The lytic replicon of bacteriophage P1 is controlled by an antisense RNA

Jochen Heinrich, Hans-Dieter Riedel, Beate Rückert, Rudi Lurz and Heinz Schuster*

Max-Planck-Institut für Molekulare Genetik, D-14195 Berlin, Germany

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ABSTRACT

The lytic replicon of phage P1 is used for DNA replication during the lytic cycle. It comprises about 2% of the P1 genome and contains the P1 C1 repressor-controlled operator–promoter element Op53-P53 and the kilA and the repL genes, in that order. Transcription of the lytic replicon of P53 and synthesis of the product of repL, but not kilA, are required for replicon function. We have identified an additional promoter, termed P53as (antisense), at the 5'-end of the kilA gene from which a 180 base transcript is constitutively synthesized and in the opposite direction to the P53 transcript. By using a promoter probe plasmid we show that transcription from P53 is strongly repressed by the C1 repressor, whereas that of P53as remains unaffected. Accordingly, the C1 repressor inhibits binding of Escherichia coli RNA polymerase to P53, but not to P53as, as shown by electron microscopy. Under non-repressed conditions transcription from P53 appears to be inhibited by P53as activity and vice versa. An inhibitory effect of P53as on the P1 lytic replicon was revealed by the construction and characterization of a P53as promoter-down mutant. Under non-repressed conditions transcription of repL and, as a consequence, replication of the plasmid is strongly enhanced when P53as is inactive. The results suggest a regulatory role for P53as on the P1 lytic replicon.

INTRODUCTION

The temperate phage P1 encodes two replicons for the replication of its genomic DNA. One, the lytic replicon, is active during the lytic cycle and requires one phage-encoded protein, RepL. The other, the prophage replicon, is responsible for maintenance of the P1 plasmid prophage and requires another phage-encoded protein, RepA. The two replicons are located about 12 map units apart on the 100 kb (100 map coordinates) circular genome of prophage P1 (1) and can act independently of each other. The prophage replicon as a recombinant plasmid is controlled by the phage incA locus and depends on the DnaA protein of the host, but is unaffected by the phage repressor for lytic functions, C1. The lytic replicon, on the other hand, is independent of the RepA and DnaA proteins and is not affected by incA. It directs high copy number replication of an otherwise replication-defective λ vector (for all but selected references on P1 and P7 see 2,3).

The components of the P1 lytic replicon (Fig. 1) are: (i) a promoter, P53, which is regulated via the C1 repressor-controlled operator Op53 and whose activity is essential for replicon function; (ii) a promoter proximal gene kilA, whose product is not essential for replicon function, but which is lethal to the bacterial cell; (iii) a promoter distal gene region, which encodes the RepL protein and contains an as yet unknown origin of replication (4–6). Recombinant plasmids whose replication solely depends on the P1 lytic replicon can only be maintained in a bacterial cell when the kilA gene is knocked out. Such plasmids, in turn, can no longer replicate when promoter P53 is shut off by the action of the C1 repressor (5). Transcription is repressed by binding of C1 to the operator Op53, whose asymmetric 17 bp sequence overlaps the promoter (7,8; Fig. 2). Thus binding of C1 prevents access of Escherichia coli RNA polymerase to the promoter. Transcription of repL is strictly required for the lytic replicon to be active. However, the promoter function of C1-regulated P53 can be replaced by other promoters (5,6). For example, when the inducible lacZ promoter of E.coli is used instead of P53, the extent of replication was shown to be proportional to the promoter activity. Surprisingly, however, the C1/P53-regulated replicon appears to be significantly more stable than the lacZ promoter-regulated replicon, although the copy number of the latter replicon is higher than is the copy number of the C1/P53-regulated replicon (5).

In the course of our studies on the C1 repressor-controlled operators of P1 we detected a second promoter in close proximity to the Op53-P53 element. We term this promoter P53as (for antisense) and its properties are described here. P53as is located at the 5'-end of the kilA gene and constitutively initiates transcription in the opposite (antisense) direction to that from P53 (Figs 1 and 2). We show that the transcriptional activity of P53as is essential for establishment of a plasmid whose replication is driven by the P1 lytic replicon. Since the lacZ promoter-regulated P1 replicon was originally constructed by deleting the region of promoters P53 and P53as (5), the instability of this replicon may be due to the absence of antisense transcription. These results suggest a crucial role for P53as in the stability of the natural P1 replicon.

