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Review

Journey from the Center of the Cell – the intra- and intercellular transport of mRNA

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Transport and localized translation of mRNA is crucial for the proper spatiotemporal organization of proteins within cells. Distribution of RNAs to subcellular domains has recently emerged as a major mechanism for establishing functionally distinct compartments and structures in the cells. There is an emerging evidence that active transport of mRNA involves cytoskeleton and membrane trafficking pathways in fungi, plants and animals, suggesting that it is a common phenomenon among eukaryotes. The important highlights are that the RNA-binding proteins recognize the cargo mRNA and that RNPs are actively transported on the cytoskeletal tracks or co-transported with membranous compartments, such as the endoplasmic reticulum and endosomes. The interest of scientists has expanded over the past years in response to the discoveries that RNA can be exported from cells to play a role in the intercellular communication. In this review, we will focus on characterization of the RNA transport both, within a cell and between cells, and on the currently proposed mechanisms for RNA targeting.

Key words: RNP, extracellular RNA, localized RNA, cytoskeleton, extracellular vesicle (EV), multivesicular body (MVB)

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INTRODUCTION

Establishment of the cell polarity is crucial for the developmental processes, including specification of the cell fates, early embryonic patterning, cell movements and specialization of cell types. Cell polarization is determined by the asymmetric segregation of organelles and various macromolecules within the cell. Distribution of RNAs to subcellular domains has recently emerged as the major mechanism for establishing functionally distinct compartments and structures in the cells. Transport and localized translation of mRNA is crucial for the proper spatio-temporal organization of proteins within cells. To ensure the spatially restricted protein synthesis, translation of mRNAs is silenced during transport and activated once they reach the final destination. mRNA localization appears to be the rule, as 70% of about 2314 mRNAs tested in *Drosophila melanogaster* embryos exhibited a clearly defined localization pattern (Martin & Ephrussi, 2009).

Transport of mRNAs to the destination domains is more suitable for a cell than transport of proteins. As a single RNA molecule can be used for translation of numerous proteins, the transport costs are reduced (Medioni *et al.*, 2012). Transport of mRNAs can prevent proteins from acting ectopically before they reach the appropriate site and can facilitate incorporation of proteins into macromolecular complexes by generating high local protein concentrations and allowing co-translation of different subunits (Mingle *et al.*, 2005). Nascent proteins may have properties distinct from the pre-existing copies due to post-translational modifications or chaperone-aided folding pathways (Dictenberg *et al.*, 2008). mRNA targeting allows for fine-tuning of gene expression in both, space and time, e.g. different splice variants are targeted to distinct cellular compartments (Medioni *et al.*, 2012; Baj *et al.*, 2011).

Recent observations suggest that active mRNA targeting involves the cytoskeleton and membrane trafficking pathways in animals (Medioni *et al.*, 2012), bacteria (Keiler, 2011), fungi (Zarnack & Feldbrügge, 2010) and plants (Crofts *et al.*, 2005), suggesting that it is a common phenomenon among eukaryotes. The inter- and intracellular transport pathways of RNA in eukaryotic cells are depicted in Fig. 1. Localization of mRNA molecules within the cytoplasm provides a basis for cell polarization, thus underlying developmental processes, such as asymmetric cell division, cell migration, neuronal maturation and embryonic patterning. Specific targeting of mRNAs in a cell can be achieved through different mechanisms. mRNA can be distributed due to (i) localized protection from degradation [e.g. *D. melanogaster* heat-shock protein-83 (Hsp83) mRNA (Aliotta *et al.*, 2010; Ding *et al.*, 1993)], (ii) passive diffusion coupled with local entrapment [e.g. *D. melanogaster* nanos mRNA (Forrest & Gavis, 2003) and *Xenopus laevis* Xcat2 mRNA (Chang *et al.*, 2004)] and (iii) finally mRNA can be actively transported to its destination domain (Besse & Ephrussi, 2008). The latter pathway is the best one characterized and will be discussed within this review. Briefly, mRNA is recognized by RNA-binding proteins, which results in formation of

