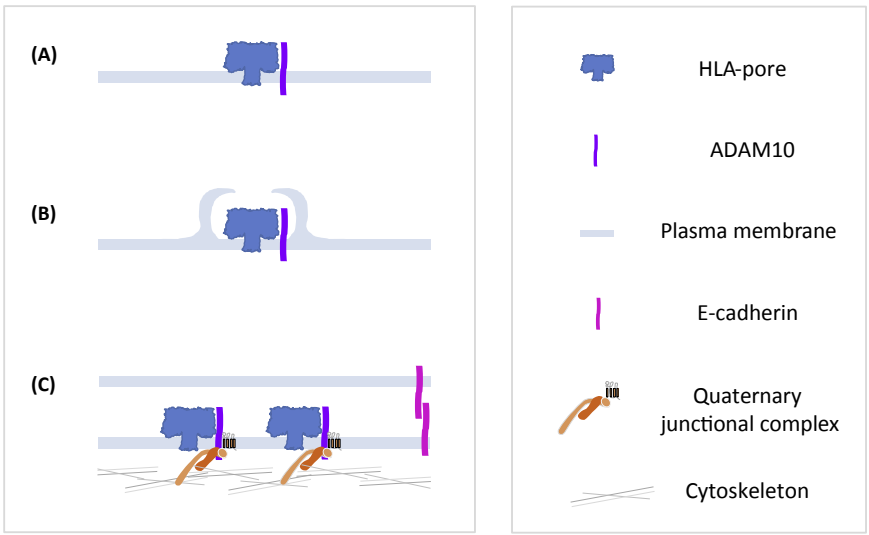

Figure 1. α-Hemolysin (HLA) Pores at Cell Junctions Escape Engulfment. (A) HLA binds to the plasma membrane via ADAM10, which leads to the formation of transmembrane pores. Uncontrolled flux of water and ions triggers multiple signaling pathways and may cause cell death. (B) Engulfment of pores by macropinocytosis, an inducible, high-capacity endocytic process, enables epithelial cells to survive an attack by HLA. (C) A quaternary junctional protein complex (comprising Tsp33, PLEKHA7, PDZD11, and afadin), organizes the clustering of ADAM10 at adherens junctions. HLA pores bound to junctional ADAM10 will escape engulfment, persist in the plasma membrane, and eventually kill the cell.

of substrates, or vice versa? Do similar interactions as those uncovered by Shah *et al.* play a role in other cell types? What is the fate of toxin monomers? Also, further details of HLA endocytosis deserve further analysis. Amiloride inhibited recovery, leading the authors to propose that uptake of HLA pores occurs via macropinocytosis. This would be in keeping with several previous observations, for example, the reduced endocytosis of HLA pores in the presence of dynasore [3], or inhibition of cellular recovery by cytochalasin D, an inhibitor of actin polymerization. Because macropinocytosis is regulated by the epidermal growth factor receptor, which is activated by HLA [9], and because it can internalize large patches of plasma membrane [10], it would seem possible that plasma membrane areas studded with HLA pores could be engulfed by this process. The concomitant intake of nutrients, a genuine function of macropinocytosis, could help cells to cope with amino acid starvation, a known consequence of membrane perforation by HLA and other pore-forming toxins [2]. Macropinocytosis could thus serve a dual function in target cells, namely, maintaining metabolic homeostasis and removing membrane pores. The paper by Shah *et al.* [7] and the foregoing work by Popov *et al.* [5] uncover how HLA targets a weak spot in cell autonomous defense (Figure 1), and they will certainly spur further research in this direction. In broader terms, their work exemplifies how investigations into host–pathogen interactions may elucidate basic cellular functions.

Acknowledgment

The authors would like to thank M. Wiedmann for critically reading the manuscript.

[1]Institute of Medical Microbiology and Hygiene, University Medical Center, Johannes Gutenberg University, Mainz, Germany

*Correspondence:
vonhoven@uni-mainz.de (G. von Hoven) and husmann@uni-mainz.de (M. Husmann).

