STAM and Hrs Down-Regulate Ciliary TRP Receptors

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Cilia are endowed with membrane receptors, channels, and signaling components whose localization and function must be tightly controlled. In primary cilia of mammalian kidney epithelia and sensory cilia of *Caenorhabditis elegans* neurons, polycystin-1 (PC1) and transient receptor polycystin-2 channel (TRPP2 or PC2), function together as a mechanosensory receptor-channel complex. Despite the importance of the polycystins in sensory transduction, the mechanisms that regulate polycystin activity and localization, or ciliary membrane receptors in general, remain poorly understood. We demonstrate that signal transduction adaptor molecule STAM-1A interacts with *C. elegans* LOV-1 (PC1), and that STAM functions with hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) on early endosomes to direct the LOV-1-PKD-2 complex for lysosomal degradation. In a *stam-1* mutant, both LOV-1 and PKD-2 improperly accumulate at the ciliary base. Conversely, overexpression of STAM or Hrs promotes the removal of PKD-2 from cilia, culminating in sensory behavioral defects. These data reveal that the STAM-Hrs complex, which down-regulates ligand-activated growth factor receptors from the cell surface of yeast and mammalian cells, also regulates the localization and signaling of a ciliary PC1 receptor-TRPP2 complex.

INTRODUCTION

Cilia are specialized organelles that function in motility (motile or nodal cilia) or sensation (sensory or primary cilia). Several human genetic diseases are linked to defects in cilia formation or function (Pazour and Rosenbaum, 2002; Badano *et al.*, 2006). Ciliary assembly via intraflagellar transport (IFT) and sensory transduction capabilities are evolutionarily conserved (Rosenbaum and Witman, 2002). These sensory devices, recently referred to as “antennae” or “nanomachines,” transduce a plethora of sensory stimuli and must be fine-tuned both temporally and spatially to execute their cellular functions (Marshall and Nonaka, 2006; Scholey and Anderson, 2006; Singla and Reiter, 2006). Significant advances have been made in understanding cilia biogenesis and the genetic basis of human ciliary disease. In contrast, little is known regarding how cilia perceive, integrate, and transduce multiple extracellular stimuli into precise developmental and physiological responses.

Sensory cilia are best known for their roles in photoreception and olfaction, which require G protein-coupled receptors (GPCRs) on the ciliary membrane (Buck and Axel, 1991; Marszalek *et al.*, 2000). Cilia also act in mechanosensory and osmotic capacities and require ciliary localization of transient receptor potential (TRP) ion channels (Tobin *et al.*, 2002; Kim *et al.*, 2003; Nauli *et al.*, 2003). Recently, vertebrate cilia have been shown to mediate not only environmental inputs, but also the Hedgehog (Hh) developmental cue that triggers translocation of the Smoothened (Smo) GPCR into the cilium (May *et al.*, 2005; Huangfu and Anderson, 2006). Vertebrate cilia also ex-

press the somatostatin receptor sst3, serotonin 5-HT₆ receptor, platelet-derived growth factor receptor α (PDGFR α) and epidermal growth factor receptor (EGFR; Brailov *et al.*, 2000; Whitfield, 2004; Ma *et al.*, 2005; Schneider *et al.*, 2005). Clearly, receptors and channels must be precisely located and regulated to endow an individual cilium with its specific properties. The mechanisms regulating ciliary membrane localization are not well understood and are likely to involve targeting, trafficking, retention, and endocytic removal. Improper ciliary receptor or channel localization could potentially cause sensory transduction defects and human ciliary diseases.

Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic disease and a major cause of end-stage renal disease (Igarashi and Somlo, 2002). Mutation in either the *PKD1* or *PKD2* gene is responsible for nearly all ADPKD cases. *PKD1* and *PKD2* gene products, PC1 and PC2, 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 Delmas, 2004; Igarashi and Somlo, 2002). TRP channels function in a broad range of sensory modalities (Clapham, 2003). PC1 and PC2 function together as a mechanosensory receptor/channel complex on kidney primary cilia and may be involved in sensing liquid shear stress and urine flow (Igarashi and Somlo, 2002; Pazour *et al.*, 2002; Yoder *et al.*, 2002; Nauli and Zhou, 2004; Delmas, 2005).

PKD1 and *PKD2* are the founding members of the polycystin gene family. In mammals, five PC1 genes (*PKD1*, *PKD1L1*, *PKD1L2*, *PKD1L3*, and *PKDREJ*) and three PC2 genes (*PKD2*, *PKD2L1*, and *PKD2L2*) have been identified. Interestingly, the PC1-PC2 gene family acts as a functional unit in numerous developmental and physiological processes, including mammalian kidney tubulogenesis (PC1 and PC2), fertilization in mammals and sea urchin (*PKDREJ* and several PC2 family members), mammalian sour taste reception (*PKD1L3* and *PKD2L1*), and *Caenorhabditis elegans* mating behavior (LOV-1 and PKD-2; Barr *et al.*, 2001; Neill *et al.*, 2004; Delmas, 2005; Ishimaru *et al.*, 2006; Sutton *et al.*,

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2006). The polycystins have been found in various subcellular compartments, including the endoplasmic reticulum (ER), plasma membrane (PM), and cilium. The knowledge of polycystin localization mechanisms is critically important for understanding their roles in health and human disease.

The transparent nematode *C. elegans* provides a powerful model to study human ciliary diseases, ciliogenesis, sensory transduction, and ciliary receptor localization in vivo (Barr, 2005). Many human disease genes that are required for normal cilia formation and function have *C. elegans* counterparts, including the ADPKD genes *lov-1* and *pkd-2*. *C. elegans* LOV-1 (PC1 family) and PKD-2 (PC2 family) colocalize in cilia of male-specific sensory neurons and act in a sensory capacity (Barr and Sternberg, 1999; Barr *et al.*, 2001). *lov-1* and *pkd-2* mutant males are defective in two sensory behaviors: response to contact with a potential mate and location of the mate's vulva (the Lov phenotype). Hence, *C. elegans* male mating behavior provides a powerful read-out for polycystin function and ciliary localization mechanisms.

In a previous study (Hu *et al.*, 2006), we proposed that cilia use a down-regulation process to adjust polycystin ciliary localization and signaling. In *C. elegans*, hyper-phosphorylation of PKD-2 promotes removal of this channel from cilia, which may be due to the overactivated channel activity (Hu *et al.*, 2006). In yeast, *Drosophila*, and mammalian cell culture, the STAM (signal-transducing adaptor molecule) and Hrs (hepatocyte growth factor regulated tyrosine kinase substrate) complex binds and sorts internalized and monoubiquitinated membrane proteins on the early endosome to the multivesicular body (MVB) for lysosomal degradation (Asao *et al.*, 1997; Komada *et al.*, 1997; Bilodeau *et al.*, 2002; Lloyd *et al.*, 2002; Raiborg *et al.*, 2002; Shih *et al.*, 2002; Takata *et al.*, 2000; Bache *et al.*, 2003; Mizuno *et al.*, 2003). Whether sensory cells use this lysosomal targeting mechanism to down-regulate ciliary receptor signaling is not known.

Herein, we demonstrate that STAM physically associates with the LOV-1 C-terminus and the STAM-Hrs complex directs internalized LOV-1 and PKD-2 on endosomes at the ciliary base to lysosomal degradation pathway. These data provide insight into a mechanism that modulates polycystin ciliary abundance and sensory signaling and demonstrate that sensory cells use receptor down-regulation and degradation to fine-tune ciliary sensory transduction.

MATERIALS AND METHODS

Strains and Alleles

Nematode culturing and genetics were performed as described (Brenner, 1974). *him-5(e1490)* was used as the wild type (Hodgkin, 1983). *stam-1(ok406)* was obtained from the *Caenorhabditis* Genetics Center (CGC). We constructed transgenic lines by injecting plasmid DNA (total 120 ng/ μ l) using standard protocols (Mello and Fire, 1995). In all experiments we used plasmid pBX1 containing the wild-type *pha-1(+)* gene as a cotransformation marker in the *pha-1(ts)* strain (Granato *et al.*, 1994). STAM-1 and HGRS-1 plasmids were injected at 0.6 ng/ μ l, 6 ng/ μ l, and 60 ng/ μ l.

