Review

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Traffic into silence: endomembranes and post-transcriptional RNA silencing

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Abstract

microRNAs (miRNAs) and small interfering RNAs (siRNAs) are small RNAs that repress gene expression at the post-transcriptional level in plants and animals. Small RNAs guide Argonaute-containing RNA-induced silencing complexes to target RNAs in a sequence-specific manner, resulting in mRNA deadenylation followed by exonucleolytic decay, mRNA endonucleolytic cleavage, or translational inhibition. Although our knowledge of small RNA biogenesis, turnover, and mechanisms of action has dramatically expanded in the past decade, the subcellular location of small RNA-mediated RNA silencing still needs to be defined. In contrast to the prevalent presumption that RNA silencing occurs in the cytosol, emerging evidence reveals connections between the endomembrane system and small RNA activities in plants and animals. Here, we summarize the work that uncovered this link between small RNAs and endomembrane compartments and present an overview of the involvement of the endomembrane system in various aspects of RNA silencing. We propose that the endomembrane system is an integral component of RNA silencing that has been long overlooked and predict that a marriage between cell biology and RNA biology holds the key to a full understanding of post-transcriptional gene regulation by small RNAs.

Keywords Argonaute; endoplasmic reticulum; microRNA; MVB; siRNA
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An introduction to RNA silencing and its cell biology

As a conserved and widespread regulatory mechanism in eukaryotes, post-transcriptional gene silencing (PTGS) impacts many biological processes such as development, defense, and stress responses. Small RNAs (sRNAs) of 20–24 nucleotides (nt) in size serve as a core component in PTGS by imparting sequence specificity to target gene repression; as such, PTGS is often referred to as post-transcriptional RNA silencing (see Box 1).

The functions of sRNAs are not restricted to PTGS. Based on their sequence origin and biogenesis, sRNAs are categorized into several classes including microRNAs (miRNAs), small interfering RNAs (siRNAs), and piwi-interacting RNAs (piRNAs) (Carthew & Sontheimer, 2009). miRNAs are final products of *MIR* genes encoded in the genome in both plants and animals (Carthew & Sontheimer, 2009; Chen, 2009). siRNAs are derived from long double-stranded RNAs that can be of exogenous or endogenous origin. In animals, exogenous siRNAs are widely used as a tool for gene silencing and are introduced into cells or animals as siRNA/siRNA* duplexes or their precursors. piRNAs derive from both transposons and genes and are only found in animals (Siomi *et al*, 2011). While miRNAs and exogenously applied siRNAs act in PTGS, piRNAs and certain endogenous siRNAs (such as heterochromatic siRNAs in *Schizosaccharomyces pombe* and plants) guide the deposition of repressive chromatin marks to result in transcriptional gene silencing (TGS) (Carthew & Sontheimer, 2009; Law & Jacobsen, 2010; Siomi *et al*, 2011). Here, we will not discuss the sRNAs involved in TGS, but we note that several factors required for piRNA biogenesis are associated with the outer surface of mitochondria (Pane *et al*, 2007; Malone *et al*, 2009; Saito *et al*, 2009, 2010; Haase *et al*, 2010; Olivieri *et al*, 2010; Watanabe *et al*, 2011; Shiromoto *et al*, 2013; Vagin *et al*, 2013), raising the possibility that aspects of piRNA biogenesis are connected to the endomembrane system. In this review, however, we focus on the connection between endomembranes (see Box 2) and the PTGS activities of miRNAs and siRNAs.

MIR genes are transcribed by RNA polymerase II (Pol II) into pri-miRNAs, which are stabilized by a 5′-cap and a 3′ poly A tail. A pri-miRNA is cropped into a stem loop pre-miRNA by the RNase III enzyme Drosha, and the pre-miRNA is further processed into a mature miRNA/miRNA* duplex by the RNase III enzyme Dicer (Carthew & Sontheimer, 2009). In plants, DICER-LIKE1 (DCL1) is responsible for both processing steps and the 3′ end nucleotides of a mature miRNA/miRNA* duplex are 2′-*O*-methylated by the methyltransferase HEN1 to promote the stability of miRNAs (Chen, 2009). In siRNA biogenesis, long double-stranded precursor RNAs formed through intramolecular base pairing or by the activities of RNA-dependent RNA polymerases are processed into mature siRNA/siRNA* duplexes by Dicer in animals or DICER-LIKE proteins in

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Box 1: RNA silencing

RNA silencing is a set of mechanistically related and conserved pathways by which the expression of one or more genes is attenuated or entirely suppressed by small non-coding RNAs. RNA silencing can occur at both post-transcriptional (PTGS) and transcriptional levels (TGS). Three primary classes of small non-coding RNAs have been identified: microRNA (miRNA), small interfering RNA (siRNA), and piwi-interacting RNA (piRNA). These small non-coding RNAs are distinguished by their tissue of origin (soma and germline for miRNAs/siRNAs and germline for piRNAs) and their biogenesis, rather than their mechanism of action. All sRNAs program a multiprotein complex, termed RISC (for RNA-induced silencing complex), invariably containing a member of the ARGONAUTE (AGO, siRNAs/miRNAs) or PIWI (piRNAs) families. RISC specifically interacts with any RNA molecule presenting sequence homology to the loaded small RNA. The outcome of this interaction is variable: mRNA deadenylation followed by exonucleolytic decay, mRNA endonucleolytic cleavage, translational inhibition, or TGS through DNA methylation or histone methylation.

miRNAs derive from long, single-stranded RNA (ssRNA) precursors that adopt a stem loop structure containing an imperfect double-stranded RNA (dsRNAs) stem. These precursors are sequentially processed by RNase III proteins of the Drosha/Dicer families, releasing 21-24 nt dsRNA. The miRNA strand of the dsRNA is loaded into an AGO protein and acts in trans by regulating mRNAs that exhibit strong complementarity to the 5′ end of the miRNA sequence.

siRNAs derive from the Dicer-mediated processing of long, perfect dsRNAs produced by transcription of inverted repeat sequences, convergent transcription of sense–antisense gene pairs, or synthesis by RNA-dependent RNA polymerases (RDRs). As multiple siRNAs from both strands of the precursor are programmed into RISC, siRNAs can potentially act both in cis and in trans by targeting the elements from which they derive as well as unrelated elements with substantial complementarity to their sequence.

The biogenesis of piRNAs is not yet fully understood, but piRNAs seem to result from single-stranded transcripts originating from specific clusters in the genome. The primary transcripts are processed through at least two nucleolytic steps into primary piRNAs, which could initiate secondary piRNA biogenesis through the ping–pong cycle (Siomi *et al*, 2011).

plants (Meister & Tuschl, 2004; Xie *et al*, 2004). The miRNA or siRNA guide strand is incorporated into an Argonaute protein (note that Argonaute will be abbreviated as AGO for general reference or for Arabidopsis proteins and as Ago for animal proteins in this article) to form the RNA-induced silencing complex (RISC), the functional entity in RNA silencing (Carthew & Sontheimer, 2009).

AGO proteins are the main effectors in RNA silencing. An AGO protein contains four functional domains, N, PAZ, MID, and PIWI. The PAZ and MID domains serve as anchors of the 3′ and 5′ ends of the bound sRNA, respectively (Jinek & Doudna, 2009). The PIWI domain is structurally similar to RNase H, and through mutational studies on the catalytic core motif DEDH or DEDD followed by biochemical analyses, it was shown that the PIWI domain of an AGO protein possesses endonuclease activity to cleave target RNAs (Liu *et al*, 2004; Baumberger & Baulcombe, 2005; Rivas *et al*, 2005; Nakanishi *et al*, 2012; Schurmann *et al*, 2013). In many organisms, multiple AGO proteins are encoded and can bind to sRNAs with differential preference for sequence or structural features. In *Drosophila*, siRNAs processed by Dicer2 have nearly perfect complementarity in the small RNA duplexes and are specifically loaded into dmAgo2, whereas miRNAs processed by Dicer1 contain mismatches in the small RNA duplexes and are loaded into dmAgo1 (Tomari *et al*, 2007). In mammals, miRNAs are associated with all four Ago proteins (Ago1–4) *in vivo*, but only Ago2 displays endonuclease activity and acts as the major effector of exogenous siRNAs in RNA silencing (Meister *et al*, 2004). In *Arabidopsis thaliana*, 5′ nucleotide identity and small RNA length are critical for the partitioning to different AGO proteins. atAGO1-associated small RNAs tend to have a 5′ U and are 21–22 nt in length, thus include almost all miRNAs and species of endogenous siRNAs that act at the level of PTGS and making atAGO1 the major effector of sRNAs in PTGS (Baumberger & Baulcombe, 2005; Mi *et al*, 2008).

RISC is recruited to targets through base pairing between the miRNA or siRNA guide and the target transcript, resulting in target gene repression. For siRISC in both plants and animals, the siRNA is fully complementary to the target transcript and siRISC cleaves the target transcript through the endonuclease activity of the AGO protein, such as atAGO1 in Arabidopsis and Ago2 in mammals (Baumberger & Baulcombe, 2005; Matranga *et al*, 2005; Rand *et al*, 2005; Leuschner *et al*, 2006). For miRISC, the mode of target recognition is different between plants and animals. Animal miRNAs show restricted complementarity to targets and the most common mode of target recognition involves the pairing of the seed sequence (nucleotides 2 to 7 or 8 from the 5′ end) with targets (Lewis *et al*, 2003, 2005). The limited pairing prevents most animal miRNAs from guiding the cleavage of their target mRNAs, and instead, they cause translational repression as well as destabilization of their target transcripts (Lim *et al*, 2005; Wu *et al*, 2006). Conversely, in plants, miRNAs exhibit a high degree of sequence complementarity to their target mRNAs and are able to guide AGO1 to cleave their target transcripts (Mallory *et al*, 2004; Fahlgren & Carrington, 2010). However, plant miRNAs are also able to inhibit the translation of their target mRNAs (Aukerman & Sakai, 2003; Chen, 2004; Gandikota *et al*, 2007; Brodersen *et al*, 2008; Dugas & Bartel, 2008; Beauclair *et al*, 2010; Yang *et al*, 2012; Li *et al*, 2013).

In the past decade, sRNA-mediated PTGS has been extensively studied and the basic molecular framework of sRNA biogenesis, RISC formation, and target repression is thus well established; however, in comparison, little is known about the subcellular localization of the PTGS machinery or its activities. Processing bodies (P-bodies), cytoplasmic foci containing enzymes acting in mRNA turnover, were first implicated as sites of RNA silencing (Jakymiw *et al*, 2005; Liu *et al*, 2005; Pillai *et al*, 2005). In animals, Ago, miRNAs, and target mRNAs were detected in P-bodies and P-bodies were proposed to be sites of sRNA-mediated translational inhibition (Jakymiw *et al*, 2005; Liu *et al*, 2005; Pillai *et al*, 2005). However, visible P-body formation was found to be dispensable for miRNA-mediated gene silencing, raising the possibility that the localization of the RNA silencing machinery and target mRNAs to P-bodies is the consequence rather than the cause of RNA silencing activity (Chu & Rana, 2006; Eulalio *et al*, 2007). In plants, P-bodies are also considered the site of mRNA turnover since these aggregates contain homologs of mammalian or yeast proteins involved in mRNA decay (Pomeranz *et al*, 2010; Xu & Chua, 2011); however, only circumstantial evidence links P-bodies to RISC action in plants (Brodersen *et al*, 2008; Pomeranz *et al*, 2010).

