The hydrolethalus syndrome protein HYLS-1 regulates formation of the ciliary gate

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Transition fibres (TFs), together with the transition zone (TZ), are basal ciliary structures thought to be crucial for cilium biogenesis and function by acting as a ciliary gate to regulate selective protein entry and exit. Here we demonstrate that the centriolar and basal body protein HYLS-1, the C. elegans orthologue of hydrolethalus syndrome protein 1, is required for TF formation, TZ organization and ciliary gating. Loss of HYLS-1 compromises the docking and entry of intraflagellar transport (IFT) particles, ciliary gating for both membrane and soluble proteins, and axoneme assembly. Additional depletion of the TF component DYF-19 in hyls-1 mutants further exacerbates TZ anomalies and completely abrogates ciliogenesis. Our data support an important role for HYLS-1 and TFs in establishment of the ciliary gate and underline the importance of selective protein entry for cilia assembly.

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rimary cilia are sensory organelles emanating from the cell surface of most eukaryotic cells to perceive various environmental cues\(^1\,^2\). A wide spectrum of human disorders, collectively termed ciliopathies, has been linked to mutations in genes involved in cilia formation and/or function\(^3\,^4\). Unlike other membrane-enclosed cellular organelles, the ciliary lumen is open to the cytoplasm at the ciliary base. Mounting evidence suggests that gating mechanisms exist to regulate selective ciliary entry and exit of membrane and soluble proteins, whose coordinated function makes the cilium a distinct functional entity\(^6\,^8\). It has been proposed that transition fibres (TFs), which develop from the distal appendages of the mother centriole during ciliogenesis, and the transition zone (TZ) at the proximal-most segment of the axoneme, two structurally distinct and highly conserved subdomains, are functional compartments of the ciliary gate\(^9\,^10\).

The TZ is characterized by Y-links that connect the axoneme to the ciliary membrane. Dozens of ciliopathy proteins, including nephronophthisis (NPHP), Joubert syndrome and Meckel-Gruber syndrome (MKS) proteins, assemble into multimeric protein modules to regulate TZ integrity and function\(^10\). Loss of TZ integrity results in loss of the diffusion barrier to ciliary entry but does not significantly affect intraflagellar transport (IFT) or axoneme assembly in *Caenorhabditis elegans*\(^10\,^11\). Immediately proximal to the TZ, TFs anchor basal bodies to the apical membrane and constitute the first visible physical barrier between the cytoplasm and ciliary lumen\(^13\). In contrast to the TZ, only six proteins, CEP164, CEP83 (CCDC41), CEP89 (CCDC123), SCLT1, CEP128 and FBF1, have been identified as components of TFs or distal appendages\(^14\,^16\). Many of these proteins are poorly conserved in ciliated invertebrates that also possess TFs, suggesting additional components await identification\(^21\). Loss of TFs has been reported to abrogate basal-body-to-membrane docking in vertebrates\(^16\) and impair recruitment of ciliary membrane proteins\(^16\,^19\,^22\,^24\). Consistent with the essential role of TFs in the context of cilia, TF components and proteins regulating TF formation have been associated with cilia-related disorders. Thus, CEP164 and CEP83 have been found to be mutated in NPHP\(^25\,^26\), whereas SCLT1 and two centriole proteins critical for appendage formation, OFD1 and C2CD3, have been linked to oral–facial–digital syndrome\(^27\,^29\), and TTBK2, an interactor of CEP164, have been linked to spinocerebellar ataxia\(^30\).

Here we set out to examine the molecular mechanisms underlying TF assembly and function using *C. elegans* as an experimental model. We identify the hydrolethalus syndrome protein HYLS-1 as a protein required for TF assembly in *C. elegans*. In addition to TF disorganization, hyls-1 mutants display defects in docking and entry of IFT particles, TZ organization, axoneme assembly and ciliary gating. Intriguingly, co-deletion of the TF component DVF-19 further exacerbates TZ anomalies and completely abrogates ciliogenesis, supporting a role for TF-mediated selective gating in cilia assembly.

