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Decoding Cilia Function: Defining Specialized Genes Required for Compartmentalized Cilia Biogenesis

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Summary

The evolution of the ancestral eukaryotic flagellum is an example of a cellular organelle that became dispensable in some modern eukaryotes while remaining an essential motile and sensory apparatus in others. To help define the repertoire of specialized proteins needed for the formation and function of cilia, we used comparative genomics to analyze the genomes of organisms with prototypical cilia, modified cilia, or no cilia and identified ∼200 genes that are absent in the genomes of nonciliated eukaryotes but are conserved in ciliated organisms. Importantly, over 80% of the known ancestral proteins involved in cilia function are included in this small collection. Using *Drosophila* as a model system, we then characterized a novel family of proteins (OSEGs: *o*uter *seg*ment) essential for ciliogenesis. We show that *osegs* encode components of a specialized transport pathway unique to the cilia compartment and are related to prototypical intracellular transport proteins.

Introduction

Cilia are microtubule-rich, hair-like cellular extensions that perform essential motile and sensory functions. In sperm and in unicellular eukaryotes, a motile form of cilia called flagellum propels cells to their destination, while in epithelial cells, multiple motile cilia beat synchronously to stir extracellular fluid (Sleigh, 1974). In vertebrate photoreceptor cells and invertebrate mechano- and chemoreceptor neurons, the entire sensory transduction machinery is housed in a specialized cellular compartment derived from the cilium (Bloodgood, 1990; Sleigh, 1974). This domain, known as the outer segment, is a hallmark of these sensory neurons and an outstanding example of subcellular compartmentalization as a strategy to optimize function (Figure 1A).

Given the wide range of cells and tissues that contain cilia, and the extraordinary diversity of roles performed by cilia (Bloodgood, 1990; Rosenbaum and Witman, 2002; Sleigh, 1974), a basic question in cell biology is how ciliogenesis is orchestrated and to what extent common mechanisms underlie this process. Cilia formation begins when the basal body, a centriole-related structure, serves as a template for the assembly of the axoneme. This process can proceed through two different mechanisms. In most motile and sensory cilia, the basal body docks to the plasma membrane, and a bud-like structure containing the axoneme and the ciliary membrane projects out from the cell body; since the ciliary membrane and the axoneme are assembled concurrently as a compartment separated from the cell body, we refer to this process as compartmentalized ciliogenesis. In a few cases, however, such as in the sperm cells of *Drosophila* (Tokuyasu et al., 1972) and the flagella of the parasite Plasmodium (Sinden et al., 1976), the entire axoneme is first assembled inside the cytosol and only later is either extruded or matures into a flagellum (i.e., cytosolic biogenesis).

Unlike cytosolic biogenesis, the process of compartmentalized ciliogenesis requires that cilia, flagella, and outer segments transport their building blocks—proteins and metabolites—from the cell soma. Genetic and biochemical studies in the biflagellated green alga Chlamydomonas have singled out kinesin II, dynein 1b, and 17 additional proteins named *i*ntra*f*lagellar *t*ransport (IFT) particle proteins as candidate proteins involved in flagella biogenesis. IFT particle proteins are proposed to function as macromolecular rafts traveling up and down the flagellum, via kinesin and dynein, transporting axonemal precursor proteins to their growing tips (Rosenbaum and Witman, 2002). Consistent with this postulate, mutations in the Chlamydomonas IFT particle proteins IFT88 and IFT52 produce very short flagella (Brazelton et al., 2001; Pazour et al., 2000). Similar results are seen in *C. elegans* mutants defective in the IFT orthologs OSM-5 and OSM-6 (Perkins et al., 1986).

We are interested in the biogenesis of cilia, with particular emphasis on the formation of sensory outer segments and developed a novel bioinformatics approach to identify genes involved in ciliogenesis. Our strategy is based on the hypothesis that the ancestral eukaryote was a ciliated unicellular organism (Cavalier-Smith, 2002), and that cilia and flagella were independently lost throughout evolution from several eukaryotic groups (Cavalier-Smith, 2002). By comparing the genomes of ciliated and nonciliated organisms, we have identified a collection of candidate genes important for cilia formation and function. In addition, by phylogenetically examining orthologs in organisms with "compartmentalized" versus "cytosolic" axonemes, we isolated a large subgroup selectively expressed in *Drosophila* sensory outer segments, but not in sperm, and characterized a novel family of proteins (OSEGs: *o*uter *seg*ment) essential for compartmentalized ciliogenesis. Together, these stud-

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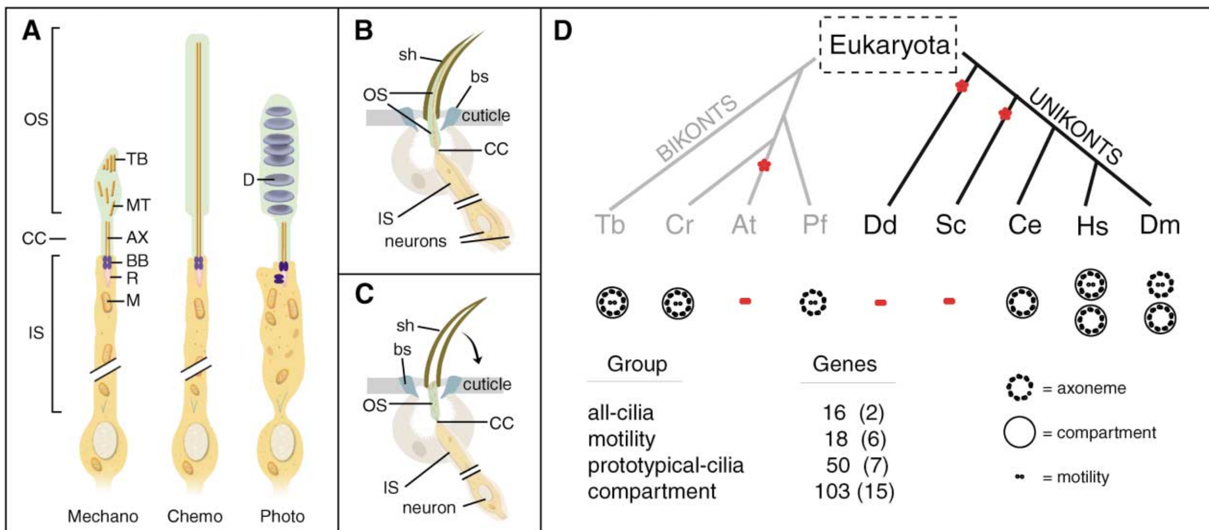

Figure 1. Sensory Cilium Structure and Evolution

(A) The sensory cilium is composed of a connecting cilium (CC) and the outer segment (OS). Cilia are anchored to the inner segment (IS) through the basal body (BB) and rootlet (R). M, mitochondria; AX, axoneme, MT, microtubules; D, discs; TB, tubular body; this is a rich array of tightly packed microtubules at the tip of the cilia. Diagram of *Drosophila* external chemosensory (B) and mechanosensory (C) organs, bristle socket (bs) and bristle shaft (sh).

(D) Ciliary genes are selectively lost from nonciliated organisms but maintained in ciliated organisms. Eukaryote phylogeny showing the two main lineages: Bikonts (gray) and Unikonts (Stechmann and Cavalier-Smith, 2002, 2003). The ancestral nature of the eukaryotic cilia is evident by its presence in organisms from both lineages: *D. melanogaster* (Dm), *H. sapiens* (Hs), *T. brucei* (Tb), and *C. reinhardtii* (Cr). In three independent events (indicated by red stars), cilia were lost in lineages leading to *A. thaliana* (At), *D. discoideum* (Dd), and *S. cerevisiae* (Sc). Similarly, compartmentalized cilia were lost in *P. falciparum* (Pf), while motile cilia were lost in *C. elegans* (Ce). Shown below the phylogenetic tree is an illustration of the various ciliary structures present in the different organisms used in the bioinformatics searches. Also shown is a summary of the screens, which provides the number of genes identified in each search (the numbers in parenthesis refer to previously known genes); Supplemental Figure S1 online shows a flowchart illustrating the four bioinformatics screens.

ies establish a compelling bioinformatics strategy to help decode gene function and lay the foundation for a comprehensive dissection of eukaryotic ciliogenesis and outer segment development.

Results and Discussion

Ciliary Genes Are Selectively Conserved in Ciliated Organisms

In order to identify specialized genes essential for cilia biogenesis and function, we undertook a phylogenetic screen that identified genes conserved in the genomes of ciliated organisms but absent in nonciliated eukaryotes. We reasoned that gene loss can be used as a powerful tactic to map gene function, particularly if the biological process in question (e.g., cilia biogenesis in this case) is conserved in distantly related species and if it was lost more than once during evolution. We chose eight species representing the two major lineages of eukaryotic evolution (Stechmann and Cavalier-Smith, 2002, 2003), and which included nodes where cilia were lost or modified during the evolution of eukaryotes (Figure 1D).

