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An IFT-A protein is required to delimit functionally distinct zones in mechanosensory cilia

Eugene Lee[1,2], **Elena Sivan-Loukianova**[3], **Daniel F. Eberl**[3], and **Maurice J. Kernan**[1,*]

1 *Department of Neurobiology & Behavior, Stony Brook University, Stony Brook, NY 11794, USA*

2 *Program in Neuroscience, Stony Brook University, Stony Brook, NY 11794, USA*

3 *Department of Biology, University of Iowa, Iowa City, IA 52242, USA*

Summary

Background—Conserved intraflagellar transport (IFT) particle proteins and IFT-associated motors are needed to assemble most eukaryotic cilia and flagella. Proteins in an IFT-A subcomplex are generally required for dynein-driven retrograde IFT, from the ciliary tip to the base. We describe novel structural and functional roles for IFT-A proteins in chordotonal organs, insect mechanosensory organs with cilia that are both sensory and motile.

Results—The *reduced mechanoreceptor potential A* (*rempA*) locus of *Drosophila* encodes the IFT-A component IFT140. Chordotonal cilia are shortened in *rempA* mutants and an IFT-B protein accumulates in the mutant cilia, consistent with a defect in retrograde IFT. A functional REMPA-YFP fusion protein concentrates at the site of the ciliary dilation (CD), a highly structured axonemal inclusion of hitherto unknown composition and function. The CD is absent in *rempA* mutants, and REMPA-YFP is undetectable in the absence of another IFT-A protein, IFT122. In a mutant lacking the IFT dynein motor, the CD is disorganized and REMPA-YFP is mislocalized. A TRPV ion channel, required to generate sensory potentials and regulate ciliary motility, is normally localized in the cilia, proximal to the CD. This channel spreads into the distal part of the cilia in dynein mutants, and is undetectable in *rempA* mutants.

Conclusions—IFT-A proteins are located at and required by the ciliary dilation, which separates chordotonal cilia into functionally distinct zones. A requirement for IFT140 in stable TRPV channel expression also suggests that IFT-A proteins may mediate preciliary transport of some membrane proteins.

Background

Eukaryotic cilia and flagella exemplify both molecular conservation and functional diversity. Their axonemal cytoskeleton, a radially symmetric array of nine microtubule doublets, is one of the most distinctive structures in the eukaryotic cell, and a large set of ciliary proteins is conserved across evolutionarily distant eukaryotes. Cilia are best known as propulsive motors, but many function as sensory probes and show diverse structural variations on the canonical, axonemal form; some cilia combine sensory functions with motility. We are interested in how

*Contact email: Maurice.Kernan@stonybrook.edu, phone (631) 632-9964/fax (631) 632-6661.

the conserved molecular mechanisms for ciliary assembly are adapted to construct specialized sensory cilia and to perform sensory functions.

Among the conserved ciliary proteins are parts of an intraflagellar transport (IFT) mechanism, which is required to extend a cilium from the cell surface (reviews: [1–3]). In *Chlamydomonas* flagella, IFT is seen as the processive movement of discrete particles along the axoneme [4]. Anterograde transport, towards the tip of the cilium, depends on kinesin-2, and retrograde transport is driven by an isoform of cytoplasmic dynein [5–8]. Uninterrupted particle movement in each direction indicates that the two motor activities are tightly regulated, and that the IFT particles are reconfigured at the base and tip of the cilium to load and unload cargoes and switch motor activities.

The component proteins of *Chlamydomonas* IFT particles associate in A and B subcomplexes [9,10]. Mutants lacking an IFT-B protein, in *Chlamydomonas* and in many other species, lack IFT and typically have very short or no cilia. In contrast, IFT-A proteins may be specifically involved in retrograde transport. *Chlamydomonas* mutants specifically defective in retrograde IFT show reductions in IFT-A proteins [11], although the specific mutated loci are unknown. Nematode [12,13] and *Tetrahymena* [14] mutants lacking IFT-A proteins have shortened or swollen cilia that accumulate material including IFT-B proteins; silencing IFT-A gene expression in trypanosomes [15] gives a similar phenotype. Other IFT-associated proteins are also implicated in retrograde IFT [13,16].

This study focuses on IFT-A proteins in chordotonal organs, ciliated mechanosensory organs in insects and crustaceans [17]. A scolopidium, the chordotonal functional unit, includes one to three sensory neurons and several specialized support cells, one of which constructs the scolopale, a fusiform, membrane-lined cavity. Each neuronal sensory process inserted at the base of the scolopale extends a cilium to its apex, where its tip is attached to an extracellular cap. The cap is connected, either directly or via another support cell, to the cuticle; a pull on the cap stretches the cilia and stimulates the neurons. Johnston's organ, an auditory chordotonal organ in the antenna, includes over two hundred scolopidia which detect and transduce airborne vibrations from nearby sound sources [17–19]. *Drosophila* mutations affecting antennal sound-evoked potentials have identified gene products implicated in chordotonal differentiation and mechanotransduction [20]. These include two TRP superfamily ion channels. The TRPV subunits Nanchung (NAN) and Inactive (IAV) form a channel which is located in chordotonal cilia and is required to generate sound-evoked potentials [21,22]. Mutants lacking NOMPC, the TRPN channel that transduces touch in bristles [23], also have reduced sound-evoked potentials [20]. The TRPN and TRPV channels also regulate a mechanical activity that increases antennal sensitivity to low-intensity stimuli [24].

Assembly of *Drosophila* sensory cilia requires anterograde IFT: mutants for the IFT-B protein IFT88/NOMPB [25] or a kinesin-2 subunit [26] lack cilia and all mechanosensory responses. Here we report that the *reduced mechanoreceptor potential A* (*rempA)* locus encodes the *Drosophila* homolog of IFT140, a component of the IFT-A subcomplex. Cilia in *rempA* mutants are shortened and accumulate IFT88, consistent with a defect in retrograde IFT. The wild type REMPA protein is localized to the ciliary dilation (CD), a characteristic, highly structured feature of chordotonal cilia. The effects of other IFT mutants on REMPA expression and localization, and of *rempA* and dynein mutants on ion channel expression and localization, indicate that the CD is an IFT-A-dependent structure which divides the chordotonal cilium into functionally distinct zones.

Results

rempA encodes IFT140

rempA^{1} mutants show moderate to severe uncoordination and reduced bristle mechanoreceptor potentials [27], and lack antennal sound-evoked potentials [20] (Figure 1). *rempA* was mapped to cytogenetic interval 21C (details in supplemental figure S1). Two other semilethal mutations in this interval, *l(2)21Ci*^{1} and *l(2)k12913*, fail to complement the behavioral and viability defects of *rempA*^{1}, although *l(2)21Ci*^{1} homozygotes or *l(2)21Ci*^{1}/*rempA*^{1} trans-heterozgotes show less severe uncoordination than *rempA*^{1} homozygotes or hemizygotes. The genomic sequence corresponding to 21C includes the predicted gene *CG11838*/*oseg3*, which encodes the *Drosophila* IFT140 homolog and was also identified as a ciliary protein by comparative genomics [28]. All three *rempA* mutations disrupt the coding region of *CG11838*: single-base nonsense mutations in *rempA*^{1} and *l(2)21Ci*^{1}, and a 3kb deletion and frameshift in *l(2)k12913* (Figure 1). An 8kbp genomic DNA fragment containing *CG11838* rescued the viability, behavioral, and electrophysiological phenotypes of *rempA* mutants, confirming its identity with *CG11838*/*oseg3*.

