A latent capacity of the C. elegans polycystins to disrupt sensory transduction is repressed by the single-pass ciliary membrane protein CWP-5

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SUMMARY

Autosomal dominant polycystic kidney disease (ADPKD) results from loss-of-function mutations in PKD1 or PKD2. The products of these genes, the polycystins PC-1 and PC-2, form a transmembrane channel that is necessary for flow sensing by renal cilia. In C. elegans, the polycystin orthologs LOV-1 and PKD-2 function in sensory neurons that mediate male mating behavior. Here, we report that the novel single-pass membrane protein CWP-5 is necessary for polycystin signaling during the response step of mating behavior. As with the polycystins, CWP-5 localizes to neuronal cilia; this localization requires LOV-1. The response defect of cwp-5 mutants does not appear to result from disruption of ciliogenesis or polycystin localization. Instead, genetic and behavioral analyses indicate that CWP-5 represses a previously undescribed antagonistic effect of the polycystins on sensory transduction. Sensory neurons required for male behavior have a latent capacity to hinder sensory transduction, and suggests that aberrant functions of the polycystins could contribute to the pathogenesis of PKD.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is a common and potentially lethal disorder that is characterized by the growth of fluid-filled cysts that disrupt renal function (Torres et al., 2007). Virtually all cases of ADPKD arise from loss-of-function mutations in either PKD1 or PKD2, which are genes that encode the polycystin proteins PC-1 and PC-2. PC-2 is a non-selective TRP (transient receptor potential)-family cation channel whose activity is thought to be regulated by PC-1, an 11-pass transmembrane protein with which PC-2 associates physically (Gonzalez-Perrett et al., 2001; Hanaoka et al., 2000). The polycystins localize to sensory cilia and intracellular structures in a variety of cell types, including renal epithelia. In cilia, the polycystin channel is necessary for the transduction of mechanical stimuli through ciliary bending (Nauli and Zhou, 2004).

Although ADPKD is inherited dominantly, its pathology is likely to arise from the reduction or loss of polycystin function at the cellular level (Harris and Torres, 2009; Torres et al., 2007). When one defective PKD1 or PKD2 allele is inherited, somatic mutation of the remaining wild-type allele is thought to account for most, or all, cyst formation. The means by which the loss of polycystin function in a single cell or clone of cells leads to cyst formation is not entirely clear. However, the prevailing view is that an insensitivity to ciliary polycystin signaling disrupts downstream signaling pathways, possibly involving cAMP, thereby triggering pathogenic changes in cell division and differentiation. Highlighting the importance of cilia in maintaining normal renal epithelial function, a related disorder, autosomal recessive PKD (ARPKD), results from inactivating mutations in PKHD1, the gene encoding fibrocystin/polyductin (FPC), a single-pass membrane protein that also localizes to renal epithelial cilia and other subcellular compartments (Harris and Torres, 2009). Although fibrocystin is likely to interact directly with the polycystin complex, the regulatory relationships between the polycystins and fibrocystin/polyductin are not well understood. In addition, many other ciliopathies, including Bardet-Biedel syndrome and Meckel syndrome, are also frequently associated with polycystic kidneys, further highlighting the connection between cilium function and cystic kidney disease.

In the nematode C. elegans, the polycystin orthologs LOV-1 (PC-1) and PKD-2 (PC-2) act in the dendritic ciliated endings of male-specific sensory neurons to mediate the transduction of mechanical and/or chemical signals that are important for male mating behavior (Barr et al., 2001; Barr and Sternberg, 1999). Loss of the gene encoding either protein significantly reduces the ability of males to carry out two key steps of this process. In the first step, called response behavior, males respond to hermaphrodite contact by placing the ventral side of the tail against their mate's body, contracting posterior ventral muscles to maintain this posture, and initiating sustained tail-first locomotion along the hermaphrodite. The second polycystin-dependent step, called vulva location, requires the male to cease reverse locomotion upon encountering the vulval opening and to begin prodding the vulva with the spicules. Polycystin function in the response step is mediated primarily through the ray RnB neurons, whereas vulva location behavior depends on polycystin function in the hook HOB neuron (Barr et al., 2001; Barr and Sternberg, 1999; Liu and Sternberg, 1995). Although null lov-1, pkd-2 and lov-1; pkd-2 mutant males are significantly impaired in both response and vulva location, these behaviors are not eliminated, suggesting that a secondary, polycystin-independent pathway can also mediate the sensory signaling required for these steps (Barr et al., 2001; Barr and Sternberg, 1999). The polycystins are also expressed in the male-
specific CEM head sensory neurons, which are important for detecting pheromones that attract males to potential mates. However, the evidence that polycystin function is important for pheromone response is conflicting (Chasnov et al., 2007; Srinivasan et al., 2008; White et al., 2007).

In both *C. elegans* and vertebrates, the two polycystins are thought to act in a single genetic pathway (Barr et al., 2001; Wu and Somlo, 2000). According to this model, the phenotypes and pathology associated with the loss of one polycystin should solely reflect the absence of signaling through the polycystin channel. However, several findings suggest that the polycystin pathway may be more complex: *lov-1; pkd-2* double-mutant males display small but consistent improvements in mating behavior compared with either of the single polycystin mutants (Barr et al., 2001; Peden and Barr, 2005), and the phenotypes of *Pkd1*+/− and *Pkd2*+/− mice are not identical to each other (Lu et al., 2001; Wu et al., 1998). This indicates that the loss of one polycystin may cause disease through mechanisms beyond simple ablation of the function of the polycystin channel. A better understanding of these effects could provide important insight into the mechanisms of cyst formation, identify genes that modify the severity of disease, and provide new opportunities for therapy.

