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Review

Right Time, Right Place: Probing the Functions of Organelle Positioning

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The proper spatial arrangement of organelles underlies many cellular processes including signaling, polarization, and growth. Despite the importance of local positioning, the precise connection between subcellular localization and organelle function is often not fully understood. To address this, recent studies have developed and employed different strategies to directly manipulate organelle distributions, such as the use of (light-sensitive) heterodimerization to control the interaction between selected organelles and specific motor proteins, adaptor molecules, or anchoring factors. We review here the importance of subcellular localization as well as tools to control local organelle positioning. Because these approaches allow spatiotemporal control of organelle distribution, they will be invaluable tools to unravel local functioning and the mechanisms that control positioning.

Trends

Many organelles have very distinct subcellular localizations, especially in cells that are polarized and/or spatially extended.

Many cellular processes, such as migration, neurite outgrowth, microvilli formation, and mitosis, correlate with well-defined organelle repositioning and indicate spatially defined activities of organelles.

Traditional methods to interfere with organelle positioning, such as disrupting cytoskeletal dynamics or motor protein activity, have many side effects but suggest a role for organelle positioning in many cellular processes.

New strategies that employ recruitment of selected motors, adaptors, or anchoring molecules to specific organelles can now be used to control organelle positioning with spatiotemporal precision and to elucidate the precise connection between organelle position and function.

Organelle Positioning often Correlates with Function

Organelles are confined compartments within cells and carry a specialized set of molecules to perform specific functions. Distinct organelle positioning and spatially defined activities of organelles occur in many cell types but they are especially apparent in cells that are either polarized, spatially extended, or both, such as epithelial cells, muscle syncytium, or neurons (Figure 1). For example, the nucleus is typically centrally located with the centrosome and Golgi apparatus in close proximity, whereas the endoplasmic reticulum (ER) often spreads throughout the cytoplasm. Elements of the secretory and endosomal systems are more dynamically located because they shuttle between the Golgi apparatus, the plasma membrane (PM), and lysosomes. In recent years evidence has accumulated that cells exploit the subcellular distribution of organelles to locally orchestrate cellular processes including signaling, polarization, and outgrowth. These developments have sparked an interest in exploring non-canonical functions of organelles that depend on proper spatial arrangement.

Local organelle positioning is often a two-step process that involves active transport followed by immobilization. Active transport is driven by cytoskeletal motor proteins that can move directionally along either of two types of cytoskeletal biopolymers: actin filaments or microtubules [1]. Actin facilitates the motility of motor proteins of the myosin superfamily, whereas microtubules serve as tracks for two families of motor proteins, kinesin and dynein, which move towards the microtubule plus end or minus end, respectively. Most cytoskeletal motor proteins associate with cargoes through their tail domain, often mediated by specific adaptor molecules that regulate particular motor–cargo interactions [2,3]. These interactions determine cargo loading as well as off-loading in space and time, and, together with the activity of controlled anchoring factors [4], define cargo distribution. Interestingly, motor proteins can also operate as anchoring

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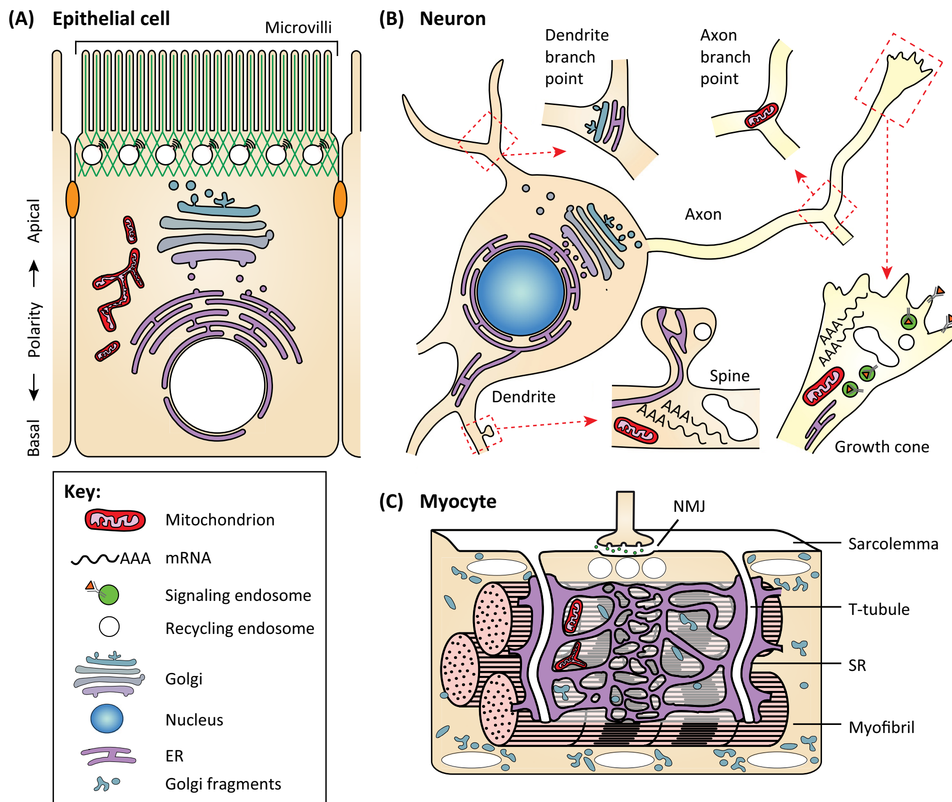

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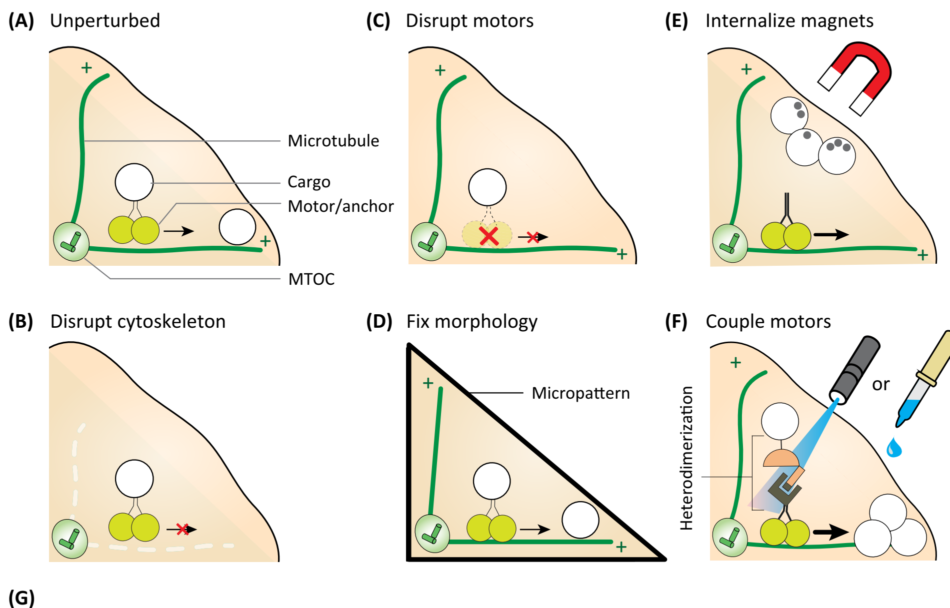
Figure 1. Functional Subcellular Organelle Positioning in Different Cell Types. (A) In epithelial cells the nucleus is located more basally, with the endoplasmic reticulum (ER) and Golgi oriented towards the apical side. In epithelial cells with microvilli, recycling endosomes and their associated kinases are positioned underneath the apical membrane to induce actin polymerization and microvilli formation. The green lines are actin. (B) In neurons, the ER can be found in the cell body, near axonal growth cones, in the dendritic shaft, and in dendritic spines. The Golgi resides in the cell body but is oriented towards the biggest dendrite or the emerging axon in developing neurons. Mitochondria localize near dendritic spines, axonal branch points, and growth cones, where they are believed to satisfy local energy demand. mRNA is locally translated in axonal growth cones and dendritic spines to allow LTP induction and growth-cone turning upon encountering extracellular cues. A pool of recycling endosomes is stored at the growth-cone tip and at the base of dendritic spines to allow rapid delivery of membranes and receptors to the surface when needed. In the opposite direction, signaling endosomes travel from the growth cone towards the cell body upon ligand-induced receptor internalization. (C) Myocytes have a specific organelle distribution, with the sarcoplasmic reticulum (SR) being aligned between the t-tubule and mitochondria to control calcium homeostasis during muscle excitation and contraction. The typical Golgi ribbon is dispersed into smaller Golgi stacks that concentrate around the nuclei that are evenly spaced along the muscle fiber. In addition, a few nuclei are anchored underneath the neuromuscular junction (NMJ), presumably to aid the local translation of specialized mRNAs.

factors. For example, dynein positions the Golgi apparatus [5], whereas several myosin motors can oppose microtubule-based transport and anchor cargoes [6–8].

