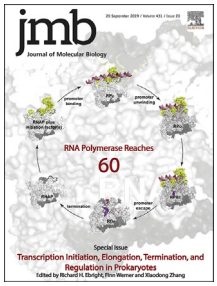

Xenogeneic Regulation of the Bacterial Transcription Machinery

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Abstract

The parasitic life cycle of viruses involves the obligatory subversion of the host's macromolecular processes for efficient viral progeny production. Viruses that infect bacteria, bacteriophages (phages), are no exception and have evolved sophisticated ways to control essential biosynthetic machineries of their bacterial prey to benefit phage development. The xenogeneic regulation of bacterial cell function is a poorly understood area of bacteriology. The activity of the bacterial transcription machinery, the RNA polymerase (RNAP), is often regulated by a variety of mechanisms involving small phage-encoded proteins. In this review, we provide a brief overview of known phage proteins that interact with the bacterial RNAP and compare how two prototypical phages of *Escherichia coli*, T4 and T7, use small proteins to "puppeteer" the bacterial RNAP to ensure a successful infection.

Introduction

Bacteriophages (phages) are obligate intracellular viruses of bacteria and represent the most abundant entities in the biosphere. Phages are found in all habitats where bacteria proliferate and phage total number is estimated to exceed 10^{31} particles. In other words, phages outnumber bacteria by 10 to 1. As such, phages drive bacterial community dynamics, evolution, and ecosystems. In general, phages can adopt two major lifestyles: A *lytic* (virulent) phage attaches to the bacterial cell, injects its genetic material, and rapidly repurposes the host's processes for the production of progeny virions, which subsequently burst out of the bacterial cell. In contrast, the genetic material of *temperate* phages is propagated as the host cell divides. In some cases, this can lead to the development of symbiotic relationships because the combination of phage and bacterial genomes, even if temporary, can provide mutual benefits [1]. Many (but not all) temperate phages can integrate their genomes into their host bacterium's genome (thus becoming a lysogen). Under certain stress conditions (and often spontaneously), the temperate phage enters the lytic cycle that leads to the demise of the infected bacterial cell. From the time of their discovery ~100 years ago, phages were considered as anti-bacterial agents. While almost completely shadowed by antibiotics, phages are now widely considered as an alternative way to treat bacterial infections, particularly those caused by antibiotic-resistant pathogens [2–9].

Much like eukaryotic and archaeal viruses, which derail the host's cellular processes to ensure viral replication, phages have evolved complex strategies to take over their bacterial hosts. The evolutionary arms race between phages and bacteria has resulted in diverse and elegant molecular strategies of attack and defence. Many phages encode specialized small proteins, which modulate, inhibit, or repurpose essential bacterial processes to

promote phage progeny production. Such phage proteins, which we here refer to as *h*ost *a*cquisition *f*actors (HAFs), can thus be considered as xenogeneic (i.e., non-bacterial) regulators of bacterial cell function. We refer readers to reviews by Roucourt and Lavingne [10] and De Smet *et al*. [11], for an extensive list of HAFs encoded from different phages that target diverse and essential physiological host processes.

A common theme by which phages modulate host physiology is through the bacterial RNAP polymerase (RNAP), the enzyme that catalyses transcription. HAFs that specifically target the bacterial RNAP have long served as a rich source of paradigms for bacterial transcriptional regulation [12,13]. The transcription of phage genes are often temporally coordinated and the genes of most phages can be categorized as early, middle, and late depending on the timing of their expression during infection. Strategies used by phages to modulate the host RNAP depends on whether the phage in question entirely or selectively depends on the host RNAP for transcription of its genes. In the case of the latter, the phage encodes its own RNAP. Therefore, many phages have evolved mechanisms to appropriate or inhibit (or a combination of both) the bacterial RNAP for the temporally coordinated transcription of their genes. The purpose of this review is to provide a brief overview of HAFs that interact with the bacterial RNAP and compare how two prototypical phages of *Escherichia coli*, T4 (which entirely relies on the host RNAP for the transcription of its genes) and T7 (which requires both, the bacterial and a phage encoded single-subunit RNAP for transcription of its genes), use specific HAFs to "puppeteer" the bacterial RNAP to coordinate phage gene transcription inside the host. Although HAFs that do not directly target the bacterial RNAP (e.g., phage-encoded endonucleases that degrade host DNA, modulators of host nucleoid binding proteins, etc.) can affect host transcription processes indirectly, they fall outside the scope of this review and are thus not discussed.

A Nexus for HAFs: The Bacterial RNAP

The multi-subunit catalytic core of bacterial RNAP (E) consists of five subunits (2α, β, β', and ω), which reversibly interacts with a sigma factor (σ) subunit to form the holoenzyme (Eσ). Promoter specificity upon the bacterial RNAP is conferred by the σ factor. The number of σ factors encoded by bacteria varies from 1 (in *Mycoplasma genitalium*) to 65 (in *Streptomyces coelicolor*). Seven different σ factors, σ^{70}, σ^{54}, σ^{38}, σ^{32}, σ^{28}, σ^{24}, and σ^{18}, have been identified in *E. coli*, and each participates in the transcription of a specific set of genes. Thus, the interchangeable nature of σ factors and the conditional control of their availability and activity represents a powerful and multi-layered regulatory "handle" for the effective execution of genetic programs in response to specific biotic and abiotic stresses, growth transitions, and morphological and lifestyle changes. For example, in *E. coli*, the predominant form of the σ factor during exponential growth under nutrient-rich conditions is σ^{70}, which is responsible for the transcription of housekeeping genes. In contrast, upon nutrient depletion, σ^{38} becomes available and results in the expression of genes required for adaptation to the stationary phase of growth. All *E. coli* σ factors, except σ^{54}, belong to the same functional class [14]. Although the β and β' subunits contain the catalytic determinants of the RNAP, all subunits are targeted by regulatory factors to fine tune RNAP performance. In fact, all subunits of the RNAP constitute interaction sites for different HAFs (Fig. 1). In this review, we will exclusively focus on how HAFs affect transcription by RNAP containing the σ^{70} class of σ factors. The σ^{54} factor belongs in a functional class of its own [15], and to the best of our knowledge, no HAF has been described to affect the RNAP containing σ^{54}. Although, a study by Ceyssens *et al*. [16] revealed that σ^{54} is required by the *Pseudomonas aeruginosa* YuA phage to successfully develop inside its host, suggesting that HAFs that affect Eσ^{54} might exist and are yet to be discovered.

The bacterial transcription cycle, which has been extensively reviewed elsewhere [17–21], can be broadly separated into four main phases: holoenzyme formation, transcription initiation, elongation, and termination. The σ^{70} factor of *E. coli* comprises four globular domains (σ_1, σ_2, σ_3, and σ_4) that make extensive contacts with the catalytic core subunits of the RNAP [22,23]. Promoter recognizing σ_2 and σ_4 interact with the β' subunit coiled-coil domain [24] and a subdomain of the β subunit called the β-flap, respectively [25]. At most σ^{70}-dependent promoters, transcription initiation begins with the σ factor-directed binding of the RNAP to consensus sequences located at position -10 (5′-TATAAT-3′) and -35 (5′-TTGACA-3′) bp upstream of the transcription start site at $+1$. The major contacts with the consensus -35 and -10 elements are facilitated by the σ^{70} domains σ_4 and σ_2, respectively. At some σ^{70}-dependent promoters, which do not contain a consensus -35 element, σ_3 interacts with a short sequence motif, called the "extended -10 element" (TGn) that is adjacent to the -10 element [26]. The carboxyl terminal domain of RNAP α subunit (α-CTD) can interact with an AT-rich sequence, called the UP element located around $-40/-60$ bp upstream of the transcription start site of some promoters [27].

