36. Li, J. *et al.* An ACAP1-containing clathrin coat complex for endocytic recycling. *J. Cell Biol.* **178**, 453–464 (2007).
37. Blot, V. & McGraw, T. E. GLUT4 is internalized by a cholesterol-dependent nystatin-sensitive mechanism inhibited by insulin. *EMBO J.* **25**, 5648–5658 (2006).
38. Pellinen, T. *et al.* Integrin trafficking regulated by Rab21 is necessary for cytokinesis. *Dev. Cell* **15**, 371–385 (2008).
39. Teckchandani, A. *et al.* Quantitative proteomics identifies a Dab2/integrin module regulating cell migration. *J. Cell Biol.* **186**, 99–111 (2009).
40. Ezratty, E. J., Bertaux, C., Marcantonio, E. E. & Gundersen, G. G. Clathrin mediates integrin endocytosis for focal adhesion disassembly in migrating cells. *J. Cell Biol.* **187**, 733–747 (2009).
41. Gu, Z., Noss, E. H., Hsu, V. W. & Brenner, M. B. Integrins traffic rapidly via circular dorsal ruffles and macropinocytosis during stimulated cell migration. *J. Cell Biol.* **193**, 61–70 (2011).
42. van Kerkhof, P. *et al.* Sorting nexin 17 facilitates LRP recycling in the early endosome. *EMBO J.* **24**, 2851–2861 (2005).
43. Temkin, P. *et al.* SNX27 mediates retromer tubule entry and endosome-to-plasma membrane trafficking of signalling receptors. *Nature Cell Biol.* **13**, 715–721 (2011).
44. Seaman, M. N., McCaffery, J. M. & Emr, S. D. A membrane coat complex essential for endosome-to-Golgi retrograde transport in yeast. *J. Cell Biol.* **142**, 665–681 (1998).
45. Seaman, M. N. Cargo-selective endosomal sorting for retrieval to the Golgi requires retromer. *J. Cell Biol.* **165**, 111–122 (2004).
46. Arighi, C. N., Hartnell, L. M., Aguilar, R. C., Haft, C. R. & Bonifacino, J. S. Role of the mammalian retromer in sorting of the cation-independent mannose 6-phosphate receptor. *J. Cell Biol.* **165**, 123–133 (2004).
47. Puthenveedu, M. A. *et al.* Sequence-dependent sorting of recycling proteins by actin-stabilized endosomal microdomains. *Cell* **143**, 761–773 (2010).
48. Parachoniak, C. A., Luo, Y., Abella, J. V., Keen, J. H. & Park, M. GGA3 functions as a switch to promote Met receptor recycling, essential for sustained ERK and cell migration. *Dev. Cell* **20**, 751–763 (2011).
49. Cancino, J. *et al.* Antibody to AP1B adaptor blocks biosynthetic and recycling routes of basolateral proteins at recycling endosomes. *Mol. Biol. Cell* **18**, 4872–4884 (2007).
50. Diaz, F. *et al.* Clathrin adaptor AP1B controls adenovirus infectivity of epithelial cells. *Proc. Natl Acad. Sci. USA* **106**, 11143–11148 (2009).
51. Deborde, S. *et al.* Clathrin is a key regulator of basolateral polarity. *Nature* **452**, 719–723 (2008).
52. Carvajal-Gonzalez, J. M. *et al.* Basolateral sorting of CAR through interaction of a canonical YXXΦ motif with the clathrin adaptors AP-1A and AP-1B. *Proc. Natl Acad. Sci. USA* **109**, 3820–3825 (2012).
53. Robinson, M. S. 100-kD coated vesicle proteins: molecular heterogeneity and intracellular distribution studied with monoclonal antibodies. *J. Cell Biol.* **104**, 887–895 (1987).
54. Ahle, S., Mann, A., Eichelsbacher, U. & Ungewickell, E. Structural relationships between clathrin assembly proteins from the Golgi and the plasma membrane. *EMBO J.* **7**, 919–929 (1988).
55. Scheiffele, P., Roth, M. G. & Simons, K. Interaction of influenza virus haemagglutinin with sphingolipid-cholesterol membrane domains via its transmembrane domain. *EMBO J.* **16**, 5501–5508 (1997).
56. Miller, E. A. *et al.* Multiple cargo binding sites on the COPII subunit Sec24p ensure capture of diverse membrane proteins into transport vesicles. *Cell* **114**, 497–509 (2003).
57. Traer, C. J. *et al.* SNX4 coordinates endosomal sorting of TfnR with dynein-mediated transport into the endocytic recycling compartment. *Nature Cell Biol.* **9**, 1370–1380 (2007).
58. Saint-Pol, A. *et al.* Clathrin adaptor epsinR is required for retrograde sorting on early endosomal membranes. *Dev. Cell* **6**, 525–538 (2004).
59. Meyer, C. *et al.* mu1A-adaptin-deficient mice: lethality, loss of AP-1 binding and rerouting of mannose 6-phosphate receptors. *EMBO J.* **19**, 2193–2203 (2000).
60. Raiborg, C. *et al.* Hrs sorts ubiquitinated proteins into clathrin-coated microdomains of early endosomes. *Nature Cell Biol.* **4**, 394–398 (2002).
61. Sachse, M., Urbe, S., Oorschot, V., Strous, G. J. & Klumperman, J. Bilayered clathrin coats on endosomal vacuoles are involved in protein sorting toward lysosomes. *Mol. Biol. Cell* **13**, 1313–1328 (2002).
62. Bonifacino, J. S. & Glick, B. S. The mechanisms of vesicle budding and fusion. *Cell* **116**, 153–166 (2004).
63. Pucadyil, T. J. & Schmid, S. L. Conserved functions of membrane active GTPases in coated vesicle formation. *Science* **325**, 1217–1220 (2009).
64. Peter, B. J. *et al.* BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. *Science* **303**, 495–499 (2004).
65. Itoh, T. *et al.* Dynamin and the actin cytoskeleton cooperatively regulate plasma membrane invagination by BAR and F-BAR proteins. *Dev. Cell* **9**, 791–804 (2005).
66. Lee, M. C. *et al.* Sar1p N-terminal helix initiates membrane curvature and completes the fission of a COPII vesicle. *Cell* **122**, 605–617 (2005).
67. Krauss, M. *et al.* Arf1–GTP-induced tubule formation suggests a function of Arf family proteins in curvature acquisition at sites of vesicle budding. *J. Biol. Chem.* **283**, 27717–27723 (2008).
68. Lundmark, R., Doherty, G. J., Vallis, Y., Peter, B. J. & McMahon, H. T. Arf family GTP loading is activated by, and generates, positive membrane curvature. *Biochem. J.* **414**, 189–194 (2008).
69. Beck, R. *et al.* Membrane curvature induced by Arf1–GTP is essential for vesicle formation. *Proc. Natl Acad. Sci. USA* **105**, 11731–11736 (2008).
70. Slot, J. W., Geuze, H. J., Gigengack, S., Lienhard, G. E. & James, D. E. Immuno-localization of the insulin regulatable glucose transporter in brown adipose tissue of the rat. *J. Cell Biol.* **113**, 123–135 (1991).
71. Dunn, K. W., McGraw, T. E. & Maxfield, F. R. Iterative fractionation of recycling receptors from lysosomally destined ligands in an early sorting endosome. *J. Cell Biol.* **109**, 3303–3314 (1989).
72. Pastan, I. & Willingham, M. C. Receptor-mediated endocytosis: coated pits, receptorsomes and the Golgi. *Trends Biochem. Sci.* **8**, 250–254 (1983).
73. Klausner, R. D., Ashwell, G., van Renswoude, J., Harford, J. B. & Bridges, K. R. Binding of apotransferrin to K562 cells: explanation of the transferrin cycle. *Proc. Natl Acad. Sci. USA* **80**, 2263–2266 (1983).
74. Cai, H., Reinisch, K. & Ferro-Novick, S. Coats, tethers, Rabs, and SNAREs work together to mediate the intracellular destination of a transport vesicle. *Dev. Cell* **12**, 671–682 (2007).
75. Collawn, J. F. *et al.* Transferrin receptor internalization sequence YXRF implicates a tight turn as the structural recognition motif for endocytosis. *Cell* **63**, 1061–1072 (1990).
76. Ohno, H. *et al.* Interaction of tyrosine-based signals with clathrin-associated proteins. *Science* **269**, 1872–1875 (1995).
77. Boll, W. *et al.* Sequence requirements for the recognition of tyrosine-based endocytic signals by clathrin AP-2 complexes. *EMBO J.* **15**, 5789–5795 (1996).
78. Cosson, P. & Letourneur, F. Coatomer interaction with di-lysine endoplasmic reticulum retention motifs. *Science* **263**, 1629–1631 (1994).
79. Letourneur, F. *et al.* Coatomer is essential for retrieval of dilysine-tagged proteins to the endoplasmic reticulum. *Cell* **79**, 1199–1207 (1994).
80. Nishimura, N. & Balch, W. E. A di-acidic signal required for selective export from the endoplasmic reticulum. *Science* **277**, 556–558 (1997).
81. Kappeler, F., Klopfenstein, D. R., Foguet, M., Paccaud, J. P. & Hauri, H. P. The recycling of ERGIC-53 in the early secretory pathway. ERGIC-53 carries a cytosolic endoplasmic reticulum-exit determinant interacting with COPII. *J. Biol. Chem.* **272**, 31801–31808 (1997).

