Marine Life Science & Technology (2023) 5:573–584
https://doi.org/10.1007/s42995-023-00209-7

REVIEW

Invasion by exogenous RNA: cellular defense strategies and implications for RNA inference

Danxu Tang[1,2] · Yan Liu[2] · Chundi Wang[1] · Lifang Li[1] · Saleh A. Al-Farraj[3] · Xiao Chen[1,4] · Ying Yan[2]

Received: 2 July 2023 / Accepted: 2 November 2023 / Published online: 24 November 2023
© Ocean University of China 2023

Abstract

Exogenous RNA poses a continuous threat to genome stability and integrity across various organisms. Accumulating evidence reveals complex mechanisms underlying the cellular response to exogenous RNA, including endo-lysosomal degradation, RNA-dependent repression and innate immune clearance. Across a variety of mechanisms, the natural anti-sense RNA-dependent defensive strategy has been utilized both as a powerful gene manipulation tool and gene therapy strategy named RNA-interference (RNAi). To optimize the efficiency of RNAi silencing, a comprehensive understanding of the whole life cycle of exogenous RNA, from cellular entry to its decay, is vital. In this paper, we review recent progress in comprehending the recognition and elimination of foreign RNA by cells, focusing on cellular entrance, intracellular transportation, and immune-inflammatory responses. By leveraging these insights, we highlight the potential implications of these insights for advancing RNA interference efficiency, underscore the need for future studies to elucidate the pathways and fates of various exogenous RNA forms, and provide foundational information for more efficient RNA delivery methods in both genetic manipulation and therapy in different organisms.

Keywords Endocytosis · Exogenous RNA · Innate immune response · RNA inference

Introduction

The invasion of exogenous RNA (e.g., derived from RNA viruses, diet derived-RNAs, artificially synthesized RNAs), can interfere with regular gene expression within the host cell through non-sexual movement of RNAs between the cell and the environment or other organisms. This process plays a vital role in maintaining cell metabolism, neurotransmitter release, fertilization, and a variety of other vital functions (Scott et al. 2014). In addition, exogenous RNA invasion can contribute to genome evolution. For example, endogenous retroviruses (ERVs), which have existed in human genomes for millions of years, may originate from RNA viruses (Koonin et al. 2021). Several recent studies have shown that ERVs act as important components of the antiviral immune response. These remnants of once-infectious retroviruses can not only regulate cellular immune activation, but also directly target invading viral pathogens (Alcazer et al. 2020; Srinivasachar Badarinarayan and Sauter 2021). It is, therefore, reasonable to assume that exogenous invasion events are also under natural selection and can influence the host's evolution (Emamalipour et al. 2020).

Special Topic: EvoDevo.

Edited by Jiamei Li.

✉ Xiao Chen
alpha67@163.com

✉ Ying Yan
yanying@ouc.edu.cn

1 Laboratory of Marine Protozoan Biodiversity and Evolution, Marine College, Shandong University, Weihai 264209, China

2 Key Laboratory of Evolution and Marine Biodiversity (Ministry of Education) and Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao 266003, China

3 Zoology Department, College of Science, King Saud University, 11451 Riyadh, Saudi Arabia

4 Suzhou Research Institute, Shandong University, Suzhou 215123, China

Exogenous RNAs are usually internalized by various endocytic pathways, which may involve macropinocytosis, phagocytosis, clathrin/caveolar/lipid raft-mediated endocytosis, or a variety of other still poorly characterized mechanisms (Mercer et al. 2010; Renard and Boucrot 2021). During their vesicular transport through the cytoplasm, several cellular defense mechanisms, such as lysosome enzymatic hydrolysis, the RNA interference-dependent defense system and innate immune responses, are activated. However, exogenous RNA can sometimes escape degradation and cause dysregulation of the host cell's gene expression (Del Pozo-Acebo et al. 2021; Gaucherand and Gaglia 2022).

Napoli et al. (1990) overexpressed chalcone synthetase (CHS) in petunia and unexpectedly found that endogenous and introduced CHS levels in transformed strains were 50 times lower than that in the wild type, leading to the hypothesis that introduced gene "co-repressing" the endogenous CHS gene is the process of RNAi. After Romano and Macino's (1992) report in *Neurospora crassa*, Guo and Kemphues (1995) also noted that the introduction of homologous RNA sequences caused "quelling" of the corresponding endogenous gene, suggesting that RNA silencing also exists in *Caenorhabditis elegans*. Since then, introducing antisense RNA has become one of the most appealing means of eliminating gene expression and led to the development of the RNAi technique (Elbashir et al. 2001; Fire et al. 1998).

RNA interference has various applications, particularly in treating viral infections, neurodegenerative disease (Germain et al. 2023), cardiovascular disease (Ruotsalainen et al. 2021) and acquired genetic diseases including various cancers (Tian et al. 2021). Naked RNA is, however, susceptible to degradation by nucleases and can stimulate the innate immune system (Kanasty et al. 2013). To improve RNAi efficiency, recent efforts have involved modifying the carrier systems or combining the use of small molecules to enhance the endosomal escape of exogenous RNA (Du Rietz et al. 2020; Nguyen and Yates 2021).

In this review, we discuss the life cycle of exogenous RNA after entering cells, how cells recognize and degrade exogenous RNA, and how they protect genome integrity using RNA interference. By summarizing recent advances in understanding cellular defense strategies in response to exogenous RNA invasion, we aim to inspire future improvements in the practice of RNA interference in both genetic research and therapy.

Cellular uptake of exogenous RNA

According to the size and properties of internalized material, exogenous RNA enters cells via different endocytosis pathways including macropinocytosis, phagocytosis and clathrin/caveolin/lipid raft-mediated endocytosis (Fig. 1).

Macropinocytosis is a non-specific process that can encapsulate foreign substances with size range from 0.5 to 5 µm, such as bacteria, mimivirus, herpes simplex virus-1 (HSV-1) and adenovirus 3 (Fig. 1IV) (Kazmierczak et al. 2020; Mercer et al. 2010). It is an endocytic process initiated by the formation of plasma membrane ruffles and is mainly driven by actin filament polymerization (Buckley and King 2017). Macropinocytosis is tightly regulated and involves the interplay of various signaling molecules and the actin cytoskeleton. The molecular mechanism of macropinocytosis has been extensively studied in recent decades, including the initiation process, actin polymerization, membrane ruffle closure, macropinosome maturation and eventually the degradation of mature macropinosomes or membrane recycling (Egami 2016; Stow et al. 2020).

Phagocytosis is a cellular process in which specialized cells, namely phagocytes, ingest or engulf other cells or particles, typically over 0.5 µm in size, such as bacteria or cellular debris (Fig. 1III) (Zachar and Boza 2020). Phagocytes can be free-living single-celled organisms, such as ciliates, or body cells such as neutrophils in peripheral blood. In some ciliates, phagocytosis acts as a feeding mechanism, while in higher eukaryotes it primarily serves as a defensive response against invasion by antigens (Allen and Fok 2000; Hartenstein and Martinez 2019).

During phagocytosis, the cell's plasma membrane is directed by cytoskeletal filaments to form pseudopodia, enabling the phagocyte to engulf the particle from the extracellular matrix. Once engulfed, the particle remains compartmentalized in an intracellular vesicle known as a phagosome. The phagosome is translocated to the perinuclear region with an abundance of lysosomes along the microtubule network, facilitated mainly by dynein motors and related effectors/adaptors. Along this journey, the phagosome continuously acidifies the internal environment to provide a suitable environment for hydrolase activity by multiple transient fusion events with endosomal membranes (Desjardins et al. 1994; Keller et al. 2017). As the phagosome approaches the lysosomes, the engulfed antigen is eventually enzymatically degraded within membrane-bound vesicles of the endolysosomal system (i.e., intracellular digestion) (Nguyen and Yates 2021; Rosales 2020).

Clathrin/caveolin/lipid raft-mediated endocytosis is the process by which cells take up smaller particles ranging from about 50 to 200 nm, including polyomavirus simian virus 40 (SV-40), human immunodeficiency virus-I (HIV-1), vesicular stomatitis virus, influenza A (IAV) and filamentous phage (Kiss and Botos 2009; Kotova et al. 2020). Although these processes are all carried out by ligands on the internalized cargo and receptors on the cell membrane, they differ in various aspects (Kazmierczak et al. 2020).

