ANNUAL REVIEWS

Annual Review of Cell and Developmental Biology

Hitchhiking Across Kingdoms: Cotransport of Cargos in Fungal, Animal, and Plant Cells

Jenna R. Christensen[1] and Samara L. Reck-Peterson[1,2,3]

[1]Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, California, USA; email: jrc039@health.ucsd.edu, sreckpeterson@health.ucsd.edu

[2]Department of Biological Sciences, Cell and Developmental Biology Section, University of California, San Diego, La Jolla, California, USA

[3]Howard Hughes Medical Institute, Chevy Chase, Maryland, USA

Annu. Rev. Cell Dev. Biol. 2022. 38:155–78

First published as a Review in Advance on July 29, 2022

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

https://doi.org/10.1146/annurev-cellbio-120420-104341

Keywords

hitchhiking, motors, cytoskeleton, dynein, kinesin, myosin

Abstract

Eukaryotic cells across the tree of life organize their subcellular components via intracellular transport mechanisms. In canonical transport, myosin, kinesin, and dynein motor proteins interact with cargos via adaptor proteins and move along filamentous actin or microtubule tracks. In contrast to this canonical mode, hitchhiking is a newly discovered mode of intracellular transport in which a cargo attaches itself to an already-motile cargo rather than directly associating with a motor protein itself. Many cargos including messenger RNAs, protein complexes, and organelles hitchhike on membrane-bound cargos. Hitchhiking-like behaviors have been shown to impact cellular processes including local protein translation, long-distance signaling, and organelle network reorganization. Here, we review instances of cargo hitchhiking in fungal, animal, and plant cells and discuss the potential cellular and evolutionary importance of hitchhiking in these different contexts.

Contents

MODES OF INTRACELLULAR TRANSPORT

Eukaryotic cells actively organize their subcellular components in space and time through transport mechanisms. Intracellular components or cargos transported include messenger RNAs (mRNAs), protein complexes, organelles, vesicles, and viruses (Ali & Yang 2020, Reck-Peterson et al. 2018, Titus 2018). Their organization is important both at the cellular level and for the development and function of the organism.

Canonical Transport

In canonical transport, a motor protein attaches to a cargo of interest and walks along actin or microtubule cytoskeletal tracks to move that cargo. Both actin filaments and microtubules are polar, and different motor proteins walk in a defined direction on these polarized tracks. On actin filament tracks, different types of myosin motor proteins walk toward either the barbed or the pointed end of actin filaments (**Figure 1*a***). On microtubule tracks, most kinesin motors walk toward the plus end of microtubules, while cytoplasmic dynein-1 (hereinafter called dynein) walks toward the minus end of microtubules (**Figure 1*a***).

In most cases, motor proteins associate with cargos via adaptor proteins. Both myosin and kinesin motors have so-called head domains that interact with the cytoskeletal track and tail domains that associate directly or indirectly with cargo adaptor proteins (**Figure 1*a***). Dynein requires the cofactors dynactin and an activating adaptor to move processively on microtubules (McKenney et al. 2014, Schlager et al. 2014) (**Figure 1*a***). Dynein, kinesin, and myosin motors associate with cargos via cargo-specific proteins such as Rab GTPases, which mark distinct membrane compartments (Christensen et al. 2021, Horgan et al. 2010, Matanis et al. 2002, Siddiqui & Straube 2017, Welz & Kerkhoff 2019).

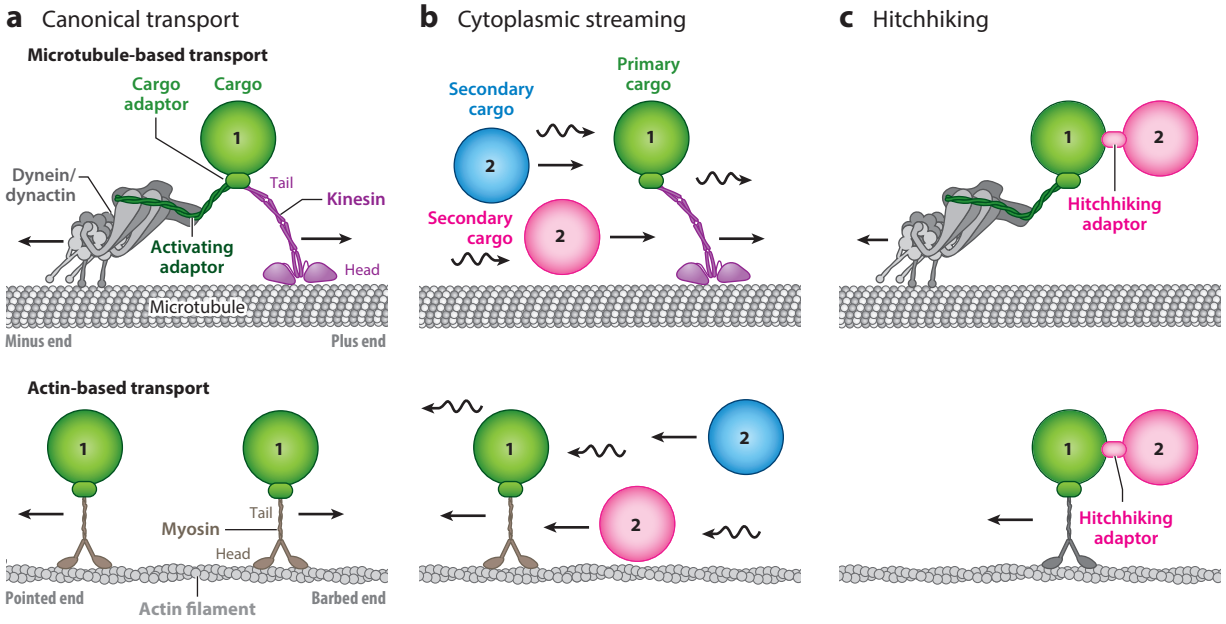

Figure 1

Modes of intracellular transport. In cells, cargo is transported via direct or indirect association with motor proteins. (*a*) In canonical transport, cargos are associated with motor proteins via cargo adaptors. (*Top*) In microtubule-based transport, activating adaptors link dynein/dynactin to cargo adaptors. Kinesin tail domains can interact directly or indirectly with cargo adaptors. (*Bottom*) In actin-based transport, myosin tail domains interact with cargo adaptors. (*b*) In cytoplasmic streaming, primary cargos (numbered 1) are transported via canonical transport. Secondary cargos (numbered 2) are cotransported via hydrodynamic flow, which results from cytoplasmic movement generated by canonically transported cargos. (*c*) In hitchhiking, primary cargos are transported via canonical transport, while secondary cargos are cotransported by directly associating with primary cargos via hitchhiking adaptor proteins.

Cytoplasmic Streaming

Though many cargos are transported via active association with a motor protein, cargos can also move through the cell via a passive form of transport known as cytoplasmic streaming (**Figure 1*b***). In cytoplasmic streaming, a motor protein carrying a cargo also moves the surrounding cytoplasm as a result of hydrodynamic flow (Esseling-Ozdoba et al. 2008). Subsequently, cargos that are located within the vicinity of the moving cargo follow the resulting cytoplasmic flow. Examples of cytoplasmic streaming have been identified across the tree of life (Allen & Allen 1978a,b; Goldstein & van de Meent 2015; Quinlan 2016).

Hitchhiking

A third form of transport known as hitchhiking was discovered more recently (Baumann et al. 2012, Guimaraes et al. 2015, Salogiannis et al. 2016). In hitchhiking, a cargo engages and is co-transported with an already-motile cargo, rather than associating directly with a motor protein (**Figure 1*c***). Here, we refer to the cargo associated with a motor protein as the primary cargo and the cargo that is cotransported as the secondary cargo. Previously, hitchhiking was proposed to be defined by three main criteria: (*a*) the comigration of the primary and secondary cargos, (*b*) an interaction between the primary and secondary cargos that does not involve membrane fusion, and (*c*) movement of the secondary cargo that requires the primary cargo but not vice versa (Salogiannis & Reck-Peterson 2017). However, it is important to state that hitchhiking is a newly

examined phenomenon, and many behaviors that could be perceived as hitchhiking-like either may not fit all three of these criteria or may require further research to elucidate whether they fit them. In this review, we explore instances of cargo hitchhiking in fungal, animal, and plant cells, with an emphasis on the current state of this emerging field. We draw connections between hitchhiking phenomena and their potential cellular and evolutionary importance, and we point out areas where further research is required to decipher whether transport is occurring by canonical modes, cytoplasmic streaming, or hitchhiking mechanisms.

Transported Cargos

As described above, intracellular transport is used to move many different cargos within the cell. In this review, we focus on the hitchhiking of mRNAs and membrane-bound organelles. In the case of mRNAs, it has not always been determined experimentally if the mRNAs are associated with ribosomes, whether these ribosomes are translationally active, or if the mRNA is part of an RNA granule. In cases in which the mRNA has been experimentally demonstrated to be associated with ribosomes or present in an RNA granule, it is displayed in the figure.

HITCHHIKING IN FUNGAL CELLS

Fungal genomes, when compared to those of plants and animals, generally have fewer encoded myosins and kinesins (Kollmar & Mühlhausen 2017, Miki et al. 2005). In fungi, hitchhiking may provide a means by which many cargos can be properly distributed despite few genome-encoded motors. Two of the primary modes of growth present in fungal species—budding and hyphal growth—both require cellular components to be appropriately distributed within a cytoplasmic space. Asexual yeast growth typically occurs through budding or fission, resulting in distributing the cytoplasmic components of the mother cell between the mother and daughter cell(s). During budding yeast division, many organelles, mRNAs, and other components are transported from the mother to the newly formed daughter bud via the unidirectional movement of myosin-V on polarized actin tracks (Bretscher 2003, Hammer & Sellers 2012). In hyphal growth, the predominant mode of growth for filamentous fungi, polarized growth occurs in one direction, requiring the transport of proteins, lipids, and other components to the site of growth (Steinberg 2007). These polarized hyphae then frequently fuse to form large syncytial networks throughout which components must be distributed. Long-distance transport in many, but not all, filamentous fungal species occurs on microtubule tracks via the motor proteins dynein and kinesin (Egan et al. 2012).

mRNA Hitchhiking in Yeast and Filamentous Fungi

Proper mRNA localization ensures that protein translation occurs at the correct time and place, resulting in the coordinated regulation of spatial events in many fungal cells. In the budding yeast *Saccharomyces cerevisiae*, mRNAs that encode polarity factors such as GTPases and their effectors are distributed from the mother cell to the growing bud tip (Aronov et al. 2007, Shepard et al. 2003). Many mRNAs are transported by the myosin-V Myo4p via an interaction with the RNA-binding protein She2p and the myosin-V adaptor She3p (reviewed in Singer-Krüger & Jansen 2014) (**Figure 2*a***).