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The authors declare no competing financial interests.

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OPINION

Intercellular communication: diverse structures for exchange of genetic information

Maria Mittelbrunn and Francisco Sánchez-Madrid

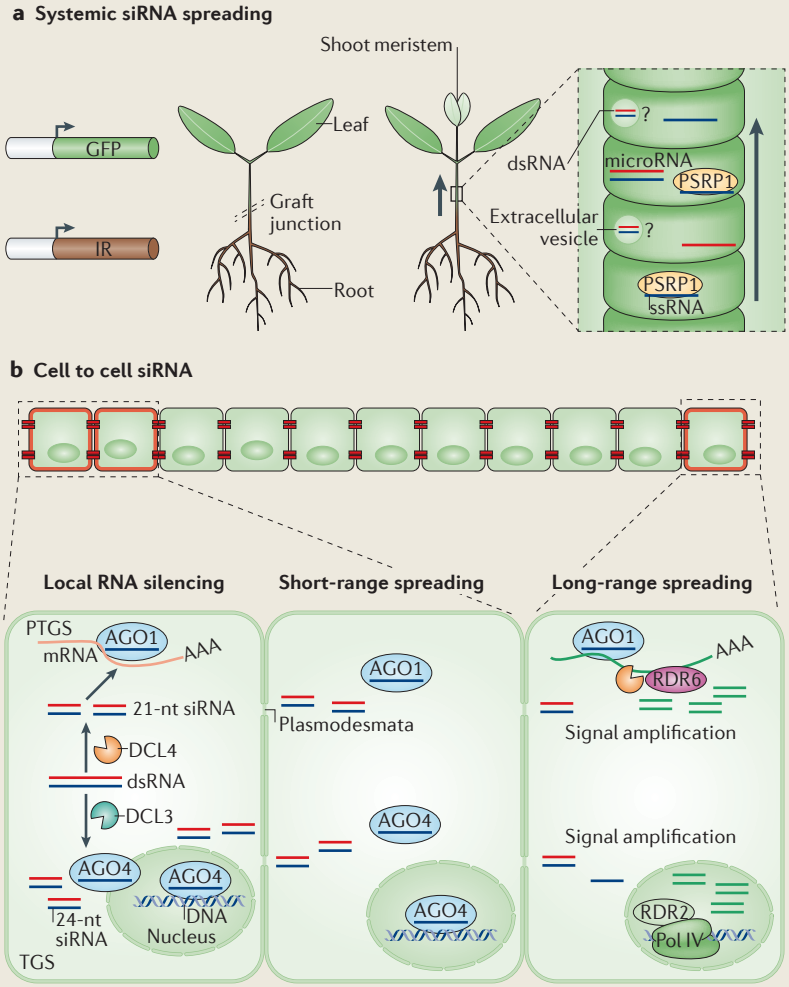
Abstract | An emerging concept is that cellular communication in mammals can be mediated by the exchange of genetic information, mainly in the form of microRNAs. This can occur when extracellular vesicles, such as exosomes, secreted by a donor cell are taken up by an acceptor cell. Transfer of genetic material can also occur through intimate membrane contacts between donor and acceptor cells. Specialized cell–cell contacts, such as synapses, have the potential to combine these modes of genetic transfer.

Cell-to-cell communication allows the coordination of cell functions, which is important for the development and environmental adaptation of multicellular organisms. Communication often involves soluble factors, such as cytokines, chemokines, growth factors and neurotransmitters, and their specific recognition by cell-surface receptors. Recent evidence indicates that cells also communicate via the direct exchange of RNA. When eukaryotic cells encounter double-stranded RNA (dsRNA), genes carrying a matching sequence are silenced through RNA interference (RNAi).

Box 1 | **Mechanisms of systemic RNA silencing in plants**

RNA interference (RNAi) can move from cell to cell through plasmodesmata and spread throughout the organism via the phloem[1,2]. Plants can transport RNA from older tissues to the root or shoot meristems along the direction of phloem flow. Long-distance spreading of RNA (which occurs via small RNAs (sRNAs)) has been demonstrated by grafting rootstocks that express an inverted repeat (IR) that targets green fluorescent protein (GFP) mRNA (see the figure, part **a**). The recipient grafted tissues (shown in dark green) overexpress GFP. Emerging new growth is uniformly silenced (shown in pale green), suggesting the propagation of a GFP-specific silencing signal. The silencing signal might be transported as single-stranded RNA (ssRNA) bound to phloem small RNA-binding protein 1 (PSRP1) or possibly inside extracellular vesicles (as double-stranded RNA (dsRNA)) (see the figure, part **a**).

Plasmodesmata are dynamic intercellular channels that establish symplasmic continuity between neighbouring cells (see the figure, part **b**). dsRNA, derived from transgenic or endogenous loci, is processed by Dicer-like enzymes (DCLs). DCL3 and DCL4 produce primary 24-nucleotide (nt) or 21-nt small interfering RNAs (siRNAs), respectively. The 24-nt siRNA can be directed to the transcriptional gene silencing (TGS) pathway by Argonaute 4 (AGO4), whereas the 21-nt siRNA binds to AGO1, which results in the cleavage of complementary mRNAs via the post-transcriptional gene silencing (PTGS) pathway (local silencing). Both 21-nt and 24-nt duplexes can move from cell to cell through plasmodesmata (short-range spreading). It is unclear whether RNAs move through plasmodesmata freely by diffusion or through a regulated mechanism involving protein interactions. Silencing signals can travel through 10 to 15 contacting cells without amplification, but RNA-dependent RNA polymerases (RDRs) can direct the synthesis of secondary siRNAs to amplify and spread the silencing information (long-range spreading). Both siRNA types can initiate signal amplification in recipient tissues via the actions of RNA polymerase IV (Pol IV) and RDRs.



