**Report**

**An acentriolar centrosome at the *C. elegans* ciliary base**

**Graphical abstract**

- PCM assembly at ciliary base
- **1. DAF-19-dependent expression of SPD-5**
- **2. Self-assembly of SPD-5 directed to ciliary base by PCMD-1**
- **3. Recruitment of client proteins (γ-tubulin,..)**
- **4. PCM maintenance via feedback loop involving PCMD-1 and SPD-5**

**Highlights**

- PCM persists at the acentriolar ciliary base in *C. elegans*
- PCM assembles in a SPD-2, AIR-1 and PLK-1-independent manner
- PCMD-1 tethers PCM at the ciliary base in the absence of centrioles
- PCM is required for neuronal morphogenesis and cillum assembly

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**In brief**

Centrioles play a critical role in mitotic centrosome assembly. Here, Garbrecht et al. show that pericentriolar material not only persists but continues to assemble in *C. elegans* ciliated sensory neurons in the absence of centrioles and known mitotic regulators, suggesting different mechanisms for mitotic and non-mitotic centrosome assembly.
An acentriolar centrosome at the *C. elegans* ciliary base

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SUMMARY

In animal cells, the functions of the microtubule cytoskeleton are coordinated by centriole-based centrosomes via $\gamma$-tubulin complexes embedded in the pericentriolar material or PCM.1 PCM assembly has been best studied in the context of mitosis, where centriolar SPD-2 recruits PLK-1, which in turn phosphorylates key scaffolding components like SPD-5 and CNN to promote expansion of the PCM polymer.2–4 To what extent these mechanisms apply to centrosomes in interphase or in differentiated cells remains unclear.5 Here, we examine a novel type of centrosome found at the ciliary base of *C. elegans* sensory neurons, which we show plays important roles in neuronal morphogenesis, cellular trafficking, and ciliogenesis. These centrosomes display similar dynamic behavior to canonical, mitotic centrosomes, with a stable PCM scaffold and dynamically localized client proteins. Unusually, however, they are not organized by centrioles, which degenerate early in terminal differentiation.6 Yet, PCM not only persists but continues to grow with key scaffolding proteins including SPD-5 expressed under control of the RFX transcription factor DAF-19. This assembly occurs in the absence of the mitotic regulators SPD-2, AIR-1 and PLK-1, but requires tethering by PCMD-1, a protein which also plays a role in the initial, interphase recruitment of PCM in early embryos.7 These results argue for distinct mechanisms for mitotic and non-mitotic PCM assembly, with only the former requiring PLK-1 phosphorylation to drive rapid expansion of the scaffold polymer.

RESULTS AND DISCUSSION

The nematode *C. elegans* represents one of the best understood experimental models for mitotic centrosome assembly. Central to PCM assembly is the coiled coil protein SPD-5, which forms a polymeric scaffold around centrioles (Figure 1A).4,8,9 PCM “client” proteins including the nucleator $\gamma$-tubulin, microtubule regulators such as TAC-1 and ZYG-9, as well as tubulin itself either dynamically concentrate within the PCM or stably bind to the polymer lattice.9,10 SPD-5 spontaneously self-assembles in vitro, a process potentiated by SPD-2 and the polo-like kinase PLK-1.11 In vivo, this process is directed to occur around centrioles, with centriole-localized SPD-2 recruiting both PLK-1 and its activator Aurora A/AIR-1.2 Laser ablation experiments have shown that centrioles are essential for mitotic PCM growth, although existing PCM polymer can be maintained by PLK-1 acting within the PCM to counter the destabilizing effect of phosphatases like PP2A.2,11 Centrioles are further essential for mitotic PCM structural integrity, potentially by acting as anchoring sites for tethering proteins such as PCMD-1, a putative ortholog of Pericentrin.2,7

An acentriolar PCM at the *C. elegans* ciliary base

*C. elegans* is well known for its stereotypical pattern of development, with precisely 959 somatic cells in the adult hermaphrodite.12 Less widely appreciated is the fact that centrioles and centrosomes appear to be lost in most of those cells, potentially reflecting their lack of further proliferative potential.13 This is also true for ciliated sensory neurons, in which centrioles degenerate soon after terminal cell division.5,14 Centriolar structural proteins including SAS-4, SAS-5 and SAS-6 are likewise lost.6 Yet, surprisingly, $\gamma$-tubulin has been shown to persist at the ciliary base in adult worms.15,16 This accumulation could simply be a consequence of $\gamma$-tubulin capping the minus ends of ciliary microtubules.17 However, further examination showed that $\gamma$-tubulin is not alone. Endogenously GFP-tagged SPD-5 similarly forms discrete foci in the amphid and phasmid sensory organs in the head and tail of the animal, respectively, which are the subject of most studies in *C. elegans* (Figure 1B,18). SPD-5 foci are also observed in cephalic and labial neurons, as well as in the sensory neurons of the male tail (Figure S1A). These foci, which are similar in size to interphase centrosomes in the early embryo, are all the more prominent given the near absence of SPD-5 signal in other somatic tissues of the adult worm. Closer inspection shows SPD-5 accumulation to be in the region of SPD-5 signal in other somatic tissues of the adult worm.

An acentriolar PCM at the *C. elegans* ciliary base

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**Report**

**A** Mitotic centrosome in early embryo

1. Initial PCM recruitment (Centrioles, PCMD-1)
2. SPD-5 scaffold expansion (SPD-2, PLK-1, AIR-1)
3. Recruitment of client proteins (TBG-1,..)
4. PCM maintenance (PLK-1)

**B** Amphid neurons

- **Merge** GFP:SPD-5
- **Merge** mCh:SAS-4

**Phasmid neurons**

**Early embryo**

- DNA
- Centrosome

**C**

- **GFP:SPD-5**
- **mCh:HYLS-1**
- **Merge**

**D** Centrosome at ciliary base

- **Cilium**
- **IFT**
- **Transition zone** (MKS-6)
- **Centriole remnant** (HYLS-1)
- **PCM**

**E**

- **GFP:SPD-5**
- **GFP:PCMD-1**
- **TBG-1:GFP**
- **TagRFP:Glp-1**
- **GIP-2:GFP**
- **mNG:MZT-1**
- **GFP:TAC-1**
- **mNG:ZYG-9**
- **NOCA-1:GFP**
- **PTRN-1:GFP**
- **PLK-1:GFP**
- **GFP:AIR-1**
- **SPD-2:GFP**

**G**

- **GFP:TBB-2**
- **GFP:TBB-2; CHE-11:mKate2**

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degradation of SAS-4 using a GFP nanobody-directed ZIF-1 degron expressed under a heat shock-inducible promoter (Phsp-70). When activated early in embryogenesis, this degron was capable of targeting both pre-existing and newly forming centrioles, resulting in highly penetrant embryonic lethality. Yet, when activated at the L1 larval stage, it did not affect the continued presence of SPD-5, nor ciliary structural integrity (Figures S1B and S1C). Thus, a type of PCM remains following cilia degeneration in the late-stage embryo and persists throughout the lifetime of the animal.

Besides SPD-5 and γ-tubulin (TBG-1 in C. elegans), the PCM in the early embryo is characterized by the presence of numerous client proteins that regulate microtubule nucleation, stability and/or organization. These include other members of the γ-tubulin complex, the GCP-2/3 homologs GIP-1 and GIP-2 and Mozart/MZT-1,21,22 and the microtubule polymerizing complex of TACC/TAC-1 and ch-TOG/ZYG-9.23 All of these proteins are also found in ciliated neurons (Figure 1E), consistent with the reported presence of a microtubule-organizing center at the dendritic tip,16 a finding we could confirm in our own hands (Figures 1F and 1G). Consistent with previous reports,7 we could also detect PCMD-1 at the ciliary base, co-localizing perfectly with SPD-5 (Figure S1D). Also present are the microtubule anchoring protein Ninein/NOCA-1 and the minus end stabilizer CAMSAP/PTRN-1, which in C. elegans are not found at the mitotic centrosome in the early embryo but have been reported to localize to non-centrosomal microtubule organizing centers at other developmental stages (Figures S1E and S1F).20,22 Another striking difference to mitotic centrosomes is the complete absence of the regulators SPD-2, AIR-1 and PLK-1,2,4,24–26 none of which could be detected at the ciliary base in contrast to their prominent localization to centrioles and/or the PCM in early embryos (Figures 1E and S1G). There are then several critical differences between the neuronal PCM and the canonical, mitotic PCM that has been the subject of most studies so far.