Clathrin-mediated endocytosis (CME) is a well-studied mechanism of endocytosis that relies on the protein clathrin

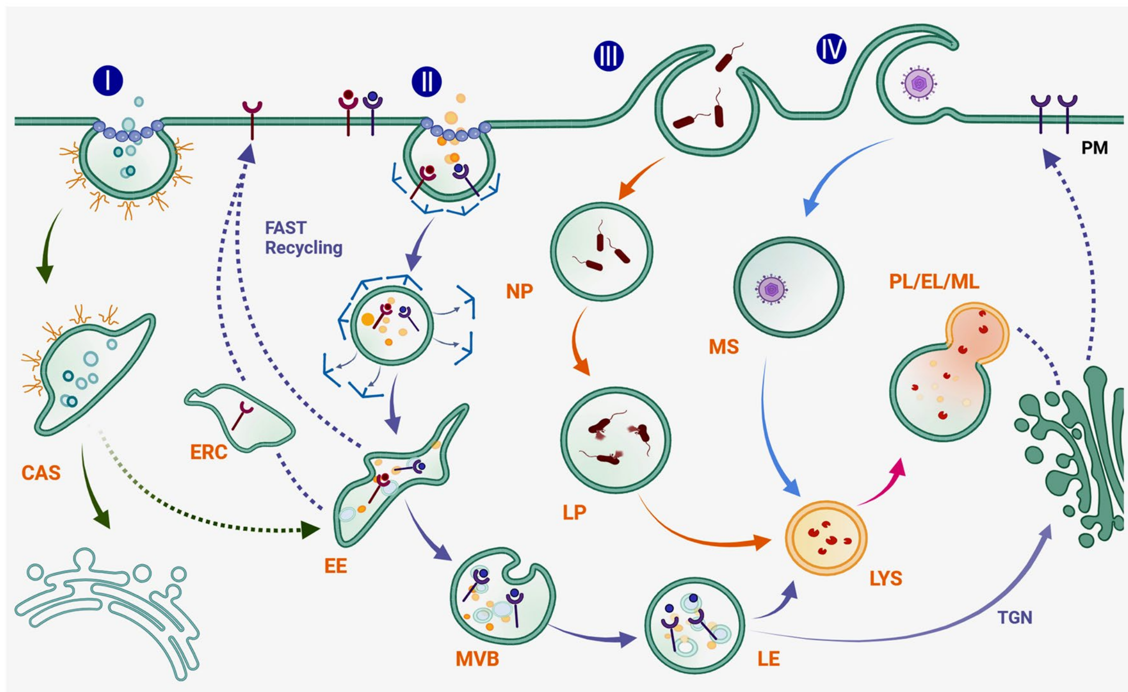

Fig. 1 Examples of cellular endocytosis processes. **I–IV** illustrate caveolin-mediated endocytosis, clathrin-mediated endocytosis, phagocytosis and macropinocytosis, respectively. *CAS* caveosome, *EE* early endosome, *ERC* endosomal recycling compartment, *LE* late endosome, *MVB* multivesicular body, *LYS* lysosomes, *NP* nascent phagosome, *LP* late phagosome, *MS* macropinosome, *PL/EL/ML* phagolysosome, endo-lysosome, and macropinosome fusion with lysosome, *PM* plasma membrane, *TGN* the trans Golgi network

(Fig. 1II). Clathrin forms a lattice-like structure that coats the plasma membrane, facilitating the formation of clathrin-coated pits. These pits invaginate and pinch off, forming clathrin-coated vesicles that transport cargo into the cell. Adaptor proteins, such as AP2, help recruit cargo molecules to the clathrin lattice. Additionally, various accessory proteins and regulatory factors participate in modulating CME dynamics and cargo selection (Mettlen et al. 2018).

Caveolin-mediated endocytosis (CavME) involves the protein caveolin, which forms flask-shaped invaginations called caveolae on the plasma membrane (Fig. 1I) (Parton and del Pozo 2013). Caveolae function as specialized lipid rafts enriched in cholesterol and sphingolipids. Caveolin interacts with signaling molecules, receptors, and lipid components to mediate endocytosis. Recently, studies have identified novel roles for caveolin in cellular processes beyond endocytosis, including lipid metabolism, mechanotransduction, and autophagy (Han et al. 2020; Hou et al. 2021; Strippoli et al. 2020). The interplay between caveolin and other endocytic pathways, such as CME and macropinocytosis, has been investigated to uncover their cooperative or competitive relationships (Parton et al. 2018).

Lipid raft-mediated endocytosis involves the selective partitioning of specific cargo molecules into lipid rafts, which serve as platforms for endocytic internalization. With the exact molecular mechanisms underlying this process still being investigated, the role of specific lipids, such as cholesterol and sphingolipids, in cargo sorting and endocytic regulation has been preliminarily explored (Li et al. 2016b; van Meer and de Kroon 2011).

Intracellular transportation of exogenous RNA

Following cellular uptake through mechanistically distinct endocytic pathways, exogenous RNA molecules are enclosed within common early/sorting endosomes and fused into the typical endosomal network. The endosomal network is a dynamic and interlinked transportation system that determines whether cargoes will ultimately be delivered to lysosomes for degradation or for polarized transport between distinct membrane-bound compartments (Elkin et al. 2016). The precise pathways and factors involved in endosomal networks can vary depending on the type of RNA and delivery method (Tokarev et al. 2009).

Endosomal maturation first plays a role in determining the degradation of the internalized RNA molecules. Endosomes undergo maturation processes that involve fusion with other endosomes or with membrane-bound compartments, leading to the formation of early endosomes (EEs), late endosomes (LEs) and ultimately, lysosomes (Huotari and Helenius

2011) (Fig. 1II). The internalized RNA molecules are captured into endocytic vesicles which undergo multiple rounds of homotypic fusion to form EEs. Tubular and cisternal structures, as well as vacuolar compartments containing intraluminal vesicles (ILVs) that are about 50 nm in diameter (in mammals), have been seen in EEs. These vacuoles gradually mature, dissociate from EEs, and are transported by multivesicular bodies (MVBs) or endosomal carrier vesicles (ECVs) to the LEs (Bissig and Gruenberg 2013; Wattiaux et al. 2000). The lysosomes then target and fuse with LEs inside which there are over 60 kinds of hydrolases that initiate the degradation of the entrapped exogenous RNAs.

Within EEs, the initial sorting decisions are made and the fates of the internalized receptors are decided. Therefore, lots of receptors internalized into EEs are frequently recycled back to the cell surface, either directly from the EEs (the fast recycling pathway) or via the endosomal recycling compartment (ERCs) in a slow recycling pathway. Examples of such receptors that undergo endosomal recycling include receptor tyrosine kinases, e.g., the epidermal growth factor receptor (ErbBs) family members, insulin-like growth factor-1 receptor (IGF1R), fibroblast growth factor receptors (FGFRs), G protein-coupled receptors, and carrier proteins such as transferrin receptors and low-density lipoprotein receptors (O'Sullivan and Lindsay 2020). Other recycling cargoes such as CI-MPR or sortilin also undergo retrograde trafficking to the trans-Golgi network (TGN) for the next round of sorting (Capitani and Baldari 2021) (Fig. 1).

Research on the molecular mechanisms of endosomal maturation has made significant progress in recent years (Margiotta et al. 2020; Wandinger-Ness and Zerial 2014; Wang et al. 2022). Rab GTPases are key regulators of endosomal maturation. Different Rab proteins associate with specific stages of endosomes, facilitating the maturation process (Egami 2016). For example, Rab5 is predominantly associated with early endosomes, whereas Rab7 is involved in the transition to late endosomes (Singer-Kruger et al. 1994; Yasuda et al. 2016). The recruitment of specific Rab effectors and effector complexes, such as Rab5 effectors (e.g., EEA1) and Rab7 effectors (e.g., RILP), plays a critical role in endosomal dynamics and fusion events (Langemeyer et al. 2018). The endosomal sorting protein complexes required for transport (ESCRT) also participate in the sorting of cargo molecules within endosomes. ESCRT complexes, including ESCRT-0, -I, -II, and -III, recruit ubiquitinated cargo proteins and facilitate their sequestration into intraluminal vesicles within late endosomes. These intraluminal vesicles can be either targeted for degradation within lysosomes or released as exosomes. The ESCRT machinery also participates in membrane remodeling events required for endosomal maturation and vesicle budding (Sardana and Emr 2021). Other processes and compounds, such as phosphoinositide lipids, the actomyosin network, the microtubule network vacuolar ATPases, and calcium signals, have also been reported to play a role in the process of endosomal maturation (Al Soraj et al. 2012; Nguyen and Yates 2021).

Once released into the cytoplasm, exogenous RNA molecules can undergo active transport mediated by molecular motor proteins, such as kinesin or dynein, along the microtubule network (Gagnon et al. 2013). These motor proteins hydrolyze ATP to provide energy and facilitate the movement specific RNA molecules or RNA carrier complexes towards specific subcellular regions, including the nucleus, specific organelles, or sites of translation, guided by specific signal sequences or localization elements. Another category of proteins that play a crucial role in cytoplasmic transport are RNA-binding proteins, which recognize specific RNA sequence motifs or structural elements. These can act as adaptors, linking RNA cargos to motor proteins or microtubules, or function as regulators, modulating the transport efficiency or specificity of RNAs (Girardi et al. 2021).