While it remains to be seen whether RNA silencing occurs in P-bodies, an unexpected theme that has emerged from recent studies is the intimate association between RNA silencing and the

Box 2: The endomembrane system and its dynamics in animals and plants

The endomembrane system of eukaryotic cells allows the spatial and temporal compartmentalization of macromolecule synthesis, sorting, delivery, and degradation. It consists of a variety of organelles that are connected either directly or through transport vesicles, the formation of which necessitates the selective recruitment of coat proteins (clathrin, COPI, COPII, and retromer), various GTPases (Sar1, Arf1, and Rabs), and the endosomal sorting complex required for transport (ESCRT) complexes (Kirchhausen, 2000; Nickel *et al*, 2002; Gabe Lee *et al*, 2009; Hurley & Hanson, 2010). The correct targeting or fusion of the transport vesicles relies on organelle-specific tethering factors and SNARE complexes (Faini *et al*, 2013). The main organelles of the endomembrane system are the endoplasmic reticulum, the Golgi complex, trans-Golgi network (TGN), endosomes, and lysosomes or vacuoles. Flows of membranes and proteins to (outward) and from (inward) the plasma membrane go through these organelles. Endosomes sit at an essential position within the endomembrane system and have a core sorting function: They are the first point of fusion for endocytic vesicles, which mediate inward flow of extracellular materials as well as the outward transport of cargoes either from the Golgi to the lysosome/vacuole or their return from lysosomes to the Golgi. Animal endosomes are classified into early, recycling, intermediate, and late endosomes/multivesicular bodies (MVBs) (Spang, 2009). Early and recycling endosomes receive and recycle both endocytosed membrane proteins back to the plasma membrane and vesicles from the vacuole back to the TGN. Intermediate and late endosomes/MVBs sort membrane proteins into endosomal intraluminal vesicles to be degraded by vacuolar/lysosomal hydrolases upon fusion with vacuoles/lysosomes. In addition, they also mediate the transport of new vacuolar proteins from the Golgi to lysosomes/vacuoles (Gruenberg & Stenmark, 2004). Endosomes coordinate the recognition, concentration, and packaging of cargo proteins by different complexes such as ESCRT (for sorting to the vacuoles/lysosomes) and the retromer (for recycling to the plasma membrane) (Seaman, 2005). Two mechanisms underpin cargo sorting in the endosomes, either direct interactions between the cargo proteins and the sorting complexes or by post-translational modifications of cargo proteins. Endocytosed cargo destined for degradation becomes ubiquitinated at the plasma membrane, and this signal causes them to be recognized by the ESCRT complex and sorted into the intraluminal vesicles of MVBs. This step is critical for the termination of signaling cascades that continue even after internalization of the receptor–ligand complex (Raiborg & Stenmark, 2009). The organelles of the plant endocytic pathway are overall similar to those present in mammalian cells. However, unlike the situation in animal cells, higher plants do not have separate TGN and early endosome compartments (Scheuring *et al*, 2011). In addition to lysosome/vacuole-mediated destruction of extracellular material and plasma membrane proteins delivered via the endocytic pathway, cytosolic material and organelles can also be delivered to the lysosome by autophagy. Autophagy is an umbrella term for different pathways: macroautophagy, microautophagy, and chaperone-mediated autophagy. In macroautophagy, a phagophore sequesters a portion of the cytoplasm, including soluble materials and organelles, to form the autophagosome, which fuses with endosomes before fusion with lysosomes. In microautophagy, domains of the cytoplasm are engulfed by inward invagination of the lysosomal membrane. Like the formation of MVBs in endosomes, this process requires the ESCRT complex. In chaperone-mediated autophagy, target proteins harboring a distinctive peptide are recognized by Hsc70 and directly translocated to the lumen of lysosomes. Autophagosomes are generated on or in close proximity to the ER and their number can increase upon starvation and other stresses (Mizushima & Komatsu, 2011).

endomembrane system (Box 2). Here, we provide an overview of the data linking RNA silencing to endomembranes in plants and animals and attempt to integrate the myriad of findings into a tentative framework that summarizes and rationalizes the impacts of membranes on intra- and intercellular RNA silencing.

Rough endoplasmic reticulum: site of translation for most cellular mRNAs?

Cellular mRNAs are partitioned into two subcellular compartments for translation: the cytosol and the endoplasmic reticulum (ER). ER with ribosomes dotting its cytosolic surface is referred to as rough ER (rER, Fig 1). The classic view holds that mRNAs encoding cytosolic or nucleoplasmic proteins are translated on cytosolic ribosomes, whereas mRNAs encoding secretory or membrane proteins are translated on the rER. The latter class of mRNAs is docked onto the rER co-translationally through the recognition of N-terminal signal peptides emerging from translating ribosomes by the ER-localized signal recognition particle (SRP) (Schwartz, 2007). However, this classic view has been challenged by observations suggesting that rER may serve as the site of translation for a broad range of mRNAs rather than a restricted set of secreted factors. In an early study, the partitioning of mRNAs between free and membrane-bound polysomes was investigated in mammalian cells (Lerner *et al*, 2003). Surprisingly, a set of mRNAs encoding soluble proteins was found associated with membrane-bound polysomes. Treatment with protein synthesis inhibitors shows that these mRNAs are bound to functionally active ribosomes and that the ER association of the mRNAs does not depend on translation. Furthermore, cDNA microarray analysis using total and ER-associated RNAs reveals that, in mammalian cells, the majority of mRNAs encoding cytosolic proteins are also present on ER-bound polysomes (Lerner *et al*, 2003).

A number of studies in cell culture and animal systems have since reported findings consistent with the notion that rER translates a broad range of cellular mRNAs (Pyhtila *et al*, 2008; Chen *et al*, 2011; Reid & Nicchitta, 2012). These studies show that a set of mRNAs encoding cytosolic or nucleoplasmic proteins is dually localized in the cytosol and at membranes, suggesting the existence of an alternative mechanism for the translocation of mRNAs to the ER besides the SRP pathway. Indeed, in stable *SRP54*-depleted cell lines in which SRP activity was compromised, the cytosol-membrane partitioning of mRNAs was not affected (Pyhtila *et al*, 2008). In fact, in *Saccharomyces cerevisiae*, several mRNAs that encode membrane/secreted proteins were shown to be targeted to the ER in a translation- and SRP-independent manner (Kraut-Cohen *et al*, 2013). Another study examined the cytosolic and rER translatome through subcellular fractionation followed by ribosomal profiling (Reid & Nicchitta, 2012) and revealed that a large portion of the transcriptome is translated on the ER. Interestingly, when two groups of mRNAs, encoding either cytosolic/nucleoplasmic proteins or ER-targeted proteins, were compared, the transcript levels as well as their ribosome loading density were similar in the ER fraction. In addition, ER-bound mRNAs showed higher levels of ribosome loading density compared to cytosolic mRNAs, which may reflect higher translational efficiency on the ER (Reid & Nicchitta, 2012).

Rough endoplasmic reticulum: site of miRNA-mediated translational repression

Translational repression is a widespread and fundamental activity of miRNAs in both animals and plants (Fabian *et al*, 2010;

Meister, 2013). For animal miRNAs, which are also known to induce target mRNA destabilization through 3′ deadenylation, 5′ decapping, and subsequent exonucleolytic decay (Wu *et al*, 2006; Fabian *et al*, 2009), it has been debated whether translational repression contributes much to target gene regulation (Guo *et al*, 2010).

Three studies examined the temporal kinetics of the two activities of miRNAs, mRNA decay and translational repression, and support that translational repression is an integral activity of miRNAs. It was found that, upon the induction or transfection of a miRNA, translational repression occurred at a time when there was no sign of target mRNA decay (Bazzini *et al*, 2012; Bethune *et al*, 2012; Djuranovic *et al*, 2012). This indicated that translational repression is a fundamental activity of animal miRNAs. Plant miRNAs pair with target mRNAs with nearly complete sequence complementarity and guide the cleavage of target mRNAs through the slicing activity of AGO1 (Mallory *et al*, 2004; Fahlgren & Carrington, 2010). Although mRNA cleavage was once thought to be the major activity of plant miRNAs, translational repression of plant miRNA targets has gradually garnered support over the years, as more and more studies examined expression of miRNA target genes at both RNA and protein levels and interrogated mutants compromised in miRNA activities (Aukerman & Sakai, 2003; Chen, 2004; Gandikota *et al*, 2007; Brodersen *et al*, 2008; Dugas & Bartel, 2008; Beauclair *et al*, 2010; Yang *et al*, 2012; Li *et al*, 2013). For example, several mutants were isolated from forward genetic screens aimed to identify players in miRNA action in *Arabidopsis* and, in some of the mutants, the expression of many miRNA target genes was elevated at the protein but not the RNA level (Brodersen *et al*, 2008; Yang *et al*, 2012; Li *et al*, 2013). This suggested that miRNAs inhibit the translation of their target mRNAs. In fact, in one such mutant, *altered meristem program 1* (*amp1*), protein synthesis from miRNA target genes was shown to be elevated, indicating that plant miRNAs indeed repress translation (Li *et al*, 2013). In addition, and confirming the observation made earlier in animal systems, atAGO1 and miRNAs were found to be associated with polysomes (Lanet *et al*, 2009; Reynoso *et al*, 2013). These results showed that translational repression is another major mode of action of miRNAs in plants but left open the question of where in the cell it takes place.

A recent study provided a location to the translational repression activity of miRNAs in plants by linking it to the rER. In Arabidopsis, the *AMP1* gene, despite its unknown molecular function, has continuously graced the scientific literature in the past 15 years as a gene that impacts almost all aspects of plant development (Conway & Poethig, 1997; Helliwell *et al*, 2001; Vidaurre *et al*, 2007; Griffiths *et al*, 2011; Shi *et al*, 2013). Recently, it was found that the molecular function of *AMP1* lies in mediating the translational repression activity of plant miRNAs (Li *et al*, 2013). The wide-ranging impacts of *AMP1* on plant development could thus reflect the importance of miRNA-induced translational repression activity. An *amp1* mutant was found to be deficient in stimulating miRNA activity, and intriguingly, miRNA target protein levels were elevated in this mutant with no corresponding changes in target mRNA levels. Pulse-labeling experiments showed that protein synthesis from miRNA target genes was reduced in a miRNA- and *AMP1*-dependent manner, demonstrating that translational repression is an activity of plant miRNAs and that this activity requires *AMP1* (Li *et al*, 2013). Although the mechanism by which *AMP1* mediates the translational repression activity of plant miRNAs remains unknown, it was found that the association of miRNA-targeted mRNAs with membrane-bound polysomes is repressed by miRNAs in an *AMP1*-dependent manner. This finding, together with the rER localization of the AMP1 protein, suggests that translational repression by plant miRNAs occurs on the rER (Fig 1).