* To whom correspondence should be addressed

*Present address: Universitätssklinik Heidelberg, D-69115 Heidelberg, Germany
Figure 1. P1 lytic replicon and plasmids used. (Center) The P1 lytic replicon is located downstream of the anti-repressor gene ant of the P1 imm1 operon and in a clockwise orientation on the P1 genetic map (1). It comprises the Cl repressor-controlled Op53 • P53 element (dot and triangle), the promoter P53as (this paper) and the genes kilA and repL (grey, arrowheaded bars). Arrowheaded wavy lines indicate the direction and maximal size of transcripts found. Only relevant restriction enzyme cleavage sites are shown. Their numbering is explained in the legend to Figure 2. (Upper and lower) P1 DNA inserts in vector DNAs are shown by horizontal lines. Numbers indicate the corresponding restriction enzyme cutting sites. The exceptions are the BgII deletion end points at positions 146 and 185 (see Fig. 2). A 426 bp deletion in the kilA gene is indicated by the interrupted line. The reading frame is not altered in the resulting truncated kilA* gene. Vectors used are pT7-6 (pAH series), pCB302a (pCB series) and pSU2719 (pSU series). a and b indicate opposite orientations of the P1 DNA insert in the vector (see Tables 1 and 2). The repL probe was used for Southern and Northern blotting. Single strand-specific RNA probes were prepared from the plasmids pAH1067a and pAH1067b as described in Materials and Methods. Plasmid pT020 is replicated via P53 • P53as-regulated synthesis of RepL protein.

Figure 2. Nucleotide sequence of the regulatory region of the P1 lytic replicon. Nucleotides are numbered from 1 (HpaI recognition site) to 360 in the kilA gene (5). The HpaI site contains the termination codon, TAA, of the ant gene (9). Nucleotide positions upstream of the HpaI site are indicated by negative numbers. Sequences of the ant and the kilA genes are framed. Upstream of kilA is a potential ribosome binding site (rbs) and the promoter P53, whose -10 and -35 regions are indicated by brackets (5). The -10 region of P53 overlaps the operator Op53 (boxed sequence). A 13 bp palindromic sequence further upstream is indicated by arrows below the sequence. The -10 and -35 regions of the promoter P53as are located at the 5'-end of the kilA gene. The base substitution in the P53as2 promoter-down mutation is marked by an arrow. The +1 positions of P53as and P53 are indicated by filled triangles and were determined by a primer extension assay (see Fig. 4). GATC dam methylation sites are shown by grey areas.

**Materials and Methods**

**Bacterial and phage strains**

The *E. coli* K12 strains used were CB454 Δ lacZ galK recA56 (10) and WM874 ara Δ(lac-pro) thi (original name CSH 26; 11). WM874 dam− was prepared by transduction of WM874 with T4GT7 phage (12) which had been grown in *E. coli* GM2199 dam− (13). Phages used were P1 Cm, P1 Cm c1.100 (14) (abbreviated to P1 and P1 c1ts respectively) and P1 C4.32 ant17 (9,15).

**Plasmid constructions**

Vectors used for the construction of plasmids, presented in Figure 1 and Table 1, were pT7-6 (S. Tabor and C. C. Richardson, personal communication), pJF118EH (16), pCB302a (10) and pSU2719 (17). Plasmid pAM2b c1+coi+ contains the 2.27 kb PvuII–BclI subfragment of P1 EcoRI fragment 7 with the c1 gene, its control region and the coi gene (18) inserted into a 2.5 kb DraI fragment of the vector pKT101 Km' (19). It was used to supply the Cl repressor. Plasmid pAM8 c1+coi-lex+ is a coi-defective derivate of pAM8 c1+coi+lex+(vector pKT101 Km') (19) that additionally contains the co-repressor lex gene (20). The coi gene
was inactivated by insertion of two bases at the Accl site (18). The plasmid was used to supply the Cl repressor and Lxc co-repressor. Plasmid pAM8 c1ts col\(^{+}\)lxc\(^{+}\) is a derivative of pAM8 c1\(^{+}\)col\(^{+}\)lxc\(^{+}\) in which the P1 BglIII→PsrI fragment containing the C-terminal part of c1\(^{+}\) is substituted by the analogous fragment of the temperature-sensitive mutant P1 cl.100 (14,21). It was used to supply the C1ts repressor and Lxc co-repressor.

