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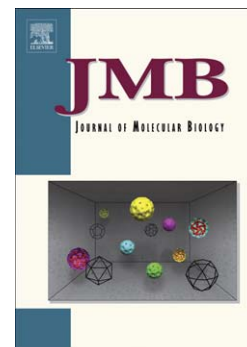
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The Xp10 bacteriophage protein P7 inhibits transcription by the major and major variant forms of the host RNA polymerase via a common mechanism

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Highlights

- Xp10 phage transcription regulator P7 inhibits transcription by RNAP containing σ^{54} .
- P7 prevents the productive engagement of the σ^{54} -RNAP with the promoter DNA.

Abstract

The σ factor is a functionally obligatory subunit of the bacterial transcription machinery, the RNA polymerase. Bacteriophage-encoded small proteins that either modulate or inhibit the bacterial RNAP to allow temporal regulation of bacteriophage gene expression often target the activity of the major bacterial σ factor, σ^{70} . Previously we showed that during *Xanthomonas oryzae* phage Xp10 infection, the phage protein P7 inhibits the host RNAP by preventing the productive engagement with the promoter and simultaneously displacing the σ^{70} factor from the RNAP. In this study, we demonstrate that P7 also inhibits the productive engagement of the bacterial RNAP containing the major variant bacterial σ factor, σ^{54} . The results suggest for the first time that the major variant form of the host RNAP can also be targeted by bacteriophage-encoded transcription regulatory proteins. Since the major and major variant σ factor interacting surfaces in the RNAP substantially overlap, but different regions of σ^{70} and σ^{54} are used for binding to the RNAP, our results further underscore the importance of the σ -RNAP interface in bacterial RNAP function, regulation and potentially for intervention by antibacterials.

Keywords

RNA polymerase, σ factor, bacteriophage, transcription regulation, bacteria.

Central to the regulation of bacterial gene expression is the bacterial RNA polymerase (RNAP), which is a complex multi-subunit enzyme responsible for the transcription of RNA from DNA template. The catalytic ‘core’ of the RNAP is composed of five-subunits $\alpha_2\beta\beta'\omega$ (E), and is reliant upon the binding of a dissociable sigma (σ) factor subunit for ‘holoenzyme’ ($\alpha_2\beta\beta'\omega\sigma$; E σ) formation and promoter specific initiation of transcription (reviewed in ¹). All bacteria have at least one essential major σ factor that serves to transcribe genes required for cell viability, and a varying number of alternate σ factors for the execution of specific transcriptional programs. *Escherichia coli*, for example, encodes six alternate σ factors in addition to the major σ^{70} factor (reviewed in ²). Transcription initiation at a prototypical σ^{70} -dependent housekeeping promoter initially involves the engagement of the E σ^{70} with conserved hexanucleotide sequences of the promoter, which are located at positions -35 and -10 with respect to the transcription initiation site at +1, and results in the formation of a short-lived E σ^{70} -promoter complex (RPc). The isomerization of the RPc to the transcriptionally proficient promoter complex RPo is accompanied by large-scale conformational rearrangements in both the DNA and RNAP, primarily in the β , β' and σ^{70} subunits. In the RPo, the DNA duplex is locally melted and the +1 site on the template strand is positioned at the catalytic centre of the RNAP; the double-stranded DNA downstream of the +1 site is cradled in the downstream DNA binding channel which consists of a trough formed by the β' jaw, β downstream lobe, β' clamp, and β' region G non-conserved domain (GNCD) (reviewed in ³). The different interfaces between σ^{70} factor and the RNAP in the holoenzyme, RPc, RPo and the transition between these

states are extensive, dynamic and functionally specialized⁴⁻⁷. In *E. coli*, all alternate σ factors (except σ^{54}) belong to the major σ^{70} class and share three regions of conserved sequences (regions 2-4, with the exception of extracytoplasmic function (ECF) σ factors which do not contain region 3): Sub-regions 2.4 and 4.2 of regions 2 and 4 of *E. coli* σ^{70} are responsible for recognition of the conserved -10 and -35 double-stranded promoter sequences, respectively^{2,5,6}. In the holoenzyme, sub-region 2.2 of σ^{70} makes extensive contact to the β' clamp helices, which comprises a coiled-coil motif and constitutes the major σ docking site in the RNAP. Region 4 makes extensive interactions with β flap domain and conserved features (notably the β' zipper and β' zinc binding domain) in the amino terminal domain of the β' subunit (hereafter called β' NTD)⁷. The interactions between region 4 of σ^{70} and the β and β' subunit are important for the binding of the holoenzyme to conserved -35 promoter sequence and during promoter clearance for the appropriate exiting of the nascent RNA from the RNAP^{5,6,8,9}.

Regulating the activity of the RNAP is a key mechanism in controlling gene expression and is often orchestrated by transcription regulators that interact with the RNAP to modulate its activity. Therefore, the RNAP often serves as a nexus for interaction of transcription regulators to fine-tune gene expression to match cellular requirements. Unsurprisingly, some bacteriophages (phages) have evolved strategies to alter the activity of host RNAP during infection to allow the temporal and coordinated usage of the host and phage RNAP for phage gene expression¹⁰. This modulation can occur in two ways, either through covalent modifications, such as phosphorylation or ADP ribosylation, of target sites on the RNAP, or through the binding of low-molecular weight phage-encoded proteins¹¹. Many phage-encoded host transcription regulators interfere with host RNAP activity by modulating the σ

