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Nucleotide Sequences of *fic* and *fic-1* Genes Involved in Cell Filamentation Induced by Cyclic AMP in *Escherichia coli*

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The nucleotide sequences of *fic-1* involved in the cell filamentation induced by cyclic AMP in *Escherichia coli* and its normal counterpart *fic* were analyzed. The open reading frame of both *fic-1* and *fic* coded for 200 amino acids. The Gly at position 55 in the Fic protein was changed to Arg in the Fic-1 protein. The promoter activity of *fic* was confirmed by fusing *fic* and *lacZ*. The gene downstream from *fic* was found to be *pabA* (*p*-aminobenzoate). There is an open reading frame (ORF190) coding for 190 amino acids upstream from the *fic* gene. Computer-assisted analysis showed that Fic has sequence similarity with part of CDC28 of *Saccharomyces cerevisiae*, CDC2 of *Schizosaccharomyces pombe*, and FtsA of *E. coli*. In addition, ORF190 has sequence similarity with the cyclosporin A-binding protein cyclophilin.

More than 30 cell division genes have been reported (5), and the number of the cell division genes is increasing, but their interaction is not still clear because of the complexity of the interaction. We have focused on cyclic AMP (cAMP) as a possible regulatory factor in cell division. Although cAMP is not essential for growth in *Escherichia coli* because the *cya* mutant is viable, much evidence supports the idea that cAMP controls cell growth or cell division in *E. coli* (3, 9, 22, 23, 26). We isolated cAMP-requiring mutants with double mutations of *cya* and *cid* (24, 25). We have found that an increase in intracellular cAMP levels induced cell filamentation in a *fic-1* mutant at high temperature (14, 23, 27). cAMP is required for anucleate cell production (9). The filamentation of *ftsZ*(Ts) is repressed in the *cya* or *crp* mutant (26). Interestingly, Hughes et al. showed that cAMP binds DnaA protein and has a regulatory role in DNA replication (8).

It is important to know the structure of the *fic* gene to understand its function in relation to cAMP. In this study we sequenced the *fic* and *fic-1* genes and found that the *fic* gene has some sequence-similar regions to other cell division genes.

Plasmid pHB3 (1, 13), containing the *fic* gene; pMK108 (13), containing the *fic-1* gene; and other subcloned plasmids used as the source of DNA for sequencing were purified by the procedure of Birnboim and Doly (2). For preparation of plasmids on a small scale, the alkaline sodium dodecyl sulfate method of Davis et al. (4, 16) was used. Restriction fragments digested by *Alu*I, *Eco*RV, *Hae*III, *Hin*cII, *Hpa*II, *Rsa*I, *Sau*3A, *Taq*I, and *Tha*I were subcloned into M13 vector mp18 or mp19 (28). We sequenced a 2.5-kilobase-pair (kb) *Ava*I-*Ava*I region which includes the *fic-1* gene and its surroundings, as reported previously (13). The sequence strategy is shown in Fig. 1. Almost all double-stranded DNAs were sequenced by the dideoxy-chain termination method (20) (shown by arrows in Fig. 1). The Maxam and Gilbert method (17) was used for the parts (dashed arrows in Fig. 1) which are difficult to analyze by the M13 chain termination method. In the base sequence (Fig. 2), we found an open reading frame consisting of 594 base pairs (bp) (base numbers from 1412 to 2011 in Fig. 2) which can encode 200 amino acid residues. We concluded that this open reading frame is for the *fic-1* gene because *fic-1* was included in the 1.6-kb *Mlu*I-*Ava*I fragment (previous result; 13). There were also three possible protein-coding frames upstream and downstream of the *fic-1* gene. Putative promoter regions (dashed lines) and Shine-Dalgarno sequences (boxed) were found, as indicated in Fig. 2. The calculated protein size from the sequence of *fic-1* was 22,930 daltons, coinciding well with the protein product of 21 kilodaltons identified by the maxicell method, as reported previously (13).

To find where in the DNA the mutation was generated in the *fic-1* gene, we sequenced, by the dideoxy-chain termination method, a 1.3-kb *Mlu*I-*Eco*RV region containing the *fic* gene. The *fic* DNA was isolated from pHB3. An enzymatically digested DNA fragment was inserted into an mp18 or mp19 vector, and sequencing was performed (detailed sequence strategy is not shown). G at position 1574 was changed to C in the *fic-1* mutant (Fig. 2). By this mutation, Gly at position 55 of the Fic protein was changed to Arg in the Fic-1 protein, so the mutation of *fic-1* was a missense mutation. This result coincided with the previous result indicating that Fic-1 and Fic proteins were the same size (13).

