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This paper is dedicated to the memory of Professor Karol Taylor

The *Escherichia coli* RNA polymerase α subunit and transcriptional activation by bacteriophage λ CII protein[o]

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Bacteriophage λ is not able to lysogenise the *Escherichia coli rpoA341* mutant. This mutation causes a single amino acid substitution Lys271Glu in the C-terminal domain of the RNA polymerase α subunit (αCTD). Our previous studies indicated that the impaired lysogenisation of the *rpoA341* host is due to a defect in transcriptional activation by the phage CII protein and suggested a role for αCTD in this process. Here we used a series of truncation and point mutants in the *rpoA* gene placed on a plasmid to investigate the process of transcriptional activation by the cII gene product. Our results indicate that amino-acid residues 265, 268 and 271 in the α subunit may play an important role in the CII-mediated activation of the p_E promoter (most probably residue 271) or may be involved in putative interactions between αCTD and an UP-like element near p_E (most probably residues 265 and 268). Measurement of the activity of p_E-*lacZ*, p_I-*lacZ* and p_{aQ}-*lacZ* fusions in the *rpoA*$^+$ and *rpoA341* hosts demonstrated that the mechanism of activation of these CII-dependent promoters may be in each case different.

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Abbreviations: e.o.l., efficiency of lysogenisation; αCTD, C-terminal domain of the RNA polymerase α subunit; IPTG, isopropyl β-D-thiogalactoside; UP, upstream promoter DNA sequence.

Bacteriophage λ serves as a paradigm of molecular mechanisms of many general biological processes, including repression and activation of transcription initiation, antitermination of transcription, site-specific recombination, DNA replication, and even the control of development (for a recent review see [1]). There are two alternative pathways for bacteriophage λ development after infection of its host, *Escherichia coli* (see [1] and references therein). The lytic cycle involves phage DNA replication and expression of the phage late genes, i.e. genes coding for head and tail proteins and for proteins causing lysis of the host cell. This pathway leads to production of phage progeny, cell lysis and liberation of virions. Alternatively, the phage genome may be incorporated into a specific site within the host genome forming a stable prophage. Such a lysogenic cell can survive for many generations carrying an integrated phage genome which is replicated as a part of the bacterial chromosome.

For the lysogenic cycle it is essential to switch off expression of the genes involved in the lytic mode of bacteriophage development. This is achieved by the action of the CI repressor which blocks transcription from the main lytic promoters, p_R and p_L, while stimulating its own synthesis by activation of the p_M promoter. However, shortly after infection the p_M promoter remains inactive because it is repressed by Cro (as the *cro* gene is the first phage gene expressed after penetration of λ DNA into the *E. coli* cell) and it requires positive regulation by CI for maximal activity. The problem of the early inactivity of p_M is circumvented by the presence of another promoter, p_E. This promoter is positively regulated by the *c*II gene product. The activity of two other CII-dependent promoters, p_I (for transcription of the λ integrase gene) and p_{aQ} (directing synthesis of an anti-*Q* transcript) is also required for lysogenisation. In the absence of CII the activity of these three lysogenic promoters is extremely low. Therefore, as *c*II is expressed from the p_R promoter, paradoxically the phage must proceed a little way along the lytic pathway in order to enter the lysogenic pathway.

It has been demonstrated previously that bacteriophage λ cannot enter the lysogenic pathway in the host harbouring the *rpoA341* mutation [2] causing a single amino acid substitution Lys271Glu in the C-terminal domain of the RNA polymerase α subunit (αCTD) [3]. Since construction of an *rpoA341* λ lysogen was possible by P1 transduction of the mutant allele into the wild-type lysogen, it was concluded that an establishment rather than maintenance of the lysogenic state is blocked in the *rpoA341* host [2]. Subsequent studies demonstrated that the levels of transcripts from CII-activated promoters (p_E, p_I and p_{aQ}) were drastically decreased in the λ-infected *rpoA341* host relative to the otherwise isogenic *rpoA*$^+$ strain [4]. Since N-dependent transcriptional antitermination was impaired also in the *rpoA341* mutant [5], it seems possible that the observed reduced level of lysogenic transcripts was due to inefficient expression of the *c*II gene, which is located downstream of the t_{R1} terminator. However, neither overproduction of CII nor stabilisation of this activator protein resulted in a bypass of the block in the lysogenic pathway indicating that the activity of CII was probably impaired [6]. In fact, activation of the p_E-*lacZ* and p_I-*lacZ* fusions by CII was found to be impaired [6] suggesting that the CII protein is not able to act as a transcriptional activator in the *rpoA341* mutant host. Since *λcin1*, which is a mutant phage containing an additional CII-independent promoter for *c*I expression, is able to lysogenise the *rpoA341* mutant, it seems that abolition of p_E activation in the mutant host is the main reason for the block in the establishment of the lysogenic state [6]. These results may suggest that CII interacts directly with the C-terminal domain of the RNA polymerase α subunit during transcription activation. On the other hand, it was demonstrated that removal of the α C-terminal domain resulted in only about five-fold reduction