Plasmids pAH1027 and pT020 contain a P1 lytic replicon and were constructed in the following way. A 16 kb Smal→HindIII fragment of P1 clts, extending from map position 48 to 64 (1) was inserted into pT7-5 (SmalHindIII) to yield plasmid pAH16 (not shown). Next, a HpaI-HpaI fragment from pAH16 was inserted into pT7-6 (Smal) to yield pAH1026 (Fig. 1). Because of the lethal effect of KilA on the bacterial cell, pAH16 and pAH1026 can only be maintained in bacteria carrying the plasmid pAM8 c1\(^{+}\)col\(^{+}\)lxc\(^{+}\), which supplies the C1 repressor and Lxc co-repressor. To inactivate the kilA gene we followed the procedure of Sternberg and Cohen (5) in deleting an AaII→Clal fragment from pAH1026 to yield plasmid pAH1027 (Fig. 1). The latter contains the ColEl replicon of the vector pT7-6, in addition to the kilA-truncated P1 lytic replicon. To eliminate the ColEl replicon, a 2226 bp BamHI→Scal fragment of pAH1027 containing the lytic replicon was ligated to a 847 bp Scal→BamHI fragment of pJF118EH to yield plasmid pT020 (Fig. 1). Replication of pT020 solely depends on the kilA-truncated lytic replicon of P1. To test the promoter activity of P53 and P53as, the P1 DNA fragments (Fig. 1, lower part) were blunt-end ligated to Smal (pCB302a)- or HincII (pSU2719)-linearized vector DNA respectively. Fragments with sticky ends were treated with T4 DNA polymerase beforehand.

**In vitro mutagenesis**

The P53as promoter-down mutant was constructed by oligonucleotide-directed mutagenesis using double-stranded DNA (22), for which plasmid pAH1020 was used, which contains the P1 HaeIII→EcoRI fragment (Fig. 1) in pT7-6 (Smal/EcoRI). The plasmid was then mutagenized using the oligonucleotide 5′-GTTGAAGGATCAACATTTTG-3′. This oligonucleotide is derived from the 1.6 kb Accl DNA fragment of plasmid repL described by Pollard et al. (24) and according to which the P1 kilA gene was inactivated by insertion of two bases at the Accl site (18). The plasmid was used to supply the C1 repressor and Lxc co-repressor. Plasmid pAM8 c1ts col\(^{+}\)lxc\(^{+}\) is a derivative of pAM8 c1\(^{+}\)col\(^{+}\)lxc\(^{+}\) in which the P1 BglIII→PsrI fragment containing the C-terminal part of c1\(^{+}\) is substituted by the analogous fragment of the temperature-sensitive mutant P1 cl.100 (14,21). It was used to supply the C1ts repressor and Lxc co-repressor.

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**Electron microscopy**

Binding of RNA polymerase (Promega) to supercoiled plasmid DNA was done with or without pre-incubation with C1 repressor as described (28). After fixation of the DNA–protein complexes with glutaraldehyde, the DNA was linearized with the restriction enzymes Dral, SacI or Scal in order to pinpoint the P1-specific RNA polymerase binding site among several binding sites in the vector DNA. Adsorption of the complexes to mica followed by electron microscopy and evaluation of the data were as described by Spiess and Lurz (29).

**RESULTS**

**Two RNA polymerase binding sites exist in the control region of the P1 lytic replicon**

We became aware of the existence of a second promoter in the Op53-P53 control region of P1 (Fig. 1) when we studied interference by the C1 repressor with binding of RNA polymerase to the known promoter P53 by electron microscopy. Two RNA polymerase binding sites were found on a 341 bp HaeIII fragment comprising the 3′-end of the ant gene, the Op53-P53 control region and the 5′-end of the kilA gene (Figs 1 and 2). The two sites can only be distinguished when the HaeIII fragment is subdivided by TaqI into two HaeIII→TaqI subfragments, 188 and 153 bp in size. The subfragments were inserted into Smal-linearized pCB302a, yielding plasmids pCB192a and pCB147b respectively (Fig. 1). As expected, binding of RNA polymerase to the 188 bp insert in pCB192a can be inhibited by the C1 repressor (Fig. 3, left half), because the overlapping Op53-P53 control element is located within the region (Figs 1 and 2). However, binding of RNA polymerase to the 153 bp insert in pCB147b was not affected by the C1 repressor (Fig. 3, right half). This RNA polymerase binding site represents a constitutive promoter which we term P53as (Figs 1 and 2). The additional three to four RNA polymerase binding sites (Fig. 3) are all located within the vector part of the recombinant plasmids.

