Cutting off ciliary protein import: intraflagellar transport after dendritic femtosecond-laser ablation

Jona Mijalkovic, Jules Girard, Jasmijn van Loo, Felix Oswald and Erwin J.G. Peterman

In preparation
Primary cilia, organelles protruding from the surface of eukaryotic cells, act as cellular antennae to detect and transmit signals from the extracellular environment. They are built and maintained by continuous cycles of intraflagellar transport (IFT), where ciliary proteins are transported between the ciliary base and tip. These proteins are derived from the cell body since cilia lack protein synthesis machinery. How input from the cell body affects IFT and ciliary function is not well understood. In this chapter, we use femtosecond laser ablation to perturb the dendritic input of proteins to chemosensory cilia in living *C. elegans*. Using fluorescence microscopy, we visualize and quantify the real-time response of ciliary proteins to dendritic ablation. We find that the response occurs in three distinct stages: primary, where IFT motors redistribute with normal velocity; secondary, where the axoneme shortens and motor velocities are reduced; and tertiary, where motors leave the cilium. We propose that such a multi-step response enables the cilium to rapidly adapt to changes in the outside environment.
6.1 Introduction

Primary cilia are conserved, membrane-enveloped, microtubule-based organelles projecting from the surface of most eukaryotic cells, with important roles in signaling and sensory perception \(^1\)-\(^3\). In cilia, no proteins are synthesized and, consequently, cilia have to receive their building blocks (axonemal precursors, tubulin) and other proteins from the cell body \(^4\). Once these ciliary proteins have entered the cilium, they undergo continuous (re-)cycling between ciliary base and tip driven by motor proteins in a bidirectional transport process called intraflagellar transport (IFT) \(^5\)-\(^9\). IFT is necessary for ciliary maintenance and assembly. While IFT has been thoroughly investigated, few studies have addressed how input from the cell body affects IFT and ciliary function.

In *C. elegans*, chemosensory cilia emanate from the dendritic endings of sensory neurons. These cilia contain axonemes with a so-called 9+0 structure: 9 microtubule doublets form the proximal or middle segment and 9 microtubule singlets extend to form the distal segment without a central pair. Two motors of the kinesin-2 family, kinesin-II and OSM-3, cooperate to drive anterograde IFT from the ciliary base to the distal tip (plus-end of microtubules) \(^10\)-\(^12\), whereas one retrograde motor, IFT dynein, returns cargo form the tip to the base (minus-end) \(^13\). Motors and IFT particles A and B form complexes, called IFT trains, that transport cargo along the ciliary axoneme. Although the *C. elegans* ciliary membrane appears to extend into the plasma membrane, there is growing evidence that the cilium is separated from the dendrite and cell body (site of protein synthesis) by a selective pericilliary diffusion barrier \(^14\). This raises questions about the role of the cilium as a compartment within the larger neuronal transport system extending from the cell body to the ciliary tip: how is ciliary trafficking regulated and how is it affected by dendritic input?

To address these questions, we perturbed the connection between cell body and cilium by femtosecond laser ablation of *C. elegans* chemosensory
dendrites. Femtosecond-laser ablation has previously been demonstrated to be a high-precision tool to mechanically perturb biological systems with minimal collateral damage\textsuperscript{15-17}. In \textit{C. elegans}, pulsed femtosecond lasers have shown to be particularly useful in investigating the role of specific motor neurons, sensory neurons and neuronal pathways. In these studies, hours or days after the severing of cell bodies\textsuperscript{18}, axons\textsuperscript{19}, or dendrites\textsuperscript{20}, worm behavior was observed and evaluated. Ciliary signaling and IFT, however, occur on much more rapid time scales (seconds). Here, we use femtosecond laser ablation of dendrites of ciliated neurons, in combination with fluorescently labeled ciliary proteins to probe the real-time ciliary response to dendritic input. We observe a rapid response to dendritic ablation that occurs in three distinct stages: a primary stage (seconds), during which IFT motors redistribute with normal velocity; a secondary stage (tens of seconds), during which the axoneme shortens and motor velocities are reduced while the motors remain in the cilium; and a tertiary stage (several minutes) characterized by motor efflux from the cilium. We propose that this multi-step response enables the cilium to rapidly adapt to changes in the extracellular environment.