Given the important roles of cytoskeletal and motor proteins, many conventional techniques have been aimed at manipulating the activity of these proteins to better understand the roles of organelle position. However, recent technical advances, such as optogenetics, are beginning to provide newer and more accurate ways to manipulate organelle positioning. We review here the importance of organelle localization and highlight several exciting strategies to manipulate organelle positioning that have recently emerged. We briefly discuss existing evidence for the importance of positioning of larger organelles such as the nucleus, ER, and Golgi apparatus, followed by a more extensive discussion about the distribution of dynamic organelles such as mRNA granules, mitochondria, endosomes and lysosomes, and inter-organelle contact sites.

Conventional Methods to Interfere with Organelle Positioning

Because the cytoskeletal network is crucial for proper cellular organization (Figure 2A), disrupting the microtubule network using microtubule-targeting agents has been frequently used to alter organelle positioning [9,10] (Figure 2B). For example, destabilizing microtubules using nocodazole results in the dispersion of lysosomes and the Golgi apparatus [11,12]. Although affecting



(G)

Tool	Advantages	Side effects	Disadvantages
Conventional techniques			
Unperturbed	–	No	Only descriptive
Disrupt cytoskeleton	Chemical inhibitors are widely available and inexpensive	Disrupts cell morphology	Typically not acute
Disrupt motors	Drug treatments and knockdowns are relatively straightforward	Affects multiple organelles	Typically not acute
Emerging techniques			
Fix morphology	Unifies the spatial organization of organelles	No	Only descriptive, additional tools needed for perturbations
Internalize magnets	Endocytosed magnetic particles can precisely mobilize targeted organelles	No	Only possible for endocytic compartments Not for *in vivo* use
Couple motors	Recruitment of specific motors to cargo of interest can drive motility in a specified direction	No	Not reversible (drugs) Can be leaky not for *in vivo* use (drugs)

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Figure 2. Different Approaches To Manipulate Subcellular Organelle Positioning. (A) In eukaryotic cells, cytoskeletal motor proteins control the transport and positioning of proteins, RNAs, and organelles. (B) Modifying cytoskeletal dynamics or microtubule post-translational modifications (PTMs) alters how motor proteins distribute organelles. (C) Changing motor protein abundance, activity, speed, or direction can reposition organelles. (D) Plating cells on a micropattern of adhesion molecules unifies organelle distribution and allows systematic analysis of organelle positioning. (E) Organelles loaded with magnetic nanoparticles can accumulate at sites where local magnetic forces are applied. (F) Physically linking motors or anchors to organelles using chemically or light-induced heterodimerization can relocate specific organelles with spatiotemporal precision. (G) Properties of organelle-repositioning tools.

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cytoskeletal build-up or breakdown is an effective method to alter cellular organization, manipulating tubulin post-translational modifications (PTMs) [13] or structures might be more subtle. PTMs are believed to affect microtubule stability and motor protein preferences. For example, treatment with the α-tubulin-specific deacetylase inhibitor tubacin redirected JIP1 (JNK-interacting protein 1) from a subset to nearly all neurite tips [14]. Similar effects have been observed upon treatment with the microtubule-stabilizing agent taxol [15,16]. Thus, modifying cytoskeletal dynamics or microtubule PTMs are methods to disrupt cargo distributions. Nevertheless, changing the cytoskeleton does not only affect specific organelles of interest but also dramatically changes cell morphology, often leading to many unwanted side effects.

Specific motor–cargo and docking factor–cargo interactions can control organelle localization and their activity can be artificially enhanced or reduced to alter organelle positioning [17,18] (Figure 2C). For example, KIF5 (kinesin family protein 5) overexpression has been used to reduce the perinuclear accumulation of lysosomes [19], whereas the knockout of the mitochondrial anchor syntaphilin was shown to increase mitochondrial motility in mouse hippocampal axons [18]. Small molecule inhibitors can also be used and are effective within minutes. For example, to study the role of RAB11-positive recycling endosomes (Ras-related protein 11) in recycling endosomes in organizing and orienting the mitotic spindle, a dynein inhibitor was used to deplete the centrosomal RAB11 pool during mitosis [20]. Thus, changing motor protein abundance or activity can relocate a subset of organelles due to specific motor–cargo interactions. Nevertheless, a single class of motor proteins generally binds more than one type of cargo, and will therefore disrupt the positioning of multiple types of organelles.

Roles of Organelle Positioning

Below we highlight recent insights into the roles of organelle positioning. Most of these insights have emerged either by observing co-occurrence of organelle movements with the process of interest or by disrupting organelle distributions through changes in cytoskeletal dynamics or motor protein activity, as described above.

Large Organelles: Nucleus, ER, Golgi Apparatus

The nucleus is one of the biggest organelles in eukaryotic cells and is often represented as a centralized and stationary organelle. However, in many cell types the nucleus is neither centrally located nor immotile [21]. Imaging of migrating cells revealed that the nucleus moves away from the leading edge to position the stationary microtubule-organizing center (MTOC) between the nucleus and the leading edge, which is needed for directed migration [22–24]. In epithelial cells nuclei are usually observed closer to the basolateral side [25] (Figure 1A), but can move apically before mitosis. Impairing nuclear migration by interfering with the typical actomyosin distribution showed that migration of the nucleus towards the apical side is necessary for reintegration of daughter cells into the developing epithelium [26]. In the multinucleated muscle syncytium most of the nuclei are evenly spaced along the fiber axis [27], but a few nuclei are anchored underneath acetylcholine receptor clusters at the post-synapse of the neuromuscular junction (NMJ) (Figure 1C), presumably to aid local translation of specialized mRNAs near the NMJ [28–31].

The ER is continuous with the outer membrane of the nuclear envelope and often spreads throughout the whole cytoplasm. In neurons, the ER enriches at dendritic branchpoints (Figure 1B). Changing the ER localization and complexity by altering the activity of an ER–microtubule anchoring protein revealed the involvement of these local ER structures in the formation of new branches [32]. ER can also be found in the axon where it processes locally synthesized proteins. Interestingly, drug-induced disruption of ER–Golgi trafficking prevented repulsive axon guidance, indicating a role for local ER-processed proteins in growth-cone responses [33]. The muscle syncytium features a specialized ER, known as the sarcoplasmic

reticulum (SR), that specifically aligns perpendicular to invaginations of the PM (Figure 1C) and is involved in Ca^{2+} signaling upon muscle excitation [34,35].