In this study we identify a novel *C. elegans* gene, *cwp-5*, that acts through an unexpected mechanism to regulate polycystin function. *cwp-5* encodes a single-pass transmembrane protein that is specifically co-expressed with the polycystins in a characteristic set of male-specific neurons. As with the polycystins, *CWP-5* localizes both to the cilia and cytoplasmic structures. *cwp-5* mutants exhibit marked impairment in the response step of male mating behavior. The loss of *cwp-5* does not further enhance the behavioral defects of *lov-1; pkd-2* mutants, indicating that *cwp-5* acts in the polycystin pathway. Interestingly, genetic interactions between *cwp-5* and the polycystins indicate that both of the polycystins have a latent potential to obstruct sensory signaling, and that *cwp-5* functions to repress this negative activity of the polycystins. Although vertebrates do not possess clear primary-sequence homologs of *cwp-5*, we propose that similar regulatory functions exist to modulate vertebrate polycystin function. Moreover, our results indicate that aberrant activities of the human polycystins could be important contributors to the pathogenesis of polycystic kidney disease.

**RESULTS**

*cwp-5* encodes a novel membrane protein present in the sensory cilia of the polycystin neurons

Using DNA microarrays, we previously identified four genes, *cwp-1* through *cwp-4*, that are specifically co-expressed with the *C. elegans* polycystins in a characteristic set of male-specific sensory neurons: sixteen ray RnB neurons (all RnB neurons apart from those of ray 6), four cephalic CEM neurons, and the tail HOB neuron (Portman and Emmons, 2004). In further analysis of this dataset, we studied the predicted gene F48C11.2 (Fig. 1A), which ranked in the first percentile of candidate ray-expressed genes. Because of its expression pattern (described below), we named this gene *cwp-5*. The predicted product of *cwp-5* is a single-pass type I membrane protein. Transcriptome data (from www.wormbase.org) indicate that *cwp-5* contains two alternative first exons that encode 11 or 14 amino acids; the use of either of these exons is predicted to generate a functional signal peptide. The extracellular region of the CWP-5 protein contains a divergent C-type lectin domain and a small mucin-like region of predicted O-glycosylation sites (Fig. 1A). Its 377-amino acid cytoplasmic domain lacks obvious similarity to other known proteins, but is rich in prolines throughout (17.5%). The predicted membrane topology of CWP-5 is shown in Fig. 1E.

We determined the expression pattern of *cwp-5* using a transcriptional yellow fluorescent protein (YFP) reporter containing the upstream promoter as well as a C-terminal translational YFP

![Fig. 1. cwp-5 is a novel gene expressed exclusively in male-specific sensory neurons. (A) The cwp-5 gene contains 11 exons and is located at +10.23 cM on the X chromosome. Transcriptome data indicate that cwp-5 has two alternative first exons (indicated with dotted lines) that encode 11 (F48C11.2a) or 14 (F48C11.2b) amino acids. Both CWP-5 products are predicted by SignalP 3.0 (Emanuelsson et al., 2007) to contain functional signal peptides (SP). CWP-5 is also predicted to contain a C-type lectin domain and a mucin-like region, as well as a single-pass transmembrane (TM) domain. The allele *tm1893* is an in-frame insertion-deletion removing most of the mucin domain and the entire transmembrane domain to yield a truncated, potentially secreted, protein. The predicted structure of the mutant transcript was confirmed by sequencing the cwp-5(tm1893) cDNA. (B) Expression of a transcriptional cwp-5::YFP reporter using the upstream (F48C11.2b) promoter was observed in 21 male-specific sensory neurons: 4 CEM neurons in the head, 16 RnB-type ray neurons (all RnBs except for those of ray 6), and the hook neuron HOB. A translational reporter, CWP-5::YFP, in which the YFP sequence was inserted at the 3′ end of cwp-5, should reflect both isoforms of CWP-5. This construct marked the same set of cells, with fluorescence visible in the cell bodies, dendrites and the cilia of CEM (C), RnB (D) and HOB (not shown) neurons. The cilia in the CEM and RnB neurons are roughly 4 and 1 μm in length, respectively. (E) A model for the membrane topology of CWP-5 and the polycystins in sensory cilia. Domains of each of these proteins are noted. As shown here, signaling through the polycystin complex is thought to be mediated by regulated Ca2+ entry.
fusión que debería reflejar la expresión de ambos promotores. La expresión de ambas de estas transgenes fue observada específicamente en el RnB, HOB y CEM neuronas del macho, y ahora, en la hembras por (Fig. 1B-D), siguiendo el mismo patrón que se ha reportado para los polycystins (Barr et al., 2001; Barr and Sternberg, 1999). El CWP-5::YFP fusión proteína era claramente enriquecido en las cilia del somaticas neuronas y era también detectable en el soma (Fig. 1 y datos no mostrados).

