Current Biology, Vol. 13, 1687–1696, September 30, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/j.cub.2003.09.025

Drosophila KAP Interacts with the Kinesin II Motor Subunit KLP64D to Assemble Chordotonal Sensory Cilia, but Not Sperm Tails

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Summary

Background: Kinesin II-mediated anterograde intraflagellar transport (IFT) is essential for the assembly and maintenance of flagella and cilia in various cell types. Kinesin associated protein (KAP) is identified as the nonmotor accessory subunit of Kinesin II, but its role in the corresponding motor function is not understood.

Results: We show that mutations in the *Drosophila* KAP (*DmKap*) gene could eliminate the sensory cilia as well as the sound-evoked potentials of Johnston's organ (JO) neurons. Ultrastructure analysis of these mutants revealed that the ciliary axonemes are absent. Mutations in *Klp64D*, which codes for a Kinesin II motor subunit in *Drosophila*, show similar ciliary defects. All these defects are rescued by exclusive expression of DmKAP and KLP64D/KIF3A in the JO neurons of respective mutants. Furthermore, reduced copy number of the *DmKap* gene was found to enhance the defects of hypomorphic *Klp64D* alleles. Unexpectedly, however, both the *DmKap* and the *Klp64D* mutant adults produce vigorously motile sperm with normal axonemes.

Conclusions: KAP plays an essential role in Kinesin II function, which is required for the axoneme growth and maintenance of the cilia in *Drosophila* type I sensory neurons. However, the flagellar assembly in *Drosophila* spermatids does not require Kinesin II and is independent of IFT.

Introduction

The type I sense organs of *Drosophila*, namely, the chordotonal organs, the mechanosensory bristles, and the taste and olfactory sensilla, are innervated by bipolar sensory neurons, each with a single dendritic cilium containing 9+0 axonemal organization of microtubules. Each chordotonal organ neuron has a single long cilium, the assembly of which begins from the distal basal bodies in the dendrite and which is attached to a tube-shaped dendritic cap at the apex. Such chordotonal organs are found in the second antennal segment, where they are required for hearing and are called Johnston's organ (JO), and in various other parts of the body, where they are required for proprioception. Mutants with auditory defects were previously found to have defective dendritic cilia in JO neurons [1–3]. The mechanisms that form and maintain such ciliary structures are only beginning to be elucidated.

Extensive studies in *Chlamydomonas* and other organisms (see [4–6] for recent reviews) have shown that flagellar and ciliary proteins are synthesized in the cell body and are then transported in preassembled IFT complexes to the distal tip of the flagella by a mechanism called "intraflagellar transport" (IFT). This process is essential for the assembly and maintenance (via turnover) of flagella [7]. Members of the Kinesin II family of motor proteins and cytoplasmic Dynein motors are known to play important roles in IFT (see [4] for a review).

Kinesin II holoenzyme was purified from sea urchin embryos as a heterotrimer of two dissimilar motor subunits and a third non-motor accessory subunit called "kinesin-associated protein" (KAP) [8]. The motor subunits contain a globular plus end-directed, microtubule-dependent ATPase domain at the N terminus, and they associate with each other via a coiled-coil stalk domain in the middle. The KAP subunit is estimated to bind to the C-terminal tail domains of the motor subunits [9]. Support for the trimeric composition of Kinesin II has been provided in various vertebrate [10–13] and invertebrate [14] organisms, and this motor has been implicated in a variety of intracellular transport processes in vivo (see [15] for a review).

Kinesin II is the motor for the anterograde IFT, and studies with different types of ciliated cells from *Chlamydomonas* to humans have shown that it is essential for ciliogenesis as well as for flagellar growth and maintenance [9]. For example, conditional mutations in the *fla10* gene of *Chlamydomonas* block anterograde IFT in the flagella when grown at nonpermissive temperatures. As a result, the flagella gradually reduce and eventually disappear [14]. FLA10 is homologous to the Kinesin II motor subunit [14], which is associated with the IFT complex subunits in the flagella (see [14, 15] for reviews). Disruption of Kinesin II activity in sea urchin cilia [10] and *Tetrahymena* flagella [16] are also shown to affect axonemal assembly. Similarly, mutations in the *osm-3* locus of *Caenorhabditis elegans* cause defective chemotaxis behavior, and the distal segments of the dendritic cilia of the chemosensory neurons are absent [17–19]. These studies suggested that Kinesin II is required for the transport of essential ciliary components in these neurons. In addition, mouse KIF3A and KIF3B play an important role in the assembly of motile cilia in embryonic nodal cells [20, 21]. KIF3A is also localized to the

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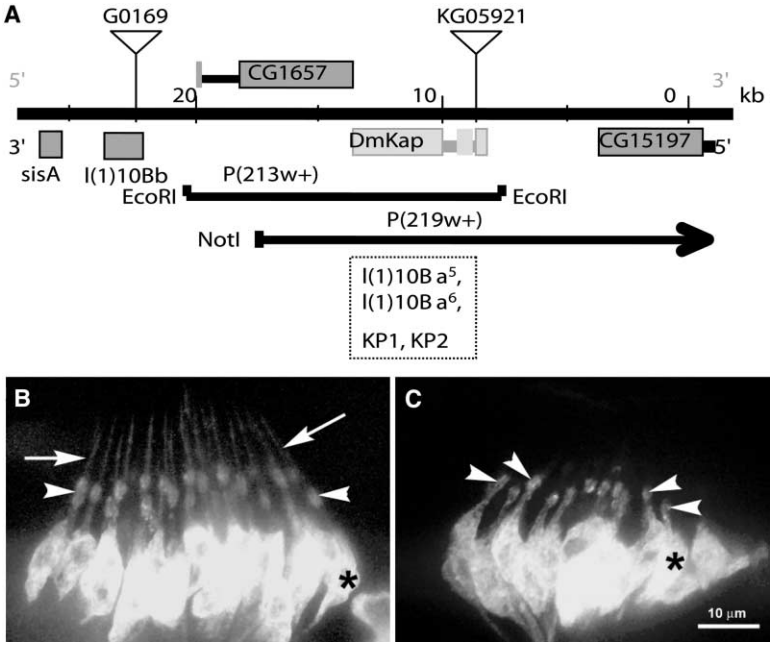

Figure 1. Mutation in *DmKap* Affects the Sensory Cilia of JO Neurons

(A) Organization of *DmKap* and its neighboring genes in the 10B region of salivary gland chromosomes. The rectangular boxes indicate the size and position of exons in each gene, and the horizontal lines indicate the relative positions and breakpoints of two genomic transgenes.

