INTRODUCTION

Broad-spectrum antibiotics that act on a wide range of disease-causing bacteria, for example, Gram-negative or Gram-positive species, have saved millions of human lives and remain among the most important drugs in modern medicine. At the same time, three major challenges highlight the need for species-specific antibiotics. First, the emergence of multidrug resistant pathogens demands new types of anti-infectives that can target resistant bacteria on the species level. Second, long-term treatment with broad-spectrum antibiotics for chronic infections or elimination of cancer-associated microbes can have severe side effects. For instance, proliferation of some life-threatening pathogens, such as Clostridium difficile, is primarily a disorder of the microbiota and difficult to manage with conventional antibiotics (Abt, Mckenney, & Pamer, 2016). Here, the use of a selective antibiotic will spare the endogenous microbiota and prevent dysbiosis. Third, microbiota research has increasingly highlighted contributions of individual microbiota members to health and disease, indicating that individual bacterial species crucially influence the host immune system, drug uptake and efficiency, as well as the onset of autoimmune diseases. Yet, these links are typically inferred from correlations between abundance of certain bacteria and disease. Proving causation will require an ability to eliminate individual species in a complex community, something that is not possible with broad-spectrum antibiotics (Figure 1).
If successful, it may open the door to a new generation of therapies editing the microbiome. Such personalized microbiota therapy, for example, could benefit nonresponsive patients in whom a drug happens to be rapidly metabolized by a resident microbiota member before it reaches the site of action.

A number of promising species-specific antimicrobials already exist. Examples include colicins that are known for their narrow killing spectrum (Cascales et al., 2007), recently developed fimbriae antagonists against some pathogenic strains of *Escherichia coli* (Spaulding et al., 2017), and several species-specific antibodies (Cattoir & Felden, 2019). Phages are highly specific for their bacterial targets, and are resurfacing as an attractive treatment option in refractory bacterial infections thanks to their activity against antibiotic-resistant pathogens and a lack of serious side effects (Hesse & Adhya, 2019). The renewed interest in phage therapy coincides with advances in our understanding of antiphage defense systems such as CRISPR-Cas which themselves, when repurposed as an antibacterial weapon (Beisel, Gomaa, & Barrangou, 2014; Bikard et al., 2014; Citorik, Mimee, & Lu, 2014), represent another strong alternative to broad-spectrum antibiotics.

An ideal scenario for species-specific antibiotics would be a class of molecules that can be synthesized chemically and programmed following universally applicable, rational rules to target any bacterial species of interest. In this regard, RNA-based antimicrobials in the form of short antisense oligonucleotides (ASOs) that...
inhibit essential genes on the RNA level are an exciting technology (Figure 2). This type of ‘programmable RNA antibiotic’ began to be pursued in E. coli decades ago, initially with tiny oligonucleotides targeting ribosomal RNA (Jayaraman, McParland, Miller, & Ts’o, 1981) and subsequently ASOs in the 9-mer to 12-mer range repressing the mRNA of the essential fatty acid biosynthesis protein, AcpP (Good, Awasthi, Dryselius, Larsson, & Nielsen, 2001). The latter study conceded that the 2 μM concentration of peptide nucleic acid (PNA) required to clear a HeLa cell culture of E. coli was ‘somewhat high compared with conventional antibiotics’ (Good et al., 2001); nonetheless, proof-of-principle was clearly established.

As compiled in a recent excellent review by others (Sully & Geller, 2016), ASO-based antimicrobials have since been tested in vitro or in vivo in many more bacteria than E. coli. The published work spans Gram-negative Acinetobacter, Brucella, Burkholderia, Campylobacter, Haemophilus, Klebsiella, Pseudomonas, and Salmonella species, and Gram-positive Enterococcus, Listeria, Staphylococcus and Streptococcus species (Geller et al., 2018; Sully & Geller, 2016). ASOs have shown efficacy in different experimental mouse infections, suggesting that they may be therapeutically useful for treating sepsis or different diseases of the lung (Barkowsky et al., 2019; Daly et al., 2018; Geller et al., 2018; Sully & Geller, 2016).

RNA is an excellent cellular target but in its unmodified form it is rather unstable, so it does not lend itself for being administered as an ASO (Kole, Krainer, & Altman, 2012). However, several classes of modified nucleic acids with improved stability and nuclease resistance are available as potential antisense antimicrobials: locked nucleic acids (LNA), phosphorodiamidate morpholino oligomers (PMO) and PNA. Of these, PMO and PNA have been most popular as an antimicrobial (Sully & Geller, 2016), with PMO having the slight disadvantage that the cost of this patented technology renders it suboptimal for high-throughput fundamental research studies. Sensu stricto, PNA is not a nucleic acid but a synthetic polymer (resistant to both nuclease and protease) with a pseudo-peptide backbone and attached nucleobases that obey classical RNA pairing rules. The universal principles guiding gene targeting allows one to leverage all of these above modalities for bacterial gene silencing. The ASO is usually designed such that it sequesters the ribosome binding site (RBS) of an mRNA, preventing its recognition by the 3OS ribosomal subunit and hence, protein synthesis (Figure 2).