\section*{6.2 Results}

\textbf{Femtosecond laser ablation of \textit{C. elegans} phasmid chemosensory dendrites}

To investigate the ciliary response to abrupt changes in dendritic input, we performed femtosecond laser ablation on the dendritic processes of phasmid chemosensory neurons in the \textit{C. elegans} tail (Supplementary Figure 6.1, Figure 6.1A, B). We chose tail neurons because of the lower density of neurons here compared to in the head. This allowed for ablation with minimal effect on surrounding neurons. \textit{C. elegans} has two pairs of phasmid cilia referred to as the left (PHAL and PHBL) and right (PHAR and PHBR) pair with overlapping distal segments (Figure 6.1A). We initially ablated both dendrites of the phasmid pair since the effects of severing only one are obscured in the distal
segment due to the overlap. To visualize IFT in real time pre- and post-ablation, we used epifluorescence microscopy of *C. elegans* strains \(^{11}\) endogenously expressing fluorescently tagged tubulin (TBB-4::EGFP), IFT dynein (XBX-1::EGFP) \(^{21}\), OSM-3 (OSM-3::mCherry) \(^{11}\) and IFT-particle sub-complex B (IFT-B; OSM-6::EGFP) \(^{11}\) (Supplementary Table 6.1). From the image sequences we obtained, we generated time-averaged fluorescence images and kymographs using *KymographClear* \(^{22}\).
IFT after dendritic femtosecond-laser ablation

A

B

cb d c

C

Pre-ablation
- 3 s 15 s 30 s 60 s
Post-ablation

IFT dynein

IFT-B

Tubulin

D

IFT dynein

Normalized cilia length (μm) Distance from ciliary base (μm)

IFT-B

Normalized cilia length (μm) Distance from ciliary base (μm)

Tubulin

Normalized cilia length (μm) Distance from ciliary base (μm)

E

IFT dynein

IFT-B

- 15 s 0 s 15 s 30 s

F

IFT dynein

G

Occupied cilia length (% of length at 0 s) Time after ablation (s)
Figure 6.1 (left): Primary response of IFT dynein, IFT-B and tubulin to femtosecond-laser ablation of C. elegans chemosensory dendrites.

A. Cartoon schematic of C. elegans tail phasmid neurons: a, axon; cb, cell body; d, sensory dendrite; *, phasmid opening. B. Zoom-in schematic of the cilium, the region indicated by the grey box in A, focussing on IFT. d, sensory dendrite; b, base; ps, proximal segment; ds, distal segment; orange, pericilliary barrier; dotted orange line: pericilliary membrane; dotted grey line, cell membrane; dotted black line, approximate position of laser cut. C. Left: cartoons showing position of the focus of the ablation laser (dotted line) in the dendrites shown right. Right: Representative summed fluorescence intensity images of IFT dynein (XBX-1::EGFP), IFT-B particle sub-complexes (OSM-6::EGFP) and tubulin (TBB-4::EGFP) in the dendrite and phasmid cilium, pre- and post-ablation. Scale bar: 2 µm. D. Averaged, normalized cilium fluorescence 3 s pre-ablation and 60 s post-ablation of IFT dynein (purple, n = 20 cilia from 19 worms), IFT-B (blue, n = 11 cilia from 11 worms) and tubulin (black, n = 20 cilia from 20 worms). Line thickness is SEM. E.-F. Representative IFT-B (E) and IFT dynein (F) kymographs showing retrograde (green) and anterograde (red) motility. Horizontal: time; vertical: position, scale bar: 2 µm. Moment of ablation is indicated by the dotted line. G. Tubulin retraction as percentage of pre-ablation cilium length, and IFT dynein and IFT-B retraction as percentage of pre-ablation occupied ciliary distance. Inset: first 60 seconds post-ablation. See also Supplementary Table 6.2 for sample size and errors.

Ciliary motors and IFT particles are actively transported to the base after dendritic ablation

First, we focused on the initial, short-term ciliary response to dendritic perturbation. Animals from the strains investigated were measured continuously at low fluorescence excitation intensity for 60–90 seconds post-ablation. Pre-ablation, IFT dynein and IFT-B are found throughout the proximal and distal segment with accumulation at the ciliary base and
transition zone (Figure 6.1C), concurrent with previous reports 11, 23. Visual inspection of the time-averaged fluorescence image sequences post-ablation reveals that IFT dynein and IFT-B retract from the distal to the middle segment and ciliary base within 60 seconds (Figure 6.1C). Also the axonemal microtubules start to retract, but less strikingly so than IFT dynein and IFT-B.