(B and C) Confocal images of 5 μm thick optical sections from pupal JO (60 hr after puparium formation) from (B) $DmKap^{V6}$/Y; *UAS-mCD8::GFP/+*; *UAS-Kap/*$Gal4^{JO15}$ and the (C) $DmKap^{V6}$/Y; *UAS-mCD8::GFP/+*; $Gal4^{JO15}$/+. The neuronal cell bodies (indicated by asterisks), the inner-dendritic segments (arrowheads), and the cilia (arrows, [B]) are indicated. (C) The cilia are absent in $DmKap^{V6}$ pupae.

connecting cilia of photoreceptor neurons in the retina, which have 9+0 organization of microtubules in the axoneme [22–24], and it has been implicated in the transport of opsin and arrestin to the outer photoreceptor compartment [25]. Although these pieces of evidence strongly indicate that Kinesin II is a good candidate for the transport of components required for the assembly and maintenance of eukaryotic cilia and flagella, little is known about the role of KAP in this process. An in vivo analysis in *C. elegans* with GFP-tagged OSM-6 and KAP has shown that the two proteins transport along the sensory cilium at a rate similar to the in vitro rate of Kinesin II; this finding indicates that KAP is associated with the IFT complex in the cilium [26].

KLP64D, KLP68D, and DmKAP are predicted to form the Kinesin II holoenzyme in *Drosophila*, and they are shown to coexpress in ciliated sensory neurons during embryogenesis [27–29]. The expression levels are particularly high in the neurons innervating the lateral chordotonal organs as well as the anterior sense organs [28, 29], and these have well-defined dendritic cilia. This indicated that *Drosophila* Kinesin II could play an important role in ciliogenesis. Therefore, to define a functional assay to study the role of KAP and other Kinesin II-associated proteins in ciliogenesis, we chose to study auditory responses of *Drosophila* carrying mutations in the *DmKap* and *Klp64D* genes. This revealed that KAP plays a critical role in Kinesin II function during ciliogenesis in these type I sensory neurons of *Drosophila*, and our genetic interaction study suggests that DmKAP interacts with a Kinesin II motor subunit in vivo. Surprisingly, we find that Kinesin II is not required for the assembly and function of sperm flagella. These results establish a new paradigm for studying the in vivo functions of IFT components in the sensory cilia of *Drosophila*.

Results

DmKap Homozygous Adults Are Sluggish and Uncoordinated

To obtain mutations in the *DmKap* gene, we used the genomic transgenes *P(213w+)* and *P(219w+)* in a chromosome walking strategy (Figure 1A). This yielded two PlacW insertion alleles, $DmKap^{KP1}$ and $DmKap^{KP2}$, and two EMS-induced alleles, $DmKap^{V5}$ and $DmKap^{V6}$. Although the *DmKap* homozygous flies die at or before the pupal stage, careful culture conditions allowed us to obtain several homozygous/hemizygous escapers as pharate adults. Some of these mutant pupae, except for $DmKap^{V5}$, even emerged as uncoordinated adults. $DmKap^{V5}$ adults never emerge by themselves, but if rescued from the pupal case, they survive for 2–4 days on moist filter paper. The uncoordinated behavior of these mutants is similar to that described earlier for the *Klp64D* alleles [28] and is reminiscent of *nomp* mutations [30]. The mutant flies cannot stand or right themselves when turned on their back, and their legs get entangled in an attempt to walk. Such behavioral defects were completely rescued by both genomic transgenes (Figure 1A), indicating that the observed phenotype arose from mutations in the *DmKap* gene.

To determine the cellular basis for this lethality and uncoordinated behavior, we constructed a *UAS-Kap* cDNA transgene. This was expressed in specific tissues with different Gal4 drivers in $DmKap^{V6}$ males and $DmKap^{V6}$/$DmKap^{V5}$ females to test for the rescue of the recessive lethality. The $Gal4^{C155}$ $DmKap^{V6}$/Y; *UAS-Kap/+* males and $Gal4^{C155}$ $DmKap^{V6}$/$DmKap^{V5}$; *UAS-Kap/+* females as well as the $DmKap^{V6}$/Y; $Gal4^{SG18.1}$/+; *UAS-Kap/+* males were perfectly motile and fertile, whereas the $DmKap^{V6}$/Y; *UAS-Kap* males (with no driver) were uncoordinated (data not shown). $Gal4^{C155}$ (elav-Gal4) expresses in all neurons during development and in the