The surprising finding is that, in some animals and plants, the transport of a silencing signal between cells allows the same gene to be specifically silenced in cells that had not encountered the primary dsRNA. This process has been best characterized in plants and *Caenorhabditis elegans*[1].

In plants, silencing RNAs move from cell to cell through plasmodesmata and over long distances through the phloem vascular tissue[2]. When a leaf is infected with a plant virus, mobile signals transmitted to other leaves confer them resistance to the virus and thus prevent the spread of the infection. Although viral-induced small interfering RNAs (siRNAs) and transgenes were known for many years to move through the plant, the movement of endogenous small RNAs (sRNAs) has only recently been demonstrated[3–5]. The mobility of endogenous sRNAs, including microRNAs (miRNAs), generates morphogenic signalling gradients that guide the patterning of leaves and roots[3]. Mobile sRNAs promote epigenetic modifications in the genome of recipient cells[5]. Furthermore, when the recipient cells are seed or pollen, mobile sRNAs induce transgenerational epigenetic changes to enhance adaptation of progeny to future stresses[6] (BOX 1). In *C. elegans*, silencing triggered by injected, ingested or locally expressed dsRNA can spread throughout the organism to silence the targeted gene in all non-neuronal cells, including the germ line, and thus transmit silencing to the next generation[7–10].

The exchange of genetic information between mammalian cells is a more recent concept. sRNAs have been detected in blood and other body fluids such as urine, saliva and milk. Most circulating sRNAs are contained within lipids or lipoprotein complexes, apoptotic bodies or exosomes, which efficiently protect them from degradation by serum ribonucleases. Recent reports indicate that RNAs, contained within exosomes, are transferred to recipient cells and modulate the function of the cell[11–14].

In this Opinion article, we discuss recent studies that describe the trafficking of genetic material in mammals, focusing on extracellular vesicles and the diverse structures that cells utilize for communication. We also discuss the potential of cellular synapses and other connective structures to act as specialized devices for mediating intercellular transfer of genetic material and explore the potential biological relevance of this mode of communication.

Genetic transfer via extracellular vesicles

Until recently, firm evidence for intercellular transfer of RNA in mammals remained elusive. The discovery that extracellular vesicles contain genetic material that can be exchanged between cells, either directly or via body fluids, supports the notion that this form of exchange of genetic information is biologically significant in mammals.

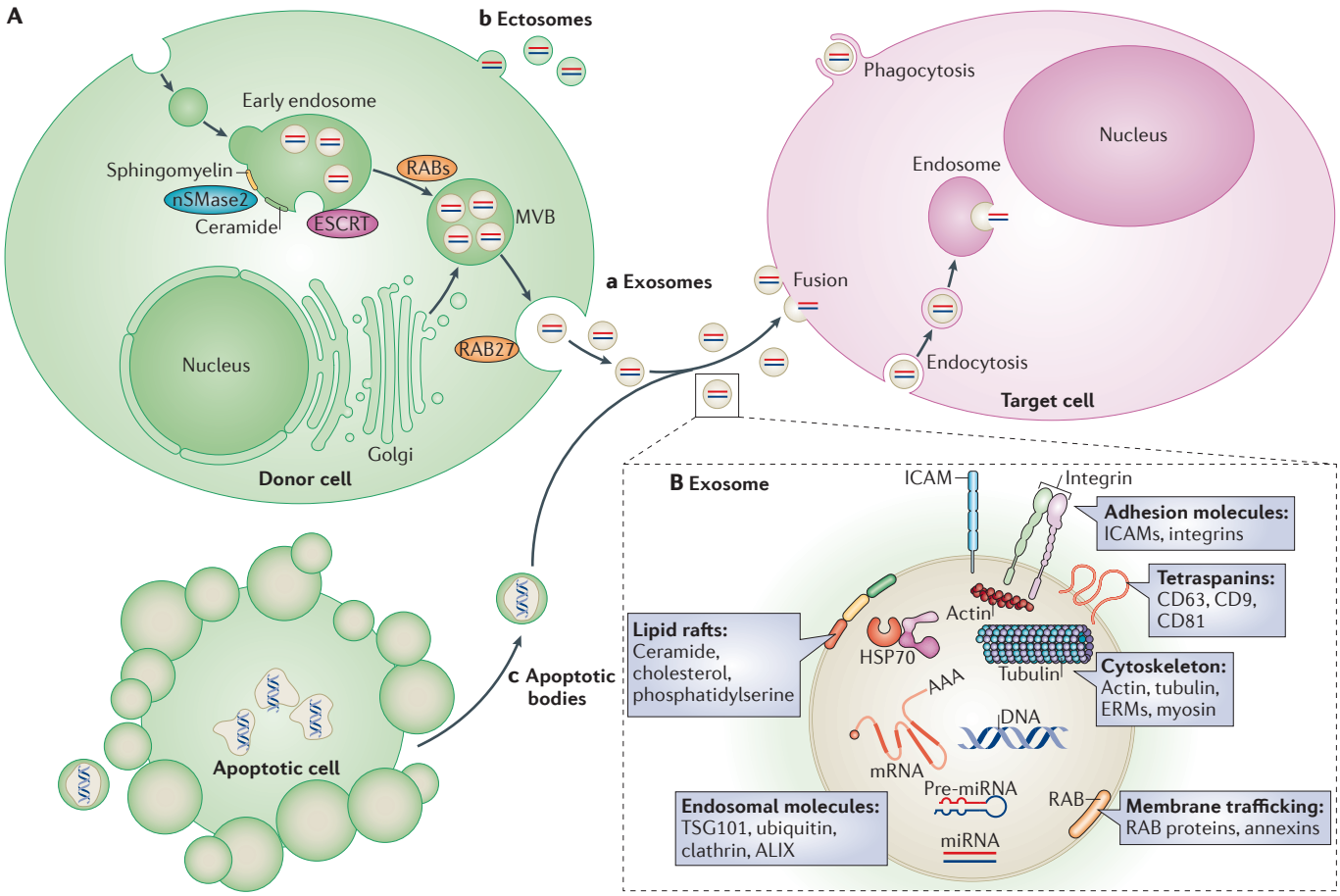


Figure 1 | **Long-distance transfer of genetic material in extracellular vesicles. A** | Extracellular vesicles originate through at least three mechanisms. **Aa** | The fusion of multivesicular bodies (MVBs) with the plasma membrane and the release of their intraluminal vesicles (ILVs) as exosomes. Neutral sphingomyelinase 2 (nSMase2) is essential for the formation of ILVs in the early endosome. Some proteins are channelled by the ESCRT (endosomal sorting complex required for transport) machinery to the MVB route. RAB proteins such as RAB11, RAB27 and RAB35, which are known to participate in vesicle trafficking among intracellular compartments, have been shown to have a role in exosome secretion. **Ab** | Blebbing of the cellular plasma membrane (ectosomes). **Ac** | Breakdown of dying cells into apoptotic bodies. Extracellular vesicles, which are secreted into the extracellular environment, contain functional mRNA, microRNA (miRNA) and DNA molecules that can be taken up by recipient cells through mechanisms including fusion with the plasma membrane, phagocytosis and endocytosis. **B** | All exosomes contain proteins involved in membrane transport and fusion (such as RAB proteins and annexins), cytoskeletal proteins, adhesion molecules and tetraspanins, as well as RNA (mainly miRNA). Exosome membranes are enriched in raft lipids such as cholesterol, ceramide and sphingolipids. ALIX, apoptosis-linked gene 2-interacting protein X; ERM, ezrin–radixin–moesin; HSP70, heat shock protein 70; ICAM, intercellular cell adhesion molecule; TSG101, tumour susceptibility gene 101.

