JOURNAL OF BACTERIOLOGY, Nov. 1993, p. 6850–6856
0021-9193/93/216850-07$02.00/0
Copyright © 1993, American Society for Microbiology
Vol. 175, No. 21

chpA and *chpB*, *Escherichia coli* Chromosomal Homologs of the *pem* Locus Responsible for Stable Maintenance of Plasmid R100

YUJI MASUDA,[1] KAZUKO MIYAKAWA,[2] YUKINOBU NISHIMURA,[2] AND EIICHI OHTSUBO[1]*

Institute of Molecular and Cellular Biosciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113,[1] and Faculty of Science, Toho University, Miyama 2-2-1, Funabashi, Chiba 274,[2] Japan

Received 17 June 1993/Accepted 26 August 1993

The *pem* locus is responsible for stable maintenance of plasmid R100 and consists of two genes, *pemI* and *pemK*. The *pemK* gene product is a growth inhibitor, while the *pemI* gene product is a suppressor of this inhibitory function. We found that the PemI amino acid sequence is homologous to two open reading frames from *Escherichia coli* called *mazE* and *orf-83*, which are located at 60 and 100 min on the chromosome, respectively. We cloned and sequenced these loci and found additional open reading frames, one downstream of each *pemI* homolog, both of which encode proteins homologous to PemK. The *pem* locus homolog at 60 min was named *chpA* and consists of two genes, *chpAI* and *chpAK*; the other, at 100 min, was named *chpB* and consists of two genes, *chpBI* and *chpBK*. The distal portion of *chpBK* was found to be adjacent to the *ppa* gene that encodes pyrophosphatase, whose map position had not been previously determined. We then demonstrated that the *chpAK* and *chpBK* genes encode growth inhibitors, while the *chpAI* and *chpBI* genes encode suppressors for the inhibitory function of the ChpAK and ChpBK proteins, respectively. These *E. coli pem* locus homologs may be involved in regulation of cell growth.

The *pem* locus has been identified as responsible for the stable maintenance of plasmid R100 (28) and consists of two genes, *pemI* and *pemK*. The *pemK* gene product inhibits growth of host cells, while the *pemI* gene product suppresses the inhibitory function of the *pemK* gene product. The two gene products are expressed coordinately from a single promoter and form a complex which lacks the ability to inhibit cell growth (25, 27). The complex, however, can function as an autoregulator to repress the expression of *pem* by binding to two sites, called *ppb*, in the *pem* promoter region (27). When the cells lose R100, selective killing of the plasmid-free segregants occurs (26, 28). Degradation of the PemI protein in the complex is proposed to lead to activation of the inhibitory function of the PemK protein (25). This killing serves to maintain the cells harboring R100 in a population. Another low-copy-number plasmid, R1, codes for the *parD* locus, which is responsible for stable maintenance of the plasmid and whose nucleotide sequence is identical to that of the *pem* locus (3).

In this report, we describe the identification and characterization of two *pem* locus homologs, here called *chpA* and *chpB*, which are located at 60 and 100 min on the *Escherichia coli* chromosome, respectively. We show that *chpA* and *chpB* are homologous to *pem* not only structurally but also functionally.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *Escherichia coli* K-12 strains used in this study are listed in Table 1. The plasmids used are listed in Table 2.

Media. The standard culture medium used was Luria (L) broth. L-rich broth (31) was used for cell cultures to prepare plasmids. Agar plates contained 1.5% agar (Difco). Antibiotics were used at the following concentrations: ampicillin (Wako), 100 μg/ml; tetracycline (Sigma), 5 μg/ml. L broth supplemented with 800 μg of ampicillin per ml was used to culture strain YM1000 harboring a plasmid.

DNA preparation. Total chromosomal DNA was prepared as described by Miura (15). Plasmid DNA was prepared as described by He et al. (10) or Sambrook et al. (22).

Nucleotide sequencing. Nucleotide sequences were determined by the dideoxynucleotide method (23) with a *Bca*BEST Dideoxy Sequencing Kit (Takara). Synthetic oligodeoxyribonucleotide 5′-CGACTGGGGAGAGCCGAA-3′ was used as a primer for sequencing of the region downstream of the *chpAI* gene. Primers M4 and RV (Takara) were used to sequence the fragments cloned into pUC119. DNA chains were labeled with [α-^{32}P]dCTP (110 TBq/mmol; Amersham) and separated by 6 or 8% polyacrylamide gels containing 8 M urea.

