Casein Kinase II and Calcineurin Modulate TRPP Function and Ciliary Localization

Jinghua Hu,*† Young-Kyung Bae,‡ Karla M. Knobel,*† and Maureen M. Barr*†

*Division of Pharmaceutical Sciences, †Laboratory of Genetics, and ‡School of Pharmacy, University of Wisconsin, Madison, WI 53705

Submitted October 11, 2005; Revised February 2, 2006; Accepted February 8, 2006

Monitoring Editor: Martin Chalfie

Cilia serve as sensory devices in a diversity of organisms and their defects contribute to many human diseases. In primary cilia of kidney cells, the transient receptor potential polycystin (TRPP) channels polycystin-1 (PC-1) and polycystin-2 (PC-2) act as a mechanosensitive channel, with defects resulting in autosomal dominant polycystic kidney disease. In sensory cilia of Caenorhabditis elegans male-specific neurons, the TRPPs LOV-1 and PKD-2 are required for mating behavior. The mechanisms regulating TRPP ciliary localization and function are largely unknown. We identified the regulatory subunit of the serine-threonine casein kinase II (CK2) as a binding partner of LOV-1 and human PC-1. CK2 and the calcineurin phosphatase TAX-6 modulate male mating behavior and PKD-2 ciliary localization. The phospho-defective mutant PKD-2<sup>2853A</sup> localizes to cilia, whereas a phospho-mimetic PKD-2<sup>2853D</sup> mutant is largely absent from cilia. Calcineurin is required for PKD-2 ciliary localization, but is not essential for ciliary gene expression, ciliogenesis, or localization of ciliary structural components. This unanticipated function of calcineurin may be important for regulating ciliary protein localization. A dynamic phosphorylation-dephosphorylation cycle may represent a mechanism for modulating TRPP activity, cellular sensation, and ciliary protein localization.

INTRODUCTION

Many eukaryotic cells possess cilia (Rosenbaum and Witman, 2002). In mammals, sensory or primary cilia can sense a wide range of environmental stimuli, including light (by photoreceptors), odorants (by olfactory cilia), and urine flow (by primary kidney cilia). All sensory (or primary) cilia share a similar internal structure consisting of a “9 + 0” axoneme that contains nine double microtubules but not the central pair of doublets found in motile cilia. Membrane-bound receptors and signaling molecules are enriched within the cilium. Defects in cilia formation or function have been implicated in many human diseases, including autosomal dominant polycystic kidney disease (ADPKD; Rosenbaum and Witman, 2002).

ADPKD affects 1 in 1000 individuals and is caused by defects in polycystin-1 (PC-1, encoded by PKD1) or polycystin-2 (PC-2, encoded by PKD2; reviewed in Igarashi and Somlo, 2002). PC-1 is a 4302 amino acid protein with a large extracellular domain, a G protein–coupled receptor proteolytic site (GPS), 11 transmembrane (TM) domains, and an intracellular C-terminus (Hughes et al., 1995). The polycystin/lipoxygenase/alpha-toxin (PLAT) domain is located in the first cytoplasmic loop between TM1 and TM2 and has been postulated to be involved in membrane–protein or protein–protein interactions (Bateman and Sandford, 1999). The PLAT domain is conserved in all PC-1 family members and also found in a variety of membrane or lipid associated proteins. PC-1 and PC-2 are members of the transient receptor protein polycystin (TRPP) family of TRP channels (Mochizuki et al., 1996) and act as a nonselective cation channel (reviewed in Igarashi and Somlo, 2002; Delmas, 2004). TRP channels have been implicated in a plethora of sensory modalities (Clapham, 2003). PC-2 forms a mechano-sensitive channel with PC-1 and localizes in kidney cilia (Nauli et al., 2003). Although our understanding of the functional role and subcellular localization of the polycystins has increased, little is known about the mechanisms that dynamically regulate this important ciliary sensory complex.