Molecular Biology Techniques

The full-length LOV-1::GFP plasmid was constructed by inserting GFP into the Eco47III site of a 13-kb genomic *lov-1* fragment (Barr and Sternberg, 1999). LOV-1::GFP is able to restore *lov-1(sy582)* response and vulva location efficiencies from ~20% to 55–60% ($n = 72$). PKD-2::GFP is described in (Bae *et al.*, 2006; Hu *et al.*, 2006). All other expression plasmids were built by cloning gene-specific promoters and cDNAs into green fluorescent protein (GFP)-tagging Fire vector pPD95.75. By using cDNAs with or without a stop codon, we generated STAM-1A and HGRS-1 GFP-tagged or untagged constructs, respectively. For the *pkd-2* promoter, a 1.3-kb genomic fragment upstream of the start codon was used (Hu and Barr, 2005). For the *stam-1* promoter, a 1.5-kb genomic fragment upstream of the *stam-1* start codon was used. For the *Ppkd-2::Ubi-PKD-2::GFP* construct, PCR was used to fuse a ubiquitin cDNA to the 5' end of the *pkd-2* cDNA, followed by cloning into the Fire vector. *Ppkd-2::STAM-1AΔUIM* was PCR-generated, removing amino acids 149–196.

Yeast Two-Hybrid Experiments

The yeast strain AH 109 (Clontech, Palo Alto, CA) was used for yeast two-hybrid (Y2H) experiments. A cDNA library derived from mixed-stage *him-5* animals was constructed in the GAL4 activation domain (DNA-AD) vector, pGADGH (Hu and Barr, 2005). Bait proteins were expressed in the GAL4 DNA-binding domain (DNA-BD) vector, pGBKT7. The C-terminus of *C. elegans* LOV-1 (amino acids: 3056–3178) was used as bait in Y2H experiments. Different *stam-1* fragments (see Figure 1) were cloned into the pGADT7 vector. Protein-protein interactions were accessed by growth rate on SD–Leu–Trp–His–Ade high stringency plates and β -galactosidase filter assays.

Mating Behavior Assay

Mating behavior assays are scored as described (Barr and Sternberg, 1999). Response efficiency reflects the percentage of males successfully responding to hermaphrodite contact within 5 min. An individual male's vulva-location ability was calculated as the number of positive vulva locations divided by the total number of vulva encounters. Vulva-location efficiency indicates the average behavior of a genotypic population. 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.

Imaging Analysis and Fluorescence Intensity Measurements

Epi-fluorescence microscopy experiments were carried out 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 (Richmond, CA). To measure cilium/cell body fluorescence intensity ratio (Hu *et al.*, 2006), L4 worms were picked and cultured at room temperature for 16–20 h. Confocal or epi-fluorescence images were taken on adults. Overexposure was avoided by making sure fluorescence was not saturated in any area on confocal images. Mean fluorescence intensities of the cilium region (including cilium proper, transition zone, and ciliary base), the corresponding cell body (including nucleus) and background (area without worm) were measured using Openlab software (Improvision, Lexington, MA). Cilium/cell body fluorescence intensity ratio was calculated by the following formula: (mean cilium fluorescence intensity – background fluorescence intensity)/(mean cell body fluorescence intensity – background fluorescence intensity).

RESULTS

C. elegans STAM Interacts with the LOV-1 Carboxy Terminus

To identify proteins that associate with LOV-1 and participate in polycystin ciliary localization and/or signaling, we performed a yeast two-hybrid screen with the 128-amino acid LOV-1 cytoplasmic C-tail as bait. Of 1×10^6 cDNAs screened, the interactor STAM-1A was isolated twice. STAM-1A does not bind the C-tail of PKD-2, demonstrating a specific association with the LOV-1 C-tail.

stam-1 (also called *pqn-19*) encodes the single STAM homolog in the *C. elegans* genome. Originally named for a role in cytokine signaling, STAM (signal transducing adaptor molecule) forms a complex with Hrs (hepatocyte growth factor regulated tyrosine kinase substrate) on the cytoplasmic face of the early endosome. The STAM-Hrs complex sorts monoubiquitinated membrane receptors to MVBs for lysosomal degradation in yeast, *Drosophila*, and mammals. The *C. elegans* genome encodes one Hrs homolog, which has been referred to as *hgrs-1* (hepatocyte growth factor-regulated tyrosine kinase substrate), *pqn-9* (www.wormbase.org), and *Cevps-27* (Roudier *et al.*, 2005).

STAM protein family members share a similar domain organization (Figure 1A). To identify the LOV-1 binding site of STAM-1A, we examined the ability of each domain to associate with the LOV-1 C-tail. As shown in Figure 1B, the C-terminal proline (P)-glutamine (Q)-rich region (amino acids 302–457) interacts with the LOV-1 C-terminus. In contrast, N-terminal fragments containing the VPS-27/Hrs/STAM (VHS), ubiquitin-interacting motif (UIM), and Src homology 3 (SH3) domains (amino acids 1–301) together or separately exhibit no binding. These results indicate that the STAM-1A C-terminus

Figure 1. STAM-1A interacts with the LOV-1 C-terminus. (A) *C. elegans* STAM-1 has two splicing isoforms, STAM-1A and STAM-1B. STAM has a shortened PQ-rich domain plus a unique 9-amino acid stretch its C-terminus (represented by a red triangle). All STAM family members share a similar structure with a VHS (VPS-27/Hrs/STAM) domain, UIM (ubiquitin-interacting motif) domain, SH3 (Src homology 3) domain, and a proline/glutamine (PQ)-rich C-terminus. The C-terminus of vertebrate STAM contains an ITAM (immunoreceptor tyrosine-based activation motif) that binds SH2-domain-containing proteins. The ITAM domain is lacking in yeast, *C. elegans*, and *Drosophila* STAM proteins. The *stam-1* genomic structure and *ok406* deletion were reproduced from Wormbase (www.wormbase.org). (B) Y2H assays define the minimal region responsible for the interaction between STAM-1 and the LOV-1 C-terminus as the 155-aa STAM-1A C-terminus. The STAM-1B C-terminus does not interact with the LOV-1 C-terminus.

is necessary and sufficient for LOV-1 binding. To narrow down the region of LOV-1 that interacts with STAM-1A, we split the LOV-1 C-terminus into two fragments corresponding to amino acids 3051-3119 and 3120-3178. Both fragments interact with STAM-1A in the Y2H assay, although the former was stronger (data not shown). These data indicate that STAM-1A may interact with the LOV-1 C-terminus at multiple sites.

stam-1 has two splicing isoforms, *stam-1a* and *stam-1b*. STAM-1B (also called PQN-19B) has a shorter C-terminus (lacking the last 59 amino acids of STAM-1A) plus a 9-amino acid extension (Figure 1A). Interestingly, the STAM-1B C-terminus (amino acids 302-397) exhibited no binding (Figure 1B), indicating that the interaction with LOV-1 is STAM-1A isoform-specific.

STAM-1A Localizes to Endosomal-like Structures in Cell Bodies, Dendrites, and Ciliary Bases in Polycystin-expressing Male-specific Sensory Neurons

stam-1 is expressed in many tissues, including the pharyngeal intestinal valve, several head neurons, and phasmids in both males and hermaphrodites throughout development (data not shown). In males, *stam-1* expression is also observed in the gonad and sensory neurons in the tail. *stam-1* and *pkd-2* are

clearly coexpressed in male-specific ciliated CEM, ray B-type (RnB), and hook HOB sensory neurons (Figure 2B).

A GFP-tagged STAM-1A fusion protein (*Pstam-1a::STAM-1A::GFP*, P stands for promoter) localized to cytoplasmic and dendritic puncta resembling endosomes, and appeared to accumulate at the ciliary base, which corresponds to the distal-most end of the dendrite and/or transition zone, but not the cilium proper (Figure 2C, enlarged panel). To more carefully examine STAM-1A subcellular distribution pattern, the *pkd-2* promoter was used to restrict STAM-1A::GFP expression (*Ppkd-2::STAM-1A::GFP*) to male-specific sensory neurons. STAM-1A::GFP labels puncta and accumulates at the ciliary bases, but is obviously excluded from the cilium proper of CEM, RnB and HOB neurons (Figure 2D). In contrast to STAM-1A, both PKD-2 and LOV-1 localize to the ciliary base and cilium proper (Figure 2A). The collocation of STAM-1A, LOV-1, and PKD-2 at the ciliary base suggests that STAM-1A may play a role in polycystin trafficking.