Secreted proteins often travel via the Golgi apparatus, and its location can bias the membrane targeted for secretion and hence influence cellular polarization. For example, Golgi outposts enrich at dendritic branchpoints (Figure 1B). Because blocking both ER-to-Golgi and late secretory trafficking led to reduced dendritic outgrowth and branching, Golgi outposts were proposed to provide membranes necessary for the newly-forming neurite and branch to grow [36]. Interestingly, Golgi outposts at dendritic branchpoints were also found to colocalize with sites of microtubule nucleation. This acentrosomal nucleation was abrogated in the absence of functional γ-tubulin or the *Drosophila* homolog of AKAP450 (A-Kinase Anchor Protein 9/AKAP9), and resulted in reduced dendritic arborization [37]. In the muscle syncytium, small and fragmented Golgi stacks are associated with ER exit sites throughout the muscle fiber, but concentrate around the nuclei [38,39] (Figure 1C). In summary, the specific positioning of nuclei, the ER, and the Golgi apparatus is important for proper polarization, tissue formation, and muscle development.

Centrosomes

Centrosomes are composed of two cylindrical centrioles surrounded by a dense mass of pericentriolar material (PCM). In many cells the centrosome serves as the major MT-organizing center (MTOC) and is thought to determine the orientation of the microtubule network and the direction of post-Golgi trafficking [40]. Because centrosome relocation was observed before initial axon formation [41,42], and centrosome inactivation mediated by the red fluorescent protein KillerRed affected axon formation [43], the centrosome has been suggested to play a role in neuronal axon formation by providing the necessary membranes and proteins. However, because mutant flies lacking centrosomes still have normal axon outgrowth [44], centrosome ablation does not prevent axon outgrowth in rat hippocampal neurons [45], and centrosome localization does not predict the site of axon formation in zebrafish retinal ganglion cells [46], the exact role of the centrosome in neuronal polarization has remained elusive.

mRNA Granules and Ribosomes

The subcellular storage of mRNA molecules and ribosomes allows local protein synthesis when circumstances require and can be used to establish gradients of protein concentration. For example, during the attractive turning of axonal growth cones, β-actin mRNA was shown to relocate towards the side of the growth cone nearest to the attractive cue (Figure 1B). Inhibition of β-actin synthesis by antisense morpholino oligonucleotides prevented the increase in actin polymerization and growth-cone turning, indicating that local mRNA translation is required for attractive growth-cone turning [47,48]. Similarly, repulsive cues induce the accumulation of mRNAs encoding for proteins involved in actin depolymerization, resulting in local growth-cone withdrawal [49,50]. In addition, it was shown that polyribosomes and a subset of mRNAs specifically localize to dendritic spines [51,52], where local translation is linked with synaptic plasticity [53–55].

Mitochondria

Mitochondria are often distributed throughout the cell and fulfill diverse functions, including ATP production, calcium buffering, and signal transduction [56]. Nevertheless, their localization is very specific and precisely regulated. Both adaptor proteins and anchoring factors have been reported to sense Ca^{2+} concentrations, resulting in mitochondria enrichment at sites with increased Ca^{2+} levels, such as presynaptic boutons [57,58] (Figure 1B). In hippocampal neurons from syntaphilin knockout mice, increased mitochondrial motility correlates with increased pulse-to-pulse variability in presynaptic strength, showing that stable mitochondria facilitate robust presynaptic transmission [59]. In addition, stalled mitochondria at axonal branchpoints

have been correlated with branch stabilization. Mito-KillerRed-mediated ablation of these mitochondria impaired branch formation, demonstrating a role for these mitochondria in branch stabilization [60,61].

In muscle cells mitochondria reside at the ER–PM junctions and take up Ca^{2+} upon muscle excitation [35] (Figure 1C). In epithelial cells, mitochondria are uniformly distributed under normal conditions [62], but enrich anteriorly in some epithelial cancer cells. Perturbing the asymmetric distribution through overexpression or knockdown of mitochondria fusion, fission, or motor adaptor molecules in these cancer cells resulted in reduced cell migration. This indicates that the apical accumulation of mitochondria is important for cancer cell migration and promotes cancer invasion and metastasis [63]. Mitochondria also enrich at the cleavage furrow during cytokinesis [64], and relocate to distinct subcellular regions upon differentiation of professional secreting cells [65], but the function of mitochondria in these processes and the importance of their positioning have not been resolved.

Early Endosomes

Endocytosis has traditionally been considered as a pathway for the degradation and deactivation of surface receptors that are internalized in response to ligand binding. However, it has become clear that the tails of internalized receptors can associate with signaling targets on the endosome, resulting in signaling regulation. In addition, multiple endosome-associated adaptors and scaffold proteins on the endosome can function as sites of signaling complex assembly to spatially regulate signaling [66–68].

In zebrafish neural precursor cells, the asymmetric distribution of Sara endosomes has been linked to asymmetric cell division. Sara endosomes are early endosomes containing Notch receptors, and are labeled by the endosomal adaptor protein Sara. Increasing the level of Sara endosome asymmetry by means of expressing a constitutively-active RAB5 resulted in more asymmetric cell divisions. This shows that directional trafficking of endosomes within the mother cell can determine whether cells divide asymmetrically and subsequently define cell fate [69,70]. In summary, early endosomes are sites of protein-complex assembly that concentrate signaling events, and the motility of these endosomes is used to propagate signals to different cellular compartments. The correct localization of early endosomes is therefore crucial for proper cellular functioning.

Recycling Endosomes

Internalized membranes and receptors can be degraded by the lysosome or recycled back to the PM, either directly or via a recycling compartment. Recycling endosomes (REs) are marked by the RAB11 GTPase, which is the key regulator of RE trafficking. Because REs can be localized close to the cell surface, cells can respond quickly to external stimuli through local exocytosis of REs, resulting in dynamic reorganization of the cell surface [71]. For example, in response to neuronal growth factor (NGF) REs and its cargo NgCAM (neuron–glia cell adhesion molecule) are redirected towards the PM in PC12 cells. Overexpression of the dominant negative form or RAB11 abrogates the relocation of REs and impairs NGF-induced neurite formation [72]. Similarly, the recycling of RE into the distal axon was shown to stimulate axon growth through the increased delivery of tropomyosin-related kinase (TRK) receptors and β1-integrins [73–75]. Moreover, the membrane protein VAMP2 (vesicle-associated membrane protein 2) is initially delivered to both axons and dendrites, but the preferential recycling of VAMP2 from the dendritic membrane results in the axonal localization of VAMP2 [76]. These results suggest that outgrowth and polarization in developing neurons depends on the selective activity of REs.

During long-term potentiation (LTP), which is the enlargement and strengthening of excitatory synapses upon repeated activation, more AMPA-type glutamate receptors (AMPAR) are

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inserted into the post-synapse. This process co-occurred with membrane trafficking from REs to the spine surface. Disrupting vesicle recycling by interfering with the actin-based motor protein myosin Vb, by overexpression of a dominant-negative form of RAB11, or by blocking membrane fusion hampered LTP-induced spine enlargement. This indicates that the relocation of REs is essential for LTP induction [77,78], consistent with recent mathematical modeling showing that positioning REs within spines increases the chance that exocytosed AMPA receptors will be incorporated into the post-synapse [79].

In addition to delivering membranes and other cargoes, REs can also function as signaling hubs. REs carry kinases and other regulatory factors, such as activators of actin nucleation, and their position within the cell can locally enhance signaling processes or the polymerization of actin. In enterocytes, REs are believed to dictate where microvilli form, and this is closely linked to myosin-V-dependent positioning of REs at the apical end of the cell (Figure 1A). Patients suffering from microvilli inclusion disease, which is associated with mutations in myosin Vb, cannot position REs apically, resulting in microvilli atrophy, nutrient malabsorption, and the appearance of microvilli inclusions near the nucleus, where $RAB11^+$ REs are enriched [80,81].