Due to the inherently poor uptake of nucleic acids by bacterial and mammalian cells, therapeutic oligonucleotides are generally tethered to a short (<30 amino acids) cell-penetrating carrier peptide (CPP), predominantly cationic or amphiphilic in nature (Xue et al., 2018), to improve delivery. For nomenclature, a peptide-ASO conjugate is typically referred to as PPNA or PPMO (Figure 2). Many CPPs are able to penetrate both mammalian and bacterial cell membranes, which increases their attractiveness for targeting intracellular pathogens.

Despite promising proof-of-concept for programmable RNA antibiotics, there are several important open questions to be addressed in advancing the technology, especially if one starts thinking about applications in the microbiota. This also acknowledges that while the abovementioned dozen bacteria targeted so far are all relatively well-studied, they represent just a tiny sliver of the >1,000 different bacteria that live in and on us. It is fair to say that the vast majority of our microbiota is ‘terra incognita’ with respect to the molecular properties important for ASO-based targeting, for example, we do not know the primary posttranscriptional mechanisms or the structure of the envelope of these many diverse bacteria.

Take the example of Fusobacterium nucleatum, a commensal-turned pathogen that has recently garnered much attention for its association with colorectal cancer and other human diseases (Brennan & Garrett, 2019; Han, 2015). To prove some of the proposed disease links, it would be desirable to selectively deplete F. nucleatum in the colon of an experimental animal, which should be possible by administrating an ASO that selectively targets the mRNA of an essential gene of this species. As it stands, however, knowledge about the transcriptome structure and membrane composition of this filamentous gram-negative rod is sparse, as it is for the entire phylum Fusobacteria.

Nonetheless, there has been much recent progress in our understanding of the RNA biology of bacteria beyond the long-standing work horse, E. coli (Storz & Papenfort, 2018). In addition, the advent of high-throughput RNA sequencing (RNA-seq) has triggered the development of many generic global approaches that can rapidly and comprehensively analyze the RNA composition of any bacterium of interest (Hör, Gorski, & Vogel, 2018; Sorek & Cossart, 2010). I will argue below that leveraging this recent mechanistic knowledge and the new global technologies of bacterial RNA biology will be important in the quest to expand programmable RNA antibiotics to the full breadth of microbes that matter in human health and disease.

1.1 | Mechanisms of action

A review of the literature on antibacterial ASOs shows that the site of translation initiation is seen as the optimal mRNA target region for antisense repression. Several studies have tested this more systematically, for example, scanning the 5’ regions of different mRNAs in E. coli (Dryselius, Aswasti, Rajarao, Nielsen, & Good, 2003) or Burkholderia cepacia (Daly et al., 2018) with PPNA or PPMO, respectively. The main conclusion from these studies is that an ASO antisense to the start codon and perhaps part of the Shine-Dalgarno sequence (SD) will be the most potent.

Going after the most conserved elements of the RBS offers a generic principle for the design of potent ASOs, but may also compromise specificity, given that the RBS is a region of low complexity. After all, the SD and start codon are similar among the genes of a given species and in bacteria in general. In other words, these elements may be suboptimal when striving for highly selective killing within bacterial communities. We may not care much about off-target effects in the same bacterium as long as it gets killed, but if the ASO happens to be taken up by another microbiota member with a similar RBS sequence, species-specific killing will be compromised. To solve this problem, can we learn from endogenous posttranscriptional control mechanisms?

The past decade has brought tremendous progress in our mechanistic understanding of endogenous antisense regulation
in bacteria (Sesto, Wurtzel, Archambaud, Sorek, & Cossart, 2013; Wagner & Romby, 2015), quite a bit of which has come from the study of small noncoding RNAs (sRNAs) in E. coli and Salmonella (Hör, Materia, Vogel, Gottesman & Storz, 2020). Most of these sRNAs have turned out to act by short base pairing on trans-encoded mRNAs and bind in 5’ regions to occlude ribosome entry, akin to the ASO approach. While pairing with the SD or start codon is also common among these sRNAs, collectively their target sites also suggests that a much larger mRNA sequence window exists for successful inhibition (Figure 3). For example, sRNAs can occlude ribosomes at sites as far as five codons into the CDS (Bouvier, Sharma, Mika, Nierhaus, & Vogel, 2008), where sequence diversity is already much higher. In Salmonella, repression was observed even further downstream, by sRNA-mediated induction of mRNA cleavage (Pfeiffer, Papenfort, Lucchini, Hinton, & Vogel, 2009). This mechanism of induced cleavage appears to be enhanced by a 5’ monophosphate status of the sRNA, at least in vitro (Bandrya et al., 2012). Whether and how different 5’ caps enable ASO-induced mRNA cleavage in other species, remains to be systematically tested. It goes without saying, though, that if ASOs could be generally directed to the CDS, this would vastly extend the target space of programmable RNA antibiotics.