Consistent with the ER being a site of miRNA-mediated translational repression in *Arabidopsis*, the major miRNA effector atAGO1 was found to be partially membrane-associated (Brodersen *et al*, 2012; Li *et al*, 2013). AGO1 partitions into soluble and membrane fractions, and its membrane association is disrupted by high salt or high pH conditions, suggesting that atAGO1 is a peripheral membrane protein, consistent with its lack of a transmembrane domain. Co-localization with an ER marker showed that atAGO1 partially resides on the ER (Li *et al*, 2013) (Fig 1). How atAGO1 becomes membrane-associated is unknown, but isoprenoid biosynthesis may play a role. A mutant in *HMG1*, which encodes 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase in the mevalonate pathway for isoprenoid biosynthesis, was isolated in a genetic screen as a miRNA action-deficient mutant (Brodersen *et al*, 2008, 2012) and the AGO1 membrane association was reduced in hypomorphic *ago1* and *hmg1* mutants (Brodersen *et al*, 2012). Intriguingly, in *Caenorhabditis elegans*, enzymes in the mevalonate pathway were found to be required for RNA silencing, pointing to an ancient and conserved involvement of isoprenoids and tentative membrane association of AGO in RNA silencing (Shi & Ruvkun, 2012).

Another study supports the functional significance of the rER in miRNA-mediated translational repression in *Drosophila* (Wu *et al*, 2013). The authors found that serum deprivation in S2 cells led to enhanced miRNA-mediated translational repression and the concomitant appearance of two non-canonical miRISCs. These are the polyribosomal miRISC (P-miRISC) and a dmAGO1-containing complex associated with membranous organelles (Fig 1). P-miRISC is composed of dmAGO1, miRNA, and Loqs-PB (a double-stranded RNA-binding protein) and devoid of GW182, an essential component in canonical miRISC (referred to as G-miRISC) (Behm-Ansmant *et al*, 2006; Eulalio *et al*, 2008). The membrane-associated dmAGO1 complex lacks both GW182 and Loqs-PB and is considered to be an intermediate in the turnover of miRISC or P-miRISC. The P-miRISC, rather than G-miRISC, was thought to mediate translational repression observed under the serum deprivation condition, as GW182 was found to be dispensable for this activity. P-miRISC is associated with polysomes and co-sediments with ER. In addition, P-miRISC is still polysome-associated upon cycloheximide treatment, suggesting that it inhibits translational elongation rather than initiation. Interestingly, it was reported that the level of miRNA-mediated translational repression is 5- to 10-fold higher under serum starvation, implying that translational repression through P-miRISC is more efficient than that of G-miRISC (Wu *et al*, 2013). Given that a wide range of the transcriptome associates with ER and that translation at rER is more efficient than in the cytosol (Reid & Nicchitta, 2012), it was proposed that miRNA-mediated translational repression on rER could allow a fast response to various environmental stimuli in both plants and animals (Ando & Leung, 2013; Li *et al*, 2013).

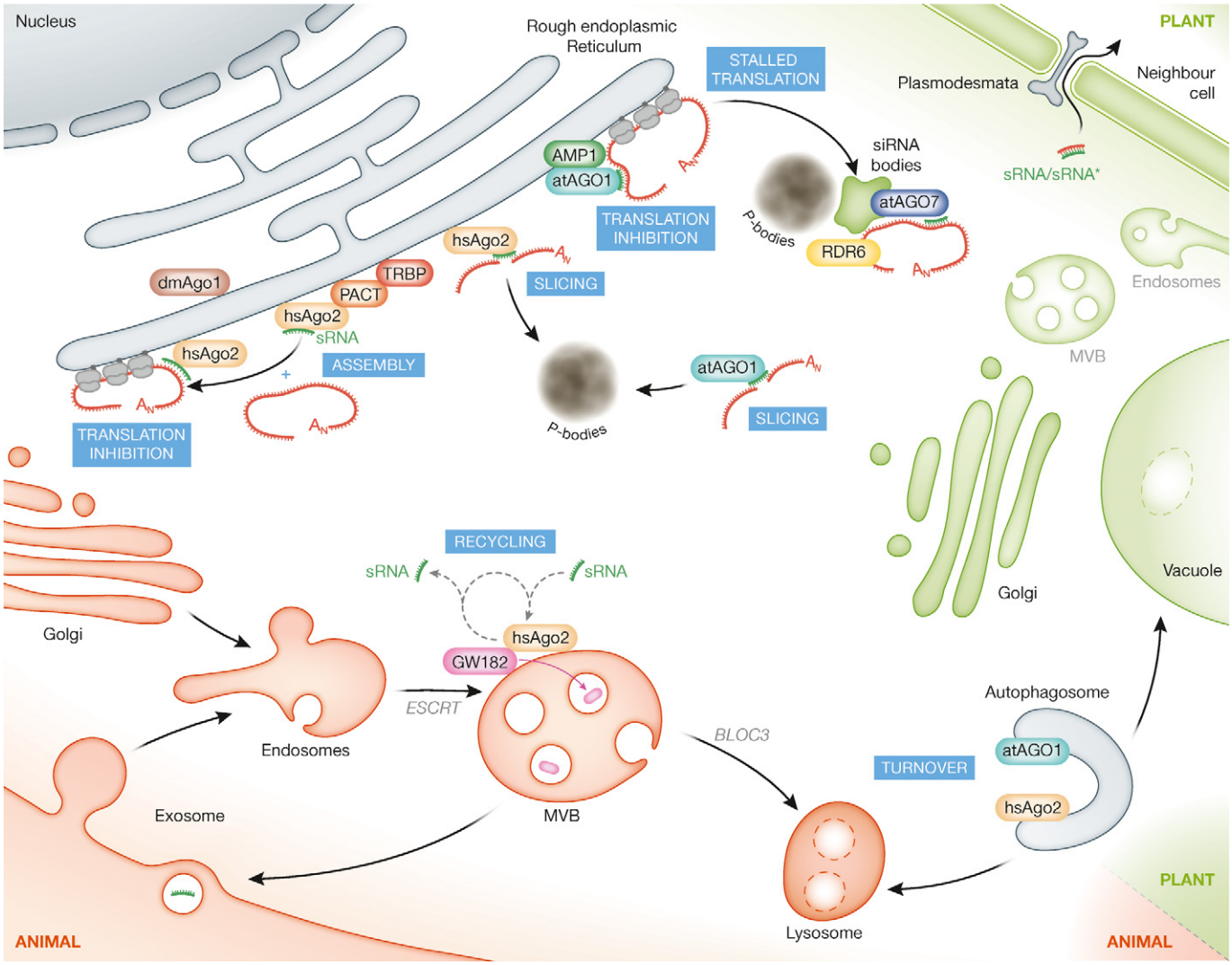

Figure 1. Connections between endomembranes and post-transcriptional gene silencing in animals and plants.
In both plant and animal cells (shaded in green and pink, respectively, in the diagram), the rough endoplasmic reticulum (rER) is probably the site of miRNA-mediated target repression. In Arabidopsis, atAGO1 is partially membrane-associated and congregates with AMP1 at the rER to inhibit the translation of target mRNAs. In Drosophila, dmAGO1 is associated with the rER. In human cells, the formation of hsAgo2 RISC, a process requiring PACT and TRBP, and target RNA slicing both occur on the rER. In animal cells, endosomes and multivesicular bodies (MVBs) are likely sites of RISC recycling. In mammalian cells, GW182 removal at MVBs by the ESCRT machinery and concomitant AGO reloading are thought to contribute to RISC recycling. In both plant and animals, autophagy regulates the turnover of AGO proteins by selectively addressing them to lysosomes (animal) or the vacuole (plants) for degradation. In plants, mRNAs stalled in translation accumulate in siRNA bodies, an intermediary compartment between the rER and Golgi where atAGO7 and RNA-dependent RNA polymerase proteins congregate. No evidence has been provided for a membrane-P-body connection, but in plants, siRNA bodies and P-bodies are in close proximity. Intercellular transfer of silencing RNAs involves, in plants, the plasmodesmata, an ER-containing organelle, and perhaps endosome/MVB-derived exosomes in animals.

Rough endoplasmic reticulum: site of RISC loading and siRNA-mediated slicing

Mammalian Ago2, an effector in RISC and the only mammalian Argonaute protein with slicer activity, was originally identified as GERp95, an ER- and Golgi-associated protein (Cikaluk *et al*, 1999). Later, it was shown that Dicer is also associated with ER and Golgi in neurons and various other cell types (Tahbaz *et al*, 2004; Barbato *et al*, 2007).

In a recent study, extensive subcellular fractionation efforts were made to document the membrane association of various RNA silencing factors (Stalder *et al*, 2013). miRNA- or siRNA-loaded human (hs) Ago2 populations co-sediment on membranes together with the RISC-loading complex (RLC) factors Dicer, protein activator of the interferon-induced protein kinase (PACT), and TAR RNA-binding protein (TRBP), but only TRBP fractionated sharply with Golgi and rER marker proteins. Fractionation and membrane co-immunoprecipitation further confirmed that siRNA-loaded hsAgo2 physically associates with the cytosolic side of the rER membranes and demonstrated that TRBP and PACT are required for anchoring RISC to ER in a target RNA-independent manner (Stalder *et al*, 2013). Moreover, loaded and active hsAgo2 was found to be mostly membrane-associated, while the slicing product was solely associated with rER fractions. Taken together, these findings led to the

conclusion that both hsAgo2 RISC formation and target RNA slicing occur on the rER and that the outer rER membrane thus acts as a central nucleation site for siRNA-mediated RNA silencing (Fig 1).

In summary, the classical view of the ER as the site of protein synthesis or folding for secreted and membrane-embedded proteins is challenged. Mounting evidence documents the association of a wide range of the transcriptome, including miRNAs and their target mRNAs, as well as RNA silencing factors with the ER. The rER is likely the site of translation for a large portion of cellular proteins and a platform for siRNA-mediated slicing and miRNA-mediated translational repression.

Endosomes: RISC assembly and turnover

Endosomes are membrane-bound compartments and an integral part of the transport pathway from the plasma membrane to the lysosome. They are a major hub for secretory and endocytic trafficking and receive cargoes from endocytosed material from the plasma membrane as well as from the trans-Golgi network (TGN). Cargoes can leave endosomes to return to the plasma membrane in recycling endosomes or travel to the TGN. Endosomes can also bud vesicles into their interior using the endosomal sorting complex required for transport (ESCRT) machinery to form multivesicular bodies (MVBs). MVBs can fuse with lysosomes or the Golgi, in a process requiring the BLOC-3 machinery, to permit the degradation of their content, or with the plasma membrane to release exosomes into the extracellular space (the reader is referred to Gruenberg & Stenmark, 2004; Li *et al*, 2004; Raiborg & Stenmark, 2009 for in-depth reviews).

