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The nucleotide sequence of the bacteriocin promoters of plasmids Clo DF13 and Col E1: role of lexA repressor and cAMP in the regulation of promoter activity

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ABSTRACT

Treatment of cells, harbouring the bacteriocinogenic plasmid Clo DF13 with mitomycin-C, which induces the cellulair SOS response, results in a significantly increased transcription of the operon encoding the bacteriocin cloacin DF13, the immunity protein and the lysis protein H. The nucleotide sequences of the promoter regions and N-terminal parts of the bacteriocin genes of Clo DF13, Col E1 and the pMB1 derivative pBR324 have been determined. A comparison of these sequences with those of corresponding regions of the lexA, recA and uvrB genes revealed that the promoter regions of the bacteriocin genes studied contain binding sites for the lexA protein, which is the repressor of the E. coli DNA-repair system. Using both, a thermosensitive lexA host strain and a host with pACYC184 into which the lexA gene had been cloned, we were able to demonstrate, that in vivo the lexA protein is involved in the regulation of bacteriocin synthesis.

From the data presented, we conclude that bacteriocin synthesis is controlled at least by the lexA repressor. It has been reported that also catabolite repression might play an essential role in the control of bacteriocin synthesis. Computer analysis of the DNA sequence data indicated that the promoter regions of both, the cloacin DF13 and colicin E1 genes contain potential binding sites for the cyclic AMP-cyclic AMP Receptor Protein complex.

INTRODUCTION

Plasmid Clo DF13, originally isolated from Enterobacter cloacae, is studied mostly in Escherichia coli (1). This small (mw 6×10^6D; 2), non-conjugative plasmid encodes at least 9 proteins. The genes encoding these proteins have been localized on the plasmid genome and functions have been assigned to 8 of their gene products (3, 4). The best known are the 3 gene products of the bacteriocin operon: the bacteriocin, cloacin DF13 (56 KD), the immunity protein (9 KD; 5) and the lysisprotein H (6 KD; 3, 6). The cloacin protein inhibits protein synthesis in sensitive bacteria by an endoribonucleolytic cleavage of a 49 nucleotide fragment from the 3'-terminus of the 16 S ribosomal RNA (7). The immunity protein has been shown to be

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an inhibitor of the biochemical activity of the cloacin protein (8).
Recently it has been reported that the genes encoding cloacin DF13 and the immunity protein are located in one operon, which is transcribed into two polycistronic mRNAs of 2200 and 2400 nucleotides (2, 9; fig. 1). The 2400 nucleotides RNA, which differs in length from the 2200 nucleotide RNA at its 3'-end, terminates at 9% on the Clo DF13 map, and also contains the message for the third protein, protein H. A high concentration of this protein in the bacterial cell results in lysis of the cell (6).

Despite their entirely different mode of action (7, 10), the synthesis of cloacin and colicin E1, just like colicin E3 and colicin Ib seems to be controlled by the same regulatory mechanisms. An increased synthesis of bacteriocins can be observed after treatment of cells with mitomycin-C or UV-light, agents which are known to induce the DNA repair system (11), or in host cells carrying a lexA$^-$ mutation (12; this paper). These phenomena suggest that the lexA protein, which is cleaved by recA protein upon DNA damage (13) is involved in the regulation of bacteriocin synthesis.
Recently it has been demonstrated that the lexA protein binds at specific DNA sequences, the so-called "SOS"-boxes (14, 15). This sequence of 20 base pairs is located within the regulatory regions of the recA and lexA genes.

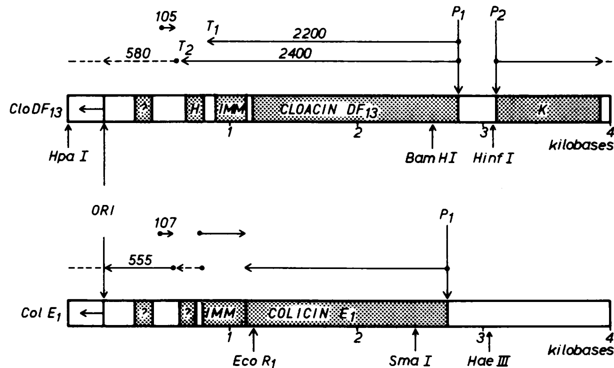

Figure 1. Transcriptional maps of the Clo DF13 and Col E1 DNA region containing the origin of replication (ORI) as well as the genes encoding the bacteriocin proteins and the immunity (IMM) proteins. The direction of transcription is indicated by arrows, whereas the estimated length of the RNAs is given in nucleotides. P1, the cloacin/colicin promoter; P2 promoter of gene K; T1 and T2, termination sites 1 and 2. This figure is based on the data presented by van den Elzen et al (2, 9) and Veltkamp and Stuitje (4).

