Organization of the lexA gene of Escherichia coli and nucleotide sequence of the regulatory region

Toru Miki, Yousuke Ebina, Fumio Kishi and Atsushi Nakazawa

Department of Biochemistry, Yamaguchi University School of Medicine, Ube, 755, Japan

ABSTRACT
     The product of the lexA gene of Escherichia coli has been shown to regu-
late expression of the several cellular functions (SOS functions) induced by
treatments which abruptly inhibit DNA synthesis. We have cloned and mapped
the lexA gene on a small segment of approximately 600 base pairs. The lexA
promoter was located by transcription R-loop analysis, and the lexA product
of 22,000 daltons was identified by protein synthesis in vitro. An unknown
gene was found which directed the synthesis of a protein of 35,000 daltons
in a region downstream from the lexA gene.
     Nucleotide sequence of the regulatory region of the lexA gene was deter-
mined. The sequence contained inverted repeats homologous to that of the
recA regulatory region. These inverted repeats may be recognized by the lexA
protein, because the protein is considered to repress both the genes as a
common repressor.

INTRODUCTION

     Treatments of the cells of Escherichia coli by agents such as ultraviolet

light and chemicals that result in DNA damage or inhibition of DNA synthesis

induce a set of cellular functions including prophage and colicin inductions,

error-prone repair, and inhibition of cell division. These functions, called

SOS functions, are known to be regulated by the products of the recA and lexA

genes (1).

     The recA gene product has been highly purified and shown to have a pro-

teolytic activity (2) in addition to biochemical activity for genetic recom-

bination (3, 4). The proteolytic activity requires polynucleotides produced

by the treatments with the agents mentioned above (2). Cleavage of λ phage

repressor by the recA protein is considered to account for the prophage in-

duction. On the other hand, the lexA gene product is regarded as a repressor

of the recA gene (5) and is subject to limited proteolysis by the recA pro-

tein similar to λ phage repressor (6). The inactivation of the lexA protein

should result in the synthesis of large amounts of the recA protein, which is

always observed during induction of SOS functions (7-10). Thus the recA and

lexA genes appear to constitute a regulatory circuit for the expression of SOS functions.

Recently, the product of the *lexA* gene was shown to regulate the expression of its own gene besides the *recA* gene (11, 12). Furthermore, it is suggested that several other genes are also regulated by the *lexA* gene directly and indirectly through the *recA* gene expression (13).

This report describes organization of the *lexA* gene by detailed restriction mapping, analysis of the transcript and proteins synthesized *in vitro*, and sequencing of the regulatory region. The physical structure of the region surrounding the *lexA* gene and construction of a plasmid which predominantly produces the *lexA* protein *in vitro* are also reported.

MATERIALS AND METHODS

Bacterial Strains and Plasmids: *E. coli* K-12, DM1187: F^-, *spr-51 lexA3 tif-1 sfiAll sup-37 thr leu his ilv^{ts} pro gal rpsL* (5) was obtained from H. Shinagawa. Plasmids pLC44-14 and pLC11-9 (14), pBR322 (15), pACYC177 and pACYC184 (16), and pAO3 (17) were obtained from K. Ueda, S. Hiraga, K. Matsubara, and A. Oka, respectively. pMCF1 and pMCR271 have been described (18, 19). Plasmid DNA was prepared as described previously (18, 19).

Restriction Enzyme Analysis: *Hae*II and *Hae*III were prepared and used according to the method of Greene *et al.* (20). *Hind*III and *Alu*I were gifts of H. Ohmori. Other restriction enzymes were purchased from Bethesda Research Laboratories, Boehringer Mannheim Co. Ltd., and Takara Shuzo Co. Ltd. (Kyoto, Japan). These enzymes were used as directed. The methods of agarose gel (19) and polyacrylamide gel (18) electrophoresis were described previously.

In Vitro Recombination: T4 DNA ligase was prepared from heat-induced cells of ED1150 (λNM989) of Murray *et al.* (21). Molecular cloning experiments in this work were carried out by standard techniques described previously (19, 22) in a P1 facility. Presence of the *lexA* gene in the plasmid was determined by complementation analysis of *spr*, a recessive mutation in the *lexA* gene. The phenotype of the plasmid-harboring cells was judged according to the method of Pacelli *et al.* (23): The spontaneous induction of phage λ occurs in an *spr* mutant, DM1187 (Spr$^-$ phenotype), but not in the mutant cells with the plasmid carrying the *lexA* gene (Spr$^+$ phenotype).

Transcription R-loop Analysis: Transcription R-loop mapping was carried out according to Brack (24). The reaction mixture contained 0.5 μg *Bam*HI-cleaved pMCR551 DNA, 2.6 μg *E. coli* RNA polymerase, 20 mM Tris-HCl (pH 8.0),

10 mM MgCl$_2$, 150 mM KCl, 0.2 mM dithiothreitol, and 0.2 mM each of ATP, GTP, CTP, and UTP in 20 µl. After incubation at 37°C for 10 min, the mixture was chilled in an ice bath, and used for R-loop formation as described (24).

In Vitro Protein Synthesis: Cell-free protein synthesis was performed by the method of Shibuya and Kaziro (25). Conditions for *in vitro* protein synthesis with ^{14}C-amino acids, sodium dodecylsulfate-polyacrylamide gel electrophoresis, and fluorography were described previously (26).