factor-RNAP interface during transcription initiation. For example, the T7 phage protein Gp2 binds in the downstream DNA binding channel and prevents the obligatory displacement of the amino-terminal domain of σ^{70} from the downstream DNA binding channel to allow RPo formation^{12,13}. The T4 phage protein AsiA binds to the region 4 of σ^{70} and structurally remodels it¹⁴. Consequently, σ^{70} region 4 can no longer bind to the conserved -35 promoter sequence of host promoters and to the β flap domain of the RNAP. This, in turn, allows another T4 protein, MotA, to interact with the far carboxyl terminal region of σ^{70} and divert the host RNAP from host promoters to T4 phage middle gene promoters, which do not contain conserved -35 promoter elements¹⁵. Recently, we demonstrated that a protein called P7, which is expressed by the *Xanthomonas oryzae* infecting Xp10 phage, inhibits the host RNAP by causing the displacement of the σ^{70} during RPc formation¹⁶. The interface between P7 and the RNAP is complex and involves three different subunits: P7 first docks onto the β' NTD, and positions itself proximal to the β flap domain. Subsequently, a new interaction surface is unveiled on P7 that interfaces with the tip helix of the β flap thereby altering the interface between σ^{70} region 4 and the β flap. Thus, upon engagement with the promoter DNA, the σ^{70} factor becomes displaced from the RNAP, which consequently prevents the formation of the RPc^{16,17}. P7 also interacts with the ω subunit of the host RNAP; however, this interaction seems to be dispensable for its role as a transcription initiation inhibitor¹⁸.

σ^{54} , which is present in many bacterial species, is the major variant bacterial σ factor and is unrelated to the σ^{70} family in sequence, structure, function and regulation (reviewed in^{19,20}). Contrasting the scenario at prototypical σ^{70} -dependent promoters, at σ^{54} -dependent promoters, the E σ^{54} forms an RPc that requires conformational remodelling by a specialized type of activator ATPase for conversion into a

transcriptionally-proficient RPo. The comparison of the $E\sigma^{70}$ and $E\sigma^{54}$ structures reveal that, overall, both σ factors occupy overlapping positions in the RNAP²¹. In the case of $E\sigma^{70}$, the region 4 of σ^{70} interacts with the β flap and β' NTD domain, respectively. In $E\sigma^{54}$, a region comprising amino acids 120-250, called the 'core binding domain' or CBD, which is obligatory for docking of σ^{54} to the RNAP, makes extensive contacts to the β' NTD and the β flap domain (Fig. 1). In other words, in $E\sigma^{54}$, the P7 and the CBD bind to substantially overlapping surfaces of the RNAP β and β' subunits (Fig. 1) and therefore, in this study, we investigated the effect of P7 on $E\sigma^{54}$ -dependent transcription.

Amino acid (aa) residues 6-9 (NLFN) of the β' subunit of *X. oryzae* RNAP are the major determinants for P7 binding²². Since the *E. coli* RNAP contains different aas at this position (KFLN) and therefore is resistant to inhibition by P7, we previously constructed a P7-sensitive version of the *E. coli* RNAP by replacing aas 6-9 of the *E. coli* β' subunit with the corresponding residues of the *X. oryzae* RNAP to study the effect of P7 on σ^{70} -dependent transcription¹⁶. We conducted an *in vitro* transcription assay using the well-characterised *Sinorhizobium meliloti nifH* promoter and the catalytic domain of the *E. coli* Phage shock protein F (PspF₁₋₂₇₅)²³ to determine the effect of P7 on $^{P7S}E\sigma^{54}$ activity. Results revealed that the amount of the UpGpGpG transcript synthesised from *S. meliloti nifH* promoter by $^{P7S}E\sigma^{54}$ was substantially reduced (by ~80%) in the presence of just equimolar amount of P7 to $^{P7S}E\sigma^{54}$ (Fig. 2a (i), lane 2). A similar effect of P7 on $^{P7S}E\sigma^{54}$ activity was observed in *in vitro* transcription reactions with two different σ^{54} -dependent promoters, *E. coli glnHp2* and *relAp4* promoters (Fig 2a (ii) and (iii), respectively). As expected, control reactions with the $^{WT}E\sigma^{54}$ confirmed that the observed reduction in the activity of $^{P7S}E\sigma^{54}$ at all three σ^{54} -dependent promoters was specific to P7 (Fig 2a (i)-(iii), lanes 5

and 6). We next investigated the step at which P7 exerts its inhibitory effect on transcription initiation by $^{P7S}E\sigma^{54}$ by adding P7 to different steps of the *in vitro* transcription reaction (Fig. 2b, schematic). The results showed that the activity of $^{P7S}E\sigma^{54}$ was reduced by ~90% when ~4-fold molar excess P7 was either added to the core RNAP prior to holoenzyme formation or to the pre-formed holoenzyme prior to RPc formation (Fig. 2b, lanes 2 and 3). However, when P7 was added to the RPc and to the RPo, the inhibitory effect of P7 on $^{P7S}E\sigma^{54}$ was reduced and $^{P7S}E\sigma^{54}$ retained ~40-60% activity compared to the reaction where no P7 was present (Fig. 2b, lanes 4 and 5). Thus, it seems that P7 is able to adversely affect the transcriptional activity of $^{P7S}E\sigma^{54}$ at all stages during transcription initiation with the maximum inhibitory effect exerted prior to RPc formation. In contrast, P7 can fully abolish the activity of $^{P7S}E\sigma^{70}$ on the *lacUV5* at any point prior to RPo formation; but once the RPo is formed, P7 has no detectable effect on the amount of ApApUpU transcript synthesized by $^{P7S}E\sigma^{70}$ from the *lacUV5* promoter (Fig. 2c). We thus considered whether P7 could have any adverse effects on the activity of the activator ATPase *per se*. To rule out this possibility, we conducted a simple EMSA based assay to monitor the ability of the activator ATPase to remodel a σ^{54} -promoter complex (which results in a super-shifted σ^{54} -promoter complex; $ss\sigma^{54}$ - ^{32}P -*nifH* in Fig. 2d) in the presence of P7²⁴. Results shown in Fig. 2d indicate that P7 did not have any detectable adverse effect on the activity of the activator ATPase. Thus, the results so far suggest that, at σ^{54} -dependent promoters, P7 does not interfere with the activity of the activator ATPase, inhibits a step(s) *en route* to RPo, and can still, to a certain degree, interact with and adversely affect the RPo once it has formed.