The binding of Eσ^{70} to the promoter results in the formation of the transcriptionally inactive "closed" promoter complex (RP_C). In a model proposed by

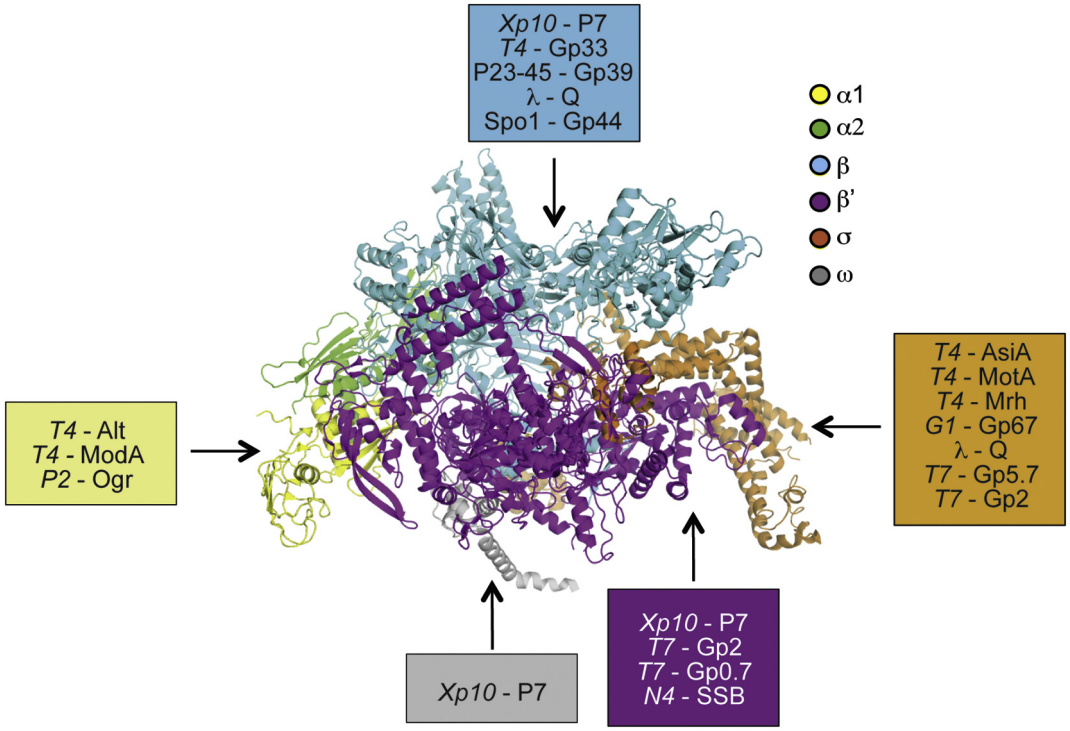

Fig. 1. All subunits of the RNAP constitute an interaction site for different HAFs. Ribbon representation of the structure of the *E. coli* RNAP containing σ^{70} in which the different subunits are color-coded as indicated. The HAFs that interact with the different subunits are shown in boxes with the corresponding color code. The phages that encode them are italicized.

Feklistov and Darst [28], the recognition of the -10 element sequence by domain σ_2 of σ^{70} nucleates promoter opening as the bases of the non-template strand are extruded from the DNA double-helix and captured by σ. This complex undergoes further large-scale conformational changes to form the "open" promoter complex (RP_O), in which a "transcription bubble" is formed by locally melting 12–14 nucleotides, approximately positions -12 to $+1$, of promoter DNA, and if ribonucleotides are present, RNA synthesis begins. Initially, the RNAP initiates several rounds of abortive nascent RNA synthesis resulting in short 2- to 9-nucleotide-long transcripts. However, once the nascent RNA reaches ~12 nucleotides in length, the RNAP undergoes a further set of conformational changes and forms the "transcription elongation complex," in which one nucleotide at a time is added to the growing RNA transcript until a transcription termination signal is reached, where RNA synthesis is stopped either in a factor (Rho)-dependent or -independent manner and RNAP core molecules become released and recycled into the transcription cycle again [29,30]. HAFs have evolved different strategies to directly interfere with host RNAP activity at different phases of the transcription cycle (Fig. 2), and their mechanisms of action (where known) are provided in Table 1.

Modulation of *E. coli* RNAP by a Phage that Entirely Depends on the Host Transcription Machinery for Transcription of Its Genes

The *E. coli* T4 phage serves as a paradigm for understanding how the bacterial RNAP is modulated by HAFs by a phage, which entirely depends on the host RNAP for transcription of its genes [57]. The T4 genome is 168,903 bp in length and encodes around 300 genes. Its double-stranded DNA contains 5-glucosylated and hydroxy-methylated cytosines, which protects it from degradation by host and T4 nucleases [57]. The entire infection cycle of T4, from infection to host lysis, takes around 25 min under standard laboratory conditions. T4 gene expression is temporally regulated during infection and genes are categorized into early, middle, and late genes. Since T4 does *not* encode its own RNAP, transcription of its genes is *entirely* dependent on the *E. coli* RNAP, and this is orchestrated by HAFs that alter the specificity and activity of the bacterial RNAP as the infection proceeds. The mechanisms of action of T4 HAFs that interact with the host RNAP are well understood at the molecular level. In this section, we provide a concise overview of T4 HAFs involved in the temporal modulation of the host RNAP. We refer

