Report

Current Biology

Loss of Centrobin Enables Daughter Centrioles to Form Sensory Cilia in *Drosophila*

Highlights

- Basal body fate is restricted to mother centrioles in *Drosophila* sensory neurons
- Depletion of Centrobin enables daughter centrioles to form sensory cilia
- Ectopic localization of Centrobin in mother centrioles inhibits cilia formation

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In Brief

Gottardo et al. show that, despite the lack of centriole maturity traits, basal body fate is reserved to mother centrioles in *Drosophila* sensory neurons. Moreover, depletion of the daughter centriole protein Centrobin enables daughter centrioles to form cilia, whereas ectopic localization of Centrobin in mother centrioles inhibits cilia formation.

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Loss of Centrobin Enables Daughter Centrioles to Form Sensory Cilia in *Drosophila*

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SUMMARY

Sensory cilia are organelles that convey information to the cell from the extracellular environment. In vertebrates, ciliary dysfunction results in ciliopathies that in humans comprise a wide spectrum of developmental disorders [1–3]. In *Drosophila*, sensory cilia are found only in the neurons of type I sensory organs, but ciliary dysfunction also has dramatic consequences in this organism because it impairs the mechanosensory properties of bristles and chaetae and leads to uncoordination, a crippling condition that causes lethality shortly after eclosion [4–7]. The cilium is defined by the ciliary membrane, a protrusion of the cell membrane that envelops the core structure known as the axoneme, a microtubule array that extends along the cilium from the basal body. In vertebrates, basal body function requires centriolar distal and subdistal appendages and satellites. Because these structures are acquired through centriole maturation, only mother centrioles can serve as basal bodies. Here, we show that although centriole maturity traits are lacking in *Drosophila*, basal body fate is reserved to mother centrioles in *Drosophila* type I neurons. Moreover, we show that depletion of the daughter-centriole-specific protein Centrobin (CNB) enables daughter centrioles to dock on the cell membrane and to template an ectopic axoneme that, although structurally defective, protrudes out of the cell and is enveloped by a ciliary membrane. Conversely, basal body capability is inhibited in mother centrioles modified to carry CNB. These results reveal the crucial role of CNB in regulating basal body function in *Drosophila* ciliated sensory organs.

RESULTS

Basal Body Fate Is Reserved to Mother Centrioles in *Drosophila* Type I Neurons

Mother centrioles in *Drosophila* present neither appendages—distal or subdistal—nor satellites, and, therefore, as far as these ultrastructural features are concerned, they are indistinguishable from daughter centrioles [8, 9]. This lack of ultrastructural dimorphism opens the question of whether in *Drosophila* either of the two centrioles of a diplosome can serve as basal body or whether, on the contrary, basal body fate is also centriole age dependent, as it is often assumed [10, 11].

We have addressed this question by examining pancentriolar, daughter-centriole-specific, and transition zone reporters in the sensory neurons of both chordotonal auditory and external olfactory sensilla. Each neuron within these sensilla presents two tandemly arranged centrioles located at the apical tip of the sensory dendrite. The most distal of each centriole pair serves as basal body from which the axoneme projects (Figure 1A) [12].

Upon coexpression of PACT-RFP and YFP-CNB (Centrobin), in agreement with previous reports [11], we found a strong PACT-RFP fluorescence signal on the distal centrioles (Figure 1B, red, arrowhead). Moreover, we also detected a weak PACT-RFP proximal signal (Figure 1B, red, arrow) that colocalizes with YFP-CNB (green). These results are consistent with published data on *Drosophila* syncytial embryos and larval neuroblasts (NBs) where PACT is found in both centrioles but is more abundant on the mother, while YFP-CNB labels only the daughter centriole [11, 13–15]. To further determine the identity of the proximal and distal centrioles in *Drosophila* sensory neurons, we performed double fluorescence labeling of CNN (Centrosomin)/CNB, ANA1/CNB, CBY (Chibby)/PACT, and CNB/CBY (Figures 1C and S1). We found that CNB, ANA1, and the weak PACT signal colocalize in the proximal centriole; ANA1, CNN, and the strong PACT signal colocalize on the distal; and the transition zone marker CBY is further distal to both centrioles (Figures 1C and S1, arrow, arrowhead, and empty arrowhead, respectively). These observations are fully consistent with the hypothesis that, despite the lack of centriole maturation features, basal body fate is reserved to mother centrioles in *Drosophila* auditory and olfactory neurons.