Nucleotide Sequence: Determination of the nucleotide sequence of DNA and RNA was carried out according to Maxam and Gilbert (27) and Simoncsitz *et al*. (28), respectively.

RESULTS

Physical Structure of the *ubiA-lexA-dnaB* Region: We have analyzed two plasmids from the *E. coli* gene bank of Clarke and Carbon (14), pLC44-14 and pLC11-9 carrying the *dnaB* gene. The *lexA* gene has been mapped near the *dnaB* gene (30). Presence of the *lexA* gene on pLC44-14 (31) was confirmed by genetic complementation. The plasmids were digested with several restriction endonucleases used singly or in pairs, and analyzed by agarose gel electrophoresis. The restriction maps of the plasmids were thus constructed. The map of pLC44-14 agreed well with that reported by Little (29). The chromosomal regions on both the plasmids were deduced as shown in Fig. 1 by comparing the maps of the plasmids and the vector plasmid ColE1 (32). The *dnaB* gene was cloned from pLC11-9 into pMCR600 (a multicopy cloning vector carrying single *Bgl*II site; T. Miki, unpublished) by using *Bgl*II. Resulting plasmid, pMCR661 carried two *Bgl*II fragments as shown in Fig. 1. The *lexA* gene was cloned from pLC44-14 into pACYC177, to produce pMCR551 and pMCR552. Restriction analysis of pMCR551 revealed the location of the *lexA* gene on the *Bam*HI-*Pst*I fragment of 3.2 kilobase pairs (kb) as shown in Fig. 1, confirming the result of Little (29). Another plasmid pMCR552 carried the additional *Bam*HI fragment (Fig. 1). The *ubiA* gene was placed as described (29).

Detailed Restriction Mapping of the *lexA* Region: pMCR551 and pACYC177 were cleaved with several combinations of restriction endonucleases and analyzed by polyacrylamide gel electrophoresis. The restriction fragments derived from the insert of pMCR551 were identified by comparing each pattern of electrophoresis. The largest *Hae*III fragment of the pMCR551 insert was purified and digested with other restriction endonucleases after labeling the 5' ends with [γ-^{32}P]ATP (New England Nuclear) (27). Resulting DNA fragments were analyzed by polyacrylamide gel electrophoresis and autoradiography. Sev-
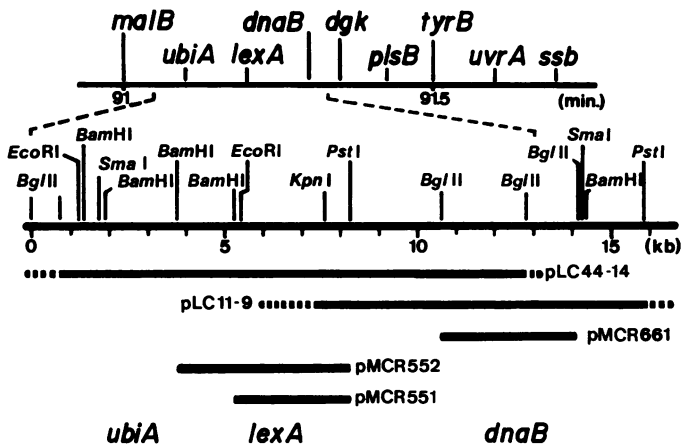
Fig. 1. Genetic and physical structure of the *ubiA-lexA-dnaB* region: Genetic map taken from Bachmann and Low (30) is shown on the top. Restriction map of the *ubiA-lexA-dnaB* region is shown in the middle. No site for *Hind*III and *Xho*I was found. Locations of the chromosomal regions of pLC44-14, pLC11-9, pMCR661, pMCR552, and pMCR551 are shown by solid thick lines under the map. Junctions between the chromosomal and ColE1 DNA in the plasmids of Clarke and Carbon collection are not clear at present and shown by dotted lines. Approximate gene positions are shown at the bottom.

eral restriction fragments derived from the pMCR551 insert were purified and digested with various combinations of restriction endonucleases. A detailed restriction map was thus established as shown in Fig. 2A. The map was further confirmed by systematic comparison of the restriction fragments produced by cleaving various plasmids shown in Fig. 2B (see later) with combinations of restriction endonucleases.

Determination of the Exact Location of the *lexA* Gene: In order to locate the *lexA* gene into a minimal region on the physical map, sets of *in vitro* recombination experiments were carried out.

