A Viral Shortcut: How a Tiny RNA Helps Viruses Take Over Bacteria

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Introduction

Bacterial infections are becoming harder to treat. Antibiotics, which once seemed like miracle drugs, are slowly losing their power as bacteria evolve ways to resist them. This growing problem, called antibiotic resistance, is a major challenge in modern medicine. It is time to start investigating new ideas or maybe go back and take a look at our old tricks. One concept that is returning to the spotlight is using viruses that infect bacteria to overcome antibiotic-resistant bacteria. When we think about viruses, we usually think about getting sick. The flu virus, COVID-19, and many others infect our cells and make us feel miserable. But viruses don't just infect humans. In fact, some viruses infect bacteria, and those viruses can be useful to us as they simply cannot infect the human cell due to their extremely specific nature. These bacteria-infecting viruses are called bacteriophages, or just phages, which literally means "bacteria eaters". Phages are incredibly common[1]. There are more phages on Earth than any other biological entity, literally millions of them in every drop of seawater, soil, or sewage sample. We can even find them in our digestive system. Wherever bacteria exist, phages are close by. Even before Alexander Fleming identified penicillin, one of the greatest medical discoveries in history, phages were already being used to treat infections, although not extensively. Unfortunately, this approach faded in the modern antibiotic era. However, phages are becoming attractive once again as candidates for treating bacterial infections, a strategy known as phage therapy[2]. Indeed, this may be the right solution against antibiotic-resistant bacteria but before phages can be safely and reliably used as treatment, we must understand exactly how they work. A phage is a virus, and like all viruses, it survives by manipulating the molecular machinery of its host. Once inside a bacterial cell, it must rapidly copy its own DNA and produce new viral particles before the cell defenses shut it down. How do they take control of bacterial cells so efficiently? What molecular tricks do they use to reproduce so quickly? This is where our research comes in[3], we uncovered an unexpected strategy: a phage uses a tiny RNA molecule to push the bacterial cell into replicating DNA faster - helping the virus copy itself in the process.

The hidden side of phage infection: RNA regulation

When a phage infects a bacterium, it follows a carefully planned sequence of steps. First, it must "land" on the bacterial surface: like a space module approaching the Moon and touching down at the precise landing site. The bacterial surface is not smooth or random; it has specific molecular "landing pads" that only certain phages can recognize. If the phage lands in the wrong place, the mission fails. Once the phage has successfully landed, it injects its genetic material into the bacterial cell, much like astronauts unloading their equipment after a successful touchdown. Inside the cell, the phage takes control of the bacterium's machinery to build many copies of itself. Finally, the bacterial cell bursts open, releasing newly assembled phages that move on to infect neighboring bacterial cells.

For decades, scientists have studied how phages manipulate bacteria. Most of what we know involves proteins - molecular machines that phages use to shut down bacterial defences or redirect the cell's resources. However, cells also use another powerful form of regulation using RNA molecules. Some RNA molecules are not used to make proteins at all. Instead, they act as regulators. These molecules, called small RNAs (sRNAs)[4], are short pieces of RNA that bind to other RNAs and change the pace of protein synthesis or

affect the stability of target RNA. Rather than acting like simple on/off switches, these sRNAs function more like rheostats, the dimming option in light fixtures, allowing cells to fine-tune protein levels. In bacteria, sRNAs are known to regulate stress responses, metabolism, and virulence[5]. This raised an intriguing question:

Could a phage also use small RNAs to manipulate its bacterial hosts?

For a long time, this question remained unanswered. Scientists lacked a global view of which bacterial and viral RNAs interact during infection. We reasoned that something was happening during viral invasion, but we needed to install a secret camera that could document all these interactions. Without this information, important regulatory networks could easily remain hidden.

To achieve this, we used a method called RIL-seq (RNA Interaction by Ligation and sequencing[6]), which allows us to capture RNA molecules that physically interact inside living cells, essentially recording molecular conversations. This method involves the entrapment of two neighbouring RNAs and based on sequencing technologies, their identification, and their location on the genome of the host and the phage.

listening in on RNA conversations

Using RIL-seq, we infected *Escherichia coli* (*E. coli*) with phage lambda, a classic model phage that has been studied for decades[7]. Next, we mapped all the RNA-RNA interactions happening inside the infected cells. What we found was surprising. Phage infection dramatically rewired the bacterial RNA interaction network. Bacterial RNAs changed their usual partners, phage RNAs interacted with other phage RNAs, and most interestingly, phage RNAs were involved in interactions with host RNAs. Among the latter, one sRNA stood out as it was found in ~50% of the identified bacteria-phage interactions. We named this previously unknown sRNA, **PreS** (phage replication enhancer sRNA). We showed that PreS is produced early during infection and binds to Hfq, a bacterial protein that acts like a matchmaker for regulatory RNAs. This immediately suggested that PreS plays an active regulatory role.

