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From observing to controlling: Inducible control of organelle dynamics and interactions

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Abstract

The dynamics and interactions of cellular organelles underlie many aspects of cellular functioning. Until recently, assessment of organelle dynamics has been primarily observational or required whole-cell perturbations to assess the implications of altered organelle motility and positioning. However, thanks to recently developed and optimized intervention strategies, we now have the ability to control organelles in their unperturbed state, altering organelle positioning, membrane trafficking pathways, as well as organelle interactions. This can be performed both globally and locally, giving fine control over the range, reversibility, and extent of organelle dynamics. Here, we describe how these tools are currently used for controlling organelles and give insight into the exciting future of this emerging field.

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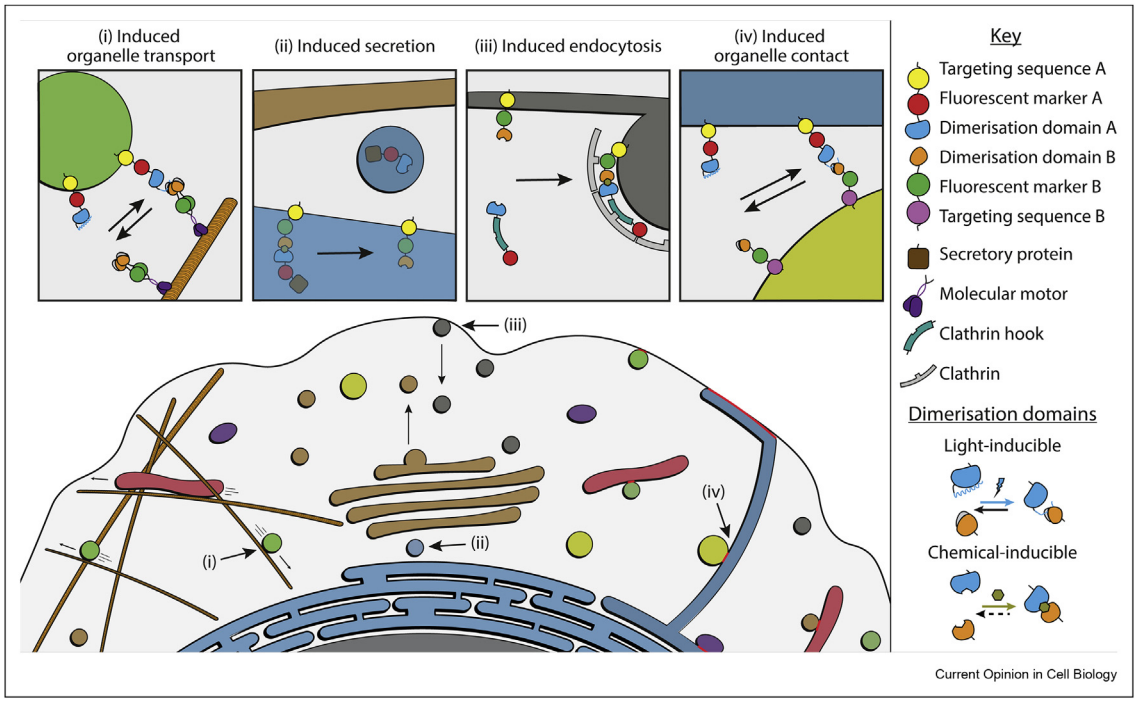
Introduction

Organelles are highly dynamic, with the capacity to constantly and rapidly change their motility, positioning, morphology, identity, and interactions as required. Organelle motility and positioning are primarily mediated by molecular motors and adaptors, linking the organelle membrane to the cytoskeleton. The recruitment of specific motors and adaptors can result in specific positioning of the organelle (for a review, see Ref. [1]). This regulated positioning of organelles is crucial for organelle inheritance in cell division, allows the efficient trafficking of intracellular cargo during exocytosis and endocytosis, and is especially important in polarized cells such as neuronal and epithelial cells. In addition, it is becoming increasingly clear that most organelles form extensive contact with each other. These interactions, typically mediated by membrane contact sites, are required for a variety of cell functions [2]. In some cases, organelle contacts also influence their dynamics and positioning, resulting in intriguing cooperation between the dynamics provided by molecular motors and the stability provided by organelle membrane contact sites.

Inducible dimerization systems, both chemical and optogenetic, have been widely used to control various aspects of cell biology. These techniques involve fusing dimerization domains with two proteins of interest, inducing dimerization through exposure to light (optogenetics) or a chemical ligand. Forcing the dimerization of two proteins has been used to regulate signaling cascades, induce transcription, or encourage post-translational modifications of proteins. Over recent years, these tools have shown many further applications, including the manipulation and observation of organelle dynamics (see Figure 1). The ability to recruit proteins to specific subcellular sites opens the possibility to induce and observe the dynamic behavior of organelles in keeping with the cell environment, with acute spatiotemporal control. This helps to uncover new biological processes, reveal the behavior of specific proteins, and describe the cause–consequence relationships of organelle dynamics and interaction. Here, we present a brief review of the use of inducible systems for the control of organelle dynamics and positioning via molecular motors. We then explore the currently emerging use of these techniques in membrane trafficking and organelle membrane contacts. We also provide an overview of available modules and a brief look at how these methods can be optimized for various experimental purposes.