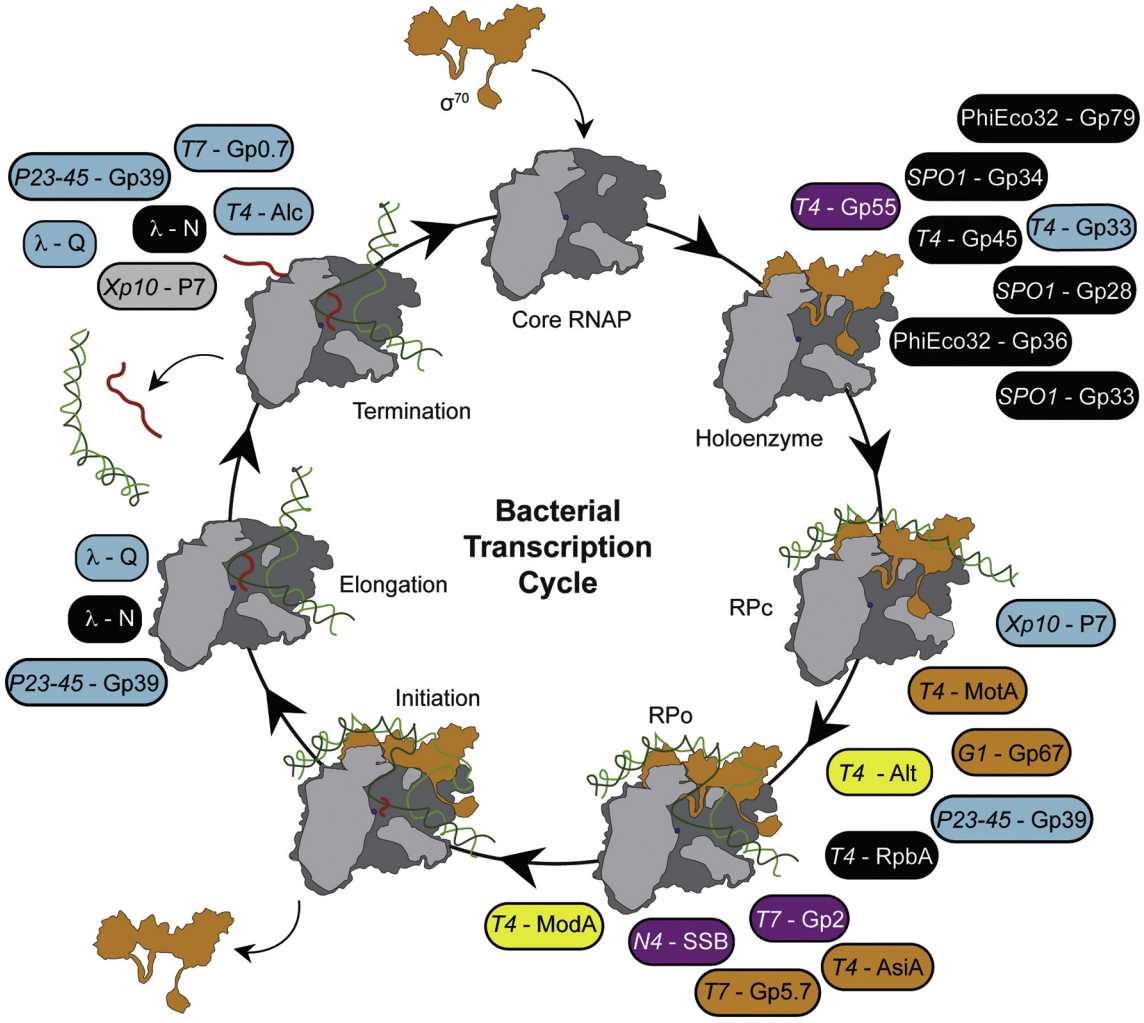

Fig. 2. A schematic representation of the bacterial transcription cycle involving the RNAP containing σ^{70}. The core RNAP subunits are shown in gray, the active site as a blue dot, and the σ^{70} in orange. The HAFs that affect RNAP activity at the different phase of the transcription cycle are indicated and color coded according to the RNAP subunit they interact with as in Fig. 1. Shown in black are HAFs for which the interaction site on the RNAP is unknown. See Table 1 for a summary of their mechanism of action (where known).

readers wishing to learn more about the molecular and structural basis of the mechanism of action of these T4 HAFs to work from the laboratories of Deborah Hinton (e.g., [38,58]) and Peter Geiduschek (e.g., [42,59,60]) who are pioneers of this subject.

Transcription of early T4 genes

Transcription of T4 early genes occurs upon the translocation of the phage DNA into bacterial cells together with the HAF Alt, a T4 late-gene product, which becomes packaged into progeny virions prior to host lysis (see below). The host RNAP, $E\sigma^{70}$, recognizes T4 early promoters, which are σ^{70}-dependent and contain UP, −35, and extended −10 (Fig. 3a). The combination of these elements makes T4 early promoters exceptionally strong allowing them to efficiently compete with host promoters for the limiting number of $E\sigma^{70}$ molecules in the exponentially growing *E. coli* cells [38] (also see later). Alt is an ADP-ribosyltransferase that ADP-ribosylates many host proteins including one of the two α subunits of the RNAP at Arg265. The post-translational modification by Alt increases $E\sigma^{70}$ activity at certain T4 early promoters by approximately 2-fold [35,38]. Since Arg265 is required for efficient interaction with the UP element or certain host transcription activators, it is thought that ribosylation of Arg265 antagonises transcription from UP element-dependent host promoters and/or those that are dependent on interactions with certain transcription activator proteins. In particular, ADP-ribosylation completely prevents UP element-dependent

Table 1. List of host RNAP-interacting HAFs for which mechanism of action is, at least partly, known

Phage	Host	HAF	Size (kDa)	Target on RNAP	Step(s) affected in transcription	Mechanism of action	Reference
T7	*E. coli*	Gp0.7[a]	30	β′	Termination	Phosphorylation of the β′ subunit at Thr^{1068} leads to increased transcription termination by $E\sigma^{70}$	[31]
		Gp2[a]	7	β′	Initiation	Inhibition of RP_O formation by $E\sigma^{70}$	[32,33]
		Gp5.7[a]	7	σ^{38}	Initiation	Inhibiting RP_O formation by $E\sigma^{38}$ at T7 early-gene promoters	[34]
T4	*E. coli*	Alt[a]	76	α	Initiation	ADP-ribosylation of Arg^{265} on one of the α subunits of host RNAP to increase activity at T4 early-gene promoters	[35]
		ModA[a]	23	α	Initiation	ADP-ribosylation of Arg^{265} on both α subunits of host RNAP to decrease activity on the T4 early-gene and host promoters	[35]
		Alc[a]	19	β (postulated)	Termination	Pre-mature transcription termination on dC-containing host DNA	[36]
		AsiA[a]	11	σ^{70}	Initiation	Inhibits transcription from most host promoters *via* appropriation of σ^{70} and increases RNAP activity at T4 middle-gene promoters	[37,38]
		MotA[a]	24	σ^{70}	Initiation	Acts together with AsiA	[39]
		RpbA	15	Unknown	Initiation (postulated)	Might affect stability of RP_O	[40]
		Gp55[a]	21	β′	Initiation	A σ^{70} domain σ_2 mimic which acts together with Gp33 for T4 late-gene transcription	[41,42]
		Gp33[a]	12	β	Initiation	A σ^{70} domain σ_4 mimic which acts together with Gp55 for T4 late-gene transcription	[43]
		Gp45[a]	21	Unknown	Initiation	A sliding clamp protein that interacts with the Gp55 and Gp33 for optimal T4 late-gene transcription	[44]
Xp10	*X. oryzae*	P7	7	β, β′, and ω	Initiation and termination	Dual role: (i) inhibitor of $E\sigma^{70}$ transcription initiation by preventing the formation of RP_C and (ii) acts as an anti-terminator by stabilizing the upstream DNA duplex in the transcription elongation complex	[45,46]
SPO1	*B. subtilis*	Gp28	26	Core	Initiation	Displaces σ^A of *B. subtilis* and directs transcription to the SPO1 middle genes	[47]
		Gp33	11	Unknown	Initiation	An accessory factor that acts together with Gp34 to replace Gp28 and direct transcription of SPO1 late genes	[47]
		Gp34	23	Core	Initiation	Displaces Gp28 together with Gp33 and directs transcription of SPO1 late genes	[47]
P23–45	*T. thermophiles*	Gp39	16	β	Initiation, elongation, and termination	Host RNAP-interacting HAF with multiple roles: (i) inhibits transcription initiation from $E\sigma^A$, (ii) stimulates transcription elongation, and (iii) acts as an anti-terminator, inhibits pausing at poly (U) tracks	[48,49]
N4	*E. coli*	SSB	28	β′	Initiation	Postulated to stabilise RP_O formation	[50]
G1	*S. aureus*	Gp67	23	σ^A	Initiation	Inhibits transcription from host promoters with UP elements by blocking interaction of α-CTD and UP element	[51]
λ	*E. coli*	Q	22	σ^{70} and β	Elongation and termination	Acts as an anti-terminator to stabilise the transcription elongation complex making it unable to respond to termination signals	[52,53]
		N	15	Unknown	Elongation and Termination	Acts as an anti-terminator by (i) increasing transcription rate while reducing RNAP pausing and (ii) inhibiting Rho-dependent and intrinsic transcription termination	[54,55]
PhiEco32	*E. coli*	Gp36	25	Core	Initiation	A phage encoded σ factor that directs transcription of selected PhiEco32 middle and late genes	[56]
		Gp79	9	Unknown	Initiation	Dual role: (i) activator of Gp36-dependent transcription and (ii) inhibitor of $E\sigma^{70}$ at host and early phage promoters	[56]