Biogenesis of extracellular vesicles. Depending on their origin, extracellular vesicles are classified as exosomes, shedding vesicles or apoptotic bodies[15] (FIG. 1). Exosomes, which have a diameter of 30-100 nm, are intraluminal vesicles (ILVs), which form inside late endosomes (also known as multivesicular bodies (MVBs))[16]. MVBs release exosomes by fusing with the plasma membrane. The endosomal origin of exosomes is reflected in their molecular composition, which includes apoptosis-linked gene 2-interacting protein X (ALIX; also known as PDCD6IP), CD63 and tumour susceptibility gene 101 (TSG101). All exosomes contain proteins that are involved in membrane transport and fusion (such as RAB GTPases, annexins and others). Cytoskeletal proteins, adhesion molecules and tetraspanin family proteins (such as tetraspanins CD81, CD82 and CD63) are also abundant. Furthermore, exosome membranes have a specific lipid composition, being enriched in lipids such as cholesterol, ceramide and sphingolipids. Ceramide is involved in the budding of ILVs into MVBs. Consistently, blockade of neutral sphingomyelinase 2 (nSMase2), which is an enzyme involved in ceramide synthesis, inhibits exosome production[17]. In mammals, the ESCRT complex (endosomal sorting complex required for transport) might not control MVB formation but could function to ensure proper ILV composition[18]. Several RAB proteins (such as RAB11, RAB27 and RAB35) contribute to exosome secretion[19]. The final step of exosome secretion, which is the fusion of MVBs with the plasma membrane, is likely to involve a complex of SNARE proteins[19]. Exosomes are released constitutively, although their secretion can be increased in response to cell activation or stress.

Ectosomes, which are also termed shedding vesicles, are usually larger than exosomes (with a diameter of 100 nm to 1 μm) and are produced by direct plasma membrane blebbing[20]. They are thought to arise from regions of the membrane enriched in lipid rafts, and they expose phosphatidylserine in the outer leaflet of

their membrane. Except for tumour cells, which constitutively shed large numbers of ectosomes, the rate of release is low and can be increased by cell activation or apoptosis.

Apoptotic bodies are larger than ectosomes or exosomes (>1 μm in diameter) and are released as blebs of apoptotic cells. They are characterized by phosphatidylserine externalization and contain fragmented DNA.

Consequences of genetic transfer

Extracellular vesicles have emerged as potent vehicles for cell-to-cell communication since the discovery that they contain functional mRNA, miRNA and DNA molecules that can be taken up by target (acceptor) cells[12–14,21,22]. The genetic information contained in extracellular vesicles can influence or even direct the fate of the target cell, for example by triggering target cell activation, migration, differentiation or de-differentiation or by promoting apoptosis or necrosis.

Early studies showed that secreted vesicles derived from embryonic stem cells or tumour cells contain mRNA[21], and that these transcripts can be delivered to target cells where they are translated into functional proteins[11]. Subsequently, extracellular vesicles were shown to also contain sRNA species[12]. Even DNA can be transferred, for example to fibroblasts and endothelial cells, through phagocytosis of tumour apoptotic bodies[22]. In addition, viral miRNAs[23,24] and retrotransposon RNA transcripts from human endogenous retroviruses[25] can be detected in extracellular vesicles and can be transferred to acceptor cells[24]. The genetic content of extracellular vesicles is not simply a straightforward reflection of the genetic content of the cell of origin; specific populations of RNAs are selectively packaged into exosomes[11,13,14], indicating the existence of an as yet unknown mechanism controlling the sorting of specific RNAs. It is likely that the ESCRT protein complex is a mediator of this process, as ESCRTII can directly bind RNA[26] and the blockade of MVB formation by ESCRT depletion impairs miRNA silencing[27,28]. Another possibility is that the nucleic acid sequences themselves directly control their trafficking to extracellular vesicles.

Once they are released, extracellular vesicles can either target a nearby cell or reach a distant cell by entering the bloodstream, but the mechanisms by which target cells take up and integrate RNA carried by extracellular vesicles are poorly understood. The functional consequences of this transfer depend on the origin and status of the donor and recipient cells. For example,

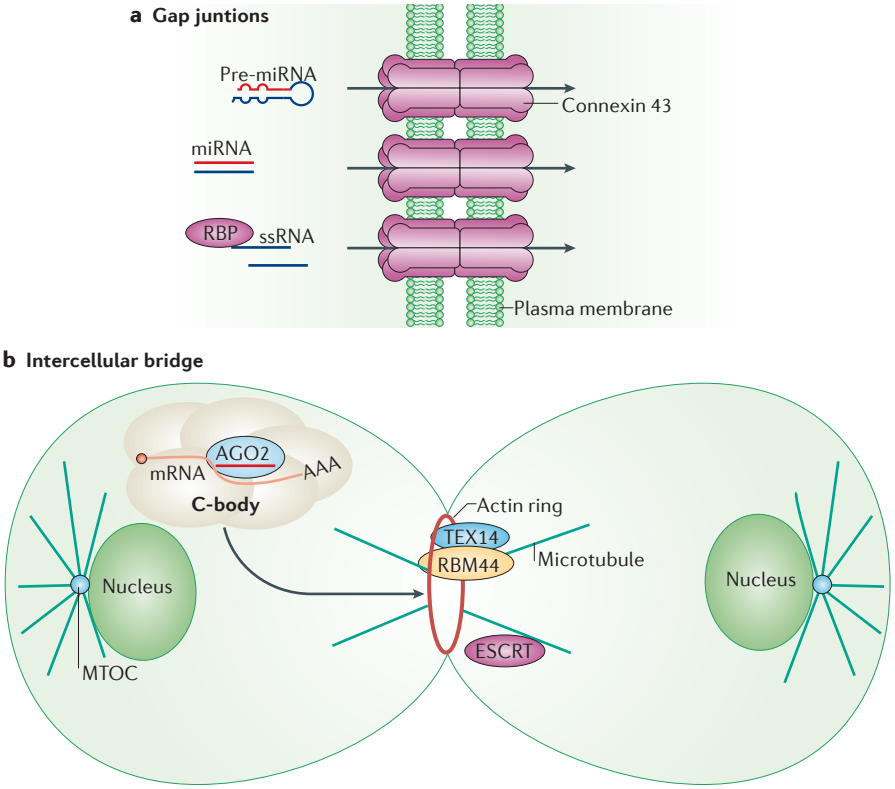


Figure 2 | **Connective structures for short-distance transfer of genetic material.** Intercellular communication can occur over short distances through the establishment of gap junctions or germ cell intercellular bridges. **a** | Gap junctions are composed of hexameric connexin oligomers that allow trafficking of small molecules between adjacent cells. The silencing signal might be transported as single-stranded RNA (ssRNA) associated with RNA-binding proteins (RBPs) or as double-stranded small RNA (microRNA (miRNA) or its precursor pre-miRNA). **b** | Intercellular bridges are formed in the germ line by incomplete cytokinesis and contain an actin ring. Testis-expressed protein 14 (TEX14) and RNA binding motif protein 44 (RBM44) locate at intercellular bridges. These bridges support cell-to-cell transfer of chromatoid bodies (C-bodies), which are cytoplasmic granules enriched in miRNAs, mRNAs and proteins of the miRNA–RISC complex. AGO2, Argonaute 2; ESCRT, endosomal sorting complex required for transport; MTOC, microtubule-organizing centre.