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Abbreviations: Ago, Argonaute protein; APC, adenomatous polyposis coli; CDS, coding sequence; CLIP-seq, ultraviolet in vivo RNA–protein cross-linking with immunoprecipitation and high-throughput sequencing; CRM1, Chromosomal Maintenance 1; DDX protein, DEAD-box protein; EJC, exon junction complex; ER, endosomal reticulum; ESCRT-II, Endosomal Sorting Complexes Required for Transport; EV, extracellular vesicle; exRNA, extracellular RNA; FMRP, Fragile X Mental Retardation Protein; hnRNP, heterogeneous nuclear RNP; Hsp83, heat-shock protein-83; mRNP, messenger ribonucleoprotein; MVB, multivesicular body; NPC, nuclear pore complex; NTP, nucleotide triphosphates; NXF1, nuclear export factor 1; NXT1, nuclear transport factor 2-like export factor 1; ORF, open reading frame; P bodies, processing bodies; RBP, RNA binding protein; RISC, RNA-induced silencing complex; RNP, ribonucleoprotein; SR protein, serine/arginine rich protein; TREX, three-prime repair exonuclease; UTR, untranslated region; Y RNA, small non-coding RNA

mRNA-protein complexes that are transported within endomembrane system and/or along cytoskeletal tracks. RNA was once thought to exist in a stable form only inside the cells. However, recent research has indicated that RNAs can play a role not only in gene expression, but also in a variety of complex cellular functions. Extracellular RNA (exRNA) can be exported from cells in extracellular vesicles or bound to lipids or proteins, to play a role in intercellular communication. exRNA circulate through the tissues and affect cells at a great distance. What is important, a cell can accept exRNAs of heterologic origin, eg. absorbed from food, from microbes, or from the environment, potentially eliciting a variety of biological responses (Raposo & Stoorvogel, 2013).

OVERVIEW OF THE mRNA-PROTEIN COMPLEX

Proteins form complexes with RNA (RNP – ribonucleoprotein) during transcription and participate in maturation of mRNA, nuclear export (via the nuclear pore complex or budding through the nuclear envelope which applies to the extra-large mega RNPs), cellular transport and localization. They control where and when translation occurs, and determine the mode and rate of degradation if it does not happen (active messenger ribonucleoprotein (mRNP) dismantling is a prerequisite for efficient degradation) (Jansen *et al*., 2014; Singh *et al*., 2015).

Dynamic remodeling of mRNP composition takes place at each step of the mRNA transport. A crucial role in differentiating the composition and function of mRNP, play alternate 5' untranslated regions (UTRs), open reading frames (ORF), and 3' UTR sequences (Patzelt *et al*., 1983; Sonenberg *et al*., 1979). RNA binding proteins (RBPs) recognize structural elements, like m^7G cap and poly A tail, or the sugar-phosphate backbone, or specific sequence motifs. They can also link up with secondary or tertiary structural elements in a sequence-independent way or simply follow the processing reactions (Kim & Dreyfuss, 2001; Singh *et al*., 2015). Binding of some RBPs is conditioned by the concentration of small ligand molecules or intermediate metabolites (Hentze & Preiss, 2010; Castello *et al*., 2015). There is also a class of proteins which link RNA with specificity for base modifications like *N*6-methyladenosine, 5-methylcytosine or pseudouridine (Carlile *et al*., 2014; Squires *et al*., 2012; Wang & He, 2014). They are capable to modify bases, recognize modifications or remove them, and as a consequence modulate gene expression (Castello *et al*., 2012).

Assembling some RNPs can affect the remodeling of others which may lead to coupling numerous steps in the regulation of gene expression. The structure and composition of mRNPs play a role both, in contemporaneous and sequential coupling events (Moore & Proudfoot, 2009). mRNPs are not confined to individual cellular compartments (Singh *et al*., 2015), as many of mRNPs formed in the nucleus may affect exploitation and metabolism in the cytoplasm (Cheng *et al*., 2006; Gebhardt *et al*., 2015; Merz *et al*., 2007; Moore & Proudfoot, 2009). Prompt co-transcriptional mRNA packaging prevents hybridization of the newly transcribed RNA to the DNA strand within the transcription bubble – which may aid in maintaining the genome integrity (Paulsen *et al*., 2009). Formation of various mRNP and heterogeneous nuclear RNP (hnRNP) prevents hybridization of the emerging RNA with the unpaired template DNA, which could result in elongation defects, high rate of recombination, and general genomic instability (Domínguez-Sánchez *et al*., 2011; Li *et al*., 2007; Paulsen *et al*., 2009).