To quantify the retraction of the ciliary components, we plotted the average fluorescence intensity along the cilium pre- and post-ablation for IFT dynein, IFT-B and tubulin. For IFT dynein and IFT-B, we observe a post-ablation decrease of the fluorescence intensity in the distal and proximal segment and a concomitant increase at the base and transition zone (Figure 6.1D). These results indicate that IFT dynein and IFT-B redistribute within the cilium in response to dendritic ablation, with the total amount of these components remaining relatively constant. For tubulin, we observe a decrease in fluorescence intensity in the distal segment, consistent with axonemal shortening (Figure 6.1D). The kinesin-2 motor OSM-3 has a markedly different distribution compared to IFT dynein and IFT-B and is located mainly in the distal segment (Supplementary Figure 6.2A) 11, 12. Therefore, we wondered whether OSM-3 would behave differently in response to ablation. Like IFT dynein and IFT-B, OSM-3 also appears to retract from the distal segment within 60 seconds, occupying the base and proximal segment in a similar distribution to IFT dynein and IFT-B and retracting at a similar rate (Supplementary Figure 6.2). The unusual positioning of OSM-3 at the ciliary base post-ablation suggests that it remains bound to IFT trains containing IFT dynein upon retraction.

To obtain insight into the dynamics of the motor and particle redistribution, we generated kymographs of IFT-dynein and IFT-B movement in the cilium, pre- and post-ablation (Figure 6.1E, F). The kymographs show a remarkably sudden reflux of both components within seconds after ablation, as can be seen by the increase in retrograde IFT frequency (more retrograde trains per second). Initially, this reflux has a similar velocity to pre-ablation, IFT-dynein driven retrograde transport (kymograph lines are parallel pre- and post-
ablation). After ~15 s post-ablation, kymograph lines become increasingly less steep, indicating a slowing down of IFT. These results suggest that ablation of dendrites of ciliated chemosensory neurons triggers the active return of IFT dynein within the cilium towards the base, together with other IFT components.

**Motor and IFT particle retraction is not triggered by axonemal collapse**

To probe whether the redistribution of motors and particles could be caused by the collapse of the ciliary axoneme, we compared axoneme length with the locations of motors and particles, obtained from the time-averaged fluorescence image sequences (Figure 6.1G, Supplementary Table 6.2). Pre-ablation, the motors (7.73 µm ± 0.20 µm, average ± s.e.m.) and IFT-B (8.05 µm ± 0.15 µm), cover the complete length of the ciliary axoneme (7.43 µm ± 0.16 µm). Post-ablation, however, IFT dynein and IFT-B rapidly retract at almost identical rates. After 15 seconds, the IFT components extend to only 76% (n = 18) and 75% (n = 11) of the pre-ablation cilium length (Supplementary Table 6.2). In comparison, the ciliary axoneme reduces only slightly, to 91% of its pre-ablation length (n = 20). After 60 seconds, IFT dynein (n = 19) and IFT-B (n = 7) retract to approximately 62%, while the axoneme has shortened to 77% (n = 19) (Figure 6.1F, Figure 6.1G, Supplementary Table 6.2). These results show that shortening of the ciliary axoneme is significantly slower than motor and IFT-B retraction, suggesting that the redistribution of IFT proteins is not triggered by the axonemal collapse. Instead, our data suggest that dendritic ablation triggers a cellular signal that stimulates the almost immediate redistribution of IFT motors and IFT particles within the cilia. The absence of motors and particles in the ciliary tip subsequently results in a depletion of components essential for axonemal maintenance, causing the axonemal microtubules to collapse and shorten.
Figure 6.2 Stability of the proximal segment in osm-3 worms in response to perturbation.

A. Cartoon schematic of intraflagellar transport (IFT) inside the cilium in osm-3 worms, which lack the distal segment. d: dendrite; b: base; ps: proximal segment; orange: pericilliary barrier; dotted orange line: pericilliary membrane; dotted grey line: cell membrane; dotted black line: position of laser cut. B. Left: cartoon showing position of dendritic laser ablation (dotted line). Right: representative summed fluorescence intensity images of IFT dynein (XBX-1::EGFP) in the dendrite and phasmid cilium pre- and post-ablation. C. IFT dynein (n=17) retraction as percentage of pre-ablation occupied ciliary distance. Error is s.e.m.