**cwp-5 es necesario para el comportamiento sensorial eficiente del macho**

Para determinar si la cwp genes tienen un papel en polycystin signaling, examinamos el comportamiento de los machos portadores de tres diferentes mutaciones de delección: cwp-2[cwp-3(ok1366), cwp-4(tm727) y cwp-5(tm1893) (ok1366) es una delección que remueve cantidades sustanciales de los vecinos cwp-2 y cwp-3 genes). Estas especies exhibieron ningún déficit aparente en el cuerpo, cola o cilia sensoriales, y no cwp-2 cwp-3 ni cwp-4 mutantes machos mostraron defectos en respuesta o vulva localización del comportamiento (datos no mostrados). Sin embargo, cwp-5 mutantes machos tuvieron un significativo y específico déficit en respuesta comportamiento: 85.9% de los machos de tipo (WT) respondieron a las hembras por arrastrando y enrollando sus cola (n=241), mientras que cwp-5 males mostró una respuesta frecuencia de 53.5% (n=170), lo que es un reducción significativa (P<1×10⁻⁴) (Tabla 1). Por otro lado, varia localización de comportamiento en estas especies era esencialmente afectado (WT, 86.8%; cwp-5, 82.9%). Así, otros genes que son importantes para el comportamiento sensorial, cwp-5 parece tener una función específica en respuesta comportamiento, indicando que la regla o función de polycystin signaling pueden diferir entre el RnB y HOB neuronas.

La tm1893 delección destruye 730 bp de cwp-5 y lo reemplaza con 4 bp inserción. Debido a esta cambio mantenga cwp-5 open reading frame, cwp-5(tm1893) es predicho para codificar un proteína secreta sin el dominio transmembrana y mucina (Fig. 1A). Como resultado, cwp-5(tm1893) quizás reduce o elimina CWP-5 función, o podría interferir con el proceso requerido para comportamiento normal. Debido a cwp-5 es en el X cromosoma, machos son hemizigóticos para este locus. Nosotros entonces preguntamos si la cwp-5(tm1893) fenotipo podría ser resuelto al introducir un nuevo tipo de cwp-5 gene. Con el propósito de que la cwp-5(tm1893) reduce o elimina cwp-5 función, hemos encontrado que un construido cwp-5::YFP fusión proteína tiene la misma frecuencia de respuesta como cwp-5(tm1893) (Tabla 1). En conjunto, una wild-type cwp-5 gene es conocido por su papel en la transducción sensorial (Barr et al., 2001; Barr and Sternberg, 1999). Para determinar si la cwp-5 funciones en el polycystin camino o un camino secundario, hemos examinado interacciones genéticas entre cwp-5, lov-1 y pkd-2. Cuando polycystin signaling es eliminado con un lov-1; pkd-2 double deficient background, hemos encontrado que el loss de cwp-5 no ha afectado la frecuencia de respuesta: 31.8% de lov-1; pkd-2 machos mostraron el mismo comportamiento (Tabla 2). En acuerdo con esto, cwp-5(RNAi) genes que son una función que se impide el comportamiento sensorial en wild-type animals (Tabla 1) falló para desactivar el lov-1; pkd-2 defecto (Tabla 2). Estos resultados indican que cwp-5 funciones en el polycystin camino para promover comportamiento sensorial.

Encontramos resultados inesperados genéticos cuando examinamos el fenotipo de cwp-5(tm1893) mutants carrying null alleles de solo uno polycystin. En ambos casos, cwp-5(tm1893) claramente aumentó los defectos de los sing polycystin mutants (Tabla 2). Loss of cwp-5 function redujo el comportamiento residual de los lov-1 mutantes por 37.6%. Además, virtualmente eliminó el comportamiento residual de pkd-2 mutants, que pkd-2; cwp-5 males casi nunca respondieron a las hembras. Genéticamente, estos resultados indican que cwp-5 mutantes revela la potencial de un polycystin subunitos a impedir la transducción sensorial. Al comparar el fenotipo de los double mutants lov-1; cwp-5 (14.3% responder) o pkd-2; cwp-5 (2.5%) con de los lov-1; pkd-2; cwp-5 triple mutant (30.6%), es aparente que los comportamientos deficientes de los double mutants puede ser aliviado por

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Response (%)</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>Wild type</td>
<td>85.8</td>
<td>241</td>
</tr>
<tr>
<td>cwp-5(tm1893)</td>
<td>53.5**</td>
<td>170</td>
</tr>
<tr>
<td>fsEx150[cwp-5::YFP]</td>
<td>83.3</td>
<td>18</td>
</tr>
<tr>
<td>cwp-5;fsEx150[cwp-5::YFP]</td>
<td>73.3*</td>
<td>30</td>
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<tr>
<td>yDp6/+</td>
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<td>yDp6/+; cwp-5</td>
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<tr>
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<td>59.2*</td>
<td>54</td>
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<tr>
<td>fsEx178[cwp-5(RNAi)]‡</td>
<td>65.7*</td>
<td>67</td>
</tr>
<tr>
<td>fsEx179[cwp-5(RNAi)]‡</td>
<td>53.2*</td>
<td>62</td>
</tr>
<tr>
<td>fsEx180[cwp-5(RNAi)]‡</td>
<td>70.3*</td>
<td>64</td>
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<tr>
<td>cwp-5;fsEx169[cwp-5(RNAi)]‡</td>
<td>63.2**</td>
<td>49</td>
</tr>
<tr>
<td>cwp-5;fsEx178[cwp-5(RNAi)]‡</td>
<td>51.7**</td>
<td>29</td>
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<tr>
<td>cwp-5;fsEx179[cwp-5(RNAi)]‡</td>
<td>53.3**</td>
<td>30</td>
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<tr>
<td>cwp-5;fsEx180[cwp-5(RNAi)]‡</td>
<td>54.8**</td>
<td>31</td>
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**P<0.0001, *P<0.01 compared with wild type.**