A model of distribution for mammalian RBPs alongside 5' UTRs, coding sequences (CDS), and 3' UTRs has been proposed (Singh *et al*., 2015). Based on the mRNA-binding data obtained e.g. by CLIP-seq (ultraviolet *in vivo* RNA–protein cross-linking with immunoprecipitation and high-throughput sequencing), it was suggested that the spliced mRNPs could be divided into three compositionally distinct domains. The 5' domain covers an area of the first exon which usually meets with the 5' UTR. This part is relatively protein free due to the ribosome-landing pad. The 3' domain includes the 3' UTR which is usually coincident with the last exon. In contrast to the opposite end, here one can find numerous RBPs, e.g. hnRNP proteins, as well as localization, translation, and decay factors. In mammalian mRNA, the CDS domain is usually rich with introns, and the protein coding region is abundant in EJCs (exon junction complex) and serine/arginine rich (SR) proteins. They are unequally distributed over ORFs, and 3' UTRs are specifically depleted in them. Besides them, there are also present translation regulators that influence the elongating ribosomes (Ji *et al*., 2016; Silverman *et al*., 2014).

After the RNP assembly, an important part in transport across the cell is the nuclear export. Correctly processed, and checked by a control mechanism, ribonucleoprotein particles move across the nucleoplasm (Percipalle *et al*., 2009). In the nuclei, mobility of RNP is limited by other particles and complexes, like nucleosomes, chromatin fibers and chromosomes in general. The accessibility of nuclear space, affected mostly by changes in the chromatin condensation, also changes during developmental states of the nuclei/cell (Wachsmuth *et al*., 2008). mRNPs are not actively transported within nucleoplasm, but by random Brownian motion (Oeffinger & Zenklusen, 2012). It was shown that mRNP particles can diffuse in the nuclei with a similar speed as in the aqueous solution, and this is enabled by existing interchromatin channels. However, the diffusion not always occurs with the maximum speed, and sometimes the particles can be retarded due to some processing events. Also, some large molecular non-chromatin structures in the nuclei can inhibit the mobility of mRNPs, which moreover can interact with these structures (Veith *et al*., 2010). Transport of RNA through NPC (nuclear pore complex) is actively supported by proteins that make up the mRNP complex (Visa *et al*., 1996). All three mRNP domains are modified even during the nuclear transport. Export receptor, the NXF1–NXT1 heterodimer, mediates the NPC transit. In the nucleus, NXF1 and NXT1 are connected by multiple adaptors, such as the TREX components, the SR proteins, the DDX (DEAD-box) protein Dbp5 or cleavage and polyadenylation specificity factor 6. Sites of recruitment occur in all three mRNP domains, so probably a single mRNP combines with various NXF1–NXT1 molecules (Silverman *et al*., 2014). The main role in the mRNP translocation from the nuclei is played by Dpb5, an ATP-dependent RNA helicase, which is involved in remodeling of the mRNP. After changing conformation of the particle, mRNP is not be able to reenter the nuclei across NPC. That important step occurs at the external site of a nuclear membrane (Siebrasse *et al*., 2012) and it could be valid to make the transport more efficient and also to control the export of special mRNP cohorts (also known as regulons, groups of mRNAs jointly controlled by one or more RBPs) (Keene, 2007; Valencia *et al*., 2008). Moreover, thanks to the export adaptors, mRNPs can transit through the

NPC in several orientations (Singh *et al*., 2015). This applies to mRNAs which have no need to release the 5' end first into the cytoplasm to bind ribosomes just during the transit through the pore but are sequestered away from the ribosomes until they reach their suitable subcellular target (Daneholt, 2001). Another export pathway uses the karyopherin CRM1 instead of NXF1 (Brennan *et al*., 2000). The protein factors responsible for budding ultralarge mRNPs *via* the nuclear envelope are still unknown (Speese *et al*., 2012). Average mRNPs, during the transport through the central NPC channel, may require only minimal remodeling (Batisse *et al*., 2009), in contrast to especially large mRNPs which need to undergo severe remodeling events, like unfolding, to transit through the pores (Skoglund *et al*., 1983).