The mRNA 5’UTR also offers ample sequence space for antisense repression. Inhibition by sRNA of 30S entry has been observed up to 50 bases upstream of the start codon (Sharma, Darfeuille, Plantinga, & Vogel, 2007). Other RBS-independent mechanisms of sRNAs include suppression of secondary structure that is required for optimal mRNA translation (Hoeckzema, Romilly, Holmqvist, & Wagner, 2019; Jagodnik, Chiuruttini, & Guiller, 2017). Thus, short base pairing sRNAs have more than one way of inhibiting protein synthesis, arguing that a substantially larger sequence space waits to be explored for the inhibition of essential genes.

1.2 | ASO design for targeting in complex communities

What kind of ASO is necessary to selectively target a single essential gene in a human microbiome with 1,000 different species? Let us make the following assumptions: each of these species has 4,000 protein-coding genes, ~10% of which will be essential and every one of them has a unique 5’ mRNA region. This will require ASOs long enough to cover 400,000 different potential target sites. Intriguingly, an ASO pool of 10-mers already covers 1,048,575 unique sequences, while 11-mers achieve a theoretical complexity of 4,194,304. Obviously, this back-of-the-envelope calculation ignores tolerance of mismatches, as well as G:U pairs, yet it does give a ballpark figure arguing that microbiome editing by species-specific RNA antibiotics may be feasible.

These numbers are well in line with studies looking at the best length for antibacterial ASOs, which together reveal an optimal length of 10–12 bases (Deere, Iversen, & Geller, 2005; Dryselius et al., 2003; Goltermann, Yavari, Zhang, Ghosal, & Nielsen, 2019). The longer the ASO, the less efficient its uptake. For example, a 10-mer anti-acpP PNA was found to kill most effectively in E. coli (Dryselius et al., 2003). However, it is important to consider that these rules largely stem from a single species (E. coli), and it will be important to repeat these experiments in representative microbiota members before making generalizations. Similarly, it will be necessary to systematically assess off-target effects. Modern RNA-seq technology is available to score changes in mRNA expression levels and decay patterns in in vitro culture of single species or communities (Chao et al., 2017; Dar et al., 2016; Sharma et al., 2010), as well as bacteria inside eukaryotic host cells or tissue (Nuss et al., 2017; Westermann et al., 2016).

It is nevertheless striking to see how well the empirically determined optimal length for ASOs echoes mRNA recognition by endogenous sRNAs, the latter of which is the result of hundreds of million years of natural evolution. With a typical length of ~ 50–200 nts, these natural sRNAs are much longer, but in substance, their target pairing is akin to ASOs. In particular, sRNAs that depend on the global RNA-binding proteins (RBPs) Hfq and ProQ (Holmqvist & Vogel, 2018), contain short ‘seed’ regions of 8–12 highly conserved bases that disproportionately contribute to target search and pairing (Gorski, Vogel, & Doudna, 2017). Systematic analyses of sRNA seed regions have demonstrated high selectivity in mRNA recognition (Balbontin, Fiorini, Figueroa-Bossi, Casadesus, & Bossi, 2010; Kawamoto, Koide, Morita, & Aiba, 2006; Papenfort, Bouvier, Mika, Sharma, & Vogel, 2010; Rutherford, Valastyan, Taillefumier, Wingreen, & Bassler, 2015), to the degree that the seed can distinguish between two similar mRNAs at the level of a single hydrogen bond whereby a G:C and G:U pair differ (Papenfort, Podkaminski, Hinton, & Vogel, 2012). The seed can be grafted onto an unrelated sRNA and will still recognize the original targets (Bouvier et al., 2008; Fröhlich, Papenfort, Fekete, & Vogel, 2013; Papenfort et al., 2010). Its functional autonomy has also been supported by structural work, revealing how an 11-base seed protrudes from a sRNA-Hfq complex, free to capture targets (Dimastrogiovanni et al., 2014).

