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An RNA biology perspective on species-specific programmable RNA antibiotics

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Abstract

Our body is colonized by a vast array of bacteria the sum of which forms our microbiota. The gut alone harbors >1,000 bacterial species. An understanding of their individual or synergistic contributions to human health and disease demands means to interfere with their functions on the species level. Most of the currently available antibiotics are broad-spectrum, thus too unspecific for a selective depletion of a single species of interest from the microbiota. Programmable RNA antibiotics in the form of short antisense oligonucleotides (ASOs) promise to achieve precision manipulation of bacterial communities. These ASOs are coupled to small peptides that carry them inside the bacteria to silence mRNAs of essential genes, for example, to target antibiotic-resistant pathogens as an alternative to standard antibiotics. There is already proof-of-principle with diverse bacteria, but many open questions remain with respect to true species specificity, potential off-targeting, choice of peptides for delivery, bacterial resistance mechanisms and the host response. While there is unlikely a one-fits-all solution for all microbiome species, I will discuss how recent progress in bacterial RNA biology may help to accelerate the development of programmable RNA antibiotics for microbiome editing and other applications.

KEYWORDS

antibiotic, microbiome, RNA-seq, small RNA

1 | 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).

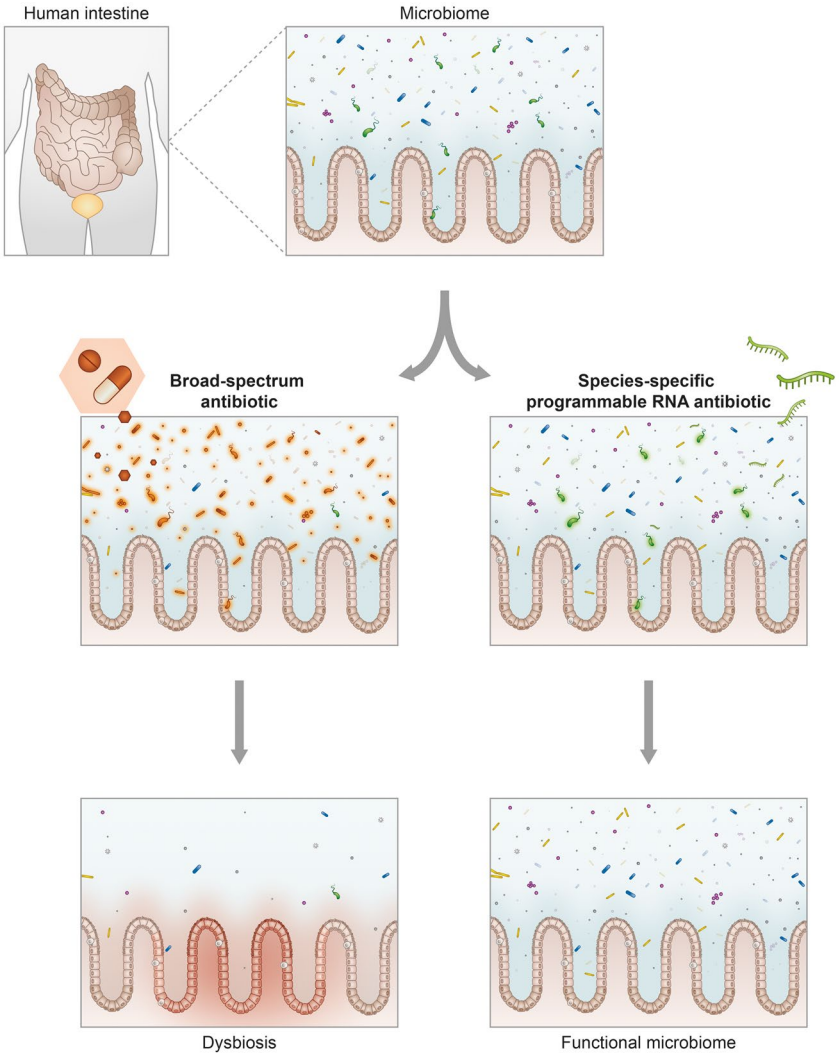

FIGURE 1 Most current antibiotics are broad-spectrum, meaning that their application to the microbiota (here shown for the intestine, but could be skin or other body part that is populated by microbes) will kill many more bacterial species than just the one of interest (bottom left). Species-specific programmable RNA antibiotics promise true microbiome editing, eliminating only the very species of interest and leaving the rest of the microbiota undisturbed (bottom right)

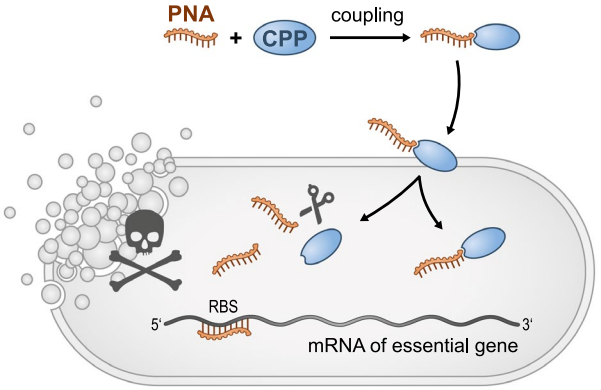

FIGURE 2 RNA-centric killing of a bacterium of interest can be achieved by delivering a short antisense oligonucleotide (ASO), here, peptide nucleic acid (PNA), to sequester the 5′ region of the mRNA of an essential gene. Such ASOs are coupled to small uptake or cell-penetrating peptides that carry them inside the bacteria. The mechanisms of transport into the bacteria and whether peptide and ASO remain attached to each other, or cleaved after entry, are incompletely understood

