CellPress

Opinion

How Our Other Genome Controls Our Epi-Genome

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Eukaryotes and prokaryotes produce extracellular nanovescicles that contain RNAs and other molecules that they exploit to communicate. Recently, inter-kingdom crosstalk was demonstrated between humans and bacteria through fecal microRNAs. We suggest here how bacteria interact with humans via RNAs within membrane vesicles to alter our epigenome, thus filling the gap and closing the circle. At the same time, there are indications that there could be a wider inter-kingdom communication network that might encompass all known kingdoms. Now that the connection with our other genome has been established, we also should begin to explore the 'social' network that we have around us.

Our Other Genome: The Gut Microbiota

Six years ago, thanks to the availability of next-generation sequencing technologies, we learned that we have 'another' genome (i.e., the microbiome) [1,2]. The discovery of this encouraged an incredible number of interdisciplinary basic studies with applications to human health, and subsequently studies on the **human microbiota** (see Glossary) have increased exponentially. The importance of the **gut microbiota** is enormous since an imbalance within the microbial composition may lead an individual to shift from physiological symbiosis to a dysbiosis and, ultimately, from health to disease [3–5]. In the perinatal period, gut microbiota can be affected by several factors such as the mode of delivery, bacterial infections, antibiotic treatments, and lifestyle. Once established, gut microbiota can be altered through eating habits and diet [6]. As an example, the modality of feeding during the first months of life (formula or breast fed) is one of the factors that explain the high variability of the colonization and development of a gut microbiota immediately after birth [7,8]. Through millennia of evolution, bacteria established beneficial host–guest associations and acquired the potential to exert both pro- and anti-inflammatory responses [9]. Interestingly, the dysbiosis of gut microbiota is most often associated with disease (i.e., inflammatory bowel disease, irritable bowel syndrome (IBS), and coeliac disease, but also allergy, asthma, metabolic syndrome, cardiovascular disease, and obesity) [10].

Therefore, our microbiota is something that we must care for, if we want to be healthy. However, to achieve this beneficial condition is not a simple matter as there can be many factors that modulate gut microbiota homeostasis and composition. A few years ago we suspected that the interplay between the gut microbiota and gene expression regulation by miRNAs was more complex that a one-sided relationship [11]. The proof of this 'host–guest dialogue' mediated by membrane vesicles came a few years later and opened the way to other interesting findings. In the following paragraphs we provide our view of a wide inter-kingdom communication and regulatory network that is based on small RNAs transported through nanovesicles. Now that we know more about our 'other genome', we think that it is time to determine how to interact with it.

Interplay between the Host and Gut Microbiota

Researchers and clinicians know very well that probiotics, functional foods, and fecal transplantation are among the ways to modulate or restore the microbiota composition [12–14].

Trends

The first evidence that outer-membrane vesicles (OMVs) are produced by *Escherichia coli* dates from 1976. After 40 years, bioengineered OMVs are considered today to be promising innovative vectors for drug delivery and cancer therapy.

The first evidence of inter-kingdom crosstalk between humans and gut microbiota through microRNAs that are contained in extracellular vesicles (EVs) was reported earlier this year.

Many bacterial small RNAs that are contained in OMVs align to histone marks in the human genome. We hypothesized that they may act as long non-coding RNAs, thus regulating our epigenome.

We still do not know how many diseases or pathological conditions may be caused by the interplay between bacterial OMVs and the human genome.

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Interestingly, in the January issue of *Cell Host & Microbe*, Liu *et al.* reported for the first time that microRNAs produced by intestinal epithelial cells modulate gene expression post-transcriptionally and can also shape the gut microbiota [15]. MicroRNAs can be released within extracellular vesicles (i.e., **exosomes**) and/or associated with high-density lipoproteins or argonaute proteins [16]. The authors demonstrated the presence of extracellular vesicles (EVs) in fecal samples and the presence of microRNAs within these particles. Therefore, their outstanding conclusion is that the host can modulate bacterial gene expression through microRNAs that are contained within EVs.

Analogous to how human cells produce EVs, bacteria also produce membrane vesicles (MVs) (in Gram-positive bacteria) or **outer-membrane vesicles** (OMVs) (in Gram-negative bacteria). In 1976, Hoekstra *et al.* discovered vesicles from *Escherichia coli* grown under normal growth conditions. They characterized the lipid content of supernatant vesicles and also reported a freeze-fracture electron micrograph of OMVs [17]. Vesicles were discovered in Gram-negative bacteria, but also in Gram-positive ones [18]. The contents of 'bleb material' isolated from cultures of *Neisseria gonorrhoeae* was reported to contain not only circular and linear DNA, but also RNA [19]. A few years later, Beveridge and others postulated that membrane vesicles, because of their metabolic 'cost' and ubiquity, should have had important biological functions that depend on the organism from which OMVs originated [20]. They emphasized the importance of OMVs as potential therapeutic vehicles for the delivery of antimicrobials in clinical settings [21]. In fact, OMVs have been shown to mediate cell-to-cell exchange not only of DNA and proteins but also of other small signaling molecules [22]. OMVs have been implicated in the pathogenesis of a broad range of infectious diseases and chronic inflammatory diseases, including *Helicobacter pylori* infection [23], and Crohn's disease [24]. OMVs that have been generated from *Pseudomonas aeruginosa* strains and isolated from the lungs of cystic fibrosis patients have been demonstrated to elicit interleukin-8 (IL-8) secretion by primary human bronchial epithelial cells [25]. However, it was in the past decade that the concept of OMVs took hold. From the concept of a bacterial by-product, the scientific community embraced OMVs as innovative nanotechnological delivery vectors [26]. Bacterial OMVs are able to modulate immunity [27] and, by proper engineering, can represent innovative vectors for cancer therapy as well [28,29]. For example, OMVs from *Burkholderia pseudomallei* have been used as vaccines to protect against lethal sepsis [30], whereas vaccination with *Klebsiella pneumoniae*-derived OMVs protect against bacteria-induced lethality via both humoral and cellular immunity [31].

