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대장균 K99섬모 유전자군중 제 3지역 발현에 관련된 조절자의 유전학적 연구

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Biogenetical study on potential regulatory factors involved in expression of region III genes of *Escherichia coli* K99 adhesion gene cluster

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초록 : 대장균 K99 섬모의 생 합성은 8개로 구성된 K99의 특이 유전자의 발현과 숙주유래 인자에 의해 조절되는 다른 유전자들의 발현에 의존된다. 본 연구에서는 K99섬모 유전자군중 제 3지역 발현에 유전조절자의 관련성 여부를 연구하였다. Gel retardation 분석 방법을 통하여 제3지역의 발현에 관련된 유전조절단위를 함유한 *fanF* 지역의 단백질 인자가 부착됨을 암시하였다. 이 분석방법을 이용한 결과는 또한 이 단백질 인자가 K99 유전자에서 유래되지 않고 대장균 염색체에서 유래됨을 지적하였다. 이를 보다 더 조사하기 위하여 대장균 염색체에 Tn10 transposon 유전자 변이 실험을 수행하였다. K99 유전자군으로부터 제 1지역과 제2지역의 유전자를 제거시키고, 제 3지역의 유전자인 *fanG*에 transposon Tn*lacZ*를 삽입한 pTL65-1 plasmid을 제작하였다. 이 pTL65-1는 다시 Tn10으로 염색체가 변이된 대장균에 주입하였다. 3개의 pTL65-1 주입된 Tn10 대장균 변이체 내에서 *fanG*의 발현이 증가되었다. 이들 변이대장균으로부터 Tn10이 어떤 염색체 유전자 부위를 변이 시켰는지 확인하기 위해서 변이부위 유전자를 cloning하여 염기서열을 분석하였다. 이중 2개의 clone이 동일하였으며 지금까지 알려지지 않은 유전자였다. 이들 2개의 변이체 내에서 *fanG*의 발현은 대조군과 비교해 약 4.2배 증가 되였다. 결론적으로 이들 2개의 clone으로부터 유래된 인자는 지금까지 알려지지 않은 제 3지역의 억제 조절자임을 나타내었다.

Key Words : *Escherichia coli*, Pilus adhesion, Gene regulation, Gene mutagenesis

Introduction

K99 positive-enterotoxigenic *Escherichia coli* (ETEC) is an important causative agent of acute diarrhea in neonatal calves, lambs and piglets[1]. K99 adhesin facilitates colonization by attachment to epithelial cells of the small intestine and then, the *E. coli* cells initiate producing enterotoxins[2,3]. The genes coding for the biosynthesis of K99 adhesin are present on a plasmid. The K99 genes have been cloned and shown to reside on a 7.1 kb BamHI fragment[4,5]. This fragment contains eight genes (*fanA-H*) and each is required for the biosynthesis of K99. A model of the regulation of the K99 gene cluster has been previously constructed[6,7]. The eight genes can transcriptionally be grouped into three: region 1

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includes *fanA-D*, region 2 includes *fanE-F*, and region III includes *fanG-H*. Using variety of molecular assays, regulatory regions for all the three region have been previously identified: regulatory elements for region I was identified proximal to *fanA* and *fanB*, a promoter for region II is located proximal to *fanE*, and three promoters for the expression of region III were identified in *fanF*[7]. The expression of the genes in region III requires *fanF in cis*, probably because *fanF* contains multiple promoters required for transcription of the region III genes[7].

Many bacterial genes are controlled by protein regulators and these regulators act to control transcription. These regulatory proteins generally act by binding to specific DNA sequences and once bound exert their effects. A number of known gene regulators such as the *E. coli* CRP protein[8], Lac repressor[9], Trp repressor[10], Fis protein[11], and Lrp protein[12] are homodimers that recognize specific regions of dyad symmetry DNA. Several significant regions of dyad symmetry were found in *fanF*[7]. It is possible that protein regulators may bind to this region and control the regulation of the region III gene expression. In trans-complementation experiments, the addition of the DNA containing *fanF-fanH* region slightly increased expression of *fanG* (unpublished data). This result suggested there is a negative regulator for the expression of the region III genes and the addition of extra *fanF-fanH* region likely decreased the concentration through the binding of the regulator to this DNA, resulting in an increase in *fanG* expression. In addition, the expression of the region III genes was increased in a *crp* negative strain[6]. This result suggests that CRP protein was directly or indirectly involved in the regulation of the expression.

