

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

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Received 21 March 1989/Accepted 22 May 1989

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 *AluI*, *EcoRV*, *HaeIII*, *HincII*, *HpaII*, *RsaI*, *Sau3A*, *TaqI*, and *ThaI* were subcloned into M13 vector mp18 or mp19 (28). We sequenced a 2.5-kilobase-pair (kb) *AvaI-AvaI* 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 *MluI-AvaI* 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 *MluI-EcoRV* 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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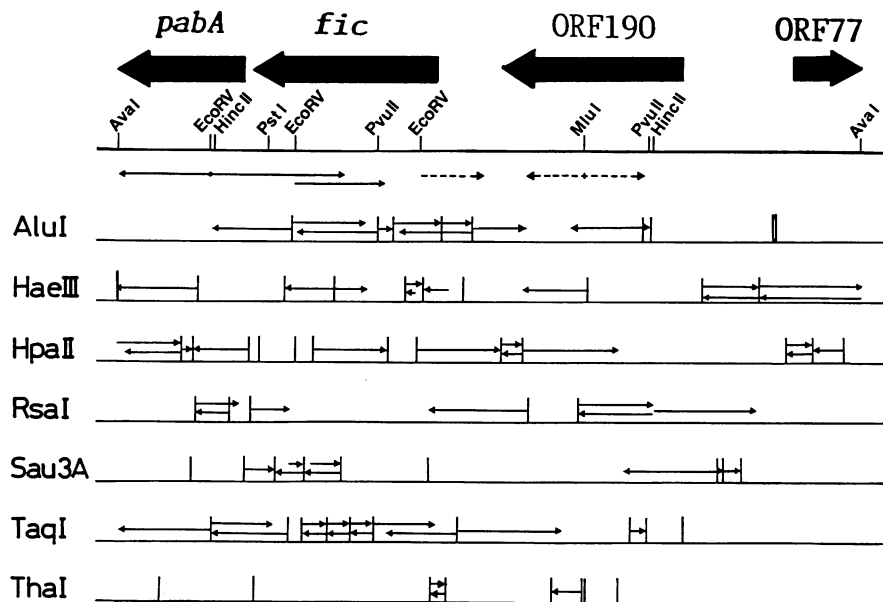


FIG. 1. Restriction map and DNA sequencing strategy for a 2.5-kb *AvaI*-*AvaI* fragment. The bold arrows represent the open reading frames of ORF77, ORF190, *fic*, and *pabA*. The nucleotide sequences were determined by the M13 dideoxy-chain termination method (solid arrows) (20) and the method of Maxam 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 hydrophathy 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 *EcoRV*-*PvuII* fragment containing the putative *fic* promoter region was ligated to a *SmaI*-digested fragment of pMC1403 (12), which is a *lacZ'* fusion vector, and the ligation mixture was used to transform MC1061 (Δlac). 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 *EcoRV*-*PvuII* fragment was ligated to the pMC1403 *SmaI* 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 *HincII*-*HincII* fragment in the *SmaI* site of pMC1403, and pYL1 contained a 1.4-kb *HincII*-*HincII* fragment in pMC1403. We examined the effects of cAMP on the pFL1 gene by measuring the β -galactosidase activity in the TP2010 ($\Delta lac \Delta cya$) (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 *EcoRI* 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-lacZ* 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