Ectopic Localization of CNB in Mother Centrioles Inhibits Cilia Formation in *Drosophila* Type I Neurons

Ultrastructural centriole dimorphism is also lacking in *Drosophila* NBs where mother and daughter centrioles, which disengage from each other soon after mitosis, are also functionally unequal [16–19]. NBs maintain a potent microtubule organizing center (MTOC) that is localized near the presumptive apical cortex throughout interphase and is organized by the daughter centriole alone. The mother centriole organizes little, if any, pericentriolar

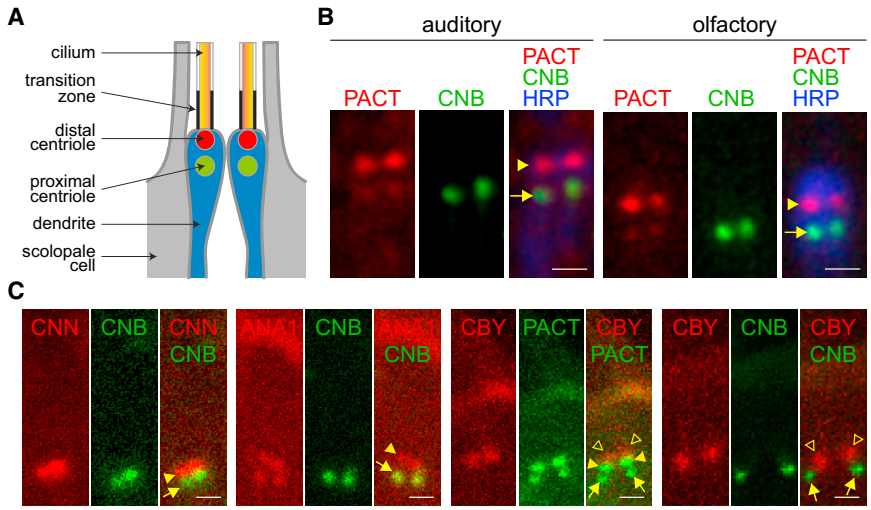

Figure 1. Axonemes Assemble from Mother Centrioles in *Drosophila* Olfactory and Auditory Neurons

(A) Graphical summary of the arrangement of centrioles and cilia in sensilla with two neurons (blue). Axonemes (orange) project from the distal centrioles (red) that serve as basal bodies. Transition zones are shown as thick black lines. Proximal centrioles are marked in green. Accessory non-neural cells within the sensilla are depicted in gray.

(B) Detail of the apical ends of the dendrites of individual auditory and olfactory sensilla, each containing two neurons. PACT-RFP (red) is pan-centriolar but significantly more abundant on the distal (arrowhead) than on the proximal (arrow) centriole. The daughter centriole marker YFP-CNB (green) is prominent on proximal centrioles and undetectable on distal centrioles. Anti-horseradish peroxidase (HRP) (blue) marks dendrite and cilium.

(C) Apical ends of pairs of olfactory neurons showing fluorescence signals corresponding to YFP-CNB/RFP-CNN, YFP-CNB/ANA1-tdTomato, GFP-PACT/CBY-Tomato, and YFP-CNB/CBY-Tomato. ANA1 and PACT mark both the proximal (arrow) and distal (arrowhead) centrioles, while CNB and CNN are specifically located at the proximal and distal centrioles, respectively; CBY (empty arrowhead) localizes distally to all these markers.

Scale bars represent 1 μm. See also Figure S1.

material and has essentially no MTOC activity during interphase. The daughter centriole protein CNB appears to be necessary and sufficient to trigger centriole asymmetry in NBs because CNB depletion impedes daughter centrioles to assemble a functional MTOC, and mother centrioles carrying ectopic CNB become active MTOCs [20]. Polo, CNN, Pericentrin-Like Protein (PLP), Partner of Inscuteable (PINS), and BLD10/CEP135 are also known to be essential for such a mother-daughter centriole asymmetry in NBs [11, 18, 20–23].