In an attempt to investigate involvement of regulatory factors for expression of the region III genes, gel retardation assay and Tn*10* mutagenesis of the chromosome were performed. Results of the gel retardation assay showed that protein factors bound to the *fanF* region which contained regulatory elements for the expression. The Tn*10* mutagenesis of the chromosome also indicated that a novel gene was involved in the down-regulation of the region III genes.

Materials and Methods

Bacterial strains, plasmids and bacteriophage

The bacterial strains used are listed in Table 1. The *E. coli* strain KS300 was used in the Tn*10* mutagenesis experiment. CA7900 was used to produce protein extracts lacking CRP for the gel-mobility shift assay. The suppresser- positive host strain LE392 was used for preparation of new λ::Tn*lacZ* and λNK1324 lysates. RH202 was used as a competent cell for transformation. A derivative plasmid of a Tn*lacZ* fusion in *fanG* (pTL65-1) (Fig. 1) was prepared to detect regulatory factors involved in the expression. The regulatory elements of region I was removed by deletion of *Bam* HI -*Pvu* II site from the plasmid. The regulatory element of region II also was removed by deletion of *Apa* I - *Spe* I site from the plasmid. Both ends were blunted with S1 nuclease and ligated. Phage λ::Tn*10*dCam (λNK1324)[13] was used for Tn*10* mutagenesis of the chromosome.

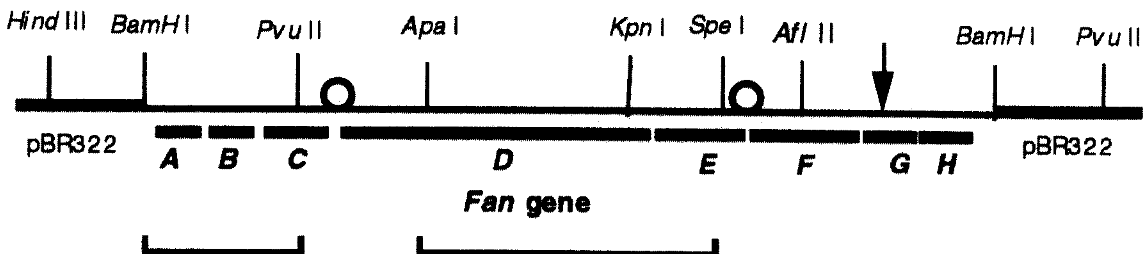

Fig. 1. A deleted derivative of Tn*lacZ* fusion in *fanG*, pTL65-1. The bottom brackets indicate the deleted regions from the fusion-plasmid. The cut ends were blunted by S1 nuclease, and ligated together using T4 DNA ligase. This derivative still remained the transcriptional terminators (0) located in-between *fanC* and *fanD*, and in-between fanE and *fanF*. The arrow indicates the location of the Tn*lacZ* fusion. The sites of restriction enzymes are indicated.

Table 1. *E. coli* strains used

Strain	Genotype	Source
KS300	F⁻, *galE, galK, lac (c74), rpsL, thi, phoA, recA1*	K. Strauch
CA7900	F⁻, *thi, crp*	J. Beckwith
RH202	F⁻, *hsdR, lacY1, supE44, thi-1, tonA21*	Helling
LE392	*hsdR514 (rk-, mk-), supE44, supF58, lacy, galK2, galT22, metB1, trp55, mcrA, lac(169), proC*::Tn5	32

Media and enzymes

Growth of bacteria for the selection of transductants and measurement of reporter gene activity was in LB. TBMM medium (10 g tryptone, 5 g NaCl, 0.2% maltose, 10 mM $MgSO_4$, 1 mg thiamine/ liter H_2O) was used to prepare new λ::Tn*lacZ* and λNK1324. Selection of Tn*10* mutants was on LB agar containing chloramphenicol (30 μg/ml).