extracellular vesicles derived from endothelial progenitors drive angiogenesis via the horizontal transfer of mRNAs to quiescent endothelial cells[29]. Apoptotic bodies can transfer miR-126 to endothelial cells; this miRNA silences the endogenous G protein signalling inhibitor RGS16 (regulator of G protein signalling 16), and this promotes an autoregulatory feedback loop in recipient cells that increases secretion of CXCL12, inducing endothelial repair and protecting against diet-induced atherosclerosis[30]. Furthermore, extracellular vesicles from patients with atherosclerosis are enriched in miR-150 and promote endothelial cell migration by downregulating the miR-150 target c-MYB[31]. miR-143 and miR-145 are enriched in extracellular vesicles produced by endothelial cells and can be transferred to smooth muscle cells[32].

This mechanism of cell-to-cell behavioural regulation is particularly important in cancer. Tumour cells produce abundant extracellular vesicles, with the potential to influence the behaviour of surrounding healthy cells in order to facilitate tumour growth, metastasis or immune evasion. Tumour-cell-derived extracellular vesicles can induce angiogenesis by delivering RNA to endothelial cells[11,33] and can also deliver DNA (including oncogenes such as *c-Myc*) or retrotransposon RNA transcripts from human endogenous retroviruses to normal cells[25]. In the immune system, exosomes from T cells or dendritic cells can fuse with the plasma membrane of target cells, releasing the cargo of functional miRNAs into their cytoplasm[13,14].

Vesicle-free circulating RNA

miRNA can also circulate in body fluids in a vesicle-independent form[34–37]. Argonaute 2 (AGO2), the key effector protein of miRNA-mediated silencing, forms circulating ribonucleoprotein complexes

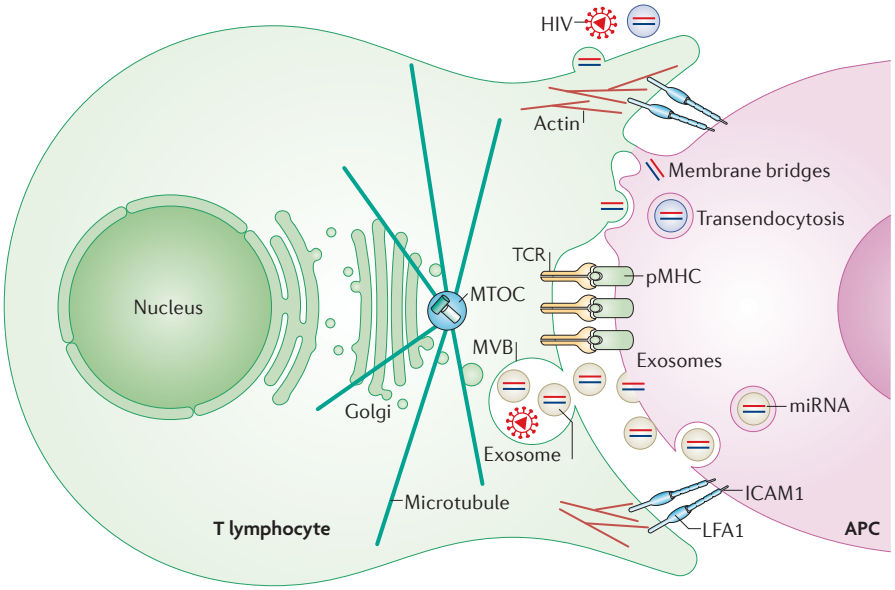


Figure 3 | **The immune synapse acts as a platform facilitating the passage of genetic material between cells.** During immune synapsis, the molecules involved in antigen recognition (such as T cell receptor (TCR) and peptide-loaded major histocompatibility complex (pMHC) molecules) move to a central cluster surrounded by a peripheral ring enriched in adhesion molecules (such as the integrin leukocyte function-associated antigen 1 (LFA1) and intercellular cell adhesion molecules (ICAMs)) and the actin cytoskeleton. The T lymphocyte orients its microtubule-organizing centre (MTOC) and secretory compartments (such as the Golgi apparatus and multivesicular bodies (MVBs)) towards the antigen presenting cell (APC). We propose that the immune synapse provides a more efficient path for the exchange of genetic material through the combination of different mechanisms, including the polarized secretion of microRNA (miRNA)-loaded exosomes, transendocytosis and membrane bridges. Pathogens, including bacteria and viruses, hijack biological synapses to spread from cell to cell.

with miRNAs[34,35]. Other associated RNA-binding proteins, such as nucleophosmin 1 (NPM1), can form complexes with miRNAs and protect them from degradation. Although the mechanism that regulates export of circulating ribonucleoprotein complexes is unknown, it seems to be an active energy-dependent process.

Several screens have identified the genes involved in dsRNA uptake and spreading in *C. elegans*. Cellular uptake of dsRNA involves SID-1 (systemic RNAi defective protein 1). SID-1, which is a multipass transmembrane protein expressed by all non-neuronal cells, allows long dsRNAs and their derivatives to move passively from cell to cell across the cell membrane[10]. The potential ability of SID-1 to transport endogenous dsRNA is supported by recent evidence showing that SID-1 imports synthetic miRNA precursors and long hairpin molecules[38]. Another gene involved in dsRNA transfer in *C. elegans* is *rsd-3*, a homologue of the mammalian protein epsin-related protein (epsinR; also known as CLINT1), which is involved in vesicle trafficking through its epsin amino-terminal homology (ENTH) domain[9]. In mammals, miRNAs can also be transported by high-density lipoproteins (HDLs) and be delivered to recipient cells to modulate their function[36]. Inhibition of nSMase2, which blocks the release of miRNA-loaded exosomes[13,17,39], increases the amount of miRNAs exported to HDLs. Thus, nSMase2 and the ceramide pathway might regulate different but coordinated pathways of miRNA secretion. HDL–miRNA delivery to recipient cells is dependent on scavenger receptor class B type I (SRBI)[36]. Mammalian homologues of *C. elegans* SID proteins exist and could also participate in lipid-modified siRNA uptake[40].

Cell-to-cell genetic transfer

Although transfer of genetic material does not require cell-to-cell contact, it has been suggested that, as occurs in plants, sRNAs move between mammalian cells through highly organized cell–cell structures such as gap junctions, intercellular bridges or synapses (FIGS 2,3).

