Regulation of phage Mu repressor transcription by IHF depends on the level of the early transcription

P.A.van Rijn, N.Goosen, S.C.H.J.Turk and P.van de Putte

Laboratory of Molecular Genetics, Department of Biochemistry, Gorlaeus Laboratoria, Leiden University, Einsteinweg 5, 2300 RA Leiden, The Netherlands

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ABSTRACT

Integration Host Factor (IHF) of *E.coli* can stimulate both early and repressor transcription of bacteriophage Mu. We introduced several mutations in the early promoter (Pe) and studied the effect of these mutations on the stimulation of early and repressor transcription by IHF. All mutant promoters are still positive regulated by IHF, but the level of stimulation is dependent on the strength of the promoter.

The strength of the early promoter has an even greater impact on the regulation of the repressor promoter by IHF: stimulation is observed in the presence of a relatively weak Pe, whereas with a strong Pe the repressor promoter Pc is inhibited by IHF. This inhibition is most probably due to an interference of the early transcription with the opposing repressor transcription. The implication of this type of regulation for the Mu life cycle is discussed.

INTRODUCTION

The temperate bacteriophage Mu uses converging promoters to regulate the lytic and lysogenic pathways (1). During lytic growth, the transposition genes are transcribed from the early promoter Pe, whereas in the lysogenic state the repressor is expressed (2,3). From *in vivo* transcription experiments two repressor promoters have been identified, the minor promoter Pc-1 and the major promoter Pc-2 (4). The Pc-2 promoter is identical to the major promoter Pcm that has been identified by *in vitro* transcription studies (5). The very weak Pc-1 promoter, which was thought to be situated outside the repressor gene is in fact inside this gene as recently has been found that the repressor is 22 amino acids longer than originally thought (E.Robinson, O.Levnah, E.Appela, J.Sussman and K.Mizuuchi cited in 6). Therefore the Pc-1 promoter can not contribute to the synthesis of the repressor protein and we will from now on refer to the major repressor promoter as Pc. Transcriptions from Pe and the major repressor promoter Pc are in opposite direction and overlapping by 36 base pairs, see figure 1 (1).

Lytic development of phage Mu is dependent on the Integration Host Factor (IHF) (7), an *Escherichia coli* protein consisting of two subunits which are the products of the *himA*- and *himD* (*hip*) genes (8,9). This heterodimer is a specific DNA binding protein and its binding site contains the consensus sequence PyAANNNTTGAT^A/_T (10,11). IHF has originally been discovered as an *E. coli* factor essential for the integration of the lambda DNA into the host genome (7). Now, it is known that IHF is involved in many different processes viz. replication, transposition, packaging and gene regulation (for a review see Friedman (12)).

It has been shown that the inability of Mu to form plaques in HimA- and HimD strains is due to a reduced early transcription in these cells (7,13). Isolated HimD independent

Mu phages all carry a T → C mutation in the -10 region of the early promoter (*pip* mutation), resulting in an increase of the early transcription (14,15). The IHF binding site (*ihf* site) involved in the stimulation of the early transcription is located approximately 50 base pairs upstream of Pe, figure 1 (5). Activation of the early transcription by IHF has been shown both *in vivo* (4) and *in vitro* (5) and requires a helix dependent orientation of IHF- and RNA polymerase binding sites on the DNA (16).

In vivo experiments with *galK* fusion plasmids revealed that also transcription from Pc is stimulated by binding of IHF to the same *ihf* site upstream of Pe. This *ihf* site is located 100 base pairs downstream of the repressor promoter and the *in vivo* experiments showed that this distance can be increased by at least another 100 basepairs without the loss of transcription activation (16). In contrast, *in vitro* experiments, using supercoiled plasmids as a substrate, showed that transcription from Pc is inhibited by IHF (5).

In this paper we have been able to dissolve this discrepancy. We show that the repressor transcription *in vivo* can either be stimulated or inhibited by IHF, depending on the strength of the early transcription.

MATERIAL AND METHODS

Bacterial strains and plasmids

Strain PP1674 is BW313 (*dut*, *ung*) (17) harbouring pGP655 (F' Tc^R) (18), was used to isolate ssDNA of M13mp11 derivatives. After site-directed mutagenesis using specific oligo's the DNA was transformed to JM101 (19). The activities of the several promoters in the presence or absence of IHF were determined in AB1157 (*galK*) (Adelberg collection) and the isogenic strain PP1954 (AB1157 *himD*). PP1954 was isolated by transduction of AB1157 with P1 lysate grown on MH5818 (*himD*, Tc^R) (20) according to standard procedures (21). The *himD* phenotype was controlled by plating of Mu and Mu *pip* phages (22).