### Results

**HYLS-1 regulates TF assembly.** We previously identified DVF-19 (the orthologue of human FBF1) as the first genuine TF component in *C. elegans*\(^17\). Loss of DVF-19 compromises the entry of IFT particles, suggesting that TFs may be a critical site for the docking and sorting of IFT particles\(^17\). However, the fact that DVF-19-depleted worm cilia still possess TFs indicated that the key factors regulating TF biogenesis remain to be identified. To further understand the role of TFs in the context of cilia, we sought to identify additional TF components. Using mCherry-tagged DVF-19 as a TF marker, we examined the localization of green fluorescent protein (GFP)-tagged versions of known or predicted ciliary proteins in *C. elegans*\(^31\,^32\). This search led us to identify three TF-associated components, GASR-8, the putative homologue of human GAS8, and K10G6.4, related to human ANKRd26, as well as HYLS-1, a conserved centriolar and basal body component\(^13\). Among them, HYLS-1 and GASR-8 localize proximal to DVF-19, suggesting they may be proteins associated with or closely adjacent to TFs, whereas K10G6.4 completely co-localizes with DVF-19 on TFs (Fig. 1a,b). GASB dysfunction causes primary ciliary dyskinesia with mild axonal disorganization in humans and disrupts hedgehog signalling in zebrafish. However, the underlying molecular mechanisms remain poorly defined\(^34\,^34\). Similarly, ANKRD26 mice displayed obesity phenotypes potentially linked to defects in primary cilia\(^35\). Finally, mutations in HYLS1 have been detected in individuals with hydrolethalus syndrome\(^38\), a putative ciliopathy, and HYLS1 and its worm homologue HYLS-1 have been reported to be required for ciliogenesis in vertebrates and *C. elegans*, respectively\(^33\).

To investigate the role of the three proteins, we obtained loss-of-function mutants of *hyls-1* (tm3067), *gasr-8* (gk1232) and *k10g6.4* (gk567). All three mutants carry large deletions in their coding region and are putative null alleles (Supplementary Fig 1a)\(^33\). In *C. elegans*, the head amphid and tail phasmid cilia take up lipophilic fluorescent dye from the external environment\(^39\). Animals with abnormal ciliogenesis fail to take up dye and are considered dye-filling defective (Dyf)\(^40\). We found that, similar to dyf-19 mutants, hyls-1 mutants showed severe dye-filling defects as previously reported\(^33\), whereas gasr-8 and *k10g6.4* mutants appeared to possess normal cilia (>200 animals analysed for each mutant; Fig. 1c). Remarkably, GFP-tagged DVF-19, GASR-8 and K10G6.4, all lost their ciliary targeting in hyls-1 mutants (Fig. 1d–i). In contrast, loss of GASR-8, K10G6.4 or DVF-19 did not affect the localization of other TF components (data not shown). These data suggest that, among identified TF or TF-associated components in *C. elegans*, HYLS-1 may be the key factor required for TF organization.

We further used serial-section transmission electron microscopy (TEM) to examine ciliary ultrastructure. The centriole-derived basal body is a near-invariant feature at the base of the cilium in most species. However, it is not so in *C. elegans*, where basal bodies largely degenerate during development via mechanisms that are currently unclear\(^33\,^40\). The only remaining basal body structures have been reported to be dense fibrous TFs\(^40\). Of note, recent cryo-tomography studies suggest that some of the electron densities observed at the ciliary base in conventional TEM studies are the result of two-dimensional projections of microtubule flares\(^41\), which may be the remnant of the degenerated basal body. Interestingly, this remnant as well as the fibrous structures universally observed at the ciliary base in wild type (WT) was missing or highly aberrant in *hyls-1* cilia (Fig. 1j and Supplementary Table 1). This probably explains why DVF-19, GASR-8 and K10G6.4 display impaired targeting in hyls-1 mutants.