Gap junctions are formed by hexameric connexin oligomers that allow transfer of small molecules (<1.2 kDa), such as ions and small metabolites, that are required for electromechanical connections between neuronal cells, smooth-muscle cells and epithelial cells. Gap junctions are true gates and exist in an open or a closed state. This state is regulated by post-translational modifications of connexins (for example, redox state or phosphorylation) or variations in transmembrane physical–chemical conditions (for example, transmembrane voltage, pH and extracellular cation concentration). siRNAs have been suggested to move through connexin-43-based gap junctions[41]. Shuttling of miRNAs through gap junctions has been described between cardiac cells[42,43], bone-marrow stromal and tumour cells[44] and glioma cells[45]. Transfer of siRNAs and miRNAs is impaired by overexpressing a dominant-negative connexin 43 mutant[42] and by gap junction channel uncouplers[43–45]. In these studies, the passage of fluorescently tagged siRNA analogues or overexpressed miRNAs was observed. However, further studies are needed to confirm physiological transfer of RNAs. The molecular pathways regulating the movement of sRNAs through gap junctions are as yet unknown.

At the end of cytokinesis, daughter mammalian cells are transiently connected by an intercellular bridge. But in the germ line, these transient structures are transformed into stable intercellular bridges that interconnect hundreds of daughter cells in a syncytium. Intercellular bridges are essential for male fertility. They are composed of general cytokinesis molecules and additional germ cell-specific factors such as testis-expressed protein 14 (TEX14). TEX14 is required for the intercellular bridge stability in gametes of both sexes. RNA binding motif protein 44 (RBM44) is found at intercellular bridges and interacts with TEX14, and it may participate in RNA transport. Intercellular bridges allow sharing of mRNA between post-meiotic haploid spermatids, keeping them phenotypically diploid[46]. Cytoplasmic granules loaded with RNA and RNA binding proteins (which in germ cells have been called chromatoid bodies (C-bodies) and are related to processing bodies (P-bodies)) can move between spermatids through the intercellular bridge[47]. Nonetheless, evidence supporting cell-to-cell movement of sRNA through gap junctions or intercellular bridges is scarce, and other mechanisms such as nanotubes[48] cannot be excluded.

Genetic exchange through synapses

Recent evidence indicates that specialized junctional structures such as synapses constitute efficient communication gateways. The intrinsic stability of these junctions promotes the exchange of vesicles and the

formation of additional structures, providing an appropriate environment for the exchange of genetic material. Immune synapses are formed at the T cell–antigen-presenting cell (APC) interface during antigen recognition, and these synapses have a central role in T cell activation and in the polarized delivery of effector molecules such as cytokines and lytic granules[49]. The T cell receptor (TCR) and associated signalling protein complexes accumulate in a central cluster (central supramolecular activating complex (cSMAC)) surrounded by a peripheral ring of adhesion molecules (pSMAC). Signalling triggers massive reorganization of the actin and microtubule cytoskeletons, with the T cell microtubule-organizing centre (MTOC) moving towards the plasma membrane at the cSMAC[50]. Both the Golgi and MVBs localize at the synapse[13,51], where exocytosis and endocytosis occur[52] (FIG. 3).

We propose that immune synapses facilitate the passage of genetic molecules between cells. The immune synapse promotes exchange of miRNA-loaded exosomes between a T lymphocyte and its cognate APC[13]. Transfer of plasma membrane-associated proteins among interacting immune cells is well established[53]. Besides promoting the exchange of exosomes, immune synapses also might facilitate transendocytosis and the formation of nanotubes, gap junctions and membrane bridges between the two cell membranes that mediate direct exchange of proteins[54–58] (FIG. 3). Whether regulatory RNA transfer between immune cells occurs through these structures needs to be addressed.

In the neural system, intercellular communication is primarily mediated by the release of neurotransmitters from the axon of the afferent neuron into the synaptic cleft and the capture of these neurotransmitters by the dendritic spines of the efferent neuron or by direct conduction through gap junctions. Within neurons, large amounts of mRNA, tRNA, ribosomal RNA (rRNA) and sRNA are transported from soma to distal growth cones or distal dendrites for local translation. In addition, Schwann cells can deliver ribosomes, and probably mRNA, to injured axons[59]. MVBs are detected in dendrites and presynaptic terminals, where they can fuse with the plasma membrane to release exosomes into the synaptic cleft under the control of glutamatergic synaptic activity[60]. Exosomes mediate transsynaptic protein transfer at *Drosophila melanogaster* neuromuscular junctions[61], raising the intriguing possibility that neurons might also exchange information in the form of RNA[62].

Pathogens hijack biological synapses to spread efficiently from cell to cell, reflecting the relevance of synapses as an entry port for exogenous genetic material. Some viruses use existing synapses to promote viral spreading, and viruses can also promote the establishment of new contacts (viral synapses) between infected and uninfected cells[63,64]. The exosomal machinery is also exploited by pathogens. For example, HIV particles hijack dendritic cell exosomes to release virus and infect T cells[65], and B cells infected with Epstein–Barr virus transfer exosomes containing viral miRNA to target cells, where the miRNA acts[23] (FIG. 3).

Conclusions and perspectives

Accumulating evidence suggests that genetic material, mainly in the form of regulatory RNAs, can be exchanged between cells as extracellular information. Movement of viral-induced siRNAs and transgenes in plants has been known since 1997. However, the movement of endogenous sRNAs and its functional implications have only recently been demonstrated[3–5]. In *C. elegans*, transfer of exogenous and transgenic RNA is well supported, but evidence for functional transfer of endogenous RNA is still lacking. In *D. melanogaster*, uninfected cells can take up viral dsRNA, a mechanism of virus-specific intracellular immunity that prevents subsequent infection and virus spread[66]. The evidence discussed here indicates that movement and intercellular transfer of regulatory RNA also occurs between mammalian cells.

Transfer of genetic material adds an exciting and novel dimension to the cell-to-cell communication modes in complex organisms. However, important questions remain. Defining the routes through which cell-to-cell genetic transfer occurs is a major area of research. Extracellular vesicles were among the first vehicles confirmed to transfer genetic material, and some of the mechanisms through which extracellular vesicles are shuttled from cell to cell have been elucidated. However, the biological significance of this transfer remains unclear. *In vitro* approaches based on overexpression of mRNA or miRNA have shown that these molecules are functional in their new location. This phenomenon is extremely interesting from the point of view of gene therapy. The outstanding challenges now are to define whether transfer occurs at endogenous levels of these molecules and to elucidate the physiological importance of this movement.

Substantial progress has been made in plants regarding the nature of the information that is transferred. RNA duplexes of 21 or 24 nucleotides have been shown to move from cell to cell through plasmodesmata, and silencing signals may also be transported as single-stranded RNA (ssRNA) through the phloem. In *C. elegans*, SID-1 and SID-2 are thought to mediate the cellular uptake of dsRNA. The nature of the sRNAs that can be transferred in mammals still needs to be defined.

A phenomenon apparently exclusive to plants and *C. elegans* is that the silencing signal can be amplified, thereby inducing systemic silencing. Amplification of the

Box 2 | The therapeutic and diagnostic potential of RNA-based communication

Circulating cell-free nucleic acids have promising potential as non-invasive diagnostic markers for pathological processes such as chronic inflammation, cancer and cardiovascular disease[70,71]. Detection of genetic biomarkers in the blood of patients with cancer might provide insight into the genetic status of individual tumours and the tissue origin of cancers of unclear primary origin[72]. Another exciting area is the existence of cell-free fetal DNA and mRNA in the blood of pregnant women, as this opens the possibility of extending the current armoury of non-invasive methods for prenatal diagnosis[73].