In this paper we present evidence that the regulation of cloacin synthesis takes place at the transcriptional level. We elucidated the DNA sequence of the cloacin and colicin E1 promoter regions. Base sequence analysis revealed that both DNA sequences contain "SOS"-boxes. The results presented in this paper indicate that the lexA repressor is directly involved in the control of bacteriocin synthesis. The analysis of the base sequences also revealed the presence of potential cAMP-CAP binding sites. The possible role of catabolite repression in the regulation of bacteriocin synthesis (16, 17) is discussed.

MATERIALS AND METHODS

Bacterial strains and plasmids

The plasmids used in this study are listed in Table 1. The host strain for Clo DF13 plasmids was *Escherichia coli* P678-54 (18), whereas *Escherichia coli* K12-294 (19) was used as a host for RSF2124 and pBR324. DM511 (*lexA*3, *tsl*-1, 20) was used to study the control of bacteriocin synthesis by *lexA*.

Media, Chemicals and Enzymes

Liquid culture (LC) medium (Difco) and Lab Lemco (LL) broth (Oxoid Ltd, London) were normally used for growth in liquid and on agar plates. Where specified, the following antibiotics were included in the media: ampicillin (Ap, 50 µg/ml), tetracyclin (Tc, 25 µg/ml), kanamycin (Km, 40 µg/ml), chloramphenicol (Cm, 25 µg/ml). Restriction endonucleases were purchased from Boehringer Mannheim (FRG) and New England Biolabs. T4 poly-

Table 1. Bacterial plasmids

Plasmid	Selection marker	Relevant characteristics	Source or reference
Clo DF13-*cop*3		copy mutant, cloacin$^+$	4
pVU30	Km^r	wt Clo DF13::Tn5	Hakkaart, unpublished
RSF2124	Ap^r	Col E1::Tn3 colicin$^+$	21,22
pBR324	Ap^r, Tc^r	pMB1 derivative colicin$^+$	23
pACYC184	Cm^r, Tc^r		24
pJA03	Cm^r, Tc^r, Ap^r	pACYC184 derivative *lexA*$^+$	Brandsma, unpublished

nucleotide kinase, DNA polymerase I and DNA polymerase I, according to Klenow, were supplied by Boehringer, Mannheim. γ-^{32}P-ATP (>2000 Ci/mmole) and α-^{32}P-dNTPs (350 Ci/mmole) were from the Radiochemical Centre, Amersham, England.

Isolation of plasmid DNA and restriction enzyme analysis

Clo DF13 plasmid DNA was isolated by the alkaline lysis procedure as described by Birnboim and Doly (25) for 10-40 ml cultures. This procedure was scaled up and used to isolate DNA from 1-3 litre cultures. If necessary, the DNA was purified further by phenol extraction or column chromatography on Bio-Gel A-50M. Plasmid DNA to be used for hybridization experiments was subjected to centrifugation in CsCl-ethidium bromide gradients.

Digestion of DNA with restriction endonucleases was carried out in reaction mixtures recommended by New England Biolabs.

Isolation of DNA restriction fragments and single-stranded DNA

DNA fragments or single-stranded DNA, separated on 5% acrylamide gels were eluted according to Maxam and Gilbert (26). After electrophoresis of DNA fragments on 1-2% agarose gels, the DNA was eluted electrophoretically in 20 mM Tris-HCl pH 7.6, 10 mM NaAc, 1 mM EDTA. The electrophoretic elution of DNA was performed using an electrophoretic sample concentrator supplied by Isco; in this way the DNA was concentrated on a dialysis membrane of 33 mm^2, after which the DNA was recovered in a final volume of 200 μl and precipitated twice with ethanol. If necessary, ethidium bromide was removed by phenol extraction before precipitation.

DNA sequence analysis procedures

Clo DF13 DNA restriction fragments, labeled at one 5'-end and single DNA strands were sequenced using the chemical degradation procedure of Maxam and Gilbert (26). Eight and twelve percent sequence gels were prepared as described previously (27).