In another example of the immense (but still unseen) impact of OMVs as a means of communication between cells and their environment, Billet *et al.* characterized OMVs in marine microbial communities that were produced by photoautotrophs, such as the cyanobacterium *Prochlorococcus* [32]. This paper suggested that vesicles, by providing binding sites or acting as reactive surfaces may mediate interactions between microorganisms. This mechanism represents a possible way of communication that merits increasing recognition in marine ecosystems.

The evidence that OMVs contained RNA motivated our group to determine whether the presence of microRNAs within bacterial OMVs could be demonstrated. In fact, we noticed that the gene expression of the intestinal tissue and the gut microbiota composition are generally correlated. This caused us to think that microRNAs may have a crucial role in this phenomenon. Therefore, we suggested that the relationships between non-coding RNAs (ncRNAs) and microbiota deserved more investigation in order to unravel their mutual role in influencing the host immune system and related processes [11]. Bacteria might release some soluble factors other than toxins or other peptides that could function as a 'communication vector' and microRNAs within the OMVs might be one of these vectors.

Glossary

Epigenetic modification: a functionally relevant change to the genomic context that does not involve directly a change in the nucleotide sequence. Such a change can be heritable, remain unaltered through cell divisions and differentiation, and might persist through subsequent generations. Epigenetic changes include chromatin modifications, such as histone acetylation, or chemical alterations to the DNA itself, such as DNA methylation.

Exosomes: exosomes or microvesicles are ~40–100 nm extracellular vesicles that are secreted by mammalian cells. They contain DNA, RNA, and proteins. They are efficient communication vectors.

Gut microbiota: a complex community of bacteria that play a fundamental role in many biological processes and may alter human health. It has been estimated that our gut contains almost 3.9×10^{13} bacteria, a number that is very close to the number of human cells, which is estimated to be approx. 3.7×10^{13}.

Human microbiota: The human microbiota is the ensemble of microorganisms (the microbiome) that lives within the human body. It resides on the surface and in deep layers of the skin, in the saliva and oral mucosa, in the conjunctiva, and in the gastrointestinal tracts. The human microbiota includes bacteria, viruses, fungi, protozoans, and archaea. Every individual human harbors 10–100 trillion symbiotic microbial cells, with gut bacteria being the most abundant.

Long intergenic non-coding RNAs (lincRNAs): are a class of long non-coding RNAs (lncRNAs, see the description below) that are transcribed from non-coding DNA sequences contained between protein-encoding genes.

Long non-coding RNAs (lncRNAs): are transcripts that are approximately 200 nt in length and are not transcribed. lncRNAs are able not only to silence or regulate gene expression through different mechanisms, but they can also modify chromatin and histones.

Outer-membrane vesicles (OMVs): are extracellular vesicles, produced by Gram-negative bacteria, that contain DNA, RNA, proteins, and

other small molecules; they are used for inter-kingdom communication. These vesicles have a size that ranges from approximately 20 nm to 250 nm. They are very similar to eukaryotes' vesicles (exosomes).

In fact, it has previously been suggested that bacteria, such as *E. coli* and *Streptococcus mutans*, produce OMVs that contain microRNA-like molecules [33,34]. However, the secondary structures of these 'microRNA-size' RNAs that the authors reported did not resemble those of eukaryotes' microRNAs. In fact, they showed several bulges and no recognizable 3′ overhang that, in humans, are two important features for inhibiting (bulges) or enhancing (3′ overhang) efficient processing. So these microRNA-size RNAs cannot interfere with the human 'miRNA machinery' and cannot directly regulate gene expression as miRNAs generally do in humans.

Nevertheless, the hypothesis that bacterial OMVs can contain 'miRNA-like' small RNAs, similar to those from eukaryotes, led us to thoroughly explore their content. Furthermore, studies of *Vibrio cholerae* and *E. coli* showed that RNA is one of many bacterial components associated with OMVs. These studies highlighted the need to evaluate the potential role of RNA-containing bacterial membrane vesicles in bacteria–host interactions [35].

It was only last year that Ghosal and colleagues characterized the extracellular RNA complement (i.e., the OMV-associated and OMV-free RNA) of the enteric bacterium *E. coli* [36]. They found that the secreted RNAs are generally smaller than 60 nt, enriched in ncRNAs, and are distinct from intracellular RNAs. By mapping RNA-Seq data against the *E. coli* genome, the authors also identified two uncharacterized ncRNAs [36].

Obviously, the authors aligned the obtained RNA-Seq reads against the bacterial genome. However, if the extracellular bacterial RNAs have a function to undertake on humans, we wondered whether they might align against the human genome as well. Therefore, the two datasets from Ghosal *et al.* [36] that are related to the extracellular bacterial RNA complement were retrieved from the Sequence Read Archive repository and aligned against the human hg19 genome.