After the initial shortening of the axoneme in the first minute post-ablation, the cilium continues to shorten but at a significantly slower rate (Figure 6.1G). After 10 minutes, the axoneme is shortened to ~60% of its pre-ablation length and does not further shorten (at least until after 60 minutes). This means that the distal segment has gradually disappeared and only the proximal segment is left. To further assess the role of the proximal segment in response to perturbation, we ablated dendrites in an osm-3 mutant strain, which lacks the distal segment (Figure 6.2A, B)\(^{12, 24}\). We determined the ciliary distance occupied by IFT-dynein motors in this strain post-ablation and found that it
does not significantly differ from the distance occupied pre-ablation (Figure 6.2C). Together, these findings show that, while the ciliary distal segment responds within tens of seconds to ablation, the proximal segment stays intact. This indicates that the proximal segment forms a stable structure that does not rapidly disassemble in response to perturbations.

**Ciliary motors slow down but do not exit the cilium after dendritic ablation**

Next, we ablated only one dendrite within a phasmid pair as a control for damage to surrounding cells and to validate the precision of the instrument. The dendrites of the phasmid pair usually overlap (Figure 6.1C), but depending on worm orientation and cellular architecture, the dendrites can be up to 3 µm apart, allowing for the ablation of a single dendrite (Figure 6.3, Supplementary Figure 3). When only a single dendrite of a pair is ablated in wildtype (Figure 3A-D) or osm-3 mutant (Supplementary Figure 6.3A-D) nematodes, IFT dynein is substantially redistributed in the cilium emanating from the ablated dendrite, but not in the second cilium. 24 hours after ablation, the unablated dendrite and emanating cilium have remained intact (Supplementary Figure 6.3E), indicating that there is minimal damage to cells surrounding the ablated neuron. In some worms, the IFT-dynein fluorescence intensity at the ciliary base and transition zone is slightly increased just after ablation (Figure 6.3A-D), which might be due to local heating caused by the ablation process. This effect is, however, very small compared to that seen in the cilia of ablated dendrites. We observed the same ciliary shortening and IFT-motor redistribution in cilia where only a single dendrite was ablated as when both were ablated (Figure 6.1, Figure 6.2), further validating our earlier findings.

We next investigated how dendritic ablation affects IFT velocity. To this end, we generated kymographs from IFT-dynein fluorescence image sequences (Figure 6.3A-D) and compared anterograde (red) and retrograde (green) movement in control cilia with that of dendrite-ablated cilia, within the same phasmid cilium pair. Pre-ablation, anterograde (IFT dynein carried as cargo by
kinesin motors) and retrograde (dynein actively driving IFT trains) velocities of control and ablated cilia are undistinguishable (Figure 6.3A, E, H). Immediately after ablation, motor velocities are unaltered but the frequency of retrograde IFT trains increases suddenly, resulting in IFT-dynein redistribution (as observed in cilia with both dendrites ablated, Figure 6.1H). Approximately 15 seconds after ablation, both retrograde and anterograde velocities start to decrease compared to control (Figure 6.3B, C, F, G, I, J). 90 seconds after ablation, IFT is still active but motors are at least twice slower, as can be seen from the increased slope of the kymograph lines (Figure 6.3D). The slowing down could be due to the loss of ATP in the cilium, suggesting that ATP is supplied from the dendrite.

**Figure 6.3 (right): IFT-dynein redistribution and slowdown.**

A.-D. Representative summed IFT dynein (XBX-1::EGFP) fluorescence images (scale bar: 2 µm) and kymographs (Time: vertical; scale bar 2 s. Position: horizontal; scale bar 1 µm) 5 s pre-ablation (A) and 30 s (B), 60 s (C) and 90 s (D) post-ablation of the ablated and non-ablated (control) cilium. Grey area indicates the dendrite. Scale bar: 2µm. E.-G. Retrograde velocity in the control (dark green) and ablated (light green) cilium. F.-J. Anterograde velocity in the control (dark red) and ablated (light red) cilium. Error is SEM. K. Ratio of control/ablated total number of IFT dyneins in the cilium pre- and post-ablation. Dot: average; error bar: 95% confidence interval; line, median.
Additionally, we used the cilium-averaged integrated fluorescence intensity to calculate the total number of dyneins in unablated and ablated cilia at different time points after ablation, using the unablated cilium as a bleaching control for each time point. We find that in wildtype worms the control / ablated motor number ratio is close to 1 up to 90 seconds after ablation (Figure 6.3K), implying that most motors remain in the cilium. Similarly, in osm-3 worms, most motors (~80%) remain in the cilium (Supplementary Figure 6.3E). These results support our previous observations of motor redistribution (Figure 6.1D, Figure 6.2B) and show that most motors, at least up to 90 seconds after ablation, do not exit the cilium but remain available at the ciliary base and proximal segment for IFT.