Many of the human cystic kidney disease genes required for ciliogenesis or sensation have counterparts in the nematode Caenorhabditis elegans (Barr, 2005). A simple approach to understanding cilia development, morphogenesis, and sensory function is to use the genetics, simple nervous system, and transparent anatomy of C. elegans. These features enable the study of TRPP function and subcellular localization in an intact, living animal. LOV-1 and PKD-2 are the C. elegans homologues of PC-1 and PC-2, respectively (Barr and Sternberg, 1999; Barr et al., 2001). lov-1 and pkd-2 mutants are specifically defective in the male mating sensory behaviors of response (Rsp) and location of vulva (Lov). lov-1 and pkd-2 are expressed in the 21 male-specific ciliated sensory neurons that mediate response (ray RnBs), vulva location (hook HOB), and possibly chemotaxis to mates (head CEMs; see Figure 1a). These male sensory neurons have one common structural feature: all have long dendrites that end in exposed ciliated sensory endings. LOV-1 and PKD-2 are concentrated in the sensory cilia of these neurons. The fact that the PC-1 PLAT domain, TRPP sensory function, and TRPP ciliary localization are evolutionarily conserved makes C. elegans an excellent model system for studying the polycystins, the molecular mechanisms underlying ADPKD, and ciliary protein localization and function (Barr and Sternberg, 1999; Barr et al., 2001; Hu and Barr, 2005; Peden and Barr, 2005).
Figure 1. CK2 interacts with the LOV-1 and PC-1 PLAT domain and enriches in the cilia of male specific neurons. (a) Anatomy of C. elegans male and PKD-2–expressing neurons. Top, DIC side view image of an adult C. elegans male oriented head (left) to ventral up tail (right). Bottom, lov-1 and pkd-2 are expressed in male head CEM neurons (left) and tail ray RnB and hook HOB neurons (right). The cilium, dendrite, cell body, and axon of the CEMD (dorsal CEM) and CEMV (ventral CEM) neuron are drawn in the male head diagram. CEMD and CEMV are arranged as left-right pairs (one side shown here). Cilia are in the nose region (dashed rectangular box). The pharynx is green. Positions of nuclei of all lov-1 and pkd-2 expressing cells in the C. elegans adult male tail, modified from (Sulston et al., 1980). Ray neurons are arranged as left-right bilateral pairs (ventral up view shown here). The male tail has nine bilaterally arranged rays (numbered 1–9, anterior to posterior) required for response and turning behaviors (Liu and Sternberg, 1995). HOB is an asymmetric ciliated hook neuron that mediates vulva location behavior (Liu and Sternberg, 1995). For simplicity, only the dendrite of R3B left and HOB is shown. The R3B cilium is indicated by a dashed rectangular box. (b) A GST pulldown assay demonstrates a direct in vitro interaction between KIN-10 and the LOV-1 PLAT domain. In vitro–translated 35S-labeled KIN-10 protein was incubated with GST or GST-PLAT (amino acids 2112–2302). Autoradiography shows 35S-labeled KIN-10 protein is specifically retained in the GST-PLAT column. A Western blot developed with an anti-GST antibody detects GST and GST-PLAT. (c) Y2H mapping reveals the CK2β signature domain of KIN-10 mediates interaction with the PLAT domains of human PC-1 and C. elegans LOV-1. Different kin-10 fragments were cloned into the Y2H AD vector pGADT7. The C. elegans and human PLAT domain (amino acids 3164–3349) were engineered into the Y2H BD vector pGBK17. Y2H interactions were accessed by growth ability on SD-Leu-Trp-His-Ade plates. (d) Confocal micrographs of Ppkd-2::PKD-2::GFP, Ppkd-2::KIN-3::GFP (using pkd-2 promoter to drive KIN-3::GFP fusion protein expression) and Ppkd-2::KIN-10::GFP (using pkd-2 promoter to drive KIN-10::GFP fusion protein expression) transgenic males. PKD-2 localizes to cilia and cell bodies (excluding nuclei) of male-specific neurons. KIN-3 and KIN-10 are localized and enriched in the cilia of the male-specific RnB, HOB and CEM neurons. KIN-3 and KIN-10 also localize weakly in dendrites and axons and strongly in cell bodies (especially in nuclei). Dashed rectangular boxes show the ciliary zone of the CEM neurons. Dashed circles show the ciliary zone of RnB neuron in ray 3. Labeled bars indicate length in micrometer (μm).
Protein phosphorylation by the coordinated activities of protein kinases and phosphatases is central to many signal transduction pathways. Here we show that casein kinase II and calcium/calmodulin/ protein phosphatase 2B (PP2B) modulate PKD-2 function and ciliary localization. We find that the regulatory subunit of casein kinase II (CKβ/KIN-10) interacts with the PLAT domain of both human PC-1 and C. elegans LOV-1. We demonstrate that CK2 and the Ca2+-activated phosphatase calcineurin act antagonistically to regulate PKD-2. We use a set of genetic and molecular manipulations to ask whether phosphorylation state affects polycystin function and ciliary localization in vivo. We find that a “phospho-defective” PKD-2 mutant protein trafficks normally to cilia but exhibits attenuated function, whereas a “phospho-mimetic” PKD-2 is defective in both function and ciliary localization. We show that tax-6 regulates PKD-2 ciliary localization but not ciliogenesis or gene expression. We propose that a dynamic phosphorylation cycle modulates normal polycystin function and ciliary distribution.

MATERIALS AND METHODS

Strains and Alleles

Nematode culturing and genetics were performed by standard techniques (Brenner, 1974). him-5(e1490) (LGV) was used as the wild-type (Hodgkin, 1983). The following mutations were used: LGL, kin-1(tem1283), kin-3(gk389); LCI1, rrf-3(pk1426), loci-1(pk562); LGL11, pha-1(e2212ts); LCV, pdk-2(y966b), tace-1(pk70); and LCV, mdpk-2(PKD-2-GFP) + cgt-1(p).

Our molecular analysis of the kinase (3gk389) deletion allele indicates that the VC928 genetic background is complex. The VC928 strain is homoygous for the gk346 deletion but PCR results show that it also contains a wild-type kin-3 copy on the chromosome. This wild-type copy could function to balance VC928 strain from lethal effects caused by gk346 allele, resulting in viable animals. Based on CK2 function in many essential processes, the lethal phenotype of the kin-1(tem1283) allele, and the kin-3 RNAi phenotypes of Embryonic lethality and Sterility, a null allele of kin-3 should be inviable. To support this prediction, a new kin-3 deletion allele, ok1516, is recently available. Unlike gk389, the kin-3(ok1516) deletion is homoygous lethal according to the Vancouer Gene Knockout Laboratory: http://aceserver.biotech.ubc.ca/cgi-bin/stable/strain.pl?

Molecular Biology Techniques

Details of plasmid constructions are available upon request. Standard procedures were used for recombinant DNA manipulations.

Transgenic Animals

We constructed transgenic lines by injecting plasmid DNA (100–200 ng/ul) using standard procedures (Mello and Fire, 1995). In all experiments the plasmid pBX containing the wild-type pha-1(+ ) gene was used as a cotransformation marker in the pha-1(ts) strain (Granato et al., 1994).

Yeast Two-Hybrid Screen

The yeast strain AH10 (Clontech, Palo Alto, CA) was used for yeast two-hybrid (Y2H) experiments. Bait proteins were expressed in the GAL4 DNA-binding domain (DNA-BD) vector pGBK77. A cDNA library derived from mix-staged him-5 animals was constructed in the GAL4 activation domain (DNA-AD) vector pGAD GH (Hu and Barr, 2005). The PLAT domain of C. elegans LOV-1 (amino acids: 2112–2302) and human PC-1 (amino acids: 3164–3349) were used as baits in Y2H hybrid assays. Protein–protein interactions were accessed by growth on SD-Leu-Trp-His-Adr high-stringency plates and β-galactosidase filter assays. KIN-10 did not interact with the cytoplasmic carboxy termini of worm LOV-1, human PC-1, worm PKD-2, or human PC-2 and various other controls (unpublished data), demonstrating specificity of the KIN-10 and PLAT domain interaction.

In Vitro Binding

In vitro–translated Y2H candidate KIN-10 protein was produced with the TNT quick-coupled transcription/translation system (Promega, Madison, WI) by using [35S]methionine (Amersham Pharmacia Biotech, Piscataway, NJ). A GST-fused LOV-1 PLAT domain was produced in the bacteria strain BL21(DE3) and incubated with in vitro–translated KIN-10 in 500 μl binding buffer (20 mM Tris-HCl, pH 7.5; 150 mM NaCl; 5 mM MgCl2; 2 mM DTT; 0.05% NP-40) at 4°C for 30 min. Glutathione-Sepharose 4B, 25 μl, was added to the reaction and incubated at 4°C for an additional 2 h or overnight. After three washes with binding buffer, bound proteins were resuspended in 50 μl 1X Laemmeli buffer and then 10-μl samples were boiled and separated by 10% SDS-PAGE. Proteins were visualized by Coomassie staining. The dried gel was exposed for autoradiography. [35S]KIN-10 binds to GST-PLAT but not GST alone, indicating that KIN-10 binds directly to the PLAT domain of LOV-1 (Figure 1b).

