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Exosomal RNA (esRNA): A New Frontier in Cellular Signalling

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A B S T R A C T

Intercellular communication in multicellular organisms is a complicated process involving several levels of regulations. So far our knowledge of cellular signaling circled around the realm of macromolecular interactions involving mainly proteins. Recently discovered exosome mediated delivery of RNAs into target cells has opened up a new avenue of research in cell-to-cell signaling. This article focuses on exosome-mediated transfer of mainly non-coding RNAs (ncRNAs) between cells and various functional roles of ncRNAs as signaling molecules.

Introduction

Exosomes were originally discovered in reticulocytes as multivesicular vacuoles. These are nothing but the extracellular cargo-filled bags emanating from cytosolic endosomes. Exosomes, which range from 30–80 nM in diameter, are formed from an inward budding of the plasma membrane, often containing vesicles inside the lumen. To clarify the terminology, 'exosomes' can also refer to multisubunit protein complexes involved in RNA degradation; however, the exosome complexes discussed in this article refer to vesicular exosomes. The target cells contain specific receptor molecules for exosomes and allow fusion of their membrane for transfer of information. The content of such exosomes can include proteins such as receptors or enzymes and nucleic acid molecules such as RNA. Such RNAs transferred by exosomes are known as exosomal RNAs (esRNAs), most of which are non-coding RNAs (ncRNAs).

The term non-coding RNA (ncRNA) is commonly assigned to RNA that does not encode a protein, but they do contain information and have specific function. Although majority of genetic information is translated into proteins, recent researches indicate that a large portion of the genomes of mammals and other complex organisms is in fact transcribed into ncRNAs. Most of which are alternatively spliced and/or processed into various smaller products, including microRNAs and snoRNAs and may be other classes of yet-to-be-discovered

small regulatory RNAs with unknown functions. These RNAs represent a secret level of cellular signals, controlling expression of various genes involved in physiology and development such as - chromatin architecture/epigenetic memory, transcription, RNA splicing, editing, translation etc.

This ability of exosomes to transfer ncRNAs to influence gene expression in distant cells presents a remarkable model for cell-to-cell signaling. The transfer of exosomal RNA offers a new perspective on intercellular communication in various fields of biology including Immunology, Neurobiology, Oncology etc. and has potential therapeutic applications, such as in diagnosis and gene delivery.

NON-CODING RNA

Only recently we came to know that these ncRNAs apart from fulfilling general functions such as translation and splicing can and do perform a wide range of biological functions and they are also involved in cis and trans regulation of gene expression mainly in higher eukaryotes.

Origin

Intronic sequences coded by at least 30% of human genome are thought to be a major putative source of regulatory ncRNAs [(1,2)], which are produced as part of protein coding and other sequences to deliver regulatory signals [(3,4)]. Introns situated in both protein-coding and non-protein-coding genes are source of majority of snoRNAs and miRNAs in animals [(5-9)]. Recent evidences clearly point out that intronic RNAs can give rise to various smaller RNAs with significant half-lives and specific subcellular locations [(10,11)]. Even ectopic expression of CFTR gene derived intronic sequences was shown to alter transcription of some genes in HeLa cells[(12)]. Findings of many ultraconserved sequences in introns [(13-17)]and detection of intronic sequences in whole genome tiling array analyses of human transcription[(18)] also suggest their involvement in production of regulatory RNAs.

Types

Noncoding RNAs can be broadly categorized into two types:

Infrastructural ncRNAs

Various infrastructural ncRNAs which include tRNAs, rRNAs, spliceosomal uRNAs or ‘snRNAs’ and the common ‘snoRNAs’ have functional roles in both translation and splicing for sequence-specific recognition of RNA substrates, and also for the catalytic process itself[(19-23)].

Small Regulatory ncRNAs

The plethora of small regulatory RNAs discovered in animals and plants [(9,24-26)]can be broadly classified into: snoRNAs and miRNAs/ siRNAs.

Biological Role

The involvement of ncRNAs in controlling chromosome dynamics, splicing, RNA editing, translational inhibition and mRNA destruction is already documented, recent researches suggest that RNA signaling play crucial roles in – i) Chromatin remodeling and epigenetic memory [(9,4,27-30)], ii) Alteration of gene expressionby promoter interference [(31)] or by altering chromatin structure [(32-34)], iii) Transcriptional silencing and alterations to DNA methylation in human cells [(35,36)], iv) Regulation of splicing by directing splice site selection [(9,4,37,38)] or

modification of sequences flanking the splice site to make them accessible or otherwise to the splicing machinery [37], v) Regulation of transcription[4,30] by regulating RNA polymerase II[39-43], interacting with transcription factors and chromatin-modifying proteins [44-50], vi) Regulation of gene expression by steroid hormones [51], vii) Regulation of activity of estrogen receptor in breast cancer cells [52], viii) Stress responses [53-58], ix) Scaffolding for the assembly of macromolecular complexes like ribosomes, (Signal Recognition Particle) SRP [27] and chromatin complexes [59], x) Nuclear transcription factor trafficking [60] etc.