Surprisingly, many reads also aligned with the human genome (Table 1) in different chromosomic regions. In particular, both datasets contained ncRNAs that ranged in length from a few nucleotides to hundreds of them (with a mean value of 61–63 nt) that aligned in regions of 14 different chromosomes (Table 1). This finding would have had no particular significance if one did not observe that the majority of these matched regions represent histone marks (i.e., H3K4Me1, H3K4Me3, and H3K27Ac), DNaseI hypersensitivity clusters or intronic regions with elevated transcription levels in different cell lines, as assayed by RNA-seq experiments from ENCODE. Some representative examples of alignments are shown in Figure 1. Although these findings surprised us, we realized immediately that this phenomenon is not unique.

Long Non-Coding RNAs and Microbiota

Interestingly, the alignment of bacterial small RNAs to the human genome revealed that their length resembled that of eukaryotes' long non-coding RNAs (lncRNAs). This suggested that bacterial RNAs that are found in OMVs could have a similar behavior [37]. We know that lncRNAs may act as signaling, decoy, guide, and scaffold molecules and can bind to transcription factors, chromatin-modifying enzymes, or be part of ribonucleoprotein complexes [37] (Figures 2 and 3).

Moreover, from a functional point of view, lncRNAs are recognized epigenetic regulators [38,39] (Figures 2 and 3). Notably, Liang *et al.* established a link between the expression of lncRNAs and gut microbiota in mice [40]. They demonstrated that lncRNA expression profiles can be used successfully to discriminate the types of microbes in the gut, and indirectly proved that various lncRNAs are present in sites where different bacteria live. However, it is still not clear whether these lncRNAs are uniquely produced by the host or by the microbiota. The impact of bacterial infections on histone modifications and chromatin remodeling is complex and widespread [41,42], and this is also true for other obligate and facultative intracellular pathogens, such as fungi and viruses [43,44].

Table 1. Alignments of Bacterial Small RNA Reads against the Canonical hg19 Human Genome[a]

Dataset #1				
Chromosome	**Coordinates (Start-End)**	**Mean Coverage**	**Region Length**	**Genomic Context (Overlap with Introns or lincRNAs[b])**
chr1	91852774-91852836	17.82	63	HFM1; probable ATP-dependent DNA helicase HFM1
chr1	237766391-237766449	21.11	59	RYR2; ryanodine receptor 2
chr1	237766500-237766554	17.67	55	RYR2; ryanodine receptor 2
chr2	33141363-33141369	6.00	7	lincRNA 486 (LINC00486)
chr2	128556522-128556572	25.50	51	WDR33; pre-mRNA 3' end processing protein WDR33 isoform 1
chr2	**133013111-133013184**	**135.98**	**74**	**ANKRD30BL; ankyrin repeat domain 30B-like**
chr3	156871336-156871387	436.55	52	CCNL1; cyclin L1
chr4	70296661-70296751	71.98	91	none
chr6	33167377-33167427	123.75	51	RXRB; retinoic acid receptor RXR-beta isoform 2
chr6	151620018-151620068	47.00	51	AKAP12; A-kinase anchoring protein 12
chr7	68527467-68527520	8.71	54	none
chr7	68527605-68527647	6.00	43	none
chr7	148660406-148660456	26.25	51	none
chr8	**70602335-70602419**	**223.06**	**85**	**SLCO5A1; solute carrier organic anion transporter family member 5A1 isoform 3**
chr8	**70602429-70602526**	**63.30**	**98**	**SLCO5A1; solute carrier organic anion transporter family member 5A1**
chr8	**70602552-70602603**	**13.58**	**52**	**SLCO5A1; solute carrier organic anion transporter family member 5A1**
chr10	34490951-34490966	14.00	16	PARD3; par-3 family cell polarity regulator
chr10	34490967-34491002	15.00	36	PARD3; par-3 family cell polarity regulator
chr11	77597504-77597618	35.64	115	AAMDC; adipogenesis associated, Mth938 domain containing INTS4; integrator complex subunit 4
chr11	111692658-111692715	12.58	58	ALG9; alpha-1,2-mannosyltransferase
chr16	33963092-33963158	25.66	67	none
chr16	33963699-33963761	24.12	63	none
chr19	24184099-24184141	6.00	43	none
chr19	36066577-36066630	17.92	54	none
chr21	**9827014-9827067**	**9.18**	**54**	**none**
chr21	**9827404-9827506**	**105.55**	**103**	**none**

Table 1. (continued)

Dataset #1				
Chromosome	**Coordinates (Start-End)**	**Mean Coverage**	**Region Length**	**Genomic Context (Overlap with Introns or lincRNAs[b])**
chrX	108297378-108297500	29.22	123	none
chrX	108297579-108297619	6.33	41	none
chrY	10035462-10035511	6.50	50	none
Dataset #2				
Chromosome	**Coordinates (Start-End)**	**Mean Coverage**	**Region Length**	**Genomic Context (Overlap with Introns or lincRNAs[b])**
chr1	91852774-91852839	30.91	66	HFM1; probable ATP-dependent DNA helicase HFM1
chr1	237766337-237766450	29.06	114	RYR2; ryanodine receptor 2
chr1	237766496-237766552	33.65	57	RYR2; ryanodine receptor 2
chr2	133012533-133012585	11.27	53	ANKRD30BL; ankyrin repeat domain 30B-like
chr2	133013077-133013187	290.41	111	ANKRD30BL; ankyrin repeat domain 30B-like
chr3	156871336-156871386	44.67	51	CCNL1; cyclin L1
chr4	70296657-70296751	134.15	95	none
chr6	33167377-33167427	9.33	51	RXRB; retinoic acid receptor RXR-beta isoform 2
chr7	68527467-68527521	21.92	55	none
chr7	68527602-68527655	15.58	54	none
chr7	148660406-148660456	8.50	51	none
chr8	70602324-70602418	213.28	95	SLCO5A1; solute carrier organic anion transporter family member 5A1
chr8	70602427-70602526	50.31	100	SLCO5A1; solute carrier organic anion transporter family member 5A1
chr8	70602551-70602603	47.92	53	SLCO5A1; solute carrier organic anion transporter family member 5A1
chr11	77597500-77597617	43.54	118	AAMDC; adipogenesis associated, Mth938 domain containing INTS4; integrator complex subunit 4
chr11	111692657-111692720	53.72	64	ALG9; alpha-1,2-mannosyltransferase
chr12	20704395-20704453	15.74	59	PDE3A; cGMP-inhibited 3' 5'-cyclic phosphodiesterase A isoform 1
chr16	33963096-33963156	16.52	61	none
chr16	33963699-33963756	54.36	58	none
chr16	33963757-33963759	6.00	3	none
chr19	24184091-24184148	49.94	58	none