After mRNAs get into the cytoplasm, they must be deprived of export receptor proteins to prevent their return. Proteins taking part in this process have been described, however, the exact mechanical details of how it works still require an explanation (Carmody & Wente, 2009; Valkov *et al*., 2012). The way of exchange of nuclear components for the cytoplasmic ones depends on their interaction strength. Passive or active remodeling is possible. Passive remodeling occurs due to mass action and is taking place only after stochastic dissociation of the weakly bound nuclear components. This frees up binding sites for proteins whose abundance is far higher in the cytoplasm (Fritzsche *et al*., 2013; Maquat *et al*., 2010). Active remodeling requires an energy input, usually obtained by hydrolysis of the nucleotide triphosphates (NTP). Such hydrolysis occurs during scanning of the small (40S) subunit along the 5' UTR and translocation of 80S ribosomes through the ORF. The first round of translation can start the active remodeling (Gehring *et al*., 2009; Huang *et al*., 2004).

Probably, most of the mRNPs are exported through NPC as singletons (Park *et al*., 2014). Nevertheless, group complexes appear in the cells as well. In the brain tissue extremely large assemblies with multiple mRNA molecules were detected (Krichevsky & Kosik, 2001). Besides neuronal granules, other large cytoplasmic mRNA aggregations were also discovered, like processing bodies (P bodies), stress granules or germ granules. High amounts of RNA degradation factors in the P bodies suggest that mRNA degradation may take place there (Anderson & Kedersha, 2006). Stress granules are formed temporarily in response to adverse conditions. Their function is to protect mRNAs and relieve ribosomes, so that they are able to synthesize proteins specific to stress, while the germ granules are unique to germlines (Voronina *et al*., 2011). Still, referring to the just mentioned P bodies and the degradation process, final disassembling still leaves a lot of questions and unsolved issues. The proteins must be finally removed so that the degradative enzymes have an access to mRNA. Half-life of mRNPs is usually set between a few minutes to more than 24 hours. Accumulation of mRNA decay intermediates decreases cell fitness (Schoenberg & Maquat, 2012). RBPs in the 5' UTR region are not stably bound and RBPs located in CDS are removed during ribosome transit, while 3' UTR is the hardest part to disassemble (Kurosaki & Maquat, 2016).

After successful export of mRNP from the nuclei, there are a few ways to deliver these particles to the destination site. During movement across the nuclear pore, there may occur changes in their conformation, which can affect the mRNA accessibility for the ribosomes. This is a direct, but not a common way, to get a given mRNA to be expressed (Percipalle *et al*., 2009). There are another ways to deliver mRNPs to the cellular periphery, where mRNA can be translated, and protein synthesis can be done. mRNP is assembled in specific structures, which helps the ribonucleoprotein particles to move to their destination, known as RNA granules. These big macromolecular complexes consist of factors necessary for active and correct transport, such as conventional kinesin and cytoplasmic dynein. Also, they contain some subunits to facilitate translation – ribosomal subunits, aminoacyl-tRNA synthases and elongation factors. After a proper assembly of this complex, the RNA granules can be assigned to specific cytoplasmic compartments, which may depend on the cell type, stage of the cell life and also on the type of mRNA cargo (Percipalle *et al*., 2009).

A classical way of RNP trafficking depends on the type of RNA which is being transported – is the RNA going to be translated or not. In correct subcellular delivery of a given RNA, the key role plays a zipcode – a *cis*-acting RNA sequence element, which is recognized by specific *trans*-acting localization factors (Cohen, 2005).

SUBCELLULAR TARGETING OF mRNA

The distance of mRNA transport can be very long, especially in highly polarized cells, e.g. neurons. The existence of special transport pathways would improve the efficiency of mRNA delivery and determine the precise spatiotemporal localization of mRNAs, regulation of translation and subsequent protein production in correct subcellular domains. To reach its subcellular destination, mRNA must be first recognized by trans-acting factors and form the mRNP complex that is involved in recruitment of the motor proteins. Then, the mRNA cargo is transported on the cytoskeletal tracks to the final location (Fig. 1). The active transport takes place both, in targeting of uniformly distributed transcripts and localizing mRNAs (Fusco *et al*., 2003; Bullock *et al*., 2006a). Both: microtubules, associated with such motor proteins as kinesins and/or dyneins, and actin filaments, associated with such motor proteins as myosins, can be involved in mRNA transport in a cell- and an mRNA-type dependent manner (St Johnston, 2005; Böhl *et al*., 2000; MacDougall *et al*., 2003; Wilkie & Davis, 2001).