Construction of pYOU plasmids carrying a *chp* gene(s). Relevant DNA fragments containing a *chp* gene(s) (Table 2) were prepared as follows. Polymerase chain reaction (PCR) was carried out in 100 μl of Cetus buffer (21) by using 1 ng of total DNA prepared from MC1000 or relevant plasmid DNA as the template, 2.5 U of AmpliTaq DNA polymerase (Perkin Elmer Cetus), and two primers at 100 pmol each. Incubations at 93°C (2 min), 50°C (2 min), and 72°C (3 min) were repeated 30 times in an automated thermal cycler and followed by incubation at 72°C for 7 min. The amplified fragments were then digested by *Eco*RI or *Bam*HI and ligated with vector plasmid, pUC119, pJG200, or pHS12 which had been digested with the same restriction enzyme. Note that the primers used here contained a cleavage site for restriction enzyme *Eco*RI or *Bam*HI (Table 3). To obtain plasmids with *chpAI* and those with *chpBI* (Table 2), the ligated samples were introduced by transformation to NM554. To obtain plasmids with *chpA* (or *chpAK*) or those with *chpB* (or *chpBK*) (Table 2), the ligated samples were introduced by transformation to NM554 harboring the plasmid with *chpAI* or with *chpBI*, respectively. Nucle-

* Corresponding author.

TABLE 1. *E. coli* K-12 strains used in this study

Strain	Genotype	Source or reference
MC1000	*araD139* Δ(*ara-leu*)*7697* Δ(*lac*)*X74 galU galK rpsL*	4
NM554	MC1000 *hsdR2* (r_K^- m_K^+) *mcrB1 recA13*	20
FS1576	C600 Thy$^-$ *recD1009*	24
YM1000	MC1000 *chpAI*::Km^r	This work

otide sequences and orientations of the fragments cloned were checked by sequencing.

All of the manipulations were carried out as described by Sambrook et al. (22), except for ligation, which was carried out with a DNA ligation kit (Takara), and transformation, which was carried out at 30°C as described by Yoshioka et al. (31).

Construction of mutant YM1000 with the Km^r fragment inserted into *chpAI*. A *Pst*I-*Sca*I fragment (about 3 kb) that contains the *relA-chpA-pyrG* region from plasmid pLC10-47 was cloned into pBR322. The resulting plasmid was digested with *Pvu*II and self-ligated to remove the *Pvu*II fragment. The resulting plasmid was digested with *Ssp*I, whose cleavage site is within *chpAI*, and then ligated with the *Hin*cII fragment (Kan^r GenBlock; Pharmacia) containing the kanamycin resistance (Km^r)-encoding gene. The resulting plasmid was linearized with *Apa*LI and introduced into strain FS1576 by transformation. The mutant *chpAI* gene with the Km^r-encoding insertion was then introduced into MC1000 by P1kc transduction to obtain YM1000.

Enzyme assay. β-Galactosidase activity was assayed as described by Miller (14).

Computer analysis. A VAX computer was used for analysis. Computer programs were supplied by the Genetics Computer Group, Inc. (7). The GenBank and EMBL nucleotide sequence data bases were searched for similarity to given amino acid sequences with the TFASTA algorithm of Pearson and Lipman (19).

Nucleotide sequence accession numbers. The nucleotide sequence data shown here have been deposited in the DDBJ, EMBL, and GenBank nucleotide sequence data bases under accession numbers D16450 and D16451.

TABLE 2. Plasmids used in this study

Plasmid	Parent	Relevant gene[a] (coordinates)	Source or reference
pUC119	pMB1		29
pJG200	pBR322		9
pBR322	pMB1		2
pHS12	pSC101		1
pLC10-47	ColE1	*chpA*	6
pDOM11	pHS12	*pemI*	28
pDOM106	pJG200	*pemK**	25
pYOU64	pUC119	*chpA* (9–695)	This work
pYOU78	pJG200	*chpAK** (284–667)	This work
pYOU84	pHS12	*chpAI* (9–381)	This work
pYOU116	pUC119	*chpB* (4′–792′)	This work
pYOU95	pJG200	*chpBK** (363′–742′)	This work
pYOU85	pHS12	*chpBI* (4′–442′)	This work

[a] *pemK**, *chpAK**, and *chpBK** indicate tripartite genes of *pemK*, *chpAK*, and *chpBK*, respectively, fused with the collagen-*lacZ* sequence. Coordinates for *chpA* and *chpB* are shown in Fig. 1. In the pUC119 derivatives, each of the coding sequences was inserted in the same direction as the *lac* promoter. In the pJG200 derivatives, each of the coding sequences inserted was under control of the λ p_R promoter.