Lysosomes

During autophagy, intracellular proteins and organelles are enclosed by a double membrane, delivered to lysosomes, and subsequently degraded. The induction of autophagy is highly regulated. In the presence of sufficient nutrients, ATP, and oxygen, the master regulator mTORC1 (mammalian target of rapamycin complex 1) is recruited to and activated at lysosomes [82–84] where it inhibits autophagy and facilitates protein synthesis and cell growth.

Nutrient-induced activation of mTORC1 was recently shown to correlate with relocation of lysosomes from the perinuclear area towards the cell periphery. Remarkably, the forced peripheral localization of lysosomes induced by overexpression of KIF2A, KIF1B-β, or the ARL8 (ADP-ribosylation factor-like 8) adaptor, enhanced mTORC1 activation upon nutrient recovery, showing that the nutrient response is modulated by the position of lysosomes [11]. Interestingly, in striatal cell lines derived from mutant huntingtin (mHTT) knock-in mice, which models Huntington's disease (HD), a severe perinuclear localization of lysosomes and an increase in mTORC1 activity were observed under basal conditions [19].

The clearance of toxic copper accumulations in hepatocytes is accomplished by the ATP7B ATPase and copper transporter that pumps copper from the cytosol into lysosomes. Upon high copper concentrations, ATP7B repositions from the Golgi to the lysosome, pumps copper, and recruits dynein to induce the relocation of lysosomes to the canalicular surface for lysosomal exocytosis. The ATP7B-mediated dynein-driven delivery of lysosomes to the canalicular surface is thus required for copper detoxification [85].

In addition to affecting autophagy and copper clearance, the centrifugal movement of lysosomes has also been shown to stimulate cell migration and motility, perhaps owing to increased delivery of adhesion molecules, signaling scaffolds, and acid hydrolases via lysosomal exocytosis [86]. Thus, nutrient-induced mTORC1 activation, cell motility, and cell spreading can all depend on the positioning of lysosomes.

Inter-Organelle Contact Sites

Most organelles are membranous compartments that carry a specialized set of molecules to perform specific biochemical reactions. However, exchange of lipids, metabolites, and Ca^{2+} between organelles is often required and can be achieved through membrane contact sites (MCS) [35]. For example, the ER contacts the PM, mitochondria, endosomes, and the Golgi [87–93], whereas contact sites between mitochondria and the same cellular structures have also

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been described [56], as well as lysosome–peroxisome [94], Golgi–PM [95] and Golgi–lysosome interactions [96].

Many different lipid species constitute the cellular membranes, with each organelle having its own characteristic lipid composition. Most lipids are made in the ER and can be trafficked to other organelles via vesicular transport or via lipid-transport proteins (LTPs), which are often targeted to MCSs and can interchange one lipid molecule at a time. For example, phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidylcholine (PC) are exchanged between the ER and mitochondria at MCSs [35]. In addition, recent studies showed that specific oxysterol-binding protein (OSBP)-related proteins (ORPs) mediated the exchange of PS and phosphatidylinositol 4-phosphate (PI4P) at ER–PM contact sites. Interestingly, artificial recruitment of ER-bound ORP5 to the PM, but not of a truncated and cytosolic form of ORP5, could change PS and PI4P levels [97,98]. These results show that MCSs are required for lipid exchange and the correct lipid distribution among organelles.

The classical example of a Ca^{2+} response in which close organelle contacts are absolutely required is excitation–contraction coupling in muscle fibers. The initial Ca^{2+} influx triggers a more pronounced Ca^{2+} release from the SR, leading to myosin activation and muscle contraction. To restore Ca^{2+} levels in the SR, the luminal Ca^{2+} sensor STIM1 (stromal interaction molecule 1) relocates to ER–PM junctions (Figure 1C) where it opens Ca^{2+} channels in the PM, which allows the SR to restore its Ca^{2+} levels [99]. A comparable response has been reported for excitatory neuronal synapses, where synaptic activity induces the relocation of STIM2 to ER–PM junctions (Figure 1B). STIM2 relocation then leads to GLUA1 (AMPA receptor subunit) phosphorylation and delivery of AMPA receptors to the surface, resulting in synapse strengthening [100].

In addition to exchanging materials, ER-marked endosome constriction sites were observed before endosome fission, and to form a diffusion barrier for endosomal cargo. Interestingly, upon overexpression of RTN4 (reticulon 4) to elongate the ER, the number of endosome fissions were significantly reduced [101]. Similarly, ER–mitochondria contact sites were shown to colocalize with sites of mitochondria fission [102] and autophagosome formation [103]. These results indicate that the position and dynamics of the ER spatiotemporally control the fission of endosomes and mitochondria, as well as autophagosome formation. The exact role of ER contact sites in these processes is not known. The ER might function as a scaffolding platform, and it may provide ER-specific lipid species or supply Ca^{2+} to drive Ca^{2+}-dependent processes [101]. Moreover, the importance of ER-mediated fission has also remained elusive, but these events might contribute to whole cell rearrangements and sorting during mitosis, cell migration, or polarization. Importantly, altered ER–mitochondria contacts have been associated with Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis [104–106], indicating that organelle contact sites are important for proper cell functioning.

Emerging Tools to Study and Manipulate Organelle Positioning

We presented evidence above for the importance of organelle positioning in many basal cellular processes. However, a substantial amount of this evidence is based on the co-occurrence of organelle movements with the process of interest, and this does not prove a causal relationship. Other evidence is based on the effects of organelle redistribution upon disrupting cytoskeletal dynamics or motor protein activity, but these treatments can have many side effects and are often not selective for the organelles of interest. Thus, to better explore the roles of specific organelle localization, more selective tools should be used. We will now highlight different recently established approaches that allow more-controlled and selective perturbation of organelle positioning.

Manipulating Cell Morphology

To systematically study the precise connection between organelle localization and function, the spatial organization of organelles should be well defined. However, strong morphological differences are observed between cells in a population, caused by differences in local cell density and cell position within a cellular islet. The presence and distribution of cell–cell and cell–extracellular matrix (ECM) contacts influence the cytoskeletal organization and consequently organelle positioning [107,108]. To standardize the spatial cellular organization, single cells can be plated on micropatterns of cell adhesion molecules in which subtle perturbations in organelle topology can be observed and quantified [107–109] (Figure 2D). For example, the nucleus–centrosome polarity axis appeared to differ between cells plated on geometrically different fibronectin adhesive patterns [107], showing that the localization of cell–ECM contacts can affect organelle positioning. In addition to standardization, micropatterns could also be used to study how geometrical constraints affect organelle distribution [110].

Manipulating Motor Protein Activity, Speed, or Directionality Using Light

Overexpression, knockdown, or inhibition of motor proteins has extensively been used to alter organelle positioning. Recently developed approaches now allow motor activation with spatio-temporal precision using light. One strategy is to photocontrol ATP hydrolysis by incorporating photochromic molecules into the ATPase domain of motor proteins, as was done to control the motility of kinesin 5 and kinesin 1 *in vitro* [111,112]. Alternatively, light-induced uncaging of motor protein activators can be used to control motor protein processivity [113]. In addition, a recent study showed that, upon exposure to blue light, both the speed and directionality of engineered myosin VI and kinesin 14 motors could be manipulated *in vitro* [114]. However, these techniques have so far not been applied to reposition organelles and, similarly to conventional methods to alter motor activity, such manipulations would in most cases affect the positioning of multiple types of organelles.

Manipulating Organelles Using Magnetic Nanoparticles (MNPs)

Focally applied magnetic forces allow the spatial accumulation of MNPs inside living cells. MNPs can be coupled to nearly any protein or protein-binding domain, enabling the local concentration of a protein or structure of interest [115–117]. For example, magnetic nanoparticles conjugated with RAN (Ras-related nuclear protein)–GTP [118] could be used to artificially and locally polymerize microtubules. Moreover, to study the role of signaling endosomes in neurite outgrowth, neurons were loaded with MNPs coupled to TrkB-agonist antibodies that were subsequently endocytosed into signaling endosomes. Application of defined magnetic forces could pull the signaling endosomes away from the growth cone, and this resulted in stalled neurite outgrowth [115]. Importantly, the transport of other organelles such as mitochondria and vacuoles remained unchanged (Figure 2E).