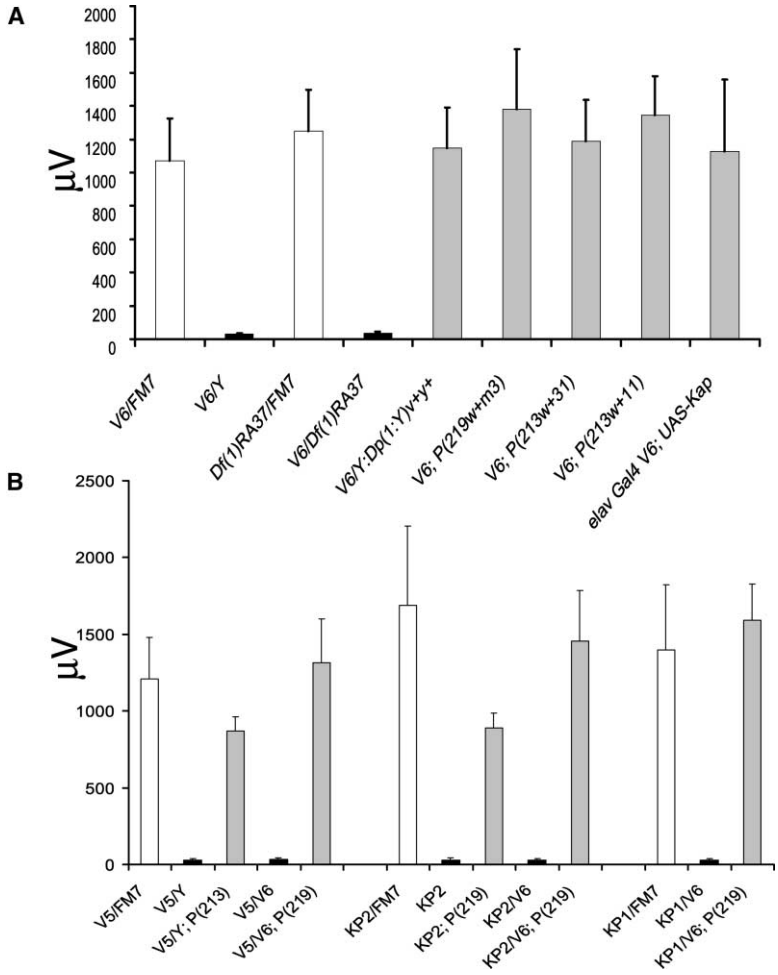

Figure 2. Mutation in the *DmKap* Locus Affects Auditory Physiology in the Adult Antenna

(A and B) The average peak voltages evoked by the sound pulse were measured from the antennal nerve of flies with various *DmKap* alleles. Each bar represents the average response of at least ten flies per genotype. The error bars indicate ±SD. Heterozygous controls are shown as white bars, mutants are shown as black bars, and rescued mutants are shown as gray bars.

adult [31], and $Gal4^{SG18.1}$ expresses in a majority of sensory neurons plus a subset of neurons in the central nervous system in larvae and adults [32, 33]. Therefore, these rescue data clearly indicate that DmKAP activity is mainly required in a subset of neurons, including the ciliated sensory neurons, and they are consistent with our earlier in situ hybridization results [29].

Mutations in the *DmKap* Locus Affect Auditory Responses from the Adult Antennae

RNA in situ analysis suggested that the *DmKap* gene expresses in the embryonic chordotonal organ neurons at a higher level in comparison to other sensory neurons [29]. These neurons contain long sensory cilia and are involved in proprioception in the larva. In adult flies, similar ciliated neurons innervate the JO in the second antennal segment [1]. Since Kinesin II motor activity is implicated in ciliogenesis in other organisms [9], we decided to investigate the cilia structures of these neurons in *DmKap* alleles. We found that the sensory cilia were absent in the mutant animals (Figure 1C) and that specific expression of the *UAS-Kap* transgene in these neurons with $Gal4^{JO15}$ rescued the phenotype (arrows, Figure 1B). This suggested that DmKAP activity in the JO neurons is required for ciliogenesis in a cell autonomous manner.

The JO neurons can detect acoustically induced antennal vibrations and were shown to respond to the male courtship song [1]. We therefore reasoned that the measurement of sound-evoked potentials from the JO neurons in wild-type and mutant animals could provide a quantitative physiological assay for the analysis of *DmKap* function in these sensory cilia. The sound-evoked potential was completely eliminated in $DmKap^{V6}$ and in $DmKapV^{6}/Df(1)RA37$ (breakpoints: 10A7–10B17) hemizygous adults, and it was rescued by the presence of genomic transgenes and the chromosomal duplication $Dp(1;Y)v^{+}y^{+}$ (Figure 2A). In addition, neuron-specific expression of *UAS-Kap* with $Gal4^{C155}$ could rescue the auditory defects in $DmKap^{V6}$ males (Figure 2A). We further observed that $DmKap^{V5}$ and $DmKap^{KP2}$ hemizygous or homozygous adults were also deaf and all failed to complement $DmKap^{V6}$ (Figure 2B). These experiments mapped the auditory defect to the *DmKap* gene. However, it is still formally possible that the auditory defects observed in homozygous *DmKap* alleles result from a general physiological defect in all neurons. To rule out this possibility, we measured the electroretinogram responses from $DmKap^{V6}$, $DmKap^{V5}$, and $DmKap^{KP2}$ hemi-