**SPD-5 is specifically expressed in ciliated neurons under control of the RFX transcription factor DAF-19**

Unlike other microtubule-organizing centers in the adult worm the acentriolar centrosome in ciliated neurons derives from a canonical, centriole-organized centrosome present at the initiation of ciliogenesis in the late-stage embryo.6 A potential explanation for the persistence of PCM is that there is no counteracting phosphatase driving polymer disassembly, as there is during mitosis.21,22 The PCM at the ciliary base could then simply be a remnant left over from the terminal cell division, surviving loss of its core organizing structure, the centriole, and the kinase that drove its assembly, PLK-1. However, further analysis showed this is not the case. Thus, our initial attempts to deplete neuronal SPD-5 using the GFP nanobody-directed ZIF-1 degron expressed under a Pdyf-7 promoter active early during neuronal differentiation were unsuccessful despite the same degron efficiently targeting other proteins25 (Figures 2A and 2B). Switching to a heat shock-inducible promoter revealed the reason why: GFP:SPD-5 signal was initially depleted but eventually recovered even after repeated cycles of degradation (Figures 2C and S2A). SPD-5 therefore continues to be expressed in ciliated neurons. Expression of ciliary genes in the worm is regulated by the RFX transcription factor DAF-19, which binds to X-box elements in the promoter of target genes.28 A closer inspection of the spd-5 promoter revealed a predicted X-box near the transcription start site (Figure 2D). Consistent with SPD-5 being under the control of DAF-19, daf-19 mutant animals displayed reduced levels of SPD-5 in sensory neurons (Figure S2B). However, loss of this master regulator of ciliogenesis and neuronal morphogenesis could perturb SPD-5 localization indirectly, via perturbing the organization of the dendritic tip. To test DAF-19 dependency more directly, we mutated the X-box element in the spd-5 promoter. This did not impair SPD-5 levels in the early embryo. On the contrary, levels were significantly increased, potentially indicating binding of a transcriptional repressor prior to DAF-19 expression. Embryonic viability was also unaffected (100%, n = 545 embryos versus parental GFP:SPD-5 strain 99.9%, n = 709). However, SPD-5 signal in ciliated neurons was strongly reduced (Figures 2E and 2F) and also no longer extended into the transition zone (Figure S2D). When combined with degron-mediated degradation targeting protein inherited from previous mitotic divisions, we were able to efficiently deplete SPD-5 using GFP nanobody-directed ZIF-1 expressed under either tissue-specific or inducible promoters (Figures 2G, 2H, and S2E). Thus, SPD-5 is continuously expressed in ciliated neurons and incorporates into an acentriolar PCM in the absence of DAF-19 expression.
Figure 2. SPD-5 is continuously expressed in ciliated neurons under control of the RFX transcription factor DAF-19

(A) Schematic of GFP nanobody-directed ZIF-1 degron system, targeting endogenously GFP-tagged proteins for ubiquitin-mediated degradation. Depending on the promoter used to drive ZIF-1 expression, protein degradation may be tissue-specific (Pdyf-7 ciliated neurons from comma stage, Posm-6 from 3-fold stage) or inducible (Phsp-16.41, heat shock-inducible).

(B) Pdyf-7-promoter-driven ZIF-mediated degradation fails to result in appreciable decrease in ciliary SPD-5 signal.

(C) Phsp-16.41 heat shock promoter-driven ZIF-mediated degradation transiently depletes ciliary SPD-5 signal. However, signal recovers within 18 h due to new protein expression.

(D) Analysis of spd-5 promoter reveals sequence motif 181bp upstream of ATG closely matching the consensus for DAF-19-regulated X-box genes (GTHNYY AT RRNAAC).

(E) Mutation of X-box motif results in a significant decrease in SPD-5 signal at the ciliary base.

(F) Quantitation of SPD-5 signal based on images as in (E). n = 14 animals control, 10 ΔX-box.

(G) Combining X-box mutation with heat shock promoter-driven ZIF-mediated degradation results in a permanent loss of ciliary SPD-5.

(H) Quantitation of heat shock degradation experiments shown in (C) and (G). Pre-heat shock signal independently normalized to 1 for wild-type and ΔX-box promoter strains. n = 13–17 animals wild-type, 6–15 ΔX-box.

Scale bars, 10 μm. Error bars, SD. *** t test, p < 0.001, n.s., not significant. See also Figure S2.
Figure 3. The centrosome at the ciliary base contributes to neuronal morphogenesis and cilium assembly

(A and B) Fluorescence recovery after photobleaching (FRAP) analysis for PCM proteins at the ciliary base of phasmid neurons. Images (A) and quantitations (B) for FRAP experiments performed on strains expressing GFP:SPD-5 (n = 7 animals), GIP-2:GFP (n = 8) and GFP:TAC-1 (n = 7). As at mitotic centrosomes, SPD-5 forms a PCM scaffold to which the γ-tubulin complex protein GIP-2 is stably bound, while TAC-1 remains in dynamic exchange with the cytoplasmic pool.

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of detectable levels of the regulators of mitotic PCM assembly, SPD-2, AIR-1, and PLK-1.

SPD-5 at the ciliary base forms a stable scaffold for the recruitment of client proteins

In the context of the early embryo, SPD-5 forms a polymeric scaffold around centrioles that exhibits little or no exchange of subunits with the surrounding cytoplasm.4,12 Certain PCM client proteins, most notably the γ-tubulin complex, stably bind to the SPD-5 lattice and likewise exhibit little cytoplasmic exchange, while others such as TAC-1 and ZYG-9 are more dynamically localized.9,12 To examine the dynamic behavior of PCM proteins at the ciliary base we performed fluorescence recovery after photobleaching (FRAP) experiments on the phasmids, where a pair of cilia is found in isolation, bleaching one or both PCM foci and comparing the kinetics of recovery with that at mitotic centrosomes. As can be seen in Figures 3A and 3B, SPD-5 exhibited essentially no recovery within the time frame of the experiment. Neither did the γ-tubulin complex protein GIP-2. In contrast, TAC-1 signal recovered relatively rapidly. While we cannot exclude that recovery kinetics are affected by the geometry of the extended dendrite and the lack of a substantial cytoplasmic pool in differentiated neurons, these results are qualitatively and quantitatively similar to those obtained for canonical centrosomes in the early embryo (Figures S3A and S3B). This is perhaps not too surprising, given that the dynamic enrichment of certain client proteins within the SPD-5 scaffold has been reproduced with purified proteins in vitro,14 indicating that it is an intrinsic property of the scaffold and client proteins themselves. Nevertheless, it argues for a similar type of scaffold being present at the ciliary base and at canonical centrosomes.

At mitotic centrosomes, SPD-5 is absolutely essential for PCM assembly. Embryos depleted of SPD-5 by RNAi accumulate no detectable PCM around centrioles and spindle assembly completely fails.8 Testing SPD-5 function in ciliated neurons by photobleaching (FRAP) experiments on the phasmids, where a pair of cilia is found in isolation, bleaching one or both PCM foci and comparing the kinetics of recovery with that at mitotic centrosomes. As can be seen in Figures 3A and 3B, SPD-5 exhibited essentially no recovery within the time frame of the experiment. Neither did the γ-tubulin complex protein GIP-2. In contrast, TAC-1 signal recovered relatively rapidly. While we cannot exclude that recovery kinetics are affected by the geometry of the extended dendrite and the lack of a substantial cytoplasmic pool in differentiated neurons, these results are qualitatively and quantitatively similar to those obtained for canonical centrosomes in the early embryo (Figures S3A and S3B). This is perhaps not too surprising, given that the dynamic enrichment of certain client proteins within the SPD-5 scaffold has been reproduced with purified proteins in vitro,14 indicating that it is an intrinsic property of the scaffold and client proteins themselves. Nevertheless, it argues for a similar type of scaffold being present at the ciliary base and at canonical centrosomes.