Because *Drosophila* contains experimentally tractable motile and sensory cilia and has an extensively annotated genome, we selected it as the anchor for these studies. We performed BLAST searches against the proteome of five ciliated (*H. sapiens* [Hs], *C. elegans* [Ce], *P. falciparum* [Pf], *C. reinhardtii* [Cr], and *T. brucei* [Tb]) and three nonciliated organisms (*A. thaliana* [At], *S. cerevisiae* [Sc], *D. discoideum* [Dd]) and searched for orthologs of the ~14,000 *Drosophila* genes in each of these species using a "reciprocal best hit" algorithm (Li et al., 2003; Remm et al., 2001). Because the *T. brucei* and *C. reinhardtii* genomes are incomplete (see Experimental Procedures), we considered a ciliary protein as conserved in Bikonts if it was present in either of those two species (Cr/Tb). Similarly, a partial draft of the *Dictyostelium discoideum* (Dd) proteome is now available; this organism displays exquisite motility, yet it lacks ciliated structures, thus providing a robust bioinformatics counterscreen.

Because all ciliated organisms have an axoneme but may differ in their mode of ciliogenesis, or whether they have motile or nonmotile cilia, we suspected that distinct sets of proteins might be required during biogenesis of the various forms of cilia. Therefore, we applied our screening strategy to four different search routines (see Supplemental Figure S1 at http://www.cell.com/cgi/content/full/117/4/527/DC1): First, to identify genes involved in processes common to all cilia, like axoneme formation, we compared all ciliated versus all nonciliated eukaryotes (i.e., genes conserved in Hs, Dm, Pf, Cr/Tb, and Ce but not in At, Sc, or Dd). Second, to identify genes involved in cilia motility (either of compartmentalized or cytosolic origin), we compared organisms with motile cilia versus those with nonmotile or no-cilia (i.e., genes conserved in Hs, Dm, Pf, and Cr/Tb but not in Ce, At, Sc, or Dd). Third, to identify genes involved in cilia compartmentalization, we compared organisms

with compartmentalized cilia biogenesis versus cytosolic biogenesis (e.g., genes conserved in Hs, Dm, Ce, and Cr/Tb but not in Pf, At, Sc, or Dd). Finally, to identify genes that may be unique to organisms that have both motile and compartmentalized cilia, we also searched for genes shared between *Drosophila* and organisms with prototypical cilia (i.e., Hs and Cr/Tb but not in Ce, Pf, At, Sc, or Dd).

From a total of 121,243 predicted transcriptional units and 141,000 ESTs (we used ESTs in Chlamydomonas due to the lack of an assembled partial proteome), we identified a total of 187 ancestral genes (Supplemental Table S1): (1) 16 conserved in all ciliated organisms, but absent in nonciliated (all-cilia subset); (2) 18 present only in organisms with motile cilia (motility subset); (3) 103 common only to organisms with compartmentalized cilia biogenesis (compartment subset); and (4) 50 shared only between organisms with prototypical cilia (both motile and compartmentalized; prototypical-cilia subset).

To evaluate the performance of the screen, we asked whether known genes implicated in ciliogenesis are indeed enriched in this collection. A search of the literature revealed that there are 36 genes that have been implicated in ciliogenesis in either flies or in other organisms and were part of the likely ancestral repertoire of genes in the primitive eukaryotic cell (e.g., conserved in organisms from both ancestral eukaryotic lineages; see Experimental Procedures). This set includes specialized genes whose primary role is in cilia biogenesis and function (e.g., dynein arms, IFTs), as well as genes that may also participate in other cellular processes (e.g., dynein light chains). A breakdown of these genes using the criteria utilized in our screen is shown in Figures 1D and 2. Remarkably, 30 out of the 36 known genes (>80%) are included in the 187 ancestral gene collection obtained in our bioinformatics screen; of the remaining six, five also function outside the cilia and were filtered out because they are present in nonciliated organisms (four dynein subunits and myosin VIIA), and one (left/right-dynein) was eliminated because it did not have an ortholog in Tb or Cr.

The selectivity of the screen is also illustrated by examining the genes in the motility subset (Figure 2): all six known ciliary genes recovered in this collection, in fact, encode proteins involved in motility (four axonemal dynein subunits, a radial spoke protein, and Mbo2, a protein important for flagella waveform). In addition, of the remaining 12 candidate motility genes in this subgroup (Supplemental Table S1), five are specifically expressed in testis (see Figure 2), a tissue highly enriched in motile cells. Taken together, these results substantiate the logic of the approach and our search criteria.

The Compartment Gene Set

We are particularly interested in the formation of sensory outer segments, therefore we focused on the genes in the cilia-compartment subset both as a platform for gene discovery and for dissecting mechanisms of outer segment biogenesis. Curation of the 103 candidates in this group (Figure 2 and Supplemental Table S1 online) suggested that several may not have a direct role in ciliogenesis, yet they cosegregated with our selection criteria. These included ion channels, signal-transduction components, transcription factors, and metabolic enzymes. In order to extract "ciliary" genes from this subset, we demanded that candidates meet two additional search criteria. First, many genes involved in sensory cilia formation share an upstream regulatory sequence known as the X box, often at 150 to 50 nucleotides upstream from the translation start site (Dubruille et al., 2002; Swoboda et al., 2000). A general search of the *D. melanogaster* and *C. elegans* genomes for the presence of the 14 nucleotides consensus X box motif demonstrated that this sequence is much too abundant to be used as a primary screen (for instance, 2449 of Dm and 1897 of Ce genes contain such a motif); however, as a secondary screen, it selected 41 candidates from the cilia-compartment subset. Notably, over 90% of the known ciliary genes in the compartment subset (14/15) are included in these 41 genes. Second, compartmentalized cilia in *Drosophila* are found only in chemo- and mechanosensory neurons. Because these neurons are scattered all over the fly body and comprise a minute fraction of the fly cells, available EST databases contain none, or very few, representatives ESTs (data not shown). Based on this premise, we searched the compartment subset for genes that contained 0-4 ESTs and identified 48 candidates. Importantly, these 48 candidates contain nearly all of the known ciliary genes in the original collection (13/15). Together, these two secondary screens identified a total of 30 genes that overlapped both the X box and EST filters (see Figure 2 and Supplemental Table S1 online); these were chosen for biological validation.

Ciliary Compartment Genes Are Expressed in *Drosophila* Sensory Neurons

Genes involved in compartmentalized ciliogenesis should satisfy two important requirements. First, the genes should be expressed in ciliated sensory cells. Second, the proteins must be essential for outer segment formation or function.

The genes selected in the compartment subset encode members of several protein groups, including IFT proteins, Bardet-Biedl syndrome (BBS)-related polypeptides, C2 domain-containing proteins, small G proteins, a group of "coil-coil" proteins, and a family of six WD-domain proteins (OSEGs). Below we provide a short summary of these families.

BBS is a heterogeneous genetic disorder that is characterized by retinal dystrophy, renal malformation, learning disabilities, and obesity. Six BBS genes have been cloned, and several were recently implicated in ciliogenesis (Ansley et al., 2003). *Drosophila* has three BBS orthologs, and all three were selected in this screen (BBS1 and BBS8 as part of the compartment subset, and BBS4 as part of the prototypical-cilia subset). Interestingly, this collection also includes two additional proteins sharing a similar domain organization (CG5142 and CG4525; Figure 2), which we suggest encode new BBS members.

The C2 domain is a ~120 amino acid sequence that functions as a Ca^{2+}-dependent membrane-targeting module in proteins involved in signal transduction (e.g., protein kinase C, cytosolic phospholipase A2) or transport processes (e.g., synaptotagmin I, rabphilin) (Shao et

Compartment Genes:

Gene ID	Name-Domain	EST	X	
CG14825	BBS1	1(0)	C	☆
CG13691	BBS8	0	CD	☆
CG30441	IFT20	2(0)	CD	
CG9595	IFT52	0	C	
CG8853	IFT57/55	0	CD	
CG12548	IFT88/ NompB	0	CD	
CG7161	Oseg1/DAF-10	3(0)	D	☆
CG13809	Oseg2//IFT172	1(0)	CD	☆
CG11838	Oseg3/CHE-11	0	CD	☆
CG9333	Oseg5/CHE-2	0	C	☆
CG6560	Arl3	2(1)	C	
CG11048	Rib74	1(0)	C	
CG2069	Oseg4	3(0)	CD	☆
CG11237	Oseg6	1(0)	D	☆
CG17599	Coiled coil	0	D	
CG1126	Coiled coil	0	C	☆
CG3259	Coiled coil	0	CD	☆
CG1399	Leucine-rich	2(0)	D	
CG5142	TPR	0	CD	☆
CG4525	TPR	4(0)	CD	
CG7735	Arl6	0	D	☆
CG18631	C2	1(0)	CD	
CG14870	C2	1(0)	C	☆
CG9227	C2	2(dc)	CD	☆
CG13178		0	C	
CG15161		1(0)	D	☆
CG14367		1(0)	D	

Compartment Genes: no X-box or few ESTs

Gene ID	Name-Domain	EST	X
CG10642	Klp64D/KIF3A	1(0)	-
CG11759	Kap	7(0)	-
CG32392	RP3	13(12)	D
CG11755		0	-
CG5195	Leucine-rich	1(0)	-
CG2006	DTW	4(0)	-
CG31249		4(2)	-

Motility Genes Group

Gene ID	Name-Domain	EST	X
CG5882	MBO2	0	D
CG8800	LC1	0	D
CG6053	IC69/IC70/DNAI2	0	D
CG9492	DNAH5/DNAH8	1(0)	-
CG3121	Radial spoke p. 4	4(2)	-
CG9313	DNAI1	29(26)	-
CG8197	Leucine-rich	11(10)	-
CG10064	WD repeats	14(13)	D
CG18675	Coiled coil	17(16)	D
CG10958	Coiled coil	24(21)	-
CG4836	GroES-like	98(93)	-
CG14358	WD	0	D
CG30259	Coiled coil	0	D