Figure 1



Control of organelle dynamics and interaction via inducible dimerization modules. (i) Control of organelle positioning can be mediated by targeting one-half of a light-inducible or chemical-inducible dimerization domain to an organelle cargo, and one-half to a molecular motor, inducing organelle transport. (ii) Secretory pathways can be induced and monitored through the release of secretory cargo following retention at the ER. (iii) Clathrin-mediated endocytosis can be induced through the recruitment of a clathrin hook to the plasma membrane. (iv) Targeting dimerization domains to opposing organelle membranes brings the organelles in close proximity, inducing organelle membrane contact sites.

Control of organelle positioning

Inducible motor recruitment

One of the earlier uses of inducible dimerization for organelle biology was to functionally examine molecular motors in induced organelle transport experiments [3,4]. Targeting one half of the dimerization module to an organelle and the other to a motor allows the inducible initiation of cargo transport (Figure 1(i)). Such systems have been used to reposition organelles both to the cell periphery and perinuclear region, providing an array of options for probing organelle dynamics [4–6].

Standardizing such repositioning experiments to a single organelle, the peroxisome, has allowed the spatial selectivity of specific motors to be uncovered; for example, revealing how microtubule-binding proteins and microtubule orientations in neurons cause certain kinesins and dynein to selectively transport cargo into the axon and dendrites, respectively [7,8]. Such induced polarized transport has also been recapitulated in the model organism *Caenorhabditis elegans* [9]. Linking motor adaptors to organelle membranes rather than the motors themselves has allowed the roles and kinetics of endogenous motors to be revealed and the specific selectivity of these adaptors [3,10,11]. Conversely, standardizing the assay to already well-characterized molecular motors and varying the membrane targeting sequence has revealed the role of specific organelles in various processes. Controlling the positioning of specific endosome populations in neuronal cells has shown their role in axonal [12] and neurite [13] outgrowth and glutamate A1 (GluA1) receptor expression at the synapse [14]. Similarly, utilizing the both chemically inducible and photocleavable dimerization module zapalog showed actin-dependent anchoring of mitochondria at the presynaptic region [15••].

Inducible recruitment of motors to organelles has also provided insights into their role in influencing organelle identity; endosome maturation in the endocytic pathway is influenced by the engagement of myosin VI [16], and transmembrane protein 55B (TMEM55B)- or c-Jun NH_2-terminal kinase-interacting protein 4 (JIP4)-mediated dynein-dependent transport of lysosomes encourages clustering of the organelles and prevents lysosome-autophagosome fusion [17]. In epithelial cells, where microtubule minus-ends are clustered near the apical surface, recruitment of the minus end-directed

kinesin KIFC3 has been used to transport peroxisomes to cilia for the delivery of cholesterol [18].

Future prospects

Such controlled positioning of organelles gives valuable insight into both organelle function and the involved molecular motors, but the effects of organelle displacement remain little studied. Following induced mislocalization, not all types of organelles recover their original distribution similarly, with varying speeds and levels of recovery per organelle, indicating such effects are physiological instead of a result of the method of induced dimerization [19, 20••]. These understudied variances will be a fascinating aspect of membrane dynamics to be further studied in the future, as well as other long-term effects of repositioning, such as the effects of altered organelle positioning on inheritance and cell cycle progression.

The act of repositioning an organelle also provides another intriguing possibility — that of an 'intracellular pull-down' assay. The induced motility of an organelle combined with fluorescently labelling a target of interest (such as a protein or mRNA) will reveal its association with the repositioned organelle. This approach has been utilized to show that early endosome antigen 1 (EEA1) mRNA transcripts associate with specific endosome subpopulations [21] and has the potential for many interesting uses in the future, including for the assessment of organelle tethering (see *Control of Organelle Interaction*).

As a result of the opportunities provided by inducible dimerization systems, organelle dynamics and positioning are no longer just observational — the functions and consequences can now be controlled with high precision, greatly furthering this fascinating aspect of organelle biology. In the future, we expect to see the continuous development of such tools and more roles of the positioning of organelles inside cells to be revealed.

Control of membrane trafficking

Membrane trafficking requires the coordinated interplay between several organelles and molecular motors to mediate the shuttling between them. The spatiotemporal dynamics of membrane trafficking has historically been difficult to quantify due to a lack of appropriate tools. The reversibility of some inducible dimerization systems opened the way to synchronize trafficking pathways for the robust assessment of membrane trafficking.