**HYLS-1 is required for IFT recruitment and entry.** To further assess the functional consequences of HYLS-1 depletion, we introduced various IFT markers into *hyls-1* mutants. Surprisingly, we found that truncated cilia still form in ~80% of *hyls-1* mutants (Fig. 2a), suggesting that the early steps of ciliogenesis, including basal body-to-membrane docking, are largely unaffected. However, fluorescence intensity analysis indicates that the ciliary levels of all IFT components examined, including the IFT-A component CHE-11, the IFT-B component OSM-6 and the BBSome component BBS-7, are decreased by 50–75% in
hyls-1 mutants compared with WT animals (Fig. 2a). Total protein levels as well as fluorescence intensities in the cell body are unchanged (Supplementary Fig. 2a–e), indicating that loss of HYLS-1 does not affect expression or stability of IFT proteins. IFT components normally show strong accumulation in the vicinity of TFs in WT worms (Fig. 2a,b). These accumulations do not occur in hyls-1 mutants (Fig. 2a,b). We previously reported that the TF component DYF-19 recruits and facilitates ciliary docking/entry of IFT particles17. To examine how loss of HYLS-1 affects IFT particles, we used bimolecular fluorescence complementation (BiFC) to analyse the assembly and transport of IFT particles by visualizing the in vivo association between IFT components in the worm11,17,42. As shown in Fig. 2c, the IFT-B component IFT-20 shows strong fluorescence complementation with the IFT-A component CHE-11, the Kinesin-2 subunit KAP-1 and the dynein subunit XBX-1 in both WT or hyls-1 mutants, indicating that assembly of the IFT-A–IFT-B complex, as well as the association of IFT particles with motors, is largely normal in hyls-1 cilia (Fig. 2c and Supplementary Fig. 2a–c). However, most fluorescence complementation signal of BiFC pairs was restricted to the ciliary base in hyls-1 mutants, suggesting that assembled IFT particles fail to efficiently enter cilia in hyls-1 cilia (Fig. 2c).

We further performed fluorescence recovery after photobleaching (FRAP) to obtain live cell imaging evidence that the ciliary entry of IFT is compromised in hyls-1 cilia. FRAP analysis was performed on phasmid cilia, bleaching the cilium proper and examining recovery of GFP signal from the cytoplasm. In WT, ~60% of the signal for the GFP-tagged IFT-B component OSM-6 recovers with a half time (t₁/₂) of 23.7 ± 3.2 s (data represented as ± s.d.), as determined from a single exponential fit to the data (Fig. 2d,e). By contrast, no detectable recovery of OSM-6:GFP fluorescence was recorded over 3 min in hyls-1 cilia (Fig. 2d,e), supporting the conclusion from BiFC experiments that the ciliary entry of IFT machinery is severely disrupted in hyls-1 cilia.

We next directly analysed IFT transport. Interestingly, although hyls-1 mutants displayed consistent albeit reduced cilia signal for individual IFT components, only ~20% of hyls-1 cilia showed active IFT particle movement in time-lapse imaging (Fig. 2f,g). This is not simply due to reduced IFT protein levels, because, first, our camera is sensitive enough to detect IFT...

