CellPress

Correspondence

Light-controlled intracellular transport in *Caenorhabditis elegans*

Martin Harterink[1,3], Petra van Bergeijk[1,3], Calixte Allier[1], Bart de Haan[1], Sander van den Heuvel[2], Casper C. Hoogenraad[1,*], and Lukas C. Kapitein[1,*]

To establish and maintain their complex morphology and function, neurons and other polarized cells exploit cytoskeletal motor proteins to distribute cargoes to specific compartments [1]. Recent studies in cultured cells have used inducible motor protein recruitment to explore how different motors contribute to polarized transport and to control the subcellular positioning of organelles [2,3]. Such approaches also seem promising avenues for studying motor activity and organelle positioning within more complex cellular assemblies, but their applicability to multicellular *in vivo* systems has so far remained unexplored. Here, we report the development of an optogenetic organelle transport strategy in the *in vivo* model system *Caenorhabditis elegans*. We demonstrate that movement and pausing of various organelles can be achieved by recruiting the proper cytoskeletal motor protein with light. In neurons, we find that kinesin and dynein exclusively target the axon and dendrite, respectively, revealing the basic principles for polarized transport. *In vivo* control of motor attachment and organelle distributions will be widely useful in exploring the mechanisms that govern the dynamic morphogenesis of cells and tissues, within the context of a developing animal.

The nematode *C. elegans* is an important model system for the study of developmental and cell biological questions. A relative weakness is its impermeable exoskeleton, which precludes acute and specific perturbations with small molecules that have been extensively used in cultured cells, for instance with rapamycin-based heterodimerization systems [4,5]. Recently introduced light-sensitive heterodimerization systems that do not require exogenous compounds provide an alternative [6–8]. To test whether light-induced heterodimerization can be used in *C. elegans*, we focused on the LOVpep–ePDZ system, in which a photosensitive LOV domain cages a small peptide that binds an engineered PDZ domain (ePDZ) after exposure to blue light [6]. Codon-optimized LOVpep