of CII-mediated activation of the p_E promoter *in vitro* [7]. This is in contrast to the complete abolition of p_E activity *in vivo* by the *rpoA341* mutation. Therefore, one might alternatively speculate that αCTD is not a contact site for CII or comprises only a part of this site, and that the Lys271Glu substitution may cause a drastic conformational change in the subunit which results in hindrance of the interaction of CII with its real target on the surface of RNA polymerase. It is worth noting that the *rpoA341* mutation also abolishes transcription activation of p_{melAB}, p_{araBAD} and p_{cysP} by the MelR, AraC and CysB proteins, respectively [8].

To investigate the mechanism of CII-mediated transcriptional activation in more detail we used a series of truncation and point mutants in the *rpoA* gene placed on a plasmid. We measured the efficiency of lysogenisation by bacteriophage λ as well as the activity of p_E-*lacZ*, p_I-*lacZ* and p_{aQ}-*lacZ* fusions. Our results indicate that CII protein may activate various promoters by different mechanisms. In the case of positive regulation of the p_E promoter, it seems that amino-acid residues 265, 268 and 271 in the RNA polymerase α subunit play an important role in this process.

MATERIALS AND METHODS

Bacterial strains, phages and plasmids. Otherwise isogenic (*araD139* Δ(*argF-lac*)*U169* Δ(*his-gnd*) *thi rpsL150 gltS*$_0$ *flbB5301 relA1 deoC1 rbsR*) *Escherichia coli rpoA*$^+$ (WAM106) and *rpoA341* (WAM105) strains were described previously [3]. A series of plasmids derived from pLAW2 (a pMB1-based replicon which contains the *lacI*^{Q} allele and the *rpoA*$^+$ gene under control of p_{lac}) [9] and bearing either truncated forms of *rpoA* or point mutations in this gene has already been described [10, 11]. Plasmid pMO23 [6] is a p15A-based replicon and contains the *cII* and *cIII* genes, each under the control of p_{tac}. Plasmid pJMH1 [2] is a pSC101 replicon carrying the *lacI*^{Q} allele. Phage λ *cI857S7* was used [12].

Gene fusions. The single copy p_E-*lacZ* fusion has already been described [6]. The p_I-*lacZ* fusion was constructed by PCR amplification of the bacteriophage λ DNA fragment encompassing the p_I promoter (from nucleotide 29001 to 29164) and cloning it into the *Eco*RI-*Sma*I sites of the pHG86 vector [13]. The following primers were used for PCR: 5′– CCC GAA CCC ATC GAC GAA and 5′– ACG AAT TCA ATA TTC GAA TTG AAG. The p_{aQ}-*lacZ* fusion was constructed by PCR amplification of the bacteriophage λ DNA fragment encompassing the p_{aQ} promoter (from nucleotide 44073 to 44286) and cloning it into the *Eco*RI-*Sma*I sites of the pHG86 vector [13]. The following primers were used for PCR: 5′- AAA CAA AAG GCT ATC AAC T and 5′- CAG AAT TCT GTT TTG GCA ATA TC. Both above mentioned PCR reactions were carried out using 30 cycles of a program of denaturation at 94°C for 30 s, annealing at 54°C for 30 s and extension at 72°C for 30 s.

Efficiency of lysogenisation. Efficiency of lysogenisation of *E. coli* hosts by bacteriophage λ*cI857S7* was measured as described previously [2].

β-Galactosidase activity. Activity of β-galactosidase was measured according to Miller [14].