Delivery of nucleic acids to target cells is a major challenge for clinical medicine, offering a possible therapeutic strategy for regenerative medicine and the treatment of cancer and viral infection. The main obstacle to achieving gene silencing by RNA interference (RNAi) technologies *in vivo* is the delivery to specific cells and tissue. Several strategies have been suggested for systemic delivery of small interfering RNA (siRNA). One strategy involves chemically modified siRNAs, synthetic nanoparticles or exosomes[74]. An advantage of exosomes as RNA delivery vehicles is that they do not activate the interferon response and are, in principle, safer and more manageable than cell therapy. Unlike soluble factors, exosomes are protected from the environment by their lipid bilayer and can reach their target cells. A second advantage is that extracellular vesicles can deliver multiple messages simultaneously, including specific subsets of mRNA, microRNA (miRNA) or proteins. This factor highlights the need to expand our knowledge of how specific genetic messages are selected for incorporation into exosomes, and how vesicles that contain mRNA and miRNA are released and circulate in the bloodstream.

RNAi signal involves RNA-dependent RNA polymerases (RDRs), which copy secondary siRNAs corresponding to flanking sequences upstream or downstream of the originally targeted sequence. The secondary siRNAs are thus able to target parts of the mRNA not targeted by the original siRNA[67,68]. The first functional mammalian homologue of RDRs was recently identified in human cells[69]. Future challenges include determining whether amplification of RNAi signals occurs in mammals and whether RDR-related molecules are involved.

The exchange of genetic material occurs mainly in two ways and, depending on the pathway used, genetic communication can occur over a distance or be confined to the local microenvironment. In one mode of transfer, particles secreted by the donor cell are taken up by an acceptor cell. These particles include extracellular vesicles such as exosomes and apoptotic bodies or ribonucleoproteins. The other mode of transfer involves the formation of intimate membrane contacts between donor and acceptor cells, such as gap junctions in animal cells and plasmodesmata in plants. Specialized contacts such as cellular synapses combine all these modes of genetic transfer. Cellular synapses and other cellular connective structures can provide specialized platforms for the intercellular transfer of genetic material. These structures could potentially be manipulated to achieve specific and efficient delivery of regulatory RNAs to either potentiate or suppress processes in the acceptor cell. The design of therapeutic delivery systems to take advantage of connective intercellular structures such as synapses holds the promise of specific delivery of 'therapeutic' exosomes to selected target cells. The therapeutic and diagnostic potential of RNA-based communication is just emerging (BOX 2).

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1. Melnyk, C. W., Molnar, A. & Baulcombe, D. C. Intercellular and systemic movement of RNA silencing signals. *EMBO J.* **30**, 3553–3563 (2011).
2. Brosnan, C. A. & Voinnet, O. Cell-to-cell and long-distance siRNA movement in plants: mechanisms and biological implications. *Curr. Opin. Plant Biol.* **14**, 580–587 (2011).
3. Carlsbecker, A. *et al.* Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature* **465**, 316–321 (2010).
4. Dunoyer, P. *et al.* Small RNA duplexes function as mobile silencing signals between plant cells. *Science* **328**, 912–916 (2010).
5. Molnar, A. *et al.* Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. *Science* **328**, 872–875 (2010).
6. Slotkin, R. K. *et al.* Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. *Cell* **136**, 461–472 (2009).
7. Fire, A. *et al.* Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806–811 (1998).
8. Jose, A. M., Garcia, G. A. & Hunter, C. P. Two classes of silencing RNAs move between *Caenorhabditis elegans* tissues. *Nature Struct. Mol. Biol.* **18**,1184–1188 (2011).
9. Whangbo, J. S. & Hunter, C. P. Environmental RNA interference. *Trends Genet.* **24**, 297–305 (2008).
10. Winston, W. M., Molodowitch, C. & Hunter, C. P. Systemic RNAi in *C. elegans* requires the putative transmembrane protein SID-1. *Science* **295**, 2456–2459 (2002).
11. Skog, J. *et al.* Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nature Cell Biol.* **10**, 1470–1476 (2008).
12. 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).
13. Mittelbrunn, M. *et al.* Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. *Nature Commun.* **2**, 282 (2011).
14. Montecalvo, A. *et al.* Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. *Blood* **119**, 756–766 (2012).
15. Thery, C., Ostrowski, M. & Segura, E. Membrane vesicles as conveyors of immune responses. *Nature Rev. Immunol.* **9**, 581–593 (2009).
16. Simons, M. & Raposo, G. Exosomes — vesicular carriers for intercellular communication. *Curr. Opin. Cell Biol.* **21**, 575–581 (2009).
17. Trajkovic, K. *et al.* Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science* **319**, 1244–1247 (2008).
18. Babst, M. MVB vesicle formation: ESCRT-dependent, ESCRT-independent and everything in between. *Curr. Opin. Cell Biol.* **23**, 452–457 (2011).
19. Bobrie, A., Colombo, M., Raposo, G. & Thery, C. Exosome secretion: molecular mechanisms and roles in immune responses. *Traffic* **12**, 1659–1668 (2011).
20. Cocucci, E., Racchetti, G. & Meldolesi, J. Shedding microvesicles: artefacts no more. *Trends Cell Biol.* **19**, 43–51 (2009).
21. Baj-Krzyworzeka, M. *et al*. Tumour-derived microvesicles carry several surface determinants and mRNA of tumour cells and transfer some of these determinants to monocytes. *Cancer Immunol. Immunother.* **55**, 808–818 (2006).
22. Ehnfors, J. *et al.* Horizontal transfer of tumor DNA to endothelial cells *in vivo*. *Cell Death Differ.* **16**, 749–757 (2009).
23. Pegtel, D. M. *et al.* Functional delivery of viral miRNAs via exosomes. *Proc. Natl Acad. Sci. USA* **107**, 6328–6333 (2010).
24. Meckes, D. G. Jr *et al.* Human tumor virus utilizes exosomes for intercellular communication. *Proc. Natl Acad. Sci. USA* **107**, 20370–20375 (2010).
25. Balaj, L. *et al*. Tumour microvesicles contain retrotransposon elements and amplified oncogene sequences. *Nature Commun.* **2**, 180 (2011).
26. Irion, U. & St. Johnston, D. bicoid RNA localization requires specific binding of an endosomal sorting complex. *Nature* **445**, 554–558 (2007).
27. Gibbings, D. J., Ciaudo, C., Erhardt, M. & Voinnet, O. Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. *Nature Cell Biol.* **11**, 1143–1149 (2009).
28. Lee, Y. S. *et al.* Silencing by small RNAs is linked to endosomal trafficking. *Nature Cell Biol.* **11**, 1150–1156 (2009).
29. Deregibus, M. C. *et al.* Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. *Blood* **110**, 2440–2448 (2007).
30. Zernecke, A. *et al.* Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. *Sci. Signal.* **2**, ra81 (2009).
31. Zhang, Y. *et al.* Secreted monocytic miR-150 enhances targeted endothelial cell migration. *Mol. Cell* **39**, 133–144 (2010).
32. Hergenreider, E. *et al.* Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. *Nature Cell Biol.* **14**, 249–256 (2012).
33. Grange, C. *et al.* Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. *Cancer Res.* **71**, 5346–5356 (2011).
34. Arroyo, J. D. *et al.* Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc. Natl Acad. Sci. USA* **108**, 5003–5008 (2011).
35. Turchinovich, A., Weiz, L., Langheinz, A. & Burwinkel, B. Characterization of extracellular circulating microRNA. *Nucleic Acids Res.* **39**, 7223–7233 (2011).
36. 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. *Nature Cell Biol.* **13**, 423–433 (2011).
37. Wang, K., Zhang, S., Weber, J., Baxter, D. & Galas, D. J. Export of microRNAs and microRNA-protective protein by mammalian cells. *Nucleic Acids Res.* **38**, 7248–7259 (2010).
38. Shih, J. D. & Hunter, C. P. SID-1 is a dsRNA-selective dsRNA-gated channel. *RNA* **17**, 1057–1065 (2011).
39. Kosaka, N., Iguchi, H. & Ochiya, T. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. *Cancer Sci.* **101**, 2087–2092 (2010).
40. Wolfrum, C. *et al.* Mechanisms and optimization of *in vivo* delivery of lipophilic siRNAs. *Nature Biotech.* **25**, 1149–1157 (2007).
41. Valiunas, V. *et al.* Connexin-specific cell-to-cell transfer of short interfering RNA by gap junctions. *J. Physiol.* **568**, 459–468 (2005).
42. Kizana, E., Cingolani, E. & Marban, E. Non-cell-autonomous effects of vector-expressed regulatory RNAs in mammalian heart cells. *Gene Ther.* **16**, 1163–1168 (2009).
43. Hosoda, T. *et al.* Human cardiac stem cell differentiation is regulated by a mircrine mechanism. *Circulation* **123**, 1287–1296 (2011).
44. Lim, P. K. *et al.* Gap junction-mediated import of microRNA from bone marrow stromal cells can elicit cell cycle quiescence in breast cancer cells. *Cancer Res.* **71**, 1550–1560 (2011).
45. Katakowski, M., Buller, B., Wang, X., Rogers, T. & Chopp, M. Functional microRNA is transferred between glioma cells. *Cancer Res.* **70**, 8259–8263 (2010).
46. Braun, R. E., Behringer, R. R., Peschon, J. J., Brinster, R. L. & Palmiter, R. D. Genetically haploid spermatids are phenotypically diploid. *Nature* **337**, 373–376 (1989).
47. Morales, C. R. *et al.* A TB-RBP and Ter ATPase complex accompanies specific mRNAs from nuclei through the nuclear pores and into intercellular bridges in mouse male germ cells. *Dev. Biol.* **246**, 480–494 (2002).
48. Davis, D. M. & Sowinski, S. Membrane nanotubes: dynamic long-distance connections between animal cells. *Nature Rev. Mol. Cell Biol.* **9**, 431–436 (2008).
49. Huse, M., Quann, E. J. & Davis, M. M. Shouts, whispers and the kiss of death: directional secretion in T cells. *Nature Immunol.* **9**, 1105–1111 (2008).
50. Vicente-Manzanares, M. & Sánchez-Madrid, F. Role of the cytoskeleton during leukocyte responses. *Nature Rev. Immunol.* **4**, 110–122 (2004).
51. Varma, R., Campi, G., Yokosuka, T., Saito, T. & Dustin, M. L. T cell receptor-proximal signals are sustained in peripheral microclusters and terminated in the central supramolecular activation cluster. *Immunity* **25**, 117–127 (2006).
52. Griffiths, G. M., Tsun, A. & Stinchcombe, J. C. The immunological synapse: a focal point for endocytosis and exocytosis. *J. Cell Biol.* **189**, 399–406 (2010).
53. Davis, D. M. Intercellular transfer of cell-surface proteins is common and can affect many stages of an immune response. *Nature Rev. Immunol.* **7**, 238–243 (2007).