To predict the function of the Fic protein, computer-assisted homology analysis was done by comparing the *fic* gene and Fic protein to the gene banks of EMBL and GenBank and the protein bank of NBRF, respectively. Homology searching was done by using the SEQFP and SEQHP programs according to the algorithm described by Goad and Kanehisa (6). It was revealed that the gene downstream from *fic* was the already sequenced *pabA* gene (10, 11). No sequence difference of *pabA* was observed between our results and already reported ones (11). The initiation codon of the *pabA* gene started only 31 bp down-

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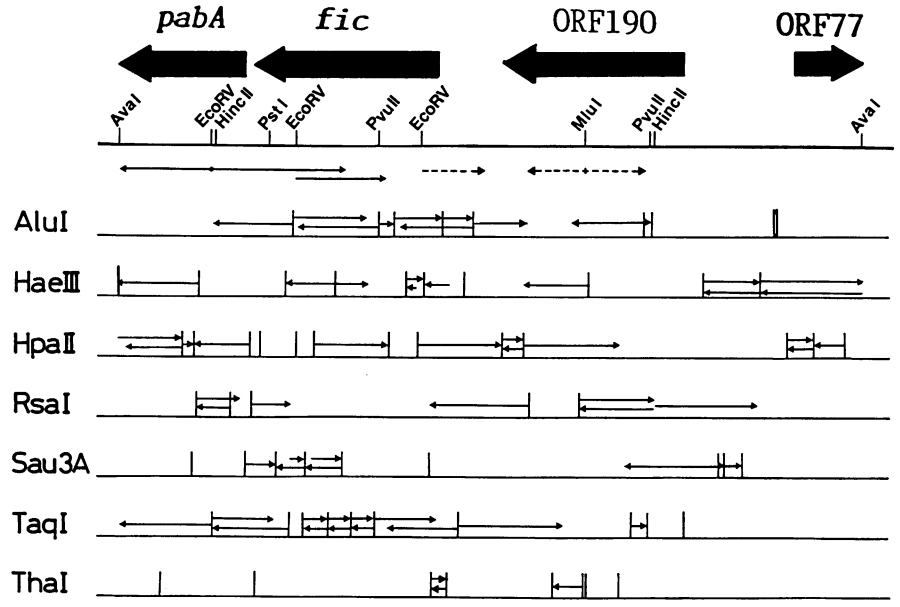

FIG. 1. Restriction map and DNA sequencing strategy for a 2.5-kb *Ava*I-*Ava*I fragment. The bold arrows represent the open reading frames of ORF77, ORF190, *fic*, and *pabA*. The nucleotide sequences were determined by the M13 dideoxy-chain termination method (solid arrows) (20) and the method of Maxam and Gilbert (dashed arrows) (17). Arrows indicate the direction and the region in which the sequence was analyzed.

stream of the *fic* termination codon. Although high-score homologous genes with *fic* were not found in the homology search, a quite interesting result was obtained. The amino acid sequence from position 11 to 67 of the *fic* gene has sequence similarity with positions 84 to 138 of CDC28 of *Saccharomyces cerevisiae*, 80 to 136 of CDC2 of *Schizosaccharomyces pombe*, and 305 to 357 of FtsA of *E. coli* (Fig. 3A). This sequence-similar region of CDC2, CDC28, and FtsA was the same as reported by Robinson et al. (19). Considering that those genes were cell division genes, the Fic protein may have some functional similarity. The hydropathy of the Fic protein, identified by the algorithm of Kyte and Doolittle (15), indicates that the Fic protein does not have any significant hydrophobic region. The upstream reading frame of the *fic* gene, which we tentatively named ORF190, codes for 190 amino acids, and its calculated molecular mass is 20,430 daltons. Homology searching for the ORF190 protein with NBRF protein bank showed that the ORF190 protein has significant sequence similarity with cyclophilin (7), a cyclosporin A-binding protein. The percentage of sequence similarity of ORF190 and Hu-Cp indicated in Fig. 3B was 35%. Furthermore, the ORF190 protein has a hydrophobic stretch in the N-terminal 22 amino acids (STLAAAMAVFALSALSPAAMAA), which is a typical character of signal peptides (18). The ORF190 protein is possibly a secreted protein. ORF77 has no significant sequence similarity with already known genes or proteins and no characteristic feature.