[a] HAFs that are discussed in detail in the text.

transcription from host ribosomal RNA promoters, which account for up to 80% of transcription in exponentially growing cells (our unpublished data). Arg265 ribosylation also weakens $E\sigma^{70}$ activity from T4 early promoters, but it is possible that the presence of the "extended −10 element" in T4 early promoters

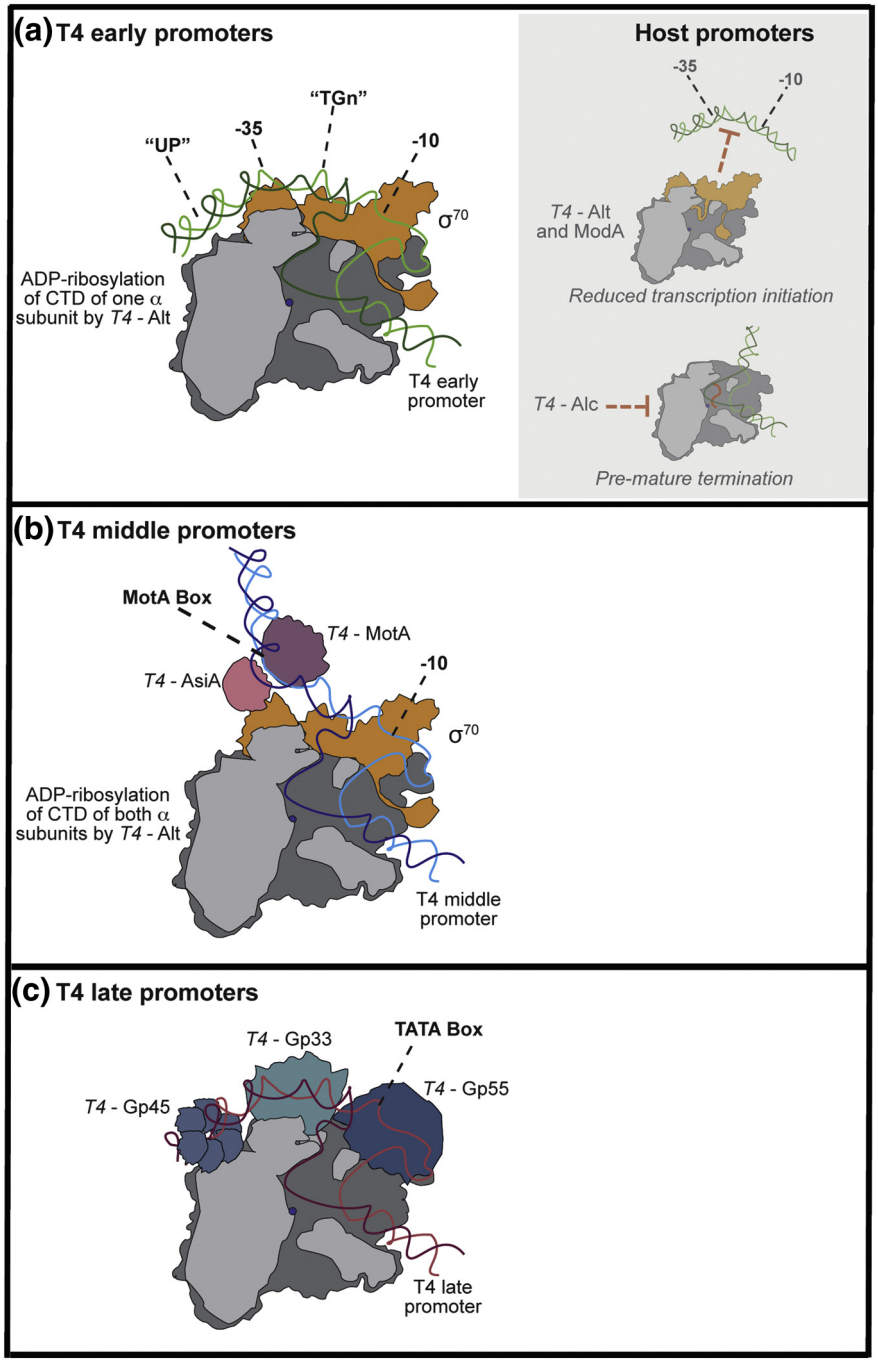

Fig. 3. Modulation of the host RNAP by the T4 phage. A schematic representation of the how the *E. coli* RNAP becomes modulated by HAFs that facilitate temporally coordinated transcription of T4 (a) early, (b) middle, and (c) late genes.

compensates for any undesired impairment of $E\sigma^{70}$ activity due to the action of Alt.

Another T4 HAF that facilitates preferential T4 early-gene transcription over host transcription is Alc, a product of an early gene. Alc terminates, *via* an as yet unknown mechanism, host transcription of DNA that contains unmodified cytosine, unlike that of T4, by interacting with a "β-lobe" domain of the β subunit [61]. Transcription of modified phage DNA is unaffected (Fig. 3a) [36]. Thus, the action of Alc is likely to increase the intracellular pool of "free" $E\sigma^{70}$ available for T4 early-gene transcription. Finally, another ADP-ribosyltransferase, ModA, also a T4 early-gene product, ribosylates both α subunits of the host RNAP at Arg265 (Fig. 3a). This results in further reduction of $E\sigma^{70}$ activity at both UP element-

and factor-dependent host promoters and at T4 early promoters. This prepares the host transcription machinery for T4 middle-gene transcription [35,38].

Transcription of middle T4 genes

The switch from T4 early to middle-gene transcription occurs within 1 min of infection [38]. T4 middle-gene promoters contain the σ^{70}-dependent -10 element and a conserved motif, called the MotA box (5′-(t/a)(t/a)(t/a)tGCTt(c/t)a-3′), which is centered at -30 bp upstream of the transcription start site instead of the canonical -35 element of σ^{70}-dependent promoters (Fig. 3b) [62,63]. Transcription initiation from T4 middle promoters requires HAFs AsiA and MotA, which are both early-gene products. AsiA binds to domain σ_4 of free σ^{70}. The binding of AsiA causes conformational changes in domain σ_4, which prevents it from efficiently interacting with the β-flap domain and, consequently, the -35 element of σ^{70}-dependent promoters [37,38]. However, AsiA-induced restructuring of $E\sigma^{70}$ creates an interaction surface for MotA on σ^{70} and thereby enables MotA to interact with the MotA box [64]: The amino terminal domain of MotA interacts with the $E\sigma^{70}$–AsiA complex *via* helix 5 (H5) of σ^{70} and its carboxyl terminal domain interacts with the MotA box [38,39]. The $E\sigma^{70}$–AsiA–MotA complex still maintains interactions with the -10 and extended -10 element, while interactions of α subunit with UP element are abolished due to the ADP-ribosylation of Arg265 by Alt and ModA (see above). Collectively, this results in the systematic reprogramming of the specificity of $E\sigma^{70}$ in favor of transcription of T4 middle genes (Fig. 3b) [38]. The process by which AsiA and MotA collaborate to change the specificity of $E\sigma^{70}$ by structurally and functionally modulating σ^{70} is referred to as σ appropriation [58]. Since homologues of AsiA and MotA are found in other T4-like phages, it is possible that σ appropriation could be a widespread mechanism used by some phages for the take-over of the host RNAP.