Nucleotide sequence determination of Col E1 and pBR324 was performed according to three published procedures as indicated in fig. 3. The Maxam and Gilbert procedure has been performed as described (28). The nick-translation/dideoxy procedure applied to 5'-^{32}P-labeled double stranded DNA fragments was performed exactly as described by Maat and Smith (27), except for the incubation time which was 30 min at 37°C. The third procedure used was the primed synthesis/dideoxy procedure described by Sanger and Coulson (29). To generate a single stranded DNA template, the Hae III - Sma I DNA fragment containing the colicin E1 promoter region (21, fig. 1) was supplied with Eco R1-linkers using T4 DNA ligase, cleaved with Eco RI and cloned into

M13 mp2 (30). M13 phage, containing the complementary strand of the Col E1-DNA sequence displayed in fig. 3, was isolated according to the procedure described by Herrmann et al (28). Either a chemically synthesized oligomer (5'-CCCAGTCACGACGTT-3') or an exonuclease-III treated DNA-fragment, generated by various restriction endonuclease cleavages was used to prime chain-elongation, catalyzed by *E. coli* DNA polymerase, according to Klenow. Treatment with exonuclease III was performed on 1-2 picomoles of DNA-fragment in 50 mM Tris-HCl pH 8.0, 1 mM $MgCl_2$, 1 mM DTT, with 5 units of exonuclease III (BRL) during 30 minutes at 37°C. The reaction was terminated by boiling for three min. The primed synthesis dideoxy procedure was performed according to Sanger et al. (29) with the exception that all dNTPs were labeled with ^{32}P on the α-position. Each reaction contained 1 μCi (or 2.5 pmole) α-^{32}P-labeled dGTP, dATP, dTTP and dCTP and 250 pmoles of the dideoxy analogue in turn.

Computer analysis of DNA base sequences

Analysis of the DNA base sequences was performed according to Staden (31), using his computer programs, which were adapted for computer HP-1000 with operating system RTE-IVB.

Isolation of RNA, electrophoresis, transfer to DBM-paper and hybridization

RNA was isolated from Clo DF13 harbouring cells, separated on 1.8% agarose gels containing methylmercuric hydroxide and transferred to diazobenzyl oxymethyl (DBM) paper as described previously (2). Hybridization with nick-translated Clo DF13 DNA (32), washing and autoradiography, was carried out according to Wahl et al. (33).

Assay for cloacin synthesis

The amount of cloacin DF13, produced by Clo DF13 harbouring cells was determined using an *in vivo* assay described by De Graaf et al. (34). For this purpose 10 ml samples of the culture were extracted with chloroform and serial twofold dilutions were made in 4 ml of LL broth, buffered with 10 mM phosphate (pH 7.0). 5 x 10^8 Cells of *Klebsiella* indicator strain were added and after 2 h growth at 37°C (shaking), killing activity was determined. One killing unit per ml is defined as the amount of cloacin DF13 required to permit 50% growth of the indicator strain.

RESULTS

Effect of mitomycin-C on Clo DF13 specific RNA synthesis

It was reported previously (6, 35) that treatment of cells, harbouring Clo DF13, with mitomycin-C results in an increased synthesis of the cloacin

protein, the immunity protein and protein H. Since it was also demonstrated that the presence of the cloacin promoter is essential for this phenomenon (6), we anticipated that this might be the result of an overproduction of the 2200 and 2400 nucleotide RNAs, the transcripts of the cloacin operon (fig. 1).

To obtain evidence for this hypothesis we grew E. coli P678-54 harbouring Clo DF13-cop3 in LC-medium to an optical density of 0.1 at 660 nanometer, after which mitomycin-C (0.5 μg/ml) was added to one half of the culture. After 0, 30, 60 and 90 min, total RNA was isolated from samples of both cultures. Fifty microgram of each RNA sample was loaded on a 1.8% agarose gel with methylmercuric hydroxide. The RNAs were transferred from the gel to DBM-paper and hybridized with 10 μg ^{32}P-labeled Clo DF13 plasmid DNA (10^7 dpm/μg).

The resulting autoradiograph (fig. 2) shows that the addition of mitomycin-C to Clo DF13 harbouring cells results, after 60 to 90 minutes, in a drastic increase in the synthesis of the 2200 and 2400 nucleotide RNAs. In fig. 2, also an increased background hybridization can be observed in tracks e and g. We suggest that this background, which is suppressed at the position of 16 S ribosomal RNA, reflects breakdown products of the 2200 and 2400 nucl. RNAs.

Densitometric scanning of the autoradiograph (not shown) revealed that, after subtracting the background, there was no significant difference in intensity of the 800, 700 and 500 nucleotide RNA bands in tracks a, c, e and g compared with tracks b, d, f and h respectively. It was also observed that mitomycin-C results in an at least 60-fold increased synthesis of the 2400 and 2200 nucleotide RNAs after 60 and 90 minutes.