RESULTS

Lysogenisation of *E. coli* hosts bearing different *rpoA* mutations by phage λ

Plasmids carrying different alleles of the *rpoA* gene under control of p_{lac} were introduced into *rpoA*$^+$ (WAM106) and *rpoA341* (WAM105) strains. We investigated the efficiency of lysogenisation of these strains with phage λ*cI857S7* (Table 1). In the control experiments we found normal efficiency of lyso-

Table 1. Efficiency of lysogenisation by bacteriophage λcI857S7 of *E. coli rpoA*$^+$ and *rpoA341* hosts bearing plasmids expressing different *rpoA* alleles

RNA polymerase α subunit expressed from a plasmid[a]	Efficiency of lysogenisation[b]	
	rpoA$^+$ host	*rpoA341*host
None	0.77	< 0.01
Wild-type α (α 329)	0.82	0.86
α 176	0.02	< 0.01
α 211+3	< 0.01	< 0.01
α 217+3	0.01	< 0.01
α 230+1	0.08	< 0.01
α 230+3	0.01	0.01
α 255+4	0.25	< 0.01
α 256	0.20	< 0.01
α 271+2	0.30	< 0.01
α 283+3	0.01	< 0.01
α 289+7	< 0.01	0.04
α 296+4	< 0.01	0.03
α 316+3	< 0.01	0.04
α 258A	0.81	0.87
α 259A	0.77	0.86
α 260A	0.75	0.85
α 263A	0.81	0.87
α 264A	0.82	0.86
α 265A	0.83	0.02
α 268A	0.80	0.06
α 269A	0.85	0.81
α 271A	0.80	0.36
α 273A	0.60	0.72

[a]The plasmids were derived from pLAW2 (see Materials and Methods). α – followed by a number denotes the length of the wild-type or truncated α derivative being expressed in each case; a number indicated after + denotes a C-terminal tail composed of amino acids not present in the wild-type α but present in the polypeptide as a result of the cloning procedure (see [10] for details). α – followed by a number and letter A denotes expression of an α derivative containing an alanine substitution at the indicated position.

[b]Bacteria were infected with phages at multiplicity of infection of 10; an efficiency of lysogenisation of 1 would represent 100% lysogens among survivors, and the presented results are referred to this value.

genisation (e.o.l.) of the *rpoA*$^+$ host devoid of plasmid and no lysogenisation of the *rpoA341* mutant, whereas the defect in the mutant host was alleviated by the *rpoA*$^+$ allele present on plasmid pLAW2. Then we measured the e.o.l. in strains bearing plasmids with truncated *rpoA* alleles. We found that in the *rpoA341* host only production of the longest mutant α subunits allowed phage λcI857S7 to form lysogens, but the e.o.l. was very low (Table 1). Interestingly, production of the truncated α polypeptides in the *rpoA*$^+$ host resulted in significant inhibition or even abolition of lysogenisation by bacteriophage λcI857S7. To find the amino acid residues in the α subunit possibly involved in the interaction with the CII activator, we used plasmids expressing *rpoA* point mutations causing substitution of individual amino-acids in αCTD by alanine. Expression of all tested alanine substitution mutants in the *rpoA*$^+$ host did not significantly change the efficiency of lysogenisation by λcI857S7. While most of these alanine substitutions in αCTD did not prevent complementation of the *rpoA341* mutation in the lysogenisation assay, one mutant allele (alanine substitution at position 271) complemented with a significantly lower efficiency and two mutant alleles (alanine substitutions at positions 265 and 268) provided very poor complementation (Table 1).

Analysis of the p_E-*lacZ* fusion

We analysed the activity of the p_E promoter using a p_E-*lacZ* fusion. As a source of the *c*II gene we used plasmid pMO23, bearing the *c*II and *c*III genes under control of p_{tac}. Since prolonged expression of these genes is lethal for *E. coli* [6], we used a compatible plasmid (pJMH1) bearing *lacI*^{Q} allele to suppress *c*II and *c*III synthesis until required. First, we looked for the optimal conditions for activation of the p_E-*lacZ* fusion by CII in the *rpoA*$^+$ host. We found that induction of *c*II and *c*III expression by addition of 0.1 mM IPTG to the culture gave the most efficient activation of the fusion. Both lower and higher IPTG concentrations caused a reduction in the effi-