TABLE 3. Primers used for PCR

Primer	Sequence (5′→3′)[a]	Position[b]
P11	GGGAaTTcGGCCGAAATTTGCTC	1–23
P12	gGgaattCCAGAATAGGAGTGAGTTAGTA	702–674
P13	TCgAAtTCAACCCAAATCAGATCGCCCA	387–360
P15	AAggaTcCCCAATCAGTACG	674–655
P16	CCGGAtccCCTCCACGAGAATAT	276–298
P9	CGGaaTTCGGTTAGTAAGGGTT	797′–776′
P10	TtgAATTCCCTCACCTTTTGCT	1′–22′
P14	GATgAAttCCAACCAGCACAATGTCTCC	450′–423′
P17	CAggaTccTCCACCACCGCCTGCAA	750′–726′
P18	CAGGATccCTGGGGTAAATCCAC	355′–377′

[a] Each primer sequence contains several bases (small letters) altered to introduce the restriction site, *Eco*RI or *Bam*HI, for cloning of the fragments amplified by PCR.

[b] Nonprimed and primed numbers are coordinates for *chpA* and *chpB* (Fig. 1), respectively.

RESULTS

Cloning and nucleotide sequencing of *pem* locus homologs *chpA* and *chpB*. By computer search, we found that the amino acid sequence of protein PemI is homologous to that of a functionally unknown protein encoded by a gene, designated *mazE*, which is located downstream of the *relA* gene at 60 min on the *E. coli* chromosome (13) (Fig. 1A). To see whether a gene homologous to *pemK* exists in the region immediately downstream of the *pemI* homolog, we determined the nucleotide sequence of plasmid pLC10-47, which carries the *relA* region (17) and found an open reading frame that encodes a protein with homology to PemK (Fig. 1A). We then amplified the fragment containing the entire coding region by PCR with synthetic primers (P11 and P12; Table 3) and total chromosomal DNA from MC1000 as the template and cloned it into pUC119. The nucleotide sequence of the fragment amplified from chromosomal DNA was identical to that of *mazE* and the *pemK*-homologous gene determined above. We designated the *pem* locus homolog *chpA* and the two frames corresponding to *pemI* and *pemK* *chpAI* and *chpAK*, respectively (Fig. 1A).

Similarly, by computer search we found another *pemI* homolog, designated *orf-83*, which is transcribed divergently from the *ileR* gene located at 100 min on the *E. coli* chromosome (30) (Fig. 1B). We also found that the nucleotide sequence downstream of *orf-83* is identical to that adjacent to the *ppa* gene, whose nucleotide sequence has previously been reported (11) but whose chromosomal location was not known. In the region between the *pemI* homolog and *ppa*, an imperfect open reading frame was found. With a single base insertion, the open reading frame would be continuous and encode a protein with homology to PemK (Fig. 1B). To prove the existence of the possible *pemK* homolog, we amplified the fragment containing the entire coding region by PCR with synthetic primers (P9 and P10; Table 3) and total chromosomal DNA from MC1000 as the template and cloned it into pUC119. Nucleotide sequencing revealed that the chromosomal sequence contained one additional guanine within the region corresponding to the *pemK* homolog (G at position 524′; Fig. 1B) and two other base differences in the noncoding region upstream of the *pemI* homolog (A for C at position 80′ and A for T at position 90′; Fig. 1B) when it was compared with that previously published by Weiss et al. (30) and Lahti et al. (11). Other fragments were independently amplified and sequenced,