**The tertiary response to dendritic ablation**

So far, we have mainly focused on the first tens of seconds following femtosecond laser ablation of phasmid dendrites and described and quantified the primary (fast redistribution of IFT components towards ciliary base) and secondary (slowing down of IFT) events. In the following, we will describe and quantify the tertiary response, in the tens of minutes afterwards. First, we determined whether the proximal segment remains stable on longer time scales (tens of minutes) after ablation. Figure 6.4A shows that the ciliary axoneme does not shorten further than 55.6% ± 5.8% (n= 6) of the pre-ablation length, 1 hour post-ablation. This confirms that the proximal segment of the axoneme, which consists of microtubule doublets, remains stable in response to the perturbation. In addition, 10 minutes after perturbation only half of the IFT dynein motors have left the cilium in both wildtype (Figure 6.4B) and osm-3 worms (Figure 6.4C). These findings highlight that, even on longer time scales, a considerable amount of IFT motors stays inside the cilium.
Figure 6.4: Tertiary response to dendritic ablation.

A. Representative summed tubulin (TBB-4::EGFP) fluorescence images pre- and post-ablation (-5, 60, 300 seconds and 1 hour). B-C. Ratio of control / ablated total number of XBX-1 in the cilium pre- and post-ablation in wildtype (B) and osm-3 worms (C). Dot: average; error bar: 95% confidence interval; line: median. D. Representative summed IFT dynein (XBX-1::EGFP) fluorescence images (scale bar: 2 µm) and corresponding kymographs of movement in the proximal segment in cilia with and without active IFT, 300 seconds post-ablation (horizontal scale bar: 1 µm, vertical scale bar: 2 s). Green: retrograde transport; red: anterograde transport. Arrow indicates position of laser
IFT after dendritic femtosecond-laser ablation

ablation. E. IFT-dynein retraction (percentage of pre-ablation distance occupied along cilia), with active IFT (grey dotted line, n=10) and without active IFT (black dotted line, n=10) after 300 seconds. Error: SEM.

Next, we assessed the long-term effects of dendritic ablation on IFT. In the first minute post-ablation, the motors move with reduced velocity in all measured cilia (Figure 6.3E-J). Remarkably, after 5 minutes, IFT resumed in some cilia but not in others (Figure 6.4D). To better understand this heterogeneity, cilia were grouped based on whether IFT reactivates or not after 5 minutes and the lengths of the regions of the cilia occupied by motors were determined. The initial rate of motor retraction does not seem to differ between the two groups of cilia (Figure 6.4E), confirming that primary and secondary response are relatively homogeneous in all ablated cilia. After 180 seconds post-ablation, however, two distinct scenarios unravel. In cilia where IFT reactivates, the IFT dynein stops retracting and remains occupying ~60 % of the cilium length (Figure 6.4E). In contrast, in cilia where IFT does not resume, the IFT dynein motors continue retracting towards the base (Figure 6.4E). It is possible that, in the group with reactivated IFT, the dendrite was only partially severed. Another possibility is that, in some cases, glycolytic enzymes can take over the production of ATP when ATP is no longer available from the dendrite, reactivating IFT in some cilia. It is currently not known if glycolytic enzymes are present in C. elegans chemosensory cilia, but their activity has been reported in the outer segments of mammalian photoreceptor cells and in Chlamydomonas flagella.

An overall picture that emerges is of a multi-step ciliary response to dendritic ablation (Figure 6.5). In a primary response, within 1-2 IFT cycles (15 seconds) dynein motors are triggered to retract from the distal segment to the base and proximal segment at normal velocity, taking with them OSM-3 motors and IFT-B particles. Within seconds, the ciliary axoneme starts to disassemble from the distal tip. In a secondary response, after 7-10 IFT cycles (60-90 seconds), motors slow down but are retained in the cilium, allowing rapid restoration of
IFT in part of the ablated cilia. If the damage to the dendrite is permanent, motors will, in a tertiary response, leave the cilium, while the proximal segment doublet microtubule structure does not shorten further.