STAM-1A and HGRS-1 Collocate with RAB-5 on Early Endosomes at the Ciliary Base

To confirm that STAM and Hrs also localize to early endosomes in *C. elegans* sensory neurons, we performed colocal-

Figure 2. STAM-1A coexpresses with LOV-1 and PKD-2 in male-specific neurons and shows an endosome-like distribution pattern. (A) Anatomy of *C. elegans* male and PKD-2-expressing neurons. Modified from (Hu *et al.*, 2006). Top, differential interference contrast (DIC) side view image of an adult *C. elegans* male oriented head (left) to ventral up tail (right). Middle, *lov-1* and *pkd-2* are expressed in male head CEM neurons (left) and tail ray B type neurons (except R6B) and the hook HOB neuron (right). Bottom, confocal micrographs of PKD-2::GFP expression pattern in male head and tail. The cilium, dendrite, cell body, and axon of the CEMD (dorsal CEM) and CEMV (ventral CEM) neuron are drawn in the male head diagram. CEM 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 nine left-right bilateral pairs, numbered 1–9, anterior to posterior (ventral up view shown here). Rays are required for response to contact with a potential mate (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) Confocal micrographs of *Pstam-1*::DsRed2 (using the *stam-1* promoter to drive DsRed2 expression) and PKD-2::GFP double-labeled *C. elegans* males. In all figures, adult males are oriented anterior to posterior, with the head pointing left and the tail pointing right. *stam-1* coexpresses with *pkd-2* in CEMs (head), RnBs, and HOB (tail, arrow points to HOB neuron). (C) With its native promoter, STAM-1A::GFP displays an endosomal-like pattern throughout the neuronal cell bodies. Note that STAM-1A also localizes to the cilia base of cilia in enlarged figure (right panel). In this and all subsequent figures, the arrow marks the ciliary base, and the arrowhead marks the approximate end of the cilium. (D) To clearly visualize the localization pattern of STAM-1A in polycystin-expressing neurons, the *pkd-2* promoter is used to drive *stam-1a* cDNA expression only in CEMs, RnBs, and HOB neurons. STAM-1A localizes to the ciliary base (arrow), but is excluded from the cilium proper (region between arrow and arrowhead). Large green puncta are observed along distal dendrites of RnB ray neurons. Enlarged figures show the cilia region of CEM and R3B. Scale bars in rightmost C and D panels, 5 μ m; in all other micrographs scale bars, 10 μ m.

ization experiments with STAM, Hrs, and the early endosomal marker RAB-5 (Sato *et al.*, 2005). STAM-1 and HGRS-1

expression completely overlaps in cell bodies and ciliary bases of polycystin-expressing neurons (*Ppkd-2*::STAM-1A::

Figure 3. STAM-1A is an early endosomal protein that localizes with RAB-5 and HGRS-1 in male-specific neurons. (A) Confocal micrographs of *Ppkd-2::STAM-1A::DsRed2* and *Ppkd-2::HGRS-1::GFP* double-labeled males. STAM-1A and HGRS-1 colocalize in cell bodies, the bases of cilia, and along RnB dendrites. Like STAM-1, HGRS-1 does not localize to the cilium proper. Arrows point to colocalization of STAM-1A and HGRS-1 at the ciliary base of CEM and R3B. (B) Confocal micrographs of *Ppkd-2::STAM-1A::GFP* and *Ppkd-2::mRFP::RAB-5* double-labeled males. RAB-5 is an early endosomal marker (Sato *et al.*, 2005). STAM-1A colocalizes with RAB-5 in the cell body and ciliary base. (C) Higher magnification images show that RAB-5 and STAM-1A collocate in CEM cell body. (D) Higher magnification fluorescence and DIC/fluorescence images of CEM cilia. RAB-5 and STAM-1A are never observed in the cilium proper. Scale bars, (A and B) 10 μ m, and (C and D) 5 μ m.

DsRed2 and *Ppkd-2::HGRS-1::GFP*, Figure 3A). Moreover, STAM-1 and RAB-5 collocate in the cell bodies and ciliary bases of polycystin-expressing neurons (*Ppkd-2::STAM-1A::GFP* and *Ppkd-2::mRFP::RAB-5*, Figure 3, B–D). Combined, these data indicate that *C. elegans* STAM-1A colocalizes with HGRS-1 on the early endosome at the ciliary base, where they may participate in endocytic sorting of LOV-1 and PKD-2.

STAM-1 Regulates Endosomal Sorting of LOV-1 and PKD-2

If STAM-1A and HGRS-1 are important for LOV-1 and PKD-2 endocytic sorting, then mutations that disrupt *stam-1* or *hgrs-1* should affect polycystin localization and/or function. *hgrs-1* null mutations are larval lethal (Roudier *et al.*, 2005), precluding analysis of adult phenotypes. RNA interference (RNAi) targeting *stam-1a* or *stam-1b* did not result in embryonic or larval lethality, suggesting that *stam-1* is not essential for viability (Fraser *et al.*, 2000; Sonnichsen *et al.*, 2005). To genetically deduce the function of *stam-1*, we analyzed the *stam-1(ok406)* putative null allele that contains a large deletion from the third to final exons (Figure 1A). The *ok406* allele deletes the majority of the STAM-1 protein (both A and B isoforms), including a portion of the VHS domain, the entire UIM and SH3 domains, and the majority of the

PQ-rich C-terminus (Figure 1A). *stam-1(ok406)* hermaphrodites produce greater than 80% unfertilized and dead eggs. Consistent with this sterility defect, *stam-1* was identified as a germline regulator of oocyte meiotic maturation in an RNAi screen (Govindan *et al.*, 2006). Transgene silencing is common in the germline and would explain why we did not detect STAM-1::GFP in sperm or oocytes. These data suggest that *stam-1* may have multiple sites of cellular action that include the germline and neurons.

The remaining viable *stam-1(ok406)* adult hermaphrodites exhibit normal locomotion, feeding, food sensation, and egg laying behaviors. Sensory cilia of *stam-1* mutants are intact as judged by fluorescent dye filling of amphid and phasmid neurons (data not shown). *stam-1(ok406)* adult males exhibit a slight, but statistically significant defect in the response step of mating behavior (response efficiency: $79.3 \pm 4.6\%$ for *stam-1(ok406)* males versus $89.1 \pm 2.1\%$ for wild-type males, $p < 0.05$). The response defect is consistent with *stam-1* regulating the polycystins.

In mice, knockout of STAM causes decreased levels of Hrs (Kanazawa *et al.*, 2003). To determine if Hrs levels are similarly affected by disruption of *stam-1* in *C. elegans*, we performed RT-PCR using mRNA isolated from *stam-1* and wild-type animals. We find that *hgrs-1* levels are normal in the *stam-1* mutant background (data not shown).

Figure 4. LOV-1 and PKD-2 accumulate at the ciliary base in the *stam-1(ok406)* mutant. Confocal micrographs of LOV-1::GFP and PKD-2::GFP transgenic males. (A) LOV-1::GFP localizes to the CEM cilium proper in wild type (left). In the *stam-1* mutant, LOV-1::GFP and small puncta (hollow arrows) accumulate at the CEM ciliary base. Small vesicle-like puncta accumulate around the CEM ciliary base (hollow arrows). (B) Fluorescence intensity ratios measure increased ciliary base accumulation of LOV-1::GFP in the *stam-1* mutant. (C) PKD-2::GFP accumulates at the CEM ciliary base in a *stam-1* mutant. Small PKD-2::GFP puncta are observed below the CEM ciliary base (hollow arrows). Right panels show a higher magnification of the CEMs in the male nose. Top panels reproduced from Hu *et al.* (2006). (D) In RnB neurons, PKD-2::GFP accumulates at the ciliary base and along the distal dendrites of *stam-1* males (bottom) compared with wild type (top). High magnifications show R3B of wild-type (top) and *stam-1* (bottom) males. In all figures, the arrowhead points to the tip of the cilium. The solid arrow points to the ciliary base. Hollow arrows point the puncta observed in the RnB distal dendrite. Scale bars in rightmost C and D, 5 μ m; in all other micrographs scale bars, 10 μ m. (E) Fluorescence intensity ratios measure increased ciliary base accumulation of PKD-2::GFP in the *stam-1* mutant.

