Extracellular ncRNAs

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Non-coding RNAs (ncRNAs) are key regulators of post-transcriptional gene expression in prokaryotic and eukaryotic organisms. These molecules can interact with mRNAs or proteins, affecting a variety of cellular functions. Emerging evidence shows that intra/inter-species and trans-kingdom regulation can also be achieved with exogenous RNAs, which are exported to the extracellular medium, mainly through vesicles. In bacteria, membrane vesicles (MVs) seem to be the more common way of extracellular communication.

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1. Introduction

Non-coding RNAs (ncRNAs) are ubiquitous gene regulatory molecules across all life domains, although with specific particularities in plants, animals, and microorganisms. In eukaryotes, ncRNAs can control gene expression by different mechanisms, including chromatin architecture, epigenetics, transcription, splicing, editing, and translation[1][2]. ncRNAs can be classified as long ncRNAs (lncRNAs) with more than 200 nucleotides, and small ncRNAs (sncRNAs) with less than 200 nt[3]. The sncRNAs can further be subdivided into microRNAs (miRNAs) and short interfering RNAs (siRNAS), usually ranging 18–22 nt and 20–24 nt long, respectively[1]. miRNAs and siRNAs can be distinguished by their role in the physiology of the eukaryotic cell. miRNAs are involved in endogenous gene expression regulation, while siRNAs are mainly responsible for protecting the cell from exogenous nucleic acid attack. miRNAs and siRNAs regulate gene expression at the post-transcriptional level by binding their target mRNA by base complementary, generally with an inhibitory effect on gene expression. These interactions depend on two major families of proteins, Dicer enzymes that excise them from their precursors, and Ago proteins that support their silencing effector functions[2][4].

miRNAs were first identified in *Caenorhabditis elegans* in 1993, and since then almost 50,000 have been discovered in 271 species[2][4] miRNAs can be generated from an exclusive gene, from clusters that encode several miRNAs and/or proteins, and even from introns[5]. In animals, miRNAs interaction with their targets mostly involve a partial complementarity that initiates with Watson–Crick base pairing of the seed region of the miRNA nucleotides 2–8 to the target mRNA[2]. Although similar to animal miRNAs, plant miRNAs are classified in hairpin-derived small RNAs (hpRNAs) and double-stranded (ds) RNA-derived small RNAs (siRNAs). The complementarity patterns of the functional miRNA/targets in plants also differ from the animal ones, where a near-perfect complementarity is achieved between miRNA and mRNA[2][6].

Originally described in plants, siRNAs were observed as a transgene- and virus-induced silencing phenomenon[7] and are categorized in trans-acting siRNAs (ta-siRNAs), endogenous siRNAs (endo-siRNAs), and exogenous siRNAs (exo-siRNAs). siRNAs are characterized by the formation of a duplex, sense-antisense, similar to cis-ncRNA in bacteria (described below), leading to the target mRNA degradation[2].

lncRNAs are the longer ncRNAs in eukaryotes and the ones that are poorly understood. Based on their location and neighborhood orientation, lncRNAs can be classified as sense/antisense, divergent/convergent, or intronic/intergenic. They can act through several mechanisms such as a direct regulation of gene expression and activity, a decoy to prevent the attachment of transcription factors to specific promoter regions, a guide to orientate proteins to their target locations, and a scaffold to assist with the assembly of pertinent molecular units[8]. lncRNAs can modulate transcription, whether repressing or activating, by sequestering factors including transcription factors, catalytic proteins, subunits of larger chromatin modifying complexes, as well as miRNAs[3]. Frequently, changes in lncRNAs expression leads to a dysregulation of cellular functions such as cell proliferation, induction of angiogenesis, resistance to apoptosis, promotion of metastasis, and evasion of tumor suppressors. Many lncRNAs have been functionally associated with human diseases such as cancer [9].