Work in *Drosophila* and mammalian cells has revealed an intriguing association of the RNA silencing machinery with the endosomal trafficking pathway. In human cells, hsAgo2 and GW182 (also called TNRC6), two essential components of RISC, have been shown to co-purify and co-localize with endosome and MVB fractions and define subcellular structures that are distinct from P-bodies (Gibbings *et al*, 2009). It was also observed in *Drosophila* that GW182 bodies are closely juxtaposed to the cytosolic face of MVBs and/or lysosomes (Lee *et al*, 2009). In agreement with this association between endomembranes and the RNA silencing machinery are the early observations mentioned above reporting the co-localization and fractionation of AGO and Dicer with membranes related to the ER and Golgi apparatus (Tahbaz *et al*, 2004). In human cells, miRNAs and target mRNAs were also shown to accumulate in endosomes/MVBs (Gibbings *et al*, 2009), suggesting that these compartments are sites of miRNA-loaded RISC accumulation and, possibly, action (Fig 1). The significance of this association between RISC components and endosomes/MVBs and its implications for RISC function were assayed by interfering with ESCRT-dependent sorting of cargoes into MVBs. Depletion of ESCRT components leads to a decrease in miRNA activity, a concomitant reduction in MVBs and an increase in GW182 abundance (Gibbings *et al*, 2009; Lee *et al*, 2009). Conversely, a *Drosophila* mutant in *HPS4*, a component of the BLOC-3 machinery that catalyzes the fusion of MVBs with lysosomes (Suzuki *et al*, 2002; Li *et al*, 2004), leads to an increase in miRNA- or siRNA-mediated RNA silencing (Lee *et al*, 2009). In fact, *hps4* mutant extracts exhibit faster RISC loading *in vitro* (Lee *et al*, 2009). Interference with ESCRT and BLOC-3 machineries thus exerts opposite effects on RISC loading and miRNA activity—ESCRT promotes RISC assembly, while BLOC-3 impinges on RISC assembly. A model coupling RISC disassembly and reloading at MVBs has been proposed (Gibbings & Voinnet, 2010): GW182 removal at MVBs by the ESCRT machinery contributes to AGO release from miRNA and target mRNA. This process is coupled to AGO reloading (by interaction with Dicer and acquisition of the miRNA–miRNA* complex), a process that also occurs on MVBs but is slowed down by BLOC-3-dependent trafficking of RISC toward lysosomes (Fig 1). The proposal that RISC loading occurs on MVBs conflicts with the model presented above placing RISC formation at the rER (Stalder *et al*, 2013). A way to reconcile observations from the different studies is to assume that upon inhibition of the BLOC-3 machinery Ago2 could be sent back to the ER from MVBs through retrograde trafficking where it would be reloaded. The endo-/lysosomal compartments would therefore act downstream of RISC loading (Fig 1).

Autophagosomes: RISC turnover

Autophagy (macroautophagy) is an intracellular degradation process contributing to cellular homeostasis in which cytoplasmic material is engulfed and then delivered to and degraded in the lysosome by autophagosomes. Autophagy-related (ATG) proteins act in a hierarchical manner to control the formation of autophagosomes. Targeting of specific components to the autophagosomes requires autophagy receptors, among them NDP52 (Thurston *et al*, 2012). More details for these processes are described extensively elsewhere (Mizushima & Komatsu, 2011). Connections between autophagy and the endosomal pathways have become apparent recently: Autophagosomes can fuse with endosomes and the ESCRT machinery regulates autophagy (Lee *et al*, 2007). In human cells, the levels of hsAgo2, hsAgo1, and DICER proteins are increased by depletion of the core autophagy components ATG5, ATG6, ATG7 or the autophagy receptor NDP52. Under the same conditions, the levels of GW182 were not affected. In accordance, hsAgo2 and DICER, but not GW182, co-purify with autophagosomes (Fig 1) and co-localize with NDP52-labeled autophagosomes, and NDP52 co-immunoprecipitates with DICER (Gibbings *et al*, 2012). Together, these data suggest that DICER and hsAgo2 are targeted for autophagic degradation through their association with NDP52. Recently, autophagy was also implicated in the modulation of miRNA-mediated silencing in *Caenorhabditis elegans* (Zhang & Zhang, 2013). Mutations in several autophagy components rescue the developmental phenotypes of animals with hypomorphic mutations in either the Dicer homolog *dcr-1*, the AGO homolog *alg-1,* or the GW182 homolog *ain-1* (ALG-1-interacting protein 1). Interestingly, in *C. elegans*, autophagy seems to regulate the effector step of miRNA-mediated silencing by selectively degrading AIN-1, unlike the situation in human cells where GW182 levels are not controlled by autophagy (Gibbings *et al*, 2012). In both mouse and *Drosophila*, existing evidence indicates that the stability of AGO protein is post-translationally controlled by miRNA availability. In mouse, mutations in the miRNA biogenesis proteins Dicer or DGCR8 led to decreased Ago2 levels by a process involving the lysosome, but not the proteasome (Martinez & Gregory, 2013). Analogously, in *Drosophila*, levels of

dmAgo1 are reduced in cells depleted of *drosha*, *pasha,* and *Dcr-1* (Smibert *et al*, 2013). However, unlike the situation in mouse, the reduced stability of dmAgo1 in these cells could be reversed by blocking the action of the proteasome, suggesting that the turnover of unloaded dmAgo1 involves the proteasome (Smibert *et al*, 2013).

Further links between autophagy and RNA silencing have been independently obtained in plants. Virus-derived small RNAs are used to program RISC to degrade the corresponding viral RNA. To counteract this line of defense, viruses have evolved viral suppressors of RNA silencing that suppress, by different strategies, the silencing response of the host. One such gene, the polerovirus P0, encodes an F-box protein that hijacks the host ubiquitin-protein ligase E3, SKP1-cullin 1-F-box protein (SCF), to promote the degradation of *Arabidopsis thaliana* AGO1 (atAGO1), the key component of RISC (Pazhouhandeh *et al*, 2006; Baumberger *et al*, 2007; Bortolamiol *et al*, 2007). However, the P0-mediated atAGO1 degradation occurs independently of the 26S proteasome pathway (Baumberger *et al*, 2007; Csorba *et al*, 2010). Derrien *et al* observed that the cysteine protease inhibitor E64d, an inhibitor of the degradation of autophagic cargo inside autolysosomes, and 3-methyladenine (3-MA) that blocks autophagosome formation via the inhibition of type III phosphatidylinositol 3-kinases (PI-3K), led to atAGO1 stabilization in the presence of P0 (Derrien *et al*, 2012). This indicates that autophagy could mediate atAGO1 turnover. A GFP-tagged version of atAGO1 co-localizes with ATG8a-positive bodies when atAGO1 degradation is impaired. Further support for the involvement of autophagy in atAGO1 turnover was provided by the over-accumulation of atAGO1 in the *amsh3* mutant in which vacuole formation is compromised and autophagosomes accumulate (Isono *et al*, 2010) and in the TOR-overexpressing mutant line G548 (Deprost *et al*, 2007) in which autophagy is reduced (Derrien *et al*, 2012). Electron microscopy imaging revealed enrichment of atAGO1 in proximity to the Golgi apparatus (Derrien *et al*, 2012), reminiscent of the situation in animal cells where both AGO and Dicer localize and fractionate with membranes of the Golgi apparatus (Cikaluk *et al*, 1999; Tahbaz *et al*, 2004). However, unlike the situation in animals, Derien *et al* did not observe localization of atAGO1 to MVBs, arguing against their involvement in routing AGO1 to the vacuole. The relevance of autophagy-mediated turnover of atAGO1 beyond antiviral responses was established by showing that atAGO1 is also degraded by the autophagy pathway when miRNA production or stability is compromised. Two conclusions arise from this observation. First, in plants, endogenous SCF(s) trigger(s) atAGO1 degradation through the autophagy pathway. Second, and in agreement with the observations made in mouse and fly (Martinez & Gregory, 2013; Smibert *et al*, 2013), defective loading of AGO by a miRNA appears to signal AGO degradation in plants.

Based on these findings, autophagy-mediated modulation of miRNA action emerges a conserved theme across phylae and kingdoms (Fig 1). Autophagy may ensure the homeostatic regulation of miRNA-mediated RNA silencing by preventing blatant perturbations that could be induced by various stresses of abiotic or biotic origin. Ramifications of this are numerous and can potentially link processes seemingly unrelated. Autophagy is modulated in response to nutrient availability, differentiation, and pathogen infection (Mizushima & Komatsu, 2011) and has also been linked to pathology. A hallmark of cancer is inhibition of autophagy (Levine, 2007), which could lead to a decrease in miRNA levels and a global impingement of the RNA silencing machinery.

Intermediary compartments and "cytoplasmic bodies"

In *Arabidopsis,* several pieces of evidence indicate that endomembranes may play a role in siRNA biogenesis. Trans-acting siRNAs (ta-siRNAs) are plant-specific endogenous siRNAs that guide the cleavage of specific mRNA targets *in trans*. ta-siRNAs result from DICER-LIKE 4 (DCL4)-mediated cleavage of a double-stranded RNA (dsRNA) produced by the cellular RNA-DEPENDENT RNA POLYMERASE 6 (RDR6) with the aid of the SGS3 RNA-binding protein (Peragine *et al*, 2004; Vazquez *et al*, 2004; Allen *et al*, 2005; Dunoyer *et al*, 2005; Gasciolli *et al*, 2005) . The RDR6/SGS3 substrate results from miRNA-mediated cleavage of a *TAS* precursor transcript. Ta-siRNA produced from the evolutionarily conserved *TAS3* precursors controls the developmental timing of the transition between juvenile and adult leaves, contributes to the specification of leaf adaxial/abaxial polarity and regulates the growth of lateral roots (Adenot *et al*, 2006; Axtell *et al*, 2006; Fahlgren *et al*, 2006; Garcia *et al*, 2006; Hunter *et al*, 2006; Nogueira *et al*, 2009; Douglas *et al*, 2010; Marin *et al*, 2010; Yoon *et al*, 2010). The processing of *TAS3* by a miR390-loaded-AGO7 complex is essential in directing the *TAS3* precursor to the siRNA pathway (Montgomery *et al*, 2008). Subcellular fractionations have shown that AGO7, miR390, and SGS3 co-purify with membranes (Jouannet *et al*, 2012). A GFP-tagged version of AGO7 congregates with RDR6 and SGS3 in cytoplasmic siRNA bodies (Jouannet *et al*, 2012) that are linked to the ER/Golgi endomembrane system since they also accumulate the viral protein 6 (VP6) of the tobacco etch virus (Skog *et al*, 2008), a membrane-associated protein marking an intermediary compartment between the ER and the cis-Golgi (Schaad *et al*, 1997; Lerich *et al*, 2011). It therefore appears that in Arabidopsis, the production of some siRNAs involves an endomembrane-associated compartment (Fig 1). It would be interesting to examine the link between these siRNA bodies and other foci implicated in RNA processing.

In plants and animals, mRNAs stalled at the translation initiation stage accumulate in so-called cytoplasmic stress granules. Although a distinctive feature of RDR6/SGS3 substrates in siRNA bodies is their poor ability to be translated (a result of their very poor coding potential, as for *TAS3* (Ben Amor *et al*, 2009), and/or the absence of a 5′-cap or 3′ polyA tail required for efficient translation), siRNA bodies and stress granules are distinct (Jouannet *et al*, 2012). Indeed, siRNA bodies, unlike stress granules, are readily detected under normal growth conditions (Weber *et al*, 2008; Jouannet *et al*, 2012) and animal stress granules are so far not known to be linked to endomembranes.