A single cDNA clone (IP14838 in the Berkeley *Drosophila* Genome Project database) matches the longer (*CG11838-PB*) of two predicted open reading frames, and encodes a 1503 amino-acid polypeptide with 33% and 27% overall sequence identity to the mammalian and *Chlamydomonas* IFT140 homologs respectively. The protein has an “oseg” domain architecture [28], which includes up to 7 WD repeats in the N-terminal half and a C-terminal part with alpha-helical tetratricopeptide repeats (TPR). The C-terminal, alpha-helical half is the more conserved, with 48% amino-acid identity to mammalian IFT140; all three mutations are predicted to remove this part of the protein.

rempA mutants have short cilia that accumulate IFT-B proteins

We visualized chordotonal neurons and cilia with cytoplasmic red fluorescent protein (RFP) (Figure 2). In wild type larval scolopidia, the cilia extend through the scolopales and into the distal extracellular caps, but in *rempA* mutants, the RFP-labeled cilia end within the scolopales, at about half the wild type length. Electron microscopy of the adult antennal chordotonal organ confirmed that the cilia are indeed present but truncated: transverse sections of mutant scolopidia show ciliary profiles at proximal, but not distal levels (Figure 2; compare to supplemental Figure S2). Thus, chordotonal cilia are present in *rempA* mutants, but are shortened and mostly disconnected from the dendritic cap. The cap-cilium connection is required for transduction [29], so its disconnection in *rempA* mutants accounts for their complete loss of antennal chordotonal responses [20].

Chordotonal cilia in *Drosophila* and other insects share a distinctive feature, the ciliary dilation (CD). This is an electron-dense inclusion within the axoneme, at about 2/3 the length of the cilium, proximal to the dendritic cap (Figure 2, supplemental figure S2). In *Drosophila* the electron-dense material appears as a hexagonal lattice or as parallel tubes at an oblique angle to the ciliary axis, depending on the plane of section (Figure 2). The microtubule doublets bend outward at this site but otherwise continue without interruption past the inclusion. Axonemal dynein arms are present in the proximal zone, but not distal to the CD [17], (supplemental figure S2). The CD is absent in *rempA* mutants; indeed, mutant cilia end at about the position where the dilation is normally located (Figure 2).

Caenorhabditis IFT140 (*che-11*) mutants [30], have distended sensory cilia [12], which lack retrograde IFT and accumulate IFT-B proteins [13]. We examined the distribution of the IFT-B protein NOMPB (IFT88) [25] in *Drosophila* IFT-A mutants. A transgene expressing NOMPB-GFP [25] was introduced into *rempA* and *oseg1* (*IFT122*) [28] strains. In wild type

and heterozygote controls, NOMPB-GFP is distributed along the cilia [25], and low levels are present in the cell body. In *rempA* and *oseg1* mutants, it is present in the truncated cilia at higher levels than in wild type (Figure 3). Thus neither IFT140 nor IFT122 is required for transport of IFT88 into the cilium, but both are probably required for its retrograde transport back to the cell body.

In contrast to their defective sensory cilia, *rempA* mutant males had motile, apparently normal sperm flagella (not shown), indicating that IFT-A proteins, like IFT-B and heterotrimeric kinesin [25] [26], are not needed in *Drosophila* spermatogenesis.

IFT140/REMPA localizes at the ciliary dilation in chordotonal organs

To view the distribution of IFT140 in sensory neurons, we examined ciliated sense organs in embryos, larvae and pupae expressing a functional REMPA-YFP fusion protein from the rescuing transgene (Figure 1). In mechanosensory bristles and campaniform sensilla, a single spot of YFP was associated with each sensillum (Supplemental figure S3), coincident with staining by the 21A6 monoclonal antibody and proximal to the dendritic sheath protein NOMPA [29]. This places REMPA in the connecting cilium, just proximal to the outer segment, consistent with a previous report of IFT140 and OSEG1/IFT122 localization in bristle neurons [28].

In the longer cilia of larval chordotonal organs, REMPA-YFP appeared at a single, well-defined focus in each scolopale, at the position of the ciliary dilation (Figure 4A–C). As these organs differentiate [31], REMPA-YFP initially appears diffusely in the neuronal cytoplasm and along the growing cilium, but ultimately concentrates at the ciliary focus and disappears from other locations (Figure 4 E–J). A similar progression during differentiation of pupal chordotonal neurons (Figure 5A) culminates in paired foci, in accordance with the paired cilia in adult scolopidia (Figures 4D and 5C). If the cilium is missing, as in *Klp64D* mutants which lack an IFT-associated kinesin-2 subunit, the REMPA focus is also absent, and a reduced amount of protein accumulates near the basal body (Supplemental Figure S4). In hypomorphic *Klp64D* mutants, which retain cilia, the REMPA-YFP foci are present but are mislocated within the dendritic caps (supplemental figure S4), as previously observed for the ciliary dilations [26].

The 21A6 antigen is the agrin/perlecan-related protein Eyes Shut (EYS) [32] or Spacemaker (SPAM) [33], which is secreted into the scolopale space where it has a mechanoprotective role under osmotic stress [34]. It was described as being located at the ciliary dilation [32], but 21A6 staining in differentiated chordotonal organs is distinct from and proximal to the REMPA-YFP focus (Figure 5E), consistent with the normal ultrastructure of the ciliary dilations in an *eys* mutant [32] [34].

IFT122 is essential for IFT 140 expression, and the *btv* dynein for IFT140 localization

The *Chlamydomonas* IFT-A subcomplex includes six polypeptides [3]. The amino acid sequences of three of these – IFT140, IFT139 and IFT122 – are published, and the nematode *ifta-1* gene product, which is required for retrograde IFT, may also be an IFT-A component [13]. All four proteins are widely conserved in ciliated eukaryotes. (Unusually, the genomes of *Drosophila melanogaster* and closely related species lack IFT139, although it is present in more distant *Drosophila* species and in other insects.) IFT122 is encoded in *D. melanogaster* by the *oseg1* locus [28]. When REMPA-YFP was crossed into an *oseg1)* mutant background, no YFP signal was detected in *oseg1* mutant cell bodies or cilia of this genotype at any stage (Figure 5). IFT122 is therefore required for stable expression of IFT140, implying that the IFT-A subcomplex must be at least partly assembled for stability and proper localization, possibly before entering the cilium.

