Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

The enigmatic epitranscriptome of bacteriophages: putative RNA modifications in viral infections

Nadiia Pozhydaieva[1,*], Maik Wolfram-Schauerte[1,*], Helene Keuthen[1] and Katharina Höfer[1,2]

RNA modifications play essential roles in modulating RNA function, stability, and fate across all kingdoms of life. The entirety of the RNA modifications within a cell is defined as the epitranscriptome. While eukaryotic RNA modifications are intensively studied, understanding bacterial RNA modifications remains limited, and knowledge about bacteriophage RNA modifications is almost nonexistent. In this review, we shed light on known mechanisms of bacterial RNA modifications and propose how this knowledge might be extended to bacteriophages. We build hypotheses on enzymes potentially responsible for regulating the epitranscriptome of bacteriophages and their host. This review highlights the exciting prospects of uncovering the unexplored field of bacteriophage epitranscriptomics and its potential role to shape bacteriophage–host interactions.

Addresses
[1] Max Planck Institute for Terrestrial Microbiology, Marburg, Germany
[2] Center for Synthetic Microbiology (SYNMIKRO), Philipps-University Marburg, Marburg, Germany

Corresponding author: Höfer, Katharina (katharina.hoefer@synmikro.mpi-marburg.mpg.de)
[*] These authors contributed equally.

Current Opinion in Microbiology 2024, **77**:102417

This review comes from a themed issue on **Host-Microbe Interactions: viruses**

Edited by **Rob Lavigne** and **Julia Frunzke**

For complete overview of the section, please refer to the article collection, "Host-Microbe Interactions: Viruses 2024"

Available online 13 January 2024

https://doi.org/10.1016/j.mib.2023.102417

Introduction

Since the discovery of bacteriophages (phages) more than 100 years ago, bacteriophage research has significantly impacted our understanding of fundamental biological processes [1]. Phages have been pivotal as model systems for understanding fundamental principles in molecular biology and discovering their biotechnological potential [2].

Lytic bacteriophages efficiently infect their bacterial host, completing the infection cycle with the release of new bacteriophage progeny through cell lysis. The infection process is highly regulated on the molecular level and typically exhibits a high degree of specificity for a given host–bacteria pair. Regardless of the specific host–bacteria pair, bacteriophage infections have consistently been observed as temporally highly regulated processes in various studies, revealing the precise timing of gene expression [3]. Therefore, the bacteriophage genes are classified into early, middle and late genes, signifying their timing in the infection cycle. To maintain efficient gene expression and, consequently, phage replication and propagation, lytic bacteriophages take control over the host's cellular machinery or its specific components.

Phage gene transcription is catalysed by either bacteriophage RNA polymerase (RNAP) or host-encoded RNAP. Based on our current textbook knowledge, RNAPs utilise the host-provided nucleotide pool consisting of uridine triphosphate (UTP), adenosine triphosphate (ATP), guanosine triphosphate (GTP) and cytidine triphosphate (CTP) to generate phage transcripts during the different infection phases. It is assumed that RNAs transcribed during each phase of phage infection are directly translated by ribosomes, resulting in proteins from the respective infection phase. However, recent multi-omics studies have revealed that the appearance of transcripts and proteins in bacteriophages does not always coincide, showing, for instance, that early transcripts contribute to the translation of late proteins [4]. This observation suggests the presence of mechanisms that allow to distinguish between bacteriophage RNAs reflected by their time point of translation.

Moreover, recent studies show that the stability and processing of host and bacteriophage RNA differ strongly during infection, indicating precise distinction and selective degradation of transcripts [4–6].

These findings suggest the existence of so-far-unknown additional mechanisms that enable the specific differentiation between phage and host transcripts and define their processing during infection, raising numerous questions, such as:

- How do RNA modifications influence the precise processing of bacteriophage and host transcripts during infection, despite their shared genetic building blocks?
- Could RNA modifications offer an additional mechanism for regulating phage infections?
- Which enzymes, supplied by both bacteriophages and bacteria, have the potential to shape the presence and function of RNA modifications?

Besides known factors influencing RNA stability and fate, such as RNA secondary structure or RNA-binding proteins, RNA modifications have been shown to regulate RNA processing in all domains of life. Incorporating chemical modifications into RNA strongly affects its biochemical properties, stability and function in cellular and biological processes [7]. These modifications can be categorised into internal (modifications on bases or nucleosides) and terminal (cap-like modifications at 5'-terminus or 3'-terminus) RNA modifications (Box 1) (reviewed in [8]). The diversity of the RNA modifications within a cell is collectively defined as the epitranscriptome. Proteins known as writers (biosynthesis of the modifications), readers (recognition) and erasers (removal) shape the epitranscriptome and its function(s) (reviewed in [8]). Their interplay results in a wide variety of more than 170 reported RNA modifications [9]. RNA modifications are found in all domains of life, with a significant focus on eukaryotes. However, the exploration and functional characterisation of RNA modifications are still evolving in bacteria, and even less is known about bacteriophages in this context.

Here, we review the current knowledge about selected RNA modifications in bacteria and explore the potential roles of known RNA modification mechanisms, encompassing writers, readers and erasers, in the context of bacteriophage infection. We focus on bacteriophage infections, with an emphasis on bacterial viruses, while excluding viruses that infect archaea. Further, we speculate on bacteriophage-encoded factors that may have potential roles in shaping bacteriophage and host epitranscriptomes during the infection (Figure 1).

Decorating RNAs: RNA modification by host and bacteriophage RNA polymerases

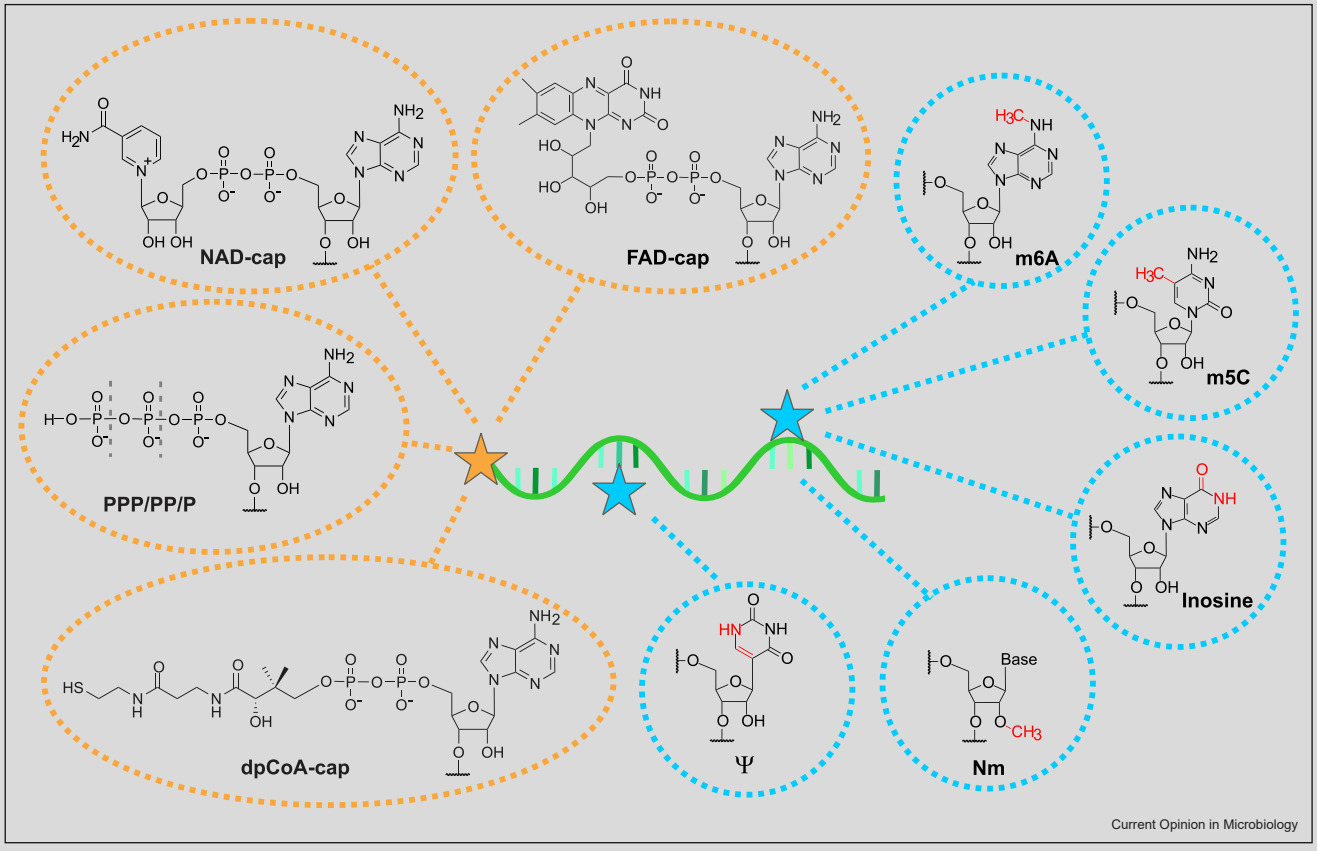
Multi-subunit RNAPs play a pivotal role in shaping the epitranscriptome. In the bacterial hosts — during transcription initiation (*ab initio*) — RNAPs can incorporate non-canonical nucleotides at the 5'-end of RNAs, including nicotinamide adenine dinucleotide (NAD), flavine adenine dinucleotide (FAD) or dephosphocoenzyme A (dpCoA) — giving rise to cofactor-capped RNAs (Box 1, Table 1) [10,11]. RNAPs from *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* have been implicated in non-canonical transcription initiation [10,12,13]. Thus, a broad range of bacterial hosts may be equipped with cofactor-capped RNA species before a potential infection by a bacteriophage (reviewed in [8,14]).