First, a plasmid pMCF1 (18, 19) was integrated into pMCR551 at the unique *Kpn*I site. pMCR551 and pMCF1 were cleaved with *Kpn*I, ligated, and used for transformation. pMCR581 was obtained from a kanamycin- and ampicillin-resistant (Km^r Ap^r) transformant (Fig. 3A). This plasmid complemented the *spr* mutation in DM1187, suggesting that *Kpn*I site is not located in the *lexA* gene. pMCR581 was cleaved with *Bam*HI or *Pst*I, diluted ten fold, and recircularized. pMCR593 and pMCR595 obtained from the Km^r transformants that were ampicillin sensitive (Ap^s) were the deletion derivatives of pMCR581. The derivatives contained parts of pMCR581 DNA (Fig. 2B, Fig. 3B). Comple-
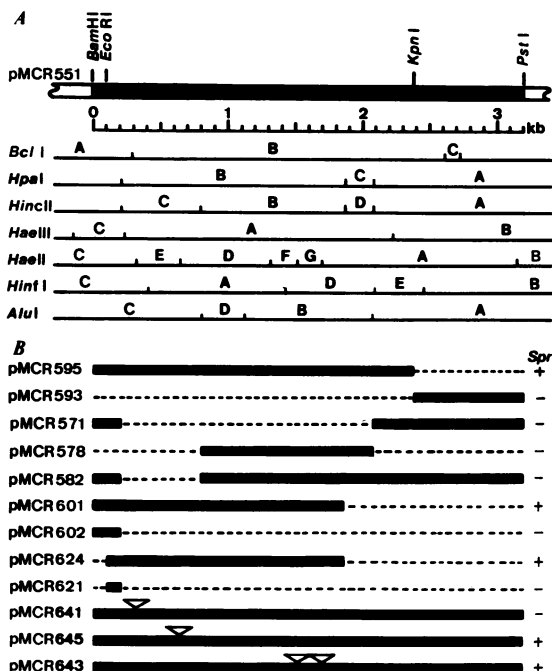
Fig. 2. Detailed restriction map of the *lexA* region and parts of the region carried by the derivatives of pMCR551: (A) The chromosomal region on pMCR551 is illustrated on the top with the sites for restriction endonucleases that cut the region once. Open and closed portions represent pACYC177 and chromosomal DNA, respectively. (B) Chromosomal region carried by each plasmid is shown by the thick line, and the portion deleted from the plasmid is shown by the dotted line. Lower three plasmids are the insertion derivatives of pMCR 551. Location of the inserted Cm^r-fragment is shown by an open triangle. It is not known which site on the map of pMCR643 is the insertion site. The ability (+) or inability (-) to confer the Spr^+ phenotype on DM1187 is shown at the right hand.

mentation analysis of the *spr* mutation revealed that pMCR595 carried the *lexA* gene, but pMCR593 did not, indicating that the *lexA* gene resides in the *Bam*HI-*Kpn*I fragment of 2.4 kb.

Second, the *Hinc*II fragments of pMCR551 were cloned into another plasmid pMCR561, a derivative of pACYC177 carrying the chloramphenicol resistance gene (Cm^r) of pACYC184 (T. Miki, unpublished). Ap^s clones were screened from the Cm^r transformants, because the unique *Hinc*II site on pMCR561 was located in the Ap^r gene. Among the plasmids obtained, pMCR571, pMCR578, and pMCR582 carried the *Hinc*II-A fragment, the *Hinc*II-B and -D fragments, and the *Hinc*II-B, -D, and -A fragments, respectively (Fig. 2B). None of the plasmids could
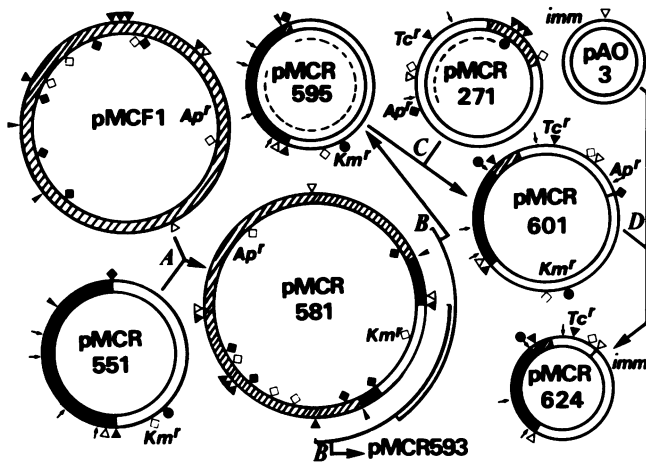
Fig. 3. Construction of the small *lexA*-plasmids: (A) pMCF1 and pMCR551 were joined by digestion with *Kpn*I and subsequent ligation, and a composite plasmid pMCR581 was isolated. (B) The deletion derivatives (pMCR593 and pMCR595) of pMCR581 were constructed. (C) The dotted portions of pMCR271 and pMCR595 were joined to produce pMCR601. (D) The *Eco*RI fragment carrying the Tc^r gene of pMCR601 was cloned into pAO3. For the detailed procedure to construct these plasmids, see text. Sites for restriction endonucleases are shown by following symbols; *Eco*RI (∇), *Hind*III ($\diamondsuit$), *Bam*HI ($\blacktriangledown$), *Pst*I ($\blacklozenge$), *Sma*I ($\bullet$), *Kpn*I ($\blacktriangledown$), and *Hinc*II ($\downarrow$). The mark $\P$ represents *Sma*I/*Hinc*II junction which can not be cleaved by either of the enzymes. *imm* denotes the gene for immunity to colicin El. Filled, hatched, and open portions represent chromosomal, pMCF1, and other DNAs, respectively.

complement the *spr* mutation, suggesting that a part of the *lexA* gene is carried by the *Hinc*II-C fragment.

Third, a part of the chromosomal region carried by pMCR595 was replaced by another DNA fragment (Fig. 3C). The *Hinc*II-*Pst*I fragment and the *Hinc*II fragment(s) of pMCR595 were removed by partial digestion with *Hinc*II and complete digestion with *Pst*I. The resulting gap of the plasmid was filled with the *Pst*I-*Sma*I fragment (shown by a dotted arc in Fig. 3C) of pMCR271 (18), a derivative of pBR322. Since both the parental fragments carried different halves of the Ap^r gene, the new plasmids could confer resistance to Ap on the cell by reassociation of the Ap^r gene. Among the new plasmids, pMCR 601 contained the *Hinc*II-B and -C fragments and could complement the *spr* mutation, whereas pMCR602 lacking both the fragments could not complement the mutation (Fig. 2B). The other two $lexA^+$-plasmids had the same structure as pMCR601, suggesting that the *Hinc*II-D and the neighboring *Hinc*II-*Kpn*I frag-

ments are dispensable for the expression of the *lexA* gene.