One could imagine exploiting the similarity of natural sRNA seeds to ASOs to learn more about the rules of specific target recognition inside bacterial cells. Indeed, synthetic sRNAs have been successfully used to target mRNAs, for example, for metabolic engineering of E. coli (Lahiry, Stimple, Wood, & Lease, 2017; Na et al., 2013). While the cost of a high-throughput, sequence-randomized ASO screen is currently prohibitive, one could use a synthetic sRNA library with a randomized seed region fused to an Hfq- or ProQ-associating backbone as a cost-effective proxy for screening. Indeed, there is an emerging class of sRNAs – those processed from mRNA 3’ ends – some of which are as short as 38 bases and could serve as excellent chassis (De Mets, Melderen, & Gottesman, 2019; Miyakoshi, Matera, Maki, Sone, & Vogel, 2019). Other good chassis could be sRNAs where everything but the seed is structured (Fröhlich et al., 2013).

Comprehensive screening would score the abilities of plasmid-expressed, randomized seed variants to regulate a suitable reporter or to simply kill their bacterial host; to be analyzed by comparative deep sequencing of libraries before and after sRNA induction, as
recently done in *Vibrio cholera* to dissect the seed-pairing domain of an envelope-stress related sRNA (Peschek, Hoyos, Herzog, Forstner, & Papenfort, 2019). Potent seeds could then be synthesized as ASOs and tested independently by delivering them to bacteria with peptidoglycans. One potential caveat to keep in mind, however, is that gene regulation by natural sRNAs often involves binding of an RBP such as Hfq in the target mRNA as well (Link, Valentin-Hansen, & Brennan, 2009; Peng, Soper, & Woodson, 2014; Zhang, Schu, Tjaden, Storz, & Gottesman, 2013). In other words, it is unclear whether a given short duplex with an mRNA will be equally productive when it forms with a natural sRNA versus an ASO. In more general terms, it is fair to say that we have a limited knowledge about possible systematic differences in the in vivo formation and stability between RNA-RNA duplexes and RNA duplexes with modified nucleic acids such as PNA or PMO.

Recently published global RNA interactomes (Han, Tjaden, & Lory, 2016; Melamed, Adams, Zhang, Zhang, & Storz, 2019; Melamed et al., 2016; Smirnov et al., 2016; Waters et al., 2017) offer yet another type of data sets to decipher the rules of productive sRNA-mRNA pairing inside bacterial cells. These interactomes...
1.3 | Carrier peptides, mechanisms of ASO uptake and side effects on eukaryotic cells

The envelopes of bacteria are nearly impenetrable by high molecular weight oligomers. An 11-mer ASO is easily in the range of 3–4 kDa, whereas porins as the main entry gates in the outer membrane exclude molecules >600 Da. Therefore, ASO delivery into the cytoplasm requires the attachment of a carrier peptide (Good et al., 2001). The conjugation of a carrier peptide is crucial as it decreases the minimal inhibitory concentration (MIC) of a toxic ASO from the millimolar to the lower micromolar or even submicromolar range, thereby endowing them with the same potency as conventional antibiotics (Andrews, 2001).

Several different peptides have been successfully used to transport ASOs into bacteria, including studies in the mouse. They are typically derived from natural CPPs and often with a sequence alternating cationic and non-polar amino acids (Sully & Geller, 2016). Natural antimicrobial peptides (AMPs) with an intracellular mode of action have also been explored (Hansen et al., 2016). However, a systematic, high-throughput analysis of the peptide efficiency and transport in individual bacterial species is yet to be conducted, especially since there are reported cases where the peptide and not the RNA-targeting part was found to be the toxic moiety (Hansen et al., 2016). More importantly, different peptides penetrate different bacteria with different efficiencies, as nicely illustrated by the clear differences seen between *Burkholderia* versus *Pseudomonas* and *Acinetobacter* (Geller et al., 2013; Greenberg et al., 2010; Howard et al., 2017). As can be expected from their generally different envelope structures, some peptides seem to work better in Gram-positive than Gram-negative species (see a recent discussion in (Barkowsky et al., 2019)). Together, this argues that species specificity in killing in complex communities can be improved by using the most selective peptide for the bacterium to be targeted.