Two transmembrane proteins, SID-1 and SID-2, play pivotal roles in facilitating the uptake and dissemination of double strand RNA (dsRNA) to induce systemic RNAi as first demonstrated in *C. elegans* (Hunter et al. 2006). SID-1 functions as a bidirectional channel that transports dsRNA across cell membranes, while SID-2 likely acts as a receptor, particularly on the luminal membrane, facilitating the uptake of dsRNA from the extracellular matrix. In humans, it appears that there are two homologs of SID-1, namely SIDT1 and SIDT2, but none for SID-2. Human SIDT1 aids in small interfering RNA (siRNA) uptake and enhances gene silencing effectiveness (Duxbury et al. 2005). Recently, it was also discovered that SIDT1 mediates dietary miRNA absorption in the mammalian stomach (Chen et al. 2021). SIDT2, which predominantly localizes to lysosomes and in part to endo-lysosomes, can transport extracellular dsRNA into the cytosol for innate immune recognition (Nguyen et al. 2017). SIDT2 also directly transports RNA into lysosomes for degradation in an unexpected ATP-dependent manner, suggesting it may not solely function as a channel (Aizawa et al. 2016).

Cellular defense strategy against exogenous RNA

RNAi-mediated immune reaction

Besides endo-lysosomal degradation, the intrinsic RNAi pathway also works as a sequence-specific RNA-mediated silencing system against invasive exogenous RNA (Fig. 2A). Wingard (1928) observed that only the initially infected tobacco leaves developed tobacco ringspot virus (TobRV) disease, while the other leaves were asymptomatic and resistant to secondary infection, which was the first evidence

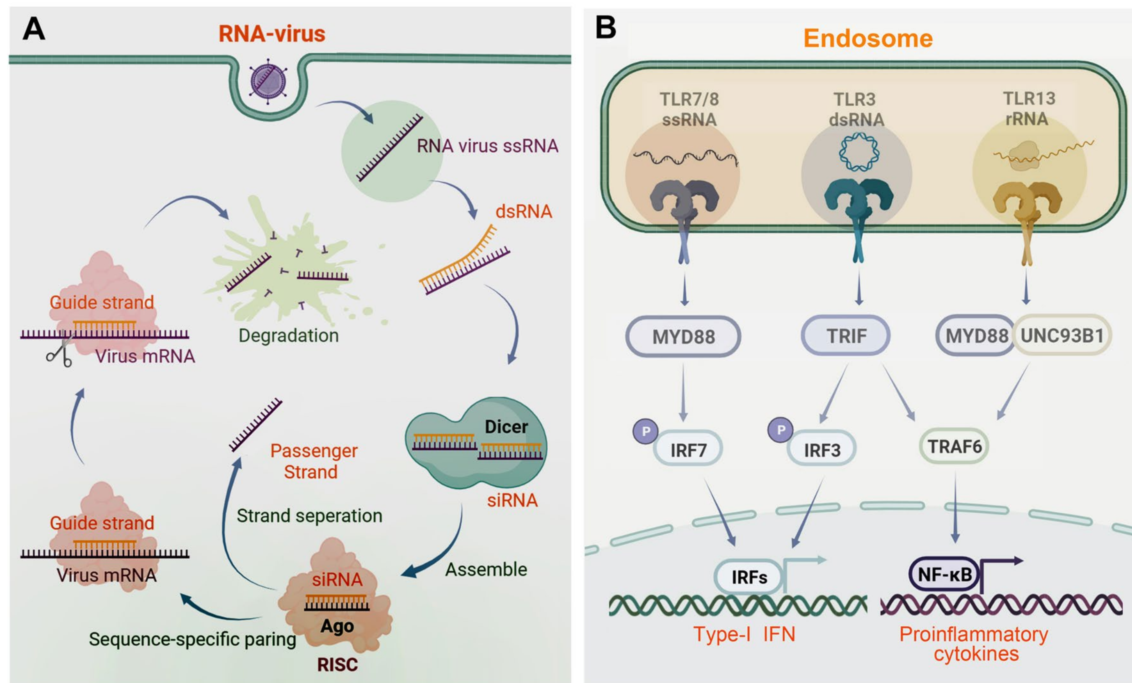

Fig. 2 Schematic representation of cellular defense systems. **A** Canonical RNAi pathway, Dicer recognizes the exogenous dsRNA and slices it into small siRNA, which is then delivered to the RISC combined with the Ago-family proteins. Finally, the anti-sense siRNA targets and degrades the homologous sequences via base-pairing. **B** Different Toll-like receptors (TLRs) that recognize the exogenous RNA within the endosome. Exogenous RNA triggers the downstream signaling pathway including the production of Type-I Interferons or cellular inflammatory responses. *IRFs* the interferon-regulatory factor family of transcription factors

for an antiviral role of RNAi in plants. Its antiviral effect was subsequently confirmed in other plants (Hamilton and Baulcombe 1999), invertebrates (Parameswaran et al. 2010) and mammals (Berkhout 2018). RNA/DNA-Virus and microorganism-derived dsRNA (by RNA-dependent-templated RNA polymerization or converging bidirectional transcription) are identified by Dicer endonucleases of the host and sliced into ~ 25 (in plants) or ~ 21–23 (in most animals) nucleotides, which are then loaded into the RNA-induced silencing complex (RISC) along with Argonaute family proteins (e.g., Ago2 in *Drosophila melanogaster*, murines and humans, RDE-1 in *C. elegans*) (Chen and Hur 2022; Hutvagner and Simard 2008; Zhao and Guo 2022). The sense or "passenger" strand RNA is released into the cytoplasm and degraded, while the anti-sense or "guide" strand is retained, matures in the RISC, and eventually targets the exogenous RNA, which is the genome of the invasive RNA virus (Schuster et al. 2019).

A growing number of studies have reported that a variety of pathogenic viruses, including IAV (Li et al. 2016a), Zika virus (Xu et al. 2019) and Enterovirus A71 (Qiu et al. 2017), induce siRNA production depending on the RNAi antiviral pathway in mammals. In a recent study, laboratory mice were infected with a viral suppressor of RNAi-deficient Nodamura virus following which a large amount of stable virus-derived siRNA was detected in muscle tissue and exosomes (Zhang et al. 2022). In subsequent protection experiments, it was shown that the virus-derived siRNA carried by the mice exosomes had the function of cleaving homologous viral RNAs (Zhang et al. 2022). In addition, Zhang et al. (2022) showed that targeted delivery of antiviral siRNA via engineered extracellular vesicles effectively alleviates microcephaly in mice caused by Zika viral infection, further demonstrating that RNAi can promote systemic immune responses to defend against viral invasion.

It is widely recognized that in nature, the potent type I-interferon (IFN-I) response is the main innate antiviral pathway in mammals, whereas RNAi seems to be an active antiviral system in pluripotent cells (e.g., undifferentiated stem cells) in which the IFN system is inactive (Petitjean et al. 2018; Schuster et al. 2019). Evidence shows that in mouse embryonic fibroblasts (MEFs) deficient in the signaling molecule mitochondrial antiviral signaling protein (MAVS) or interferon receptor (IFNAR1), though defective in sensing non-self RNA and responding to IFN, long dsRNA can nevertheless induce sequence-specific gene silencing in a RNAi-dependent manner (Maillard et al. 2016). In another study, depletion of IFN-I in mice led to small-RNA mediated silencing that triggered effective suppression of IAV infection, resulting in a 5-log attenuation

in depleted strains (Benitez et al. 2015). These data support the assertion that RNAi can be used as an effective defensive system in mammals in the absence of an IFN response.

Innate immune reactions

The innate immune system is generally considered to be sequence non-specific and able to identify exogenous RNAs via pattern-recognition receptors (PRRs) that can recognize both conservative pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) from a wide range of pathogens. PRRs produce inflammatory cytokines or interferon to mediate downstream cellular responses, such as programmed cell death (Carty et al. 2021; Takeuchi and Akira 2010). PRRs, currently known to be activated by RNA virus invasion, are strategically located either intracellularly, on the membrane, or in secretions. All these localizations have been recognized as crucial for early viral detection and immune response (Jensen and Thomsen 2012).