A

```
1
GGGAGTTAGGCCGAAATTTGCTCGTATCTACAATGTAGATTGATATATACTGTATCTACATATGATAGCGGTTTGAGGAAAGGGTTATGATCCACAGTAGCGTAAAGCGTTGGGGAAATT
                                          -10  -10                          SD         M I H S S V K R W G N S
                                                                                       chpAI (mazE)
121
CACCGGCGGTGCGGATCCCGGCTACGTTAATGCAGGCGCTCAATCTGAATATTGATGATGAAGTGAAGATTGACCTGGTGGATGGCAAATTAATTATTGAGCCAGTGCGTAAAGAGCCCG
 P A V R I P A T L M Q A L N L N I D D E V K I D L V D G K L I I E P V R K E P V

241
TATTTACGCTTGCTGAACTGGTCAACGACATCACGCCGGAAAACCTCCACGAGAATATCGACTGGGGAGAGCCGAAAGATAAGGAAGTCTGGTAATGGTAAGCCGATACGTACCCGATAT
 F T L A E L V N D I T P E N L H E N I D W G E P K D K E V W *
                                                                                 SD        M V S R Y V P D M
361                                                                                        chpAK
GGGCGATCTGATTTGGGTTGATTTTGACCCGACAAAAGGTAGCGAGCAAGCTGGACATCGTCCAGCTGTTGTCCTGAGTCCTTTCATGTACAACAACAAAACAGGTATGTGTCTGTGTGT
 G D L I W V D F D P T K G S E Q A G H R P A V V L S P F M Y N N K T G M C L C V

481
TCCTTGTACAACGCAATCAAAAGGATATCCGTTCGAAGTTGTTTTATCCGGTCAGGAACGTGATGGCGTAGCGTTAGCTGATCAGGTAAAAAGTATCGCCTGGCGGGCAAGAGGAGCAAC
 P C T T Q S K G Y P F E V V L S G Q E R D G V A L A D Q V K S I A W R A R G A T

601
GAAGAAAGGAACAGTTGCCCCAGAGGAATTACAACTCATTAAAGCCAAAATTAACGTACTGATTGGGTAGTGTTACTAACTCACTCCTATTCTGGTCACGCTT
 K K G T V A P E E L Q L I K A K I N V L I G *
```

B

```
1'
TGTAATTCCCTCACCTTTTGCTTTTCTCTCCGAGCCGCTTTCCATATCTATTAACGCATAAAAAACTCTGCTGGCATTCACAAATGCGCAGGGGTAAAACGTTTCCTGTAGCACCGTGAG
                                                                                                  -10
121'
TTATACTTTGTATAACTTAAGGAGGTGCAGATGCGTATTACCATAAAAAGATGGGGGAACAGTGCAGGTATGGTCATTCCCAATATCGTAATGAAAGAACTTAACTTACAGCCGGGGCAG
  -10            SD        M R I T I K R W G N S A G M V I P N I V M K E L N L Q P G Q
                        chpBI (orf-83)
241'
AGCGTGGAGGCGCAAGTGAGCAACAATCAACTGATTCTGACACCCATCTCCAGGCGCTACTCGCTTGATGAACTGCTGGCACAGTGTGACATGAACGCCGCGGAACTTAGCGAGCAGGAT
S V E A Q V S N N Q L I L T P I S R R Y S L D E L L A Q C D M N A A E L S E Q D

361'
GTCTGGGGTAAATCCACCCCTGCGGGTGACGAAATATGGTAAAGAAAAGTGAATTTGAACGGGGAGACATTGTGCTGGTTGGCTTTGATCCAGCAAGCGGCCATGAACAGCAAGGTGCTG
V W G K S T P A G D E I W *
                     SD        M V K K S E F E R G D I V L V G F D P A S G H E Q Q G A G
481'                        chpBK . ▼
GTCGACCTGCGCTTGTGCTCTCCGTTCAAGCCTTTAATCAACTGGGAATGACGCTGGTGGCCCCCATTACGCAGGGCGGAAATTTTGCCCGTTATGCCGGATTTAGCGTTCCTTTACATT
 R P A L V L S V Q A F N Q L G M T L V A P I T Q G G N F A R Y A G F S V P L H C

601'
GCGAAGAAGGCGATGTGCACGGCGTGGTGCTGGTGAATCAGGTGCGGATGATGGATCTACACGCCCGGCTGGCAAAGCGTATTGGTCTGGCTGCGGATGAGGTGGTGGAAGAGGCGTTAT
 E E G D V H G V V L V N Q V R M M D L H A R L A K R I G L A A D E V V E E A L L

721'
TACGCTTGCAGGCGGTGGTGGAATAAGGTGTGTTTATTTATCGCGGGCATAAAAAAACCCTTACTAACCGAAGCCCG
 R L Q A V V E *
```

FIG. 1. DNA sequences of the *chpA* (A) and *chpB* (B) loci. The predicted amino acid sequences encoded by the *pemI* and *pemK* homologs in each locus are shown below the DNA sequences. The *chpAI* gene corresponds to *mazE* (13), and *chpBI* corresponds to *orf-83* (30). Boldface letters represent −10 regions of possible promoters (13, 30) and putative ribosome binding sequences (SD). Nonprimed and primed numbers are coordinates given to *chpA* and *chpB*, respectively. The arrowhead indicates one additional guanine.

confirming the presence of the base changes. The presence of an additional base confirmed an intact open reading frame from the chromosomal DNA homolog to *pemK*, as suspected from the previous sequence. We designated this *pem* locus homolog *chpB* and the two frames corresponding to *pemI* and *pemK* *chpBI* and *chpBK*, respectively (Fig. 1B).