Controlling exocytosis

The retention using selective hooks (RUSH) assay utilizes this reversibility to stably 'hook' a secretory reporter protein of interest to the ER membrane [22] (Figure 1(ii)). Heterodimerization of the reporter protein to the hook is established using streptavidin, which then dissociates in the presence of biotin. The procession of reporter protein from the ER to the Golgi apparatus, capture in post-Golgi vesicles, and finally secretion at the plasma membrane can be tracked in real-time, with simultaneous imaging of fluorescent-tagged markers of interest.

Enhancement of this technique with optogenetics has allowed finer control over the forward trafficking of secretory proteins. This spatial control has allowed local assessment of secretory dynamics in neurons by releasing the reporter protein in only the dendrites or the soma of the neuron. The secretory glycoprotein vesicular stomatitis virus glycoprotein (VSVG), when released from the ER near dendritic branch points using the UV-sensitive homodimer UV-B receptor 8 (UVR8), appears to traffic to the local dendritic Golgi outposts rather than disperse globally [23]. Taking this further, Bourke and colleagues increased spatiotemporal resolution by developing a photocleavable zapalog-mediated ER trap (zapERtrap) [24]. Using this system to examine the secretory dynamics of the synaptic proteins neuroligin 1 (NL1) and GluA1, they demonstrated differences in the timing and amount of surface delivery between these proteins and observed that these kinetics varied with the site of release within the neuron, be it from the soma or dendrites.

Controlling endocytosis

Membrane trafficking in endocytosis has been probed with both chemical and optogenetic techniques. Wood and colleagues described how clathrin-mediated endocytosis can be 'hot-wired', inducing dimerization of a clathrin-binding protein fragment to the plasma membrane causes a clathrin pit to form [25] (Figure 1(iii)). This can occur both globally with the rapalog system and locally utilizing optogenetics.

Future prospects

Due to the acute spatiotemporal control of membrane trafficking provided by these techniques, it is now possible to reveal the specific timings, localizations, and specificity of the exocytic/secretory and endocytic pathways with unprecedented precision. In the future, this will reveal more specific trafficking pathways, as well as the regulatory systems and organelle dynamics that underlie them.

Control of organelle interactions

While the compartmentalization of cells into distinct organelles is crucial to allow simultaneous regulation of multiple metabolic pathways at once, it is now clear that no organelle is as independent as previously thought. It is apparent that there is a level of interaction between organelles beyond membrane trafficking; direct contacts have been observed between most, if not all, organelles

Table 1

Overview of available systems for controlled dimerization.

Mode of action	System core	Cofactor	Activation method	Activation time	Inactivation method	Inactivation time	Example	References
Induced blue-light sensitive heterodimerization	LOV domains	Flavin[a]	Blue light	Seconds	Darkness	Seconds to hours (variants available)	TULIP	[9,12,16,25,45]
							iLID	[20••, 31,34,40,46]
							LOVTRAP	[47]
							Magnets	[48]
							eMags	[35•]
	Cryptochromes	Flavin[a]	Blue light	Seconds	Darkness	Minutes	CRY2/CIB	[5,13,36,41,49]
Induced blue-light sensitive homodimerization	LOV domains	Flavin[a]	Blue light	Seconds	Darkness	Seconds to hours (variants available)	VIVID	[20••, 50]
UV-light sensitive homodimerization	UV receptors	None	UV light	Milliseconds	Darkness	Hours	UVR8/UVR8	[23]
Red/Far-red-light sensitive heterodimerization	Plant phytochromes	PCB[b]	Red light	Milliseconds	Far-red light	Milliseconds	PhyB/PIF3	[6]
	Bacterial phytochromes	Biliverdin[c]	Far-red light	Seconds	Red light	Seconds	BphP1/Q-PAS1	[51]
Chemically induced heterodimerization	mTOR/rapamycin	None	Rapalog addition	Seconds to minutes	Irreversible	N/A	FKBP/FRB	[3,4,7,8,10,14,16–18,21, 25,30,32,33,42,52]
	GID1/gibberellin	None	Gibberellin addition	Seconds to minutes	Irreversible	N/A	GID1/GAI	[10,53]
Chemically induced homodimerization	mTOR/rapamycin	None	Rapalog addition	Seconds to minutes	Irreversible	N/A	FKBP/FKBP	[43,44]
Chemically induced heterotrimerization	mTOR/rapamycin	None	Rapalog addition	Seconds to minutes	Irreversible	N/A	Split-FRB/FKBP Split-FKBP/FRB	[37•]
Chemically reversed heterodimerization	Streptavidin/biotin	None	Biotin addition[d]	Seconds to minutes	Irreversible	N/A	RUSH	[22]
							RAMP	[19]
Photocleavable chemically induced heterodimerization	cTMP-Htag	None	UV light	Milliseconds	Irreversible	N/A	Halo/DFHR	[54]
	Zapalog	None	UV light	Milliseconds	Influx of unlysed compound	Seconds	FKBP/DFHR	[15••, 24]

[a] Endogenously available in all mammalian systems.