On the other hand, bacterial non-coding RNAs were first described in *Escherichia coli*, in the 1960s. The majority of bacterial ncRNAs range from 50 to 400 nucleotides long, being capable of driving the fastest transcriptional regulation[10][11][12][13]. As far as it is known, bacterial ncRNAs are mainly located in intergenic regions and represent about 5% of the total number of bacterial genes[12][14]. Bacterial ncRNAs can target multiple molecular structures, being involved in the regulation of diverse cellular processes, including replication, transcription, translation, energetic and general metabolism, peptidoglycan synthesis, and bacterial virulence[10][15]. Bacterial ncRNAs can play a role in cellular metabolism, iron homeostasis, quorum-sensing (QS), stress response, environmental adaptation, as well as in mechanisms related to bacterial pathogenesis[16]. Mainly acting by antisense base-pairing, bacterial ncRNAs can be classified as cis- or trans-encoded, sharing a full or partial complementarity with their mRNA targets, respectively. Due to their nature, trans-encoded ncRNAs are less specific and usually target multiple mRNAs[15][17]. The majority of the bacterial ncRNAs bind the 5′ untranslated region (5′-UTR) of the target mRNA, although interactions with the 3′-UTR and the coding region have been reported. The interaction between ncRNA and mRNA can result in a translation suppression, with or without RNase E-mediated mRNA degradation. There are also cases reporting that this interaction can lead to an activation of gene expression[15][18]. The regulatory function of a bacterial ncRNA can also be impaired by other RNA molecules such as mRNA and ncRNA[19][20]. Bacterial ncRNAs can also interact with proteins, and particularly important are the interactions with RNA chaperones. RNA chaperones play a crucial role in the regulatory mechanisms of some ncRNAs, by stabilizing the RNA molecules and/or mediating the interaction between sRNAs and their targets. Proteins of the Hfq family are the best characterized bacterial RNA chaperones, being present across kingdoms and highly conserved among bacterial genomes[21][22][23][24] [21,22,23,24]. Another recently characterized RNA chaperone is the ProQ, a chaperone that seems to be involved in interactions with more structured ncRNAs[25].

2. Extracellular ncRNAs in Eukaryotes: Release Mechanisms and Relevance in Bacterial Infections

siRNAs were the first ncRNAs described to be involved in intercellular interactions, as a way to disturb other cells, to promote cell-cell communication, or as a defense mechanism against exogenous RNAs. However, an increasing panoply of ncRNAs is being found in the extracellular space, including among others, eukaryotic miRNAs, lncRNAs, siRNAs, piwi-interacting RNAs, and bacterial ncRNAs[26][27]. miRNAs have also already been found in diverse extracellular environments such as blood, urine, saliva, and ascitic fluid[26]. These facts may open the door to engineer the use of miRNAs as biomarkers and, in a research perspective, to pave the way for the understanding of the possible intra- and interspecies communication through exogenous miRNAs.

At least five mechanisms of miRNAs release to the extracellular environment have been described: (i) miRNA bound to RNA-protein complexes. A couple of studies have identified the secretion of miRNAs linked to proteins of the Argonaute family, such as Argonaute 2 (Ago2), a type of protein that is associated to RNA-inducing silencing complex (RISC)[28]. RISC is involved in most of miRNAs regulation[29]. In fact, the majority of miRNAs found in human plasma are bounded to Argonaut proteins, but this seems to be mainly related to cell death and not to a selective secretion of miRNAs[30]; (ii) transport via lipid or lipoprotein particles. In addition to being crucial transporters of steroids, triglycerols, cholesterol, and fat-soluble vitamins, low-density lipoproteins (LDL) and mainly high-density lipoproteins (HDL) can also play a role in miRNAs intercellular communication[31][32]. The loading mechanism seems to involve divalent cation bridging between miRNAs and HDL[31]; (iii) inside microvesicles. Microvesicles are formed by plasma membrane by budding or fission, and therefore, their lipid content is quite similar to the parent cell membrane [33]. Microvesicles can be a way of secretion of many types of molecules, including nucleic acids, being also responsible for the cell-to-cell communication by miRNAs exportation in several clinical conditions [34]. Although there is evidence of the involvement of microvesicles miRNAs on intracellular communication, their relevance in infection conditions remains unknown, as well as the sorting and loading processes [35]; (iv) inside vesicles from apoptotic bodies. Apoptosis is a natural process of controlled cell death by eukaryotic cells. In this process, the release of apoptotic bodies, the greatest vesicles secreted by eukaryotic cells, is common [36]. As with microvesicles, the content can be vast, including miRNAs, mRNAs, and DNA fragments, but it can also be selective, and under specific conditions, some miRNAs can be highly represented on these vesicles [37]. Again, the sorting mechanism remains unknown[35]; (v) inside exosomes. Despite their origin or structure, extracellular vesicles (EV) seem to be the most usual mechanism to selectively export ncRNAs to the extracellular space [27]. Exosomes seem to play a special role in cell-to-cell communication on infection conditions[35]. Exosomes are generated inside endosomes or multivesicular bodies (MVBs) and released through fusion of these exosome-enriched late endosomes with the plasma membrane (Figure 1) [38]. Although the process of sorting and loading of ncRNAs is still poorly characterized, is about exosomes that we know the most. In animals, the heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1) was described to control the exosomal loading of miRNAs by binding to specific “EXOmotifs” on these miRNAs[39]. The most detailed example found in the literature is about colorectal cancer cells. In this case, the KRAS-MEK signaling pathway