To identify the mechanism by which P7 inhibits $E\sigma^{54}$ activity, we conducted EMSAs with ^{32}P labeled *nifH* promoter probe to determine if P7, like at σ^{70} -dependent

promoters, inhibits transcription initiation by preventing RPe formation by $E\sigma^{54}$. As shown in Fig. 3a, the wild-type and P7-sensitive core RNAP (in the absence of σ^{54}) migrate as two complexes (C1 and C2) under our conditions (lanes 4 and 13). We note that the C1 complex is more prominent in the reaction containing the wild-type core RNAP than it is in the reaction with P7-sensitive core RNAP and suggest that this possibly indicates conformational differences between the two enzymes. In the presence of σ^{54} , the C1 complex disappeared and a third complex, C3, appeared (Fig. 3a, lanes 6 and 15). However, the C2 complex remains, although to a much lesser extent in the reactions with wild-type RNAP compared to reactions with P7-sensitive RNAP (Fig. 3a, lanes 6 and 15). In the presence of P7, the radioactivity in complex C3 disappeared and we detected the formation of complex C4 (Fig. 3a, lane 16 and 17). As expected, this P7-induced disappearance and formation of C3 and C4, respectively, was not seen in control reactions with the $^{WT}E\sigma^{54}$ (Fig. 3a, lanes 7 and 8).

To determine whether complexes C2-C4 contain σ^{54} , we repeated the EMSAs with ^{32}P labeled *nifH* probe and holoenzymes reconstituted with Alexa488-fluorophore labeled versions of σ^{54} (σ^{54*}) and analyzed the gels by autoradiography and fluorescence imaging (the same reactions were split and electrophoresed on two separate gels run in the same gel tank). The fluorescence image of the gel containing reactions with wild-type RNAP revealed that the C2 complex did not contain σ^{54*} (Fig. 3b, (i), lanes 6 and 6'). Since <5 nM of σ^{54*} (=maximum amount of σ^{54*} that could potentially be in complex C2) is within the detection limit of our fluorimeter, we are confident that C2 is a σ^{54} -free complex. As can be clearly seen in the autoradiographs and fluorescence images of gels containing both the wild-type and P7-sensitive RNAP, complex C3 is composed of the core RNAP, *nifH* probe and σ^{54*}

and thus we consider this complex to be the RPc (Fig. 3b, (i) and (ii), compare lanes 6 and 6'). We note that the RPc migrates at the same position as the $E\sigma^{54*}$ complex (Fig. 3b, (i) and (ii), compare lanes 6, 6' and 9') under our conditions. Since complex C3, i.e. the RPc, is not present in reactions containing P7 (Fig. 3b, (ii), lanes 7 and 8), we conclude that P7 inhibits transcription initiation at σ^{54} -dependent promoters by preventing RPc formation. Control reactions with the wild-type core RNAP, as expected, show that C3 is unaffected by the presence of P7 (Fig. 3b, lanes 7 and 8). We note the presence of a fluorescence band (originating from σ^{54*} ; indicated as complex CX in Fig. 3b, (ii), lanes 7', 8' & 10') on the gel containing the P7-sensitive RNAP migrates at the same position as C3 (=RPc; Fig. 3b, (ii), compare lanes 6 & 6' with 7', 8' & 10') and since P7 inhibits RPc formation (see above) and the RPc and $E\sigma^{54*}$ complexes co-migrate at the same position under our conditions (see above), we propose that the slower migrating fluorescent band (= complex CX) seen in lanes 7', 8' & 10' could be the $E\sigma^{54*}$ and/or $E\sigma^{54*}$ -P7 complexes (see below). Importantly, we clearly observe that σ^{54} is not present in complex C4 (Fig. 3b, (ii), compare lanes 7 and 8 with 7' and 8').

We next conducted EMSAs with ^{32}P labeled *nifH* probe and Alexa488-fluorophore labeled P7 (P7*) to determine if P7 is present in the various complexes seen in Fig. 3a and 3b. Results shown in Fig. 4a clearly indicated that P7 is present in complex C4 (compare lanes 6 and 7 with 6' and 7') (whereas σ^{54} is not (Fig. 3b, (ii); see above)). The results also revealed that P7 was present in complex CX seen in Fig. 3b, (ii) (lanes 7' 8' and 10'), which confirms that neither a ternary complex consisting of core RNAP, σ^{54} , *nifH* probe, *ipso facto*, the RPc, nor a quaternary complex consisting of core RNAP, σ^{54} , *nifH* probe and P7 can exist in the presence of P7 and thus P7 inhibits transcription at σ^{54} -dependent promoters by inhibiting RPc formation.

However, it seems that P7 does not detectably affect the stability of the $E\sigma^{54}$ as a ternary complex consisting of RNAP, σ^{54} and P7 can clearly exist (compare Fig. 3b, (ii), lanes 9' and 10' and Fig. 4a, lanes 9' and 10'). We also note that P7 is present in complex C5, which indicates that this complex contains the core RNAP, *nifH* probe and P7 (Fig. 3a, lane 14 and Fig. 4a, lane 5 and 5'). Thus, it seems that although complexes C4 and C5 consist of the same three components (core RNAP, *nifH* probe and P7) they clearly seem to assume different conformations. Put simply, the RNAP-*nifH*-probe-P7 (=C4) complex that forms as a result of P7 action during RPc formation appears to be conformationally different to the ternary RNAP-*nifH*-probe-P7 complex (=C5) that forms in the absence of any DNA. Finally, we conducted EMSAs with ^{32}P labeled *nifH* probe and P7* to determine if P7 can disrupt pre-formed RPc (in other words, P7 was added to the RPc (=complex C3) prior to separation of the complexes on the native gel). Results in Fig. 4b show that P7 can disrupt the preformed RPc (lane 4) as efficiently as when added prior to RPc formation (lanes 2 and 3) and causes the formation of complex C4 (compare lanes 2-4 with 2'-4'). Overall, the results strongly indicate that P7 prevents RPc formation and can destabilize preformed RPc. Put simply, like at σ^{70} -dependent promoters¹⁶, in the presence of the promoter DNA, P7 seems to cause the dissociation of σ^{54} from the holoenzyme resulting in the formation of a ternary complex core RNAP-*nifH*-probe-P7 complex (=C4) and the $E\sigma^{54}$ -P7 and/or $E\sigma^{54}$ complexes, but never the RPc (core RNAP- σ^{54} -*nifH*-probe complex) or a complex consisting of core RNAP, σ^{54} , *nifH* probe and P7.