Retrograde IFT is powered by a processive dynein, related to the cytoplasmic dynein that drives cellular minus-end directed transport on microtubules. The *Drosophila* IFT dynein heavy chain is encoded by the *beethoven* (*btv*) locus [35]; *btv* mutants have extremely reduced antennal sound-evoked potentials [20]. Cilia in *btv* chordotonal organs are variably disrupted: some retain their connections to the dendritic caps, but their ciliary dilations are always disorganized [20]. In chordotonal cilia of *btv* mutant larvae and pupal legs, REMPA-YFP is delocalized and redistributed toward the distal tip of the scolopidium. In antennal chordotonal organs, *btv* mutants show ectopic deposits of REMPA-YFP along the dendritic caps, (Figure 5F, G) suggesting either a failure to retract ciliary material during differentiation, or leakage from disrupted cilia.

A ciliary TRPV ion channel requires IFT140 for expression and IFT dynein for localization

The TRPV channel subunits encoded by the *nanchung* (*nan*) and *inactive* (*iav*) loci are expressed specifically in chordotonal neurons and localized in their cilia, proximal to the ciliary dilations [21,22]. Mutations in either *nan* or *iav* eliminate antennal sound-evoked potentials, and each subunit requires the other for ciliary expression, suggesting that they form a heteromeric channel required to depolarize the chordotonal neurons enough to fire action potentials [22]. The channel is also needed to regulate active motility of the distal antennal segments, which vibrate with higher amplitude in *iav* or *nan* mutants [24].

To determine if the restricted TRPV ion channel distribution in *Drosophila* requires IFT-A or IFT dynein function, we introduced a functional IAV-GFP fusion transgene [22], under the control of its native promoter, into *rempA* and *btv* mutant flies (Figure 6). Surprisingly, instead of accumulating in chordotonal cilia like NOMPB-GFP, IAV-GFP was completely undetectable in *rempA* mutants. This appears to be a posttranscriptional depletion, as quantitative RT-PCR showed no significant difference in *iav* or *nan* transcript levels between *rempA* mutants and controls (supplemental table 1). Thus, IFT140 may be essential for stability of the expressed TRPV channel. However, REMPA-YFP is normally expressed and localized in chordotonal organs of *iav* mutants (supplemental figure S5), indicating that the ciliary dilation does not depend on TRPV channel expression or activity, nor is it affected by the increased antennal vibration in *iav* mutants [24].

In contrast to its absence from *rempA* mutants, IAV-GFP is still expressed in the chordotonal cilia of *btv* mutants, consistent with the small antennal potentials that can still be evoked in *btv* flies by high-amplitude sound stimuli [20]. However, in *btv* mutant cilia, some IAV-GFP signal penetrates beyond the ciliary dilation, into the distal zone from which it is normally excluded (Figure 6). This implies either that BTV dynein activity normally retrieves the TRPV channel from the distal zone, or that the disrupted ciliary dilations in the *btv* mutant cilia are insufficient to exclude it.

Discussion

rempA encodes the *Drosophila* homolog of IFT140, a conserved WD-TPR protein first identified in the IFT-A subcomplex. IFT-A proteins in other species are required for dynein-driven, retrograde transport within cilia and flagella, and the *rempA* mutant phenotypes are consistent with defective retrograde transport in developing chordotonal cilia. But the data also indicate unexpected functions for the *Drosophila* IFT-A proteins, as required elements of a sensory-specific structure, the ciliary dilation, and for stable expression of a TRPV ion channel. They show that IFT proteins, generally regarded as elements of the conserved IFT mechanism, can be reconfigured in some cells for specialized sensory functions, an important new perspective for studies both of IFT and of mechanosensory transduction.

IFT140 and IFT dynein function in differentiating ch and es organs

During the differentiation of chordotonal neurons, when REMPA/IFT140 is distributed along the growing cilia, it may function in retrograde transport. In contrast to IFT-B or kinesin-2 null mutants, which do not extend cilia beyond the basal body [25,26], chordotonal cilia in *rempA* mutants are substantially longer and accumulate the IFT-B protein IFT88, indicating that anterograde IFT can still operate. These results are consistent with a specific defect in retrograde IFT, as in nematode and *Tetrahymena* IFT-A mutants [12–14,36].

However, if the *Drosophila* IFT-A proteins are required only for retrograde IFT, then mutants lacking the IFT-associated dynein should show the same phenotype as *rempA*. Indeed, nematode and *Chlamydomonas* IFT dynein mutants have a similar phenotype, with shortened, distended cilia and flagella that accumulate IFT proteins and other material [8,16,37]. But the *Drosophila* IFT dynein, BTV, has a less severe mutant phenotype in chordotonal organs than *rempA: btv* mutant cilia retain their distal segments, and most are still connected to the dendritic caps. Moreover, BTV appears not to be required for the operation of other ciliary mechanosensors: unlike *rempA* mutants, *btv* mutants have normal bristle receptor potentials [20], and their mildly uncoordinated behavior, similar to that of the TRPV channel mutants *iav* and *nan*, is consistent with a chordotonal-specific loss of mechanotransduction. This suggests that the IFT-A proteins have additional functions, not wholly dependent on dynein-driven transport, in chordotonal and other cilia.

The ciliary dilation delimits structurally and molecularly distinct ciliary segments

As chordotonal cilia complete differentiation, REMPA-YFP concentrates to a focus at the same time and site as the CD forms. A distal shift of the REMPA-YFP signal in kinesin-2 hypomorphs parallels the displacement of the CD previously seen in these mutants [26]. The CD is missing in *rempA* mutants, and the REMPA-YFP focus is missing in mutants lacking IFT122, the other IFT-A protein in *Drosophila*. Thus IFT-A proteins are localized at the CD, and are probably both required for its formation. In addition, OSEG4, the homolog of the retrograde IFT-associated protein IFTA-1 [13], also localizes to the CD [34].

As a characteristic feature of chordotonal cilia, the CD has been proposed to have a role in mechanotransduction [17], but its precise function is unknown. Chordotonal cilia are unusual among sensory cilia in that they are potentially motile [38,39]: their axonemal microtubules bear extensions similar to dynein arms (supplementary figure 2). They are a likely source of the mechanical energy that increases antennal compliance and sensitivity to low-intensity vibrations, and causes the antennae to oscillate even in the absence of any stimulus [40]. Mutants with cilia that are structurally defective (*btv*, *tilB*) or disconnected from the distal antennal segments (*nompA*) lack active antennal mechanics, implying that the cilia themselves are indeed the motor elements [41]. The active mechanics are also absent and the sensitivity reduced in *nompC* mutants, but the TRPV mutants *iav* or *nan* show much larger antennal oscillations than wild type [24]. These different mutant phenotypes imply opposing functions for the TRPN and TRPV ion channels in regulating ciliary motility: the NOMPC/TRPN channel may be required to trigger motility whereas the TRPV channel normally reduces it.