Bacteriophages have different ways of controlling transcription through RNAPs (reviewed in [15,16]). On the one hand, bacteriophages such as T4 make use of the host's transcriptional apparatus throughout infection [17], as their genome does not encode an enzyme that can catalyse RNA biosynthesis. On the other hand, bacteriophages can encode their own (set of) RNAP(s) that they use to transcribe their own genes [15,16]. In the latter case, one can distinguish between virion-associated [18] and non-virion-associated RNAPs [16]. The virion- associated RNAP is co-injected with the bacteriophage genome into the host cell and ensures early transcription of bacteriophage genes not excluding its involvement in later stages of infection. Subsequently, non-virion RNAP is synthesised from early bacteriophage genes during infection and drives middle and late transcription. Bacteriophages that only partially rely on the host RNAP may encode the non-virion RNAP only, which is transcribed during infection by the host RNAP.

Drawing from the elucidated mechanisms of RNA synthesis during phage infection, one can investigate whether host and/or bacteriophage RNAPs incorporate non-canonical building blocks, alongside the canonical RNA bases U, A, C and G, into phage transcripts.

Some bacteriophages, such as bacteriophage T4, utilise the host RNAP for the transcription of their own genes [17]. In this particular case, *E. coli* RNAPs can incorporate cofactor-caps upon transcription initiation, as described above, thereby likely defining the bacteriophage/host epitranscriptome during infection. Here, one may consider the various strategies of bacteriophages to hijack the host's transcriptional apparatus and its yet- unknown effect on the epitranscriptional regulation [15]. For instance, three adenosine diphosphate (ADP)-ribosyltransferases (ARTs) of bacteriophage T4 post-translationally modify host proteins with ADP-ribose from the substrate NAD to modulate cellular processes such as transcription [19–21]. The ARTs Alt and ModA ADP-ribosylate the host RNAP to direct its specificity towards bacteriophage genes [19,22]. It is so far unknown whether this post-translational protein modification (ADP-ribosylation) also influences the host RNAP's ability to initiate transcription with cofactors. One could imagine that ADP-ribosylation of RNAP could provide a means to incorporate RNA modifications to distinguish newly synthesised bacteriophage messenger RNAs (mRNAs) from host RNA.

Box 1 Terminal (orange) and internal (blue) RNA modifications in bacteria.



For bacterial RNA, the 5′-terminus defines its origin. In primary transcripts, the initiating nucleoside triphosphate gives rise to the triphosphorylated 5′-end of the RNA. Through nucleolytic RNA processing, 5'-P-RNA is formed, and recent research has revealed the formation of diphosphorylated RNA as well [92]. However, the 5'-end of RNA can carry functional groups different from phosphates, due to transcription initiation with non-canonical initiating nucleotides. Here, nucleotide-based cofactors such as NAD (here used to refer to the oxidised NAD^+), FAD and dpCoA can be accepted by RNAPs to initiate transcription, leading to the generation of 5'-capped RNAs. These 5'-RNA-caps, such as the NAD-cap, are assumed to protect the modified RNA from degradation by 5′-end-dependent endonucleases such as RNase E [48,49].

The role of internal RNA modifications differs strongly based on the type of the modified RNA. For bacterial RNA, m^6A, 5-methylcytidine (m^5C), inosine (I), pseudouridine (Ψ) and 2′-O-methylation (Nm) are the most common internal RNA modifications [8]. However, only two of them were detected to date in bacterial mRNA–m6A and inosine [93].

Whilst the role of m^6A in bacterial rRNA is well-studied and has been determined to play a role in folding and stability, translational control and cell fitness [94–96], the presence of m^6A in bacterial mRNA, where m^6A is predominantly found within open reading frames (ORFs), remains relatively unexplored [93].

Inosine is a product of C6 deamination of adenosine, which occurs either by spontaneous hydrolysis or enzymatic conversion [97]. Inosine has been identified in mRNA of several bacteria, where it exerts a regulatory function. Its presence has a significant regulatory impact in bacteria and has been described to be involved in oxidative stress tolerance and the induction of biofilm formation [75,98].

Nm was described to have an impact on transcript stability and translation efficiency. Both, m5C and Ψ, were described as being present in tRNA, contributing to its structural stabilisation and influence on the translational fidelity of the ribosome [99].

The T7 bacteriophage is a well-studied example for a phage that requires the host RNAP only during the early phase of phage infection — in particular — until its own RNAP (T7 RNAP) is generated. T7 RNAP transcribes T7 genes in middle- and late- infection phase [23]. The T7 RNAP is probably one of the most famous single-subunit RNAPs (ssRNAPs) and is widely used for *in vitro* transcription applications and protein expression systems [23,24]. Moreover, the T7 RNAP stands out with its capability to incorporate non-canonical building blocks co-transcriptionally [25]. It caps RNAs with various co-factors, for example, NAD, with up to 50% efficiency *in vitro* [25] and accepts modified nucleotides such as pseudouridine as substrates to generate internal RNA modifications (Box 1, Table 1) [26]. Pseudouridine is a critical building block of the Covid-19 mRNA vaccines that are generated using large-scale *in vitro* transcriptions with T7 RNAP [27]. Besides its ability to incorporate

Table 1

Comparison of (hypothetical) biological roles of RNA modifications in bacteriophage infections.

A	*Biosynthesis of 5'-modified transcripts* *RNAP (s)*	
	Host	*Bacteriophage*
Biological role	• Non-canonical transcription initiation (e.g. NAD, FAD and dephospho-CoA) *in vitro* and *in vivo* [10,11]	• Non-canonical transcription initiation (e.g. NAD, FAD and dephospho-CoA) *in vitro* [25] • Incorporation of modified nucleotides (e.g. pseudouridine) *in vitro* [25]
Potential role during bacteriophage infection	• Capping of host transcripts to protect them from degradation by bacteriophage enzymes • Exploitation of host RNAP for transcription of bacteriophage genes and capping of bacteriophage RNA to increase their stability	• Capping of bacteriophage transcripts to protect them from degradation by the host • Incorporation of the modified nucleotides during bacteriophage infection to enhance stability or avoid immune recognition of bacteriophage transcripts
B	*RNA processing and degradation* *Nudix hydrolases*	
	Host	*Bacteriophage*
Biological role	• Decapping of capped RNAs (e.g. NAD-capped RNA) *in vitro* and *in vivo* by Nudix hydrolases [49] • Hydrolysis of PP from the 5′-PPP primary transcripts by pyrophosphohydrolases (e.g. RppH)[46,92]	• No knowledge about bacteriophage Nudix hydrolases involved in RNA processing in infections
Potential role during bacteriophage infection	• Decapping of bacteriophage transcripts by Nudix hydrolases to destabilise bacteriophage transcripts and induce their degradation • Recruitment of host Nudix hydrolases by bacteriophages to induce the degradation of (capped) host transcripts	• Expression of own bacteriophage Nudix hydrolases to control the stability and degradation of 5′-capped host and bacteriophage transcripts
Endo- and exonucleases		
Biological role	• Cofactor-caps as shields for RNA to protect against 5'-P-end-dependent nucleases such as RNase E [48]	• Unknown impact of RNA modifications on bacteriophage nucleases
Potential role during bacteriophage infection	• RNA modifications as potential epitranscriptomic marks for nucleases to distinguish own (host) from invader (bacteriophage) RNAs	• RNA modifications as distinct features of host or bacteriophage RNAs to trigger their cleavage
C	*Post-translational protein modification* *ART(s)*	
Biological role	• No RNAylation observed in any bacteria	• RNAylation of the host's translational apparatus by the bacteriophage T4 ART ModB [55]
Potential role during bacteriophage infection	• Potential functions of bacterial ARTs in RNAylation to counteract bacteriophage infection	• RNAylation to modulate protein function during infection • Bacteriophage-mediated RNAylation of host proteins as a means to take control over the host cell