Table 1. (continued)

Dataset #1				
Chromosome	**Coordinates (Start-End)**	**Mean Coverage**	**Region Length**	**Genomic Context (Overlap with Introns or lincRNAs[b])**
chr19	36066577-36066630	36.18	54	none
chr21	9827027-9827056	6.00	30	none
chr21	9827405-9827503	103.33	99	none
chrX	108297370-108297436	46.87	67	none
chrX	108297443-108297498	43.79	56	none
chrX	108297580-108297622	8.17	43	none
chrX	108297623-108297630	6.50	8	none
chrY	10035461-10035512	12.71	52	none

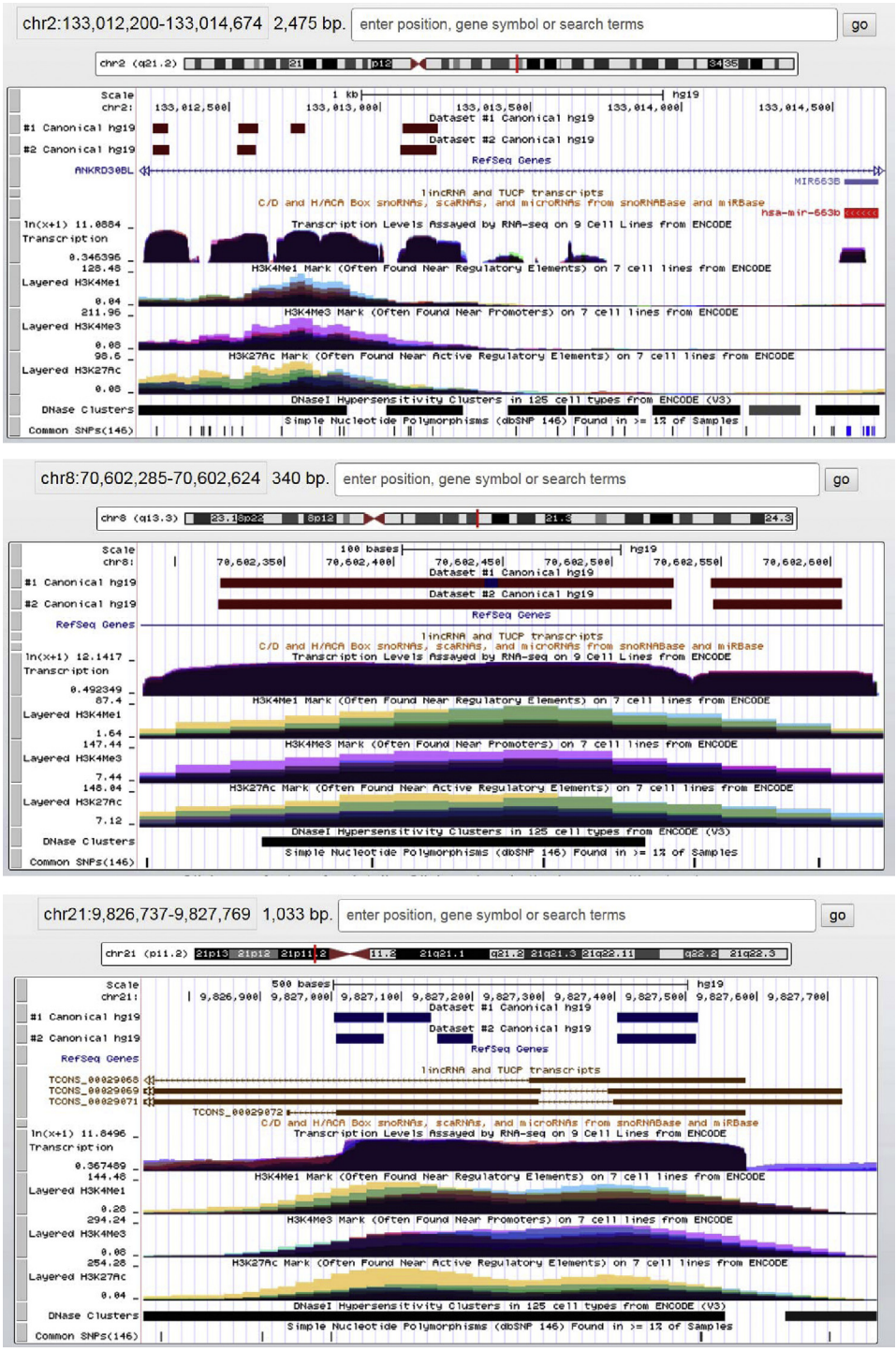
[a]Two datasets from Ghosal *et al.* [36] were used. The alignment has been performed with Bowtie2 with default parameters. Regions indicated in bold have been depicted in Figure 1.
[b]lincRNAs, **long intergenic non-coding RNAs**, are a class of long non-coding RNAs that are transcribed from non-coding DNA sequences contained between protein-encoding genes.