<table>
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<tr>
<td>cwp-5(tm1893)</td>
<td>53.5**</td>
<td>170</td>
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<tr>
<td>fsEx150[cwp-5::YFP]</td>
<td>83.3</td>
<td>18</td>
</tr>
<tr>
<td>cwp-5;fsEx150[cwp-5::YFP]</td>
<td>73.3*</td>
<td>30</td>
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<tr>
<td>yDp6/+</td>
<td>90.4</td>
<td>21</td>
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<tr>
<td>yDp6/+; cwp-5</td>
<td>77.0**</td>
<td>87</td>
</tr>
<tr>
<td>fsEx169[cwp-5(RNAi)]‡</td>
<td>59.2*</td>
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<tr>
<td>cwp-5;fsEx169[cwp-5(RNAi)]‡</td>
<td>63.2**</td>
<td>49</td>
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<tr>
<td>cwp-5;fsEx178[cwp-5(RNAi)]‡</td>
<td>51.7**</td>
<td>29</td>
</tr>
<tr>
<td>cwp-5;fsEx179[cwp-5(RNAi)]‡</td>
<td>53.3**</td>
<td>30</td>
</tr>
<tr>
<td>cwp-5;fsEx180[cwp-5(RNAi)]‡</td>
<td>54.8**</td>
<td>31</td>
</tr>
</tbody>
</table>

**P<0.0001, *P<0.01 compared with wild type.**

Polycystin signaling in C. elegans
removing the remaining functional polycystin. Thus, in cwp-5 mutants, a lone polycystin subunit can impede, rather than facilitate, normal sensory function.

Such lone polycystin subunits might also accumulate when the stoichiometry of normal polycystin expression is disrupted. To determine whether an excess of one polycystin over the other might disrupt sensory transduction in a wild-type background, we assayed the behavior of males stably expressing a PKD-2::GFP fusion protein driven by the pkd-2 promoter (myIs4). Although this myIs4 transgene rescues the mating defects of pkd-2(sy606) mutants (Bae et al., 2006), we found that it significantly reduced response in a wild-type background (60.4%, 48, P<0.0002 compared with WT males). Thus, an excess of functional PKD-2 can interfere with ray neuron function, consistent with the notion that the polycystins can both promote and antagonize sensory signaling.

cwp-5 is not essential for ciliogenesis or cilium maintenance

We next explored the mechanisms by which CWP-5 promotes RnB sensory transduction and how a lone polycystin can inhibit it. Several possibilities can be imagined: CWP-5 could be required for normal ciliogenesis, correct trafficking of cilium proteins, the assembly of a functionally competent polycystin complex, or for transduction of the sensory signal itself. Although not all of these possibilities can be easily evaluated in C. elegans sensory neurons, we assessed the first two possibilities by examining ciliogenesis and protein trafficking in cwp-5 and polycystin mutants.

The structural integrity of RnB and HOB sensory cilia is known to be important for response behavior (Bae et al., 2006) (R. Lints and P. Koo, personal communication; D. Hurd, R.M.M., L. Nuñez and D.S.P, manuscript submitted). To assess ciliogenesis and cwp-5 mutants, we first examined the localization of OSM-6::GFP in a single ray. This suggests that the sensory cilia of the RnA and RnB neurons, whose dendrites run parallel to each other inside each ray, were both intact (data not shown). We also found that the localization of two motor proteins that are important for cilium structure, KLP-6 and OSM-3 (Peden and Barr, 2005; Snow et al., 2004), was not altered in cwp-5 mutants (Fig. 2C,D and data not shown). Finally, the localization of TBA-6, an α-tubulin isoform that marks the cilia of the RnB neurons (D. Hurd, R.M.M., L. Nuñez and D.S.P, manuscript submitted), was not defective in cwp-5 mutants (Fig. 2E,F). Because both KLP-6 and TBA-6 allow specific visualization of RnB neuron sensory cilia, these results suggest that the loss of cwp-5 does not disrupt cilium assembly or maintenance.

Behavioral defects in cwp-5 mutants are unlikely to result from defects in protein trafficking

A potential cause of the response defect of cwp-5 males is mislocalization of PKD-2, as is seen in animals bearing mutations in the kinesin-like gene klp-6 (Peden and Barr, 2005) and the phosphoinositide [PI] 5-phosphatase gene cil-1 (Bae et al., 2009). However, we found that the localization of PKD-2::GFP in cwp-5(tm1893) males was indistinguishable from that in wild-type males (Fig. 2G,H). Although we were unable to examine LOV-1 localization directly, mislocalization of this polycystin in cwp-5 mutants seems unlikely (see Discussion). Thus, we do not favor the possibility that polycystin mislocalization underlies the response defects of cwp-5 mutants.

Table 2. cwp-5 functions in the polycystin pathway to promote response behavior

<table>
<thead>
<tr>
<th>Genotype</th>
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<th>Vulva location (%)</th>
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<tr>
<td>Wild type</td>
<td>85.8</td>
<td>86.8</td>
<td>241</td>
</tr>
<tr>
<td>lov-1(sy582)</td>
<td>22.9*</td>
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<td>61</td>
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<tr>
<td>pkd-2(sy606)</td>
<td>34.0*</td>
<td>28.6*</td>
<td>103</td>
</tr>
<tr>
<td>cwp-5(tm1893)</td>
<td>53.5*</td>
<td>82.9</td>
<td>170</td>
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<tr>
<td>lov-1; cwp-5</td>
<td>14.3</td>
<td>36.4</td>
<td>63</td>
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<td>pkd-2; cwp-5</td>
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</tr>
<tr>
<td>lov-1; pkd-2</td>
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<td>lov-1; pkd-2; transgene</td>
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<td>lov-1; pkd-2; transgene; cwp-5(RNAi)</td>
<td>38.7</td>
<td>n.d.</td>
<td>31</td>
</tr>
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</table>

*P<0.0001 compared with him-5; †P<0.0001 compared with pkd-2. n.d., not determined.