The type and number of active molecular motors recruited to a target mRNA implicate the choice of the cytoskeletal tracks (actin filaments or microtubules) used for the mRNA transport, the type of movement (uni- or bidirectional), and the properties (e.g. speed, processivity) of mRNA transport (Gagnon & Mowry, 2011; Marchand *et al*., 2012; Medioni *et al*., 2012). The RNA-binding proteins are controlling the kinetics of mRNA transport by limiting the number of motor proteins bound to mRNP and the balance between these motors (Bullock *et al*., 2006a, 2006b). It has been confirmed that e.g. yeast ASH1 mRNA is transported by actin, whereas *D. melanogaster* oskar mRNA and *X. laevis* Vg1 mRNA are transported by tubulin. Yeast mutants that prevent bundling of actin cables have been shown to result in mislocalization of ASH1 mRNA (Takizawa *et al*., 1997). The efficiency of monodirectional ASH1 mRNA transport on actin filaments in yeast is enhanced by the recruitment of several molecules of the myosin motor (Chung & Takizawa, 2010). Due to the recruitment of opposite acting motors, RNPs transported in dendrites exhibit a microtubule-dependent bidirectional movement (Doyle & Kiebler, 2011). The RBP FMRP binds to transcripts transported in

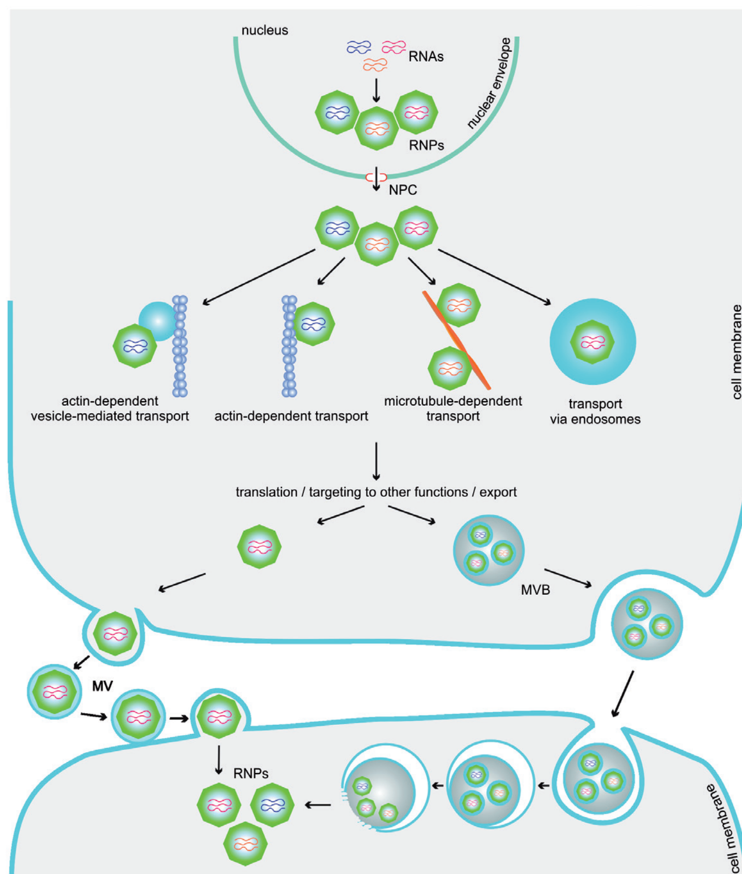

Figure 1. Schematic of the RNA transfer in eukaryotic cells. RNAs form complexes with proteins (RNPs) that are exported from the nucleus to the cytoplasm through NPC.
RNA cargo can be delivered to the destination domain on cytoskeletal tracks (actin or tubulin) or can be selectively incorporated into the MVBs or MVs budding from the plasma membrane. MVBs fuse with the plasma membrane releasing exosomes into the extracellular environment. MVs and exosomes may bind to the plasma membrane of a target cell and fuse directly with the plasma membrane or may be endocytosed. As a result, the RNA is delivered into the cytoplasm of the target cell.

dendrites, and also to a component of the plus-end motor Kinesin-1 and to the dynein-interacting BicD protein (Dictenberg *et al*., 2008; Bianco *et al*., 2013). This would allow RNPs to ensure a constant reassessment and fine-tuning of the directional transport (Medioni *et al*., 2012).