Several mRNAs that encode polarity factors may hitchhike on the endoplasmic reticulum (ER) during its transport to the bud (Aronov et al. 2007, Schmid et al. 2006). Myo4p and its adaptor She3p both cofractionate with the ER, and deletion of Myo4p or She3p disrupts the movement of the ER to the bud tip, suggesting the involvement of She3p/Myo4p in transport of the ER (Estrada

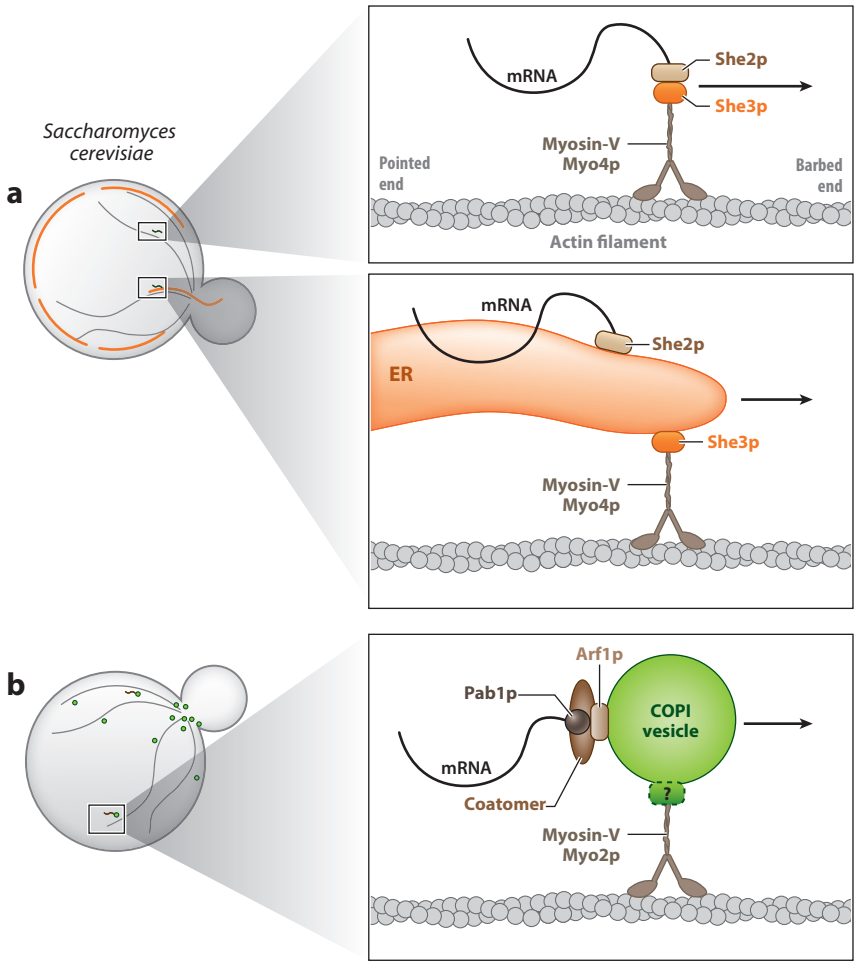

Figure 2

Messenger RNA hitchhiking in the budding yeast, *Saccharomyces cerevisiae*. (*a*) Two potential mechanisms for messenger RNA (mRNA) transport via She-dependent mechanisms in budding yeast. (*Top*) The myosin-V motor Myo4p transports mRNA on actin cables via a canonical transport mechanism. Myo4p associates directly with the cargo adaptor She3p, which forms a complex with mRNA-bound She2p. (*Bottom*) Myo4p transports mRNA via hitchhiking on the endoplasmic reticulum (ER). Myo4p associates with the ER cargo adaptor She3p. mRNA associates with the ER via the hitchhiking adaptor She2p. (*b*) A potential model for mRNA movement via hitchhiking is on coatomer-coated vesicles called COPI vesicles in budding yeast. The myosin-V motor Myo2p associates with COPI vesicles via an unknown cargo adaptor. Arf1p, coatomer, Pab1p, and mRNA form a complex on the surface of COPI vesicles, resulting in mRNA cotransport with COPI vesicles.

et al. 2003). However, deletion of either of these components also disrupts mRNA transport to the bud site, demonstrating a link between mRNA and ER transport (Aronov et al. 2007, Shepard et al. 2003).

She2p, which binds to polarity-associated mRNAs (Aronov et al. 2007), can form a complex with Myo4p and She3p. This finding initially suggested that a She2p/She3p/Myo4p complex may associate with and directly transport mRNAs by a canonical transport mechanism (Böhl et al. 2000, Long et al. 2000, Takizawa & Vale 2000) (**Figure 2*a***). However, disrupting She2p affects

the distribution of mRNA but not that of the ER (Aronov et al. 2007, Estrada et al. 2003). Furthermore, She2p is capable of binding ER membranes in the absence of She3p/Myo4p (Genz et al. 2013). Therefore, there may be two distinct mechanisms by which mRNAs are transported via She2p/She3p/Myo4p—one canonical mechanism in which the three proteins form a complex that binds mRNA and transports it along actin filaments (**Figure 2*a***), and the other via hitchhiking of mRNA associated with She2p on the ER (Fundakowski et al. 2012, Shepard et al. 2003) (**Figure 2*a***).

In contrast to mRNA transport requiring She2p/She3p/Myo4p, some mRNAs in *S. cerevisiae* may be transported by a mechanism that involves an association with vesicles called COPI vesicles. The poly-A-binding protein Pab1p associates with the 3′ end of mRNAs in budding yeast. Pab1p has been demonstrated to form a complex with the GTP-bound form of Arf1p GTPase and coatomer on the surface of COPI vesicles (Trautwein et al. 2004) (**Figure 2*b***). Knockout of secretory pathway components including Arf1 or coatomer showed defects in mRNA distribution. One interpretation of these data is that the distribution of a subset of mRNAs may occur by hitchhiking on COPI vesicles. This form of mRNA hitchhiking could exist as an additional mechanism to prevent the diffusion of mRNAs away from the bud tip (Trautwein et al. 2004).

The hitchhiking of mRNAs also occurs during hyphal growth in some fungi. *Candida albicans* is a fungus that can grow in a yeast-like budding manner, a pseudohyphal state, and a pathogenic hyphal mode (Sudbery et al. 2004). In *C. albicans*, *SEC2* mRNA associates with the Sec2 protein on secretory vesicles, suggesting that *SEC2* mRNA might hitchhike on secretory vesicles (Caballero-Lima et al. 2014) (**Figure 3*a***). Much higher levels of *SEC2* mRNA associate with Sec2p in the hyphal state versus in the yeast state of *C. albicans*, suggesting that *SEC2* mRNA hitchhiking may occur preferentially during hyphal growth. During hyphal growth, secretory vesicles are transported to the hyphal tip, likely via myosin-V as in budding yeast (Pruyne et al. 1998) (**Figure 3*a***).

In *Ustilago maydis*, mRNAs and their associated ribosomes hitchhike on early endosomes for long-distance transport (Higuchi et al. 2014) (**Figure 3*b***). Unlike the myosin-V-driven cargos of yeast, early endosomes in *U. maydis* associate with the microtubule-associated motor proteins dynein and kinesin-3 via the Hook adaptor Hok1 and associated proteins Fts1 and Fhp1 (Bielska et al. 2014). The machinery linking mRNAs to endosomes involves components that bind mRNA and the endosome. mRNAs associate with Rrm4, a protein containing three RNA-recognition motifs as well as two Mademoiselle (MLLE) domains that interact with PAM2-like motifs. Rrm4 then interacts with the PAM2-like motifs present in the *U. maydis* PAM2 proteins Upa1 and Upa2. The direct association of Rrm4 with early endosome–associated Upa1 facilitates association of the mRNA complex with early endosomes (Pohlmann et al. 2015). Upa2 serves as a scaffold for the entire complex (Jankowski et al. 2019).

Rrm4 binds thousands of mRNAs (Olgeiser et al. 2019). While the absence of Rrm4, Upa1, or Upa2 results in decreased mRNA motility, endosomal movement is not affected (Baumann et al. 2012, Jankowski et al. 2019). One of the Rrm4-binding RNAs, the *cdc3* septin mRNA, was shown to be transported on endosomes. The septin proteins Cdc3, Cdc10, Cdc11, and Cdc12 also shuttle on early endosomes (Zander et al. 2016). The absence of one septin component affects the localization and transport of the others, and the regions required for septin heterodimerization are required for endosomal localization (Zander et al. 2016). These findings suggest that endosomes are platforms for septin mRNA translation and hetero-oligomer assembly. However, how the septin hetero-oligomers associate with endosomes and whether they associate via a hitchhiking tether or via direct association with the membrane are unclear.

Whether mRNAs hitchhike in other filamentous fungi remains to be determined. All three components of the Rrm4/Upa1/Upa2 complex are conserved within the Basidiomycota (Müller et al. 2019). Homologs of Rrm4 and Upa1 containing the required domains for RNA binding, early

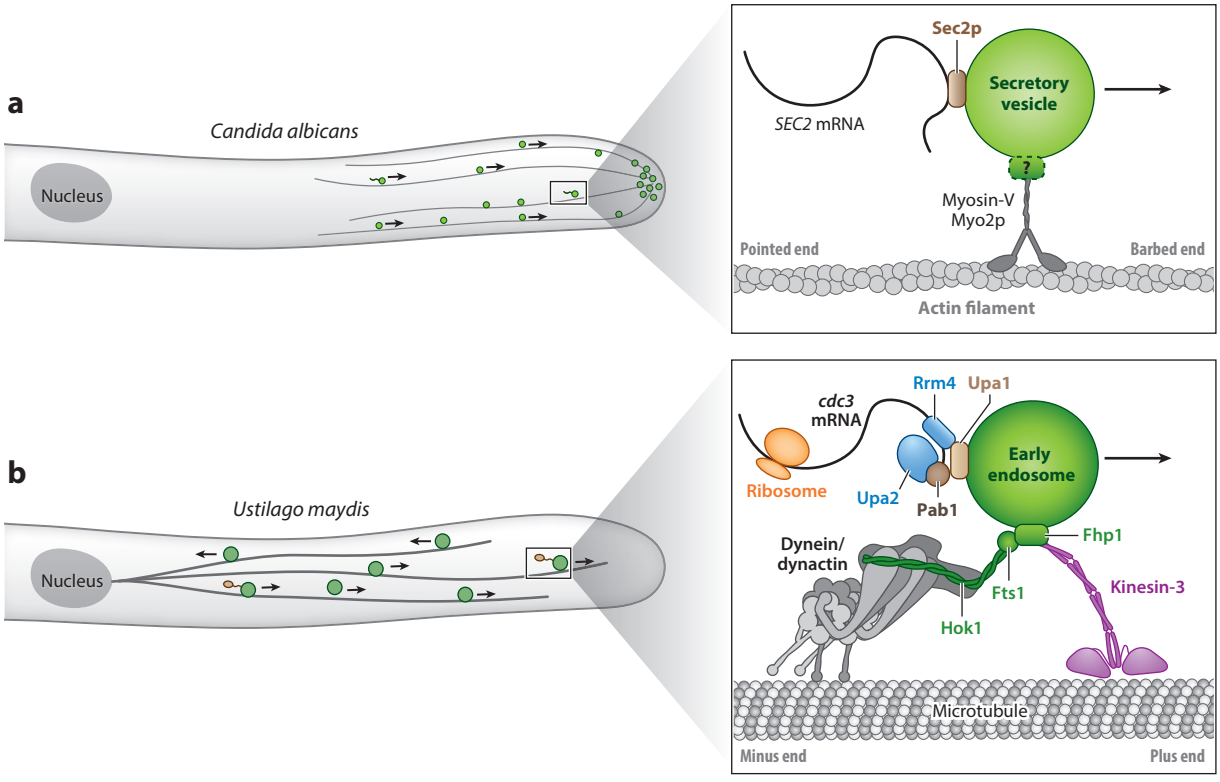

Figure 3

Messenger RNA hitchhiking in fungal hyphae. (*a*) In the hyphae of *Candida albicans*, *SEC2* messenger RNA (mRNA) hitchhikes on secretory vesicles. Secretory vesicles are transported by the myosin-V motor Myo2p via an unknown cargo adaptor. The encoded protein of *SEC2* mRNA, Sec2p, acts as a hitchhiking adaptor for *SEC2* mRNA. (*b*) In the hyphae of *Ustilago maydis*, many mRNAs, including mRNA encoding *cdc3* and its associated ribosomes, hitchhike on early endosomes. Early endosomes associate with dynein/dynactin and kinesin motors via the activating adaptor Hok1 in complex with the cargo adaptors Fts1 and Fhp1. A hitchhiking adaptor complex composed of Rrm4, Upa1, Upa2, and Pab1 links mRNA to early endosomes for cotransport.

endosome association, and complex formation are also found within the Mucoromycota phylum, and Rrm4 homologs from species within this phylum are capable of shuttling on early endosomes when expressed in *U. maydis* (Müller et al. 2019). This conservation suggests that mRNA transport may occur by a similar mechanism in these distantly related fungi. However, though an Upa1 homolog is present within the Ascomycota, the phylum that includes *Aspergillus nidulans*, this homolog does not include the required domains for Rrm4 association, and Rrm4 and Upa2 homologs are absent, suggesting that if mRNA hitchhiking occurs in *A. nidulans*, it likely uses a different mechanism.