Exosome

Majority of cells release different types of membrane microvesicle and nanovesicle, with variety of important physiological functions. Microvesicles which differ from nanovesicles in terms of size and mechanism of formation [61-64] are released from the plasma membrane by shedding or budding, usually larger than 0.2 μm in size and have been referred to as microparticles or ectosomes. Whereas, nanovesicles, including exosomes, are between 30–100 nm in diameter, are of endocytic origin and formed by the reverse budding of the peripheral membrane of multivesicular bodies (MVBs) or late endosomes. Exosomes are mainly extracellular vesicles (<100 nm in size), originate from the endocytic compartment of the cell. They are released by most, if not all, nucleated cells, reticulocytes and platelets, and are present in most bodily fluids. Although, some of the nanovesicles seem to be derived from the plasma membrane [65]. Recently, different families of molecules have been shown to allow intracellular formation of exosomes and their subsequent secretion, indicating the existence of different sub-types of exosomes.

Biogenesis and trafficking of Exosomes

Exosomes originate by the inward budding of an endosomal membrane which is pinched off and released as intraluminal vesicles (ILVs) inside the endosomes, and are called MVBs [61,63] (FIG. 1), which can then follow either the secretory pathway or the lysosomal pathway. Following the secretory pathway, MVBs fuse with the plasma membrane and the release the ILVs as exosomes with concomitant incorporation of the peripheral membrane of the MVBs into the plasma membrane. In the lysosomal pathway, MVBs fuse with lysosomes and release ILVs into the lysosomal lumen for degradation.

During exosome formation first the exosome-targeted contents are sorted into the endosomal membrane, followed by the delivery of the exosome cargo into nascent ILVs and the excision of ILVs. The contents are captured by the endosomal sorting complex for transport (ESCRT) machinery [66,67], involving ESCRT0, ESCRTI, ESCRTII and ESCRTIII, each of which is composed of different subunits and accessory molecules. ESCRT0 is recruited by the lipid phosphatidyl-inositol-3-phosphate on the endosomal membrane, followed by subsequent recruitment of ESCRTI and ESCRTII; these two proteins together initiate the reverse budding of the MVB membrane. Next ESCRTII recruits the components of ESCRTIII, which catalyze the vesicle cleavage.

However, generation of MVBs in the absence of key subunits of ESCRTs also indicates the existence of alternative mechanisms of exosome biogenesis[68]. Next a concerted effort of cytoskeleton, molecular motors and vesicle fusion machinery is required to drive the trafficking of the MVBs to the cell periphery, fusion with the

cell membrane and release of their ILV cargo (FIG. 1). This trafficking process is controlled by the RAB family of small GTPases including RAB27A required for docking and the fusion of MVBs to the cell membrane and RAB27B, which directs the transfer of vesicles from the Golgi to MVBs and mobilizes MVBs to the actin-rich cortex under the plasma membrane [(69)].

After secretion the extracellular vesicles bind to neighbouring cells or to the extracellular matrix, or passively traffic through the bloodstream or through other bodily fluids.

Exosome Mediated Transfer of Non-Coding RNA

Apart from carrying protein antigens exosomes are also known to mediate the transfer of genetic information between cells in the form of RNAs [(62,69,70)]. RNA content of exosomes includes functional mRNAs and small ncRNAs, including miRNAs [(71-77)] but not rRNAs [(72)]. The RNAs thus transported are protected from RNase degradation [(72)]. Existence of a preferential sorting of certain RNA sequences is evident from the fact that some mRNAs and miRNAs are detected in both extracellular vesicles and parent cells, whereas others are identified in either extracellular vesicles or in parent cells [(72,79)]. RNAs are transported from the lumen of extracellular vesicles into the target cells by release of vesicular content into the acceptor-cells (Fig.2). The process involves the docking of extracellular vesicles on target cells (predominantly at cholesterol-rich micro domains) and the fusion of their membranes.