When mRNAs reach their final destination, they must be stably retained at this subcellular domain. Generally, the anchorage of mRNA is controlled by actin, actin binding proteins and the motor proteins (Delanoue & Davis, 2005). In recent years, alternative actin-independent mechanisms have been discovered (Medioni *et al*., 2012): in mammalian migrating cells accumulation of transcripts in protrusions depends on the tumor-suppressor APC (Mili *et al*., 2008); in *D. melanogaster* blastoderm embryos apical anchoring of pair-rule transcripts requires a motor activity-independent function of dynein (Delanoue & Davis, 2005), and in ascidian eggs transcripts are associated with a sub-domain of the cortical ER (endoplasmic reticulum) (Paix *et al*., 2011). In some cells, no static anchor is pre-localized and subcellular targeting of mRNA can be achieved *via* continuous rounds of short-range active transport, as shown for bicoid mRNA localization at the anterior pole of the late-stage *D. melanogaster* oocytes (Weil *et al*., 2006).

There is an emerging evidence that localized transport of mRNA is related to membrane trafficking. The important highlights are that the RNA-binding proteins recognize the cargo mRNA synergistically and that mRNAs are co-transported with membranous compartments, such as the (ER) and endosomes. The novel concept of endosome-coupled translation that loads shuttling endosomes with a septin cargo was studied in a fungal model system (Jansen *et al*., 2014). In *Sacharomyces cerevisiae,* the core factors of the mRNA SHE trafficking machinery include the myosin motor Myo4p, the adaptor protein She3p, and the RNA-binding protein She2p (Heym & Niessing, 2012). This machinery transports approximately 30 types of transcripts along actin tracks from the mother cell to the tip of the budding daughter cell during cell division and polar growth (Hermesh & Jansen, 2013). This mechanism allows for accumulation of mRNA at the growth pole of the daughter cells, resulting in a specific subcellular localized expression in the bud. It was proven that She2p is both, an RNA an lipid-binding protein, with preference to bind to membranes of high curvature, as e.g. ER, and that it interacts more strongly with ER than with other membranes (Genz *et al*., 2013). An RNA live imaging confirmed that the co-transport of mRNA and ER is dependent on the SHE machinery in *S. cerevisiae* (Schmid *et al*., 2006). Most of the mRNAs transported in an ER-dependent manner encode proteins translated at the ER membrane and secreted proteins or proteins required for establishment of polarized secretion (Fundakowski *et al*., 2012)

In filamentous fungi, e.g. *Ustilago maydis*, the polar extension at the hyphal growth pole depends on effective vesicular transport on microtubule tracks (Riquelme, 2013). mRNAs encoding mainly the membrane-associated proteins shuttle on dynamic endosomes and constitute a pathway for long-distance membrane and mRNA trafficking (Koepke *et al*., 2011). The RBP Rrm4-mRNA complex that is important for hyphal growth, was found in endosomes shuttling along microtubules in the endosomal motors Kin3- and Dyn1/2-dependent manner (Zarnack & Feldbrügge, 2010; Baumann *et al*., 2012). There is a hypothesis that mRNPs may shuttle bidirectionally on moving endosomes in order to distribute mRNAs and ribosomes, as well as to deliver the translated products (Jansen *et al*., 2014). An emerging evidence is accumulating that the RNA and membrane trafficking is also tightly interwoven in higher eukaryotes, suggesting that this is a common phenomenon. In *D. melanogaster* oocytes, oskar mRNA-containing RNPs are transported along microtubules with a random orientation. Mammalian neurons exhibit extensive shuttling of mRNPs but without significant accumulation at specific subcellular sites (Zimyanin *et al*., 2008). Genomic RNA of HIV-1 is transported on endosomes and Endosomal Sorting Complexes Required for Transport (ESCRT-II) components are crucial for trafficking (Ghoujal *et al*., 2012; Molle *et al*., 2009). Membrane trafficking also seems to be in-

volved in RNA silencing in plants and animals (Kim *et al*., 2014). The RNA-induced silencing complex (RISC) controls stability and translation of target mRNAs. RISC is mainly formed by Argonaute (Ago) proteins and small RNAs (small interfering RNAs or microRNAs). Ago was identified as a membrane-associated protein with unknown function, GERp95 (Golgi–ER p95) (Kim *et al*., 2014). It was discovered that Ago and small RNAs associate with the ER, Golgi, endosomes, multivesicular bodies (MVBs), autophagosomes, and secretory vesicles (Kim *et al*., 2014). In animal cells, an active RISC is assembled at the cytoplasmic part of the ER (Stalder *et al*., 2013), and in plants, an ER-associated RISC regulates translation of target mRNAs (Li *et al*., 2013).