The enzymes responsible for the elimination of aberrant RNAs such as ones lacking a 5′-cap or 3′ polyA tail accumulate in discrete cytoplasmic P-bodies. It is interesting to note that although siRNA bodies never co-localize with P-bodies (Jouannet *et al*, 2012; Moreno *et al*, 2013), these two sets of bodies are usually found juxtaposed, are highly dynamic, and move together in the cytoplasm (A. Maizel, unpublished observation). The dynamic nature of membrane-containing siRNA bodies and their association with P-bodies suggests that they are sites of mRNA triage, wherein mRNAs could be sorted for degradation by P-bodies or enter the siRNA pathway (Fig 1).

Endomembranes and small RNA's intercellular transfer

One of the most fascinating aspects of RNA silencing is its ability to spread from cell to cell. The spread of RNA silencing was first reported in plants and in *C. elegans* (Palauqui *et al*, 1997; Voinnet & Baulcombe, 1997; Fire *et al*, 1998; Timmons & Fire, 1998). Intriguingly, non-cell autonomy of silencing bears point of convergence with the endomembrane system.

The observation made in animals and in plants that RISC associates with endosomes, a compartment acting as a hub for cargo traffic between cells and their exterior environment, raises the possibility that RISC may be secreted from cells (Fig 1). In animals, different populations of miRNAs have been consistently retrieved in biological fluids derived from healthy or cancerous individuals, making these miRNAs tentative diagnostic and predictive biomarkers of cancers (for review, see Turchinovich *et al*, 2012). Reports indicate that small RNAs are present in exosomes exported from cultured cells (Valadi *et al*, 2007; Skog *et al*, 2008) and that purified exosome-like vesicles contain single-stranded, mature miRNAs in addition to high levels of GW182 and low levels of hsAgo2 (Gibbings *et al*, 2009). One may speculate that a controlled targeting of miRNAs and/or RISC components by factors modulating endosomal trafficking could serve as a mechanism for the selective secretion in exosomes. In agreement with this idea, a ceramide-dependent secretory mechanism that induces endosome sorting into MVBs (Trajkovic *et al*, 2008) was found to actively regulate the release of exosomal miRNAs (Kosaka *et al*, 2010). The exosome-associated RISCs may have the potential to be internalized by recipient cells, where they modulate gene expression and trigger functional effects (Valadi *et al*, 2007; Skog *et al*, 2008; Pegtel *et al*, 2010; Zhang *et al*, 2010; Mittelbrunn *et al*, 2011; Hergenreider *et al*, 2012; Montecalvo *et al*, 2012). However, the view that exosome-associated RISC and/or miRNAs is the sole form of extracellular miRNAs is challenged by observations that extracellular miRNAs are not only membrane-vesicle-free but are also associated with AGO (Wang *et al*, 2010; Arroyo *et al*, 2011; Turchinovich *et al*, 2011). In addition to secretion/uptake-mediated intercellular small RNA transfer, siRNAs and miRNAs have also been implicated to travel through immune synapses in mammalian cells (Rechavi *et al*, 2009). However, few studies have investigated the intercellular transfer of small RNAs and its functional consequence under physiologically relevant conditions, and the extent and impact of small RNA intercellular transfer in mammals are still debatable. In *C. elegans,* the systemic nature of RNA silencing has long been established (Winston *et al*, 2002), and four SID proteins have been implicated in the uptake and spreading of dsRNAs (Hinas *et al*, 2012). Whereas SID-1, SID-2, and SID-3 are associated with the plasma membrane, SID-5 is endosome-associated (Hinas *et al*, 2012), implicating the endomembrane system in the systemic spreading of RNA silencing in *C. elegans*.

On the contrary, the functional implications of non-cell autonomous RNA silencing in plants are substantial (a topic reviewed elsewhere Melnyk *et al*, 2011). The long-distance movement of RNA silencing through the vasculature not only forms the cornerstone of systemic antiviral defense (Schwach *et al*, 2005), but also has been shown to direct epigenetic changes systemically (Molnar *et al*, 2010) and be implicated in miRNA-regulated stress responses (Buhtz *et al*, 2008; Pant *et al*, 2008). Many species of small RNAs have been found in the phloem sap, suggesting that the phloem is one of the routes for the long-distance transport of small RNAs (Buhtz *et al*, 2008; Varkonyi-Gasic *et al*, 2010). In addition to the systemic movement of RNA silencing, short-range movement of RNA silencing (by a few to tens of cell layers) patterns leaves and roots (Chitwood *et al*, 2009; Schwab *et al*, 2009; Carlsbecker *et al*, 2010) (for review, see Furuta *et al*, 2012). Plant cells are connected by plasmodesmata (PD), channels traversing the cell walls of plant cells, ensuring cytoplasmic continuity between cells and enabling transport and communication between them. The center of the PD contains a tube of appressed endoplasmic reticulum that runs between two adjacent cells (Maule, 2008) and accumulation of the glucose polymer callose at the PD controls permeability to cargoes (Simpson *et al*, 2009). Genetic screens have not yet revealed plasmodesmata-associated proteins that would influence the symplastic movement of silencing RNA, presumably since mutants affecting symplastic communications between cells would suffer from limited or no viability. However, evidence points toward PD being the channel by which small RNAs transit between cells (Fig 1) (Vatén *et al*, 2011): A dominant mutation in the *CALS3* gene that directs callose synthesis causes the accumulation of callose in PD and inhibits the intercellular transit of proteins and small RNAs through PD (Vatén *et al*, 2011). As trafficking to and through the PD requires the endomembrane system (Wright *et al*, 2006; Tagami & Watanabe, 2007), it is tempting to speculate that links may exist between PD-mediated intercellular traffic of silencing and the congregation of RNA silencing factors on endomembranes.

Concluding remarks

We have come a long way from the simple view of RNA silencing as a solely cytosolic process. The studies discussed above show that many aspects of RNA silencing, such as RISC formation, action, and turnover, involve endomembranes (Fig 1). Although overwhelming and convincing, the results obtained highlight that our understanding of the role of endomembranes in RNA silencing is still very lacunar. We can, however, speculate about the potential implications of this RNA silencing-membrane connection. Endomembranes could serve as a platform for partitioning silencing processes within the cell. The association of the silencing complexes with a specific type of endomembranes would ensure their sorting and allow the encounter with the proper set of targets or partners. It could also determine the ability or magnitude of intercellular transfer for a particular sRNA. The differential mobility of plant siRNAs and miRNAs (Felippes *et al*, 2010) could find its origin in the type of endomembranes associated with the two populations of sRNAs. It is essential for future research to elucidate the intracellular dynamics of various RNA silencing complexes. The rER emerges as a nexus for genome expression where a large portion of the transcriptome is translated and where miRNA-mediated translational repression occurs. Several competing models for miRNA-mediated translational repression have been proposed: inhibition of initiation, disruption of the mRNA loop, competition with eIF4E, or inhibition of elongation (Filipowicz *et al*, 2008). The important role of the rER should be taken into consideration when these models are evaluated. Beyond the obvious parallels between plants and animals described above, it is tempting to surmise that the central role of endomembranes in partitioning RNA processes in distinct subcellular

compartments is evolutionarily ancient, since work in bacteria has shown the RNA degradation machinery to congregate at the bacterial membrane, perhaps ensuring its separation from transcription and translation (Liou *et al*, 2001). The intricate association of RNA silencing—a fundamental coordinator of gene expression in eukaryotes—with the endomembrane system could ensure its coupling to signaling pathways and allow prompt responses to changes in the environment (Gibbings & Voinnet, 2010). In a broader perspective, this draws an interesting parallel to the emerging paradigm in cellular signaling that the biological context can influence the outcome of a ligand–receptor interaction (Barton & Kagan, 2009).

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Author contributions

YK, AM, and XC wrote and revised the manuscript; AM created the figure.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Adenot X, Elmayan T, Lauressergues D, Boutet S, Bouche N, Gasciolli V, Vaucheret H (2006) DRB4-dependent TAS3 trans-acting siRNAs control leaf morphology through AGO7. *Curr Biol* 16: 927 – 932

Allen E, Xie Z, Gustafson AM, Carrington JC (2005) microRNA-directed phasing during trans-acting siRNA biogenesis in plants. *Cell* 121: 207 – 221

Ando Y, Leung AKL (2013) Does an emergency visit to the ER make MicroRNAs stronger during stress? *Mol Cell* 52: 1 – 3

Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF, Pogosova-Agadjanyan EL, Stirewalt DL, Tait JF, Tewari M (2011) Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci USA* 108: 5003 – 5008

Aukerman MJ, Sakai H (2003) Regulation of flowering time and floral organ identity by a MicroRNA and its APETALA2-like target genes. *Plant Cell* 15: 2730 – 2741

Axtell MJ, Jan C, Rajagopalan R, Bartel DP (2006) A two-hit trigger for siRNA biogenesis in plants. *Cell* 127: 565 – 577

Barbato C, Ciotti MT, Serafino A, Calissano P, Cogoni C (2007) Dicer expression and localization in post-mitotic neurons. *Brain Res* 1175: 17 – 27

Barton GM, Kagan JC (2009) A cell biological view of Toll-like receptor function: regulation through compartmentalization. *Nat Rev Immunol* Nature Publishing Group 9: 535 – 542

Baumberger N, Baulcombe DC (2005) Arabidopsis ARGONAUTE1 is an RNA Slicer that selectively recruits microRNAs and short interfering RNAs. *Proc Natl Acad Sci USA* 102: 11928 – 11933

Baumberger N, Tsai C-H, Lie M, Havecker E, Baulcombe DC (2007) The polerovirus silencing suppressor P0 targets argonaute proteins for degradation. *Curr Biol* 17: 1609 – 1614

Bazzini AA, Lee MT, Giraldez AJ (2012) Ribosome profiling shows that miR-430 reduces translation before causing mRNA decay in zebrafish. *Science* 336: 233 – 237

Beauclair L, Yu A, Bouche N (2010) microRNA-directed cleavage and translational repression of the copper chaperone for superoxide dismutase mRNA in *Arabidopsis*. *Plant J* 62: 454 – 462

Behm-Ansmant I, Rehwinkel J, Doerks T, Stark A, Bork P, Izaurralde E (2006) MRNA degradation by miRNAs and GW182 requires both CCR4: NOT deadenylase and DCP1: DCP2 decapping complexes. *Gene Dev* 20: 1885 – 1898

Ben Amor B, Wirth S, Merchan F, Laporte P, d'Aubenton-Carafa Y, Hirsch J, Maizel A, Mallory A, Lucas A, Deragon JM, Vaucheret H, Thermes C, Crespi M (2009) Novel long non-protein coding RNAs involved in *Arabidopsis* differentiation and stress responses. *Genome Res* 19: 57 – 69

Bethune J, Artus-Revel CG, Filipowicz W (2012) Kinetic analysis reveals successive steps leading to miRNA-mediated silencing in mammalian cells. *EMBO Rep* 13: 716 – 723

Bortolamiol D, Pazhouhandeh M, Marrocco K, Genschik P, Ziegler-Graff V (2007) The polerovirus F box protein P0 targets argonaute1 to suppress RNA silencing. *Curr Biol* 17: 1615 – 1621

