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The Role of the T7 Gp2 Inhibitor of Host RNA Polymerase in Phage Development

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SUMMARY

Bacteriophage T7 relies on its own RNA polymerase (RNAP) to transcribe its middle and late genes. Early genes, which include the viral RNAP gene, are transcribed by the host RNAP from three closely spaced strong promoters -- A1, A2, and A3. One middle T7 gene product, gp2, is a strong inhibitor of the host RNAP. Gp2 is essential and is required late in infection, during phage DNA packaging. Here, we explore the role of gp2 in controlling host RNAP transcription during T7 infection. We demonstrate that in the absence of gp2 early viral transcripts continue to accumulate throughout the infection. Decreasing transcription from early promoter A3 is sufficient to make gp2 dispensable for phage infection. Gp2 also becomes dispensable when an antiterminating element *boxA*, located downstream of early promoters, is deleted. The results thus suggest that antiterminated transcription by host RNAP from the A3 promoter is interfering with phage development and that the only essential role for gp2 is to prevent this transcription.

Keywords

bacteriophage T7; RNA polymerase; RNA polymerase inhibitor; transcription regulation

INTRODUCTION

Infection of *Escherichia coli* by bacteriophage T7 has been extensively studied (for a review, see Molineux, 2006). After adsorption, about 2% of the T7 genome enters the infected cell by an apparently enzymatic process catalyzed by the virion proteins gp15 and gp16.^{2, 3} The remaining DNA is brought in by transcription, first by *E. coli* and then by T7

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RNAp.⁴ The leading ~2.5% of the genome, corresponding to the genetic left end, contains four early promoters recognized by the host σ^{70} RNAp holoenzyme ($E\sigma^{70}$). The A0 (also known as D) promoter is in the reverse orientation to all T7 genes⁵ and its function is unknown. The early genes of the phage are transcribed from closely spaced strong promoters A1, A2, and A3, whose transcription start points are located, respectively, at positions 498, 626, and 750 from the left end of the phage genome. Transcription by *E. coli* RNAp from the early promoters causes at least 7 kbp of the ~40 kbp T7 genome to enter the bacterial cell.⁴ mRNAs produced from these promoters code for several phage proteins involved in the shut off of host defenses and macromolecular synthesis, such as gp0.3, an antirestriction protein⁶; 7⁷ 8, and gp0.7, a protein kinase that phosphorylates the β' subunit of host RNAp and affects its termination properties.⁹ In addition, *E. coli* RNAp transcribes gene *I*, which encodes a single-subunit, rifampicin-resistant viral RNAp. This enzyme normally completes the genome internalization process by transcription from its promoters, which are located throughout the genome.⁴

Since T7 RNAp transcribes the middle and late genes of the phage, there appears to be no function for *E. coli* RNAp late in infection, after gene *I* has been transcribed and T7 RNAp synthesized. Bacteriophage T7 and its relatives encode an inhibitor of host RNAp, the product of phage gene 2, gp2 (reviewed in Ref. 1). Gp2 is a 7 kDa protein that binds to the host RNAp β' subunit downstream jaw domain and prevents promoter recognition by $E\sigma^{70}$.¹⁰ Gp2 has no effect on transcription by T7 RNAp.¹¹ Thus, gp2 appears to be well suited for the role of a switch factor that abolishes transcription of early viral and host genes by host RNAp but allows continued transcription of the middle and late genes by T7 RNAp.

T7 gene 2 is essential. Analysis of infection of non-permissive cells by T7 gene 2 amber ($T7^{2am}$) mutants demonstrated, surprisingly, that the infection process is not blocked between the early and middle stages of viral transcription as would be expected if the biological role of gp2 was that of a switch factor between the two RNAs. Instead, $T7^{2am}$ infections are blocked at the stage of packaging of concatemeric viral DNA into virion heads,¹¹ long after the beginning of transcription by viral RNAp. This unexpected observation suggests that *i*) uninhibited host RNAp becomes deleterious late in infection and *ii*) the essential function of gp2 is to disable host RNAp at late stages of infection. An alternative explanation, that gp2 has an essential function unrelated to host RNAp inhibition, is unlikely since allele-specific suppression data show that non-permissive cells carrying mutations in the gp2-binding site of RNAp β' are productively infected by T7 carrying compensatory mutations in gene 2 that restore gp2 binding.¹² 13¹⁴ 10

In this work, we examined the role of gp2 *in vivo* by monitoring the fate of selected host and viral transcripts during T7 phage infection in the presence or in the absence of functional gp2. Our data reveal, unexpectedly, that gp2 is not involved in shutting off transcription of host genes, and that its essential role is to abolish transcription from just one early promoter, A3.