To better understand the individual impact and activities of the carrier peptides, it seems timely to leverage the power of bacterial RNA-seq approaches (Hör et al., 2018) to start asking whether or not they are truly neutral, and which cellular pathways they themselves may trigger. The goal would be to find functionally neutral peptides that do not induce envelope or other type of stress, so leave the microbiota members generally undisturbed. Another important aspect yet to be addressed in ASO research are persisters, that is, cells within a population that survive intensive antibiotic treatment without being genetically resistant, leading to relapse of the infection once treatment stops (Balaban et al., 2019). Dual RNA-seq allows one to study bacterial persisters even when these form inside eukaryotic cells (Stapels et al., 2018). This will be important against the backdrop of foreseeable obstacles for ASO-based treatment of persisters: these cells are metabolically largely inactive, so uptake may be an issue. In addition, if they are contained within biofilms, we have little knowledge if and how this type cellular matrix can be penetrated by ASOs.

It is also becoming clear that bacterial mRNA expression shows some degree of spatial organization. There is recent RNA-seq data reporting cytoplasmic versus membrane or other subcellular localization (Kannaiah, Livny, & Amster-Choder, 2019), which could tell us why some mRNAs are better targets than others. This may then also inform the choice of peptides that deliver an ASO to near its desired target mRNA to increase efficacy.

There is yet another, burgeoning area of bacterial RNA biology that may benefit the development of programmable RNA antibiotics: extracellular RNA. While the extent to which bacteria release RNA molecules is currently unclear, there is excitement that extracellular vesicles may delivery RNA from one cell to another as a form of interspecies communication (Franz et al., 2019; Koeppen et al., 2016; Lee, 2019). As interest increases, other and more defined molecular mechanisms for transport of nucleic acids across bacterial envelopes may be found. In this regard, the Cossart laboratory has just made the pioneering discovery of a secreted protein of *Listeria monocytogenes* that carries RNA species from the bacterial cytosol into the lumen of host cells (Pagliuso et al., 2019). The precise export mechanism is yet unknown, but one is tempted to speculate that where there is export, there is import, too, and that proteins that transport RNA or even naturally modified nucleic acids into bacterial cells may be found in the future.

1.4 | Resistance mechanisms and host response

Resistance is a concern for any antibiotic but is poorly understood with respect to peptide-ASO conjugates. The only systematic analyses published thus far concluded that some PPNA or PPMO conjugates enter *E. coli* through the inner membrane peptide transporter, SbmA, whose gene is nonessential and so easily yields viable resistant mutants (Ghosal, Vitali, Stach, & Nielsen, 2013; Puckett et al., 2012). It is important to stress that SbmA is necessary for only a subset of peptide-ASO conjugates, and that the transport mechanism(s) of many other constructs used in the field remains to be elucidated (Ghosal et al., 2013; Hansen et al., 2016). RNA-seq again may offer a potent approach to learn more about bacterial resistance acquisition. On the level of gene expression, it could reveal particular transporters or stress response pathways that respond to peptide-ASO conjugates or their individual parts. This should involve a comparison of resistance formation against ASO drugs with that induced by sub-inhibitory concentrations of conventional antibiotics (Ebral et al., 2019; Dar et al., 2016), to discover the potential for synergistic or antagonistic interactions. Dual RNA-seq, which simultaneously profiles gene expression changes in both a bacterium and infected...
eukaryotic cells (Westermann et al., 2016), lends itself to determining how peptide-ASO conjugates alter the host response, regardless of their successful intracellular killing. Moreover, considering the much larger size of human transcriptsomes, ASOs of 10–12 nucleotides in length would surely have targets on mRNAs in host cells as well. Likewise, there is the possibility that ASO{s reach the nucleus and induce DNA damage. Given the growing feasibility of Dual RNA-seq (Westermann, Barquist, & Vogel, 2017) and new protocols that work with very little starting material (Penaranda & Hung, 2019), the hope is that next generation sequencing based transcriptomics will provide us with a fine-grained picture of the wanted and unwanted activities of ASO antimicrobials as they get administered.

To briefly return to the matter of extracellular RNA, we currently know little about possible transport mechanisms or nucleic acid secretion/uptake systems in general (with the exception of type IV secretion systems), neither in the model species of modern microbiology, nor in the many more, largely uncharacterized members of the human microbiota. If there are more secreted RBPs among bacterial effector proteins (Pagliuso et al., 2019), most of them will lack recognizable RNA-binding domains, rendering in silico prediction difficult (Tawk, Sharan, Eulalio, & Vogel, 2017). In this regard, bacterial RNA biology approaches may reveal potential routes of nucleic acids transport in bacteria from resistant mutants to RNA-like antibiotics.