COC GAG AAT GAA CAT CCG CGC CGA GAA CAG CGC CAG GCT GTG GCT GAA CAT CAA ACC GGC AAC CGC CAG CAC CAT CAG GAG AAA 84
 Gly Leu Ile Phe Met Ala Ala Ser Phe Leu Ala Leu Ser His Ser Phe Met Leu Gly Ala Val Ala Leu Val Met Leu Leu Phe
 CCG AAA ACG TAA CTG COT TTT CAA CGG GAC GAT TTC CAT CAG CCA GGC GTT CAG GAA GAT AGA GAT TAA AAT GCC GCC GTT CAG 168 **ORF77**
 Gly Phe Arg Leu Gln Thr Lys Leu Pro Val Ile Glu Met Leu Trp Ala Asn Leu Phe Ile Ser Ile Leu Ile Gly Ala Asn Leu
 GAA CGT GAA GCT ATT ACT CAT ACT GGA AAC AGG CAG ATT GAA ATA ATC CGC GAT ATT TCC CAT CACGATCCCTGACATAAOCAGCA 258
 Phe Thr Phe Thr Asn Ser Met Ser Ser Val Pro Leu Asn Phe Tyr Asp Ala Ile Asn Gly Met
 CCAGTCAGTGGCTAGGAGAGAAAGCTAAATCCATGTGAGCTTGATCCGATTCTGTTAGTCTGATGATGGCTTCCAAAATAAAATGGCATAGCCGGATATGCCAGGGC 369
 CGSATTITAGGTGATTTTGATCTGTTTAAATGTTTTATGCAATCGGTTGCTAAATGCAATTTAAGAGGGTGAATTTGATCAGCGAATAAAAAGTGCATCGTCAGGTTA 480
 CATATATTTTCAGATACTAAAATAGCTAAAGGGATGGCCCTGTTCTTGAAGGCTATTTAGAATCTCTTCACTTGGCTTTTTTCTGCTCTGTTTCTTAAAGCAATCTCATG 591
 Met
 TTC AAA TCG ACC CTG CGC GCG ATG GCT GCT GTT TTC GCT CTT TCT GCT CTT TCT CCC GCT GCA ATG GCA GCG AAA GGG GAC CCG 675
 Phe Lys Ser Thr Leu Ala Ala Met Ala Val Phe Ala Leu Ser Ala Leu Ser Pro Ala Ala Met Ala Ala Lys Gly Asp Pro
 CAC GTA TTG TTG ACA ACC TCA CCT GGT AAC ATC GAA CTG GAG CTG GAT AAA GAA AAA GGC CCA CTG TCT GTG CAA AAC TTT GTC 759
 His Val Leu Leu Thr Thr Ser Ala Gly Asn Ile Glu Leu Asp Lys Gln Lys Ala Pro Val Ser Val Gln Asn Phe Val
 GAT TAT GTG AAC AGC GGT TTT TAT AAC AAC ACT ACC TTT CAC CGC GTC ATT CCT GGC TTT ATG ATT CAG GGC GGC GGT TTC ACC 843
 Asp Tyr Val Asn Ser Gly Phe Tyr Asn Asn Thr Thr Phe His Arg Val Ile Pro Gly Phe Met Ile Gln Gly Gly Phe Thr
 GAG CAG ATG CAG CAG AAA AAA CCA AAC CCG CCA ATC AAA AAT GAA GCC GAT AAC GGC CTG CCG AAC CCG CCG ACC ATC CCG 927 **ORF190**
 Glu Gln Met Gln Gln Lys Lys Pro Asn Pro Pro Ile Lys Asn Glu Ala Asp Asn Gly Leu Arg Asn Thr Arg Gly Thr Ile Ala
 ATG GCA CGT ACC GCT GAC AAA GAC AGC GCC ACC AGC CAG TTC TTT ATC AAC GTT GCC GAT AAC GCC TTC CTT GAC CAT GGT CAG 1011
 Met Ala Arg Thr Ala Asp Lys Asp Ser Ala Thr Ser Gln Phe Phe Ile Asn Val Ala Asp Asn Ala Phe Leu Asp His Gly Gln
 CGT GAT TTC GGT TAC GCG GTA TTT GGT AAA GTG GTG AAA GGC ATG GAC GTT GCC GAT AAG ATT TCC CAG GTG CCG ACT CAT GAC 1095
 Arg Asp Phe Gly Tyr Ala Val Phe Gly Lys Val Val Lys Gly Met Asp Val Ala Asp Lys Ile Ser Gln Val Pro Thr His Asp
 GTT GGT CCG TAC CAG AAT GTG CCG TCA AAA CCG GTA GTT ATC CTT TCC GCT AAA GTC CTG CCG TAATGATTTCTAATCTTCCGCCGACTT 1185
 Val Gly Pro Tyr Gln Asn Val Pro Ser Lys Pro Val Val Ile Leu Ser Ala Lys Val Leu Pro ***
 CTGCTCTCCGGCGTAAACCCGATTTCCCGCTTATCTTGTGCAATGGACACGTTCAAGGAGCCCAACTGAAGAACTCACCGATAACCAAAGTCCCGTCTCTGGGAG 1296
 CTTCAGCGTAATGTAATTTTCAGGCGCAGTCCCGCTTTCAGAGGCGTCGAGATGCCCTTTACTCACTCTTACTGCCGAGAGGCTTTAGCCGCGCTTGAAGAGCTCAGGAG 1407
 CACTATG AGC GAT AAA TTC GGC GAA GGG CCG GAT CCG TAT CTT TAT CCA GGC CTT GAT ATC ATG CGT AAC CCG CTG AAC ATC CCG 1492