In summary, we conclude that both the major and major variant forms of the bacterial RNAP are inhibited by P7 by a mechanism that involves inhibition of RPc formation. Since the functional homologue of P7 in T7 phage, Gp2, does not inhibit

transcription initiation by $E\sigma^{54}$ ²⁵, this study demonstrates for the first time that phage-encoded transcription regulators can also potentially target the major variant form of the bacterial RNAP. Although the results clearly show that P7 inhibits R_{Pc} formation by $E\sigma^{54}$, the precise mechanism underpinning this process is unknown. We propose that the binding of P7 to the β flap/ β' NTD domains could allosterically affect other parts of the RNAP and σ^{54} associated with promoter recognition and R_{Pc} formation. This view is consistent with the previous finding that a mutant form of $E\sigma^{54}$ reconstituted with a mutant variant of the core RNAP containing a deletion of the β flap tip helix displayed defects at several steps after holoenzyme formation *en route* to the transcriptionally-proficient R_{Po}²⁶. Equally, it is possible that P7 repositions the CBD of σ^{54} , which is obligatory for docking of σ^{54} to the RNAP and makes extensive contacts to the β' NTD and the β flap domain (=P7 binding regions) and thereby indirectly affects promoter DNA binding by the $E\sigma^{54}$. Intriguingly, whereas P7 has no detectable effect on $E\sigma^{70}$ activity after the R_{Po} has formed (Fig. 2c, lane 4), P7 clearly detrimentally affects the activity of the $E\sigma^{54}$ -R_{Po} to some degree (Fig. 2a, lane 5). This observation could possibly indicate conformational differences in the R_{Po} formed by $E\sigma^{70}$ and $E\sigma^{54}$: whereas the P7 interacting regions are accessible for P7 binding in the $E\sigma^{54}$ -R_{Po}, this seems to be not the case in the $E\sigma^{70}$ -R_{Po}.

The transcriptional programme of the Xp10 phage clearly relies on the coordinated activity of both the host and Xp10 RNAPs. During early stages of infection, Xp10 relies on the *X. oryzae* $E\sigma^{70}$ because several σ^{70} -dependent promoters drive the transcription of early Xp10 genes. The host RNAP becomes dispensable for the transcription of late Xp10 genes and P7 facilitates the switching between the host and phage RNAP^{27, 28}. The results presented here, although derived from using an altered version of the *E. coli* RNAP in which the amino acid (aa) residues 6-9 (NLFN)

of the β' subunit is substituted with the corresponding residues from the *X. oryzae* β' subunit (=the major determinant for P7 binding), suggests that P7 can inhibit RPC formation by the major and major variant forms of the *X. oryzae* RNAP, thus suggesting that the Xp10 transcription programme might require or involve the inactivation of the host transcription machinery containing σ^{54} . The use of σ^{54} by phages for the execution of their transcriptional programme, although rare, is not unprecedented since the development of the *Pseudomonas aeruginosa* phage YuA is strictly dependent on the host σ^{54} factor²⁹.

Our results further indicate that, regardless of the nature of the σ factor- β flap- β' NTD interface, P7 is able to indiscriminately prevent the productive and efficient engagement of the RNAP with the promoter and thereby underscores the significance of the β flap/ β' NTD domains for bacterial RNAP function and regulation. Since the RNAP is a proven antibacterial target, the σ factor- β flap/ β' NTD interface is potentially an Achilles' heel in the bacterial RNAP for intervention by small molecules to inhibit bacterial transcription.

Figure legends

Figure 1. Structural models of P7 bound to the major and major variant forms of the bacterial RNAP. (*above*) Schematic representation of the domain organisations of σ^{70} and σ^{54} . In yellow are domains that are proximal to P7 interacting surfaces on the core RNAP (see text for details). (*below*) Surface representation of the structural models of P7 bound to the *E. coli* σ^{70} and σ^{54} holoenzymes (derived from PDB 4YG2 and PDB 5BYH respectively ^{7, 21}). The β flap, P7 and σ factors are coloured as indicated in the key.

Figure 2. P7 inhibits transcription initiation by the σ^{54} containing RNAP. (a) An autoradiograph of a 20% (w/v) denaturing gel showing synthesis of the transcript by $^{P7S}E\sigma^{54}$ (lanes 1-5) or $^{WT}E\sigma^{54}$ (lanes 7 & 8) in the absence and presence of increasing amount of P7 from the following σ^{54} -dependent promoters: (i) *nifH* (transcript = UpGpGpG), (ii) *glnH* P2 (transcript = UpGpU) and (iii) *relA* P4 (transcript CpUpGpG). All transcripts are indicated by an arrow where the underlined nucleotides are ^{32}P labelled. P7 was added to the reactions prior to holoenzyme formation as is indicated in the schematic. All data obtained in at least 3 independent experiments fell within 5% of the relative %A value shown. (b) An autoradiograph of a 20% (w/v) denaturing gel showing synthesis of the transcript UpGpGpG (indicated by the arrow, where the underlined nucleotides are ^{32}P labelled) from the *nifH* promoter by $^{P7S}E\sigma^{54}$ in the absence and presence of P7. P7 was added to the reaction at different stages, indicated by the numerals I-IV: I = prior to holoenzyme formation, II = prior to R_{Pc} formation, III = prior to R_{Po} formation and IV = after R_{Po} formation. The percentage of UpGpGpG synthesized (%A) indicates the activity of the RNAP in the presence of P7 compared to reactions with no P7 present (lane 1).