A) Biosynthesis of 5'-modified transcripts, *B)* RNA processing and degradation and *C)* post-translational modifications.
Modifier B (ModB).

non-canonical nucleotides into RNA, T7 RNAP exhibits several other advantageous properties for biotechnological applications. These include characteristics such as its single-subunit nature (unlike bacterial multi-subunit RNAPs), high specificity for the short T7 promoter (18 nt), higher transcriptional speed, independence from auxiliary transcription factors, ease of engineering, possible application as a parallel expression system and the ability to produce long transcripts [28–30]. This emphasises the potential and broad applicability of bacteriophage RNAPs in regard to synthesising and modifying RNA. T7 RNAP can even replicate small RNAs or use deoxynucleotides as artificial RNA building blocks (reviewed in [31]). In addition, single-point mutations in T7 RNAP or its homologues can cause acceptance of deoxynucleotides or 2'-fluoro-ribonucleotides as alternative substrates for transcription [32–34]. However, *in vivo*, evidence for T7 RNAP function in installing RNA modifications during T7 infection is still missing. T7-like ssRNAPs are found in diverse bacteriophages with various host ranges. These include T7-like RNAPs in *Pseudomonas aeruginosa* bacteriophages [35], *Klebsiella* bacteriophages K11 [36] and KP34 [34], *Salmonella* bacteriophage SP6 [37] and *Synechococcus* bacteriophage Syn5 [38]. However, whether these RNAPs may exhibit similar activities as T7 RNAP towards installing RNA modifications *in vitro*, remains elusive.

The larger bacteriophages, so-called Jumbo phages (genome usually > 200 kb), display additional interesting features of transcription (reviewed in [16]). Some Jumbo bacteriophages form a nucleus-like structure (pseudonucleus),

enabling the compartmentalisation of phage DNA from the bacterial cytoplasm. This results in locally separated phage gene transcription within the host [39,40]. Upon infection, a pseudonucleus is formed to protect the bacteriophage DNA from bacterial nucleases and to allow transcription of the phage transcripts by the phage-encoded RNAP [41–43]. To the best of our knowledge, these nucleus-like compartments have not yet been investigated in terms of their exact molecular composition. It is plausible that this compartment created by the phage differs from the bacterial cytosol in terms of the abundance of nucleotides and epitranscriptional writers. This difference may create distinct transcriptional environments that either promote or hinder the incorporation of specific RNA modifications by the relevant RNAPs. On the other hand, one may argue that transcriptional environments in the cytosol and pseudonuclei may be similar to each other allowing to equip phage transcripts similar to host transcripts. These hypotheses might be exciting prospects for future studies of RNA biosynthesis in Jumbo bacteriophages.

Altogether, bacterial RNAPs can shape cofactor-capped transcriptomes of bacterial hosts and — depending on the bacteriophage's transcriptional strategy — might contribute to cofactor-capping of bacteriophage transcripts, although evidence is lacking so far. *In vitro*, bacteriophage RNAPs possess the capabilities to cofactor-cap transcripts and directly incorporate internal RNA modifications. It is likely that our current knowledge only scratches the surface of bacteriophage RNAP diversity such as single- and multi-subunit organisation, infection phase-specific occurrence as virion and non-virion RNAPs. Given the diverse features of RNAPs and their capabilities to install RNA modifications, it is possible that RNA modifications may exist in and even regulate bacteriophage infections.

Cleaning up: removal of modifications by Nudix hydrolases

Bacterial Nudix hydrolases have been described to play an important function in the removal of bacterial RNA modifications (reviewed in [8]) [44]. Nudix enzymes generally hydrolyse nucleoside diphosphates linked to a moiety X within their diphosphate moiety, thereby releasing a nucleoside monophosphate and a monophosphate-X group. In *E. coli*, 13 Nudix hydrolases are described, which vary in their substrate spectrum, ranging from nucleoside-based cofactors to modified RNA species [45]. A well-characterised Nudix hydrolase known to interact with RNA *in vivo* is *E. coli* RppH (EcRppH). It processes primary transcripts harbouring a 5'-triphosphate to 5'-monophosphorylated RNAs (5'-P-RNAs) (Table 1), favouring their degradation by 5'-end-dependent nucleases such as RNase E [45,46].

Bacteriophage T4 infection of *E. coli* represents a well-studied scenario for this. Here, EcRppH was suggested to promote mRNA decay by generating 5'-P-RNA that activates RNase E-mediated RNA decay [46,47]. Experimental evidence showing how far this mechanism regulates the presence of host and bacteriophage transcripts is missing.

Another bacterial Nudix hydrolase, described to interact with bacterial transcripts, is *E. coli* NudC (EcNudC). EcNudC is known to hydrolyse cofactor-caps from RNA, thereby decapping NAD-RNAs *in vivo* [48] and several cofactor-capped RNAs, such as NAD-, FAD- or CoA-RNAs *in vitro* [49–51]. Interestingly, in other bacteria, different enzymes are involved in NAD-RNA decapping. For instance, due to a missing NudC homologue in *Bacillus subtilis*, BsRppH performs decapping of NAD-RNAs [13].

In the context of bacteriophage infection, one could speculate that Nudix hydrolases may also be involved in processing both bacteriophage and host RNA modifications during infection. It needs to be investigated whether distinct host Nudix hydrolases may positively or negatively contribute to bacteriophage infections, for example, as a required prerequisite in the host cell or as a host defence strategy. Bacterial hosts employ various strategies to defend themselves from bacteriophage infection. For instance, the Thoeris system senses and aborts bacteriophage infection by depleting essential NAD from the cellular metabolite pool *via* conversion to cyclic-ADP-ribose [52]. One may hypothesise that the Thoeris and related systems may also act on NAD-capped RNA or may influence the NAD-capping of transcripts by RNAPs during phage infections by reducing the NAD pool. Also, it could be speculated that bacteriophages could sequester NAD in NAD-capped RNA to protect a minimum level of NAD in the cell.

Given the widespread conservation of Nudix hydrolases across all domains of life, one might question whether these enzymes are also found in bacteriophages that could potentially influence the epitranscriptome during the phage–host interaction. Interestingly, Nudix hydrolases have been predicted in various bacteriophages [53]. However, only two bacteriophage-derived Nudix hydrolases have been characterised [53,54]. The T4-like *Vibrio* bacteriophage KVP40 possesses the Nudix hydrolase domain protein NatV active on NAD, NADH and ADP-ribose, thereby regulating cellular NAD levels during infection [53]. The bacteriophage T4-encoded enzyme NudE.1 — named based on its sequence homology to *E. coli* Nudix hydrolase NudE — has been described to hydrolyse substrates such as ADP-ribose and FAD, both cofactor(-derived) compounds, as well as Ap_3A, an alarmone involved in stress signalling [54]. Notably, the activity of bacteriophage Nudix hydrolases on cofactors may indicate that they could similarly be active on cofactor-capped RNAs, such as in the case of EcNudC (Table 1).

However, it cannot be generalised that a Nudix hydrolase, active on a cofactor, can equally process a cofactor-capped RNA. For instance, EcNudE hydrolyses NAD, but is inactive on NAD-capped RNA [49], demonstrating distinct substrate specificities. Further, the occurrence of Nudix hydrolases in various bacteriophage genomes suggests yet-unexplored roles in nucleotide metabolism and potential functions in processing or removal of RNA modifications.

Taking control: RNA modifications in host take-over

As mentioned above, the identity, synthesis and removal of cofactor-caps (terminal modifications) such as the NAD-cap are characterised in bacteria. The general notion persists that cofactor-caps such as NAD-caps stabilise host transcripts compared with their 5'-triphosphorylated counterparts that are more easily degraded by 5'-end-dependent nucleases in bacteria [12,13,48,49]. The existence of cofactor-capped bacteriophage mRNAs has not been studied yet. In general, one might speculate that both internal and external RNA modifications could provide an additional regulatory layer for the bacteriophage to evade bacterial defence systems or to take-over the host cell. A recent study has indicated that bacteriophages may also use existing RNA modifications of the host to regulate cellular processes during infection [55]. Modifier B (ModB) — one of the ARTs from bacteriophage T4 — not only accepts NAD but also NAD-capped RNA as a substrate to attach entire RNA chains to host proteins in a covalent manner [55]. Through ModB-mediated 'RNAylation', bacteriophage T4 targets the host's translational apparatus resulting in an efficient bacteriophage infection (Table 1) [55]. The concept of 'RNAylation' suggests a direct connection between RNA modification and post-translational protein modification, which may be present in additional bacteriophage–host interactions. ModB homologues appear to exist in various other bacteriophages targeting *Escherichia*, *Klebsiella* or *Salmonella* as indicated by Blast search (Supplementary Table 1).