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The centrosome at the ciliary base contributes to neuronal morphogenesis and cillum assembly

The continued expression of SPD-5 and maintenance of a centrosomal microtubule-organizing center specifically in ciliated neurons suggests that it plays an important role in their development and/or function. To examine centrosome function, we took advantage of the tissue-specific degradation system described earlier. Depletion of SPD-5 early but not late in neuronal differentiation resulted in pronounced defects in the dyefill assay, which monitors uptake of the lipophilic dye DiI by surface-exposed ciliated nerve endings31 (Figure S3D). Defects were rescued by expression of a non-degradable form of SPD-5, confirming specificity of the phenotype. Conceptually, a dyefill phenotype could arise from a failure of dendrite extension or cillum assembly, or indeed a failure to form the neuron in the first place. An examination of the IFT marker CHE-11 in the phasmid neurons of SPD-5-depleted animals (Figures 3E and 3F) showed that terminal cell division was largely unaffected. However, neuronal morphology was affected in a subset of animals, with collapsed dendrites indicating a failure of retrograde extension.32,33 Cilia were also frequently truncated or entirely missing. While the majority of phasmids displayed both fully elongated dendrites and cilia with IFT occurring at roughly normal rates (Figures S3E and S3F), in many of those neurons intracellular trafficking was clearly perturbed, with CHE-11 particles accumulating along the dendrites (Figures 3E and 3F). The functional consequences for ciliogenesis could also be observed at the ultrastructural level, with transmission electron micrographs revealing missing axonomes as well as axonomes with less than 9 or incomplete doublet microtubules (Figures 3G and S3G). Thus, loss of the neuronal centrosome perturbs both neuronal morphogenesis and the trafficking that sustains cillum assembly and function.

Distinct mechanisms of mitotic and non-mitotic PCM assembly

What is most remarkable about the neuronal centrosome is that it not only persists throughout the lifetime of the animal but new scaffold continues to assemble despite the absence of the key regulator PLK-1 and the degeneration of the centriole-derived basal body. In contrast, the mitotic PCM requires centrioles for further scaffold incorporation as well as mechanical stability,2 while PLK-1 phosphorylation is essential for SPD-5 polymer formation as well as maintenance.4 The C. elegans genome contains two further PLK1 homologs, PLK-2, which functions partially redundantly with PLK-1 in the germline and early embryo,2,34,35 and PLK-3, a putative pseudogene. We therefore considered the possibility that one of these paralogs sustains neuronal

\[(C \text{ and } D)\] Tissue-specific degradation of SPD-5 early (Pdyf-7) or late (Posm-6) in neuronal differentiation results in a loss of GIP-1 from the amphid ciliary base. Images (C) and quantifications (D) for strains expressing GFP:SPD-5 and TagRFP:GIP-1 alone (n = 18/19 animals) or ΔX-box GFP:SPD-5 and TagRFP:GIP-1 in combination with Pdyf-7 (n = 24/23) or Posm-6 (n = 28/22) promoter-driven GFP-nanobody degrons.

\[(E \text{ and } F)\] Range of defects observed using CHE-11:mKate2 IFT reporter following Pdyf-7 promoter-driven degradation of SPD-5 in phasmid neurons. Defects include an accumulation of IFT particles along dendrites (arrowhead) and abnormal neuronal morphology. Both phenotypes were accompanied by a failure of terminal cell division (n = 14 animals control, 33 Pdyf-7 degron).

\[(G)\] Transmission electron micrographs of amphid cilia of wild-type and Pdyf-7 degron SPD-5-depleted animals. Cross sectional views reveal missing cilia in the amphid channel (10 cilia extending into the channel in wild type), missing and incomplete doublet microtubules in the proximal segment of the axoneme and, rarely, disorganized ciliary transition zones. The centriolar remnant previously referred to as transitional fibers located below the transition zone is apparently unaffected. However, the periciliary membrane is frequently distended (arrowhead). Defects are quantitated in Figure S3G.

Scale bars, 2 μm (A), 10 μm (C, E), 500 nm (G, amphid channel), 200 nm (G, others). Error bars are 90% CI (B), SD (D). *** t test, p < 0.001. See also Figure S3.
Figure 4. Distinct mechanisms of mitotic and non-mitotic PCM assembly

(A and B) PLK-1 inhibition does not affect de novo recruitment of SPD-5 following heat shock promoter-driven ZIF-mediated degradation. Images (A) and quantitation (B) of amphid GFP:SPD-5 signal before and immediately after heat shock, as well as after 24 h of recovery in 40 μM BI2536 in M9, 2% DMSO in M9 (No (legend continued on next page)
PCM incorporation. However, we were unable to detect PLK-2 at the ciliary base (not shown) and co-deletion of both plc-2 and plc-3 did not appreciably affect neuronal SPD-5 localization (Figure S4A). Treatment of worms with the specific inhibitor BI2536 which is expected to inhibit all PLK1 homologs and results in mitotic PCM polymer disassembly in the early embryo further did not affect the ability of SPD-5 to reassemble at the ciliary base following heat shock-induced ZIF-1-mediated degradation (Figures 4A and 4B). PLK-1 phosphorylation sites on SPD-5 have previously been mapped, with phosphorylation on S653 and S658 accounting for the stimulating effect of PLK-1 on SPD-5 oligomerization in vitro and in vivo. Mutating these sites to alanine in the context of an RNAi-resistant endogenous promoter GFP transgene resulted in a failure of mitotic PCM expansion and significant embryonic lethality following depletion of the endogenous protein (Figure S4B, 0% viability, n = 207 embryos spd-5(RNAi); 99.7%, n = 289 embryos spd-5(RNAi) WT rescue, 58.6%, n = 186 embryos spd-5(RNAi) S653A S658A rescue). However, the same transgene was fully functional in re-forming a PCM at the ciliary base following shock-induced degradation of both it and endogenous, wild-type, SPD-5, the latter being prevented from neuronal expression by mutation of the X-box element in its promoter (Figures 4C and 4D). While we cannot exclude the possibility that another kinase drives SPD-5 oligomerization in ciliated neurons, the most straightforward explanation is that PCM assembly occurs via spontaneous self-assembly of SPD-5, as also observed in vitro. Could this also be the case elsewhere? While most attention in the C. elegans early embryo has been on the dramatic expansion of the PCM in mitosis, a small amount of SPD-5 is already present at sperm-derived centrioles soon after exit from meiosis II, contributing among other things to polarity establishment. PLK-2, PLK-1 and AIR-1 are already present at this stage. Yet, simultaneous inhibition of both Aurora A and PLK-1 with MLN8237 and BI2536 coupled with RNAi-mediated depletion of SPD-2 leave initial PCM recruitment largely unaffected (Figure 4E). Thus, in the absence of any external signals, PCM assembly mechanisms may be not so much between neuron and early embryo as between interphase/G0 and mitosis, with only the rapid expansion upon mitotic entry requiring PLK-1 (Figures S4C and S4D).

**PCMD-1 tethers SPD-5 to the ciliary base**

The lack of centrioles as a core organizing structure presents another question: what maintains SPD-5 at the ciliary base and gives the PCM its structural integrity? The answer appears to lie in the recently described ortholog of Pericentrin, PCMD-1, which plays a critical role in the initial recruitment of PCM to sperm-derived centrioles in the early embryo. Like Pericentrin, PCMD-1 localizes close to the centriole barrel in the early embryo, with weaker signal extending into the PCM. Unlike other centriolar proteins PCMD-1 is retained at the ciliary base. In both locations, fluorescence recovery after photobleaching shows the protein to be stably bound, with little exchange with the cytoplasmic pool (Figures S4E and S4F). Surprisingly, despite its important role in PCM recruitment in interphase, pcmd-1 null mutants act in a temperature-sensitive manner. At lower temperatures mitotic PCM expansion and cell division appear mostly unaffected, such that pcmd-1 mutants can be maintained in their homozygous state. At the L4 larval stage these animals display dyefill phenotypes similar to those following SPD-5 depletions (Figure S4G). An examination of the IFT marker CHE-11 in the phasmid neurons of pcmd-1 mutant animals shows these to stem from a similar spectrum of underlying defects, including IFT accumulation in dendrites and impaired ciliogenesis (Figure S4H).