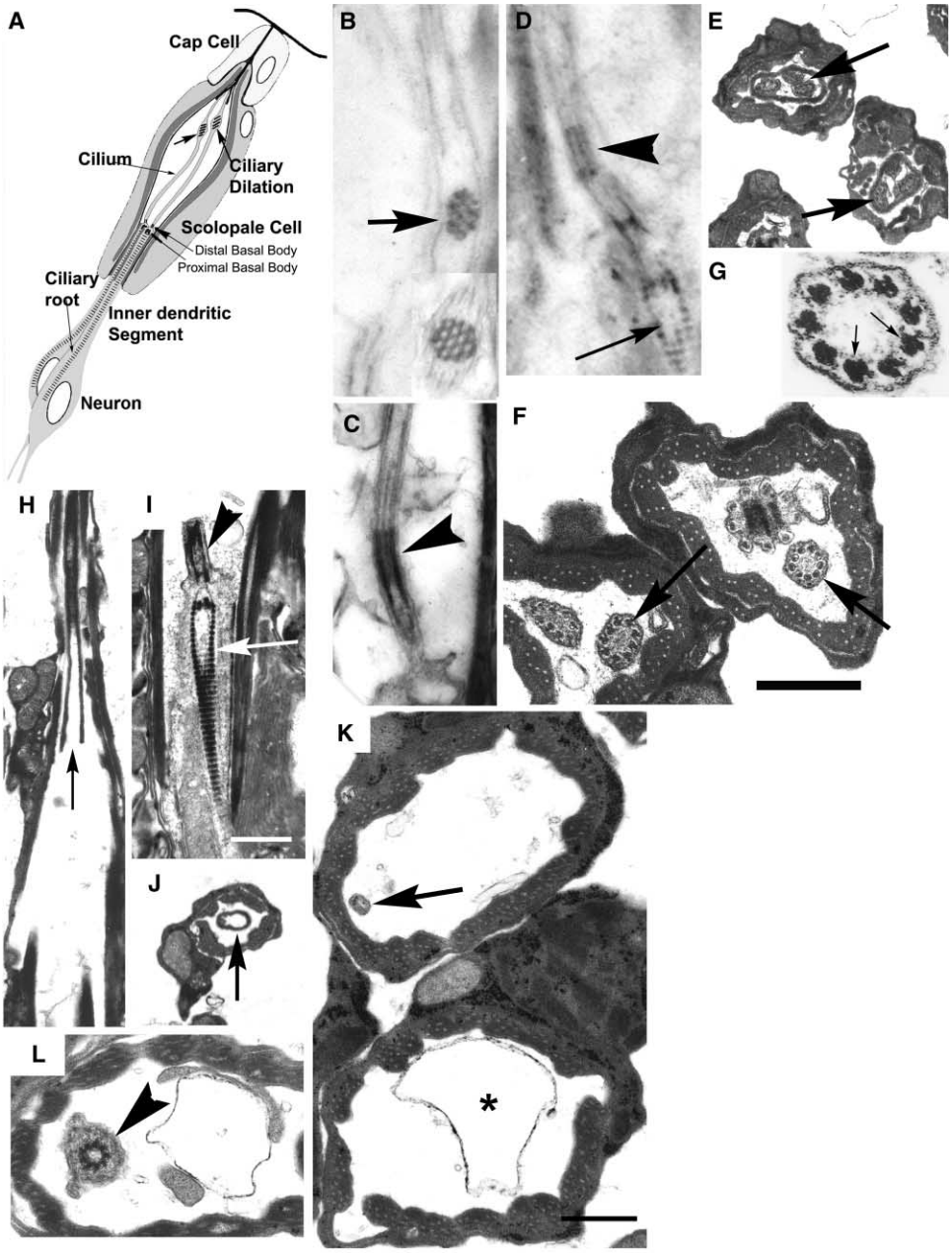

Figure 3. *DmKap* Is Essential for Axoneme Growth in JO Neuron Cilia

(A) The organization of a typical JO chordotonal organ (scolopidium) labeled according to Field and Matheson [45]. The inner-dendritic segment of the neurons includes the region from the cell body to the basal bodies, while the outer-dendritic segment, beyond the basal bodies, is the cilium.

(B–G) Sections through the JO of control animals show (B) ciliary dilation (arrow; the inset is another section of the same ciliary dilation) and (C and D) distal and proximal basal bodies (arrowheads). The fine arrow in (D) indicates the ciliary root. (E) The extracellular dendritic cap structure (arrows), where the cilia are attached, is visible in cross-sections from the apical parts of the scolopales. (F) A section from the middle of a scolopidium reveals the sensory cilia (arrows); (G) the axoneme contains the circular 9+0 organization of microtubule doublets (fine arrows). The scale bar in (F) represents 0.5 μm for (B)–(F).

(H–L) Sections through scolopidia of *DmKap*^{v6} homozygous animals show various structural defects. Longitudinal sections of a scolopidium show empty dendritic caps (arrow, [H]) but intact basal bodies (arrowhead, [I]) and ciliary roots (white arrow, [I]). This cilium terminates at the distal basal bodies. (J) The dendritic caps (arrow) also appear empty in transverse sections through the apical part of the scolopale. (K) Most sections through the middle of scolopales reveal no well-defined axoneme structures. Sometimes a stunted membranous structure is visible (arrow, [K]), and in some cases, membranes devoid of axoneme are visible (asterisk, [K]). (L) Transverse sections through the lower part of scolopale always show the normal electron-dense material around the distal basal body structures (arrowhead). The scale bar in (I) indicates 1.0 μm for (H)–(J), and the scale bar in (K) indicates 0.5 μm for (K) and (L).

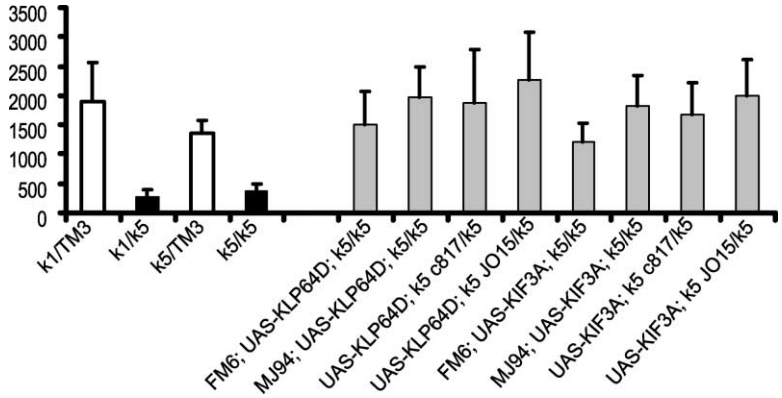

Figure 4. KLP64D Activity in JO Neurons Is Essential for Maintaining Auditory Function

Sound-evoked potentials from various *Klp64D* mutants and rescued combinations. The following abbreviations are used in the figure: k1, k5 are *Klp64D* alleles; TM3 for *TM3Ser*<*y*+>; and c817, MJ94, and JO15 for Gal4^{c817}, Gal4^{MJ94}, Gal4^{JO15}, respectively. The white bars are heterozygous controls, the black bars are mutants, and the gray bars are rescue genotypes. The error bars indicate ±SD, and N ≥ 10 for each bar.

zygous adults and found that all these mutants responded like the wild-type control (see Figure S1 in the Supplemental Data available with this article online). Unlike vertebrate photoreceptor cells, the photoreceptor neurons of *Drosophila* have no connecting cilium, and therefore this result further indicated that the recessive auditory response defects of *DmKap* mutants are caused by loss of sensory cilia in the JO neurons.

Mutation in the *DmKap* Locus Eliminates the Ciliary Axoneme of JO Neurons

JO is a large chordotonal organ containing nearly 200 scolopidial units [1]. Each scolopidium is innervated by two or three bipolar sensory neurons (Figure 3A). Each sensory neuron grows a single slender dendritic cilium. The cilia are encapsulated in the extracellular scolopale space formed by the scolopale cell. The apical ends of the cilia are attached to the extracellular dendritic cap formed by the scolopale cell. At about 3/4th the length of the cilium from basal bodies, the cilium contains an electron-dense matrix called the ciliary dilation (arrow, Figure 3B, and inset). The cilium is supported by an axoneme of nine microtubule doublets (fine arrows, Figure 3G) that assemble from electron-dense basal bodies (arrowheads, Figures 3C and 3D) at the base of the cilium. The basal body is anchored to the ciliary root (arrow, Figure 3D), which contains the protein rootletin [34]. A cross-section from the middle of the scolopale reveals two cilia in each JO scolopidium (arrows, Figure 3F). Electron microscopic observations of ultrathin sections of JO from *DmKap* homozygous mutant animals revealed deformities in the ciliary substructure (Figures 3H–3L). Almost all sections through the dendritic cap were devoid of cilia (arrows, Figures 3H and 3J), and sections at the midscolopale level (Figure 3K) showed lack of axonemal profiles; however, in more proximal sections, the basal body structures (arrowheads, Figures 3I and 3L) and roots (white arrow, Figure 3I) were visible in many scolopales. Very rarely we found scolopales with thin, deformed membranous cilia extending from a distal basal body. Together, all these observations established that DmKAP is required for axoneme formation in the cilia.