Imaging Analysis and Fluorescence Quantitation

Epifluorescence microscopy experiments were carried out by using a Zeiss Axioplan2 Imaging system (Thornwood, NY) and photographed with an Orca-ER camera. Confocal experiments were carried out on a Bio-Rad MRC-1024 laser scanning confocal microscope (Richardson, CA). An approximate cilium/cell body fluorescence intensity ratio, L4 animals were picked and cultured at 16°C for 20–24 h. Confocal or epifluorescence images were taken of adults. Overexpression was avoided by ensuring fluorescence was not saturated in any areas of the image so mean fluorescence intensities of the cilium (including the axoneme and transition zone), the corresponding cell body (including the nucleus), and background (area without the animal) were quantified using OpenLab software (Improvision, Lexington, MA). Cilium/cell body fluorescence intensity ratios were calculated using the following formula [(mean cilium fluorescence intensity – background fluorescence intensity) / (mean cell body fluorescence intensity – background fluorescence intensity)]

RNA Interference and Mating Behavior Assays

Multiple RNA interference (RNAi) methods were used. For heat shockinducible RNAi mediated by the inverted-repeat (IR) genes (HS-IR-RNAi), we constructed IR genes as described (Tavernarakis et al., 2000; Wang and Barr, 2005). Transgenic lines carrying IR plasmids were generated. Mix-staged transgenic animals were heat shocked for 4 h at 35°C, before returning to 16°C. After 63–67 h at 15°C, L4 males were transferred to new plates for culturing another 14 h at 15°C. Mating behaviors were scored as described (Barr and Sternberg, 1999). For tissue-specific RNAi (TS-IR-RNAi), we constructed IBs whose transcription is under the control of the prd-2 promoter. Transgenic lines were generated, and males were scored for mating behaviors. For feeding RNAi, RNAi bacteria clones were obtained from MRC Geneservice (Cambridge, UK). The kin-3 RNAi feeding clone was generated by inserting the 600-base pair kin-3 cDNA into the NcoI site of the plasmid pL4440, followed by transformation into the Escherichia coli HT115(DE3) strain. Double-stranded RNA (dsRNA) producing bacteria were grown in LB with 50 ng/ml ampicillin overnight at 37°C and seeded onto NGM agar plates including additives (1 mM IPTG, 50 mg/ml ampicillin). The following day, 8–12 hr, RNAi sensibilization (Simmer et al., 2002) were transferred onto feeding plates and incubated ~72 h at 15°C. L4 males (n = 20–50) were picked to new feeding plates seeded with feeding RNAi bacteria and cultured 14 h at 15°C, and the mating behaviors of adult males were scored. In all experiments, at least 24 animals were scored per experimental trial. Triplicate trials were performed for each line to obtain statistical data. All behavioral assays were done with the experimenter completely blinded to the sample.

Immunoprecipitation

HA-tagged PKD-2 or HA-tagged PKD-2S534A expressing HEK 293T cells were lysed in buffer supplemented with protease and phosphatase inhibitors (50 mM Tris-HCl, pH 8.0, 1% NP-40, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml Na3PO4, 1 μM EDTA). For immunoprecipitation, whole cell lysates were prepared with cell protein-A–agarose beads (Amersham Pharmacia Biotech). A mouse anti-HA IgG1 (HA11, clone 16B12, Covance, Madison, WI) was used to preclear whole cell lysates and incubated overnight at 4°C. Protein-A–agarose beads, 30 μl, were added to each of the incubations for 4 h at 4°C. Control immunoprecipitation with mouse IgG or beads alone was also performed. The immunoprecipitated complexes were washed three times in phosphate-buffered saline, and Western blotting was performed.

In Vitro Kinase and Phosphatase Assays

After immunoprecipitation, HA-PKD-2, HA-PKD-2S534A, or HA-PKD-2S534A was incubated in CK2 buffer (20 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM MgCl2, 200 mM ATP, 100 μM Na3VO4 with 100 μCi/ml [γ-32P]ATP (Amersham Pharmacia, 3000 Ci/mM) and 300 U recombinant CK2 (NEB BioLabs, Beverly, MA; P0570S) for 30 min at 30°C. Control reaction without immunoprecipitation complex was also done. The kinase assays were stopped by adding 2X Laemmli sample loading buffer and boiled for 5 min. For phosphatase assays, the HA-PKD2-2S534A– or HA-PKD-2S534A–conjugated protein A beads were washed three times with phosphate buffer (50 mM Tris-HCl, pH 7.0, 0.1 mM CaCl2, 0.1 mM PMSF), and incubated in the kinase assay system (50 mM Tris-HCl, pH 7.0, 0.1 mM CaCl2, 1 mM NiCl2, 125 μg/ml BSA, 10 μg/ml calmodulin) with 1 U/ml calcineurin/PP2B (Upstate, Cat. 14-446) for 30 min at 37°C. The reactions were stopped by adding 2X Laemmli sample loading buffer and boiled for 5 min. The samples were then centrifuged and loaded onto a 10% SDS-polyacrylamide gel. After electrophoresis, the gel was dried and autoradiography was performed. Western blotting with anti-HA antibody was performed to quantitate protein levels.
RESULTS

The CK2 β Regulatory Subunit Interacts with C. elegans LOV-1 and Human PC-1

The PLAT domain is a highly conserved structure in all PC-1 family members, yet its function remains unknown. We reported previously that overexpression of the PLAT domain of the C. elegans PC-1 homolog, LOV-1, dominantly interferes with C. elegans male sensory behaviors but is insufficient for ciliary localization (Hu and Barr, 2005). We sought to determine the functional role of the PLAT domain by identifying binding partners via a yeast two-hybrid screen (Hu and Barr, 2005). We found that KIN-10, the regulatory β subunit of casein kinase II (CK2), interacts with the PLAT domain of LOV-1 in a yeast two-hybrid assay (Figure 1c) and GST pulldown assay (Figure 1b). KIN-10 also interacts with the PLAT domain of human PC-1 (Figure 1c) but not the carboxy cytoplasmic tails of LOV-1 or PKD-2 (unpublished data).