An examination of PCM organization reveals the reason why: strongly reduced SPD-5 and GIP-2 signal at the ciliary base (Figures 4F and 4G). ZIF-1-mediated degradation late in neuronal differentiation shows that acute PCMD-1 loss also affects SPD-5 maintenance, albeit to a lesser extent (Figures S4I and S4J), and results in ciliary structural defects similar to those following acute depletion of SPD-5 (Figures S4K–S4M). PCMD-1 is therefore required to establish and maintain the PCM in ciliated neurons. Consistent with an ongoing role of PCMD-1, PCMD-1 signal at the ciliary base recovers following heat shock-induced degradation, revealing ongoing neuronal expression (Figures S4N and S4O). With a potential X-box motif located ~2 kb upstream of the transcription start site and PCMD-1 signal reduced in daf-19 mutants (Figure S4P), pcmd-1 expression like that of spd-5 may be DAF-19 regulated. However, this remains to be confirmed experimentally. How is
this new protein targeted to the correct cellular location? Acute degradation shows PCMD-1 localization to be partially dependent on SPD-5 (Figure 4H). There is then a positive feedback loop between PCMD-1 and SPD-5, with PCMD-1 helping to recruit and stabilize the SPD-5 matrix, which in turn helps to recruit and maintain PCMD-1. While initially directed to occur around centrioles, such a feedback loop could stabilize the PCM following loss of its core organizing structure, the centriole. It would also explain why the PCM is robust to loss of specific ciliary structures, such as the IFT docking machinery in hyls-1 mutants, the transition zone in ccep-290;mkrs-2;nhph-4 triple mutants (Figure S4Q), or indeed the entire cilogenesis program in daf-19 mutants, where residual SPD-5 and PCMD-1 inherited from prior cell cycles continue to form clear PCM foci despite the failure to form any ciliary structures (Figures S2B and S4P).

In summary, we show that an acentriolar centrosome persists at the ciliary base in C. elegans sensory neurons. This centrosome is no mere remnant, but is actively continued by continued expression of the scaffolding component SPD-5 under control of the RFX transcription factor DAF-19, contributing to neuronal morphogenesis and the cellular trafficking that sustains cilium assembly and maintenance (see model, Figure 4I). In contrast to mitotic PCM expansion, assembly and maintenance of the non-mitotic PCM, both in neurons and elsewhere in the worm, is independent of the mitotic regulator PLK-1. Instead, PCM assembly is likely driven by the self-assembly properties of SPD-5. At canonical centrosomes, centrioles act as the focal point for PCM assembly, while in differentiated tissues other cellular structures can assume that role. Here, this is the ciliary base, which originally contained a centriole-derived basal body, but in other cases there is a transfer of PCM and consequently microtubule-organizing capacity to other cellular locations, such as the nuclear envelope in muscle and the cell cortex in polarized epithelial cells. A critical protein in maintaining the PCM scaffold at the ciliary base is Pericentrin/PCMD-1. PCMD-1 also tethers the growing PCM to centrioles containing the PCM scaffold at the ciliary base in C. elegans and S4P.)

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2021.03.023.

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AUTHOR CONTRIBUTIONS

J.G. and T.L.: Conception and Design, Acquisition of Data, Analysis and Interpretation of Data, Drafting or Revising the Article; E.H. and M.D.: Contributing Unpublished Essential Data or Reagents; A.D.: Conception and Design, Analysis and Interpretation of Data, Drafting or Revising the Article.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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# STAR★METHODS

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**       |        |            |
| Rabbit polyclonal anti-ceSAS-4 | N/A | 46 |
| Rabbit polyclonal anti-ceSPD-5 | N/A | 46 |
| **Chemicals, Peptides, and Recombinant Proteins** | | |
| Agar 100 Resin      | Agar Scientific | Cat# AGR1031; CAS: 25038-04-4 |
| Alisertib (MLN8237) | Selleckchem | Cat# S1133; CAS: 102848-01-2 |
| B1 2536             | Axon MedChem | Cat# 1129; CAS: 755038-02-9 |
| Dil                 | Invitrogen | Cat# V2285 |
| Glutaraldehyde 25% EM grade | Agar Scientific | Cat# AGR1020; CAS: 111-30-8 |
| Hoechst 33342       | Sigma-Aldrich | Cat# B2261; CAS: 875756-97-1 |
| Osmium Tetroxide 4% Solution | Electron Microscopy Sciences | Cat# 19170; CAS: 20816-12-0 |
| Tetramisole hydrochloride | Sigma-Aldrich | Cat# L9756; CAS: 16595-80-5 |
| Recombinant Cas9::NLS | Vienna Biocenter Core Facilities | N/A |
| **Critical Commercial Assays** | | |
| MEGAscript T3 Transcription Kit | Invitrogen | Cat# AM1338 |
| MEGAscript T7 Transcription Kit | Invitrogen | Cat# AM1334 |
| MEGAclean™ Transcription Clean-Up Kit | Invitrogen | Cat# AM1908 |
| **Experimental Models: Organisms/Strains** | | |
| C. elegans: Strain N2: C. elegans wild-type (ancestral) | Caenorhabditis Genetics Center | N2 |
| C. elegans: Strain DAM181: vieSi18[pAD154; Psas-4::gfp::sas-4::reencoded; cb unc-119(+)]; sas-4(tm3951) II; unc-119(ed3) III? | | DAM181 |
| C. elegans: Strain DAM703: unc-119(ed3) III?; lts69[pAA191; Ppie-1::mCherry::spd-2; unc-119(+)]; lts37[pAA64; Ppie-1::mCherry::his-58; unc-119(+)] IV; air-1[10v10g][g::air-1] V | This study, 47, 48 | DAM703 |
| C. elegans: Strain DAM763: spd-2(vie4[spd-2::gfp +loxP]) II; unc-119(ed3) III?; lts37[pAA64; Ppie-1::mCherry::his-58; unc-119(+)] IV; tjs222[Ppie-1::mCherry::air1; unc-119(+)] | This study, 10, 49, 48 | DAM763 |
| C. elegans: Strain DAM827: lts69[pOD1110; CEP73608 tbg-1::mCherry; cb-unc-119(+)] I; unc-119(ed3) III?; lts37[pAA64; Ppie-1::mCherry::his-58; unc-119(+)] IV | This study, 4, 48 | DAM827 |
| C. elegans: Strain DAM831: plk-1[t117][plk-1:: superfoldergfp +loxP] III; lts69[pAA191; Ppie-1::mCherry::spd-2; unc-119(+)]; lts37[pAA64; Ppie-1::mCherry::his-58; unc-119(+)] IV | This study, 47, 50, 48 | DAM831 |
| C. elegans: Strain DAM859: tac-1[vie12][gfp::tac-1] II | 2 | DAM859 |
| C. elegans: Strain DAM873: unc-119(ed3) III; vieSi70[pAD675; pche-11::che-11::mKate2] IV | This study, 6 | DAM873 |
| C. elegans: Strain DAM930: plk-1[t117][plk-1:: superfoldergfp +loxP] III; vieSi16[pAD390; Phyls1::mCherry::hyls-1; cb unc-119(+)] IV | This study, 30, 50 | DAM930 |
| C. elegans: Strain DAM931: spd-2(vie4[spd-2::gfp +loxP]) II; unc-119(ed3) III?; vieSi16[pAD390; Phyls1::mCherry::hyls-1; cb unc-119(+)] IV | This study, 10, 33 | DAM931 |
| C. elegans: Strain DAM994: plk-2(ok936); spd-5(vie26[gfp::spd-5 +loxP]) II; plk-3(ok2812) IV | This study, 51, 34 | DAM994 |