Because CNB localization exerts such a determining role in establishing functional differences between mother and daughter centrioles in *Drosophila* NBs, we tested whether CNB could play a similar role in centriole function in sensory neurons. To this end, we first examined the effect of localizing CNB ectopically on mother centrioles. Transverse sections through the cilia of wild-type olfactory and auditory sensilla containing three and two sensory neurons, respectively, show the corresponding number of axonemes (Figures 2A, 2B, and S2A). In contrast, axonemes were missing in most (23 out of 24) olfactory and (25 out of 27) auditory sensilla expressing YFP-CNB-PACT, henceforth referred to as CNB-PACT (Figures 2C, 2D, and S2B). Unlike in wild-type sensilla where electron microscopy (EM) sections through centrioles are relatively easy to obtain following the axonemes, it is very difficult to identify centrioles in non-ciliated CNB-PACT sensilla. We have, however, identified two centrioles in two different CNB-PACT-expressing auditory neurons and one in an olfactory neuron, strongly suggesting that failed ciliogenesis in these cells is not due to the lack of centrioles. Indeed, expression of CNB-PACT does not affect centriole number in other cell lineages in *Drosophila* [20]. Unlike wild-type basal bodies, which are made of doublets (Figures 2E and 2F), the only two centrioles for which we have obtained transverse EM section in CNB-PACT-expressing neurons (one olfactory and one auditory) presented singlets or incomplete doublets (Figures 2G and 2H, respectively), as is often the case in wild-type proximal centrioles. Interestingly, the centriole from the CNB-PACT olfactory neuron was found close to the nucleus (Figure 2I, arrow), many centriole diameters away from the tip of the dendrite where centrioles are located in wild-type cells. This result strongly suggests that upon CNB-PACT expression, centrioles may become scattered over the cell body, as they are in *Plp* mutant auditory neurons [11], thus rendering their identification extremely difficult. No rootlets were found in CNB-PACT-expressing neurons.

Consistent with the absence of cilia, we found that CNB-PACT-expressing flies are uncoordinated, cannot fly, get stuck to the food, and die shortly after eclosion. On the contrary, control flies expressing either YFP-PACT or YFP-CNB hatch, feed, and mate normally, showing that overexpression of PACT (without CNB) or overexpression of CNB (without PACT) does not produce the phenotypic traits brought about by expression of YFP-CNB-PACT.

These results strongly suggest that ectopic CNB localization impedes mother centrioles to function as basal bodies in *Drosophila* type I sensory neurons.

Loss of CNB Enables Daughter Centrioles to Function as Basal Bodies in *Drosophila* Type I Neurons

We then investigated cilia formation in sensilla depleted of CNB. As reported previously [12, 24], wild-type olfactory (Figures 3A–3E) and auditory (Figure S3A) neurons present a pair of tandemly arranged centrioles, one distal (d) that serves as basal body from which the cilium (c) is assembled and one proximal (p). Rootlets (r) that emerge from the distal centriole enclose the proximal centriole and extend further proximally. Ciliary rootlets differ in different types of sensilla [12, 25]. In olfactory neurons (Figure 3A), the rootlet bundle is thinner than that of chordotonal auditory sensilla (Figure S3A), which shows a characteristic periodic cross-striation [12, 26–29].

We have found that CNB depletion causes a major disruption of the mono-axial arrangement of cilium and centrioles observed

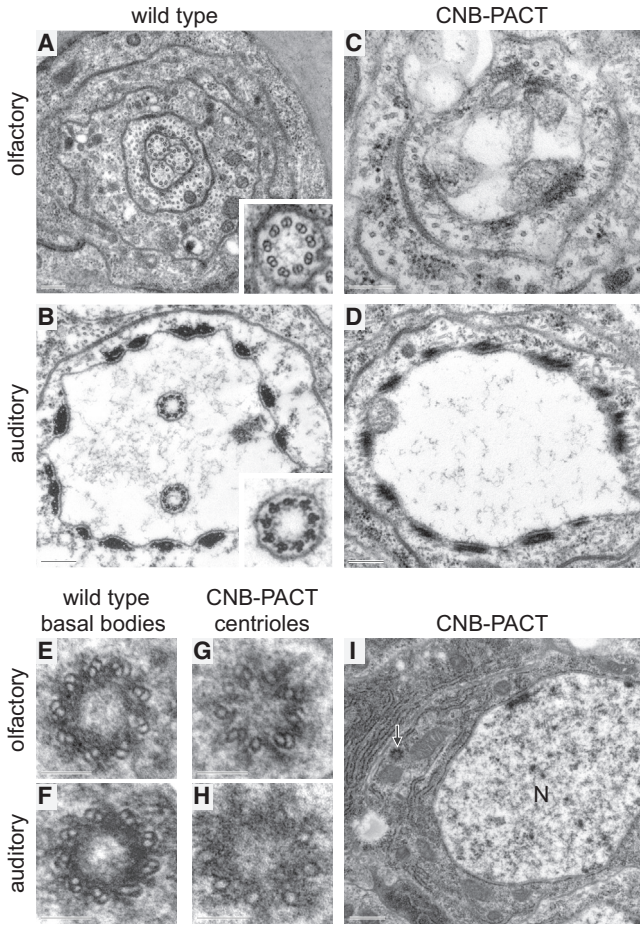

Figure 2. Pancentriolar CNB Localization Inhibits Axoneme Assembly in Type I Neurons

(A and B) Transverse sections through olfactory (A) and auditory (B) wild-type sensilla containing three and two neurons, respectively. Each neuron protrudes a cilium that contains a fully assembled axoneme (insets).