Future Directions

In the future further information is needed about the functional relevance of the alignment of bacterial small RNAs to the human genome. It is still premature to say what the impact of such findings will be, without a functional validation. However, we envisage that our observation will lead to many discoveries in various fields. We now discuss a few perspectives that we hope will be extended on in the near future.

We know that inflammatory conditions may increase blood–brain barrier (BBB) permeability to circulating compounds, such as extracellular vesicles [45]. Notably, OMVs also can affect BBB permeability [46]. Therefore, the inter-kingdom transfer of genetic material by OMVs can represent an important process that underlies brain development or diseases, such as multiple sclerosis [47] or other progressive diseases where the BBB integrity is disrupted. Moreover, we know that **epigenetic modification** by histone acetylases and deacetylases is a process that dynamically modulates the gene expression in synaptic plasticity processes and brain development [48,49], as well as learning and memory [50]. Therefore, we hypothesize that many brain-related processes and disease onset may be regulated by small RNAs within extracellular vesicles.

There are many other conditions where small ncRNAs that are contained in OMVs may have an effect. In fact, a differential methylation pattern of gene promoters linked to lipid metabolism and obesity has been already observed [51]. Therefore, it is tempting to speculate that bacterial OMVs may be involved in this association between bacterial predominance and epigenetic profiles. Moreover, the gut microbiome of obese individuals is altered, in comparison to that of lean individuals [52], and their spermatozoa have differentially methylated genes and an altered expression of small ncRNAs [53]. Finally, the interactions of gut microbiota and dietary factors may also affect particular epigenetic processes that influence not only human health and disease but also cancer onset and the response to treatment [54]. Is it reasonable to think that even in these cases OMVs also may perform their function?



Trends in Microbiology

Figure 1. UCSC Genome Browser Representative Examples of Three Genomic Regions That Align with Bacterial Small RNAs. See Table 1 for details. Brown boxes represent bacterial reads aligned (Bowtie2 with default parameters) against the human genome; the multi-view composite tracks (colored regions) reported below indicate the occurrence of ENCODE Histone Modification Tracks (Transcription, H3K4Me1, H3K4Me3, and H3K27Ac) found in different cell types (colored in cyan, green, yellow, red, magenta, and violet). Human genome assembly as of February 2009 (GRCh37/hg19).

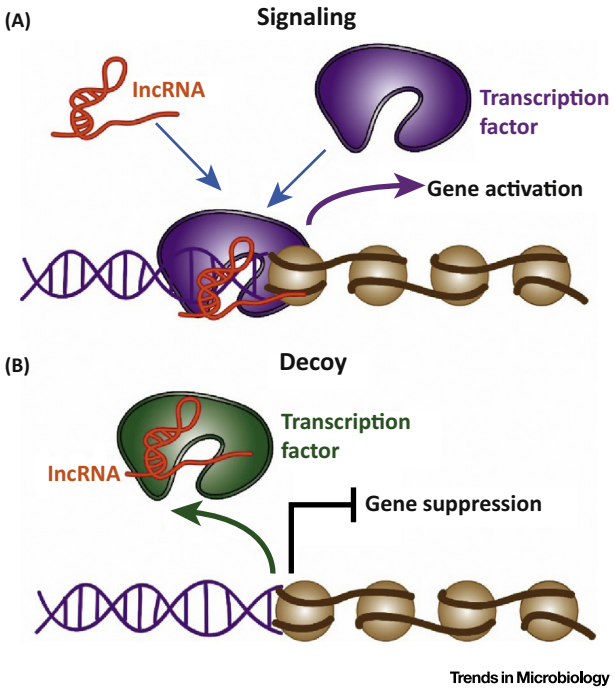


Figure 2. Long Non-Coding RNAs (lncRNA) Acting as (A) Gene Activators (Signaling Archetype) or (B) Gene Suppressor (Decoy Archetype).

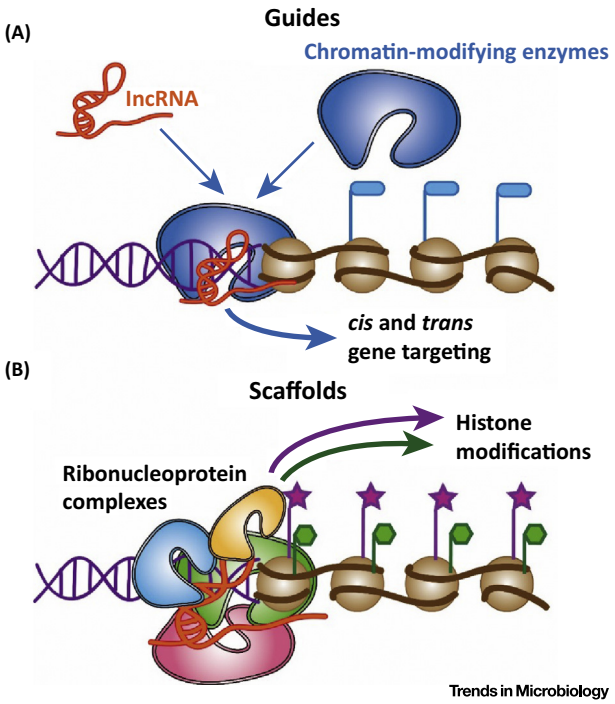


Figure 3. Long Non-Coding RNAs (lncRNAs) Acting as (A) *cis* and *trans* Gene Expression Regulators (Guide Archetype) or (B) Chromatin Modificators (Scaffold Archetype).