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 30S 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 cenocepacia* (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, Matera, 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 (Bandyra 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 (Hoekzema, Romilly, Holmqvist, & Wagner, 2019; Jagodnik, Chiaruttini, & Guillier, 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

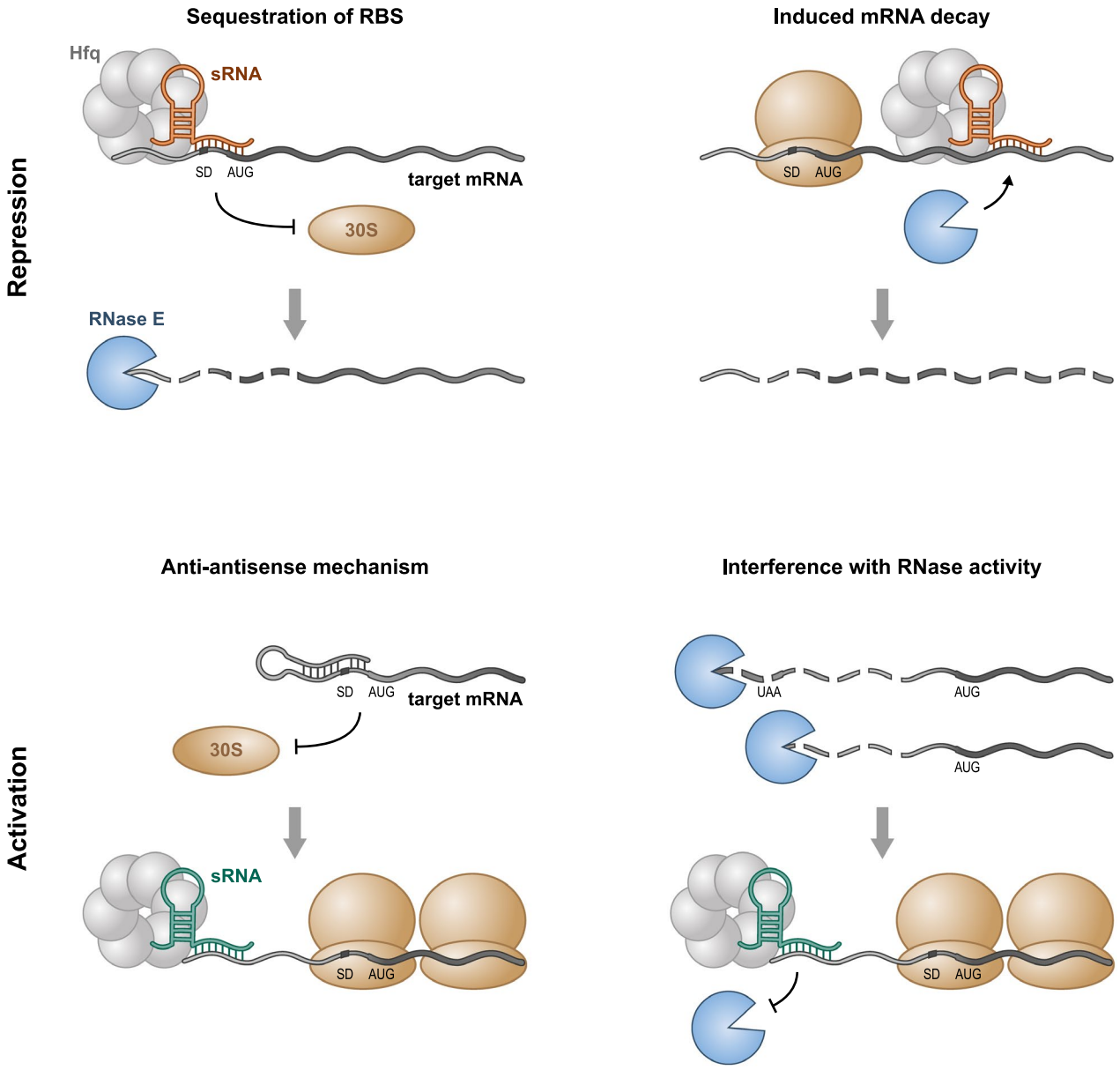

FIGURE 3 Studies with endogenous sRNAs have revealed a diversity of mechanisms of RNA-centric gene regulation on the posttranscriptional level. Shown are a few examples of mechanisms whereby Hfq-associated sRNAs of *E. coli* and *Salmonella* (Hör et al., 2020) repress translation initiation, induce target mRNA decay by binding deep in the coding sequence, activate suboperonic genes by stabilizing shorter mRNAs as longer polycistronic transcripts get processed, or activate mRNAs translationally by resolving inhibitory structure around the start codon (clockwise from top left). The mechanisms to the right operate in a much larger sequence window on bacterial mRNAs than just at the start codon or Shine Dalgarno sequence, and so promise a much larger sequence space and more sequence diversity for the design of highly selective ASOs. See the main text for more details

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 peptides. 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

representing thousands of RNA–RNA interactions that form stably in the cell may also provide excellent training sets for machine learning-based algorithms developed to predict productive ASO-mRNA pairing. Taken together, endogenous sRNAs offer a great knowledge source that awaits to be tapped for better ASO design.

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 (Frantz 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 (Cabral 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 transcriptomes, ASOs of 10–12 nucleotides in length would surely have targets on mRNAs in host cells as well. Likewise, there is the possibility that ASOs 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 *wundertüte* of bacterial RNA biology has more to offer for ASO-based applications, as several different new sRNA 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?

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