RNA As Inter Cellular Signalling Molecule

1. Delivery of miRNAs - miR-148a and miR-451 by Dendritic Cell (DC)-derived exosomes to acceptor DCs indicates a means of horizontal propagation of post-transcriptional regulation among APCs [(80)], or between APCs and other cell types. Moreover, antigen-induced formation of immunological synapse directs the polarization of T cell MVBs to the point of APC–T cell contact, and triggers the transfer of exosomal miR-335, in acceptor APCs [(79)]. During EBV infection, infected B-Cells deliver exosome-derived viral miRNAs to DCs, which silences mRNA transcripts that encode immune-stimulatory molecules [(81)].
2. Exosomes derived from mast cell line MC/9, bone marrow derived mast cells, and human mast cell line HMC-1, contain substantial amount of intact functional RNA, [(82)] that can be translated to protein and subsequently alter the recipient cells protein production.
3. The expression of long noncoding RNA (lncRNA) is altered in hepatocellular cancer (HCC). lncRNA with highly conserved sequences are called ultraconserved lncRNA (ucRNA) that are transcribed and altered in expression in HCC. Extracellular vesicles, such as exosomes and microvesicles, are released from tumor cells and can transfer biologically active proteins and RNA across cells. The most abundant ucRNA in HCC cell–derived extracellular vesicles was found to be a 1,198-bp ucRNA, termed TUC339, which modulates tumor cell growth and adhesion. Suppression of TUC339 by siRNA reduced HCC cell proliferation, clonogenic growth, and growth in soft agar. Thus, intercellular transfer of TUC339 represents a unique signaling mechanism by which tumor cells can promote HCC growth and spread.

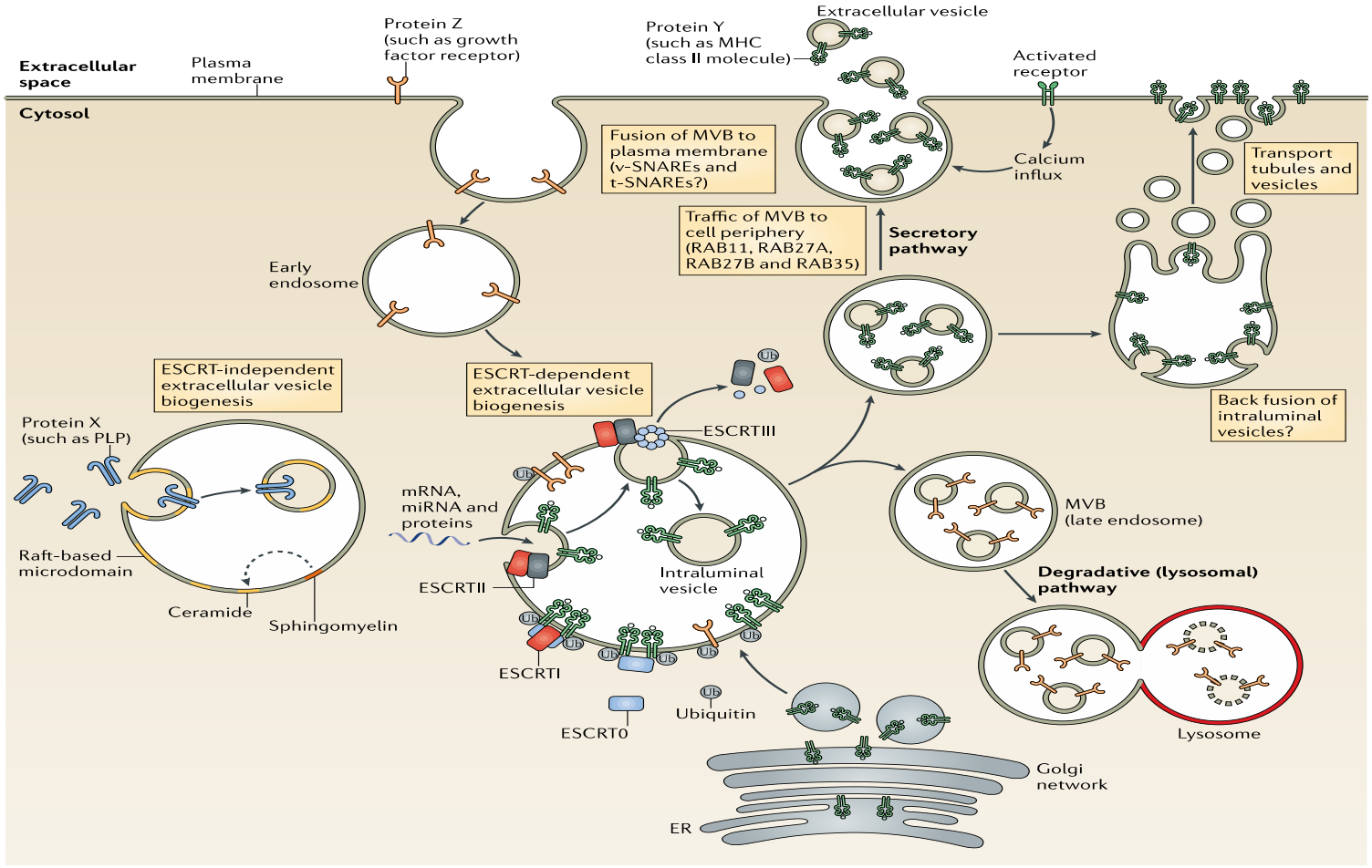

Figure.1 Biogenesis of Exosome:
Courtesy- Paul D. Robbins and Adrian E. Morelli: Regulation of immune responses by extracellular vesicles. NATURE REVIEWS | IMMUNOLOGY VOLUME 14 | MARCH 2014 | 195 -208.[89]