Organelle Hitchhiking in Filamentous Fungi

In both *U. maydis* and *A. nidulans*, early endosomes are the only known platform for hitchhiking cargos. Early endosomes move on microtubules via the motor proteins dynein and kinesin-3 and the Fts-Hook-FHIP adaptor complex (Bielska et al. 2014, Yao et al. 2014, Zhang et al. 2014) (**Figure 4**). In *U. maydis*, peroxisomes, lipid droplets, and the ER all hitchhike on early endosomes (Guimaraes et al. 2015) (**Figure 4*a***). No hitchhiking tethers or regulators for the hitchhiking of

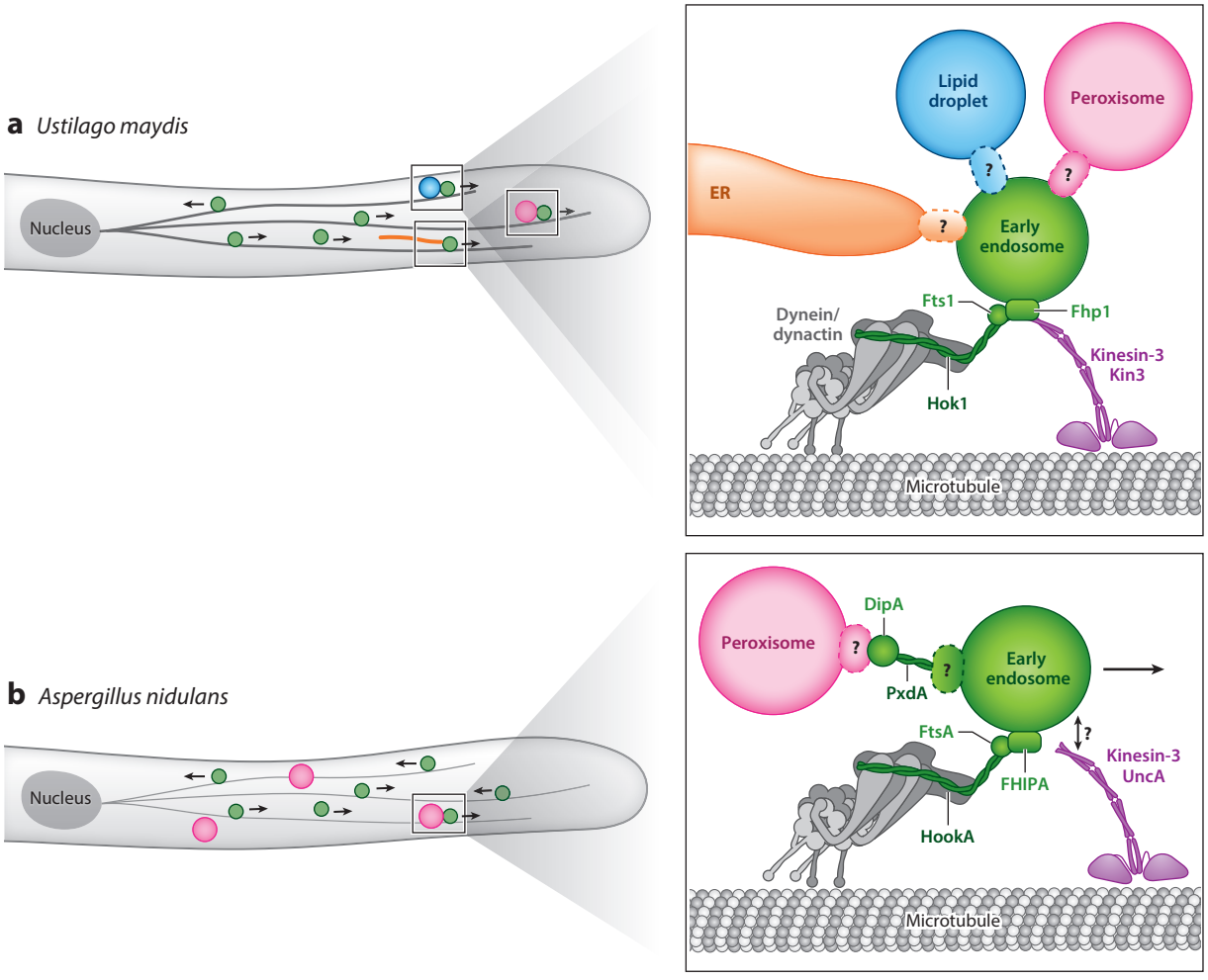

Figure 4

Organelle hitchhiking in filamentous fungi. (*a*) In *Ustilago maydis* hyphae, endoplasmic reticulum (ER), lipid droplets, and peroxisomes hitchhike on early endosomes, but the tethers mediating those interactions are unknown. Early endosomes are transported bidirectionally via the Fts1-Hok1-Fhp1 complex, which associates with dynein/dynactin and kinesin-3. (*b*) In *Aspergillus nidulans* hyphae, peroxisomes hitchhike on early endosomes via the putative hitchhiking adaptors peroxisome distribution mutant A (PxdA) and DipA. Early endosomes are transported bidirectionally. Dynein/dynactin interacts with early endosomes via the FtsA-HookA-FHIPA complex. How kinesin-3 interacts with early endosomes and whether this interaction involves the FtsA-HookA-FHIPA complex are unknown.

those organelles have been identified. As multiple organelles hitchhike in *U. maydis*, this fungus may use hitchhiking as a sort of conveyor belt to properly distribute many organelles within the hyphal network, perhaps compensating for having few motors or motor adaptors encoded in the genome. In this model, early endosomes would act as a singular primary cargo, moving up and down the hyphae via association with dynein and kinesin-3 (Bielska et al. 2014). Secondary cargos could then associate and dissociate from the early endosome, promoting their distribution across the hyphae.

In *A. nidulans*, peroxisomes are the only organelle that have been demonstrated to hitchhike on early endosomes, while mitochondria, pre-autophagosomes, and lipid droplets do not hitchhike

on early endosomes (Salogiannis et al. 2016, 2021). In *A. nidulans*, two proteins required for peroxisome hitchhiking on early endosomes have been identified: peroxisome distribution mutant A (PxdA) (Salogiannis et al. 2016) and the phosphatase DipA (Salogiannis et al. 2021) (**Figure 4*b***). Both PxdA and DipA associate with early endosomes. PxdA associates with early endosomes via tandem coiled-coil regions, while DipA depends on PxdA for its association with early endosomes (Salogiannis et al. 2016, 2021). Although PxdA has been proposed to act as a tether between endosomes and peroxisomes (Salogiannis et al. 2016), it is still unclear whether PxdA's interaction with early endosomes is direct or mediated via other binding partners, and if or how PxdA and DipA associate with peroxisomes. Furthermore, whether DipA's phosphatase activity regulates hitchhiking remains to be determined. As peroxisomes are the only cargo that has been demonstrated to hitchhike in *A. nidulans*, there may be a cellular requirement for the hitchhiking of peroxisomes specifically.

HITCHHIKING IN ANIMAL CELLS

Unlike fungi that typically exist as unicellular or hyphal networks, animal cells exist in a multicellular context. Differences in cell shape, gene expression, cytoskeletal organization, and cellular role within the organism may necessitate different transport mechanisms, including noncanonical mechanisms such as hitchhiking (Burute & Kapitein 2019). In many animal cells, much of the long-distance transport of cargos occurs on microtubules using the motor proteins dynein and kinesin.

mRNA Hitchhiking in Polarized Animal Cells

In animal cells, as in fungi, mRNA polarization is crucial for certain cellular processes and development, and mRNA hitchhiking may be one mechanism by which mRNA polarity is established in varying cell types. mRNA distribution in the oocytes of the model organisms *Drosophila melanogaster* and *Xenopus laevis* has been correlated with the transport of cargos such as COPII vesicles, recycling endosomes, and the ER, though the mechanisms driving this transport are unclear (Chang et al. 2004, Dollar et al. 2002, Jankovics et al. 2001, Wilhelm et al. 2005).

Recently, several studies have identified a link between mRNA and motile endosomes and mitochondria in neurons. Neurons are long, polarized cells in which the cell body is far away from the axon terminals (**Figure 5*a***). Therefore, as in the long hyphae of filamentous fungi, the long-distance transport of mRNAs along the axon may allow for protein translation at sites distant from the nucleus, a process critical for proper neuronal function (Fernandez-Moya et al. 2014). In animal cells, Rab5-positive early endosomes link to dynein/dynactin via the Fts-Hook-FHIP complex (Christensen et al. 2021, Guo et al. 2016). A subset of mRNAs and their associated ribosomes have been found to associate with Rab5-positive early endosomes via the five-subunit endosomal Rab5 and RNA/ribosome intermediary (FERRY) complex (Quentin et al. 2021, Schuhmacher et al. 2021). The FERRY complex consists of five subunits, designated Fy-1 through Fy-5. Fy-2 serves as the central scaffolding protein (Quentin et al. 2021) (**Figure 5*b***) and interacts directly with GTP-bound Rab5a to link the complex to endosomes (Quentin et al. 2021). mRNA association likely occurs at multiple sites across the FERRY complex but predominantly on Fy-2. The FERRY complex associates with a subset of mRNAs, including many that encode nuclear-encoded mitochondrial proteins. Both Rab5a- and Rab7a-associated motile endosomes have been demonstrated to be platforms for the transport of mRNAs and their associated ribosomes (Cioni et al. 2019). Furthermore, Rab7a-associated late endosomes harboring mRNAs are frequently found near mitochondria and can serve as platforms for the translation of mitochondrial mRNAs (Cioni et al. 2019). Together, these studies suggest that when mRNAs associate with motile early or late