Brodersen P, Sakvarelidze-Achard L, Bruun-Rasmussen M, Dunoyer P, Yamamoto YY, Sieburth L, Voinnet O (2008) Widespread translational inhibition by plant miRNAs and siRNAs. *Science* 320: 1185 – 1190

Brodersen P, Sakvarelidze-Achard L, Schaller H, Khafif M, Schott G, Bendahmane A, Voinnet O (2012) Isoprenoid biosynthesis is required for miRNA function and affects membrane association of ARGONAUTE 1 in *Arabidopsis*. *Proc Natl Acad Sci USA* 109: 1778 – 1783

Buhtz A, Springer F, Chappell L, Baulcombe DC, Kehr J (2008) Identification and characterization of small RNAs from the phloem of *Brassica napus*. *Plant J* 53: 739 – 749

Carlsbecker A, Lee J-Y, Roberts CJ, Dettmer J, Lehesranta S, Zhou J, Lindgren O, Moreno-Risueno MA, Vatén A, Thitamadee S, Campilho A, Sebastian J, Bowman JL, Helariutta Y, Benfey PN (2010) Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature* 465: 316 – 321

Carthew RW, Sontheimer EJ (2009) Origins and mechanisms of miRNAs and siRNAs. *Cell* 136: 642 – 655

Chen Q, Jagannathan S, Reid DW, Zheng TL, Nicchitta CV (2011) Hierarchical regulation of mRNA partitioning between the cytoplasm and the endoplasmic reticulum of mammalian cells. *Mol Biol Cell* 22: 2646 – 2658

Chen XM (2004) A microRNA as a translational repressor of APETALA2 in Arabidopsis flower development. *Science* 303: 2022 – 2025

Chen XM (2009) Small RNAs and their roles in plant development. *Annu Rev Cell Dev Biol* 25: 21 – 44

Chitwood DH, Nogueira FTS, Howell MD, Montgomery TA, Carrington JC, Timmermans MCP (2009) Pattern formation via small RNA mobility. *Genes Dev* 23: 549 – 554

Chu CY, Rana TM (2006) Translation repression in human cells by microRNA-induced gene silencing requires RCK/p54. *PLoS Biol* 4: 1122 – 1136

Cikaluk DE, Tahbaz N, Hendricks LC, DiMattia GE, Hansen D, Pilgrim D, Hobman TC (1999) GERp95, a membrane-associated protein that belongs to a family of proteins involved in stem cell differentiation. *Mol Biol Cell* 10: 3357 – 3372

Conway LJ, Poethig RS (1997) Mutations of *Arabidopsis thaliana* that transform leaves into cotyledons. *Proc Natl Acad Sci USA* 94: 10209 – 10214

Csorba T, Lózsa R, Hutvagner G, Burgyán J (2010) Polerovirus protein P0 prevents the assembly of small RNA-containing RISC complexes and leads to degradation of ARGONAUTE1. *Plant J* 62: 463 – 472

Deprost D, Yao L, Sormani R, Moreau M, Leterreux G, Nicolaï M, Bedu M, Robaglia C, Meyer C (2007) The Arabidopsis TOR kinase links plant growth, yield, stress resistance and mRNA translation. *EMBO Rep* 8: 864 – 870

Derrien B, Baumberger N, Schepetilnikov M, Viotti C, De Cillia J, Ziegler-Graff V, Isono E, Schumacher K, Genschik P (2012) Degradation of the antiviral component ARGONAUTE1 by the autophagy pathway. *Proc Natl Acad Sci USA* 109: 15942 – 15946

Djuranovic S, Nahvi A, Green R (2012) miRNA-mediated gene silencing by translational repression followed by mRNA deadenylation and decay. *Science* 336: 237 – 240

Douglas RN, Wiley D, Sarkar A, Springer N, Timmermans MCP, Scanlon MJ (2010) Ragged seedling 2 encodes an ARGONAUTE7-like protein required for mediolateral expansion, but not dorsiventrality, of maize leaves. *Plant Cell* 22: 1441 – 1451

Dugas DV, Bartel B (2008) Sucrose induction of Arabidopsis miR398 represses two Cu/Zn superoxide dismutases. *Plant Mol Biol* 67: 403 – 417

Dunoyer P, Himber C, Voinnet O (2005) DICER-LIKE 4 is required for RNA interference and produces the 21-nucleotide small interfering RNA component of the plant cell-to-cell silencing signal. *Nat Genet* 37: 1356 – 1360

Eulalio A, Behm-Ansmant I, Schweizer D, Izaurralde E (2007) P-body formation is a consequence, not the cause, of RNA-mediated gene silencing. *Mol Cell Biol* 27: 3970 – 3981

Eulalio A, Huntzinger E, Izaurralde E (2008) GW182 interaction with Argonaute is essential for miRNA-mediated translational repression and mRNA decay. *Nat Struct Mol Biol* 15: 346 – 353

Fabian MR, Mathonnet G, Sundermeier T, Mathys H, Zipprich JT, Svitkin YV, Rivas F, Jinek M, Wohlschlegel J, Doudna JA, Chen CY, Shyu AB, Yates JR III, Hannon GJ, Filipowicz W, Duchaine TF, Sonenberg N (2009) Mammalian miRNA RISC recruits CAF1 and PABP to affect PABP-dependent deadenylation. *Mol Cell* 35: 868 – 880

Fabian MR, Sonenberg N, Filipowicz W (2010) Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem* 79: 351 – 379

Fahlgren N, Carrington JC (2010) miRNA target prediction in plants. *Methods Mol Biol* 592: 51 – 57

Fahlgren N, Montgomery TA, Howell MD, Allen E, Dvorak SK, Alexander AL, Carrington JC (2006) Regulation of AUXIN RESPONSE FACTOR3 by TAS3 ta-siRNA affects developmental timing and patterning in Arabidopsis. *Curr Biol* 16: 939 – 944

Faini M, Beck R, Wieland FT, Briggs JAG (2013) Vesicle coats: structure, function, and general principles of assembly. *Trends Cell Biol* 23: 279 – 288

Felippes F, Ott F, Weigel D (2010) Comparative analysis of non-autonomous effects of tasiRNAs and miRNAs in *Arabidopsis thaliana*. *Nucleic Acids Res* 39: 2880 – 2889

Filipowicz W, Bhattacharyya SN, Sonenberg N (2008) Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 9: 102 – 114

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391: 806 – 811

Furuta K, Lichtenberger R, Helariutta Y (2012) The role of mobile small RNA species during root growth and development. *Curr Opin Cell Biol* 24: 211 – 216

Gabe Lee MT, Mishra A, Lambright DG (2009) Structural mechanisms for regulation of membrane traffic by Rab GTPases. *Traffic* 10: 1377 – 1389

Gandikota M, Birkenbihl RP, Höhmann S, Cardon GH, Saedler H, Huijser P (2007) The miRNA156/157 recognition element in the 3′ UTR of the Arabidopsis SBP box gene SPL3 prevents early flowering by translational inhibition in seedlings. *Plant J* 49: 683 – 693

Garcia D, Collier SA, Byrne ME, Martienssen RA (2006) Specification of leaf polarity in Arabidopsis via the trans-acting siRNA pathway. *Curr Biol* 16: 933 – 938

Gasciolli V, Mallory AC, Bartel DP, Vaucheret H (2005) Partially redundant functions of Arabidopsis DICER-like enzymes and a role for DCL4 in producing trans-acting siRNAs. *Curr Biol* 15: 1494 – 1500

Gibbings D, Mostowy S, Jay F, Schwab Y, Cossart P (2012) Selective autophagy degrades DICER and AGO2 and regulates miRNA activity. *Nat Cell Biol* 14: 1314 – 1321

Gibbings D, Voinnet O (2010) Control of RNA silencing and localization by endolysosomes. *Trends Cell Biol* 20: 491 – 501

Gibbings DJ, Ciaudo C, Erhardt M, Voinnet O (2009) Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. *Nat Cell Biol* 11: 1143 – 1149

Griffiths J, Barrero JM, Taylor J, Helliwell CA, Gubler F (2011) ALTERED MERISTEM PROGRAM 1 is involved in development of seed dormancy in Arabidopsis. *PLoS ONE* 6: e20408

Gruenberg J, Stenmark H (2004) The biogenesis of multivesicular endosomes. *Nat Rev Mol Cell Biol* 5: 317 – 323

Guo H, Ingolia NT, Weissman JS, Bartel DP (2010) Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature* 466: 835 – 840

Haase AD, Fenoglio S, Muerdter F, Guzzardo PM, Czech B, Pappin DJ, Chen CF, Gordon A, Hannon GJ (2010) Probing the initiation and effector phases of the somatic piRNA pathway in *Drosophila*. *Gene Dev* 24: 2499 – 2504

Helliwell CA, Chin-Atkins AN, Wilson IW, Chapple R, Dennis ES, Chaudhury A (2001) The Arabidopsis AMP1 gene encodes a putative glutamate carboxypeptidase. *Plant Cell* 13: 2115 – 2125

Hergenreider E, Heydt S, Tréguer K, Boettger T, Horrevoets AJG, Zeiher AM, Scheffer MP, Frangakis AS, Yin X, Mayr M, Braun T, Urbich C, Boon RA, Dimmeler S (2012) Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. *Nat Cell Biol* 14: 249 – 256

Hinas A, Wright AJ, Hunter CP (2012) SID-5 is an endosome-associated protein required for efficient systemic RNAi in *C. elegans*. *Curr Biol* 22: 1938 – 1943

Hunter C, Willmann MR, Wu G, Yoshikawa M, de la Luz Gutiérrez-Nava M, Poethig SR (2006) Trans-acting siRNA-mediated repression of ETTIN and ARF4 regulates heteroblasty in Arabidopsis. *Development* 133: 2973 – 2981

Hurley JH, Hanson PI (2010) Membrane budding and scission by the ESCRT machinery: it's all in the neck. *Nat Rev Mol Cell Biol* 11: 556 – 566

Isono E, Katsiarimpa A, Müller IK, Anzenberger F, Stierhof Y-D, Geldner N, Chory J, Schwechheimer C (2010) The deubiquitinating enzyme AMSH3 is required for intracellular trafficking and vacuole biogenesis in *Arabidopsis thaliana*. *Plant Cell* 22: 1826 – 1837

Jakymiw A, Lian SL, Eystathioy T, Li SQ, Satoh M, Hamel JC, Fritzler MJ, Chan EKL (2005) Disruption of GW bodies impairs mammalian RNA interference. *Nat Cell Biol* 7: 1267 – 1274

Jinek M, Doudna JA (2009) A three-dimensional view of the molecular machinery of RNA interference. *Nature* 457: 405 – 412

Jouannet V, Moreno AB, Elmayan T, Vaucheret H, Crespi MD, Maizel A (2012) Cytoplasmic Arabidopsis AGO7 accumulates in membrane-associated siRNA bodies and is required for ta-siRNA biogenesis. *EMBO J* 31: 1704 – 1713

Kirchhausen T (2000) Three ways to make a vesicle. *Nat Rev Mol Cell Biol* 1: 187 – 198