Protein kinase CK2 is a ubiquitous serine/threonine kinase comprised of two catalytic α and two regulatory β subunits (Meggio and Pinna, 2003). The CK2β subunit determines kinase substrate specificity (Pinna, 2002; Litchfield, 2003). C. elegans CK2 α catalytic and β regulatory subunits are encoded by kin-3 and kin-10, respectively (http://www.wormbase.org). We mapped the region of KIN-10 region responsible for the interaction with the PLAT domain of both human PC-1 and C. elegans LOV-1. The C-terminus of KIN-10 (amino acids 109–174), which contains a conserved CK2 β signature domain, is the minimal PLAT binding region (Figure 1c). Based on the crystal structures of CK2 holoenzyme and the CK2β dimer, the CK2 β signature domain has been proposed to mediate β subunit dimerization and form a protein–protein interaction site (Chantalat et al., 1999; Niefind et al., 2001).

CK2 Is Present in Cilia

A prerequisite for a physiologically relevant interaction between CK2 and the polycystins is coexpression and localization in the same subcellular compartment. kin-3/CK2α and kin-10/CK2β are widely expressed throughout development in both males and hermaphrodites, predominantly in many head neurons and the intestine. kin-3 and kin-10 are coexpressed with lov-1 and pkd-2 in the male-specific CEM head neurons and ray RnB and hook HOB tail neurons (Supplementary Figure 1). We next examined the subcellular localization of CK2 using the 1.3-kb pkd-2 promoter to restrict expression of KIN-3:GFP and KIN-10::GFP (Ppkd-2::KIN-3::GFP and Ppkd-2::KIN-10::GFP) to only the polycystin-expressing cells. In pkd-2–expressing cells, KIN-3::GFP and KIN-10::GFP are enriched in cilia and also found in cell bodies (including nuclei), dendrites, and axons (Figure 1d). Although CK2 has been found in nearly every compartment of the eukaryotic cell (reviewed in Faust and Montenarh, 2000), this is the first description of CK2 enrichment within a primary cilium, a restricted subcellular compartment dedicated to sensory functions.

CK2 Modulates TRPP-dependent Male Sensory Behaviors

The role of CK2 in polycystin-mediated response and vulva location behaviors was examined. kin-10 and kin-3 null mutants are homozygous lethal, precluding adult behavioral analysis (refer to Materials and Methods for strain information). To study the function of CK2 in C. elegans male mating behavior, kin-3 and kin-10 were knocked down by using multiple RNAi methods (Hu and Barr, 2005; Wang and Barr, 2005). Feeding RNAi directed to either kin-3 or kin-10 produced similar phenotypes: Emb (embryonic lethality), Gro (Slow growth), and pVul (protruding vulva; unpublished data; Fraser et al., 2000). For those males that survived to adulthood, kin-3 and kin-10 feeding RNAi caused Rsp but not Lov mating behavior defects (Figure 2a). IR plasmids producing dsRNA are effective at knocking down gene function in neurons and are particularly valuable for temporal or tissue-specific gene knockdown using appropriate promoters (Tavernarakis et al., 2000). Either the heat shock promoter (HS-IR-RNAi for heat shock–inducible inverted repeat RNAi) or pkd-2 promoter (TS-IR-RNAi, for tissue-specific expressed inverted repeat RNAi) was used to drive expression of kin-10 or kin-3 cDNA IRs. Knockdown of kin-10 or kin-3 function by either HS-IR-RNAi or TS-IR-RNAi consistently resulted in both Rsp and Lov defects (Figure 2b and c). Negative controls to unrelated genes do not affect male mating behaviors (unpublished data). These results indicate that CK2 functions in a cell autonomous manner to modulate response and vulva location behavior.

HS-IR kin-3 RNAi was more potent in reducing response than TS-IR kin-3 RNAi (compare Figure 2b and c). The heat shock promoter is expressed broadly, suggesting that KIN-3/CK2α may function in both polycystin-expressing and polycystin-nonexpressing cells to modulate response behavior. These data also indicate that there are cells in addition to the polycystin-sensory neurons (CEMs, RnBs, and HOB) that control the response step of mating behavior.

Laser ablation of the neurons expressing lov-1 and pkd-2 causes more severe behavioral defects than mutations in lov-1 and pkd-2 (for example, 0% vulva location efficiency for HOB ablated animals vs. ~25% vulva location efficiency for lov-1 and pkd-2 mutants; Liu and Sternberg, 1995; Barr and Sternberg, 1999; Barr et al., 2001). If CK2 and the polycystins act in different pathways or if knocking down CK2 expression causes general developmental defects of the targeted sensory neurons, we would observe an additive effect with more severe Rsp and Lov defects than loss of either alone. To determine if CK2 and the polycystins function in the same or a parallel genetic pathway, the effects of kin-3 and kin-10 RNAi were examined in lov-1 and pkd-2 null mutant backgrounds. The behavioral defects of lov-1 and pkd-2 mutants are not exacerbated by kin-3 or kin-10 TS-IR RNAi (Figure 2, d and e). These results indicate that CK2, lov-1, and pkd-2 act in the same genetic pathway regulating mating behavior and that the interaction between KIN-10 and LOV-1 is physiologically relevant.

To determine if Lov-1 or PKD-2 are required for CK2 localization, we quantified the ciliary expression levels in wild-type and CK2 RNAi (compare Figure 2, b and c). The heat shock promoter is expressed broadly, suggesting that KIN-3/CK2α may function in both polycystin-expressing and polycystin-nonexpressing cells to modulate response behavior. These data also indicate that there are cells in addition to the polycystin-sensory neurons (CEMs, RnBs, and HOB) that control the response step of mating behavior.

To determine if Lov-1 or PKD-2 are required for CK2 localization, we quantified the ciliary expression levels of KIN-3:GFP and KIN-10:GFP in wild-type and lov-1 or pkd-2 mutants. The levels of Ppkd-2:KIN-3:GFP and Ppkd-2:KIN-10:GFP in cilia are similar in wild-type and lov-1 or pkd-2 mutant males (unpublished data). Therefore, lov-1 and pkd-2 are not required for CK2 localization in cilia. To determine if CK2 is required for PKD-2:GFP localization, we quantified the ciliary expression levels in wild-type and CK2 RNAi-treated males. PKD-2:GFP localization in wild-type and kin-3 or kin-10 TS-IR RNAi-treated animals is comparable (unpublished data), indicating that CK2 modulates PKD-2 function but is not essential for PKD-2 ciliary localization.