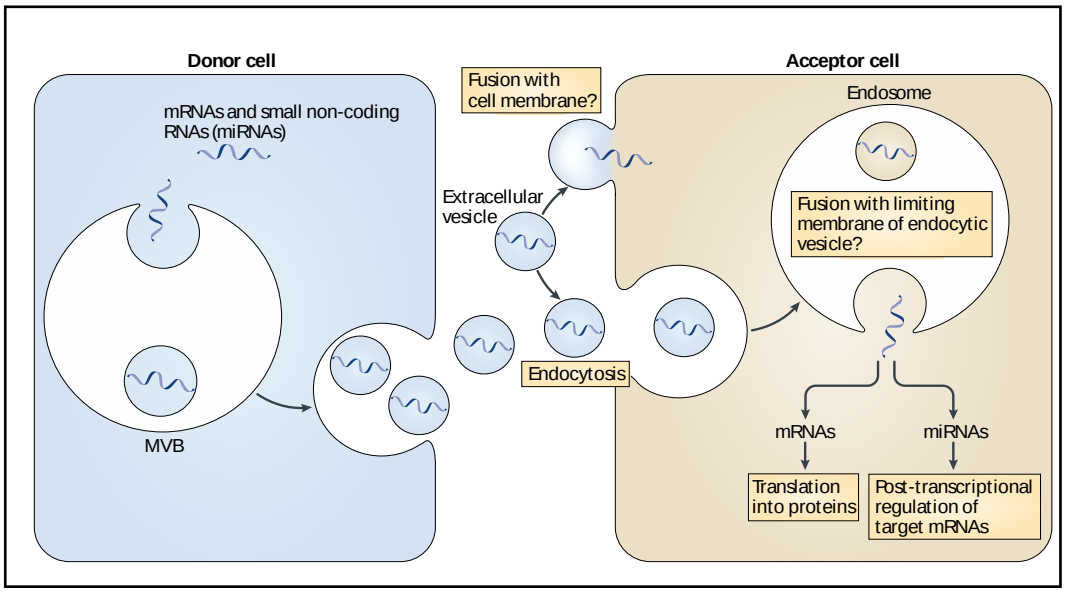

Fig.2 Mechanism of transfer of exosomal shuttle RNAs (esRNAs) between cells.
Courtesy- Paul D. Robbins and Adrian E. Morelli: Regulation of immune responses by extracellular vesicles. NATURE REVIEWS | IMMUNOLOGY VOLUME 14 | MARCH 2014 | 195 -208.[89]

These findings expand the potential roles of ucRNA in HCC, support the existence of selective mechanisms for lncRNA export from cells, and implicate extracellular vesicle–mediated transfer of lncRNA as a mechanism by which tumor cells can modulate their local cellular environment. Intercellular transfer of functionally active RNA molecules by extracellular vesicles provides a mechanism that enables cells to exert genetic influences on other cells within the microenvironment [83]. Human salivary exosomes can be used as biomarkers for disease diagnosis as miRNAs can be found in salivary samples [84]. Glioblastoma tumor cells are found to release exosomes containing mRNA, miRNA, and angiogenic proteins [85].

4. Recently, exosomes electroporated with gene-specific siRNA (*BACE1*) were used to down-regulate specific genes in brain cells [86], pointing out potential application of exosomes in gene delivery, and opening up avenues for therapeutic applications.
5. Exosomal miRNAs can be used for significant enrichment of genes in gene ontology (GO) terms, including genes for protein phosphorylation, RNA splicing, chromosomal abnormality, and angiogenesis [87] indicating potential regulatory roles of exosomal miRNAs.
6. In mammalian systems RNA can move between cells via vesicles. Gastrointestinal nematode *Heligmosomoides polygyrus*, which infects mice, secretes vesicles containing microRNAs (miRNAs) and Y RNAs as well as a nematode Argonaute protein. These vesicles are of intestinal origin and are enriched for homologues of mammalian exosome proteins. These exosomal RNAs can suppress Type 2 Th2-like innate responses and eosinophilia induced by the allergen Alternaria in mice probably by suppressing Dusp1 gene. Discovery of miRNAs from the filarial nematode *Litomosoides sigmodontis* in the serum

of infected mice, suggests that miRNA secretion into host tissues is conserved among parasitic nematodes and these results reveal exosomes as another mechanism by which helminths manipulate their hosts and provide a mechanistic framework for RNA transfer between animal species [88].