All Ciliary Genes Group

Gene ID	Name-Domain	EST	X	
CG5359	LC2/Tctex-2	3(1)	C	☆
CG6971	DNALI1/P28	6(5)	C	
CG5987	Tubulin ligase	23(22)	-	

Prototypical Cilia Genes Group

Gene ID	Name-Domain	EST	X	
CG13232	BBS4	0	D	☆
CG14905	p66/Oda1	0	-	
CG8407	Dlc4	1(1)	D	
CG5526	Dhc36C/ DNAH7	3(3)	-	
CG14271	PF2/Gas8	5(4)	-	
CG7293	Klp68D	9(0)	D	
CG7264	Rib43A	10(8)	-	
CG11449	Coiled coil	0	-	
CG10061	T complex 10	2(0)	D	
CG16789	ATPase	3(0)	D	
CG31652	WD40 Coiled coil	7(6)	D	
CG31345	EF-hand	1(0)	-	
CG15120	ARM repeat	1(0)	-	
CG15027		2(0)	-	
CG16896	WD,Ypt/Rab-GAP	4(0)	-	
CG7742	Ypt/Rab-GAP	20(16)	-	

Figure 2. Candidate Ciliary Genes

Genes previously implicated as ciliary and isolated in the four bioinformatics screens described in this paper are highlighted by the gray boxes. EST refers to the total number of ESTs, with the number in testis in parenthesis. X box refers to the presence of an X box in either flies (D) or worms (C). Novel candidate genes that meet the EST and X box criteria are highlighted in yellow. Genes selected for promoter fusions are indicated by the star symbol. See Supplemental Figure S1 online for a summary of the bioinformatics screens.

al., 1996). Our analysis identified three novel C2 domain-containing proteins (CG18631, CG9227, and CG14870). Given the central role of calcium in regulating cilia function (Tamm, 1994)—as well as processes as diverse as membrane fusion, protein transport, and protein breakdown—these are worthy candidates for sensors of the calcium signals. Small G proteins are known to function as universal molecular switches in a wide range of intracellular processes. Recently, Leishmania ARL3 (LdARL-3A) was implicated in flagellum biogenesis (Cuvillier et al., 2000). Notably, our screen identified ARL3 and ARL6, two Arf-like proteins, as components of the compartment group (see below). The cilia-compartment subset also contains orthologs of all seven known IFT particle proteins. In addition, this group also contains two novel WD domain-containing proteins (OSEGs) and three novel coiled-coiled candidate IFT members (see Figure 2). OSEGs are a family of six related polypeptides sharing the same predicted topology (Figure 4D) and signature sequences: an N terminus with seven tandem WD repeats (~300 residues), a β sheet rich interdomain (~300 residues), and multiple TPR-like repeats (tetratricopeptide repeats; ~300 residues). WD repeats are 44–60 residue sequence motifs that fold as parts of two adjacent blades of a typically seven blade propeller structure. TPR-like repeats comprise a TPR-related sequence motif that folds into two antiparallel α helices; these in turn assemble into large right-handed helices. WD- and TPR-like-repeats are often found in large macromolecular assemblies and are thought to function as structural platforms for reversible protein-protein interactions (Das et al., 1998; Neer and Smith, 1996) (see below).

To identify the cells that express the candidate ciliary compartment genes, we selected 15 genes representing the various gene families (indicated by a red star in Figure 2), plus a control each from the all-cilia (Tctex2) and prototypical-cilia subsets (BBS4), and generated

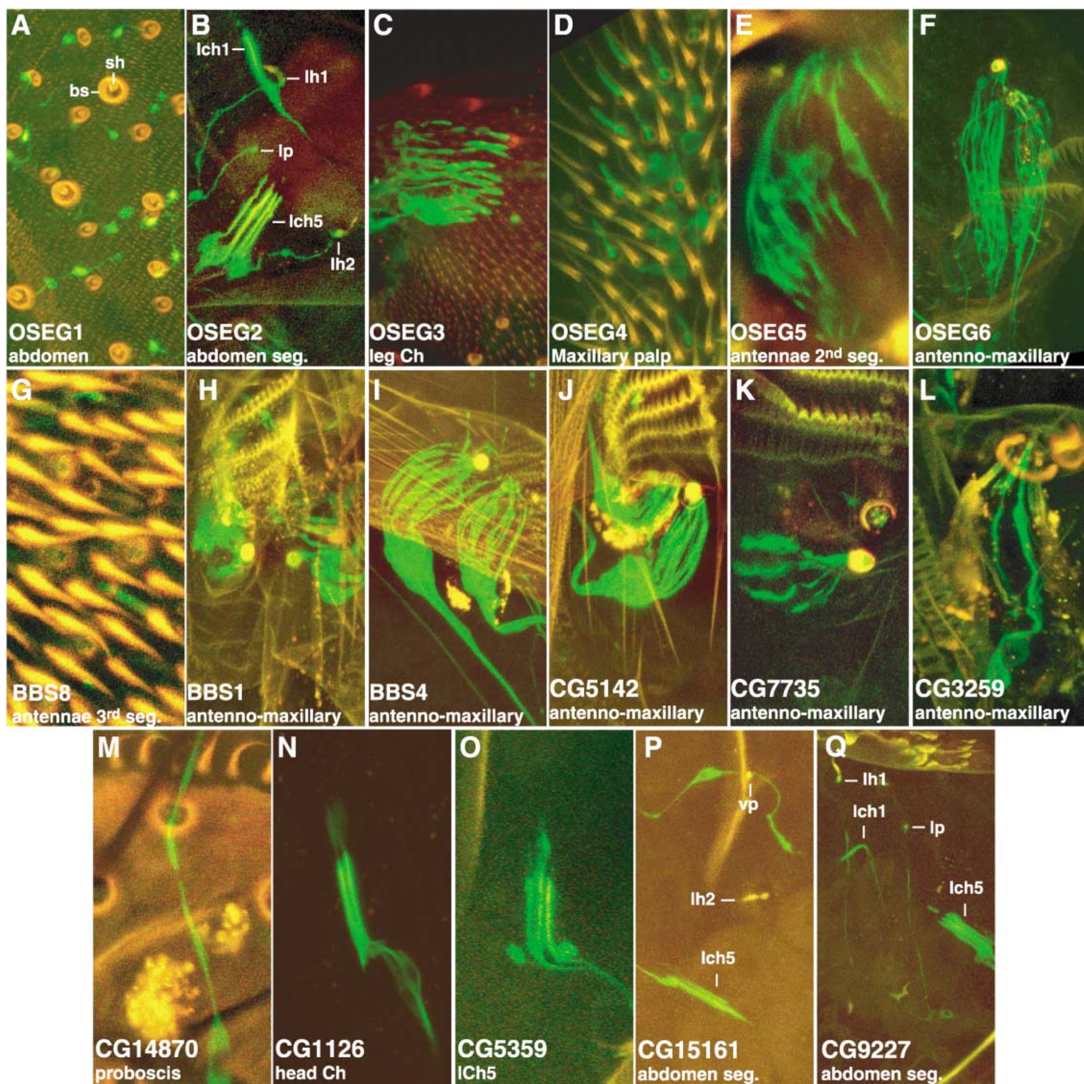

Figure 3. Ciliary Compartment Genes Are Selectively Expressed in Ciliated Sensory Neurons

Upstream regulatory regions of candidate ciliary compartment genes were fused to Gal4 and expressed in animals carrying UAS-GFP or UAS-GFP-α1tub84B reporters; *oseg1* (A); *oseg2* (B); *oseg3* (C); *oseg4* (D); *oseg5* (E); *oseg6* (F); BBS8 (G); BBS1 (H); BBS4 (I); CG5142 (J); CG7735 (K); CG3259 (L); CG14870 (M); CG1126 (N); CG5359 (O); CG15161 (P); CG9227 (Q). lch, lateral chordotonal organs; lh, lateral hairs; lc, lateral campaniform organ. See Table 1 for a compilation of expression data.

transgenic flies expressing Gal4 promoter fusions. Individual lines were crossed to flies containing UAS reporters and examined for GFP expression in larvae and adult animals.

