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Polycystic Kidney Disease: Lessons Learned from *Caenorhabditis elegans* Mating Behavior

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Abstract

Male worm mating requires *lov-1* and *pkd-2* (homologs of the human polycystic kidney disease genes, *PKD1* and *PKD2*), which are expressed in male-specific neurons. Transcriptomic analysis of these neurons now catalogs molecules involved in signaling and ectosome biogenesis, with implications for human PKD.

Polycystic kidney disease (PKD) represents one of the most common human genetic diseases, affecting 1 in 800 individuals, and is a major cause of renal failure [1]. There are two autosomal dominant genes responsible for the disease — *PKD1* (OMIM:601313) and *PKD2* (OMIM:613095), encoding polycystin-1 (PC1) and polycystin-2 (PC2), respectively. PKD is characterized by the slow development of fluid-filled cysts in both kidneys, secondary to epithelial dedifferentiation, increased proliferation, apoptosis and the acquisition of a secretory phenotype. Both PC1 and PC2 have been localized to the primary cilia of renal epithelia and both are secreted on urinary exosome-like vesicles, 100 nm diameter membrane vesicles that are generated by the multivesicular body pathway and secreted from the apical aspect of the cell [2,3]. In the worm, the homologs of PC1 and PC2 — LOV-1 and PKD-2(LOV-2) — are needed for efficient male mating [4,5]. Male worms have 48 specialized sex-specific neurons, termed extracellular vesicle releasing neurons (EVNs), that are ciliated and produce 100 nm diameter membranous extracellular vesicles (EVs). These vesicles are secreted by an ectosomal pathway from the plasma membrane close to the base of the cilium. Cilia as well as EVs are needed for the acquisition of a hermaphrodite and subsequent mating. LOV-1 and PKD-2(LOV-2) localize to the cilia and EVs of male EVNs, showing that polycystin subcellular localization has been conserved over an evolutionary distance of 10^9 years. A study by Wang *et al.* [6] published in this issue of *Current Biology* now identifies new components of the polycystin-mediated signaling pathways in *Caenorhabditis elegans* that will be relevant to PKD.

Topologically, both LOV-1 and PKD-2(LOV-2) appear very similar to PC1 and PC2, respectively, with an identical arrangement of transmembrane domains and intra- and extracellular loops (http://www.kumc.edu/documents/kidney/PC1_LOV1.pdf). LOV-1 has two blocks of 20–25% identity in the last six transmembrane domains of PC1 and has a PLAT (polycystin-1, lipoxygenase and alpha-toxin) domain and a G-protein-coupled

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receptor proteolytic site (GPS). Cleavage at the GPS separates PC1 into carboxy- and amino-terminal portions and the same is predicted to occur in LOV-1. The extracellular domain of LOV-1 is mainly composed of a serine/threonine-rich mucin-like region, in contrast to PC1, which is a mosaic of leucine-rich repeat (LRR), carbohydrate-binding wall integrity and stress response component (WSC), C-type lectin, receptor for egg jelly (REJ) and 17 PKD1 domains. PKD-2(LOV-2) is a good homolog of PC2, with 36% identity across its six transmembrane domains (http://www.kumc.edu/documents/kidney/PC2_LOV2.pdf). The carboxy-terminal six transmembrane domains of PC1/LOV-1 and the six transmembrane domains of PC2/PKD-2(LOV-2) have homology to TRP cation channels and co-expression of PC1 and PC2 has been shown to generate cation channel activity [7]. Despite the differences in the first extracellular region of PC1 and LOV-1, the worm and human proteins are remarkably conserved [4]. Thus, male mating behavior presents a robust and tractable system for dissecting the biology of a *bona fide* polycystin complex, assessable using the power of worm genetics. The *lov-1* and *pkd-2* genes are involved in the generation of three behaviors: sex drive, which is the tendency of a male to leave a food supply and search for a hermaphrodite; response, which is the stereotyped circling behavior of a male when he finds a potential mate; and finally the location of the vulva (lov) [8]. All three behaviors are defective in *lov-1* and *pkd-2* mutants.