(Continued on next page)
Continued

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| C. elegans: Strain DAM1062: spd-5[vie26(gfp::spd-5 +loxP)]; unc-119(ed3); vieSi16[pAD390::Phyls-1::mCherry::hyls-1; cb unc-119(+)]; IV | This study, DAM1062 |
| C. elegans: Strain DAM1068: spd-5[vie26(gfp::spd-5 +loxP)]; daf-19(m86); vieSi16[pAD390::Phyls-1::mCherry::hyls-1; cb unc-119(+)]; IV; daf-12(sa204) X | This study, DAM1068 |
| C. elegans: Strain DAM1075: spd-5[vie26(gfp::spd-5 +loxP)]; I; lociSi139[pPhsp-16.41::vhhGFP4::zif-1::operon linker::mCherry::his-11::tbb-2 3’UTR]; unc-119(ed3) III | This study, DAM1075 |
| C. elegans: Strain DAM1086: spd-5[vie26(gfp::spd-5 +loxP)]; unc-119(ed3); vieSi16[pAD390::Phyls-1::mCherry::hyls-1; cb unc-119(+)]; IV | This study, DAM1086 |
| C. elegans: Strain DAM1103: spd-5[vie26(gfp::spd-5 +loxP)]; lociSi139[pPhsp-16.41::vhhGFP4::zif-1::operon linker::mCherry::his-11::tbb-2 3’UTR]; unc-119(ed3) III | This study, DAM1103 |
| C. elegans: Strain DAM1108: spd-5[vie26(gfp::spd-5 +loxP)]; lociSi139[pPhsp-16.41::vhhGFP4::zif-1::operon linker::mCherry::his-11::tbb-2 3’UTR]; unc-119(ed3) III | This study, DAM1108 |
| C. elegans: Strain DAM1124: spd-5[vie26(gfp::spd-5 +loxP)]; lociSi139[pPhsp-16.41::vhhGFP4::zif-1::operon linker::mCherry::his-11::tbb-2 3’UTR]; unc-119(ed3) III | This study, DAM1124 |
| C. elegans: Strain DAM1138: spd-5[vie26(gfp::spd-5 +loxP)]; lociSi139[pPhsp-16.41::vhhGFP4::zif-1::operon linker::mCherry::his-11::tbb-2 3’UTR]; unc-119(ed3) III | This study, DAM1138 |
| C. elegans: Strain DAM1195: pmcd-1(vie975) | This study, DAM1195 |
| C. elegans: Strain DAM1196: spd-5[vie26(gfp::spd-5 +loxP)]; lociSi139[pPhsp-16.41::vhhGFP4::zif-1::operon linker::mCherry::his-11::tbb-2 3’UTR]; unc-119(ed3) III | This study, DAM1196 |
| C. elegans: Strain DAM1198: pmcd-1(vie986); lociSi139[pPhsp-16.41::vhhGFP4::zif-1::operon linker::mCherry::his-11::tbb-2 3’UTR]; unc-119(ed3) III | This study, DAM1198 |
| C. elegans: Strain DAM1197: pmcd-1(vie986); lociSi139[pPhsp-16.41::vhhGFP4::zif-1::operon linker::mCherry::his-11::tbb-2 3’UTR]; unc-119(ed3) III | This study, DAM1197 |
| C. elegans: Strain DAM1206: spd-5[vie26(gfp::spd-5 +loxP)]; lociSi139[pPhsp-16.41::vhhGFP4::zif-1::operon linker::mCherry::his-11::tbb-2 3’UTR]; unc-119(ed3) III | This study, DAM1206 |
| C. elegans: Strain DAM1211: vieSi131[pAD782::Pmzt-1::mNeonGreen::mzt-1; cb unc-119(+)]; III | This study, DAM1211 |
| C. elegans: Strain DAM1220: spd-5[vie26(gfp::spd-5 +loxP)]; vieSi70[pAD675::che-11::mKate2]; cb unc-119(+)]; IV | This study, DAM1220 |
| C. elegans: Strain DAM1221: spd-5[vie26(gfp::spd-5 +loxP)]; vieSi70[pAD675::che-11::mKate2]; cb unc-119(+)]; IV; gip-1([wow25];TagRFP::gip-1) | This study, DAM1221 |
| C. elegans: Strain DAM1222: spd-5[vie26(gfp::spd-5 +loxP)]; vieSi70[pAD675::che-11::mKate2]; cb unc-119(+)]; IV; gip-1([wow25];TagRFP::gip-1) | This study, DAM1222 |
| REAGENT or RESOURCE SOURCE | RESOURCE IDENTIFIER |
|----------------------------|---------------------|
| **C. elegans**: Strain DAM1229: pcmd-1(syb486[gfp::pcmd-1]) I; lucSi10[Phsp-16.41::vhGFP4::zif-1::operon linker::mCherry::his-11::tbb-2 3 UTR] II; unc-119(ed3) III? | This study; DAM1229 |
| **C. elegans**: Strain DAM1232: unc-119(ed3) III; vieEx48[pAD787; Pzyg-9::NeonGreen::zyg-9] | This study |
| **C. elegans**: Strain DAM1233: spd-5(vie26[gfp::spd-5 +loxP]) I; vuaSi21[pBP39; Pmks-6::mks-6::mCherry; cb unc-119(+)] II; unc-119(ed3) III; vieSi70[pAD675; pche-11::che-11::mKate2] IV; hyls-1(tm3067) V | This study; DAM1233 |
| **C. elegans**: Strain DAM1234: ccep-290(tm4927); spd-5(vie26[gfp::spd-5 +loxP]) I; unc-119(ed3) III?; mksr-2(tm2452); vieSi70[pAD675; pche-11::che-11::mKate2] IV; nphp-4(tm925) V | This study; DAM1234 |
| **C. elegans**: Strain DAM1236: spd-5(vie26[gfp::spd-5 +loxP]) I; unc-119(ed3) III?; vieSi70[pAD675; pche-11::che-11::mKate2] IV; hyls-1(tm3067) V | This study; DAM1236 |
| **C. elegans**: Strain DAM1240: spd-5(vie26[gfp::spd-5 +loxP]) I; unc-119(ed3) III?; vieSi20[pAD389; Paas-4::mCherry::sas-4; cb unc-119(+)] IV | This study; DAM1240 |
| **C. elegans**: Strain DAM1249: pcmd-1(syb486[gfp::pcmd-1]) I; daf-19(m86) II; vieSi16[pAD390; Phys-1::mCherry::hyls-1; cb unc-119(+)] IV;daf-12(sa204) X | This study; DAM1249 |
| **C. elegans**: Strain DAM1251: spd-5(vie26[gfp::spd-5 +loxP]) I; unc-119(ed3) III?; vieSi20[pAD389; Paas-4::mCherry::sas-4; cb unc-119(+)] IV; itts37[pPA664; Ppje-1::mCherry::his-58; unc-119(+)] IV | This study; DAM1251 |
| **C. elegans**: Strain DAM1252: pcmd-1(syb975) I; vieSi15[pAD395; Pspd-5::gfp::spd-5 reencoded; cb unc-119(+)] II; unc-119(ed3) III?; vieSi70[pAD675; pche-11::che-11::mKate2] IV | This study; DAM1252 |
| **C. elegans**: Strain DAM1254: spd-5(vie26[gfp::spd-5 +loxP]) I; vieSi15[pAD395; Pspd-5::gfp::spd-5 reencoded; cb unc-119(+)] II; unc-119(ed3) III?; vieSi137[pAD834; Phsp-16.41::vhGFP4::ZIF-1::tbb-2 3'UTR; cb unc-119(+)] IV | This study; DAM1254 |
| **C. elegans**: Strain DAM1256: spd-5(vie26[gfp::spd-5 +loxP]) I; vieSi140[pAD796; Pspd-5::gfp::spd-5 S653A S658A reencoded; cb unc-119(+)] II; unc-119(ed3) III?; vieSi137[pAD834; Pspd-5::gfp::spd-5 S653A S658A reencoded; cb unc-119(+)] IV | This study; DAM1256 |
| **C. elegans**: Strain DAM1260: itts569[pOD1110; CEP3608 tbg-1::mCherry; cb unc-119(+)] I; vieSi15[pAD395; Pspd-5::gfp::spd-5 reencoded; cb unc-119(+)] II; unc-119(ed3) III?; vieSi70[pAD675; pche-11::che-11::mKate2] IV | This study; DAM1260 |
| **C. elegans**: Strain DAM1262: itts569[pOD1110; CEP3608 tbg-1::mCherry; cb unc-119(+)] I; vieSi15[pAD395; Pspd-5::gfp::spd-5 reencoded; cb unc-119(+)] II; unc-119(ed3) III?; itts37[pPA664; Ppje-1::mCherry::his-58; unc-119(+)] IV | This study; DAM1262 |
| **C. elegans**: Strain DAM1263: spd-5(vie26[gfp::spd-5 +loxP]) I; vieSi140[pAD796; Pspd-5::gfp::spd-5 S653A S658A reencoded; cb unc-119(+)] II; unc-119(ed3) III?; vieSi137[pAD834; Pspd-5::gfp::spd-5 S653A S658A reencoded; cb unc-119(+)] IV | This study; DAM1263 |
| **C. elegans**: Strain DAM1266: spd-5(vie26[gfp::spd-5 +loxP]) I; vieSi140[pAD796; Pspd-5::gfp::spd-5 S653A S658A reencoded; cb unc-119(+)] II; unc-119(ed3) III?; vieSi70[pAD675; pche-11::che-11::mKate2] IV | This study; DAM1266 |
| **C. elegans**: Strain DAM1279: pcmd-1(syb975) I; gip-1[wow25;TagRFP::gip-1] III | This study; DAM1279 |
| **C. elegans**: Strain DAM1290: spd-5(vie42[Pspd-5(deltaBox)::gfp::spd-5 +loxP]) I; vuaSi21[pBP39; Pmks-6::mks-6::mCherry; cb unc-119(+)] II | This study; DAM1290 |
| **C. elegans**: Strain DAM1291: tbb-2[geh26[gfp::tbb-2]; cb unc-119(+)] III; vieSi70[pAD675; pche-11::che-11::mKate2] IV | This study; DAM1291 |
| **C. elegans**: Strain DAM1292: pcmd-1(syb486[gfp::pcmd-1]) I; itts914[pOD2048; Posm-6::vhGFP4::zif-1::operon linker::mCherry::his-11::tbb-2 3'UTR; cb unc-119(+)] II; unc-119(ed3) III?; vieSi70[pAD675; pche-11::CHE-11::mKate2] IV | This study; DAM1292 |
Continued