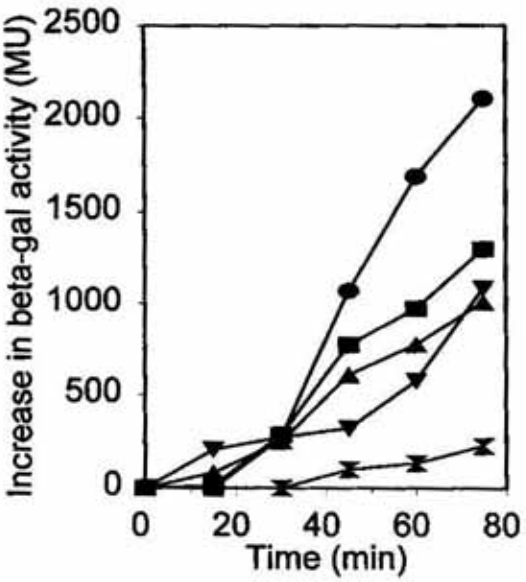

Figure 1. Activation of the p_E-*lacZ* fusion by different levels of CII protein at 30°C.

The *rpoA*$^+$ strain harbouring the p_E-*lacZ* fusion and pMO23 and pJMH1 plasmids was induced for cII expression by addition of IPTG into the culture to final concentration of 0.01 mM (◆), 0.05 mM (▼), 0.1 mM (●), 0.2 mM (■) and 0.5 mM (▲). The increase in β-galactosidase activity (in Miller units, MU) was measured at indicated times following IPTG induction.

ciency of activation indicating that high concentrations of CII have a negative effect on the activity of p_E (Fig. 1).

We analysed the activity of the p_E-*lacZ* fusion in the *rpoA*$^+$ and *rpoA341* hosts harbouring pMO23, pJMH1 and plasmids bearing one of the various *rpoA* alleles listed in Table 1. We found that the defect in CII-mediated activation of p_E in the *rpoA341* host may be alleviated partially or almost completely by some *rpoA* alleles expressed from a plasmid (examples are provided in Fig. 2). However, surprisingly, we found that the results obtained in this assay did not always completely correspond with the results obtained in the lysogenisation assay (Table 1) i.e. sometimes the p_E activity was relatively low in strains harbouring a plasmid with an *rpoA* allele which allowed for efficient lysogenisation. For example, the presence of pLAW2 plasmid (expressing wild-type *rpoA* allele) in the *rpoA341* host resulted in the efficiency of lysogenisation comparable to that observed in the *rpoA*$^+$ host, but in the analogous system in the p_E-*lacZ* fusion assay the activity of β-galactosidase increased only to about 350 units. The possible reasons for this discrepancy are presented in the Discussion.

Analysis of the p_I-*lacZ* and p_{aQ}-*lacZ* fusions

Apart from the p_E promoter, the CII protein activates two other phage λ promoters, p_I and p_{aQ}. We have shown previously that activation of the p_I promoter is also impaired in the *rpoA341* mutant [6] but the fusion used in that work was of low activity (the p_I-*lacZ* fusion was constructed by obtaining a double lysogen containing λ*imm*21*lacW205*Dt$_I$53) and λ*cI857S7* thus a possible low efficiency of p_I activation could be overlooked. Here we constructed p_I-*lacZ* and p_{aQ}-*lacZ* fusions of high activity and measured the β-galactosidase activity of these fusions in *rpoA*$^+$ and *rpoA341* hosts harbouring pMO23 and pJMH1 plas-

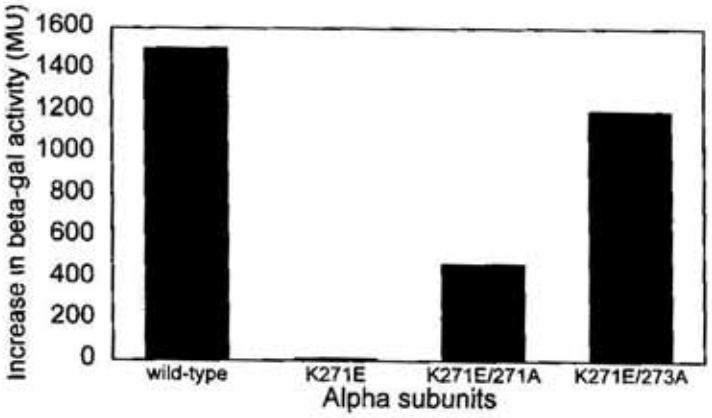

Figure 2. Activation of the p_E-*lacZ* fusion by CII in bacteria bearing different *rpoA* alleles on the chromosome and on plasmids at 30°C (the nature of the alleles expressed from the plasmids is given after the slash).