Mutations in *Klp64D* also Reduce Auditory Response

KLP64D and KLP68D have been identified as the two motor subunits of Kinesin II in *Drosophila* [28], and *DmKap* is shown to coexpress with *Klp64D* in the ciliated sensory neurons [29]. If DmKAP functions as part of the Kinesin II motor in the sensory cilia of JO neurons, then loss of KLP64D function should also affect the auditory response from JO. To test this hypothesis, we recorded the sound-evoked potentials from several viable combinations of *Klp64D* mutants. The *Klp64D^{k5}* homozygous mutant animals, and those with heteroallelic combinations *Klp64D$^{k1/k5}$* and *Klp64D$^{l4/k5}$*, survive as uncoordinated adults [28, 35]. Recordings from all three genotypes produced drastically reduced responses to the standard pulse stimulus (Figure 4, also see below and Figure S2 in the Supplemental Data), while the heterozygous control flies responded normally. To confirm that the *Klp64D* mutations were responsible for the reduced response, we simultaneously tested mutant flies rescued by the *UAS-Klp64D* and *UAS-Kif3A* (mouse homologue of KLP64D) transgenes. We found that, even in the absence of a Gal4 driver, these two transgenes could partially rescue the response. The two transgenes were previously shown to rescue the lethality and the behavioral defects caused by mutations in the *Klp64D* gene [28]. The amplitudes of sound-evoked potentials from the *UAS-Klp64D; Klp64D$^{k5/k5}$* and *UAS-Kif3A; Klp64D$^{k5/k5}$* flies were enhanced by introducing any of three different Gal4 drivers in the background (Figure 4). The *Gal4^{MJ94}* line expresses in chordotonal organs and a subset of other Type I sense organs, as well as in some CNS neurons [36]. The *Gal4^{C817}* expresses in chordotonal organs and in a subset of CNS neurons, while the *Gal4^{JO15}* line mainly expresses in a subset of chordotonal organ neurons in JO [37]. The homozygous *Klp64D$^{k5/k5}$* produced an average maximum potential of 370 μV, which is increased to 1513 μV and 1198 μV, respectively, by introducing *UAS-Klp64D* and *UAS-Kif3A* transgenes (Figure 4). The response index is further significantly enhanced to 2277 μV ($p \leq 0.0113$) and 1992 μV ($p \leq 0.0001$), respectively, in the presence of *Gal4^{JO15}*. This suggests that *Klp64D* gene activity is required in the JO neurons to maintain the sound reception ability.

Electron Microscopic Analysis of JO in *Klp64D* Mutants

The *Klp64D* mutant alleles significantly reduce the sound-evoked potentials but still have some residual response to sound stimulus, probably because we can only test alleles that allow some animals to survive to

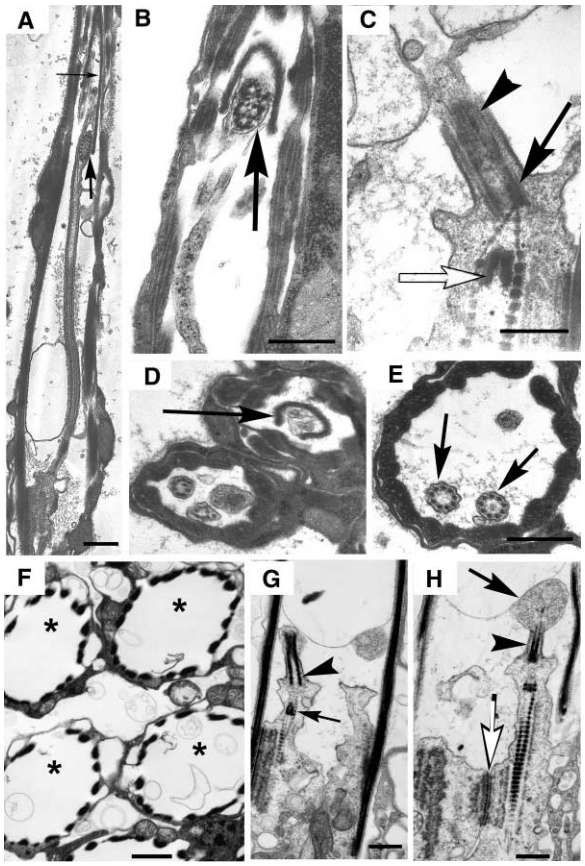

Figure 5. KLP64D Is Essential for Maintaining the Axonemal Organization in the Cilia of JO Neurons

(A–H) Sections from (A–E) *Klp64D*^{k5} homozygous and (F–H) *Klp64D*$^{k1/A8.n123}$ adult males are shown in this figure. (A) A longitudinal section through a scolopale shows a complete cilium structure with the distal basal bodies (arrowhead) and ciliary dilations (arrow). The ciliary dilations are deformed (arrow), and they are found to be in the same plane as the dendritic caps (fine arrow). (B) Approximate longitudinal section through the apical region of the scolopale shows abnormal organization in ciliary dilation (arrow). (C) The proximal (arrow) and distal (arrowhead) basal body and ciliary root (open arrow) structures appear normal. (D) Transverse sections through the scolopales also reveal dendritic caps (arrow) filled with ciliary structures including ciliary dilation material and (E) cilia (arrows) with apparently normal axonemes at the mid-length of the scolopales. The scale bar represnts 1.0 μm in (A), 0.5 μm in (B) and (E), and 0.3 μm in (C). (F–H) Ciliary structures are missing in the scolopales of *Klp64D*$^{k1/A8.n123}$ adults. (F) Cross-sections through the mid-region of scolopales show no discernable cilia (asterisk), while (G and H) longitudinal sections show normal looking proximal and distal basal body structures (arrowheads, [G] and [H]), ciliary roots (arrow, [G]), and desmosomal junctions (open arrow, [H]). (H) In certain sections, we observed a little membranous stump (arrow) at the distal end of the basal body. The scale bar in (F) equals 1.0 μm, and the scale bar in (G) and (H) equals 0.5 μm.