From the result of this experiment we conclude that transcription of the bacteriocin operon is activated strongly by the addition of mitomycin-C.

Nucleotide sequence analysis of the bacteriocin promoter regions of plasmids Clo DF13, RSF2124 and pBR324

The results of our hybridization experiment indicates that mitomycin-C at least affects the expression of the cloacin operon at the transcriptional level. To get more insight into the nature of the regulatory sequences, we analysed the DNA base sequence of the cloacin promoter and the DNA regions of plasmids RSF2124 and pBR324 containing the colicin promoters of Col E1 (22) and pMB1 (23).

Previously it was reported (2) that the cloacin promoter is located at about 31% on the Clo DF13 genome (fig. 1). In this paper we present the

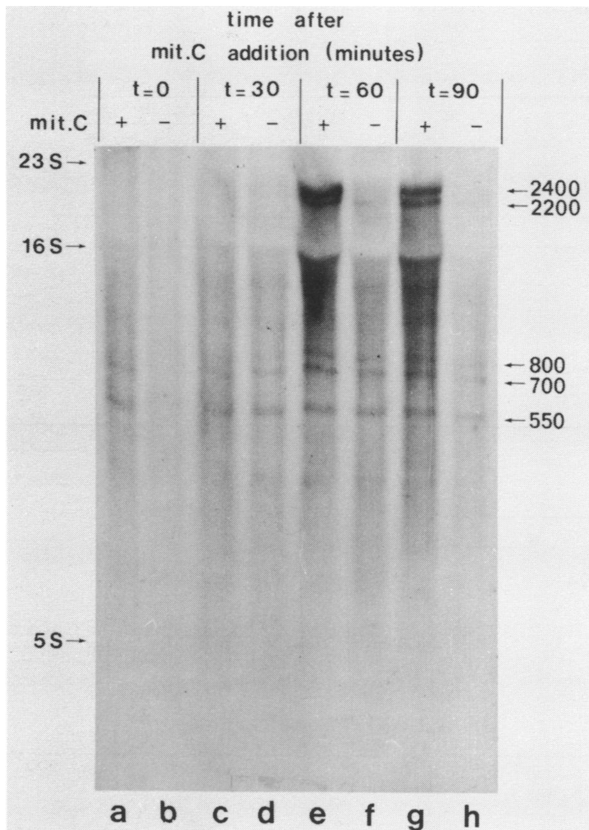

Figure 2. Effect of mitomycin-C on plasmid specific RNA-synthesis in E. coli cells harbouring Clo DF13-cop3. RNA isolation, electrophoresis, transfer to DBM-paper and hybridization with Clo DF13 DNA was performed as described in Results and Materials and Methods. Indicated are, the length of the RNA molecules in nucleotides, as well as the position of the ribosomal RNAs (16, 23 and 5 S) in the gel.

nucleotide sequence of the entire DNA region between 29% (Bam HI site) and 34% (Hinf I site). The sequence strategy applied, using the chemical degradation procedure of Maxam and Gilbert, is presented in fig. 3.

The N-terminal part of the colicin gene on plasmid Col E1 has been localized previously between 0.77 and 0.78 map units on the plasmid genome (21), which is approximately 1450 bp from the Eco RI site (fig. 1). The Hae III - Sma I fragment (fig. 1) was therefore isolated from plasmid RSF2124 (Col E1::Tn3; 22), cloned in M13 mp2 and sequenced as described in