The strains bearing the p_E-*lacZ* fusion and pMO23, pJMH1 and (in some cases) plasmids producing mutant α polypeptides were used. The increase in β-galactosidase activity (in Miller units, MU) was measured 60 min after induction by IPTG (0.1 mM).

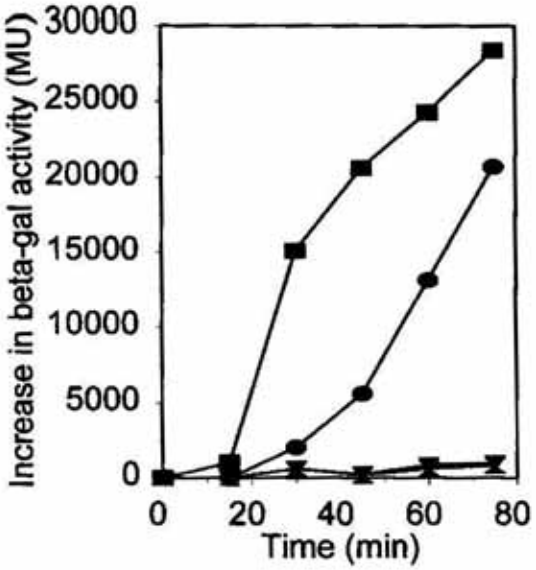

Figure 3. Activation of the p_I-*lacZ* fusion by CII in *rpoA*$^+$ (■ and ▼) and *rpoA341* (● and ◆) hosts at 30°C.

Bacteria harbouring the p_I-*lacZ* fusion and pMO23 and pJMH1 plasmids were either untreated (◆ and ▼) or induced with 0.1 mM IPTG (■ and ●). The increase in β-galactosidase activity (in Miller units, MU) was measured at indicated times following IPTG induction.

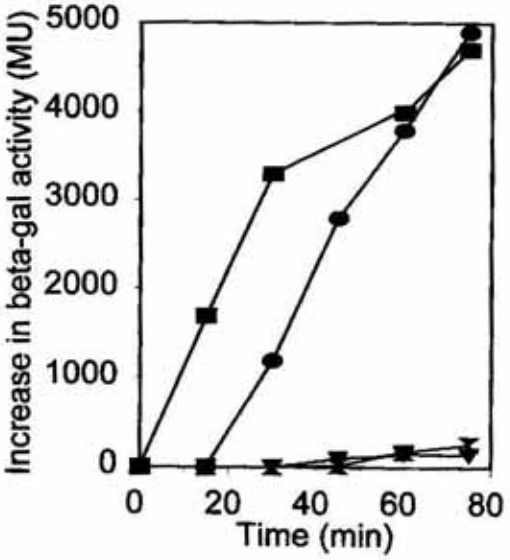

Figure 4. Activation of the p_{aQ}-*lacZ* fusion by CII in *rpoA*$^+$ (■ and ▼) and *rpoA341* (● and ◆) hosts at 30°C.

Bacteria harbouring the p_{aQ}-*lacZ* fusion and pMO23 and pJMH1 plasmids were either untreated (◆ and ▼) or induced with 0.1 mM IPTG (■ and ●). The increase in β-galactosidase activity (in Miller units, MU) was measured at indicated times following IPTG induction.

mids. In accordance with our previous observations, we found that activation of the p_I promoter was impaired in the *rpoA341* mutant, though in our new system we were able to measure substantial p_I activity in the mutant background (Fig. 3). Perhaps surprisingly we found that activation of the p_{aQ} promoter was efficient in both *rpoA*$^+$ and *rpoA341* strains, though a delay in activation in the *rpoA341* host was observed (Fig. 4). These results indicate that mechanisms of activation of p_E, p_I and p_{aQ} promoters by the CII protein must be different.

