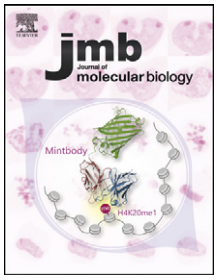

The Xp10 Bacteriophage Protein P7 Inhibits Transcription by the Major and Major Variant Forms of the Host RNA Polymerase via a Common Mechanism

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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 the 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 displaces 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}, with its cognate promoter. 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 and regulation and potentially for intervention by antibacterials.

Central to the regulation of bacterial gene expression is the bacterial RNA polymerase (RNAP), which is a complex multisubunit enzyme responsible for the transcription of RNA from the 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" ($\alpha_2\beta\beta'\omega\sigma$; Eσ) formation and promoter-specific initiation of transcription (reviewed in Ref. [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 Ref. [2]). Transcription initiation at a prototypical σ^{70}-dependent housekeeping promoter initially involves the engagement of the $E\sigma^{70}$ 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 $E\sigma^{70}$–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 the 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, which is downstream of the +1 site, is cradled in the downstream DNA binding channel that consists of a trough formed by the β′ jaw, β downstream lobe, β′ clamp, and β′ region G non-conserved domain (GNCD) (reviewed in Ref. [3]). The different interfaces between the σ^{70} factor and the RNAP in the holoenzyme, RPc, and RPo, and the transition between these states are extensive, dynamic, and functionally specialised [4–7]. 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 that do not contain region 3].

Subregions 2.4 and 4.2 of regions 2 and 4 of *E. coli* σ^{70} are responsible for the recognition of the conserved −10 and −35 double-stranded promoter sequences, respectively [2,5,6]. In the holoenzyme, subregion 2.2 of σ^{70} makes extensive contact to the β′ clamp helices, which comprise of a coiled-coil motif and constitutes the major σ docking site in the RNAP. Region 4 makes extensive interactions with β flap domain and the 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 expression [10]. 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 proteins [11]. 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 it [14]. 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 elements [15]. Recently, we demonstrated that a protein called P7, which is expressed by the *Xanthomonas orzyae* infecting Xp10 phage, inhibits the host RNAP by causing the displacement of the σ^{70} during RPc formation [16]. 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 inhibitor [18].

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 Refs [19,20]). Contrasting the scenario at prototypical σ^{70}-dependent promoters and at σ^{54}-dependent promoters, the $E\sigma^{54}$ forms an RPc that requires conformational remodelling by a specialised type of activator ATPase for conversion into a transcriptionally proficient RPo. The comparison of the $E\sigma^{70}$ and $E\sigma^{54}$ structures reveals that, overall, both σ factors occupy overlapping positions in the RNAP [21]. 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" (CBD), which is obligatory for the 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.

Residues 6–9 Asparagine, Leucine, Phenylalanine, Asparagine (NLFN) of the β′ subunit of *X. oryzae* RNAP are the major determinants for P7 binding [22]. Since the *E. coli* RNAP contains different amino acids at this position Lysine, Phenylalanine, Leucine and Asparagine (KFLN) and is therefore resistant to inhibition by P7, we previously constructed a P7-sensitive version of the *E. coli* RNAP by replacing 6–9 aa 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 transcription [16]. 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 ($PspF_{1-275}$) [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 an 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