What does this sRNA do?

As previously mentioned, sRNAs act by physically binding to other RNAs, resulting in their regulation. When taking a closer look at who PreS was interacting with on Hfq, we noticed that it binds to the RNA encoded by the bacterial gene *dnaN*. What really caught our attention was that *dnaN* only interacted with PreS. The *dnaN* gene encodes the β-sliding clamp[8], an essential protein for DNA replication. The sliding clamp acts like a stabilizing ring, holding the DNA polymerase, the enzyme that copies DNA, in place so it can copy DNA quickly and efficiently. Without sliding clamps, cells do not survive as DNA replication is almost impossible.

Here is the clever part: phage lambda does not bring its own DNA replication machinery. Instead, it relies on the bacterium's system. That means anything that boosts bacterial DNA replication also helps the phage copy its own genome, and this is exactly what PreS does.

We found that PreS, while bound to Hfq, increases the production of the DnaN protein. It does so by helping ribosomes, the cell's protein-making machines, start translating the *dnaN* mRNA more efficiently. This happens due to changes in the folding and structure of *dnaN* mRNA supporting our hypothesis.

In other words, PreS acts like an RNA switch that puts *dnaN* translation into high gear, just by binding to it. But we still needed to show that DNA replication is alleviated by PreS, so we synthetically overexpressed PreS from a plasmid in cells without any phage and showed that DNA synthesis was enhanced compared to an empty plasmid (Figure 1A). To neatly connect all the dots, we show that when infecting cells that have a mutant *danN,* fewer phages are produced and phage DNA quantity is lower. When PreS is present: The bacterium makes more sliding clamps, DNA replication becomes more efficient, phage DNA is copied faster, resulting in the production of more phage particles. Alternatively, when PreS is removed or mutated, everything slows down. Phage DNA replication is delayed (Figure 1B), and fewer viruses are made (Figure 1C).

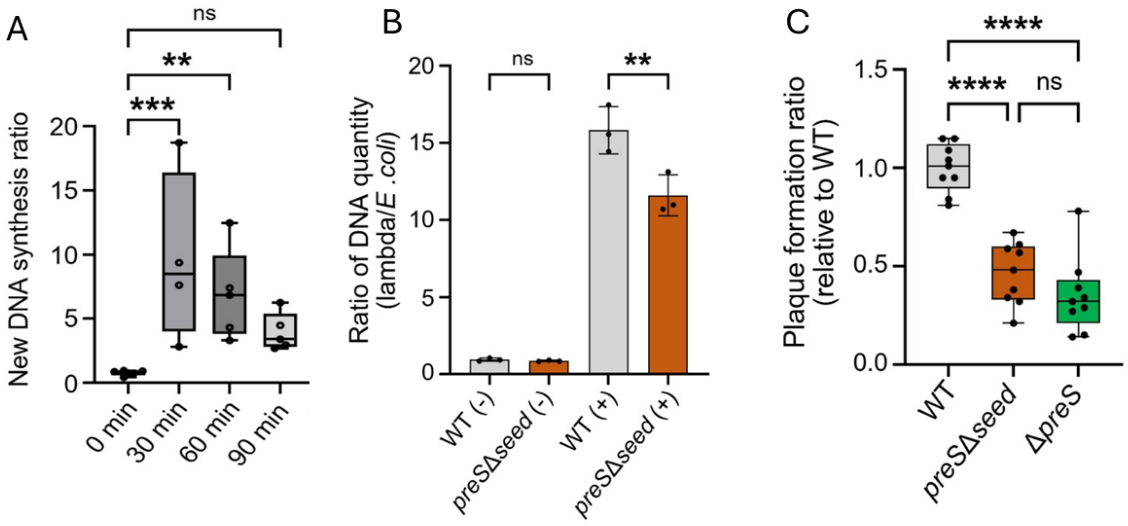

Figure 1 – PreS overexpression in *E. coli* results in enhanced DNA synthesis measured by BrdU incorporation into newly synthesized DNA (A). Results are relative to new DNA synthesis with an empty vector. Deletion of *preS* seed sequence resulted in reduced phage DNA quantity following induction of the lytic cycle by UV irradiation (B). Deletion of *preS* from lysogens results in reduced efficiency as measured by plaque assay of spontaneous lytic induction (C). All panels were adapted from Silverman A. *et. al., 2025* [3]

Why this discovery matters

This work shows for the first time that a phage can directly control bacterial DNA replication using an sRNA. It reveals a completely new strategy that viruses use to hijack their hosts. Instead of smashing the system with brute force, the phage uses a subtle RNA-based trick, fine-tuning an essential bacterial process to serve its own needs (Figure 2). This changes how we think about phage biology. It suggests that many more phage-encoded sRNAs are waiting to be discovered, quietly pulling the strings inside infected cells.