Conclusions and Future Perspectives

As already established in this article, exosomes can be viewed as unique method of cell-cell communication, but the function of exosomes in cellular physiology and signaling is yet to be clearly understood. Further investigations are necessary in topics like - the signals that direct the packaging of specific miRNA and other informational molecules into exosomes and their trafficking to various extracellular destinations etc. The mechanisms of exosome incorporation into targeted cells still remain a mystery. Although, the exosome mediated cell signaling adds yet another layer in the complexity of eukaryotic communication networks.

There are still many unanswered questions like – How the exosomes are precisely targeted to the destination cells? How specific contents are assigned to various exosomes? and What triggers exosome release in different cell types? Answers to some of these questions can help design strategies for drug delivery and gene therapy using exosomes, as they are natural transporters derived from cells and can potentially be better tolerated by the immune system. Exosomes can also be used for diagnosis as presence of viral miRNA in the plasma of patients can indicate infections. Changes in miRNA, protein, or mRNA profiles of organ-specific exosomes in the blood plasma or urine of patients can be used as indicator of organ dysfunctions. However, a major focus of future exosome research should be in the context of human diseases, such as cancer.

References

1. Mattick, J.S. and Gagen, M.J. (2001) The evolution of controlled multitasked gene networks: the role of introns and other non-coding RNAs in the development of complex organisms. Mol. Biol. Evol., 18, 1611 – 1630.
2. Mattick, J.S. (1994) Introns: evolution and function. Curr. Opin. Genet. Dev., 4, 823–831.
3. Mattick, J.S. (2001) Non-coding RNAs: the architects of eukaryotic complexity. EMBO Rep., 2, 986–991.
4. Mattick, J.S. (2003) Challenging the dogma: the hidden layer of non-protein-coding RNAs in complex organisms. Bioessays, 25, 930 – 939.
5. Rodriguez, A., Griffiths-Jones, S., Ashurst, J.L. and Bradley, A. (2004) Identification of mammalian microRNA host genes and transcription units. Genome Res., 14, 1902–1910.
6. Cai, X., Hagedorn, C.H. and Cullen, B.R. (2004) Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. RNA, 10, 1957 – 1966.
7. Baskerville, S. and Bartel, D.P. (2005) Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. RNA, 11, 241 – 247.
8. Ying, S.Y. and Lin, S.L. (2005) Intronic microRNAs. Biochem. Biophys. Res. Commun., 326, 515 – 520.
9. Mattick, J.S. and Makunin, I.V. (2005) Small regulatory RNAs in mammals. Hum. Mol. Genet., 14, R121–R132.
10. Clement, J.Q., Qian, L., Kaplinsky, N. and Wilkinson, M.F. (1999) The stability and fate of a spliced intron from vertebrate cells. RNA, 5,206 – 220.
11. Clement, J.Q., Maiti, S. and Wilkinson, M.F. (2001) Localization and stability of introns spliced from the Pem homeobox gene. J. Biol. Chem., 276, 16919–16930.