In *Drosophila*, there are three types of ciliated cells: sperm, mechanosensory, and chemosensory neurons. Mechanosensory and chemosensory cilia are assembled through compartmentalized ciliogenesis, while the sperm tail is assembled via cytoplasmic ciliogenesis. Figure 3 and Table 1 demonstrate refined specificity in the anatomical sites of expression of all 17 genes: each transgene is restricted to ciliated cells, with BBS4 and the 15 candidate compartment genes expressed exclusively in neurons of mechanosensory and chemosensory organs. The remaining one, Tctex2/LC2 (a dynein light chain subunit from dynein arms and cytosolic dyneins), was also expressed in sperm cells (data not shown; see Patel-King et al. [1997]). No other sites of expression were observed for any of the transgenes. Taken together, these results strongly authenticate our bioinformatics strategy, provide a new perspective into the evolution of cilia, and set the foundation for a comprehensive use of this approach in other biological processes.

oseg Genes Are Specifically Required for Outer Segment Formation

To gain insights into the biology of outer segment biogenesis, we next screened for mutants defective in candidate cilia-compartment genes. *Drosophila* mutants with outer segment defects are expected to be mechanosensory defective; we therefore screened mutagenized F3 lines for the presence of mechanoinsensitive flies (Han et al., 2003; Kernan et al., 1994) and recorded

Table 1. Expression Pattern of Ciliary Compartment Genes

	Larvae			Adult		Adult
	A-M	Ch	ES	Ch	ES	T
oseg1	√	√	√	√	√	–
oseg2	√	√	√	√	√	–
oseg3	√	√	–	√	√	–
oseg4	√	√	√	√	√	–
oseg5	–	√	–	√	√	–
oseg6	√	√	ND	√	√	–
CG7735	√	√	ND	√	√	–
CG9227	√	√	√	√	√	–
CG14870	–	–	–	–	√	–
CG5142	√	√	√	√	√	–
CG1126	√	√	–	–	√	–
CG3259	√	√	–	√	√	–
CG15161	√	√	√	√	√	–
CG14825	√	√	√	√	√	–
CG13691	√	√	√	–	√	–
CG13232	√	√	√	√	√	–
CG5359	√	√	√	–	√	√

ND: not determined.

Flies expressing promoter fusions for all 17 candidate genes were examined for anatomical sites of GFP expression in larvae and adult animals (see also Figure 3). There was no expression of the reporter in nonciliated cells. A-M, larval anteno-maxilary complex; Ch, chordotonal organs; ES, external sensory organs; T, testis.

mechanoreceptor currents (MRC; Figure 5A) and transepithelial potentials (TEP; Figure 5B) from candidate lines. Mutations that affect the cilia are predicted to show defective MRC (Walker et al., 2000). In contrast, mutations that affect the function or development of the support and accessory cells should abolish both the MRC and the TEP (Barolo et al., 2000). We recorded MRCs and TEPs from multiple bristles in various uncoordinated lines and selected complementation groups with normal TEP but defective MRC and tested them in chemosensory and sperm motility assays. Two complementation groups with abnormal mechano- and chemosensory responses but normal sperm motility mapped near the location of *oseg1* and *oseg2*, respectively (Figures 5A–5C). We expected that mutant alleles would carry missense or nonsense mutations, and that introduction of the wild-type gene into mutant animals should rescue their behavioral and physiological phenotype. Indeed, *oseg1*^{179} and *oseg1*^{10} alleles had stop codons in *oseg1* (Figure 4A), and the *oseg2* allele contained a nonconservative substitution in the *oseg2* gene (Figure 4B). More importantly, introduction of the wild-type *oseg1* and *oseg2* genes by germline transformation rescued the uncoordinated and MRC defects of *oseg1* and *oseg2* mutants (Figure 4C and data not shown).

To analyze the phenotype of *oseg1* and *oseg2* mutants in detail, we examined the ultra-structure of the sensory cilium by EM serial section analyses. Wild-type mechano- and chemosensory dendrites contain a striated rootlet, two basal bodies, a connecting cilium, and the outer segment (Figures 5D and 5H). *oseg1* and *oseg2* mutants have normal inner segments and an intact rootlet, basal bodies, and connecting cilium (Figures 5E–5F). However, both mutants display dramatic defects in outer segment morphology (Figures 5I and 5J): In mechanoreceptor neurons, *oseg1* has a striking reduction of the distal-most end of the outer segment (the dendritic tip and tubular body), while *oseg2* has a total loss of the tubular body. In chemoreceptors, *oseg1* and *oseg2* both show severely shortened outer segments (data not shown). Together, these results firmly implicate *oseg* genes in ciliogenesis, and outer segment formation.

OSEGs Are Required for Selective Intraciliary Transport

HMM analyses and secondary structure predictions indicate that OSEGs are related to α- and β'-coatomers (Figure 4D), two proteins involved in intracellular trafficking (Kirchhausen, 2000). Significantly, clathrin heavy chain (Chc) also displays prominent domain similarity to OSEG family members (Figure 4D). Because outer segments (and cilia) are separated from the rest of the cell by a connecting cilium, they need to import their proteins from the cell soma and therefore might be expected to require specialized machinery to assemble a functional compartment.

If the OSEG proteins were essential for the transport of selective macromolecules into ciliary compartments, we would expect them to meet several criteria. First, in contrast to structural or signal transduction components of the outer segment, OSEGs should travel in and out of the outer segment, while concentrating primarily at the base of the cilia. This region of the cell is considered the cilium's "hub," a strategic place between the cell soma and the outer segment, and is hypothesized to function as the site where molecules targeted to the cilium are loaded and transported via the microtubule-based motors (Cole et al., 1998; Dwyer et al., 2001; Marszalek et al., 2000; Pazour et al., 1999). Second, ciliary cargo should be transported normally from the cell soma to the cilia base of *oseg* mutants, but it should be unable to enter the cilia and therefore may accumulate near the cilia base.

To examine the subcellular localization of OSEG proteins, we engineered translational fusions between all six OSEG family members and GFP. Each fusion protein was then targeted to ciliated sensory cells using a panneuronal promoter. In order to mark the position of the cilium, we co-labeled the cells with mab21A6 (Fujita et al., 1982), a monoclonal antibody that labels the base of the cilium at the inner/outer segment boundary. As predicted, all GFP-tagged OSEG proteins localize primarily at the base of the cilium (Figure 6) and can be found inside the sensory cilia (Figures 6G–6I).

To examine transport into outer segments, we needed to identify a candidate cargo protein, ideally one that requires either of the available mutants (*oseg1* or *oseg2*). Mechanosensory outer segments contain at their distal-most end a unique microtubule-rich structure known as the tubular body; this is the proposed site of channel anchoring and force generation in mechanosensory bristles (Figure 1A). The α-tubulin isoform in the tubular body is encoded by the α1tub84B gene in *Drosophila*. Therefore, we hypothesized that α1tub84B would be an ideal OSEG cargo. Figure 7A demonstrates that overexpression of a plain GFP reporter, or even a membrane tagged-GFP (data not shown), does not label the outer segment of ciliated neurons. However, if GFP is linked to α1tub84B (i.e., a GFP-α1tub84B fusion protein), it is

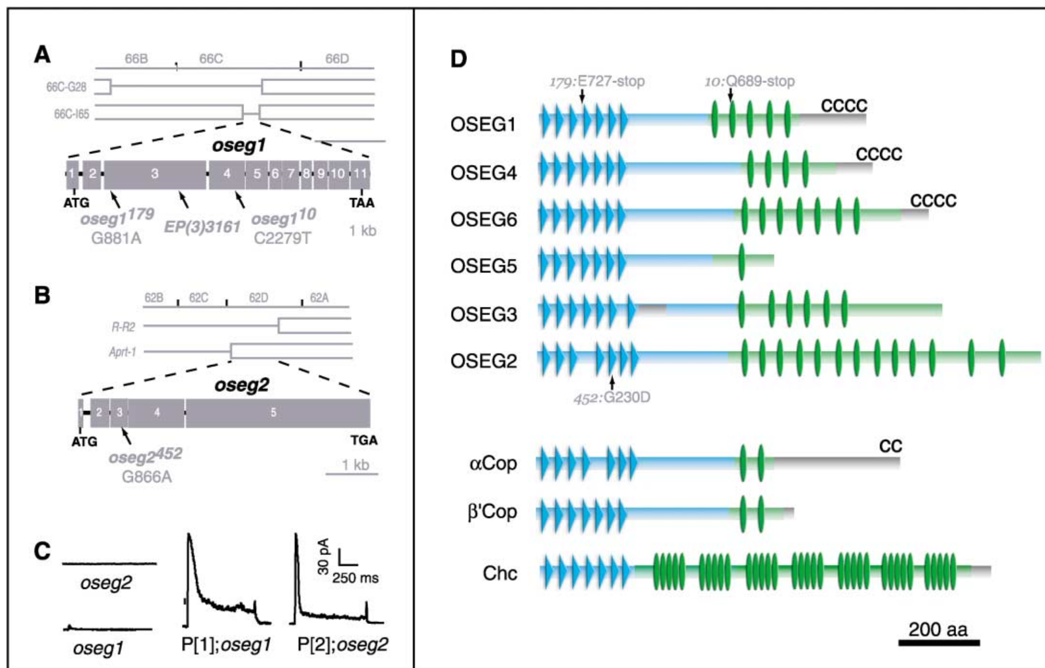

Figure 4. A Subgroup of Ciliary Compartment Genes, *osegs*, Encodes a Family of Proteins Related to Prototypical Intracellular Transport Proteins

(A and B) Genetic maps of *oseg1* and *oseg2* illustrating the chromosomal deficiencies used to localize both loci, the gene structures, and the nature of the mutations in the various alleles.

(C) *oseg1* and *oseg2* MRC defects are rescued by introduction of wild-type transgenes.

(D) Predicted structure and topology of OSEG family members (see also Supplemental Figure S1 online). Blue triangles, WD repeats; blue bar, β sheet rich domain; green ovals, TPR-like repeats; green bar, α helix rich domain; c, CxxC repeats. Arrows indicate the positions of the mutations.

now co-targeted with tubulin and functions as a robust reporter of α1tub84B transport into the outer segment (Figure 7B).