2 | CONCLUSION

There is ample proof-of-concept that ASO drugs can effectively eliminate diverse bacteria, including demonstrated efficacy in animal models. Yet, many fundamental questions remain to be answered for this technology to reach its true potential for a precision editing of the microbiome. I hope to have conveyed in this brief essay that the accumulated knowledge and RNA-centric techniques provided by basic microbiology can help to accelerate the development of new species-specific RNA antibiotics. I foresee a wide range of applications beyond the killing of pathogens, which has been the focus over the past two decades. Microbes contribute to our health in so many other ways: they impact on how we digest our food, what allergens are produced or how drugs are metabolized. As human medicine pays more attention to these aspects, this will increase the demand for precise ways of manipulating individual bacterial species. Some of these potential applications may not require mRNA repression but activation of genes. In this regard, the wunder{tüte of bacterial RNA biology has more to offer for ASO-based applications, as several different new rRNA mechanisms of mRNA activation (Figure 3) have been identified over the past few years (Papenfort, Sun, Miyakoshi, Vanderpool, & Vogel, 2013; Papenfort & Vanderpool, 2015; Salvail, Caron, Belanger, & Masse, 2013; Sedlyarova et al., 2016), including ones involving the mRNA 3’ UTR (Ruiz de los Mozos et al., 2013).

Applications can also be envisaged beyond human-associated microbes, including general ecology. Outside laboratories, sterile animals do not exist. If one wanted to test the importance of a given bacterial species for the development and health of a beetle or insect, or even a zebra fish, it may be hard to find the right antibiotic in the pharmacy. But why not try an ASO?

ACKNOWLEDGMENT

I would like to wholeheartedly thank Pascale Cossart for her strong support in my career since I began to work with bacteria, and in more general terms, for her unremitting promotion of microbiology. The present text is based on a lecture I wrote in her honor when she received the Heinrich Wieland Prize in 2018. Lars Barquist, Franziska Faber and Kristina Popova were so kind to provide comments on the manuscript. Work on RNA-centric antibiotics in the Vogel lab is supported by a Bayresq.net grant and by a Gottfried Wilhelm Leibniz award (DFG Vo875/18).

ORCID

Jörg Vogel https://orcid.org/0000-0003-2220-1404

REFERENCES

Abt, M. C., McKenney, P. T., & Pamer, E. G. (2016). Clostridium difficile colitis: Pathogenesis and host defence. Nature Reviews Microbiology, 14, 609–620. https://doi.org/10.1038/nrmicro.2016.108

Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy, 48(Suppl 1), 5–16. https://doi.org/10.1093/jac/48.suppl_1.5

Balaban, N. Q., Helaine, S., Lewis, K., Ackermann, M., Aldridge, B., Andersson, D. I., … Zinkernagel, A. (2019). Definitions and guidelines for research on antibiotic persistence. Nature Reviews Microbiology, 17, 441–448. https://doi.org/10.1038/s41579-019-0196-3

Balbontin, R., Fiorini, F., Figueroa-Bossi, N., Casadesus, J., & Bossi, L. (2010). Recognition of heptameric seed sequence underlies multi-target regulation by RybB small RNA in Salmonella enterica. Molecular Microbiology, 78, 380–394. https://doi.org/10.1111/j.1365-2958.2010.07342.x

Bandyra, K. J., Said, N., Pfeiffer, V., Gorna, M. W., Vogel, J., & Luisi, B. F. (2012). The seed region of a small RNA drives the controlled destruction of the target mRNA by the endoribonuclease RNase E. Molecular Cell, 47, 943–953. https://doi.org/10.1016/j.molcel.2012.07.015

Barkowsky, G., Lemster, A. L., Pappesch, R., Jacob, A., Kruger, S., Schroder, A., … Patenge, N. (2019). Influence of di&erent cell-penetrating peptides on the antimicrobial e&iciency of RNAs in Streptococcus pneumoniae. Molecular Therapy Nucleic Acids, 18, 444–454. https://doi.org/10.1016/j.omtn.2019.09.010

Beisel, C. L., Gomaa, A. A., & Barrangou, R. (2014). A CRISPR design for next-generation antimicrobials. Genome Biology, 15, 516. https://doi.org/10.1186/s13059-014-0516-x

Bikard, D., Euler, C. W., Jiang, W., Nussenzweig, P. M., Goldberg, G. W., Dupportet, X., … Marraffini, L. A. (2014). Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. Nature Biotechnology, 32, 1146–1150. https://doi.org/10.1038/nbt.3043

Bouvier, M., Sharma, C. M., Mika, F., Nierhaus, K. H., & Vogel, J. (2008). Small RNA binding to 5’ mRNA coding region inhibits translational initiation. Molecular Cell, 32, 827–837. https://doi.org/10.1016/j.molc.2008.10.027

Brennan, C. A., & Garrett, W. S. (2019). Fusobacterium nucleatum—Symbiont, opportunistand oncobacterium. Nature Reviews Microbiology, 17, 156–166. https://doi.org/10.1038/s41579-018-0129-6