seems to be responsible for the regulation of exosomal loading of the RISC component Argonaute 2, an RNA-binding protein and a key effector of miRNA-guided RNA silencing process[40]. In addition, during the sorting and loading of ncRNAs in exosomes, other RNA-binding proteins are also suggested to participate, such as the Y-box protein required for the miR-233 secretion by human embryonic kidney (HEK)293T cells[41], and the SYNCRIP protein required for miRNA sorting in hepatocytes. miRNAs found in those exosomes possess an extra-seed sequence (hEXO motif) that binds to these RNA-binding proteins[42]. Still, in the hepatic system, the RNA-binding protein Vps4A was found to mediate the flux of miRNAs through exosomes. Vps4A facilitates the secretion of oncogenic miRNAs in exosomes while promoting the accumulation and uptake of tumor suppressor miRNAs in cells. A downregulation of this protein was observed in hepatocellular carcinoma (HCC) cells[43]. In addition, the transcriptional regulation of miRNAs expression or of their targets also implies a miRNA sorting regulation on exosome secretion, as shown in macrophages and endothelial cell communication[44]. The mechanism of sorting and loading of miRNAs in infection conditions needs to be investigated.

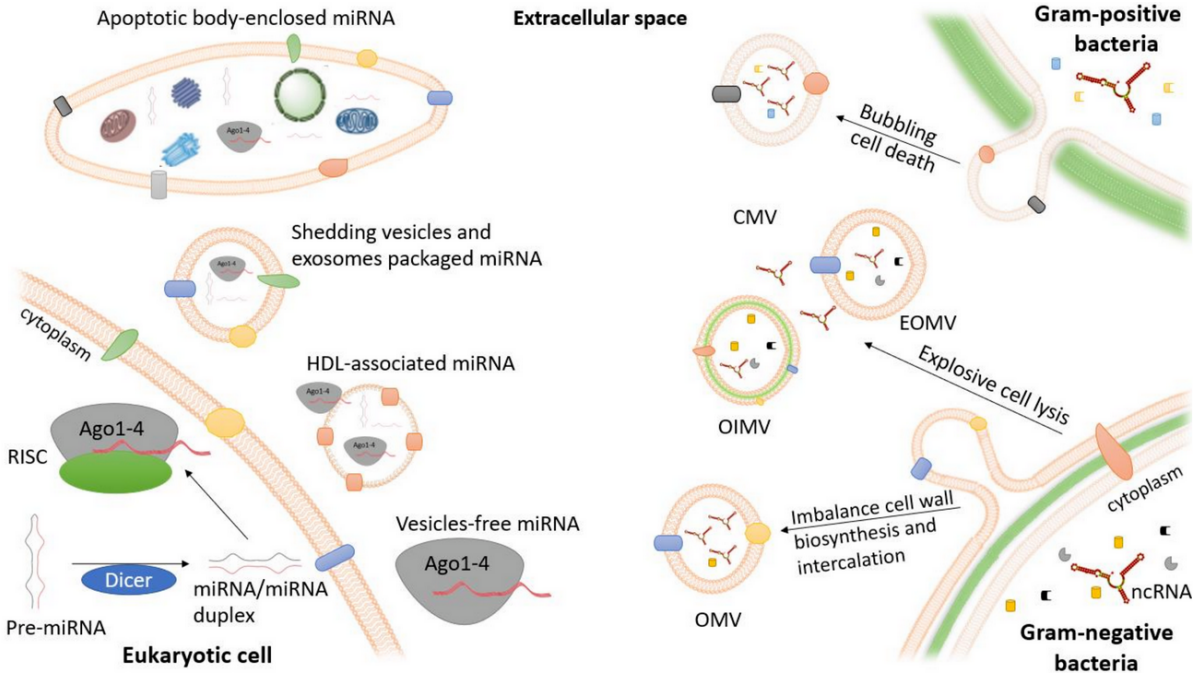

Figure 1. Modes of extracellular miRNA packaging in eukaryotic cells and routes leading to the formation of different membrane vesicle types in bacteria. Extracellular miRNAs can be cargo in membranous vesicles or can be vesicle free and associated with either Argonaute (Ago) proteins alone or incorporated into HDL particles. Apoptotic bodies, shedding vesicles, and exosomes are three types of membranous vesicles that contain these extracellular miRNAs. Apoptotic bodies can also contain various cellular organelles including mitochondria and nucleic acids[28]. In bacteria, distinct membrane vesicles are formed by Gram-negative and Gram-positive bacteria. Blebbing of the outer membrane and explosive cell lysis are the two main routes for vesicle formation in Gram-negative bacteria. The membrane vesicles from these bacteria can be divided into outer-inner membrane vesicles (OIMVs), explosive outer membrane vesicles (EOMVs), and traditional OMVs according to their formation routes, structures, and compositions[45]. In Gram-positive bacteria, membrane vesicles are formed by a mechanism involving the prophage-encoded endolysin that generates holes in the peptidoglycan cell wall, allowing the cytoplasmic membrane material to protrude into the extracellular space and release the cytoplasmic membrane vesicles (CMVs). CMVs can contain membrane and cytoplasmic components[46].