RESULTS

The absence of gp2 delays the inhibition of β galactosidase induction during T7 infection

It is generally accepted that T7 shuts down host transcription during its development. For example, McAllister and Barrett¹⁵ showed that in T7 infected cells, incorporation of uridine into *lacZ* transcripts is blocked and, consequently, no β -galactosidase synthesis occurs after IPTG induction. Gp2, a strong inhibitor of host RNAp, is a natural candidate for a factor responsible for host transcription shut-off. However, no direct experiments demonstrating that gp2 plays a role in this shut-off have been reported. We find, in agreement with published data, that while robust β -galactosidase synthesis occurs upon IPTG addition of

uninfected cells, no β -galactosidase activity is detected when IPTG is added along with wild-type T7 (Fig. 1). Gp2 clearly contributes to a decrease in levels of β -galactosidase during T7 infection since wild-type cells infected by T7^{2am} induced β -galactosidase to the same levels as uninfected cells at the early stages post infection (Fig. 1). However, after 15 minutes post-infection, accumulation of β -galactosidase stops even in T7^{2am} infected cells. A gp2-independent process must therefore be able to inhibit expression of *lacZ* in infected cells late in infection.

Dynamics of host and viral transcripts during T7 infection

We used primer extension assays to follow the fates of several cellular mRNAs (*lacZ*, *ompA*, and *aceE*) and early T7 transcripts at various times after phage infection and/or IPTG induction. Primer extensions determine steady-state transcript levels, which depend on both the transcript synthesis and decay rates. To determine the decay rate of host and phage transcripts, primer extension reactions were also performed with RNA prepared from cells at various times after addition of rifampicin, an inhibitor of *E. coli* RNAP. When necessary, rifampicin was added five minutes after IPTG induction and/or phage infection.

In the absence of T7 infection, the expected induction of *lacZ* mRNA by IPTG was observed (Fig. 2A, left panel; note that there are two primer extension products corresponding to the *lacZ* transcript, since two different *lacZ*-specific primers were used). The abundance of *aceE* and *ompA* transcripts was unaffected by IPTG. The addition of rifampicin (Fig. 2A, right panel) led to the rapid disappearance of *lacZ* and *aceE* mRNAs, showing that these transcripts are unstable. In contrast, the *ompA* mRNA level decreased slowly if at all, indicating that this transcript is stable, in agreement with published data.¹⁶

T7 infection produced several changes in the pattern of primer extension products (Fig. 2B). Induction of *lacZ* proceeded normally during the first 5 minutes of infection but thereafter transcript levels strongly decreased. The levels of *aceE* mRNA also decreased rapidly. The decrease of *lacZ* and *aceE* transcripts abundance observed in infected cells closely paralleled that observed in cells treated with rifampicin five minutes post-infection (Fig. 2B, compare corresponding lanes of left- and right-hand panels), indicating that the synthesis of these transcripts is shut off during T7 infection.

With respect to early phage transcripts, primer extension experiments revealed that the T7 A1, A2, and A3 transcripts, as well as the divergent A0 transcript accumulate very rapidly early in infection (within the first two minutes) and then their abundance gradually decreases. The addition of rifampicin five minutes post-infection did not change the pattern of early phage transcript accumulation. The result implies that a shut-off of early phage transcription occurs during T7 infection and the timing of early phage transcription shut-off appears to coincide with the host transcription shut-off. In addition to the early phage transcripts generated by host RNAP, a primer extension product corresponding to T7 RNAP transcript initiating at the *phiOL* promoter was observed. This transcript became detectable five minutes post-infection.

The high stability of the *ompA* transcript in uninfected cells made it unsuitable for the following of the transcription shut-off during T7 infection. Interestingly, the abundance of the *ompA* transcript decreased as the infection progressed, but concomitantly, the appearance and gradual increase of a shorter *ompA*-specific primer extension product (*ompA**) was observed. The *ompA** product was not detectable in uninfected cells. Late in infection, almost all *ompA* primer extension products corresponded to *ompA**. The 5' end of the *ompA** primer extension product was mapped and found to be located 38 bases downstream of the *ompA* transcription initiation start site (therefore 95 bases upstream of the first codon of the *ompA* reading frame, Fig. 3). The *ompA** 5' end is not preceded by a recognizable T7

promoter. If *ompA** transcript were initiated by host RNAP, we would expect to observe it in uninfected cells, from which it was completely absent. We therefore conclude that the *ompA** primer extension product is most likely a result of processing that occurs during the T7 infection.