Concluding Remarks

We are imagining many other diseases or conditions in which bacterial MVs can release their ncRNA content in a host's cells and in which bacteria receive, at the same time, a 'feedback' response from the host through the miRNAs of EVs (Figure 4, Key Figure). It may also be reasonable to think that commensal bacteria [55], pathogens, and viruses may exert their

Outstanding Questions

Can OMVs be exploited for their potential role as epigenetic modulators?

Are there additional infectious/noninfectious diseases where OMVs could play a role?

Could MVs be used therapeutically against pathogens or diseases such as tumors?

How many kingdoms exploit MVs to communicate (intra- and inter-kingdom communication)?

Key Figure

The Vitruvian Man and the New 'Holobiont'

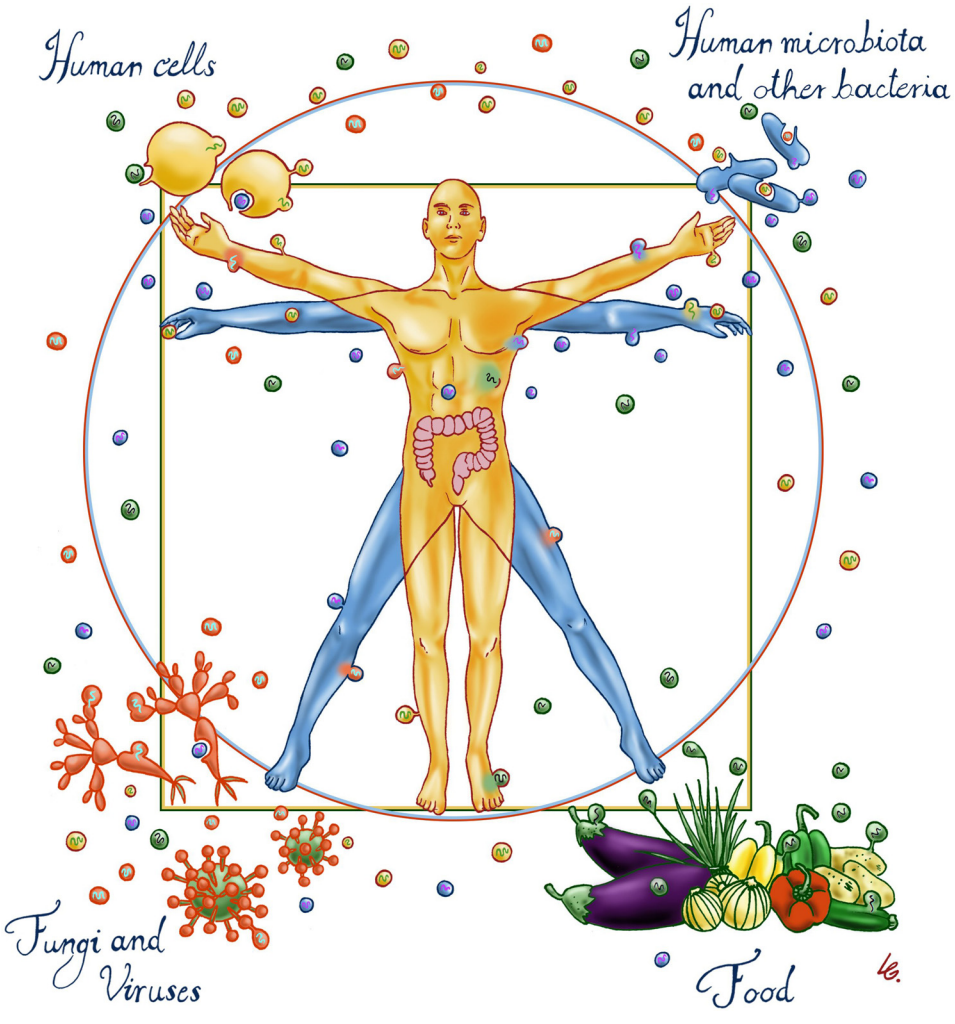


Figure 4. The picture represents our vision of inter-kingdom communication mediated by vesicles (small colored spheres). Human cells convey exosomes to the gut microbiota that also produce OMVs that enter into host cells. Fungi and viruses also produce vesicles that may convey their 'message' to other cells. Finally, food produces small RNA molecules that have been detected in human serum, thus suggesting an additional, but still unexplored, regulatory level. Artistic composition by Laura Galeazzo.

multifaceted effect by this mechanism and contribute to the onset of different diseases (see Outstanding Questions). A few years ago we suggested that an equilibrated diet, by integrating beneficial substances, could minimize negative side effects due to the exposure of xenobiotics (i.e., inorganic arsenic) from food [56]. Now we know that arsenic exposure alters the gut microbiome community at the abundance level and strongly disturbs its metabolic profiles at the functional level [57], thus we are prone to think that MVs may also exert a role in these processes. A new inter-kingdom communication concept in the field of circulating miRNAs is emerging, that is represented by the regulation of human mRNAs by exogenous miRNAs (or xenomiRs) that are most likely absorbed when food is ingested [58]. Exogenous miRNAs from vegetal origin are very stable and bioavailable even after extensive cooking [59]. Therefore, as circulating miRNAs are generally contained in microvesicles, why not assume that plants and vegetables also may contribute with their 'vesicles' to inter-kingdom communication? On the plant side, emerging studies have revealed that plant microbiomes are structured and form complex, interconnected microbial networks [60]. We can therefore apply the same considerations illustrated above and hypothesize that the plant-associated microorganisms might also utilize MVs to communicate with their plant hosts. If all of these living organisms produce vesicles, a question spontaneously arises in our mind: are we all surrounded by vesicles?