To determine whether *stam-1* regulates polycystin trafficking, we compared the localization of functional LOV-1::GFP and PKD-2::GFP fusion proteins in wild-type and *stam-1* mutant males. *lov-1* encodes a predicted 3178-amino acid protein, making cDNA structure–function–localization studies prohibitively difficult. Hence, a full-length LOV-1::GFP reporter was generated by inserting GFP into a rescuing *lov-1* genomic clone between amino acids 3119 and 3220 (Barr and Sternberg 1999). LOV-1::GFP and PKD-2::GFP are able to rescue the response and Lov defects of the *lov-1*(sy582) and *pkd-2*(sy606) single mutants, respectively (Materials and Methods, Bae *et al.*, 2006). Faint LOV-1::GFP expression is detected in CEM cilia (Figure 4A, left), but only rarely observed in hook HOB and ray RnB cilia (data not

shown), which may be due to mosaicism or very low *lov-1* expression levels. PKD-2::GFP is more strongly expressed than LOV-1::GFP, facilitating analysis of STAM-1 function in polycystin ciliary localization. In wild-type animals, PKD-2::GFP primarily localizes to the cell body, ciliary base, and cilium proper (Figure 4, C and D) and is observed moving bidirectionally along the dendrite (Bae *et al.*, 2006).

In *stam-1(ok406)* males, LOV-1::GFP and PKD-2::GFP localize normally to the cilium proper of CEM neurons (Figure 4, A and C). However, both LOV-1::GFP and PKD-2::GFP accumulate at the ciliary base (arrowhead in Figure 4, A and C) and are often observed in discrete puncta in the ciliary base region (thin arrows in Figure 4, A and C). In RnB neurons of *stam-1* mutant males, many PKD-2::GFP-labeled

Figure 5. Overexpression of STAM-1A or HGRS-1 interferes with polycystin mediated male sensory behaviors and polycystin ciliary localization. STAM-1A (A) or HGRS-1 (B) are injected into wild-type animals. Greater behavioral and targeting effects are observed with increasing concentrations of injected *Ppkd-2::STAM-1A::GFP* or *Ppkd-2::HGRS-1::GFP* DNA (0.6, 6, and 60 ng/ μ l). *myEx* stands for extra-chromosomal array in transgenic animals. (C) To rule out potential effects of a GFP tag on STAM-1A and HGRS-1 in A and B, tagless *Ppkd-2::STAM-1A* and *Ppkd-2::HGRS-1* constructs were injected at 60 ng/ μ l into wild-type animals. Similar defects in response and vulva location behaviors are observed with tagged and untagged versions of STAM-1A and HGRS-1. (D) Transgenic animals from C were crossed with another transgenic line containing an integrated, functional *PKD-2::GFP* to generate the strains *PKD-2::GFP; myEx(Ppkd-2::STAM-1A)* and *PKD-2::GFP; myEx(Ppkd-2::HGRS-1)*. Fluorescence intensity ratios show that overexpression of STAM-1A or HGRS-1 dramatically reduces *PKD-2* ciliary localization. (E) As negative controls, *Ppkd-2::STAM-1AΔUIM* and *Ppkd-2::STAM-1B* constructs were injected at 60 ng/ μ l into wild-type animals. No response or location of vulva (Lov) defect is observed. (F) Transgenic animals obtained from E were crossed with *PKD-2::GFP*. Fluorescence intensity ratios show no change in *PKD-2::GFP* distribution with STAM-1AΔUIM or STAM-1B overexpression. Mating behavior assays are described in *Materials and Methods*. Figures were generated using Sigmaplot5 (Jandel Scientific, San Rafael, CA). Data are represented as mean $\pm$ SEM. **p < 0.01 compared with control.

Figure 6. *stam-1* is involved in down-regulating ubiquitinated or hyper-phosphorylated PKD-2 from sensory cilia. (A) Ubi-PKD-2::GFP is observed in neuronal cell bodies but virtually absent from cilia. (B) Ubi-PKD-2 cannot rescue the response and vulva location defects of *pkd-2(sy606)* mutants. (C) In the *stam-1(ok406)* mutant, Ubi-PKD-2 accumulates at the ciliary base, which is similar to the PKD-2::GFP distribution pattern in the *stam-1(ok406)* mutant. In RnB ray neurons, Ubi-PKD-2::GFP puncta are observed along the distal dendrites. Right panels show a magnified view of the cilia region of the CEMs and R3B. (D) Fluorescence intensity ratios show an increase of Ubi-PKD-2 at the ciliary base in *stam-1(ok406)* mutant compared with Ubi-PKD-2 in wild type. (E) In wild type, PKD-2^{S534D}::GFP is observed in neuronal

puncta accumulate along the distal dendrite (Figure 4D, bottom). PKD-2 dendritic motility is intact, indicating that *stam-1* is not required for transportation between the neuronal cell body and ciliary base. LOV-1 and PKD-2 subcellular distribution at ciliary base in *stam-1(ok406)* animals is similar to the STAM-1A::GFP localization pattern in wild-type male sensory neurons (compare Figure 4, A, C, and D, with Figure 2D), indicating that the polycystins may accumulate in endosomes in the absence of STAM. The finding that LOV-1 and PKD-2 accumulate at ciliary bases in a *stam-1* mutant background is consistent with the evolutionarily conserved role for STAM in sorting membrane receptors for lysosomal degradation.

Accelerating Endosomal Sorting Affects PKD-2 Ciliary Function and Localization

If STAM-1A and HGRS-1 function to sort endocytosed LOV-1 and PKD-2 to the MVBs for lysosomal degradation, then accelerating this process may affect polycystin ciliary abundance and function. To test this hypothesis, we overexpressed each protein in male-specific sensory neurons and scored effects on male mating behavior and polycystin localization. We observed greater behavioral and targeting effects with increasing concentrations of injected *Ppkd-2::STAM-1A::GFP* or *Ppkd-2::HGRS-1::GFP* DNA (Figure 5).

STAM-1A or HGRS-1 overexpression resulted in response and location of vulva (Lov) defects, similar to that of polycystin mutant males (Figure 5, A and B). Overexpression of GFP-tagged or untagged STAM-1A or HGRS-1 resulted in consistent and comparable response and Lov defects (Figure 5C). STAM-1A or HGRS-1 overexpression dramatically reduces the amount of PKD-2 in the cilium and ciliary base (Figure 5D). As a negative control, the STAM-1B isoform that does not interact with LOV-1 was overexpressed and has no effect on male mating behavior (Figure 5E) or PKD-2::GFP ciliary localization patterns (Figure 5F). This data indicates STAM-1A, but not STAM-1B, specifically acts in the polycystin down-regulation process. We propose that endocytosed PKD-2 may either be recycled or degraded and that overexpression of STAM-1A or HGRS-1 accelerates the latter process, thereby reducing PKD-2 ciliary signaling and membrane distribution.

STAM-1A Sorts Phosphorylated and Ubiquitinated PKD-2 for Lysosomal Degradation

The UIM domain of STAM and Hrs binds the ubiquitin moiety of ubiquitinated membrane receptors is critical for sorting and down-regulation (Bilodeau *et al.*, 2002; Polo *et al.*, 2002; Shih *et al.*, 2002; Bache *et al.*, 2003; Fisher *et al.*, 2003; Hicke and Dunn, 2003; Mizuno *et al.*, 2003; Swanson *et al.*, 2003; Urbe *et al.*, 2003; Komada and Kitamura, 2005). The UIM itself is required for monoubiquitination of sorting machinery proteins (such as Epsin, Eps15, Hrs, and STAM),

suggesting that the UIM may function other than as a sorting determinant (Oldham *et al.*, 2002; Polo *et al.*, 2002). We generated a STAM-1AΔUIM truncation protein lacking the UIM domain. Overexpression of STAM-1AΔUIM does not affect male mating behaviors or PKD-2 ciliary localization (Figure 5, E and F) nor does STAM-1AΔUIM rescue the PKD-2::GFP localization defects of a *stam-1* mutant (data not shown), demonstrating an essential role for the UIM domain in polycystin down-regulation.