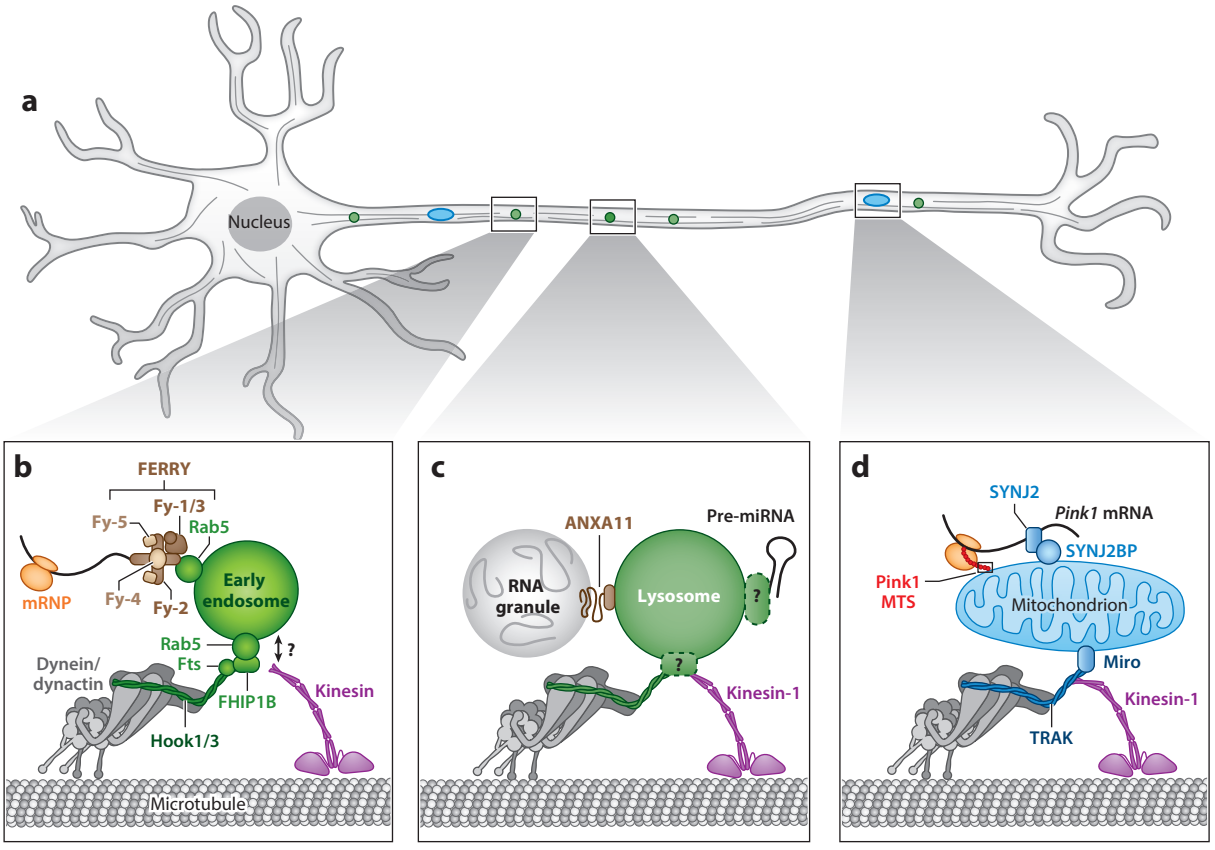

Figure 5

Messenger RNA hitchhiking in neurons. (*a*) Early endosomes, lysosomes, and mitochondria in axons are transported long distances on microtubules. Messenger RNAs (mRNAs) hitchhike on these organelles by several mechanisms. (*b*) The five-subunit endosomal Rab5 and RNA/ribosome intermediary (FERRY) complex links many mRNAs and their associated ribosomes to early endosomes via a direct interaction between the FERRY complex component Fy-2 and Rab5. Rab5 also links dynein/dynactin to early endosomes via a direct interaction with FHIP1B, in complex with Fts and Hook1 or Hook3. (*c*) RNA granules hitchhike on lysosomes via ANXA11. Precursor microRNAs (pre-miRNAs) also hitchhike on lysosomes via an unknown tether. (*d*) *Pink1* mRNA and associated ribosomes hitchhike on mitochondria via two mechanisms. *Pink1* mRNA is translated while associated with mitochondria, and the translated mitochondrial targeting sequence (MTS) associates with the mitochondria, linking the mRNA beginning to be translated and its associated ribosomes to the mitochondria as well. *Pink1* mRNA associates with synaptojanin-2 (SYNJ2), which binds mitochondria-associated synaptojanin 2–binding protein (SYNJ2BP). Mitochondria are transported via dynein/dynactin and kinesin-1 via the TRAK adaptor and Miro GTPase.

endosomes, the endosomal cargo could both assist in transporting the mRNAs to specific sites, such as mitochondria, and serve as a platform for the translation of those mRNAs (Cioni et al. 2019, Schuhmacher et al. 2021).

RNA granules have also been found to hitchhike on lysosomes using the linker protein ANXA11 (Liao et al. 2019) (**Figure 5*c***). The N-terminus of ANXA11 is capable of phase separating into RNA granules, while the annexin domains within the C-terminus of ANXA11 bind lysosomes (Liao et al. 2019). Inhibition of the dynein cofactor dynactin prevented lysosome and RNA granule cotransport (Liao et al. 2019), demonstrating the requirement of the dynein/dynactin complex for long-distance transport. Mutations in ANXA11 have been found in patients with amyotrophic lateral sclerosis (Smith et al. 2017). These mutations affected the phase transition

properties of ANXA11, the ability of ANXA11 to associate with RNA granules, and the ability of RNA granules to hitchhike on lysosomes, but they did not affect lysosome motility itself, suggesting that ANXA11 is a true hitchhiking tether (Liao et al. 2019).

In addition to mRNAs, precursor microRNAs (miRNAs) in neurons also hitchhike on motile endolysosomal cargos, predominantly on late endosomes and lysosomes (Corradi et al. 2020) (**Figure 5*c***). They are transported through the axon to growth cones, where they are processed locally to repress the translation of certain genes and influence growth cone steering (Corradi et al. 2020). One possibility is that the hitchhiking of mRNAs and miRNAs could act antagonistically, with mRNA transport positively affecting protein translation and miRNA hitchhiking inhibiting it.

Mitochondria can also serve as platforms for mRNA hitchhiking via mitochondria-specific RNA-binding proteins (RBPs) (Qin et al. 2021). Dynein/dynactin and kinesin-1 transport mitochondria via the TRAK1/2 adaptors and the mitochondria-associated GTPase Miro (Fenton et al. 2021, Henrichs et al. 2020). mRNA for *Pink1*, a protein crucial for mitophagy, and its associated ribosomes have been shown to hitchhike on motile mitochondria (Harbauer et al. 2022) (**Figure 5*d***). *Pink1* mRNA is recruited to mitochondria by two mechanisms. The translated mitochondria-targeting sequence in Pink1 recruits *Pink1* mRNA to mitochondria, suggesting a mechanism whereby *Pink1* mRNA and its associated ribosomes are recruited cotranslationally. Additionally, the RNA-binding region in synaptojanin-2 (SYNJ2) binds *Pink1* mRNA. SYNJ2 is bound by synaptojanin 2–binding protein (SYNJ2BP), a protein localized to the outer mitochondrial membrane (Harbauer et al. 2022). As the Pink1 protein is rapidly degraded in neurons, the current model is that hitchhiking and local translation of *Pink1* mRNA ensure that mitophagy can occur at sites far from the nucleus (Harbauer et al. 2022). SYNJ2 also binds mRNA transcripts that encode other mitochondrial factors, suggesting that other mitochondrial mRNAs may also hitchhike on mitochondria (Harbauer et al. 2022). Overall, the hitchhiking of RNAs on motile endosomal or mitochondrial cargos could promote the fine-tuning of protein translation in different locations and contexts—on endosomes (Popovic et al. 2020), at mitochondria (Müntjes et al. 2021), at the axon terminus (Corradi et al. 2020), and potentially at other sites.

Organelle Hitchhiking in Animal Cells

In recent years, our understanding of the importance of organelle contact sites has greatly expanded. Contact sites are involved in many cellular processes including signaling, organelle fission and fusion, and lipid transport (reviewed in Prinz et al. 2020). In addition, many of these cellular processes are regulated by their spatial organization in the cell. Consistent with a role in their spatial distribution, the microtubule network mediates many of these organelle contacts. In COS-7 cells, disruption of the microtubule network destabilizes the majority of organelle interactions (Valm et al. 2017). As there are many identified instances of contact site formation in animal cells related to different cellular processes, we focus here on instances in which an interaction between the primary and secondary cargo is linked to cargo motility.

Endoplasmic Reticulum–Endosome Contacts

In animal cells, the ER acts as a central player for many organelle contacts. These contact sites serve a variety of roles, including lipid exchange, calcium regulation, and organelle fission/fusion (reviewed in Perkins & Allan 2021). The VAMP-associated protein (VAP) family mediates the formation of many contact sites between the ER and other organelles (reviewed in James & Kehlenbach 2021). The VAP family includes VAPA, VAPB, MOSPD1, MOSPD2, and MOSPD3 (Neefjes & Cabukusta 2021). These proteins associate with the ER membrane and bind proteins

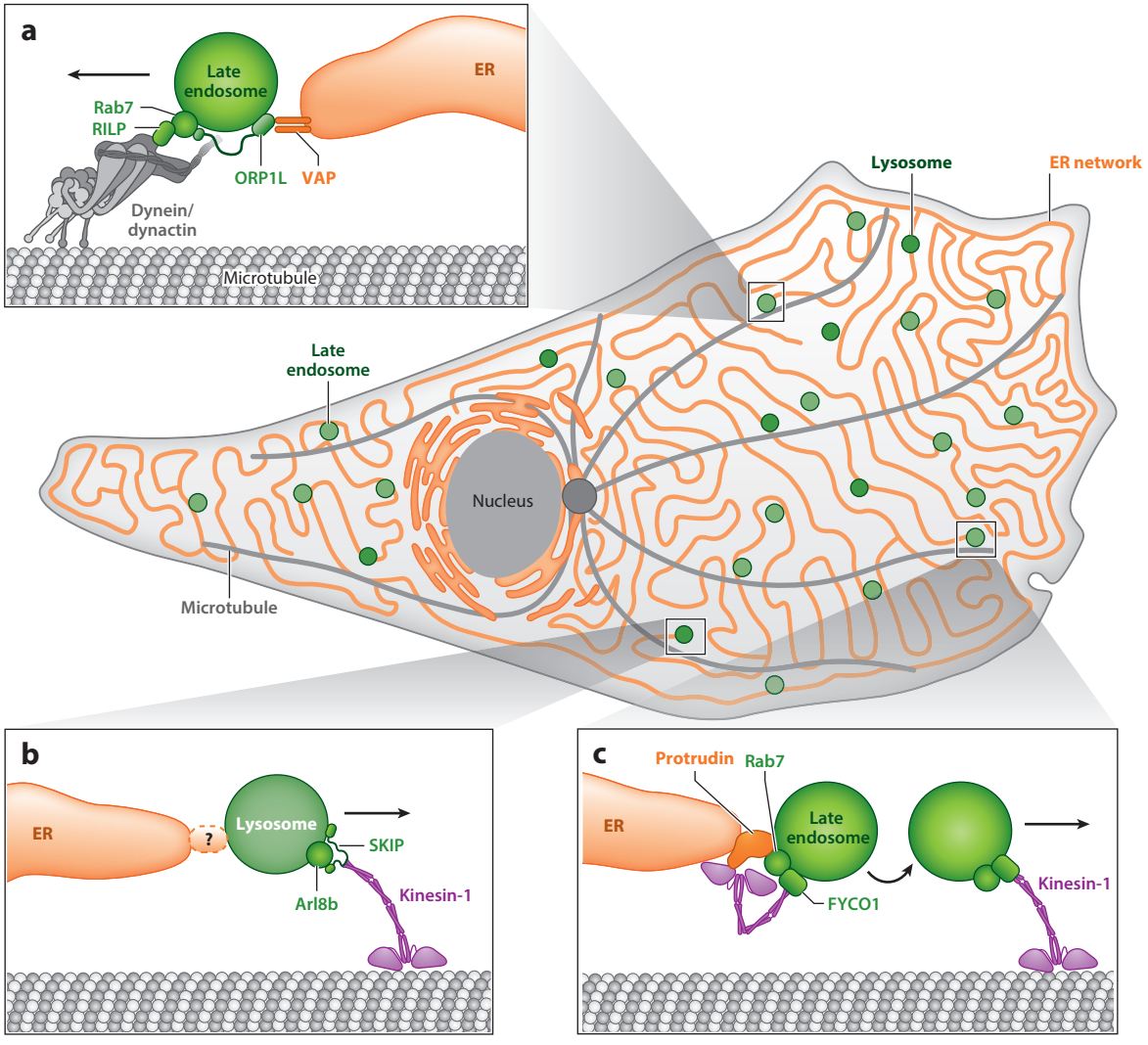

Figure 6

Potential instances of endoplasmic reticulum–endosome hitchhiking in animal cells. (*a*) The endoplasmic reticulum (ER) hitchhikes on late endosomes. The ER-associated VAMP-associated protein (VAP) interacts with ORP1L on late endosomes. ORP1L interacts with the surface of late endosomes as well as with Rab7. Rab7 also links late endosomes to dynein/dynactin via RILP. (*b*) The ER hitchhikes on kinesin-1-transported lysosomes via an unknown tether. Kinesin-1 is associated with lysosomes via Arl8b and SKIP. (*c*) The ER-associated protein Protrudin links the ER to late endosomes by binding lipids on the surface of late endosomes and Rab7. Protrudin also binds the kinesin-1 heavy chain and facilitates the transfer of kinesin-1 to FYCO1 present on late endosomes. FYCO1 then mediates the kinesin-1-dependent transport of late endosomes.

that contain FFAT/FFNT domains within other organelle membranes (Cabukusta et al. 2020, Loewen & Levine 2005). In some instances, these contacts mediate the cotransport of the ER with other organelles. Super-resolution multicolor imaging of interorganelle contacts and dynamics revealed that ER tubules contact and can be cotransported with motile late endosomes, lysosomes, and mitochondria (Guo et al. 2018) (**Figure 6**).

Multiple studies have demonstrated co-movement of the ER with endosomes (Lu et al. 2020, Özkan et al. 2021, Spits et al. 2021, Zajac et al. 2013). Though early endosomes (Rab5 positive), late endosomes (Rab7 positive), and lysosomes (Rab7 or LAMP1 positive) can all be cotransported with the ER, ER-endosome contacts have been shown to increase as endosomes mature (Friedman et al. 2013), which may result in increased co-movement of the ER with Rab7-positive late endosomes or lysosomes in comparison to early endosomes. The motility of late endosomes and lysosomes can directly impact the organization of the ER network. Overexpression of the dynein/dynactin adaptors RILP or TMEM55B activates Rab7-positive endosome movement to the centrosome and the corresponding retrograde movement and relocalization of the ER to a perinuclear region (Spits et al. 2021) (**Figure 6*a***). Similarly, depleting the kinesin-1 adaptors Arl8B or SKIP decreases the movement of lysosomes to the cell periphery and correspondingly decreases the number of tubular ER domains (Lu et al. 2020) (**Figure 6*b***).