Figure 6.5: Multi-step ciliary response to dendritic ablation.
A cartoon schematic showing the three steps of the ciliary response to dendritic ablation: primary (motor redistribution), secondary (slowdown of IFT) and tertiary response (motor efflux).
6.3 Discussion and conclusion

Cilia are built and maintained by continuous cycles of IFT between base and tip, requiring the input of ciliary proteins and ATP from the dendrite across the pericilliary barrier. Here, we probed the real-time ciliary response to the sudden loss of dendritic input using a combination of dendritic femtosecond laser ablation and fluorescence microscopy.

We found that dendritic ablation triggers a ciliary response within seconds. Dynein-driven IFT trains return, at normal velocity, from the tip towards the ciliary base, resulting in the redistribution of IFT dynein, OSM-3 and IFT-B from the distal segment to the proximal segment and base. We propose that this sudden increase of retrograde transport is caused activation or disruption of a signaling pathway in response to the ablation. As sensory organelles, cilia play important roles in a myriad of signaling processes. Ion channels, TGF-β receptors, receptor tyrosine kinases (RTK) and GPCRs are expressed in various types of primary cilia at the base, tip or ciliary membrane. In C. elegans, inositol 1,4,5-triphosphate (IP₃), Ca²⁺ and G-protein signaling have been reported in chemosensory neurons. It is therefore likely that the rapid activation and redistribution of IFT-dynein motors on the sub-second and seconds timescale are induced by a signaling cue. Further work on IFT-dynein regulation and dendritic signal transduction will be necessary to elucidate the mechanism of this response.

Tens of seconds after ablation, in a secondary response, the velocity of IFT decreases, which could be due to depletion of ATP. Most likely, IFT and other processes in the cilium require a constant supply of ATP from the dendrite, since they are thought to not contain mitochondria. Without external supply of ATP, we would expect that ATP is almost completely converted into ADP within ~2 seconds (assuming that only a fraction (~10%) of ciliary motors are active (~240 of the in total ~2400 kinesin-II, OSM-3 and IFT dynein motors in total) at a given time, with an ATP-hydrolysis rate of 10–30 s⁻¹ and given that the ATP concentration in the cilium is ~2mM, and the volume of the cilium
is \( \sim 4 \times 10^{-19} \) L). This appears to be an order of magnitude faster than we observed (~tens of seconds). It could be that in vivo IFT trains, which are composed of tens of motors, can continue moving longer after ATP depletion than individual motors, or that dendrite severing does not completely block ATP supply. In addition to modulation by ATP concentration, motor velocity can also be affected by intracellular signaling. For example, dauer pheromone and Ga\(\alpha\)-protein signals have been shown to alter kinesin-II and OSM-3 velocities in C. elegans larvae. \(^{35}\)

An important feature of the secondary response to dendritic ablation is that, although the distal segment retracts, most (at least \(\sim 80\%\)) motors remain in the ciliary compartment in the first 90 seconds. Retaining the motors and their track could allow the cilium to reactivate IFT if the perturbation is temporary or reversible, or continue disassembly (at a slower rate) in the case of an irreversible perturbation such as our and others’ \(^{20}\) dendritic ablations. The C. elegans chemosensory cilium appears to be ideally equipped for such a bimodal response: it consists of a proximal segment, composed of microtubule doublets in a stabilized axonomal structure and a distal segment built of dynamic microtubule singlets that can rapidly disassemble. \(^{7}\) Chlamydomonas cilia lack such a bipartite structure, yet appear to also respond in a step-wise manner to chemical perturbations: after addition of sodium chloride or sodium pyrophosphate, Chlamydomonas cilia first retract to approximately 50\% of their length, followed by a slower, long-term retraction (although both happen at longer timescales than in our experiments). \(^{39}\) This suggests that the rate of ciliary shortening in response to stimuli is not necessarily determined by axonemal structure, but could also be influenced by other factors, such as the number of motors.

In any femtosecond laser-ablation experiment, caution needs to be taken with collateral damage to surrounding cells. We have shown that we can ablate an individual dendrite of the phasmid pair without inducing damage to the second dendrite 1-3 \(\mu\)m away. We observed retraction of the severed dendritic ends post-ablation, indicating successful severing. \(^{20,40}\) To ensure that the dendrite
is ablated, however, the laser damage spot size slightly exceeds dendritic diameter and some damage to surrounding cells is unavoidable. In addition, we cannot exclude effects due to local, laser-induced heat dissipation and pressure, which could act as additional ciliary stimuli.