These findings exemplify that bacteriophages may exploit their proteins, such as in the case of ModB, to 'read' RNA modifications, facilitating host take-over or possibly immunity against bacteria, thereby underlying the epitranscriptome as an important aspect of bacteriophage infection.

Another RNA modification that could significantly impact RNA fate and stability during phage infection involves the addition of polyA tails at the 3´-end of RNA. In contrast to eukaryotes, where polyA tails are important features of mature mRNAs, polyA tails in prokaryotes actively promote RNA degradation (reviewed in [56]). Bacterial polyA polymerases such as *Ec* polyA RNAP I attach multiple adenosines to the 3'-end of transcripts, thereby destabilising the RNA (reviewed in [56]). PolyA tails of phage RNAs can occur on primary transcripts and after initial nucleolytic processing of the transcripts. Thus, polyA tails are located within or at the ends of protein-coding regions of RNA, indicating that they are added to a later stage in the life cycle of a phage RNA [57,58]. Importantly, polyA tailing is vital in order for some RNA fragments to be degraded [57]. However, it is unclear whether polyA tails are directly involved in overall destabilisation or even stabilisation of phage RNAs during infection [58]. One may speculate that the polyA tailing machinery of the host may either be used by the host to counteract phage infection by initiating RNA degradation. Alternatively, the phage may recruit this machinery to selectively enhance the degradation or stabilisation of phage and/or host transcripts.

Molecular duel: potential role of internal RNA modifications in bacteriophage infection

Until now, the functions of most RNA modifications in bacteria remain largely unknown — especially for mRNAs [8]. Ribosomal RNA (rRNA) and transfer RNA (tRNA) modifications play key roles in regulation and fine-tuning of translation. In particular, rRNA modifications impact the mRNA decoding efficiency [59–61], whereupon tRNA modifications are crucial for ensuring the stability, abundance and optimal affinity of tRNAs for the ribosomes [62–64]. tRNAs are highly modified RNA species decorated with various modifications, including — amongst others — pseudouridine and inosine (Box 1), which are installed by more than 20 different modifying enzymes in *E. coli* (reviewed in [65]). Intriguingly, hypomodification of tRNAs (modification at lower levels than usual) triggers their degradation in *Vibrio cholera*, exemplifying the importance of RNA modifications for RNA stability and decay [66]. Despite such important roles for rRNA and tRNA modifications, they have not yet been studied in bacteriophage infections. Host tRNA pools are often downregulated upon bacteriophage infections [4,67,68]. Could this be triggered by mechanisms of tRNA hypomodification or downregulation of tRNA-modifying enzymes in the host as a response to infection? Some bacteriophages even encode their own tRNAs [67,69] that are expressed during infection [4,68]. Do these bacteriophage-encoded tRNAs also contain modifications such as pseudouridine or inosine? Do host enzymes install these modifications, do bacteriophages direct these enzymes to specifically act on these tRNAs or do they encode their own modifying enzymes? These questions only exemplify which variety and mechanisms of RNA modifications could play a role in bacteriophage infections and how they might shape central processes such as translation.

Pseudouridine may have become one of the most famous internal RNA modifications (Box 1). It is an

integral part of mRNA-based vaccines, which prevents recognition of the RNA by the innate immune system of human cells [27,70]. Vaccines without RNA modification trigger the innate immune response and are thus less effective [27]. Key players in the innate immune response are toll-interleukin-1 receptor (TIR) domain proteins that sense the immunogenic material and activate signalling cascades (reviewed in [71]). Interestingly, homologues of eukaryotic TIR domains are also found in bacterial defence systems, as recently demonstrated for the Thoeris system [52]. Based on the conservation of such innate immune recognition systems across all domains of life, one may speculate that immune recognition of RNA modifications may take place in bacteriophage–host interactions as well. For instance, when bacteriophages with single- or double-stranded RNA genomes [72] (reviewed in [73]) infect their bacterial hosts. One could imagine that these genomes are decorated with RNA modifications that trigger anti-phage defence mechanisms. On the other hand, bacteriophages may install host-like RNA modifications on their transcripts/genomes in order to avoid immune recognition and anti-bacteriophage defence.

Another RNA-modifying event in bacteria is A-to-I editing (Box 1). This has been observed in various bacterial species, including *E. coli*. For example, in *E. coli*, the enzyme TadA is responsible for converting adenosine 34 in the anticodon of tRNAs to inosine [74]. Interestingly, TadA was shown to perform A-to-I editing in a small set of mRNAs, too, which changes distinct codons, as inosine is read as guanosine [75]. As a result, different amino acids are incorporated into the encoded proteins impacting protein function, for instance, increasing the toxicity of the HokB protein [75]. Blast search indicates that bacteriophages may possess TadA homologues in their genomes (Supplementary Table 1). Thus, one may hypothesise that bacteriophages could fine-tune protein expression and diversity through targeted A-to-I editing of mRNAs with self- or host-encoded factors. Additionally, it can be assumed that inosine is also present within phage mRNAs. While no information is currently available for bacteriophages, A-to-I conversions have been observed in eukaryotic viruses. These conversions are host-dependent deamination and advantageous for the virus in evading the host immune response and reducing virus toxicity [76,77]. Interestingly, A-to-I editing also occurs as an anti-phage defence mechanism in some bacteria. The *r*estriction by an *a*denosine *d*eaminase *a*cting on *R*NA (RADAR) system senses phage infection and converts (deoxy)ATP to (deoxy)inosine triphosphate (ITP) by deamination of adenosine to inosine [78]. This limits phage infection by inhibiting phage DNA replication and creating an imbalance in the cellular nucleotide pool [78,79]. The RADAR system is able to bind and translocate RNA [79], however, A-to-I editing of RNA by the RADAR system has not yet been observed *in vitro* and *in vivo* [78,79]. Nevertheless, this exemplifies the importance of nucleotides and their modifications during phage infections.

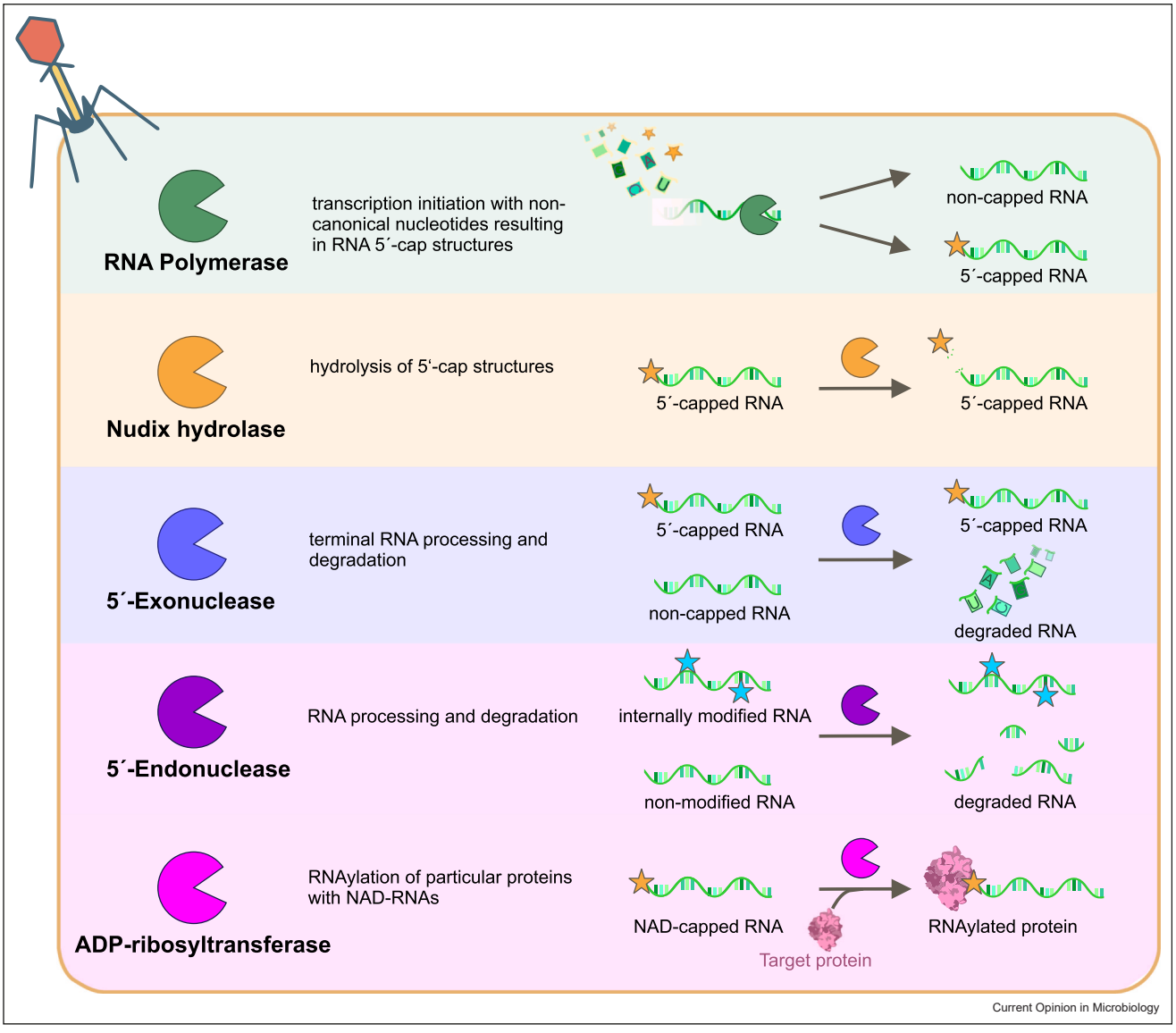
Along this line, also other RNA modifications have been detected in eukaryotic viruses. For instance, N6-methyladenosine (m^6A) has been found in transcripts of eukaryotic DNA viruses and in the genomes of RNA viruses [80]. These modifications play a role in the regulation of viral replication and protection from the innate immune response of the host [80]. Similar protective mechanisms against the host's innate immune response can be expected in bacteriophages, given their constant evolutionary race with bacteria [81].

