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REVIEW ARTICLE

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Control mechanisms of bacteriophage ϕ29 DNA expression

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Summary The phage ϕ29 regulatory protein p4 activates the late promoter A3 by stabilizing the binding of *Bacillus subtilis* RNA polymerase (RNAP) as a closed complex. Interaction between the two proteins occurs through amino acid Arg120 in protein p4 and the C-terminal domain of the RNAP α subunit (α-CTD). In addition to its role as activator of the late transcription, protein p4 represses early transcription from the A2b and A2c promoters, that are divergently transcribed. Binding of p4 to its recognition site at the A3 promoter displaces the RNAP from promoter A2b, both by steric hindrance and by the curvature induced upon p4 binding. At the A2c promoter, the RNAP cooperates with p4 binding in such a way that promoter clearance is prevented. Interestingly, amino acid Arg120 in p4 and the α-CTD in *B. subtilis* RNAP are involved in the interactions that lead to transcription repression at promoter A2c. To investigate how this interaction leads to activation at PA3 and to repression at PA2c, mutant promoters were constructed. In the absence of a –35 consensus box for σ^A-RNAP activation was observed, while in its presence repression occurred. The results support the idea that overstabilization of RNAP at the promoter over a threshold level leads to repression.

Key words Bacteriophage ϕ29 · RNA polymerase · Transcription repression · Transcription activation · Protein regulation

Introduction

The *Bacillus subtilis* phage ϕ29 has a linear, double-stranded DNA 19,285 bp long with a terminal protein covalently linked to the 5' ends that acts as a primer for the initiation of replication [14]. Early ϕ29 genes, located at the two DNA ends, are transcribed from several early promoters that have the consensus –10 and –35 regions and are recognized by the *B. subtilis* RNA polymerase with the major σ^A subunit. Late genes, located at the middle of the genome, are transcribed from a single promoter, A3, that has the –10 consensus sequence but lacks the –35 region, and requires, in addition to the *B. subtilis* σ^A RNA polymerase, the product of the viral early gene 4 [15].

Gene 4 has been cloned and protein p4 was overexpressed and purified. The protein, 125 amino acids long, is a dimer in solution and binds to DNA as a tetramer. It activates the late promoter A3 and represses the early promoters A2b and A2c.

Transcription activation of the late promoter A3

Protein p4 interacts with the late promoter A3 in a region from position –58 to –104 from the transcription start point and recognizes an 8 bp inverted sequence [1, 11]. This DNA region is intrinsically bent and the curvature increases from about 45° to 80° upon p4 binding [13].

RNA polymerase does not bind by itself to the late promoter, but the presence of p4 promotes the binding of RNA polymerase as a closed complex by interaction of both proteins, p4 and RNA polymerase [10]. By deletion mutagenesis we had shown that the carboxy-terminal region of p4 is involved in the interaction [13]. By site-directed mutagenesis at this region we showed that Arg 120 in protein p4 is critical for the interaction with the RNA polymerase [5].

On the other hand, by deletion mutagenesis of the *B. subtilis* α subunit we showed that the 15 carboxy-terminal amino acids are required for the interaction with p4, as shown by gel

retardation assays [3]. We also reconstituted the *B. subtilis* RNA polymerase with the wild-type or deleted α subunits. Transcription assays indicated that the RNA polymerase reconstituted with an α subunit containing a deletion of 15 amino acids at the carboxy-terminal domain (CTD) cannot be activated by protein p4 [3].

Since the *Escherichia coli* RNA polymerase cannot be activated by p4, we asked the question whether the α-CTD of the *B. subtilis* RNA polymerase was enough to provide specificity to the *E. coli* RNA polymerase for activation by protein p4. Thus, we constructed a chimerical α subunit containing the N-terminal domain of the *E. coli* RNA polymerase α subunit and the α-CTD of the *B. subtilis* enzyme. The *E. coli* RNA polymerase was reconstituted with the chimerical α subunit and with either the σ^{70} from *E. coli* or the σ^{A} from *B. subtilis*. The two reconstituted RNA polymerases could be activated by protein p4 at the late A3 promoter to a similar extent as the *B. subtilis* RNA polymerase, whereas the *E. coli* RNA polymerase was inactive. These results indicate that the α-CTD of the *B. subtilis* RNA polymerase is enough to provide specificity to the *E. coli* enzyme for transcription activation by p4 [4].

Transcription repression of the early A2b and A2c promoters

The protein p4 binding site at the late A3 promoter overlaps with the –35 region of the early A2b promoter, which is divergently transcribed. It has been shown that binding of p4 to its recognition site at the A3 promoter displaces the RNA polymerase from the A2b to the A3 promoter, both by steric hindrance and by the curvature induced upon p4 binding, giving rise to transcription repression by protein p4 [12].

Interestingly, upstream from the early A2c promoter, there is a predicted p4 binding site. By methylation interference assays protein p4 was shown to bind to the A2c promoter at guanine residues at positions –26 and –52, whereas in the presence of RNA polymerase, guanines at positions –58 and –84 are the ones recognized by p4. Site-directed mutagenesis was carried out at either of these two sites, named 1 and 2, respectively.

Gel retardation assays showed that, as expected, in the absence of RNA polymerase, mutations at site 2 did not essentially affect p4 binding, whereas mutation at site 1 reduced the p4 binding ability. On the contrary, in the presence of RNA polymerase, mutations at site 2 had a drastic effect, whereas that of mutation at site 1 was small [6].