Disrupting the interactions between the ER and late endosomes/lysosomes also results in a restructuring of the ER. ER mobility and distribution are negatively affected in COS7 cells when the known ER-endosome tethers VAPA, VAPB, and MOSPD2 are depleted (Spits et al. 2021). This ER reorganization can have negative cellular consequences, as expressing a mutant VAPA incapable of binding the FFAT motif of lysosomal proteins resulted in a discontinuous ER network in *Xenopus* retinal ganglion cell axons and a corresponding growth defect (Lu et al. 2020).

In addition to a direct impact on the reorganization of the ER network, ER-endosome contact sites affect endosome and lysosome transport and dynamics. The ER and lysosomes are cotransported in the pre-axonal region of neurons (Özkan et al. 2021). These ER-lysosome contacts enhance lysosome fission and their translocation into the axon (Özkan et al. 2021). In addition to lysosome contacts, the ER is also connected to late endosomes via the protein Protrudin (Raiborg et al. 2015) (**Figure 6*c***). Protrudin has both a FYVE domain that interacts with PI(3)P (phosphatidylinositol 3-phosphate) on the late endosome surface and a low-complexity region that interacts with Rab7. Protrudin also interacts with kinesin-1 (Matsuzaki et al. 2011). Though the ER and lysosomes move together on microtubules, this motility is slow, and it is unclear if this co-movement represents a true hitchhiking phenomenon. Instead, the primary role for this contact site may be to load kinesin-1 onto lysosomes for translocation into the axon and corresponding neurite outgrowth (Raiborg et al. 2015). Together, there are likely multiple mechanisms in place to link the ER with endosomal cargos of varying compositions and to cotransport these organelles. As these interactions and their coordinated motilities facilitate proper organelle distribution and neuronal functioning, further research is necessary to define how the cotransport of these organelles may be regulated.

Endoplasmic Reticulum Contacts with Other Organelles

In addition to contacting endosomes, the ER has been demonstrated to contact other organelles including mitochondria, peroxisomes, lipid droplets, and the Golgi (Guo et al. 2018, Valm et al. 2017). In some cases, these contact sites are known to be linked to the underlying transport machinery; however, whether these examples display true hitchhiking behaviors that result in the cotransport of these organelle pairs remains to be determined. ER contacts with the mitochondria have been shown to recruit Miro1, a protein that links mitochondria to the kinesin-1 KIF5B for active transport (Qin et al. 2020). As a result, increased mitochondrial tubulation occurs at ER-mitochondria contact sites (Qin et al. 2020). Similarly, short-range movements of peroxisomes and ER appear to be linked, and these movements are decreased when Miro1 and Miro2 are knocked out (Covill-Cooke et al. 2020). Future work into how and when motors are recruited to contact site locations will provide further insight into which types of organelle contacts mediate true hitchhiking and what the role of hitchhiking is on various cellular processes.

HITCHHIKING IN PLANT CELLS

Studying intracellular transport in plants is different from that of fungal or animal cells in several ways. Some of these differences are related to plant cells themselves—in many plant cell types, a vacuole takes up a large amount of cytoplasmic space, resulting in the remaining cytoplasmic components being pushed toward the cell periphery (Tan et al. 2019). This constraining of cytoplasmic material makes studying mechanisms of organelle cotransport more difficult. Furthermore, in plants, several technical innovations not yet applied to studying cotransport of cargos in fungi or animal cells, such as optical tweezers (Ketelaar et al. 2014, Sparkes et al. 2018) and light-induced motility (Banaś et al. 2012), have been used to probe the cotransport of cargos in plant cells. Several key cellular components also differ in plants cells, resulting in mechanistic differences in hitchhiking. Land plant genomes do not encode a cytoplasmic dynein, and long-distance transport occurs primarily via myosin motors on actin cytoskeleton tracks. Additionally, chloroplasts, an organelle found only in plants, introduce another potential platform for contacts and cotransport.

mRNA Hitchhiking in Plant Cells

Much like in animal cells, in plants, mRNA transport occurs in a multicellular context. Different plant cells have different roles and cellular requirements, and as a result, subsets of mRNAs are transported by distinct mechanisms for precise local translation. In the endosperm of the rice *Oryza sativa*, mRNAs encoding the energy storage proteins glutelin and prolamine are transported to different regions of the ER for translation (Chou et al. 2019). RBPs identify and bind so-called zip code sequences on these mRNAs. Glutelin and prolamine mRNAs are bound by two RBPs, RBP-P and RBP-L (Tian et al. 2018, 2019). RBP-P and RBP-L interact with each other and also form a complex with *N*-ethylmaleimide-sensitive factor (NSF) and a GTP-bound version of the GTPase Rab5a, which associates with the endosome surface (Tian et al. 2018, 2020) (**Figure 7*a***). The movement of these mRNAs is inhibited upon disruption of the actin cytoskeleton, suggesting that these endosomes may associate with a myosin motor, though the identity of the myosin or other actin-based transport mechanism is unknown (Hamada et al. 2003) (**Figure 7*a***).

mRNA localization in plants, as in fungi and animals, affects the proper functioning of many cellular processes (Tian et al. 2020). However, in plants, unlike in fungi and animals, the intracellular transport of mRNAs is also connected to the intercellular transport of mRNAs throughout the plant via cell–cell channels known as plasmodesmata. Many mRNAs localize to plasmodesmata (Otero et al. 2016), and this localization has been correlated with their movement through the phloem, part of the plant vasculature (Luo et al. 2018). Recently, the *FLOWERING LOCUS T* mRNA was shown to hitchhike on Rab5-associated cargos for their transport to plasmodesmata (Luo et al. 2022) (**Figure 7*b***). This transport requires both the actin and microtubule cytoskeletons. Therefore, the actin cytoskeleton may be required for the long-distance transport of mRNAs on endomembrane cargos, while the microtubule cytoskeleton is required for the anchoring of mRNAs at plasmodesmata.

FLOWERING LOCUS T mRNA is bound by rotamase cyclophilin (ROC) RNA-binding proteins. ROCs associate with the surface of Rab5-associated cargos via an unknown mechanism. Four closely related cyclophilins, ROC1, 2, 3, and 5, likely act redundantly in mRNA transport, as a quadruple mutant of these four *roc* genes was required to impair transport of *FLOWERING LOCUS T* mRNA (Luo et al. 2022). In addition to mRNAs, viral proteins may also hitchhike on motile cargos for their transport to plasmodesmata via a "grab a Rab" model (Oparka 2004). In this model, cellular factors that need to be transported to a certain region of the cell could associate with Rabs or other parts of a motile cargo destined for that region. When the destination is the

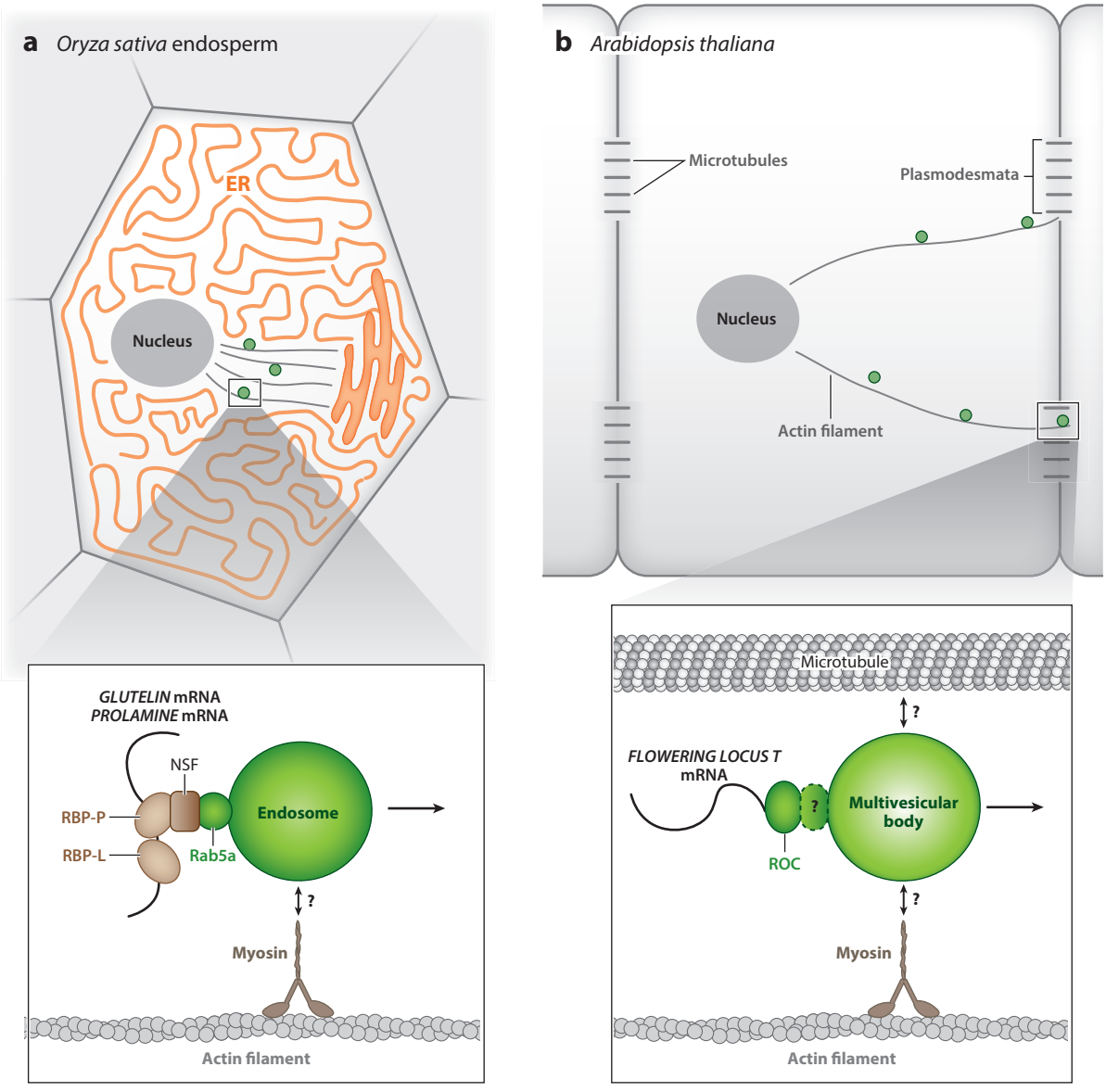

Figure 7

Messenger RNA hitchhiking in plant cells. (*a*) In the endosperm of the rice *Oryza sativa*, messenger RNAs (mRNAs) encoding the storage proteins glutelin and prolamine hitchhike on endosomes on the way to the endoplasmic reticulum (ER). The RNA-binding proteins RBP-P and RBP-L bind these mRNAs and associate with N-ethylmaleimide-sensitive factor (NSF), which binds Rab5a on the surface of endosomes. These endosomes require actin for their movement, potentially via a myosin motor. (*b*) In *Arabidopsis thaliana*, *FLOWERING LOCUS T* mRNA hitchhikes on multivesicular bodies to the plasmodesmata. *FLOWERING LOCUS T* mRNA directly associates with a member of the rotamase cyclophilin (ROC) family of proteins on the surface of multivesicular bodies. The long-distance transport of *FLOWERING LOCUS T* mRNA also requires actin and is potentially transported via a myosin motor. Anchoring of ROC mRNA at the plasmodesmata requires microtubules.

plasmodesmata, hitchhiking that occurs at the level of the individual cell could affect movement not only locally but also systemically.