To confirm that the *fic* gene was actually expressed, we constructed a *fic-lacZ* fusion gene (Fig. 4). The 752-bp *Eco*RV-*Pvu*II fragment containing the putative *fic* promoter region was ligated to a *Sma*I-digested fragment of pMC1403 (12), which is a *lacZ'* fusion vector, and the ligation mixture was used to transform MC1061 (Δ*lac*). Transformants were selected on MacConkey-lactose agar plates containing ampicillin. It was predicted from the DNA sequence that the reading frames of *fic* and *lacZ* would match when the 752-bp *Eco*RV-*Pvu*II fragment was ligated to the pMC1403 *Sma*I site. As predicted, red colonies were isolated. The restriction enzyme digestion analysis revealed that red colonies contained a *fic-lacZ* fusion plasmid (pFL1) ligated in the proper orientation of the reading frame. The result showed that the *fic* gene was exactly expressed and that this open reading frame was correctly operating. In another experiment, *argD-lacZ* fusion plasmids, pAL1 and pYL1, were constructed. Both fusion plasmids coded for active β-galactosidase. Plasmid pAL1 contained a 1.5-kb *Hin*cII-*Hin*cII fragment in the *Sma*I site of pMC1403, and pYL1 contained a 1.4-kb *Hin*cII-*Hin*cII fragment in pMC1403. We examined the effects of cAMP on the pFL1 gene by measuring the β-galactosidase activity in the TP2010 (Δ*lac* Δ*cya*) (12) strain. The β-galactosidase activity was the same when TP2010(pFL1) was incubated in the presence or absence of cAMP (data not shown). To circumvent the possibility that the supply of cyclic AMP receptor protein (CRP) is not enough for detecting the regulation of *fic-lacZ* by cAMP-CRP in plasmid pFL1, we constructed another plasmid, called pFLCR1, in which *crp* gene was cloned in the *Eco*RI site of pFL1. In pFLCR1 the gene dosages of *crp* and *fic-lacZ* are the same. We examined the effect of cAMP on the expression of *fic-lacZ*, but we could not find any significant effect of cAMP on *fic-lacZ* expression. In addition, we could not find the consensus CRP-binding sequence upstream of the *fic* gene. These results would indicate that the *fic* gene is not controlled by cAMP. Furthermore we could not find a consensus protein homology of a cAMP-binding protein such as CRP (1), DnaA (7), or protein kinase. The effects of cAMP on the *fic-1* gene seem to be indirect.

At present we do not know how Fic protein acts in cell division, but a mutation of Gly-55 to Arg-55 in the *fic* gene causes an inhibitory effect on cell division induced by cAMP