DNase I footprinting showed that the RNA polymerase binds to the A2c promoter, both in the absence and presence of protein p4. Thus, the inhibitory effect of p4 should be exerted at a step following formation of the closed complex. On the other hand, potassium permanganate footprinting showed that RNA polymerase can efficiently form an open complex at promoter A2c in the absence and presence of p4. Moreover, protein p4 did not significantly reduce the overall amount of abortive transcripts produced at PA2c under conditions in which formation of a run-off transcript was inhibited. In addition, in the presence of the initiating dinucleotide GpU, and ATP and GTP, that allows the RNA polymerase to transcribe up to position +10, the footprint corresponding to the protected area moved from position +20 to +30, both in the absence or presence of protein p4 or its mutant Arg 120 → Gln (R120Q). When the 4 NTPs were supplied in the absence of protein p4, RNA polymerase was not detected at the promoter, indicating the formation of elongation complexes. However, in the presence of p4, RNA polymerase remained bound at the promoter, in agreement with the idea that protein p4 allows the RNA polymerase to advance up to the formation of the initial transcribing complex, but impairs promoter clearance. When the p4 mutant R120Q was used, a significant proportion of the RNA polymerase could leave the promoter. In agreement with the latter result, the positive control mutant R120Q, that is unable to activate the A3 promoter, is also unable to repress the A2c promoter [8].

By gel retardation assays protein p4 was shown to bind to the α subunit at the A2c promoter, but not to the deleted α lacking the 15 C-terminal amino acids. In agreement with the repression results, the p4 mutant R120Q was not able to interact with the α subunit at PA2c. Transcription assays indicated that the RNA polymerase reconstituted with the wild-type α subunit is able to repress the A2c promoter, whereas the enzyme reconstituted with the α deletion derivative lacking the 15 C-terminal amino acids repressed very poorly [9].

Control of ϕ29 DNA transcription

At the beginning of the infection by phage ϕ29, the early promoters A2b and A2c, as well as promoter C2, are transcribed by the host RNA polymerase with the major σ^{A} subunit. Transcription from the A2b and A2c promoters gives rise to the synthesis of the early proteins p6, p5, p3, p2 and p1, involved in DNA replication, and p4 involved in control of transcription. The C2 promoter gives rise to the synthesis of p17, also involved in the viral DNA synthesis, and several small proteins of as yet unknown function. Synthesis of protein p6 strongly represses the C2 promoter, and thus, synthesis of protein p17 stops. This is in agreement with the role of p17 in DNA replication very early after infection [2].

Synthesis of protein p4 represses the A2b promoter by steric hindrance and by the curvature it induces on the DNA upon binding. On the other hand, protein p4 binds to the A2c

promoter in the presence of RNA polymerase, and interaction between the two proteins occurs through residue Arg 120 in p4 and the 15 C-terminal amino acids in the α subunit of *B. subtilis* RNA polymerase. This leads to a very stable complex that can form initiated transcription complexes, but cannot leave the promoter in the presence of NTPs. The synthesis of protein p4, in turn, activates the late promoter A3 by promoting the binding of the RNA polymerase as a closed complex. Interaction between the two proteins also occurs through Arg 120 in p4 and the 15 C-terminal amino acids in the α subunit. When NTPs are added, the RNA polymerase is able to escape from the promoter and to proceed to elongation.

Molecular basis for transcription activation and repression by phage ϕ29 protein p4

An intriguing question is why protein p4 activates the late A3 promoter and represses the early A2c promoter using the same residue (Arg 120) in protein p4 and the same region (15 C-terminal amino acids) in the *B. subtilis* RNA polymerase α subunit. As already indicated, the mechanism of activation by p4 is to promote the binding of the RNA polymerase as a closed complex and the repression mechanism is to prevent the escape of the RNA polymerase from the promoter.

The main differences between the A3 and A2c promoters are the following: the A3 promoter lacks the –35 region and has the p4 binding site centred at position –82 relative to the transcription start site. The A2c promoter contains –35 region and has the p4 binding site centred at position –71.

By site-directed mutagenesis two independent changes were produced at the A3 promoter: a –35 region was introduced (A3-35$^+$) and the p4 binding site was located at the –72 position (A3Δ10). The effect of protein p4 on these mutant A3 promoters was studied by in vitro run-off assays. The A3Δ10 promoter was activated by p4 to the same extent as the wild-type A3 promoter. However, the A3-35$^+$ promoter was repressed by protein p4 instead of being activated by the protein [7].

In the case of the A2c promoter two independent changes were also made: the –35 region was removed (A2c-35$^-$) and the p4 binding site was located at position –81 (A2c∇10). In vitro run-off assays indicated that the A2c∇10 promoter was still repressed by protein p4. On the contrary, the A2c-35$^-$ promoter was activated by p4 instead of being repressed [7].

Thus, the introduction of a –35 consensus sequence converts a promoter from activatable to repressible, whereas the removal of the –35 region changes a promoter from repressible to activatable. Since one of the key elements that determine the strength of a promoter is the –35 region, transcription activation or repression by phage ϕ29 protein p4 depends on the strength of the RNA polymerase-promoter interactions.

Conclusions

Bacteriophage ϕ29 turned out to be a very good model system to study control mechanisms of gene expression. Protein p4, the regulatory protein, is an activator of the late promoter A3 and a repressor of the early promoter A2c. In the first case, the mechanism of transcription activation consists in promoting the binding of the RNA polymerase to the late promoter as a closed complex. In the A2c promoter, the repression by p4 is due to the inability of the RNA polymerase to leave the promoter. In both cases, the same amino acid in p4 (R120) and the same region in the *B. subtilis* RNA polymerase α subunit (15 C-terminal amino acids) are involved in the interaction. It has been shown that the absence or presence of a –35 sequence determines the activation or repression by protein p4, thus depending on the strength of the RNA polymerase-promoter interactions.

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