In conclusion, we have shown that dendritic ablation triggers a rapid molecular response in *C. elegans* chemosensory cilia, resulting in IFT-protein redistribution towards the ciliary base, reduced motor velocity and distal segment retraction. By visualizing and quantifying the real-time ciliary response to perturbation at the molecular level, we provide new insights into ciliary trafficking and function.

### 6.4 Methods

#### *C. elegans* maintenance and strains

*C. elegans* maintenance and genetic crosses were done using standard procedures. The *C. elegans* strains used in this study are listed in Supplementary Table 6.1. All strains were constructed using Mos1 Mediated Single Copy Insertion (MosSCI) as described previously. Integration was confirmed by PCR on the regions spanning the insertion.

#### Femtosecond laser ablation and image acquisition

The instrument used (Supplementary Figure 6.1) was built around a Nikon Eclipse Ti-E inverted microscope, equipped with a computer-controlled sample stage, perfect focus and a Nikon Plan Apo IR, NA 1.27, 60x water immersion objective. For ablation, we used a mode-locked, Ti:Sapphire laser system (Coherent, Mira 900 Femto / Verdi 10W) with wavelength tuned to 860–870 nm with pulse durations of approximately 150 fs. The beam was expanded using telescopes to fill the back aperture of the microscope objective and positioned using a piezoelectric tip/tilt scanning mirror (Physik Instrumente S-334). Epifluorescence microscopy measurements were
performed using the same objective, using a 488 nm or 561 nm laser (Coherent Compass) for excitation and a sCMOS camera (Hamamatsu Orca Flash 4.0 V2) for detection.

We performed femtosecond laser ablation on phasmid dendrites in the tails of *C. elegans* young adult hermaphrodites. The worms were anaesthetized in 5 mM levamisole in M9 and immobilized on a 2% agarose in M9 pad covered with a 22 × 22 mm cover glass and sealed with VaLaP. The focus of the femtosecond laser was positioned 1-3 µm from the ciliary base in all worms. After ablation, each strain was imaged continuously for 60 or 90 seconds and then at 180s, 300s and 600s (200 frames; 152ms per frame).

**Image analysis**

Images were analyzed using open source tools KymographDirect and KymographClear 22.

**6.5 Acknowledgements**

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**6.6 Author contributions**

6.7 References

### 6.8 Supplementary Tables

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**Supplementary Table 6.1: Strains used in this study.**

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**Supplementary Table 6.2: Tubulin, IFT-B and IFT dynein retraction post-ablation.**

Tubulin (TBB-4::EGFP), IFT-B (OSM-6::EGFP) and IFT dynein (XBX-1::EGFP) retraction as a percentage of the occupied pre-ablation length. Error is SEM. n= number of cilia; one cilium per worm.
**6.9 Supplementary Figures**

**Supplementary Figure 6.1: Schematic of the experimental set-up for femtosecond laser ablation.**

Femtosecond pulses from the Ti:Sapphire mode-locked laser fill the back aperture of a water immersion objective (Plan Apo IR, NA 1.27, 60x). A mechanical shutter was used to control the number of pulses for the experiments. The epifluorescence imaging system consists of two fluorescence excitation lasers (488 nm and 561 nm), excitation and emission filters, and a sCMOS camera.
Supplementary Figure 6.2: OSM-3 dynamics after femtosecond laser ablation of C. elegans chemosensory dendrites.

A. OSM-3::mCherry representative summed fluorescence images pre- and post-ablation (15 s, 30 s and 60 s). B. Average, normalized cilium fluorescence 5 s pre-ablation and 60 s post-ablation for OSM-3 (n=5 cilia from 5 worms). Line thickness is SEM. C. OSM-3 (n=5) retraction as percentage of pre-ablation occupied ciliary distance. Error is SEM.
Supplementary Figure 6.3 IFT dynein redistribution in osm-3 worms.

A–D. XBX-1::EGFP representative summed fluorescence images and cilium intensity pre-ablation (A) and 30 s (B), 60 s (C), 90 s (D) and 24 hours (E) post-ablation of the ablated and non-ablated (control) cilium in osm-3 worms. Grey area indicates the dendrite. Scale bar 2µm. (F) Ratio of control/ablated XBX-1 number in the cilium pre- and post-ablation in the osm-3 mutant background. Dot, average; error bar, 95% confidence interval; line, median.