[b] Needs to be supplemented in mammalian systems.

[c] Endogenously available in some mammalian systems.

[d] Needs to be depleted from most mammalian systems.

[26]. Membrane contacts play major roles in signaling pathways, lipid transport, metabolic channeling, and organelle dynamics, with direct implications to health and disease [2]. Organelle membrane contact sites are typically around 30 nm, with the opposing membranes brought together by tethering and spacing proteins [27]. Therefore, methods to bring together opposing membranes while avoiding membrane fusion are extremely useful to assess the function of organelle contact sites and interaction. For an overview of other techniques used to observe membrane contact sites, see Ref. [28]. Bridging the gap between two membranes to induce contact has been achieved using artificial chimeric tethers composed of two organelle adaptor domains [29], but these could be improved using inducible or photocleavable tethers (Figure 1(iv)).

Calcium signaling

The extensive contacts of the ER with other organelles are critically involved in calcium ion (Ca^{2+}) signaling. Inducible linkage of ER to the plasma membrane (PM) has revealed the role of ER-PM contact during store-operated calcium entry (SOCE), demonstrating that the ER-resident calcium sensor stromal interaction molecule 1 (STIM1) interacts with the PM-resident Ca^{2+} channel calcium release-activated calcium channel protein 1 (ORAI1) to facilitate Ca^{2+} uptake by the cell [28,30]. Prior to activation during SOCE, STIM1 tracks the ends of growing microtubules as part of the tip attachment complex, where it plays a role in ER remodeling. STIM1 is activated by oligomerization, which releases STIM1 from the microtubule plus ends to migrate into ER−PM contacts. Inducing STIM1 oligomerization allowed further characterization of STIM1-microtubule and STIM1-PM interactions [31]. Mitochondria are also involved in Ca2+ signaling, and inducing ER-mitochondria contacts revealed an ER-mitochondria interface microdomain for Ca^{2+} transfer between the organelles [32].

Lipid trafficking

Membrane contact sites are critical in lipid trafficking, exchanging lipids between organelles via lipid transfer proteins (LTPs) localized at the contact sites. The accumulation of lipid at sites of organelle interaction has been visualized by chemically inducing ER-mitochondria contacts and monitoring the transfer of fluorescently labelled phosphatidylserine [33]. Optogenetic ER-mitochondria tethering may provide further insight into the ER-mitochondria lipid exchange in the future [34]. Benedetti and colleagues recently introduced the optimized blue light-sensitive heterodimerization module eMags, demonstrating its capability to induce ER-lysosome, ER-mitochondria, and mitochondria-lysosome contact sites [35•]. In addition, the authors engineered an optogenetic ER-organelle contact site inducer 'Opto-VAP' by restoring a split version of the ER-organelle tether vesicle-associated membrane protein-associated protein B (VAPB) using eMags. One-half of Opto-VAP contains the VAP major sperm protein (MSP) domain, which binds to phenylalanine-phenylalanine-acidic-tract (FFAT)-motif-containing proteins to facilitate ER-organelle membrane contact sites. Activating Opto-VAP induces ER-organelle contact sites, as demonstrated by the exchange of trans-Golgi network (TGN)-derived PI4P for cholesterol from the ER at ER-TGN contacts [35•]. By using local illumination, optogenetic systems can be used to induce spatially restricted stimuli. For example, recruiting the phosphatase inositol polyphosphate 5-phosphatase (OCRL) to the PM depletes PI(4,5)P2 locally, thereby destabilizing the ER-PM tethering component E-Syts, demonstrating a role of lipids, as well as Ca^{2+} in ER-PM junctions [36].

Inducible trimerization

As all possible contact pairs have been demonstrated between the ER, PM, and mitochondria, it follows that there may be the presence of a triple contact site for these three organelles and that this may play a role in Ca^{2+} signaling and/or lipid homeostasis. In order to provide tools for the induction of triple membrane contact sites, Wu and colleagues developed the first chemically-inducible heterotrimerization system [37•], modifying the rapalog system by splitting either one of the respective heterodimer FK506 binding protein (FKBP) and FKBP-rapamycin binding domain (FRB) modules into its own heterodimer. The authors demonstrated that the system could stably induce triple ER-PM-mitochondria contact sites. In addition, this method allows the recruitment of proteins of interest to an induced contact site, demonstrated by recruiting the yeast phosphatase phosphatidylinositol 4,5-bisphosphate 5-phosphatase (Inp54p) to the ER-PM contact site for local depletion of PI(4,5)P2 [37•].