Plasmids used in this study are schematically presented in figure 2. Plasmids pCA95 (23), pGP133, pGP134 and pGP139 (1), pGP182 and pGP185 (16) have been described. pGP188 was constructed by ligation of the *EcoRI*-*Clal* fragment of pGP134 (position 818 to 1063 of the Mu sequence) into pGP182, thereby placing the *galK* gene under the control of the δ Pe promoter. After introduction of the Pe mutations, the same fragment (position 818 to 1063 of the Mu sequence) was inserted in pGP182 resulting in pGP732 (δ Pe-*pip*), pGP750 (*wtPe*) and pGP758 (*wtPe-pip*). Plasmids containing the *galK* gene under control of the repressor promoter have the Mu sequence from 818 to 1242 inserted in pCA95 resulting in pGP740 (δ Pe-*pip*) and pGP752 (*wtPe*). Plasmid pGP740 was constructed by ligation of the *NheI*-*SmaI* fragment and pGP752 by ligation of the *HindIII* fragment of the mutated insert of the M13 derivative in pGP139. Plasmid pGP772 was made by insertion of the *EcoRI*-*Clal* fragment of pGP752 in pCA95. Introduction of the transcription terminator T1 of the *rmB* operon was done by insertion of the *EcoRI* fragment of pKK232-8 (24) in the *EcoRI* site of pGP133 and pGP752 (pGP773 and pGP774, respectively). All cloning procedures were carried out essentially as described by Maniatis (25).

Oligonucleotide directed mutagenesis

Site-directed mutagenesis was carried out as described by Kunkel (17). M13mp11 phage containing the *EcoRI*-*SmaI* fragment of pGP134 was grown on PP1674 in L broth containing 25 μ g/ml tetracyclin and enriched with 0.05 w/w% uracil. Usually we obtained a difference of plating efficiency on PP1674 and JM101 of 10⁻⁵ to 10⁻⁶. ssDNA was isolated as described (27). The oligonucleotide used to convert the δ Pe promoter to the *wtPe* promoter by introduction of one base pair at position 1000 was 5'-AAGCTTTTGTAAAGCTGC-3'.

The oligonucleotide 5'-CTAAAAGGATAATTA-3' was used to introduce the *pip* mutation at position 1020.

Hybridization was carried out in 20 μ l ligation buffer, containing 50 ng kinated oligonucleotide and about 300 ng ssDNA, by heating at 90 °C for 10 minutes and cooling down slowly until room temperature was reached. The second-strand synthesis was carried out with 2 units Klenow Large Fragment of DNA polymerase I and dNTP's (30 μ M each) in 30 μ l for 30 minutes at room temperature followed by ligation (0.2 units ligase and 1 μ l 8 mM ATP) in 40 μ l for at least 2 hours. After transformation to JM101 screening for mutations was carried out by sequencing with the dideoxy-method according to Sanger *et al.* (26).

Galactokinase assay

Galactokinase (GalK) activities of cells containing *galK* fusion plasmids were determined essentially as described (28). [14-C]-galactose was supplied by Amersham. All samples were measured in duplo and each experiment was done at least twice. GalK activity is expressed in units (u) which represents nanomoles galactose phosphorylated/min/ 10^8 cells at 32 °C. The ratio of GalK activities determined in AB1157 (*himD*⁺) and PP1954 (*himD*) represents the factor of transcription stimulation by IHF.

RESULTS

Deviations in the DNA sequence of the early promoter of phage Mu

The regulation of transcription from both the early promoter (Pe) and the repressor promoter (Pc) of phage Mu have been studied using *galK* fusion plasmids (4,16). We showed in these studies that both Pe and Pc transcriptions are stimulated by IHF, which binds to a site just upstream Pe.