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**Figure 1 | HYLS-1 promotes TF assembly.** (a) Localization of DYF-19, HYLS-1, GASR-8 and K10G6.4 in tail phasmid cilia. Each phasmid organ contains two cilia bundled together40. Images show the ciliary base of one set of two phasmid cilia expressing mCherry-tagged DYF-19 and GFP-tagged candidate proteins as indicated. HYLS-1 and GASR-8 localize proximal to DYF-19 in the region of the degenerated basal body, whereas K10G6.4 completely co-localizes with DYF-19 on TFs. (b) Cartoon illustrating the relative localization of DYF-19, HYLS-1, GASR-8 and K10G6.4 at the ciliary base. (c) Dye-filling of phasmid cilia was used to analyse cilia integrity. hyls-1 and dyf-19 mutants are dye-fill defective, whereas gasr-8 and k10g6.4 mutants possess apparently normal cilia. More than 200 worms analysed for each genetic background. (d–i) HYLS-1 is required for proper localization of DYF-19, GASR-8 and K10G6.4. Phasmid cilia expressing fluorescently tagged DYF-19 (d), GASR-8 (f) and K10G6.4 (h) in WT and hyls-1 mutants. NPHP-1 (d, f) and MKS-5 (h) were used to label the TZ. Quantification of relative fluorescence intensities of DYF-19 (e), GASR-8 (g) and K10G6.4 (i) in cilia of WT and hyls-1 mutants. n represents number of cilia analysed. (j) BB remnant and TFs are missing at the base of hyls-1 amphid cilia. Scale bars, 200 nm (j), 1 μm (other panels). Error bars indicate s.d. Student’s t-test indicates significant differences; *P<0.01 and ***P<0.001.
movement in cilia with even weaker IFT signal and, second, even *hyls-1* cilia with higher residual signal frequently showed no IFT movement at all. In addition, in *hyls-1* cilia with detectable IFT movement, the frequency of IFT transport was significantly lower (Fig. 2g). These results further confirm the defective ciliary entry of IFT machinery in *hyls-1* mutants and also suggest that individual IFT components leak into cilia without being incorporated into functional IFT particles. Interestingly, we found that reduced frequency of IFT transport correlates with defects in cilia elongation (Fig. 2h). We thus conclude that, in *hyls-1* cilia, impaired entry of functional IFT complexes results in defective ciliogenesis.

**Figure 2**

**a** Phasmid cilia. WT and *hyls-1* cilia with detectable IFT movement were analyzed for the intensity of OSR-6::GFP, CHE-11::GFP, and BBS-7::GFP. The frequency of IFT transport was significantly lower in *hyls-1* cilia. **b** BBS-7::GFP and MKS-5::mCherry Merge. WT and *hyls-1* cilia were analyzed for the intensity of BBS-7::GFP. **c** CHE-11::VN + VC-IFT-20. WT and *hyls-1* cilia were analyzed for the normalized fraction of cilia with BiFC fluorescence. **d** Pre-bleach and After bleach experiments of WT and *hyls-1* cilia for OSR-6::GFP. **e** Relative fluorescence intensity of WT and *hyls-1* mutants after bleach. **f** Pre-bleach and After bleach experiments of WT and *hyls-1* cilia for OSR-6::GFP. **g** Percentage of cilia with IFT movement. **h** IFT particle flux.
Deletion of DYF-19 in *hyls-1* mutants abrogates ciliogenesis. The ciliogenesis defect in *hyls-1* mutants is variable, with cilia lengths ranging from mildly reduced to severely stunted. When visualized with mCherry-tagged β-tubulin TBB-4 as an axonemal marker, >98% of WT cilia are longer than 6 μm. By contrast, ~60% of *hyls-1* cilia are between 1 and 6 μm in length and ~20% are below 1 μm in length or are absent (Fig. 3a,b). We previously noted that, despite severe TF defects, ~10% DYF-19 still localizes to the ciliary base in *hyls-1* mutants (Fig. 1e). As DYF-19/FBF1 is a key TF component promoting the ciliary entry of IFT complexes17, we hypothesized that the remaining DYF-19 could facilitate residual IFT entry and contribute to ciliogenesis in *hyls-1* mutants. Consistent with this, in *hyls-1* mutant worms co-expressing mCherry-tagged TBB-4 and GFP-tagged DYF-19, DYF-19 signal was indeed stronger in mildly truncated cilia than in severely stunted cilia (Fig. 3c,d), indicating that the level of the remaining DYF-19 at the ciliary base correlates with the severity of ciliogenesis defect.