†n indicates the number of animals assayed for response behavior. The n for vulva location is lower, since vulva location can only be assayed in those animals that successfully execute response.
appropriate model for investigating the mechanism and function of polycystin signaling.

**CWP-5 localization depends on the polycystins**

The finding that *cwp-5(tm1893)* suppresses the frequency of aberrant cytoplasmic PKD-2 localization in *lov-1* mutants (Fig. 3C,D) suggests that CWP-5 helps to stabilize or anchor the mislocalized PKD-2 seen in *lov-1* mutants. This raises the possibility that CWP-5 might functionally interact with the polycystins, potentially as part of a larger polycystin signaling complex. Consistent with this possibility, we found that the dendritic and ciliary localization of CWP-5::YFP in the ray neurons depends on *lov-1* (Fig. 3E,F). Interestingly, in *lov-1* mutants, CWP-5::YFP accumulated in perinuclear domains reminiscent of those seen with PKD-2::GFP. The simultaneous loss of both polycystins suppressed CWP-5 mislocalization to these structures (Fig. 3G,H). This indicates that the substrate for cytoplasmic sequestration in *lov-1* mutants could be a CWP-5:PKD-2 complex, further supporting the possibility that CWP-5 functionally interacts with the polycystins.

**DISCUSSION**

The simplicity and genetic tractability of *C. elegans* male mating behavior (Barr and Garcia, 2006) provides a powerful system to investigate the mechanism and function of polycystin signaling. Using this model, we have identified CWP-5, a novel single-pass transmembrane protein that co-localizes with the polycystins in the sensory cilia of a specific set of sensory neurons in *C. elegans* adult males. We find that CWP-5 functions in the polycystin pathway to promote sensory behavior. Genetic interactions between *cwp-5* and the polycystins revealed an unexpected aspect of the function and regulation of the *C. elegans* polycystins: both *lov-1* and *pkd-2* have the potential to hinder, rather than facilitate, sensory signaling, and *cwp-5* represses these negative influences.

In the simplest genetic model for *cwp-5* and polycystin function (Fig. 4), the canonical LOV-1/PKD-2 complex acts directly or indirectly to transduce a sensory stimulus. Because *lov-1*; *pkd-2* mutants still exhibit significant response behavior (Barr et al., 2001), a secondary, as-yet-unidentified pathway (‘X’) also contributes to the transduction of stimuli that trigger this step. *cwp-5* is unlikely to function in this secondary pathway, since loss of *cwp-5* has no effect in a *lov-1*; *pkd-2* mutant background. Instead, because the phenotype of *lov-1*; *cwp-5* and *pkd-2*; *cwp-5* double mutants is more severe than that of the triple mutant *lov-1*; *pkd-2*; *cwp-5*, we propose that wild-type CWP-5 acts to block a toxic activity of the remaining polycystin subunit. Because the enhanced response defects of these double mutants are suppressed by removing the remaining polycystin, we conclude that the polycystins act downstream of *cwp-5*. Since response defects are also apparent in *cwp-5(tm1893)* single mutants, it seems likely that CWP-5 actively functions to prevent the negative effects of free polycystins in wild-type animals. This possibility is also supported by the observation that the *lov-1* single mutant phenotype is consistently more severe than that of the *lov-1*; *pkd-2* double mutant (Table 1) (Barr et al., 2001; Peden and Barr, 2005), which suggests that PKD-2 can modestly inhibit sensory signaling in the absence of LOV-1, even when CWP-5 is present. Although our results cannot rule out the possibility that CWP-5 also has some functions downstream of, or in parallel with, the polycystins, we have no evidence that activation of CWP-5 by the polycystins is necessary for sensory signaling.
The most likely site of action of CWP-5 is in the ray RnB neurons. Because RnB-specific rescue of cwp-5 is challenging, we have not demonstrated this directly. However, as the primary mediators of response behavior (Liu and Sternberg, 1995), the RnBs are the most likely site for cwp-5 function. Because cwp-5(tm1893) mutants exhibit wild-type vulva location behavior, we consider it unlikely that cwp-5 has an important role in the HOB neuron. However, since HOB can play a role in response behavior (Liu and Sternberg, 1995), it is possible that CWP-5 has a response-specific function in HOB in addition to a role in RnB.

CWP-5 is not likely to act primarily in the CEM neurons, as cwp-5 mutant males have no defects in pheromone-response assays (R.M.M. and D.S.P., unpublished data). Moreover, ceh-30 mutant males, which specifically lack the CEM neurons, exhibit robust response behavior (R.M.M. and D.S.P., unpublished data), indicating that the CEMs are not likely to be important for response. These results also highlight an interesting cell-type specificity in the function of the C. elegans polycystin pathway, such that it is functionally specialized to respond to different stimuli in different cells. Although cwp-5 is expressed in all C. elegans polycystin neurons, it may play a role in this functional specialization. Consistent with this possibility, there are also cell-type differences in protein trafficking: Bae et al. have shown that the requirements for PKD-2 localization to cilia differ by neuron type (Bae et al., 2006), and we find that the frequency of CWP-5 mislocalization in lov-1 and lov-1; pkd-2 mutants differs between RnB, HOB and CEM neurons (R.M.M. and D.S.P., unpublished data). The mechanisms that tune these different cell types to respond to different stimuli in a polycystin-dependent manner is a promising area for future study.