**Two promoters, P53 and P53as, initiate transcription in opposite and convergent directions**

We verified the existence of the two promoters P53 and P53as, arranged in opposite directions to each other, by two methods: (i) the +1 transcript initiation sites were determined by primer extension of in vitro transcripts from appropriate plasmids (Fig. 4); (ii) transcriptional activities were determined by measuring β-galactosidase activity using the promoter probe plasmid pCB302a (Table 1). Transcripts from promoter P53 were found to start at three positions located in close proximity to each other. The transcriptional activity of P53 on supercoiled pSU20A3 DNA (Fig. 1) is comparable with that on linearized pSU20A3 DNA. Both are strongly inhibited in the presence of the C1 repressor (Fig. 4, left half). Transcripts from P53as, on the other hand, start at a unique position and transcription is not inhibited by the C1 repressor, but is strongly stimulated when linearized instead of supercoiled pSU20A3 DNA (Fig. 1) is used (Fig. 4, right half). These findings are discussed in detail below. The data are supported by the results of the β-galactosidase assay (Table 1). In this assay the enzyme activity was measured with recombinant promoter probe plasmids.
from P53 promoter transcription from pCB192a is strongly inhibited in the presence of both the Cl promoter probe plasmids pCB20a and pCB20b are used (Table 1). The repression of sense transcription from P53 and constitutive pCB147b remains unaffected by Cl, with or without Lxc. The antisense transcription from P53as are also observed when the promoter probe plasmids pCB20a and pCB20b are used (Table 1).

In E. coli CB454, P53 promoter transcription from pCB192a and pCB147b; Fig. 1) or both promoters together (pCB20a and pCB20b; Fig. 1). In E. coli CB454, P53 promoter transcription from pCB192a is strongly inhibited in the presence of both the Cl repressor and the Lxc co-repressor (supplied from the PI prophage), but less strongly in the presence of C1 alone (supplied from plasmid pAM2b). In contrast, P53as transcription from pCB147b remains unaffected by C1, with or without Lxc. The repressibility of sense transcription from P53 and constitutive antisense transcription from P53as are also observed when the promoter probe plasmids pCB20a and pCB20b are used (Table 1).

Table 1. Transcriptional activities of P53 and P53as

<table>
<thead>
<tr>
<th>Promoter</th>
<th>E. coli O157</th>
<th>5'-GATC' activity (µM)</th>
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<tr>
<td></td>
<td>P53</td>
<td>P53as</td>
</tr>
<tr>
<td>pCB192a</td>
<td>540</td>
<td>3</td>
</tr>
<tr>
<td>pCB192b</td>
<td>210</td>
<td>180</td>
</tr>
<tr>
<td>pCB147a</td>
<td>2350</td>
<td>200</td>
</tr>
<tr>
<td>pCB147b</td>
<td>510</td>
<td>500</td>
</tr>
<tr>
<td>pAM2b</td>
<td>360</td>
<td>300</td>
</tr>
</tbody>
</table>

Escherichia coli strains of the CB454 and the WM874 series were transformed with one of the plasmids shown in the first column. Relevant PI immunity genes, which are present on the prophage or plasmid of the recipient strain, are indicated. The letters a and b of the plasmid (first column) mark the relative position of the lacZ gene (arrowhead, second column) for P1 DNA fragments inserted into the promoter probe vector. Bacteria were grown at 37°C. At A600 = 0.5, bacteria were prepared for the β-galactosidase assay as described (11). Activity is expressed as Miller units.

It is striking that the sequence 5'-GATC-3', which is recognized specifically by the methylase coded for by the bacterial dam gene (30), occurs three times close to and within the -35 region of P53 and once overlapping the -35 region of P53as (Fig. 2). 6-Methyladenosine (6-meAde) is the product of the reaction catalyzed by the bacterial methylase and a lack of 6-meAde has been correlated with decreased replication of replicons (30). In E. coli WM874 dam+, P53 promoter transcription from pCB192a and pCB279a is 3- to 4-fold stronger than in the corresponding dam- strain. In contrast, P53as transcription is not affected by methylation when P53as is the only promoter on the plasmid (pCB147b), however, an ~2-fold reduction is found when P53 is present on the same plasmid (pCB279b) (Table 1). We assume that this is due to the increase in activity of P53, which negatively affects P53as transcription.