(C and D) Axonemes are missing in olfactory and auditory sensilla expressing the CNB-PACT fusion protein, which drives CNB to both mother and daughter centrioles.

(E and F) Basal bodies from wild-type olfactory (E) and auditory (F) sensilla contain doublets and a few triplets.

(G and H) Centrioles from olfactory (G) and auditory (H) neurons expressing CNB-PACT are mostly made of singlets.

(I) Low-magnification view of the section containing the centriole shown in (G) (arrow), which is located close to the nucleus (N), away from the apical dendrite.

Scale bars of (A)–(D) represent 250 nm; scale bars of (E)–(H) represent 100 nm; and the scale bar of (I) represents 500 nm. See also Figure S2.

in wild-type sensory neurons. Upon expression of RNAi-*Cnb*, most olfactory (13 out of 15; Figures 3F–3J) and auditory (11 out of 15; Figures S3B, S3C, and S2C) neurons are biciliated (c1 and c2) and present two centrioles that are arranged in parallel, not in tandem, both serving as basal bodies (d1 and d2) from which axonemes assemble, and centrioles proximal to the basal bodies are lacking. The same result was observed in olfactory neurons that carry the hypomorph *Cnb* mutant allele *PBac{RB}Cnb*^{e00267} over *Def(3L)ED4284*, which uncovers *Cnb* (Figure S3G).

In all 14 cells for which we have transverse section (8 olfactory; 6 auditory), we found that one of the two cilia present in RNAi-*Cnb*-expressing sensory neurons was shorter than normal and had defects in the 9-fold symmetry of the axoneme, with doublets that were incomplete or missing (Figure 3G, c2; Figure S3C, c2; Figures S3D and S3E; Figure S3G, c2). The two basal bodies, however, presented a rather normal ultrastructure (Figure 3H, d1 and d2; Figure S3F). Judged by both the abundance of doublets rather than singlets and the electron-dense stripes between doublets that could be observed at their distal ends, which are thought to be the *Drosophila* equivalent of Y linkers, the two basal bodies of CNB-depleted cells present the appearance of a wild-type distal centriole. Consistently, PACT is equally distributed on each centriole in *PBac{RB}Cnb*^{e00267}/*Def(3L)ED4284* mutant olfactory neurons (Figures S3H and S3I). However, the two basal bodies can still be told apart by the distribution of ciliary rootlets that were always (n = 12; 7 olfactory and 5 auditory) attached to only one of the two basal bodies (Figure 3F, d1; Figure S3B, d1).

Neither flies expressing RNAi-*Cnb* nor *PBac{RB}Cnb*^{e00267}/*Def(3L)ED4284* mutant flies are uncoordinated, and their performance in negative geotaxis tests (climbing assays) is not significantly different from that of wild-type strains (data not shown).

The presence of a pair of cilia and basal bodies and the corresponding lack of proximal centrioles strongly suggest that CNB depletion enables daughter centrioles to function as basal bodies in *Drosophila* type I neurons.

DISCUSSION

A graphical summary of our findings and working hypothesis on the role of CNB in ciliogenesis in *Drosophila* type I sensory neurons is shown in Figure 4. In wild-type sensory neurons (Figure 4A), axoneme (a), basal body (d), from which rootlets (r) emanate, and proximal centriole (p), where CNB localizes, are arranged along one axis. This mono-axial arrangement is critically dependent on normal CNB function. CNB ectopic localization on mother centrioles eliminates this axis altogether; axonemes do not grow, and centrioles are not tandemly arranged (Figure 4B). Likely, these cells contain two centrioles, as it has been shown to be the case in other *Drosophila* cell lineages upon expression of the CNB-PACT fusion protein [20]. In turn, CNB depletion (Figure 4C) brings about a biaxial arrangement made of two axonemes (a1 and a2) with their corresponding basal bodies (d1 and d2), which are not associated to proximal centrioles. Only one of the two basal bodies observed in these cells presents rootlets (r). Therefore, CNB seems to be required for both keeping mother-daughter centriole alignment and preventing daughter centriole docking on the cell membrane. We do not know whether these two functions operate independently or depend on each other.