https://doi.org/10.1016/j.tim.2018.11.010

References

1. Berube, B.J. and Bubeck Wardenburg, J. (2013) *Staphylococcus aureus* alpha-toxin: nearly a century of intrigue. *Toxins (Basel)* 5, 1140–1166
2. Bischofberger, M. *et al.* (2012) Pathogenic pore-forming proteins: function and host response. *Cell Host Microbe* 12, 266–275
3. Husmann, M. *et al.* (2009) Elimination of a bacterial pore-forming toxin by sequential endocytosis and exocytosis. *FEBS Lett.* 583, 337–344
4. Wilke, G.A. and Bubeck Wardenburg, J. (2010) Role of a disintegrin and metalloprotease 10 in *Staphylococcus aureus* alpha-hemolysin-mediated cellular injury. *Proc. Natl. Acad. Sci. U. S. A.* 107, 13473–13478
5. Popov, L.M. *et al.* (2015) The adherens junctions control susceptibility to *Staphylococcus aureus* alpha-toxin. *Proc. Natl. Acad. Sci. U. S. A.* 112, 14337–14342
6. Virreira Winter, S. *et al.* (2016) Genome-wide CRISPR screen reveals novel host factors required for *Staphylococcus aureus* alpha-hemolysin-mediated toxicity. *Sci. Rep.* 6, 24242
7. Shah, J. *et al.* (2018) A dock-and-lock mechanism clusters ADAM10 at cell–cell junctions to promote alpha-toxin cytotoxicity. *Cell Rep.* 25, 2132–2147 e7
8. von Hoven, G. *et al.* (2016) Dissecting the role of ADAM10 as a mediator of *Staphylococcus aureus* alpha-toxin action. *Biochem. J.* 473, 1929–1940
9. Haugwitz, U. *et al.* (2006) Pore-forming *Staphylococcus aureus* alpha-toxin triggers epidermal growth factor receptor-dependent proliferation. *Cell. Microbiol.* 8, 1591–1600
10. Watanabe, S. and Boucrot, E. (2017) Fast and ultrafast endocytosis. *Curr. Opin. Cell Biol.* 47, 64–71

Spotlight

Planting the Microbiome

Jennifer K. Spinler,[1,2,@] Vivekanudeep Karri,[3] and Kendal D. Hirschi[3,*,@]

Plant-derived microRNAs stabilized by species-specific lipid nanoparticles mediate interkingdom communication through bacterial intermediates and impact consumer health. Ingested by distinct gut bacteria, these microRNA-containing particles alter bacterial gene expression to affect host immunity.

This three-kingdom interplay provides compelling approaches for health-directed dietary interventions for consumers.

A large portion of the world lives on plant-based diets, and these plants contain hundreds of thousands of microRNAs (miRNAs), a subset of small RNAs that are 19–24 nucleotides in length. In both plants and animals, an miRNA can impact gene expression by annealing to mRNA and inhibiting translation or stability. One current model suggests that exosomes (lipid-based nanoparticles) encapsulate miRNAs and are shed from almost all cell types to interact with specific target cells. In animals, gut epithelial cells excrete miRNAs in exosomal vesicles, making endogenous miRNAs normal components of feces that can regulate specific gut-associated bacterial gene transcripts [1]. Plant-derived exosome-like nanoparticles (ELNs) have also been characterized, are structurally similar to mammalian exosomes [2], and mediate transport of various compounds including proteins and RNA [3]. However, the bioavailability of plant-derived miRNA is controversial. A well publicized study showed that a rice-derived miRNA survives digestion, circulates through the body, and modulates gene expression in the liver of the animal consuming the rice [4]. Unfortunately, replication of this observation has not been straightforward, causing diminished (or lost) enthusiasm for this concept [5]. In fact, public databases strongly suggest contamination and experimental errors as sources of exogenous miRNAs [6]. Despite this controversy, edible nanoparticles have been previously characterized [7] and set the stage to examine if ELNs are bioavailable to gut bacteria and whether diet-derived ELN RNA regulates gut bacterial gene expression [8].

In their recent publication in *Cell Host & Microbe*, Teng and colleagues [8] provide evidence supporting the bioavailability of plant-derived miRNA, its sequestration within edible ELNs, and how ELN composition drives target specificity to impact microbiome structure and function (Figure 1). While previous studies have shown that edible ELNs can be absorbed in the mammalian gastrointestinal (GI) tract, and that ginger-derived vesicles protect against the development of alcohol-induced liver damage [9], the bioactive components of edible ELNs have remained largely enigmatic. Through next-generation sequencing of ginger ELNs (GELNs) and bioinformatic analysis, Teng *et al*. showed that various miRNAs within ginger-derived nanoparticles could potentially bind to bacterial mRNAs. The