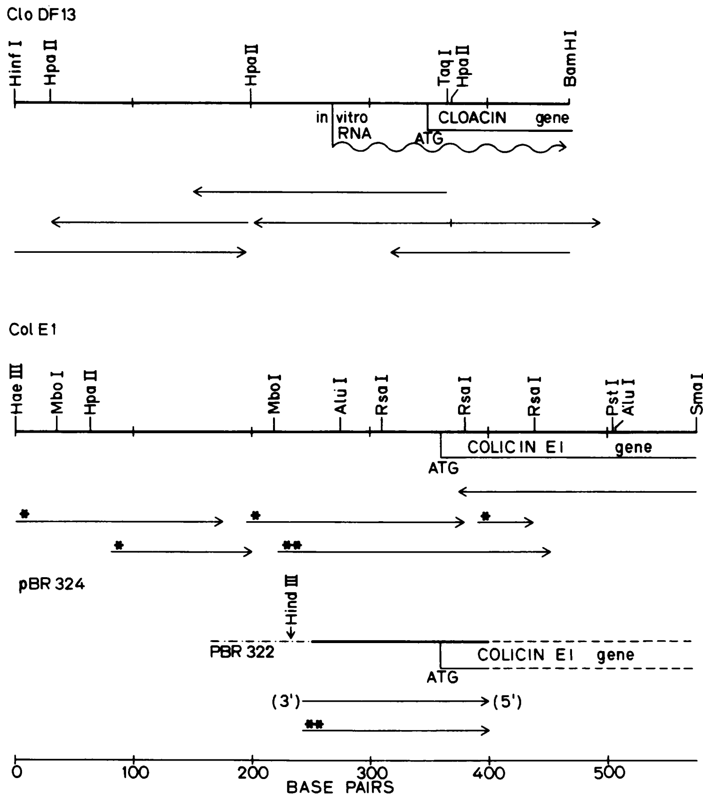

Figure 3. Sequencing strategy for the bacteriocin promoter regions of Clo DF13, Col E1 and pBR324. The arrows indicate at which site the radioactive label was introduced as well as the length of the sequence that was determined. Unless indicated otherwise, the sequence was read from 5' → 3'. The DNA sequence methods used, were the chemical degradation method (⟶) of Maxam and Gilbert (26), the primed synthesis/dideoxy procedure (*⟶) of Sanger (29) and the nick translation/dideoxy procedure (**⟶) described by Maat and Smith (27). DNA sequences of pBR322, present in pBR324 are indicated (·—·—·—·).

Materials and Methods. The DNA sequence strategy is presented in figure 3.

Plasmid pBR324 is a pBR322 derivative, into which the entire bacteriocinogenic region of the Col E1 related plasmid pMB1, has been cloned, namely in the filled-in Eco RI site of pBR322 (23). The colicin promoter region present on pBR324 was sequenced using the Hind III site of pBR322 as indicated in figure 3.

In fig. 4 the complete DNA base sequence of the cloacin and colicin promoter regions are presented. They are aligned in such a way that maximum

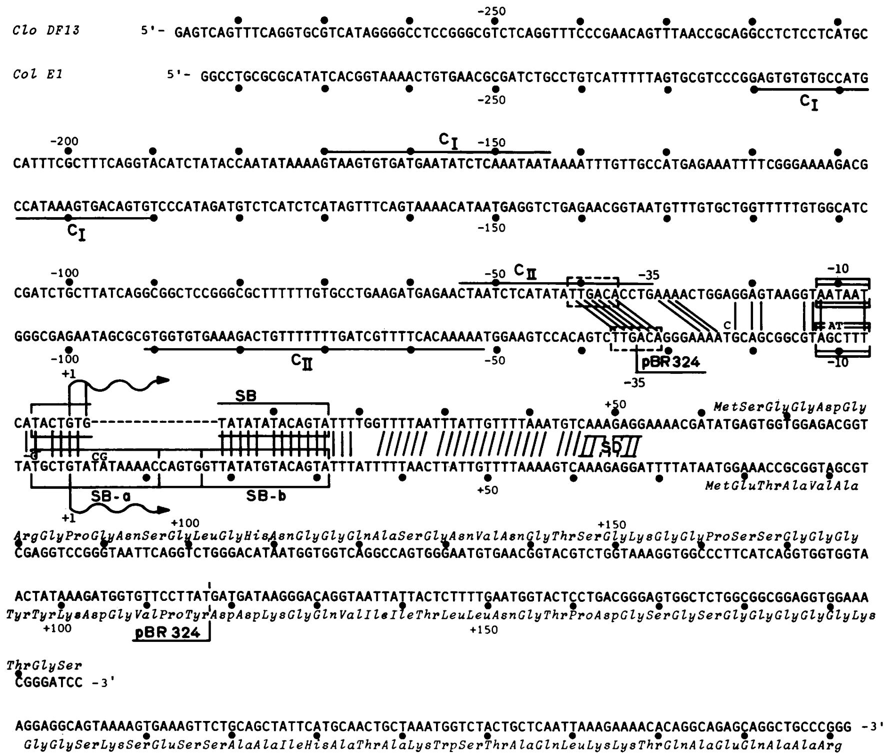

Figure 4. Nucleotide sequence of the cloacin DF13 promoter region and the promoter regions of colicin E1, as derived from RSF2124 (Col E1) and pBR324 (pMB1). Both sequences are aligned in such a way that maximum homology is obtained and are numbered from the presumed transcription initiation site (+1). The nucleotide sequence of the colicin promoter region of pMB1 as present in pBR324 starts at position -33 of Col E1. This sequence is preceded by the filled-in Eco RI site of pBR322 (23,40) and is determined untill Col E1 residue 117. Nucleotides different from the Col E1 sequence are given in small capitals above the Col E1 sequence. Indicated are: SD (Shine and Dalgarno sequence; 39), potential ribosome binding site; Pribnow box ("-10") and RNA polymerase recognition site ("-35") by boxes; SB, presumed "SOS"-box; CI and CII, putative interaction sites for cAMP-CRP complex.