Future prospects

Similar to the aforementioned intracellular pull-down assay (see '*Control of Organelle Positioning*'), a different approach to visualize organelle membrane contact sites is to use inducible organelle redistribution to track the response of any possible connected organelles. It is well-known that the ER maintains contact sites with many different organelles [38,39]. We recently demonstrated that optogenetically induced lysosome repositioning remodels the ER structure through membrane anchoring [40]. This situation highlights an interesting cooperation between the seemingly antagonistic organelle motility and tethering to regulate cell processes.

The combination of newly optimized inducible/cleavable dimerization systems and the emerging field of organelle contact sites results in some exciting prospects

for future research. Similar to the field of membrane trafficking, these tools push the study of membrane contact sites beyond observational, to elucidate their distinct roles in various processes. We expect that it will become increasingly common to apply such dimer/trimerization systems to induce organelle membrane contact, expanding our currently limited view on exactly which organelles maintain contact and under which conditions they do so.

Optimization of experiments

Optimizing dimerization domains

With multiple uses emerging, and multiple dimerization domains being discovered, engineered, and optimized, there are many factors influencing the choice and optimization of constructs used for control of organelle positioning, membrane trafficking, and organelle interaction. First and foremost is the choice of dimerization module, where tunable differences in dynamic range, reversibility, and dark-state activation need to be considered (Table 1). Broadly, chemical-based systems provide a more high-throughput workflow and are less leaky, but rely on the diffusion of sometimes costly compounds for activation, are poorly reversible, and lacking in spatiotemporal control. Optogenetic systems provide local activation and reversibility but show higher degrees of unwanted preinduction due to ambient light and require more specific microscopy setups. When designing new experiments, it may be beneficial to start with chemical-induced systems for robustness and switch to optogenetics for finer control.

Optimizing organelle targeting

The requirement of many of these applications to target at least one-half of a dimer pair to an organelle membrane means that these targeting sequences need to be carefully chosen and validated to minimize mislocalization and dual-localization, and limit artifacts such as clustering or morphological changes of the labelled organelles. For the assessment of membrane contact sites, be aware of the size of the induced dimer; the spacing, depending on the proteins targeted to the respective membranes, may not allow other proteins to enter and be functional at the induced contact site [30,32]. Finally, for the assessment of motor behavior, it is important to be aware that targeting different cargoes may produce differences in performance due to variances in load on the motor or different effects from the pulling forces. Indeed, optogenetic recruitment of motors to mitochondria can cause repositioning but also has the capability to remodel the mitochondrial membrane and induce morphological changes [41].

Optimizing motor selection

For optimizing repositioning of organelles via dimerization to a molecular motor, several things can be considered. Both monomeric and dimeric versions of a motor of interest can be investigated; these single-headed or double-headed kinesins have different mechanics, speeds, and pulling forces [42]. An additional optogenetic step that combines inducible dimerization of the molecular motor with recruitment to an organelle (opto-kinesin) helps to prevent premature activation and improves responsiveness [20••]. Kinesins have also been engineered that are chemically inhibitable, providing more options for the assessment of motor-cargo behavior [43,44].

Optimizing expression

Further improvements can be made by carefully modulating the expression levels of the constructs. Levels can either be measured and the response recorded, polycistronic vectors can be used for equimolar expression levels of the dimerization pair, or ideally, stable cell lines can be generated [20••]. As well as expression levels, titration of the inducer can also aid optimization; varying the concentration of chemical/cofactor or experimenting with light intensity/duration can both be useful.

Conclusions

While previous work has laid a valuable foundation to get insight into the extent and function of organelle dynamics and interaction, recent advances in the development of inducible and cleavable dimerization systems are allowing for a lot more control over these processes. There is now a vast array of options and uses, giving countless possibilities for control, and this is only set to increase over the next few years. We envisage future tools that combine the advantages of the above and limit the drawbacks, to make spatially activatable, stably inducible, and reversible systems with the increased capability of prolonged activation/stimulation. These techniques can also be used at the tissue and whole organism scale, providing further insights *in vivo*. An exciting prospect is the possibility of a closed-feedback loop setup — simultaneous reversible dimerization combined with monitoring by imaging software could allow these processes to be interrogated in an automated way to establish specific organelle distributions. The advancement of these fields by the tools presented in this review is certainly only just beginning.

Conflict of interest statement

Nothing declared.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. van Bergeijk P, Hoogenraad CC, Kapitein LC: **Right time, right place: probing the functions of organelle positioning**. *Trends Cell Biol* 2016, **26**:121–134.

2. Prinz WA, Toulmay A, Balla T: **The functional universe of membrane contact sites**. *Nat Rev Mol Cell Biol* 2020, **21**:7–24.