adulthood. To study whether the auditory defect has a correlation to ciliary structure, we analyzed the JO scolopidia from *Klp64D* mutant combinations. Often the scolopidia from *Klp64D*$^{k5/k5}$ adults contain complete sets of cilia (Figures 5A, 5D, and 5E). The basal body structures (arrowhead, Figure 5C), ciliary roots (open arrow, Figure 5C), and desmosomal junctions between inner-dendritic segments appeared normal. However, the ciliary dilations were deformed and disorganized (arrow, Figure 5B) and were often located in the same plane as the dendritic caps (arrow, Figure 5A). This might happen if the distal-most axoneme extension is compromised. The sensory cilia were absent (Figures 5F–5H) in most of the JO scolopidia from *Klp64D*$^{k1/A8.n123}$ hemizygous adults, but the inner-dendritic segment and desmosomal junctions (open arrow, Figure 5H) appeared normal. Together, these observations show that severe mutations in both *DmKap* and *Klp64D* would cause identical defects in JO neurons, while weaker hypomorphic mutations in the *Klp64D* locus, e.g., *Klp64D*^{k5}, would cause moderate levels of ciliary and axonemal damage. Interestingly, the levels of ciliary and axonemal defects in different *Klp64D* and *DmKap* alleles were directly correlated to the reduction of the auditory response. Since both *DmKap* and *Klp64D* functions are cell autonomous and map to the JO neurons, these gene products are involved in ciliogenesis in the JO neurons.

Reduced *DmKap* Dose Enhances the Auditory Defects of *Klp64D* Homozygous Adults

To further test the functional interaction between the two Kinesin II subunits DmKAP and KLP64D during ciliogenesis, we used a dominant genetic interaction paradigm in which sound-evoked responses were recorded from flies carrying one copy of a *DmKap* mutation and a viable combination of *Klp64D* mutations. Reduction to a single copy of *DmKap*^{V6} significantly enhanced the recessive lethality of different *Klp64D* combinations (Table 1). This made it difficult to obtain viable adults carrying one copy of a *DmKap* mutant allele and two *Klp64D* alleles. Finally, we obtained a combination of *DmKap*^{V6}/+; *Klp64D*^{l4}/*Klp64D*^{k5} females, which survived to the adult stage with a reduced viability compared to that of *Klp64D*^{l4}/*Klp64D*^{k5} females (Table 1). The *Klp64D*^{l4}/*Klp64D*^{k5} adults respond to a sound stimulus with intermediate efficiency (Figure 6A); the average amplitude of the sound-evoked potential is 426 μV (Figure 6B). In contrast, we found that the sound-evoked response is completely absent in *DmKap*^{V6}/+; *Klp64D*^{l4}/*Klp64D*^{k5} females (Figures 6A and 6B). This suggests that mutation in the *DmKap* locus is haplo-insufficient to compromised *Klp64D*, and therefore these two gene products are likely to interact with each other in JO neurons.

DmKap^{V6} Dominantly Enhances the Ciliary Defects in *Klp64D* Mutant Background

The interaction between DmKAP and KLP64D is further established at the ultrastructure level. We found that *Klp64D*$^{l4/k5}$ has the highest viability amongst the *Klp64D* mutant alleles and that the JO of these mutant adults have an almost indistinguishable set of defects (see Figure S3 in the Supplemental Data) from those seen in *Klp64D*$^{k5/k5}$ animals (Figures 5A–5E). These ciliary defects were significantly enhanced in *DmKap*^{V6}/+; *Klp64D*^{l4}/*Klp64D*^{k5} animals (Figures 6C–6E). Once again, we found that the proximal basal body structures (big arrows, Figures 6C and 6D) were normal and that the desmosomes between inner-dendritic segments were present (arrows, Figures 6E). However, the cilia appear greatly deformed and disappear apically (fine arrow, Figure 6C).

Table 1. *DmKap* Enhances the Lethality of *Klp64D* Transheterozygous Flies

Genotype	% Emerged	Activity
ru Klp64D^{l4}/Klp64D^{k5} h	97.6% (164/168)	++
DmKap^{V6}/w; ru Klp64D^{14}/Klp64D^{k5} h	74.0% (139/188)	+
DmKap^{V6}/Y; +/+	50.0% (184/368)	+
DmKap^{V6}/Y; ru Klp64D^{l4}/Klp64D^{k5} h	0.0% (1/188)	−

This table indicates the percent viability of various combinations of *DmKap* and *Klp64D* mutants. The mutant larvae of appropriate genotypes were collected at the first instar stage and were allowed to grow in sparsely populated vials. "% Emerged" indicates the (number of adults emerged/total number of larvae collected) × 100.

Transverse sections through central and distal levels of the scolopales showed variable presence of the axonemes within the dendritic caps, and some ciliary membranes appeared inflated. This further established that DmKAP interacts with KLP64D for axoneme growth from the distal basal body in JO neurons.

Kinesin II Is Not Required for the Assembly or Maintenance of Sperm Flagella

The Kinesin II subunits KRP85 and SpKAP115 localize to the mid-piece and flagellum of sea urchin and sand dollar sperm [38]. Additionally, Polaris, the mouse homolog of IFT88, is present in mature spermatids [39]. These observations indicated that Kinesin II and the IFT particles are involved in the maintenance and the growth of sperm flagella [4]. Therefore, to determine the universality of this hypothesis, we examined the testes from *DmKap^{V6}* (N = 17) hemizygous and *Klp64D$^{k1/A8n123}$* (N = 10) males. We were surprised to find that seminal vesicles of these mutants had vigorously motile sperm (see Supplemental Movies 1 and 2 in the Supplemental Data). In addition, the *DmKap^{V6}* males, rescued with neuronally expressed *UAS-Kap* (with the Gal4^{C155} driver), were as fertile as the wild-type. We verified that *Gal4^{C155}* does not express in the germline cells of the testis by using the UAS-mCD8::GFP reporter element. Hence, this observation established that the sperm axoneme growth is not affected in *DmKap* mutants. We also examined the morphology of sperm axonemes in *Klp64D* mutants. Unlike sensory cilia, *Drosophila* sperm tails contain the classical 9+2 microtubule arrangement. Ultrastructural