In conclusion, we are inclined to imagine the Vitruvian man by Leonardo da Vinci (Figure 4) as the new 'holobiont' who is connected to multifaceted kingdoms that interact with him in a feed-forward/feed-back cycle for the purpose of maintaining a universal 'homeostasis' and, ultimately, contributing to sustaining the complex network of Life.

References

1. Qin, J. *et al.* (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464, 59–65
2. Human Microbiome Jumpstart Reference Strains Consortium *et al.* (2010) A catalog of reference genomes from the human microbiome. *Science* 328, 994–999
3. Althani, A. *et al.* (2016) Human microbiome and its association with health and diseases. *J. Cell. Physiol.* 231, 1688–1694
4. Biedermann, L. and Rogler, G. (2015) The intestinal microbiota: its role in health and disease. *Eur. J. Pediatr.* 174, 151–167
5. Martin, R. *et al.* (2014) The role of metagenomics in understanding the human microbiome in health and disease. *Virulence* 5, 413–423
6. Del Chierico, F. *et al.* (2014) Mediterranean diet and health: food effects on gut microbiota and disease control. *Int. J. Mol. Sci.* 15, 11678–11699
7. Putignani, L. *et al.* (2014) The human gut microbiota: a dynamic interplay with the host from birth to senescence settled during childhood. *Pediatr. Res.* 76, 2–10
8. Gensollen, T. *et al.* (2016) How colonization by microbiota in early life shapes the immune system. *Science* 352, 539–544
9. Round, J.L. and Mazmanian, S.K. (2009) The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* 9, 313–323
10. Carding, S. *et al.* (2015) Dysbiosis of the gut microbiota in disease. *Microb. Ecol. Health Dis.* 26, 26191
11. Masotti, A. (2012) Interplays between gut microbiota and gene expression regulation by miRNAs. *Front. Cell. Infect. Microbiol.* 2, 137
12. Montalban-Arques, A. *et al.* (2015) Selective manipulation of the gut microbiota improves immune status in vertebrates. *Front. Immunol.* 6, 512
13. Laparra, J.M. and Sanz, Y. (2010) Interactions of gut microbiota with functional food components and nutraceuticals. *Pharmacol. Res.* 61, 219–225
14. Cammarota, G. *et al.* (2014) Gut microbiota modulation: probiotics, antibiotics or fecal microbiota transplantation? *Intern. Emerg. Med.* 9, 365–373
15. Liu, S. *et al.* (2016) The host shapes the gut microbiota via fecal microRNA. *Cell. Host Microbe* 19, 32–43
16. Arroyo, J.D. *et al.* (2011) Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc. Natl. Acad. Sci. U.S.A.* 108, 5003–5008
17. Hoekstra, D. *et al.* (1976) Release of outer membrane fragments from normally growing *Escherichia coli*. *Biochim. Biophys. Acta* 455, 889–899
18. Brown, L. *et al.* (2015) Through the wall: extracellular vesicles in Gram-positive bacteria, mycobacteria and fungi. *Nat. Rev. Microbiol.* 13, 620–630
19. Dorward, D.W. *et al.* (1989) Export and intercellular transfer of DNA via membrane blebs of *Neisseria gonorrhoeae*. *J. Bacteriol.* 171, 2499–2505
20. Beveridge, T.J. (1999) Structures of Gram-negative cell walls and their derived membrane vesicles. *J. Bacteriol.* 181, 4725–4733
21. Kadurugamuwa, J.L. and Beveridge, T.J. (1998) Delivery of the non-membrane-permeative antibiotic gentamicin into mammalian cells by using *Shigella flexneri* membrane vesicles. *Antimicrob. Agents Chemother.* 42, 1476–1483
22. Kadurugamuwa, J.L. and Beveridge, T.J. (1996) Bacteriolytic effect of membrane vesicles from *Pseudomonas aeruginosa* on other bacteria including pathogens: conceptually new antibiotics. *J. Bacteriol.* 178, 2767–2774
23. Keenan, J. *et al.* (2000) A role for the bacterial outer membrane in the pathogenesis of *Helicobacter pylori* infection. *FEMS Microbiol. Lett.* 182, 259–264
24. Rolhion, N. *et al.* (2005) Strong decrease in invasive ability and outer membrane vesicle release in Crohn's disease-associated adherent-invasive *Escherichia coli* strain LF82 with the yfgL gene deleted. *J. Bacteriol.* 187, 2286–2296
25. Bauman, S.J. and Kuehn, M.J. (2006) Purification of outer membrane vesicles from *Pseudomonas aeruginosa* and their activation of an IL-8 response. *Microbes Infect.* 8, 2400–2408
26. Schwechheimer, C. and Kuehn, M.J. (2015) Outer-membrane vesicles from Gram-negative bacteria: biogenesis and functions. *Nat. Rev. Microbiol.* 13, 605–619