The DNA sequence of the Mu regulatory region has been determined in two different laboratories (5,28). The published sequences differ however at two positions (positions 999 and 1000, see figure 3A). Since these differences are located in a very important part of the regulatory region viz. the spacer between the -35 and -10 regions of Pe, we wanted to find out which of these sequences correspond to our *galK* constructs. Surprisingly the Pe sequence of our constructs corresponded to neither one of the published sequences. The spacing between the -35 and -10 regions in our *galK* constructs consists of 16 base pairs instead of the 17 base pairs as determined by the other authors (figure 3A). To find out which of these sequences is the correct one, we sequenced the early promoter region of the phage Mu_{cts62}, from which all the clones in the different laboratories were derived. This revealed that the sequence as determined by Krause *et al.* is the correct wild type promoter sequence, and that the sequence determined by Priess *et al.*, which is in the EMBL data bank, contains two base substitutions and that our *galK* constructs were deleted for one base pair. As it is not certain in which way the presence of the point deletion could have affected the results of the IHF studies, we decided to repeat some of the regulation studies using the wild type promoter.

Instability of plasmids carrying the wild type early promoter

The correct wild type early promoter (*wrPe*) was constructed by introduction of the missing base pair in our early promoter (δ Pe) by oligo-directed mutagenesis (M & M). Then we cloned the Mu fragment corresponding to the *TaqI-BalI* fragment (position 818 to 1242, see figure 1) in the *galK* expression plasmid pGP182, see M & M. The resulting plasmid (pGP772) has the *galK* gene under transcriptional control of *wrPe* (figure 2). All the cloning experiments were done using an IHF⁻ recipient strain (PP1954). However when pGP772 was introduced in an IHF⁺ background (AB1157) no transformants were found. There

FIGURE 1. Schematic representation of the regulatory region of Mu. The reading frames of the repressors Ner and c are represented by open boxes. The IHF binding site is indicated by a filled box. The positions of the different promoters are shown and the directions of transcription are indicated by arrows. Relevant sites are T=*TaqI* and B=*BallI*. The line at the bottom indicates distance (in base pairs) from the left end of the Mu sequence.

are two possible explanations for this: either a high expression of *galK* by the IHF stimulated *wtPe* could be lethal for the cell, or the *wtPe* directed transcription could be interfering with plasmid replication. When the insert of pGP772 was inverted (pGP752, figure 2),

FIGURE 2. Schematic representation of the *galK* fusion plasmids. The open box indicates the reading frame of the *galK* gene. The IHF binding site is represented by a filled box. The arrows indicate the direction of the transcription from Pe and Pc promoters. Relevant sites are E=*EcoRI*, C=*ClaI*, H=*HindIII*, S=*SmaI* and N=*NheI*. Plasmids pGP185, pGP772, pGP188, pGP133, pGP773 and pGP134 carry the δ Pe promoter and pGP732 and pGP740 the δ Pe-*pip* promoter. Plasmids pGP750, pGP752 and pGP774 carry the *wtPe* promoter and pGP758 carries *wtPe-pip* promoter.

placing the *galK* gene under control of the much weaker promoter Pc, lethality was still observed. Apparently it is not the level of GalK expression which causes the inability to transform IHF⁺ cells, but the Pe transcription itself. This was confirmed by the insertion of the T1 transcription terminator of the *rnnB* operon (24) downstream *wrPe* (pGP774, see figure 2) leading to suppression of the instability, and normal transformation of IHF⁺ cells.

Since instability of plasmids containing δ Pe in IHF⁺ strains has never been observed, the properties of the *wrPe* plasmids already indicate that the *wrPe* promoter is much stronger than the δ Pe promoter. Surprisingly we observed however that when a shorter DNA fragment (positions 818–1063) containing *wrPe* was fused to *galK* (pGP750, figure 2) stable transformants in IHF⁺ could be obtained. The start of the early transcription is at position 1028, this indicates that sequences further downstream than 35 base pairs from the start of transcription are important for the expression from the early promoter. To measure more quantitatively the influence of the downstream sequences, we determined the GalK activities using the δ Pe promoter in pGP185 with the Mu fragment from position 818 to 1242 and pGP188 (positions 818–1063) in the IHF⁺- and IHF⁻ strains. In the IHF⁺ strain the GalK activity of the plasmid with the largest insert, pGP185 (346u) appears to be about 4.5 times higher than the GalK activity of pGP188 (80u) in which the downstream sequences are missing. Also in an IHF⁻ background the GalK activity of pGP185 (45u) is higher than the GalK activity of pGP188 (4u). This indicates that the downstream sequences are not required for the activation of the early transcription by IHF. The mechanism by which the downstream sequences cause a higher level of Pe directed expression is at present unknown (see discussion).