By targeting *dnaN*, PreS pushes the bacterial replication system into high gear without completely disrupting the cell. This intermediate exploitation strategy allows the phage to extract maximum benefit from the host before the cell is destroyed. Understanding these strategies is also important for applied science. As phage therapy gains momentum, knowing how phages optimize their replication could help in selecting or engineering a phage that could work more efficiently against dangerous bacteria.

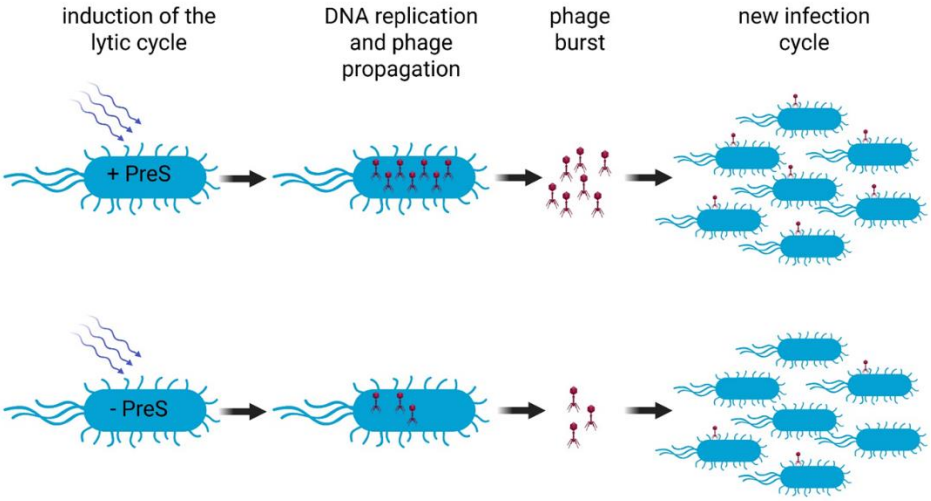

Figure 2 – Phage lambda can infect bacteria from the outside resulting in lysis of the bacterial cell or integrate its genome into the host chromosome, a state known as lysogeny. When lysogens are induced or when a phage undergoes a lytic cycle, its genome is transcribed as well as the PreS sRNA. PreS binds Hfq and targets *dnaN*, resulting in the opening of an inhibitory secondary structure, producing more DnaN protein enabling enhanced DNA replication, and therefore increased phage propagation. Phages are assembled, and new progeny burst out of the cell to initiate a new infection cycle. Figure was created using BioRender (BioRender.com).

What's next?

There are still important pieces of the puzzle that we are trying to solve. First, we focused on one model bacterium (*E. coli*) and one well-studied phage (lambda). These are the "lab mice" of microbiology - they are extremely useful for discovery, but they do not represent all bacteria or all phage species found in nature. While we found that PreS-like sequences appear in related phages, we can only estimate how common this RNA-based strategy is across the enormous diversity of phages in the environment.

Second, although PreS interacts with many bacterial RNAs, and we have explored a few of its targets, there are many more RNA–RNA interactions. It is very likely that other sRNAs influence and contribute to phage biology, such as host-encoded sRNAs that could potentially create a counter-response to the infection. In other words, we have identified the control panel, but we have only tested a few of the buttons. There is still a lot to learn, and the complete control panel, although encrypted, is available in our research paper.

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Dr. Sahar Melamed

Dr. Melamed is a principal investigator at the Hebrew University of Jerusalem. He also serves at the director of the Microbiology track in the Faculty of Medicine at the Hebrew University of Jerusalem. Dr. Melamed is leading a group of researchers who are excited about RNA biology and love to do science. They aim to understand the roles played by regulatory RNAs in the relationships of bacteria with their environment.

Aviezer Silverman

Aviezer is a PhD student the Melamed lab. His research work lead to their publication in Molecular Cell. The above layman summary is based on these published research findings. Please read more about the authors in the Mentor-Grad Student Spotlights, MGS, series.

Reviewers:

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