Organelle Hitchhiking in Plant Cells

The motility of many organelles, including the ER, Golgi bodies, endosomes, mitochondria, and peroxisomes, has been linked to the motility of a myosin XI, myosin XI-K in plants (Avisar et al. 2008, 2012; Ueda et al. 2010). However, myosin XI-K does not often appear to interact directly with those organelles. Instead, when fluorescently tagged, myosin XI-K is observed on a vesicle population that resembles beads on a string (Peremyslov et al. 2012) (**Figure 8*a***) and associates with a family of proteins termed the myosin-binding (MyoB) family (Peremyslov et al. 2013). Therefore, much of the organelle movement in plants may be indirect via association with MyoB vesicles (Perico & Sparkes 2018). MyoB vesicles may act as a primary cargo, affecting the motility of other organelles via sustained hitchhiking or transient interactions, cytoplasmic streaming, or a combination of mechanisms (Geitmann & Nebenführ 2015, Peremyslov et al. 2015, Sparkes et al. 2008) (**Figure 8*a***). Therefore, the exact links between myosin XI-K and the transport of organelles, including those mentioned later in this section, remain to be determined.

Endoplasmic Reticulum–Golgi Interactions

As in both animal and fungal cells, the ER in plant cells contacts multiple organelles, and movement of the ER has been demonstrated to affect the movement of the Golgi, peroxisomes, and mitochondria (Liu & Li 2019, Stefano et al. 2014). Unlike many animal cells, in which the Golgi apparatus is perinuclear, in many plant cells, including leaf epidermis, Golgi bodies are closely associated with ER exit sites throughout the cell, forming a mobile secretory unit (daSilva et al. 2004) (**Figure 8*b***). Golgi bodies have also been shown to move along ER tubules (Boevink et al. 1998). An early indication of a physical interaction between the ER and Golgi came from experiments using photoactivatable green fluorescent protein (GFP)-tagged ER in *Nicotiana tabacum* leaves. When ER proteins were photoactivated, they moved at a similar rate to Golgi bodies nearby, suggesting potential cotransport (Runions et al. 2006). Furthermore, inhibition of actin polymerization via latrunculin B or cytochalasin D slowed movement of both the ER and Golgi bodies (Boevink et al. 1998, Runions et al. 2006).

Some of the most convincing evidence concerning a physical link between the ER and Golgi came from optical tweezers experiments in *Arabidopsis thaliana* and *N. tabacum* leaf epidermal cells (Hawes et al. 2010; Sparkes et al. 2009, 2018). Upon movement of the Golgi via optical tweezers, ER tubules followed. These ER tubules retracted upon release of the Golgi from the optical trap (Sparkes et al. 2009). Furthermore, Golgi bodies that were picked up by the optical trap and displaced from the ER could be reattached to other regions of the ER. These experiments suggest a physical link between the ER and Golgi.

More recently, AtCASP was identified as a potential tether for the ER to the Golgi in *A. thaliana*. In cells expressing a dominant negative version of AtCASP lacking its coiled-coil domains, Golgi movement was substantially decreased (Osterrieder et al. 2017). Furthermore, optical tweezers experiments demonstrated that the ER-Golgi connection was more easily disrupted when the coiled-coil domains of AtCASP were removed, suggesting that AtCASP is involved in mediating the tethering between the ER and Golgi (Osterrieder et al. 2017) (**Figure 8*b***).

Links Between Chloroplasts and Other Organelles

Chloroplasts are organelles that are found exclusively in plants. Chloroplasts are one type of plastid, an organelle that is involved in biosynthetic pathways in plants. In leaves, chloroplast

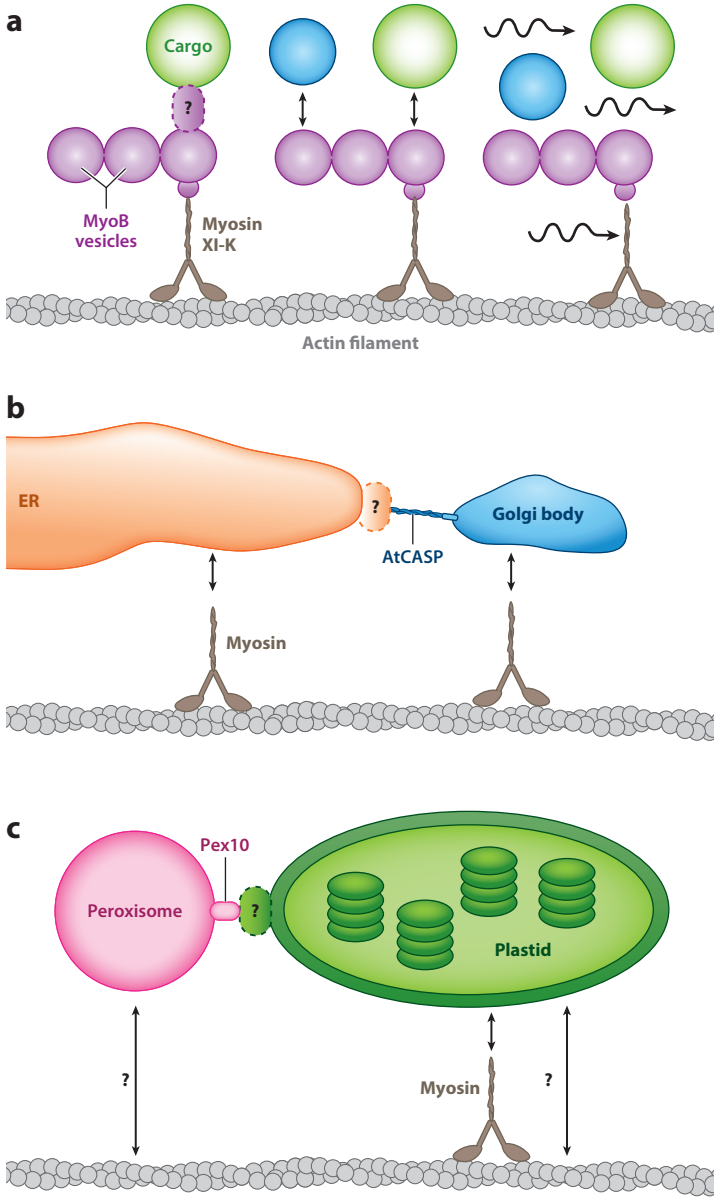

Figure 8

Potential instances of organelle hitchhiking in plant cells. (*a*) Myosin XI-K associates with the MyoB receptor on the surface of vesicles resembling beads on a string. MyoB vesicles have been implicated in the transport of multiple cargos by an unclear mechanism. Other cargos may hitchhike on MyoB vesicles via (*left*) a direct, sustained interaction or (*middle*) transient interactions. Alternatively, other cargos may move by (*right*) cytoplasmic streaming generated from the transport of MyoB vesicles. (*b*) The endoplasmic reticulum (ER) directly interacts with Golgi bodies via AtCASP. The coiled-coil region of AtCASP is required for this interaction. A myosin motor associated with either the ER or Golgi body may be involved in this hitchhiking. (*c*) Peroxisomes are cotransported with plastids such as chloroplasts. The peroxin Pex10 is required for this cotransport. Plastid movement is dependent upon the actin cytoskeleton and potentially a myosin motor. The actin cytoskeleton also influences peroxisome movement and its contact with plastids.

movements are correlated with light cues and are actin dependent (Suetsugu et al. 2010). The light-induced movement of chloroplasts or plastids has also been correlated with the movement of other organelles, suggesting a potential hitchhiking mechanism. Nuclei in *Arabidopsis* leaf pavement cells are nearly always colocalized with small plastids, and the cotransport of nuclei with plastids occurs upon their light-induced movement (Higa et al. 2014). Mutants with defects in plastid movement showed corresponding defects in nuclear movement. In mutants in which nuclei were not associated with plastids, nuclei did not exhibit light-induced movement (Higa et al. 2014). As plastids have their own genome, close association with the nucleus may assist in signaling or information exchange between these organelles (Jung & Chory 2010). However, the degree to which they are transported, and the role and mechanism of that transport, remains to be determined.

Similarly, in *Arabidopsis* palisade mesophyll cells, peroxisomes are nearly always in contact with chloroplasts (Oikawa et al. 2003, 2015) (**Figure 8*c***). When chloroplasts are mislocalized in chloroplast unusual positioning (chup) mutants, peroxisomes are mislocalized as well, though still associated with chloroplasts (Oikawa et al. 2003), suggesting that their movement may be coordinated to some degree. As chloroplasts and peroxisomes are both involved in photosynthesis, their close proximity may promote the exchange of metabolites during this process. In accordance with this idea, Pex10 is required for both the peroxisome-chloroplast interaction and the transfer of metabolites (Schumann et al. 2007) (**Figure 8*c***). The degree to which nuclei, peroxisomes, and chloroplasts each interact with actin and/or myosin and whether their coordinated movements constitute bona fide hitchhiking mechanisms remain to be determined.

FUTURE DIRECTIONS

Though much has been elucidated in the past 10 years regarding the cotransport of cargos across diverse organisms, much also remains to be discovered. In fungi, animals, and plants, identifying the tethers involved in known hitchhiking examples and deciphering how these attachment sites are regulated remain a clear direction for future research (Eisenberg-Bord et al. 2016). Differences among cell types, contexts, and experimental techniques may also provide distinct insights into these diverse hitchhiking behaviors. As organelle contacts have been associated with neurological diseases (Herker et al. 2021, Wilson & Metzakopian 2021), understanding the regulation of hitchhiking in animal cells may improve our understanding of the molecular basis of these diseases and the development of therapeutics. The tubular geometry of fungal cells or neurons may promote the mathematical modeling of hitchhiking behaviors (Mogre et al. 2020), while the use of optical tweezers experiments in plants could allow for probing the ability of hitchhiking cargos to withstand forces. Finally, uncovering the physiological and evolutionary relevance of hitchhiking as well as other novel forms of intracellular transport will further our understanding of the dynamic interplay between different transport phenomena and the processes they regulate in cells.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Soojin Kim, Aga Kendrick, D. Alexander Stevens, and Livia Songster for critical reading of the manuscript. J.R.C. is funded by a MOSAIC (Maximizing Opportunities for Scientific and Academic Independent Careers) award (K99/R00) from the National Institutes of Health (NIH) (K99GM140269). S.L.R.-P. is supported by the Howard Hughes Medical Institute and the NIH (1R35GM141825).

LITERATURE CITED

Ali I, Yang W-C. 2020. The functions of kinesin and kinesin-related proteins in eukaryotes. *Cell Adhes. Migr.* 14(1):139–52

Allen NS, Allen RD. 1978a. Cytoplasmic streaming in green plants. *Annu. Rev. Biophys. Bioeng.* 7:497–526

Allen RD, Allen NS. 1978b. Cytoplasmic streaming in amoeboid movement. *Annu. Rev. Biophys. Bioeng.* 7:469–95

Aronov S, Gelin-Licht R, Zipor G, Haim L, Safran E, Gerst JE. 2007. mRNAs encoding polarity and exocytosis factors are cotransported with the cortical endoplasmic reticulum to the incipient bud in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 27(9):3441–55

Avisar D, Abu-Abied M, Belausov E, Sadot E. 2012. Myosin XIK is a major player in cytoplasm dynamics and is regulated by two amino acids in its tail. *J. Exp. Bot.* 63(1):241–49

Avisar D, Prokhnevsky AI, Makarova KS, Koonin EV, Dolja VV. 2008. Myosin XI-K is required for rapid trafficking of Golgi stacks, peroxisomes, and mitochondria in leaf cells of *Nicotiana benthamiana*. *Plant Physiol.* 146(3):1098–108

Banaś AK, Aggarwal C, Łabuz J, Sztatelman O, Gabryś H. 2012. Blue light signalling in chloroplast movements. *J. Exp. Bot.* 63(4):1559–74

Baumann S, Pohlmann T, Jungbluth M, Brachmann A, Feldbrügge M. 2012. Kinesin-3 and dynein mediate microtubule-dependent co-transport of mRNPs and endosomes. *J. Cell Sci.* 125(11):2740–52

Bielska E, Schuster M, Roger Y, Berepiki A, Soanes DM, et al. 2014. Hook is an adapter that coordinates kinesin-3 and dynein cargo attachment on early endosomes. *J. Cell Biol.* 204(6):989–1007

Boevink P, Oparka K, Cruz SS, Martin B, Betteridge A, Hawes C. 1998. Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network. *Plant J.* 15(3):441–47