To further test our hypothesis that residual DYF-19 at the ciliary base supports incomplete ciliogenesis in *hyls-1* mutants, we constructed *hyls-1*; *dyf-19* double mutants. Strikingly, double mutants showed no cilia at all (Fig. 3e,f), TEM analysis showed that the axoneme terminates immediately distal to the TZ with only a few microtubules observed in *hyls-1*; *dyf-19* double mutants, indicating that axoneme elongation is almost completely blocked (Fig. 3g and Supplementary Fig. 3a). We did not observe any genetic interactions between *hyls-1* and gasr-8 or k10g6.4 (Supplementary Fig. 3b,c). Based on these observations we propose that, in the absence of HYLS-1, TFs are disorganized but residual DYF-19 at the ciliary base still supports partial ciliogenesis by recruiting and facilitating the ciliary entry of IFT particles. Additional deletion of DYF-19 in *TF-deficient cilia that result from *hyls-1* knockout completely abrogates ciliogenesis.

Loss of HYLS-1 disrupts TZ integrity and function. Although HYLS-1 does not localize to the TZ, an unexpected discovery was that *hyls-1* mutants exhibit profound irregularities in the TZ: the B-tubules of the outer microtubule doublets are frequently missing and some Y-links, especially those on incomplete doublets, are missing or not intact (Fig. 4a). These axonemal anomalies are unique and distinct from those of known TZ mutants and in other ciliogenesis mutants in *C. elegans* in which the doublet microtubules of the TZ are usually unaffected11,40,43. Despite the severe disruption of TZ architecture, most TZ components examined, including MKS-3, MKS-6, MKSR-2, NPHP-4 and CCEP-290, show normal targeting in *hyls-1* mutants (Supplementary Fig. 4). However, MKS-5, which is proposed to be the central component required for TZ formation43-45, is mislocalized both above and below the TZ in *hyls-1* mutants (Fig. 4c).

Given that HYLS-1 was first identified as a centriolar protein interacting with SAS-4 (ref. 33), one explanation is that TZ anomalies stem from impaired templating of axonemal microtubules by the basal body. Alternatively, defective TF formation might cause deregulated cilia entry of key factors required for axonemal microtubule formation and/or stability. To distinguish between these possibilities, we examined the TZ in *hyls-1*; *dyf-19* double mutants. Strikingly, more B-tubules are missing in the TZ of *hyls-1*; *dyf-19* cilia compared with *hyls-1* single mutants (Fig. 4a,b). DYF-19 is a specific TF component and does not directly affect TZ organization and axonemal microtubules17. We reason that depletion of *hyls-1* mutants further impairs TF function, exacerbating impaired ciliary entry of key proteins required for TZ organization. We thus propose that TZ defects in *hyls-1* mutants are at least in part due to defects in TF function.

TZO have been linked to cell-matrix anchorage/adhesion during dendrite extension, with loss of multiple TZ modules resulting in drastically shortened dendrites11,43,46,47. Except for mks-5, mutations in mks genes (mks-1, mks-2, mks-3, mks-6, mks-1 and mks-2) or ccep-290 do not affect dendrite extension unless combined with mutations in nphp genes (nphp-1 and nphp-4), conditions which result in severely disorganized TZO and dendrite collapse11,43,46,47. Interestingly, we found that dendrite collapse could be observed in *hyls-1*; nphp-1 and *hyls-1*; mks-5 double mutants, but not in *hyls-1* single mutants or *hyls-1*; mks-1 ans *hyls-1*; mks-3, or *hyls-1*; mks-6 double mutants (Fig. 4d and Supplementary Fig. 4b). The synergistic effect in dendrite extension in *hyls-1*; nphp-1 and *hyls-1*; mks-5 double mutants may be caused by aggravated TZ disorganization, as depletion of HYLS-1 alone is sufficient to cause MKS-5 mislocalization and TZ anomalies. Alternatively, TFs may directly contribute to cell-matrix adhesion.