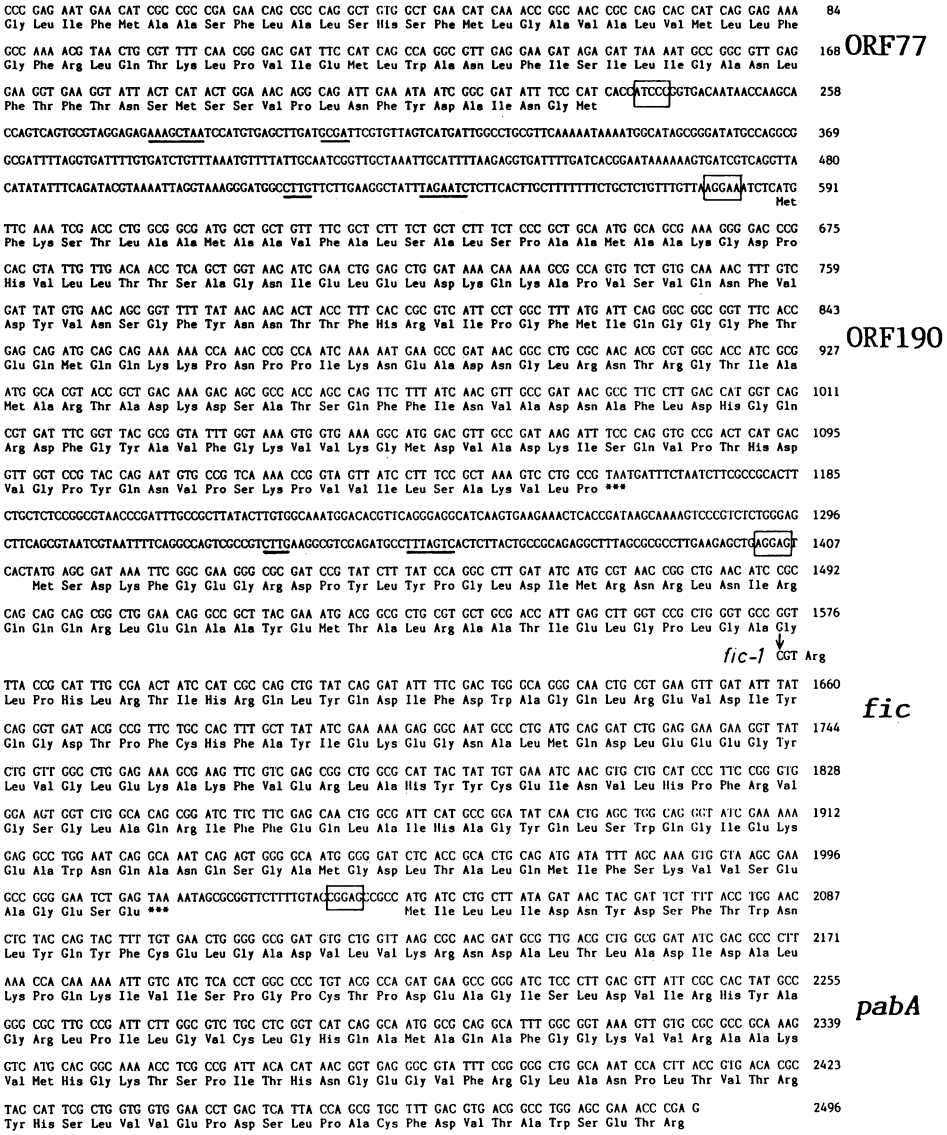

FIG. 2. Nucleotide sequence and deduced amino acid sequence in the 2496-bp *Ava*I-*Ava*I region. Putative promoters are underlined, and ribosome-binding sites are boxed. The location of the *fic-1* mutation is shown at position 1574. Asterisks indicate the stop codon.

A

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CDC2   80LYLVFE-FLDMDLKKYMDRI-SETGATSLDPR-LVQKF-TYQLVNG-VNFC--HSRRIIHRDLK136
CDC28  84LYLVFE-FLDLDLKRYMEGIPKDQP-LGAD---IVKKF-MMQLCKG-IAYC--HSHRILHRDLK138
FIC    11PYL-YP-GLDIMRNR-LN-I-RQQQRLE-QAAYEMTALRAATIELGPLGAGLPHL-RTIHRQLY67
FTSA  305LNLVNEEILQLQ-EK----L-RQQGVKHHLAAGIVLTGGARQIE-G-LAAC---AQRVFHTQVR357
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B

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ORF190 38GNIELELDKQKAPVSVQNF--VDYVNSGF-YNNTTFHRVIPGFMIQGGGFT-EQMQQKKPNPPIK98
Hu-Cp  18GRVSFELFADKVPKTAENFRALSTGEKGFGYKGSCFHRIIPGFMCQGGDFTRHNGTGGKSIYGEK82

ORF190 99NEADN-GLRNT-RGTIAMARTADKDSATSQFFINVADNAFLDHGQRDFGYAVFGKVVKGMD-V-A159
Hu-Cp  83FEDENFILKHTGPGILSMAN-AGPNTNGSQFFICTAKTEWLD-G-KH---VVFGKVKEGMNIVEA141
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FIG. 3. Alignment of the Fic protein amino acid sequence with FtsA of *E. coli*, CDC2 of *S. pombe*, and CDC28 of *S. cerevisiae* (A) and alignment of the amino acid sequence of ORF190 with human cyclophilin (Hu-Cp) (B). The sequences are aligned for maximum sequence similarity. Amino acid identities are marked with two asterisks, and conserved amino acids are marked with one asterisk. Intermediate dashes indicate insertions needed to maintain the alignment. Related amino acids are grouped as follows: P, A, G, S, and T; Q, N, E, and D; H, K, and R; L, I, V, and M; F, Y, and W.

with temperature sensitivity. Considering that cells of a *fic*-deleted strain are short rods (13) and that the Fic protein has some sequence similarities with the other cell division proteins, Fic protein surely has some role in cell division. An alternate possible speculation about the role of *fic*, which is not favorable, is that the mutation in *fic* merely has an inhibitory effect on cell division, such as in the case of the SOS system (5), and normal *fic* is not directly involved in cell division. The role of *fic* will be clarified by constructing double mutants carrying *fic* and other cell division mutations. Since the *fic-1* mutation was fortuitously found in PA3092, the *fic-1* mutation probably still has a leaky phenotype in cell division. We are now isolating another type of mutation occurring in the *fic* gene. Interestingly, the location of the *fic* gene was close upstream from *pabA* gene. Kaplan et al. sequenced the *pabA* region of several species of bacteria and indicated the possibility of the existence of a *pabA* upstream gene in several bacteria, such as *Salmonella typhimurium*, *Klebsiella aerogenes*, and *E. coli* (10). The *fic* gene might be conserved among bacteria and have some important functions. The study of the relationship of the *pabA* and *fic* genes will be necessary.

