Review

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EMBO reports

Linking transport and translation of mRNAs with endosomes and mitochondria

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Abstract

In eukaryotic cells, proteins are targeted to their final subcellular locations with precise timing. A key underlying mechanism is the active transport of cognate mRNAs, which in many systems can be linked intimately to membrane trafficking. A prominent example is the long-distance endosomal transport of mRNAs and their local translation. Here, we describe current highlights of fundamental mechanisms of the underlying transport process as well as of biological functions ranging from endosperm development in plants to fungal pathogenicity and neuronal processes. Translation of endosome-associated mRNAs often occurs at the cytoplasmic surface of endosomes, a process that is needed for membrane-assisted formation of heteromeric protein complexes and for accurate subcellular targeting of proteins. Importantly, endosome-coupled translation of mRNAs encoding mitochondrial proteins, for example, seems to be particularly important for efficient organelle import and for regulating subcellular mitochondrial activity. In essence, these findings reveal a new mechanism of loading newly synthesised proteins onto endocytic membranes enabling intimate crosstalk between organelles. The novel link between endosomes and mitochondria adds an inspiring new level of complexity to trafficking and organelle biology.

Keywords endosomes; local translation; microtubules; mitochondria; organelle; RNA transport

Subject Categories Membranes & Trafficking; RNA Biology; Translation & Protein Quality

DOI 10.15252/embr.202152445 | Received 12 January 2021 | Revised 6 July 2021 | Accepted 27 July 2021 | Published online 17 August 2021

EMBO Reports (2021) 22: e52445

See the Glossary for abbreviations used in this article.

Introduction

A major evolutionary invention is the subcellular organisation of the eukaryotic cell, relying on defined compartments for division of labour. The nucleus, for example, harbours the genetic information, the endoplasmic reticulum (ER) is essential for protein secretion, and mitochondria act as cellular powerhouses. However, despite the apparent separation by different functionalities, there is intensive transport and communication between organelles. For example, most mRNAs encoding mitochondrial proteins are synthesised in the nucleus and translated in the cytosol, and subsequently, the translation products are targeted to distinct mitochondrial subcompartments (Pfanner *et al*, 2019). Furthermore, interorganelle contacts contribute to the synthesis of numerous lipids that undergo bidirectional transfers between the ER and mitochondria (Tamura *et al*, 2020).

To orchestrate communication between organelles, the cells heavily depend on intracellular trafficking of membranes, proteins and mRNAs. Outbound membrane and protein trafficking is mediated by secretion via the ER, Golgi apparatus and secretory vesicles. For inbound trafficking or endocytosis, protein-containing plasma membrane portions are internalised via early and late endosomes for degradation in the vacuole/lysosome compartment or recycling to the plasma membrane (Huotari & Helenius, 2011). Importantly, the intracellular trafficking is tightly intertwined with the transport of mRNAs. For instance, mRNAs are targeted to the ER and their membrane-coupled translation results in the entry of proteins into the ER to follow the secretory pathway (Akopian *et al*, 2013; Kramer *et al*, 2019). mRNA delivery and local translation on the surface of mitochondria can occur by distinct pathways ensuring import of a specific subset of mitochondrial proteins (Hansen & Herrmann, 2019; Bykov *et al*, 2020). However, the currently known examples appear to be only the tip of the iceberg and new cases are being uncovered steadily.

In this review, we describe novel mechanistic insights and implications for the co-transport of mRNAs with endosomes and discuss how local translation at the cytoplasmic surface of early and late endosomes is linked to mitochondrial protein import. Originally, endosomal mRNA trafficking was found as an alternative hitchhiking pathway for long-distance transport in infectious hyphae of the plant pathogen *Ustilago maydis* (Haag *et al*, 2015; Box 1). However, endosome-coupled RNA transport seems to be more widespread than previously anticipated, as the process is important for plant endosperm development and neuronal functions (Tian *et al*, 2020a; Fernandopulle *et al*, 2021). Malfunction of membrane-coupled mRNA transport has been implicated in neuronal diseases such as Charcot-Marie-Tooth type 2B neuropathy (Cioni *et al*, 2019; Liao *et al*, 2019). These biological processes exemplify how membrane, protein and mRNA trafficking communicate intensively. Thus,

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Glossary

ALS	amyotrophic lateral sclerosis
BicD	bicaudal D
Cdc	cell division control
CMT2B	charcot-marie-tooth type 2B
CSRP1	cysteine and glycine-rich protein 1
EEA1	early endosomal antigen 1
Egl	egalitarian
EJC	exon junction complex
ELAV	embryonic lethal abnormal vision
ER	endoplasmic reticulum
FERRY	five-subunit endosomal Rab5 and RNA/ribosome intermediary
FMRP	fragile X mental retardation protein
FYVE	Fab 1, YOTB, Vac 1, and EEA1
IDRs	intrinsically disordered regions
KH	K homology domain
LAMP1	Lysosomal-associated membrane protein 1
LB2	Lamin B2
MAMs	mitochondria-associated ER membranes
MLLE	MademoiseLLE domain
mRNPs	messenger ribonucleoproteins
MTS	mitochondrial targeting sequence
NAC	nascent chain-associated complex
nRCCs	Mitochondrial respiratory chain complexes
NSF	*N*-ethylmaleimide-sensitive factor
NTC	NineTeen complex
PAM2	PAB1C-associated motif
PARKIN	RBR E3 ubiquitin protein ligase
PI3P	phosphatidylinositol 3-phosphate
PINK1	PTEN-induced serine/threonine kinase 1
RBP	RNA-binding protein
RRMs	RNA recognition motifs
SFPQ	splicing factor proline- and glutamine-rich
STRIPAK	Striatin-interacting phosphatase and kinase complex
SYN2	synaptojanin 2
SYNJ2BP	SYN2 binding protein
TOM	translocase of the mitochondrial outer membrane complex
UTR	untranslated region
vRNA	viral genomic RNA

these seemingly separate fields of research studying endocytosis, post-transcriptional regulation and mitochondrial biology need to converge in the future. For more information on specific topics in the individual fields, we refer to excellent recent reviews (Holt & Bullock, 2009; Das *et al*, 2019; Hansen & Herrmann, 2019; Pfanner *et al*, 2019; Bykov *et al*, 2020; Das *et al*, 2021; Fernandopulle *et al*, 2021).

Membrane-free versus membrane-coupled mRNA transport

As noted above, the transport of mRNAs is a pronounced subcellular mechanism to orchestrate spatiotemporal protein expression. Currently, the main mechanisms described for mRNA transport function independent of membranes. Key RNA-binding proteins (RBPs) recognize specific RNA localisation elements within cargo mRNAs. Such transporter RBPs join with accessory RBPs to form large ribonucleoprotein complexes called mRNPs, which are the actual transport units (Martin & Ephrussi, 2009; Das *et al*, 2019; Abouward & Schiavo, 2020). The transport mRNPs are linked to molecular motors by tethering proteins for their active transport along the actin or microtubule cytoskeleton (Tekotte & Davis, 2002; Mofatteh & Bullock, 2017; Das *et al*, 2019).

Elegant *in vitro* studies deciphering minimal transport systems in fungi, animal and mammalian systems revealed important fundamental principles. In *Saccharomyces cerevisiae*, actin-dependent mRNA transport is important to regulate mating type-switching during cell division (Niessing *et al*, 2018). The minimal system for processive mRNA transport consists of the RNA-binding proteins She2p and She3p that cooperate to bind RNA localisation elements within the *ASH1* cargo mRNA. Binding of a She2p tetramer induces a pronounced conformational change in the mRNA and the presence of a She3p dimer is essential for specific RNA recognition (Edelmann *et al*, 2017). The dimeric She3p configuration allows recruitment of two Myo4p myosin motors forming a dimeric myosin complex that is essential for processive movement along actin filaments towards the daughter cell (Heym *et al*, 2013; Sladewski *et al*, 2013).

In *Drosophila melanogaster*, microtubule-dependent mRNA transport is crucial during embryogenesis to express determinants for patterning of embryonic segments locally (Bullock & Ish-Horowicz, 2001; Wilkie & Davis, 2001). The RNA-binding protein Egalitarian (Egl), for example, binds defined RNA localisation elements in pair-rule transcripts. The binding of two Egl per cargo mRNA promotes the engagement of BicD and releases the autoinhibition of this adaptor protein. Activated BicD recruits the dynein motor and its accessory complex dynactin more efficiently resulting in enhanced processive movement along microtubules with velocities observed *in vivo* (McClintock *et al*, 2018; Sladewski *et al*, 2018).