The untranslated 5' leader of *ompA* mRNA can be folded into a structure containing two imperfect stem loops hp1 and hp2 separated by single-stranded region ss1.¹⁶ Two RNase E cleavage sites in *ompA* leader are located in ss1 and in ss2, a single-stranded region located downstream of hp2. Processing at these sites is not detected in either infected or uninfected cells by our method (Fig. 2). Hp1 stabilizes the *ompA* RNA.¹⁶ The T7 induced processing site is located in a bubble inside hp1. Cleavage at the T7-induced processing site destroys the stem-loop structure of the hp1 element and may influence *ompA* mRNA stability. However, our time-course experiments (i.e., Fig. 2B) suggest that the *ompA** transcript is stable during the infection.

Early T7 transcripts are overproduced in the absence of gp2

E. coli JE1134 harbors a deletion in the *rpoC* gene that removes β' residues 1149–1190.¹⁷ The deleted residues form the downstream jaw, which contains the gp2-binding site.¹⁰ Gp2 does not bind to and does not inhibit RNAP purified from JE1134¹⁰ and, as a consequence, T7 does not grow normally on JE1134. However, JE1134 is efficiently infected and lysed by T7 wild type, although no progeny phage result. The results of primer extension analysis of phage and selected host transcripts during the non-productive infection of JE1134 by the wild-type phage are shown in Fig. 4A. The behavior of *E. coli* transcripts was qualitatively similar to that seen in the wild-type cell infection, indicating that gp2 is not required for host transcription shut-off. However, the behavior of early phage transcripts was markedly different, as they continued to accumulate during the entire course of infection.

The infection of wild-type cells by T7 ^{2^{am}} (wild-type target, mutant inhibitor) and the reciprocal infection of JE1134 by T7⁺ (mutant target, wild-type inhibitor) should be blocked at the same stage and both host and viral transcription should proceed in the same manner in both infections. Indeed, when wild-type cells were infected with T7 ^{2^{am}} (a non-productive infection), inhibition of host transcripts and abnormal accumulation of early viral transcripts was observed (Fig. 4B). From these results, we conclude that the transcriptional shut-off of host genes in T7-infected cells proceeds normally whether gp2 is functioning or not. However, shut-off of early viral transcription requires gp2 binding to the host RNAP downstream jaw. In the absence of this interaction, host RNAP continues to transcribe phage DNA at late stages of infection, and this transcription may lead to abortive infection. This notion is supported by earlier observations that the addition of rifampicin to cells infected with T7^{2^{am}} phage midway through infection leads to a productive infection.^{18; 19} Indeed, we observed normal production of T7^{2^{am}} progeny phage when wild-type host cells were treated with rifampicin 5 minutes post-infection (data not shown), i.e., at conditions when no excess early transcription was taking place (Fig. 4A, right panel).

Mutations in the T7 A3 promoter make gp2 dispensable for T7 development

We selected spontaneous T7 mutants that formed large plaques at 25°C on JE1134. The lower temperature increased the stringency of the selection by minimizing formation of small plaques by the wild-type phage that escaped the block imposed by the loss of gene 2 activity. Many of the mutants were found to contain deletions in the early promoter region. However, two mutants appeared to have the early promoter region intact, as judged by electrophoretic analysis of a PCR product obtained using a pair of primers annealing outside of the early promoter area. Sequence analysis of amplified fragments revealed that each mutant had only one change from the wild-type sequence (Fig. 5). Mutant T7 A3(–35), has

a T->C point mutation in the -35 promoter element, while T7 A3(-10) has the same change in the -10 element of T7 A3. Both mutations decrease the similarity of the A3 promoter sequence to consensus. Each mutation was transferred to wild type phage background and was shown to allow growth on JE1134, thereby confirming that the mutations in A3 were sufficient to cause the phenotype.