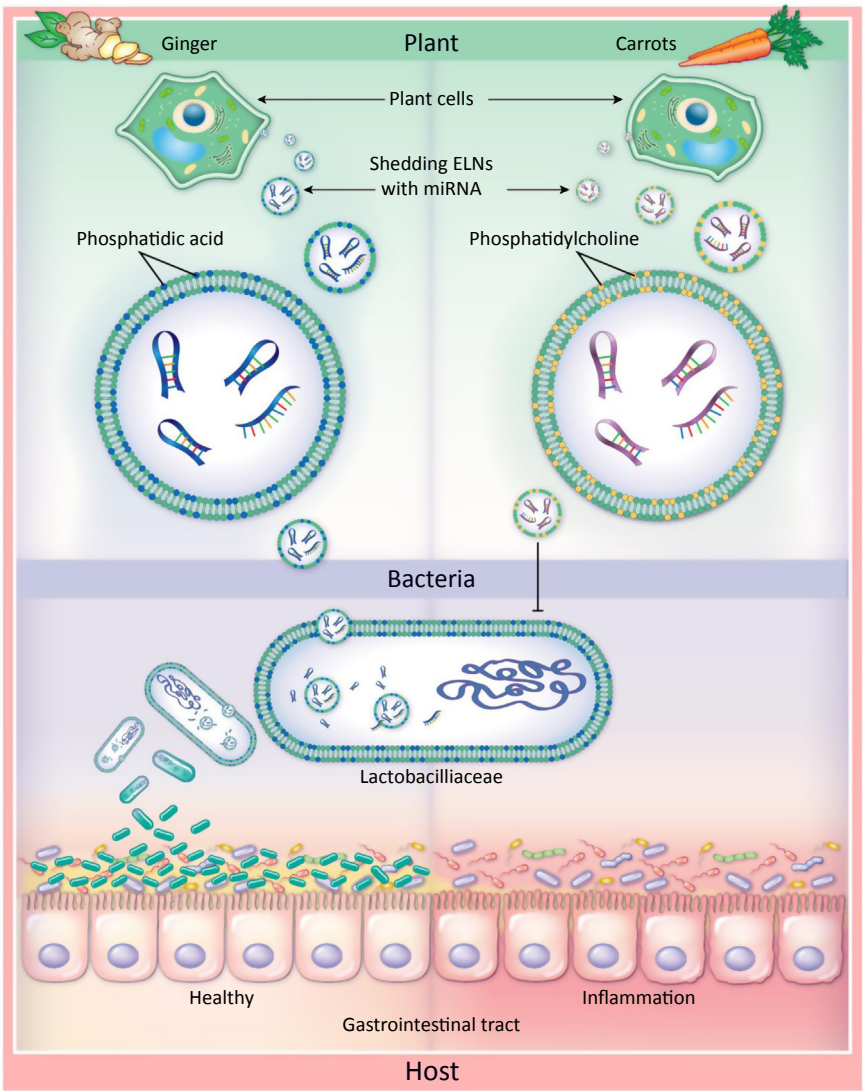

Figure 1. Interkingdom Communication between Plant, Bacteria, and Host. Plant-specific exosome-like nanoparticles (ELNs) harboring plant-derived miRNA are differentiated by phospholipid content which drives preferential uptake by target bacteria. After entering the bacterial cell, ELN-transported miRNA affects bacterial populations and ultimately gut health.

feasibility of this regulation was extended by spatial localization of GELN-specific miRNAs to the gut of mice [8]. To directly address microbiome impact, GELNs gavaged to mice altered the GI microbiome composition as monitored by 16S rRNA gene sequencing. Lactobacilliaceae and Bacteroidales S24-7 populations increased in the GELN-treated mice while Clostridiaceae populations decreased. These GELN-induced changes occurred in both male and female mice and did not cause any mouse abnormalities. These findings were validated in a small human study in which healthy subjects administered oral GELNs for a week showed increased relative abundance of Lactobacilliaceae and Bacteroidaceae, with decreased Clostridiaceae in feces compared to subjects fed normal saline, suggesting that this dietary regulation of gut microbiota is clinically relevant. GELN-directed increases in the relative abundance of Lactobacilliaceae in the GI tract of both humans and mice were further substantiated *in vitro* when GELN treatment of *Lactobacillus rhamnosus* GG (LGG), a member of the Lactobacilliaceae family and a probiotic bacterium, directly promoted LGG growth. Conversely, treatment with grapefruit-derived ELNs reduced LGG growth *in vitro*, suggesting that ELN-directed microbiome shifts could be plant-species-specific.