27. Kaparakis-Liaskos, M. and Ferrero, R.L. (2015) Immune modulation by bacterial outer membrane vesicles. *Nat. Rev. Immunol.* 15, 375–387
28. Gujrati, V. *et al.* (2014) Bioengineered bacterial outer membrane vesicles as cell-specific drug-delivery vehicles for cancer therapy. *ACS Nano* 8, 1525–1537
29. Gujrati, V.B. and Jon, S. (2014) Bioengineered bacterial outer membrane vesicles: what is their potential in cancer therapy? *Nanomedicine (Lond)* 9, 933–935
30. Nieves, W. *et al.* (2014) A *Burkholderia pseudomallei* outer membrane vesicle vaccine provides protection against lethal sepsis. *Clin. Vaccine Immunol.* 21, 747–754
31. Lee, W.H. *et al.* (2015) Vaccination with *Klebsiella pneumoniae*-derived extracellular vesicles protects against bacteria-induced lethality via both humoral and cellular immunity. *Exp. Mol. Med.* 47, e183
32. Biller, S.J. *et al.* (2014) Bacterial vesicles in marine ecosystems. *Science* 343, 183–186
33. Kang, S.M. *et al.* (2013) Identification of microRNA-size, small RNAs in *Escherichia coli*. *Curr. Microbiol.* 67, 609–613
34. Lee, H.J. and Hong, S.H. (2012) Analysis of microRNA-size, small RNAs in *Streptococcus mutans* by deep sequencing. *FEMS Microbiol. Lett.* 326, 131–136
35. Sjostrom, A.E. *et al.* (2015) Membrane vesicle-mediated release of bacterial RNA. *Sci. Rep.* 5, 15329
36. Ghosal, A. *et al.* (2015) The extracellular RNA complement of *Escherichia coli*. *Microbiologyopen* 4, 252–266
37. Da Sacco, L. *et al.* (2012) Bioinformatics tools and novel challenges in long non-coding RNAs (lncRNAs) functional analysis. *Int. J. Mol. Sci.* 13, 97–114
38. Cao, J. (2014) The functional role of long non-coding RNAs and epigenetics. *Biol. Proced. Online* 16, 11
39. Mercer, T.R. and Mattick, J.S. (2013) Structure and function of long noncoding RNAs in epigenetic regulation. *Nat. Struct. Mol. Biol.* 20, 300–307
40. Liang, L. *et al.* (2015) Long noncoding RNA expression profiles in gut tissues constitute molecular signatures that reflect the types of microbes. *Sci. Rep.* 5, 11763
41. Bierne, H. *et al.* (2012) Epigenetics and bacterial infections. *Cold Spring Harb Perspect. Med.* 2, a010272
42. Hamon, M.A. and Cossart, P. (2008) Histone modifications and chromatin remodeling during bacterial infections. *Cell. Host Microbe* 4, 100–109
43. Niller, H.H. and Minarovits, J. (2016) Patho-epigenetics of infectious diseases caused by intracellular bacteria. *Adv. Exp. Med. Biol.* 879, 107–130
44. Enguita, F.J. *et al.* (2016) Transcriptomic crosstalk between fungal invasive pathogens and their host cells: opportunities and challenges for next-generation sequencing methods. *J. Fungi* 2, 1
45. Ridder, K. *et al.* (2014) Extracellular vesicle-mediated transfer of genetic information between the hematopoietic system and the brain in response to inflammation. *PLoS Biol.* 12, e1001874
46. Wispelwey, B. *et al.* (1989) *Haemophilus influenzae* outer membrane vesicle-induced blood-brain barrier permeability during experimental meningitis. *Infect. Immun.* 57, 2559–2562
47. Lee, Y.K. *et al.* (2011) Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. U.S.A.* 108 (Suppl 1), 4615–4622
48. Stefanko, D.P. *et al.* (2009) Modulation of long-term memory for object recognition via HDAC inhibition. *Proc. Natl. Acad. Sci. U.S.A.* 106, 9447–9452
49. Kavalali, E.T. *et al.* (2011) Role of MeCP2, DNA methylation, and HDACs in regulating synapse function. *J. Neurodev Disord.* 3, 250–256
50. Haettig, J. *et al.* (2011) HDAC inhibition modulates hippocampus-dependent long-term memory for object location in a CBP-dependent manner. *Learn. Mem.* 18, 71–79
51. Kumar, H. *et al.* (2014) Gut microbiota as an epigenetic regulator: pilot study based on whole-genome methylation analysis. *mBio* 5, http://dx.doi.org/10.1128/mBio.02113-14
52. Turnbaugh, P.J. *et al.* (2009) A core gut microbiome in obese and lean twins. *Nature* 457, 480–484
53. Donkin, I. *et al.* (2016) Obesity and bariatric surgery drive epigenetic variation of spermatozoa in humans. *Cell. Metab.* 23, 369–378
54. Paul, B. *et al.* (2015) Influences of diet and the gut microbiome on epigenetic modulation in cancer and other diseases. *Clin. Epigenetics* 7, 112
55. Ruff, W.E. and Kriegel, M.A. (2015) Autoimmune host-microbiota interactions at barrier sites and beyond. *Trends Mol. Med.* 21, 233–244
56. Sacco, L.D. *et al.* (2013) Diet's role in the toxicity of inorganic arsenic (iAs): A journey from soil to children's mouth. *J. Geochem. Explor.* 131, 45–51
57. Lu, K. *et al.* (2014) Arsenic exposure perturbs the gut microbiome and its metabolic profile in mice: an integrated metagenomics and metabolomics analysis. *Environ. Health Perspect.* 122, 284–291
58. Fabris, L. and Calin, G.A. (2016) Circulating free xeno-microRNAs–The new kids on the block. *Mol. Oncol.* 10, 503–508
59. Philip, A. *et al.* (2015) Determination of the potential bioavailability of plant microRNAs using a simulated human digestion process. *Mol. Nutr. Food Res.* 59, 1962–1972
60. Agler, M.T. *et al.* (2016) Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biol.* 14, e1002352