The ORF190 protein contains a signal-sequence-like sequence and has sequence similarity with human cyclophilin, which is the cyclosporin A-binding protein (7). Recently it was found that cyclophilin is identical with peptidyl-prolyl *cis-trans* isomerase (21). Peptidyl-prolyl *cis-trans* isomerase activity has been detected in *E. coli*, yeast, insects, and mammals (21). Cyclophilin is widely conserved in many organisms. It is possible that ORF190 has the same property of cyclophilin (peptidyl-prolyl *cis-trans* isomerase). Further investigation of ORF190 will be necessary.

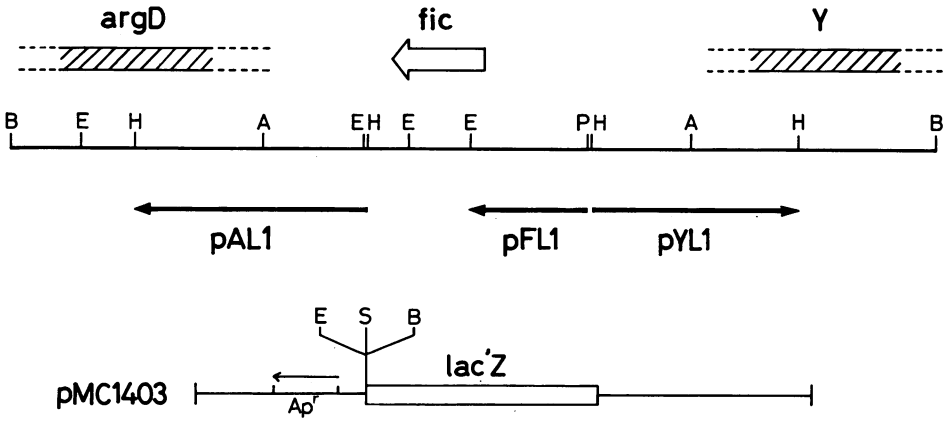

FIG. 4. Structures of the fusion genes pFL1, pAL, and pYL. Plasmid pFL was constructed by ligating the 0.8-kb *Eco*RV-*Pvu*II fragment containing the *fic* promoter with *Sma*I-digested pMC1403. pAL1 was constructed by inserting the *Hin*cII 1.5-kb fragment containing the *argD* promoter in the pMC1403 *Sma*I site. pYL1 was constructed by inserting the 1.4-kb fragment in the pMC1403 *Sma*I site. All three plasmids coded for active fusion β-galactosidase.