Finally, a fragment containing a *Cm^r* gene was randomly inserted into a *Hae*II site of pMCR551. pMCR551 was cleaved partially with *Hae*II to obtain linear molecules, mixed with the *Cm^r*-fragments of 0.9 kb purified from *Hae*II-digested pACYC184, and ligated. Insertion derivatives were obtained from Cm^r Km^r transformants. Location of the inserted fragment on each derivative was determined by cleaving each of the plasmid with *Alu*I, *Hinf*I, and *Hinc*II (Fig. 2B). No *Hae*II fragment was found to be deleted from the derivatives. Insertion of the *Cm^r*-fragment into the *lexA* gene would inactivate the gene function. pMCR643 and pMCR645 could complement the *spr* mutation, but pMCR641 could not. Therefore, the functional *lexA* gene should require the *Hae*II-C and -E fragments. Since a small *Bam*HI-*Eco*RI fragment was dispensable for the expression of the *lexA* gene (see later), the minimal region for the *lexA* gene seems to be the DNA segment of 0.58 kb between the unique *Eco*RI site and the second *Hae*II site from the *Eco*RI site. The minimal coding region for the *lexA* protein, however, is calculated to be 0.6 kb, since our estimation of the molecular weight of the protein is 22,000 (see later). Thus the *Cm^r*-fragment insertion on pMCR645 may have occurred in the *lexA* gene. Ability of pMCR645 to complement the *spr* mutation suggests a possibility that the carboxy-terminal region of the *lexA* protein is not important for the function.

In Vitro Synthesis of Proteins Directed by the *lexA*-Plasmids: The proteins directed by the *lexA*-plasmids were analyzed by a cell-free protein synthesizing system. pMCR551 directed the synthesis of 35-kilodalton (kd), 27-kd, and 22-kd proteins (Fig. 4, lane B). The 27-kd protein is probably the *Km^r* gene product (aminoglycoside 3'-phosphotransferase I), because a protein of this size was also directed by pACYC177 (lane A), and the molecular weight agreed well with the published value of the *Km^r* gene product (33). The 30-kd protein was specified by pACYC177, but not by pMCR551 which lacked the *Ap^r* gene (lane A). The other plasmids carrying the *Ap^r* gene also produced a 30-kd protein (lane C, D, and E). This protein is considered to be the product of the *Ap^r* gene (the precursor of β-lactamase). Although the *Ap^r* gene product is known to undergo post-translational processing (34), the mature form of β-lactamase (27 kd) could not be detected in our *in vitro* system. The size of the unique protein common to all the *lexA$^+$*-plasmids is 22 kd (lane B, E, and H), suggesting that the protein is the *lexA* gene product. The synthesis of a 35-kd protein was also directed by pMCR551 (lane B). Inability of pACYC177 to direct the 35-kd protein (lane A) indicated that the protein is
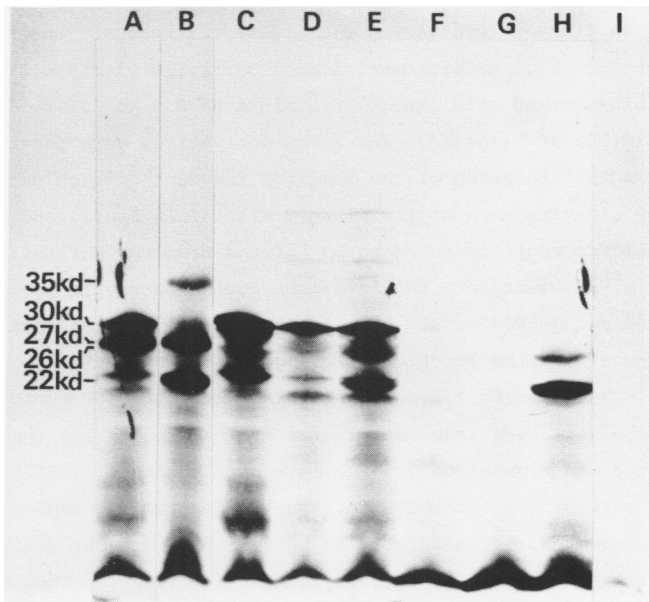
Fig. 4. In vitro protein synthesis directed by the lexA-plasmids: Templates used were (A) pACYC177 (10.2 μg), (B) pMCR551 (10.0 μg), (C) pBR322 (9.8 μg), (D)pMCR602 (10.2 μg), (E) pMCR601 (12.0 μg), (F) pAO3 (3.6 μg), (G) pMCR621 (9.9 μg), (H) pMCR624 (9.5 μg), and (I) without DNA. Molecular weight markers were bovine serum albumin (67,000), hen egg albumin (45,000), chymo-trypsinogen A (25,000), and lysozyme (14,300).

encoded by the insert of pMCR551. pMCR601 did not direct the 35-kd protein, but the plasmid produced a new protein of 26 kd. pMCR602 did not direct ei-ther of the proteins, suggesting that the 26-kd protein is encoded by the chromosomal region of pMCR601. Since pMCR601 lacked the right half of the insert of pMCR551 (see Fig. 2B), a part of the gene for the 35-kd protein might have been deleted on pMCR601 and the 26-kd protein might be a truncated form of the 35-kd protein. If that is the case, the HincII site between the HincII-B and -D fragments (see Fig. 2A) is considered to be located in the gene for the 35-kd protein. The coding regions of the 22-kd and 35-kd prote-ins are shown in Fig. 6.