In summary, although the presence and impact of RNA modifications within the phage transcriptome have not been reported, the existence of RNA modifications in eukaryotic viruses and the potential discovery of homologues of bacterial and eukaryotic RNA-modifying enzymes, such as TadA, Mettl3 and RluF in phages (Supplementary Table 1), suggest that internal RNA modifications are likely present in phages. In such a scenario, the presence of internal modifications within phage transcripts and possibly varying levels of these modifications would help to address one of initial questions of this review: how do RNA modifications influence the precise processing and degradation of bacteriophage and host transcripts during infection?

Housekeeping nucleases: RNA modulation in phage–host crosstalk

RNA synthesis, processing and its selective degradation are key processes during bacteriophage infection. Based on available time-resolved transcriptomic studies during phage infection, a rapid degradation of host RNA is observed, while phage transcripts are actively transcribed and remain preserved [55,82]. As the abundance of host transcripts is already strongly decreased within the first minutes of infection, it suggests the presence of selective RNA degradation mechanisms during the infection, for example, nuclease-based degradation. The degradation of RNA by bacterial nucleases has been reported to be impacted by terminal RNA modifications. Nevertheless, research regarding the influence of RNA modifications in the context of phage–host interaction is currently lacking [48]. Both bacteriophage and host possess their own set of nucleases, which might selectively process and degrade RNA throughout the infection process. Here the following questions arise: how do these nucleases distinguish between phage and bacterial RNA when both types of transcripts are composed of the same four nucleotide building blocks? And what constitutes the molecular basis for discerning between host and phage RNA? In addition to the sequence specificity

Figure 1



Putative modulators of the bacteriophage and host epitranscriptome during infection.

of nucleases and the impact of RNA secondary structure motifs on recognition and cleavage, RNA modifications can exert an impact on RNA stability and its susceptibility to nucleases [48,83,84].

Studies that characterise the processing of transcripts during phage infection in the context of RNA modifications are missing to date. However, initial insights into the potential impact of RNA modifications on RNA processing have been gleaned from previous studies, particularly in the case of certain nucleases. For example, in *E. coli*, RNase E plays a central role in RNA processing and has also been implicated in the bacteriophage T4 infection cycle through the processing of gene-32 mRNA, which is crucial for T4 DNA replication and repair [57,82,85–88]. Nevertheless, cap structures, such as the NAD-cap, can protect transcripts from degradation mediated by RNase E, thereby providing another level of post-transcriptional regulation [48]. As described above, EcRNAP performs NAD-capping of RNA [10] and bacteriophage T4 relies on EcRNAP to express its genes [17]. Thus, it is conceivable that bacteriophages may utilise cofactor-capping of their transcripts [8] to modulate RNA stability against host nucleases such as RNase E.

Another potential strategy employed by bacteriophages to counteract host nucleases through RNA modifications is illustrated by bacteriophage tRNAs. It is conceivable

that phage-encoded tRNAs have evolved to withstand the impact of host anticodon nucleases that deactivate tRNAs by cleaving within the anticodon. Notably, the anticodon of tRNAs is a heavily modified RNA region [65] and one might speculate that RNA modifications might influence its cleavage by nucleases, including VapC, PrrC, Colicin D and Colicin E5. The latter might play a fundamental role in the host's defence against viral infections [67].

With numerous instances of nucleases actively processing both phage and host RNA during the infection process, it becomes increasingly clear that understanding how these nucleases differentiate between phage and host transcripts is of paramount importance. This inquiry is particularly promising when considering the role of RNA modifications in this selective process, as it sheds light on the intricate mechanisms at play during phage infection.

Conclusions

In this review, we illuminated the known and potential writers, readers and erasers of RNA modifications in both bacteria and bacteriophages. The field of epitranscriptomics during phage infection remains understudied and the presence of RNA modifications in bacteriophages has yet to be proved. This review highlights that both bacteria and bacteriophages harbour a variety of genes that could encode potential writers, erasers and readers of internal and terminal RNA modifications.

The observed resilience of bacteriophages in maintaining infections and effectively hijacking their host, regardless of the presence of numerous anti-phage defence systems, strongly suggests the involvement of RNA modifications in bacteriophage infections. The modifications of phage RNA potentially offer an additional protective layer to phage RNA, making it less susceptible to degradation by the bacterial immune system. Moreover, phage RNA modifications can act as a factor distinguishing between phage and host RNA during infection, a process observed but not yet explored on a molecular level [4]. On the other hand, it is plausible that phages do not just modify their RNA but also target host RNA, potentially altering its function through these modifications. It is also conceivable that bacteriophages may reprogram host nucleases to hinder the introduction of modifications or exploit them for their own advantage. Therefore, both terminal and internal modifications, as discussed in this review, could significantly impact bacteriophage infections, enabling phages to manipulate their host or, conversely, contributing to anti-phage defence mechanisms.

All these possibilities become more credible and of higher relevance of investigation, when considering the current research in the field of the epitranscriptome of eukaryotic viruses. For eukaryotic viruses, the impact of RNA modifications on the regulation of host take-over during the infection was already observed, strongly contributing to efficient infection, for example, the substitution of uridine by pseudouridine enhances RNA stability and decreases anti-RNA immune response [89], or the introduction of the FAD-cap protects viral RNA from innate immune recognition [90]. Therefore, given the features of the RNA modifications and their prevalence across all domains of life, and across eukaryotic viruses, it is reasonable to speculate their existence in bacteriophages and potential roles in infection regulation.

Identifying modified RNA building blocks using well-established methods (reviewed in [8]) is the key to addressing this question. Such studies will reveal valuable insights into bacteriophage infections, provide mechanistic details of infection regulation and potentially unveil novel bacteriophage and host immune systems. Moreover, the rapid advancements in sequencing technologies, especially third-generation methods such as direct RNA-sequencing [91], hold the promise of simultaneously determining both the transcriptome and epitranscriptome in the future, which would be immensely beneficial for this research field.

CRediT authorship contribution statement

Nadiia Pozhydaieva, Maik Wolfram-Schauerte and Helene Keuthen: Conceptualization, Writing – original draft, Writing – review & editing. **Katharina Höfer**: Conceptualization, Funding acquisition, Writing – review & editing.

Data Availability

NCBI BLAST+ was used to perform a homology search for Modifier B (ModB) (UniProtID: P39423), TadA (UniProtID: P68398), METTL3 (UniProtID: F1R777) and RluF (UniProtID: P32684) on the EMBL website (https://www.ebi.ac.uk/Tools/sss/ncbiblast/, accessed on 26/10/2023). UniProtKB Viruses database was used for the search.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships that may be considered as potential competing interests: K.H. is in the process of applying for a patent (PCT/EP2021/071295) covering the RNAylation that lists K.H. as inventor. The remaining authors declare no competing interests.

Acknowledgements

This project has received funding from the European Research Council, Belgium under the European Union's Horizon 2020 research and

innovation programme (ERC-2023-STG grant 101114948, NAD-ART to K.H.), from the German Research Council (DFG: SPP 2330 project number 464500427, RTG 2355 project 11 and RTG 2927 project 06 to K.H.), from the Max Planck Society (Max Planck Research Group Leader funding to KH), from the Studienstiftung des Deutschen Volkes e.V. (PhD scholarship to M.W.S.) and from the Joachim Herz Stiftung (Add-on Fellowships for Interdisciplinary Life Science to M.W.S.).

Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mib.2023.102417.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Clokie MR, Millard AD, Letarov AV, Heaphy S: **Phages in nature**. *Bacteriophage* 2011, **1**:31-45.

2. Keen EC: **A century of phage research: bacteriophages and the shaping of modern biology**. *Bioessays* 2015, **37**:6-9.

3. Yang H, Ma Y, Wang Y, Yang H, Shen W, Chen X: **Transcription regulation mechanisms of bacteriophages: recent advances and future prospects**. *Bioengineered* 2014, **5**:300-304.

4. • Wolfram-Schauerte M, Pozhydaieva N, Viering M, Glatter T, Höfer K: **Integrated omics reveal time-resolved insights into T4 phage infection of E. coli on proteome and transcriptome levels**. *Virus-Basel* 2022, **14**:2502.

This study provides in-depth insights into the dual-transcriptome and proteome of T4 phage infection and thereby points out connection points between two layers of gene expression during infection of *E. coli*. Further, it is the first study of its kind in the field of phage-host interactions and indicates the diverse regulatory mechanisms, which contribute to the regulation of infection but are not yet understood.

5. Ueno H, Yonesaki T: **Phage-induced change in the stability of mRNAs**. *Virology* 2004, **329**:134-141.

6. • Wicke L, Ponath F, Coppens L, Gerovac M, Lavigne R, Vogel J: **Introducing differential RNA-seq mapping to track the early infection phase for Pseudomonas phage ϕKZ**. *RNA Biol* 2021, **18**:1099-1110.

Wicke and colleagues applied differential RNA-seq to determine transcription start sites of both phage and host during early infection of Pseudomonas phage PhiKZ. Thereby, they shed light onto transcriptional organization and architecture early during infection. This study is the first of its kind and emphasizes that novel technologies are just started to being applied to investigate phage-host interactions.

7. Shi H, Chai P, Jia R, Fan X: **Novel insight into the regulatory roles of diverse RNA modifications: re-defining the bridge between transcription and translation**. *Mol Cancer* 2020, **19**:78.

8. Schauerte M, Pozhydaieva N, Höfer K: **Shaping the bacterial epitranscriptome-5'-terminal and internal RNA modifications**. *Adv Biol (Weinh)* 2021, **5**:e2100834.

9. •• Boccaletto P, Stefaniak F, Ray A, Cappannini A, Mukherjee S, Purta E, Kurkowska M, Shirvanizadeh N, Destefanis E, Groza P, *et al.*: **MODOMICS: a database of RNA modification pathways. 2021 update**. *Nucleic Acids Res* 2022, **50**:D231-D235.

MODOMICS is a state-of-the-art database for RNA modifications, their structures, occurrences, and relevance. This database serves as a central collection point for these information and will serve as a potential site for the deposition of phage RNA modifications.

10. Bird JG, Zhang Y, Tian Y, Panova N, Barvik I, Greene L, Liu M, Buckley B, Krasny L, Lee JK, *et al.*: **The mechanism of RNA 5 ' capping with NAD(+), NADH and desphospho-CoA**. *Nature* 2016, **535**:444.

11. Julius C, Yuzenkova Y: **Bacterial RNA polymerase caps RNA with various cofactors and cell wall precursors**. *Nucleic Acids Res* 2017, **45**:8282-8290.

12. Morales-Filloy HG, Zhang Y, Nübel G, George SE, Korn N, Wolz C, Jäschke A: **The 5' NAD cap of RNAIII modulates toxin production in staphylococcus aureus isolates**. *J Bacteriol* 2020, **202**:e00591-19.

13. Frindert J, Zhang Y, Nübel G, Kahloon M, Kolmar L, Hotz-Wagenblatt A, Burhenne J, Haefeli WE, Jäschke A: **Identification, biosynthesis, and decapping of NAD-capped RNAs in B. subtilis**. *Cell Rep* 2018, **24**:1890-1901 e1898.

14. Wolfram-Schauerte M, Höfer K: **NAD-capped RNAs - a redox cofactor meets RNA**. *Trends Biochem Sci* 2023, **48**:142-155.

15. Tabib-Salazar A, Mulvenna N, Severinov K, Matthews SJ, Wigneshweraraj S: **Xenogeneic regulation of the bacterial transcription machinery**. *J Mol Biol* 2019, **431**:4078-4092.

16. Sokolova ML, Misovetc I, VS K: **Multisubunit RNA polymerases of jumbo bacteriophages**. *Viruses* 2020, **12**:1064.

17. Miller ES, Kutter E, Mosig G, Arisaka F, Kunisawa T, Rüger W: **Bacteriophage T4 genome**. *Microbiol Mol Biol Rev* 2003, **67**:86-156 (table of contents).

18. Falco SC, Laan KV, Rothman-Denes LB: **Virion-associated RNA polymerase required for bacteriophage N4 development**. *Proc Natl Acad Sci USA* 1977, **74**:520-523.

19. Tiemann B, Depping R, Gineikiene E, Kaliniene L, Nivinskas R, Rüger W: **ModA and ModB, two ADP-ribosyltransferases encoded by bacteriophage T4: catalytic properties and mutation analysis**. *J Bacteriol* 2004, **186**:7262-7272.

20. Depping R, Lohaus C, Meyer HE, Rüger W: **The mono-ADP-ribosyltransferases Alt and ModB of bacteriophage T4: target proteins identified**. *Biochem Biophys Res Commun* 2005, **335**:1217-1223.

21. Tiemann B, Depping R, Rüger W: **Overexpression, purification, and partial characterisation of ADP-ribosyltransferases modA and modB of bacteriophage T4**. *Gene Expr* 1999, **8**:187-196.

22. Koch T, Raudonikiene A, Wilkens K, Rüger W: **Overexpression, purification, and characterisation of the ADP-ribosyltransferase (gpAlt) of bacteriophage T4: ADP-ribosylation of E. coli RNA polymerase modulates T4 "early" transcription**. *Gene Expr* 1995, **4**:253-264.

23. Summers WC, Siegel RB: **Control of template specificity of E. coli RNA polymerase by a phage-coded protein**. *Nature* 1969, **223**:1111-1113.

24. Borkotoky S, Murali A: **The highly efficient T7 RNA polymerase: a wonder macromolecule in biological realm**. *Int J Biol Macromol* 2018, **118**:49-56.

25. Huang F: **Efficient incorporation of CoA, NAD and FAD into RNA by in vitro transcription**. *Nucleic Acids Res* 2003, **31**:e8.

26. •• Chen TH, Potapov V, Dai N, Ong JL, Roy B: **N(1)-methyl-pseudouridine is incorporated with higher fidelity than pseudouridine in synthetic RNAs.** *Sci Rep* 2022, **12**:13017.

Chen et al. investigated the fidelity of incorporating uridine analogs, specifically pseudouridine and N1-methyl-pseudouridine, into synthetic mRNAs during *in vitro* transcription. The research reveals that N1-methyl-pseudouridine is incorporated more accurately than pseudouridine, and introduces a method to improve uridine analog incorporation fidelity, providing guidance for selecting RNAPs for *in vitro* production of modified uridine containing mRNAs.

27. Morais P, Adachi H, Yu YT: **The critical contribution of pseudouridine to mRNA COVID-19 vaccines**. *Front Cell Dev Biol* 2021, **9**:789427.

28. Yin YW, Steitz TA: **The structural mechanism of translocation and helicase activity in T7 RNA polymerase**. *Cell* 2004, **116**:393-404.

29. Wang W, Li Y, Wang Y, Shi C, Li C, Li Q, Linhardt RJ: **Bacteriophage T7 transcription system: an enabling tool in synthetic biology**. *Biotechnol Adv* 2018, **36**:2129-2137.

30. Kortmann M, Kuhl V, Klaffl S, Bott M: **A chromosomally encoded T7 RNA polymerase-dependent gene expression system for Corynebacterium glutamicum: construction and comparative evaluation at the single-cell level**. *Micro Biotechnol* 2015, **8**:253-265.

31. McAllister WT, Raskin CA: **The phage RNA polymerases are related to DNA polymerases and reverse transcriptases**. *Mol Microbiol* 1993, **10**:1-6.

32. Zhu B, Hernandez A, Tan M, Wollenhaupt J, Tabor S, Richardson CC: **Synthesis of 2'-Fluoro RNA by Syn5 RNA polymerase**. *Nucleic Acids Res* 2015, **43**:e94.

33. Padilla R, Sousa R: **Efficient synthesis of nucleic acids heavily modified with non-canonical ribose 2'-groups using a mutant T7 RNA polymerase (RNAP)**. *Nucleic Acids Res* 1999, **27**:1561-1563.