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| C. elegans: Strain DAM1294: pcmd-1(syb975) I; unc-119(ed3) III; vieSi70[pDA675; pche-11::che-11::mKate2] IV | This study, 6, 7 | DAM1294 |
| C. elegans: Strain DAM1296: vieSi18[pAD154; Psas-4::gfp::sas-4::reencoded; cb unc-119(+)] II; vieSi137[pAD834; phsp-16.41::vhhGFP4::zif-1::ttb-2 3 UTR; cb unc-119(+)] IV | This study, 6, 47 | DAM1296 |
| C. elegans: Strain DAM1304: spd-5(vie26[gfp::spd-5 +loxP]) I; ebp-2(or1954[ebp-2::mKate2]) II | This study, 2, 54 | DAM1304 |
| C. elegans: Strain DAM1305: spd-5(vie26[gfp::spd-5 +loxP]); pcmd-1(vie3504[mCherry::pcmd-1]) I | This study, 2 | DAM1305 |
| C. elegans: Strain DAM1307: spd-5(vie42[Pspd-5(deltaXbox)::gfp::spd-5 +loxP]; pcmd-1(vie3504[mCherry::pcmd-1]) I; itsi914[pOD2048; Posm-6::vhhgfp4::zif-1::operon linker::mCherry::his-11::ttb-2 3 UTR; cb-unc-119(+)] II; unc-119(ed3) III? | This study, 6 | DAM1307 |
| C. elegans: Strain JLF353: gip-1(wow25[TagRFP:gip-1]) III | 22 | JLF353 |
| C. elegans: Strain JT6924: daf-19(m86) II; daf-12(sa204) X | 28 | JT6924 |
| C. elegans: Strain OD523: itsi63[pOD1111; CEOP3608 ttg-1::gfp; cb unc-119(+)] II; unc-119(ed3) III | 20 | OD523 |
| C. elegans: Strain OD2115: itsi569[pOD1110; CEOP3608 ttg-1::mCherry; cb unc-119(+)] I; itsi540[pOD1343; Pnoca-1 de::noca-1 de::superfoldergfp; cb unc-119(+)] II; unc-119(ed3) III | 20 | OD2115 |
| C. elegans: Strain OD2116: itsi569[pOD1110; CEOP3608 ttg-1::mCherry; cb unc-119(+)] I; itsi542[pOD1506; Pptrn-1::ptrn-1cDNA::superfoldergfp; cb unc-119(+)] II; unc-119(ed3) III | 20 | OD2116 |
| C. elegans: Strain OD2509: gip-2(f19[gip-2::gfp +loxP]) I; unc-119(ed3) III | 20 | OD2509 |
| C. elegans: Strain PHX3504: pcmd-1(vie3504[mCherry::pcmd-1]) I | SunyBiotech | PHX3504 |
| C. elegans: Strain TMD119: pcmd-1(vie3504[gfp::pcmd-1]) I | 7 | TMD119 |

Oligonucleotides

| ptr-2(C32E8.8) dsRNA: 1.7mg/mL, amplified from N2 genomic DNA; forward primer: TAATACGACTCACTATAAGGTGTCGCCATCATCAAGCCTGATT | 2 | AD1168 |
| ptr-2 reverse primer: AATTAACTACCATTAAAGGTGGTGCCCATCAAAGCTGATT | 2 | AD1169 |
| spd-2(F32H2.3) dsRNA: 5.4mg/mL, amplified from N2 cDNA; forward primer: TAATACGACTCACTATAAGGTGTCGCCATCATCAAGCCTGATT | 2 | AD60 |
| spd-2 reverse primer: TAATACGACTCACTATAAGGTGTCGCCATCATCAAGCCTGATT | 2 | AD61 |
| spd-5(F56A3.4) dsRNA: 2.5mg/mL, amplified from N2 genomic DNA; forward primer: TAATACGACTCACTATAAGGTGTCGCCATCATCAAGCCTGATT | This study | AD1088 |
| spd-5 reverse primer: TAATACGACTCACTATAAGGTGTCGCCATCATCAAGCCTGATT | This study | AD1089 |
| spd-5 CRISPR crRNA (target sequence: GUUUCAGAUAGUAAAGAU) | This study | N/A |
| spd-5 CRISPR repair template: CGGTCTGCCAGCTCCCATTGGCAGCTGAGCAGCAGCGCACGACGACGGCCGTCCGGGGCACAGTTCCTGGTCTTTCCTGGTGTTTTATTACGAGTATTACGAGTATTAC | This study | N/A |

(Continued on next page)
### RESOURCE AVAILABILITY

#### Lead Contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Alexander Dammermann (alex.dammermann@univie.ac.at).

#### Materials Availability
*C. elegans* strains and plasmids generated in this study are available upon request from the Lead Contact.

#### Data and Code Availability
The published article includes all datasets generated or analyzed during this study.

### EXPERIMENTAL MODEL AND SUBJECT DETAILS

#### C. elegans strains and culture conditions
*C. elegans* strains expressing endogenously tagged EBP-2:mKate2, GFP:PCMD-1, GFP:SPD-5 and GFP:TAC-1, GFP:TBB-2, GIP-2:GFP, PLK-1:GFP, SPD-2:GFP and TagRFP:GIP-1, endogenous promoter-driven CHE-11:mKate2, GFP:SAS-4, GFP:SPD-5 and mCherry:SPD-2, mCherry:AIR-1, mCherry:HYLS-1, MKS-6:mCherry, NOCA-1 de:GFP, PDRN-1:GFP and TBG-1:GFP, and TBG-1:mCherry, the Pdyf-7 and Posm-6 promoter-driven GFP nanobody-directed ZIF-1 degrons, Ppie-1 promoter-driven mCherry:H2B, and the loss of function alleles ccep-290(tm4927), mksr-2(tm2452) and npo-4(tm925), daf-19(m86), hyls-1(tm3067), pmcd-1(syb975), plk-2(ok1936), plk-3(ok2812) and sas-4(tm3951) have been described previously.

A strain with a mutated X-box sequence in the spd-5 promoter was generated by Cas9-mediated homologous recombination using *in vitro*-synthesized Cas9-crRNA-tracrRNA complexes with endogenously tagged GFP:SPD-5 as the starting point, replacing the original X-box motif (GTTTCC AT AGTAAC) with a reshuffled version unable to bind DAF-19 (TCTCGA GA TCATAT). The latter sequence also includes an XhoI restriction site (CTCGAG) facilitating identification of mutant animals. Endogenously tagged GFP:AIR-1 was similarly generated by Cas9-mediated homologous recombination using a plasmid-based protocol. Successful integration was detected by single worm PCR (GFP:AIR-1)/PCR and Xhol digest (ΔX-box GFP:SPD-5) and confirmed by PCR.