To test the hypothesis that ubiquitination plays a role in polycystin down-regulation, we directly coupled a 76-amino acid ubiquitin tag to the amino terminus of PKD-2 (Ubi-PKD-2::GFP). A similar approach was used by Kaplan and colleagues to show that direct conjugation of ubiquitin to the *C. elegans* glutamate receptor GLR-1 promotes endocytic removal at synapses (Burbea *et al.*, 2002). Ubi-PKD-2::GFP exhibits a completely normal cell body localization pattern but is largely absent from the cilium and ciliary base (Figure 6A) and cannot rescue the response and Lov defects of a *pkd-2* mutant (Figure 6B). Ubi-PKD-2::GFP dendritic transport and ciliary targeting are intact (data not shown), suggesting that ciliary Ubi-PKD-2::GFP is targeted for degradation.

In general, ubiquitinated proteins may be degraded via the proteasome or lysosome. Efficient degradation of ubiquitinated proteins by the proteasome requires the formation of polyubiquitin chains containing at least four ubiquitin moieties at ubiquitin K48 site (Thrower *et al.*, 2000). Conversely, monoubiquitination is sufficient for sorting into MVBs and subsequent lysosomal degradation, a mechanism mediated by the STAM-Hrs complex. We tagged PKD-2 with the ubiquitin mutant Ubi(K48R), which cannot be polyubiquitinated. Ubi(K48R)-PKD-2::GFP and Ubi-PKD-2::GFP localization patterns are identical (data not shown), suggesting that PKD-2 degradation does not require K48 polyubiquitination and is likely due to STAM-Hrs-mediated lysosomal degradation. Next, we reasoned that by blocking STAM-1, Ubi-PKD-2 should accumulate at the ciliary base. Consistent with this hypothesis, Ubi-PKD-2::GFP localizes to the ciliary bases of CEM and RnB neurons in the *stam-1* mutant (Figure 6C). In the *stam-1* mutant, fluorescence intensity values for Ubi-PKD-2::GFP at the ciliary base increased 10-fold compared with a wild-type background (Figure 6D). In RnB neurons, Ubi-PKD-2::GFP puncta are also observed in the distal dendrite. Unfortunately, attempts at detecting ubiquitinated PKD-2 in vivo have been unsuccessful, likely due to the difficulty of detecting endogenous PKD-2 on a Western blot. These data suggest that STAM-1A acts on early endosomes located at the ciliary base and sorts LOV-1 and PKD-2 for lysosomal degradation.

We next determined whether retrograde transport by the CHE-3 dynein or cytoplasmic dynein retrograde motor (Signor *et al.*, 1999; Wicks *et al.*, 2000; Koushika *et al.*, 2004) is required for STAM-1-mediated polycystin down-regulation. We examined Ubi-PKD-2::GFP in *che-3(e1379)* and *dhc-1(or195)* (dynein heavy chain) single mutants. Similar to the wild-type background, Ubi-1::PKD-2::GFP levels are greatly reduced in *che-3* and *dhc-1* mutant males (data not shown). These observations suggest that *che-3* and *dhc-1* either act upstream of *stam-1* or do not function at all in the PKD-2 down-regulation process.

To further explore the effects of posttranslational modifications on ciliary receptor down-regulation, we made use of the "phosphomimetic" PKD-2^{S534D} mutant. Casein kinase 2 (CK2) and calcineurin (TAX-6) act in concert to regulate the phosphorylation state of PKD-2 at the S534 site (Hu *et al.*, 2006). When S534 is constitutively phosphorylated via the

Figure 6 (cont). cell bodies, but mostly absent from cilia (reproduced from Hu *et al.*, 2006). (F) In the *stam-1(ok406)* mutants, PKD-2^{S534D} accumulates at the ciliary base, which is similar to the Ubi-PKD-2 localization pattern in *stam-1(ok406)* mutants. In RnB neurons, small PKD-2^{S534D}::GFP puncta distribute along the distal dendrites. Right panels show a higher magnification of the cilia region of CEM and R3B neurons. (G) Fluorescence intensity ratios demonstrate an increase of PKD-2^{S534D} at the ciliary base in *stam-1(ok406)* mutants when compared with PKD-2^{S534D} in wild type. Data are represented as mean $\pm$ SEM. ***p* < 0.01 compared with control. Scale bars in rightmost C and F panels, 5 μ m; in all other micrographs scale bars, 10 μ m.

Figure 7. Model of the TRPP down-regulation and degradation process. In *C. elegans* sensory cilia, LOV-1 and PKD-2 might assemble into a receptor-channel complex as established for the mammalian polycystins in renal primary cilia (Nauli *et al.*, 2003). Activation of polycystins causes an increase in intracellular Ca^{2+} . Polycystin signaling and localization is controlled by a dynamic phosphorylation cycle (Cai *et al.*, 2004; Kottgen *et al.*, 2005; Hu *et al.*, 2006). Here we show that LOV-1 and PKD-2 signaling and localization are also regulated by ubiquitination and internalization at the ciliary base. The LOV-1 C-tail serves as a recognition signal to recruit the STAM-1-HGRS-1 (STAM-Hrs) sorting machinery to the polycystin complex. The STAM-Hrs machinery then sorts the polycystin complex the lysosome for degradation.

S534D mutation, PKD-2 ciliary abundance, but not initial ciliary targeting, is dramatically reduced. We proposed that PKD-2^{S534D} may represent an overactivated channel whose activity is signaling down-regulated via removal from cilia. To test this possibility, we examined PKD-2^{S534D}::GFP localization in the *stam-1(ok406)* mutant. In *stam-1* animals, PKD^{S534D}::GFP localizes to the ciliary base, and the fluorescence intensity ratios for PKD-2^{S534D}::GFP increase about fivefold (Figure 6F). Strikingly, wild-type PKD-2::GFP, Ubi-PKD-2::GFP, and PKD-2^{S534D}::GFP accumulate at the *stam-1(ok406)* ciliary base in a similar pattern (compare Figure 3, C and D, with Figure 6, C and E, respectively). These data suggest that PKD-2^{S534D} and Ubi-PKD-2 are down-regulated and degraded via the same mechanism. Cumulatively, our data indicate that STAM-1A binds, sorts, and targets the polycystin complex for lysosomal degradation (Figure 7).

DISCUSSION

Receptor down-regulation protects an organism from uncontrolled signaling, cell growth, and proliferation (Katzmann *et al.*, 2002; Staub and Rotin, 2006). For example, ligand-activated EGFRs on the plasma membrane are internalized and sorted for lysosomal degradation (Schlessinger, 2000). Our report is the first to show that ciliary receptors and TRP channels may be down-regulated in a similar manner. Here, we demonstrate that down-regulation of the TRP polycystin complex involves phosphorylation, ubiquitination, endocytic sorting, and lysosomal degradation. Whether a similar TRPP down-regulation mechanism acts in mammals is not known, although the HECT ubiquitin ligase AIP4 has been recently shown to regulate the cell surface expression of TRPV4 and TRPC4, but not TRPP2 (Wegierski *et al.*, 2006). We propose that STAM-Hrs-mediated down-regulation controls ciliary TRPP receptor abundance and signaling. Too much or too little polycystin signaling results in a sensory defect in *C. elegans* males, and in mammals, may potentially result in PKD.

The STAM-Hrs complex acts on the early endosomes to sort internalized membrane proteins to MVB (Bilodeau *et al.*, 2003; Mizuno *et al.*, 2003; Raiborg *et al.*, 2003). Our report provides evidence that a membrane receptor (LOV-1) is cargo of the STAM-Hrs complex. Surprisingly, the LOV-1-

STAM-1 interaction does not require the UIM domain. How cargo proteins are recognized by STAM-Hrs complex is not well understood. One model involves binding of ubiquitinated cargo to the UIM domain found in STAM, Hrs, and many other components of the endocytic sorting machinery. Other lines of evidence hint at the existence of alternative recognition and sorting mechanisms (Raiborg *et al.*, 2002) and suggest that the UIM may have functions other than as a sorting determinant. We propose that LOV-1 functions as an adaptor to recruit STAM-1 and Hrs in close proximity to ubiquitinated PKD-2. From here, the LOV-1-PKD-2 complex may be trafficked to the MVB. Without the LOV-1-interacting PQ-domain, STAM-1 is not recruited to the polycystin unit; without the UIM domain, STAM-1 cannot function to down-regulate PKD-2. We failed to detect an interaction between the STAM-1 UIM and the PKD-2 C-tail (data not shown), which indicates that 1) STAM-1 and PKD-2 do not directly interact, 2) an interaction requires PKD-2 to be ubiquitinated, or 3) the interaction is transient or occurs outside the PKD-2 region tested.

