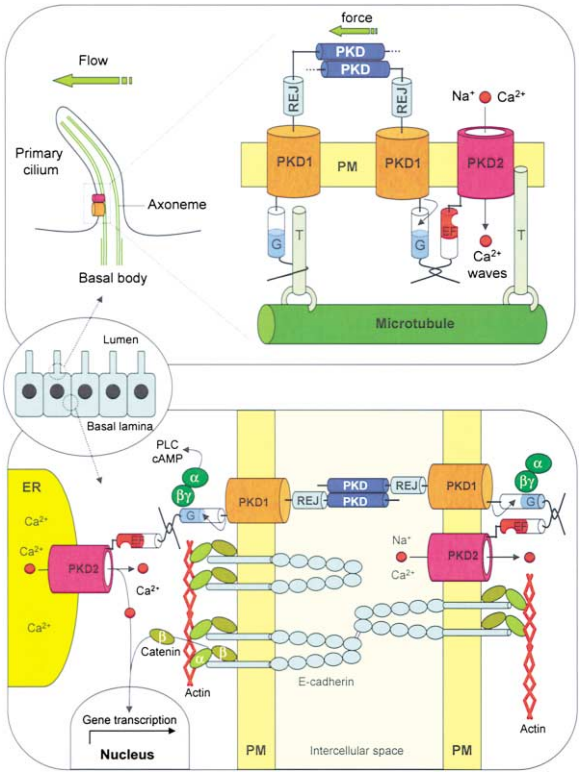

Figure 2. Models for Polymodal Polycystin Complexes

Polycystin complexes are found in the luminal cilium (upper panel) and in adherens junctions (lower panel). Upper panel: here the mechanosensitive channel is formed of a microtubule-polycystin complex. In this model, either PKD1 or PKD2 is proposed to be anchored to the microtubules composed of α- and β-tubulin via tethering molecules (T). In addition, PKD1 may form *cis* dimers via PKD domain interaction. The fluid shear force (arrow) produces conformational change in the microtubule-polycystin complex, thereby activating PKD2 channels and Ca^{2+}-dependent intracellular cascades that may regulate cell growth. Lower panel: polycystin complexes found at the cell-cell contacts are formed of PKD1 located in the plasma membrane (PM), while PKD2 may be located either in the PM or in the ER. PKD1 may form bonds between adjacent cells via *trans*-interaction and couples to G protein pathways. Polycystin complexes also interact with the adherens junction proteins α-, β- and γ-catenin, and E-cadherin, which may regulate cell-cell adhesion and gene expression via the Wnt pathway, suggesting transcriptional control of proliferation and epithelial differentiation.

attention to the regulation of Ca^{2+} influx as a possible misstep that initiates cystogenesis. Functional support for this model comes from the recent finding that PKD1 and PKD2 form functionally associated subunits of a receptor-ion channel complex in which PKD1 acts as a receptor that controls the gating of PKD2 channels (Delmas et al., 2004). Therefore, the polycystin complex may be envisioned as a mechanotransducer, which is used to signal relevant intratubular informations such as flow rates. Failure to do so would result in aberrant cell growth and kidney disease.

A Bona Fide Mechanically Gated Polycystin Complex?

Although a mechanically based gating mechanism of polycystin complexes cannot yet be defined, the above representation of polycystin mechanotransduction provides a tempting framework for such speculation. For the polycystin complex to meet the criteria for a bona fide mechanically gated channel, it must be sensitive to a membrane property that changes with mechanical deformation. Typically, and assuming a simple two-state channel model, a shift in the equilibrium between closed and open channel conformations may be provoked by changes in bilayer tension, local curvature, or by direct anchoring of the mechanosensitive channel to cytoskeletal or extracellular tethers. This implies that PKD2, the pore-forming subunit of the polycystin complex, is held in a higher-energy closed state at rest and relaxes into its lower-energy open state upon mechanical stimulation. However, this is at odds with the constitutive activity seen for homomeric PKD2 channels in reconstitution experiments, which, if occurring in native cells, would lead to Ca^{2+} overload unless this activity is silenced by oligomerization with PKD1.

Recently, it has been shown that antibodies directed against the REJ domain of PKD1 causes activation of PKD2 channels possibly via conformational coupling (Delmas et al., 2004) (Figure 1A). This molecular cascade has fascinating structural and functional parallels with

the acrosome reaction (AR) in sea-urchin spermatozoa during fertilization (Figure 1B). The AR occurs when the membrane forming the head of spermatozoa contacts the jelly layer surrounding the egg, triggering a set of events culminating in Na^+/Ca^{2+} influx and exocytosis of the acrosomal vesicle. This reaction requires the activation of suREJ3, a PKD1 homolog expressed on the surface of spermatozoa and that binds the sea urchin homolog of PKD2 (suPKD2) in the acrosome plasma membrane (Neill et al., 2004). Here again, antibodies directed against the REJ domain induce the AR by opening Ca^{2+}-permeant channels (Moy et al., 1996), suggesting a similar mechanism of gating of polycystin complexes in sea-urchin sperm and mammalian kidneys (Figure 1B).