34. Lu X, Wu H, Xia H, Huang F, Yan Y, Yu B, Cheng R, Drulis-Kawa Z, Zhu B: **Klebsiella phage KP34 RNA polymerase and its use in RNA synthesis**. *Front Microbiol* 2019, **10**:2487.

35. Lammens EM, Feyaerts N, Kerremans A, Boon M, Lavigne R: **Assessing the orthogonality of phage-encoded RNA polymerases for tailored synthetic biology applications in Pseudomonas species**. *Int J Mol Sci* 2023, **24**:7175.

36. Rong MQ, Castagna R, McAllister WT: **Cloning and purification of bacteriophage K11 RNA polymerase**. *Biotechniques* 1999, **27**:690 692, 694.

37. Krieg PA, Melton DA: **In vitro RNA synthesis with SP6 RNA polymerase**. *Methods Enzym* 1987, **155**:397-415.

38. Zhu B, Tabor S, Raytcheva DA, Hernandez A, King JA, Richardson CC: **The RNA polymerase of marine cyanophage Syn5**. *J Biol Chem* 2013, **288**:3545-3552.

39. Al-Shayeb B, Sachdeva R, Chen LX, Ward F, Munk P, Devoto A, Castelle CJ, Olm MR, Bouma-Gregson K, Amano Y, *et al.*: **Clades of huge phages from across Earth's ecosystems**. *Nature* 2020, **578**:425-431.

40. Chaikeeratisak V, Nguyen K, Khanna K, Brilot AF, Erb ML, Coker JK, Vavilina A, Newton GL, Buschauer R, Pogliano K, *et al.*: **Assembly of a nucleus-like structure during viral replication in bacteria**. *Science* 2017, **355**:194-197.

41. Malone LM, Warring SL, Jackson SA, Warnecke C, Gardner PP, Gumy LF, Fineran PC: **A jumbo phage that forms a nucleus-like structure evades CRISPR-Cas DNA targeting but is vulnerable to type III RNA-based immunity**. *Nat Microbiol* 2020, **5**:48-55.

42. Lavysh D, Sokolova M, Minakhin L, Yakunina M, Artamonova T, Kozyavkin S, Makarova KS, Koonin EV, Severinov K: **The genome of AR9, a giant transducing Bacillus phage encoding two multisubunit RNA polymerases**. *Virology* 2016, **495**:185-196.

43. Takahashi I, Marmur J: **Replacement of thymidylic acid by deoxyuridylic acid in the deoxyribonucleic acid of a transducing phage for Bacillus subtilis**. *Nature* 1963, **197**:794-795.

44. Sharma S, Grudzien-Nogalska E, Hamilton K, Jiao X, Yang J, Tong L, Kiledjian M: **Mammalian Nudix proteins cleave nucleotide metabolite caps on RNAs**. *Nucleic Acids Res* 2020, **48**:6788-6798.

45. McLennan AG: **The Nudix hydrolase superfamily**. *Cell Mol Life Sci* 2006, **63**:123-143.

46. Deana A, Celesnik H, Belasco JG: **The bacterial enzyme RppH triggers messenger RNA degradation by 5' pyrophosphate removal**. *Nature* 2008, **451**:355-358.

47. Qi D, Alawneh AM, Yonesaki T, Otsuka Y: **Rapid degradation of host mRNAs by stimulation of RNase E Activity by Srd of bacteriophage T4**. *Genetics* 2015, **201**:977-987.

48. Cahova H, Winz ML, Höfer K, Nübel G, Jäschke A: **NAD captureSeq indicates NAD as a bacterial cap for a subset of regulatory RNAs**. *Nature* 2015, **519**:374-377.

49. Höfer K, Li S, Abele F, Frindert J, Schlotthauer J, Grawenhoff J, Du J, Patel DJ, Jäschke A: **Structure and function of the bacterial decapping enzyme NudC**. *Nat Chem Biol* 2016, **12**:730-734.

50. Zhou W, Guan Z, Zhao F, Ye Y, Yang F, Yin P, Zhang D: **Structural insights into dpCoA-RNA decapping by NudC**. *RNA Biol* 2021, **18**:244-253.

51. Vvedenskaya IO, Bird JG, Zhang Y, Zhang Y, Jiao X, Barvik I, Krasny L, Kiledjian M, Taylor DM, Ebright RH, *et al.*: **CapZyme-seq comprehensively defines promoter-sequence determinants for RNA 5' capping with NAD.** *Mol Cell* 2018, **70**:553-564 e559.

52. Ofir G, Herbst E, Baroz M, Cohen D, Millman A, Doron S, Tal N, Malheiro DBA, Malitsky S, Amitai G, *et al.*: **Antiviral activity of bacterial TIR domains via immune signalling molecules**. *Nature* 2021, **600**:116-120.

53. Lee JY, Li Z, Miller ES: **Vibrio phage KVP40 encodes a functional NAD(+) salvage pathway**. *J Bacteriol* 2017, **199**:e00855-16.

54. Xu W, Gauss P, Shen J, Dunn CA, Bessman MJ: **The gene e.1 (nudE.1) of T4 bacteriophage designates a new member of the Nudix hydrolase superfamily active on flavin adenine dinucleotide, adenosine 5'-triphospho-5'-adenosine, and ADP-ribose**. *J Biol Chem* 2002, **277**:23181-23185.

55. •• Wolfram-Schauerte M, Pozhydaieva N, Grawenhoff J, Welp LM, Silbern I, Wulf A, Billau FA, Glatter T, Urlaub H, Jäschke A, *et al.*: **A viral ADP-ribosyltransferase attaches RNA chains to host proteins**. *Nature* 2023, **620**:1054-1062.

This is the first study focusing on the function of an RNA modification in the context of phage-host interaction. Wolfram-Schauerte et al. identify that the T4 phage ART ModB cannot only accept NAD but also NAD-capped RNA as substrate. Thereby, it attaches entire RNA chains to host proteins in a covalent manner in an 'RNAylation' reaction. The T4 phage RNAylates host proteins of the translational apparatus with *E. coli* NAD-capped transcripts. T4 phages with inactive ModB show delayed lysis and reduced progeny indicating the importance of ModB function for efficient infection. This is the first study describing the function of an RNA modification in phage infection. As several phages and viruses encode ARTs, the RNAylation may be a broader phenomenon in other phages and viruses. Further, it opens new avenues towards the creation of RNA-protein conjugates.

56. Hajnsdorf E, Kaberdin VR: **RNA polyadenylation and its consequences in prokaryotes**. *Philos Trans R Soc Lond B Biol Sci* 2018, **373**:20180166.

57. Goodrich AF, Steege DA: **Roles of polyadenylation and nucleolytic cleavage in the filamentous phage mRNA processing and decay pathways in Escherichia coli**. *RNA* 1999, **5**:972-985.

58. Johnson MD, Popowski J, Cao GJ, Shen P, Sarkar N: **Bacteriophage T7 mRNA is polyadenylated**. *Mol Microbiol* 1998, **27**:23-30.

59. Burakovsky DE, Prokhorova IV, Sergiev PV, Milon P, Sergeeva OV, Bogdanov AA, Rodnina MV, Dontsova OA: **Impact of methylations of m2G966/m5C967 in 16S rRNA on bacterial fitness and translation initiation**. *Nucleic Acids Res* 2012, **40**:7885-7895.

60. Valadon C, Namy O: **The importance of the Epi-transcriptome in translation fidelity**. *Noncoding RNA* 2021, **7**:51.

61. Hughes KJ, Chen X, Burroughs AM, Aravind L, Wolin SL: **An RNA repair operon regulated by damaged tRNAs**. *Cell Rep* 2020, **33**:108527.

62. Noguchi S, Nishimura Y, Hirota Y, Nishimura S: **Isolation and characterisation of an Escherichia coli mutant lacking tRNA-guanine transglycosylase. Function and biosynthesis of queuosine in tRNA.** *J Biol Chem* 1982, **257**:6544-6550.

63. Vicens Q, Paukstelis PJ, Westhof E, Lambowitz AM, Cech TR: **Toward predicting self-splicing and protein-facilitated splicing of group I introns**. *RNA* 2008, **14**:2013-2029.

64. Motorin Y, Helm M: **tRNA stabilisation by modified nucleotides**. *Biochemistry* 2010, **49**:4934-4944.

65. de Crecy-Lagard V, Jaroch M: **Functions of bacterial tRNA modifications: from ubiquity to diversity**. *Trends Microbiol* 2021, **29**:41-53.

66. Kimura S, Waldor MK: **The RNA degradosome promotes tRNA quality control through clearance of hypomodified tRNA**. *Proc Natl Acad Sci USA* 2019, **116**:1394-1403.