Next, we introduced the GFP-α1tubulin 84B reporter into *oseg* mutant backgrounds and examined its localization. Figures 7E–7G show that the GFP-α1tub84B cargo completely fails to enter the outer segment of *oseg2* mutants, but is efficiently transported to the outer segments of controls and *oseg1* mutants. Furthermore, EM examination of *oseg2* mutant cells revealed a dramatic accumulation of microtubules at the base of the cilium (Figures 7K and 7M). These results prove that *oseg2*, but not *oseg1,* is essential for tubulin transport into the cilium and illustrate an important aspect of OSEGs function: OSEGs may play distinct roles, and different cargo are likely to be matched to specific OSEG members. Notably, the N-terminal WD domains of α-coatomers and clathrin have been implicated in cargo recognition and sorting (Eugster et al., 2000; ter Haar et al., 2000). The identification of six OSEG members with distinct N-terminal WD domains may provide the structural basis for selective cargo recognition within this family.

Our bioinformatics approach also identified two kinesin II subunits as cilia-compartment genes (see Figure 2). Kinesin II has been shown to be required for cilia assembly in a variety of organisms and was proposed to function as the anterograde motor carrying cargo from the base of the cilia to its distal tip (Cole et al., 1998; Marszalek et al., 2000; Piperno and Mead, 1997). If OSEGs mediate the kinesin-based intraciliary transport, and if this transport were specifically required for outer segment formation, we reasoned that mutations in *klp64D*, the central component of *Drosophila* kinesin II (Ray et al., 1999), should generate in vivo phenotypes that resemble *oseg* defects. Thus, we generated flies defective in *klp64D* function and examined mechano- and chemosensory physiology and the transport and accumulation of α1tub84B into sensory cilia. Figures 5 and 7 demonstrate that *klp64D* mutant animals share all of the hallmarks of *oseg2* mutants: (1) severe chemoinsensitivity, (2) a total loss of mechanoreceptor currents, (3) GFP-α1tub84B completely failing to enter the outer segments, and (4) microtubules dramatically accumulating at the base of the cilia (Figure 7L). Furthermore, *klp64D* animals, just like *oseg2* mutants, have an almost complete loss of the tubular body, but have normal basal bodies and connecting cilia (Figures 5G and 5K); thus, kinesin II is also not essential for the assembly of the proximal ciliary structures, including axoneme components. Together, these results substantiate kinesin II as a critical player in OSEG function and validate the fundamental importance of intraciliary transport in outer segment (compartmentalized cilia) biogenesis.

Concluding Remarks

In this study, we used a novel bioinformatics screen relying on evolutionary gene conservation and gene loss

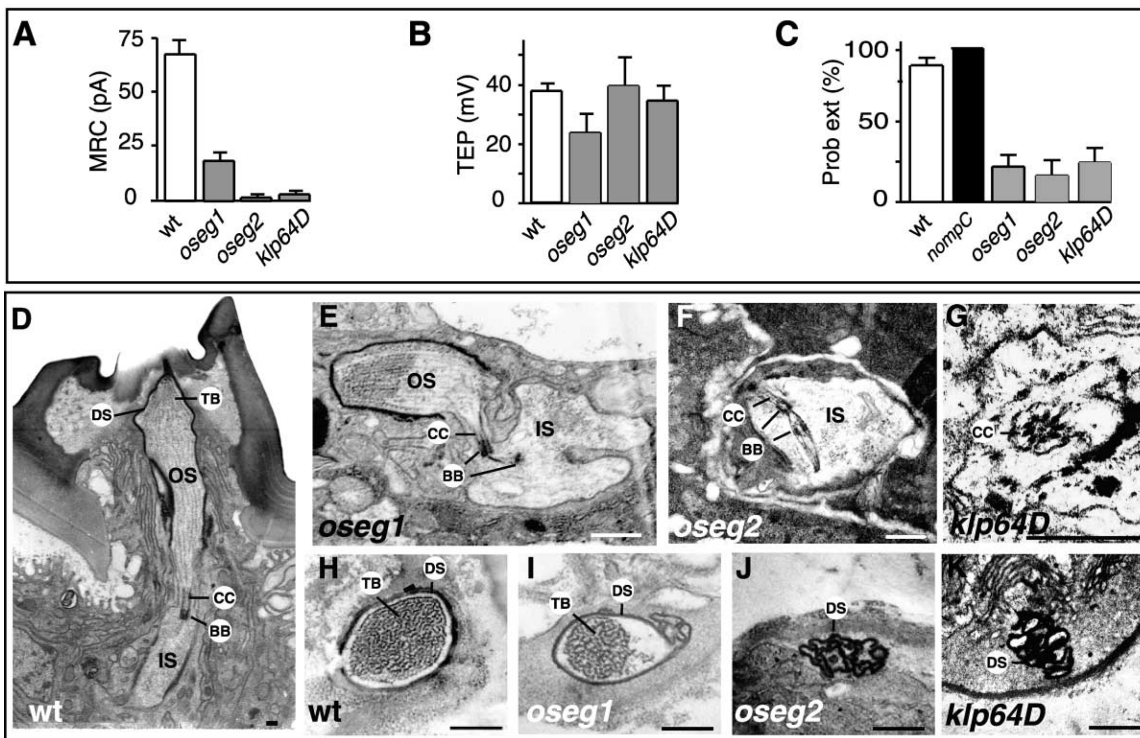

Figure 5. *oseg* Genes Are Required for Compartmentalized Cilia Formation and Function

oseg1, *oseg2*, and *klp64D* mutants have a dramatic loss of MRC (A) but have a robust TEP (B). They also have strongly attenuated chemosensory responses to sucrose (C), while responses of the mechanoinsensitive mutant *nompC* (Walker et al., 2000) are indistinguishable from controls (wt). Electron micrographs of wild-type (D and H), *oseg1* (E and I), *oseg2* (F and J), and *klp64D* (G and K) mechanoreceptor cells showing the IS, CC, OS, and TB. Note the severe reduction in microtubule content in *oseg1* (I) and the total loss of microtubules in the outer segment of *oseg2* (J) and *klp64D* (K), while the connecting cilium remains unaffected (E–G). Similar defects are seen in *oseg1* and *oseg2* chemosensory neurons (data not shown). DS, dendritic sheath; CC, connecting cilium; BB, basal body. Bar, 0.5 μM.

as a paradigm to discover loci selectively involved in cilia formation and function. We showed that this strategy efficiently identified a wide spectrum of known ciliary proteins and dramatically enriched the repertoire of candidate ciliary genes. Because we focused on identifying ciliary genes of the ancestral eukaryotic cell (e.g., by selecting ciliary genes found in both Bikonts and Unikonts lineages), we did not expect to recover genes unique to specific lineages. However, by using selective combinations of genomes in our search algorithm, we were also able to define and distinguish between genes involved in cilia motility versus cilia compartmentalization; as additional genomes are completed, it should be possible to target new categories.

Approximately 200 genes (Table S1 on *Cell* website) were selected in the four searches described in this paper. We analyzed in detail the cilia-compartment subset and identified 27 genes as strong ciliary compartment candidates. We selected 15 for detailed in vivo expression studies and demonstrated that all were specifically expressed in compartmentalized cilia. Using a spectrum of curation strategies, we also examined many of the genes in the motility and prototypical-cilia subsets (see Experimental Procedures). Our analysis identified an additional collection of novel candidate ciliary genes (Figure 2). It will be of great interest to determine whether mutations in the human orthologs of these genes underlie cilia-based sensory, developmental, or reproductive disorders.

Ciliary genes that serve multiple cellular functions were not selected in this screen, mainly because they are still present in organisms that have lost ciliated structures. For example, dyneins are critical components of the ciliary motility apparatus, yet many were filtered out in our screens because they are also involved in intracellular transport in nonciliated organisms. Indeed, we suggest that the reason so few candidate genes were recovered in the "all ciliated organisms" subgroup is because proteins common to all cilia, like those involved in axoneme assembly, are also required in basic cellular processes and therefore conserved in nonciliated organisms (e.g., α-tubulin, β-tubulin, γ-tubulin, centrin, pericentrin, etc.).

What do cilia-compartment genes do? At a basic level, these genes should encode components of the intraciliary transport system and the cilia pore, a supramolecular structure that forms the gate into the cilia (Rosenbaum and Witman, 2002). Indeed, our screen identified all of the known IFT homologs found in *Drosophila*, including novel OSEG members. By extension, we suggest that the compartment group also contains the molecular components of the cilia pore complex.