3. Hoogenraad CC, Wulf P, Schiefermeier N, Stepanova T, Galjart N, Small JV, Grosveld F, de Zeeuw CI, Akhmanova A: **Bicaudal D induces selective dynein-mediated microtubule minus end-directed transport**. *EMBO J* 2003, **22**:6004–6015.

4. Kapitein LC, Schlager MA, van der Zwan WA, Wulf PS, Keijzer N, Hoogenraad CC: **Probing intracellular motor protein activity using an inducible cargo trafficking assay**. *Biophys J* 2010, **99**: 2143–2152.

5. Duan L, Che D, Zhang K, Ong Q, Guo S, Cui B: **Optogenetic control of molecular motors and organelle distributions in cells**. *Chem Biol* 2015, **22**:671–682.

6. Adrian M, Nijenhuis W, Hoogstraaten RI, Willems J, Kapitein LC: **A phytochrome-derived photoswitch for intracellular transport**. *ACS Synth Biol* 2017, **6**:1248–1256.

7. Kapitein LC, Schlager MA, Kuijpers M, Wulf PS, van Spronsen M, MacKintosh FC, Hoogenraad CC: **Mixed microtubules steer dynein-driven cargo transport into dendrites**. *Curr Biol* 2010, **20**:290–299.

8. Lipka J, Kapitein LC, Jaworski J, Hoogenraad CC: **Microtubule-binding protein doublecortin-like kinase 1 (DCLK1) guides kinesin-3-mediated cargo transport to dendrites**. *EMBO J* 2016, **35**:302–318.

9. Harterink M, van Bergeijk P, Allier C, de Haan B, van den Heuvel S, Hoogenraad CC, Kapitein LC: **Light-controlled intracellular transport in Caenorhabditis elegans**. *Curr Biol* 2016, **26**:R153–R154.

10. Janssen AFJ, Tas RP, van Bergeijk P, Oost R, Hoogenraad CC, Kapitein LC: **Myosin-V induces cargo immobilization and clustering at the axon initial segment**. *Front Cell Neurosci* 2017, **11**.

11. French AR, Sosnick TR, Rock RS: **Investigations of human myosin VI targeting using optogenetically controlled cargo loading**. *Proc Natl Acad Sci Unit States Am* 2017, **114**:E1607.

12. van Bergeijk P, Adrian M, Hoogenraad CC, Kapitein LC: **Optogenetic control of organelle transport and positioning**. *Nature* 2015, **518**:111–114.

13. Nguyen MK, Kim CY, Kim JM, Park BO, Lee S, Park H, Heo WD: **Optogenetic oligomerization of Rab GTPases regulates intracellular membrane trafficking**. *Nat Chem Biol* 2016, **12**: 431–436.

14. Esteves da Silva M, Adrian M, Schätzle P, Lipka J, Watanabe T, Cho S, Futai K, Wierenga Corette J, Kapitein Lukas C, Hoogenraad Casper C: **Positioning of AMPA receptor-containing endosomes regulates synapse architecture**. *Cell Rep* 2015, **13**:933–943.

15. •• Gutnick A, Banghart MR, West ER, Schwarz TL: **The light-sensitive dimerizer zapalog reveals distinct modes of immobilization for axonal mitochondria**. *Nat Cell Biol* 2019, **21**: 768–777.
Chemically inducing dimerization has the advantage of allowing stable dimers without prolonged light exposure and phototoxicity but is irreversible. For overcoming this, this study presents the light-sensitive heterodimer zapalog, which combines the stability of chemically inducible systems with the reversibility of optogenetics.

16. Ritt M, Sivaramakrishnan S: **Engaging myosin VI tunes motility, morphology and identity in endocytosis**. *Traffic* 2018, **19**: 710–722.

17. Willett R, Martina JA, Zewe JP, Wills R, Hammond GRV, Puertollano R: **TFEB regulates lysosomal positioning by modulating TMEM55B expression and JIP4 recruitment to lysosomes**. *Nat Commun* 2017, **8**:1580.

18. Miyamoto T, Hosoba K, Itabashi T, Iwane AH, Akutsu SN, Ochiai H, Saito Y, Yamamoto T, Matsuura S: **Insufficiency of ciliary cholesterol in hereditary Zellweger syndrome**. *EMBO J* 2020, **39**, e103499.

19. Guardia CM, De Pace R, Sen A, Saric A, Jarnik M, Kolin DA, Kunwar A, Bonifacino JS: **Reversible association with motor proteins (RAMP): a streptavidin-based method to manipulate organelle positioning**. *PLoS Biol* 2019, **17**, e3000279.

20. •• Nijenhuis W, van Grinsven MMP, Kapitein LC: **An optimized toolbox for the optogenetic control of intracellular transport**. *J Cell Biol* 2020, **219**:e201907149.
Dark-state pre-activation and the perturbation of endogenous transport are common limitations to the optogenetic repositioning of organelles; this study limits these due to the creating a double-step optogenetic activation method using opto-kinesin and the use of the plant-derived retrograde motor ppKin14VIb.