Isolation of chromosome and plasmid DNA

Promega magic mini-prep (Promega, Madison, WI, USA) and cesium chloride-ethidium bromide density gradient centrifugation were used to prepare plasmid DNA and chromosome for cloning, sequencing, and producing deletions.

Isolation of Tn*lacZ* fusion in *fanG* and Tn*10* fusions in chromosome of *E. coli*. Fresh λ::Tn*lacZ* was prepared in *E. coli* LE392 and was added to *E. coli* KS300 containing pIX12. Other conditions of isolation of Tn*lacZ* fusion were as described in a previous report[7]. For Tn*10* fusions, lysates containing λ::Tn*10*dCam (λNK1324) were also prepared in *E. coli* strain LE392[13]. *E. coli* KS300 was grown in TBMM medium, collected by centrifugation, and resuspended in 1/10 volume LB. Fresh λ-Tn*10* lysates (1×10^9 Pfu) were added to 0.1 ml of concentrated cells. The mixed tube was incubated at room temperature for 15 min, then incubated at 37℃ for 15 min. 100 ml aliquots of the grown cultures were plated onto LB agar containing chloramphenicol (30 μg/ml), then incubated at 39℃ overnight. Transductants were collected and 100 pools were prepared. Each pool contained 50 transductants.

Selection of Tn*10*-mutant strains affecting expression of *fanG*

Each Tn*10* mutant pool was inoculated into 50 ml of LB and incubated at 37℃ in shaker-incubator for 4 hr. The cells were placed on ice for 10 min., collected by centrifugation at 4℃, 2,500 rpm for 10 min. The pellets was resuspended in 25 ml cold calcium chloride solution (50 mM $CaCl_2$, 10 mM Tris, pH 8.0), placed on ice for 10 min and centrifuged at 2,500 rpm for 10 min at 4℃. The pellet was resuspended in 4 ml cold calcium chloride solution and placed on ice for 2 hr. Approximately 100 ng of plasmid pTL65-1 was transformed into 100 ml of competent cells, and plated onto LB agar containing 150 μg/ml kanamycin, 100 μg/ml ampicillin, 30 μg/ml chloramphenicol and 20 μg/ml X-gal. Cells producing light blue and dark blue-colored colonies were picked. The β-galactosidase activities of the cells were measured.

β-galactosidase assay

Bacterial stocks of Tn*10*-mutant strains affecting expression of *fanG* were freshly grown overnight in LB medium with appropriate antibiotics. 20 μℓ of the overnight cultures were reinoculated in 2ml LB and were grown to mid-log phase. The reaction was initiated by adding o-nitrophenyl-β-D-galactoside (ONPG) at 4 mg/ml, absorbance was measured at 420 and 590 nm, and units of β-galactosidase activity were calculated according to Miller[14]. One unit of activity was defined as one nanomole of substrate hydrolyzed per minute.

Cloning chromosomal DNA fragment mutagenized with Tn*10*

100 ml TB was inoculated with each of the selected cells and incubated overnight at 37℃ in a shaker. The cells were collected by centrifugation and the pellet was resuspended with 2 ml of lysis solution (0.1 M glucose, 0.05 M Tris, pH 8.0, 0.01 M EDTA). RNase (100 mg/ml) and 40 ml lysozyme solution (2.5 mg/ml) were added. The mixture was incubated on ice for 5 min. 40 ml lysozyme solution and 20 ml 0.5 M EDTA were added. The mixture