LITERATURE CITED

1. **Aiba, H., S. Fujimoto, and N. Ozaki.** 1982. Molecular cloning and nucleotide sequencing of the gene for *E. coli* cAMP receptor protein. Nucleic Acids Res. **10**:1345–1361.
2. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
3. **D'Ari, R., A. Jaffé, P. Bouloc, and A. Robin.** 1988. Cyclic AMP and cell division in *Escherichia coli*. J. Bacteriol. **170**:65–70.
4. **Davis, R. W., D. Botstein, and J. R. Roth (ed.).** 1980. Advanced bacterial genetics, p. 120–121. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Donachie, W. D., K. J. Begg, and N. F. Sullivan.** 1984. Morphogenes of *Escherichia coli*, p. 27–62. *In* R. Losick and L. Shapiro (ed.), Microbial development. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Goad, W. B., and M. Kanehisa.** 1982. Pattern recognition in nucleic acid sequences. I. A general method for finding local homologies and symmetries. Nucleic Acids Res. **10**:247–263.
7. **Harding, M. W., R. E. Handschumacher, and D. W. Speicher.** 1986. Isolation and amino acid sequence of cyclophilin. J. Biol. Chem. **261**:8547–8555.
8. **Hughes, P., A. Landouisi, and M. Kohiyama.** 1988. A novel role for cAMP in the control of the activity of the *E. coli* chromosome replication initiator protein, DnaA. Cell **55**:343–350.
9. **Jaffé, A., R. D'Ari, and V. Norris.** 1986. SOS-independent coupling between DNA replication and cell division in *Escherichia coli*. J. Bacteriol. **165**:66–71.
10. **Kaplan, J. B., W. K. Merkel, and B. P. Nichols.** 1985. Evolution of glutamine amidotransferase genes. Nucleotide sequences of the *pabA* genes from *Salmonella typhimurium*, *Klebsiella aerogenes* and *Serratia marcescens*. J. Mol. Biol. **183**:327–340.
11. **Kaplan, J. B., and B. P. Nichols.** 1983. Nucleotide sequence of *Escherichia coli pabA* and its evolutionary relationship to *trp(G)D*. J. Mol. Biol. **168**:451–468.
12. **Kawamukai, M., J. Kishimoto, R. Utsumi, M. Himeno, T. Komano, and H. Aiba.** 1985. Negative regulation of adenylate cyclase gene (*cya*) expression by cyclic AMP-cyclic AMP receptor protein in *Escherichia coli*: studies with *cya-lac* protein and operon fusion plasmids. J. Bacteriol. **164**:872–877.
13. **Kawamukai, M., Y. Matsuda, W. Fujii, T. Nishida, Y. Izumoto, M. Himeno, R. Utsumi, and T. Komano.** 1988. Cloning of the *fic-1* gene involved in cell filamentation induced by cyclic AMP and construction of a Δ*fic Escherichia coli* strain. J. Bacteriol. **170**:3864–3869.
14. **Kawamukai, M., K. Murao, R. Utsumi, M. Himeno, and T. Komano.** 1986. Cell filamentation in an *Escherichia coli* K-12 *fic* mutant caused by theophylline or an adenylate cyclase gene (*cya*)-containing plasmid. FEMS Microbiol. Lett. **34**:117–120.
15. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
16. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
18. **Oliver, D.** 1985. Protein secretion in *Escherichia coli*. Annu. Rev. Microbiol. **39**:615–648.
19. **Robinson, A. C., J. F. Collins, and W. D. Donachie.** 1987. Prokaryotic and eukaryotic cell-cycle proteins. Nature (London) **328**:766.
20. **Schreier, P. H., and R. Cortese.** 1979. A fast and simple method for sequencing DNA cloned in the single-stranded bacteriophage M13. J. Mol. Biol. **129**:169–172.
21. **Takahashi, N., T. Hayano, and M. Suzuki.** 1989. Peptidylprolyl *cis-trans* isomerase is the cyclosporin A-binding protein cyclophilin. Nature (London) **337**:473–475.
22. **Utsumi, R., M. Kawamukai, H. Aiba, M. Himeno, and T. Komano.** 1986. Expression of the adenylate cyclase gene during cell elongation in *Escherichia coli* K-12. J. Bacteriol. **168**: 1408–1414.
23. **Utsumi, R., Y. Nakamoto, M. Kawamukai, M. Himeno, and T. Komano.** 1982. Involvement of cyclic AMP and its receptor protein in filamentation of an *Escherichia coli fic* mutant. J. Bacteriol. **151**:807–812.
24. **Utsumi, R., M. Noda, M. Kawamukai, and T. Komano.** 1987. Effects of *cya* and *cid* mutations on cell growth in *Escherichia coli*. Agric. Biol. Chem. **51**:465–469.
25. **Utsumi, R., M. Noda, M. Kawamukai, and T. Komano.** 1988. Isolation of a cAMP-requiring mutant in *Escherichia coli* K-12: evidence of growth regulation via N-acetylglucosamine metabolism controlled by cAMP. FEMS Microbiol. Lett. **50**:217–221.
26. **Utsumi, R., M. Noda, M. Kawamuakai, and T. Komano.** 1989. Control mechanism of the *Escherichia coli* K-12 cell cycle is triggered by the cyclic AMP-cyclic AMP receptor protein complex. J. Bacteriol. **171**:2909–2912.
27. **Utsumi, R., H. Tanabe, Y. Nakamoto, M. Kawamukai, H. Sakai, M. Himeno, T. Komano, and Y. Hirota.** 1981. Inhibitory effect of adenosine 3′,5′-phosphate on cell division of *Escherichia coli* K-12 mutant derivatives. J. Bacteriol. **147**:1105–1109.
28. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of M13mp18 and pUC19 vectors. Gene **33**:103–119.