DISCUSSION

Bacteriophage λ is unable to lysogenise the *E. coli rpoA341* mutant [2] due to impaired activation of the p_E promoter by the phage CII protein [6]. As it has become apparent that the RNA polymerase α subunit serves as a contact site for many transcriptional activators (for reviews see [15] and [16]) the most obvious hypothesis explaining our observations is that the C-terminal domain of the α subunit is a contact site for the CII activator. Nevertheless, *in vitro* studies have demonstrated that some CII-mediated transcriptional activation is possible when RNA polymerase is devoid of αCTD [7]. To investigate transcription activation by CII in more detail, we employed a series of plasmids coding for truncated α polypeptides and for α subunits with single amino acids changed for alanine. Since the *rpoA341* mutation is recessive, a strain carrying this allele on the chromosome can be used to assess the ability of other *rpoA* alleles present on a plasmid to complement the defective phenotypes of *rpoA341* [3, 10]. Using this system we found that the full-length (329 amino-acid residues) α subunit, but not truncated derivatives, is necessary for efficient lysogenisation by bacteriophage λ. Since the *rpoA341*-mediated block in lysogenisation is due to the abolition of CII-dependent activation of the p_E promoter [6], one may conclude that the full-

length α subunit is also necessary for efficient activation of p_E by CII *in vivo*. Our results with the alanine substitution mutants may also indicate that amino-acid residues 265, 268 and 271 are important for this activation. It seems that the best candidate for a contact site with CII is residue 271 of αCTD, as Lys271Glu substitution caused by the *rpoA341* allele impairs also other activation systems [8] and this allele may be partially complemented in both lysogenisation and p_E-*lacZ* fusion assays by the Lys271Ala mutant. On the other hand, residues 265 and 268 (but not 271) are known for their role in the activation of certain promoters by interaction of αCTD with the so called UP-element, a DNA sequence rich in AT base pairs located upstream of the promoter (for a review see [15] and [16]). Although there is no sequence resembling the UP-element near the p_E promoter [6], recent studies demonstrated that the p_M promoter could be stimulated by sequence nonspecific interactions of αCTD with a DNA region located upstream of the promoter [17]. An alternative explanation of our results would be that truncated and some of alanine substitution α mutants are defective in N-dependent transcriptional antitermination leading to inefficient expression of cII. This explanation, however, seems to be unlikely, as although the *rpoA341* mutation results in impaired N-dependent transcriptional antitermination [5], it was demonstrated that expression of α polypeptides devoid of the C-terminal domain has a positive effect on the antitermination efficiency *in vivo* [18]. The possibility also remains that the Lys271Glu substitution in αCTD, caused by the *rpoA341* allele, provokes a drastic conformational change in the α subunit which prevents interaction with some activators or even interaction with a sequence nonspecific UP-like element. In this regard it is worth mentioning that although transcription activation by the MelR activator is impaired in the *rpoA341* host, the Lys271Glu substitution does not define an activation contact site for MelR [10].

Unexpected results were also obtained with the truncated α polypeptides expressed in the *rpoA*$^+$ host. Expression of the shortest and the longest truncated α polypetides resulted in almost complete abolition of lysogenisation, whereas expression of the polypeptides of medium length allowed for some lysogenisation. The α polypeptides from 176 to 230+1 are defective in the assembly of α subunit dimers [10]. One might speculate that CII contacts the N-terminal domain of α (αNTD). If so, then expression of these truncated derivatives could titrate CII causing absence of the activity of this protein in the wild-type cells infected by λ phage. The α derivatives from 230+3 to 271+2 are essentially devoid of the C-terminal domain (α 271 has a small bit of it) but are assembly proficient [10]. Decreased efficiency of lysogenisation of the *rpoA341* host expressing these truncated polypeptides indicates again that αCTD is also required for CII-mediated transcription activation. Perhaps the presence of some wild-type α homodimers and some heterodimers results in relatively low, but still significant, efficiency of lysogenisation. The α polypetides from 283+3 to 316+3 contain an incomplete (and perhaps unstructured) C-terminal domain. This probably may interefere with the activity of CII due to incorrect spatial orientation. The presence of some wild-type homodimers in the *rpoA*$^+$ host would mean that CII could retain some activity. However, this could be below the threshold required for lysogenisation. If this explanation were true, one should assume that α polypetides 283+3 to 316+3 form heterodimers with wild-type α more efficiently than do α polypeptides 230+3 to 271+2, or that heterodimers containing wild-type α and the shorter derivatives (230+3 to 271+2), but not those containing wild-type α and the longer derivatives (283+3 to 316+3), retain some activity during CII-mediated stimulation of transcription.