Transcriptional activity of Pe mutants in IHF⁺- and IHF⁻ background

We studied the transcriptional activity of different Pe mutants and the effect of IHF on this activity. For this purpose derivatives were used in which the downstream sequences of Pe have been deleted, since these plasmids can be stably maintained in both IHF⁻- and IHF⁺ strains (see previous section). First we compared the transcription of the wild type promoter and the promoter with the one base pair deletion, measuring GalK activity of pGP188 (δ Pe) and pGP750 (*wrPe*) in IHF⁺- and IHF⁻ backgrounds.

In addition two other early promoter mutants were constructed by introducing a point mutation in the -10 region (TATCTT $\rightarrow$ TATCCT) of this promoter in pGP188 and pGP750 resulting in pGP732 (δ Pe-*pip*) and pGP758 (*wrPe*-*pip*) respectively, see figure 2. This so called *pip* mutation is expected to be a promoter-up mutation, since Mu phages carrying this mutation show an increase in early transcription, thereby allowing the phage to form plaques on a *himD* strain (12). The GalK activities of the four different promoter variants (see figure 3B) were determined in IHF⁻- and IHF⁺ backgrounds. From the results presented in table 1 several conclusions can be drawn.

First, the comparison of the basic transcriptional level (in an IHF⁻ background) of pGP188 (δ Pe) and pGP750 (*wrPe*) reveals that deletion of one base pair between the -10 and -35 region results in a reduction of transcription by a factor of 10. This reduction is comparable to results obtained by Stephano *et al.* (29) who showed that changing the spacing region of the *lac* promoter from 17 to 16 base pairs also results in a decrease of transcription by a factor of 10.

Secondly, the introduction of the *pip* mutation causes an increase of the basic transcriptional level (in an IHF⁻ background) by a factor of 2. The effect of the *pip* mutation is independent on the strength of the promoter, since both the weak variant of Pe (δ Pe) and the *wrPe* are stimulated to the same extent. Since the -10 region is thought

FIGURE 3. A: DNA sequences of the early promoter region of: (a) published by Krause *et al.* (2), (b) published by Priess *et al.* (29), (c) the sequence of the *galK* fusion plasmids. B: DNA sequences of the Pe mutants. The -35 and -10 regions are indicated by bold symbols. Mutations are underlined and the transcription start is doubly underlined.

to play an important role in open complex formation (30), the *pip* mutation might facilitate this step in the transcription initiation process (see Discussion).

Finally it is clear from table 1 that all four promoters are still activated by IHF. The extent of activation however is highly dependent on the strength of the early promoter. The weakest promoter (δPe) is stimulated by a factor of 20, whereas the strongest promoter (wtPe-pip) is only stimulated by a factor of 3.

Effect of the Pe mutations on the level of repressor transcription

Next we determined the level of repressor transcription in the presence of the different Pe mutations. For this purpose DNA fragments with the different Pe mutations were introduced in *galK* fusion plasmids with the *galK* gene under control of the Pc promoter. The resulting plasmids pGP133 (δPe), pGP740 (δPe-pip) and pGP752 (wtPe) are indicated in figure 2. Since, as we have shown in the previous section, the very strong Pe transcription of pGP752 in the presence of IHF causes instability of this plasmid in IHF⁺ strains we used the derivative pGP774 carrying a transcription terminator to measure Pc activity. As a control the transcription terminator was also inserted in pGP133, resulting in pGP773 (δPe). The different constructs were introduced in IHF⁺- and IHF⁻ strains and the GalK activities were determined.

The presence of the terminator fragment has no effect on the basic level (in an IHF⁻ strain) of Pc transcription in the δPe construct (table 2). The terminator might have a slight effect on Pc transcription in the presence of IHF, but it is clear however that in both δPe constructs Pc transcription is activated by IHF approximately to the same extent.