Fig. 3. Protein trafficking in cwp-5 and polycystin mutants. (A-C) Panels show fluorescence (above) and DIC (below) of young adult males of the indicated genotypes carrying the PKD-2::GFP fusion protein transgene myIs4 (Bae et al., 2006). White arrowheads indicate the localization of PKD-2::GFP to RnB cilia; the dashed gray arrowhead indicates the absence of ray cillum localization. Yellow arrowheads indicate aberrant perinuclear inclusions of PKD-2::GFP. (D) Quantitation of perinuclear inclusions of PKD-2::GFP in animals of the indicated genotypes. Individual cells were scored for the presence or absence of intense cytoplasmic inclusions by epifluorescence microscopy. In wild-type animals, 173 cells in 13 animals were scored; in lov-1 mutants, 285 cells in 23 animals were scored; in lov-1; cwp-5 mutants, 272 cells in 24 animals were scored. Statistical comparisons were made using Fisher’s exact test. Asterisks (***) indicate P<10^{-4}. (E-G) Panels show fluorescence (above) and DIC (below) of young adult males of the indicated genotypes carrying the CWP-5::YFP fusion protein transgene fsEx150. White arrowheads indicate the localization of CWP-5::YFP to RnB cilia; the dashed gray arrowhead indicates the absence of ray cillum localization. Yellow arrowheads indicate aberrant perinuclear inclusions of CWP-5::YFP. (H) Quantitation of perinuclear inclusions of CWP-5::YFP in animals of the indicated genotypes. Individual cells were scored for the presence or absence of intense cytoplasmic inclusions by epifluorescence microscopy. In wild-type animals, 121 cells in 13 animals were scored; in lov-1 mutants, 393 cells in 32 animals were scored; in lov-1; pkd-2 mutants, 30 cells in 24 animals were scored. Statistical comparisons were made using Fisher’s exact test. Asterisks (***) indicate P<10^{-4}. 

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was altered in cwp-5 mutants, nor was the localization of PKD-2 to the RnB neuron cilia. Because visualizing LOV-1 localization is technically difficult, we were unable to assess the localization of LOV-1 directly. However, we consider its mislocalization to be unlikely for the following reason: since lov-1 is necessary for the ciliary trafficking of PKD-2 (Fig. 2) (Bae et al., 2006), any mislocalization of LOV-1 would probably result in a secondary disruption of PKD-2::GFP ciliary trafficking. Because PKD-2::GFP localization appears normal in cwp-5(tm1893) mutants, we infer that lov-1 function is unlikely to be compromised, and that LOV-1 is not likely to be mislocalized in these animals. Nevertheless, we are unable to rule out the possibility that LOV-1 trafficking is disrupted in cwp-5 mutants.

Our results do indicate that CWP-5 has some involvement in protein trafficking: the cytoplasmic mislocalization of PKD-2 in lov-1 mutants is partially suppressed by cwp-5(tm1893). One possible explanation for this is that CWP-5 contributes to a surveillance mechanism that prevents inappropriate trafficking of lone polycystin subunits to the cilium, where they might disrupt ciliary function. This possibility is challenging to test directly in C. elegans. However, two results suggest that this may not be the most important role of CWP-5: first, its ciliary localization seems somewhat at odds with surveillance of trafficking as a primary function. In addition, the observation that cwp-5 single mutants have significant response defects would imply that CWP-5 prevents a substantial amount of misfolded or otherwise defective polycystins from reaching the cilium in wild-type animals. However, we detected no increase in ciliary PKD-2 (or decrease in cytoplasmic PKD-2) in cwp-5 mutants. An alternative interpretation for this result is that CWP-5 and PKD-2 comprise a complex that is the substrate for cytoplasmic inclusion by a surveillance pathway that is active in a lov-1 mutant. Since either component can be trafficked normally when the other is missing, this would suggest that CWP-5 and PKD-2 are the targets, rather than the components, of an important surveillance mechanism. Nevertheless, surveillance of polycystin trafficking by CWP-5 may be an important part of the mechanism by which this factor promotes sensory transduction.

A final possibility is that CWP-5 facilitates signaling through the polycystin complex more directly. At the molecular level, several functions can be imagined. First, CWP-5 could tether the polycystin complex to an extracellular matrix. In this model, deformation of this link as the cilium bends could be necessary for sensory signaling. In support of this idea, the cilia of the RnB neurons are surrounded by an electron-dense glycocalyx that could be important for putative mechanosensory capacities of these cells (Chow et al., 1995). Second, CWP-5 could be important for the assembly or stability of the polycystin complex. Third, CWP-5 could be a co-receptor for a signal transduced by the polycystins. Fourth, if the polycystin complex is not the primary transducer of a signal, CWP-5 could act between a primary receptor and the polycystin complex to facilitate signal transduction. In all of these possible models, CWP-5 may interact directly with the polycystin complex itself. Unfortunately, this is difficult to address directly in C. elegans. The colocalization of CWP-5 with the polycystins is consistent with the possibility of direct interaction, as are the findings that CWP-5 localization requires LOV-1, and that efficient sequestration of both PKD-2 and CWP-5 to cytoplasmic bodies in lov-1 mutants depends on each other. Interestingly, the extracellular N-terminus of LOV-1 contains a large mucin-like domain of predicted O-linked glycosylation sites that could serve as a site for direct interaction with the C-type lectin domain of CWP-5. Because the LOV-1 N-terminus is highly divergent from the comparable region of mammalian PC-1 (Barr and Sternberg, 1999), a functional mammalian CWP-5 ortholog that interacts with the PC-1 N-terminus may exist but be indiscernible by primary sequence alone. Consistent with this possibility, both polycystins are highly divergent in the diplogasterid nematode P. pacificus (Sommer, 2006), and no cwp-5 sequence ortholog is recognizable.