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| dpy-10 cri-CRISPR crRNA (target sequence: GCUACCAUAG GCACCGGAG) | 55 | N/A |
| dpy-10 cri-CRISPR repair template | 55 | N/A |
| tracrRNA: AACAGCAUAAGCGAAGUUAAUAAGCGUCAGUCGU UACUAACUGAAAAGUCAGCAGUCUCGU | Dharmacon | Cat# U-002005-200 |
| CRISPR repair template for GFP-AIR-1 (1.5kb flanking sequences) in pUC57 vector | This study | pAD609 |
| Cas9-SV40 NLS expression vector Peft-3::cas9-SV40-NLS::tbb-2utr | Addgene | Plasmid # 46168 |
| Mos Co-injection marker pCFJ104 - Pmyo-3::mCherry::unc-54 | Addgene | Plasmid # 19328 |
| Mos Co-injection marker pGH8 - pRAB-3::mCherry::unc-54utr | Addgene | Plasmid # 19359 |
| Mos Co-injection marker pCFJ90 - Pmyo-2::mCherry::unc-54utr | Addgene | Plasmid # 19327 |
| Mos Negative selection marker pMA122 - Phsp-16.41::peel-1::tbb-2utr | Addgene | Plasmid # 34873 |

### Software and Algorithms

| Software | SOURCE | IDENTIFIER |
|----------|--------|------------|
| ImageJ version 2.0.0 | NIH | https://imagej.nih.gov/ij/ |
| MetaMorph 7.7.5 | Molecular Devices | N/A |
| GraphPad Prism 6 | GraphPad | N/A |
| Python 3.8.5 | N/A | https://www.python.org |
| Sci-kit image package 0.17.1 | N/A | https://scikit-image.org |
and sequencing. Endogenously tagged mCherry:PCMD-1 was generated by SunyBiotech. Strains expressing endogenous promoter-driven mNeonGreen:MZT-1, mCherry:SAS-4, GFP:SPD-5 (S653A S658A), mNeonGreenSPD-5 and mNeonGreen:ZYG-9 were generated by cloning the corresponding genomic locus including 5' and 3' regulatory sequences and N-terminal GFP/mNeonGreen/mCherry into the targeting vector pCFJ151 followed by Mos1-mediated transposition / gonad injection to form extrachromosomal arrays (mNeonGreen:ZYG-9, \( [60] \)). Both GFP:SPD-5 (S653A S658A) and the corresponding wild-type control from which it is derived were rendered RNAi-resistant by replacing the region between nucleotides 500 to 1079 in the spd-5 coding region to change the nucleotide sequence without altering the encoded amino acid sequence or codon usage. For inducible degradation of GFP:PCMD-1 and GFP:SPD-5, strains expressing a GFP nanobody:ZIF-1 fusion, \( [20] \) with and without mCherry:histone H2B as a visible marker coexpressed via an operon linker under the hsp-16.41 heat shock promoter, were generated by Mos1 transposon insertion as above.

Dual color strains and strains carrying mutant alleles and ZIF-1 degrons were constructed by mating. The genotypes of all strains used are listed in the Key Resources Table. All strains were maintained at 23°C with the exception of strains carrying the temperature-sensitive allele \( \text{pcmd-1}(syb975) \), which were kept at 16°C.

### METHOD DETAILS

#### Dye-fill assays

All dye-fill assays were performed on L4 stage animals. For each condition, \( \sim 100 \) worms were picked into 250μl of M9 0.1% Triton, washed 3x with M9 and incubated for 1h in 0.2% DiI (Invitrogen) in M9. After incubation, worms were allowed to destain for at least 30min on a seeded NGM plate before analysis. Dye accumulation in the cell bodies of amphid and phasmid neurons was scored on a Zeiss Axio Imager Z2 microscope equipped with a 63x 1.4NA Plan Apochromat objective and Lumencor SOLA SE. II light source. For illustration purposes 1μm z series were acquired using a Photometrics CoolSNAP-HQ2 cooled CCD camera controlled by ZEN 2 blue software (Zeiss).

#### Live imaging of embryos

Live imaging of early embryos was performed primarily on a Yokogawa CSU X1 spinning disk confocal mounted on a Zeiss Axio Observer Z1 inverted microscope equipped with a 63x 1.4NA Plan Apochromat objective, 120mW 405nm and 100mW 488nm and 561nm solid-state lasers, 2D-VisiFRAP Galvo FRAP module, Photometrics CoolSNAP-HQ2 cooled CCD and Hamamatsu ImagEM X2 EM-CCD cameras and controlled by VisiView software (Visitron Systems). Embryos were dissected in meiosis medium (60% Leibowitz L-15 media, 25μM HEPES pH7.4, 0.5% Inulin, 20% heat-inactivated fetal bovine serum) and filmed without compression. Low laser illumination (max power of 0.32mW) was used to minimize photobleaching. For quantitative analysis of PCM recruitment, 12x1μm GFP/mCherry z series as well as single plane DIC images were acquired at irregular intervals from nuclear envelope breakdown until cytokinesis onset. For the experiments presented in Figures S2A and S2B another DeltaVision microscope equipped with a CoolSNAP-HQ2 cooled CCD camera (Photometrics) but the same 60x objective and 7-Color SSI module was used. Worms were anesthetized using 10mM tetramisole in M9 for 5min before mounting on 2% agarose pads and imaging. 0.1-0.3μm GFP/mCherry z series as well as single plane DIC images were collected for the amphid and phasmid neurons closest to the objective. For analysis of CHE-11 IFT trafficking, single-plane mKate2 images of phasmid cilia in L4 larvae were acquired every half second. Live imaging of amphid and phasmid neurons was performed primarily using a 60x 1.42NA Plan Apochromat objective for Figures 1C and S2A and 100x 1.4NA Upian S Apochromat objective) on a DeltaVision 2 Ultra microscope, equipped with 7-Color SSI module and sCMOS camera and controlled by Acquire Ultra acquisition software (GE Healthcare). For the experiments presented in Figures S2A and S2B another DeltaVision microscope equipped with a CoolSNAP-HQ2 cooled CCD camera (Photometrics) but the same 60x objective and 7-Color SSI module was used. Worms were anesthetized using 10mM tetramisole in M9 for 5min before mounting on 2% agarose pads and imaging. 0.1-0.3μm GFP/mCherry z series as well as single plane DIC images were collected for the amphid and phasmid neurons closest to the objective. For analysis of CHE-11 IFT trafficking, single-plane mKate2 images of phasmid cilia in L4 larvae were acquired every half second. Live imaging of EBP-2 comets was performed on the Yokogawa CSU X1 spinning disk confocal described above, with single plane GFP and mKate2 images acquired using a Photometrics Prime sCMOS camera with no binning every 0.8s. Image stacks were imported into MetaMorph (Molecular Devices) or Fiji for post-acquisition processing.

#### Fluorescence recovery after photobleaching

To examine the dynamics of PCM proteins in early embryos, embryos in the first mitotic division expressing the GFP fusion of interest were followed from early prophase, with 6x1μm z series acquired at irregular intervals as described above. Photobleaching was performed in prometaphase using the galvanometer point scanner to target a region encompassing the centrosome with the 405nm laser at 10mW power and embryos imaged until completion of cytokinesis. Embryos were analyzed only if centrosome signal was completely eliminated throughout the entire z-volume. To examine PCM dynamics in ciliated neurons, L4 stage worms were anesthetized using 10mM tetramisole and mounted on 2% agarose pads. Photobleaching was performed on phasmid neurons targeting one or both PCM foci at the ciliary base using the spinning disk confocal set-up described above. Before and after photobleaching images 6x0.5μm z series were acquired at irregular intervals using the 63x objective and CCD camera with 2x2 binning. As for early embryos, low laser illumination (max power of 0.32mW)
was used to minimize photobleaching during acquisition. Pharyngeal pumping was monitored to ensure animals remained healthy during the ~10 min following photobleaching. Image stacks were imported into MetaMorph (Molecular Devices) for post-acquisition processing.