21. Popovic D, Nijenhuis W, Kapitein LC, Pelkmans L: **Co-translational targeting of transcripts to endosomes**. *bioRxiv* 2020. 2020.07.17.208652.

22. Boncompain G, Divoux S, Gareil N, de Forges H, Lescure A, Latreche L, Mercanti V, Jollivet F, Raposo G, Perez F: **Synchronization of secretory protein traffic in populations of cells**. *Nat Methods* 2012, **9**:493–498.

23. Chen D, Gibson ES, Kennedy MJ: **A light-triggered protein secretion system**. *JCB (J Cell Biol)* 2013, **201**:631–640.

24. Bourke AM, Schwartz SL, Bowen AB, Gutnick A, Schwarz TL, Kennedy MJ: **Local neuronal secretory trafficking dynamics revealed with zapERtrap: a light-inducible ER release system**. *bioRxiv* 2020. 2020.2009.2002.278770.

25. Wood LA, Larocque G, Clarke NI, Sarkar S, Royle SJ: **New tools for "hot-wiring" clathrin-mediated endocytosis with temporal and spatial precision**. *JCB (J Cell Biol)* 2017, **216**:4351–4365.

26. Valm AM, Cohen S, Legant WR, Melunis J, Hershberg U, Wait E, Cohen AR, Davidson MW, Betzig E, Lippincott-Schwartz J: **Applying systems-level spectral imaging and analysis to reveal the organelle interactome**. *Nature* 2017, **546**:162–167.

27. Scorrano L, De Matteis MA, Emr S, Giordano F, Hajnóczky G, Kornmann B, Lackner LL, Levine TP, Pellegrini L, Reinisch K, *et al.*: **Coming together to define membrane contact sites**. *Nat Commun* 2019, **10**:1287.

28. Jing J, Liu G, Huang Y, Zhou Y: **A molecular toolbox for interrogation of membrane contact sites**. *J Physiol* 2020, **598**: 1725–1739.

29. Csordás G, Renken C, Várnai P, Walter L, Weaver D, Buttle KF, Balla T, Mannella CA, Hajnóczky G: **Structural and functional features and significance of the physical linkage between ER and mitochondria**. *J Cell Biol* 2006, **174**:915–921.

30. Várnai P, Tóth B, Tóth DJ, Hunyady L, Balla T: **Visualization and manipulation of plasma membrane-endoplasmic reticulum contact sites indicates the presence of additional molecular components within the STIM1-orai1 complex**. *J Biol Chem* 2007, **282**:29678–29690.

31. Ma G, He L, Liu S, Xie J, Huang Z, Jing J, Lee Y-T, Wang R, Luo H, Han W, *et al.*: **Optogenetic engineering to probe the molecular choreography of STIM1-mediated cell signaling**. *Nat Commun* 2020, **11**:1039.

32. Csordás G, Várnai P, Golenár T, Roy S, Purkins G, Schneider TG, Balla T, Hajnóczky G: **Imaging interorganelle contacts and local calcium dynamics at the ER-mitochondrial interface**. *Mol Cell* 2010, **39**:121–132.

33. Komatsu T, Kukelyansky I, McCaffery JM, Ueno T, Varela LC, Inoue T: **Organelle-specific, rapid induction of molecular activities and membrane tethering**. *Nat Methods* 2010, **7**: 206–208.

34. Shi F, Kawano F, Park S-hE, Komazaki S, Hirabayashi Y, Polleux F, Yazawa M: **Optogenetic control of endoplasmic reticulum–mitochondria tethering**. *ACS Synth Biol* 2018, **7**: 2–9.

35. • Benedetti L, Marvin JS, Falahati H, Guillén-Samander A, Looger LL, De Camilli P: **Optimized Vivid-derived Magnets photodimerizers for subcellular optogenetics**. *eLife* 2020, **9**, e63230.

This study presents in-depth optimization of the photodimerizer eMags. Using eMags, the authors introduce the optogenetic ER-organelle contact site inducer Opto-VAP, revealing lipid transport dynamics at the ER-trans-Golgi interface.

36. Giordano F, Saheki Y, Idevall-Hagren O, Colombo SF, Pirruccello M, Milosevic I, Gracheva Elena O, Bagriantsev Sviatoslav N, Borgese N, De Camilli P: **PI(4,5)P_2-Dependent and Ca^{2+}-regulated ER-PM interactions mediated by the extended synaptotagmins**. *Cell* 2013, **153**:1494–1509.

37. • Wu HD, Kikuchi M, Dagliyan O, Aragaki AK, Nakamura H, Dokholyan NV, Umehara T, Inoue T: **Rational design and implementation of a chemically inducible heterotrimerization system**. *Nat Methods* 2020, **17**:928–936.