12. Hill, A.E., Hong, J.S., Wen, H., Teng, L., McPherson, D.T., McPherson, S.A., Levasseur, D.N. and Sorscher, E.J. (2006) Micro-RNA-like effects of complete intronic sequences. Front. Biosci., 11, 1998–2006.
13. Bejerano, G., Pheasant, M., Makunin, I., Stephen, S., Kent, W.J., Mattick, J.S. and Haussler, D. (2004) Ultraconserved elements in the human genome. Science, 304, 1321 – 1325.
14. Dermitzakis, E.T., Reymond, A., Scamuffa, N., Ucla, C., Kirkness, E., Rossier, C. and Antonarakis, S.E. (2003) Evolutionary discrimination of mammalian conserved non-genic sequences (CNGs). Science, 302, 1033 – 1035.
15. Thomas, J.W., Touchman, J.W., Blakesley, R.W., Bouffard, G.G., Beckstrom-Sternberg, S.M., Margulies, E.H., Blanchette, M., Siepel, A.C., Thomas, P.J., McDowell, J.C. et al. (2003) Comparative analyses of multi-species sequences from targeted genomic regions. Nature, 424, 788 – 793.
16. Siepel, A., Bejerano, G., Pedersen, J.S., Hinrichs, A.S., Hou, M., Rosenbloom, K., Clawson, H., Spieth, J., Hillier, L.W., Richards, S. et al. (2005) Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. Genome Res., 15, 1034–1050.
17. Sironi, M., Menozzi, G., Comi, G.P., Cagliani, R., Bresolin, N. and Pozzoli, U. (2005) Analysis of intronic conserved elements indicates that functional complexity might represent a major source of negative selection on non-coding sequences. Hum. Mol. Genet., 14, 2533 – 2546.
18. Cheng, J., Kapranov, P., Drenkow, J., Dike, S., Brubaker, S., Patel, S., Long, J., Stern, D., Tammana, H., Helt, G. et al. (2005) Transcriptional maps of 10 human chromosomes at 5-nucleotide resolution. Science, 308, 1149 – 1154.
19. Gesteland, R.F., Cech, T.R. and Atkins, J.F. (eds) (2006) The RNA World, 3rd edn. Cold Spring Harbor Laboratory Press.
20. Steitz, T.A. and Moore, P.B. (2003) RNA, the first macromolecular catalyst: the ribosome is a ribozyme. Trends Biochem. Sci., 28, 411–418. 25.
21. Noller, H.F. (2005) RNA structure: reading the ribosome. Science, 309, 1508 – 1514.26.
22. Nilsen, T.W. (2003) The spliceosome: the most complex macromolecular machine in the cell? Bioessays, 25, 1147–1149. 27.
23. Butcher, S.E. and Brow, D.A. (2005) Towards understanding the catalytic core structure of the spliceosome. Biochem. Soc. Trans., 33, 447 – 449.
24. Steitz, T.A. and Moore, P.B. (2003) RNA, the first macromolecular catalyst: the ribosome is a ribozyme. Trends Biochem. Sci., 28, 411–418.
25. Noller, H.F. (2005) RNA structure: reading the ribosome. Science, 309, 1508 – 1514.
26. Nilsen, T.W. (2003) The spliceosome: the most complex macromolecular machine in the cell? Bioessays, 25, 1147–1149.
27. Bernstein, E. and Allis, C.D. (2005) RNA meets chromatin. Genes Dev., 19, 1635 – 1655.
28. Morey, C. and Avner, P. (2004) Employment opportunities for non-coding RNAs. FEBS Lett., 567, 27–34.
29. Bayne, E.H. and Allshire, R.C. (2005) RNA-directed transcriptional gene silencing in mammals. Trends Genet., 21, 370–373.
30. Corey, D.R. (2005) Regulating mammalian transcription with RNA. Trends Biochem. Sci., 30, 655–658.
31. Martens, J.A., Laprade, L. and Winston, F. (2004) Intergenic transcription is required to repress the Saccharomyces cerevisiae SER3 gene. Nature, 429, 571–574.
32. Rank, G., Prestel, M. and Paro, R. (2002) Transcription through intergenic chromosomal memory elements of the Drosophila bithorax complex correlates with an epigenetic switch. Mol. Cell. Biol., 22, 8026 – 8034.
33. Schmitt, S., Prestel, M. and Paro, R. (2005) Intergenic transcription through a polycomb group response element counteracts silencing. Genes Dev., 19, 697–708.
34. Vakoc, C.R., Mandat, S.A., Olenchock, B.A. and Blobel, G.A. (2005) Histone H3 lysine 9 methylation and HP1gamma are associated with transcription elongation