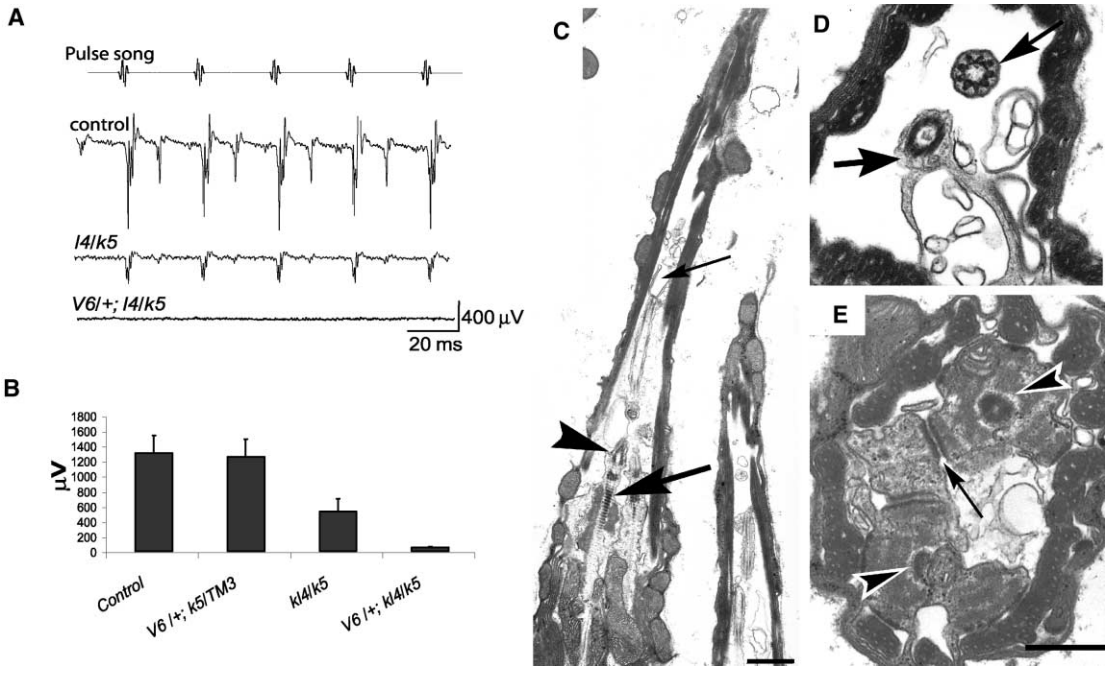

Figure 6. Mutation in the *DmKap* Locus Acts as a Dominant Enhancer of Klp64D Phenotypes

(A) Typical sound-evoked potential traces as recorded from the antennal nerve of control and mutant genotypes.

(B) The histogram of sound-evoked potentials from these mutants shows the quantitative analysis. The error bars indicate ±SD and N ≥ 10 for all bars.

(C–E) Sections through the second antennal segment from *DmKap^{V6}/+*, *Klp64D$^{l4/k5}$* adults. (C) The basal bodies (arrowhead) and ciliary root (arrow) structures are present and appear normal, but the cilium (fine arrow) is deformed. (D and E) Transverse sections through the proximal part of the scolopale show normal microtubule organization at the level of the distal basal bodies (arrows, [D]) and the desmosomes (fine arrow, [E]) between the inner-dendritic segments appear normal. The arrowheads in (E) indicate proximal basal bodies within the inner-dendritic segment. The scale bar in (A) equals 1.0 μm, and the scale bar in (C) equals 0.25 μm for (B) and (C).

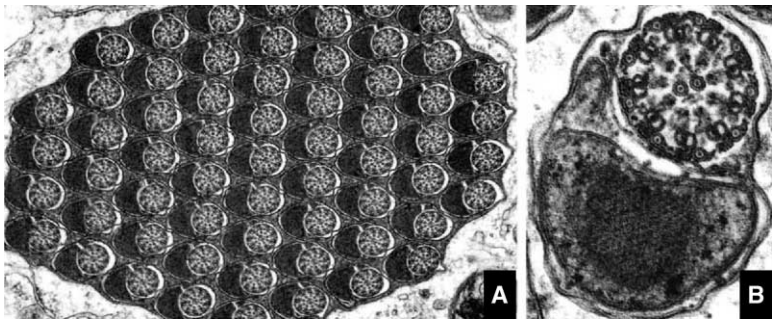

Figure 7. Sperm Tails in *Klp64D*$^{k1/A8.n123}$ Male Testis Show Normal Flagella and Axonemes

(A) A complete cyst containing 64 fully mature spermatids shows normal organization.
(B) An enlarged view of a mature sperm flagellum shows normal organization of axoneme and mitochondrial derivatives, and these are vigorously motile (see Movie 1 in the Supplemental Data).

analysis revealed normal axoneme and other sperm tail structures in these mutants (Figure 7). This suggests that in *Drosophila*, Kinesin II is not required to generate or maintain sperm flagella. This result is consistent with the finding that the *Drosophila nompB* gene product, a homolog of the *Chlamydomonas* IFT88 protein, is also not required to generate motile sperm [40]. This indicates that sperm development in *Drosophila* occurs by an IFT-independent mechanism.

Discussion

The vertebrate homologs of KAP protein are known to associate with the Kinesin II motor subunits [11, 13, 41], which are implicated in ciliogenesis in various cell types (see [9] for a review). However, the precise role of KAP in this process was unknown. We have now shown that mutations in the *DmKap* locus are haplo-insufficient in *Klp64D* hypomorphic backgrounds and enhance both the auditory reception defects as well as the ciliogenesis defects of the *Klp64D* alleles. This established that KAP plays a critical role in Kinesin II motor function in vivo. A recent study has further shown that mutations in the *nompB* locus of *Drosophila*, which encodes an IFT88/Tg737/OSM-5 homologous protein, also affect auditory responses of JO neurons and that mutations in *Klp64D* reduce the GFP-NOMPB localization in the cilia [40]. Therefore, the auditory system of *Drosophila* can be used to further study in vivo interactions between various IFT components.