In neurons, mRNA transport is vital for a variety of functions ranging from directed axonal outgrowth to synaptic plasticity (Das *et al*, 2019; Holt *et al*, 2019; Dalla Costa *et al*, 2021; Fernandopulle *et al*, 2021). The nucleus in the soma of these highly polarised cells is separated substantially from distal dendrites and axons. This holds particularly true for axons that can extend up to a metre in length. Thus, long-distance mRNA transport needs to provide all parts of the neuronal cells with transcripts, and localised translation is a crucial determinant for the local proteome (Zappulo *et al*, 2017; Cagnetta *et al*, 2018; Das *et al*, 2019; Poulopoulos *et al*, 2019; Wang *et al*, 2019). Among the best-studied examples is the transport of β-actin mRNA taking place in both dendrites and axons (Zhang *et al*, 2001; Tiruchinapalli *et al*, 2003). Newly translated actin is most likely important for morphological changes of expanding synapses and dendritic spines (Yoon *et al*, 2016). The first *in vitro* transport-competent minimal system from humans revealed that the neuronal RNA-binding protein APC (adenomatous polyposis coli; Preitner *et al*, 2014) binds specific guanine-rich RNA sequences. The resulting RNA/protein complex is tethered to kinesin-2 via the adaptor protein KAP3 for microtubule-dependent movement of, e.g., β-actin mRNAs towards the plus ends of microtubules. Both, APC and cargo mRNAs, promote transport efficiency (Baumann *et al*, 2020). Thus, various

Box 1: The eukaryotic model organism *Ustilago maydis*

Ustilago maydis is the causative agent of corn smut disease and serves as a fungal model system for biotrophic phytopathogens, DNA repair, and cell biology (Holliday, 2004; Vollmeister *et al*, 2012; Lanver *et al*, 2017). The fact that highly polarised growth of infectious hyphae is strongly dependent on endosomal transport along microtubules makes it an ideal system to study membrane-coupled mRNA transport and translation (Haag *et al*, 2015). The dimeric key transcription factor regulating formation of infectious hyphae is called bEast (bE) and bWest (bW; in analogy with the reunion of Berlin). This central regulator is only active as heterodimer with the respective subunits derived from different mating partners (Vollmeister *et al*, 2012). Thereby, only after cell fusion an active bE/bW heterodimer can be formed. The regulation by heterodimerisation elegantly links transcription factor activation with mating. The resulting transcriptional activator is necessary and sufficient to arrest the cell cycle and elicit the hyphal growth programme with a defined axis of polarity: i.e. cells elongate at the apical growth pole and insert septa at the basal pole (Fig 3). This knowledge was used to design laboratory strains expressing an active bE/bW heterodimer under control of promoters regulatable by the nitrogen or carbon source in the medium (Brachmann *et al*, 2001). Thus, hyphal growth can be efficiently and highly reproducibly elicited in culture, independent of a mating partner. The resulting monokaryotic hyphae resemble their dikaryotic counterpart in all aspects of polarised hyphal growth (Brachmann *et al*, 2001; Baumann *et al*, 2012; Pohlmann *et al*, 2015).

in vitro studies with minimal systems recapitulate the natural transport processes independent of membranes with physiological run length and velocities comparable to those observed in living cells (Edelmann *et al*, 2017; McClintock *et al*, 2018; Sladewski *et al*, 2018; Baumann *et al*, 2020).

However, *in vivo* the process of mRNA transport appears to be more complex, since (i) higher-order mRNP transport granules are formed and (ii) alternative membrane-dependent transport pathways exist (Béthune *et al*, 2019; preprint: Cohen *et al*, 2021; preprint: Harbauer *et al*, 2021, see below). The formation of higher-order RNP granules in the form of biomolecular condensates is known for neuronal mRNA transport (Brangwynne *et al*, 2009; Kato & McKnight, 2018). Such RNP transport granules are comparable to other cellular biomolecular condensates like the nucleolus or RNP stress granules. All are formed by liquid–liquid phase separation involving RNA/RNA interactions (Tauber *et al*, 2020). Assembly is supported by RBPs acting as RNA chaperones. They often contain intrinsically disordered regions (IDRs) that promote formation of additional protein/RNA and protein/protein interactions (Protter & Parker, 2016). These larger mRNP granules might constitute key units of transport *in vivo* (Fernandopulle *et al*, 2021) and could explain how numerous mRNAs and RBPs are co-transported in the cell with a limited set of motor and adaptor proteins (Pushpalatha & Besse, 2019). Interestingly, these membrane-less RNP granules can be linked to membrane-enclosed organelles (Liao *et al*, 2019; Pushpalatha & Besse, 2019 see below), providing even higher-order transport structures consisting of both membrane-less and membrane-enclosed subcellular units.

Hitchhiking on membrane-enclosed organelles is an alternative transport mode and has been described for various organelle membranes like ER, mitochondria as well as endosomes (Béthune *et al*, 2019; preprint: Cohen *et al*, 2021; preprint: Harbauer *et al*, 2021; see below). These alternative modes of mRNA transport will be extensively discussed in the remaining part of the review with a special focus on endosomal transport. In summary, in a living cell, there are various transport mechanisms for mRNA delivery that are dependent and independent of membranes. Outstanding questions are what the individual contributions of each transport pathway to cellular RNA trafficking are, and what the function of the close association with membranes is. To address these critical questions, we need to uncover the specific routes cargo mRNAs take *in vivo* and to disclose the underlying mechanisms in the most detailed fashion possible.

Mechanistic insight into endosomal mRNA transport and membrane-coupled translation in *U. maydis*

The birth of an important new concept in mRNA transport has been the discovery of co-transport of mRNAs with membranes. Among the best-studied systems is endosomal mRNA transport in fungi (Haag *et al*, 2015; Béthune *et al*, 2019; Fig 1) that was disclosed during polar growth of infectious hyphae from *U. maydis* (Vollmeister *et al*, 2012, Box 1). In this fungus, the switch from yeast to hypha is an essential prerequisite for infection. The resulting highly polarised cells depend heavily on active motor-mediated transport. Loss of microtubule-dependent transport causes defects in the unipolar hyphal growth programme. A characteristic feature of aberrant growth is the formation of a second growth pole, resulting in bipolar hyphae (Vollmeister & Feldbrügge, 2010; Haag *et al*, 2015).

Major carriers for long-distance transport are endosomes that shuttle bidirectionally along microtubules throughout the hyphae, between the basal septum and the hyphal tip, passing the nucleus in the centre (Fig 1A). Microtubules are bundled in antiparallel arrays stretched throughout the whole hyphae, with unipolar regions at the plus ends of hyphal poles (Steinberg *et al*, 2001, Fig 1A and B). Processive movement of endosomes is mainly achieved by the plus-end directed Kinesin-3 type motor protein Kin3 (Schuster *et al*, 2011b). Minus-end-directed transport is mediated by cytoplasmic split dynein Dyn1/2 (Straube *et al*, 2001). The adaptor protein Hok1 coordinates motor attachment to endosomes (Bielska *et al*, 2014b), and the conventional kinesin Kin2 is needed to transport dynein back to the plus ends of microtubules for motor recycling (Lenz *et al*, 2006; Schuster *et al*, 2011a; Fig 1B). As expected, loss of any of these motors results in aberrant bipolar growth due to stalling of endosomal movement (Steinberg, 2012).

The shuttling endosomes display characteristics of early endosomes, because they are positive for the small GTPase Rab5a and the SNARE Yup1 (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors; Wedlich-Söldner *et al*, 2000). Early endosomes are classically known to function during endocytosis (Steinberg, 2014; Haag *et al*, 2017). However, according to a more modern view, early endosomes function as multipurpose platforms also involved in signalling and transport of protein complexes, such as peroxisomes and mRNPs (Bielska *et al*, 2014a; Guimaraes *et al*, 2015; Salogiannis & Reck-Peterson, 2016; Béthune *et al*, 2019). These functions need to be precisely regulated. The ESCRT regulator Did2 coordinates accurate maturation of endosomes in

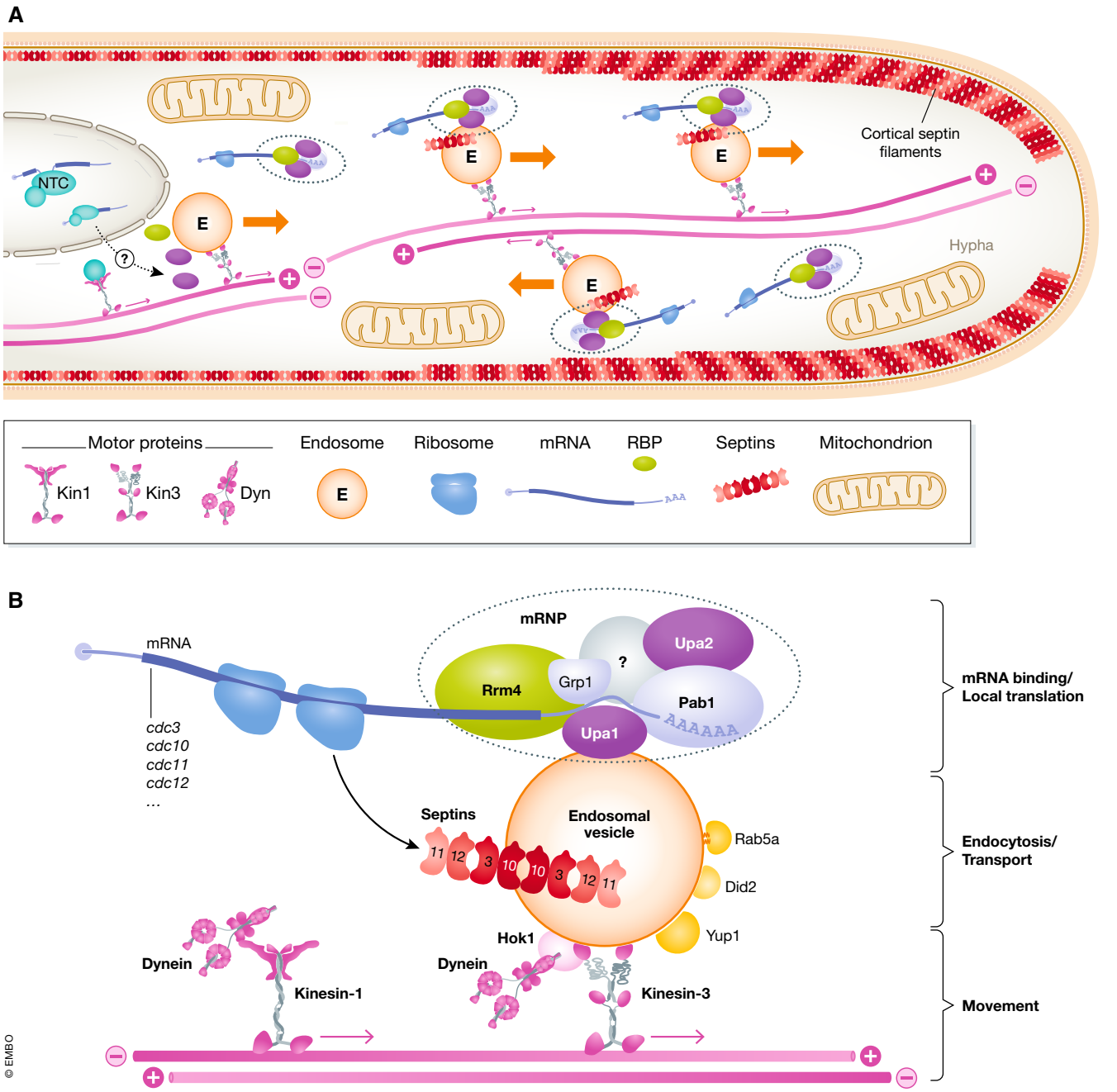

Figure 1. Model depicting endosomal mRNA transport in *Ustilago maydis*.