The TZ is known to form part of the ciliary gate restricting entry of non-ciliary membrane-associated proteins43. We therefore tested whether deletion of HYLS-1 also affects ciliary gating. RPI-2, the *C. elegans* orthologue of human X-linked retinitis pigmentosa 2, which associates with the plasma membrane in sensory neurons, and the transmembrane protein TRAM-1 are excluded from the ciliary membrane in WT but abnormally leak into cilia in *hyls-1* mutants17,43. As shown in Fig. 4e and Supplementary Fig. 4c, we observed abnormal ciliary entry of both RPI-2 and TRAM-1 in *hyls-1* cilia, indicating that the diffusion barrier for membrane proteins is compromised in HYLS-1-deficient cilia. We further examined the localization of...
the ciliary sensory receptor OSM-9, which specifically targets to OLQ neuronal cilia in WT worms. This ciliary enrichment is disrupted in *hyls-1* mutants, with a strong mislocalization to below the ciliary base (Fig. 4f). A compromised diffusion barrier at cilia base could lead to the lateral leak (diffusion) of proteins that should be enriched inside the cilia. An alternative

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**Figure 3 | Additional deletion of DYF-19 in *hyls-1* mutants completely abrogates ciliogenesis.** (a) *hyls-1* mutants exhibit variable defects in axoneme elongation. Phasmid cilia were labelled with the mCherry-tagged axoneme marker β-tubulin TBB-4. Asterisks indicate cilia base. (b) Quantification of cilia length in WT and *hyls-1* mutants. Average of three independent experiments. *n* > 100 cilia for each genetic background in each experiment. (c) Representative images of phasmid cilia co-labelled with mCherry-tagged TBB-4 and GFP-tagged DYF-19. The severity of cilia truncation in *hyls-1* mutants is correlated with the level of residual DYF-19 at the cilia base. (d) Quantification of DYF-19 signal in WT and *hyls-1* mutants. *n* represents the number of cilia analysed. (e) Additional deletion of DYF-19 in *hyls-1* mutants completely abrogates ciliogenesis. Cilia are labelled with TBB-4. Asterisks indicate cilia base. (f) Quantification of phasmid neurons without visible axoneme based on OSM-6::GFP in WT, *hyls-1*, *dyf-19* and *hyls-1; dyf-19* double mutants, respectively. *n* represents the number of phasmid neurons analysed. (g) TEM analysis of the proximal axoneme (immediately distal to the TZ) of amphid neurons. Compared with WT and *dyf-19* axonemes that consist of nine doublet microtubules, *hyls-1* mutants frequently display missing B-tubules (red stars). *dyf-19; hyls-1* double mutants show severely compromised and stunted axonemes that possess only one or two microtubules and immediately terminate only one TEM section (~80 nm) beyond the TZ. Scale bars, 200 nm (g), 5 μm (a,c,e). Error bars indicate s.d. Student’s t-test indicates significant differences; *P* < 0.01 and ***P* < 0.001.
explanation is that selective import of cilia-specific sensory receptors is disrupted in hyls-1 cilia. We next investigated whether gating for soluble proteins is also affected in hyls-1 mutants. PICC-1, the orthologue of human CCDC85A, and DAF-21, the orthologue of HSP90, do not enter cilia in WT animals (Fig. 4g and Supplementary Fig. 4d). In contrast, significant amounts of both proteins gain access to the cilia lumen in hyls-1 mutants (Fig. 4g and Supplementary Fig. 4d), indicating that loss of HYLS-1 also disrupts ciliary gating for soluble proteins. Collectively, these results indicate that HYLS-1 is essential for gating of both membrane and soluble proteins by regulating the proper architecture of TFs and/or TZ.

Discussion

Taken together, we found that HYLS-1 is required for formation of the ciliary gate. In hyls-1 cilia, the docking/import of IFT machinery and the formation of the TZ and axoneme are
compromised (Fig. 5). In addition, ciliary gating for both membrane and soluble proteins is disrupted, resulting in mislocalization of both ciliary and non-ciliary proteins. Interestingly, residual amount of the TF component DYF-19 continue to target to hyls-1 cilia and facilitate minimal IFT entry and axonemal elongation. Co-deletion of DYF-19 in hyls-1 cilia further exacerbates TZ malformation and totally abrogates ciliogenesis. The formation of truncated cilia in hyls-1 mutants correlates with residual targeting of the TF component DYF-19 to the ciliary base. Depletion of DYF-19 in hyls-1 mutants abrogates ciliogenesis, potentially by abolishing IFT entry. TZ structural anomalies are also exacerbated in hyls-1; dyf-19 double mutants, suggesting that TFs contribute to ciliary entry of proteins required for TZ integrity.