In an IHF⁻ background the different mutations in Pe have no influence on the level of Pc transcription. Apparently, in the absence of IHF, the opposing transcription from

TABLE 1. GalK activities of Pe-*galK* fusion plasmids carrying different Pe mutations in IHF⁺- and IHF⁻ strains.

plasmid	Pe	AB1157 IHF ⁺	PP1954 IHF ⁻	factor of stimulation
pGP188	δPe	80	4	20.0
pGP732	δPe-pip	147	8	18.4
pGP750	wtPe	195	38	5.1
pGP758	wtPe-pip	270	84	3.2

TABLE 2. GalK activities of Pc-*galK* fusion plasmids carrying different Pe mutations in IHF⁺- and IHF⁻ strains.

plasmid	present Pe	terminator	AB1157 IHF ⁺	PP1954 IHF ⁻	factor of stimulation
pGP133	δ Pe	—	35	6	5.8
pGP773	δ Pe	+	25	6	4.2
pGP740	δ Pe- <i>pip</i>	—	6	7	0.86
pGP774	<i>wt</i> Pe	+	3	7	0.43

the early promoter in all constructs is not strong enough to inhibit Pc transcription. In the presence of IHF however there is a drastic effect of the strength of the Pe promoter on Pc transcription. In the presence of the slightly stronger δ Pe-*pip* promoter the Pc transcription is reduced about sixfold compared to Pc transcription in the presence of the relatively weak δ Pe. In the presence of the even stronger *wt*Pe promoter Pc transcription is further reduced. Apparently, in the presence of IHF the Pe directed transcription reaches a level high enough to inhibit the opposing Pc transcription. As a result the net effect of IHF on Pc transcription is an activation in the presence of a weak Pe, no effect in the presence of a slightly stronger Pe and an inhibition in the presence of a very strong Pe promoter.

DISCUSSION

The early promoter Pe and the major repressor promoter Pc play an important role in the Mu regulatory system (1). When the repressor promoter is turned on and the repressor expressed, Pe is shut off thereby preventing lytic development resulting in lysogeny. When the early promoter is turned on the transposition functions A and B and the Ner protein are expressed. Ner shuts down repressor synthesis and as a result the lytic state is maintained. Both Ner and repressor proteins also regulate their own synthesis.

In this paper we show that expression from the early promoter is relatively strong, especially when the region 35 base pairs beyond the transcription start is present. As a consequence plasmids harbouring the entire regulatory region are unstable in strains where the negative regulators of Pe transcription (Ner and repressor) are absent and the activator of Pe transcription (IHF) is present. This instability is most probably due to an interference of a very high level of Pe directed transcription with plasmid replication. The instability of plasmids with the regulatory region presumably caused the selection of the one base pair deletion between the -10 and -35 region in our original Pe-*galK* fusion constructs. This deletion results in a reduction of early transcription, which apparently is low enough to allow stable maintenance of the plasmids in the cell. In the DNA sequence of the early Mu region as published by Priess *et al.* (29) two base substitutions compared to the original Mu sequence are present within the early promoter. It is conceivable that also these authors selected for promoter-down mutations upon subcloning of the Mu DNA, although it is not known to us whether these mutations actually decrease the early transcription.

The tenfold stimulation of Pe transcription by the presence of the region 35 base pairs beyond the transcription start of this promoter is an interesting observation. The high transcription levels measured in the presence of the downstream region could be a reflection of an increased messenger stability caused by this region. We however regard this as unlikely since we have shown that the presence of the downstream region in combination with *wt*Pe causes instability of plasmids in an IHF⁺ background. As stated above this instability is most probably due to an interference of Pe transcription with plasmid replication and can

not be ascribed to lethality caused by the messenger. So we suggest that the downstream region activates Pe directed transcription. We envisage two possible explanations for this activation. First, the downstream region might speed up the elongation of transcription, thereby making the promoter sequences sooner available for a new round of transcription initiation. This putative effect on transcription elongation might be due to the presence of the translation start of the Ner protein in the activating region, which makes that the leader transcript of the *galK* messenger is translated when the activating region is present. In the absence of the activating region, when the leader transcript is not translated, RNA polymerase might pause at certain sites. In the presence of the Ner translation start, due to the coupling of translation and transcription a faster transcription through these pause sites might occur. Alternatively the activating region might stimulate the initiation of transcription from Pe. Since the activating sequence is located at least 35 base pairs downstream the transcription start, which is at least 15 base pairs beyond the region normally protected by RNA polymerase (5), it is not likely that the sequence itself would influence RNA polymerase binding. The sequence is also not involved in the activation of early transcription by IHF, since Pe transcription is still stimulated by IHF in the absence of the downstream region. Therefore, if the activating sequence is involved in initiation of transcription, this would probably occur through the binding of an additional activator protein.