Construction of a Plasmid Which Predominantly Produces the lexA Protein In Vitro: The use of the cells harboring the lexA-plasmid as a starting ma-terial would facilitate the purification of the lexA protein. In order to construct a plasmid which produces the lexA protein more efficiently, the

small segment containing the *lexA* gene was recloned into a plasmid with a higher copy number than pACYC177. The *Eco*RI fragment containing the *lexA* and tetracycline resistance (Tc^r) genes was excised from pMCR601. This fragment and the corresponding *Eco*RI fragment of pMCR602 were then cloned on pAO3, a small multicopy plasmid consisting of a quarter part of ColE1 plasmid (17) (Fig. 3D). pMCR601 and pAO3 were digested with *Eco*RI, ligated, and used for transformation. pMCR624 was a plasmid isolated from the Tc^r transformants that were immune to colicin E1. Corresponding region of pMCR602 was cloned into pAO3, to produce pMCR621 (see Fig. 2B). pMCR621 had the same structure as pMCR624 except lacking the *Hinc*II-B and -C fragments. pMCR624 complement-ed the *spr* mutation, indicating that the small *Bam*HI-*Eco*RI fragment is dis-pensable for the Spr^+ phenotype. As shown in Fig. 4, lane H, the *lexA* prote-in was predominantly synthesized *in vitro*. The 26-kd protein, presumably a truncated form of the 35-kd protein, was also observed. Tc^r protein was not detected in this system, despite the high expression of the *lexA* gene.

Location of the Promoter and Direction of the Transcription: Transcrip-tion of pMCR551 was analyzed by the transcription R-loop method of Brack (24). The *Bam*HI-cleaved plasmid DNA was transcribed with RNA polymerase *in vitro*, the RNA was hybridized *in situ* to the template, and analyzed by an electron microscopy. Seventy three percent of the *Bam*HI-cleaved pMCR551 formed R-loop at a region proximal to an end of the molecule. *Bam*HI-cleaved pACYC177, how-ever, did not form such R-loop frequently (data not shown). Since *Bam*HI cleaved an insert-vector junction of pMCR551, the right half of the *Bam*HI-cleaved pMCR551 molecule contained the major *Bam*HI-*Pst*I fragment of pACYC177. Therefore, most of the R-loops appeared to be located on the insert of pMCR 551, indicating that a strong promoter resided at the region proximal to the unique *Bam*HI site, and the transcription proceeded towards the *Pst*I site (Fig. 5). Assuming that the length of pMCR551 DNA is 6.2 kb, the initiation site of the transcription was calculated to be 0.175 (standard deviation = 0.06) kb from the *Bam*HI site and corresponded to the region between the *Eco*RI site and the neighboring *Hae*III site (Fig. 2A and see also Fig. 6). Although there may be some other weak promoters including the Km^r gene promoter, the direction of the transcription from these promoters could not be determined.

Nucleotide Sequence of the Regulatory Region: On the basis of the map-ping data of the promoter region, nucleotide sequence of the region was de-termined by the method of Maxam and Gilbert (27). Sequence is shown in Fig. 7A, and the strategy used for sequencing is shown in Fig. 7B. In order to determine the exact position of the initiation of the transcription, the
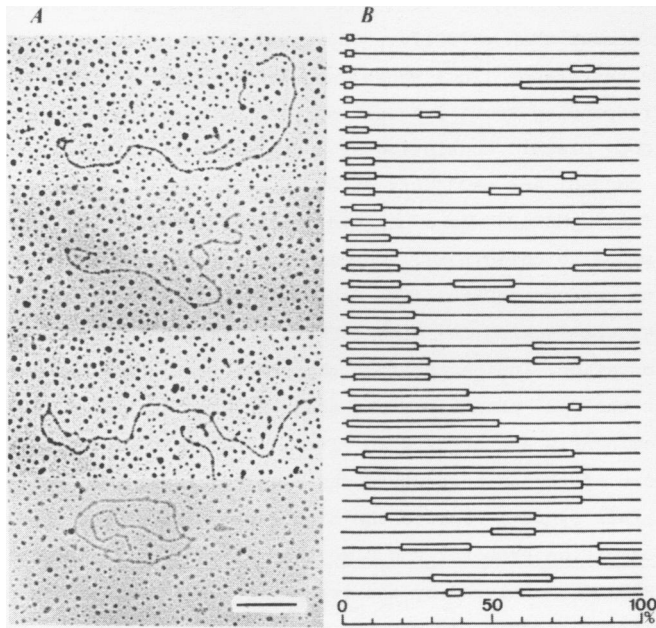
Fig. 5. Transcription R-loop analysis of *Bam*HI-cleaved pMCR551: (A) Examples of the electron micrographs of the R-loop molecules. Bar represents the length of 1 kb. (B)A schematic representation of the R-loop molecules. Altogether 37 molecules were analyzed and the length of the R-loop molecule is represented as percent that of the average of seven open circular molecules of pMCR551 (6.2 kb). Orientation of the lower 10 molecules is tentative.