The transport of MNP-targeted vesicles can be controlled very precisely; however, only endocytic compartments are suitable for ligand-mediated MNP loading. Alternative methods can deliver smaller MNPs into the cytosol and could potentially be targeted to other organelles for subsequent manipulation.

Coupling Molecular Motors or Anchors to Specific Organelles

Most cytoskeletal motor proteins associate with cargoes through their tail domain, often mediated by specific adaptor molecules that mediate specific motor–cargo interactions [2,3] These interactions determine cargo loading and offloading in space and time, and thus define cargo distribution. Therefore, physically linking a selected motor or adaptor molecule to an organelle of interest can induce organelle displacements. The role of mitochondria in axonal degeneration was studied in *C. elegans* by expressing a fusion construct of full-length kinesin 1 and the outer mitochondrial membrane protein TOM7. This approach forced mitochondria to

move from the cell body into the axon [119]. However, as with motor protein overexpression, these manipulations last for the lifetime of an organism and are not suited to achieve temporal control over organelle relocations.

The recruitment of molecular motors to cargoes using chemically induced heterodimerization does allow the controlled initiation of cargo transport. In short, organelle membrane-targeting signals can be fused to FKBP (FK506 binding protein) that, upon the addition of rapalog, can crosslink to FRB-fused motor proteins or adaptors fused to a FRB (FKBP rapamycin binding domain) [120–122] (Figure 2F). By recruiting different types of motor proteins, organelles could be forced to move anterogradely, retrogradely, or become immobilized [7,121]. Because the uptake of rapalog takes several minutes, it is difficult to precisely control the start of cargo movement. A recently developed photocaged linker that connects dihydrofolate reductase (DHFR) to the Halo-tag was recently used to instantly couple DHFR-tagged motor proteins to Halo-tagged organelles upon exposure to 385–405 nm light [123]. However, similarly to rapalog-induced heterodimerization, this interaction could not be reversed [121,124] and resulted in persistent motor–cargo interactions [123].

Table 1. Properties of Protein Heterodimerization Systems

Dimerization System[a]	Exogenous Compound	Dimerization Speed	Reversibility	Activation Light	Refs
Chemical-induced protein heterodimerization					
FKBP–rapalog–FRB	Rapalog	Minutes	No	–	[120]
GID–gibberellin–GAI	Gibberellin	Minutes	No	–	[129]
SNAPtag–CoreM–HaloTag	CoreM	Minutes	No	–	[130]
14-3-3 protein–fusicoccin–PMA	Fusicoccin	Minutes	Minutes	–	[131]
Photocaged-induced protein heterodimerization					
FKBP–photocaged rapamycin–FRB	Photocaged rapamycin	Minutes	Not shown	365–405 nm	[132,133]
SNAPtag–photo-cleavable MeNV–HaloTag	Photocleavable MeNV	Minutes	1 round, seconds	365–405 nm	[134]
DHFR–photocaged TMP/ HaloTag– Haloenzyme	Photocaged TMP/HaloTag	Seconds	Minutes (after addition of free TMP)	365–405 nm	[124]
Photo-induced protein heterodimerization					
Phytochrome–PIF	PCB	Seconds	Seconds (750 nm illumination) or hours (dark reversion)	650 nm (on) 750 nm (off)	[135,136]
FKF1–GIGANTEA	–	Seconds	No/very slow	450 nm	[137]
UVR8–COP1	–	Seconds	No	280–315 nm	[138]
VIVID A–VIVID B	–	Seconds	Seconds to hours (different variants)	470 nm	[139]
Cryptochrome–CIB	–	Seconds	Minutes	405–488 nm	[126,135]
LOVpep–ePDZ	–	Seconds	Tens of seconds to minutes for different variants	440–473 nm	[125,135]

[a]Abbreviations: COP1, constitutively photomorphogenic 1; FKF, flavin binding, kelch repeat, F-box 1; GAI, gibberellin-insensitive; GID, gibberellin-insensitive dwarf1; MeNV, methyl-6-nitroveratryl; PCB, phycocyanobilin; PIF, phytochrome-interacting factor; UVR 8, UV resistance locus 8; TMP, trimethoprim. For further abbreviations see text.

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Light-controlled motor recruitment would be ideal, because this interaction would be reversible and does not require exogenous co-factors. The blue light-induced interaction of a LOV2 (light-oxygen-voltage-sensing) domain with an engineered PDZ domain (PSD95/discs large/zonula occludens 1) [125], and the interaction between cryptochrome 2 and CIB1 (cryptochrome-interacting basic-helix-loop-helix 1) [126], have recently been exploited to recruit motors to specific organelles with spatiotemporal control [127]. Using these two systems, the motility of peroxisomes, mitochondria, and recycling endosomes could be started or stopped [127]. Targeted laser illumination could specifically deplete selected areas from peroxisomes or mitochondria, whereas recycling endosomes could be forced into specific dendritic spines, or targeted away or towards axonal growth cones. Surprisingly, the addition of recycling endosomes stimulated outgrowth, revealing that growth-cone dynamics are sensitive to endosome positioning [127]. Subsequent work extended this optogenetic approach to lysosomes [128]. Different (optogenetic) heterodimerization systems differ in sensitivity to blue light, dissociation speed, compatibility with N-terminal versus C-terminal fusions, and in their oligomerizing versus homodimerizing properties (summarized in Table 1), and can be used to manipulate organelle positioning with different dynamics.

Concluding Remarks

In recent years it has become increasingly clear that many organelles have functions that depend on proper positioning. However, in many cases the precise local functions and the molecular pathways underlying localization have remained unclear due to a lack of tools to perturb the placement of a selected organelle without off-target effects. Nonetheless, novel approaches are now emerging that use (light-sensitive) coupling of motor proteins or anchoring factors to selected organelles to spatiotemporally control organelle positioning. These tools will be valuable in addressing many outstanding questions, for example in addressing how stationary mitochondria contribute to axon branching and synapse function, how recycling endosomes contribute to polarization and axon outgrowth, and how lysosomes at the surface stimulate cell migration and motility. In addition, future research might uncover more correlations between aberrant organelle distributions and pathological conditions (see Outstanding Questions).

The majority of organelle positioning studies have so far been conducted in simple 2D cell cultures, and the importance of organelle localization in a multicellular context might be underestimated in such models. Therefore, using controlled organelle repositioning in 3D models or *in vivo* might uncover (additional) roles for proper spatial arrangements in processes such as asymmetric cell division, stem cell maintenance, and tissue formation. For all these questions, further development of the optogenetic modules will be necessary to combine on–off control of organelle transport with multi-spectral imaging without the need for exogenous compounds. Despite these challenges, future work using these exciting techniques will undoubtedly illuminate the many local functions of organelles.

Outstanding Questions

What mechanisms control organelle positioning?

How do mitochondria contribute to axon branching and synapse function?

How do recycling endosomes signal during polarization and axon outgrowth?

What is the exact role of Golgi outposts in the dendrite?

How does the centrifugal transport of lysosomes contribute to cell migration and motility?

How do ER contact sites contribute to fission of endosomes and mitochondria?

How can optical manipulation of organelle redistribution be combined with multicolor imaging?

Can organelle positioning be controlled *in vivo*?