Promoter P53as is active in non-induced and induced PI prophage

To determine the transcripts in the Op53-P53 control region of an intact PI genome, the PI wild-type and a PI cits lysogen were used. Northern blot analysis using single-stranded sense and antisense RNA probes revealed two sets of transcripts. (i) A 180 base long antisense RNA in the PI wild-type and a PI cits lysogen at 28°C, the amount of which increases dramatically during PI cits induction (Fig. 5, left half). As expected, synthesis of this RNA is initiated at the promoter P53as and terminates in the palindromic terminator structure, as judged from its size (Fig. 2). No such RNA is observed in a non-lysogen. (ii) Sense transcripts ranging in size from 240 to 2300 bases are found only upon induction of the PI cits prophage (Fig. 5, right half). Most probably they represent the mRNAs of the whole repL operon, the
described in Materials and Methods. The 153 bp and TaqI-HaeIII activity by a factor of -1.5 (Table 2).

mutation in pCB279a, on the other hand, leads to an increase in P53 mutation in pCB 147b reduces P53as activity to -15%. The same As judged by (β-galactosidase activity, the P53as2 promoter-down to yield the plasmids pCB 147b and pCB279a respectively (Fig. 1). The transcripts were used as antisense and sense probes respectively Total cellular RNA (20 μg) was loaded in each lane. P1, P1 wild-type lysogen, (+), non-lysozymic

*klA and repL genes individually and, possibly, some degradation products.

Promoter P53as down-regulates the P1 lytic replicon

To investigate the effect of transcription from P53as on the P1 lytic replicon we first constructed the plasmids pAH1027 and pT020 as described in Materials and Methods. Replication of pT020 solely depends on the P1 lytic replicon, with an (unknown) origin of replication which appears to reside in the repL region, between the ClaI and HpaI cleavage sites (5; Fig. 1). Transcription from P53 is a prerequisite for the lytic replicon to function. Since the C1 repressor blocks the P53 promoter, plasmid pT020 cannot be maintained in cells containing the C1 repressor. However, plasmid pAH1027 carries a CoIE1 replicon on its pT7-6 vector DNA, in addition to the P1 lytic replicon. Therefore, the function of the latter can be turned off by repressor C1 without impairing replication driven by the CoIE1 replicon. In both plasmids the lethal effect of KilA on the bacterial cell is prevented by deleting 52% of the kilA gene, without impairing the reading frame.

Next we constructed a P53as promoter-down mutation as described in Materials and Methods. The 153 bp TaqI-HaeIII and the 279 bp Hpal-HaeIII DNA fragments of P1 carrying this mutation were inserted into the promoter probe plasmid pCB302a to yield the plasmids pCB147b and pCB279a respectively (Fig. 1). As judged by β-galactosidase activity, the P53as2 promoter-down mutation in pCB147b reduces P53as activity to -15%. The same mutation in pCB279a, on the other hand, leads to an increase in P53 activity by a factor of -1.5 (Table 2).

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<th>Plasmid</th>
<th>β-Galactosidase activity (U)</th>
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<tr>
<td>pCB147b</td>
<td>730</td>
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<tr>
<td>pCB279a</td>
<td>800</td>
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</table>

We then tried to insert the P53as2 promoter-down mutation into plasmids pAH1027 and pT020 by replacing the BstXI-BstXI346 wild-type fragment with the corresponding mutant fragment of plasmid pAH1020-2. Since the two BstXI recognition sequences differ from each other (Fig. 2), reinsertion of the fragment is only possible in the natural orientation. The ligation mixtures were used to transform WM874 and WM874/pAM8 c1<sup>cor</sup>-lxc<sup>+</sup> bacteria. Only recombinants from mixtures of pAH1027 and pAH1020-2, but not of pT020 and pAH1020-2, yielded transformants of C1 repressor-containing bacteria. Transformants of WM874 bacteria were not obtained. The C1 repressor requirement of the recombinant plasmid was proven by repeating the transformation. Compared with plasmid pAH1027 (wild-type), mutant plasmid pAH1027-2 again yielded transformants only of C1 repressor-containing bacteria. Transformants of WM874 bacteria were grown at 28°C At A550 = 0.6 the temperature of the PI cits lysogenic bacteria was shifted to 42 °C by addition of an equal volume of 54 °C pre-warmed medium Incubation was then continued at 42°C for the indicated times before the bacteria were harvested. P1 wild-type and non-lysozymic WM874 bacteria were harvested at 28 °C at A550 = 0.6. For Northern blots single-stranded RNA was transcribed with T7 RNA polymerase in vitro (27) using EcoRI-linearized plasmid pAH1067a (b) to yield a run-off sense (antisense) transcript (Fig. 1) The transcripts were used as antisense and sense probes respectively Total cellular RNA (20 μg) was loaded in each lane. P1, P1 wild-type lysogen, (+), non-lysozymic