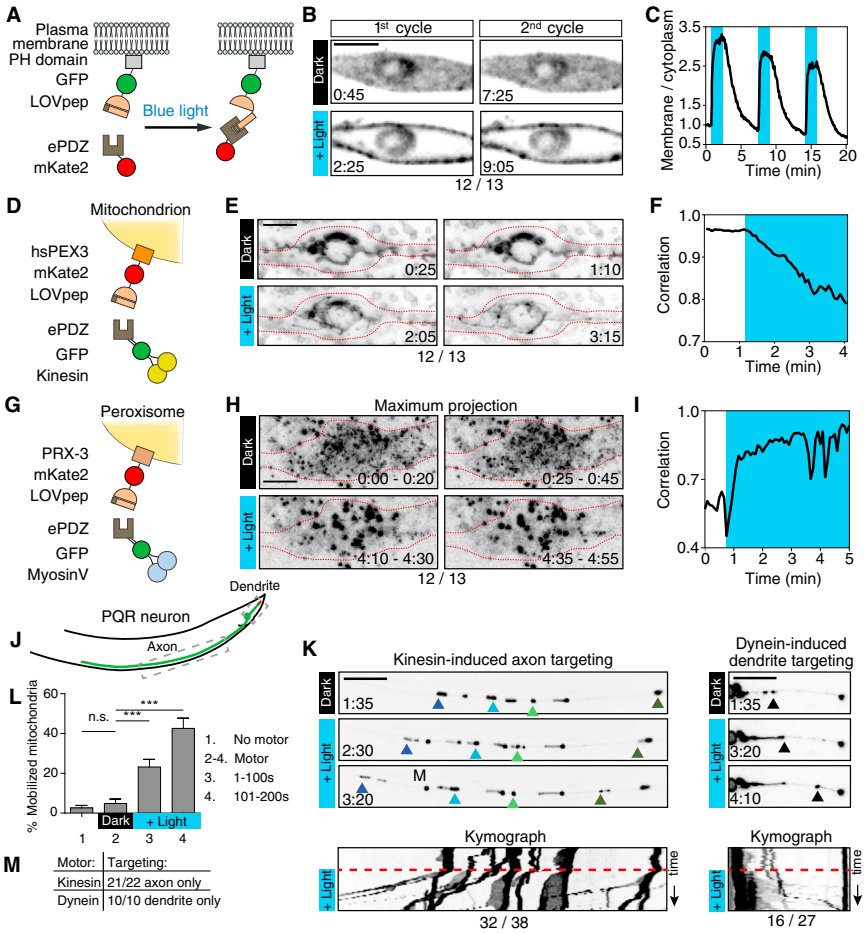

Figure 1. Light-controlled organelle transport in C. elegans.
(A) Assay: LOVpep binds to ePDZ upon exposure to blue light. (B) mKate2–ePDZ distribution in the absence of blue light (upper panels) or during exposure to blue light in a *C. elegans* seam cell. (C) Membrane-to-cytoplasm ratio of mKate2–ePDZ fluorescence for the cell shown in (B), normalized to the start. Blue boxes mark blue light exposure. (D) Assay: hsPEX-3 targets mitochondria. (E) Mitochondria before (upper panels) or during (lower panels) exposure to blue light in a *C. elegans* seam cell. Red lines indicate cell outline. (F) Time-trace of the correlation index (frame-to-frame differences in mitochondria recordings) of the cell shown in (E). Blue box: blue light illumination. (G) Assay: PRX-3 targets peroxisomes. (H) Maximum intensity projections of five subsequent frames of peroxisomes (5 seconds intervals) in the absence of blue light (upper panels) or during blue-light exposure (lower panels) in a *C. elegans* seam cell. Red line: cell outline. (I) Correlation time-trace of the cell shown in (H). Blue box: blue-light illumination. (J) PQR neuron with anterior axonal process and posterior dendritic process with sensory cilium (red). Gray dashed boxes are the imaged areas in (K). (K) Stills and kymograph depicting the distribution of mitochondria (labeled by TOMM-20(aa1–41)::mKate2::LOVpep), before and after light-induced coupling of UNC-116(aa1–381)::GFP::ePDZ (left) or BICD-1(aa1–513)::GFP::ePDZ (right). The cell body is to the right/left for the kinesin/dynein panels, respectively. Arrowheads track individual mitochondria. (L) Percentage of mobilized mitochondria (labeled by TOMM-20(aa1–41)::mKate2::LOVpep) without (#1) or with (#2–4) the kinesin construct (UNC-116(aa1–381)::GFP::ePDZ), before (#2) and during the first (#3) or second (#4) 100 s interval of blue light exposure. Mitochondria were counted in the first frame and scored positive if they would displace or undergo fission during the following 100 s (n=10/13 for control/motor). (M) Quantification of axon/dendrite targeting of mitochondria by kinesin-1 (UNC-116(aa1–381)) and BICD-1(aa1–513). Only movies with induced transport from the cell body where axon and dendrite could both be seen were analyzed. Numbers below images indicate the number of successful/total light-induced experiments. All images have inverted contrast and scale bars represent 5 µm.

was fused to the lipid-binding pleckstrin homology (PH) domain for targeting to the plasma membrane, and co-expressed with codon-optimized ePDZ fused to the red fluorescent protein mKate2, under the control of the seam-cell-specific *wrt-2* promoter. Multiple rounds of (subcellular) exposure to blue light resulted in a rapid relocalization of cytosolic mKate2–ePDZ to the illuminated plasma membrane, which was reversed within minutes after stopping the illumination (Figures 1A–C and S1A–C, Movie S1). Similarly, we successfully recruited mKate2–ePDZ to a subset of histone-bound LOVpep by illuminating one half of the nucleus (Figure S1D–F, Movie S1). Thus, light-induced protein dimerization can be locally and reversibly induced in *C. elegans*.

To address whether organelle transport could be triggered with light, LOVpep was targeted to the mitochondrial membrane in seam cells and co-expressed with a fusion construct of ePDZ and a truncated form of the microtubule plus-end-directed motor kinesin-1 (UNC-116). Upon exposure to blue light, kinesin-1 motors were recruited to mitochondria, which then began to move and became scattered throughout the cell. The increased mitochondrial dynamics upon illumination resulted in a drop in the frame-to-frame correlation index, which measures the similarity between subsequent frames (Figure 1D–F, Movie S1). Conversely, we were able to slow down rapidly moving peroxisomes by coupling them to a myosin-V (HUM-2) fragment, resulting in an increased correlation index (Figure 1G–I, Movie S1). Together, these data show that organelles can be repositioned within individual cells of a living animal using light.

We next switched to neurons to test how different motors behaved in highly polarized cells. We chose the tail-localized PQR neuron, which possesses one axon and one small ciliated dendrite (Figure 1J). We first tested several fragments and full-length kinesin-1, as well as a motor fragment of kinesin-3/UNC-104 (Figure S1G,H), which are both microtubule plus-end-directed motor proteins. Exposure to blue light triggered rapid motor recruitment to LOVpep-labeled mitochondria (Figure S1I), followed by efficient anterograde motility of axonal mitochondria for all kinesin-1 fusion proteins, except the shortest one (Figures 1K,L and S1G–M, Movie S2 and S3). Kinesin-3–ePDZ was also able to move mitochondria, but to a lesser extent than kinesin-1 (Figure S1H). The induced transport was more efficient halfway along the axon compared with proximal to the soma, because active motor constructs depleted themselves from the proximal axon by walking towards the axon tip (Figure S1J, Movie S3). Nonetheless, even in *ric-7* mutants, in which mitochondria accumulate in the soma and are largely absent from the axon [9], light-induced kinesin recruitment successfully redistributed mitochondria to the axon tip (Figure S1K–M).