Using a genetic screen relying on uncoordinated behavior and electrophysiological recordings of sensory

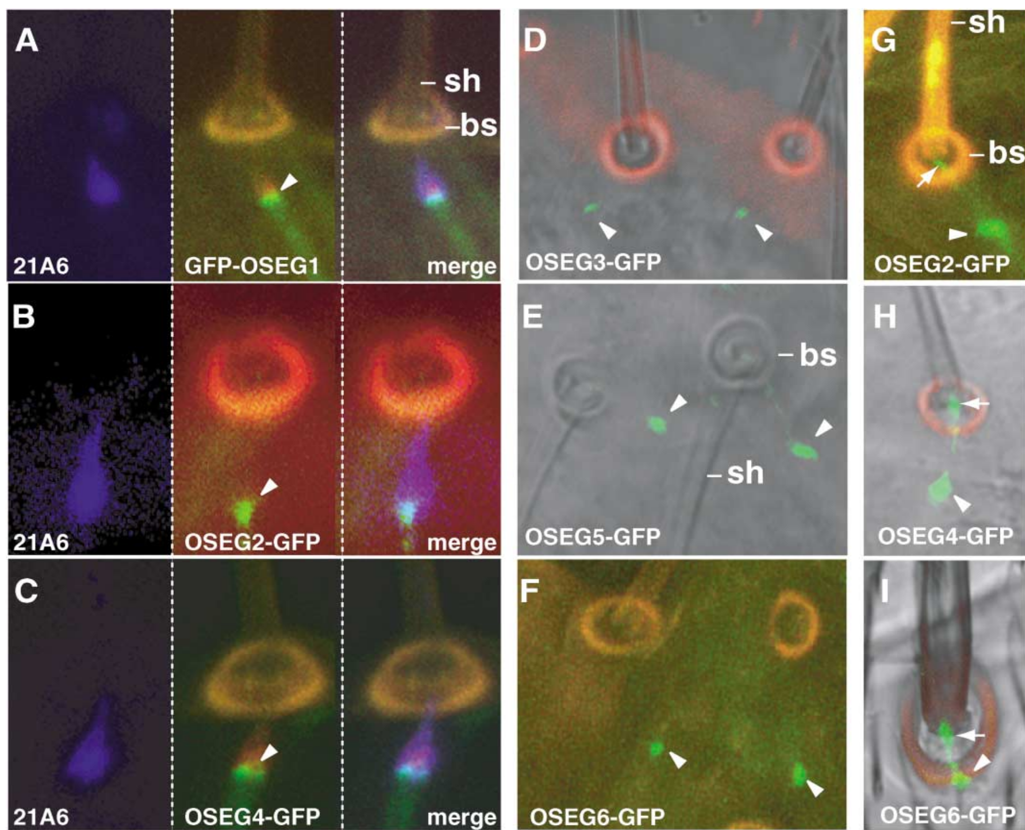

Figure 6. *osegs* Are Primarily Localize at the Base of the Cilia

(A–F) Confocal imaging of chemosensory organs expressing GFP-OSEG1 (A), OSEG2-GFP (B), OSEG4-GFP (B), OSEG3-GFP (C), OSEG5-GFP (E), and OSEG6-GFP (F) protein fusions. (A–C) To unequivocally identify the subcellular location of the OSEG-GFP fusions, whole-mounts were co-stained with mab21A6, an antibody that selectively labels the base of the cilium. Left panels show mab21A6 (blue), middle panels, OSEG-GFPs (green), right panels, merged images.

(G–I) OSEG-GFP labeling inside the outer segment of chemosensory organs (G and H) and mechanosensory organs (I). Orange labeling highlights the bristle socket (bs) and shaft (sh).

bristles, we isolated and characterized mutations in two *oseg* family members. We showed that *oseg1* and *oseg2* have distinct roles in ciliogenesis, but neither *oseg1* nor *oseg2*, or even kinesin II, are required for formation of the connecting cilium. These results demonstrate that the assembly of outer segment is orchestrated independently of the connecting cilia (and its axoneme). It will be of great interest to determine which cilia-compartment genes have a role in the biogenesis of this structure.

OSEGs are characterized by the presence of two major protein-protein interaction domains, WD and TPR repeats, implicated in the assembly of multiprotein complexes. Significantly, the most closely related proteins outside of the family are α- and β'-coatomer, two cargo-carrying proteins intimately involved in intracellular trafficking (Kirchhausen, 2000). Furthermore, clathrin heavy chains display striking domain similarity to the OSEG family: an N terminus consisting of 7 WD repeats and a C terminus consisting of $\sim$35 TPR-like repeats known as CHCR motifs (ter Haar et al., 2000; Ybe et al., 1999). Interestingly, coatomers and clathrin-mediated transport systems use small G proteins of the Arf subfamilies as regulators of the transport process. Notably, our screen also identified ARL3 and ARL6, two Arf-like proteins, as components of the ciliary compartment group, with ARL6 expression restricted to mechano- and chemosensory neurons.

What do OSEGS do? The *Drosophila oseg2* gene shares significant similarity with a 20 amino acid tryptic peptide from Chlamydomonas IFT172 (Cole et al., 1998). IFTs were originally identified as a group of proteins enriched in the flagella of Chlamydomonas dynein-1b mutants and absent in the flagella of kinesin II mutants. Because anterograde transport is blocked in kinesin mutants, and retrograde transport is abolished in dynein mutants, IFT particle proteins were proposed to function as molecular rafts transporting cargo up and down the axoneme. Multiple lines of evidence strongly support the proposal that OSEGs function as ciliary transport proteins. First, OSEGs are specifically expressed in ciliated cells, and the proteins are selectively localized to the cilia and cilia base. Second, OSEGs share structural similarity to prototypical intracellular transport proteins (e.g., clathrin, COP1). Third, *oseg2* mutants have specific defects in intraciliary transport. Fourth, *Drosophila* OSEGS are required for compartmentalized ciliogenesis (sensory cilia) but not for cytosolic ciliogenesis (sperm tail). Finally, flies defective in *oseg2* have nearly the same phenotype as mutants defective in *klp64D*, the ciliary motor. While there is very limited available data on *oseg*

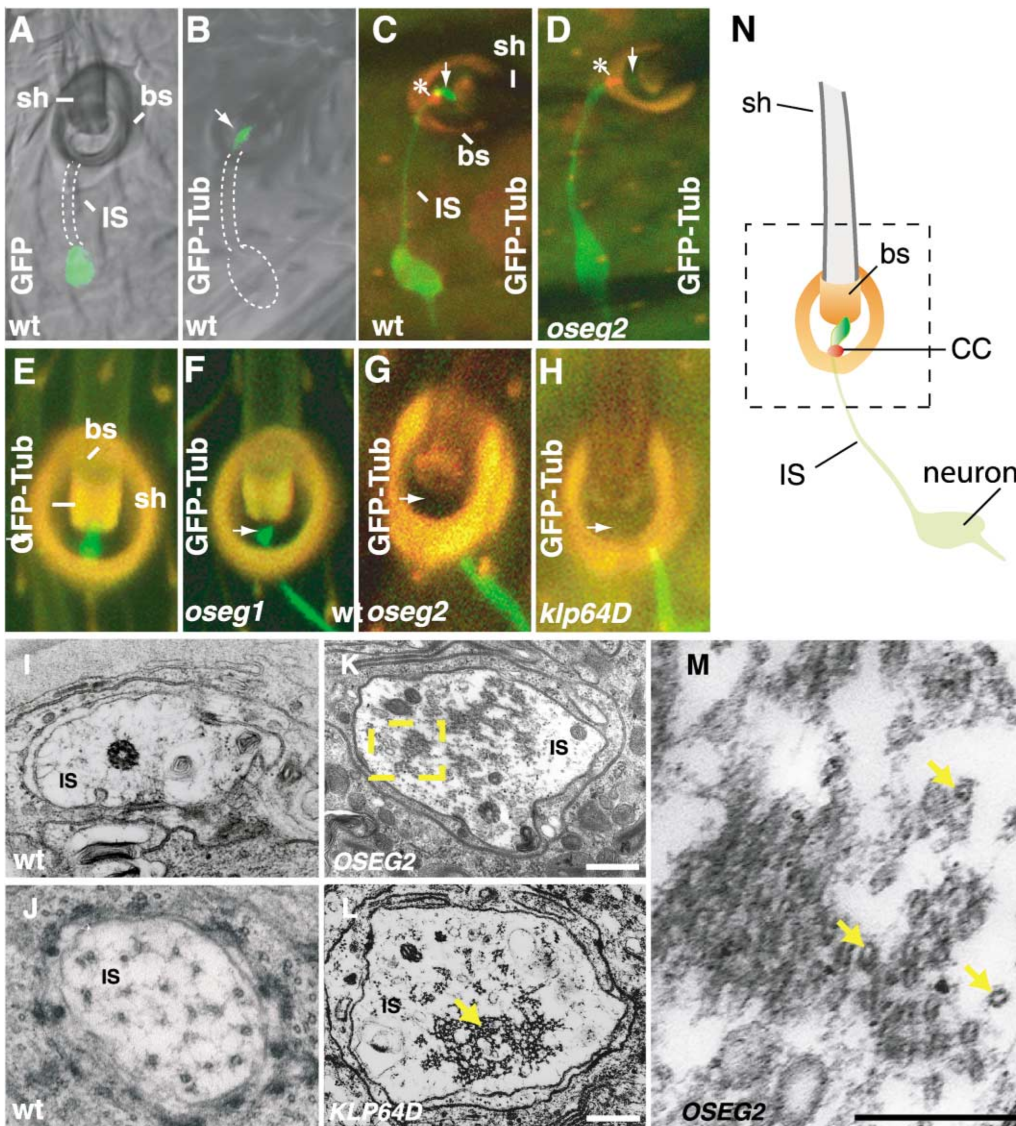

Figure 7. Outer Segment Cargo Is Mislocalized in *oseg2* and Kinesin II Mutants

(A and B) Confocal images of mechanoreceptor neurons expressing GFP (A) and GFP-α1tub84B (B). Note selective labeling of outer segments by GFP-α1tub84B (arrow). Dotted lines illustrate the location of the neurons and their projections.