An important insight from our work is that the polycystins have the capacity to hinder, as well as facilitate, sensory behavior. How could a lone polycystin disrupt signaling through a secondary pathway? One possibility is that the polycystins mislocalized to cytoplasmic bodies could have an inhibitory effect. However, this mislocalization is suppressed by cwp-5 loss, whereas behavioral defects are enhanced. Thus, it seems more likely that these antagonistic effects are manifest in cilia. For example, when either LOV-1 or PKD-2 is absent, the remaining polycystin may be able to form complexes with other transmembrane proteins, particularly TRP channels, to generate species that interfere with sensory signaling. This possibility is especially interesting given the recent findings that mammalian PC-2 can interact with other classes of TRP channels to form functional heteromeric complexes (Bai et al., 2008; Kottgen et al., 2008). In addition, physiological studies have provided evidence for a mutually inhibitory relationship between mammalian PC-1 and PC-2 function in vitro (Delmas et al., 2004). The ability to measure Ca2+ signaling in vivo in C. elegans should help evaluate the physiological consequences of the loss of cwp-5 and the basis for the antagonistic effects of the polycystins. In addition, we now have a means to genetically dissect these inhibitory effects, as mutations in components of this pathway might restore the ability of pkd-2; cwp-5 males to exhibit response behavior.
Implications for polycystic kidney disease
In humans, ADPKD has been proposed to result from the disruption of the flow-sensing ability of primary cilia in renal epithelia (Nauli and Zhou, 2004). Because of the cellular-recessive mechanism of this disorder, cystogenic cells lack one polycystin subunit but contain two functional alleles encoding the other. Thus, our genetic dissection of polycystin signaling in *C. elegans* raises an interesting possibility for human pathology: in addition to the loss of positive signaling through the polycystin complex, aberrant inhibitory functions of the remaining polycystin may augment the severity of the ADPKD phenotype. Indeed, overexpression of *Pkd1* in mice can cause polycystic kidney disease (Pritchard et al., 2000; Thivierge et al., 2006). Additional evidence demonstrating that disruption of the stoichiometry of the polycystin complex can have deleterious effects (Sharif-Naeini et al., 2009) appeared while this manuscript was in press. According to this view, interventions designed to block aberrant PC-1 or PC-2 activity might have therapeutic value. An important test of this prediction will be the examination of genetic interactions between *Pkd1* and *Pkd2* in mouse models: our model suggests that kidney-specific conditional loss of both *Pkd1* and *Pkd2* may cause less severe pathology than the loss of one polycystin alone.

It is notable that CWP-5 shares several features with fibrocystin/polyductin (FPC), the product of the *PKHD1* gene; mutations in this gene cause the autosomal recessive form of PKD (ARPKD) (Onuchic et al., 2002; Ward et al., 2002). As with CWP-5, FPC is a single-pass transmembrane protein whose loss causes a phenotype similar to polycystin mutants. FPC may be a component of the polycystin complex (Wu et al., 2006) and has been implicated in flow-mediated calcium signaling in renal epithelia (Wang et al., 2007). Heterozygosity of *Pkd1* enhances the severity of cystic defects in *Pkhd1* null mice (Garcia-Gonzalez et al., 2007), and loss of *Pkhd1* enhances the cystic phenotype of *Pkd2* mutant animals (Kim et al., 2008), reminiscent of the genetic interactions between *cwp-5* and the polycystins. However, FPC has been implicated in ciliogenesis (Kim et al., 2008), whereas this does not seem to be the case for CWP-5. Because CWP-5 and FPC share no primary sequence similarity, it is unlikely that they are functionally interchangeable orthologs. However, our results raise the possibility that some aspects of FPC and CWP-5 function may be analogous, and that therapies designed to block toxic polycystin functions may have value in ARPKD. Our results point to specific genetic tests in mouse models that could be carried out to investigate this possibility.

**METHODS**

**Nematode culture and strains**

Animals were cultured on nematode growth media (NGM) agar plates seeded with *E. coli* OP50, as described previously (Brenner, 1974). The following mutants were used in this work: linkage group (LG) II: *lov-1*(sy582); LG III: *pha-1*(e2123ts), *tra-1*(e1099); LG IV: *pdk-2*(sy606), *unc-31*(e169); LG V: *him-5*(e1490), *cwp-4*(tm727), *cwp-2&cwp-3*(ok1366); LG X: *cwp-5*(tm1893); LG unknown: *yDp6(XA)* (Akerib and Meyer, 1994). Standard genetic methods were used to construct strains containing multiple mutations (Brenner, 1974); PCR genotyping was used to follow the presence of deletion alleles that lacked obvious phenotypes. The *cwp-5*(tm1893) and *cwp-4*(tm727) deletion alleles were obtained from the Japanese National Bioresource Project (S. Mitani, Tokyo, Japan). The *cwp-2&cwp-3*(ok1366) deletion was obtained from the *C. elegans* Gene Knockout Consortium (Oklahoma). All strains were out-crossed to wild-type animals at least five times prior to the behavioral assays. Unless stated otherwise, all strains carried *him-5*(e1490) to increase the number of males in self-fertilizing cultures.