In the new study, Wang *et al.* [6] used an RNA-seq strategy to compare the transcriptome of the 27 *klp-6* (*klp-6*::GFP) positive EVNs with the rest of the male worm: KLP-6 is a kinesin-3 homolog that is required for the release of bioactive *pkd-2*::GFP-containing EVs. These authors found that 335 genes were over-represented between 2- and 11-fold, including the known EVN markers *klp-6*, *lov-1*, *pkd-2*, *cil-7* and the *cwp* genes. Gene ontology showed an enhancement of transcripts involved in neuropeptide signaling, ciliogenesis and synaptic transmission. A major finding is that the TNF-receptor-associated factors (TRAFs) TRF-1 and TRF-2 are involved in LOV-1/PKD-2(LOV-2) signaling, are not functionally redundant and are involved in all LOV-1/PKD-2(LOV-2)-controlled behaviors. The disruption of *trf-1* or *trf-2* had no effect on EV secretion or the localization of LOV proteins to the cilium. TRAFs have been shown to act downstream of Toll receptors, but disrupting members of the Toll pathway did not generate a defect in mating behavior.

Wang *et al.* [6] provide evidence that the three *lov-1/pkd-2*-dependent mating behaviors can be genetically dissected from one another, suggesting a bifurcation of the signaling pathway downstream of *trf-1/trf-2*. For example, mutants in the five-transmembrane-spanning, epidermal growth factor (EGF) and C-type lectin domain containing protein F25D7.5 could suppress the defect seen in the sex drive of *pkd-2* mutant worms, implying that the wild-type allele is an inhibitor of sex drive. The same mutant alleles had no effect on the other *pkd-2* mutant phenotypes, i.e. response and location of vulva. The two-transmembrane-spanning von Willebrand factor A domain-containing protein F31F7.2 was necessary for vulval location but not response or sex drive. These data show that LOV-1/PKD-2(LOV-2) signaling, and by inference PC1/PC2 signaling in mammals, is likely to be complex and that multiple downstream pathways may be involved.

Proteins involved in EV biogenesis were also discovered. The stress-activated p38 MAP kinase homolog *pmk-1* interfered with the biogenesis and release of *pkd-2*::GFP-positive

EVs from the head of male worms, but did not interfere with the localization of the protein to the cilium. These animals had a normal sex drive, but were defective in response and vulva location. Transmission EM studies on *pmk-1* mutants showed that there was an absence of EVs around the shaft of the cilium and that the cilium itself appeared to have fewer doublets than usual, suggesting that the pathways for loading PKD-2(LOV-2) onto the cilium and EVs are distinct and that EVs are necessary for non-drive mating behaviors. Finally, the investigators report two classes of EV cargo: two acid-sensing/ameloride-sensitive ion channels (ASICs), ASIC-2 and EGAS-1; and F14D7.11, a member of a class of antimicrobial peptides containing a cysteine-rich transmembrane (CYSTM) domain. CYSTM domains are thought to oligomerize in target membranes, generating a pore lined with cysteine residues. Mammalian exosomes also contain a range of antimicrobial peptides, defensins and azurocidin and can induce lysis of *Escherichia coli* [9].

Both worms and mammals secrete EVs containing the PC1–PC2 complex. The EVs in the worm are necessary for mate recognition and location of vulva but are not involved in sex drive. The integrity of the worm cilium is required for both of these functions, and so the EVs may be involved in delivering proteins directly to the cilium from the plasma membrane. This would account for the necessity of EV biogenesis in mating behavior. However, it does not explain why EVs are shed from the head of the worm. The most likely possibility is that the EVs are involved in signaling between animals and may act as a decoy for males competing for the same hermaphrodites. In this scenario, the EVs ‘jam’ the hermaphrodite-sensing ability of competing males. Indeed, the application of EVs to male worms causes them to transiently recognize themselves, generating a characteristic tail-chasing contortion, similar to the response seen when a male encounters a hermaphrodite. One would predict that, if males from divergent backgrounds were challenged in this way, this response might be considerably greater. From a teleological point of view, EVs might confer an advantage for the secreting worm by blinding the hermaphrodite-sensing ability of competing genetically disparate males, driving them into a persistent futile bout of self-recognition and/or clumping with other males [10]. If EVs can deliver a ‘package’ to a competitor, the presence of membrane-disrupting proteins, such as F14D7.11, may be responsible for the shortened lifespan seen in males in pooled populations versus those cultured alone [11].

So what are the functions of exosome-like vesicles in mammals? These vesicles have been shown to interact with the primary cilia of epithelial cells, but their physiological role is obscure [12]. This is because there are so many possible pathways that could be activated by these vesicles, given that they contain PC1/PC2 cation channels, the transmembrane Hedgehog-signal transducer Smoothened, seven-transmembrane spanners such as RAIG-1, 2 and 3, heterotrimeric G proteins, and microRNAs [13]. Perhaps these vesicles are involved in a ‘urocrine’ signaling pathway where upstream epithelial cells can signal to downstream cells via the urine flow, a process that might be particularly important in an injured kidney. Further studies of worm mating behavior will give us more clues.

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