through mammalian chromatin. Mol. Cell, 19, 381 – 391.
35. Morris, K.V., Chan, S.W., Jacobsen, S.E. and Looney, D.J. (2004) Small interfering RNA-induced transcriptional gene silencing in human cells. Science, 305, 1289 – 1292.
36. Imamura, T., Yamamoto, S., Ohgane, J., Hattori, N., Tanaka, S. and Shiota, K. (2004) Non-coding RNA directed DNA demethylation of Sphk1 CpG island. Biochem. Biophys. Res. Commun., 322, 593 – 600.
37. Kishore, S. and Stamm, S. (2006) The snoRNA HBII-52 regulates alternative splicing of the serotonin receptor 2C. Science, 311, 230 – 232.
38. Holliday, R. and Murray, V. (1994) Specificity in splicing. Bioessays, 16, 771 – 774.
39. Yang, Z., Zhu, Q., Luo, K. and Zhou, Q. (2001) The 7SK small nuclear RNA inhibits the CDK9/cyclin T1 kinase to control transcription. Nature, 414, 317 – 322.
40. Michels, A.A., Fraldi, A., Li, Q., Adamson, T.E., Bonnet, F., Nguyen, V.T., Sedore, S.C., Price, J.P., Price, D.H., Lania, L. et al. (2004) Binding of the 7SK snRNA turns the HEXIM1 protein into a P-TEFb (CDK9/cyclin T) inhibitor. EMBO J., 23, 2608 – 2619.
41. Yik, J.H., Chen, R., Pezda, A.C. and Zhou, Q. (2005) Compensatory contributions of HEXIM1 and HEXIM2 in maintaining the balance of active and inactive positive transcription elongation factor b complexes for control of transcription. J. Biol. Chem., 280, 16368 – 16376.
42. Li, Q., Price, J.P., Byers, S.A., Cheng, D., Peng, J. and Price, D.H. (2005) Analysis of the large inactive P-TEFb complex indicates that it contains one 7SK molecule, a dimer of HEXIM1 or HEXIM2, and two P-TEFb molecules containing Cdk9 phosphorylated at threonine 186. J. Biol. Chem., 280, 28819 – 28826.
43. Haaland, R.E., Herrmann, C.H. and Rice, A.P. (2005) siRNA depletion of 7SK snRNA induces apoptosis but does not affect expression of the HIV-1 LTR or P-TEFb-dependent cellular genes. J. Cell Physiol., 205, 463 – 470.
44. Shi, Y. and Berg, J.M. (1995) Specific DNA – RNA hybrid binding by zinc finger proteins. Science, 268, 282 – 284.
45. Ladomery, M. (1997) Multifunctional proteins suggest connections between transcriptional and post-transcriptional processes. Bioessays, 19, 903 – 909.
46. Akhtar, A., Zink, D. and Becker, P.B. (2000) Chromodomains are protein-RNA interaction modules. Nature, 407, 405 – 409.
47. Muchardt, C., Guilleme´, M., Seeler, J., Trouche, D., Dejean, A. and Yaniv, M. (2002) Coordinated methyl and RNA binding is required for heterochromatin localization of mammalian HP1. EMBO Rep., 3, 975 – 981.
48. Jeffery, L. and Nakielny, S. (2004) Components of the DNA methylation system of chromatin control are RNA-binding proteins. J. Biol. Chem., 279, 49479–49487.
49. Krajewski, W.A., Nakamura, T., Mazo, A. and Canaani, E. (2005) A motif within SET-domain proteins binds single-stranded nucleic acids and transcribed and supercoiled DNAs and can interfere with assembly of nucleosomes. Mol. Cell. Biol., 25, 1891–1899.
50. Brown, R.S. (2005) Zinc finger proteins: getting a grip on RNA. Curr. Opin. Struct. Biol., 15, 94–98.
51. Lanz, R.B., Razani, B., Goldberg, A.D. and O'Malley, B.W. (2002) Distinct RNA motifs are important for coactivation of steroid hormone receptors by steroid receptor RNA activator (SRA). Proc. Natl Acad. Sci. USA, 99, 16081 – 16086.
52. Chooniedass-Kothari, S., Hamedani, M.K., Troup, S., Hube, F. and Leygue, E. (2006) The steroid receptor RNA activator protein is expressed in breast tumor tissues. Int. J. Cancer, 118, 1054–1059.
53. Espinoza, C.A., Allen, T.A., Hieb, A.R., Kugel, J.F. and Goodrich, J.A. (2004) B2 RNA binds directly to RNA polymerase II to repress transcript synthesis. Nat. Struct. Mol. Biol., 11, 822–829.
54. Allen, T.A., Von Kaenel, S., Goodrich, J.A. and Kugel, J.F. (2004) The SINE-encoded

mouse B2 RNA represses mRNA transcription in response to heat shock. Nat. Struct. Mol. Biol., 11, 816–821.

55. Martignetti, J.A. and Brosius, J. (1993) BC200 RNA: a neural RNA polymerase III product encoded by a monomeric Alu element. Proc. Natl Acad. Sci. USA, 90, 11563–11567.
56. Valgardsdottir, R., Chiodi, I., Giordano, M., Cobianchi, F., Riva, S. and Biamonti, G. (2005) Structural and functional characterization of non-coding repetitive RNAs transcribed in stressed human cells. Mol. Biol. Cell, 16, 2597–2604.
57. Lakhotia, S.C., Rajendra, T.K. and Prasanth, K.V. (2001) Developmental regulation and complex organization of the promoter of the non-coding hsr(omega) gene of Drosophila melanogaster. J. Biosci., 26, 25–38.
58. Prasanth, K.V., Rajendra, T.K., Lal, A.K. and Lakhotia, S.C. (2000) Omega speckles—a novel class of nuclear speckles containing hnRNPs associated with non-coding hsr-omega RNA in Drosophila. J. Cell. Sci., 113, 3485 – 3497.
59. Nagai, K., Oubridge, C., Kuglstatter, A., Menichelli, E., Isel, C. and Jovine, L. (2003) Structure, function and evolution of the signal recognition particle. EMBO J., 22, 3479 – 3485.
60. Willingham, A.T., Orth, A.P., Batalov, S., Peters, E.C., Wen, B.G., Aza-Blanc, P., Hogenesch, J.B. and Schultz, P.G. (2005) A strategy for probing the function of non-coding RNAs finds a repressor of NFAT. Science, 309, 1570–1573.
61. Thery, C., Zitvogel, L. & Amigorena, S. Exosomes: composition, biogenesis and function. *Nature Rev. Immunol.* 2, 569–579 (2002). This is an excellent early review of the structure and the function of exosomes.
62. Andaloussi, S. E. L., Mager, I., Breakefield, X. O. & Wood, M. J. Extracellular vesicles: biology and emerging therapeutic opportunities. *Nature Rev. Drug Discov.* 12, 347–357 (2013).
63. Thery, C., Ostrowski, M. & Segura, E. Membrane vesicles as conveyors of immune responses. *Nature Rev. Immunol.* 9, 581–593 (2009).
64. Yang, C. & Robbins, P. D. Immunosuppressive exosomes: a new approach for treating arthritis. *Int. J. Rheumatol.* 2012, 573–528 (2012).
65. Booth, A. M. *et al.* Exosomes and HIV Gag bud from endosome-like domains of the T cell plasma membrane. *J. Cell Biol.* 172, 923–935 (2006). This report describes the release of small 30–100 nm vesicles, similar in size to exosomes, from the plasma membrane.
66. Raiborg, C. & Stenmark, H. The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. *Nature* 458, 445–452 (2009).
67. Hurley, J. H. & Hanson, P. I. Membrane budding and scission by the ESCRT machinery: it's all in the neck. *Nature Rev. Mol. Cell Biol.* 11, 556–566 (2010).
68. Ostrowski, M. *et al.* Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nature Cell Biol.* 12, 19–30 (2010). This is the first paper to show an essential role for two specific RAB proteins in extracellular vesicle secretion.
69. Alvarez-Erviti, L. *et al.* Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nature Biotech.* 29, 341–345 (2011).
70. Ohno, S. *et al.* Systemically injected exosomes targeted to EGFR deliver antitumor microRNAto breast cancer cells. *Mol. Ther.* 21, 185–191 (2013).
71. Kog, J. *et al.* Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nature Cell Biol.* 10, 1470–1476 (2008).
72. Valadi, H. *et al.* Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nature Cell Biol.* 9, 654–659 (2007).
    This manuscript is the first to report that mRNAs and ncRNAs, including miRNAs, contained in extracellular vesicles could be functionally exchanged between cells.
73. Luo, S. S. *et al.* Human villous trophoblasts express and secrete placenta-specific microRNAs into maternal circulation via exosomes. *Biol. Reprod.* 81, 717–729 (2009).