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References

1. Vale, R.D. (2003) The molecular motor toolbox for intracellular transport. *Cell* 112, 467–480
2. Schlager, M.A. and Hoogenraad, C.C. (2009) Basic mechanisms for recognition and transport of synaptic cargos. *Mol. Brain* 2, 25
3. Fu, M-M. and Holzbaur, E.L.F. (2014) Integrated regulation of motor-driven organelle transport by scaffolding proteins. *Trends Cell Biol.* 24, 564–574
4. Sheng, Z-H. (2014) Mitochondrial trafficking and anchoring in neurons: new insight and implications. *J. Cell Biol.* 204, 1087–1098
5. Yadav, S. and Linstedt, A.D. (2011) Golgi positioning. *Cold Spring Harb. Perspect. Biol.* 3, a005322
6. Woolner, S. and Bement, W.M. (2009) Unconventional myosins acting unconventionally. *Trends Cell Biol.* 19, 245–252
7. Kapitein, L.C. *et al.* (2013) Myosin-V opposes microtubule-based cargo transport and drives directional motility on cortical actin. *Curr. Biol.* 23, 828–834
8. McIntosh, B.B. *et al.* (2015) Control of the initiation and termination of kinesin-1-driven transport by myosin-Ic and nonmuscle tropomyosin. *Curr. Biol.* 25, 523–529

9. De Brabander, M.J. *et al.* (1976) The effects of methyl (5-(2-thienylcarbonyl)-1H-benzimidazol-2-yl) carbamate (R 17934; NSC 238159), a new synthetic antitumoral drug interfering with microtubules, on mammalian cells cultured in vitro. *Cancer Res.* 36, 905–916
10. Dumontet, C. and Jordan, M.A. (2010) Microtubule-binding agents: a dynamic field of cancer therapeutics. *Nat. Rev. Drug Discov.* 9, 790–803
11. Korolchuk, V.I. *et al.* (2011) Lysosomal positioning coordinates cellular nutrient responses. *Nat. Cell Biol.* 13, 453–460
12. Cole, N.B. *et al.* (1996) Golgi dispersal during microtubule disruption: regeneration of Golgi stacks at peripheral endoplasmic reticulum exit sites. *Mol. Biol. Cell* 7, 631–650
13. Song, Y. and Brady, S.T. (2015) Post-translational modifications of tubulin: pathways to functional diversity of microtubules. *Trends Cell Biol.* 25, 125–136
14. Reed, N.A. *et al.* (2006) Microtubule acetylation promotes kinesin-1 binding and transport. *Curr. Biol.* 16, 2166–2172
15. Kapitein, L.C. *et al.* (2010) Mixed microtubules steer dynein-driven cargo transport into dendrites. *Curr. Biol.* 20, 290–299
16. Hammond, J.W. *et al.* (2010) Posttranslational modifications of tubulin and the polarized transport of kinesin-1 in neurons. *Mol. Biol. Cell* 21, 572–583
17. Hirokawa, N. *et al.* (2010) Molecular motors in neurons: transport mechanisms and roles in brain function, development, and disease. *Neuron* 68, 610–638
18. Kang, J-S. *et al.* (2008) Docking of axonal mitochondria by syntaphilin controls their mobility and affects short-term facilitation. *Cell* 132, 137–148
19. Erie, C. *et al.* (2015) Altered lysosomal positioning affects lysosomal functions in a cellular model of Huntington's disease. *Eur. J. Neurosci.* 42, 1941–1951
20. Hehnly, H. and Doxsey, S. (2014) Rab11 endosomes contribute to mitotic spindle organization and orientation. *Dev. Cell* 28, 497–507
21. Gundersen, G.G. and Worman, H.J. (2013) Nuclear positioning. *Cell* 152, 1376–1389
22. Gomes, E.R. *et al.* (2005) Nuclear movement regulated by Cdc42, MRCK, myosin, and actin flow establishes MTOC polarization in migrating cells. *Cell* 121, 451–463
23. Luxton, G.W.G. and Gundersen, G.G. (2011) Orientation and function of the nuclear-centrosomal axis during cell migration. *Curr. Opin. Cell Biol.* 23, 579–588
24. Etienne-Manneville, S. (2013) Microtubules in cell migration. *Annu. Rev. Cell Dev. Biol.* 29, 471–499
25. Koeneman, B.A. *et al.* (2010) Toxicity and cellular responses of intestinal cells exposed to titanium dioxide. *Cell Biol. Toxicol.* 26, 225–238
26. Strzyz, P.J. *et al.* (2015) Interkinetic nuclear migration is centrosome independent and ensures apical cell division to maintain tissue integrity. *Dev. Cell* 32, 203–219
27. Bruusgaard, J.C. *et al.* (2003) Number and spatial distribution of nuclei in the muscle fibres of normal mice studied in vivo. *J. Physiol.* 551, 467–478
28. Englander, L.L. and Rubin, L.L. (1987) Acetylcholine receptor clustering and nuclear movement in muscle fibers in culture. *J. Cell Biol.* 104, 87–95
29. Burke, B. and Roux, K.J. (2009) Nuclei take a position: managing nuclear location. *Dev. Cell* 17, 587–597
30. Elhanany-Tamir, H. *et al.* (2012) Organelle positioning in muscles requires cooperation between two KASH proteins and microtubules. *J. Cell Biol.* 198, 833–846
31. Chakkalakal, J.V. and Jasmin, B.J. (2002) Localizing synaptic mRNAs at the neuromuscular junction: It takes more than transcription. *Bioessays* 25, 25–31
32. Cui-Wang, T. *et al.* (2012) Local zones of endoplasmic reticulum complexity confine cargo in neuronal dendrites. *Cell* 148, 309–321
33. Merianda, T.T. *et al.* (2009) A functional equivalent of endoplasmic reticulum and Golgi in axons for secretion of locally synthesized proteins. *Mol. Cell. Neurosci.* 40, 128–142
34. Block, B.A. *et al.* (1988) Structural evidence for direct interaction between the molecular components of the transverse tubule/sarcoplasmic reticulum junction in skeletal muscle. *J. Cell Biol.* 107, 2587–2600
35. Helle, S.C.J. *et al.* (2013) Organization and function of membrane contact sites. *Biochim. Biophys. Acta* 1833, 2526–2541
36. Horton, A.C. *et al.* (2005) Polarized secretory trafficking directs cargo for asymmetric dendrite growth and morphogenesis. *Neuron* 48, 757–771
37. Ori-McKenney, K.M. *et al.* (2012) Golgi outposts shape dendrite morphology by functioning as sites of acentrosomal microtubule nucleation in neurons. *Neuron* 76, 921–930
38. Ralston, E. (1993) Changes in architecture of the Golgi complex and other subcellular organelles during myogenesis. *J. Cell Biol.* 120, 399–409
39. Lu, Z. *et al.* (2001) Golgi complex reorganization during muscle differentiation: visualization in living cells and mechanism. *Mol. Biol. Cell* 12, 795–808
40. Sütterlin, C. and Colanzi, A. (2010) The Golgi and the centrosome: building a functional partnership. *J. Cell Biol.* 188, 621–628
41. Zmuda, J.F. and Rivas, R.J. (1998) The Golgi apparatus and the centrosome are localized to the sites of newly emerging axons in cerebellar granule neurons in vitro. *Cell Motil. Cytoskeleton* 41, 18–38
42. de Anda, F.C. *et al.* (2005) Centrosome localization determines neuronal polarity. *Nature* 436, 704–708
43. de Anda, F.C. *et al.* (2010) Centrosome motility is essential for initial axon formation in the neocortex. *J. Neurosci.* 30, 10391–10406
44. Basto, R. *et al.* (2006) Flies without centrioles. *Cell* 125, 1375–1386
45. Stiess, M. *et al.* (2010) Axon extension occurs independently of centrosomal microtubule nucleation. *Science* 327, 704–707
46. Zolessi, F.R. *et al.* (2006) Polarization and orientation of retinal ganglion cells in vivo. *Neural Dev.* 1, 2
47. Leung, K-M. *et al.* (2006) Asymmetrical beta-actin mRNA translation in growth cones mediates attractive turning to netrin-1. *Nat. Neurosci.* 9, 1247–1256
48. Yao, J. *et al.* (2006) An essential role for beta-actin mRNA localization and translation in Ca^{2+}-dependent growth cone guidance. *Nat. Neurosci.* 9, 1265–1273
49. Wu, K.Y. *et al.* (2005) Local translation of RhoA regulates growth cone collapse. *Nature* 436, 1020–1024
50. Piper, M. *et al.* (2006) Signaling mechanisms underlying Slit2-induced collapse of *Xenopus* retinal growth cones. *Neuron* 49, 215–228
51. Steward, O. and Levy, W.B. (1982) Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus. *J. Neurosci.* 2, 284–291
52. Steward, O. *et al.* (1998) Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites. *Neuron* 21, 741–751
53. Kang, H. and Schuman, E.M. (1996) A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. *Science* 273, 1402–1406
54. Huber, K.M. *et al.* (2000) Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science* 288, 1254–1257
55. Holt, C.E. and Schuman, E.M. (2013) The central dogma decentralized: new perspectives on RNA function and local translation in neurons. *Neuron* 80, 648–657
56. Klecker, T. *et al.* (2014) Making connections: interorganelle contacts orchestrate mitochondrial behavior. *Trends Cell Biol.* 24, 537–545
57. Wang, X. and Schwarz, T.L. (2009) The mechanism of Ca^{2+}-dependent regulation of kinesin-mediated mitochondrial motility. *Cell* 136, 163–174
58. MacAskill, A.F. *et al.* (2009) Miro1 is a calcium sensor for glutamate receptor-dependent localization of mitochondria at synapses. *Neuron* 61, 541–555