**Figure 5 | Working model of HYLS-1 function in ciliogenesis.** Ciliogenesis involves a hierarchy of steps: TF maturation, basal body docking, TZ formation, IFT anchoring and entry and axoneme elongation. TFs and the TZ are proposed as important parts of the ciliary gate. HYLS-1 is essential for basal body stability and TF assembly. hyls-1 mutants also show defects in TZ integrity, compromised cilia gating and truncated axonemes. The formation of truncated cilia in hyls-1 mutants correlates with residual targeting of the TF component DYF-19 to the ciliary base. Depletion of DYF-19 in hyls-1 mutants abrogates ciliogenesis, potentially by abolishing IFT entry. TZ structural anomalies are also exacerbated in hyls-1; dyf-19 double mutants, suggesting that TFs contribute to ciliary entry of proteins required for TZ integrity.

Recent cryo-tomography analysis suggests that the TF-like structures observed by conventional TEM in C. elegans, at least in part, are two-dimensional projected images of microtubule flares at the ciliary base, which may be the remnant of the degenerated basal body. The apparent loss of these flares in hyls-1 mutants suggest additional basal body defects, which we are currently investigating. Nevertheless, the presence of fibrous connections between those microtubules and the plasma membrane (see Fig. 4c and Supplementary Fig. 2c), and the localization of DAF-19/FBF1, a bona fide TF component in vertebrates, to precisely this region support the conservation of key aspects of TF structure in worms. It will be interesting to determine the extent of this conservation and relate it to the three-dimensional architecture of TFs in worms and other species.

HYLS-1 is unlikely to be a structural component of TFs based on the fact that it was identified as a core centriolar component and only partially co-localizes with TF components in C. elegans (Fig. 1a). Instead, we speculate that HYLS-1 may regulate TF assembly by conditioning the distal end of the centriole to enable
structural components to dock, to form TFs. Currently, four other ciliopathy associated proteins, OFD1, ODF2, C2CD3 (C2 calcium-dependent domain containing 3) and DZIP1 (DAZ-interacting zinc finger protein 1), have been implicated in TF formation by acting on the outer wall of the mother centriole to promote fibre-appendage assembly. It will be interesting to investigate the functional relationship between HYLS1 and these proteins. Intriguingly, HYLS-1 is the only protein besides C2CD3 to have a homologue in the C. elegans genome, suggesting that it may be a key factor in regulating TF formation across species.

In sum, we provide mechanistic insights into how the ciliopathy protein HYLS-1 contributes to ciliogenesis and establishment of the ciliary gate. In C. elegans, the TZ is required for gating of membrane proteins but dispensable for IFT machinery through TFs. Therefore, the TZ and TFs appear to serve distinct functions in ciliary gating, with the TZ acting primarily as a passive barrier to ciliary entry, whereas TFs help to actively load components enriched in the ciliary compartment.

**Methods**

**Strains.** Worms were cultured, maintained and crossed using standard procedures. All C. elegans strains and reporters used in this study are listed in Supplementary Table 2.

**Dye-filling assays.** Worms were incubated in 1% Dío (Molecular Probes) in M9 buffer for 2 h at room temperature and allowed to destain for ~30 min on a seeded Nematode Growth Medium (NGM) plate before analysis. Cell bodies with positive dye filling in phasmid cilia were scored under a Nikon TE 2000-U microscope with a Plan Apochromat ×60, 1.49 numerical aperture (NA) objective.