Transcription of late T4 genes

Unlike the T4 early and middle promoters, late T4 promoters lack canonical -35 and -10 elements. Instead, T4 late promoters contain an 8-bp TATA box (5′-TATAAATA-3′) replacing the -10 element, which is recognized by Gp55, a T4 late promoter specificity subunit (Fig. 3c). Gp55 is a distant homologue of σ^{70}, with similarity between the two proteins limited to the domain σ_2 of σ^{70}. Therefore, Gp55 interacts with the same surface of the β′ subunit of the *E. coli* RNAP as σ_2 [41]. The displacement of σ^{70} from the RNAP by Gp55 completely abrogates $E\sigma^{70}$-dependent host transcription. The Gp55–RNAP complex displays weak basal transcription activity from late T4 promoters but requires another HAF, a co-activator called Gp33. Gp33 binds to the β-flap of the *E. coli* RNAP, which is also the attachment site of σ^{70} domain σ_4. However, Gp33 does not share any structural homology with σ_4 and does not bind to DNA [43,65]. Interestingly though, Gp33 alone represses basal transcription of Gp55–RNAP [44]. However, Gp33 is strictly required for activated late T4 transcription because it interacts with T4 sliding clamp protein Gp45 (Fig. 3c). Although the precise mechanism by which Gp45 functions is beyond the scope of this review and discussed elsewhere [66], the binding of Gp45 to the Gp33–Gp55–RNAP increases transcription from T4 late promoters by over 1000-fold [42,44] and couples T4 late transcription with phage DNA replication.

In summary, the modulation of *E. coli* RNAP activity by T4 HAFs involves three major themes: (i) post-transcriptional modification of the α subunit, (ii) appropriation of σ^{70}, and (iii) σ^{70} factor replacement by two T4 proteins, which can interact with the host RNAP as a "split" σ factor. The strategies employed by T4 allow for complete takeover of the host transcription machinery for phage transcriptional requirements in just 1 min (under standard laboratory conditions) following infection. Hence, the efficiency and efficacy by which T4 HAFs act could also be applicable to HAFs made by many other phages that, like T4, entirely depend on their hosts' RNAP for the transcription of their genes.

Modulation of *E. coli* RNAP by a Phage that Depends on the Host's and a Phage-Encoded RNAP for the Transcription of Its Genes

T7 is an *E. coli* lytic phage. The T7 genome is much smaller than that of T4 and consists of 39,937 bp of linear double-stranded DNA. Like in the T4 phage, the expression of T7 genes is temporally coordinated, and the genes are designated as early, middle, and late based on the timing of their expression during the infection. However, unlike the T4 phage, the T7 phage depends on both the bacterial and a phage-encoded single-subunit RNAP for transcription of its genes. The T7 RNAP is a 98-kDa single-subunit enzyme that recognizes a specific promoter sequence (5′-TAATACGACTCACTATAG-3′) that is distinct from bacterial promoters.

The expression of the T7 genome proceeds uniformly from left to right (Fig. 4a, top panel). Early and middle T7 genes mainly encode proteins required for phage DNA transcription and replication and HAFs (including ones involved in the shut-off of the *E. coli* RNAP), whereas late T7 genes encode structural components for progeny T7 particle

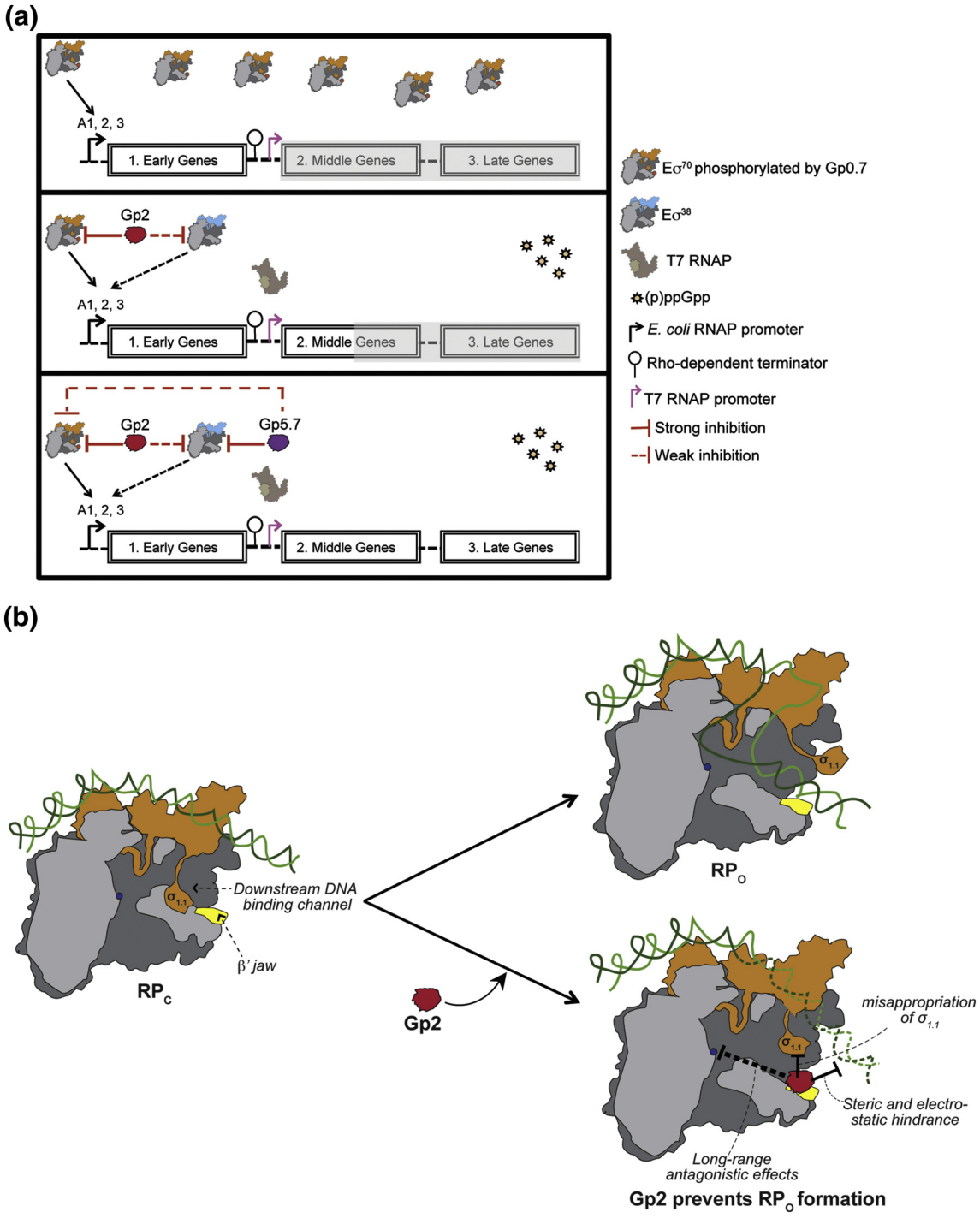

Fig. 4. Inhibition of the host RNAP by the T7 phage in exponentially growing *E. coli*. (a) A schematic representation of the steps involved in the inhibition of the host RNAP during T7 development in exponentially growing *E. coli* cells. (b) Cartoon representation summarizing how Gp2 inhibits $E\sigma^{70}$ RP_O formation.