59. Sun, T. *et al.* (2013) Motile axonal mitochondria contribute to the variability of presynaptic strength. *Cell Rep.* 4, 413–419
60. Spillane, M. *et al.* (2013) Mitochondria coordinate sites of axon branching through localized intra-axonal protein synthesis. *Cell Rep.* 5, 1564–1575
61. Tao, K. *et al.* (2014) AMP-activated protein kinase mediates activity-dependent axon branching by recruiting mitochondria to axon. *Dev. Neurobiol.* 74, 557–573
62. Fox, R.M. and Andrew, D.J. (2015) Changes in organelle position and epithelial architecture associated with loss of CrebA. *Biol. Open* 4, 317–330
63. Desai, S.P. *et al.* (2013) Mitochondrial localization and the persistent migration of epithelial cancer cells. *Biophys. J.* 104, 2077–2088
64. Lawrence, E.J. and Mandato, C.A. (2013) Mitochondria localize to the cleavage furrow in mammalian cytokinesis. *PLoS ONE* 8, e72886
65. Jin, R.U. and Mills, J.C. (2014) RAB26 coordinates lysosome traffic and mitochondrial localization. *J. Cell Sci.* 127, 1018–1032
66. Di Fiore, P.P. and De Camilli, P. (2001) Endocytosis and signaling: an inseparable partnership. *Cell* 106, 1–4
67. Seto, E.S. *et al.* (2002) When cell biology meets development: endocytic regulation of signaling pathways. *Genes Dev.* 16, 1314–1336
68. Sadowski, L. *et al.* (2009) Signaling from endosomes: location makes a difference. *Exp. Cell Res.* 315, 1601–1609
69. Bökel, C. *et al.* (2006) Sara endosomes and the maintenance of Dpp signaling levels across mitosis. *Science* 314, 1135–1139
70. Kressmann, S. *et al.* (2015) Directional Notch trafficking in Sara endosomes during asymmetric cell division in the spinal cord. *Nat. Cell Biol.* 17, 333–339
71. van Ijzendoorn, S.C.D. (2006) Recycling endosomes. *J. Cell Sci.* 119, 1679–1681
72. Shirane, M. and Nakayama, K.I. (2006) Protrudin induces neurite formation by directional membrane trafficking. *Science* 314, 818–821
73. Ascaño, M. *et al.* (2009) Axonal targeting of Trk receptors via transcytosis regulates sensitivity to neurotrophin responses. *J. Neurosci.* 29, 11674–11685
74. Eva, R. *et al.* (2010) Rab11 and its effector Rab coupling protein contribute to the trafficking of beta 1 integrins during axon growth in adult dorsal root ganglion neurons and PC12 cells. *J. Neurosci.* 30, 11654–11669
75. Takano, T. *et al.* (2012) LMTK1/AATYK1 is a novel regulator of axonal outgrowth that acts via Rab11 in a Cdk5-dependent manner. *J. Neurosci.* 32, 6587–6599
76. Sampo, B. *et al.* (2003) Two distinct mechanisms target membrane proteins to the axonal surface. *Neuron* 37, 611–624
77. Park, M. *et al.* (2006) Plasticity-induced growth of dendritic spines by exocytic trafficking from recycling endosomes. *Neuron* 52, 817–830
78. Wang, Z. *et al.* (2008) Myosin Vb mobilizes recycling endosomes and AMPA receptors for postsynaptic plasticity. *Cell* 135, 535–548
79. Kusters, R. *et al.* (2013) Shape-induced asymmetric diffusion in dendritic spines allows efficient synaptic AMPA receptor trapping. *Biophys. J.* 105, 2743–2750
80. Szperl, A.M. *et al.* (2011) Functional characterization of mutations in the myosin Vb gene associated with microvillus inclusion disease. *J. Pediatr. Gastroenterol. Nutr.* 52, 307–313
81. Dhekne, H.S. *et al.* (2014) Myosin Vb and Rab11a regulate phosphorylation of ezrin in enterocytes. *J. Cell Sci.* 127, 1007–1017
82. Sancak, Y. *et al.* (2010) Ragulator–Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell* 141, 290–303
83. Russell, R.C. *et al.* (2014) Autophagy regulation by nutrient signaling. *Cell Res.* 24, 42–57
84. Menon, S. *et al.* (2014) Spatial control of the TSC complex integrates insulin and nutrient regulation of mTORC1 at the lysosome. *Cell* 156, 771–785
85. Polishchuk, E.V. *et al.* (2014) Wilson disease protein ATP7B utilizes lysosomal exocytosis to maintain copper homeostasis. *Dev. Cell* 29, 686–700
86. Pu, J. *et al.* (2015) BORC, a multisubunit complex that regulates lysosome positioning. *Dev. Cell* 33, 176–188
87. de Brito, O.M. and Scorrano, L. (2008) Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* 456, 605–610
88. Friedman, J.R. *et al.* (2010) ER sliding dynamics and ER-mitochondrial contacts occur on acetylated microtubules. *J. Cell Biol.* 190, 363–375
89. Alpy, F. *et al.* (2013) STARD3 or STARD3NL and VAP form a novel molecular tether between late endosomes and the ER. *J. Cell Sci.* 126, 5500–5512
90. Friedman, J.R. *et al.* (2013) Endoplasmic reticulum–endosome contact increases as endosomes traffic and mature. *Mol. Biol. Cell* 24, 1030–1040
91. Ladinsky, M.S. *et al.* (1999) Golgi structure in three dimensions: functional insights from the normal rat kidney cell. *J. Cell Biol.* 144, 1135–1149
92. Mogelsvang, S. *et al.* (2004) Predicting function from structure: 3D structure studies of the mammalian Golgi complex. *Traffic* 5, 338–345
93. English, A.R. and Voeltz, G.K. (2013) Endoplasmic reticulum structure and interconnections with other organelles. *Cold Spring Harb. Perspect. Biol.* 5, a013227
94. Chu, B-B. *et al.* (2015) Cholesterol transport through lysosome–peroxisome membrane contacts. *Cell* 161, 291–306
95. Sytnyk, V. *et al.* (2002) Neural cell adhesion molecule promotes accumulation of TGN organelles at sites of neuron-to-neuron contacts. *J. Cell Biol.* 159, 649–661
96. Wang, T. and Hong, W. (2002) Interorganellar regulation of lysosome positioning by the Golgi apparatus through Rab34 interaction with Rab-interacting lysosomal protein. *Mol. Biol. Cell* 13, 4317–4332
97. Moser von Filseck, J. *et al.* (2015) Phosphatidylserine transport by ORP/Osh proteins is driven by phosphatidylinositol 4-phosphate. *Science* 349, 432–436
98. Chung, J. *et al.* (2015) PI4P/phosphatidylserine countertransport at ORP5- and ORP8-mediated ER–plasma membrane contacts. *Science* 349, 428–432
99. Lam, A.K.M. and Galione, A. (2013) The endoplasmic reticulum and junctional membrane communication during calcium signaling. *Biochim. Biophys. Acta* 1833, 2542–2559
100. Garcia-Alvarez, G. *et al.* (2015) STIM2 regulates PKA-dependent phosphorylation and trafficking of AMPARs. *Mol. Biol. Cell* 26, 1141–1159
101. Rowland, A.A. *et al.* (2014) ER contact sites define the position and timing of endosome fission. *Cell* 159, 1027–1041
102. Friedman, J.R. *et al.* (2011) ER tubules mark sites of mitochondrial division. *Science* 334, 358–362
103. Hamasaki, M. *et al.* (2013) Autophagosomes form at ER–mitochondria contact sites. *Nature* 495, 389–393
104. Area-Gomez, E. *et al.* (2012) Upregulated function of mitochondria-associated ER membranes in Alzheimer disease. *EMBO J.* 31, 4106–4123
105. Guardia-Laguarta, C. *et al.* (2014) α-Synuclein is localized to mitochondria-associated ER membranes. *J. Neurosci.* 34, 249–259
106. Stoica, R. *et al.* (2014) ER-mitochondria associations are regulated by the VAPB–PTPIP51 interaction and are disrupted by ALS/FTD-associated TDP-43. *Nat. Commun.* 5, 3996
107. Thery, M. *et al.* (2006) Anisotropy of cell adhesive microenvironment governs cell internal organization and orientation of polarity. *Proc. Natl. Acad. Sci. U.S.A.* 103, 19771–19776
108. Schauer, K. *et al.* (2010) Probabilistic density maps to study global endomembrane organization. *Nat. Methods* 7, 560–566
109. Schauer, K. *et al.* (2014) A novel organelle map framework for high-content cell morphology analysis in high throughput. *J. Biomol. Screen* 19, 317–324
110. Roth, S. *et al.* (2012) How morphological constraints affect axonal polarity in mouse neurons. *PLoS ONE* 7, e33623