The effect of the mutant α derivatives on p_E-*lacZ* expression in many cases did not corre-

spond with their effect on the efficiency of lysogenisation. In some cases a low β-galactosidase activity was observed in strains producing an α derivative which efficiently complemented the *rpoA341* mutant in the lysogenisation assay, and *vice versa*. There are several possible reasons for this discrepancy. In the lysogenisation assay we used strains harbouring only plasmids with *rpoA* alleles. Although these plasmids harbour also the $lacI^Q$ allele, effective expression of the *rpoA* allele (being under control of p_{lac}) from the plasmid occurred even without induction, as efficient complementation of the chromosomal *rpoA341* allele was observed in these conditions. On the other hand, in experiments with the p_E-*lacZ* fusion we used, in addition, pMO23 (for expression of *c*II) and pJMH1 (bearing the $lacI^Q$ allele). The presence of pJMH1 was necessary to keep expression of *c*II repressed until required (prolonged expression of *c*II is lethal for *E. coli*), but introduction of another $lacI^Q$ allele (apart from that located on a pLAW2 derivative) also prevented expression of the *rpoA* alleles from a plasmid. Therefore, in these experiments, expression of mutant *rpoA* alleles was induced simultaneously with expression of *c*II at the beginning of the experiment. This could lead to significantly different production of mutant α polypeptides in the fusion experiments than in the lysogenisation experiments. Furthermore, it was demonstrated that truncated α polypeptides of more than 271 residues in length are sensitised to proteolysis near Lys 271 [10]. This, together with above mentioned differences in expression of *rpoA* alleles, would result in significantly different conditions in two assays used by us. In fact, when the lysogenisation experiments were repeated using IPTG-induced strains harbouring pMO23 and pJMH1 together with pLAW2 derivatives, in many cases we observed a significantly lower efficiency of lysogenisation of both *rpoA341* and $rpoA^+$ hosts. Nevertheless, we were able to demonstrate that complete inhibition of CII-mediated activation of the p_E-*lacZ* fusion may be alleviated to different extents by expression of different *rpoA* alleles. This is compatible with our proposal that αCTD plays an important role in the positive regulation of p_E activity.

Perhaps surprisingly we found that the p_E, p_I and p_{aQ} promoters, which are all dependent on CII for activity, respond differentially to CII in the *rpoA341* host as measured by the activity of appropriate *lacZ* fusions. The p_E promoter seems to be completely unresponsive to CII in the *rpoA341*strain, whereas p_I responds to CII with a decreased efficiency in the mutant host, and p_{aQ} is activated as efficiently in *rpoA341* bacteria as in the $rpoA^+$ host (though a delay in the activation was observed in the *rpoA341* mutant). Therefore, we conclude that the mechanisms of activation of these promoters by CII must differ considerably.

The problem remains why we observed drastically decreased levels of transcripts from all CII-dependent promoters in the λ-infected *rpoA341* host relative to the $rpoA^+$ strain [4] whereas, according to the reporter gene fusion experiments, only transcription from p_E is abolished in the mutant host. The possible explanation of this phenomenon is that expression of the *c*II gene is inefficient in the λ-infected *rpoA341* mutant. This gene is under control of the p_R promoter but is located downstream of the t_{R1} terminator, therefore it requires N-dependent transcriptional antitermination for maximal expression. It was demonstrated that N-dependent transcriptional antitermination is impaired in the *rpoA341* mutant [5], thus *c*II expression may be relatively depressed in this host. Moreover, CII protein is rapidly degraded in *E. coli* by the HflB/FtsH protease, but it is partially stabilised by the *c*III gene product which negatively regulates the activity of HflB/FtsH [19]. The *c*III gene is under control of the p_L promoter and is located downstream of the t_{L1} terminator. Thus the expression of this gene may also be decreased in the *rpoA341* host due to impaired N-dependent transcriptional antitermination. Therefore, the combined ef-

fect of decreased production and stability of CII may be a reason for the low level of p_I- and p_{aQ}-derived transcripts observed in the λ-infected *rpoA341* host. In our experiments with gene fusions, the *cII* gene was expressed from p_{tac} promoter which is active in both *rpoA*$^+$ and *rpoA341* strains [6].

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