homology is obtained. In vitro, transcription initiated at the cloacin promoter, starts at about 195 nucleotides from the Bam HI site (2; fig. 1). By varying the levels of ATP and GTP in our in vitro transcription assay (2), as described by Johnson and Reznikoff (36), it could be demonstrated that the first incorporated nucleotide is guanosine (data not shown). From these data we conclude that transcription of the cloacin gene starts at +1 and/or

+3 (fig. 4). The presumed Pribnow box and RNA polymerase recognition site (37, 38) are indicated. In both the Clo DF13 and Col E1 sequences (fig. 4) only one open reading frame is observed. The amino acids corresponding to the open reading frames, which very likely represent the bacteriocin genes, are indicated, as well as the possible ribosome binding sites (38, 39). The sequence of the 20 N-terminal amino acids of colicin E1, as determined by Suit, Sauer and Luria (S.E. Luria, personal communication) corresponds with that of the first 20 amino acids derived from the DNA base sequence, except for residue 19 (glutamic acid instead of glutamine).

A computer search for DNA sequence homology between both bacteriocin encoding regions was performed. This analysis revealed that there is no significant homology between the DNA sequences coding for cloacin DF13 and colicin E1. However, extensive homology can be observed between part of the Clo DF13 promoter region (residue -5 till +53) with the corresponding DNA sequence of both colicin promoter regions.

We anticipated that the lexA protein might be the repressor of the bacteriocin genes. Therefore we examined whether both bacteriocin promoter regions contain sequences homologous to the base sequence of the binding sites for lexA protein. These binding sites ("SOS"-boxes) have been identified in the promoter regions of the recA and lexA genes (14, 15). It was observed that both bacteriocin promoter regions contain stretches of 20 basepairs, which share significant homology with the "SOS"-boxes, identified in lexA and recA (14, 15) as well as with the DNA region containing the uvrB promoter (41). The Clo DF13 derived DNA sequence contains one such a 20 bp sequence (fig. 4, 5), whereas within the colicin promoter regions at least one but probably two partially overlapping "SOS"-boxes could be identified (fig. 4, 5). The observed "SOS"-boxes are located in a DNA region that seems to be duplicated in Col E1 and pMB1.

The homology between both promoter regions enabled us to predict the precise start site of transcription of the colicin genes as well as the presumed Pribnow box and RNA polymerase recognition site (fig. 4).

From the analysis of the DNA sequence we learned that the pMB1 part of pBR324 starts at nucleotide -33. This sequence has been determined untill residue +116, and is homologous with that of Col E1 except for a few bases, indicated in fig. 4.

In vivo effect of lexA gene product on cloacin synthesis

From the homology between the nucleotide sequences of the cloacin DF13 and colicin E1 promoter region with the binding sites of lexA protein within

the regulatory regions of recA and lexA genes, we infer that the lexA protein probably is the repressor of both bacteriocin genes.

It is known from results obtained in our laboratory (P. Andreoli, unpublished results) and in other laboratories (12) that plasmids Clo DF13 and Col E1 synthesize more bacteriocin in lexA$^-$ host cells. In this study we quantitated the bacteriocin production directed by plasmid Clo DF13 in E. coli DM511 (lexA3, tsl-1; 20) at 30°C and 43°C.

For this purpose, a culture of DM511, harbouring pVU30 was grown in LL broth, supplemented with 0.5% lactate (17, 42) at 30°C. When the culture had reached an optical density of 0.1 at 660 nm, one half was shifted to 43°C. Four h after the shift, the total activity of the cloacin produced, was determined as described in Materials and Methods. It has to be noted that the culture grown at 43°C started to lyse at about 2 h after the shift, (not shown) probably due to an overproduction of protein H (6, fig. 1). This lysis was not observed in the culture which was grown at 30°C.

From our results presented in table 2 we conclude that the inactivation of the lexA gene product at 43°C, results in a 800-fold stimulation of cloacin production, comapred with the cloacin produced at 30°C.

To study the effect of a wild type lexA gene on the cloacin synthesis in DM511 at 43°C, we made use of plasmid pJA03, a pACYC184 derivative carrying the lexA gene. Although plasmids Clo DF13 and pACYC184 are compatible plasmids (van den Elzen and Hakkaart, unpublished), the copy number of Clo DF13 might be affected by the presence of a pACYC184 derivative. For this reason we also analysed the total cloacin production in DM511 harbouring pVU30 and pACYC184 at 43°C.