was reincubated on ice for 30 min. 620 ml Triton-lytic solution (3% Triton X-100, 200 mM EDTA, 150 mM Tris-Cl, pH 8.0) was added. The mixture was reincubated on ice for 20 min, and 13.6 ml proteinase K solution (20 mg/ml) was added. After reincubating at 55℃ for 1 hr, an equal volume of H_2O-saturated phenol was added to the mixture. After centrifuging for 2 min, the top aqueous layer was collected. 8 g CsCl and 2.4 ml ethidium bromide solution (1 mg/ml) were added to 5.5 ml of the aqueous layer. This mixture was transferred to a ultra centrifuge tube (16 × 76 mm), and centrifuged at 58,000 rpm for 20 hr at 20℃ using 50 Ti rotor (Beckman, Fullerton, Calif. USA). The DNA layer was collected and precipitated with 2 volume of cold ethanol. Ethidium bromide was removed using H_2O-saturated butanol. The chromosomal DNA was digested with Hind III, Pst I or Sal I endonucleases whose sites does not exist in the mini-Tn*10*, and ligated in the vector pGEM-4Z after treated with the same enzymes. The recombinant DNA was transformed into a competent cell RH202 by electroporation.

DNA sequencing and sequence analysis

Nucleotide sequences were determined. Primer TNCAT (5'-GGAGGTAATAATTGACGATA-3') was used to sequence the cloned chromosome inserted with Tn*10*. This primer binds to 120 bp downstream region of 5' end of Tn*10* sequences. To localize the Tn*10* insertion in the chromosome the sequences were analyzed by computer using BLAST.

Gel-Mobility-Shift Assay

Two oligonucleotide primers, GPF1 (5'-AAACATCAC GGTAACAGCA-3') and GPF2 (5'-G AATGTTTCTATTT TACCAGT-3') were used for PCR amplification of a DNA fragment containing the *fanF* region. The amplified fragment was purified using Geneclean II kit (Bio101 Inc., La Jolla, Calif., USA). The product was labeled with ^{32}P. Protein extracts were prepared by passage of appropriate *E. coli* cells through a French press[15]. For the binding assay, samples contained the following in a total volume of 20 μl: 5 ng of ^{32}P-labeled DNA fragment; 20 μg of protein extract; 3 μg of calf thymus DNA; 20 mM Tris-Cl buffer (pH 7.9) containing 0.1 mM EDTA, 0.1 mM dithiothreitol, 4 mM magnesium acetate, 50 mM NaCl and 12.5 % glycerol. After incubation at room temperature for 10 min, samples were run by electrophoresis through 4 % polyacrylamide gel in a low-ionic strength buffer (6.7 mM Tris acetate [pH 7.5], 3.3 mM sodium acetate, 1 mM EDTA). Gels were dried and subjected to radioautography.

Results

Gel-mobility-shift assay of the *fanF* region using *E. coli* protein extracts.

The expression of *fanG* decreases as the *fanF* region was sequentially removed while the deletion of the fanE gene did not seem to affect the activity[7]. Several significant dyad regions of symmetry in the *fanF* sequence were found. The addition of DNA fragment containing *fanF-fanH* region slightly increased the expression of *fanG* (unpublished data) and the expression of the region III genes increased slightly in a *crp* negative strain6. From these results, two hypotheses were developed. 1) A protein (s) from the *fanF-fanH* region could positively activate the expression of *fanG* in trans, or 2) the regulation of the *fanG* expression involves a host regulatory factor that negatively controls the expression of *fanG*. Because of the increased expression in *crp* mutants, the CRP-cAMP complex may be directly or indirectly involved in the process. When the *trans*-complementation was performed, the presence of extra *fanF* containing the regulatory region for the region III genes would bind to the regulatory protein(s) and decrease the concentration available to bind to the regulatory elements in front of *fanG*. This would allow for greater expression of *fanG*.