 Met Ser Asp Lys Phe Gly Glu Gly Arg Asp Pro Tyr Leu Tyr Pro Gly Leu Asp Ile Met Arg Asn Arg Leu Asn Ile Arg
 CAG CAG CAG CCG CTG GAA CAG GCC GCT TAC GAA ATG ACC CCG CTG CGT GCT CCG ACC ATT GAG CTT GGT CCG CTG GGT GCC GGT 1576
 Gln Gln Gln Arg Leu Glu Gln Ala Ala Tyr Glu Met Thr Ala Leu Arg Ala Ala Thr Ile Glu Leu Gly Pro Leu Gly Ala Gly
fic-1 ↓ CGT Arg
 TTA CCG CAT TTG CCA ACT ATC CAT CCG CAG CTG TAT CAG GAT ATT TTC GAC TGG GCA GGG CAA CTG CGT GAA GTT GAT ATT TAT 1660
 Leu Pro His Leu Arg Thr Ile His Arg Gln Leu Tyr Gln Asp Ile Phe Asp Trp Ala Gly Gln Leu Arg Glu Val Asp Ile Tyr **fic**
 CAG GGT GAT ACG CCG TTC TGC CAC TTT GCT TAT ATC GAA AAA GAG GGC AAT GCC CTG ATG CAG GAT CTG GAG GAA GAA GGT TAT 1744
 Gln Gly Asp Thr Pro Phe Cys His Phe Ala Tyr Ile Glu Lys Glu Gly Asn Ala Leu Met Gln Asp Leu Glu Glu Gly Tyr
 CTG GTT GGC CTG GAG AAA CCG AAG TTC GTC GAG CCG CTG CCG CAT TAC TAT TGT GAA ATC AAC GTG CTG CAT CCC TTC CCG GTG 1828
 Leu Val Gly Leu Glu Lys Ala Lys Phe Val Glu Arg Leu Ala His Tyr Tyr Cys Glu Ile Asn Val Leu His Pro Phe Arg Val
 GCA AGT GCT CTG CCA CAG CCG ATC TTC TTC GAG CAA CTG CCG ATT CAT CCG GGA TAT CAA CTG AGC TGG CAG GGT ATC GAA AAA 1912
 Gly Ser Gly Leu Ala Gln Arg Ile Phe Phe Glu Gln Leu Ala Ile His Ala Gly Tyr Gln Leu Ser Trp Gln Gly Ile Glu Lys
 GAG GCC TGG AAT CAG GCA AAT CAG AGT GGG GCA ATG GGG GAT CTC ACC CCA CTG CAG ATG ATA TTT ACC AAA GIG GTA AGC GAA 1996
 Glu Ala Trp Asn Gln Ala Asn Gln Ser Gly Ala Met Gly Asp Leu Thr Ala Leu Gln Met Ile Phe Ser Lys Val Val Ser Glu
 GCC GGG GAA TCT GAG TAA AATAGCGCGGTCTTTGTATCCGGAGCGGCC ATG ATC CTG CTT ATA GAT AAC TAC GAT TCT TTT ACC TGG AAC 2087
 Ala Gly Glu Ser Glu *** Met Ile Leu Leu Ile Asp Asn Tyr Asp Ser Phe Thr Trp Asn
 CTC TAC CAG TAC TTT TGT GAA CTG GGG CCG GAT GTG CTG GTT AAG CCG AAC GAT CCG TTG ACG CTG CCG GAT ATC GAC GCC CTT 2171
 Leu Tyr Gln Tyr Phe Cys Glu Leu Gly Ala Asp Val Lys Arg Asn Asp Ala Leu Thr Leu Ala Asp Ile Asp Ala Leu
 AAA CCA CAA AAA ATT GTC ATC TCA CCT GGC CCC TGT ACC CCA GAT GAA GCC GGC ATC TCC CTT GAC GTT ATT CGC CAC TAT GCC 2255
 Lys Pro Gln Lys Ile Val Ile Ser Pro Gly Pro Cys Thr Pro Asp Glu Ala Gly Ile Ser Leu Asp Val Ile Arg His Tyr Ala **pabA**
 GGG CCG TTG CCG ATT CTT GGC GTC TGC CTC GGT CAT CAG CCA ATG GCG CAG GCA TTT GGC GGT AAA GTT GIG CCG GCC GCA AAG 2339
 Gly Arg Leu Pro Ile Leu Gly Val Cys Leu Gly His Gln Ala Met Ala Gln Ala Phe Gly Gly Lys Val Val Arg Ala Ala Lys
 GTC ATG CAC GGG AAA ACC TGG CCG ATT ACA CAT AAC GGT GAG GCG GTA TTT CCG GCG CTG GCA AAT CCA CTT ACC GIG ACA GCG 2423
 Val Met His Gly Lys Thr Ser Pro Ile Thr His Asn Gly Glu Gly Val Phe Arg Gly Leu Ala Asn Pro Leu Thr Val Thr Arg
 TAC CAT TGG CTG GTG GAA CCT GAC TCA TTA CCA GCG TCC TTT GAC CTG ACC GCC TGG ACC GAA ACC CCA G 2496
 Tyr His Ser Leu Val Val Glu Pro Asp Ser Leu Pro Ala Cys Phe Asp Val Thr Ala Trp Ser Glu Thr Arg