To establish if the specificity of the microbiome responses were RNA dependent, RNAs from various plant-derived ELNs (carrot, ginger, and grapefruit) were separately encapsulated within GELN-derived lipid nanoparticles (GNVs). When these GNVs were fed to mice, the composition of the gut microbiota fluctuated based on RNA populations housed within the lipid nanoparticles. For example, ginger-derived miRNAs caused changes in Lactobacilliaceae levels where carrot-derived RNAs did not. This regulation of the microbiome population appeared to be established in part via preferential uptake of GELNs by Lactobacilliaceae (Figure 1). Exposure of fluorescently labeled GELNs to LGG established that GELNs target members of the Lactobacilliaceae family. Conversely, when similar studies were done with fluorescently labeled garlic or grapefruit ELNs, Ruminococcaceae uptake was enhanced. A comparative lipid profile of the different plant-derived ELNs showed heterogeneity in lipid content, with GELNs being enriched in phosphatidic acid (PA). Depletion of the PA content in labeled GELNs diminished uptake by LGG, and the reintroduction of PA into these labeled GELNs re-established LGG uptake. These observations strongly suggest that plant-specific lipid signatures in ELN populations target certain bacteria.

This interplay between plant and bacteria driven by GELN-RNAs ultimately benefits the host by enhancing gut barrier function. Mice fed GNVs containing GELN-specific RNAs had superior protection against DSS-induced mouse colitis compared to mice fed GNVs containing scrambled RNA. Similar experiments in a germ-free mouse colitis model validated the requirement of the microbiome intermediate for protection against DSS-induced colitis. Inflammatory cytokines were significantly decreased in colon tissue of germ-free mice treated with LGG and GNVs/GELN-RNAs as compared to mice receiving LGG alone. Deeper probing with bioinformatics and standard molecular biology tools enabled Teng *et al*. to validate their conjecture, showing that various individual ginger miRNAs modified specific LGG genes that are known to have anti-inflammatory effects in the GI tract of mammals.

The work by Teng and colleagues is a tour de force and has transformed our understanding of the relationship between diet and gut health [8]. This work is the strongest evidence to date that plant dietary miRNAs are bioavailable and bioactive. The preferential uptake of ELNs by gut microbiota will interest a wide array of scientists, including bacteriologists and plant biologists that seek to understand how plants communicate with microbes in a variety of environments. Molecular biologists will also be interested in working to unravel the themes that prevail in eukaryotic miRNA regulation of prokaryotic genes. With billions of people consuming a wide variety of plant-based diets, and each person harboring 100–200 different bacterial species consisting of >2 million genes [10], studying interactions between plant-derived ELNs and bacterial gene regulation in consumers forms a Gordian knot. However, if work centers on dissecting the specificity of ELN uptake by bacteria, biologists can focus on utilizing plant-derived nanovectors and synthetic miRNAs as precision tools in dietary therapeutics to manipulate the microbiome for the health of the consumer.

Acknowledgments

We thank Karen Prince for designing the figure.

[1]Texas Children's Microbiome Center, Department of Pathology, Texas Children's Hospital, Houston, TX, USA
[2]Department of Pathology & Immunology, Baylor College of Medicine, One Baylor Plaza, Houston, TX, USA
[3]Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX, USA
@Twitters: @DrJKSpinler, @kendalhirschi

*Correspondence: kendalh@bcm.edu (K.D. Hirschi).

https://doi.org/10.1016/j.tim.2018.12.001

References

1. Liu, S. *et al.* (2016) The host shapes the gut microbiota via fecal microRNA. *Cell Host Microbe* 19, 32–43
2. Deng, Z. *et al.* (2017) Broccoli-derived nanoparticle inhibits mouse colitis by activating dendritic cell AMP-activated protein kinase. *Mol. Ther.* 25, 1641–1654
3. Rutter, B.D. and Innes, R.W. (2018) Extracellular vesicles as key mediators of plant–microbe interactions. *Curr. Opin. Plant Biol.* 44, 16–22
4. Zhang, L. *et al.* (2012) Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. *Cell Res.* 22, 107–126
5. Dickinson, B. *et al.* (2013) Lack of detectable oral bioavailability of plant microRNAs after feeding in mice. *Nat. Biotechnol.* 31, 965–967
6. Witwer, K.W. (2018) Alternative miRNAs? Human sequences misidentified as plant miRNAs in plant studies and in human plasma. *F1000Research* 7, 244

7. Mu, J. *et al.* (2014) Interspecies communication between plant and mouse gut host cells through edible plant derived exosome-like nanoparticles. *Mol. Nutr. Food Res.* 58, 1561–1573
8. Teng, Y. *et al.* (2018) Plant-derived exosomal microRNAs shape the gut microbiota. *Cell Host Microbe* 24, 1–16
9. Zhuang, X. *et al.* (2015) Ginger-derived nanoparticles protect against alcohol-induced liver damage. *J. Extracell. Vesicles* 4, 28713
10. Faith, J.J. *et al.* (2013) The long-term stability of the human gut microbiota. *Science* 341, 1237439

Forum

Highly Pathogenic Avian H7N9 Influenza Viruses: Recent Challenges

Mai-Juan Ma,[1,*] Yang Yang,[2] and Li-Qun Fang[1,*]

Novel highly pathogenic avian influenza (HPAI) H7N9 viruses of the fifth epidemic wave infect humans and poultry. Recently, HPAI H7N9 viruses have evolved into different subtypes and genotypes, exhibited heightened virulence in mammals, and extended their host range, thereby posing a potential threat to public health and the poultry industry.