A subset of toll-like receptors (TLRs), which are a typical class of PRRs, play a critical role in the early recognition of nucleic acids both in host cells and in exogenous invaders (Lind et al. 2022). TLRs are type I transmembrane proteins characterized by an ectodomain with leucine-rich repeats, a transmembrane region, and a cytoplasmic tail that contains a conserved region called the Toll/IL-1 receptor (TIR) domain (Fig. 2B) (Asami and Shimizu 2021). The function of TLRs in defense usually depends on the MyD88 pathway, resulting in the production of inflammatory cytokines, and a MyD88-independent pathway associated with the stimulation of IFN-β (Kawai and Akira 2011). Four widely known mammalian TLRs are thought to be associated with recognition of foreign RNA, and four are normally located on endosomal membranes. Studies on the evolution of TLRs have revealed species-specific variations in TLR types among 11 mammalian species (Bagheri and Zahmatkesh 2018; Zhang et al. 2016). Notably, while TLR3 exhibits a more widespread presence across all 11 species, *Oryctolagus cuniculus* (rabbit) lacks TLR7 and TLR8, and only *Mus musculus* (mouse), *Rattus norvegicus* (rat), and *Tupaia belangeri chinensis* (Chinese tree shrews) possess TLR13. TLR3 recognizes double-stranded RNA, TLR7 and TLR8 can recognize extremely short fragments of RNAs, even single nucleotides in some cases, and TLR13 specializes in the recognition of fragments of single-stranded RNA (Reniewicz et al. 2016; Shibata et al. 2016). This specificity not only enables broad recognition by virtue of the ubiquity of nucleic acids but also introduces the possibility of an overactive immune response or even an autoinflammatory reaction (Lind et al. 2022). Therefore, an additional balancing mechanism that involves exporting excessive RNAs from endosomes to the cytosol is utilized to regulate the concentration of endosomal RNAs. A typical example is SIDT1 and SIDT2, which are present in the endosome of mice and facilitate the export dsRNA from endosomes to reduce the TLR3 response (Nguyen et al. 2017).

Another class of PRRs is RIG-I like receptors (RLRs) that belong to RNA helicases, which can recognize and bind the nonself signature of viral RNAs (Hur 2019). RLRs usually have three components, namely retinoic acid inducible gene I (RIG-I), melanoma differentiation associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2) (Fig. 3) (Rehwinkel and Gack 2020). RIG-I and MDA5 can recognize the dsRNA by their typical helicase domains and the C-terminal domain (Wu and Hur 2015). RIG-I primely recognizes the short dsRNA with its 5’ triphosphate (5’ppp) or 5’ diphosphate (5’pp) (Chen et al. 2017), while MDA5 can bind to long dsRNA (longer than 2000 nt) (Kato et al. 2008).

In addition to the typical PRRs, some other interferon-inducible RNA sensors also contribute to the innate immune reactions. Protein kinase R (PKR) is a dsRNA-dependent protein kinase consisting of two tandem repeats of dsRNA-binding domains (dsRBDs) and a kinase domain. Once dsRBD binds to one face of dsRNA through phosphate and ribose backbones of two adjacent minor grooves, it converts the PKR to an active state. This leads to the global shutdown of protein synthesis via phosphorylates eIF2α, an essential cap-dependent translation initiation factor, and the inhibition of cellular activity and viral replication (Fig. 3) (Pfaller et al. 2011). Additionally, the OASes family of enzymes can synthesize 2’-5’-phosphodiester-linked oligoadenylates by binding to dsRNA, leading to the cleavage of exogenous dsRNA (Fig. 3) (Nogimori et al. 2019).

Some helicases in superfamily-2 are also known to be exogenous RNA sensors and induce antiviral responses and inflammatory signaling pathways. For example, DDX60 is thought to play a role in the defense against exogenous RNA either by assisting RLRs or by degrading them directly. DHX15, DHX33 and the complex of DDX1-DDX21-DHX36 were also reported to contribute to innate immune reactions mediated by exogenous RNA (Leitao et al. 2015). In addition, recent studies in humans identified a novel nuclear RNA sensor, namely scaffold attachment factor A (SAFA), which can recognize dsRNA derived from virus replication. When SAFA senses dsRNA, it oligomerizes and induces IFN-β production by interacting with DNA topoisomerase I (TOPI) (Cao et al. 2019; Li et al. 2021).

Implication of improving RNA interference

Throughout the process of intracellular transport, exogenous RNA is subjected to dynamic changes in its microenvironment, including typical Rab GTPase conversions,

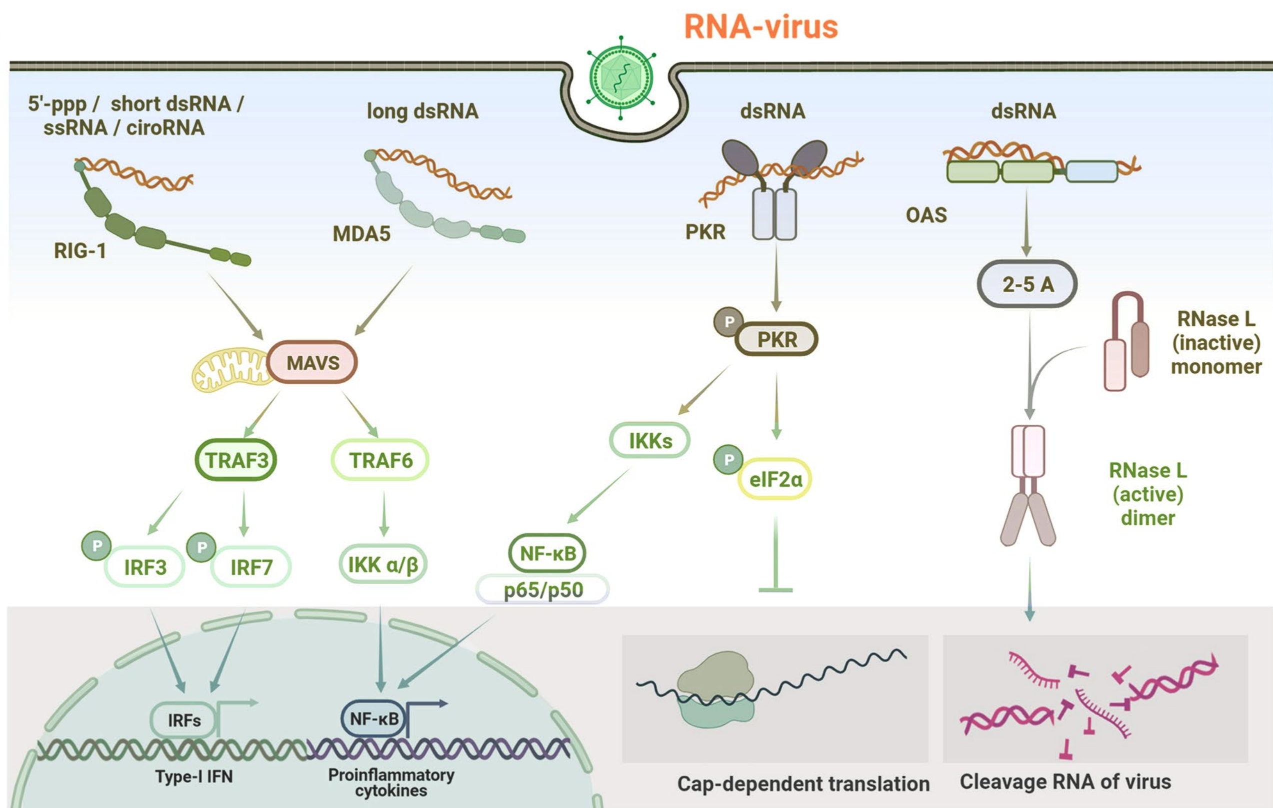

Fig. 3 Schematic representation of cellular defense systems, focusing on various exogenous RNA sensors in the cytoplasm. RIG-1 and MDA5 belong to the RIG-I-like receptors (RLRs) family. These two RLRs recognize most exogenous RNAs via their CTD and helicase domain, then activate the MAVS, which leads to the downstream production of the IFN-I or NF-κB pathway. Protein kinase R (PKR) is a dsRNA-dependent protein kinase that can facilitate the NF-κB pathway and also might inhibit the cap-dependent translation by phosphorylating eIF2α, an elongation factor of translation. OASes can bind the exogenous RNA and leading to the production of 2-5A, an activator of RNase L, which degrades the exogenous RNA

sorting into intraluminal vesicles, acidification, and ultimately, fusion with lysosomes for its degradation (Borchers et al. 2021; Sardana and Emr 2021). Therefore, how to effectively deliver and release functional exogenous RNA to the intended target has become the biggest obstacle for the application of RNA interference in basic research and therapeutics.

Many species can take up dsRNA from their food and achieve efficient RNAi throughout the body. Examples of feeding-induced RNAi have been reported in *C. elegans*, *Penaeus orientalis*, different ciliate species including *Paramecium tetraurelia*, *Blepharisma japonicum* and *Spirostomum minus*, and various insects (Carradec et al. 2015; Hunter et al. 2006; Itsathitphaisarn et al. 2017; Sobierajska et al. 2011; Zhang et al. 2023). Although this form of RNAi is efficient, its effectiveness can vary significantly even among closely related species, with multiple factors potentially contributing to these differences (Zhu and Palli 2020). Previous studies have highlighted the crucial role of the dsRNA transport-related proteins SID-1 and SID-2 in regulating the efficiency of RNAi in *C. elegans*, *Penaeus orientalis*, and *D. melanogaster* (Feinberg and Hunter 2003; Labreuche et al. 2010; Shih and Hunter 2011). In addition, factors such as dsRNA stability, endosome capture, core RNAi machinery expression level, and RNAi dissemination efficiency have substantial impacts on the effectiveness of RNAi in different organisms (Alshaer et al. 2021; Zhu and Palli 2020). Therefore, further research is needed to elucidate this phenomenon and advance RNAi application.