All data obtained in at least 3 independent experiments fell within 5% of the relative %A value shown. (c) An autoradiograph of a 20% (w/v) denaturing gel showing synthesis of the transcript ApApUpU (indicated by the arrow, where the underlined nucleotides are ^{32}P -labelled) from the *lacUV5* promoter by $^{70}\text{E}\sigma^{70}$ where P7 was added to the reaction at different stages, indicated by the numerals I-III: I = prior to holoenzyme formation, II = prior to RPc formation and III = after RPo formation. The percentage of ApApUpU synthesized (%A) indicates the activity of the RNAP in the presence of P7 compared to reactions with no P7 present (lane 1). In at least 3 independent experiments all data obtained fell within 5% of the %A value shown. (d) Autoradiograph of 4.5% (w/v) native polyacrylamide gel showing results from an EMSA to determine whether P7 affects the formation of an activation-dependent (here using PspF₁₋₂₇₅ and 4 mM dGTP) remodelled σ^{54} -DNA complex (indicated as super-shifted or ss σ^{54} - ^{32}P -*nifH*). In lanes 5-9, the presence of increasing amounts of P7 had no detectable effect on ss σ^{54} - ^{32}P -*nifH* formation. In (a) to (d) the schematic above autoradiograph images indicates the concentration of reaction components, the point in time they were added to the reactions and the incubation times; the migration positions of protein and DNA components in each lane are indicated and the assays were conducted essentially as previously described. The experiments in (a-d) were conducted as described in ²⁶, ³⁰ and ²⁴ respectively.

Figure 3. P7 prevents RPc formation by the σ^{54} containing RNAP. (a) Autoradiograph of a 4.5% (w/v) native polyacrylamide gel showing results from EMSA experiment with ^{32}P -labelled *nifH* promoter probe to demonstrate that P7 inhibits RPc formation by the σ^{54} holoenzyme conducted as previously described ^{16,30}. The components present in each lane are indicated above each image of the gel and

the schematic indicates the concentration of reaction components, time of addition and incubation time. (b) As in (a) but the assays were conducted with Alexa488-labelled σ^{54} (σ^{54*}) to determine presence or absence of σ^{54} in the different complexes detected in (a) by fluorescence imaging. The Alexa488-labelled version of σ^{54} was prepared as described in ³¹. In (a) and (b), the migration positions of the different protein-protein and protein-DNA complexes are indicated (see text for details). We note that we could not clearly distinguish the free σ^{54*} and σ^{54*} -³²P-*nifH* complex in the gels shown on the right in Fig. 3b. We explain this by suggesting that the excess of free σ^{54*} (800 nM) may mask the amount of σ^{54*} -³²P-*nifH* complexes formed (maximum 10 nM) under our experimental conditions. The gels analysed by radiography were dried prior to exposure to the phosphorimaging plate, whilst gels analysed by fluorescence were not dried.

Figure 4. P7 inhibits R_{Pc} formation by the σ^{54} containing RNAP, but does not fully dissociate σ^{54} -RNAP holoenzyme. Autoradiograph and fluorescent image of a 4.5% (w/v) native polyacrylamide gel showing results from EMSA experiment with ³²P-labelled *nifH* promoter probe to demonstrate that P7 inhibits R_{Pc} formation by the σ^{54} holoenzyme conducted as previously described ^{16, 30}. (a) and (b) essentially completed as in Fig. 3a. but the assays were conducted with Alexa488-labelled P7 (P7*) to determine presence or absence of P7 in the different complexes detected in Fig 3a. The Alexa488-labelled version of P7 was prepared as described in ³¹. The components present in each lane are indicated above each image of the gel and the schematic indicates the concentration of reaction components, time of addition and incubation time. In (a) and (b), the migration positions of the different protein-protein

and protein-DNA complexes are indicated (see text for details). Note that gels analysed by radiography were dried prior to exposure to the phosphorimaging plate, whilst gels analysed by fluorescence were not dried.

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References

1. Haugen SP, Ross W, Gourse RL. (2008). Advances in bacterial promoter recognition and its control by factors that do not bind DNA. *Nat Rev Microbiol.* 6, 507-19.
2. Paget MS. (2015). Bacterial Sigma Factors and Anti-Sigma Factors: Structure, Function and Distribution. *Biomolecules.* 5, 1245-65.
3. Saecker RM, Record MT, Jr., Dehaseth PL. (2011). Mechanism of bacterial transcription initiation: RNA polymerase - promoter binding, isomerization to initiation-competent open complexes, and initiation of RNA synthesis. *J Mol Biol.* 412, 754-71.
4. Gruber TM, Markov D, Sharp MM, Young BA, Lu CZ, Zhong HJ, et al. (2001). Binding of the initiation factor sigma(70) to core RNA polymerase is a multistep process. *Mol Cell.* 8, 21-31.
5. Murakami KS, Masuda S, Campbell EA, Muzzin O, Darst SA. (2002). Structural basis of transcription initiation: an RNA polymerase holoenzyme-DNA complex. *Science.* 296, 1285-90.
6. Murakami KS, Masuda S, Darst SA. (2002). Structural basis of transcription initiation: RNA polymerase holoenzyme at 4 Å resolution. *Science.* 296, 1280-4.
7. Murakami KS. (2013). X-ray crystal structure of Escherichia coli RNA polymerase sigma70 holoenzyme. *J Biol Chem.* 288, 9126-34.
8. Mekler V, Kortkhonjia E, Mukhopadhyay J, Knight J, Revyakin A, Kapanidis AN, et al. (2002). Structural organization of bacterial RNA polymerase holoenzyme and the RNA polymerase-promoter open complex. *Cell.* 108, 599-614.
9. Kuznedelov K, Minakhin L, Niedziela-Majka A, Dove SL, Rogulja D, Nickels BE, et al. (2002). A role for interaction of the RNA polymerase flap domain with the sigma subunit in promoter recognition. *Science.* 295, 855-7.
10. Nechaev S, Severinov K. (2008). The elusive object of desire--interactions of bacteriophages and their hosts. *Curr Opin Microbiol.* 11, 186-93.
11. Nechaev S, Severinov K. (2003). Bacteriophage-induced modifications of host RNA polymerase. *Annu Rev Microbiol.* 57, 301-22.
12. James E, Liu M, Sheppard C, Mekler V, Camara B, Liu B, et al. (2012). Structural and mechanistic basis for the inhibition of Escherichia coli RNA polymerase by T7 Gp2. *Mol Cell.* 47, 755-66.
13. Bae B, Davis E, Brown D, Campbell EA, Wigneshweraraj S, Darst SA. (2013). Phage T7 Gp2 inhibition of Escherichia coli RNA polymerase involves misappropriation of sigma70 domain 1.1. *Proc Natl Acad Sci U S A.* 110, 19772-7.