In vitro, a promoter fragment containing T7 A3(-10) was inactive in abortive transcription initiation assays, but a fragment containing T7 A3(-35) retained ~80% activity (data not shown). The relative (compared to other early promoters) activity of T7 A3(-35) was also lower in the course of wild-type cells infection by the mutant phage (compare the corresponding lanes of Fig. 5A and Fig. 2B). Most importantly, in the course of the JE1134 host infection, no accumulation of transcripts from T7 A3(-35) was observed, while A0, A1, and A2 transcripts accumulated throughout the infection (Fig. 5B). Thus, while down-mutations in T7 A3 are sufficient to make gp2 function dispensable for phage development, the absence of early phage transcription shut-off *per se* is not detrimental for T7 development. Therefore, the data strongly suggest that binding of *E. coli* RNAP to (or transcription from) the A3 promoter is the only physiological target of gp2.

Deletion of antiterminating element *boxA* makes gp2 dispensable for T7 development

An additional T7 mutant, originally constructed for other purposes, was also found to grow on JE1444. The mutant contains a deletion of T7 nucleotides 814–836, removing a sequence identical to *E. coli boxA*, a *cis*-acting element that interacts with several cellular factors to allow host RNAP to bypass phage λ 20 and host *rrn* transcription terminators.²¹ T7 *boxA* also antiterminates *E. coli* RNAP-catalyzed early phage transcription (WPR and IJM, in preparation). Primer extension analysis of productive infection of JE1134 cells with T7 $\Delta boxA$ revealed that transcripts from all three rightward early promoters accumulated throughout the infection, apparently without the deleterious effect associated with uninhibited transcription during infection of JE1134 by T7⁺ or wild-type cells infection by 2^{am} phage (Fig. 6). Combining these primer extension data with those obtained with the A3 mutant, we suggest that only A3-initiated, *boxA*-antiterminated transcription needs to be abolished by gp2 in order for a productive infection to occur.

DISCUSSION

In this work, we demonstrate that bacteriophage T7 gp2 is required for the shut-off of T7 early transcription. When gp2 is rendered non-functional, either by mutation in gene 2 or by alteration of the gp2-binding site in the *E. coli* RNAP β' subunit, early phage transcripts continue to accumulate throughout the infection.

The principal result of this work is the demonstration that gene 2 is not required when the infecting phage contains down mutations in the early promoter A3. The mutations either sufficiently diminish the A3 function altogether or prevent excessive accumulation of A3 transcripts in the absence of gp2. However, the A0, A1, and A2 transcripts continue to accumulate. The results strongly argue that continuous transcription from A3, but not from other early promoters, is detrimental to phage development. It therefore follows that the only essential function of gp2 is to prevent excessive transcription from A3 late in infection. A second type of mutation that abolishes the requirement for gp2 lies in *boxA*, a DNA sequence that encodes an RNA element required for antiterminated transcription by *E. coli* RNAP past terminators located between the early and middle genes of the phage. Deletion of the *boxA* element abolishes antiterminated transcription, including that initiated at A3. Our results reveal that during the productive infection by *boxA*⁻ mutant phage of cells whose RNAP lacks the gp2-binding site, transcription from all early promoters continues late into infection. It therefore follows that the biologically significant role of gp2 is to prevent

antiterminated transcription by host RNAP into areas of the phage genome that are normally transcribed by the viral RNAP. *In vitro* data and structural considerations show that gp2, a strong inhibitor of open promoter complex formation, cannot bind to a transcription elongation complex and therefore gp2 cannot affect antiterminated transcription directly.^{10; 22} Instead, gp2 must affect antiterminated transcription indirectly, presumably by preventing promoter complex formation. Our data strongly suggest that during a normal infection, only A3-initiated transcripts are antiterminated and the biological function of gp2 is to prevent such transcription. The exact mechanism by which host RNAP transcription into the middle and late regions of the T7 genome interferes with productive infection is not known. Qimron and Richardson proposed an attractive model that posits that the relatively slow moving host RNAP impedes transcription by the faster moving viral RNAP, causing the latter to pause.²³ A paused T7 RNAP transcription complexes can recruit phage DNA packaging machinery which introduces double-stranded breaks in phage DNA at and around the site of transcription pausing, causing the generation of less than unit size phage genomes that are indeed observed during infections in the absence of functional gp2.^{24; 25}