In the context of infectious processes, miRNAs have been increasingly implicated in the eukaryotic response to viruses, nematodes, and bacterial pathogens[47]. Specific miRNAs, such as miR-155, miR-146a, miR-21, and the let-7 family of miRNAs have been demonstrated to be involved in the regulation of the immune response to infection (the mechanisms involved were recently reviewed by Kumar et al. [48]. Immune cells are able to release microRNA-containing exosomes that can be uptake by recipient cells. During antigen recognition, when antigen-specific T cells form an immunologic synapse with antigen-presenting cells (APCs), miRNAs are unidirectionally transferred between cells by exosomes[49]. More recently, increasing evidence has also suggested that infections with pathogenic organisms lead to significant changes in the miRNA exosome content (miRNA abundance and profile)[50][51]. Some of these exosome-delivered miRNAs seem to immunomodulate the inflammatory response, however, their specific role in host vesicles derived from bacterially infected host cells is not completely understood. For instance, higher levels of miR-18a, a miRNA that promotes the intracellular *Mycobacterium tuberculosis* survival by counteracting autophagy, were detected in macrophages infected by *M. tuberculosis* and in their derived exosomes[52]. However, the miR-18a impact in exosome-receiving cells remains unclear. On the other hand, miR-155, a prototype multifunctional miRNA that exhibits crucial roles during innate or adaptive immune responses, was shown to be loaded in exosomes derived from *Helicobacter pylori*-

infected macrophages. This miRNA exacerbates inflammatory responses in recipient macrophages by promoting the expression of inflammatory cytokines, such as TNF-α, IL-6, and IL-23, which help to inhibit the proliferation of *H. pylori*[53]. Exosomes containing miR-146a and miR-155 were also described to be secreted by murine bone marrow-derived dendritic cells (BMDC) after exposure to LPS. Both miRNAs seem also to be efficiently transferred to recipient cells, modulating the expression of inflammatory genes and cell responses to endotoxins[54].