14. Baxter K, Lee J, Minakhin L, Severinov K, Hinton DM. (2006). Mutational analysis of sigma70 region 4 needed for appropriation by the bacteriophage T4 transcription factors AsiA and MotA. *J Mol Biol.* 363, 931-44.
15. Hinton DM, Pande S, Wais N, Johnson XB, Vuthoori M, Makela A, et al. (2005). Transcriptional takeover by sigma appropriation: remodelling of the sigma70 subunit of Escherichia coli RNA polymerase by the bacteriophage T4 activator MotA and co-activator AsiA. *Microbiology.* 151, 1729-40.
16. Liu B, Shadrin A, Sheppard C, Mekler V, Xu Y, Severinov K, et al. (2014). A bacteriophage transcription regulator inhibits bacterial transcription initiation by sigma-factor displacement. *Nucleic Acids Res.* 42, 4294-305.
17. Liu B, Shadrin A, Sheppard C, Mekler V, Xu Y, Severinov K, et al. (2014). The sabotage of the bacterial transcription machinery by a small bacteriophage protein. *Bacteriophage.* 4, e28520.
18. Esyunina D, Klimuk E, Severinov K, Kulbachinskiy A. (2015). Distinct pathways of RNA polymerase regulation by a phage-encoded factor. *Proc Natl Acad Sci U S A.* 112, 2017-22.
19. Wigneshweraraj S, Bose D, Burrows PC, Joly N, Schumacher J, Rappas M, et al. (2008). Modus operandi of the bacterial RNA polymerase containing the sigma54 promoter-specificity factor. *Mol Microbiol.* 68, 538-46.
20. Zhang N, Buck M. (2015). A perspective on the enhancer dependent bacterial RNA polymerase. *Biomolecules.* 5, 1012-9.
21. Yang Y, Darbari VC, Zhang N, Lu D, Glyde R, Wang YP, et al. (2015). TRANSCRIPTION. Structures of the RNA polymerase-sigma54 reveal new and conserved regulatory strategies. *Science.* 349, 882-5.
22. Yuzenkova Y, Zenkin N, Severinov K. (2008). Mapping of RNA polymerase residues that interact with bacteriophage Xp10 transcription antitermination factor p7. *J Mol Biol.* 375, 29-35.
23. Bordes P, Wigneshweraraj SR, Schumacher J, Zhang X, Chaney M, Buck M. (2003). The ATP hydrolyzing transcription activator phage shock protein F of Escherichia coli: identifying a surface that binds sigma 54. *Proc Natl Acad Sci U S A.* 100, 2278-83.
24. Cannon WV, Gallegos MT, Buck M. (2000). Isomerization of a binary sigma-promoter DNA complex by transcription activators. *Nat Struct Biol.* 7, 594-601.
25. Wigneshweraraj SR, Burrows PC, Nechaev S, Zenkin N, Severinov K, Buck M. (2004). Regulated communication between the upstream face of RNA polymerase and the beta' subunit jaw domain. *EMBO J.* 23, 4264-74.
26. Wigneshweraraj SR, Kuznedelov K, Severinov K, Buck M. (2003). Multiple roles of the RNA polymerase beta subunit flap domain in sigma 54-dependent transcription. *J Biol Chem.* 278, 3455-65.

27. Semenova E, Djordjevic M, Shraiman B, Severinov K. (2005). The tale of two RNA polymerases: transcription profiling and gene expression strategy of bacteriophage Xp10. *Mol Microbiol.* 55, 764-77.
28. Djordjevic M, Semenova E, Shraiman B, Severinov K. (2006). Quantitative analysis of a virulent bacteriophage transcription strategy. *Virology.* 354, 240-51.
29. Ceysens PJ, Mesyanzhinov V, Sykilinda N, Briers Y, Roucourt B, Lavigne R, et al. (2008). The genome and structural proteome of YuA, a new *Pseudomonas aeruginosa* phage resembling M6. *J Bacteriol.* 190, 1429-35.
30. Camara B, Liu M, Reynolds J, Shadrin A, Liu B, Kwok K, et al. (2010). T7 phage protein Gp2 inhibits the *Escherichia coli* RNA polymerase by antagonizing stable DNA strand separation near the transcription start site. *Proc Natl Acad Sci U S A.* 107, 2247-52.
31. Sheppard C, Camara B, Shadrin A, Akulenko N, Liu M, Baldwin G, et al. (2011). Reprint of: inhibition of *Escherichia coli* RNAP by T7 Gp2 protein: role of negatively charged strip of amino acid residues in Gp2. *J Mol Biol.* 412, 832-41.