Earlier work has demonstrated that rifampicin, a drug that prevents promoter clearance by host RNAP, can substitute for gp2 *in vivo* (when added to T7 2^{am} phage infected cells after the synthesis of early transcripts,^{18; 19}) and *in vitro* (when added to packaging extracts prepared from T7 2^{am} phage infected cells¹¹). It is likely that rifampicin rescues infections by mimicking the action of gp2 (preventing antiterminated transcription from early promoters, including A3), however, it does so by acting at a later stage than gp2, by preventing promoter complexes formed at A3 from leaving the promoter. The result suggests that the biologically significant consequence of gp2 function is not the prevention of promoter complex formation at A3 *per se* (a direct effect of gp2 binding to RNAP). Rather, the critical biological consequence of gp2 action is that RNAP initiating at the A3 promoter does not go into antitermination mode by accretion of additional components at *boxA* and does not elongate transcripts downstream. Currently, it is unclear what makes A3-initiated transcripts special. It is possible that transcripts initiated from the two upstream promoters, A1 and A2, contain 5' proximal elements that prevent transcription antitermination and/or promoter termination.

Despite its clear ability to inhibit host RNAP when overexpressed²⁶, gp2 does not play an exclusive role in the shut-off of host transcription. Moreover, the host enzyme is clearly able to recognize, and initiate transcription at, early viral promoters (strong $E\sigma^{70}$ promoters) throughout the infection, provided that gp2 is absent. This result indicates that a mechanism to distinguish the recognition of host and early viral promoters exists in T7, but it remains elusive despite extensive prior studies. Gp0.7, a T7 kinase that phosphorylates host RNAP is not involved, since during infection with a mutant phage lacking 0.7 the shut off occurs normally (data not shown). It is possible, however, that this mechanism is a passive one, i.e., that it is a simple consequence of host DNA degradation during T7 infection.

METHODS

Bacterial strains and bacteriophages and their growth

Cells and phages used are listed in Table 1. All strains were grown in LB media at 30°C (unless otherwise indicated). Bacteriophage T7, and all mutant variants were CsCl-purified before use. In all infections, phage T7 was added at an MOI of 10 when the cell culture reached an A_{600} of 0.5.

Primer extension reactions

Cells were grown to $A_{600} = 0.5$ at 30°C. After the 0 min sample was collected, phage [or an equal volume of SM buffer [100 mM NaCl, 50 mM Tris-HCl (pH 7.8)]] was added and *lacZ* was induced 15 sec later with 0.5 mM IPTG. For cultures treated with rifampicin, 100 µg/ml was added immediately after removing the 5 min sample. Cells were harvested by centrifugation at 5,000 rpm for 10 min. Total RNA was isolated from cell pellets using RNeasy RNA isolation kits (Qiagen); the protocol provided by the manufacturer was used with slight modification (a 3-minute treatment with lysozyme). Total RNA samples were quantified via spectrophotometry and examined by gel electrophoresis. Equal amounts of total RNA isolated from each sample were used for all primer extension reactions. All buffers and enzymes were either included in the Superscript III first strand synthesis kit (Invitrogen) or purchased separately. For the 20 µl annealing reactions, 5X FS buffer (Invitrogen), 3 mM dNTPs, total RNA, and [γ - 32 P]ATP-labeled primers were cooled down from 65°C to 45°C during the course of 20 min and then transferred to 25°C and incubated for additional 10 min. For primer extension reactions, RNase Out enzyme, SSIII reverse transcriptase enzyme, 5X FS buffer (Invitrogen), and 15 mM DTT, were added to the annealing reaction and the mixture was incubated at 50°C for 50 min, 85°C for 5 min, 4°C for 5 min (when 1 µl of RNase H was added), and then 37°C for 20 min. Reactions were chloroform-extracted, and ethanol-precipitated using Glycoblue (Ambion). The pellets were resuspended in a solution containing formamide, bromophenol blue, xylene cyanol, and saturated urea, then boiled for 1 min prior to loading onto an 8% acrylamide gel. The gel was fixed, dried, and exposed on a phosphor screen overnight. Quantifications were done using ImageJ (available on the NIH website).

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Figure 1. Induction of β -galactosidase activity during wild-type and gp2-deficient T7 infections
 Levels of β -galactosidase activity (in Miller units) at various times post-infection. *lacZ* was induced with 0.5 mM IPTG at the time of phage addition. The multiplicity of infection was 10 and less than 5% of the cells survived the infection. A representative result from four independent experiments is shown.