Many membrane proteins are down-regulated. The internalization of some membrane proteins, such as CXCR4, EGFR, and Ste3, is ubiquitination-independent (Levkowitz *et al.*, 1998; Thien *et al.*, 2001; Chen and Davis, 2002; Marchese *et al.*, 2003), whereas other studies show that ubiquitination is essential for the rapid internalization of growth hormone receptor and Ste2 (Govers *et al.*, 1999; Dunn and Hicke, 2001). After internalization, these receptors may traverse one of two routes: recycling back to membrane or sorting to the lysosome for degradation. Cell- or receptor-specific mechanisms may exist to ensure the specificity and efficiency of the down-regulation. Thus, other adaptor proteins may be involved in targeting endocytosed membrane proteins to the MVB. On the early endosome, STAM-1A may bind LOV-1 and recruit the sorting machinery to the polycystin complex. Potential interactions between UIMs of other sorting components and the ubiquitinated polycystins may ensure efficient sorting and degradation.

Our data suggest that a phosphorylated and/or ubiquitinated polycystin complex is down-regulated via the STAM-Hrs machinery (Figure 7). The subcellular localization point at which these post-translational modifications occur is not known. Overexpression of STAM-1A or HGRS-1 results in similar phenotypes: male response and Lov defects and a

dramatic reduction of PKD-2 levels in the ciliary region. Conversely, knockout of *stam-1* has the opposite effect on PKD-2 distribution: an accumulation at the ciliary base and distal dendrite. We interpret our results to mean that STAM-1 and HGRS-1 act to promote ciliary receptor down-regulation and that blocking this pathway results in a failure to degrade LOV-1 and PKD-2. A similar process is observed in *Drosophila*, where Hrs acts to promote degradation of activated receptors (Lloyd *et al.*, 2002). In contrast, Hrs over-expression in mammalian cells inhibits lysosomal trafficking of ubiquitinated receptors (Chin *et al.*, 2001; Petiot *et al.*, 2003). The reasons for these experimental discrepancies are not known, but may reflect differences between in vivo and cultured cell systems.

Interestingly, ciliary and flagellar proteomes contain components of the ubiquitination machinery (Pazour *et al.*, 2005; Liu *et al.*, 2007). PC-1 interacts with the seven in Absentia (Siah-1) E3 ligase (Kim *et al.*, 2004), but the physiological relevance of this association is not known. Although sharing no obvious sequence homology, the short C-terminal tails of both human PC-1 and *C. elegans* LOV-1 are essential for their respective roles in kidney epithelial cells and worm sensory neurons (Barr and Sternberg, 1999; Vidorpe *et al.*, 2001; Nickel *et al.*, 2002; Aguiari *et al.*, 2003; Hooper *et al.*, 2003; Chauvet *et al.*, 2004; Kim *et al.*, 2004). The *C. elegans* EGFR LET-23, GPCR ODR-10, and TRPP signal transduction pathways are negatively regulated by the AP-1 mu1 clathrin adaptor UNC-101 (Lee *et al.*, 1994; Dwyer *et al.*, 2001; Bae *et al.*, 2006). Whether the STAM-Hrs complex acts in a common ciliary receptor down-regulation pathway remains to be seen.

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REFERENCES

- Aguiari, G., Campanella, M., Manzati, E., Pinton, P., Banzi, M., Moretti, S., Piva, R., Rizzuto, R., and del Senno, L. (2003). Expression of polycystin-1 C-terminal fragment enhances the ATP-induced Ca(2+) release in human kidney cells. *Biochem. Biophys. Res. Commun.* 301(3), 657–664.
- Asao, H., Sasaki, Y., Arita, T., Tanaka, N., Endo, K., Kasai, H., Takeshita, T., Endo, Y., Fujita, T., and Sugamura, K. (1997). Hrs is associated with STAM, a signal-transducing adaptor molecule. Its suppressive effect on cytokine-induced cell growth. *J. Biol. Chem.* 272(52), 32785–32791.
- Bache, K. G., Raiborg, C., Mehlum, A., and Stenmark, H. (2003). STAM and Hrs are subunits of a multivalent ubiquitin-binding complex on early endosomes. *J. Biol. Chem.* 278(14), 12513–12521.
- Badano, J. L., Mitsuma, N., Beales, P. L., and Katsanis, N. (2006) The ciliopathies: an emerging class of human genetic disorders. *Annu. Rev. Genomics Hum. Genet.* 7, 125–148.
- Bae, Y. K., Qin, H., Knobel, K. M., Hu, J., Rosenbaum, J. L., and Barr, M. M. (2006). General and cell-type specific mechanisms target TRPP2/PKD-2 to cilia. *Development* 133(19), 3859–3870.
- Barr, M. M. (2005). *Caenorhabditis elegans* as a model to study renal development and disease: sexy cilia. *J. Am. Soc. Nephrol.* 16(2), 305–312.
- Barr, M. M., DeModena, J., Braun, D., Nguyen, C. Q., Hall, D. H., and Sternberg, P. W. (2001). The *Caenorhabditis elegans* autosomal dominant polycystic kidney disease gene homologs *lov-1* and *pkd-2* act in the same pathway. *Curr. Biol.* 11(17), 1341–1346.
- Barr, M. M., and Sternberg, P. W. (1999). A polycystic kidney-disease gene homologue required for male mating behaviour in *C. elegans*. *Nature* 401(6751), 386–389.
- Bilodeau, P. S., Urbanowski, J. L., Winistorfer, S. C., and Piper, R. C. (2002). The Vps27p Hse1p complex binds ubiquitin and mediates endosomal protein sorting. *Nat. Cell Biol.* 4(7), 534–539.
- Bilodeau, P. S., Winistorfer, S. C., Kearney, W. R., Robertson, A. D., and Piper, R. C. (2003). Vps27-Hse1 and ESCRT-I complexes cooperate to increase efficiency of sorting ubiquitinated proteins at the endosome. *J. Cell Biol.* 163(2), 237–243.
- Brailov, I., Bancila, M., Brisorgueil, M. J., Miquel, M. C., Hamon, M., and Verge, D. (2000). Localization of 5-HT(6) receptors at the plasma membrane of neuronal cilia in the rat brain. *Brain Res.* 872(1–2), 271–275.
- Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* 77(1), 71–94.
- Buck, L., and Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 65(1), 175–187.
- Burbea, M., Dreier, L., Dittman, J. S., Grunwald, M. E., and Kaplan, J. M. (2002). Ubiquitin and AP180 regulate the abundance of GLR-1 glutamate receptors at postsynaptic elements in *C. elegans*. *Neuron* 35(1), 107–120.
- Cai, Y. *et al.* (2004). Calcium dependence of polycystin-2 channel activity is modulated by phosphorylation at Ser812. *J. Biol. Chem.* 279(19), 19987–19995.
- Chauvet, V. *et al.* (2004). Mechanical stimuli induce cleavage and nuclear translocation of the polycystin-1 C terminus. *J. Clin. Invest.* 114(10), 1433–1443.
- Chen, L., and Davis, N. G. (2002). Ubiquitin-independent entry into the yeast recycling pathway. *Traffic* 3(2), 110–123.
- Chin, L. S., Raynor, M. C., Wei, X., Chen, H. Q., and Li, L. (2001). Hrs interacts with sorting nexin 1 and regulates degradation of epidermal growth factor receptor. *J. Biol. Chem.* 276(10), 7069–7078.
- Clapham, D. E. (2003). TRP channels as cellular sensors. *Nature* 426(6966), 517–524.
- Delmas, P. (2004). Polycystins: from mechanosensation to gene regulation. *Cell* 118(2), 145–148.
- Delmas, P. (2005). Polycystins: polymodal receptor/ion-channel cellular sensors. *Pfluegers Arch.* 451(1), 264–276.
- Dunn, R., and Hicke, L. (2001). Multiple roles for Rsp5p-dependent ubiquitination at the internalization step of endocytosis. *J. Biol. Chem.* 276(28), 25974–25981.
- Dwyer, N. D., Adler, C. E., Crump, J. G., L'Etoile, N. D., and Bargmann, C. I. (2001). Polarized dendritic transport and the AP-1 mu1 clathrin adaptor UNC-101 localize odorant receptors to olfactory cilia. *Neuron* 31(2), 277–287.
- Fraser, A. G., Kamath, R. S., Zipperlen, P., Martinez-Campos, M., Sohrmann, M., and Ahringer, J. (2000). Functional genomic analysis of *C. elegans* chromosome I by systematic RNA interference. *Nature* 408(6810), 325–330.
- Fisher, R. D., Wang, B., Alam, S. L., Higginson, D. S., Robinson, H., Sundquist, W. I., and Hill, C. P. (2003). Structure and ubiquitin binding of the ubiquitin-interacting motif. *J. Biol. Chem.* 278(31), 28976–28984.
- Govindan, J. A., Cheng, H., Harris, J. E., and Greenstein, D. (2006). Galpho/i and Galphas signaling function in parallel with the MSP/Eph receptor to control meiotic diapause in *C. elegans*. *Curr. Biol.* 16(13), 1257–1268.
- Govers, R., ten Broeke, T., van Kerkhof, P., Schwartz, A. L., and Strous, G. J. (1999). Identification of a novel ubiquitin conjugation motif, required for ligand-induced internalization of the growth hormone receptor. *EMBO J.* 18(1), 28–36.
- Granato M, Schnabel H, and Schnabel R. (1994). pha-1, a selectable marker for gene transfer in *C. elegans*. *Nucleic Acids Res.* 22(9), 1762–1763.
- Hicke, L., and Dunn, R. (2003). Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. *Annu. Rev. Cell Dev. Biol.* 19, 141–172.
- Hodgkin, J. (1983). Male phenotypes and mating efficiency in *Caenorhabditis elegans*. *Genetics* 103, 43–64.
- Hooper, K. M., Unwin, R. J., and Sutters, M. (2003). The isolated C-terminus of polycystin-1 promotes increased ATP-stimulated chloride secretion in a collecting duct cell line. *Clin. Sci. (Lond.)* 104(3), 217–221.
- Hu, J., Bae, Y. K., Knobel, K. M., and Barr, M. M. (2006). Casein kinase II and calcineurin modulate TRPP function and ciliary localization. *Mol. Biol. Cell* 17(5), 2200–2211.
- Hu, J., and Barr, M. M. (2005). ATP-2 interacts with the PLAT domain of LOV-1 and is involved in *Caenorhabditis elegans* polycystin signaling. *Mol. Biol. Cell* 16(2), 458–469.
- Huangfu, D., and Anderson, K. V. (2006). Signaling from Smo to Ci/Gli: conservation and divergence of Hedgehog pathways from *Drosophila* to vertebrates. *Development* 133(1), 3–14.