Previous research on improving the efficiency of RNAi has largely focused on modifying RNA delivery systems to facilitate endosomal escape. This includes the possibility of using macropinocytosis as a means to deliver RNAi molecules, such as siRNA or short hairpin RNA, into cells to silence specific genes (Cavalli et al. 2017; Paunovska et al. 2022). One approach to enhance the uptake of RNAi molecules via macropinocytosis is to engineer RNAi therapeutics with macropinocytosis-inducing agents, such as growth factors or peptides. This method has been shown to improve cellular uptake and RNAi-mediated gene silencing efficiency, thereby enhancing the therapeutic potential of RNAi (Wang et al. 2010). Other studies have focused on

modulating signaling pathways associated with macropinocytosis to promote its efficiency. Manipulating key signaling molecules, such as Ras, Rac or PKC, can influence macropinocytic activity (Sahay et al. 2010). It has been shown that increased cellular uptake of RNAi molecules, and improved gene silencing effects, can be achieved by utilizing small molecules or genetic approaches to enhance macropinocytosis-inducing signaling pathways (Hu et al. 2022; Sahay et al. 2008; Xiao et al. 2021).

Recent research has also highlighted the potential of modifying the cytoplasmic transport process to enhance RNAi efficiency, mainly by developing improved RNA carrier systems or by manipulating RNA molecules or regulatory factors (Vocelle et al. 2020). The carrier systems often utilize lipid-based nanoparticles, polymers, or non-viral vectors to protect and efficiently deliver RNAi molecules to target cells. Modifications of these carriers, such as adjusting particle size, materials (e.g., lipid, polymeric and inorganic), chemical modifications, surface functionalization, or the incorporation of targeting ligands, can improve delivery to desired cell types or subcellular compartments (Zylberberg et al. 2017). Regulatory factors have also been targeted to enhance RNAi efficiency by facilitating the cytoplasmic localization and stability of RNAi molecules (Mettlen et al. 2018).

Another proposed mechanism involves the pH-sensitive disruption of endosomal membranes by exogenous RNA carriers. pH-responsive components, such as protonatable amino groups or acid-labile linkages, undergo conformational changes or protonation, leading to membrane destabilization and the subsequent release of the RNA payload. For example, pH-sensitive peptides, such as the histidine-rich fusogenic peptides, can undergo conformational changes in response to low pH, promoting membrane fusion and endosomal escape. Certain membrane-disrupting agents including cationic detergents (e.g., Triton X-100), pore-forming peptides (e.g., melittin), or lysosomotropic agents (e.g., chloroquine) are also thought to facilitate endosomal escape of exogenous RNA (Regen 2020). These agents can destabilize the endosomal membrane leading to leakage or rupture and the subsequent release of the encapsulated RNA cargo. Some viral proteins, such as influenza virus hemagglutinin protein, also possess membrane-disrupting properties and thus have been utilized for membrane fusion and RNA release (Benton et al. 2020; Blijleven et al. 2016). In addition, calcium-dependent fusogenic liposomes or calcium-responsive polymers can undergo structural changes in response to elevated calcium levels within endosomes, which can trigger membrane fusion events resulting in endosomal escape of RNA molecules (Gu et al. 2020). However, a large degree of endosome capture is still unavoidable. Currently, most gene therapies focus on the combination of small molecule drugs and gene carriers, which has shown good efficacy. The majority of the small-molecule drugs used in recent research are cationic amphiphilic drugs (CADs), which are relatively lipophilic in their unprotonated form, allowing them to penetrate lipid membranes with a distinct pharmacological activity. However, due to their amphiphilic and weak basic characteristics, they tend to accumulate in substantial proportions in acidic compartments (Kornhuber et al. 2011). The accumulated CADs are subsequently protonated in acid lysosomes, thereby restricting diffusion back into the cytoplasm and inducing them to insert hydrophobic fragments into the lipid membrane, releasing the membrane-attached acid sphingomyelinase (ASM). Eventually, the decrease of ASM-mediated sphingomyelin hydrolysis leads to the accumulation of sphingomyelin and cholesterol, inducing lysosomal swelling and instant membrane permeabilization (Joris et al. 2018; Petersen et al. 2013).

Studies have demonstrated that co-administration of chloroquine and cholesterol-conjugated siRNA could increase the efficiency of siRNA knockdown and diminish the effective siRNA concentration both in cultured cells and tumor spheroids (e.g., Du Rietz et al. 2020). In a few situations, siramesine can improve knock-down efficiency but is not as effective as chloroquine owing to differences in compartments targeting small molecules (Chernikov et al. 2019). In addition, various adjuvants can also be used to enhance the endo-lysosome escape of nucleotides with noncarriers. These include: carvedilol, a β-blocker; ketotifen, an asthma medication; loperamide, an anti-peristaltic agent; nortriptyline, an antidepressant; and desloratadine, which can form pores in the lysosome membrane with a diameter of 10–40 nm and release dextran with a maximum molecular weight of 150 KDa (Joris et al. 2018; Shaabani et al. 2021). Furthermore, up to 96 CADs have been shown to boost endosomal escape, which can raise the efficacy of gene knockdown by 3–6 times in various cancer cells. Hit-rates of up to 13.7% in the screening of CADs have shown that their effectiveness depends on their physical and chemical properties rather than intermolecular interactions (Van de Vyver et al. 2020). In general, CADs have preferences for various customized nanocarriers, cargos, cell types, and even cell compartments, all of which should therefore be considered prior to their application. Furthermore, a previous study demonstrated that combining two types of CADs and administering them in a phased manner can result in an additive effect of endosome-lysosome escape (Joris et al. 2018).

Concluding remarks

RNA interference is an adaptable and versatile genetic manipulation technique that can be used in reverse genetic studies, specific gene repression, and in targeted therapies. However, its efficiency in several species remains

problematic due to factors such as dsRNA instability, incomplete dsRNA internalization, defects in the core RNAi machinery, limited systemic dissemination within the body, and multiple functions of various categories of RNAs in different organisms (Cooper et al. 2019). dsRNA instability is attributed to RNases and variations in intracellular pH levels. Incomplete dsRNA internalization is also a complex issue that may result from insufficient endosomal escape, RNAi repressors, or possibly different internalization mechanisms. In addition, the composition, expression, regulation, and function of the core RNAi machinery genes vary considerably among different species, having significant effects on RNAi efficiency.

Despite recent successes in modifying the RNAi delivery system and enhancing endosomal escape in laboratory studies, targeted delivery to specific cells/tissues and the systemic expansion of RNAi still poses significant challenges in the context of therapy. It is crucial to avoid both renal and reticuloendothelial clearance, while simultaneously enhancing extravasation (Traber and Yu 2023). Consequently, further development of metabolically stable RNAi triggering factors and coupling ligands is necessary to ensure efficient RNAi therapy. Additionally, careful consideration should be given to the safety of RNAi delivery carriers and the long-term impact of RNAi on biological effects. Some of these issues may benefit from understanding synergistically with complex biological pathways such as those responsible for intracellular cargo sorting and trafficking following endocytosis.

In this review, we summarized the recent findings reported in the accumulating literature covering the complete cellular response to exogenous RNA, including cellular entry, intracellular transportation, RNA-mediated silencing, and immune-inflammatory reactions. Based on these new discoveries, we propose potential strategies that may enhance the efficiency of RNA interference. Future studies should focus on a systematic understanding of the routes and fates of different forms of exogenous RNA, which will shed light on more efficient RNA delivery approaches for genetic manipulation and therapeutic applications in humans and other organisms.

Acknowledgements This work was supported by funding from: the National Natural Science Foundation of China to YY (Project Number: 32270558), to XC (Project Number: 32270512) and to CW (Project number: 32100382); Young Taishan Scholar Program of Shandong Province to YY; Natural Science Foundation of Jiangsu Province (Project Number: BK20220268) and the Program of Qilu Young Scholars of Shandong University to XC; and the King Saud University, Saudi Arabia (Project number: RSP2023R7).

Author contributions DT: writing the original draft, reviewing and editing the manuscript. YL, CW, and LL: editing the manuscript. SA: language polishing. YY and XC: conceptualization, supervision, writing, reviewing and editing the manuscript. All authors read and approve the final version of the manuscript.

Data availability The data used to support the views of this review are included within the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Animal and human rights statement We declare that all applicable international, national, and/or institutional guidelines for experimental use of organisms for the study have been followed and all necessary approvals have been obtained (not applicable for review articles).