Serine 534 Is an Important Residue for PKD-2 Function and Ciliary Localization

The basic cellular function of CK2 is to phosphorylate and regulate substrates. There are multiple predicted CK2 phosphorylation sites in LOV-1 and PKD-2. We focused on PKD-2 for two reasons: First, CK2 has been demonstrated to phosphorylate and regulate human PC-2 (Cai et al., 2004; Kottgen et al., 2005). Second, the LOV-1 protein is 3125 amino acids and PKD-2 is only 716 amino acids (K. M. Knobel and M. M. Barr, unpublished results), making the latter more easily manipu-
lated. GFP-tagged wild-type PKD-2 rescues pkd-2 null mutant mating behavior defects and localizes to cilia, similar to endogenous PKD-2 (Barr et al., 2001; Bae, Y. K., Qin, H., Knobel, K. M., Hu, J., Rosenbaum, J. L., and Barr, M. M., unpublished results). Therefore, male mating behavior and ciliary localization are powerful readouts of polycystin function (Barr and Sternberg, 1999; Barr et al., 2001; Peden and Barr, 2005). The cytoplasmic regions of PKD-2 contain 7 predicted CK2 sites: 2 in the N-terminus (S58, S73) and 5 in the C-terminus (S534, T577, S606, S656, S709; Figure 3a). We generated “phospho-defective” PKD-2 mutants by changing individual serine or threonine sites to alanine (Greif et al., 2004). Individual GFP-tagged mutantized clones were analyzed for ability to complement pkd-2 mutant mating behavior defects and to localize to cilia. For the latter, we measured the levels of different GFP-tagged proteins by quantitative fluorescence, comparing the relative levels of PKD-2::GFP in the cilium versus cell body and obtaining a fluorescence intensity ratio (see Materials and Methods). In this manner, we can also measure overall expression levels for each transgene by comparing fluorescence intensity values for cell bodies and cilia.

Only PKD-25534A fails to fully rescue pkd-2 defects (Figure 3b), suggesting that S534 is an important functional residue. The expression level and ciliary localization of GFP-tagged PKD-25534A is comparable to wild-type PKD-2::GFP in male-specific neurons (compare Figures 1d and 3c). Alignment of C. elegans PKD-2 with C. briggsae, sea urchin, zebrafish, mouse, rat, and human PC-2- and PC-2-like proteins reveals that S534 is the only conserved predicted CK2 phosphorylation site (S681 in mouse PC-2 and T683 in human PC-2; Figure 3a). Evolutionary conservation of the S534 CK2 site suggests selective pressure and an important cellular function. Interestingly, Dro-
PKD-2S534 is an important site. (a) Multiple amino acid alignment of human, mouse, and C. elegans polycystin-2 C-termini. Red bars indicate the five predicted C. elegans PKD-2 CK2 phosphorylation sites: S534, T577, S606, S656, and S709. Only the predicted S534 CK2 phosphorylation site is conserved among human, mouse, and C. elegans. (b) Different CK2 phosphodefective variants of C. elegans PKD-2 were generated by site-mutagenesis of Serine (S) or Threonine (T) to Alanine (A), injected into pkl-2 null animals, and the behavior of transgenic lines scored for rescue. Only S534A does not fully rescue pkl-2 mutant defects. In all experiments, at least 24 animals were scored per trial. For each line, experiment trials were done in triplicate trials to obtain statistical data. Figures were generated using Sigmaplot5 (Jandel Scientific). Data are scored for rescue. Only S534A does not fully rescue PKD-2S534A than wild-type HA-PKD-2 (Figure 3d). HA-cytophilates PKD-2, an in vitro kinase assay was performed. HA-PKD-2 and HA-PKD-2S709A, a phosphorylation site mutant defects. In all experiments, at least 24 animals were scored per trial. For each line, experiment trials were done in triplicate trials to obtain statistical data. Figures were generated usingSigmaplot5 (Jandel Scientific). Data are represented as mean ± SEM. * p < 0.01 compared with control. (c) In these confocal images, PKD-2S534A::GFP exhibits normal localization in cilia and cell bodies of male-specific neurons (compare to wild-type PKD-2::GFP in Figure 1d). Dashed rectangular boxes show the ciliary zone of the CEM neurons. Labeled bars indicate the ciliary zone of HOB neuron. Dashed diamond boxes show the ciliary zone of RnB neuron in ray 3. Numbers indicate the corresponding rays. Labeled bars indicate length in micrometers (μm). (d) S534 is one of CK2 phosphorylation sites on PKD-2. HA-tagged PKD-2 or HA tagged PKD-2S534A were transfected and expressed in HEK 293 cells. Immunoprecipitated HA-PKD-2 or HA-PKD-2S534A was incubated with human recombinant CK2 kinase and [γ-32P]ATP at 30°C for 30 min, followed by electrophoresis and autoradiography. A Western blot with an anti-HA antibody was used to quantify protein concentration in each lane. With equal amounts of wild-type or S534A mutant PKD-2 protein, phosphorylation of PKD-2S534A is significantly lower than wild-type PKD-2.

TAX-6 Calcineurin Is Required for PKD-2 Ciliary Localization

PKD-2 phosphorylation state appears to modulate its function and ciliary localization, with S534A and S534D reflecting two extreme states. Calcineurin (CaN, protein phosphatase 2B, PP2B) is a calcium-activated serine/threonine protein phosphatase that regulates the activity of several ligand-gated channels and membrane receptors (Kuhara et al., 2002; Misonou et al., 2004; Mohapatra and Nau, 2005; Wu et al., 2005). Human TRPP channels conduct calcium (Chen et al., 1999; Hanaoka et al., 2000), making calcineurin an attractive candidate phosphatase. In C. elegans, tax-6 encodes the catalytic subunit of calcineurin (Bandyopadhyay et al., 2002; Kuhara et al., 2002) and regulates several behaviors (Kuhara et al., 2002; Lee et al., 2004, 2005; Gottschalk et al., 2005). In the hermaphrodite, tax-6 is expressed in ciliated sensory neurons (Kunitomo et al., 2005). In the male, we observed tax-6 expression in the CEM, HOB, and PKD-2 phosphorylation site. As a control, we also compared phosphorylation level by CK2 between HA-PKD-2 and HA-PKD-2S709A, a phosphorylation site mutant that does not visibly affect PKD-2 function or subcellular localization. No difference in phosphorylation levels between wild-type PKD-2 and PKD-2S709A was observed (unpublished data). Our physiological and biochemical data corroborate, showing that S534 is an important site. Endogenous PKD-2 and our PKD-2::GFP transgenes are expressed in a small number of neurons (~5% of the somatic cells in the male), and we have been unable to detect the protein by Western analysis, prohibiting in vivo analysis of PKD-2 phosphorylation.

sophila PKD2 does not possess this site and does not function in sensory cilia (Gao et al., 2003, 2004; Watnick et al., 2003).

