require better proxies for past atmospheric CO_2 concentrations, especially in the Precambrian.

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The PKD protein qilin undergoes intraflagellar transport

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Cilia play diverse roles in motility and sensory reception, and defects in their formation and function underlie cilia-related human diseases [1]. One such disease is polycystic kidney disease (PKD), a heritable nephropathy associated with defects in the formation and function of sensory (also known as primary) cilia within renal tubules of the kidney [2]. Because the assembly and maintenance of these sensory cilia depends upon the intraflagellar transport (IFT) of axoneme and ciliary membrane components, such as polycystins [3], defective IFT is one of the factors that can contribute to PKD. Qilin is a ciliary, PKD-associated protein of as yet unknown function; here, we show that qilin does in fact undergo IFT to build sensory cilia on *Caenorhabditis elegans* neurons.

Qilin was identified recently in a forward genetic screen for PKD-related genes in zebrafish [4]. *qilin* mutants can assemble sensory cilia, suggesting that qilin is associated with PKD via an IFT-independent pathway [4]. This interpretation is complicated, however, because several zebrafish IFT particle proteins are maternally loaded and may support ciliogenesis even in the presence of loss-of-function mutations in the corresponding gene [4]. Subsequent genomic analysis uncovered qilin homologs in the human ciliary proteome and in the model flagellate *Chlamydomonas* [5]. *Chlamydomonas* qilin did not match any of the IFT particle polypeptides so far identified biochemically [4], but genome-wide transcriptome analysis revealed that it is upregulated during flagellar regeneration, suggesting that it has a role in flagellar assembly [6]. It therefore remains possible that qilin is associated with PKD because it participates in IFT.

To address this possibility, we have used time-lapse microscopy and genetics to determine whether qilin participates in IFT in the nematode *C. elegans* [7], which lacks a bona fide kidney yet has emerged as a valuable model for studying the basic cilia-related mechanisms associated with PKD [8]. First, we identified a qilin homolog in *C. elegans* as C04C3.5 (*E* value $2.1e^{-46}$; identity = 35%; positives = 57%). We asked if any of the *C. elegans* chemosensory ciliary mutants (defective in *che*motaxis; *osm*otic avoidance; *da*uer larva *f*ormation, and *dy*e-*f*illing [9]) contain a molecular lesion within C04C3.5 and found that such a mutant, *dyf-3*, had, in fact, recently been cloned [10]. Careful fluorescence and electron microscopy of sensory ciliary structure in the *dyf-3* mutant had revealed a complete loss of distal segments and truncated middle segments of the cilia [10]. This morphology phenocopies mutations in IFT subcomplex B polypeptides, but is distinct from *osm-3* and *bbs-7/bbs-8* mutants (which have fully intact middle segments), indicating that *C. elegans* qilin/DYF-3 contributes to the assembly of both middle and distal ciliary segments as a component of IFT particle B (Figure 1A,B). In the residual truncated middle segment, no IFT can be detected using time-lapse fluorescence microscopy of the IFT particle subunit OSM-6::GFP in a *dyf-3* mutant background, suggesting that qilin is required for normal IFT within these sensory cilia. This finding is distinct from *osm-3* and *bbs-7/bbs-8* mutants, which support IFT along the middle segments, but leaves open the question of how the middle segment is maintained within *dyf-3* mutants in the absence of IFT.

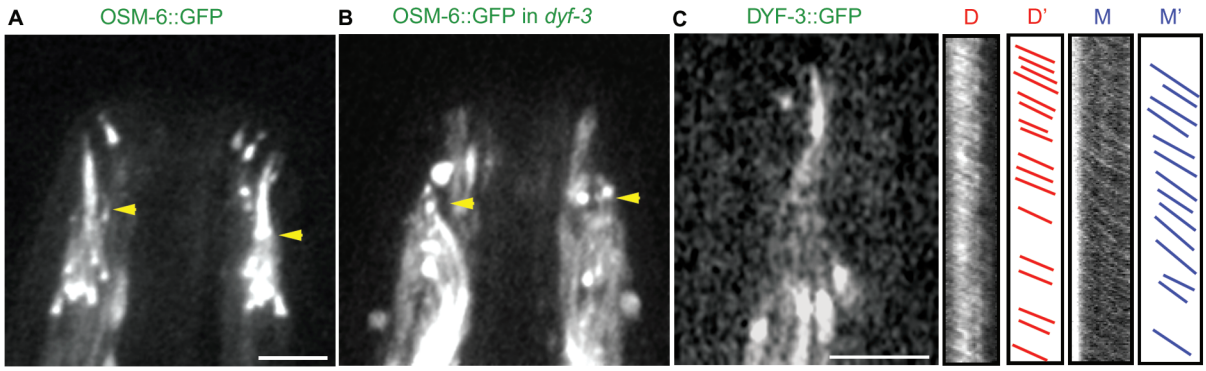

Figure 1. The qilin homolog, DYF-3, is an essential component of the IFT machinery that builds sensory cilia.

The *dyf-3 (m185)* mutant has truncated chemosensory cilia and displays no detectable IFT. (A) Wild-type axonemes are 7.5 μm long, containing a 1 μm long basal body, the transition zone, a 4 μm middle segment, and a 2.5 μm distal segment. Arrowheads show the middle–distal segment junctions. Fluorescence micrographs show the distribution of IFT particles (OSM-6::GFP) along the wild-type sensory cilia. (B) The *dyf-3* mutant loses its distal segments and has only a truncated middle segment and, moreover, no IFT can be detected in the remaining middle segment. (C) Motility of DYF-3::GFP within sensory cilia. Left panel shows fluorescence micrograph of sensory cilia. Right panels are kymographs with corresponding cartoons showing the lines representing selected DYF-3 particle trajectories along the middle segments (M, M') and the distal segment (D, D'). Kymographs show that motility along the distal segment is faster than along middle segments. Bar = 5 μm.

The cilia-specific localization of DYF-3, together with its mutant ciliary phenotype are consistent with the idea that *C. elegans* qilin might function in the movement of the IFT particle B complex. If this is indeed the case, a fluorescent DYF-3::GFP fusion protein should be able to be seen participating in IFT along chemosensory cilia using time-lapse spinning disc confocal microscopy [7]. We tested this prediction and observed that DYF-3::GFP does indeed undergo IFT, moving at 0.73 +/– 0.13 μm/sec (*n* = 243) along the middle segment of the sensory ciliary axoneme then accelerating to 1.25 +/– 0.16 μm/sec (*n* = 255) along the distal segment (Figure 1C and Movie S1 in Supplemental Data available with this article online). These two transport velocities are very similar to those previously observed for IFT particles [7]. This indicates that DYF-3::GFP is associated with IFT particles that are moved along the middle segment by kinesin-II and OSM-3-kinesin acting together, at a speed intermediate between each motor's characteristic speed, but are moved along the distal segment only by the faster motor, OSM-3-kinesin, acting alone [7].

Thus, based on these simple, straightforward observations of the transport properties of DYF-3::GFP along *C. elegans* sensory cilia, in combination with previous observations of the ciliary structure in the *dyf-3* mutant, we propose that *C. elegans* DYF-3 is a homolog of the zebrafish PKD protein qilin and is a conserved intraflagellar transport protein that plays an essential role in building sensory cilia.

Supplemental data

Supplemental data consisting of a movie showing transport of DYF-3::GFP along sensory cilia of the rescued *dyf-3 (m185)* mutant are available at http://www.current-biology.com/cgi/content/full/15/11/R410/DC1/

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