From the results presented in table 2, it can be concluded that cloacin production in DM511 at 43°C, was suppressed by the presence of pACYC184 to about 38%. However cloacin synthesis directed by pVU30 in DM511 harbouring pJA03 was reduced to 7%.

These data supported the prediction, based on the identification of"SOS"-boxes in the bacteriocin promoter regions, that the lexA protein is the repressor of bacteriocin synthesis.

DISCUSSION

In previous papers (2, 9) we demonstrated that the genes encoding the cloacin protein, the immunity protein as well as protein H are organized in one operon. Hakkaart et al. (6) reported that the addition of mitomycin-C results in a markedly increased synthesis of these proteins after 60 to 90

Table 2. Production of cloacin DF13 controlled by lexA.

Plasmid(s)[a]	Growth temp.[b]	Killing activity (kU/ml)[e]
pVU30	30	54
pVU30	42	43,200
pVU30, pACYC184	42	16,400
pVU30, pJA03	42	2,890

[a] Plasmids indicated were (co)transformed to strain DM511 (lexA3,tsl-1; 20).

[b] Cells harboring Clo DF13 were grown to an optical density of 0.1 at 660 nm, subsequently the culture was shifted to the temperature indicated.

[c] After 4 h the killing activity was determined as described in Materials and Methods.

minutes, in cells harbouring plasmid Clo DF13.

The hybridization experiments described in this paper indicate that mitomycin-C affects the regulation of transcription of the bacteriocin operon. According to our data, the addition of mitomycin-C results after about 60 minutes in a derepressed transcription of this operon, which is in good agreement with the observation of Hakkaart et al.(6).

Unlike the cloacin gene, the colicin E1 gene seems not to be located in one operon with the immunity gene (43, 44; fig. 1), whereas also the mode of action of both bacteriocins differs entirely (7, 10).

Our DNA sequencing results confirm that at least the N-terminal parts of both bacteriocins are different. The only similarity might be the high percentage of glycine residues and the low percentage of charged amino acids in the first 42 amino acids (fig. 4).

In view of the fact that the N-terminal parts of both bacteriocin genes are entirely different, it is remarkable that an extremely AT-rich region of about 60 nucleotides between the start sites of transcription and translation is conserved almost entirely (fig. 4). Within this region of homology sequences of 20 bp could be identified which are largely homologous with the "SOS"-boxes present within the regulatory regions of the recA and lexA genes (14, 15; fig. 4,5). Whereas Clo DF13 seems to contain one such a "SOS"-box, the colicin E1 promoter region might contain two lexA binding sites (a and b in fig. 4 and 5), which are partially overlapping. For this reason we speculate that a duplication has occurred within this part of the colicin promoter region.

In fig. 5 the sequences of Clo DF13, Col E1 and pBR324 containing the presumed "SOS"-boxes are compared with the operater regions of lexA, recA and the presumed operater of uvrB (41). From this comparison we conclude that the "SOS"-boxes observed in the bacteriocin promoter regions probably represent the binding sites for lexA protein. We were also able to predict a consensus sequence for lexA binding sites (fig. 5), which, in principle, consists of an inverted repeat. This inverted repeat is almost entirely conserved in recA and Clo DF13. The two putative "SOS"-boxes within the colicin E1 promoter regions are conserved to a lesser extent.

The conclusion that the lexA protein is the repressor of the bacteriocin gene is also supported by in vivo experiments reported in this paper. According to our results, derepression of cloacin synthesis by inactivation of the lexA repressor results in a 800-fold increased bacteriocin synthesis. The presence of the pACYC184 derivative, plasmid pJA03, containing the