To further explore the function of phosphorylation, we generated the GFP-tagged phospho-mimetic PKD-2S534D mutant. Serine or threonine to aspartate (D) substitutions might mimic phosphorylation by introducing a negative charge (Greif et al., 2004). The PKD-2S534D construct was expressed in pkl-2 null animals. In all eight PKD-2S534D::GFP transgenic lines examined, none is fully rescued (Figure 4c). Strikingly, the expression level of the GFP-tagged PKD-2S534D protein in the cilium is greatly reduced (Figure 4, a and b, compared with Figures 1d and 5c). Because the ciliary localization of PKD-2 is critical for function, the reduction of PKD-2S534D in cilia may account for the failure to fully rescue the pkl-2 mutant. Alternatively, PKD-2S534D may represent a dysregulated channel.

To genetically determine whether PKD-2S534 is a direct target of CK2, we combined RNAi of CK2 with the PKD-2S534D point mutant. If CK2 functions on sites in addition to PKD-2 S534, we have been unable to detect the protein by Western analysis, prohibitive in vivo analysis of PKD-2 phosphorylation.
ray RnB neurons, with expression noticeably absent from ray 6, which is very similar to lov-1 and pkd-2 expression patterns (Figure 5a). TAX-6::GFP also localizes to cilia of these polycystin-expressing neurons (Supplementary Figure 2).

Overexpression of TAX-6::GFP results in Rsp and Lov defects, a behavioral phenotype similar to that caused by CK2 RNAi or a PKD-2S534 phosphorylation mutation (unpublished data). Next, we examined male mating behavior and PKD-2 localization in two tax-6 mutant backgrounds. tax-6(p675) is a loss-of-function allele whereas tax-6(jh107) is a gain-of-function allele lacking the calmodulin-binding site and autoinhibitory region (Kuhara et al., 2002; Lee and Ahnn, 2004). Both loss-of-function and gain-of-function tax-6 alleles exhibit Rsp and Lov defects (Figure 5d). However, their PKD-2 ciliary localization phenotypes are distinct. Gain-of-function tax-6(jh107) does not affect PKD-2 localization. Similarly, rcn-1, a negative regulator of calcineurin (Lee et al., 2003), is also required for response and vulva location behaviors (Figure 5d) but not PKD-2::GFP ciliary localization (unpublished data). In contrast, loss-of-function tax-6(p675) exhibit greatly decreased PKD-2::GFP ciliary expression (Figure 5, b and c), mirroring the PKD-2S534D phenotypes.

To determine whether calcineurin dephosphorylates PKD-2, an in vitro phosphatase assay was performed. As shown in Figure 5e, CK2-phosphorylated PKD-2 is dephosphorylated by calcineurin. Equivalent phosphorylation levels between PKD-2 and PKD-2S534A after calcineurin treatment indicate that S534 in wild-type PKD-2 is dephosphorylated by calcineurin. Residual phosphorylation indicates that not all CK2 in vitro phosphorylation sites on the PKD-2 protein are dephosphorylated by calcineurin. These data biochemically substantiate the assertion that CK2 and calcineurin function antagonistically to regulate PKD-2 phosphorylation state.

Constitutive phosphorylation of PKD-2 (by tax-6 loss-of-function or the phospho-mimetic PKD-2S534D) results in a loss of PKD-2 from cilia. Alternatively or perhaps additionally, tax-6 may regulate pkd-2 gene expression or ciliogenesis. To determine if cilia are structurally normal, we used GFP-tagged ciliary markers. Amphid and phasmid cilia were labeled with Posm-6::OSM-6::GFP, an intraflagellar transport (IFT) Complex B polypeptide that localizes to the ciliary base and moves along the axoneme (Collet et al., 1998; Orozco et al., 1999). In the tax-6(p675) loss-of-function mutant, cilia development, morphology, and OSM-6 motility were intact in amphid, phasmid, and male-specific sensory neurons (unpublished data). Hence, we can rule out an essential role for calcineurin in ciliogenesis. These results also indicate that tax-6 is not required for OSM-6 expression, protein stability, ciliary localization, or motility.
Figure 5. The C. elegans calcineurin TAX-6 is required for male sensory behaviors and PKD-2 ciliary localization. (a) Confocal micrographs of the head (top) and tail (bottom) of a transgenic male expressing P\textit{tax-6}:DsRed2 (using \textit{tax-6} promoter to drive DsRed2 expression) and P\textit{pkd-2}:PKD-2::GFP. \textit{tax-6} is coexpressed with \textit{pkd-2}. Like \textit{lov-1} and \textit{pdk-2}, \textit{tax-6} is never expressed in ray 6 (R6B). (b) Confocal micrographs and (c) quantification of PKD-2::GFP localization in \textit{tax-6 (p675)} loss-of-function and \textit{tax-6 (jh107)} gain-of-function mutants. PKD-2::GFP ciliary localization is normal in \textit{tax-6 (jh107)} gain-of-function mutant, but is significantly reduced in \textit{tax-6 (p675)} loss-of-function mutant. Dashed rectangular boxes show the ciliary zone of CEMs neuron. Dashed circles show the ciliary zone of HOB neuron. Dashed diamond boxes show the ciliary zone of RnB neuron in ray 3. Labeled bars indicate length in micrometers (\mu m). (c) Quantification of the fluorescence intensity ratio of PKD-2::GFP–expressing cilium to the corresponding cell body. (d) Both \textit{tax-6 (jh107)} and \textit{tax-6 (p675)} mutant males are Rsp and Lov defective. Knocking down \textit{rcn-1} expression in wild-type males also causes response and vulva location behaviors defects. (e) Calcineurin dephosphorylates PKD-2. Immunoprecipitated HA-PKD-2 or HA-PKD-2\textit{S534A} was incubated with human recombinant CK2 kinase and [\gamma-\textit{32P}]ATP at 30°C for 30 min, washed with phosphatase buffer three times, and then incubated with calcineurin at 37°C for 1.5 h, followed by electrophoresis and autoradiography. A Western blot with an anti-HA antibody was used to quantify protein concentration to insure equivalent protein input in each level. With equal amounts of wild-type or mutant S534A PKD-2 protein, both PKD-2 and PKD-2\textit{S534A} are dephosphorylated by calcineurin to equivalent phosphorylation levels. (f) Quantification of the fluorescence intensity ratio of PKD-2\textit{S534A}::GFP in wild-type and \textit{tax-6 (p675)} mutant.
Next, we examined the effects of the tax-6(p675) loss-of-function mutation on pkd-2 expression. We found that Ppkd-2::GFP expression is slightly higher in wild-type (~1.5-fold) than in the tax-6(p675) mutant (as determined by comparing cell body fluorescence intensity). Hence, calcineurin is not essential but does play a subtle role in the regulation of pkd-2 expression. However, this result cannot explain the nearly 10-fold reduction of PKD-2 in tax-6 (p675) cilia (Figure 5c).