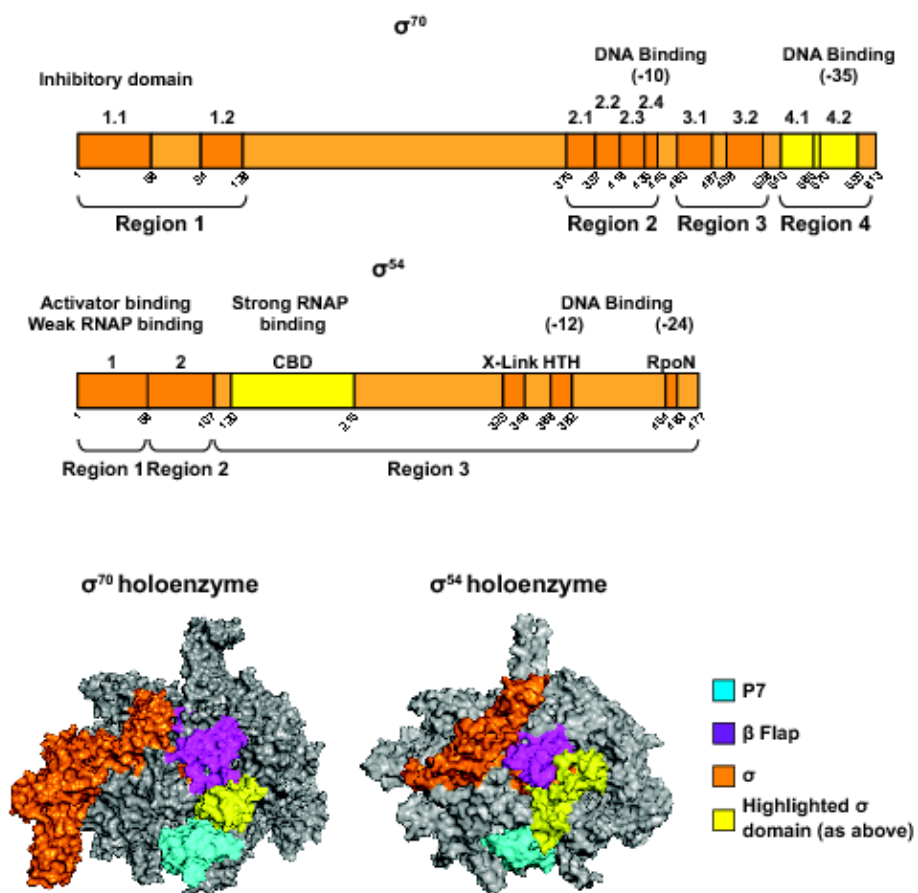


Figure 1

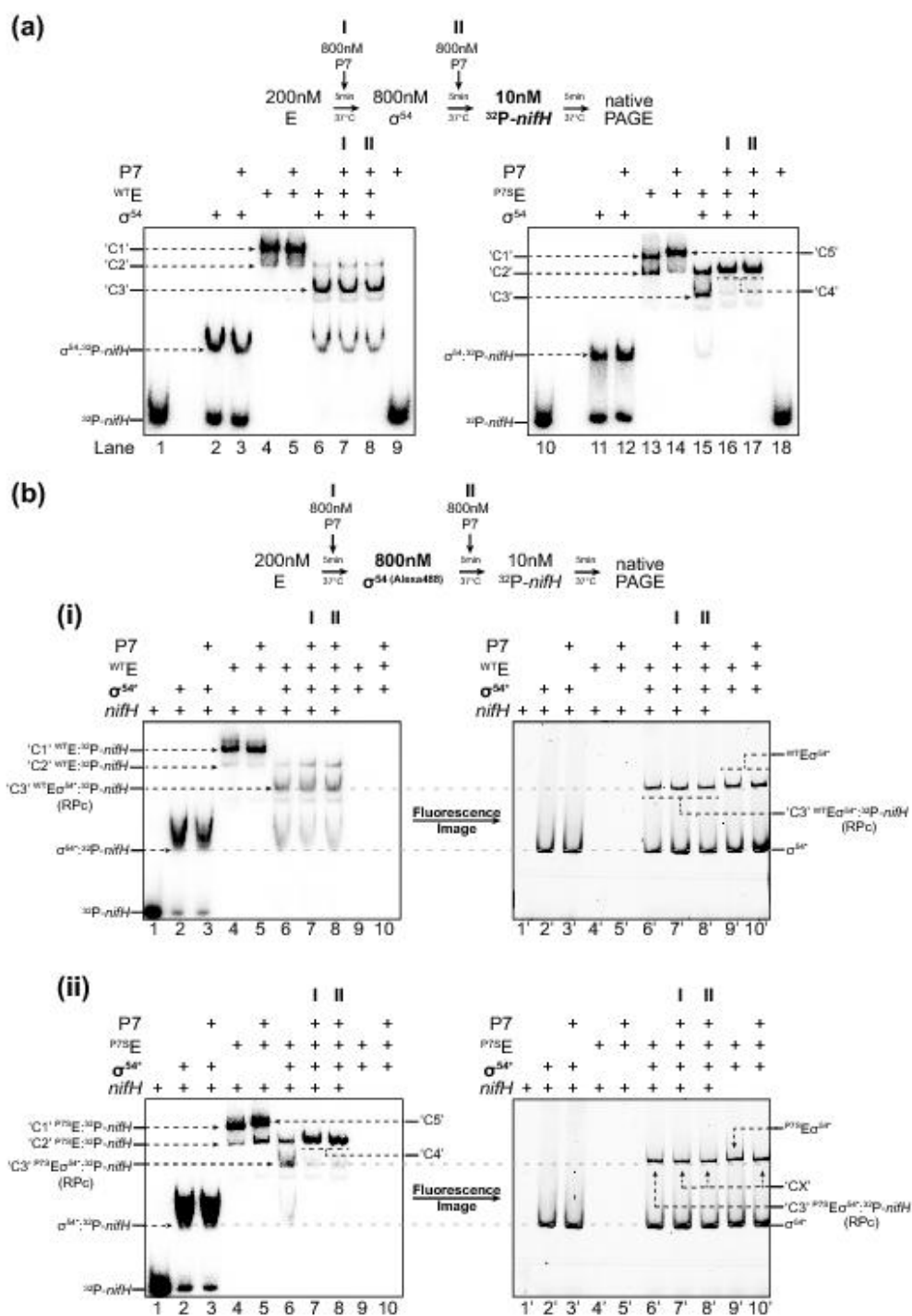


Figure 3