(C–H) Confocal images of mechanoreceptors expressing GFP-α1tub84B in wild-type (C and E), *oseg1* (F), *oseg2* (D and G), and *klp64D* (H) mutants. Note the mislocalization of GFP-α1tub84B in *oseg*2 and klp64D. (C) and (D) were also co-labeled with mab21A6 to mark the base of the cilium (red staining, star). Orange labeling highlights the bristle socket (bs) and shaft (sh).

(K–M) EM pictures demonstrating the dramatic accumulation of microtubules (yellow arrow and box) at the cilia base of *oseg2* (K and M) and *klp64D* (L); no microtubule accumulation is seen in the proximal part of the inner segment of mutant cells or in ciliated cells of wild-type controls (I and J).

(N) Diagram of a mechanosensory neuron labeled with GFP-α1tub84B (green) and mab21A6 (red). The dashed box illustrates the approximate plane of the confocal images shown in (E)–(H); bristle socket (bs), shaft (sh), connecting cilium (CC).

orthologs in Chlamydomonas, several of the *oseg* orthologs in *C. elegans* genes map at, or near, the location of worm mutations leading to sensory cilia defects and implicated in cilia formation and maintenance (Perkins et al., 1986). For example, OSEG2 and OSEG5 are orthologs of OSM-1 (Signor et al., 1999) and CHE-2 (Fujiwara et al., 1999), and OSEG1 and OSEG3 are probably orthologs of DAF-10 and CHE-3 (Qin et al., 2001), respectively. Surprisingly, the integration of these proteins into a group of genes related to the main families of intracellular transport proteins had escaped notice. Our results illustrate a common foundation in the organization of intracellular transport systems, whether mediating internalization of surface proteins, transferring cargo between organelles, or delivering components from the cell body to distal ciliary compartments.

Experimental Procedures

Bioinformatics

Genome sequence information used in this study was obtained in March 2003. These include *Drosophila* Genome Project, BDGP, (http://www.fruitfly.org), release 3 (17,878 protein sequences corre-

sponding to 13,759 genes) from UC Berkeley; WormBase (http://www.wormbase.org), release 100 (21,565 protein sequences); *T. brucei*, 15,300 protein sequences from Sanger Institute (http://www.sanger.ac.uk/Projects/T_brucei); The Hs (27,625 protein sequences); and At (27,242), Sc (6,333), Pf (5,300) protein sequences from the National Center for Biotechnology Information, NCBI, (http://www.ncbi.nih.gov). For the incomplete genome of Chlamydomonas, we used 141,000 sequence entries derived from EST, genomic, and cDNA sources and compared them using TBLASTN/BLASTX.

Bidirectional BLAST was used in the process of identifying orthologs and corresponding ESTs for *Drosophila* genes (http://www.ncbi.nlm.nih.gov/BLAST). After bidirectional BLASTP between two proteomes, an automatic clustering program, "Inparanoid," was used to identify the main orthologs (http://www.cgb.ki.se/inparanoid; Remm et al., 2001); only reciprocal best hits were considered as putative orthologs. Co-orthologs were identified by searching the OrthoMCL database (http://www.cbil.upenn.edu/gene-family; Li et al., 2003). A gene was considered an "ancestral candidate" if it was present in Unikonts (*Drosophila*) and Bikonts; the presence of a main ortholog or a co-ortholog in a nonciliated organism was sufficient to eliminate the candidate gene or move it from one subset to the other.

Putative X boxes (consensus: GTTGGCCATGGCAAC) were identified in the 500 bp upstream of the initiator ATG from Dm and Ce genes using an in-house Perl program that scores pattern positional matches (A.M.M., unpublished data). Only putative X boxes that did not differ from the consensus by more than four nucleotides were accepted. ESTs for Dm genes (a total of 253,545 EST of which 30,384 were derived from testis) were identified using sequences from BDGP and Brian Oliver's testis EST project (Andrews et al., 2000) using reciprocal BLASTN. Only hits with at least 90% identity over the entire length of the EST were counted in our analysis

Gene structures were predicted by FGENESH and validated by direct genomic and cDNA sequence analyses. Hidden Markov Model homology searches used MEME (Bailey and Elkan, 1994) and MAST (Karplus et al., 1998). Domain analysis of OSEGs and α- and β'-coatomers proteins used SAM-T2 (http://www.soe.ucsc.edu/research/compbio/HMM-apps/T02-query.html), SMART (http://smart.embl-heidelberg.de/), and superfamily (http://supfam.mrc-lmb.cam.ac.uk/SUPERFAMILY/) programs. Gene function was predicted based on annotation, expression pattern, and mutant phenotype available in FlyBase (http://flybase.bio.indiana.edu), WormBase (http://www.wormbase.org), and Ensembl (http://www.ensembl.org). The 36 genes known to be involved, or implicated, in ciliogenesis were identified by detailed searches of PubMed, OMIM, and the Protein databases.

Transgenic Constructs

The generation of the 17 OSEG promoter-Gal4 transgenes was performed by cloning upstream elements immediately adjacent to the predicted initiator methionine of individual *oseg* genes in front of a modified CasSper-AUG-Gal4 vector (Vosshall et al., 2000). The oseg1 fragment was 6842 nucleotides; oseg2, 3115 nucleotides; oseg3, 1021 nucleotides; oseg4, 640 nucleotides; oseg5, 452 nucleotides; oseg6, 5002 nucleotides; BBS1, BBS2, BBS8, CG5142, CG7735, CG3259, CG14870, CG1126, CG5359, CG15161, CG9227, 1500 or 2000 nucleotides. Gal4 drivers, transformation rescue constructs, and GFP fusion proteins were cloned into the pP{UAST} vector. For translation fusions, GFP was fused at the C terminus of OSEG2, 3, 4, and 6 and at the N terminus of the OSEG1. We used an Elav-Gal4 driver (Lin and Goodman, 1994) as a pan-neuronal promoter in all our translational gene fusion experiments. In order to increase the signal from OSEG-GFP fusions inside the outer segments of chemo- or mechanosensory neurons, transgenic constructs were examined in animals carrying two copies of the Elav-Gal4 driver and the fusion construct.

Experimental Animals

Drosophila stocks containing ELAV-Gal4 (c1115), UAS-GFP, *Df(3L)66C-G28*, *Df(3L)66C-I65*, and *klp64D^{k1}* were obtained from the Bloomington Stock Center; *Df(3L)Aprt-1* and *Df(3L)R-R2* from L. Goldstein; *EP(3)3161* from G. Rubin; UAS-GFP-α1tub84B from A. Spradling (Grieder et al., 2000). Our control lines were isogenized *cn*, *bw*, and *bw*; *st* flies. P element-mediated germline transformations and fly manipulations were performed according to standard techniques. Rescue of *oseg1* and *oseg2* were assayed in ELAV-Gal4; UAS-OSEG1/Cyo; *oseg1* and ELAV-Gal4; UAS-OSEG2/Cyo; *oseg2^{452}*, respectively.

Genetic Screen for Candidate Ciliary Mutants

Mutagenized lines were generated using isogenized *bw*; *st* flies; homozygous mutants are white eyed while heterozygous siblings are brown eyed. To identify pharate adult lethal lines, lightly populated vials were screened for the presence of white-eyed nonbalanced pupae, but also the absence of viable white-eyed adult flies. To test uncoordinated behavior, mutant pupae were transferred to humidified chambers and examined by visual inspection as described previously (Kernan et al., 1994). Electrophysiological recordings (Walker et al., 2000) and proboscis extension assays (Galindo and Smith, 2001) were performed within 8–24 hr after eclosion; for MRC recordings, the TEP was held at 40 mV. A minimum of four flies were examined for proboscis extension assays and a minimum of five bristles from three different flies were used for all electrophysiological recordings.

Imaging and Immunofluorescence Staining

Whole-mount preparations of pupal thorax or adult tissues were fixed in 4% formaldehyde in PBS, permeablized by 0.3% Triton-X100, and blocked using 1% bovine serum albumin in PBS plus 0.3% Triton-X100 (blocking buffer). Samples were incubated in a 1:100 dilution of anti-GFP or 1:50 dilution of mab21A6 (Fujita et al., 1982) in blocking buffer for 12–18 hr at 4°C and detected using fluorescein-conjugated donkey anti-rabbit or Red-X-conjugated anti-mouse secondary antibodies (Jackson Immunolaboratory). Cuticular bristle organ structures (socket and shaft) were visualized by exciting the tissue with blue light and collecting autofluorescence signals in the red and green channels. Images were obtained using a BioRad MRC1024 confocal microscope with an argon-krypton laser. The identity of cells expressing GFP was established based on their morphology, location, innervation patterns, and, in the case of chordotonal organs, aided by the number of scolopeles.

Tissue Processing for Electron Microscopy

For EM analysis of mechanosensory macrochaetae, we studied the scutelar bristles; for chemosensory bristles, we examined the outer surface of the proboscis. Tissue was fixed either in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, with 5% sucrose, or in 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer with 2 mM calcium chloride and 1% tannic acid, pH 7.3, for 3–4 hr at 4°C. After washing in 0.1 M cacodylate or phosphate buffers, respectively, the specimens were postfixed in 1% OsO4, *en bloc* stained with 1% uranyl acetate, dehydrated in ethanol series, and embedded in either Spurr or Polybed/Araldite (Polysciences). Ultrathin sections were post-stained with 2% aqueous uranyl acetate, triple lead stain, and examined with JEOL 1200 transmission electron microscope. A minimum of four bristles from three flies were examined for each mutant backgrounds.