References

Aizawa S, Fujiwara Y, Contu VR, Hase K, Takahashi M, Kikuchi H, Kabuta C, Wada K, Kabuta T (2016) Lysosomal putative RNA transporter SIDT2 mediates direct uptake of RNA by lysosomes. Autophagy 12:565–578

Al Soraj M, He L, Peynshaert K, Cousaert J, Vercauteren D, Braeckmans K, De Smedt SC, Jones AT (2012) siRNA and pharmacological inhibition of endocytic pathways to characterize the differential role of macropinocytosis and the actin cytoskeleton on cellular uptake of dextran and cationic cell penetrating peptide octaarginine (R8) and HIV-Tat. J Control Release 161:132–141

Alcazer V, Bonaventura P, Depil S (2020) Human endogenous retroviruses (HERVs): shaping the innate immune response in cancers. Cancers 12:610

Allen RD, Fok AK (2000) Membrane trafficking and processing in *Paramecium*. Int Rev Cytol 198:277–318

Alshaer W, Zureigat H, Al Karaki A, Al-Kadash A, Gharaibeh L, Hatmal MM, Aljabali AAA, Awidi A (2021) siRNA: mechanism of action, challenges, and therapeutic approaches. Eur J Pharmacol 905:174178

Asami J, Shimizu T (2021) Structural and functional understanding of the toll-like receptors. Protein Sci 30:761–772

Bagheri M, Zahmatkesh A (2018) Evolution and species-specific conservation of toll-like receptors in terrestrial vertebrates. Int Rev Immunol 37:217–228

Benitez AA, Spanko LA, Bouhaddou M, Sachs D, tenOever BR (2015) Engineered mammalian RNAi can elicit antiviral protection that negates the requirement for the interferon response. Cell Rep 13:1456–1466

Benton DJ, Gamblin SJ, Rosenthal PB, Skehel JJ (2020) Structural transitions in influenza haemagglutinin at membrane fusion pH. Nature 583:150–153

Berkhout B (2018) RNAi-mediated antiviral immunity in mammals. Curr Opin Virol 32:9–14

Bissig C, Gruenberg J (2013) Lipid sorting and multivesicular endosome biogenesis. Cold Spring Harb Perspect Biol 5:016816

Blijleven JS, Boonstra S, Onck PR, van der Giessen E, van Oijen AM (2016) Mechanisms of influenza viral membrane fusion. Semin Cell Dev Biol 60:78–88

Borchers AC, Langemeyer L, Ungermann C (2021) Who's in control? Principles of Rab GTPase activation in endolysosomal membrane trafficking and beyond. J Cell Biol 220:e202105120

Buckley CM, King JS (2017) Drinking problems: mechanisms of macropinosome formation and maturation. FEBS J 284:3778–3790

Cao L, Liu S, Li Y, Yang G, Luo Y, Li S, Du H, Zhao Y, Wang D, Chen J, Zhang Z, Li M, Ouyang S, Gao X, Sun Y, Wang Z, Yang L, Lin R, Wang P, You F (2019) The nuclear matrix

protein SAFA surveils viral RNA and facilitates immunity by activating antiviral enhancers and super-enhancers. Cell Host Microbe 26:369–384
Capitani N, Baldari CT (2021) F-Actin dynamics in the regulation of endosomal recycling and immune synapse assembly. Front Cell Dev Biol 9:670882
Carradec Q, Gotz U, Arnaiz O, Pouch J, Simon M, Meyer E, Marker S (2015) Primary and secondary siRNA synthesis triggered by RNAs from food bacteria in the ciliate *Paramecium tetraurelia*. Nucleic Acids Res 43:1818–1833
Carty M, Guy C, Bowie AG (2021) Detection of viral infections by innate immunity. Biochem Pharmacol 183:114316
Cavalli R, Primo L, Sessa R, Chiaverina G, di Blasio L, Alongi J, Manfredi A, Ranucci E, Ferruti P (2017) The AGMA1 polyamidoamine mediates the efficient delivery of siRNA. J Drug Target 25:891–898
Chen YG, Hur S (2022) Cellular origins of dsRNA, their recognition and consequences. Nat Rev Mol Cell Biol 23:286–301
Chen YG, Kim MV, Chen X, Batista PJ, Aoyama S, Wilusz JE, Iwasaki A, Chang HY (2017) Sensing self and foreign circular RNAs by intron identity. Mol Cell 67:228–238
Chen Q, Zhang F, Dong L, Wu H, Xu J, Li H, Wang J, Zhou Z, Liu C, Wang Y, Liu Y, Lu L, Wang C, Liu M, Chen X, Wang C, Zhang C, Li D, Zen K, Wang F et al (2021) SIDT1-dependent absorption in the stomach mediates host uptake of dietary and orally administered microRNAs. Cell Res 31:247–258
Chernikov IV, Vlassov VV, Chernolovskaya EL (2019) Current development of siRNA bioconjugates: from research to the clinic. Front Pharmacol 10:444
Cooper AM, Silver K, Zhang J, Park Y, Zhu KY (2019) Molecular mechanisms influencing efficiency of RNA interference in insects. Pest Manag Sci 75:18–28
Del Pozo-Acebo L, de Las L, Hazas MC, Margolles A, Davalos A, Garcia-Ruiz A (2021) Eating microRNAs: pharmacological opportunities for cross-kingdom regulation and implications in host gene and gut microbiota modulation. Br J Pharmacol 178:2218–2245
Desjardins M, Huber LA, Parton RG, Griffiths G (1994) Biogenesis of phagolysosomes proceeds through a sequential series of interactions with the endocytic apparatus. J Cell Biol 124:677–688
Du Rietz H, Hedlund H, Wilhelmson S, Nordenfelt P, Wittrup A (2020) Imaging small molecule-induced endosomal escape of siRNA. Nat Commun 11:1809
Duxbury MS, Ashley SW, Whang EE (2005) RNA interference: a mammalian SID-1 homologue enhances siRNA uptake and gene silencing efficacy in human cells. Biochem Biophys Res Commun 331:459–463
Egami Y (2016) Molecular imaging analysis of Rab GTPases in the regulation of phagocytosis and macropinocytosis. Anat Sci Int 91:35–42
Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T (2001) Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411:494–498
Elkin SR, Lakoduk AM, Schmid SL (2016) Endocytic pathways and endosomal trafficking: a primer. Wien Med Wochenschr 166:196–204
Emamalipour M, Seidi K, ZununiVahed S, Jahanban-Esfahlan A, Jaymand M, Majdi H, Amoozgar Z, Chitkushev LT, Javaheri T, Jahanban-Esfahlan R, Zare P (2020) Horizontal gene transfer: from evolutionary flexibility to disease progression. Front Cell Dev Biol 8:229
Feinberg EH, Hunter CP (2003) Transport of dsRNA into cells by the transmembrane protein SID-1. Science 301:1545–1547
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
Gagnon JA, Kreiling JA, Powrie EA, Wood TR, Mowry KL (2013) Directional transport is mediated by a dynein-dependent step in an RNA localization pathway. PLoS Biol 11:e1001551
Gaucherand L, Gaglia MM (2022) The role of viral RNA degrading factors in shutoff of host gene expression. Annu Rev Virol 9:213–238
Germain ND, Chung WK, Sarmiere PD (2023) RNA interference (RNAi)-based therapeutics for treatment of rare neurologic diseases. Mol Aspects Med 91:101148
Girardi E, Pfeffer S, Baumert TF, Majzoub K (2021) Roadblocks and fast tracks: how RNA binding proteins affect the viral RNA journey in the cell. Semin Cell Dev Biol 111:86–100
Gu Z, Da Silva CG, Van der Maaden K, Ossendorp F, Cruz LJ (2020) Liposome-based drug delivery systems in cancer immunotherapy. Pharmaceutics 12:1054
Guo S, Kemphues KJ (1995) par-1, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. Cell 81:611–620
Hamilton AJ, Baulcombe DC (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. Science 286:950–952
Han M, Pioronska W, Wang S, Nwosu ZC, Sticht C, Wang S, Gao Y, Ebert MP, Dooley S, Meyer C (2020) Hepatocyte caveolin-1 modulates metabolic gene profiles and functions in non-alcoholic fatty liver disease. Cell Death Dis 11:104
Hartenstein V, Martinez P (2019) Phagocytosis in cellular defense and nutrition: a food-centered approach to the evolution of macrophages. Cell Tissue Res 377:527–547
Hou K, Li S, Zhang M, Qin X (2021) Caveolin-1 in autophagy: a potential therapeutic target in atherosclerosis. Clin Chim Acta 513:25–33
Hu Y, Jiang K, Wang D, Yao S, Lu L, Wang H, Song J, Zhou J, Fan X, Wang Y, Lu W, Wang J, Wei G (2022) Core-shell lipoplexes inducing active macropinocytosis promote intranasal delivery of c-Myc siRNA for treatment of glioblastoma. Acta Biomater 138:478–490
Hunter CP, Winston WM, Molodowitch C, Feinberg EH, Shih J, Sutherlin M, Wright AJ, Fitzgerald MC (2006) Systemic RNAi in *Caenorhabditis elegans*. Cold Spring Harb Symp Quant Biol 71:95–100
Huotari J, Helenius A (2011) Endosome maturation. EMBO J 30:3481–3500
Hur S (2019) Double-stranded RNA sensors and modulators in innate immunity. Annu Rev Immunol 37:349–375
Hutvagner G, Simard MJ (2008) Argonaute proteins: key players in RNA silencing. Nat Rev Mol Cell Biol 9:22–32
Itsathitphaisarn O, Thitamadee S, Weerachatyanukul W, Sritunyalucksana K (2017) Potential of RNAi applications to control viral diseases of farmed shrimp. J Invertebr Pathol 147:76–85
Jensen S, Thomsen AR (2012) Sensing of RNA viruses: a review of innate immune receptors involved in recognizing RNA virus invasion. J Virol 86:2900–2910
Joris F, De Backer L, Van de Vyver T, Bastiancich C, De Smedt SC, Raemdonck K (2018) Repurposing cationic amphiphilic drugs as adjuvants to induce lysosomal siRNA escape in nanogel transfected cells. J Control Release 269:266–276
Kanasty R, Dorkin JR, Vegas A, Anderson D (2013) Delivery materials for siRNA therapeutics. Na Mater 12:967–977
Kato H, Takeuchi O, Mikamo-Satoh E, Hirai R, Kawai T, Matsushita K, Hiiragi A, Dermody TS, Fujita T, Akira S (2008) Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. J Exp Med 205:1601–1610
Kawai T, Akira S (2011) Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 34:637–650