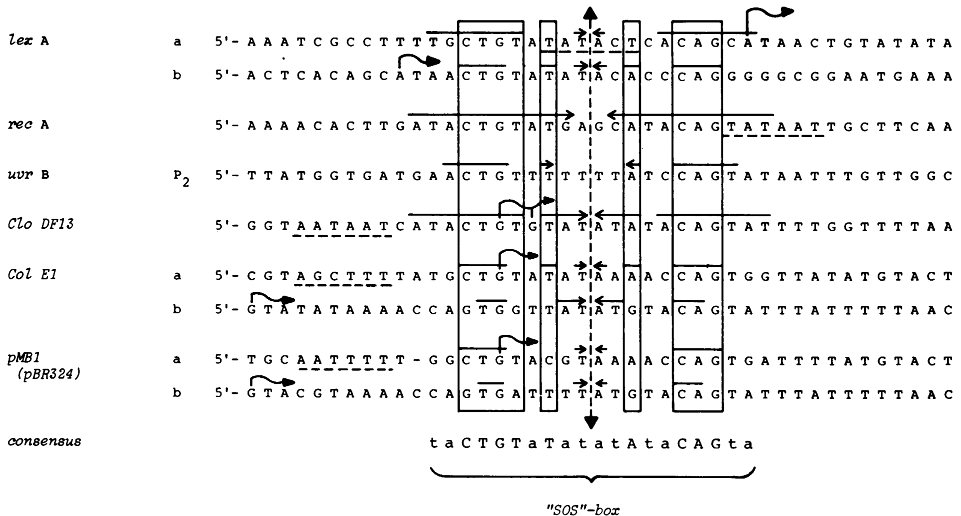

Figure 5. Comparison of the nucleotide sequences comprising the lexA binding sites ("SOS"-boxes,14,24) of the lexA and recA genes, with the presumed lexA binding sites of the uvrB gene (41) and the bacteriocin genes of Clo DF13, Col E1 and pMB1 (pBR324) (this paper). The start sites of transcription of lexA and recA, as well as the presumed start sites of Clo DF13, Col E1 and pMB1 are indicated by wavy arrows. Straight arrows represent the inverted repeats, pointing towards the central axis. The consensus sequence is given below. Capitals indicate the most conserved bases as shown by the boxes. The extend of the "SOS"-box as defined by Little et al. (15) is indicated.

intact lexA gene, significantly represses bacteriocin synthesis in DM511 at 43°C. The negative effect of pACYC184 on the cloacin synthesis is not due to incompatibility but might be caused by a decreased copy number of pVU30.

Ohkubo et al. (45) suggested that a Col E1 specified gene product is the repressor of colicin E1 synthesis, since Col E1 derivatives were able to repress colicin synthesis directed by their constitutive mutant. We suggest that this phenomenon is caused by incompatibility between both, related plasmids.

It has been suggested that catabolite repression might play a regulatory role in the synthesis of colicin E1 (46), colicin I (47) and cloacin DF13, although the effect is less pronounced in the latter system (17). We have tried to identify DNA sequences, which might be involved in the binding of the complex of cAMP and cAMP Receptor Protein (CRP). For this purpose we performed a computer search for homology with consensus sequences derived from known binding sites of cAMP-CRP complex. Several slightly different consensus sequences have been proposed, such as: AAAGTGTGACA, of which GTGA seems to be the most conserved part (48), $^{A}/_{G}$ - - - TGT$^{G}/_{C}$ACA - - - $^{C}/_{T}$ (49) and TGTGN$_8$CACA (50). Using these consensus sequences we were able to localize at least 2 DNA regions within the DNA sequence of Col E1 (around -215 and -70; fig. 4, CI and CII) that show a high degree of homology with all consensus sequences. We also localized one such a region within the DNA sequence of Clo DF13 around -160 (fig. 4, CI). Around the RNA polymerase recognition site ("-35" region) of the cloacin promoter a second cAMP-CRP interaction site (fig. 5, CII) might be located, although the homology with the consensus sequences is less significant.

With respect to the regulation of bacteriocin synthesis, pBR324 seems to be an interesting plasmid. The nucleotide sequence of the regulatory region of the pMB1 derived colicin gene probably starts at position -33 (fig. 4) and therefore does not contain the potential cAMP-CRP binding sites. Moreover within both "SOS"-boxes some changes have occurred (fig. 5.). It was observed that the synthesis of colicin , in cells harbouring pBR324 could not be induced by mitomycin-C, whereas in a recA$^-$ strain, which normally represses colicin synthesis directed by Col E1, pBR324 gives rise to a normal synthesis of colicin (J. Maat, unpublished results). We suggest that the lexA protein does not repress colicin synthesis directed by pBR324 to a normal level, although it is not as yet clear whether this is due to the different "SOS"-boxes or the lack of possibly regulatory sequences located proximal to the -33 position (for instance the lack of

binding sites for cAMP-CRP complex).

Whether the potential cAMP-CRP interaction sites play an essential, for instance, positive role in the regulation of bacteriocin synthesis remains to be clarified.

During the preparation of this manuscript, Ebina et al. (51) published the nucleotide sequence of the same DNA region of Col E1 as is presented in this paper. Our DNA sequence data confirm those of Ebina et al. except for Thymine (-280) which is not present in their sequence.

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