Current control of organelle dynamics and interaction involve heterodimerization modules; this study introduces the first chemically inducible heterotrimerisation system. Being able to induce triple organelle contact sites and recruit proteins to induced contact sites in one step opens up many possibilities for future investigation.

38. Rowland Ashley A, Chitwood Patrick J, Phillips Melissa J, Voeltz Gia K: **ER contact sites define the position and timing of endosome fission**. *Cell* 2014, **159**:1027–1041.

39. Guo Y, Li D, Zhang S, Yang Y, Liu J-J, Wang X, Liu C, Milkie DE, Moore RP, Tulu US, *et al.*: **Visualizing intracellular organelle and cytoskeletal interactions at nanoscale resolution on millisecond timescales**. *Cell* 2018, **175**:1430–1442. e1417.

40. Lu M, van Tartwijk FW, Lin JQ, Nijenhuis W, Parutto P, Fantham M, Christensen CN, Avezov E, Holt CE, Tunnacliffe A, *et al.*: **The structure and global distribution of the endoplasmic reticulum network is actively regulated by lysosomes**. *Sci Adv* 2020, **6**, eabc7209.

41. Song Y, Huang P, Liu X, Cui B, Duan L: **Light-inducible deformation of mitochondria in live cells**. *bioRxiv* 2020. 2020.11.01.363663.

42. Schimert KI, Budaitis BG, Reinemann DN, Lang MJ, Verhey KJ: **Intracellular cargo transport by single-headed kinesin motors**. *Proc Natl Acad Sci Unit States Am* 2019, **116**:6152.

43. Engelke MF, Winding M, Yue Y, Shastry S, Teloni F, Reddy S, Blasius TL, Soppina P, Hancock WO, Gelfand VI, *et al.*: **Engineered kinesin motor proteins amenable to small-molecule inhibition**. *Nat Commun* 2016, **7**:11159.

44. Engelke MF, Waas B, Kearns SE, Suber A, Boss A, Allen BL, Verhey KJ: **Acute inhibition of heterotrimeric kinesin-2 function reveals mechanisms of intraflagellar transport in mammalian cilia**. *Curr Biol* 2019, **29**:1137–1148. e1134.

45. Strickland D, Lin Y, Wagner E, Hope CM, Zayner J, Antoniou C, Sosnick TR, Weiss EL, Glotzer M: **TULIPs: tunable, light-controlled interacting protein tags for cell biology**. *Nat Methods* 2012, **9**:379–384.

46. Guntas G, Hallett RA, Zimmerman SP, Williams T, Yumerefendi H, Bear JE, Kuhlman B: **Engineering an improved light-induced dimer (iLID) for controlling the localization and activity of signaling proteins**. *Proc Natl Acad Sci Unit States Am* 2015, **112**:112.

47. Wang H, Vilela M, Winkler A, Tarnawski M, Schlichting I, Yumerefendi H, Kuhlman B, Liu R, Danuser G, Hahn KM: **LOVTRAP: an optogenetic system for photoinduced protein dissociation**. *Nat Methods* 2016, **13**:755–758.

48. Kawano F, Suzuki H, Furuya A, Sato M: **Engineered pairs of distinct photoswitches for optogenetic control of cellular proteins**. *Nat Commun* 2015, **6**:6256.

49. Kennedy MJ, Hughes RM, Peteya LA, Schwartz JW, Ehlers MD, Tucker CL: **Rapid blue-light–mediated induction of protein interactions in living cells**. *Nat Methods* 2010, **7**:973–975.

50. Nihongaki Y, Suzuki H, Kawano F, Sato M: **Genetically engineered photoinducible homodimerization system with improved dimer-forming efficiency**. *ACS Chem Biol* 2014, **9**:617–621.

51. Redchuk TA, Omelina ES, Chernov KG, Verkhusha VV: **Near-infrared optogenetic pair for protein regulation and spectral multiplexing**. *Nat Chem Biol* 2017, **13**:633–639.

52. Kapitein Lukas C, van Bergeijk P, Lipka J, Keijzer N, Wulf Phebe S, Katrukha Eugene A, Akhmanova A, Hoogenraad Casper C: **Myosin-V opposes microtubule-based cargo transport and drives directional motility on cortical actin**. *Curr Biol* 2013, **23**:828–834.

53. Miyamoto T, DeRose R, Suarez A, Ueno T, Chen M, Sun T-p, Wolfgang MJ, Mukherjee C, Meyers DJ, Inoue T: **Rapid and orthogonal logic gating with a gibberellin-induced dimerization system**. *Nat Chem Biol* 2012, **8**:465–470.

54. Ballister ER, Ayloo S, Chenoweth DM, Lampson MA, Holzbaur ELF: **Optogenetic control of organelle transport using a photocaged chemical inducer of dimerization**. *Curr Biol* 2015, **25**:R407–R408.