Cabral, D. J., Penumutchu, S., Reinhart, E. M., Zhang, C., Korry, B. J., Wurster, J. I., … Belenky, P. (2019). Microbial metabolism modulates antibiotic susceptibility within the murine gut microbiome. Cell Metabolism, 30(800–823), e807. https://doi.org/10.1016/j.cmet.2019.08.020
membrane vesicles. PLoS Pathogens, 12, e1005672. https://doi.org/10.1371/journal.ppat.1005672

Kole, R., Krainer, A. R., & Altman, S. (2012). RNA therapeutics: Beyond RNA interference and antisense oligonucleotides. Nature Reviews Drug Discovery, 11, 125–140. https://doi.org/10.1038/ndr3625

Lahiry, A., Simple, S. D., Wood, D. W., & Lease, R. A. (2017). Retargeting a dual-acting sRNA for multiple mRNA transcript regulation. ACS Synthetic Biology, 6, 648–658. https://doi.org/10.1021/acssynbio.6b00261

Lee, H. J. (2019). Microbe-host communication by small RNAs in extracellular vesicles: Vehicles for transkingdom RNA transportation. International Journal of Molecular Sciences, 20(6), 1487.

Link, T. M., Valentin-Hansen, P., & Brennan, R. G. (2009). Structure of Escherichia coli Hfq bound to polyriboadenylate RNA. Proceedings of the National Academy of Sciences of the United States of America, 106, 19292–19297. https://doi.org/10.1073/pnas.0908744106

Malamed, S., Adams, P. P., Zhang, A., Zhang, H., & Storz, G. (2019). RNA-RNA interacstomes of ProQ and Hfq reveal overlapping and competing roles. Molecular Cell, 77, 411–425.e7. https://doi.org/10.1016/j.molcel.2019.10.022

Malamed, S., Peer, A., Faigenbaum-Romm, R., Gatt, Y. E., Reiss, N., Bar, A., … Margalit, H. (2016). Global mapping of small RNA-target interactions in bacteria. Molecular Cell, 63, 884–897. https://doi.org/10.1016/j.molcel.2016.07.026

Miyakoshi, M., Mattera, G., Maki, K., Sone, Y., & Vogel, J. (2019). Functional expansion of a TCA cycle operon mRNA by a 3’ end-degrded small RNA. Nucleic Acids Research, 47, 2075–2088. https://doi.org/10.1093/nar/gky1243

Na, D., Yoo, S. M., Chung, H., Park, H., Park, J. H., & Lee, S. Y. (2013). Metabolic engineering of Escherichia coli using synthetic small regulatory RNAs. Nature Biotechnology, 31, 170–174. https://doi.org/10.1038/nbt.2461

Nuss, A. M., Beckstette, M., Pimenova, M., Schmuhl, C., Opitz, W., Pisano, F., … Dersch, P. (2017). Coding sequence targeting by MicC RNA reveals bacterial mRNA silencing downstream of translational initiation. Nature Structural & Molecular Biology, 16, 840–846. https://doi.org/10.1038/nsmb.1631

Puckett, S. E., Reese, K. A., Mitev, G. M., Mullen, V., Johnson, R. C., Pomraning, K. R., … Geller, B. L. (2012). Bacterial resistance to antisense peptide phosphorodiamidate morpholino oligomers. Antimicrobial Agents and Chemotherapy, 56, 6147–6153. https://doi.org/10.1128/AAC.00850-12

Puhui, M., Pappoe, K., Lucchini, S., Hinton, J. C., & Vogel, J. (2009). Coding sequence targeting by MicC RNA reveals bacterial mRNA silencing downstream of translational initiation. Nature Structural & Molecular Biology, 16, 840–846. https://doi.org/10.1038/nsmb.1631

Rutherford, S. T., Valastyan, J. S., Taillefumier, T., Wingreen, N. S., & Bassler, B. L. (2015). Comprehensive analysis reveals how single nucleotides contribute to noncoding RNA function in bacterial quorum sensing. Proceedings of the National Academy of Sciences of the United States of America, 112, E6038–E6047. https://doi.org/10.1073/pnas.1518958112

Salvai, H., Caron, M. P., Belanger, J., & Masse, E. (2013). Antagonistic functions between the RNA chaperone Hfq and an sRNA regulate sensitivity to the antibiotic colicin. EMBO Journal, 32, 2764–2778. https://doi.org/10.1038/emboj.2013.205

Sedlyarova, N., Shamovsky, I., Bharati, B. K., Epstein, V., Chen, J., Gottesman, S., … Nuider, L. (2016). sRNA-mediated control of transcription termination in E. coli. Cell, 167, 111–121 e113.