(A) Overview of an infectious hypha. Endosomes (E) shuttle along antiparallel microtubule bundles (pink). Plus-end directed transport is mediated by Kin3, whereas dynein transports endosomes to the minus ends. Cargo mRNPs (dashed ovals) are assembled most likely close to the nucleus involving components of the NineTeen complex (NTC, light blue) deposited during splicing. Local translation (ribosomes in dark blue) of cargo mRNAs like septins results in septin complex formation (red octamer). These complexes are transported towards the growth pole to generate higher-order septin filaments with gradients emanating from the growing tip (red line). Endosomal mRNP transport appears to be also important for import of proteins into mitochondria (see Fig 3). (B) Close-up of endosomes shown above. Components important for movement, endocytosis, transport and localised translation are shown (further details are given in the text).

U. maydis (Haag *et al*, 2017). Loss of Did2 renders Rab5a-positive early endosomes Rab7-positive, so that they adopt the membrane identity of late endosomes. This changes the motor composition and results in a switch from motile early endosomes to static late endosomes. Thus, without Did2, endocytosis and mRNP transport are disturbed, indicating that the ESCRT regulator orchestrates these two vital functions of the endosomal multipurpose platform (Haag *et al*, 2017; Fig 1B).

The core components of endosomal mRNA transport in *U. maydis*

The link between endosome and mRNA transport was discovered by studying the key RNA-binding protein Rrm4. This post-transcriptional regulator shuttles intensively along microtubules and binds thousands of mRNAs (see below; Baumann *et al*, 2012; Baumann *et al*, 2014; Olgeiser *et al*, 2019). Importantly, Rrm4 hitchhikes on the cytoplasmic surface of Rab5a-positive endosomes and loss of Rrm4 function results in the formation of aberrant bipolar hyphae. Comparable defects in hyphal growth are also observed during malfunction of microtubules or endosomal movement suggesting that the transport of mRNPs constitutes an essential function of endosomes during regulation of highly polarised growth (Becht *et al*, 2006; König *et al*, 2009). Endosomal mRNA transport is mediated by a defined core machinery consisting of factors like Rrm4, whose loss of function results in disturbed mRNA transport causing distinct defects in hyphal growth. The key factor Rrm4 contains three N-terminal RRMs (RNA recognition motifs) with a spacing typical for embryonic lethal abnormal vision (ELAV)-type RBPs. These RRMs are functionally important for RNA binding (Becht *et al*, 2006; Olgeiser *et al*, 2019), and RRM3 recognises specifically the core sequence UAUG in cargo mRNAs (Olgeiser *et al*, 2019). At its C-terminus, Rrm4 contains two MademoiseLLE domains (MLLE, also known as PABC, poly(A)-binding protein C-terminal domain, Becht *et al*, 2006; Müller *et al*, 2019), which serve as a protein-protein interaction pocket for specific interaction with two PAM2-like motifs (PAB1C-associated motif, Xie *et al*, 2014) present in the adaptor protein Upa1. The second core component Upa1 tethers mRNPs containing Rrm4 to the cytoplasmic surface of endosomes (Pohlmann *et al*, 2015, Fig 1). Attachment is mediated via its FYVE (Fab 1, YOTB, Vac 1, and EEA1) zing finger domain for specific interaction with PI(3)P lipids (phosphatidylinositol 3-phosphate, Stenmark *et al*, 2002). Loss of Upa1 causes defects in Rrm4 shuttling, which in turn results in disturbed mRNA transport and aberrant bipolar hyphal growth (Pohlmann *et al*, 2015). Besides the two PAM2-like motifs, Upa1 contains a single PAM2 motif for the interaction with the poly(A)-binding protein Pab1, an RBP with four RRMs that also carries an MLLE domain at its C-terminus. Notably, the MLLE domains of Rrm4 and Pab1 have different specificities for the PAM2 or the PAM2-like motifs in Upa1 (Pohlmann *et al*, 2015).

An additional core component of endosomal mRNP transport is the scaffold protein Upa2 containing four PAM2 motifs for interaction with Pab1 and a conserved C-terminal GWW sequence for interaction with a currently unknown component on the endosomal surface. Loss of Upa2 also interferes with mRNP transport resulting in the formation of bipolar hyphae (Jankowski *et al*, 2019). Similarly, the small glycine-rich protein Grp1 shuttles intensively on transport endosomes. However, Grp1 is not an essential core component of endosomal mRNPs and rather serves as RNA chaperone accompanying cargo mRNAs (Olgeiser *et al*, 2019). Loss of Grp1 results in the formation of unipolar hyphae with defects in response to cell wall stress (Olgeiser *et al*, 2019). Both Upa2 and Grp1 contain intrinsically disordered regions, opening up the possibility that biomolecular condensation is important for the formation of endosomal transport mRNPs in *U. maydis*. This assumption is supported by recent findings demonstrating that the fungal RNA-binding protein Whi3 from *Ashbya gossypii* containing glutamine-rich disordered regions forms biomolecular condensates associated with ER membranes. Importantly, membrane association of Whi3 controls the size of RNP condensates suggesting that interaction with lipid bilayers is crucial for regulating the precise formation and composition of these condensates *in vivo* (preprint: Snead *et al*, 2021). Similarly, Upa2 and Grp1 containing intrinsically disordered regions might influence formation of such RNP condensates at endosomal surfaces. In summary, the core endosomal mRNP consists of the key factors Rrm4, Upa1 and Upa2 and accessory RBPs such as Grp1 and Pab1 (Figs 1B and 2A).

Potential loading mechanism of mRNPs for endosomal transport in *U. maydis*

Currently, it is unclear how mRNPs are loaded on endosomes in *U. maydis*. Interestingly, a potential link between splicing and mRNP transport was discovered by studying Num1 (Kellner *et al*, 2014; Zhou *et al*, 2018). This regulatory protein is a homologue of splice factor SPF27 in humans that functions as core component of the evolutionarily conserved Prp19/CDC5 complex during intron removal (also called NTC, NineTeen complex; Grote *et al*, 2010, Fig 1A). Besides its nuclear function, Num1 also localises in the cytoplasm in the vicinity of microtubules and interacts with conventional kinesin Kin1 (Kellner *et al*, 2014; Zhou *et al*, 2018; Fig 1A). Loss of Num1 causes defects in processive movement of endosomes and associated mRNPs (Kellner *et al*, 2014). Although its precise function in endosomal mRNP transport is not solved, an attractive hypothesis is that Num1 is loaded onto mRNPs during splicing in the nucleus. Upon export to the cytoplasm, Num1 interacts with the kinesin motor close to microtubules and participates in loading of mRNPs onto endosomes (Fig 1A).

The link to the nuclear history of mRNPs and their cytoplasmic remodelling is well-known from transport processes in other organisms (Mofatteh & Bullock, 2017). For example, in *S. cerevisiae*, mRNP transport is initiated in the nucleus when the nuclear RBP Loc1p interacts with localisation elements of *ASH1* mRNA (Long *et al*, 2001; Niedner *et al*, 2013). During entry into the cytoplasm, the mRNP is remodelled and the SHE machinery containing She2p/She3p takes over the interaction with the RNA localisation elements during transport (Long *et al*, 2001; Niedner *et al*, 2013). In *D. melanogaster*, the exon junction complex (EJC) is deposited on mRNAs during splicing (Obrdlik *et al*, 2019). EJC deposition is particularly important for the transport of *oskar* mRNPs, since splicing creates the *oskar* mRNA localisation element by exon/exon junction and deposition of the EJC is needed for correct kinesin-mediated transport (Ghosh *et al*, 2012; Simon *et al*, 2015). Hence, these examples illustrate how post-transcriptional processes like splicing and mRNA transport are tightly intertwined to orchestrate spatiotemporal expression.