Often, one of the two cilia of CNB-depleted cells is shorter than normal and has defects in the axoneme's 9-fold symmetry and in the ciliary membrane. Such defects may reflect yet unknown roles of CNB in ciliogenesis. Alternatively, they may arise from the dilution of one or more critical ciliogenesis factors whose concentration in normally monociliated cells like type I sensory neurons may be rate limiting for the assembly of only one fully formed cilium. A dilution phenotype that affects ciliary function

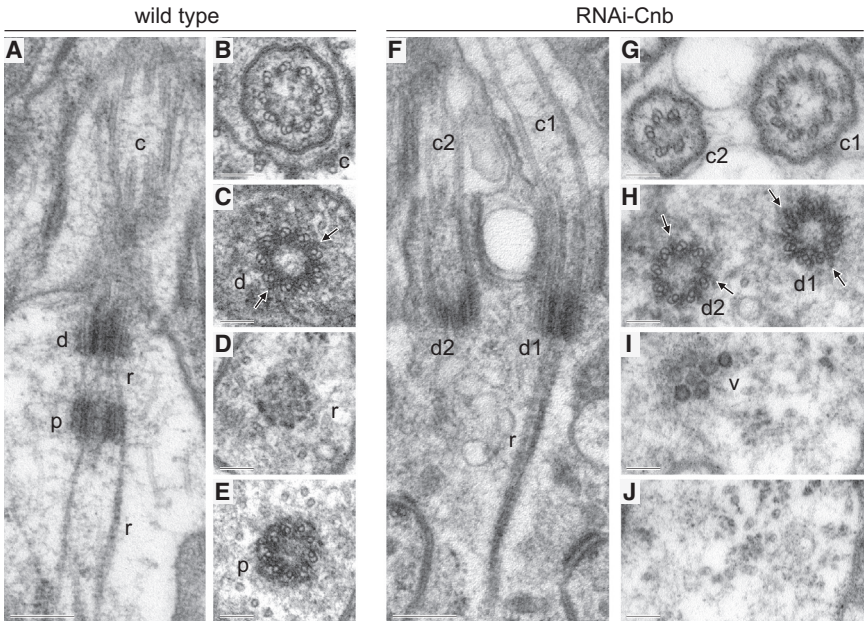

Figure 3. CNB Depletion Results in Biciliated Type I Olfactory Neurons

(A–E) Wild-type. Longitudinal (A) and serial transverse sections from distal to proximal (B–E) reveal the mono-axial arrangement of cilium (c), basal body (distal centriole, d), and proximal centriole (p). Centriolar rootlets (r) originate in the basal body, envelop the proximal centriole, and extend further proximally. Electron-dense stripes corresponding to putative Y linkers (C, arrows) are visible in between the doublets of the basal body. (F–J) Olfactory neurons depleted for CNB by expression of RNAi-*Cnb*. Longitudinal (F) and serial transverse sections from distal to proximal (G–J) reveal two cilia (c1 and c2), two basal bodies (d1 and d2), and the lack of proximal centrioles. Centriolar rootlets (r) originate from only one of the two basal bodies (d1). Cilium c2 presents a defective axoneme (G). Both basal bodies bear doublets (H) and electron-dense stripes similar to those observed in wild-type basal bodies (H, arrows). Dense vesicle-like bodies (v), can be observed proximal to d2 (I). Identical structures are found proximal to d1 and to centrioles in wild-type olfactory neurons (data not shown).

Scale bars of (A) and (F) represent 250 nm; scale bars of (B)–(E) and (G)–(J) represent 100 nm. See also Figure S3.

has been reported in IMCD-3 cells and in cells mutant for the tuberous sclerosis gene *TSC2* that carry supernumerary centrioles. When these cells form more than one cilium, ciliary concentration of Smoothened in response to Sonic hedgehog (Shh) stimulation is reduced [30]. Nonetheless, despite the presence of malformed cilia present in CNB-depleted *Drosophila* type I sensory neurons, the fully formed cilia that are also present seem to be capable of sustaining normal ciliary function because adult flies bearing CNB-depleted sensilla perform just like wild-type flies in climbing assays.