67. •• van den Berg DF, van der Steen BA, Costa AR, Brouns SJJ: **Phage tRNAs evade tRNA-targeting host defenses through anticodon loop mutations**. *Elife* 2023, **12**:e85183.
Van den Berg et al. investigated the role of tRNAs in bacteriophage genomes and introduced a new hypothesis suggesting that phage-encoded tRNAs serve to counteract the host tRNA-depleting strategies, enhancing their defence against viral infection. The research indicates that phage tRNAs are designed to be insensitive to host anticodon nucleases.

68. Yang JY, Fang W, Miranda-Sanchez F, Brown JM, Kauffman KM, Acevero CM, Bartel DP, Polz MF, Kelly L: **Degradation of host translational machinery drives tRNA acquisition in viruses**. *Cell Syst* 2021, **12**:771-779 e775.

69. Bailly-Bechet M, Vergassola M, Rocha E: **Causes for the intriguing presence of tRNAs in phages**. *Genome Res* 2007, **17**:1486-1495.

70. Kariko K, Buckstein M, Ni H, Weissman D: **Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA**. *Immunity* 2005, **23**:165-175.

71. O'Neill LA, Bowie AG: **The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling**. *Nat Rev Immunol* 2007, **7**:353-364.

72. Yang Y, Lu S, Shen W, Zhao X, Shen M, Tan Y, Li G, Li M, Wang J, Hu F, *et al.*: **Corrigendum: characterisation of the first double-stranded RNA bacteriophage infecting Pseudomonas aeruginosa**. *Sci Rep* 2017, **7**:46853.

73. Callanan J, Stockdale SR, Callanan A, Draper LA, Ross RP, Hill C: **RNA phage biology in a metagenomic era**. *Viruses* 2018, **10**:386.

74. Rafels-Ybern A, Torres AG, Grau-Bove X, Ruiz-Trillo I, Ribas de Pouplana L: **Codon adaptation to tRNAs with Inosine modification at position 34 is widespread among Eukaryotes and present in two Bacterial phyla**. *RNA Biol* 2018, **15**:500-507.

75. Bar-Yaacov D, Mordret E, Towers R, Biniashvili T, Soyris C, Schwartz S, Dahan O, Pilpel Y: **RNA editing in bacteria recodes multiple proteins and regulates an evolutionarily conserved toxin-antitoxin system**. *Genome Res* 2017, **27**:1696-1703.

76. Zhu TT, Niu GY, Zhang YS, Chen M, Li CY, Hao LL, Zhang Z: **Host-mediated RNA editing in viruses**. *Biol Direct* 2023, **18**:12.

77. Volchkova VA, Dolnik O, Martinez MJ, Reynard O, Volchkov VE: **RNA editing of the GP gene of Ebola virus is an important pathogenicity factor**. *J Infect Dis* 2015, **212**:S226-S233.

78. •• Duncan-Lowey B, Tal N, Johnson AG, Rawson S, Mayer ML, Doron S, Millman A, Melamed S, Fedorenko T, Kacen A, *et al.*: **Cryo-EM structure of the RADAR supramolecular anti-phage defence complex**. *Cell* 2023, **186**:987-998 e915.
Duncan-Lowey and colleagues present detailed molecular insights into the RADAR defence system and show how it is involved in anti-phage defence by A-to-I editing of nucleotides. Even though it does not act on RNA — according to their findings — it nicely demonstrates the importance of nucleoside modifications in the context of phage-host interactions.

79. •• Gao Y, Luo X, Li P, Li Z, Ye F, Liu S, Gao P: **Molecular basis of RADAR anti-phage supramolecular assemblies**. *Cell* 2023, **186**:999-1012 e1020.
This study investigated the molecular basis of the RADAR system demonstrating its ability to bind and recruit RNA to the complex. Even though they could not proof A-to-I editing on RNA itself, it showcases the potential involvement of anti-phage defence on the level of RNA and its modification.

80. Li HA, Guo Y, Qi WB, Liao M: **N-methyladenosine modification of viral RNA and its role during the recognition process of RIG-I-like receptors**. *Front Immunol* 2022, **13**:1031200.

81. Georjon H, Bernheim A: **The highly diverse antiphage defence systems of bacteria**. *Nat Rev Microbiol* 2023, **21**:686-700.

82. Uzan M, Miller ES: **Post-transcriptional control by bacteriophage T4: mRNA decay and inhibition of translation initiation**. *Virol J* 2010, **7**:360.

83. Feng Y, Vickers TA, Cohen SN: **The catalytic domain of RNase E shows inherent 3' to 5' directionality in cleavage site selection**. *Proc Natl Acad Sci USA* 2002, **99**:14746-14751.

84. Zhang DL, Liu YX, Wang Q, Guan ZY, Wang J, Liu J, Zou TT, Yin P: **Structural basis of prokaryotic NAD-RNA decapping by NudC**. *Cell Res* 2016, **26**:1062-1066.

85. Lambrecht SJ, Stappert N, Sommer F, Schroda M, Steglich C: **A cyanophage MarR-type transcription factor regulates host RNase E expression during infection**. *Microorganisms* 2022, **10**:2245.

86. Kokoska RJ, Steege DA: **Appropriate expression of filamentous phage f1 DNA replication genes II and X requires RNase E-dependent processing and separate mRNAs**. *J Bacteriol* 1998, **180**:3245-3249.

87. Mudd EA, Prentki P, Belin D, Krisch HM: **Processing of unstable bacteriophage T4 gene 32 mRNAs into a stable species requires Escherichia coli ribonuclease E**. *EMBO J* 1988, **7**:3601-3607.

88. Uzan M: **RNA processing and decay in bacteriophage T4**. *Prog Mol Biol Transl Sci* 2009, **85**:43-89.

89. Martinez Campos C, Tsai K, Courtney DG, Bogerd HP, Holley CL, Cullen BR: **Mapping of pseudouridine residues on cellular and viral transcripts using a novel antibody-based technique**. *RNA* 2021, **27**:1400-1411.

90. •• Sherwood AV, Rivera-Rangel LR, Ryberg LA, Larsen HS, Anker KM, Costa R, Vagbo CB, Jakljevic E, Pham LV, Fernandez-Antunez C, *et al.*: **Hepatitis C virus RNA is 5'-capped with flavin adenine dinucleotide**. *Nature* 2023, **619**:811-818.
Sherwood et al. show that the RNA of hepatitis C virus is 75 % FAD-capped and a strategy employed by HCV to evade RIG-I-mediated antiviral immunity. This finding demonstrates the importance of cofactor-capped RNAs in virus-host interactions, potentially also for phages.

91. Jain M, Abu-Shumays R, Olsen HE, Akeson M: **Advances in nanopore direct RNA sequencing**. *Nat Methods* 2022, **19**:1160-1164.

92. Luciano DJ, Vasilyev N, Richards J, Serganov A, Belasco JG: **A novel RNA phosphorylation state enables 5' end-dependent degradation in Escherichia coli**. *Mol Cell* 2017, **67**:44-54 e46.

93. Deng X, Chen K, Luo GZ, Weng X, Ji Q, Zhou T, He C: **Widespread occurrence of N6-methyladenosine in bacterial mRNA**. *Nucleic Acids Res* 2015, **43**:6557-6567.

94. Golovina AY, Dzama MM, Osterman IA, Sergiev PV, Serebryakova MV, Bogdanov AA, Dontsova OA: **The last rRNA methyltransferase of E. coli revealed: the yhiR gene encodes adenine-N6 methyltransferase specific for modification of A2030 of 23S ribosomal RNA**. *RNA* 2012, **18**:1725-1734.

95. Sergiev PV, Serebryakova MV, Bogdanov AA, Dontsova OA: **The ybiN gene of Escherichia coli encodes adenine-N6 methyltransferase specific for modification of A1618 of 23 S ribosomal RNA, a methylated residue located close to the ribosomal exit tunnel**. *J Mol Biol* 2008, **375**:291-300.

96. Ma H, Wang X, Cai J, Dai Q, Natchiar SK, Lv R, Chen K, Lu Z, Chen H, Shi YG, *et al.*: **N(6-)Methyladenosine methyltransferase ZCCHC4 mediates ribosomal RNA methylation.** *Nat Chem Biol* 2019, **15**:88-94.

97. Alseth I, Dalhus B, Bjoras M: **Inosine in DNA and RNA**. *Curr Opin Genet Dev* 2014, **26**:116-123.

98. Nie W, Wang S, He R, Xu Q, Wang P, Wu Y, Tian F, Yuan J, Zhu B, Chen G: **A-to-I RNA editing in bacteria increases pathogenicity and tolerance to oxidative stress**. *PLoS Pathog* 2020, **16**:e1008740.

99. Edelheit S, Schwartz S, Mumbach MR, Wurtzel O, Sorek R: **Transcriptome-wide mapping of 5-methylcytidine RNA modifications in bacteria, archaea, and yeast reveals m5C within archaeal mRNAs**. *PLoS Genet* 2013, **9**:e1003602.