Sesto, N., Wurtzel, O., Archambaud, C., Sorek, R., & Cossart, P. (2013). The excludon: A new concept in bacterial antisense RNA-mediated gene regulation. Nature Reviews Microbiology, 11, 75–82. https://doi.org/10.1038/nrmicro2934

Sharma, C. M., Darfeuille, F., Plantinga, T. H., & Vogel, J. (2007). A small RNA regulates multiple ABC transporter mRNAs by targeting C/A-rich elements inside and upstream of ribosome-binding sites. Genes & Development, 21, 2804–2817. https://doi.org/10.1101/gad.447207

Sharma, C. M., Hoffmann, S., Darfeuille, F., Reignier, J., Findeiss, S., Sittka, A., … Vogel, J. (2010). The primary transcriptome of the major human pathogen Helicobacter pylori. Nature, 464, 250–255. https://doi.org/10.1038/nature08756

Sminov, A., Forstner, K. U., Holmquist, E., Otto, A., Gunster, R., Becker, D., … Vogel, J. (2016). Grad-seq guides the discovery of ProQ as a major small RNA-binding protein. Proceedings of the National Academy of Sciences of the United States of America, 113, 11591–11596. https://doi.org/10.1073/pnas.1609981113

Sorek, R., & Cossart, P. (2010). Prokaryotic transcriptomics: A new view on regulation, physiology and pathogenicity. Nature Reviews Genetics, 11, 9–16. https://doi.org/10.1038/nrg2695

Spaulding, C. N., Klein, R. D., Ruer, S., Kau, A. L., Schreiber, H. L., Cusumano, Z. T., … Hultgren, S. J. (2017). Selective depletion of uro-pathogenic E. coli from the gut by a FimH antagonist. Nature, 546, 528–532. https://doi.org/10.1038/nature22972

Stapels, D. A. C., Hill, P. W. S., Westermann, A. J., Fisher, R. A., Thurston, T. L., Saliba, A. E., … Helaine, S. (2018). Salmonella persisters under-mine host immune defenses during antibiotic treatment. Science, 362, 1156–1160.

Storz, G., & Papenfort, K. (Eds.). (2018). Regulating with RNA in bacteria and archaea (1st ed.). ASM Press. 575 p.
Sully, E. K., & Geller, B. L. (2016). Antisense antimicrobial therapeutics. *Current Opinion in Microbiology*, 33, 47–55. https://doi.org/10.1016/j.mib.2016.05.017

Tawk, C., Sharan, M., Eulalio, A., & Vogel, J. (2017). A systematic analysis of the RNA-targeting potential of secreted bacterial effector proteins. *Scientific Reports*, 7, 9328. https://doi.org/10.1038/s41598-017-09527-0

Wagner, E. G. H., & Romby, P. (2015). Small RNAs in bacteria and archaea: Who they are, what they do, and how they do it. *Advances in Genetics*, 90, 133–208.

Waters, S. A., McAteer, S. P., Kudla, G., Pang, I., Deshpande, N. P., Amos, T. G., ... Tree, J. J. (2017). Small RNA interactome of pathogenic *E. coli* revealed through crosslinking of RNase E. *EMBO Journal*, 36, 374–387.

Westermann, A. J., Barquist, L., & Vogel, J. (2017). Resolving host-pathogen interactions by dual RNA-seq. *PLoS Pathogens*, 13, e1006033. https://doi.org/10.1371/journal.ppat.1006033

Westermann, A. J., Forstner, K. U., Amman, F., Barquist, L., Chao, Y., Schulte, L. N., ... Vogel, J. (2016). Dual RNA-seq unveils noncoding RNA functions in host-pathogen interactions. *Nature*, 529, 496–501. https://doi.org/10.1038/nature16547

Xue, X. Y., Mao, X. G., Zhou, Y., Chen, Z., Hu, Y., Hou, Z., ... Luo, X. X. (2018). Advances in the delivery of antisense oligonucleotides for combating bacterial infectious diseases. *Nanomedicine*, 14, 745–758. https://doi.org/10.1016/j.nano.2017.12.026

Zhang, A., Schu, D. J., Tjaden, B. C., Storz, G., & Gottesman, S. (2013). Mutations in interaction surfaces differentially impact *E. coli* Hfq association with small RNAs and their mRNA targets. *Journal of Molecular Biology*, 425, 3678–3697. https://doi.org/10.1016/j.jmb.2013.01.006

How to cite this article: Vogel J. An RNA biology perspective on species-specific programmable RNA antibiotics. *Mol Microbiol*. 2020;113:550–559. https://doi.org/10.1111/mmi.14476