Kazmierczak Z, Szostak-Paluch K, Przybylo M, Langner M, Witkiewicz W, Jedruchniewicz N, Dabrowska K (2020) Endocytosis in cellular uptake of drug delivery vectors: molecular aspects in drug development. Bioorg Med Chem 28:115556

Keller S, Berghoff K, Kress H (2017) Phagosomal transport depends strongly on phagosome size. Sci Rep 7:17068

Kiss AL, Botos E (2009) Endocytosis via caveolae: alternative pathway with distinct cellular compartments to avoid lysosomal degradation? J Cell Mol Med 13:1228–1237

Koonin EV, Dolja VV, Krupovic M (2021) The healthy human virome: from virus-host symbiosis to disease. Curr Opin Virol 47:86–94

Kornhuber J, Muehlbacher M, Trapp S, Pechmann S, Friedl A, Reichel M, Muhle C, Terfloth L, Groemer TW, Spitzer GM, Liedl KR, Gulbins E, Tripal P (2011) Identification of novel functional inhibitors of acid sphingomyelinase. PLoS ONE 6:e23852

Kotova A, Timonina K, Zoidl GR (2020) Endocytosis of connexin-36 is mediated by interaction with Caveolin-1. Int J Mol Sci 21:5401

Labreuche Y, Veloso A, de la Vega E, Gross PS, Chapman RW, Browdy CL, Warr GW (2010) Non-specific activation of antiviral immunity and induction of RNA interference may engage the same pathway in the Pacific white leg shrimp *Litopenaeus vannamei*. Dev Comp Immunol 34:1209–1218

Langemeyer L, Frohlich F, Ungermann C (2018) Rab GTPase function in endosome and lysosome biogenesis. Trends Cell Biol 28:957–970

Leitao AL, Costa MC, Enguita FJ (2015) Unzippers, resolvers and sensors: a structural and functional biochemistry tale of RNA helicases. Int J Mol Sci 16:2269–2293

Li Y, Basavappa M, Lu J, Dong S, Cronkite DA, Prior JT, Reinecker HC, Hertzog P, Han Y, Li WX, Cheloufi S, Karginov FV, Ding SW, Jeffrey KL (2016a) Induction and suppression of antiviral RNA interference by influenza A virus in mammalian cells. Nat Microbiol 2:16250

Li Y, Gao L, Tan X, Li F, Zhao M, Peng S (2016b) Lipid rafts-mediated endocytosis and physiology-based cell membrane traffic models of doxorubicin liposomes. Biochim Biophys Acta 1858:1801–1811

Li S, Cao L, Zhang Z, Kuang M, Chen L, Zhao Y, Luo Y, Yin Z, You F (2021) Cytosolic and nuclear recognition of virus and viral evasion. Mol Biomed 2:30

Lind NA, Rael VE, Pestal K, Liu B, Barton GM (2022) Regulation of the nucleic acid-sensing toll-like receptors. Nat Rev Immunol 22:224–235

Maillard PV, Van der Veen AG, Deddouche-Grass S, Rogers NC, Merits A, Reis e Sousa C (2016) Inactivation of the type I interferon pathway reveals long double-stranded RNA-mediated RNA interference in mammalian cells. EMBO J 35:2505–2518

Margiotta A, Frei DM, Sendstad IH, Janssen L, Neefjes J, Bakke O (2020) Invariant chain regulates endosomal fusion and maturation through an interaction with the SNARE Vti1b. J Cell Sci 133:244624

Mercer J, Schelhaas M, Helenius A (2010) Virus entry by endocytosis. Annu Rev Biochem 79:803–833

Mettlen M, Chen PH, Srinivasan S, Danuser G, Schmid SL (2018) Regulation of clathrin-mediated endocytosis. Annu Rev Biochem 87:871–896

Napoli C, Lemieux C, Jorgensen R (1990) Introduction of a chimeric chalcone synthase gene into *Petunia* results in reversible co-suppression of homologous genes in trans. Plant Cell 2:279–289

Nguyen JA, Yates RM (2021) Better together: current insights into phagosome-lysosome fusion. Front Immunol 12:636078

Nguyen TA, Smith BRC, Tate MD, Belz GT, Barrios MH, Elgass KD, Weisman AS, Baker PJ, Preston SP, Whitehead L, Garnham A, Lundie RJ, Smyth GK, Pellegrini M, O'Keeffe M, Wicks IP, Masters SL, Hunter CP, Pang KC (2017) SIDT2 transports extracellular dsRNA into the cytoplasm for innate immune recognition. Immunity 47:498–509

Nogimori T, Nishiura K, Kawashima S, Nagai T, Oishi Y, Hosoda N, Imataka H, Kitamura Y, Kitade Y, Hoshino SI (2019) Dom34 mediates targeting of exogenous RNA in the antiviral OAS/RNase L pathway. Nucleic Acids Res 47:432–449

O'Sullivan MJ, Lindsay AJ (2020) The endosomal recycling pathway at the crossroads of the cell. Int J Mol Sci 21:6074

Parameswaran P, Sklan E, Wilkins C, Burgon T, Samuel MA, Lu R, Ansel KM, Heissmeyer V, Einav S, Jackson W, Doukas T, Paranjape S, Polacek C, dos Santos FB, Jalili R, Babrzadeh F, Gharizadeh B, Grimm D, Kay M, Koike S et al (2010) Six RNA viruses and forty-one hosts: viral small RNAs and modulation of small RNA repertoires in vertebrate and invertebrate systems. PLoS Pathog 6:e1000764

Parton RG, del Pozo MA (2013) Caveolae as plasma membrane sensors, protectors and organizers. Nat Rev Mol Cell Biol 14:98–112

Parton RG, Tillu VA, Collins BM (2018) Caveolae. Curr Biol 28:R402–R405

Paunovska K, Loughrey D, Dahlman JE (2022) Drug delivery systems for RNA therapeutics. Nat Rev Genet 23:265–280

Petersen NH, Olsen OD, Groth-Pedersen L, Ellegaard AM, Bilgin M, Redmer S, Ostenfeld MS, Ulanet D, Dovmark TH, Lonborg A, Vindelov SD, Hanahan D, Arenz C, Ejsing CS, Kirkegaard T, Rohde M, Nylandsted J, Jaattela M (2013) Transformation-associated changes in sphingolipid metabolism sensitize cells to lysosomal cell death induced by inhibitors of acid sphingomyelinase. Cancer Cell 24:379–393

Petitjean O, Montavon T, Pfeffer S (2018) To have and have not, RNA interference as an antiviral defense system in mammals. Virologie 22:251–260

Pfaller CK, Li Z, George CX, Samuel CE (2011) Protein kinase PKR and RNA adenosine deaminase ADAR1: new roles for old players as modulators of the interferon response. Curr Opin Immunol 23:573–582

Qiu Y, Xu Y, Zhang Y, Zhou H, Deng YQ, Li XF, Miao M, Zhang Q, Zhong B, Hu Y, Zhang FC, Wu L, Qin CF, Zhou X (2017) Human virus-derived small RNAs can confer antiviral immunity in mammals. Immunity 46:992–1004