Studies in *Chlamydomonas* have established that the Kinesin II motor subunits associate with a soluble, protein-rich IFT complex, which they transport toward the distal ends of flagella [42, 43]. This anterograde IFT seems to play a critical role in maintaining the flagellar length and activity (see [4] for a review). The electron microscopic data presented in this paper show that both *DmKap* and *Klp64D* gene functions are critical for proper axoneme growth in the dendritic cilia of the JO neurons. This suggests that Kinesin II may transport essential axonemal components into the dendritic cilia for the growth and maintenance of the axoneme structure. Thus, Kinesin II activity in the sensory cilia of *Drosophila* and in the motile cilia and flagella of other organisms appears to be conserved. In contrast, the spermatogenesis in *Drosophila* seems to be independent of anterograde IFT. This indicates the presence of hitherto unknown mechanisms of axonemal assembly operating in constructing these unusually long flagella.

Conclusions

DmKap interacts with *Klp64D*, and these two gene products are involved in axonemal assembly in the sensory cilia of JO neurons, but not in sperm. Our genetic interaction study suggests that DmKAP plays an important role in Kinesin II motor activity in vivo. This work has established a genetic interaction paradigm to further study the in vivo functions of Kinesin II and IFT proteins by using auditory function as an assay.

Experimental Procedures

Transgenes

The genomic transgenes *P(213w+)* and *P(219w+)* were constructed by cloning ~12 kb EcoRI and a ~24 kb NotI/XhoI genomic DNA fragments, respectively, into PCaSpeR vector. Sequencing 5′ and 3′ ends of these cloned fragments and comparing their positions with the genome sequence revealed that *P(213w+)* includes complete coding regions of *DmKap* and CG1657 (Figure 1A) and that *P(219w+)* excludes the 1925 bp from the 5′ of the CG1657 ORF (Figure 1A). Hence, these two transgenes could only overlap for the *DmKap* gene. The *UAS-Kap* transgene was constructed by subcloning the 3.7 kb EcoRI/XhoI cDNA fragment containing the complete DmKAP coding sequence from LD13502 [29] into the pUAST vector [44]. All these were transformed into *Drosophila* embryos by using standard techniques.

Fly Stocks and Mutagenesis

Detailed descriptions of the stocks obtained from the *Drosophila* stock center are available at www.flybase.org. The PlacW element in the *l(1)10Bb*^{G0169} stock was remobilized in heterozygous females with one copy of the *Sb ry*^{506} <*Pry*$^{+}$Δ*2-3*> chromosome in the background, and the X chromosomes of putative remobilized F_1 progeny were balanced over the *y w B FM7aGFP* balancer chromosome. About 300 such balanced stocks were screened for recessive lethality that could be rescued by both *P(219w+)* and *P(213w+)*. This yielded two recessive lethal stocks, *KP1* and *KP2*, which were allelic to each other, and *KP2* was rescued by the genomic transgenes. A similar screen with a collection of EMS-induced lethal mutations in the 10B region showed that lethality in *l(1)10Ba*^{5} (V5) and *l(1)10Ba*^{6} (V6) also maps in the DmKap locus. Finally a genetic complementation test showed that *KP1*, *KP2*, *l(1)10Ba*^{5}, *l(1)10Ba*^{6}, and *KG05921* are allelic to each other, and the recessive lethality in all these stocks, except *KP1*, was rescued by the genomic transgenes *P(213w+)* and *P(219w+)*. The alleles are mentioned as *DmKap*^{KG05921}, DmKap^{KP1}, *DmKap*^{KP2}, *DmKap*^{V5}, and *DmKap*^{V6}. *Klp64D* mutations are described previously [28]. *Klp64D*^{l4} is a recessive lethal allele with a Gly101Asp change in the predicted KLP64D protein sequence [35].

Electrophysiology

Auditory recordings from mutants and wild-type controls were performed 2 days after eclosion as described previously [1]. Pulse song stimulus traces consisted of five pulses at 35-ms intervals; the first pulse began at 15 ms. The responses of ten consecutive stimuli were averaged, and the maximum amplitude of this average response from each fly was used in assembling the histograms.

Electron Microscopy

Fly heads, with proboscis removed to facilitate infiltration, were fixed by immersion overnight at 4°C in a fixative containing 2.5% glutaraldehyde, 2.0% paraformaldehyde, and 0.04% $CaCl_2$ in 0.1 M phosphate buffer (PB) at pH 7.4. The $CaCl_2$ provides increased membrane stabilization. Heads were washed in PB, postfixed with OsO_4, dehydrated in an ethanol series, and embedded in Polybed 812. Ultrathin sections (75 nm) were stained with aqueous uranyl acetate and lead citrate and were examined with a Hitachi 7000 electron microscope.

Supplemental Data

Supplemental Data including the ERG traces from hemizygous *DmKap* adults, sound-evoked potential traces of homozygous *Klp64D* adults, electron micrographs of sensory cilia of JO neurons from $Klp64D^{l4/lk5}$ adults, and sperm motility movies from $DmKap^{V6}/Y$ and $Klp64D^{k1/A8.n123}$ males are available at http://www.currentbiology.com/cgi/content/full/13/19/1687/DC1/.

Acknowledgments

We thank Prof. K.S. Krishnan, Tata Institute of Fundamental Research (TIFR), for allowing us to use the ERG recording facility and S. Kaushal and S. Maiti of the Biophotonics Laboratory, TIFR, for the confocal images. We are grateful to F. Rudolf Turner for electron microscopy for some of the panels and Vikash K. Singh for microinjections. A part of the collaborative work is supported by a travel fellowship from the Journal of Experimental Biology to R.S. E.C.R. is supported by National Institutes of Health (NIH) grant GM56493. K.R. is supported by an intramural grant from TIFR, India, DST grant SP/SO/D-76/98, and NIH RO3 grant TWO5784. D.F.E. is supported by NIH grant DC04848 and by a grant from the Whitehall Foundation.

Received: June 6, 2003
Revised: August 13, 2003
Accepted: August 29, 2003
Published: September 30, 2003

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