Therefore, to test these hypotheses, gel retardation assay was performed using the *fanF* region which likely has the regulatory elements and regulatory factor-binding sites required for the expression of the region III genes. The *fanF* DNA fragment for this assay was amplified by PCR and was labeled with 32P. Protein extracts were prepared from *E. coli* KS300 containing pIX12, the *fanD-fanH* region, pBR322 (as a vector control), and KS300 with no plasmid and a *crp* mutant strain. These extracts were mixed with the labeled *fanF* DNA. Calf thymus DNA was added to block non-specific binding. These mixtures were incubated at room temperature for 10 min and run by electrophoresis in a 4% polyacrylamide gel. The results are shown in Fig. 2. All extracts except from the *crp* mutant strain and the control in which no protein was added caused retardation of the *fanF* DNA. These results suggest that *fanF* DNA

binds one or more proteins and that all extracts tested contained these factors except the *crp* mutant strain. This assay also showed that there was no different between these strains with or without K99 genes, suggesting that the protein factors were not encoded by K99-specific genes.

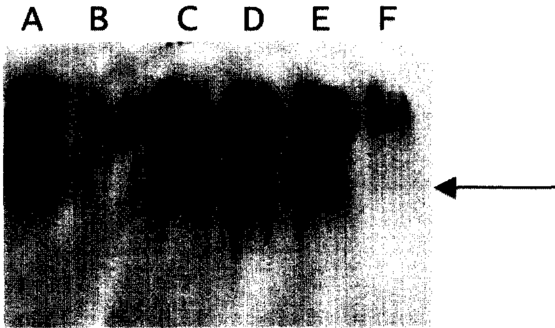

Fig. 2. Gel-mobility-shift assay measuring the binding of protein extracts from a variety of *E. coli* strains to the *fanF* DNA fragment containing the regulatory element for expression of region III. The DNA fragment contained the region between *Spe* I site in fanE and the 3' end of *fanF*. Lanes: A, KS300; B, a *crp*⁻ strain; C, KS300 containing pBR322; D, KS300 containing *fanD-H* fragment; E, KS300 containing pIX12; F, no protein extract. Arrow indicates retardations with protein extracts from the strains except extract from a *crp*⁻ strain and control.

Detection of potential chromosomally encoded regulatory factors for region III using Tn*10* mutagenesis.

Gel-mobility-shift assays demonstrated that protein factors from chromosomally encoded genes bind to the *fanF* DNA region and these factors may be involved in the regulation of the gene expression of region III. To further investigate on the factors, Tn*10* mutagenesis of the chromosome was performed using the mini-Tn*10* derivative 105 on phage vehicle λ NK1324[13]. Plasmid pTL65-1 was constructed by deleting the regulatory elements for region I and II from a *fanG*::Tn*lacZ* fusion-plasmid so that the expression of *fanG* should be regulated only by the regulatory element for region III. In addition, the two transcriptional terminators were still remained upstream of the regulatory element for region III so that these terminators would terminate any possible transcriptional activity from the upstream of *fanF*. Tn*10* fusions in chromosome of a *E. coli* KS300 were prepared and then pTL65-1 was transformed to the Tn*10* mutagenized-cells. Colonies showing significant color shade difference compared to the color shade of non-mutated cells were picked. β-galactosidase activities of the cells were measured to confirm that the mutation in chromosome affected the expression of *fanG*. Chromosome fragments inserted with Tn*10* were cloned and sequenced. The sequence was analyzed using BLAST Network Service in National Center for Biotechnology Information. From the search analysis the location of the transposon insertion in the chromosome was determined. The chromosome fragments from two light blue colonies and three dark blue colonies were cloned and sequenced. Mutations in factors that up regulate expression should yield light blue colonies and mutations in factors that suppress expression should yield dark blue colonies.