Calcineurin may affect PKD-2 ciliary localization by regulating trafficking to cilia or away from cilia. To distinguish between these models and test the hypothesis that calcium and neuronal activity are regulating PKD-2 trafficking, we took several experimental approaches. First, we examined the subcellular distribution patterns of PKD-2 in wild-type and tax-6(p675) late L4 larval males. Although pkd-2 is expressed in the late L4 larval stage (just preceding adulthood), L4 males are sexually inactive (Simon and Sternberg, 2002; Lipton et al., 2004). Wild-type late L4 males and wild-type adult males exhibit distinct PKD-2 ciliary localization patterns. In wild-type adult males, PKD-2 is distributed along the ciliary axoneme and accumulates in the transition zone between the cillum and dendrite (Figure 1d). In ray neurons of wild-type late L4 males, the majority of the PKD-2 protein localizes to the tip of the cillum and around the transition zone, whereas the staining along the ciliary axoneme is very weak (Figure 6a). In both wild-type and tax-6(p675) late L4 males, PKD-2 localization and expression levels in cilia and cell bodies are similar (Figure 6a). These data show that PKD-2 is transcribed, translated, and localized normally in tax-6(p675) L4 males. In wild-type L4 males, we also observe indistinguishable PKD-2 and PKD-2534S43 ciliary localization phenotypes, with both wild-type and the S534D phosphomimetic localizing to the ciliary tip and transition zone (Figure 6a). These results demonstrate that PKD-2534S43 is transcribed, translated, and localized normally to cilia in L4 males. In the adult worm, decreased ciliary localization of PKD-2534S43 or PKD-2 in tax-6(p675) mutant may due to the changes in a pathway that maintains PKD-2 ciliary localization or regulates PKD-2 removal from cilia.

To further examine the effects of phosphorylation and neuronal activity on PKD-2 ciliary localization, we generated a PKD-2 mutant that does not form a normal channel but still localizes to cilia. The carboxy terminus of human neuronal activity on PKD-2 ciliary localization, we generated a PKD-2 mutant (as determined by comparing cell body fluorescence intensity). Hence, calcineurin is not essential but does play a subtle role in the regulation of pkd-2 expression. However, this result cannot explain the nearly 10-fold reduction of PKD-2 in tax-6 (p675) cilia (Figure 5c).

Calcineurin may affect PKD-2 ciliary localization by regulating trafficking to cilia or away from cilia. To distinguish between these models and test the hypothesis that calcium and neuronal activity are regulating PKD-2 trafficking, we took several experimental approaches. First, we examined the subcellular distribution patterns of PKD-2 in wild-type and tax-6(p675) late L4 larval males. Although pkd-2 is expressed in the late L4 larval stage (just preceding adulthood), L4 males are sexually inactive (Simon and Sternberg, 2002; Lipton et al., 2004). Wild-type late L4 males and wild-type adult males exhibit distinct PKD-2 ciliary localization patterns. In wild-type adult males, PKD-2 is distributed along the ciliary axoneme and accumulates in the transition zone between the cillum and dendrite (Figure 1d). In ray neurons of wild-type late L4 males, the majority of the PKD-2 protein localizes to the tip of the cillum and around the transition zone, whereas the staining along the ciliary axoneme is very weak (Figure 6a). In both wild-type and tax-6(p675) late L4 males, PKD-2 localization and expression levels in cilia and cell bodies are similar (Figure 6a). These data show that PKD-2 is transcribed, translated, and localized normally in tax-6(p675) L4 males. In wild-type L4 males, we also observe indistinguishable PKD-2 and PKD-2534S43 ciliary localization phenotypes, with both wild-type and the S534D phosphomimetic localizing to the ciliary tip and transition zone (Figure 6a). These results demonstrate that PKD-2534S43 is transcribed, translated, and localized normally to cilia in L4 males. In the adult worm, decreased ciliary localization of PKD-2534S43 or PKD-2 in tax-6(p675) mutant may due to the changes in a pathway that maintains PKD-2 ciliary localization or regulates PKD-2 removal from cilia.

To further examine the effects of phosphorylation and neuronal activity on PKD-2 ciliary localization, we generated a PKD-2 mutant that does not form a normal channel but still localizes to cilia. The carboxy terminus of human PC-2 is important for its interaction with PC-1, channel (Koulen et al., 2002; Bildl et al., 2004), and for receptor internalization as a mechanism for modulating channel properties, for attiqution of sensory signals, for clustering of channels, receptors, and signaling molecules, or for receptor internalization. In C. elegans, an imbalance between inactive and active PKD-2 states culminates in functional defects represented by a reduction in male mating behavior. CK2 and calcineurin have been individually implicated in other behaviors such as Drosophila circadian rhythms and mammalian learning and memory, respectively (Blau, 2003; Lee and Ahnn, 2004) as well as the regulation of ion channel activity and/or localization (Kuhara et al., 2002; Bildl et al., 2004; Cai et al., 2004; Misonou et al., 2004; Kottgen et al., 2005; Mohapatra and Nau, 2005; Wu et al., 2005). Our results provide the first demonstration of a coordinated and antagonistic relationship between the two.

CK2 is constitutively active in most systems. The model proposed in Figure 6d may be initiated by structural changes in LOV-1 protein or the entire polycystin complex after a putative mechanical stimulation. A conformational change may place PC-2 in close proximity to PKD-2534S43, resulting in phosphorylation. Alternatively, it has been demonstrated that the regulated timing and localization of CK2 activity are important for phospho-regulation of diverse processes ranging from cell division to circadian rhythms (Allende and Allende, 1995; Lin et al., 2002). CK2 activity is regulated by protein kinase C and inositol phosphates and increased several fold following neuronal-induced elevation of cytosolic calcium levels (Charriaut-Marlangue et al., 1991; Blanquet, 1998, 2000; Boehning and Snyder, 2003; Solyakov et al., 2004). Recently, PP2A has been shown to regulate CK2 activity to modulate the phosphorylation status of the Ca2+-activated K+ channel (Bildl et al., 2004). Finally, the activity of CK2 may be controlled by the regulatory b subunit (Pinna, 2002). In the LOV-1/PKD-2 pathway, CK2 may be similarly regulated. However, we have no direct evi
dence proving that CK2 activation occurs downstream of calcium entry through LOV-1/PKD-2 after mating.