Endosomal transport of translationally active mRNAs in *U. maydis*

Studying cargo mRNAs of Rrm4 by combining *in vivo* UV crosslinking (iCLIP) and fluorescent protein (FP)-based RNA live imaging in *U. maydis* (Box 2) revealed that key cargo mRNAs encode the four septins Cdc3, Cdc10, Cdc11 and Cdc12 (König *et al*, 2009; Zander

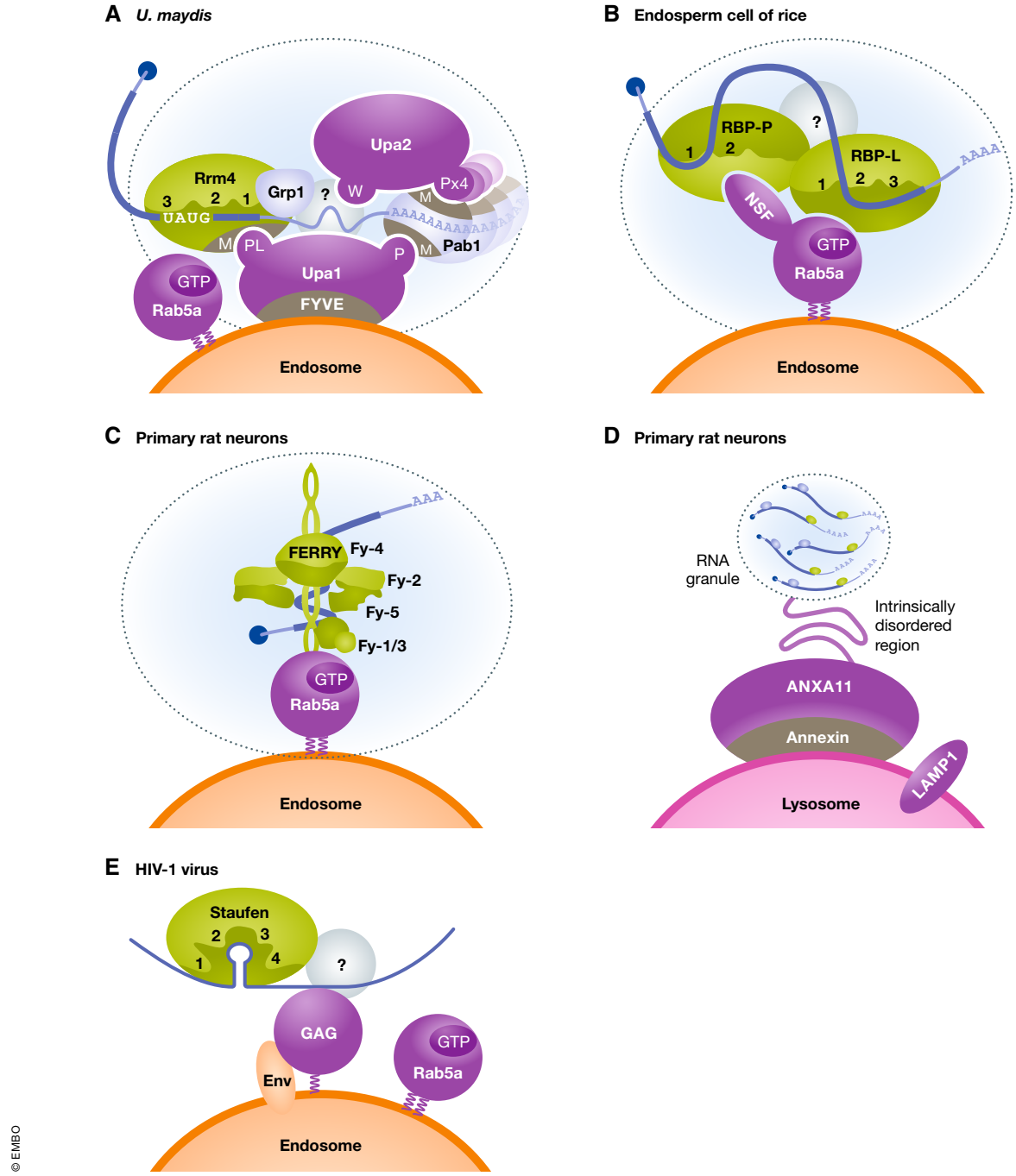

Figure 2. Schematic comparison of endosomal RNA transport in fungi, plants and animals.

(A–E) On the cytoplasmic surface of transport endosomes or lysosomes, mRNPs are attached by different factors (purple) to endosomes. Key RNA-binding proteins (green) interact with cargo RNA (blue). mRNAs are symbolised by CAP (blue circle) and a poly(A) tail (B and D are adapted from Tian *et al*, 2020b and Liao *et al*, 2019, respectively. Panel (C) is adapted from Schuhmacher *et al*, 2021 and Quentin *et al*, 2021 (both preprints). Panels (D, E) are proposed based on current literature (further details are given in the text).

et al, 2016; Olgeiser *et al*, 2019). Besides these septin mRNAs, their translation products are also shuttling on transport endosomes, (Baumann *et al*, 2012; Baumann *et al*, 2014; Zander *et al*, 2016; Fig 1). Interestingly, endosomal septin transport is needed for assembly of the four septin subunits into hetero-octameric complexes on the surface of endosomes. The resulting complexes are transported towards the hyphal tip and form higher-order septin filaments with a gradient emanating from the growth pole (Baumann *et al*, 2014;

Zander *et al*, 2016; Fig 1). In the absence of endosomal septin mRNA transport, hetero-octameric septin complexes no longer shuttle on endosomes and the formation of septin filaments as well as the tip gradient is disturbed. Instead, aberrant accumulations of small septin rings are visible in the cell (Zander *et al*, 2016).

The fact that septin mRNAs, translation products and ribosomes associate with endosomes in an Rrm4-dependent manner strongly suggests that endosome-coupled translation mediates septin complex formation and their long-distance transport (Baumann *et al*, 2014; Zander *et al*, 2016; Fig 1B). Aberrant cytoplasmic translation of septin subunits with promiscuous membrane-binding properties causes mistargeting and problems in complex formation (Zander *et al*, 2016). In essence, one critical function of endosomal mRNP transport is the loading of newly synthesised proteins onto the cytoplasmic surface of endosomes. Hence, membrane-coupled co-translation and co-movement of complex subunits promote formation and transport of heteromeric septin complexes.

Spatiotemporal regulation of complex assembly by co-translational mechanisms is an ancient process already operational in prokaryotes. The bacterial organisation of polycistronic mRNAs promotes the local translation of subunits and likely favours the co-translational assembly of these subunits (Shieh *et al*, 2015; Kramer *et al*, 2019). Eukaryotes follow a different strategy to reach the same goal. Monocistronic mRNAs of eukaryotes are spatially and temporally organised in RNA operons and regulons by the action of RBPs binding preferentially in the 3′UTR to orchestrate co-localisation of mRNAs and cognate translation products like subunits of protein complexes (Keene, 2007; Blackinton & Keene, 2014). Thereby, co-translational assembly of complexes is promoted. Consistently, in *S. cerevisiae*, ribosome profiling revealed that nine out of 12 multi-protein complexes, like fatty acid synthase (FAS) and translation initiation factor eIF2, are co-translationally assembled (Shiber *et al*, 2018; Schwarz & Beck, 2019). In the case of the proteasomal subunits Rpt1 and Rpt2, mRNAs and translation products co-localise in defined assembly particles for co-translational complex formation (Panasenko *et al*, 2019). Thus, co-translational assembly of protein complexes promoted by mRNA co-localisation appears to be a widespread mechanism.

Box 2: Main techniques used to study RNA transport and local translation

Monitoring RNA localisation and transport

i Apex-Seq: Proximity labelling of mRNAs combined with RNA sequencing or mass spectrometry analysis to examine their localisation and interaction with proteins (Fazal *et al*, 2019; Padron *et al*, 2019). In this technique, the ascorbate peroxidase APEX2 is targeted to subcellular compartments or fused to known RBPs for specific and direct biotin labelling of nearby mRNAs using biotin-phenol.

ii FISH: RNA fluorescence *in situ* hybridisation (RNA-FISH) or single-molecule FISH (smFISH) enable the specific and quantitative detection of endogenous mRNAs by using fluorescently labelled antisense oligonucleotides. Most of the knowledge about the subcellular localisation of mRNAs has been obtained using FISH methodology. However, these methods only provide a static view of mRNA localisation because cells need to be fixed prior to analysis (Bassell *et al*, 1994; Maekiniemi *et al*, 2020).

iii Fluorescent protein (FP)-based RNA live imaging: Enables *in vivo* labelling of specific single mRNAs and provides dynamic spatiotemporal information. mRNAs are visualised with bacteriophage-derived RBPs fused to fluorescent proteins that specifically bind to unique RNA hairpins integrated into the 3′ UTR (Buxbaum *et al*, 2015). Three systems are mainly used that exploit the RNA-binding characteristics of these RBPs to distinctive RNA hairpins: the MS2 (Bertrand *et al*, 1998), PP7 (Larson *et al*, 2011) and λN system (Daigle & Ellenberg, 2007). For the application of this method, it must be ensured that the integrated hairpins and the tagging do not affect the stability or translation of the mRNAs.

iv Molecular Beacon technology for live imaging: Molecular beacons (MBs) offer another method to analyse the localisation of specific endogenous mRNAs *in vivo* without any modification of the mRNA itself. MBs are single-stranded antisense oligonucleotide probes, which can form a stem-loop structure with a fluorophore and a quencher (Tyagi & Kramer, 1996). Upon hybridisation to the mRNA of interest, a conformational change releases the quencher and fluorescent signal appears (Bratu *et al*, 2003; Donlin-Asp *et al*, 2021). Although MBs are not yet widely exploited, they give great insight into the localisation of mRNAs especially for low abundant RNA molecules (Bratu, 2003).

v Cy3-UTP labelling for live imaging: Fluorescently labelled uridine-5′-triphosphate (UTP) analogs, e.g. Cy3-UTP or Cy5-UTP, can be injected into cells to label endogenous nascent RNAs *in vivo*. The UTP analogs are incorporated into RNA (including mRNA and rRNA) during its synthesis and can subsequently be monitored by fluorescence microscopy (Piper *et al*, 2015; Cioni *et al*, 2019). This method also allows the imaging of RNAs without major modification and reduces, e.g. the possibility of influencing the stability of the RNA. However, the feasibility to inject UTP analogs is a prerequisite for the application of this method.