References

1. Ledda, B.; Ottaggio, L.; Izzotti, A.; Sukkar, S.G.; Miele, M. Small RNAs in eucaryotes: New clues for amplifying microRNA benefits. Cell Biosci. 2020, 10, 1–13, doi:10.1186/s13578-019-0370-3.
2. Carthew, R.W.; Sontheimer, E.J. Origins and Mechanisms of miRNAs and siRNAs. Cell 2009, 136, 642–655, doi:10.1016/j.cell.2009.01.035.
3. Fang, Y.; Fullwood, M.J. Roles, Functions, and Mechanisms of Long Non-coding RNAs in Cancer. Genom. Proteom. Bioinforma. 2016, 14, 42–54, doi:10.1016/j.gpb.2015.09.006.
4. Fehlmann, T.; Backes, C.; Pirritano, M.; Laufer, T.; Galata, V.; Kern, F.; Kahraman, M.; Gasparoni, G.; Ludwig, N.; Lenhof, H.-P.; et al. The sncRNA Zoo: A repository for circulating small noncoding RNAs in animals. Nucleic Acids Res. 2019, 47, 4431–4441, doi:10.1093/nar/gkz227.
5. Lu, J.; Shen, Y.; Wu, Q.; Kumar, S.; He, B.; Shi, S.; Carthew, R.W.; Wang, S.M.; Wu, C.-I. The birth and death of microRNA genes in Drosophila. Nat. Genet. 2008, 40, 351–355, doi:10.1038/ng.73.
6. Axtell, M.J. Classification and Comparison of Small RNAs from Plants. Annu. Rev. Plant Biol. 2013, 64, 137–159, doi:10.1146/annurev-arplant-050312-120043.
7. Mello, C.C.; Conte, D. Revealing the world of RNA interference. Nat. Cell Biol. 2004, 431, 338–342, doi:10.1038/nature02872.
8. Biswas, S.; Chakrabarti, S. Increased Extracellular Matrix Protein Production in Chronic Diabetic Complications: Implications of Non-Coding RNAs. Non-Coding RNA 2019, 5, 30, doi:10.3390/ncrna5010030.
9. Gutschner, T.; Diederichs, S. The hallmarks of cancer. RNA Biol. 2012, 9, 703–719, doi:10.4161/rna.20481.
10. Mikulík, K. Structure and functional properties of prokaryotic small noncoding RNAs. Folia Microbiol. 2003, 48, 443–468, doi:10.1007/bf02931326.
11. Hindley, J. Fractionation of 32P-labelled ribonucleic acids on polyacrylamide gels and their characterization by fingerprinting. J. Mol. Biol. 1967, 30, 125–136, doi:10.1016/0022-2836(67)90248-3.
12. Nitzan, M.; Rehani, R.; Margalit, H. Integration of Bacterial Small RNAs in Regulatory Networks. Annu. Rev. Biophys. 2017, 46, 131–148, doi:10.1146/annurev-biophys-070816-034058.
13. Pita, T.; Feliciano, J.R.; Leitão, J.H. Small Noncoding Regulatory RNAs from Pseudomonas aeruginosa and Burkholderia cepacia Complex. Int. J. Mol. Sci. 2018, 19, 3759, doi:10.3390/ijms19123759.
14. Papenfort, K.; Pfeiffer, V.; Mika, F.; Lucchini, S.; Hinton, J.C.D.; Vogel, J. ΣE-dependent small RNAs of Salmonella respond to membrane stress by accelerating global omp mRNA decay. Mol. Microbiol. 2006, 62, 1674–1688, doi:10.1111/j.1365-2958.2006.05524.x.
15. Klein, G.; Raina, S. Small regulatory bacterial RNAs regulating the envelope stress response. Biochem. Soc. Trans. 2017, 45, 417–425, doi:10.1042/bst20160367.
16. Michaux, C.; Verneuil, N.; Hartke, A.; Giard, J.-C. Physiological roles of small RNA molecules. Microbiology 2014, 160, 1007–1019, doi:10.1099/mic.0.076208-0.
17. Brantl, S. Chapter 4 Small Regulatory RNAs (SRNAs): Key Players in Prokaryotic Metabolism, Stress Response, and Virulence. In Regulatory RNAs; Mallick, B., Ed.; Springer: Berlin/Heidelberg, Germany, 2012; pp. 73–108.
18. Park, H.; Yoon, Y.; Suk, S.; Lee, J.Y.; Lee, Y. Effects of different target sites on antisense RNA-mediated regulation of gene expression. BMB Rep. 2014, 47, 619–624, doi:10.5483/bmbrep.2014.47.11.257.
19. Figueroa-Bossi, N.; Valentini, M.; Malleret, L.; Bossi, L. Caught at its own game: Regulatory small RNA inactivated by an inducible transcript mimicking its target. Genes Dev. 2009, 23, 2004–2015, doi:10.1101/gad.541609.
20. Azam, M.S.; Vanderpool, C.K. Talk among yourselves: RNA sponges mediate cross talk between functionally related messenger RNA s. EMBO J. 2015, 34, 1436–1438, doi:10.15252/embj.201591492.
21. Malabirade, A.; Morgado-Brajones, J.; Trépout, S.; Wien, F.; Marquez, I.; Seguin, J.; Marco, S.; Velez, M.; Arluison, V. Membrane association of the bacterial riboregulator Hfq and functional perspectives. Sci. Rep. 2017, 7, 1–12, doi:10.1038/s41598-017-11157-5.