Figure 2. Primer extension analysis of host and viral transcripts after wild-type T7 infection

A. Wild-type *E. coli* cells were induced at time 0 with 0.5 mM IPTG. Total RNA was purified from cells collected at the indicated times post-induction and primer extension reactions were carried out. In each right-hand panel, rifampicin (100 µg/ml) was added to induced cells 5 minutes post-induction (indicated by a downward red arrow). Individual primer extension products are labeled at the right hand side of the figure. Two different primers were used to reveal the *lacZ* transcript, resulting in two different primer extension products.

B. As in **A** but cells were infected with wild-type T7 phage (MOI of 10) at the time of the induction. Additional primers designed to reveal transcripts initiated from the early promoter region of the phage were added to the primer extension reactions. Promoters to which specific primer extension products correspond are identified on the left hand side of the figure. The “*ompA**” label identifies an *ompA* primer-specific extension product that is only seen in infected cells (see text for details). Under the conditions of the experiment, cell lysis occurs 35 minutes post-infection. Less than 5% of the cells survive the infection.

A predicted structure of *ompA* 5'-UTR between the transcription start point and the initiating codon of the *ompA* ORF, the T7-induced processing site generating *ompA**, and known RNase E sites indicated. The schematic is based on Fig. 1B of reference 16.

Figure 4. Primer extension analysis of host and viral transcripts during non-productive infections when the gp2-host RNAP interaction is disrupted

The experiment was conducted as described in the Figure 2 legend.

A. Wild-type *E. coli* cells were infected with T7^{2am}.

B. JE1134 *E. coli* cells (containing *rpoC*^{Δ1149–1190} deletion) were infected with the wild-type phage.

A.

-35 -10

wtT7A3 AAACAAACGGTTGACAACATGAAGTAAACACGGTACCGATGTACCACA →

T7A3(-35) AAACAAACGGCTTGACAACATGAAGTAAACACGGTACCGATGTACCACA

T7A3(-10) AAACAAACGGTTGACAACATGAAGTAAACACGGCACCGATGTACCACA

B.

Figure 5. Down mutations in the A3 promoter allow productive infection of JE1134 cells
A. T7 A3 promoter mutations that allow productive infections of JE1134 host. The wild type sequence is shown at the top. Promoter consensus elements are labeled and highlighted by color. The transcription start site is shown by a rightward arrow. Sequences of T7 A3 promoters from phages that productively infect JE1134 are shown below. The sites of mutations are highlighted with red color.
B. Primer extension analysis of host and viral transcripts during productive infections of wild-type (left panel) and JE1134 (right panel) cells with T7 A3(–35) phage. The experimental design was as described in the Figure 2 legend.

Figure 6. Primer extension analysis of host and viral transcripts during productive infection of JE1134 cells with the T7^{boxA} phage

The experiment was conducted as described in the Figure 2 legend.

Table 1

Bacterial strains and bacteriophages used in this study

Name	Description
<i>Bacterial strains</i>	
MG1655	<i>E. coli</i> K-12, wild type
JE1134	MG1655 <i>rpoC</i> $\Delta^{1149-1190}$ (Ref. 17)
<i>T7 strains</i>	
T7 ⁺	wild type
2am64	2 ^{am} ; amber mutation in gene 2, codon 16
3490 (T7 Δ g1)	gene 1 deletion mutant (Ref. 27)
T7 Δ boxA	deletion of bp 814–836 (WPR and IJM, to be published)
T7 A3(–35)	T714 C; –35 region of A3 promoter (WPR and IJM, to be published)
T7 A3(–10)	T737 C; –10 region of A3 promoter (WPR and IJM, to be published)

Table 2

Primers used

Promoters monitored	Primer Name	Sequence	Length
<i>Viral primers</i>			
A0	PreE T7-1	CCTTGAGTGTCTCTCTGTGTCCCTATCTGT	30
phiOL, A1,	A3rev2	GTCATGCACTCAAGAGCTATTTACCAGATT	30
A2, A3	836rev ^a	CTAGTCATGCACTCAAGAGC	20
<i>Host primers</i>			
<i>PaceE</i>	aceE	ACGCTCAACACCTTCTTCACGG	22
<i>PompA</i>	ompA	ATCCAAAATACGCCATGAATATCTCC	26
<i>PlacZ</i>	lacZdir	CAAGGCGATTAAGTTGGGTA	20
<i>PlacZ</i>	NEB M13	GTTTCCCAGTCACGAC	17

^aPrimer 836rev was used for only the T7 *Abx*A primer extensions, as the A3rev2 primer overlapped the deleted sequence.