**Transgenes and transgenic strains**

To produce the *cwp-5b::YFP* transcriptional reporter, 1.2 kb of sequence upstream of the predicted F48C11.2b start site was amplified and fused to YFP using overlap-extension PCR (Boulin et al., 2006). To produce the *CWP-5::YFP* translational reporter, DNA from 1.2 kb upstream of the predicted F48C11.2b start site to the penultimate codon of CWP-5 was amplified and fused in-frame to YFP. Fused PCR products were co-injected with pBX1 into a *pha-1*(e2123ts); *him-5*(e1490) strain (Granato et al., 1994), or co-injected with *unc-122::GFP* into young adult *him-5* hermaphrodites. Several stable transgenic lines were obtained for each construct.

*myls4*[PKD-2::GFP] and *myEx648*[Pklp-6::KLP-6::GFP] were generously provided by Maureen Barr (Rutgers University, NJ). OSM-6::GFP DNA was kindly supplied by Robert Herman (University of Minnesota) and was co-injected into young adult hermaphrodites, at 50 ng/µl, with *unc-122::GFP* to generate *fsEx146*. The TBA-6::YFP fusion construct *fsEx262* will be described elsewhere (D. Hurd, R.M.M., L. Nuñez and D.S.P., manuscript submitted).

To produce the *cwp-5* stem-loop RNA interference (RNAi) construct, two overlapping fragments of *cwp-5* (571 bp and 639 bp) were amplified by standard PCR techniques. The two fragments were directionally cloned end-to-end into pPD49.78, then subsequently moved into pPD49.26 behind the *pkl-2* promoter. The construct was sequenced and injected into young adult *him-5* hermaphrodites at 50 ng/µl.

**Microscopy**

All images, except for those in Fig. 2A,B, were acquired with Nomarski DIC and epifluorescence optics using a Zeiss Axioplan 2 and Axioskop software. A Leica confocal microscope was used to produce the images in Fig. 2A,B. Image files were imported into Adobe Photoshop CS3 for adjustment of black, white and contrast levels.

**Behavioral assays**

Male behavioral assays were carried out according to described protocols (Hart, 2006; Liu and Sternberg, 1995). In all assays, the experimenter was blinded to the genotype of the animals until all observations were complete. Male nematodes were isolated at the L4 larval stage and allowed to reach sexual maturity overnight (16-20 hours). Young adult males were placed on a 1-cm lawn of *E. coli* OP50 with ~8 paralyzed *unc-31*(e169) hermaphrodites and observed for a 10-minute period. For a given strain, the response frequency was calculated as the percentage of males that exhibited response behavior, defined as sustained backing along the body of the hermaphroditic with the male tail in a ventrally arched posture, within 10 minutes of being placed on the bacterial lawn. Vulva location efficiency was calculated as the ratio of the number of successful vulva location events to the number of times that the
Translational impact

Clinical issue

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by the growth of fluid-filled renal cysts that can ultimately lead to organ failure. ADPKD is inherited dominantly by loss-of-function mutations in the PKD1 or PKD2 genes, which encode the human polycystin proteins PC1 and PC2. These two proteins combine to form a heterodimeric TRP (transient receptor potential)-family cation channel that localizes to sensory cilia in the renal epithelium. These channels are necessary for the flow-sensing ability of kidney cells. ADPKD pathology is thought to arise through a cellular-recessive mechanism in which the disruption of one allele of either PKD1 or PKD2 predisposes a cell to the somatic loss of the remaining functional allele. When this occurs, the current models of ADPKD hold that compromised flow sensing disrupts homeostasis in the renal epithelium, increasing cell proliferation and inducing the formation of cysts.

Results

In C. elegans, the polycystins LOV-1 (PC1) and PKD-2 (PC2) act in the ciliated endings of male sensory neurons to transduce signals that are important for the execution of mating behavior. In the current work, the authors identify CWP-5, a novel single-pass membrane protein that colocalizes with the polycystins to a characteristic set of male-specific sensory neurons. Males carrying cwp-5 mutations show significant defects in initiating mating behavior, a phenotype reminiscent of lov-1 and pkd-2 mutants. Genetic interactions between cwp-5 and the polycystins indicate that cwp-5 acts in the polycystin signaling pathway. However, these studies also reveal that LOV-1 and PKD-2 can interfere with sensory behavior in mutants lacking both cwp-5 and a single polycystin. CWP-5 may normally repress interference by preventing toxic polycystin forms from reaching the cilia and/or by blocking their aberrant proteins in the cilium itself.

Implications and future directions

These genetic interactions between CWP-5 and the C. elegans polycystins raise the possibility that the dominant view of ADPKD, in which cystogenesis results from the loss of polycystin function, may not adequately account for its pathogenesis. Instead, cells lacking PKD1 or PKD2 may also suffer from unmasked deleterious properties of the mutant polycystin. There are also intriguing parallels between CWP-5 and fibrocystin/polyductin (FPC), the ciliary protein linked to autosomal recessive polycystic kidney disease, that lead to more speculative suggestions about how aberrant polycystin functions could also contribute to ARPKD. Both of these ideas imply that interventions to block aberrant polycystin properties could have therapeutic value in polycystic kidney disease. Further studies in mice, where polycystic kidney disease pathology can be modeled more faithfully, will be important for testing the predictions of this toxic polycystin model.

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