Regen SL (2020) Membrane-disrupting molecules as therapeutic agents: a cautionary note. JACS Au 1:3–7

Rehwinkel J, Gack MU (2020) RIG-I-like receptors: their regulation and roles in RNA sensing. Nat Rev Immunol 20:537–551

Renard HF, Boucrot E (2021) Unconventional endocytic mechanisms. Curr Opin Cell Biol 71:120–129

Reniewicz P, Zyzak J, Siednienko J (2016) The cellular receptors of exogenous RNA. Postepy Hig Med Dosw 70:337–348

Romano N, Macino G (1992) Quelling: transient inactivation of gene expression in *Neurospora crassa* by transformation with homologous sequences. Mol Microbiol 6:3343–3353

Rosales C (2020) Neutrophils at the crossroads of innate and adaptive immunity. J Leukoc Biol 108:377–396

Ruotsalainen AK, Makinen P, Yla-Herttuala S (2021) Novel RNAi-based therapies for atherosclerosis. Curr Atheroscler Rep 23:45

Sahay G, Batrakova EV, Kabanov AV (2008) Different internalization pathways of polymeric micelles and unimers and their effects on vesicular transport. Bioconjug Chem 19:2023–2029

Sahay G, Alakhova DY, Kabanov AV (2010) Endocytosis of nanomedicines. J Control Release 145:182–195

Sardana R, Emr SD (2021) Membrane protein quality control mechanisms in the endo-lysosome system. Trends Cell Biol 31:269–283

Schuster S, Miesen P, van Rij RP (2019) Antiviral RNAi in insects and mammals: parallels and differences. Viruses 11:448

Scott CC, Vacca F, Gruenberg J (2014) Endosome maturation, transport and functions. Semin Cell Dev Biol 31:2–10

Shaabani E, Sharifiaghdam M, Lammens J, De Keersmaecker H, Vervaet C, De Beer T, Motevaseli E, Ghahremani MH, Mansouri P, De Smedt S, Raemdonck K, Faridi-Majidi R, Braeckmans K, Fraire JC (2021) Increasing angiogenesis factors in hypoxic diabetic wound conditions by siRNA delivery: additive effect of LbL-gold nanocarriers and desloratadine-induced lysosomal escape. Int J Mol Sci 22:9216

Shibata T, Ohto U, Nomura S, Kibata K, Motoi Y, Zhang Y, Murakami Y, Fukui R, Ishimoto T, Sano S, Ito T, Shimizu T, Miyake K (2016) Guanosine and its modified derivatives are endogenous ligands for TLR7. Int Immunol 28:211–222

Shih JD, Hunter CP (2011) SID-1 is a dsRNA-selective dsRNA-gated channel. RNA 17:1057–1065

Singer-Kruger B, Stenmark H, Dusterhoft A, Philippsen P, Yoo JS, Gallwitz D, Zerial M (1994) Role of three rab5-like GTPases, Ypt51p, Ypt52p, and Ypt53p, in the endocytic and vacuolar protein sorting pathways of yeast. J Cell Biol 125:283–298

Sobierajska K, Joachimiak E, Bregier C, Fabczak S, Fabczak H (2011) Effect of phosducin silencing on the photokinetic motile response of *Blepharisma japonicum*. Photochem Photobiol Sci 10:19–24

Srinivasachar Badarinarayan S, Sauter D (2021) Switching sides: how endogenous retroviruses protect us from viral infections. J Virol 95:e02299-20

Stow JL, Hung Y, Wall AA (2020) Macropinocytosis: insights from immunology and cancer. Curr Opin Cell Biol 65:131–140

Strippoli R, Sandoval P, Moreno-Vicente R, Rossi L, Battistelli C, Terri M, Pascual-Anton L, Loureiro M, Matteini F, Calvo E, Jimenez-Heffernan JA, Gomez MJ, Jimenez-Jimenez V, Sanchez-Cabo F, Vazquez J, Tripodi M, Lopez-Cabrera M, Del Pozo MA (2020) Caveolin1 and YAP drive mechanically induced mesothelial to mesenchymal transition and fibrosis. Cell Death Dis 11:647

Takeuchi O, Akira S (2010) Pattern recognition receptors and inflammation. Cell 140:805–820

Tian Z, Liang G, Cui K, Liang Y, Wang Q, Lv S, Cheng X, Zhang L (2021) Insight into the prospects for RNAi therapy of cancer. Front Pharmacol 12:644718

Tokarev AA, Alfonso A, Segev N (2009) Overview of intracellular compartments and trafficking pathways. In: Segev N (ed) Trafficking inside cells: pathways, mechanisms and regulation. Springer, New York, pp 3–14

Traber GM, Yu AM (2023) RNAi-based therapeutics and novel RNA bioengineering technologies. J Pharmacol Exp Ther 384:133–154

Van de Vyver T, Bogaert B, De Backer L, Joris F, Guagliardo R, Van Hoeck J, Merckx P, Van Calenbergh S, Ramishetti S, Peer D, Remaut K, De Smedt SC, Raemdonck K (2020) Cationic amphiphilic drugs boost the lysosomal escape of small nucleic acid therapeutics in a nanocarrier-dependent manner. ACS Nano 14:4774–4791

Van Meer G, de Kroon AI (2011) Lipid map of the mammalian cell. J Cell Sci 124:5–8

Vocelle D, Chan C, Walton SP (2020) Endocytosis controls siRNA efficiency: implications for siRNA delivery vehicle design and cell-specific targeting. Nucleic Acid Ther 30:22–32

Wandinger-Ness A, Zerial M (2014) Rab proteins and the compartmentalization of the endosomal system. Cold Spring Harb Perspect Biol 6:a022616

Wang J, Lu Z, Wientjes MG, Au JL (2010) Delivery of siRNA therapeutics: barriers and carriers. AAPS J 12:492–503

Wang R, Miao G, Shen JL, Fortier TM, Baehrecke EH (2022) ESCRT dysfunction compromises endoplasmic reticulum maturation and autophagosome biogenesis in *Drosophila*. Curr Biol 32:1262–1274

Wattiaux R, Laurent N, Wattiaux-De Coninck S, Jadot M (2000) Endosomes, lysosomes: their implication in gene transfer. Adv Drug Deliv Rev 41:201–208

Wu B, Hur S (2015) How RIG-I like receptors activate MAVS. Curr Opin Virol 12:91–98

Xiao F, Li J, Huang K, Li X, Xiong Y, Wu M, Wu L, Kuang W, Lv S, Wu L, Zhu X, Guo H (2021) Macropinocytosis: mechanism and targeted therapy in cancers. Am J Cancer Res 11:14–30

Xu YP, Qiu Y, Zhang B, Chen G, Chen Q, Wang M, Mo F, Xu J, Wu J, Zhang RR, Cheng ML, Zhang NN, Lyu B, Zhu WL, Wu MH, Ye Q, Zhang D, Man JH, Li XF, Cui J et al (2019) Zika virus infection induces RNAi-mediated antiviral immunity in human neural progenitors and brain organoids. Cell Res 29:265–273

Yasuda S, Morishita S, Fujita A, Nanao T, Wada N, Waguri S, Schiavo G, Fukuda M, Nakamura T (2016) Mon1-Ccz1 activates Rab7 only on late endosomes and dissociates from the lysosome in mammalian cells. J Cell Sci 129:329–340

Zachar I, Boza G (2020) Endosymbiosis before eukaryotes: mitochondrial establishment in protoeukaryotes. Cell Mol Life Sci 77:3503–3523

Zhang Z, Ohto U, Shibata T, Krayukhina E, Taoka M, Yamauchi Y, Tanji H, Isobe T, Uchiyama S, Miyake K, Shimizu T (2016) Structural analysis reveals that toll-like receptor 7 is a dual receptor for guanosine and single-stranded RNA. Immunity 45:737–748

Zhang Y, Dai Y, Wang J, Xu Y, Li Z, Lu J, Xu Y, Zhong J, Ding SW, Li Y (2022) Mouse circulating extracellular vesicles contain virus-derived siRNAs active in antiviral immunity. EMBO J 41:e109902

Zhang J, Qin W, Hu C, Gu S, Chai X, Yang M, Zhou F, Wang X, Chen K, Yan G, Wang G, Jiang C, Warren A, Xiong J, Miao W (2023) Giant proteins in a giant cell: molecular basis of ultrafast $Ca^{(2+)}$-dependent cell contraction. Sci Adv 9:eadd6550

Zhao JH, Guo HS (2022) RNA silencing: from discovery and elucidation to application and perspectives. J Integr Plant Biol 64:476–498

Zhu KY, Palli SR (2020) Mechanisms, applications, and challenges of insect RNA-Interference. Annu Rev Entomol 65:293–311

Zylberberg C, Gaskill K, Pasley S, Matosevic S (2017) Engineering liposomal nanoparticles for targeted gene therapy. Gene Ther 24:441–452

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.