To induce microtubule minus-end-directed transport we used the dynein-binding and activating amino terminus of BICD-1 [2]. Exposure to blue light triggered the unidirectional transport of mitochondria towards the dendrite tip (Figure 1K, Movie S2). Despite this acute induction, mitochondria were already accumulating at the tip prior to illumination (Figure 1K), which was largely caused by premature heterodimerization, since accumulation was less pronounced when overexpressing the BICD-1 amino terminus without ePDZ (Figure S1K,L). Importantly, in all 10 worms where the beginning of the axon and dendrite could be imaged simultaneously, BICD-1 exclusively targeted mitochondria to the dendrite upon exposure to blue light. In contrast, coupling of kinesin-1 resulted in selective transport to the axon in 21 of the 22 worms tested in the same way (Figure 1M). The unidirectionality of dendritic dynein-driven transport is consistent with a dendritic microtubule organization in which all minus ends are pointing outwards, which also explains why the kinesins selectively targeted the axon, where plus ends point outwards [10]. These findings highlight the traffic rules that govern polarized sorting in neurons and can be further exploited to alter organelle distributions in a controlled manner. In summary, our optogenetic strategy to control organelle transport and positioning within a multicellular organism should be widely applicable in further unravelling the mechanisms that govern cellular processes such as division, polarization and migration.

SUPPLEMENTAL INFORMATION

Supplemental Information includes experimental procedures, one figure and three movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.12.016.

ACKNOWLEDGEMENTS

We are grateful to Rik Korswagen for the gift of the *wrt-2* promoter gateway entry clone, to Henrik Bringmann for the gift of codon-optimized mKate2, and to WormBase. Several strains were provided by the CGC, which is funded by the NIH Office of Research Infrastructure Programs (P40 OD010440). This work is supported by the Netherlands Organisation for Scientific Research (NWO) (NWO-ALW-VENI to M.H., NWO-ALW-VICI to C.C.H., NWO-ALW-VIDI to L.C.K.), the Dutch Technology Foundation STW, which is part of the NWO (to C.C.H and L.C.K.), and the European Research Council (ERC Starting Grant to L.C.K., ERC Consolidator Grant to C.C.H.).

REFERENCES

1. Vale, R.D. (2003). The molecular motor toolbox for intracellular transport. Cell *112*, 467–480.
2. Kapitein, L.C., Schlager, M.A., Kuijpers, M., Wulf, P.S., van Spronsen, M., MacKintosh, F.C., and Hoogenraad, C.C. (2010). Mixed microtubules steer dynein-driven cargo transport into dendrites. Curr. Biol. *20*, 290–299.
3. van Bergeijk, P., Adrian, M., Hoogenraad, C.C., and Kapitein, L.C. (2015). Optogenetic control of organelle transport and positioning. Nature *518*, 111–114.
4. DeRose, R., Miyamoto, T., and Inoue, T. (2013). Manipulating signaling at will: chemically-inducible dimerization (CID) techniques resolve problems in cell biology. Pflugers Archiv. *465*, 409–417.
5. Kapitein, L.C., Schlager, M.A., van der Zwan, W.A., Wulf, P.S., Keijzer, N., and Hoogenraad, C.C. (2010). Probing intracellular motor protein activity using an inducible cargo trafficking assay. Biophys. J. *99*, 2143–2152.
6. Strickland, D., Lin, Y., Wagner, E., Hope, C.M., Zayner, J., Antoniou, C., Sosnick, T.R., Weiss, E.L., and Glotzer, M. (2012). TULIPs: tunable, light-controlled interacting protein tags for cell biology. Nat. Methods *9*, 379–384.
7. Kennedy, M.J., Hughes, R.M., Peteya, L.A., Schwartz, J.W., Ehlers, M.D., and Tucker, C.L. (2010). Rapid blue-light-mediated induction of protein interactions in living cells. Nat. Methods *7*, 973–975.
8. Yumerefendi, H., Dickinson, D.J., Wang, H., Zimmerman, S.P., Bear, J.E., Goldstein, B., Hahn, K., and Kuhlman, B. (2015). Control of protein activity and cell fate specification via light-mediated nuclear translocation. PLoS One *10*, e0128443.
9. Rawson, R.L., Yam, L., Weimer, R.M., Bend, E.G., Hartwieg, E., Horvitz, H.R., Clark, S.G., and Jorgensen, E.M. (2014). Axons degenerate in the absence of mitochondria in C. elegans. Curr. Biol. *24*, 760–765.
10. Maniar, T.A., Kaplan, M., Wang, G.J., Shen, K., Wei, L., Shaw, J.E., Koushika, S.P., and Bargmann, C.I. (2012). UNC-33 (CRMP) and ankyrin organize microtubules and localize kinesin to polarize axon-dendrite sorting. Nat. Neurosci. *15*, 48–56.

[1]Cell Biology, Faculty of Science, Utrecht University, 3584 CH, Utrecht, The Netherlands. [2]Developmental Biology, Faculty of Science, Utrecht University, 3584 CH, Utrecht, The Netherlands.
[3]These authors contributed equally to this work.
*E-mail: l.kapitein@uu.nl (L.C.K.), c.hoogenraad@uu.nl (C.C.H.)