22. Brennan, R.G.; Link, T.M. Hfq structure, function and ligand binding. Curr. Opin. Microbiol. 2007, 10, 125–133, doi:10.1016/j.mib.2007.03.015.

23. Feliciano, J.R.; Grilo, A.M.; I Guerreiro, S.; Sousa, S.A.; Leitão, J.H. Hfq: A multifaceted RNA chaperone involved in virulence. Futur. Microbiol. 2016, 11, 137–151, doi:10.2217/fmb.15.128.

24. Feliciano, J.R.; Seixas, A.M.; Pita, T.; Leitão, J.H. Comparative Genomics and Evolutionary Analysis of RNA-Binding Proteins of Burkholderia cenocepacia J2315 and Other Members of the B. cepacia Complex. Genes 2020, 11, 231, doi:10.3390/genes11020231.

25. Smirnov, A.; Foerstner, K.U.; Holmqvist, E.; Otto, A.; Günster, R.; Becher, D.; Reinhardt, R.; Vogel, J. Grad-seq guides the discovery of ProQ as a major small RNA-binding protein. Proc. Natl. Acad. Sci. USA 2016, 113, 11591–11596, doi:10.1073/pnas.1609981113.

26. Cajal, S.R.y.; Segura, M.F.; Hümmer, S. Interplay between ncRNAs and Cellular Communication: A Proposal for Understanding Cell-Specific Signaling Pathways. Front. Genet. 2019, 10, 281, doi:10.3389/fgene.2019.00281.

27. Yang, S.; Li, X. Recent advances in extracellular vesicles enriched with non-coding RNAs related to cancers. Genes Dis. 2018, 5, 36–42, doi:10.1016/j.gendis.2017.12.001.

28. Turchinovich, A.; Weiz, L.; Burwinkel, B. Extracellular miRNAs: The mystery of their origin and function. Trends Biochem. Sci. 2012, 37, 460–465, doi:10.1016/j.tibs.2012.08.003.

29. Filipowicz, W.; Bhattacharyya, S.N.; Sonenberg, N. Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight? Nat. Rev. Genet. 2008, 9, 102–114, doi:10.1038/nrg2290.