The β-galactosidase activities of the colonies and sequence analysis are shown in Table 2. Two chromosomal genes were identified that resulted in dark blue colonies

Table 2. Potential regulatory factors on K99 region III

Mutant	Color on X-gal	Activity (U)[a]	Chromosomal gene inserted with Tn*10*
TLBC1	dark blue	8355	unknown gene, uncharacterized
TLBC2	dark blue	6320	*gltA* (citrate synthase)
TLBC4	dark blue	8460	Same gene of TLBC1
TLWC3	light blue	802	*groES* (GroES)
TLWC6	light blue	632	unknown gene, 92.8 to 0.01 min.
Control	blue	2015	

[a]Average of at least 5 determinants of β-galactosidase activity. One unit of activity was defined as one nanomole of substrate hydrolyzed per minute.

(TLBC1 and TLBC4). The activities were approximately 4.2-fold higher than that of the control without the Tn*10* mutation. The sequences from these two chromosome fragments are matched, indicating that these genes are identical. However, the gene has not been previously identified. The other chromosomal gene from the dark blue colony (TLBC2) was shown to be *gltA*. The two chromosomal mutations from the light blue colonies (TLWC3 and TLWC6) were shown to be *groES* and a known sequence but unknown function, respectively. The unknown gene was located between 92.8 and 0.01 min on the *E. coli* chromosome.

Discussion

Our previous results suggested that the expression of the region III genes requires specific regulatory factors including CRP[6,7]. To examine this possibility, gel-mobility-shift assay was performed. The *fanF* region was selected for the target DNA since it has the regulatory elements and regulatory factor-binding sites required for the expression[7]. All protein extracts used in the gel-mobility-shift assays except the proteins from the *crp* mutant strain caused retardation of the *fanF* DNA. These results suggest that *crp* positive cells contain a specific protein (s) that binds to the *fanF* region. Therefore, CRP-cAMP complex could be directly or indirectly involved in the binding to the *fanF* DNA and mediates the regulation of the region III genes. The complex is involved in activation or inactivation of many genes[16,17,18]. The complex controls genes either by an interaction with RNA polymerase bound to the promoter or by structural changes in the DNA from CRP-cAMP induced bending[19,20]. The complex is also known to function in the regulation of *E. coli* pilus genes. The *pap* genes in uropathogenic *E. coli* is regulated by the complex[21,22] as are K99 genes[6,7]. It was hypothesized that a protein(s) encoded within the *fanF-fanH* region also could positively activate the expression of *fanG in trans*, since the addition of the *fanF-fanH* fragment to the *fanG′* Tn*phoA* fusions increased *fanG* expression (data not shown). However, using the gel-mobility assay there was no difference between these strains with or without K99 genes, suggesting that the *fanF-H* region does not encode a protein that binds to the region III regulatory sequences. More likely, the reason for the increase in *fanG* expression is due to the binding of the protein regulators to the added *fanF* DNA, thereby reducing the concentration of the putative repressor so that the expression of *fanG* was increased. To examine whether CRP-cAMP complex directly binds to the *fanF* region, the fan DNA was analyzed by computer search to find putative CRP binding sites and gel-mobility-shift assays were carried out using purified CRP protein and cAMP. No significant CRP binding sites was found in the *fanF* region. The purified CRP protein did not retard the DNA suggesting that the CRP affect is indirect (data not shown). The production of the regulatory factor is possibly regulated by CRP. This regulator is likely involved in the down-regulation of the expression, since the expression of *fanG* and *fanH* in a *crp* negative strain was increased.

The results of gel-mobility-shift assays were consistent with the hypothesis that chromosomally encoded regulatory factors was involved in the expression of region III. Gene expression is modulated by positive or negative control via binding to DNA regulatory sequences[23]. In bacteria most regulators bind at a distance close enough to the promoter region to allow protein-protein contact with RNA polymerase, to prevent RNA polymerase from binding to promoters, or to dissociate RNA polymerase in process of RNA polymerization from the DNA template[24,25]. To identify these factors, Tn*10* mutagenesis of the *E. coli* chromosome was performed. A plasmid containing *lacZ* gene insertion in *fanG* was transformed to Tn*10* mutagenized *E. coli* cells and colonies with increased *fanG* expression identified based on the amount of β-galactosidase produced. Three clones were identified that resulted in increased *fanG* expression. Two clones of the clones were identical by the sequence analysis. These clones did not corresponded to any previously sequenced genes based on accessions to Genbank. The protein factor from this gene may be directly involved in the down-regulation of the gene expression of region III. The region III genes are expressed by multiple promoters and the gene products are minor subunits for the biosynthesis of K99 adhesin[26,27]. Therefore, it is not necessary to produce large amounts of *FanG* or FanH. In minicell assays the products of *fanG-H* were relatively small amount compared to the other products[27]. It is plausible that the *fanG* and fanH genes have a down-regulation system to control the high transcriptional activities from the multiple promoters. The results from a *crp* negative strain and gel-mobility-shift assay indicated that CRP may be involved