Böhl F, Kruse C, Frank A, Ferring D, Jansen R-P. 2000. She2p, a novel RNA-binding protein tethers ASH1 mRNA to the Myo4p myosin motor via She3p. *EMBO J.* 19(20):5514–24

Bretscher A. 2003. Polarized growth and organelle segregation in yeast. *J. Cell Biol.* 160(6):811–16

Burute M, Kapitein LC. 2019. Cellular logistics: unraveling the interplay between microtubule organization and intracellular transport. *Annu. Rev. Cell Dev. Biol.* 35:29–54

Caballero-Lima D, Hautbergue GM, Wilson SA, Sudbery PE. 2014. In *Candida albicans* hyphae, Sec2p is physically associated with SEC2 mRNA on secretory vesicles. *Mol. Microbiol.* 94(4):828–42

Cabukusta B, Berlin I, van Elsland DM, Forkink I, Spits M, et al. 2020. Human VAPome analysis reveals MOSPD1 and MOSPD3 as membrane contact site proteins interacting with FFAT-related FFNT motifs. *Cell Rep.* 33(10):108475

Chang P, Torres J, Lewis RA, Mowry KL, Houliston E, King ML. 2004. Localization of RNAs to the mitochondrial cloud in *Xenopus* oocytes through entrapment and association with endoplasmic reticulum. *Mol. Biol. Cell* 15(10):4669–81

Chou H-L, Tian L, Washida H, Fukuda M, Kumamaru T, Okita TW. 2019. The rice storage protein mRNAs as a model system for RNA localization in higher plants. *Plant Sci.* 284:203–11

Christensen JR, Kendrick AA, Truong JB, Aguilar-Maldonado A, Adani V, et al. 2021. Cytoplasmic dynein-1 cargo diversity is mediated by the combinatorial assembly of FTS–Hook–FHIP complexes. *eLife* 10:e74538

Cioni J-M, Lin JQ, Holtermann AV, Koppers M, Jakobs MAH, et al. 2019. Late endosomes act as mRNA translation platforms and sustain mitochondria in axons. *Cell* 176(1–2):56–72.e15

Corradi E, Dalla Costa I, Gavoci A, Iyer A, Roccuzzo M, et al. 2020. Axonal precursor miRNAs hitchhike on endosomes and locally regulate the development of neural circuits. *EMBO J.* 39(6):e102513

Covill-Cooke C, Toncheva VS, Drew J, Birsa N, López-Doménech G, Kittler JT. 2020. Peroxisomal fission is modulated by the mitochondrial Rho-GTPases, Miro1 and Miro2. *EMBO Rep.* 21(2):e49865

daSilva LLP, Snapp EL, Denecke J, Lippincott-Schwartz J, Hawes C, Brandizzi F. 2004. Endoplasmic reticulum export sites and Golgi bodies behave as single mobile secretory units in plant cells. *Plant Cell* 16(7):1753–71

Dollar G, Struckhoff E, Michaud J, Cohen RS. 2002. Rab11 polarization of the *Drosophila* oocyte: a novel link between membrane trafficking, microtubule organization, and *oskar* mRNA localization and translation. *Development* 129(2):517–26

Egan MJ, McClintock MA, Reck-Peterson SL. 2012. Microtubule-based transport in filamentous fungi. *Curr. Opin. Microbiol.* 15(6):637–45

Eisenberg-Bord M, Shai N, Schuldiner M, Bohnert M. 2016. A tether is a tether is a tether: tethering at membrane contact sites. *Dev. Cell* 39(4):395–409

Esseling-Ozdoba A, Houtman D, Van Lammeren AAM, Eiser E, Emons AMC. 2008. Hydrodynamic flow in the cytoplasm of plant cells. *J. Microsc.* 231(2):274–83

Estrada P, Kim J, Coleman J, Walker L, Dunn B, et al. 2003. Myo4p and She3p are required for cortical ER inheritance in *Saccharomyces cerevisiae*. *J. Cell Biol.* 163(6):1255–66

Fenton AR, Jongens TA, Holzbaur ELF. 2021. Mitochondrial adaptor TRAK2 activates and functionally links opposing kinesin and dynein motors. *Nat. Commun.* 12:4578

Fernandez-Moya SM, Bauer KE, Kiebler MA. 2014. Meet the players: local translation at the synapse. *Front. Mol. Neurosci.* 7:84

Friedman JR, Dibenedetto JR, West M, Rowland AA, Voeltz GK. 2013. Endoplasmic reticulum-endosome contact increases as endosomes traffic and mature. *Mol. Biol. Cell* 24(7):1030–40

Fundakowski J, Hermesh O, Jansen R-P. 2012. Localization of a subset of yeast mRNAs depends on inheritance of endoplasmic reticulum. *Traffic* 13(12):1642–52

Geitmann A, Nebenführ A. 2015. Navigating the plant cell: intracellular transport logistics in the green kingdom. *Mol. Biol. Cell* 26(19):3373–78

Genz C, Fundakowski J, Hermesh O, Schmid M, Jansen R-P. 2013. Association of the yeast RNA-binding protein She2p with the tubular endoplasmic reticulum depends on membrane curvature. *J. Biol. Chem.* 288(45):32384–93

Goldstein RE, van de Meent J-W. 2015. A physical perspective on cytoplasmic streaming. *Interface Focus* 5(4):20150030

Guimaraes SC, Schuster M, Bielska E, Dagdas G, Kilaru S, et al. 2015. Peroxisomes, lipid droplets, and endoplasmic reticulum "hitchhike" on motile early endosomes. *J. Cell Biol.* 211(5):945–54

Guo X, Farías GG, Mattera R, Bonifacino JS. 2016. Rab5 and its effector FHF contribute to neuronal polarity through dynein-dependent retrieval of somatodendritic proteins from the axon. *PNAS* 113(36):E5318–27

Guo Y, Li D, Zhang S, Yang Y, Liu J-J, et al. 2018. Visualizing intracellular organelle and cytoskeletal interactions at nanoscale resolution on millisecond timescales. *Cell* 175(5):1430–42.e17

Hamada S, Ishiyama K, Choi S-B, Wang C, Singh S, et al. 2003. The transport of prolamine RNAs to prolamine protein bodies in living rice endosperm cells. *Plant Cell* 15(10):2253–64

Hammer JA, Sellers JR. 2012. Walking to work: roles for class V myosins as cargo transporters. *Nat. Rev. Mol. Cell Biol.* 13(1):13–26

Harbauer AB, Hees JT, Wanderoy S, Segura I, Gibbs W, et al. 2022. Neuronal mitochondria transport *Pink1* mRNA via synaptojanin 2 to support local mitophagy. *Neuron* 110(9):1516–31.e9

Hawes C, Osterrieder A, Sparkes IA, Ketelaar T. 2010. Optical tweezers for the micromanipulation of plant cytoplasm and organelles. *Curr. Opin. Plant Biol.* 13(6):731–35

Henrichs V, Grycova L, Barinka C, Nahacka Z, Neuzil J, et al. 2020. Mitochondria-adaptor TRAK1 promotes kinesin-1 driven transport in crowded environments. *Nat. Commun.* 11:3123

Herker E, Vieyres G, Beller M, Krahmer N, Bohnert M. 2021. Lipid droplet contact sites in health and disease. *Trends Cell Biol.* 31(5):345–58

Higa T, Suetsugu N, Kong S-G, Wada M. 2014. Actin-dependent plastid movement is required for motive force generation in directional nuclear movement in plants. *PNAS* 111(11):4327–31

Higuchi Y, Ashwin P, Roger Y, Steinberg G. 2014. Early endosome motility spatially organizes polysome distribution. *J. Cell Biol.* 204(3):343–57

Horgan CP, Hanscom SR, Jolly RS, Futter CE, McCaffrey MW. 2010. Rab11-FIP3 links the Rab11 GTPase and cytoplasmic dynein to mediate transport to the endosomal-recycling compartment. *J. Cell Sci.* 123(Part 2):181–91

James C, Kehlenbach RH. 2021. The interactome of the VAP family of proteins: an overview. *Cells* 10(7):1780

Jankovics F, Sinka R, Erdélyi M. 2001. An interaction type of genetic screen reveals a role of the *Rab11* gene in *oskar* mRNA localization in the developing *Drosophila melanogaster* oocyte. *Genetics* 158(3):1177–88

Jankowski S, Pohlmann T, Baumann S, Müntjes K, Devan SK, et al. 2019. The multi PAM2 protein Upa2 functions as novel core component of endosomal mRNA transport. *EMBO Rep*. 20(9):e47381

Jung H-S, Chory J. 2010. Signaling between chloroplasts and the nucleus: can a systems biology approach bring clarity to a complex and highly regulated pathway? *Plant Physiol.* 152(2):453–59

Ketelaar T, de Ruijter N, Niehren S. 2014. Optical trapping in plant cells. In *Plant Cell Morphogenesis: Methods and Protocols*, ed. V Žárský, F Cvrčková, pp. 259–65. Totowa, NJ: Humana Press

Kollmar M, Mühlhausen S. 2017. Myosin repertoire expansion coincides with eukaryotic diversification in the Mesoproterozoic era. *BMC Evol. Biol.* 17:211

Liao Y-C, Fernandopulle MS, Wang G, Choi H, Hao L, et al. 2019. RNA granules hitchhike on lysosomes for long-distance transport, using annexin A11 as a molecular tether. *Cell* 179(1):147–64.e20

Liu L, Li J. 2019. Communications between the endoplasmic reticulum and other organelles during abiotic stress response in plants. *Front. Plant Sci.* 10:749

Loewen CJR, Levine TP. 2005. A highly conserved binding site in vesicle-associated membrane protein-associated protein (VAP) for the FFAT motif of lipid-binding proteins. *J. Biol. Chem.* 280(14):14097–104

Long RM, Gu W, Lorimer E, Singer RH, Chartrand P. 2000. She2p is a novel RNA-binding protein that recruits the Myo4p–She3p complex to ASH1 mRNA. *EMBO J.* 19(23):6592–601

Lu M, van Tartwijk FW, Lin JQ, Nijenhuis W, Parutto P, et al. 2020. The structure and global distribution of the endoplasmic reticulum network are actively regulated by lysosomes. *Sci. Adv.* 6(51):eabc7209

Luo K-R, Huang N-C, Chang Y-H, Yu T-S. 2022. Arabidopsis cyclophilins direct plasmodesmata-targeting of mobile mRNA via organelle hitchhiking. Res. Square. **https://doi.org/10.21203/rs.3.rs-1088339/v1**

Luo K-R, Huang N-C, Yu T-S. 2018. Selective targeting of mobile mRNAs to plasmodesmata for cell-to-cell movement. *Plant Physiol*. 177(2):604–14

Matanis T, Akhmanova A, Wulf P, Nery ED, Weide T, et al. 2002. Bicaudal-D regulates COPI-independent Golgi–ER transport by recruiting the dynein–dynactin motor complex. *Nat. Cell Biol.* 4(12):986–92

Matsuzaki F, Shirane M, Matsumoto M, Nakayama KI. 2011. Protrudin serves as an adaptor molecule that connects KIF5 and its cargoes in vesicular transport during process formation. *Mol. Biol. Cell* 22(23):4602–20

McKenney RJ, Huynh W, Tanenbaum ME, Bhabha G, Vale RD. 2014. Activation of cytoplasmic dynein motility by dynactin-cargo adapter complexes. *Science* 345(6194):337–41

Miki H, Okada Y, Hirokawa N. 2005. Analysis of the kinesin superfamily: insights into structure and function. *Trends Cell Biol*. 15(9):467–76

Mogre SS, Christensen JR, Niman CS, Reck-Peterson SL, Koslover EF. 2020. Hitching a ride: mechanics of transport initiation through linker-mediated hitchhiking. *Biophys. J.* 118(6):1357–69

Müller J, Pohlmann T, Feldbrügge M. 2019. Core components of endosomal mRNA transport are evolutionarily conserved in fungi. *Fungal Genet. Biol.* 126:12–16

Müntjes K, Devan SK, Reichert AS, Feldbrügge M. 2021. Linking transport and translation of mRNAs with endosomes and mitochondria. *EMBO Rep*. 22(10):e52445