The opposite effects of the TRPN and TRPV channels on motility could result from different locations relative to the ciliary motors. The TRPV channel is normally restricted proximal to the ciliary dilation [22]. The apparent axonemal dynein arms are also located only in the proximal zone [17,42] (supplementary figure 2). Thus potential drivers and negative regulators of ciliary motility are both restricted to the proximal part of the cilium. Conversely, new immunostaining data (Dr. Y.D. Chung, personal communication) show the TRPN/NOMPC channel to be located specifically in the distal zone of chordotonal cilia, and bounded proximally by the CD. The CD is therefore located between ciliary segments with different

channel populations and axonemal structures. In *btv* mutants, in which the CD is disorganized, the partitioning is compromised, and some of the TRPV channel is located in the distal zone, suggesting that an intact CD and/or localized dynein activity is required to maintain the localization, and possibly to sort specific channels into each segment.

A role for IFT-A in preciliary transport?

In differentiating wild-type neurons, REMPA-YFP was observed in the cell body and inner dendrite as well as in the nascent cilia, but it is undetectable at any of these sites, at any stage, in *oseg1* mutants. This suggests that IFT140 requires IFT122 for stable expression, even before arrival at the basal body and entry into the cilium. A similar interdependence was observed in *Chlamydomonas*, in which levels of multiple IFT-A proteins are reduced in each of three retrograde IFT mutants [11,43].

Similarly, the lack of expression of the IAV channel subunit in *rempA* mutants indicates that TRPV protein expression or stability also requires the IFT-A proteins. (There is, however, no reciprocal requirement: IFT140 is normally localized at the ciliary dilation in TRPV mutants.) We speculate that the IFT-A proteins may be required for the extraciliary, vesicular transport of specific membrane proteins to the basal body region, before insertion into the ciliary membrane. Both OSEG1/IFT22 and REMPA/IFT140 share a common WD/TPR domain architecture with coat proteins (COP) of the coated vesicle transport pathways [28], from which IFT may be derived [44]. Prior evidence for preciliary, vesicular transport of ciliary membrane proteins includes the AP1-dependent transport of ODR-10 [45] and PKD-2 [46] in *C. elegans*, the presence of IFT20 at the Golgi apparatus [47], and the Rab8-dependent transport of rhodopsin [48] and BBS module proteins [49] to the cilium. Preciliary transport could be driven by a minus end-directed microtubule motor such as dynein, as the microtubules in the inner dendritic segment have their minus ends oriented distally [50]. It cannot, however, be the IFT dynein, as the IFT-A proteins accumulate in the cilia, not in the cell bodies or inner segments, of a *btv* mutant.

Conclusion

In conclusion, the involvement of REMPA/IFT140 in forming the ciliary dilation and localizing ciliary ion channels demonstrates an unexpected versatility for the IFT-A proteins. It will be interesting to see if reconfigurations and adaptations of the IFT proteins also underlie the many diverse forms of other sensory cilia.

Experimental Procedures

Genetic alleles, stocks, and transgene constructs

rempA^{1} [27] and *btv*^{5P1} [20] were isolated as described. The *rempA* allele *P{lacW}ex*^{k12913} and the *Oseg1* allele *P[*^{32}*]Oseg1*^{EP3616} were obtained from the Bloomington *Drosophila* stock center. The *rempA* allele *l(2)21Ci*^{1} and other lethal mutations in the 21C region were obtained from Dr. P Heitzler. Transgenic stocks expressing GFP fused to NOMPB/IFT88 [25] and to the TRPV channel subunit Inactive [22] were described previously. Construction of the rescuing fusion transgene *P{rempA*$^{+}$*-YFP}* is described in supplemental figure 1B.

Sensory electrophysiology

Antennal sound-evoked potentials [20], and bristle transepithelial potentials and mechanoreceptor potentials [27], were recorded from adult flies as previously described.

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 in 0.1 M phosphate buffer (PB) at pH 7.4. 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.

Fluorescence microscopy and immunostaining

Fluorescently tagged or immunostained proteins were visualized by scanning confocal microscopy in embryos, larvae, and late pupae or pharate adults. Embryos were collected 13–15 hours after egglaying and dechorionated in dilute bleach solution. Heterozygous sibs were distinguished from mutants by GFP-expressing balancer chromosomes. Pupal antennae and legs were dissected 24–48 hours after pupariation in PBT (0.2% Triton-X in PBS) and fixed in 4% formaldehyde in PBT for 20 min, then washed three times in PBT for 10 minutes. For immmunostaining, samples were first blocked for 1hr in 5% normal goat serum in PBT before incubating with primary antibodies overnight at 4°C in the blocking solution, then washed in PBT as before and incubated with secondary antibody for 2 hours. After washing with PBT for 3 times 20 minutes, samples were mounted in Vectashield (Vectorlabs, CA) and imaged with a laser scanning confocal microscope (Leica TCS SP2).