FIG. 2. Nucleotide sequence and deduced amino acid sequence in the 2496-bp *AvaI-AvaI* 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.

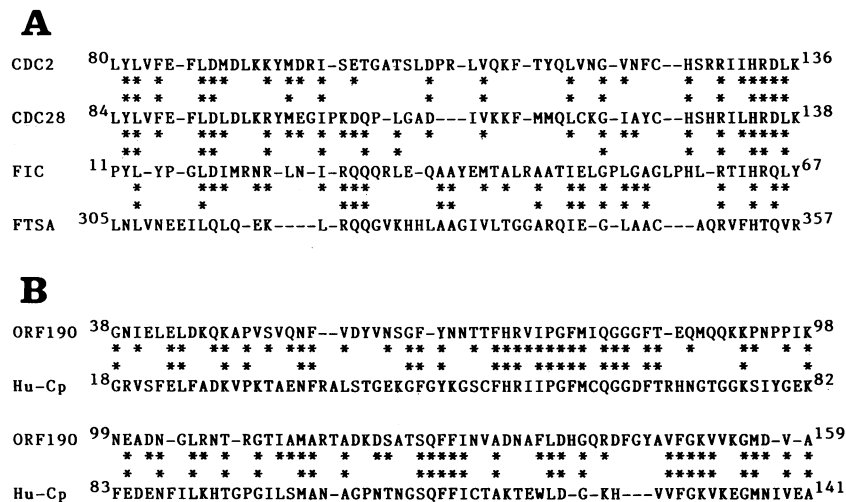


FIG. 3. Alignment of the Fic protein amino acid sequence with FtsA of *E. coli*, CDC2 of *S. pombe*, and CDC28 of *S. cerevisiae* (A) and alignment of the amino acid sequence of ORF190 with human cyclophilin (Hu-Cp) (B). The sequences are aligned for maximum sequence similarity. Amino acid identities 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.

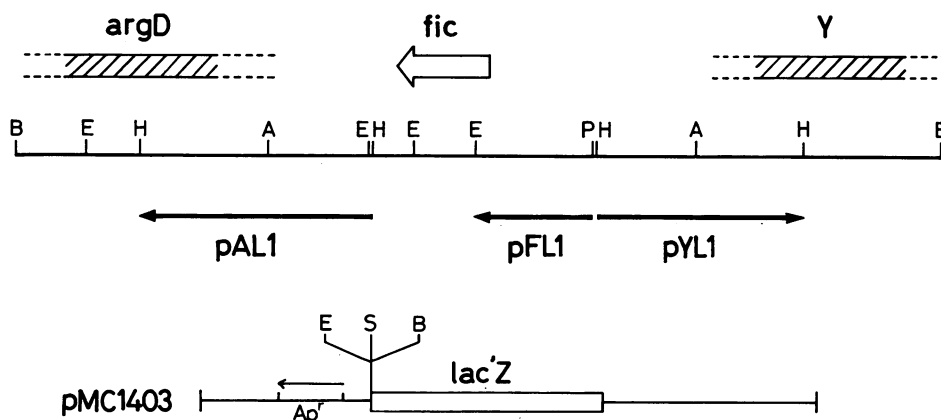


FIG. 4. Structures of the fusion genes pFL1, pAL, and pYL. Plasmid pFL was constructed by ligating the 0.8-kb *EcoRV-PvuII* fragment containing the *fic* promoter with *SmaI*-digested pMC1403. pAL1 was constructed by inserting the *HincII* 1.5-kb fragment containing the *argD* promoter in the pMC1403 *SmaI* site. pYL1 was constructed by inserting the 1.4-kb fragment in the pMC1403 *SmaI* site. All three plasmids coded for active fusion β -galactosidase.

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