*Eco*RI-*Hae*III fragment of 116 base pairs isolated from pMCR624 was transcribed *in vitro* in the presence of $[\gamma-^{32}P]$ATP or $[\gamma-^{32}P]$GTP. The products were analyzed by electrophoresis. Two RNA species of approximately 30-40 nucleotides long were labeled by $[\gamma-^{32}P]$ATP but not by $[\gamma-^{32}P]$GTP (Fig. 8A). Both of the
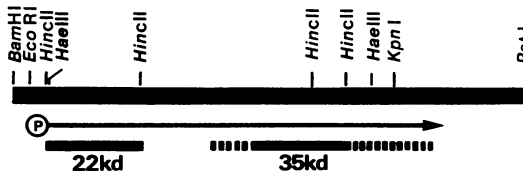

Fig. 6. Organization of the *lexA* region: Map of the insert of pMCR551 is shown on the top. Location of the *lexA* promoter is shown by Ⓟ, and direction of the transcription is shown by an arrow. Transcription seems to continue through the *Kpn*I site (Fig. 4). The possibilities for weak promoter, attenuator, and ρ-dependent terminator are ommited in this figure. The coding region for the 22-kd and 35-kd proteins are shown by thick lines. Dotted thick lines show uncertain portion for the coding region.
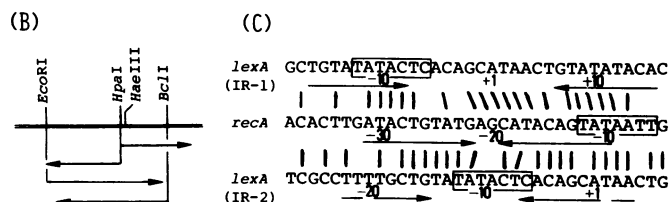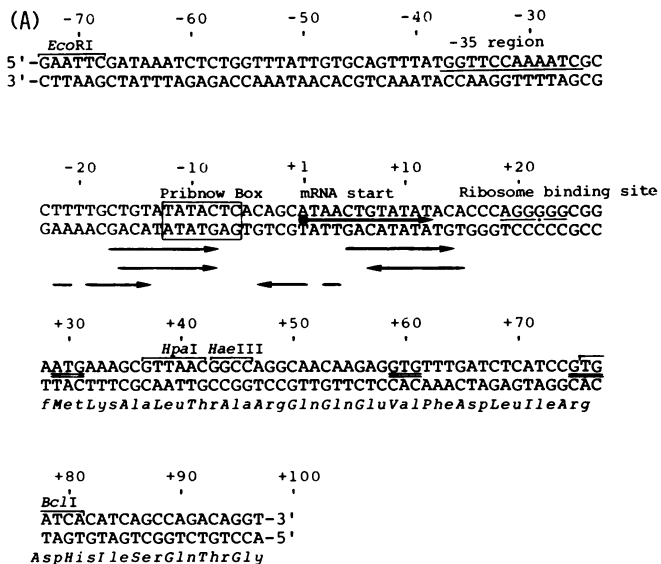
(A)   -70   -60   -50   -40   -30
        EcoRI   -35 region
5'-GAATTCGATAAATCTCTGGTTTATTGTGCAGTTTATGGTTCCAAAATCGC
3'-CTTAAGCTATTTAGAGACCAAATAACACGTCAAATACCAAGGTTTTAGCG

    -20   -10   +1   +10   +20
                Pribnow Box   mRNA start   Ribosome binding site
CTTTTGCTGTATATACTCACAGCATAACTGTATATACACCCAGGGGGCGG
GAAAACGACATATATGAGTGTCGTATTGACATATATGTGGGTCCCCCGCC

    +30   +40   +50   +60   +70
                HpaI HaeIII
AATGAAAGCGTTAACGGCCAGGCAACAAGAGGTGTTTGATCTCATCCGTG
TTACTTTCGCAATTGCCGGTCCGTTGTTCTCCACAAACTAGAGTAGGCAC
fMetLysAlaLeuThrAlaArgGlnGlnGluValPheAspLeuIleArg

    +80   +90   +100
Bcl I
ATCACATCAGCCAGACAGGT-3'
TAGTGTAGTCGGTCTGTCCA-5'
AspHisIleSerGlnThrGly

(B)   (C)

                lexA   GCTGTATATACTCACAGCATAACTGTATATACAC
                (IR-1)

                recA   ACACTTGATACTGTATGAGCATACAGTATAATTG

                lexA   TCGCCTTTTGCTGTATATACTCACAGCATAACTG
                (IR-2)

Fig. 7. (A) Nucleotide sequence of the regulatory region of the *lexA* gene:
Nucleotides are numbered from the site corresponding to 5' end of the tran-
script synthesized *in vitro*. "Pribnow box" heptamer (35), "-35 region" (36),
and ribosome binding site (37) are indicated. Direct and inverted repeats
are shown by arrows. Initiator codons (ATG and GTG) are indicated by double
underlines. Since the first initiator triplet is associated by a ribosome
binding site, the amino acids sequence of the protein started from this
triplet is shown in italics. (B) Strategy used for sequencing: Extent and
direction of sequencing are shown by arrows. Sequencing was carried out in
triplicate. (C) Sequence homology of the inverted repeats of the *lexA* and
recA regulatory regions. The same bases are joined by vertical lines. In-
verted repeats and "Pribnow boxes" are indicated by arrows and boxes, respec-
tively. The sequence of the *recA* gene is taken from Horii *et al.* (38), and
numbered from the predominant site for transcription initiation.

labeled mRNAs were extracted from the gel and the nucleotide sequence was
determined by the enzymatic method described by Simoncsitz *et al.* (28). The
sequence of the 5' region of both the mRNAs were identical; pppAUAACUGUAUAU...