Kosaka N, Iguchi H, Yoshioka Y, Takeshita F (2010) Secretory mechanisms and intercellular transfer of microRNAs in living cells. *J Biol Chem* 285: 17442 – 17452

Kraut-Cohen J, Afanasieva E, Haim-Vilmovsky L, Slobodin B, Yosef I, Bibi E, Gerst JE (2013) Translation- and SRP-independent mRNA targeting to the endoplasmic reticulum in the yeast *Saccharomyces cerevisiae*. *Mol Biol Cell* 24: 3069 – 3084

Lanet E, Delannoy E, Sormani R, Floris M, Brodersen P, Crété P, Voinnet O, Robaglia C (2009) Biochemical evidence for translational repression by Arabidopsis microRNAs. *Plant Cell* 21: 1762 – 1768

Law JA, Jacobsen SE (2010) Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat Rev Genet* 11: 204 – 220

Lee J-A, Beigneux A, Ahmad ST, Young SG, Gao F-B (2007) ESCRT-III dysfunction causes autophagosome accumulation and neurodegeneration. *Curr Biol* 17: 1561 – 1567

Lee YS, Pressman S, Andress AP, Kim K, White JL, Cassidy JJ, Li X, Lubell K, Lim DH, Cho IS, Nakahara K, Preall JB, Bellare P, Sontheimer EJ, Carthew RW (2009) Silencing by small RNAs is linked to endosomal trafficking. *Nat Cell Biol* 11: 1150 – 1156

Lerich A, Langhans M, Sturm S, Robinson DG (2011) Is the 6 kDa tobacco etch viral protein a *bona fide* ERES marker? *J Exp Bot* 62: 5013 – 5023

Lerner RS, Seiser RM, Zheng T, Lager PJ, Reedy MC, Keene JD, Nicchitta CV (2003) Partitioning and translation of mRNAs encoding soluble proteins on membrane-bound ribosomes. *RNA* 9: 1123 – 1137

Leuschner PJ, Ameres SL, Kueng S, Martinez J (2006) Cleavage of the siRNA passenger strand during RISC assembly in human cells. *EMBO Rep* 7: 314 – 320

Levine B (2007) Cell biology: autophagy and cancer. *Nature* 446: 745 – 747

Lewis BP, Burge CB, Bartel DP (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 120: 15 – 20

Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB (2003) Prediction of mammalian microRNA targets. *Cell* 115: 787 – 798

Li S, Liu L, Zhuang X, Yu Y, Liu X, Cui X, Ji L, Pan Z, Cao X, Mo B, Zhang F, Raikhel N, Jiang L, Chen X (2013) MicroRNAs inhibit the translation of target mRNAs on the endoplasmic reticulum in Arabidopsis. *Cell* 153: 562 – 574

Li W, Rusiniak ME, Chintala S, Gautam R, Novak EK, Swank RT (2004) Murine Hermansky-Pudlak syndrome genes: regulators of lysosome-related organelles. *BioEssays* 26: 616 – 628

Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 433: 769 – 773

Liou GG, Jane WN, Cohen SN, Lin NS, Lin-Chao S (2001) RNA degradosomes exist *in vivo* in *Escherichia coli* as multicomponent complexes associated with the cytoplasmic membrane via the N-terminal region of ribonuclease E. *Proc Natl Acad Sci USA* 98: 63 – 68

Liu JD, Carmell MA, Rivas FV, Marsden CG, Thomson JM, Song JJ, Hammond SM, Joshua-Tor L, Hannon GJ (2004) Argonaute2 is the catalytic engine of mammalian RNAi. *Science* 305: 1437 – 1441

Liu JD, Valencia-Sanchez MA, Hannon GJ, Parker R (2005) MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. *Nat Cell Biol* 7: 719 – U118

Mallory AC, Reinhart BJ, Jones-Rhoades MW, Tang G, Zamore PD, Barton MK, Bartel DP (2004) MicroRNA control of PHABULOSA in leaf development: importance of pairing to the microRNA 5′ region. *EMBO J* 23: 3356 – 3364

Malone CD, Brennecke J, Dus M, Stark A, McCombie WR, Sachidanandam R, Hannon GJ (2009) Specialized piRNA pathways act in germline and somatic tissues of the *Drosophila* ovary. *Cell* 137: 522 – 535

Marin E, Jouannet V, Herz A, Lokerse AS, Weijers D, Vaucheret H, Nussaume L, Crespi MD, Maizel A (2010) miR390, Arabidopsis TAS3 tasiRNAs, and their AUXIN RESPONSE FACTOR targets define an autoregulatory network quantitatively regulating lateral root growth. *Plant Cell* 22: 1104 – 1117

Martinez NJ, Gregory RI (2013) Argonaute2 expression is post-transcriptionally coupled to microRNA abundance. *RNA* 19: 605 – 612

Matranga C, Tomari Y, Shin C, Bartel DP, Zamore PD (2005) Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. *Cell* 123: 607 – 620

Maule AJ (2008) Plasmodesmata: structure, function and biogenesis. *Curr Opin Plant Biol* 11: 680 – 686

Meister G (2013) Argonaute proteins: functional insights and emerging roles. *Nat Rev Genet* 14: 447 – 459

Meister G, Landthaler M, Patkaniowska A, Dorsett Y, Teng G, Tuschl T (2004) Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. *Mol Cell* 15: 185 – 197

Meister G, Tuschl T (2004) Mechanisms of gene silencing by double-stranded RNA. *Nature* 431: 343 – 349

Melnyk CW, Molnar A, Baulcombe DC (2011) Intercellular and systemic movement of RNA silencing signals. *EMBO J* 30: 3553 – 3563

Mi SJ, Cai T, Hu YG, Chen Y, Hodges E, Ni FR, Wu L, Li S, Zhou H, Long CZ, Chen S, Hannon GJ, Qi YJ (2008) Sorting of small RNAs into Arabidopsis argonaute complexes is directed by the 5 terminal nucleotide. *Cell* 133: 116 – 127

Mittelbrunn M, Gutiérrez-Vázquez C, Villarroya-Beltri C, González S, Sánchez-Cabo F, González MÁ, Bernad A, Sánchez-Madrid F (2011) Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. *Nat Comms* 2: 282

Mizushima N, Komatsu M (2011) Autophagy: renovation of cells and tissues. *Cell* 147: 728 – 741

Molnar A, Melnyk CW, Bassett A, Hardcastle TJ, Dunn R, Baulcombe DC (2010) Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. *Science* 328: 872 – 875

Montecalvo A, Larregina AT, Shufesky WJ, Stolz DB, Sullivan MLG, Karlsson JM, Baty CJ, Gibson GA, Erdos G, Wang Z, Milosevic J, Tkacheva OA, Divito SJ, Jordan R, Lyons-Weiler J, Watkins SC, Morelli AE (2012) Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. *Blood* 119: 756 – 766

Montgomery TA, Howell MD, Cuperus JT, Li D, Hansen JE, Alexander AL, Chapman EJ, Fahlgren N, Allen E, Carrington JC (2008) Specificity of ARGONAUTE7-miR390 interaction and dual functionality in TAS3 trans-acting siRNA formation. *Cell* 133: 128 – 141

Moreno AB, Martínez de Alba AE, Bardou F, Crespi MD, Vaucheret H, Maizel A, Mallory AC (2013) Cytoplasmic and nuclear quality control and turnover of single-stranded RNA modulate post-transcriptional gene silencing in plants. *Nucleic Acids Res* 41: 4699 – 4708

Nakanishi K, Weinberg DE, Bartel DP, Patel DJ (2012) Structure of yeast Argonaute with guide RNA. *Nature* 486: 368 – 374

Nickel W, Brügger B, Wieland FT (2002) Vesicular transport: the core machinery of COPI recruitment and budding. *J Cell Sci* 115: 3235 – 3240

Nogueira FTS, Chitwood DH, Madi S, Ohtsu K, Schnable PS, Scanlon MJ, Timmermans MCP (2009) Regulation of small RNA accumulation in the maize shoot apex. *PLoS Genet* 5: e1000320

Olivieri D, Sykora MM, Sachidanandam R, Mechtler K, Brennecke J (2010) An *in vivo* RNAi assay identifies major genetic and cellular requirements for primary piRNA biogenesis in *Drosophila*. *EMBO J* 29: 3301 – 3317

Palauqui JC, Elmayan T, Pollien JM, Vaucheret H (1997) Systemic acquired silencing: transgene-specific post-transcriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions. *EMBO J* 16: 4738 – 4745

Pane A, Wehr K, Schupbach T (2007) zucchini and squash encode two putative nucleases required for rasiRNA production in the *Drosophila* germline. *Dev Cell* 12: 851 – 862

Pant BD, Buhtz A, Kehr J, Scheible W-R (2008) MicroRNA399 is a long-distance signal for the regulation of plant phosphate homeostasis. *Plant J* 53: 731 – 738

Pazhouhandeh M, Dieterle M, Marrocco K, Lechner E, Berry B, Brault V, Hemmer O, Kretsch T, Richards KE, Genschik P, Ziegler-Graff V (2006) F-box-like domain in the polerovirus protein P0 is required for silencing suppressor function. *Proc Natl Acad Sci USA* 103: 1994 – 1999

Pegtel DM, Cosmopoulos K, Thorley-Lawson DA, van Eijndhoven MAJ, Hopmans ES, Lindenberg JL, de Gruijl TD, Würdinger T, Middeldorp JM (2010) Functional delivery of viral miRNAs via exosomes. *Proc Natl Acad Sci USA* 107: 6328 – 6333

Peragine A, Yoshikawa M, Wu G, Albrecht HL, Poethig RS (2004) SGS3 and SGS2/SDE1/RDR6 are required for juvenile development and the production of trans-acting siRNAs in Arabidopsis. *Genes Dev* 18: 2368 – 2379

Pillai RS, Bhattacharyya SN, Artus CG, Zoller T, Cougot N, Basyuk E, Bertrand E, Filipowicz W (2005) Inhibition of translational initiation by Let-7 microRNA in human cells. *Science* 309: 1573 – 1576

Pomeranz MC, Hah C, Lin P-C, Kang SG, Finer JJ, Blackshear PJ, Jang J-C (2010) The Arabidopsis tandem zinc finger protein AtTZF1 traffics between the nucleus and cytoplasmic foci and binds both DNA and RNA. *Plant Physiol* 152: 151 – 165

Pyhtila B, Zheng T, Lager PJ, Keene JD, Reedy MC, Nicchitta CV (2008) Signal sequence- and translation-independent mRNA localization to the endoplasmic reticulum. *RNA* 14: 445 – 453

Raiborg C, Stenmark H (2009) The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. *Nature* 458: 445 – 452

Rand TA, Petersen S, Du F, Wang X (2005) Argonaute2 cleaves the anti-guide strand of siRNA during RISC activation. *Cell* 123: 621 – 629

Rechavi O, Erlich Y, Amram H, Flomenblit L, Karginov FV, Goldstein I, Hannon GJ, Kloog Y (2009) Cell contact-dependent acquisition of cellular and viral nonautonomously encoded small RNAs. *Gene Dev* 23: 1971 – 1979