A fundamental issue then is the identification of the membrane parameters that confer mechanosensitivity on the polycystin complex. Typically, two models have been used to depict gating of mechanosensitive channels. Microbial mechanosensitive channels are gated by change in membrane tension that develops in the lipid bilayer upon osmotic imbalance. As an alternative, the more sophisticated eukaryotic systems seem to rely more on a tethered model, in which cytoskeletal proteins are believed to play a key role in transducing mechanical deformation. In this respect, the recent identification of TRP channels as core components of mechanoreceptors in *C. elegans*, *Drosophila melanogaster* and in vertebrates may offer clues to the conservative mechanoreceptive structural elements of mechanotransducers.

In the fruit fly, bristle mechanosensation is ensured by ciliated sensory neurons, located in mechanoreceptive organs such as the chordotonal organs, and that extend their dendrites into the fly's bristle hair shaft. Mechanoelectrical responses in bristle sensory neurons occur rapidly upon deflection of the bristle hair shaft and result from the opening of the NOMPC channel (for *no m*echanoreceptor *p*otential), a member of the TRP superfamily. Importantly, NOMPC has 6 membrane-spanning domains and a particularly long intracellular amino-terminal tail harboring 29 ankyrin repeats. Ankyrin repeats are considered to provide anchoring of the channel to the cytoskeleton and may mediate the protein-protein interaction of a tethered mechanism that might be required for mechanical gating. In a similar vein, the nematode *C. elegans* senses nose touch by stimulation of ciliated nociceptive sensory neurons, which detect, among others, mechanical and osmotic stimuli. The OSM-9 channel is thought to be the mechanosensitive channel because *osm-9* mutants are defective in osmotic avoidance and in sensitivity to nose touch. OSM-9 is a predicted six membrane-spanning channel homolog to the members of the TRPV-related family of TRP channels with 3–4 ankyrin-repeat domains at their amino-terminal domains. OSM-9 may be prone to heteromultimerization with other OSM9/capsaicin-related channels such as OCR-1, OCR-2, and OCR-4, making the ensuing channels multifunctional and involved in a variety of sensory modalities. Like its invertebrate TRP cousins, TRPV4 has three ankyrin repeat domains and is implicated in vertebrate mechanosensation in that it can sense hypoosmotic stress (Alessandri-Haber et al., 2003). Though its expression pattern includes tissues with relation to osmotic and mechanical sensing, such as epithelial cells of kidney tubules, cochlear hair cells, vibrissal merkel cells, and sensory dorsal root neurons, its broader distribution in other unconnected systems suggests polymodal functioning. With regard to mechanical gating, it is noteworthy that TRPV4 requires the amino-terminal domain with the three ankyrin repeats to sense physical challenges (Liedtke et al., 2000). Importantly, neither PKD1 nor PKD2 display ankyrin repeats that would allow tight interaction between the channel complex and the cytoskeleton. On this last issue, however, intermediate filament proteins such as vimentin and cytokeratins have been identified as PKD1-interacting partners (Xu et al., 2001), but they are most likely to provide structural support for trafficking and storage at desmosomal junctions rather than mechanosensation in the solitary cilium. With higher faithfulness to the criteria for mechanosensitivity, PKD2 has been shown to connect indirectly with the cytoskeletal network via Hax-1, an actin cytoskeletal-associated protein (Gallagher et al., 2000) and via tropomyosin-1, a well-characterized component of the actin microfilament present in virtually all eukaryotic cells (Li et al., 2003). It remains to be shown whether these actin-based elements play a role in cilia mechanotransduction, given that cilia are primarily the domain of microtubules rather than actin filaments (Figure 2).

It is worth considering an alternative model in which mechanical force is transmitted indirectly to the channel protein, perhaps through a genuine sensor. This would imply that the polycystin complex acts as a nonmechanosensitive amplifier of a true mechanically gated channel, with cytosolic Ca^{2+} acting as a suitable activator of PKD2 (Koulen et al., 2002). However, the dual nature of PKD2 activation, whereby channel opening is not only dependent on mechanical stimulus but also modulated by calcium and PKD1, remains to be established. Thus, the basic distinction between physical and chemical mechanisms of polycystin mechanotransduction is yet to be made.