- Igarashi, P., and Somlo, S. (2002). Genetics and pathogenesis of polycystic kidney disease. *J. Am. Soc. Nephrol.* 13(9), 2384–2398.
- Ishimaru, Y., Inada, H., Kubota, M., Zhuang, H., Tominaga, M., and Matsunami, H. (2006). Transient receptor potential family members PKD1L3 and PKD2L1 form a candidate sour taste receptor. *Proc. Natl. Acad. Sci. USA* 103(33), 12569–12574.
- Kanazawa, C., Morita, E., Yamada, M., Ishii, N., Miura, S., Asao, H., Yoshimori, T., and Sugamura, K. (2003). Effects of deficiencies of STAMs and Hrs, mammalian class E Vps proteins, on receptor downregulation. *Biochem. Biophys. Res. Commun.* 309(4), 848–856.
- Katzmann, D. J., Odorizzi, G., and Emr, S. D. (2002). Receptor downregulation and multivesicular-body sorting. *Nat. Rev. Mol. Cell Biol.* 3(12), 893–905.
- Kim, H., Jeong, W., Ahn, K., Ahn, C., and Kang, S. (2004). Siah-1 interacts with the intracellular region of polycystin-1 and affects its stability via the ubiquitin-proteasome pathway. *J. Am. Soc. Nephrol.* 15(8), 2042–2049.
- Kim, J. *et al.* (2003). A TRPV family ion channel required for hearing in *Drosophila*. *Nature* 424(6944), 81–84.
- Komada, M., and Kitamura, N. (2005). The Hrs/STAM Complex in the Downregulation of receptor tyrosine kinases. *J. Biochem. (Tokyo)* 137(1), 1–8.
- Komada, M., Masaki, R., Yamamoto, A., and Kitamura, N. (1997). Hrs, a tyrosine kinase substrate with a conserved double zinc finger domain, is localized to the cytoplasmic surface of early endosomes. *J. Biol. Chem.* 272(33), 20538–20544.
- Kottgen, M. *et al.* (2005). Trafficking of TRPP2 by PACS proteins represents a novel mechanism of ion channel regulation. *EMBO J.* 24(4), 705–716.
- Koushika, S. P., Schaefer, A. M., Vincent, R., Willis, J. H., Bowerman, B., and Nonet, M. L. (2004). Mutations in *Caenorhabditis elegans* cytoplasmic dynein components reveal specificity of neuronal retrograde cargo. *J. Neurosci.* 24(16), 3907–16.
- Lee, J., Jongeward, G. D., and Sternberg, P. W. (1994). unc-101, a gene required for many aspects of *Caenorhabditis elegans* development and behavior, encodes a clathrin-associated protein. *Genes Dev.* 8(1), 60–73.
- Levkowitz, G., Waterman, H., Zamir, E., Kam, Z., Oved, S., Langdon, W. Y., Beguinot, L., Geiger, B., and Yarden, Y. (1998). c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. *Genes Dev.* 12(23), 3663–3674.
- Liu, K. S., and Sternberg, P. W. (1995). Sensory regulation of male mating behavior in *Caenorhabditis elegans*. *Neuron* 14(1), 79–89.
- Liu, Q., Tan, G., Levenkova, N., Li, T., Pugh, E. N. Jr., Rux, J., Speicher, D. W., and Pierce, E. A. (2007) The proteome of the mouse photoreceptor sensory cilium complex. *Mol. Cell Proteomics Epub ahead of print.*
- Lloyd, T. E., Atkinson, R., Wu, M. N., Zhou, Y., Pennetta, G., and Bellen, H. J. (2002). Hrs regulates endosome membrane invagination and tyrosine kinase receptor signaling in *Drosophila*. *Cell* 108(2), 261–269.
- Ma, R., Li, W. P., Rundle, D., Kong, J., Akbarali, H. I., and Tsiokas, L. (2005). PKD2 functions as an epidermal growth factor-activated plasma membrane channel. *Mol. Cell. Biol.* 25(18), 8285–8298.
- Marchese, A., Raiborg, C., Santini, F., Keen, J. H., Stenmark, H., and Benovic, J. L. (2003). The E3 ubiquitin ligase AIP4 mediates ubiquitination and sorting of the G protein-coupled receptor CXCR4. *Dev. Cell* 5(5), 709–722.
- Marshall, W. F., and Nonaka, S. (2006). Cilia: tuning in to the cell's antenna. *Curr. Biol.* 16(15), R604–R614.
- Marszalek, J. R., Liu, X., Roberts, E. A., Chui, D., Marth, J. D., Williams, D. S., and Goldstein, L. S. (2000). Genetic evidence for selective transport of opsin and arrestin by kinesin-II in mammalian photoreceptors. *Cell* 102(2), 175–187.
- May, S. R., Ashique, A. M., Karlen, M., Wang, B., Shen, Y., Zarbalis, K., Reiter, J., Ericson, J., and Peterson, A. S. (2005). Loss of the retrograde motor for IFT disrupts localization of Smo to cilia and prevents the expression of both activator and repressor functions of Gli. *Dev. Biol.* 287(2), 378–389.
- Mello, C., and Fire, A. (1995). DNA transformation. *Methods Cell Biol.* 48, 451–482.
- Mizuno, E., Kawahata, K., Kato, M., Kitamura, N., and Komada, M. (2003). STAM proteins bind ubiquitinated proteins on the early endosome via the VHS domain and ubiquitin-interacting motif. *Mol. Biol. Cell* 14(9), 3675–3689.
- Mochizuki, T. *et al.* (1996). PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* 272(5266), 1339–1342.
- Nauli, S. M. *et al.* (2003). Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat. Genet.* 33(2), 129–137.
- Nauli, S. M., and Zhou, J. (2004). Polycystins and mechanosensation in renal and nodal cilia. *Bioessays* 26(8), 844–856.
- Neill, A. T., Moy, G. W., and Vacquier, V. D. (2004). Polycystin-2 associates with the polycystin-1 homolog, suREJ3, and localizes to the acrosomal region of sea urchin spermatozoa. *Reprod. Dev.* 67(4), 472–477.
- Nickel, C., Benzing, T., Sellin, L., Gerke, P., Karihaloo, A., Liu, Z. X., Cantley, L. G., and Walz, G. (2002). The polycystin-1 C-terminal fragment triggers branching morphogenesis and migration of tubular kidney epithelial cells. *J. Clin. Invest.* 109(4), 481–489.
- Oldham, C. E., Mohney, R. P., Miller, S. L., Hanes, R. N., and O'Bryan, J. P. (2002). The ubiquitin-interacting motifs target the endocytic adaptor protein epsin for ubiquitination. *Curr. Biol.* 12(13), 1112–1116.
- Pazour, G. J., Agrin, N., Leszyk, J., and Witman, G. B. (2005). Proteomic analysis of a eukaryotic cilium. *J. Cell Biol.* 170(1), 103–113.
- Pazour, G. J., and Rosenbaum, J. L. (2002). Intraflagellar transport and cilia-dependent diseases. *Trends Cell Biol.* 12(12), 551–555.
- Pazour, G. J., San Agustin, J. T., Follit, J. A., Rosenbaum, J. L., and Witman, G. B. (2002). Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in orpk mice with polycystic kidney disease. *Curr. Biol.* 12, R378–R380.
- Petiot, A., Faure, J., Stenmark, H., and Gruenberg, J. (2003). PI3P signaling regulates receptor sorting but not transport in the endosomal pathway. *J. Cell Biol.* 162(6), 971–979.
- Polo, S., Sigismund, S., Faretta, M., Guidi, M., Capua, M. R., Bossi, G., Chen, H., De Camilli, P., and Di Fiore, P. P. (2002). A single motif responsible for ubiquitin recognition and monoubiquitination in endocytic proteins. *Nature* 416(6879), 451–455.
- Raiborg, C., Bache, K. G., Gillooly, D. J., Madhus, I. H., Stang, E., and Stenmark, H. (2002). Hrs sorts ubiquitinated proteins into clathrin-coated microdomains of early endosomes. *Nat. Cell Biol.* 4(5), 394–398.
- Raiborg, C., Rusten, T. E., and Stenmark, H. (2003). Protein sorting into multivesicular endosomes. *Curr. Opin. Cell Biol.* 15(4), 446–455.
- Rosenbaum, J. L., and Witman, G. B. (2002). Intraflagellar transport. *Nat. Rev. Mol. Cell Biol.* 3(11), 813–825.
- Roudier, N., Lefebvre, C., and Legouis, R. (2005). CeVPS-27 is an endosomal protein required for the molting and the endocytic trafficking of the low-density lipoprotein receptor-related protein 1 in *Caenorhabditis elegans*. *Traffic* 6(8), 695–705.
- Sato, M., Sato, K., Fonarev, P., Huang, C. J., Liou, W., and Grant, B. D. (2005). *Caenorhabditis elegans* RME-6 is a novel regulator of RAB-5 at the clathrin-coated pit. *Nat. Cell Biol.* 7(6), 559–569.
- Schlessinger, J. (2000). Cell signaling by receptor tyrosine kinases. *Cell* 103(2), 211–225.
- Schneider, L., Clement, C. A., Teilmann, S. C., Pazour, G. J., Hoffmann, E. K., Satir, P., and Christensen, S. T. (2005). PDGFR α signaling is regulated through the primary cilium in fibroblasts. *Curr. Biol.* 15(20), 1861–1866.
- Scholey, J. M., and Anderson, K. V. (2006). Intraflagellar transport and cilium-based signaling. *Cell* 125(3), 439–442.
- Shih, S. C., Katzmann, D. J., Schnell, J. D., Sutanto, M., Emr, S. D., and Hicke, L. (2002). Epsins and Vps27p/Hrs contain ubiquitin-binding domains that function in receptor endocytosis. *Nat. Cell Biol.* 4(5), 389–393.
- Signor, D., Wedaman, K. P., Orozco, J. T., Dwyer, N. D., Bargmann, C. I., Rose, L. S., and Scholey, J. M. (1999). Role of a class DHC1b dynein in retrograde transport of IFT motors and IFT raft particles along cilia, but not dendrites, in chemosensory neurons of living *Caenorhabditis elegans*. *J. Cell Biol.* 147(3), 519–530.
- Singla, V., and Reiter, J. F. (2006). The primary cilium as the cell's antenna: signaling at a sensory organelle. *Science* 313(5787), 629–633.
- Sonnichsen, B. *et al.* (2005). RNAi profiling of early embryogenesis in *Caenorhabditis elegans*. *Nature* 434(7032), 462–469.
- Staub, O., and Rotin, D. (2006). Role of ubiquitylation in cellular membrane transport. *Physiol. Rev.* 86(2), 669–707.
- Sulston, J. E., Albertson, D. G., and Thomson, J. N. (1980). The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. *Dev. Biol.* 78(2), 542–576.
- Sutton, K. A., Jungnickel, M. K., Ward, C. J., Harris, P. C., and Florman, H. M. (2006). Functional characterization of PKDREJ, a male germ cell-restricted polycystin. *J. Cell. Physiol.* 209(2), 493–500.
- Swanson, K. A., Kang, R. S., Stamenova, S. D., Hicke, L., and Radhakrishnan, I. (2003). Solution structure of Vps27 UIM-ubiquitin complex important for endosomal sorting and receptor downregulation. *EMBO J.* 22(18), 4597–4606.