30. Arroyo, J.D.; Chevillet, J.R.; Kroh, E.M.; Ruf, I.K.; Pritchard, C.C.; Gibson, D.F.; Mitchell, P.S.; Bennett, C.F.; Pogosova-Agadjanyan, E.L.; Stirewalt, D.L.; et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc. Natl. Acad. Sci. USA 2011, 108, 5003–5008, doi:10.1073/pnas.1019055108.

31. Vickers, K.C.; Palmisano, B.T.; Shoucri, B.M.; Shamburek, R.D.; Remaley, A.T. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat. Cell Biol. 2011, 13, 423–433, doi:10.1038/ncb2210.

32. Niculescu, L.S.; Simionescu, N.; Sanda, G.M.; Carnuta, M.G.; Stancu, C.S.; Popescu, A.C.; Popescu, M.R.; Vlad, A.; Dimulescu, D.R.; Simionescu, M.; et al. MiR-486 and miR-92a Identified in Circulating HDL Discriminate between Stable and Vulnerable Coronary Artery Disease Patients. PLoS ONE 2015, 10, e0140958, doi:10.1371/journal.pone.0140958.

33. Lee, Y.; El Andaloussi, S.; Wood, M.J.A. Exosomes and microvesicles: Extracellular vesicles for genetic information transfer and gene therapy. Hum. Mol. Genet. 2012, 21, R125–R134, doi:10.1093/hmg/dds317.

34. Mause, S.F.; Weber, C. Microparticles. Circ. Res. 2010, 107, 1047–1057, doi:10.1161/circresaha.110.226456.

35. Sohel, M.H. Extracellular/Circulating MicroRNAs: Release Mechanisms, Functions and Challenges. Achiev. Life Sci. 2016, 10, 175–186, doi:10.1016/j.als.2016.11.007.

36. Turiák, L.; Misják, P.; Szabó, T.G.; Aradi, B.; Pálóczi, K.; Ozohanics, O.; Drahos, L.; Kittel, Ágnes; Falus, A.; Buzás, E.I.; et al. Proteomic characterization of thymocyte-derived microvesicles and apoptotic bodies in BALB/c mice. J. Proteom. 2011, 74, 2025–2033, doi:10.1016/j.jprot.2011.05.023.

37. Hergenreider, E.; Heydt, S.; Tréguer, K.; Boettger, T.; Horrevoets, A.J.G.; Zeiher, A.M.; Scheffer, M.P.; Frangakis, A.S.; Yin, X.; Mayr, M.; et al. Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. Nat. Cell Biol. 2012, 14, 249–256, doi:10.1038/ncb2441.

38. Kourembanas, S. Exosomes: Vehicles of Intercellular Signaling, Biomarkers, and Vectors of Cell Therapy. Annu. Rev. Physiol. 2015, 77, 13–27, doi:10.1146/annurev-physiol-021014-071641.

39. Villarroya-beltri, C.; Gutie, C.; Martin-cofreces, N.; Martinez-herrera, D.J.; Pascual-montano, A. Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. Nat. Commun. 2013, 4, 2980, doi:10.1038/ncomms3980.

40. McKenzie, A.J.; Hoshino, D.; Hong, N.H.; Cha, D.J.; Franklin, J.L.; Coffey, R.J.; Patton, J.G.; Weaver, A.M. KRAS-MEK Signaling Controls Ago2 Sorting into Exosomes. Cell Rep. 2016, 15, 978–987, doi:10.1016/j.celrep.2016.03.085.

41. Shurtleff, M.J.; Temoche-Diaz, M.M.; Karfilis, K.V.; Ri, S.; Schekman, R. Y-box protein 1 is required to sort microRNAs into exosomes in cells and in a cell-free reaction. eLife 2016, 5, e19276, doi:10.7554/elife.19276.

42. Santangelo, L.; Giurato, G.; Cicchini, C.; Alonzi, T.; Weisz, A.; Tripodi, M.; Santangelo, L.; Giurato, G.; Cicchini, C.; Montaldo, C.; Mancone, C.; Tarallo, R. The RNA-Binding Protein SYNCRIP Is a Component of the Hepatocyte Exosomal Machinery Controlling MicroRNA Sorting. Cell Rep. 2016, 17, 799–808, doi:10.1016/j.celrep.2016.09.031.