Polymodal Functioning of Polycystins

Polycystin complexes are not restricted to the cilia, suggesting multiple-gating mechanisms and functions. For example, PKD1/PKD2 complex has been localized to lateral cell junctions of renal tubular cells where it participates in cell-cell junction and cell-extracellular matrix adhesion through interactions with neighboring cells (Figure 2). At this location, however, it is unclear whether PKD2 is in the plasma membrane or in the ER in close apposition with the plasma membrane. Through multiple interactions with other proteins and signals from external stimuli, which may include *cis/trans*-homotypic interaction, cleavage at the GPS and as yet unidentified ligands, polycystin complexes can stimulate various intracellular-signaling cascades that influence gene transcription. Among these, polycystin complex has been shown to regulate heterotrimeric G proteins, the transcription factor AP-1, and the JAK-STAT pathway (Bhunia et al., 2002; Delmas et al., 2002; Parnell et al., 2002). Importantly, polycystin complexes also interact with the adherens junction protein E-cadherin and its associated cytoplasmic catenins, suggesting a role in the stabilization of adherens junctions and the maintenance of a fully differentiated polarized renal epithelium (Roitbak et al., 2004). The E-cadherins play important

roles in cell-cell adhesion during tissue differentiation and are connected to the actin cytoskeleton by association with the αβ-catenins. β-catenin has an additional role in the Wnt/Wingless signal transduction pathway in which it transmits signals to the cell nucleus and is known to be an oncogene in many forms of human cancer following acquisition of stabilizing mutations. The C terminus of PKD1 itself has been shown to modulate the Wnt-signaling pathway via stabilization of β-catenin (Kim et al., 1999). Thus, subtle interplay between polycystin and E-cadherin/β-catenin complexes in lateral membranes may regulate adhesion, differentiation, and maturation, which are all essential steps of kidney morphogenesis.

At this point, the information does not entirely support the candidacy of polycystin complexes as the core component of the primary cilium's mechanosensitive apparatus but rather suggests gating promiscuity. Notwithstanding, it is an amazing set of coincidences that keeps placing polycystin proteins in the center of mechanoreceptive structures. Recently, PKD2 has been shown to play a central role in the establishment of the left-right (LR) asymmetry of visceral organs in mouse embryos (McGrath et al., 2003). The generation of LR asymmetry occurs during early embryonic development when the nodal gene, initially expressed throughout the node, becomes limited to the left margin of the node. The node is a triangular-shaped structure at the distal tip of ~E7 embryos, consisting of endodermally derived cells, each carrying a single cilium on their apical surface. Importantly, the monocilia located at the center of the node express dynein, a microtubular motor protein, and are motile, producing rotational movement that creates a leftward fluid flow across the node. This leftward nodal flow is critical for the sidedness of asymmetric gene expression since mice with immotile cilia develop laterality defects. In contrast, monocilia located in the periphery of the node are immobile (lacking dynein) and act as sensors of directional nodal flow by generating an asymmetric Ca^{2+} signal. PKD2 is expressed in both motile and immotile monocilia, yet perinodal Ca^{2+} signal was absent in PKD2$^{-/-}$ mice embryos, suggesting that PKD2 functions as a mechanotransducer in immotile monocilia, transducing leftward nodal flow into an increase in Ca^{2+} at the left border of the node. This function would be key for the establishment of a morphogenic gradient at the embryonic node and consistent with the observation that targeted disruption in PKD2 causes right isomerism in addition to the hallmark cardiac and kidney defects. It is interesting, in this regard, that the homolog of PKD2 encoded by the *amo* gene (*amo*st there) in *Drosophila melanogaster* is localized to the distal tip of the sperm flagella and plays a critical role for directional movement inside the female reproductive tract (Watnick et al., 2003). This suggests that PKD2 in *Drosophila* sperm is part of a complex or a signaling pathway involved in detection of directional cues that are necessary for recognition and entry into the female storage organs, supporting a common role for PKD2 in both motile and immotile axonemal-based structures.

Conclusions

The notion that polycystin proteins act as cellular sensors has become an established concept. Understanding which and how information arising from different stimuli activates polycystin proteins and is integrated into cellular functions will be the next challenge. Much of the exciting research involves understanding the role of polycystin proteins in human diseases. However, insight into the relevance of polycystin pathways will come from studies of biological problems, spanning different functions and species. Defining a molecular mechanism of gating of polycystin ion channel complex presupposes the solution of many interrelated problems: what kind of stimuli constitutes the trigger for activation? How are mechanical stimuli, ligand binding, and other cues sensed by the channels? Given that a single cell may express multiple polycystin complexes at different location (e.g., extraciliary sites), how do cells extract the specific information regarding the relevant stimulus, while filtering out extraneous stimuli? Thus, although much remains to be clarified, polycystins and related proteins are emerging as widely distributed polymodal sensors in various cell types.

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