- Takata, H., Kato, M., Denda, K., Kitamura, N. (2000). A hrs binding protein having a Src homology 3 domain is involved in intracellular degradation of growth factors and their receptors. *Genes Cells* 5(1), 57–69.
- Thien, C. B., Walker, F., and Langdon, W. Y. (2001). RING finger mutations that abolish c-Cbl-directed polyubiquitination and downregulation of the EGF receptor are insufficient for cell transformation. *Mol. Cell* 7(2), 355–365.
- Thrower, J. S., Hoffman, L., Rechsteiner, M., and Pickart, C. M. (2000). Recognition of the polyubiquitin proteolytic signal. *EMBO J.* 19(1), 94–102.
- Tobin, D., Madsen, D., Kahn-Kirby, A., Peckol, E., Moulder, G., Barstead, R., Maricq, A., and Bargmann, C. (2002). Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in *C. elegans* neurons. *Neuron* 35(2), 307–318.
- Urbe, S., Sachse, M., Row, P. E., Preisinger, C., Barr, F. A., Strous, G., Klumperman, J., and Clague, M. J. (2003) The UIM domain of Hrs couples receptor sorting to vesicle formation. *J. Cell Sci.* 116(Pt 20), 4169–4179.
- Vandorpe, D. H., Chernova, M. N., Jiang, L., Sellin, L. K., Wilhelm, S., Stuart-Tilley, A. K., Walz, G., and Alper, S. L. (2001). The cytoplasmic C-terminal fragment of polycystin-1 regulates a Ca²⁺-permeable cation channel. *J. Biol. Chem.* 276(6), 4093–4101.
- Wegierski, T., Hill, K., Schaefer, M., and Walz, G. (2006). The HECT ubiquitin ligase ALP4 regulates the cell surface expression of select TRP channels. *EMBO J.* 25(24), 5659–5669.
- Whitfield, J. F. (2004). The neuronal primary cilium—an extrasynaptic signaling device. *Cell Signal* 16(7), 763–767.
- Wicks, S. R., de Vries, C. J., van Luenen, H. G., and Plasterk, R. H. (2000). CHE-3, a cytosolic dynein heavy chain, is required for sensory cilia structure and function in *Caenorhabditis elegans*. *Dev. Biol.* 221(2), 295–307.
- Yoder, B. K., Hou, X., and Guay-Woodford, L. M. (2002). The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. *J. Am. Soc. Nephrol.* 13(10), 2508–2516.