Monoclonal antisera 21A6 (used at a dilution of 1:250) and 22C10 (1:100) were obtained from the Developmental Studies Hybridoma Bank (DSHB, http://dshb.biology.uiowa.edu/). Phalloidin-Alexa568 (1:1000) and the secondary antibodies Alexa647 conjugated goat anti-rabbit (1:1000), Alexa647 conjugated goat anti-mouse (1:1000), Alexa568 conjugated goat anti-mouse (1:500), and Alexa546 goat anti-mouse (1:1000) were obtained from Molecular Probes/Invitrogen (http://probes.invitrogen.com/) and used at the dilutions indicated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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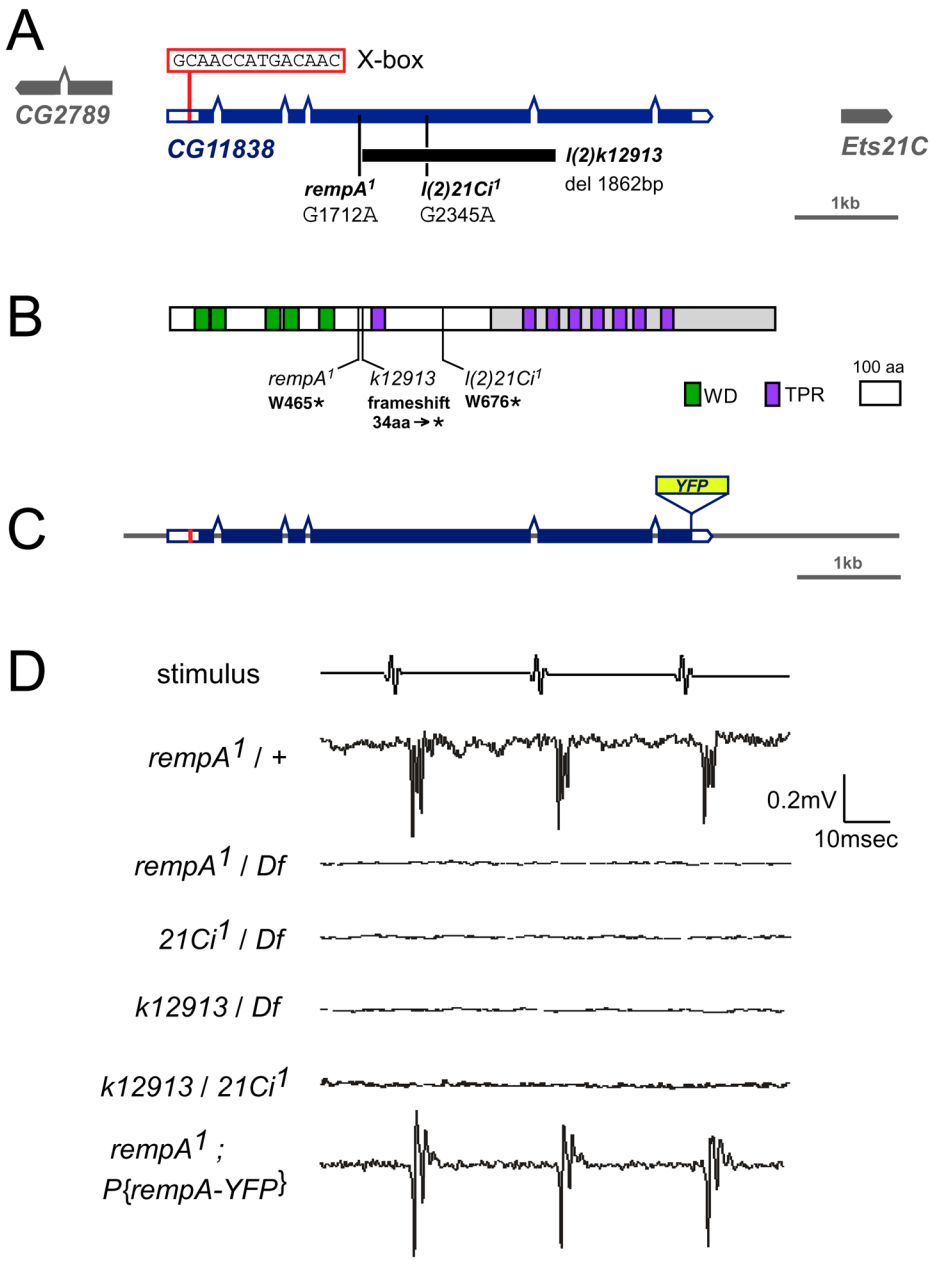

Figure 1. *rempA* encodes the IFT-A protein IFT140

A, Genomic region including *CG11838*/*rempA*, showing the deletion in *l(2)k12913* (bar) and the positions of two nonsense mutations. Boxes, exons; filled boxes: coding region. The X box (Rfx transcription factor binding site) may not be functional [51]. B., Predicted protein structure and mutant changes. Domains were predicted by the REP repeat prediction algorithm [52]; gray shading indicates the more conserved region. The deletion in *l(2)k12913* causes a frameshift and premature termination. C, Genomic rescue construct, showing the C-terminal insertion of the yellow fluorescent protein (YFP) coding region. D., Compound potentials recorded from the antennal nerve in response to a pulsed sound stimulus. Each trace is the averaged response to ten trials. $rempA^{1}/rempA^{+}$ heterozygotes show a wild-type response; but homozygotes, hemizygotes and trans-heterozygotes for *rempA* alleles lack sound-evoked potentials. A single insertion of the rescue fusion construct restores the response.