Fig. 8. Nucleotide sequence of 5' portion of the lexA mRNA: (A) Autoradio-
graph of RNAs transcribed in vitro from the EcoRI-HaeIII fragment. RNA syn-
thesis was carried out in a reaction mixture (30 μl) containing 10 mM $MgCl_2$,
100 mM KCl, 1 mM dithiothreitol, 50 mM Tris-HCl (pH. 7.9), 0.5 mM [γ-^{32}P]ATP
or [γ-^{32}P]GTP and 10 mM each of the three other NTPs, 1 pmol of DNA, and 3
pmol of E.coli RNA polymerase. After incubation for 30 min at 37°C, the
samples were prepared and electrophoresed as described (38). Labeled NTP is
indicated at the top. RNA-I (110 bp) of pAO3 (17) was used as a marker.
(B) Sequencing of in vitro transcripts by the method of Simoncsitz et al.
(28): RNA-1 (indicated by an arrow in A) was extracted and partially digest-
ed by [lane G] RNase T1 (Sankyo, Japan), [lane A] RNase U2 (Sankyo), [lane U+
C] RNase A (Boehringer Mannheim Co. Ltd.), and [lane -C] RNase Phy I (P-L
Biochemicals). Digested RNA was electrophoresed and subjected to autoradio-
graphy. RNA-2 gave the same pattern (not shown) as RNA-1.

(Fig. 8B). The minor RNA product formed might be resulted by the premature

transcription termination. Predominant site of transcripiton initiation is

shown in Fig. 7A. "Pribnow box" (35), "-35 region" (RNA polymerase recogni-

tion site) (36), ribosome binding site (37), and possible amino acid sequence of the amino-terminal region of the *lexA* protein are shown in Fig. 7A. Direct and inverted repeats are also shown in Fig. 7A. Noteworthy is the finding that both of the inverted repeats have high sequence homologies to one of those of the *recA* gene regulatory region determined by Horii *et al.* (29). (Fig. 7C).

DISCUSSION

The *lexA* gene of *E. coli* was physically defined by the following steps. i) A restriction map of the *ubiA-lexA-dnaB* region was established and the approximate position of each gene was assigned on the restriction map. ii) The *lexA* gene was further cloned and a detailed restriction map of the insert of the new *lexA*-plasmid was constructed. iii) The exact position of the *lexA* gene was determined on the restriction map. iv) The proteins encoded by the *lexA* region were identified *in vitro*. v) Location of the *lexA* promoter and direction of the transcription were determined. vi) Nucleotide sequence of the regulatory region was determined.

The product of the *lexA* gene was identified by Little and Harper (11), and Brent and Ptashne (12), to be a protein of 24 kd by using ultraviolet light-irradiated cells and maxi-cells, respectively. The 24-kd protein should be the same as the 22-kd protein of our identification by *in vitro* protein synthesis. An additional protein of 35 kd which was not identified by others was found to be the product of an unknown gene near *lexA*. The protein was synthesized to a lesser extent than the *lexA* protein *in vitro* (Fig. 4). If the 26-kd protein produced from pMCR601 and pMCR624 is regarded as a truncated form of the 35-kd protein, direction of transcription of the gene for the 35-kd protein should be the same as that of the *lexA* protein. Since the transcription from the *lexA* promoter *in vitro* seems not to stop at the unique site in the insert of pMCR551 (Fig. 5), the gene for the 35-kd protein may be cotranscribed with the *lexA* gene. Another possibility is that a transcript which could not be detected under conditions in this study starts from a putative weak promoter upstream from the gene for the 35-kd protein. However failure to detect the 35-kd protein *in vivo* by Little and Harper (11) suggests the existence of the control signal for transcription termination (*e.g.*, ρ-dependent terminator or attenuator) between the two genes. Because the regulatory factors are diluted in the *in vitro* protein synthesizing system, the transcript may not terminate at the end of the *lexA* gene and proceed through the gene for the 35-kd protein. Thus the 35-kd protein is synthesized.

Kenyon and Walker (13) recently isolated a set of insertion mutations of the *E. coli* genes whose expression is induced in response to treatment with the DNA-damaging agent, mitomycin C. One of the insertion mutations, called *dinF*, is tightly linked to the *lexA* gene, but not located within the *lexA* gene itself. Since the repression of the *recA* gene expression by the *lexA* protein is considered to be released on the mitomycin C treatment, the *lexA* gene expression which is under the control by its own gene product might be also enhanced by the agent. Therefore, a possibility was proposed that the *dinF* gene is positioned distal to the *lexA* structural gene in the same transcriptional unit (13). If that is the case, the *dinF* gene is one of the candidates of the gene for the 35-kd protein identified in this study. Approximate map position of the *dinF* locus agreed with our mapping result of the gene for 35-kd protein (clockwise from the *lexA* gene).

Nucleotide sequence of the regulatory region of the *lexA* gene indicated that the region contained two inverted repeats both of which have sequence homologies to one of those of the *recA* regulatory region, besides the typical sequences common to the *E. coli* promoters. Since expression of both the genes is considered to be regulated by the *lexA* protein, these inverted repeats may serve as operators of the *lexA* gene. Lesion of the *lexA* gene induces the increased expression of the colicin El gene (39) as well as the *recA* gene. We have also determined the nucleotide sequence of the regulatory region of the *colEl* gene and found that one of the inverted repeats on the region also had a sequence homology to that of the *recA* regulatory region (Ebina *et al.*, submitted).