Reid DW, Nicchitta CV (2012) Primary role for endoplasmic reticulum-bound ribosomes in cellular translation identified by ribosome profiling. *J Biol Chem* 287: 5518 – 5527

Reynoso MA, Blanco FA, Bailey-Serres J, Crespi M, Zanetti ME (2013) Selective recruitment of mRNAs and miRNAs to polyribosomes in response to rhizobia infection in *Medicago truncatula*. *Plant J* 73: 289 – 301

Rivas FV, Tolia NH, Song JJ, Aragon JP, Liu JD, Hannon GJ, Joshua-Tor L (2005) Purified Argonaute2 and an siRNA form recombinant human RISC. *Nat Struct Mol Biol* 12: 340 – 349

Saito K, Inagaki S, Mituyama T, Kawamura Y, Ono Y, Sakota E, Kotani H, Asai K, Siomi H, Siomi MC (2009) A regulatory circuit for piwi by the large Maf gene traffic jam in *Drosophila*. *Nature* 461: 1296 – U1135

Saito K, Ishizu H, Komai M, Kotani H, Kawamura Y, Nishida KM, Siomi H, Siomi MC (2010) Roles for the Yb body components Armitage and Yb in primary piRNA biogenesis in *Drosophila*. *Gene Dev* 24: 2493 – 2498

Schaad MC, Jensen PE, Carrington JC (1997) Formation of plant RNA virus replication complexes on membranes: role of an endoplasmic reticulum-targeted viral protein. *EMBO J* 16: 4049 – 4059

Scheuring D, Viotti C, Krüger F, Künzl F, Sturm S, Bubeck J, Hillmer S, Frigerio L, Robinson DG, Pimpl P, Schumacher K (2011) Multivesicular bodies mature from the trans-golgi network/early endosome in Arabidopsis. *Plant Cell* 23: 3463 – 3481

Schurmann N, Trabuco LG, Bender C, Russell RB, Grimm D (2013) Molecular dissection of human Argonaute proteins by DNA shuffling. *Nat Struct Mol Biol* 20: 818 – 826

Schwab R, Maizel A, Ruiz-Ferrer V, Garcia D, Bayer M, Crespi M, Voinnet O, Martienssen RA (2009) Endogenous TasiRNAs mediate non-cell autonomous effects on gene regulation in *Arabidopsis thaliana*. *PLoS ONE* 4: e5980

Schwach F, Vaistij FE, Jones L, Baulcombe DC (2005) An RNA-dependent RNA polymerase prevents meristem invasion by potato virus X and is required for the activity but not the production of a systemic silencing signal. *Plant Physiol* 138: 1842 – 1852

Schwartz TU (2007) Origins and evolution of cotranslational transport to the ER. *Adv Exp Med Biol* 607: 52 – 60

Seaman MNJ (2005) Recycle your receptors with retromer. *Trends Cell Biol* 15: 68 – 75

Shi HT, Ye TT, Wang YP, Chan ZL (2013) Arabidopsis ALTERED MERISTEM PROGRAM 1 negatively modulates plant responses to abscisic acid and dehydration stress. *Plant Physiol Biochem* 67: 209 – 216

Shi Z, Ruvkun G (2012) The mevalonate pathway regulates microRNA activity in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 109: 4568 – 4573

Shiromoto Y, Kuramochi-Miyagawa S, Daiba A, Chuma S, Katanaya A, Katsumata A, Nishimura K, Ohtaka M, Nakanishi M, Nakamura T, Yoshinaga K, Asada N, Nakamura S, Yasunaga T, Kojima-Kita K, Itou D, Kimura T, Nakano T (2013) GPAT2, a mitochondrial outer membrane protein, in piRNA biogenesis in germline stem cells. *RNA* 19: 803 – 810

Simpson C, Thomas C, Findlay K, Bayer E, Maule AJ (2009) An Arabidopsis GPI-anchor plasmodesmal neck protein with callose binding activity and potential to regulate cell-to-cell trafficking. *Plant Cell* 21: 581 – 594

Siomi MC, Sato K, Pezic D, Aravin AA (2011) PIWI-interacting small RNAs: the vanguard of genome defence. *Nat Rev Mol Cell Biol* 12: 246 – 258

Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT, Carter BS, Krichevsky AM, Breakefield XO (2008) Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol* 10: 1470 – 1476

Smibert P, Yang J-S, Azzam G, Liu J-L, Lai EC (2013) Homeostatic control of Argonaute stability by microRNA availability. *Nat Struct Mol Biol* 20: 789 – 795

Spang A (2009) On the fate of early endosomes. *Biol Chem* 390: 753 – 759

Stalder L, Heusermann W, Sokol L, Trojer D, Wirz J, Hean J, Fritzsche A, Aeschimann F, Pfanzagl V, Basselet P, Weiler J, Hintersteiner M, Morrissey DV, Meisner-Kober NC (2013) The rough endoplasmatic reticulum is a central nucleation site of siRNA-mediated RNA silencing. *EMBO J* 32: 1115 – 1127

Suzuki T, Li W, Zhang Q, Karim A, Novak EK, Sviderskaya EV, Hill SP, Bennett DC, Levin AV, Nieuwenhuis HK, Fong C-T, Castellan C, Miterski B, Swank RT, Spritz RA (2002) Hermansky-Pudlak syndrome is caused by mutations in HPS4, the human homolog of the mouse light-ear gene. *Nat Genet* 30: 321 – 324

Tagami Y, Watanabe Y (2007) Effects of brefeldin A on the localization of Tobamovirus movement protein and cell-to-cell movement of the virus. *Virology* 361: 133 – 140

Tahbaz N, Kolb FA, Zhang H, Jaronczyk K, Filipowicz W, Hobman TC (2004) Characterization of the interactions between mammalian PAZ PIWI domain proteins and dicer. *EMBO Rep* 5: 189 – 194

Thurston TLM, Wandel MP, von Muhlinen N, Foeglein A, Randow F (2012) Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. *Nature* 482: 414 – 418

Timmons L, Fire A (1998) Specific interference by ingested dsRNA. *Nature* 395: 854

Tomari Y, Du T, Zamore PD (2007) Sorting of *Drosophila* small silencing RNAs. *Cell* 130: 299 – 308

Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, Schwille P, Brügger B, Simons M (2008) Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science* 319: 1244 – 1247

Turchinovich A, Weiz L, Burwinkel B (2012) Extracellular miRNAs: the mystery of their origin and function. *Trends Biochem Sci* 37: 460 – 465

Turchinovich A, Weiz L, Langheinz A, Burwinkel B (2011) Characterization of extracellular circulating microRNA. *Nucleic Acids Res* 39: 7223 – 7233

Vagin VV, Yu Y, Jankowska A, Luo YC, Wasik KA, Malone CD, Harrison E, Rosebrock A, Wakimoto BT, Fagegaltier D, Muerdter F, Hannon GJ (2013) Minotaur is critical for primary piRNA biogenesis. *RNA* 19: 1064 – 1077

Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO (2007) Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 9: 654 – 659

Varkonyi-Gasic E, Gould N, Sandanayaka M, Sutherland P, MacDiarmid RM (2010) Characterisation of microRNAs from apple (Malus domestica "Royal Gala") vascular tissue and phloem sap. *BMC Plant Biol* 10: 159

Vatén A, Dettmer J, Wu S, Stierhof Y-D, Miyashima S, Yadav SR, Roberts CJ, Campilho A, Bulone V, Lichtenberger R, Lehesranta S, Mähönen AP, Kim J-Y, Jokitalo E, Sauer N, Scheres B, Nakajima K, Carlsbecker A, Gallagher KL, Helariutta Y (2011) Callose biosynthesis regulates symplastic trafficking during root development. *Dev Cell* Elsevier Inc 21: 1144 – 1155

Vazquez F, Gasciolli V, Crété P, Vaucheret H (2004) The nuclear dsRNA binding protein HYL1 is required for microRNA accumulation and plant development, but not posttranscriptional transgene silencing. *Curr Biol* 14: 346 – 351

Vidaurre DP, Ploense S, Krogan NT, Berleth T (2007) AMP1 and MP antagonistically regulate embryo and meristem development in Arabidopsis. *Development* 134: 2561 – 2567

Voinnet O, Baulcombe DC (1997) Nature. *Nature* 389: 553

Wang K, Zhang S, Weber J, Baxter D, Galas DJ (2010) Export of microRNAs and microRNA-protective protein by mammalian cells. *Nucleic Acids Res* 38: 7248 – 7259

Watanabe T, Chuma S, Yamamoto Y, Kuramochi-Miyagawa S, Totoki Y, Toyoda A, Hoki Y, Fujiyama A, Shibata T, Sado T, Noce T, Nakano T, Nakatsuji N, Lin HF, Sasaki H (2011) MITOPLD is a mitochondrial protein essential for nuage formation and piRNA biogenesis in the mouse germline. *Dev Cell* 20: 364 – 375

Weber C, Nover L, Fauth M (2008) Plant stress granules and mRNA processing bodies are distinct from heat stress granules. *Plant J* 56: 517 – 530

Winston WM, Molodowitch C, Hunter CP (2002) Systemic RNAi in *C. elegans* requires the putative transmembrane protein SID-1. *Science* 295: 2456 – 2459

Wright KM, Wood NT, Roberts AG, Chapman S, Boevink P, MacKenzie KM, Oparka KJ (2006) Targeting of TMV movement protein to plasmodesmata requires the actin/ER network; evidence from FRAP. *Traffic* 8: 21 – 31

Wu LG, Fan JH, Belasco JG (2006) MicroRNAs direct rapid deadenylation of mRNA. *Proc Natl Acad Sci USA* 103: 4034 – 4039

Wu P-H, Isaji M, Carthew RW (2013) Functionally diverse microRNA effector complexes are regulated by extracellular signaling. *Mol Cell* 52: 113 – 123

Xie Z, Johansen LK, Gustafson AM, Kasschau KD, Lellis AD, Zilberman D, Jacobsen SE, Carrington JC (2004) Genetic and functional diversification of small RNA pathways in plants. *PLoS Biol* 2: E104

Xu J, Chua NH (2011) Processing bodies and plant development. *Curr Opin Plant Biol* 14: 88 – 93

Yang L, Wu G, Poethig RS (2012) Mutations in the GW-repeat protein SUO reveal a developmental function for microRNA-mediated translational repression in Arabidopsis. *Proc Natl Acad Sci USA* 109: 315 – 320

Yoon EK, Yang JH, Lim J, Kim SH, Kim S-K, Lee WS (2010) Auxin regulation of the microRNA390-dependent transacting small interfering RNA pathway in Arabidopsis lateral root development. *Nucleic Acids Res* 38: 1382 – 1391

Zhang P, Zhang H (2013) Autophagy modulates miRNA-mediated gene silencing and selectively degrades AIN-1/GW182 in *C. elegans*. *EMBO Rep* 14: 568 – 576

Zhang Y, Liu D, Chen X, Li J, Li L, Bian Z, Sun F, Lu J, Yin Y, Cai X, Sun Q, Wang K, Ba Y, Wang Q, Wang D, Yang J, Liu P, Xu T, Yan Q, Zhang J *et al* (2010) Secreted monocytic miR-150 enhances targeted endothelial cell migration. *Mol Cell* 39: 133 – 144