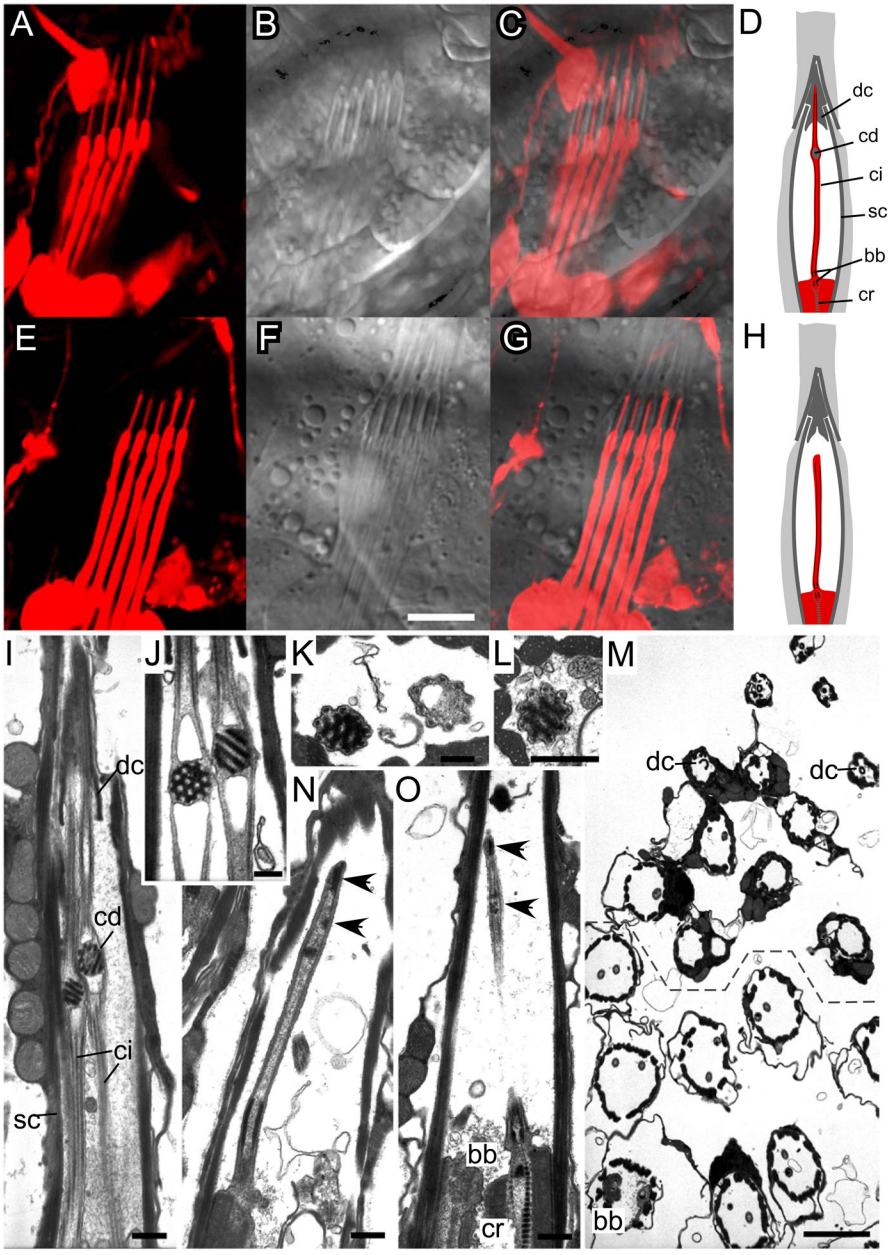

Figure 2. Disrupted ciliary morphology in *rempA* mutants

A–C and G–F, Confocal fluorescence (A, E), DIC (B, F) and merged (C, G) images of a pentascolopidial chordotonal organ in a larva expressing red fluorescent protein (RFP) in neurons. D, H, schematics of single scolopidia. Wild-type cilia (A–D, *rempA*^{1}/+ heterozygote) extend through the scolopale into a dendritic cap; mutant cilia (E–H, *rempA*^{1} homozygote) end within the scolopale cavity. Scale bar, 5μm. I–O; electron micrographs of wild type (I–L) and *rempA* mutant (M–O) antennal scolopidia. I–L, Longitudinal (I, J) and transverse (K, L) sections of wild type scolopidia, showing the subterminal ciliary dilations (cd) proximal to the dendritic cap. M, transverse section of scolopidia in a *rempA*^{1} mutant. Moving from the bottom toward the top of the image, scolopidia are sectioned at progressively more apical levels, from basal body (bb) to dendritic cap (dc). In scolopidia above the dashed line, one or both ciliary profiles are absent; no ciliary dilations are observed in any section. N, O, longitudinal sections

of scolopidia in *rempA*^{1}(N) and *rempA*^{k12913} (O) mutants. No ciliary dilations are observed; arrowheads indicate disorganized electron-dense material in the lumen of the axoneme. Other abbreviations: ci, cilium, cr, ciliary rootlet, sc, scolopale rods. Scale bars, 0.5μm (I–L, N, O); 2 μm (M).

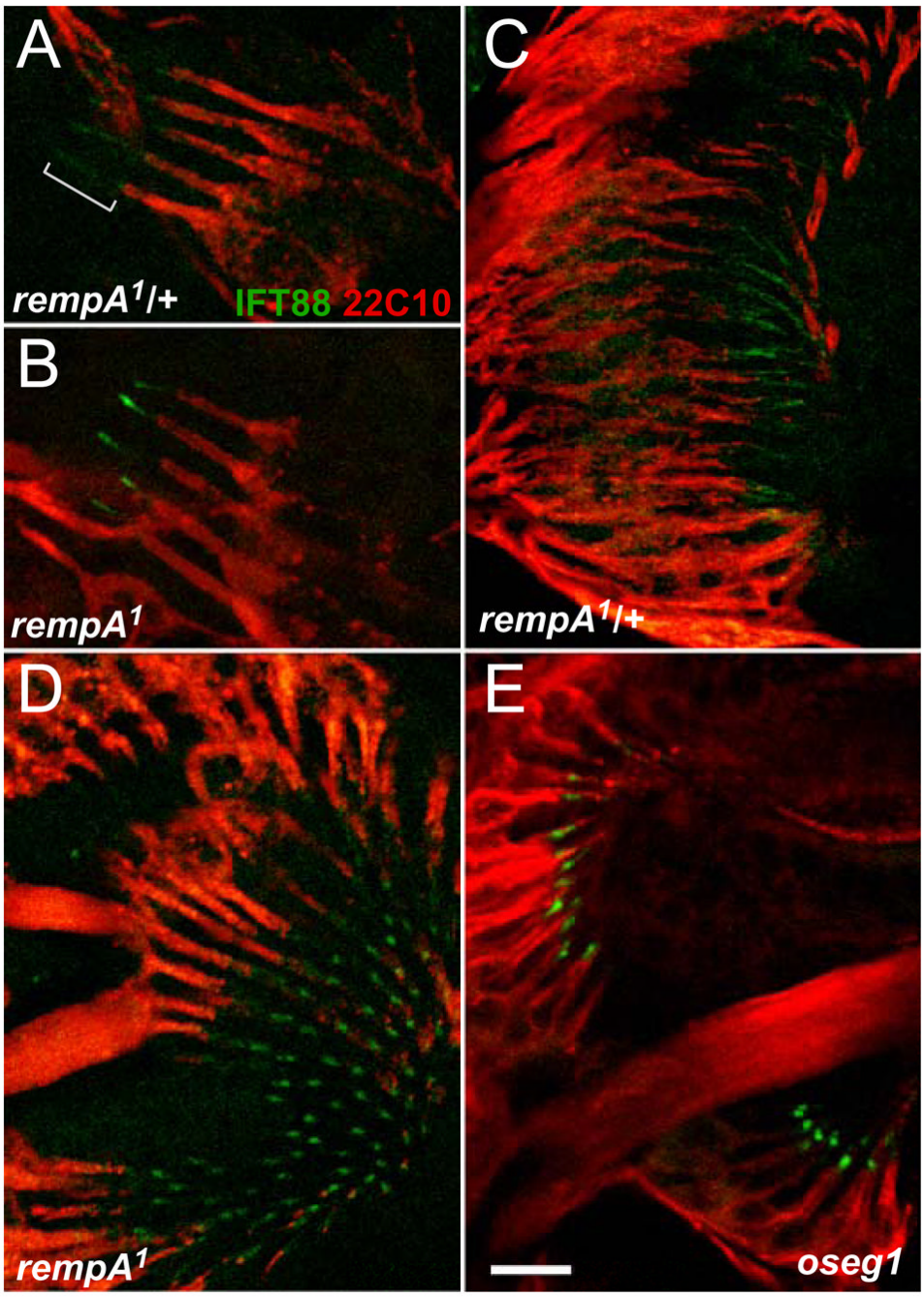

Figure 3. An IFT-B protein accumulates in IFT-A mutant cilia

Confocal projections of larval abdominal ch organs (A, B) and pupal antennal (C–E) ch organs in *rempA*^{1}/+ heterozygotes (A, C), *rempA*^{1} homozygotes (B, D) and an *oseg1*^{EP3616} homozygote (E), all expressing functional, GFP-tagged NOMPB/IFT88 (green). Neurons are counterstained with MAb22C10 (red), which detects MAP1B/Futsch throughout all neurons, except for their cilia. IFT88 is distributed along wild type cilia (extent indicated by bracket in A), but accumulates at or near the tips of the truncated cilia in the mutants. Scale bar (C–E): 10μm.