Analysing local translation

i Proximity-based ribosome profiling: Enables ribosome profiles for specific organelles such as ER or mitochondria. For this purpose, the deep-sequencing-based technique is combined, for example, with the expression of a biotin ligase (BirA), which can be fused to a localisation element specific for organelles such as ER or mitochondria. As a result, ribosomes located in close proximity are labelled and can be specifically purified. mRNAs that have been protected from nuclease digestion by these ribosomes can subsequently be identified using RNA sequencing. This method allows to quantify the level of new protein synthesis in close proximity to, e.g., organelles and gives information on the ribosome position on bound mRNAs (Ingolia *et al*, 2009).

ii SunTag approach: Enables the visualisation of translation of mRNA molecules of interest *in vivo*. FP-based RNA live imaging is combined with fluorescent tagging of the nascent peptide chain. To this end, a repeating peptide array is fused to the ORF of interest recruiting a fluorescently labelled single-chain variable antibody fragment (Tanenbaum *et al*, 2014). Thereby, imaging of single mRNA molecules simultaneously with their nascent peptides is possible and the technique is successfully used in different cell types (Wang *et al*, 2016; Cioni *et al*, 2019).

iii Image-based transcriptomic analysis: Combination of smFISH, puromycin treatment and indirect immunofluorescence to study the localisation of translationally active mRNAs in proximity of membranous organelles. Puromycin dislocates mRNAs from translating ribosomes by initiating premature termination. Therefore, translation-dependent mRNA localisation can be studied (preprint: Popovic *et al*, 2020).

Distributive function of endosomal mRNA transport in *U. maydis*

Generating a transcriptome-wide view revealed that Rrm4 binds thousands of mRNAs mainly in the 3′UTR (Baumann *et al*, 2014; Olgeiser *et al*, 2019). All four septin mRNAs, for example, are bound in the 3′ UTR, which is consistent with co-translational assembly and transport. The fact that thousands of mRNAs can be bound by Rrm4 suggests intensive bulk endosomal transport of mRNAs. Consistently, the polyA-binding protein Pab1 also shows intensive endosomal co-shuttling (König *et al*, 2009). Bulk mRNA transport reflects an important distributive function of endosomal mRNP transport, avoiding gradients of mRNAs around the nucleus and supplying all areas of the hyphae with sufficient transcripts. The function in mRNA distribution also explains why most cargo mRNAs shuttle bidirectionally along microtubules, a very common feature of long-distance transport also observed during oocyte development and neuronal transport.

In oocytes from *D. melanogaster*, mRNAs are moving back and forth along microtubules with a small bias towards the posterior cortex (Zimyanin *et al*, 2008). Furthermore, at later stages of oocyte development, gradient formation is avoided by intensive kinesin-dependent ooplasmic streaming actively mixing the complete ooplasm (Lu *et al*, 2018). In neurons, bulk mRNA transport is also very prominent. Most mRNPs move bidirectionally in dendrites and axons (Cajigas *et al*, 2012; Shigeoka *et al*, 2016; Zappulo *et al*, 2017; Tushev *et al*, 2018; Das *et al*, 2019). This transport phenomenon was explained in dendrites by the sushi belt model: mRNPs are transported bidirectionally at all times to supply synapses with mRNAs for local translation. Hence "hungry" synapses are served on demand by deposition of specific mRNPs (Doyle & Kiebler, 2011).

It was also demonstrated in *U. maydis* for the first time that translationally active polyribosomes are co-transported intensively on endosomes (Baumann *et al*, 2014; Higuchi *et al*, 2014). Thereby, not only mRNAs but also ribosomes are transported over long distances to distal areas in highly polarised cells. This supports the hypothesis of a distributive function of endosomal transport to enable efficient translation throughout the entire cell (Baumann *et al*, 2014; Higuchi *et al*, 2014). Noteworthy, distal ribosomes can also be remodelled locally after deposition in neurons, as described recently for distinct ribosomal proteins, which were locally translated at growth poles of axons (Shigeoka *et al*, 2016).

In summary, main functions of endosomal mRNA transport are the distribution of mRNAs throughout cells, the transport of translation products and associated ribosomes, as well as the loading of newly synthesised proteins onto the surface of transport endosomes. Thereby, the entire cell is supplied with vital components for optimal spatiotemporal protein expression at all times.

Conservation and biological functions of endosomal mRNA transport

How widespread is endosomal transport and translation of mRNAs? Besides *U. maydis*, endosome-mediated mRNA trafficking is also operational in other fungi (Müller *et al*, 2019; Stein *et al*, 2020), plants (Tian *et al*, 2020b) and humans (Cioni *et al*, 2019; Liao *et al*, 2019). The phylogenetic analysis of the core endosomal mRNP transport machinery from *U. maydis* revealed that homologues of the components are present in other Basidiomycota. In addition, the orthologues are also found in distantly related Chytridiomycota and Mucoromycota (Müller *et al*, 2019). Interestingly, Rrm4 and Upa1 orthologues of *Rhizophagus irregularis*—a distantly related symbiont forming arbuscular mycorrhiza within plant cells (Mucoromycota)—shuttle on transport endosomes when expressed in hyphae of *U. maydis*. This suggests that core endosomal mRNP transport is widespread and functionally conserved in fungi (Müller *et al*, 2019).

By contrast, no orthologues of the core endosomal transport machinery are found in Ascomycota, while RNA transport is well-known in *S. cerevisiae* and *C. albicans* (Elson *et al*, 2009; Niessing *et al*, 2018). Thus, endosomal transport might be carried out by different factors in Ascomycetes. One such transport component could be the RNA-binding protein Gul1/Ssd1 that shuttles on endosomes in *Sordaria macrospora* and its close relative *Neurospora crassa* (Herold *et al*, 2019; Stein *et al*, 2020). Since the poly(A)-binding protein Pab1 also shuttles on endosomes, mRNP transport via endosomes is highly likely in these fungi (Stein *et al*, 2020). Remarkably, we also identified a Gul1 orthologue as a potential interaction partner of Rrm4 that shuttles on mRNP transport endosomes in *U. maydis* (M Tulinski, K Müntjes, M Feldbrügge; unpublished observation). This points towards a conserved, ancient and widespread endosomal transport machinery in fungi. Noteworthy, Gul1 was identified as a target of the STRIPAK "striatin-interacting phosphatase and kinase" complex, an evolutionarily conserved signalling complex implicated in several human diseases, like different types of cancer and cardiovascular disorders (Kück *et al*, 2019; Stein *et al*, 2020). In humans, the STRIPAK complex has been hypothesised to modulate dynein activity in axons and might thereby influence vesicle and autophagosome transport (Neisch *et al*, 2017). These findings provide new hints on how post-translational regulation might influence endosomal mRNP transport.

In endosperm cells of developing rice seeds, mRNAs encoding the storage proteins glutelin and prolamine are segregated to distinct, specific subdomains of the cortical ER, thereby enabling the localisation of the encoded storage proteins in separate endomembrane compartments in the mature seed (Tian *et al*, 2020a). Specific localisation elements present in the glutelin and prolamine mRNAs are recognised by the RNA-binding proteins RBP-P and RBP-L, which might mediate mRNP transport (Hamada *et al*, 2003; Washida *et al*, 2009; Washida *et al*, 2012). These proteins contain two and three RRMs, respectively, and carry a glycine-rich intrinsically disordered region at their C-terminus (Tian *et al*, 2018). Both RBPs interact with each other and in addition, RBP-P binds to *N*-ethylmaleimide-sensitive factor (NSF), an ATPase involved in the regulation of membrane fusion. RBP-L interacts with the GTP-bound form of the small GTPase Rab5a, a marker for early endosomes resulting in a quaternary complex containing mRNA (Fig 2B). The mRNP complex is formed at the cytoplasmic surface of early endosomes containing the glutelin cargo mRNA. Loss of function mutations in *rab5a* results in mistargeting of the mRNP complex verifying that endosomal transport of translationally inactive mRNAs is crucial for the efficient targeting of the mRNA to the cortical ER (Tian *et al*, 2020b). This uncovers an alternative mode of mRNP attachment to endosomes via direct Rab5a interaction (Fig 2B). In essence, endosomal RNA transport in plants also include RRM proteins and is needed for organelle communication between endosomes and ER.

Endosomal mRNA transport and endosome-coupled translation are also quite common in animal cells. Initially, this process was found in axons of retinal ganglion cells from *Xenopus laevis*, but it is also operational in rat and zebrafish neurons, as well as in various types of cultured mammalian cells (Cioni *et al*, 2019; Liao *et al*, 2019; preprint: Popovic *et al*, 2020 preprint: Schuhmacher *et al*, 2021). Studies of mRNP transport in axons of retinal ganglion cells showed that Cy3-UTP injections resulted in labelling of endogenous mRNAs (Box 2) that were co-transported with Rab5a-positive, early and Rab7a-positive, late endosomes (Cioni *et al*, 2019). Movement was mainly observed on motile late endosomes. Interfering with Rab5a and Rab7a function did not strongly influence the bulk mRNP transport (Cioni *et al*, 2019), suggesting the presence of alternative transport pathways. Importantly, the authors found evidence for membrane-coupled translation at the surface of late endosomes. Prominent examples are mRNAs encoding mitochondrial proteins, suggesting a function as endosomal platform to orchestrate mitochondrial protein import (Cioni *et al*, 2019). Consistently, studying human Rab5 effector proteins revealed the presence of the novel FERRY protein complex potentially linking endosomal mRNA transport, the translation machinery and mitochondrial protein import in neurons (preprint: Schuhmacher *et al*, 2021). This stable five-membered complex, consisting of Fy1 to Fy5, interacts *in vitro* specifically with the activated GTP-bound form of Rab5a. Structural studies revealed a clamp-like elongated structure with arm-like appendages protruding from a central Fy4 dimer. One of the extensions composed of the Fy2 coiled-coil region interacts with Rab5 most likely on the endosomal surface (preprint: Quentin *et al*, 2021; Fig 2C).