43. Wei, J.; Lv, L.; Wan, Y.; Cao, Y.; Li, G.; Lin, H.; Zhou, R. Vps4A functions as a tumor suppressor by regulating the secretion and uptake of exosomal microRNAs in human hepatoma cells. Hepatology 2015, 61, 1284–1294, doi:10.1002/hep.27660.
44. Squadrito, M. L.; Maderna, C.; Gilfillan, G.D.; Lyle, R.; Baer, C.; Ibberson, M.; Palma, M.D. Endogenous RNAs Modulate MicroRNA Sorting to Exosomes and Transfer to Acceptor Cells. Cell Rep. 2014, 8, 1432–1446, doi:10.1016/j.celrep.2014.07.035.
45. Toyofuku, M.; Nomura, N.; Eberl, L. Types and origins of bacterial membrane vesicles. Nat. Rev. Microbiol. 2019, 17, 13–24, doi:10.1038/s41579-018-0112-2.
46. Toyofuku, M.; Cárcamo-Oyarce, G.; Yamamoto, T.; Eisenstein, F.; Hsiao, C.-C.; Kurosawa, M.; Gademann, K.; Pilhofer, M.; Nomura, N.; Eberl, L. Prophage-triggered membrane vesicle formation through peptidoglycan damage in Bacillus subtilis. Nat. Commun. 2017, 8, 1–10, doi:10.1038/s41467-017-00492-w.
47. Eulalio, A.; Schulte, L.; Vogel, J.; Eulalio, A.; Schulte, L. N.; Vogel, J. The mammalian microRNA response to bacterial infections. RNA Biol. 2012, 9, 742–750, doi:10.4161/rna.20018.
48. Kumar, M.; Kundu, M.; Basu, J. The Role of MicroRNAs in Bacterial Infections. In AGO-Driven Non-Coding RNAs; Mallick, Bibekanand, Ed.; Academic Press: Cambridge, MA, USA, 2019; pp. 57–73.
49. Lai, F.W.; Lichty, B.D.; Bowdish, D.M.E. Microvesicles: Ubiquitous contributors to infection and immunity. J. Leukoc. Biol. 2014, 97, 237–245, doi:10.1189/jlb.3ru0513-292rr.
50. Fleming, A.; Sampey, G.; Chung, M.-C.; Bailey, C.; Van Hoek, M.L.; Kashanchi, F.; Hakami, R.M. The carrying pigeons of the cell: Exosomes and their role in infectious diseases caused by human pathogens. Pathog. Dis. 2014, 71, 109–120, doi:10.1111/2049-632x.12135.
51. Zhang, W.; Jiang, X.; Bao, J.; Wang, Y.; Liu, H.; Tang, L. Exosomes in Pathogen Infections: A Bridge to Deliver Molecules and Link Functions. Front. Immunol. 2018, 9, 90, doi:10.3389/fimmu.2018.00090.
52. Yuan, Q.; Chen, H.; Yang, Y.; Fu, Y.; Yi, Z. miR-18a promotes Mycobacterial survival in macrophages via inhibiting autophagy by down-regulation of ATM. J. Cell. Mol. Med. 2019, 24, 2004–2012, doi:10.1111/jcmm.14899.
53. Wang, J.; Deng, Z.; Wang, Z.; Wu, J.; Gu, T.; Jiang, Y.; Li, G. MicroRNA-155 in exosomes secreted from Helicobacter pylori infection macrophages immunomodulates inflammatory response. Am. J. Transl. Res. 2016, 8, 3700–3709.
54. Alexander, M.; Hu, R.; Runtsch, M.C.; Kagele, D.A.; Mosbruger, T.L.; Tolmachova, T.; Seabra, M.C.; Round, J.L.; Ward, D.M.; O'Connell, R.M. Exosome-delivered microRNAs modulate the inflammatory response to endotoxin. Nat. Commun. 2015, 6, 7321, doi:10.1038/ncomms8321.