Immunofluorescence and Fixed Imaging

*C. elegans* immunofluorescence experiments were performed as previously described using directly labeled affinity-purified antibodies to SAS-4 and SPD-5. Embryos were permeabilized by freeze-crack, fixed in −20°C methanol for 20 min, rehydrated in PBS, blocked in AbDil (PBS, 2% BSA, 0.1% Triton X-100) for 20 min, incubated with directly labeled antibodies at 1 μg/mL in AbDil for 1.5 h, incubated with 1 μg/mL Hoechst 33342 in PBS for 5 min, washed in PBST, and mounted in 0.5% p-phenylenediamine, 20 mM Tris, pH 8.8, and 90% glycerol. 3D widefield datasets were acquired using the 60x objective on the DeltaVision 2 Ultra microscope described above and imported into Fiji for post-acquisition processing.

RNA-mediated interference

RNAi experiments were performed by soaking. dsRNAs were prepared by performing T3 and T7 in vitro transcription reactions (MEGAscript, Invitrogen) using DNA templates generated by PCR from N2 genomic DNA using oligonucleotides containing either T3 or T7 promoters (see Key Resources Table) and reactions cleaned up using the MEGAClear kit (Invitrogen). T3 and T7 reactions were then mixed in equal proportions with 3x soaking buffer (32.7 mM Na₂HPO₄, 16.5 mM KH₂PO₄, 6.3 mM NaCl, 14.1 mM NH₄Cl), annealed by incubating at 68°C for 10 min followed by 37°C for 30 min, aliquoted and stored at −80°C. For RNAi, L4 stage worms were collected from plates, washed with M9 and transferred using an eyelash tool into a drop containing 5 μL dsRNA and 0.5 μL of a 1:1 mixture of 1% gelatin and 63 mM spermidine. Worms were kept in this solution for 24 h at 20°C in a humidified chamber before being transferred onto seeded NGM plates to recover. Standard RNAi conditions of 48 h at 16°C were used for spd-2 and spd-5. For egg shell permeabilization by *ptr-2(RNAi)*, recovery time was reduced to 24 h at 20°C to minimize phenotypes beyond embryo permeability.

Degron-mediated depletion

To deplete SPD-5 and PCMD-1 specifically in ciliated neurons, we employed the GFP nanobody directed ZIF-1-mediated protein degradation system to target endogenously GFP-tagged SPD-5 and PCMD-1, expressing the GFP nanobody::ZIF-1 fusion under the tissue-specific promoters *Pdyf-7* and *Posm-6*, active at the time of dendrite elongation (comma-stage) and after initiation of ciliogenesis (3-fold stage), respectively. For inducible degradation of centrosomal proteins at different developmental stages, ZIF-1 was expressed under the heat shock-inducible promoter *Phsp-16.41*, activated by a temperature upshift to 30°C for 1 h (for degradation in early embryos) or 5 h (all other stages). Embryonic viability was assessed for embryos laid by gravid adults in the 90Min time window from the beginning of the heat shock until 30 min after, while cilium structural integrity was assessed by dye-fill on worms 24 h after heat shock as L1.

Inhibitor experiments

To inhibit AIR-1 and PLK-1 in early embryos, *ptr-2(RNAi)* permeabilized embryos were dissected in meiosis medium (60% Leibowitz L-15 media, 25 μM HEPES pH 7.4, 0.5% Inulin, 20% heat-inactivated fetal bovine serum) containing 20 μM MLN8237 (Aurora A inhibitor, Selleckchem) and 20 μM BI2536 (PLK1 inhibitor, Axon MedChem). To inhibit PLK-1 in ciliated neurons following transient depletion of SPD-5, L2-stage larvae expressing *hsps-16.41* promoter-driven GFP-nanobody::ZIF-1 and endogenously GFP-tagged SPD-5 were first heat-shocked for 30°C to degrade existing SPD-5 at the ciliary base. Worms were then either allowed to recover for 24 h at 20°C (Control) or removed from the plate, washed with PBS and transferred with an eyelash tool to a drop containing 40 μM BI2536 or 2% DMSO (No food control) in M9 and incubated in a humidified chamber for 24 h at 20°C.

Transmission electron microscopy

L4-stage worms were prepared by chemical fixation as previously described. Worms were fixed in 2.5% glutaraldehyde in cytoskeleton buffer (100 mM methyl ester sulfonate, 150 mM NaCl, 5 mM EGTA, 5 mM MgCl₂, 5 mM glucose in ddH₂O, pH 6.1) overnight at 4°C. Samples were then washed 3× in the same buffer and post-fixed for 30 min in 0.5% osmium tetroxide in buffer, washed 3× in buffer and 1× in ddH₂O. Finally, samples were dehydrated for 15 min each in 40%, 60%, 80%, 2× in 95% and finally 3× in 100% acetone. Samples were embedded in Agar100 resin after fixation and dehydration. 70 nm serial sections were then prepared, post-stained with aqueous uranyl acetate and lead citrate and examined with a Morgagni 268D microscope (FEI) equipped with an 11-megapixel Morada CCD camera (Olympus-SIS) and operated at 80 kV.

QUANTIFICATION AND STATISTICAL ANALYSIS

Image analysis

Quantification of PCM signal in early embryos was performed in Metamorph as previously described. GFP signal was measured on single planes for both centrosomes at each time point. Two variable size concentric regions were drawn around each centrosome, a smaller one encompassing the centrosome, and a larger one including the surrounding cytoplasm as background. The integrated GFP intensity was then calculated by subtracting the mean fluorescence intensity in the area between the two boxes (mean
background) from the mean intensity in the smaller box and multiplying by the area of the smaller box. To compensate for centrosome movement in z within the embryo, measurements for both centrosomes at each time point were averaged. All measurements are normalized to their respective controls.

Quantification of PCM signal at the ciliary base in amphids was performed in Fiji. GFP signal was measured on single planes with two concentric regions as described for early embryos, with the inner box encompassing the amphid ciliary cluster, the outer box the surrounding background, avoiding regions of high autofluorescence. The encompassing regions were drawn on the image plane with the highest signal intensity. All measurements are normalized to their respective controls. For the PLK-1 inhibition experiment, measurements were normalized to levels before heat shock. GIP-1 Shannon Entropy was calculated in Python using the entropy function from the sci-kit image package.

For FRAP analysis in early embryos and phasmid ciliated neurons, GFP signal for the bleached centrosome was quantified on single planes and the integrated fluorescence intensity calculated as described above. Measurements taken after photobleaching were normalized to the mean intensity of measurements in the 30 s preceding photobleaching. To account for additional PCM recruitment during centrosome maturation in early embryos, recruitment profiles were generated for unbleached embryos during the same cell cycle stage and the calculated signal increase subtracted from the measured FRAP recovery profiles. GraphPad Prism was then used to fit the data to the exponential equation $Y = A(1-\exp(-kX))+B$ where $A$ is the mobile fraction, $B$ is the background left after the bleach, and the half life $t_{1/2} = \ln(0.5)/-k$. $R^2$ values are the correlation coefficient obtained by fitting experimental data to the model. Data points on the graphs are the mean of the normalized GFP intensity measurements collected during the 40s interval centered on that point.

For analysis of CHE-11 dynamics in phasmid cilia the time-lapse sequence of images was first corrected for sample drift using the Correct 3D Drift plugin in Fiji. Next, a segmented line with a width of 3 pixels was drawn along the cilium and the KymographBuilder plugin used to generate a kymograph. The velocity of CHE-11 IFT particles was then calculated from the slopes of individual lines on the kymograph. For IFT flux rate analysis, the number of particles passing through the proximal segment of the cilium was counted over the course of 30s. Analysis of EBP-2 comets was performed similarly to CHE-11, with a segmented line with a width of 3 pixels drawn along the dendrite near the ciliary base and the KymographBuilder plugin used to generate a kymograph. To estimate the nucleation rate of the centrosome, the number of EBP-2 particles emanating from the ciliary base was counted over the course of 24s for 8 animals.

**Statistical Analysis**

All error bars are standard deviation unless otherwise indicated. To compare samples in a specific experiment, unpaired t tests were conducted using GraphPad Prism. For entropy measurements in Figure S3C Welch’s correction was applied. *, **, *** represent p values of < 0.05, 0.01 and 0.001, respectively. Tests are comparing indicated condition to control unless otherwise specified.