It is remarkable that although centriolar functions in sensory neurons and NBs are notably different, in both cell types, daughter centrioles acquire mother centriole traits upon CNB depletion, and mother centrioles become daughter centriole like upon CNB binding [20]. Something similar applies to PLP. In NBs and in type I sensory neurons, PLP is enriched on the mother centriole ([11, 21] and our own results). Upon loss of PLP function, mother centrioles behave like daughters in NBs [21], and cilia are lacking in sensory neurons, which indeed causes uncoordination in adult flies [31]. However, despite these tantalizing similarities, the molecular pathways that make centrioles functionally unequal in NBs and in type I sensory neurons present conspicuous differences. For instance, in NBs, where CNB is essential to recruit CNN [20], CNN specifically localizes on the daughter centrosome. In sensory neurons, on the contrary, CNB and CNN do not colocalize: CNN is associated with the basal body (Figures 1C and S1; [32]), while CNB marks the proximal centriole. Moreover, in *Bld10* mutant NBs, mother centrioles recruit CNN [23], which is a critical daughter centriole trait in wild-type cells, but *Bld10* mutant adults display no obvious sign of uncoordination [22].

Biciliated cells have been described in nature in fish [33], frogs [34], rodents [35–37], and primates [38]. In rodents and primates, biciliated cells account for more than 80% of the cells lining the central canal epithelium in the adult brain and are highly proliferative. Notably, these biciliated cells present two basal bodies that are not associated with daughter centrioles [37, 38]. This observation strongly suggests that biciliation in these cells does not result from an additional round of centriole duplication but from centriole splitting and maturation of the daughter centriole as basal body [37, 38]. The same applies to biciliated cells experimentally induced in *Xenopus* embryos by high Foxj1 misexpression [34] or in the MCF10A cell line following an unscheduled pulse of PLK1 activity [39]; in both cases, daughter centrioles precociously mature, and ultrastructural, normally age-dependent differences between centrioles are erased.

Our findings demonstrate that in *Drosophila* ciliated sensory organs, both internal and external, CNB localization plays a key role in the regulation of centriole conversion into basal bodies.

EXPERIMENTAL PROCEDURES

Details on experimental procedures are described in Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and three figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.07.038.

AUTHORS CONTRIBUTIONS

M.G., G.P., S.L., J.R., M.G.R., G.C., and C.G. conceived and designed the experiments and analyzed the data. S.L. and J.R. generated transgenic fly strains. S.L. and G.P. performed immunofluorescence microscopy. M.G., M.G.R., and G.C. performed electron microscopy. G.P. and C.G. wrote the paper.

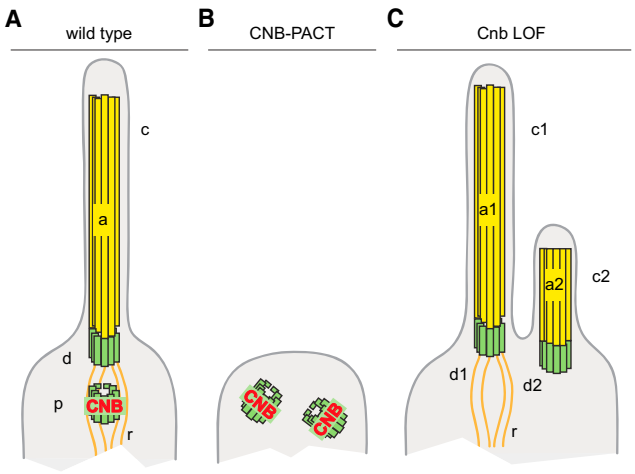

Figure 4. Graphical Summary of the Role of CNB Function in Cilium Assembly in *Drosophila* Type I Olfactory and Auditory Neurons

(A) In wild-type sensory neurons, axoneme (a), basal body (distal centriole, d), and proximal centriole (p) are arranged along one axis. Ciliary rootlets (r) that emanate from the basal body extend proximally. CNB localizes only on the proximal centriole.

(B) Pancentriolar CNB localization driven by the expression of a CNB-PACT fusion inhibits axoneme growth. The presence of two centrioles, like the absence of rootlets, in CNB-PACT cells is consistent with current data but still hypothetical.

(C) Depletion of CNB results in biciliated cells (c1, c2) containing two axonemes (a1, a2) with their corresponding basal bodies (d1, d2) and no proximal centrioles. In most cases, one of the two cilia is shorter (c2) and may display ultrastructural abnormalities. Ciliary rootlets are associated only to the basal body of the cilia that presents normal morphology.

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