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111. Ishikawa, K. *et al.* (2014) Photocontrol of mitotic kinesin Eg5 facilitated by thiol-reactive photochromic molecules incorporated into the loop L5 functional loop. *J. Biochem.* 155, 195–206
112. Kamei, T. *et al.* (2012) A photochromic ATP analogue driving a motor protein with reversible light-controlled motility: controlling velocity and binding manner of a kinesin-microtubule system in an in vitro motility assay. *Chem. Commun.* 48, 7625–7627
113. Goguen, B.N. *et al.* (2011) Light-triggered myosin activation for probing dynamic cellular processes. *Angew. Chem. Int. Ed. Engl.* 50, 5667–5670
114. Nakamura, M. *et al.* (2014) Remote control of myosin and kinesin motors using light-activated gearshifting. *Nat. Nanotechnol.* 9, 693–697
115. Steketee, M.B. *et al.* (2011) Nanoparticle-mediated signaling endosome localization regulates growth cone motility and neurite growth. *Proc. Natl. Acad. Sci. U.S.A.* 108, 19042–19047
116. Etoc, F. *et al.* (2013) Subcellular control of Rac-GTPase signalling by magnetogenetic manipulation inside living cells. *Nat. Nanotechnol.* 8, 193–198
117. Etoc, F. *et al.* (2015) Magnetogenetic control of protein gradients inside living cells with high spatial and temporal resolution. *Nano Lett.* 15, 3487–3494
118. Hoffmann, C. *et al.* (2013) Spatiotemporal control of microtubule nucleation and assembly using magnetic nanoparticles. *Nat. Nanotechnol.* 8, 199–205
119. Rawson, R.L. *et al.* (2014) Axons degenerate in the absence of mitochondria in C. elegans. *Curr. Biol.* 24, 760–765
120. Clackson, T. *et al.* (1998) Redesigning an FKBP-ligand interface to generate chemical dimerizers with novel specificity. *Proc. Natl. Acad. Sci. U.S.A.* 95, 10437–10442
121. Kapitein, L.C. *et al.* (2010) Probing intracellular motor protein activity using an inducible cargo trafficking assay. *Biophys. J.* 99, 2143–2152
122. Hoogenraad, C.C. *et al.* (2003) Bicaudal D induces selective dynein-mediated microtubule minus end-directed transport. *EMBO J.* 22, 6004–6015
123. Ballister, E.R. *et al.* (2015) Optogenetic control of organelle transport using a photocaged chemical inducer of dimerization. *Curr. Biol.* 25, R407–R408
124. Ballister, E.R. *et al.* (2014) Localized light-induced protein dimerization in living cells using a photocaged dimerizer. *Nat. Commun.* 5, 5475
125. Strickland, D. *et al.* (2012) TULIPs: tunable, light-controlled interacting protein tags for cell biology. *Nat. Methods* 9, 379–384
126. Kennedy, M.J. *et al.* (2010) Rapid blue-light-mediated induction of protein interactions in living cells. *Nat. Methods* 7, 973–975
127. van Bergeijk, P. *et al.* (2015) Optogenetic control of organelle transport and positioning. *Nature* 518, 111–114
128. Duan, L. *et al.* (2015) Optogenetic control of molecular motors and organelle distributions in cells. *Chem. Biol.* 22, 671–682
129. Miyamoto, T. *et al.* (2012) Rapid and orthogonal logic gating with a gibberellin-induced dimerization system. *Nat. Chem. Biol.* 8, 465–470
130. Erhart, D. *et al.* (2013) Chemical development of intracellular protein heterodimerizers. *Chem. Biol.* 20, 549–557
131. Skwarczynska, M. *et al.* (2013) Activation of NF-κB signalling by fusicoccin-induced dimerization. *Proc. Natl. Acad. Sci. U.S.A.* 110, E377–E386
132. Umeda, N. *et al.* (2011) A photocleavable rapamycin conjugate for spatiotemporal control of small GTPase activity. *J. Am. Chem. Soc.* 133, 12–14
133. Karginov, A.V. *et al.* (2011) Light regulation of protein dimerization and kinase activity in living cells using photocaged rapamycin and engineered FKBP. *J. Am. Chem. Soc.* 133, 420–423
134. Zimmermann, M. *et al.* (2014) Cell-permeant and photocleavable chemical inducer of dimerization. *Angew. Chem. Int. Ed. Engl.* 53, 4717–4720
135. Tischer, D. and Weiner, O.D. (2014) Illuminating cell signalling with optogenetic tools. *Nat. Rev. Mol. Cell Biol.* 15, 551–558
136. Levskaya, A. *et al.* (2009) Spatiotemporal control of cell signalling using a light-switchable protein interaction. *Nature* 461, 997–1001
137. Yazawa, M. *et al.* (2009) Induction of protein–protein interactions in live cells using light. *Nat. Biotechnol.* 27, 941–945
138. Crefcoeur, R.P. *et al.* (2013) Ultraviolet-B-mediated induction of protein–protein interactions in mammalian cells. *Nat. Commun.* 4, 1779
139. Kawano, F. *et al.* (2015) Engineered pairs of distinct photoswitches for optogenetic control of cellular proteins. *Nat. Commun.* 6, 6256