in the down-regulation of region III. CRP may activate the repressor. Therefore, in *crp* negative cells the repressor was not well produced so that expression of region III genes increased.

The other chromosomal mutation that increased *fanG* expression was shown to be in *gltA*. *gltA* encodes citrate synthase[28,29]. How this affects the expression of K99 genes is not known. The increased expression may be due to a general shift of metabolism.

Two chromosomal mutations generated colonies that had decreased *fanG* expression. The mutations were in *groES* and a unknown gene, respectively. The expression of both colonies were slightly lower than that of the control. GroES has been known as a *E. coli* chaperonin that facilitates proper protein folding and prevent protein from aggregating[30,31]. GroES may exert its effect on the *fanG-lacZ* fusion by being required for the correct folding of *LacZ*. If this is so, GroES may not be important for *fanG* expression. This possibility could be tested by directly measuring *fanG* transcripts from *groES* negative and positive cells or by screening expression of other fan gene in the groES mutant. The other mutation that decreased *fanG* expression was shown to be located between 92.8 and 0.01 min on *E. coli* chromosome. However, the function of this sequence has not been determined. Additional genetic and biochemical studies are required to characterize the genes encoding the regulatory factors and their products. Such studies should provide new insights about general understanding of the regulation of K99.

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References

1. Jones GW, Isaacson RE. Proteinaceous bacterial adhesins. *Crit Rev Microbiol*, 10:229-260, 1983.
2. Cheng K, Costerton J. Microbial adhesion and colonization within the digestive tract. *Soc Appl Bacteriol Symp Series*, 13:239-261, 1986.
3. Sharon N. Bacterial lectins, cell-cell recognition and infectious disease. *FEBS Lett*, 217:145-157, 1987.
4. De Graaf FK, Krenn BE, Klaasen P. Organization and expression of genes involved in the biosynthesis of K99 fimbriae. *Infect Immun*, 43:508-514, 1984.
5. van Embden JDA, de Graaf FK, Schouls LM, *et al.* Cloning and expression of a deoxyribonucleic acid fragment that encodes for the adhesive antigen K99. *Infect Immun*, 29:1125-1133, 1980.
6. Inoue OJ, Lee JH, Isaacson RE. Transcriptional organization of the *Escherichia coli* pilus adhesin K99. *Mol Microbiol*, 10:607-613, 1993.
7. Lee JH, Isaacson RE. Expression of the gene cluster associated with the *E. coli* pilus-adhesin K99. *Infect Immun*, 63: 4143-4149, 1995.
8. MaKay DB, Steitz T. Structure of catabolite gene activator protein at 2.9 A8 resolution suggests binding to left-handed B-DNA. *Nature*, 290: 744-749, 1981.
9. Kaptein R, Zuiderweg ER, Scheek RM *et al.* A protein structure from nuclear magnetic resonance data. *J Mol Biol*, 182: 179-82, 1985.
10. Schevitz RW, Otwinowski Z, Joachimiak A, *et al.* The three dimensional structure of Trp repressor. *Nature*, 317: 782-786,1985.
11. Kostrewa D, Granzin J, Koch C, *et al.* Three-dimensional structure of the *E. coli* DNA-binding protein FIS. *Nature*, 349: 178-180, 1991.
12. Wang Q, Wu J, Friedberg D, *et al.* Regulation of the *Escherichia coli lrp* gene. *J Bacteriol*, 176: 1831-1839, 1994.
13. Kleckner N, Bender J, Gottesman S. Uses of transposons with emphasis on Tn*10*. *Methods Enzym*, 204: 139-180, 1991.
14. Miller JH. Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold spring Harbor, NY, 1971.
15. Ricca E, Aker D, Calvo JM. A protein that binds to the regulatory region of the *Escherichia coli ilvIH* operon. *J Bacteriol*, 171:1658-1664, 1989.
16. Cossart P, Gjcquel-Sanzey B. Regulation of expression of the *crp* gene of *Escherichia coli* K-12. *J Bacteriol*, 161: 454-457, 1985.
17. Inouye S, Franceschini K, Inouye M. Structural similarities between the development-specific protein A from a gram-negative bacterium, *Myxococcus xanthus*, and *calmodulin*. *Proc Natl Acad Sci USA*, 80: 6829- 6833, 1983.
18. Short SA, Singer JT. Studies on *deo* operon regulation