**Microscopy and imaging.** Young adult worms were mounted on a 3% agarose pad, immobilized with 10 mM levamisole and imaged using a Nikon TE 2000-U microscope for IFT component IFT-20::VC155 and the kinesin II motor component IFT-B complex formation and motor association in living worms. For this, worms were cultured, maintained and crossed using standard procedures by the EM core facility at the Mayo Clinic. Worm heads and the IFT component IFT-20::VC155 and the dynein component XBX-1::VN173; and the IFT component IFT-20::VC155 and the kinesin II motor component KAP-1::VN173. Fluorescent signals were visualized using the YFP filter under a Nikon TE 2000-U microscope.

Fluorescence intensity measurement. Identical exposure conditions were used for samples from the same experiment. Quantification of fluorescence intensities was performed using Nikon’s NIS-Elements microscopy imaging software. Cilia signal was determined using the manual measurement tool, drawing an area around the cilia proper, excluding the cilia base. Background signal was measured in the area surrounding the cilia and subtracted. The average value of signals in WT was normalized to one.

Fluorescence recovery after photobleaching. FRAP assays were carried out on a Zeiss LSM 510 confocal microscope with a ×100, 1.46 NA oil objective at 25 °C. A 488 nm laser (25 mW) at 80% power was used for photobleaching and images acquired every 4 s. All images were acquired using the same settings. After back-ground subtraction, the data were normalized to the pre-bleach fluorescence. The recovery curve was fitted to a single-exponential equation $R(t) = R_0 + (R_f - R_0) e^{-kt}$, where $R(t)$ is the total fluorescence at time t after the bleach, k is the constant describing the rate of recovery, $R_f$ is the fluorescence immediately post bleach (0 min time point) and $R_0$ is the maximum recovered fluorescence. The recovery half-time was calculated by $t_{1/2} = ln(2)/k$.

**Immunoprecipitation and western blotting.** WT and hyls-1 mutant worms expressing OSM-6::GFP were washed off twenty 10 cm plates and transferred into an Eppendorf tube filled with M9 buffer with 0.1% Triton X-100, pelleted at 100–200 g for 3 min, washed 3 times with 1 ml M9 with 0.1% Triton, then lysed in binding buffer (25 mM Tris·Cl pH 7.6, 150 mM NaCl, 1 mM EDTA and 0.5% Triton X-100) by grinding in liquid nitrogen. Lysate (1/10) was kept to load on gel to blot for β-actin. OSM-6::GFP was immunoprecipitated from the remaining 9/10 of the lysate with anti-GFP monoclonal antibody (A-11120, Invitrogen) then incubated with 30 μl protein G Sepharose beads in binding buffer with Complete Protease Inhibitor Cocktail (Roche) overnight at 4 °C. After washing three times with binding buffer, about half of immunoprecipitates was loaded on a 7.5% SDS–PAGE gel and immunoblotted. The primary antibodies used were mouse monoclonal anti-GFP (A-11120, Invitrogen, diluted 1:500) and β-actin antibody (C4) (sc-47778, Santa Cruz, 1:5000) as a loading control. Proteins were detected using anti-GFP or anti-β-actin antibodies conjugated to horseradish peroxidase (Jackson Immunoresearch) and SuperSignal West Fermo Luminol Enhanced ECL detection kit (Thermo Scientific). Visualization of protein bands was performed using the ChemiDoc XRS imaging system (Bio-Rad). Band intensities were measured using ImageJ (National Institutes of Health).

**Transmission electron microscopy.** TEM on amphid channel cilia was performed using standard procedures by the EM core facility at the Mayo Clinic. Worm heads and the IFT component IFT-20::VC155 and the kinesin II motor component IFT-B complex formation and motor association in living worms were fixed in 2.5% glutaraldehyde in cacodylate buffer and postfixed in 1% osmium tetroxide in cacodylate buffer. Samples were dehydrated and embedded in Embed®8 resin according to standard procedures. Serial sections (~80 nm thickness) from the anterior tip of worm head were collected and viewed on an electron microscope (JEM-1400; JEOL). More than ten worms were cut for each strain.

**Data availability.** All relevant data supporting the findings of this study are either included within the article and its Supplementary Information files or available upon request from the corresponding author.

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