formation. After adsorption of T7 phage on to the *E. coli* cell, an 850-bp-long segment of the left end of the T7 genome initially enters the bacterial cell, which contains three strong $E\sigma^{70}$-dependent promoters (T7 A1p, T7 A2p, and T7 A3p) (Fig. 4a, top panel). The translocation of the following ~7000 bp of the T7 genome into the *E. coli* cell occurs concomitantly with the co-directional transcription of early T7 genes and is in fact facilitated by $E\sigma^{70}$ [10,67]. One of the first early T7 genes to be

expressed is *gp1*, which encodes the T7 RNAP. The transcription of the middle and late T7 genes and the concomitant translocation of the remaining T7 genome into the *E. coli* cell are likely to be catalyzed by the T7 RNAP [68] (Fig. 4a, middle panel). Late in the infection process, T7 DNA accumulates in the form of concatemers (molecules that comprise T7 chromosomes joined in a head to tail arrangement). During packaging of progeny virions, the T7 DNA concatemers are cleaved into unit-length genomes that are packaged into virion proheads. Qimron and colleagues [69] posited that the processing of T7 concatemers requires pausing of the T7 RNAP at concatemer junctions to allow for recruitment of T7 processing and packaging factors. Since the T7 RNAP has a 5-fold faster transcription elongation rate than the *E. coli* RNAP [70], any inadequate shut-off of the host RNAP during the transition between early- and middle-gene transcription can lead to the slower moving *E. coli* RNAP entering into regions of T7 genome normally transcribed by the faster phage RNAP and hindering it. This would lead to pausing of T7 RNAP molecules at aberrant sites on the T7 genomes, consequently leading to the premature recruitment of the T7 DNA packaging machinery and the generation of less than unit-size phage genomes—all of which will ultimately result in aborted infection [69]. Therefore, T7 has evolved three different HAFs (Gp0.7, Gp2, and Gp5.7), each with a distinct mechanism of action, to ensure adequate and temporally coordinated shut-off of the host RNAP during T7 development in *E. coli*.

Early T7 gene product Gp0.7

T7 Gp0.7 is a 30-kDa serine/threonine kinase. It is dispensable for T7 development under standard laboratory conditions, but it is essential at elevated temperatures, in nutrient-poor media or when Gp2 (see below) is compromised [69,71]. Over 90 *E. coli* proteins are phosphorylated by Gp0.7, and these include components of the RNAP, translation apparatus (elongation factor G and ribosomal protein S6), and RNA processing enzymes (RNAse III and RNase E), suggesting that Gp0.7 has diverse roles in T7 development [72–74]. The phosphorylation of the *E. coli* RNAP by Gp0.7 occurs on a threonine residue at position 1068 (Thr1068) of the β' subunit. Thr1068 is located in a loop, called the β' I6 loop, that is proximal to the downstream DNA binding channel where the double-stranded DNA downstream of the catalytic center is positioned within the RP_O. However, in the structures of the *E. coli* RNAP (e.g., [75–78]), this region is not in close contact with the DNA, and experimental evidence indicates that phosphorylation at Thr1068 does not affect RNAP functions dependent on interactions with the downstream DNA [31]. However, phosphorylation at β' Thr1068 by Gp0.7 results in increased Rho-dependent transcription termination at specific sites located between the early and middle regions on the T7 genome [31] (Fig. 4a, top panel). It has been proposed that increased termination helps minimize read-through transcription by the *E. coli* RNAP into the region of the T7 genome containing the middle genes and thus decrease interference with T7 RNAP transcription (see above). Although the precise mechanism by which Thr1068 phosphorylation leads to increased Rho-dependent transcription termination remains unknown, it may involve conformational signaling from the β' I6 domain at the downstream face of the RNAP to the RNA exit channel at its upstream face [31].

Early middle T7 gene product Gp2

Gp2 is an essential 7-kDa phage protein encoded by one of the first middle genes transcribed by the T7 RNAP [79]. Gp2 is a potent and specific inhibitor of $E\sigma^{70}$ (but not the *E. coli* RNAP containing alternative σ factors such as σ^{38} [32] or σ^{54} [80]) and the structural and molecular basis underpinning its mechanism of action has been extensively studied by us [32,33,81–86] and reviewed in detail by Sheppard *et al*. [87]. Briefly, the mechanism by which Gp2 inhibits $E\sigma^{70}$ is multifaceted. Gp2 binds to the structurally conserved domain of the β' subunit, called the β' jaw domain (Fig. 4b). The β' jaw domain represents one side of the wall that constitutes the downstream DNA binding channel and makes extensive contacts with the downstream DNA in the RP_O (Fig. 4b). Gp2 interacts with the β' jaw domain in such a manner that a negatively charged strip of amino acids on Gp2 face the downstream DNA binding channel [83]. Thus, the presence of Gp2 on the β' jaw domain sterically and electrostatically hinders the binding of downstream DNA in the downstream DNA binding channel, antagonizing the RP_O formation (Fig. 4b). In the RP_C, a negatively charged subdomain of σ_1 ($\sigma_{1.1}$) occupies the downstream DNA in the downstream DNA binding channel and is displaced by the incoming DNA during RP_O formation [32] (Fig. 4b). In other words, the appropriate displacement of $\sigma_{1.1}$ from the downstream DNA binding channel is obligatory for efficient RP_O formation. In the crystal structure of the Gp2-$E\sigma^{70}$ complex, domain $\sigma_{1.1}$ of σ^{70} is displaced from its normal position and interacts with the β' jaw domain, suggesting that the negatively changed Gp2 electrostatically repulses the negatively changed domain $\sigma_{1.1}$ of σ^{70} and thereby prevents the normal displacement of $\sigma_{1.1}$ during RP_O formation (Fig. 4b) [32,33]. Furthermore, it seems that the binding of Gp2 to the β' jaw domain has long-range antagonistic effects on RNAP–promoter interactions around the RNAP catalytic center [32,84]. Although the mechanism of the latter effect of Gp2 is unknown,

it is possible that it is linked to the interaction between Gp2 and $\sigma_{1.1}$ [32]. Collectively, the misappropriation of $\sigma_{1.1}$ by Gp2 and its physical presence in the downstream DNA binding channel efficiently inhibits RP_O formation at $E\sigma^{70}$-dependent early-gene T7 promoters (T7 A1p, T7 A2p, and T7 A3p). Transcription from some $E\sigma^{70}$-dependent host promoters is also inhibited by Gp2 [88], although this is unlikely to be of any physiological importance for T7 development. Therefore, the role of Gp2 is to facilitate the switch from $E\sigma^{70}$-dependent early T7 gene transcription to T7 RNAP-dependent middle and late T7 gene transcription once T7 RNAP becomes available (Fig. 4a, middle panel). This, alongside the action of Gp0.7 (see above), minimizes anti-terminated transcription (Fig. 4a, top and middle panels). Consistent with this, a T7 Δ*gp2* mutant fails to form normal concatemeric structures during the processing of newly synthesized T7 DNA, which is probably due to interference by anti-terminated $E\sigma^{70}$ activity [79].