Posttranslational modification of receptors is a common theme in the regulation of vertebrate sensory signal transduction. This study demonstrates a role for calcineurin in regulating ciliary protein localization and elucidates a novel mechanism for targeting CK2 to the TRPP complex. Interestingly, CK2 has been implicated in the regulation of mammalian PC-2 activity and trafficking to the plasma membrane (Cai et al., 2004; Kottgen et al., 2005) and PC-1 has been shown to activate a calcineurin/NFAT (nuclear factor of activated T-cells) signaling pathway (Puri et al., 2004). Mam-
malian PC-2<sup>28912</sup> is constitutively phosphorylated in vivo (Cai et al., 2004). This CK2 site (S812) is not conserved in C. elegans PKD-2. Walz and colleagues have shown that trafficking of PC-2 from the ER to the plasma membrane involves CK2 phosphorylation at S812 in an acidic cluster region and PACS proteins (Kottgen et al., 2005). There are no data to suggest that S812 is critical for PC-2 ciliary localization nor is there an acidic cluster region found in C. elegans PKD-2. Distinct mechanisms are likely required for localizing polycystin-2 to the ER, plasma membrane, and cilium. Our studies address the latter. The PKD-2 S534 CK2 phosphorylation site is conserved between C. elegans and mammalian PC-2 (Harteneck et al., 2000; Figure 3a). A role for the comparable C. elegans PKD-2 S534 site in mammalian PC-2 (S661 in mouse and T683 in human) has not been explored.

Our data suggest that PKD-2 phosphorylation state and TAX-6/calcineurin play pivotal roles in modulating ciliary sensory receptor localization, possibly be regulating PKD-2 recycling and turnover. Interestingly, the PKD-2 carboxy terminus interacts with an ubiquitin family member in the yeast two-hybrid system (D. R. Braun, J. Hu, and M. M. Barr, unpublished results) and human PC-1 and is regulated by Siah-1 (seven in absentia homolog) through the ubiquitin-proteasome pathway (Kim et al., 2004). Whether or not the polycystin complex or other ciliary sensory receptors are down-regulated by ubiquitylation in vivo is an open and intriguing question.

These studies also raise the question of how natural sensory stimuli regulate polycystin subcellular distribution. Although the nature of the LOV-1/PKD-2 polycystin ligand is not known, chemical and mechanical cues appear to be required for vulva location (M. M. Barr and Paul W. Sternberg, unpublished results) and human PC-1 binds and is regulated by Siah-1 (seven in absentia homolog) through the ubiquitin-proteasome pathway (Kim et al., 2004). Whether or not the polycystin complex or other ciliary sensory receptors are down-regulated by ubiquitylation in vivo is an open and intriguing question.

These studies also raise the question of how natural sensory stimuli regulate polycystin subcellular distribution. Although the nature of the LOV-1/PKD-2 polycystin ligand is not known, chemical and mechanical cues appear to be required for vulva location (M. M. Barr and Paul W. Sternberg, unpublished results) and human PC-1 binds and is regulated by Siah-1 (seven in absentia homolog) through the ubiquitin-proteasome pathway (Kim et al., 2004). Whether or not the polycystin complex or other ciliary sensory receptors are down-regulated by ubiquitylation in vivo is an open and intriguing question.

These studies also raise the question of how natural sensory stimuli regulate polycystin subcellular distribution. Although the nature of the LOV-1/PKD-2 polycystin ligand is not known, chemical and mechanical cues appear to be required for vulva location (M. M. Barr and Paul W. Sternberg, unpublished results) and human PC-1 binds and is regulated by Siah-1 (seven in absentia homolog) through the ubiquitin-proteasome pathway (Kim et al., 2004). Whether or not the polycystin complex or other ciliary sensory receptors are down-regulated by ubiquitylation in vivo is an open and intriguing question.

These studies also raise the question of how natural sensory stimuli regulate polycystin subcellular distribution. Although the nature of the LOV-1/PKD-2 polycystin ligand is not known, chemical and mechanical cues appear to be required for vulva location (M. M. Barr and Paul W. Sternberg, unpublished results) and human PC-1 binds and is regulated by Siah-1 (seven in absentia homolog) through the ubiquitin-proteasome pathway (Kim et al., 2004). Whether or not the polycystin complex or other ciliary sensory receptors are down-regulated by ubiquitylation in vivo is an open and intriguing question.

These studies also raise the question of how natural sensory stimuli regulate polycystin subcellular distribution. Although the nature of the LOV-1/PKD-2 polycystin ligand is not known, chemical and mechanical cues appear to be required for vulva location (M. M. Barr and Paul W. Sternberg, unpublished results) and human PC-1 binds and is regulated by Siah-1 (seven in absentia homolog) through the ubiquitin-proteasome pathway (Kim et al., 2004). Whether or not the polycystin complex or other ciliary sensory receptors are down-regulated by ubiquitylation in vivo is an open and intriguing question.

These studies also raise the question of how natural sensory stimuli regulate polycystin subcellular distribution. Although the nature of the LOV-1/PKD-2 polycystin ligand is not known, chemical and mechanical cues appear to be required for vulva location (M. M. Barr and Paul W. Sternberg, unpublished results) and human PC-1 binds and is regulated by Siah-1 (seven in absentia homolog) through the ubiquitin-proteasome pathway (Kim et al., 2004). Whether or not the polycystin complex or other ciliary sensory receptors are down-regulated by ubiquitylation in vivo is an open and intriguing question.

These studies also raise the question of how natural sensory stimuli regulate polycystin subcellular distribution. Although the nature of the LOV-1/PKD-2 polycystin ligand is not known, chemical and mechanical cues appear to be required for vulva location (M. M. Barr and Paul W. Sternberg, unpublished results) and human PC-1 binds and is regulated by Siah-1 (seven in absentia homolog) through the ubiquitin-proteasome pathway (Kim et al., 2004). Whether or not the polycystin complex or other ciliary sensory receptors are down-regulated by ubiquitylation in vivo is an open and intriguing question.