Another example for membrane-coupled mRNA transport was uncovered in neurons from rats and zebrafish, studying LAMP1-positive endosomes of the lysosomal and endolysosomal system, referred to as lysosomes in short. Highly comparable to mRNA transport on the cytoplasmic surface of endosomes, RNP granules hitchhike on LAMP1-positive lysosomal vesicles (Fig 2D). Transport mRNPs containing cargo like β-actin mRNA are linked to lysosomes by annexin ANXA11 (Liao *et al*, 2019). This membrane-associated factor contains an N-terminal intrinsically disordered region for interaction with RNP granules and a C-terminal annexin domain for calcium-dependent lipid binding. Remarkably, a new concept was disclosed by demonstrating that the IDR of ANAX11 enables co-transport of mRNPs in form of biomolecular condensates. Hence, ANAX11 serves as a novel tethering factor linking membrane-less RNP granules with membrane-bound organelles (Liao *et al*, 2019; Fig 2D). Mutations associated with amyotrophic lateral sclerosis (ALS) in ANXA11 interfere with mRNP transport suggesting a link to neuronal disease (Liao *et al*, 2019).

Importantly, the link between mRNAs and endosomes is not restricted to neurons, since image-based transcriptomic analysis (Box 2) of candidate mRNAs demonstrated endosomal association also in cultured mammalian cells (preprint: Popovic *et al*, 2020). Studying a selection of mRNAs encoding various endosomal and endomembrane regulators revealed the presence of twelve mRNAs specifically associated with endosomes. Biochemical purification of endosomes combined with RNAseq experiments verified that all twelve mRNAs were present in the endosomal fraction (preprint: Popovic *et al*, 2020). Endosomal localisation of mRNAs encoding FYVE proteins like EEA1 was dependent on translation, and the cysteine and glycine-rich protein 1 (CSRP1) appears to be involved in translational regulation (preprint: Popovic *et al*, 2020). Hence, local translation might function in efficient subcellular endosomal targeting of EEA1, a process that is comparable with local translation of the membrane-associated septins in *U. maydis* (see above).

Investigating membrane association of mRNPs in animal and human systems revealed the presence of mRNAs on the cytoplasmic surface of Rab5-positive early endosomes, Rab7-positive late endosomes and LAMP1-positive lysosomal vesicles (Cioni *et al*, 2019; Liao *et al*, 2019; preprint: Popovic *et al*, 2020; preprint: Schuhmacher *et al*, 2021; see below). For LAMP1-positive lysosomal vesicles, the association of cargo mRNAs via the adaptor protein ANAX11 is important for membrane-coupled long-distance transport in highly polarised axonal cells (Liao *et al*, 2019). These examples indicate that mRNAs are associated with numerous types of membranes of the endocytic transport pathway. However, the precise biological function appears to be specific in the different systems. In case of early and late endosomes, local translation might be more important to load newly synthesised proteins on membranes of the endocytic system, whereas LAMP1-positive vesicles are used for long-distance mRNA trafficking. Furthermore, since it has been observed that some neuronal LAMP1-positive lysosomes lack major lysosomal hydrolases, the presence of different subpopulations of lysosomes with variable functions is conceivable (Cheng *et al*, 2018). This is clearly an emerging research field and additional examples and more mechanistic insights are needed to draw final conclusions.

Previously, it was found that viral RNAs hijack the endosomal machinery for maturation and transport towards the plasma membrane (Basyuk *et al*, 2020). This indicates that the endosomal transport is not restricted to mRNAs. The genomic RNA of Murine Leukaemia Virus (MLV) is co-transported with early endosomes, where the membrane-associated viral Gag protein recognises the genomic vRNA and functions as a tethering factor (Basyuk *et al*, 2003; Fig 2E). Similarly, the HIV-1 genomic vRNA travels intracellularly on a dynein-dependent LAMP1-positive endosomes. Membrane association is mediated by myristoylated Gag protein (Lehmann *et al*, 2009) and cellular factors like the double-strand RNA-binding protein Staufen 1 might form vRNPs resembling natural occurring mRNPs (Mouland *et al*, 2001; Chatel-Chaix *et al*, 2004; Fig 2E). It is noteworthy that the transport of vRNA on endosomal membrane directly influences viral production (Lehmann *et al*, 2009).

More recently, it was shown that also precursors of miRNAs (pre-miRNAs) are transported on late endosomes/lysosomes in axons of retinal ganglion cells from *X. laevis* (Corradi & Baudet, 2020; Corradi *et al*, 2020). The transported pre-miRNAs are delivered to the growth cone, where they are spatially processed to active miRNAs after exposure with repellent cues. Thereby, the translation of specific transcripts encoding, for example, tubulin beta 3 class III (TUBB3) is silenced. This process is needed for growth cone steering and correct formation of neuronal circuits (Corradi & Baudet, 2020; Corradi *et al*, 2020). Interestingly, small RNAs also shuttle bidirectionally suggesting a distributive function via these trafficking mechanism.

In summary, endosomal RNA transport is a widespread mechanism present in fungi, plants and animals. Cargos are various types of RNAs, like mRNAs, vRNAs and pre-miRNAs. Evidence is provided that mRNAs are translated at the cytoplasmic surface of

endosome to determine precise subcellular targeting of the cognate translation products. The new link of mitochondrial proteins in *X. laevis* and *U. maydis* to endosome-coupled translation (see below) opens up new possibilities to interlink different organelles and regulate subcellular mitochondrial functions.

Targeting of proteins to mitochondria in a pre-, co-, or post-translational manner

More than 99% of all mitochondrial proteins are encoded in the nucleus and many of those are synthesised in the cytosol and transported post-translationally to distinct locations within mitochondria using N-terminal, C-terminal or internal targeting signals (Pfanner *et al*, 2019). There is clear evidence that a substantial subset of mitochondrial proteins is targeted to this organelle of endosymbiotic origin via pre-translational and co-translational pathways. Efficient targeting of mitochondrial proteins optimises most likely mitochondrial function locally and dynamically (Williams *et al*, 2014; Fazal *et al*, 2019). The corresponding pathways are briefly introduced as follows: (i) the classical co-translational pathway depends on the interaction of an N-terminal mitochondrial targeting sequence (MTS) in the nascent mitochondrial protein with mitochondrial binding partners during translation, (ii) direct mRNA anchoring to the mitochondrial surface by mitochondria-associated RNA-binding proteins followed by *in situ* translation, (iii) the ER surface-mediated protein-targeting pathway (ER-SURF), (iv) and endosome-coupled translation in the vicinity of mitochondria.

The classical co-translational targeting pathway was suggested already in the 1970s by Ron Butow *et al* based on the observation of cytosolic 80S ribosomes that were located on the mitochondrial outer membrane where mRNAs encoding mitochondrial proteins are translated (Kellems & Butow, 1972; Kellems *et al*, 1974). In *S. cerevisiae*, the mitochondrial outer membrane protein OM14 is one receptor for ribosomes that interacts with the nascent chain-associated complex (NAC) and thereby supports co-translational import (Lesnik *et al*, 2014; Béthune *et al*, 2019). In this setting, the MTS emerging from the ribosome exit tunnel directly interacts with the translocase of the outer membrane (TOM) complex, so that the protein import occurs co-translationally. Consistently, electron cryotomography showed that ribosomes at mitochondrial membranes face with their exit tunnels towards the mitochondrial membrane (Gold *et al*, 2017). Active translation of mRNAs by such ribosomes was demonstrated by proximity-based ribosome profiling studies (Vardi-Oknin & Arava, 2019; Williams *et al*, 2014; Fazal *et al*, 2019; Box2).

Besides this pathway, there is increasing evidence that mRNAs can be targeted to mitochondria by mitochondria-associated RNA-binding proteins. Consistently, it has been demonstrated that localisation elements in the 3′ UTR are crucial for targeting ATP2 and Oxa1 to mitochondria (Gadir *et al*, 2011; Béthune *et al*, 2019). The mitochondrial-associated RBP Puf3p from *S. cerevisiae*, for example, binds numerous mRNAs encoding mitochondrial proteins in their 3′ UTR and thereby promotes mitochondrial protein import (Gerber *et al*, 2004; Bykov *et al*, 2020). This was further supported by a systematic approach using proximity labelling of mRNAs with APEX-Seq (Box 2) demonstrating the presence of mRNAs encoding distinct sets of mitochondrial proteins in the vicinity of mitochondria in a manner that is independent of translation (Fazal *et al*, 2019).

Targeting mRNAs to mitochondria also enables precise control of translation. In *D. melanogaster*, mRNAs of nuclear-encoded subunits of mitochondrial respiratory chain complexes (nRCCs) are translationally repressed in the cytosol. These mRNAs are recruited to mitochondria by PINK1, which interacts with Tom20, a major mitochondrial import receptor at the outer membrane of mitochondria. Upon mRNA binding, PINK1 together with PARKIN stimulate the degradation of translational repressors and thereby activate translation of the nRCC mRNAs. Thereby, PINK1 might not only regulate translation of specific mitochondrial proteins but also ensures that translation occurs in the vicinity of the TOM complex (Gehrke *et al*, 2015). Since PINK1 stabilisation and PARKIN recruitment is taking place at the outer membrane of dysfunctional mitochondria, it appears that this PINK1-dependent mechanism might be of particular importance for localised mitochondrial quality control. It could also contribute to the pathogenesis of Parkinson's disease beyond the known roles of PINK1 and PARKIN in mitophagy (Kumar & Reichert, 2021).