While this manuscript was in preparation, we learned that Horii and Ogawa had independently determined the nucleotide sequence of the *lexA* gene. Their sequence data for the regulatory region agreed well with ours.

REFERENCES
1. Witkin, E.M. (1976) Bacteriol. Rev. 40, 869-907
2. Craig, N.L. and Roberts, J.W. (1980) Nature 283, 26-30
3. Weinstock, G.M., McEntee, K., and Lehman, I.R. (1979) Proc. Natl. Acad. Sci. USA 76, 126-130

4. Shibata, T., DasGupta, C., Cunningham, R.P., and Radding, C.M. (1979) Proc. Natl. Acad. Sci. USA 76, 1638-1642
5. Mount, D.W. (1977) Proc. Natl. Acad. Sci. USA 74, 300-304
6. Little, J.W., Edmiston, S.H., Pacelli, L.Z., and Mount, D.W. (1980) Proc. Natl. Acad. Sci. USA 77, 3225-3229
7. Inouye, M. and Pardee, A.B. (1970) J. Biol. Chem. 245, 5813-5819
8. Gudas, L.J. and Pardee, A.B. (1976) J, Mol. Biol. 101, 459-477
9. McEntee, K. (1977) Proc. Natl. Acad. Sci. USA 74, 5275-5279
10. Little, J.W. and Kleid, D.G. (1977) J. Biol. Chem. 252, 6251-6252
11. Little, J.W. and Harper, J.E. (1979) Proc. Natl. Acad. Sci. USA 76, 6147-6151
12. Brent, R. and Ptashne, M. (1980) Proc. Natl. Acad. Sci. USA 77, 1932-1936
13. Kenyon, C.J. and Walker, G.C. (1980) Proc. Natl. Acad. Sci. USA 77, 2819-2823
14. Clarke, L. and Carbon, J. (1976) Cell 9, 91-99
15. Bolivar, F., Rodoriguez, R.L., Greene, P.J., Betlach, M.C., Heyneker, H. L., and Boyer, H.W. (1977) Gene 2, 95-113
16. Chang, A.C.Y. and Cohen, S.N. (1978) J. Bacteriol. 134, 1141-1156
17. Oka, A., Nomura, N., Morita, M., Sugisaki, H., Sugimoto, K., and Takanami, M. (1979) Mol. Gen. Genet. 172, 151-159
18. Miki, T. (1978) Ann. Rep. Inst. Virus Res., Kyoto Univ. 21, 1-26
19. Miki, T., Hiraga, S., Nagata, T., and Yura, T. (1978) Proc. Natl. Acad. Sci. USA 75, 5099-5103
20. Greene, P.J., Heyneker, H.L., Bolivar, F., Rodoriguez, R.L., Betlach, M. C., Covarrubias, A.A., Backman, K., Russel, D.J., Tait, R., and Boyer, H.W. (1978) Nucleic Acids Res. 5, 2373-2380
21. Murray, N.E., Bruce, S.A., and Murray, K. (1979) J. Mol. Biol. 132, 493-505
22. Miki, T., Kimura, M., Hiraga, S., Nagata, T., and Yura, T. (1979) J. Bacteriol. 140, 817-824
23. Pacelli, L.Z., Edmiston, S.H., and Mount, D.W. (1979) J. Bacteriol. 137, 568-573
24. Brack, C. (1979) Proc. Natl. Acad. Sci. USA 76, 3164-3168
25. Shibuya, M. and Kaziro, Y. (1979) J. Biochem. 86, 403-411
26. Ebina, Y., Kishi, F., Nakazawa, T., and Nakazawa, A. (1979) Nucleic Acids Res. 7, 639-649
27. Maxam, A. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560-564
28. Simoncsitz, A., Brownlee, G.G., Brown, R.S., Rubin, J.R., and Guilley, H. (1977) Nature 269, 833-836
29. Little, J.W. (1980) Gene 10, 237-247
30. Bachmann, B.J. and Low, K.B. (1980) Microbiol. Rev. 44, 1-56
31. Mount, D.W., Little, J.W., and Edmiston, S.H. (1980) Molec. Gen. Genet. 177, 477-483
32. Dougan, G., Saul, M., Warren, G., and Sherratt, D. (1978) Molec. Gen. Genet. 158, 325-327
33. Meagher, R.B., Tait, R.C., Betlach, M., and Boyer, H.W. (1977) Cell 10, 521-536
34. Sutcliffe, J.G. (1978) Proc. Natl. Acad. Sci. USA 75, 3737-3741
35. Pribnow, D. (1975) Proc. Natl. Acad. Sci. USA 72, 784-788
36. Gilbert, W. (1976) in RNA Polymerase, Losik, R. and Chamberlin, M. Eds., pp. 193-205 Cold Spring Harbor Laboratory, New York
37. Shine, J. and Dalgarno, L. (1975) Nature 254, 34-38
38. Horii, T., Ogawa, T., and Ogawa, H. (1980) Proc. Natl. Acad. Sci. USA 77, 313-317
39. Tessmann, E.S., Gritzmacher, C.A., and Perterson, P.K. (1978) J. Bacteriol. 135, 29-38