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References

Andrews, J., Bouffard, G.G., Cheadle, C., Lu, J., Becker, K.G., and Oliver, B. (2000). Gene discovery using computational and microarray analysis of transcription in the Drosophila melanogaster testis. Genome Res. *10*, 2030–2043.

Ansley, S.J., Badano, J.L., Blacque, O.E., Hill, J., Hoskins, B.E., Leitch, C.C., Kim, J.C., Ross, A.J., Eichers, E.R., Teslovich, T.M., et al. (2003). Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome. Nature *425*, 628–633.

Bailey, T.L., and Elkan, C. (1994). Fitting a mixture model by expectation maximization to discover motifs in biopolymers. Proc. Int. Conf. Intell. Syst. Mol. Biol. *2*, 28–36.

Barolo, S., Walker, R.G., Polyanovsky, A.D., Freschi, G., Keil, T., and Posakony, J.W. (2000). A notch-independent activity of suppressor of hairless is required for normal mechanoreceptor physiology. Cell *103*, 957–969.

Bloodgood, R.A. (1990). Ciliary and Flagellar Membranes (New York: Plenum Press).

Brazelton, W.J., Amundsen, C.D., Silflow, C.D., and Lefebvre, P.A. (2001). The bld1 mutation identifies the Chlamydomonas osm-6 homolog as a gene required for flagellar assembly. Curr. Biol. *11*, 1591–1594.

Cavalier-Smith, T. (2002). The phagotrophic origin of eukaryotes and phylogenetic classification of Protozoa. Int. J. Syst. Evol. Microbiol. *52*, 297–354.

Cole, D.G., Diener, D.R., Himelblau, A.L., Beech, P.L., Fuster, J.C., and Rosenbaum, J.L. (1998). Chlamydomonas kinesin-II-dependent intraflagellar transport (IFT): IFT particles contain proteins required for ciliary assembly in Caenorhabditis elegans sensory neurons. J. Cell Biol. *141*, 993–1008.

Cuvillier, A., Redon, F., Antoine, J.C., Chardin, P., DeVos, T., and Merlin, G. (2000). LdARL-3A, a Leishmania promastigote-specific ADP-ribosylation factor-like protein, is essential for flagellum integrity. J. Cell Sci. *113*, 2065–2074.

Das, A.K., Cohen, P.W., and Barford, D. (1998). The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions. EMBO J. *17*, 1192–1199.

Dubruille, R., Laurencon, A., Vandaele, C., Shishido, E., Coulon-Bublex, M., Swoboda, P., Couble, P., Kernan, M., and Durand, B. (2002). Drosophila regulatory factor X is necessary for ciliated sensory neuron differentiation. Development *129*, 5487–5498.

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*, 277–287.

Eugster, A., Frigerio, G., Dale, M., and Duden, R. (2000). COP I domains required for coatomer integrity, and novel interactions with ARF and ARF-GAP. EMBO J. *19*, 3905–3917.

Fujita, S.C., Zipursky, S.L., Benzer, S., Ferrus, A., and Shotwell, S.L. (1982). Monoclonal antibodies against the Drosophila nervous system. Proc. Natl. Acad. Sci. USA *79*, 7929–7933.

Fujiwara, M., Ishihara, T., and Katsura, I. (1999). A novel WD40 protein, CHE-2, acts cell-autonomously in the formation of C. elegans sensory cilia. Development *126*, 4839–4848.

Galindo, K., and Smith, D.P. (2001). A large family of divergent Drosophila odorant-binding proteins expressed in gustatory and olfactory sensilla. Genetics *159*, 1059–1072.

Grieder, N.C., de Cuevas, M., and Spradling, A.C. (2000). The fusome organizes the microtubule network during oocyte differentiation in Drosophila. Development *127*, 4253–4264.

Han, Y.G., Kwok, B.H., and Kernan, M.J. (2003). Intraflagellar transport is required in Drosophila to differentiate sensory cilia but not sperm. Curr. Biol. *13*, 1679–1686.

Karplus, K., Barrett, C., and Hughey, R. (1998). Hidden Markov models for detecting remote protein homologies. Bioinformatics *14*, 846–856.

Kernan, M., Cowan, D., and Zuker, C. (1994). Genetic dissection of mechanosensory transduction: mechanoreception-defective mutations of Drosophila. Neuron *12*, 1195–1206.

Kirchhausen, T. (2000). Three ways to make a vesicle. Nat. Rev. Mol. Cell Biol. *1*, 187–198.

Li, L., Stoeckert, C.J., Jr., and Roos, D.S. (2003). OrthoMCL: identification of ortholog groups for eukaryotic genomes. Genome Res. *13*, 2178–2189.

Lin, D.M., and Goodman, C.S. (1994). Ectopic and increased expression of Fasciclin II alters motoneuron growth cone guidance. Neuron *13*, 507–523.

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*, 175–187.

Neer, E.J., and Smith, T.F. (1996). G protein heterodimers: new structures propel new questions. Cell *84*, 175–178.

Patel-King, R.S., Benashski, S.E., Harrison, A., and King, S.M. (1997). A Chlamydomonas homologue of the putative murine t complex distorter Tctex-2 is an outer arm dynein light chain. J. Cell Biol. *137*, 1081–1090.

Pazour, G.J., Dickert, B.L., and Witman, G.B. (1999). The DHC1b (DHC2) isoform of cytoplasmic dynein is required for flagellar assembly. J. Cell Biol. *144*, 473–481.

Pazour, G.J., Dickert, B.L., Vucica, Y., Seeley, E.S., Rosenbaum, J.L., Witman, G.B., and Cole, D.G. (2000). Chlamydomonas IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. J. Cell Biol. *151*, 709–718.

Perkins, L.A., Hedgecock, E.M., Thomson, J.N., and Culotti, J.G. (1986). Mutant sensory cilia in the nematode Caenorhabditis elegans. Dev. Biol. *117*, 456–487.

Piperno, G., and Mead, K. (1997). Transport of a novel complex in the cytoplasmic matrix of Chlamydomonas flagella. Proc. Natl. Acad. Sci. USA *94*, 4457–4462.

Qin, H., Rosenbaum, J.L., and Barr, M.M. (2001). An autosomal recessive polycystic kidney disease gene homolog is involved in intraflagellar transport in C. elegans ciliated sensory neurons. Curr. Biol. *11*, 457–461.

Ray, K., Perez, S.E., Yang, Z., Xu, J., Ritchings, B.W., Steller, H., and Goldstein, L.S. (1999). Kinesin-II is required for axonal transport of choline acetyltransferase in Drosophila. J. Cell. Biol. *147*, 507–518.

Remm, M., Storm, C.E., and Sonnhammer, E.L. (2001). Automatic clustering of orthologs and in-paralogs from pairwise species comparisons. J. Mol. Biol. *314*, 1041–1052.

Rosenbaum, J.L., and Witman, G.B. (2002). Intraflagellar transport. Nat. Rev. Mol. Cell Biol. *3*, 813–825.

Shao, X., Davletov, B.A., Sutton, R.B., Sudhof, T.C., and Rizo, J. (1996). Bipartite Ca2+-binding motif in C2 domains of synaptotagmin and protein kinase C. Science *273*, 248–251.

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*, 519–530.

Sinden, R.E., Canning, E.U., and Spain, B. (1976). Gametogenesis and fertilization in Plasmodium yoelii nigeriensis: a transmission electron microscope study. Proc. R. Soc. Lond. B. Biol. Sci. *193*, 55–76.

Sleigh, M.A. (1974). Cilia and Flagella (London: Academic Press).

Stechmann, A., and Cavalier-Smith, T. (2002). Rooting the eukaryote tree by using a derived gene fusion. Science *297*, 89–91.

Stechmann, A., and Cavalier-Smith, T. (2003). Phylogenetic analysis of eukaryotes using heat-shock protein Hsp90. J. Mol. Evol. *57*, 408–419.

Swoboda, P., Adler, H.T., and Thomas, J.H. (2000). The RFX-type transcription factor DAF-19 regulates sensory neuron cilium formation in C. elegans. Mol. Cell *5*, 411–421.

Tamm, S. (1994). Ca2+ channels and signalling in cilia and flagella. Trends Cell Biol. *4*, 305–310.

ter Haar, E., Harrison, S.C., and Kirchhausen, T. (2000). Peptide-in-groove interactions link target proteins to the beta- propeller of clathrin. Proc. Natl. Acad. Sci. USA *97*, 1096–1100.

Tokuyasu, K.T., Peacock, W.J., and Hardy, R.W. (1972). Dynamics of spermiogenesis in Drosophila melanogaster. I. Individualization process. Z. Zellforsch. Mikrosk. Anat. *124*, 479–506.

Vosshall, L.B., Wong, A.M., and Axel, R. (2000). An olfactory sensory map in the fly brain. Cell *102*, 147–159.

Walker, R.G., Willingham, A.T., and Zuker, C.S. (2000). A Drosophila mechanosensory transduction channel. Science *287*, 2229–2234.

Ybe, J.A., Brodsky, F.M., Hofmann, K., Lin, K., Liu, S.H., Chen, L., Earnest, T.N., Fletterick, R.J., and Hwang, P.K. (1999). Clathrin self-assembly is mediated by a tandemly repeated superhelix. Nature *399*, 371–375.