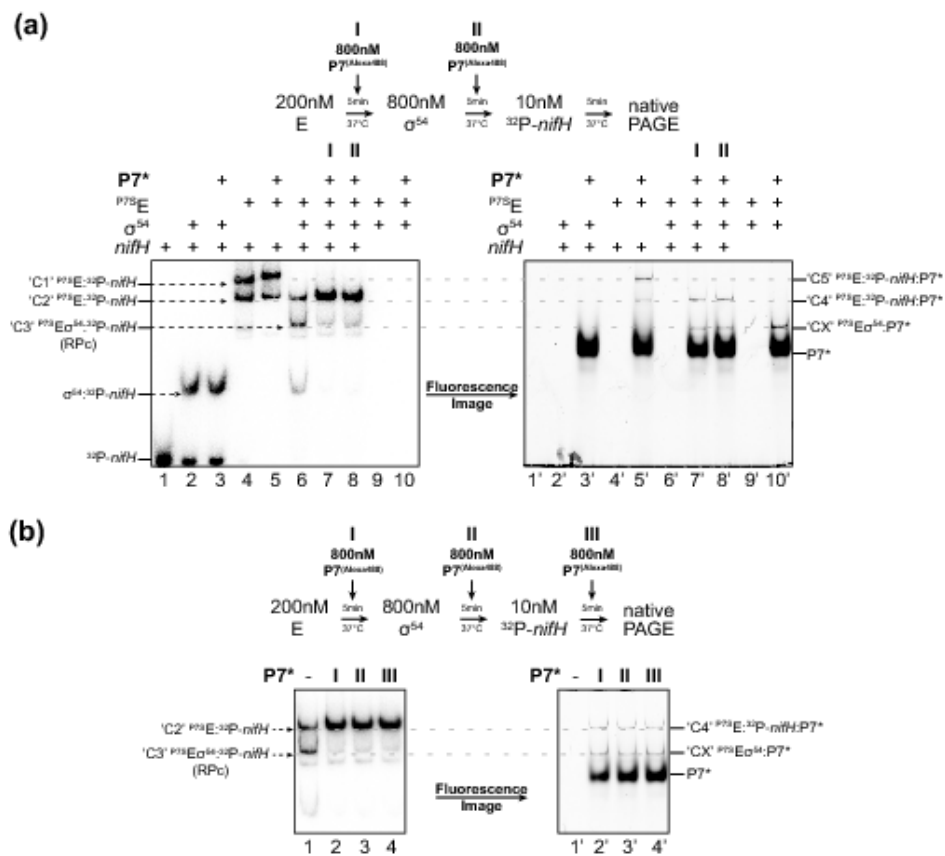
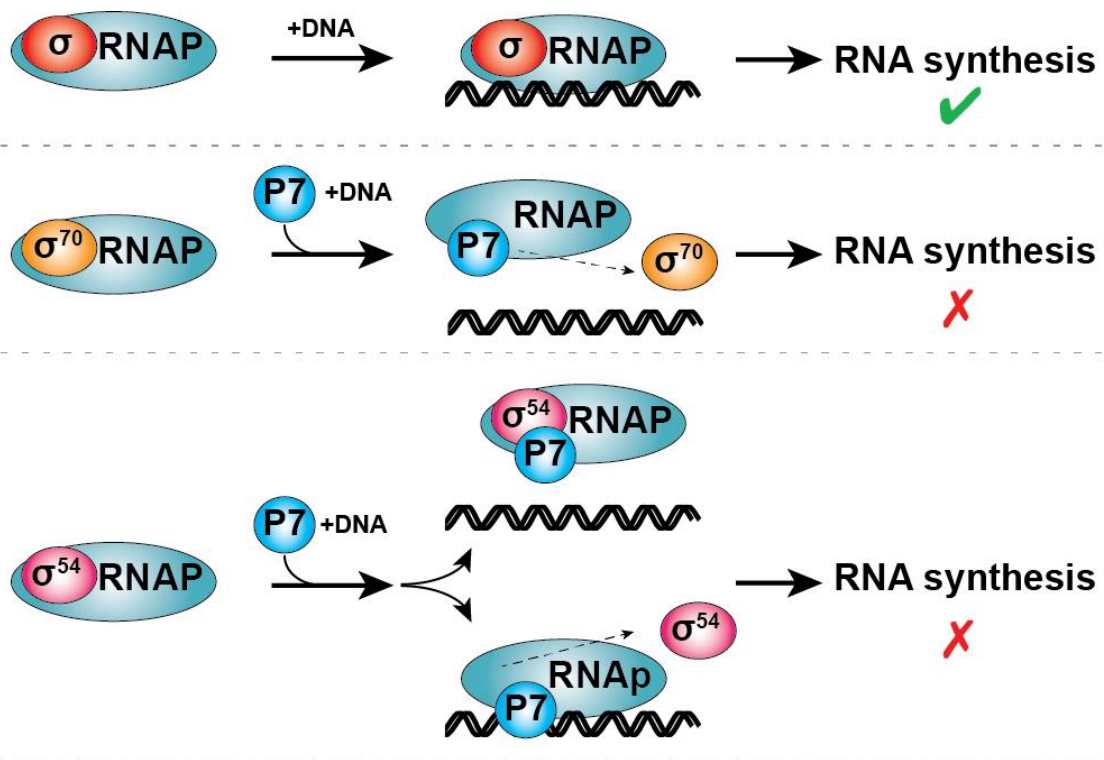


Figure 4



Graphical abstract