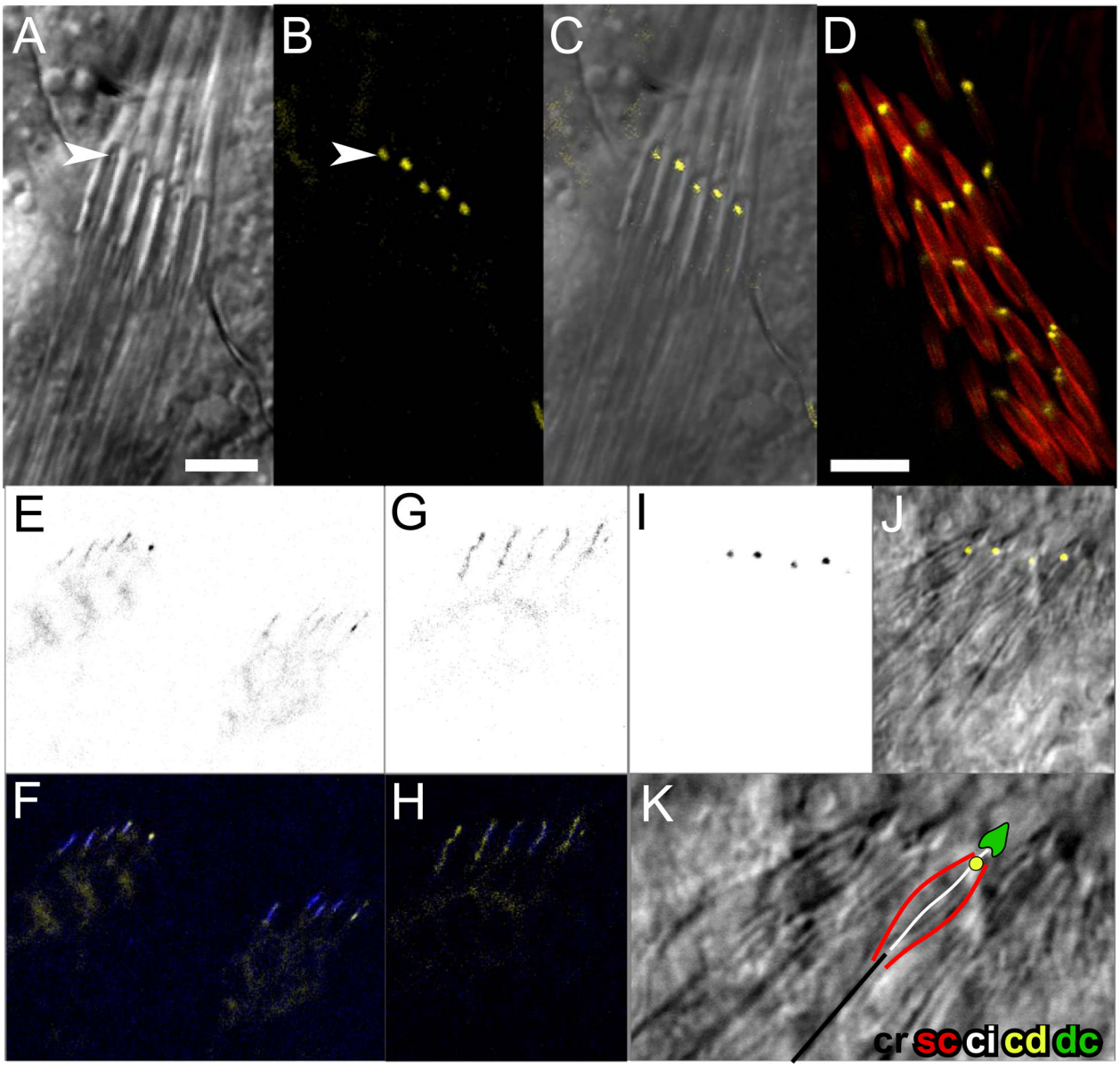

Figure 4. REMPA localizes to the ciliary dilation in chordotonal organs

A–C, DIC (A), confocal fluorescence (B) and merged (C) images of an abdominal chordotonal organ in a 3rd instar larva expressing the *P{rempA-YFP}* rescue construct. The YFP signal is concentrated at the site of the ciliary dilation (arrowheads in A and B). D, femoral chordotonal organ in a pupa expressing *P{rempA-YFP}*, showing paired REMPA-YFP foci corresponding to the paired cilia and ciliary dilations in adult scolopidia. Scolopale rods stained with rhodamine-phalloidin (red). E–K, REMPA-YFP expression in differentiating embryonic chordotonal organs. E–H, inverted YFP fluorescence images (E, G) and merged fluorescence images counterstained with MAb 21A6 (F, H) showing expression of REMPA-YFP in the cell body at embryonic stage 15 (E, F; two organs shown) and concentration in the cilium at stage 16 (G, H). I–K, stage 17 organ, in inverted YFP fluorescence (I), and merged with DIC (J). K is an enlarged detail from J with DIC only, overlaid with a schematic of a single scolopidium. Abbreviations as in Figure 2. Scalebars: 5 μm (A–C); 10 μm (D).