Neefjes J, Cabukusta B. 2021. What the VAP: the expanded VAP family of proteins interacting with FFAT and FFAT-related motifs for interorganellar contact. *Contact* 4. **https://doi.org/10.1177/25152564211012246**

Oikawa K, Kasahara M, Kiyosue T, Kagawa T, Suetsugu N, et al. 2003. CHLOROPLAST UNUSUAL POSITIONING1 is essential for proper chloroplast positioning. *Plant Cell* 15(12):2805–15

Oikawa K, Matsunaga S, Mano S, Kondo M, Yamada K, et al. 2015. Physical interaction between peroxisomes and chloroplasts elucidated by *in situ* laser analysis. *Nat. Plants* 1:15035

Olgeiser L, Haag C, Boerner S, Ule J, Busch A, et al. 2019. The key protein of endosomal mRNP transport Rrm4 binds translational landmark sites of cargo mRNAs. *EMBO Rep*. 20:e46588

Oparka KJ. 2004. Getting the message across: how do plant cells exchange macromolecular complexes? *Trends Plant Sci*. 9(1):33–41

Osterrieder A, Sparkes IA, Botchway SW, Ward A, Ketelaar T, et al. 2017. Stacks off tracks: a role for the golgin AtCASP in plant endoplasmic reticulum-Golgi apparatus tethering. *J. Exp. Bot.* 68(13):3339–50

Otero S, Helariutta Y, Benitez-Alfonso Y. 2016. Symplastic communication in organ formation and tissue patterning. *Curr. Opin. Plant Biol.* 29:21–28

Özkan N, Koppers M, van Soest I, van Harten A, Jurriens D, et al. 2021. ER–lysosome contacts at a pre-axonal region regulate axonal lysosome availability. *Nat. Commun.* 12:4493

Peremyslov VV, Cole RA, Fowler JE, Dolja VV. 2015. Myosin-powered membrane compartment drives cytoplasmic streaming, cell expansion, and plant development. *PLOS ONE* 10(10):e0139331

Peremyslov VV, Klocko AL, Fowler JE, Dolja VV. 2012. *Arabidopsis* myosin XI-K localizes to the motile endomembrane vesicles associated with F-actin. *Front. Plant Sci.* 3:184

Peremyslov VV, Morgun EA, Kurth EG, Makarova KS, Koonin EV, Dolja VV. 2013. Identification of myosin XI receptors in *Arabidopsis* defines a distinct class of transport vesicles. *Plant Cell* 25(8):3022–38

Perico C, Sparkes I. 2018. Plant organelle dynamics: cytoskeletal control and membrane contact sites. *New Phytol.* 220(2):381–94

Perkins HT, Allan V. 2021. Intertwined and finely balanced: endoplasmic reticulum morphology, dynamics, function, and diseases. *Cells* 10(9):2341

Pohlmann T, Baumann S, Haag C, Albrecht M, Feldbrügge M. 2015. A FYVE zinc finger domain protein specifically links mRNA transport to endosome trafficking. *eLife* 4:e06041

Popovic D, Nijenhuis W, Kapitein LC, Pelkmans L. 2020. Co-translational targeting of transcripts to endosomes. bioRxiv 208652. **https://doi.org/10.1101/2020.07.17.208652**

Prinz WA, Toulmay A, Balla T. 2020. The functional universe of membrane contact sites. *Nat. Rev. Mol. Cell Biol.* 21(1):7–24

Pruyne DW, Schott DH, Bretscher A. 1998. Tropomyosin-containing actin cables direct the Myo2p-dependent polarized delivery of secretory vesicles in budding yeast. *J. Cell Biol.* 143(7):1931–45

Qin J, Guo Y, Xue B, Shi P, Chen Y, et al. 2020. ER-mitochondria contacts promote mtDNA nucleoids active transportation via mitochondrial dynamic tubulation. *Nat. Commun.* 11:4471

Qin W, Myers SA, Carey DK, Carr SA, Ting AY. 2021. Spatiotemporally-resolved mapping of RNA binding proteins via functional proximity labeling reveals a mitochondrial mRNA anchor promoting stress recovery. *Nat. Commun.* 12:4980

Quentin D, Schuhmacher JS, Klink BU, Lauer J, Shaikh TR, et al. 2021. Structure of the human FERRY Rab5 effector complex. bioRxiv 449265. **https://doi.org/10.1101/2021.06.21.449265**

Quinlan ME. 2016. Cytoplasmic streaming in the *Drosophila* oocyte. *Annu. Rev. Cell Dev. Biol.* 32:173–95

Raiborg C, Wenzel EM, Pedersen NM, Olsvik H, Schink KO, et al. 2015. Repeated ER–endosome contacts promote endosome translocation and neurite outgrowth. *Nature* 520(7546):234–38

Reck-Peterson SL, Redwine WB, Vale RD, Carter AP. 2018. The cytoplasmic dynein transport machinery and its many cargoes. *Nat. Rev. Mol. Cell Biol.* 19(6):382–98

Runions J, Brach T, Kühner S, Hawes C. 2006. Photoactivation of GFP reveals protein dynamics within the endoplasmic reticulum membrane. *J. Exp. Bot.* 57(1):43–50

Salogiannis J, Christensen JR, Songster LD, Aguilar-Maldonado A, Shukla N, Reck-Peterson SL. 2021. PxdA interacts with the DipA phosphatase to regulate peroxisome hitchhiking on early endosomes. *Mol. Biol. Cell* 32(6):492–503

Salogiannis J, Egan MJ, Reck-Peterson SL. 2016. Peroxisomes move by hitchhiking on early endosomes using the novel linker protein PxdA. *J. Cell Biol.* 212(3):289–96

Salogiannis J, Reck-Peterson SL. 2017. Hitchhiking: a non-canonical mode of microtubule-based transport. *Trends Cell Biol.* 27(2):141–50

Schlager MA, Hoang HT, Urnavicius L, Bullock SL, Carter AP. 2014. *In vitro* reconstitution of a highly processive recombinant human dynein complex. *EMBO J.* 33(17):1855–68

Schmid M, Jaedicke A, Du T-G, Jansen R-P. 2006. Coordination of endoplasmic reticulum and mRNA localization to the yeast bud. *Curr. Biol.* 16(15):1538–43

Schuhmacher JS, tom Dieck S, Christoforidis S, Landerer C, Hersemann L, et al. 2021. The novel Rab5 effector FERRY links early endosomes with the translation machinery. bioRxiv 449167. **https://doi.org/10.1101/2021.06.20.449167**

Schumann U, Prestele J, O'Geen H, Brueggeman R, Wanner G, Gietl C. 2007. Requirement of the C3HC4 zinc RING finger of the *Arabidopsis* PEX10 for photorespiration and leaf peroxisome contact with chloroplasts. *PNAS* 104(3):1069–74

Shepard KA, Gerber AP, Jambhekar A, Takizawa PA, Brown PO, et al. 2003. Widespread cytoplasmic mRNA transport in yeast: identification of 22 bud-localized transcripts using DNA microarray analysis. *PNAS* 100(20):11429–34

Siddiqui N, Straube A. 2017. Intracellular cargo transport by kinesin-3 motors. *Biochemistry* 82(7):803–15

Singer-Krüger B, Jansen R-P. 2014. Here, there, everywhere. *RNA Biol*. 11(8):1031–39

Smith BN, Topp SD, Fallini C, Shibata H, Chen H-J, et al. 2017. Mutations in the vesicular trafficking protein annexin A11 are associated with amyotrophic lateral sclerosis. *Sci. Transl. Med*. 9(388):eaad9157

Sparkes IA, Ketelaar T, de Ruijter NCA, Hawes C. 2009. Grab a Golgi: laser trapping of Golgi bodies reveals *in vivo* interactions with the endoplasmic reticulum. *Traffic* 10(5):567–71

Sparkes IA, Teanby NA, Hawes C. 2008. Truncated myosin XI tail fusions inhibit peroxisome, Golgi, and mitochondrial movement in tobacco leaf epidermal cells: a genetic tool for the next generation. *J. Exp. Bot*. 59(9):2499–512

Sparkes IA, White RR, Coles B, Botchway SW, Ward A. 2018. Using optical tweezers combined with total internal reflection microscopy to study interactions between the ER and Golgi in plant cells. *Methods Mol. Biol*. 1691:167–78

Spits M, Heesterbeek IT, Voortman LM, Akkermans JJ, Wijdeven RH, et al. 2021. Mobile late endosomes modulate peripheral endoplasmic reticulum network architecture. *EMBO Rep*. 22(3):e50815

Stefano G, Renna L, Brandizzi F. 2014. The endoplasmic reticulum exerts control over organelle streaming during cell expansion. *J. Cell Sci*. 127(5):947–53

Steinberg G. 2007. Hyphal growth: a tale of motors, lipids, and the spitzenkörper. *Eukaryot. Cell* 6(3):351–60

Sudbery P, Gow N, Berman J. 2004. The distinct morphogenic states of *Candida albicans*. *Trends Microbiol*. 12(7):317–24

Suetsugu N, Dolja VV, Wada M. 2010. Why have chloroplasts developed a unique motility system? *Plant Signal. Behav*. 5(10):1190–96

Takizawa PA, Vale RD. 2000. The myosin motor, Myo4p, binds Ash1 mRNA via the adapter protein, She3p. *PNAS* 97(10):5273–78

Tan X, Li K, Wang Z, Zhu K, Tan X, Cao J. 2019. A review of plant vacuoles: formation, located proteins, and functions. *Plants* 8(9):327

Tian L, Chou H-L, Fukuda M, Kumamaru T, Okita TW. 2020. mRNA localization in plant cells. *Plant Physiol*. 182(1):97–109

Tian L, Chou H-L, Zhang L, Hwang S-K, Starkenburg SR, et al. 2018. RNA-binding protein RBP-P is required for glutelin and prolamine mRNA localization in rice endosperm cells. *Plant Cell* 30(10):2529–52

Tian L, Chou H-L, Zhang L, Okita TW. 2019. Targeted endoplasmic reticulum localization of storage protein mRNAs requires the RNA-binding protein RBP-L. *Plant Physiol*. 179(3):1111–31

Titus MA. 2018. Myosin-driven intracellular transport. *Cold Spring Harb. Perspect. Biol.* 10(3):a021972

Trautwein M, Dengjel J, Schirle M, Spang A. 2004. Arf1p provides an unexpected link between COPI vesicles and mRNA in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 15(11):5021–37

Ueda H, Yokota E, Kutsuna N, Shimada T, Tamura K, et al. 2010. Myosin-dependent endoplasmic reticulum motility and F-actin organization in plant cells. *PNAS* 107(15):6894–99

Valm AM, Cohen S, Legant WR, Melunis J, Hershberg U, et al. 2017. Applying systems-level spectral imaging and analysis to reveal the organelle interactome. *Nature* 546(7656):162–67

Welz T, Kerkhoff E. 2019. Exploring the iceberg: prospects of coordinated myosin V and actin assembly functions in transport processes. *Small GTPases* 10(2):111–21

Wilhelm JE, Buszczak M, Sayles S. 2005. Efficient protein trafficking requires trailer hitch, a component of a ribonucleoprotein complex localized to the ER in *Drosophila*. *Dev. Cell* 9(5):675–85

Wilson EL, Metzakopian E. 2021. ER-mitochondria contact sites in neurodegeneration: genetic screening approaches to investigate novel disease mechanisms. *Cell Death Differ*. 28(6):1804–21

Yao X, Wang X, Xiang X. 2014. FHIP and FTS proteins are critical for dynein-mediated transport of early endosomes in *Aspergillus*. *Mol. Biol. Cell* 25(14):2181–89

Zajac AL, Goldman YE, Holzbaur ELF, Ostap EM. 2013. Local cytoskeletal and organelle interactions impact molecular-motor-driven early endosomal trafficking. *Curr. Biol.* 23(13):1173–80

Zander S, Baumann S, Weidtkamp-Peters S, Feldbrügge M. 2016. Endosomal assembly and transport of heteromeric septin complexes promote septin cytoskeleton formation. *J. Cell Sci.* 129(14):2778–92

Zhang J, Qiu R, Arst HN, Peñalva MA, Xiang X. 2014. HookA is a novel dynein–early endosome linker critical for cargo movement in vivo. *J. Cell Biol.* 204(6):1009–26