INTERCELLULAR TARGETING OF RNA

The exchange of extracellular vesicles carrying a protein and an RNA cargo seems to be an evolutionarily conserved mechanism of cell–cell communication (Ratajczak *et al*., 2006; Valadi *et al*., 2007). A classical secretory pathway addresses the release of ER-Golgi transported proteins into the extracellular space. Recent observations suggest that secretion of the proteins/RNA through extracellular vesicles (EVs) may serve as an important pathway in the cell development and communication (Rodrigues *et al*., 2008; Meyer *et al*., 2009). The major categories of cell membrane-derived vesicles are currently distinguished on the basis of size, density, and characteristic marker protein expression, and are divided into exosomes and ectosomes (Keerthikumar *et al*., 2015; Samuel *et al*., 2015). Exosomes are secreted microvesicles (30–150 nm in diameter) of endocytic origin that are conserved across various species and cell types (Lötvall *et al*., 2014; Gangoda *et al*., 2015). Exosomes are released into the extracellular space via exocytosis after fusion of the MVB with the plasma membrane (Cocucci *et al*., 2009; Simons & Raposo, 2009). Ectosomes or shedding microvesicles are generally larger (100–1000 nm in diameter), and bud off directly from the plasma membrane (Keerthikumar *et al*., 2015). The budding of microvesicles involves cortical actin reorganization followed by the outward protrusion of plasma membrane domains and subsequent detachment. There is a growing evidence of vesicular transport of RNA. A wide range of RNA species, including mRNA, was detected in the vesicles (Roberts & Kurre, 2013). Analysis of RNA from EVs (extracellular vesicle) by unbiased deep sequencing approaches demonstrated that, in addition to mRNA and miRNA, EVs also contain a large variety of other small noncoding RNA species, including RNA transcripts overlapping with protein coding regions, repeat sequences, structural RNAs, tRNA fragments, vault RNA, Y RNA, and small interfering RNAs (Bellingham *et al*., 2012; Nolte-'t Hoen *et al*., 2012). The ExoCarta database (http://www.exocarta.org) and the Vesiclepedia compendium (http://microvesicles.org/index.html) index the RNA and other molecules that have been identified in the EVs from different sources (Raposo & Stoorvogel, 2013). The RNAs are selectively incorporated into EVs, as it was found that many RNAs that were isolated with EVs were enriched relative to the RNA profiles of the originating cells (Ratajczak *et al*., 2006; Valadi *et al*., 2007; Skog *et al*., 2008; Nolte-'t Hoen *et al*., 2012). RNAs in EVs share specific sequence motifs that may potentially function as cis-acting elements for targeting to EVs (Batagov *et al*., 2011). Evidence that MVBs are sites of miRNA-loaded RISC accumulation (Gibbings *et al*., 2009), and that exosome-like vesicles are considerably enriched in GW182 and AGO2, implicate functional roles of these proteins in the RNA sorting to exosomes (Raposo & Stoorvogel, 2013). The EV function in physiological and pathological processes depends on their ability to interact with the recipient cells to deliver their cargo. Target cell specificity for EV binding is probably determined by adhesion molecules, such as integrins, that are present in the EVs. While bound to the recipient cells, EVs may remain stably associated with the plasma membrane or dissociate, directly fuse with the plasma membrane, or be internalized through distinct endocytic pathways (Fig.1). When endocytosed, EVs may subsequently fuse with the endosomal membrane or be targeted to the lysosomes for degradation (Raposo & Stoorvogel, 2013). Vesicle trafficking that involves direct cell–cell delivery of RNA may influence the transcriptional control of cellular programs in the target cell. Vesicle trafficking may have a far-reaching effect as the cytoplasmic exchange of RNA or protein might reprogram the cells, alter tissue-specific gene expression, and modulate the cell fates (Ratajczak *et al*., 2006; Aliotta *et al*., 2010).

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