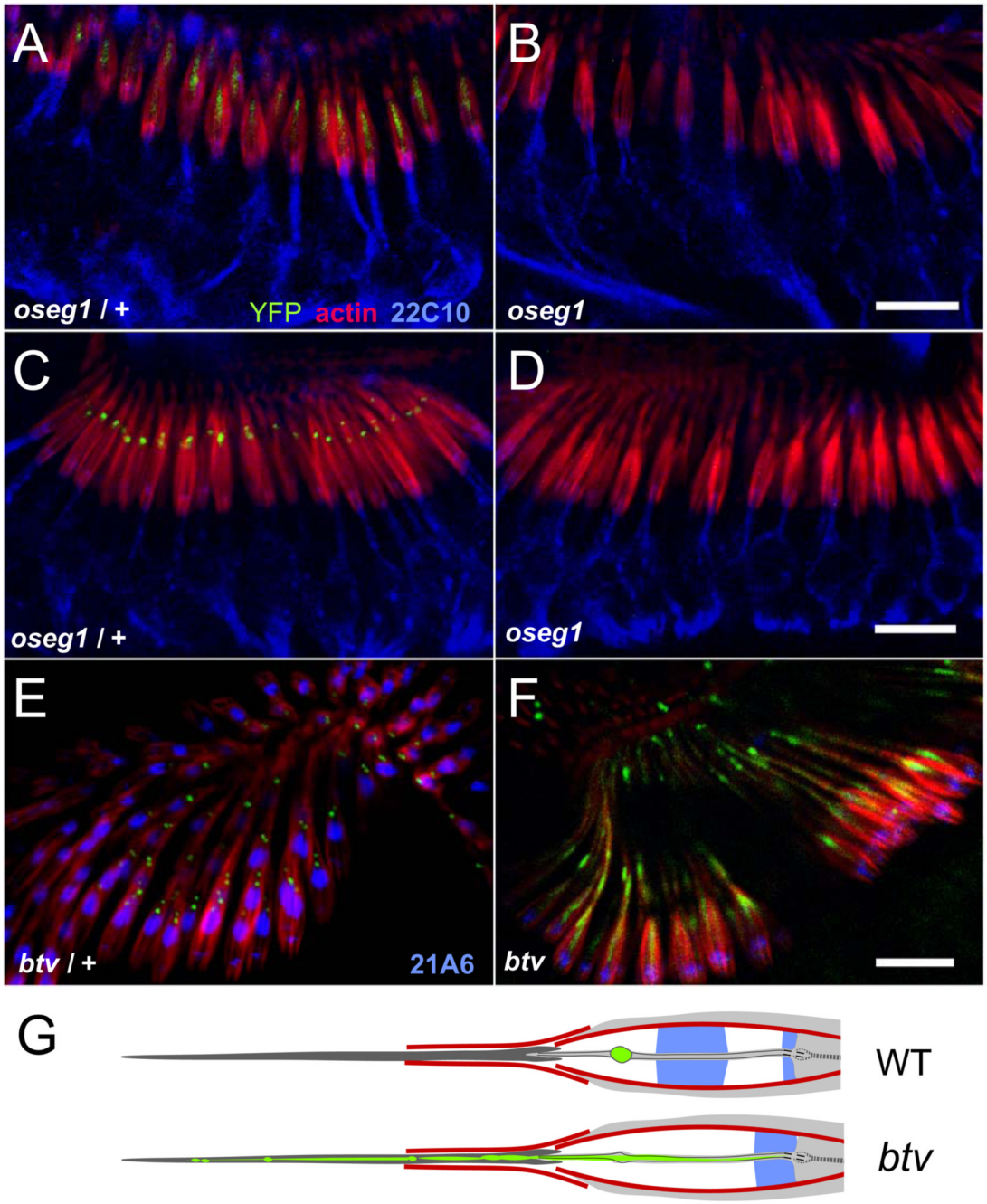

Figure 5. REMPA requires IFT122 for expression and IFT dynein for normal localization

A–D, Differentiating antennal chordotonal organs in *oseg1*^{EP3616}/+ heterozygotes (A, C) and *oseg1*^{EP3616} homozygotes (B, D) with a REMPA-YFP transgene. A and B show an earlier stage, indicated by the parallel arrangement of the scolopales (stained with phalloidin, red) and the distribution of YFP along cilia in A; In C and D the YFP signal resolves to foci and the apical ends of the scolopidia are drawn together. No REMPA-YFP signal was detectable in any stage in the *oseg1* mutants. E, F, Antennal chordotonal organs in a *btv*^{5P1}/+ heterozygote and a *btv*^{5P1} homozygote, expressing REMPA-YFP and counterstained with MAb21A6 (blue) and phalloidin (red). G, schematic interpretation of E and F; with the elongated dendritic tips in the antennal scolopidia; only one of the two cilia in a scolopale is drawn. In the heterozygote,

the paired REMPA-YFP foci are located just distal to the major zone of 21A6 staining. In *btv* mutants, most 21A6 staining is shifted to the base of the scolopale, and the YFP signal is redistributed along the cilium and throughout the length of the tubular dendritic cap. Scalebars = 10μm.

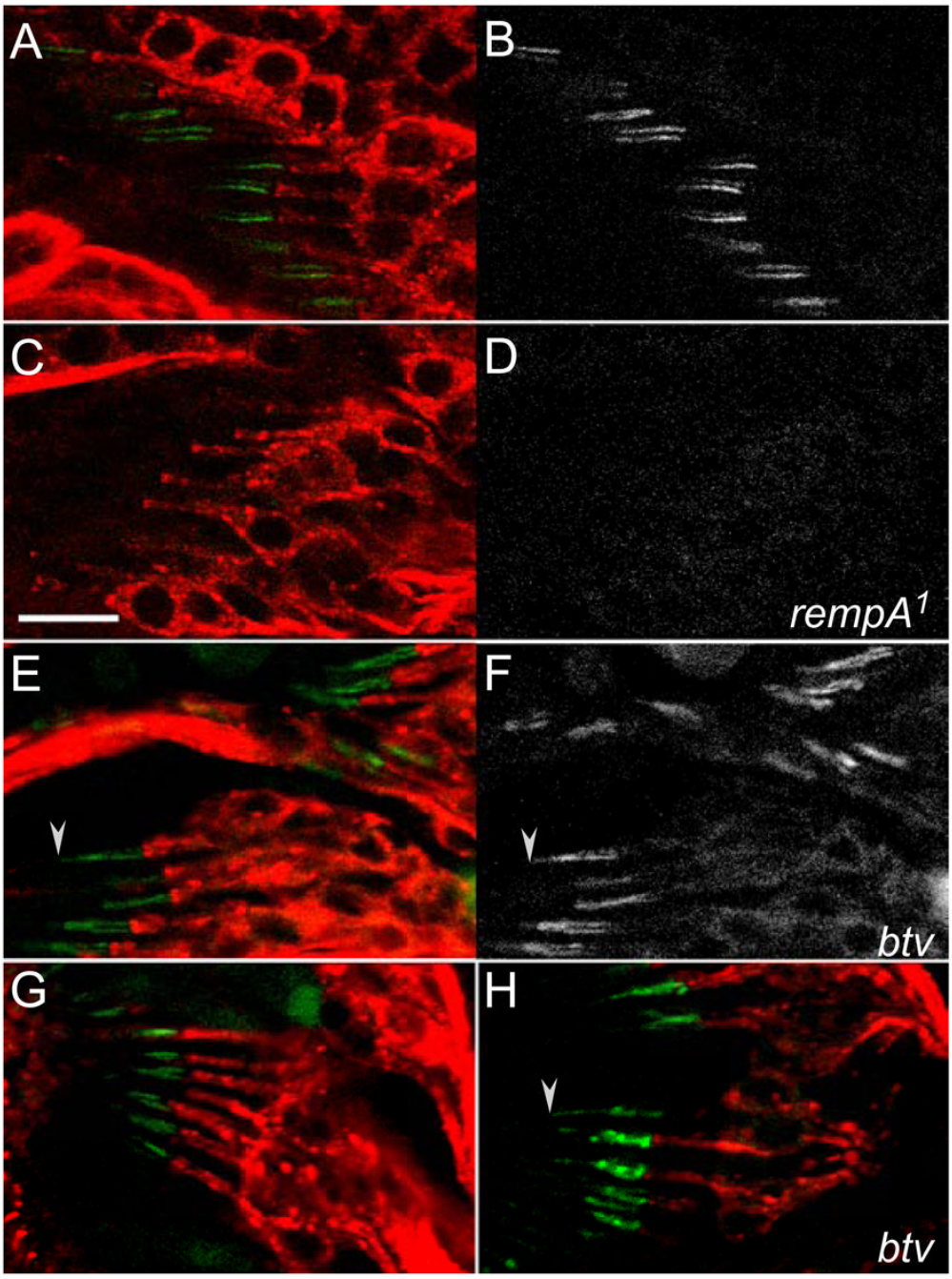

Figure 6. The channel subunit IAV is not expressed in *rempA* mutants, and is delocalized in *btv* mutants

Scolopidia in femoral (A–F) and antennal (G, H) chordotonal organs, from pupae expressing the GFP-tagged IAV subunit (B, D, F), and counterstained with MAb 22C10 (A, C, E, G, H). A, B, G; wild type; C, D; *rempA*^{1} homozygotes; E–H, *btv*^{5P1} homozygotes. The IAV channel is normally restricted to the proximal part of chordotonal cilia, but is undetectable in the cilia of *rempA* mutants. In *btv* mutants, the channel is expressed and appears to extend further along the cilia, spreading into the zone distal to the ciliary dilation (arrowheads). Scalebar = 10μm; all panels at same scale.