An important question is how mitochondrial functions are regulated in highly polarised cells like neurons and hyphae. Recent findings indicate that organelle co-transport is important. One example is mRNA hitchhiking on mitochondria as shown for *PINK1* mRNA (preprint: Harbauer *et al*, 2021). As elaborated above, the short-lived PINK1 protein needs to accumulate on the surface of mitochondria to orchestrate mitophagy. How is a constant supply of newly synthesised protein guaranteed for mitochondria that are far away from the nucleus? A neuron-specific variant of Synaptojanin 2 (SYN2) containing an RRM type RNA-binding domain binds *PINK1* mRNA in complex with the SYN2 binding protein (SYNJ2BP). Mitochondrial association of the PINK1 mRNA depends on translation as well as the synergistic action of the MTS and sequence elements at the beginning of the ORF. Therefore, mRNA transport in dendrites and axons is achieved (preprint: Harbauer *et al*, 2021). Interestingly, mitochondrial hitchhiking is not restricted to *PINK1* mRNA, as Cox7c mRNA (encoding cytochrome c oxidase subunit 7c; respiration chain complex IV) is also actively transported on the surface of mitochondria in an MTS-dependent manner in axons (preprint: Cohen *et al*, 2021). One critical RBP-binding mRNAs encoding mitochondrial proteins is CLUH that was shown to couple mitochondrial biogenesis with mitophagy and mTORC1 signalling (Pla-Martin *et al*, 2020).

Besides mRNA co-transport on the surface of mitochondria, new insights disclosed an intimate link of mitochondrial protein import with communicating organelles like ER and endosomes. In case of the ER-SURF pathway, certain proteins with the final destination mitochondria are found initially at the ER surface (Hansen *et al*, 2018; Bykov *et al*, 2020). Targeting of mitochondrial proteins might be supported by ER-coupled translation, since mRNAs encoding mitochondrial proteins are found at the ER (Jan *et al*, 2014). Here, they are translated and the resulting proteins are transferred to mitochondria. The ER-localised Hsp40/*DnaJ*-like protein Djp1 is required for protein translocation into the mitochondria (Hansen *et al*, 2018). Protein transfer from ER to mitochondria presumably occurs at ER-mitochondrial contact sites, also termed as mitochondria-associated ER membranes (MAMs). MAMs are well-known interorganelle sites that are needed, e.g. for lipid transport

and Ca^{2+} exchange (Lackner, 2019). In addition to mitochondrial and ER *surfing*, another post-translational pathway promoting mitochondrial protein import depends on local translation of mitochondrial proteins from nuclear-encoded mRNAs at the cytoplasmic surface of endosomes (see next section).

Endosome-coupled translation and mitochondrial protein import

Endosome-coupled translation constitutes a novel concept mediating efficient mitochondrial protein import. This process appears to be particularly important in highly polarised cells like fungal hyphae and neurons (Cioni *et al*, 2019; Olgeiser *et al*, 2019; preprint: Schuhmacher *et al*, 2021), where subcellular mitochondrial function needs to be maintained at a large distance from the nucleus (Fig 3). Local mitochondrial activity might be particularly important in neurons. This notion is based on the observation that local translation of mitochondrial proteins at activated synapses mediates efficient mitochondrial protein import to fuel high local energy demands in dendrites (Rangaraju *et al*, 2019; Kuzniewska *et al*, 2020).

In axons of retinal ganglion cells from *X. laevis* local mitochondrial activity is needed to coordinate branching. Focal hotspots of translation have been identified in the vicinity of mitochondria throughout axon length (Spillane *et al*, 2013), and mRNAs are co-transported with early and late endosomes. The latter serve as platforms of local translation in axons (Cioni *et al*, 2019; Fig 3). Consistently, ribosomal proteins as well as the RBP SFPQ (splicing factor proline- and glutamine-rich) co-localise with Rab7a-positive endosomes (Cioni *et al*, 2019). SFPQ controls an RNA regulon involved in axon functionality (Cosker *et al*, 2016).

Notably, late endosomes carrying mRNAs encoding mitochondrial proteins often pause at mitochondria (Cioni *et al*, 2019; Fig 3). For example, an important target mRNA for endosomal transport

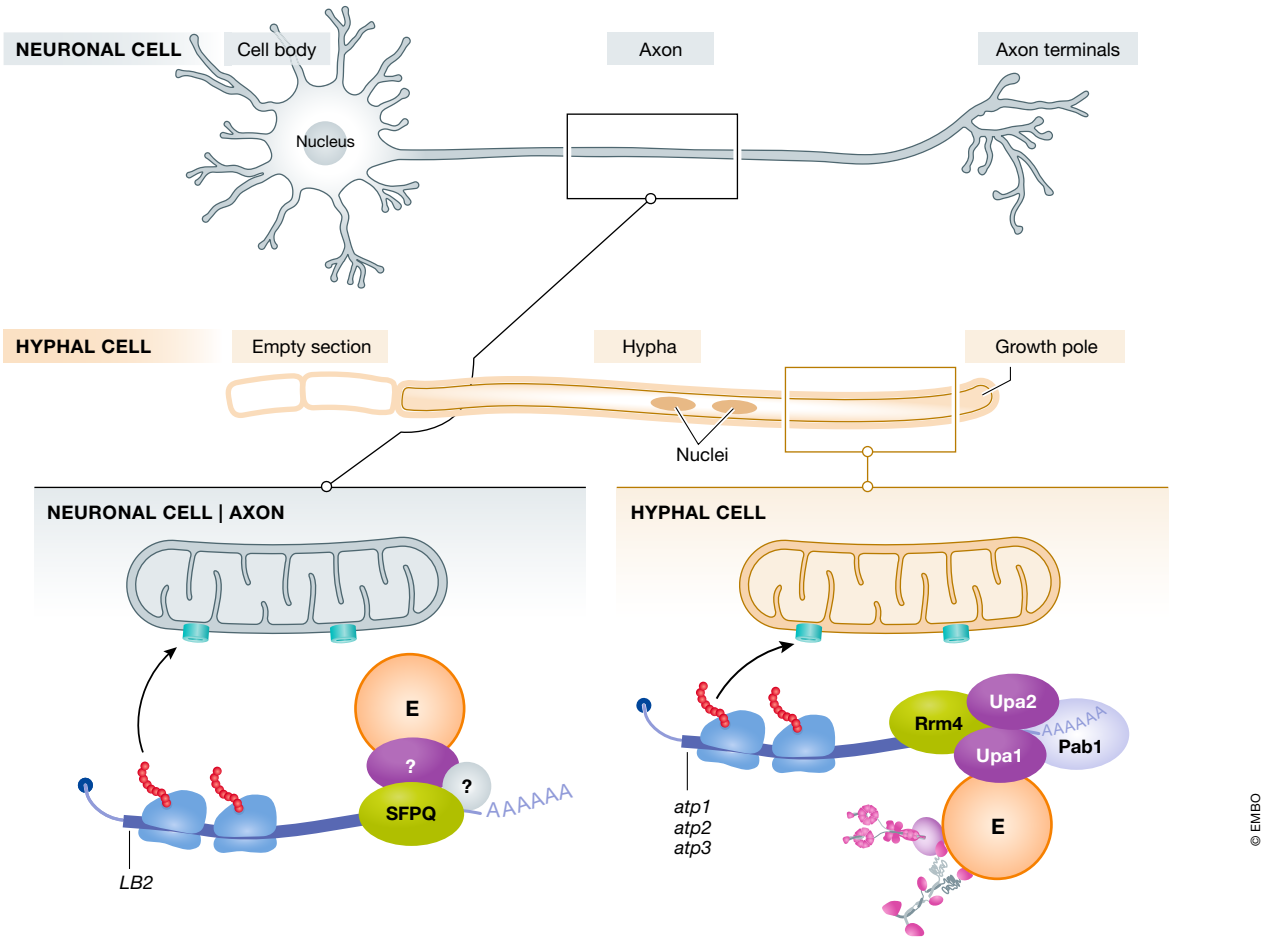

Figure 3. Schematic comparison of endosome-coupled translation and mitochondrial protein import.

At the top, a highly polarised neuron and a fungal hypha are depicted. In both cases, a defined axis of polarity is established with the growth pole indicated on the right. Local translation of mRNAs encoding mitochondrial proteins at the surface of late endosomes (E) in neurons (left) is compared to membrane-coupled translation at the surface of early endosomes (E) in hyphae (right). The TOM complex is depicted as a pore in the mitochondrial membrane. Symbols are used as in the other figures, and details are given in the text.

encodes the intermediate filament protein lamin B2 (LB2), an essential factor for axon maintenance. LB2 conventionally localises at the nuclear membrane. However, in axons, LB2 is targeted to mitochondria where it determines mitochondrial activity (Yoon *et al*, 2012). According to results obtained with the *in vivo* SunTag fluorescent tagging system (Wang *et al*, 2016; Wu *et al*, 2016; Yan *et al*, 2016; Box 2), *LB2* mRNA is associated with late endosomes and translationally active on these endosomes in the vicinity of mitochondria (Cioni *et al*, 2019). This observation suggests that endosomal-coupled translation of *LB2* mRNA targets the translation product to mitochondria. A second example is the mRNA encoding voltage-dependent anion-selective channel protein 2 (VDAC2), which functions in exchanging solutes across the outer mitochondrial membrane (Naghdi & Hajnoczky, 2016). VDAC2 is translated on endosomes near mitochondria. Hence, it appears to be a common process that mRNAs encoding mitochondrial proteins are translated on the surface of endosomes often in proximity to mitochondria (Cioni *et al*, 2019; Fig 3). Remarkably, Rab7a mutations characteristic for CMT2B Charcot-Marie-Tooth type 2B disease interfere with endosome-coupled translation at late endosomes (Cioni *et al*, 2019). Expressing these mutant alleles resulted in highly elongated mitochondria and altered mitochondrial membrane potential indicating a functional influence on mitochondrial morphology and bioenergetics.