in Escherichia coli: cloning and expression of the *deoR* structural gene. *Gene*, 31: 205-211, 1984.

19. Botsford J, Harman JG. Cyclic AMP in prokaryotes. *Microbiol Rev*, 56: 100-122, 1992.
20. Kolb A, Busby S, Buc H *et al.* Transcriptional regulation by cAMP and its receptor protein. *Annu Rev Biochem*, 62: 749-795, 1993.
21. Forsman K, Sonden B, Goransson M, *et al.* Antirepression function in *Escherichia coli* for the cAMP-cAMP receptor protein transcriptional activator. *Proc Natl Acad Sci USA*, 89: 9880-9884, 1992.
22. Goransson M, Forsman K, Nilsson P, *et al.* Upstream activating sequences that are shared by two divergently transcribed operons mediate cAMP-CRP regulation of pilus-adhesin in *Escherichia coli. Mol Microbiol*, 3: 1557-1565, 1989.
23. Ptashne M, Gann AF. Activators and targets. *Nature*, 346:329-331, 1990.
24. Adhya S, Garges S. Positive control. *J Biol Chem*, 265: 10797-10800, 1990.
25. Collado-Vides J, Magasanik B, Gralla JD. Control site location and transcriptional regulation in *Escherichia coli. Microbiol Rev*, 55: 371-394, 1991.
26. Roosendaal E, Jacobs AAC, Rathman P, *et al.* Primary structure and subcellular localization of two fimbrial subunit-like proteins involved in the biosynthesis of K99 fibrillae. *Microbial Pathogenesis*, 1: 211-217, 1987.
27. Simons, LH, Willemsen PTJ, Bakker D, *et al.* Localization and function of FanH and *FanG*, minor components of K99 fimbriae of enterotoxigenic *Escherichia coli. Microbial Pathogenesis*, 11: 325-326, 1991.
28. Park SJ, McCabe J, Turna J, *et al.* Regulation of the citrate synthase (*gltA*) gene of *Escherichia coli* in response to anaerobiosis and carbon supply: role of the *arcA* gene product. *J Bacteriol*, 176: 5086-5092, 1994.
29. Patton AJ, Hough DW, P. Towner P, *et al.* Does possess a second citrate synthase gene? *Eur J Biochem*, 214: 75-81, 1993.
30. Hendrick JP, Hartl FU. Molecular chaperone functions of heat-shock proteins. *Annu Rev Biochem*, 62: 349-384, 1993.
31. Lund P. The chaperonin cycle and protein folding. *Bioassays*, 16: 229-231, 1994.
32. Bastard PJ Jr, T. J. Silhavy TJ, Beckwith JR. Use of gene fusion to study secretion of maltose-binding protein *Escherichia coli* periplasm. *J Bacteriol*, 139: 19-31, 1979.