54. Chauveau, A., Aucher, A., Eissmann, P., Vivier, E. & Davis, D. M. Membrane nanotubes facilitate long-distance interactions between natural killer cells and target cells. *Proc. Natl Acad. Sci. USA* **107**, 5545–5550 (2010).
55. Mendoza-Naranjo, A. *et al.* Functional gap junctions accumulate at the immunological synapse and contribute to T cell activation. *J. Immunol.* **187**, 3121–3132 (2011).
56. Qureshi, O. S. et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. *Science* **332**, 600–603 (2011).
57. Stinchcombe, J. C., Bossi, G., Booth, S. & Griffiths, G. M. The immunological synapse of CTL contains a secretory domain and membrane bridges. *Immunity* **15**, 751–761 (2001).
58. Ueda, H., Morphew, M. K., McIntosh, J. R. & Davis, M. M. $CD4^+$ T-cell synapses involve multiple distinct stages. *Proc. Natl Acad. Sci. USA* **108**, 17099–17104 (2011).
59. Court, F. A., Hendriks, W. T., MacGillavry, H. D., Alvarez, J. & van Minnen, J. Schwann cell to axon transfer of ribosomes: toward a novel understanding of the role of glia in the nervous system. *J. Neurosci.* **28**, 11024–11029 (2008).
60. Lachenal, G. *et al.* Release of exosomes from differentiated neurons and its regulation by synaptic glutamatergic activity. *Mol. Cell. Neurosci.* **46**, 409–418 (2011).
61. Korkut, C. *et al. Trans*-synaptic transmission of vesicular Wnt signals through Evi/Wntless. *Cell* **139**, 393–404 (2009).
62. Dinger, M. E., Mercer, T. R. & Mattick, J. S. RNAs as extracellular signaling molecules. *J. Mol. Endocrinol.* **40**, 151–159 (2008).
63. Igakura, T. *et al.* Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. *Science* **299**, 1713–1716 (2003).
64. Jolly, C., Kashefi, K., Hollinshead, M. & Sattentau, Q. J. HIV-1 cell to cell transfer across an Env-induced, actin-dependent synapse. *J. Exp. Med.* **199**, 283–293 (2004).
65. Izquierdo-Useros, N. *et al.* HIV and mature dendritic cells: Trojan exosomes riding the Trojan horse? *PLoS Pathog.* **6**, e1000740 (2010).
66. Saleh, M. C. *et al.* Antiviral immunity in *Drosophila* requires systemic RNA interference spread. *Nature* **458**, 346–350 (2009).
67. Sijen, T. *et al.* On the role of RNA amplification in dsRNA-triggered gene silencing. *Cell* **107**, 465–476 (2001).
68. Vaistij, F. E., Jones, L. & Baulcombe, D. C. Spreading of RNA targeting and DNA methylation in RNA silencing requires transcription of the target gene and a putative RNA-dependent RNA polymerase. *Plant Cell* **14**, 857–867 (2002).
69. Maida, Y. *et al.* An RNA-dependent RNA polymerase formed by TERT and the RMRP RNA. *Nature* **461**, 230–235 (2009).
70. Cortez, M. A. *et al.* MicroRNAs in body fluids — the mix of hormones and biomarkers. *Nature Rev. Clin. Oncol.* **8**, 467–477 (2011).
71. Small, E. M. & Olson, E. N. Pervasive roles of microRNAs in cardiovascular biology. *Nature* **469**, 336–342 (2011).
72. Rosenfeld, N. et *al.* MicroRNAs accurately identify cancer tissue origin. *Nature Biotech.* **26**, 462–469 (2008).
73. Hung., E. C., Chiu, R. W. & Lo, Y. M. Detection of circulating fetal nucleic acids: a review of methods and applications. *J. Clin. Pathol.* **62**, 308–313 (2009).
74. Alvarez-Erviti, L. *et al.* Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nature Biotech.* **29**, 341–345 (2011).

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Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

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