74. Michael, A. *et al.* Exosomes from human saliva as a source of microRNA biomarkers. *Oral Dis.* 16, 34–38 (2010).
75. Nolte-'t Hoen, E. N. *et al.* Deep sequencing of RNA from immune cell-derived vesicles uncovers the selective incorporation of small non-coding RNA biotypes with potential regulatory functions. *Nucleic Acids Res.* 40, 9272–9285 (2012).
76. Rabinowits, G., Gercel-Taylor, C., Day, J. M., Taylor, D. D. & Kloecker, G. H. Exosomal microRNA: a diagnostic marker for lung cancer. *Clin. Lung Cancer* 10, 42–46 (2009).
77. Ohshima, K. *et al.* Let-7 microRNA family is selectively secreted into the extracellular environment via exosomes in a metastatic gastric cancer cell line. *PLoS ONE* 5, e13247 (2010).
78. Huan, J. *et al.* RNA trafficking by acute myelogenous leukemia exosomes. *Cancer Res.* 73, 918–929 (2013).
79. Mittelbrunn, M. *et al.* Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen- presenting cells. *Nature Commun.* 2, 282 (2011).
80. Montecalvo, A. *et al.* Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. *Blood* 119, 756–766 (2012). This manuscript indicates that DCs release exosomes with different miRNAs depending on the maturation of the DCs. Moreover, it shows that exosomes fuse with the target DCs to release their exosome content into the DC cytosol.
81. Pegtel, D. M. *et al.* Functional delivery of viral miRNAs via exosomes. *Proc. Natl Acad. Sci. USA* 107, 6328–6333 (2010).
82. ValadiH,EkstromK,BossiosA,SjostrandM,LeeJJ,LotvallJO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9:654-9.
83. Takayuki Kogure, Irene K.Yan,Wen-Lang Lin, and Tushar Patel : Extracellular Vesicle–Mediated Transfer of a Novel Long Noncoding RNA TUC339: A Mechanism of Intercellular Signaling in Human Hepatocellular Cancer . Genes & Cancer 4(7-8) 261–272
84. Michael A, Bajracharya SD, Yuen PS, Zhou H, Star RA, Illei GG, et al. Exosomes from human saliva as a source of microRNA biomarkers. Oral diseases. 2010; 16:34–38.
85. Skog J, Wurdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nature cell biology. 2008; 10:1470–1476.
86. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nature biotechnology. 2011; 29:341–345.
87. Huang et al. : Characterization of human plasma-derived exosomal RNAs by deep sequencing. BMC Genomics 2013, 14:319
88. Buck AH et al.: Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity. Nat Commun. 2014 Nov 25;5:5488.
89. Paul D. Robbins and Adrian E. Morelli: Regulation of immune responses by extracellular vesicles. Nature reviews, Immunology. March 2014; 14:195 -208.