The Emergence of HPAI H7N9 Infecting Poultry and Humans

Initially, the avian influenza A(H7N9) virus that emerged in early 2013 in China had low pathogenicity to chickens but could replicate and spread efficiently among them. Previous studies have shown that low pathogenic avian influenza (LPAI) viruses can become highly pathogenic via the insertion of multiple basic amino acids at the cleavage site of the hemagglutinin (HA) protein, which was the case for the H5 and H7 subtype avian influenza viruses (AIVs). In July 2016, a novel HPAI H7N9 variant possessing multiple basic amino acids at the cleavage site of the HA protein emerged [1] (Figure 1) and had caused 32 human infections by November 2018[i] as well as 27 outbreaks in poultry farms in 13 provinces of China[ii], raising serious concerns about its epidemic and pandemic potential.

Evolution and Diversity of HPAI H7N9 Viruses

Recent studies by Shi *et al.* and Qi *et al.* [1–3] unveiled the evolution of HPAI H7N9 viruses. They found that the novel HPAI H7N9 variants from both poultry and humans clustered together and formed a distinct cluster in both the hemagglutinin and neuraminidase phylogenetic trees, indicating a single origin. Of note, the hemagglutinin mutants may have arisen from two different H7N9 viruses, one of which subsequently reassorted with others to form two more genotypes [3]. A more recent analysis of the HPAI H7N9 viruses showed that the viruses evolved rapidly and had reassorted with other AIV subtypes within a few months after its emergence in early 2017, forming nine H7 genotypes, including a new 'G9' genotype – a novel HPAI H7N2 strain that was generated by the reassortment between the HPAI H7N9 viruses and other unknown duck viruses in unvaccinated domestic ducks [2] (Figure 1). Additionally, HPAI H7N9 and H7N2 viruses detected in provinces other than Guangdong are descendants of the earlier Guangdong HPAI H7N9 viruses, rather than mutated derivatives of the local LPAI H7N9 viruses. The internal genes of the novel HPAI H7N9 variants, however, are scattered across the phylogenetic tree and formed several clusters but are closely related to the LPAI H7N9/H9N2 strains circulating locally among humans and chickens in the past waves. These observations suggest that these HPAI H7N9 variants have undergone complex reassortment with LPAI H7N9/H9N2 strains, and that other subtypes of AIVs are cocirculating in poultry and forming multiple genotypes.

Replication, Pathogenicity, and Transmissibility of HPAI H7N9 Viruses in Mammals

To date, although no sustained transmission of HPAI H7N9 viruses among humans has been documented, further adaptation of these AIVs to humans may result in transmissible viruses with pandemic potential. Therefore, timely and comprehensive evaluation of the replication, pathogenicity, and transmissibility of HPAI H7N9 viruses in mammals is critical to the understanding of their evolutionary characteristics and determinants for the emergence of potential pandemic strains. Recent studies have shown that the HPAI H7N9 viruses isolated from humans could have different consequences for mammals compared to those isolated from poultry [1,3,4] (Figure 1). In the mouse model, human strains (A/Guangdong/17SF003/2016-like virus) were lethal and more virulent than LPAI H7N9 viruses [1,4]. In ferrets, A/Guangdong/17SF003/2016-like viruses not only killed the animals but also transmitted through respiratory droplets with comparable efficiency to LPAI H7N9 viruses [4]. Replication of the viruses in extrapulmonary tissues, including in brains, was detected in mice and ferrets. In contrast, a chicken strain (A/chicken/Guangdong/SD008/2017) was not lethal to mice or ferrets nor transmitted through ferrets [3]. Therefore, human-isolated HPAI H7N9 viruses were more pathogenic in mice and more transmissible in ferrets than those isolated from poultry. However, when bearing the mammalian-adapted 627K or 701N mutation in its polymerase basic 2 (PB2) protein, the A/chicken/Guangdong/SD008/2017 virus became highly lethal in mice and efficiently transmissible in ferrets [3], which may be due to the avian-isolated HPAI H7N9 with such mutations were easily recovered [1,3]. In the recent study of Shi *et al.* [2], all 18 tested HPAI H7 subtype strains of avian-isolated viruses (including the