Late middle T7 gene product Gp5.7

Gp5.7 is a 7-kDa protein and is one of the last middle genes to be expressed. Tabib-Salazar *et al*. [34] recently discovered that Gp5.7 is an *E. coli* RNAP binding protein, which displayed, relative to Gp2, weak inhibitory activity against $E\sigma^{70}$. Strikingly, and unlike Gp2, the inhibitory activity of Gp5.7 is restricted to $E\sigma^{70}$-dependent T7 early-gene promoters (T7 A1p, T7 A2p, and T7 A3p). Structural studies revealed that Gp5.7 folds as a winged helix–turn–helix motif and interacts with a region partially overlapping the −35 element of $E\sigma^{70}$-dependent T7 A1 promoter [89]. These observations partially explain the promoter specificity of Gp5.7. The following model for the biological role of Gp5.7 (one of the two as it later transpires; see below) was posited by Tabib-Salazar *et al*.: During the course of T7 development in *E. coli*, the host chromosome becomes degraded [90]. The degradation of the host chromosome consequently results in the release of *transcribing* RNAP (which cannot be inhibited by Gp2 since the Gp2 binding site is already occupied by the downstream DNA; see above). This leads to an intracellular surge in "free" RNAP molecules "searching" for promoters to bind to. At this point in the T7 development process, the strong $E\sigma^{70}$-dependent T7 early-gene promoters (T7 A1p, T7 A2p, and T7 A3p) on the progeny T7 genomes become the only available binding targets for these host RNAP molecules. Thus, Tabib-Salazar *et al*. suggest that Gp5.7 could provide a further layer of control to *specifically* inhibit the host RNAP activity on T7 early promoters that have escaped inhibition by Gp2. This model is contingent on intracellular levels of Gp2 being insufficient to cope with the surge in promoter-seeking RNAP molecules. The existence of two inhibitors of the host RNAP that are expressed at different times during the T7 development process in *E. coli* underscores the diversity and complexity of the mechanisms phages like T7 have evolved to temporally inhibit host RNAP activity to ensure optimal infection outcome.

Gp5.7 is a strong inhibitor of $E\sigma^{38}$

Although the initial study by Tabib-Salazar *et al*. [89] provided a plausible reason for why T7 has evolved two different proteins to inhibit the host RNAP, unlike Gp2, Gp5.7 is dispensable for T7 development and is only required for optimal progeny development. This led Tabib-Salazar *et al*. to question whether Gp5.7 has an additional role during T7 development in *E. coli*. Previous work by Friesen and Fill [91] noted the accumulation of the stress-signaling nucleotide guanosine pentaphosphate, (p)ppGpp, in *E. coli* in response to T7 infection. Although the host RNAP is a major target of (p)ppGpp, *in vitro*, (p)ppGpp has little effect on $E\sigma^{70}$ activity on the T7 A1 promoter [92]. However, one of the major consequences of (p)ppGpp accumulation is that it directly contributes to the accumulation of σ^{38} (the primary σ active in stationary phase *E. coli* cells), which can initiate transcription from T7 A1 promoter as efficiently as $E\sigma^{70}$ [34] but, unlike the latter, is poorly inhibited by Gp2 (Fig. 4a, middle panel) [32]. Thus, transcription initiation by $E\sigma^{38}$ from T7 early promoters can have, as detailed above, detrimental consequences on T7 development. However, Gp5.7 is a more potent inhibitor of $E\sigma^{38}$ than $E\sigma^{70}$ (Fig. 4a, bottom panel). The mechanism of $E\sigma^{38}$ inhibition by Gp5.7, although not fully understood, may involve interfering with RP_O formation through misappropriation of σ^{38} domain σ_4. Thus, an additional role of Gp5.7 appears to be the inhibition of $E\sigma^{38}$, which collaterally accumulates during T7 infection in *E. coli* due to the accumulation of (p)ppGpp. These new observations raise important new questions: Does (p)ppGpp bind to T7 phage proteins and have direct effect on T7 physiology? Does T7 phage require $E\sigma^{38}$-dependent gene expression for development in *E. coli*, which, when no longer needed, is inhibited by Gp5.7?

Host RNAP Inhibition in Nutrient-Starved Bacteria

Conditions that sustain constant bacterial growth are seldom found in nature. Hence, many bacteria spend the majority of their time starved of essential nutrients and are growth attenuated. This is widely considered as a relevant physiological state. The adaptive transcriptional response to nutrient-starvation in *E. coli* involves alternative σ factors, notably σ^{38}, to express genes to cope with nutrient limitation. How phages such as T4 and T7 develop inside

nutrient-starved *E. coli*, where $E\sigma^{38}$ is the predominant RNAP, remains poorly understood. The use of stationary phase bacteria might be more reflective of how phages modulate host RNAP activity and develop inside their bacterial host in a more natural physiological context, for example, the nutrient-starved state. For example, the T4 phage, initially thought to only infect exponentially growing *E. coli*, has been shown to successfully infect *E. coli* in the stationary phase of growth [93]. Although the mechanistic details remain unknown, T4 appears to achieve this in two ways: First, T4 enters a "hibernation mode" in which middle- and late-gene expression is halted until the nutrient status improves [93]. Therefore, it is plausible that host RNAP-interacting HAFs responsible for middle-gene transcription (AsiA and MotA) are unable to efficiently repurpose $E\sigma^{38}$, which is likely to be the predominant form of the RNAP in stationary phase *E. coli*. In support of this view, AsiA is unable to inhibit a chimera of σ^{70}, which contains domain σ^4 of σ^{38} [94]. Second, T4 becomes a "scavenger" and utilizes whatever is left of the host's resources to produce very small quantities of progeny virions [93], consistent with a view that modulation of $E\sigma^{38}$ is likely to be inefficient compared to $E\sigma^{70}$.

Similar to the T4 phage, T7 can also infect and develop in stationary phase *E. coli* [34]. Since $E\sigma^{38}$ would be the predominant form of the RNAP in stationary phase *E. coli*, which is specifically inhibited by Gp5.7, Tabib-Salazar *et al*. speculated that Gp5.7 *also* represents a HAF that allows T7 to efficiently develop in stationary phase *E. coli*. To investigate this, the authors developed the T7 plaque growth (TPG) assay. A phage-plaque is a clearing in a bacterial lawn and plaques form *via* an outward diffusion of progeny virions that prey on surrounding bacteria. The premise of the TPG assay is that a major barrier for plaque growth is the nutrient status of the bacteria on the lawn, which concomitantly become nutrient-starved with the aging of the lawn (Fig. 5). The rate of plaque formation by wild-type T7 and T7 Δ*gp5.7* was initially indistinguishable. However, as the *E. coli* lawn aged, the plaques produced by the wild-type T7 continued to enlarge, but plaque growth by T7 *Δgp5.7* slowed then ceased. This led Tabib-Salazar *et al*. to propose a model shown in Fig. 6. Overall, it seems that Gp5.7, to the best of our knowledge, is the only HAF that is required for optimal phage development inside nutrient-starved bacteria.