Initiation of transcription occurs in three well defined steps (32). First, RNA polymerase binds to a promoter to form a 'closed complex'. Second, part of the promoter DNA is melted out to form an 'open complex' and finally binding of the first ribonucleoside triphosphate converts the open complex into an 'initiation complex'. The -10 region of a promoter is thought to play an important role in open complex formation (30). Drew *et al.* (33) showed that a change of the -10 region of the *tyrT* promoter from TATGAA to TATGAT not only increases the strength of the promoter, but also the probability of DNA unwinding of this region. We found that the change of the -10 region of the early promoter from TATCTT to TATCCT (the *pip* mutation) increases the strength of the promoter by a factor of 2. This factor of stimulation is independent whether Pe is relatively weak (δ Pe) or relatively strong (*wt*Pe). In contrast, the stimulation of Pe caused by the presence of the activator IHF is much higher when Pe is relatively weak (δ Pe) than when the early promoter is relatively strong (*wt*Pe). This might implicate that the *pip* mutation and the IHF activation affect two different steps of the transcription initiation process. Since the activation of early transcription by IHF requires a helix dependent orientation of IHF and RNA polymerase on the DNA (16), it is very likely that IHF stimulates the initial binding (closed complex formation) of RNA polymerase by protein-protein interactions. Stimulation of the initial RNA polymerase binding by IHF is also supported by the strongly reduced stimulation of early promoters with the optimal spacing of 17 base pairs. Since the *pip* mutation is probably involved in a different step of the initiation process, it might to facilitate the local melting of the DNA, leading to an increased open complex formation.

In previous publications (4,16), we showed that IHF also stimulates transcription from Pc *in vivo*. These results were inconsistent with the data published by Krause *et al.* (5) who showed that *in vitro* Pc transcription is inhibited by IHF. From the experiments described in this paper it is clear that the discrepancy can be explained by the different early promoter constructs used in both type of experiments. In our original *in vivo* experiments we used a mutated early promoter (δ Pe) that is relatively weak. In the presence

of this δ Pe promoter IHF activates Pc transcription. IHF has also been shown to stimulate repressor transcription in the absence of the early promoter (4). In the presence of the wild type promoter which is relatively strong however Pc transcription is inhibited by IHF. This is apparently not due to a directed inhibition of Pc transcription by IHF as postulated by Higgins *et al.* (34), but to the activation of the opposing early transcription, which now reaches a level high enough to interfere with repressor transcription. Below a certain level of Pe transcription no interference with Pc transcription is observed. In the absence of IHF Pc transcription is the same in the presence of *w*Pe and δ Pe, although the activity of both Pe variants differ by a factor of 10. Analogous results have been obtained by Adhya and Gottesman (35). They showed that transcription from a promoter can inhibit transcription from a downstream promoter (which in their case initiated transcription in the same direction) by what was referred to as 'promoter occlusion'. This promoter occlusion was also shown to be dependent on the strength of the upstream promoter. We argue that also in the case of the two convergent Pe- and Pc transcriptions, interference is not a result of two colliding RNA polymerases but due to the inhibition of Pc initiation by the presence of an elongating RNA polymerase from Pe. Therefore the interference is only observed when the time that Pc is not occupied by an elongating RNA polymerase is relatively short; that is when the frequency with which Pe is initiated is high enough.

Since we have shown that the stimulation of Pc transcription by IHF can only be observed when constructs with a mutated Pe are used, the question arises what the biological significance of the Pc activation by IHF might be. When the Mu DNA enters the host cell upon infection, both Pe and Pc can be turned on. In the presence of sufficient levels of IHF, Pe transcription will be activated and as a consequence Pc transcription will be reduced. Ner protein will be synthesized from the early transcript shutting off Pc and the phage will enter the lytic state. However occasionally (maybe due to some unknown factors) upon infection transcription from Pc reaches a level high enough to synthesize a small amount of repressor protein. It has been shown *in vitro* (5) that in the presence of a low concentration of repressor protein the operator of Pe (O_2) is occupied, whereas the operator of Pc (O_3) is still free. This could mean that now a situation is created whereby the early transcription is reduced by repressor and subsequently Pc transcription can be activated by IHF leading to a stable immune state. In this way a small shift in the balance between Pe- and Pc transcription can be made irreversible by IHF, resulting in a firm decision for lysogeny or for lytic development.

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