Intriguingly, a novel link between endosomes, RNA and mitochondrial biology has been recently discovered studying the novel Rab5a effector complex FERRY (preprint: Quentin *et al*, 2021; preprint: Schuhmacher *et al*, 2021; Fig 2C). Co-purification experiments using the FERRY complex as molecular handle revealed the presence of mRNAs and ribosomal proteins on endosomes suggesting a role in endosome-coupled transport and translation of mRNAs (preprint: Schuhmacher *et al*, 2021). No specific RNA-binding domain has been identified yet, but several RNA contact sites are present along the elongated structure of the FERRY complex (preprint: Quentin *et al*, 2021). mRNAs that encode mitochondrial proteins, like *mrpl41* mRNA encoding ribosomal protein 41 of the large subunit of 70S mitochondrial ribosomes, are enriched on endosomes. Based on results obtained with smFISH experiments, mRNAs, endosomes and mitochondria are found in close proximity in primary rat hippocampal neurons. Although endosomal mRNA movement has not been demonstrated, the FERRY complex has the potential to serve as a novel anchor for endosomal mRNA transport in neurons. In three of five cases, namely Fy1 (Tbck), Fy2 (Ppp1r21) and Fy3 (C12orf4), mutations in these human proteins cause brain malfunctions (preprint: Quentin *et al*, 2021; preprint: Schuhmacher *et al*, 2021; Fig 2C). In summary, early and late endosome-coupled translation might determine mitochondrial function and be linked to neuronal diseases.

Studying the proteome of membrane-associated fractions in hyphae from *U. maydis* showed that loss of endosomal mRNA transport altered the amount of mitochondrial proteins. Accumulation of the complex I component Nuo2 and the F_1F_O ATP synthase subunit Atp4 was reduced in comparison with wildtype, while ATPase family gene 3 (Afg3) was increased in *rrm4Δ* strains lacking endosomal mRNA transport (Koepke *et al*, 2011). Afg3 is a subunit of the m-AAA protease located in the inner membrane and functions in the quality control of mitochondrial complex assembly by mediating degradation of non-assembled mitochondrial inner membrane proteins (Arlt *et al*, 1998). This exemplifies a molecular link between endosomal mRNA transport and the biogenesis of mitochondrial respiratory chain complexes. Consistently, uncovering transcriptome-wide Rrm4 target mRNAs revealed that mRNAs encoding mitochondrial proteins such as most subunits of the F_1F_O ATP synthase are recognised by Rrm4. Intriguingly, the mRNAs are predominantly bound by Rrm4 precisely at the stop codon linking transport with translational termination (Olgeiser *et al*, 2019).

Thus, mRNAs encoding mitochondrial proteins might be co-translationally transported exposing the N-terminal MTS for a potential direct handover to the mitochondrial import apparatus (Fig 3), yet might be delayed for translational termination. Based on this hypothesis, Rrm4 would pause translation just before termination by binding to the stop codon, until the translation product reaches its destination. Hence, spatial regulation of translation would be another function for endosome-coupled transport to mitochondria (Fig 3). Consistently, it was found in *S. cerevisiae* that translation speed was important for mRNA localisation to mitochondria. The coding sequence of mitochondrial protein TIM50 contains seven consecutive prolines slowing down translation, which increases the possibility for the MTS to interact with its receptor on the surface. Therefore, the localisation to mitochondria is enhanced (Tsuboi *et al*, 2020). In essence, we observe a link of endosomal mRNA transport to mitochondria both in axons and fungal hyphae suggesting evolutionary conversation (Fig 3). The linkage could either be due to convergent or divergent evolution. At present, this is unknown, but we favour the idea that endosomal mRNA transport is an ancient process, since it is present in all fungal phyla, plants and mammalian cells. Therefore, also the link to mitochondrial biology might be ancient and more widespread than currently anticipated.

Consistently, potential homologues of components of the FERRY complex were found in fungi (preprint: Schuhmacher *et al*, 2021). Along this line, we speculate that the process of membrane-coupled transport of mRNAs to mitochondria is not restricted to polarised cells. During the oogenesis of *X. laevis*, nuclear mRNAs destined to the "vegetal pole" are enriched at a peculiar mitochondrial cloud (Kloc *et al*, 1996; Chang *et al*, 2004). This region is rich in active mitochondria and located between germinal vesicles and the plasma membrane (Wilding *et al*, 2001). A target mRNA encodes XNOA36 (Vaccaro *et al*, 2012), the homolog of the mammalian NOA36, which associates dynamically with outer membranes of mitochondria (de Melo *et al*, 2009). Since the subcellular region is also rich in ER, another potential link between RNA, membrane trafficking and mitochondrial function is conceivable.

Concluding remarks and outlook

mRNP trafficking constitutes a highly complex intracellular process with various transport solutions for distinct sets of cargo mRNAs. Besides the classical system consisting of membrane-free mRNP transport, it now becomes apparent that alternative pathways exist like organelle hitchhiking on mitochondria, the ER and endosomes (Béthune *et al*, 2019; preprint: Cohen *et al*, 2021; preprint: Harbauer *et al*, 2021; preprint: Schuhmacher *et al*, 2021). Why are several sophisticated modes of transport systems operational? In this context, it is important to reiterate that each translation product has a well-defined final subcellular location: β-actin is needed at the

cellular growth poles, septins need to assemble in heteromeric complexes delivered to specific subcellular sites and mitochondrial proteins end up in mitochondria. Hence, the most parsimonious explanation for the existence of various transport pathways is the presence of tailor-made solutions that are best adapted to the individual need of the cargo mRNA. In analogy, the cell can choose between car, truck, plane or ship type vehicles for transportation. Another advantage of using additional membrane-coupled pathways is to avoid traffic jams for protein import into organelles by fully exploiting other membrane surfaces. In this way, mRNAs or co-translational intermediates can be stored or parked nearby.

One of the emerging alternative transport mechanisms is endosomal mRNA transport, an ancient, conserved mechanism, present in fungi, plants and animals. Various types of RNAs are transported, and the main function is spatiotemporal expression of proteins. More precisely, endosomal transport and translation of mRNAs are needed for (i) equal distribution of mRNAs and ribosomes, (ii) heteromeric complex assembly, (iii) ER targeting and (iv) mitochondrial protein import. The use of organellar membranes as local platforms for membrane-coupled translation (Béthune *et al*, 2019) mediates targeting to other organelles, and this appears to be an efficient mechanism regulating local activities in the eukaryotic cell. Currently, it is restricted to ER and endosomal membranes; however, links to secretory vesicles and mitochondrial membranes have been described (Zabezhinsky *et al*, 2016; Hansen *et al*, 2018). We believe that the link between mRNA transport, localised translation and organelle communication is a widespread phenomenon. Outstanding questions that need to be addressed are listed in Box 3.

Finally, we would like to point out that the intimate link of subcellular organelles like ER and endosomes with mitochondria of endosymbiont origin is also inspiring to reflect on the origin of endosymbiosis. It is now well accepted that genetic information of the initial endosymbiont was transferred to the nucleus and that protein-targeting signals enable the necessary import of proteins into the endosymbiotic organelle. Additionally, RNA-centric targeting signals are also required to direct RNAs in the vicinity of mitochondria for efficient protein import to orchestrate organelle function. Surprisingly, import of distinct mitochondrial proteins is promoted by membrane-coupled translation of cognate mRNAs at the surface of neighbouring organelles such as endosomes, demonstrating intensive networking of intracellular organelles. It is conceivable that for a successful incorporation of an endosymbiont in the local environment of its host, it is beneficial to establish intricate modes of communication with the host intracellular organelles like nucleus, ER and endosomes. In essence, studying the complex life of RNAs does not only increase our understanding of fundamental processes of the eukaryotic cell but also bridges numerous research areas ranging from RNA, membrane and mitochondrial biology to evolutionary biology.

Box 3: In need of answers

i How widespread is endosome-coupled mRNA transport and translation? Additional examples of membrane-coupled trafficking in other cell types and organisms are needed to answer this question.

ii What is the diversity of subcellular organelles endosomes communicate with? Besides peroxisomes and mitochondria, the link to other subcellular organelles should be analysed.

iii What is the composition of the endosome-associated transcriptome in different cell types and at different subcellular locations? The respective inventory of endosomal mRNAs using tailored biochemical purification and RNA sequencing approaches need to be determined.

iv How are mRNPs molecularly linked to endosomes? Structure function analyses of adaptor proteins or RBPs are required.

v How is endosomal RNA biology regulated and dynamically adapted to different growth and stress conditions? We need to identify key post-translational modifications in RBPs and study the action of the modifying enzymes.

vi How are mRNPs loaded and unloaded from endosomes? What are the interaction partners of key RBPs and how are these factors post-translationally regulated?

vii How does membrane-coupled translation orchestrate subcellular mitochondrial function? Biosensors and optogenetics can be used to study the influence of translation on local mitochondrial activity.

Acknowledgements

We thank laboratory members for critical reading of the manuscript. The work was funded by grants from the Deutsche Forschungsgemeinschaft under Germany's Excellence Strategy EXC-2048/1—Project ID 39068111 to MF; Project ID 267205415 – SFB 1208 to MF and ASR, DFG-FOR2333 FE448/10-2, DFG-FOR5116 FE448/15-1 and DFG-FE 448/11-1 to MF as well as DFG-GRK2576, DFG-GRK 2578 and DFG-RE1575/2-1 to ASR. Open Access funding enabled and organized by ProjektDEAL.

Author contributions

KM was responsible for the figures. All authors contributed to writing and revising of the manuscript and approved the final version.

Conflict of interest

The authors declare that they have no conflict of interest.

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