Perspectives

"Though she be but little, she is fierce" is a quote from William Shakespeare's *A Midsummer Night's Dream*, which perhaps best describes small phage gene products that specifically and systematically perturb large macromolecular machineries in their bacterial prey for the benefit of the phage. However, considering that at the time of writing there are estimated to be in excess of a million phage gene products, of which many remain functionally uncharacterised. Hence, the HAFs that bind to the host RNAP described in this review, which represent decades of research, are likely to signify the tip of the iceberg. Despite the status of T4 and T7 as model phages of *E. coli*, the biological roles of many gene products of T4 (~144 out of ~300) and T7 (18 out of 57) still remain unknown. Thus, whether T4 and T7 encode any more HAFs to modulate the host RNAP remains to be discovered. However, the recent observations with Gp5.7 indicate that more RNAP-interacting HAFs might be encoded by these phages that act under specific growth conditions. The

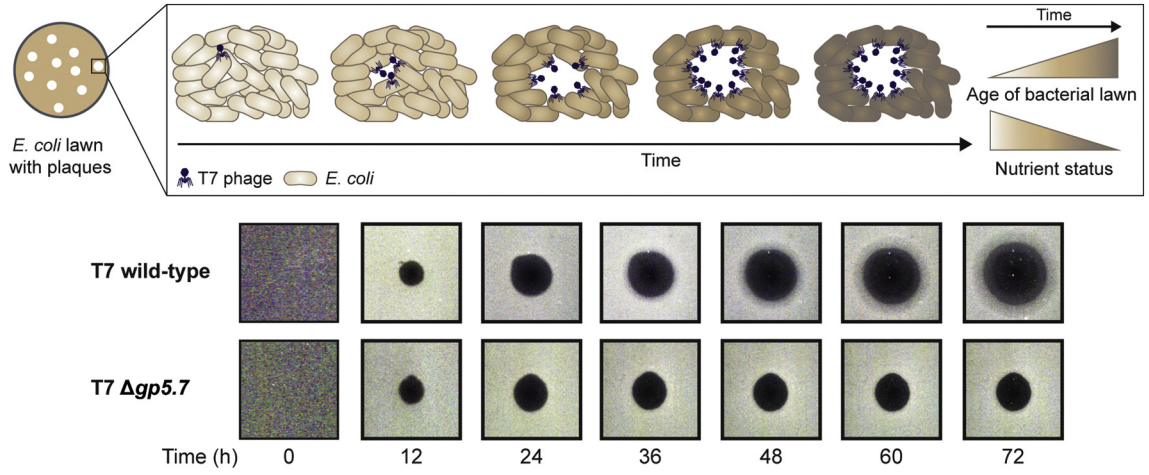

Fig. 5. TPG assay. A schematic description of the basis of the TPG assay showing the ability of T7 wild-type and T7 Δ*gp5.7* phage to develop in aging *E. coli*.

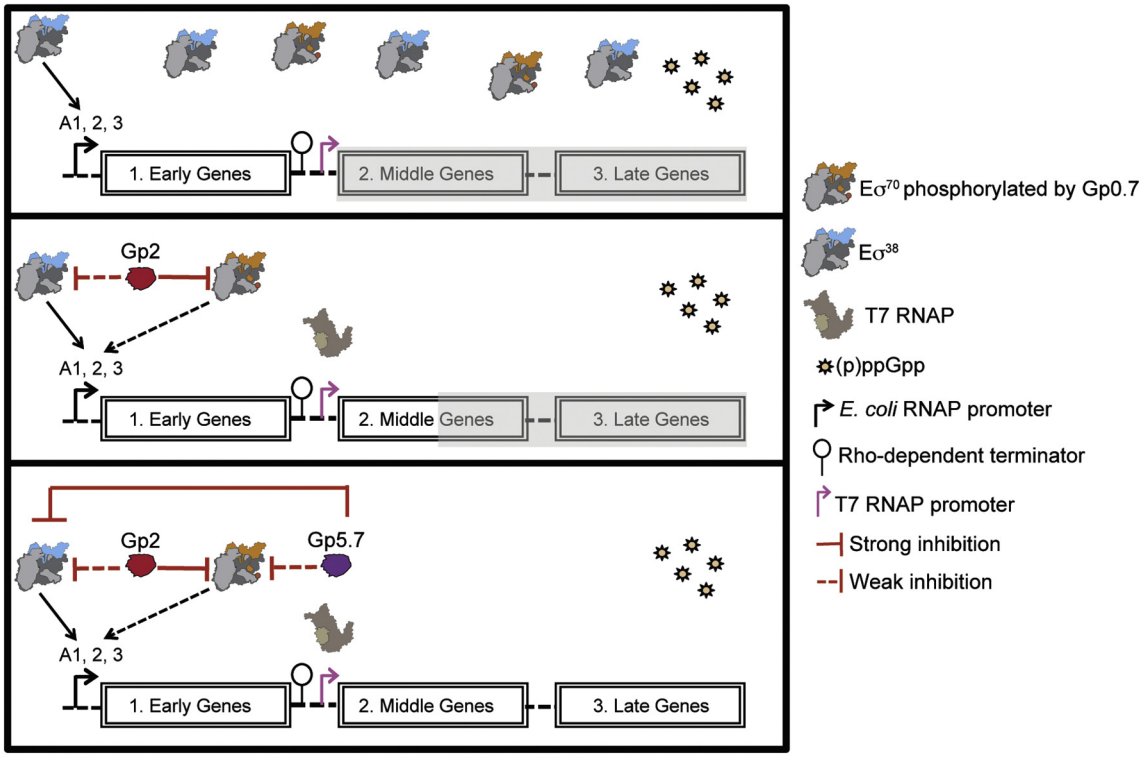

Fig. 6. Inhibition of the host RNAP by the T7 phage in stationary phase *E. coli*. A schematic representation describing the role of Gp5.7 in inhibiting $E\sigma^{38}$, which is the predominant form of the RNAP (compare with Fig. 4).

mechanisms of action of many host RNAP-interacting HAFs identified to date (Figs. 1 and 2) conceptually resemble many transcription regulatory strategies present in bacteria. However, the mechanism of action of several others, including the ones discussed here in detail, are truly xenogeneic in nature. Since HAFs have been selected in the evolutionary arms race between phages and bacteria, host RNAP-interacting HAFs will certainly continue to help uncover paradigm-defining ways by which the bacterial RNAP and thus transcription can be controlled. Furthermore, HAFs, in general, represent one of the largest untapped resources to interrogate bacterial cell function. For example, Sarkar *et al*. [88] used Gp2 as a xenogeneic probe of RNAP function to investigate how *E. coli* would cope with perturbations to the transcriptome under standard laboratory conditions. Strikingly, they discovered that adaptation to conditions that directly perturb bacterial RNAP performance can confer the "adapted" bacterial cells an enhanced ability to tolerate diverse antibacterial stresses. Importantly, the results imply that while synthetic transcriptional rewiring may confer bacteria with the intended desirable properties, such approaches may also collaterally allow them to acquire undesirable traits. Since many host RNAP-interacting HAFs are potent inhibitors of host transcription, their potential to uncover novel "Achilles heels" in the bacterial RNAP—a frontline antibacterial target—should not be underestimated. For HAFs, due to their small size, potency, and multifaceted mode of action, they might just represent the much-needed molecular framework for novel antibiotic discovery. HAFs: they might be small but they are truly mighty.

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Abbreviations used:
HAFs, host acquisition factors; RNAP, RNA polymerase; RP_C, closed promoter complex; RP_O, open promoter complex; TPG, T7 plaque growth.

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