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<th>Plasmid</th>
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<tr>
<td>pAH1027</td>
<td>100</td>
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<tr>
<td>pAH1027-2</td>
<td>&lt;0.02</td>
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</table>

<sup>a</sup>100% = 4200 transformants

Figure 5. Sense and antisense transcription in the lytic replicon of prophage P1 Escherichia coli WM874 bacteria and their P1 wild-type and P1 c1ts lysogenes were grown at 28°C At A550 = 0.6 the temperature of the P1 c1ts lysogenic bacteria was shifted to 42°C by addition of an equal volume of 54°C pre-warmed medium Incubation was then continued at 42°C for the indicated times before the bacteria were harvested. P1 wild-type and non-lysozymic WM874 bacteria were harvested at 28 °C at A550 = 0.6. For Northern blots single-stranded RNA was transcribed with T7 RNA polymerase in vitro (27) using EcoRI-linearized plasmid pAH1067a (b) to yield a run-off sense (antisense) transcript (Fig. 1) The transcripts were used as antisense and sense probes respectively Total cellular RNA (20 μg) was loaded in each lane. P1, P1 wild-type lysogen, (+), non-lysozymic

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Table 2. Effect of a P53as promoter-down mutation on the transcriptional activities of P53 and P53as

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<td>pCB279a</td>
<td>800</td>
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Escherichia coli CB454 was transformed with the plasmids pCB147b or pCB279a, which carry the P53as wild-type or P53as2 promoter-down mutant Growth of bacteria and β-galactosidase assay were done as described in the legend to Table 1

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<tbody>
<tr>
<td>pAH1027</td>
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<tr>
<td>pAH1027-2</td>
<td>&lt;0.02</td>
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</tbody>
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<sup>a</sup>100% = 4200 transformants

Table 3. Requirement of C1 repressor and Lxc co-repressor for the maintenance of plasmid pAH1027-2

<table>
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<tr>
<th>Plasmid</th>
<th>WM874</th>
<th>WM874/pAM8 c1&lt;sup&gt;cor&lt;/sup&gt;-lxc&lt;sup&gt;+&lt;/sup&gt;</th>
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<td>pAH1027</td>
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<tr>
<td>pAH1027-2</td>
<td>&lt;0.02</td>
<td>11</td>
</tr>
</tbody>
</table>

<sup>a</sup>100% = 4200 transformants

![Figure 5](image-url)
of 31 and only seven codons respectively, neither initiation codon is preceded by a potential ribosome binding site. Moreover, no protein was detected upon expression of a P1 DNA EcoRI:14 fragment (positions -1226 to +657) containing expression vector pT7-6 or pTacl in which the T7φ10 or the tac promoter is superimposed on P53as.

The identification and characterization of promoter P53as reveals a second control element of the P1 lytic replisome aside from the C1-regulated operator–promoter element Op53-P53. Both elements have a negative effect on transcription of the P1 lytic replisome, apparently without affecting each other. Stimulation of P53 activity by methylation (Table 1), on the other hand, indicates that synthesis of repL protein is related to replication of the P1 DNA. What might be the function of the P53as transcript? Keeping in mind that P53as activity is much stronger on linear than on supercoiled DNA (Fig 4), we suggest that transcription from P53as serves to down-regulate P1 DNA replication at two stages of the P1 life cycle: (i) at the very beginning, upon infection, before the linear DNA is circularized by the phage-specific lxo–cre recombination system (3); (ii) during the late stage of infection, when replication of supercoiled P1 DNA is superceded by rolling circle replication and linear P1 DNA concatemers accumulate (32). In accordance with this hypothesis is the finding that the amount of P53as transcript increases strongly in the late stage of P1 development (Fig. 5, left part). In contrast, the amount of repL transcripts, which is roughly the same at 10 and 30 min after induction (Fig. 5, right part), is expected to decrease per P1 DNA molecule.

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