Sequence comparison among *pem* locus homologs. The products of the *pemI*, *chpAI*, and *chpBI* genes are 85, 82, and 83 amino acid residues, respectively, and their amino acid sequences are similar to one another (Fig. 2A): PemI and ChpAI share 34% identical and 69% conserved residues, ChpAI and ChpBI share 33% identical and 82% conserved residues, and PemI and ChpBI share 37% identical and 77% conserved residues. The products of the *pemK*, *chpAK*, and *chpBK* genes are 110, 111, and 116 amino acid residues, respectively, and their amino acid sequences are similar to one another (Fig. 2B): PemK and ChpBK share 43% identical and 81% conserved residues, ChpAK and ChpBK share 35% identical and 66% conserved residues, and PemK and ChpAK share 26% identical and 64% conserved residues.

We have previously reported that expression of *pem* is negatively regulated at its promoter region by the complex formed between proteins PemI and PemK, which binds to two sequences, named *ppbA* and *ppbB* (Fig. 3) (27). We thus compared the nucleotide sequences of the regions upstream of three loci, *pem*, *chpA*, and *chpB*, and found that the region preceding *chpA* or *chpB* contained a match to the *ppb* consensus.

Analysis of functions of the two genes in *chpA*. To see whether the *chpAK* gene in *chpA* encodes a growth inhibitor

FIG. 2. Alignments of the amino acid sequences, showing homology among the PemI, ChpAI, and ChpBI proteins (A) and among the PemK, ChpAK, and ChpBK proteins (B). Amino acids in each protein are numbered by assigning the first Met in each coding sequence position 1. Amino acids identical in two or three homologous proteins are shaded.

like *pemK* in *pem*, we constructed plasmid pYOU78 carrying a heat-inducible *chpAK* gene (Table 2) by using expression vector pJG200. In this construct, *chpAK* was fused in frame with the collagen-*lacZ* coding region located downstream of the bacteriophage λ p_R promoter. The fusion gene (here called *chpAK**) encodes a tripartite protein, ChpAK–collagen–β-galactosidase (here called ChpAK*). The ChpAK* protein produced upon heat induction in NM554 did not inhibit cell growth (Fig. 4Aa), while the PemK* protein, which was produced in cells harboring pDOM106 with tripartite gene *pemK**, inhibited it (Fig. 4B). We suspected that the product from the *chpAI* gene present on the *E. coli* chromosome might suppress the inhibitory function of ChpAK. To test this, we used a mutant strain with an insertion of the Km^r-encoding fragment in *chpAI* on the chromosome, i.e., YM1000 (see Materials and Methods). The strain itself grew normally; the insertion into *chpAI* may exhibit a polar effect on expression of the downstream *chpAK* gene, circumventing inhibition. The ChpAK* protein produced in this mutant inhibited cell growth (Fig. 4Ab) like the PemK* protein (Fig. 4B).

The results described above also indicate that the product of the *chpAI* gene on the *E. coli* chromosome is responsible for suppression of the inhibitory function of the ChpAK protein. To verify the suppressor function of the ChpAI protein, we introduced plasmid pYOU84 (Table 2), a pHS12 derivative carrying *chpAI* which is constitutively expressed from its own promoter, into YM1000 harboring *chpAK**-carrying plasmid pYOU78. Growth of cells harboring both pYOU78(*chpAK**) and pYOU84 (*chpAI*) was not inhibited, while growth of cells harboring both pYOU78 (*chpAK**) and pHS12 with no *chpAI* gene was (Fig. 5A). This shows that the ChpAI protein suppresses the inhibitory function of ChpAK like the PemI protein suppresses that of PemK (Fig. 5B).

As described in the previous section, both proteins PemI and ChpBI have homology to ChpAI. To determine whether PemI or ChpBI can suppress the inhibitory function of ChpAK, we introduced pDOM11 (*pemI*) or pYOU85 (*chpBI*) (Table 2), from which either *pemI* or *chpBI* is constitutively expressed from its own promoter, respectively, into YM1000 harboring pYOU78(*chpAK**). The growth of the cells harboring both pYOU78(*chpAK**) and pDOM11(*pemI*) [or pYOU85(*chpBI*)] was inhibited (Fig. 5A), showing that neither protein PemI nor ChpBI can suppress the inhibitory function of ChpAK*. In addition, protein ChpAI did not suppress the inhibitory function of PemK, since growth of the cells harboring both pDOM106(*pemK**) and pYOU84(*chpAI*) was inhibited (Fig. 5B).

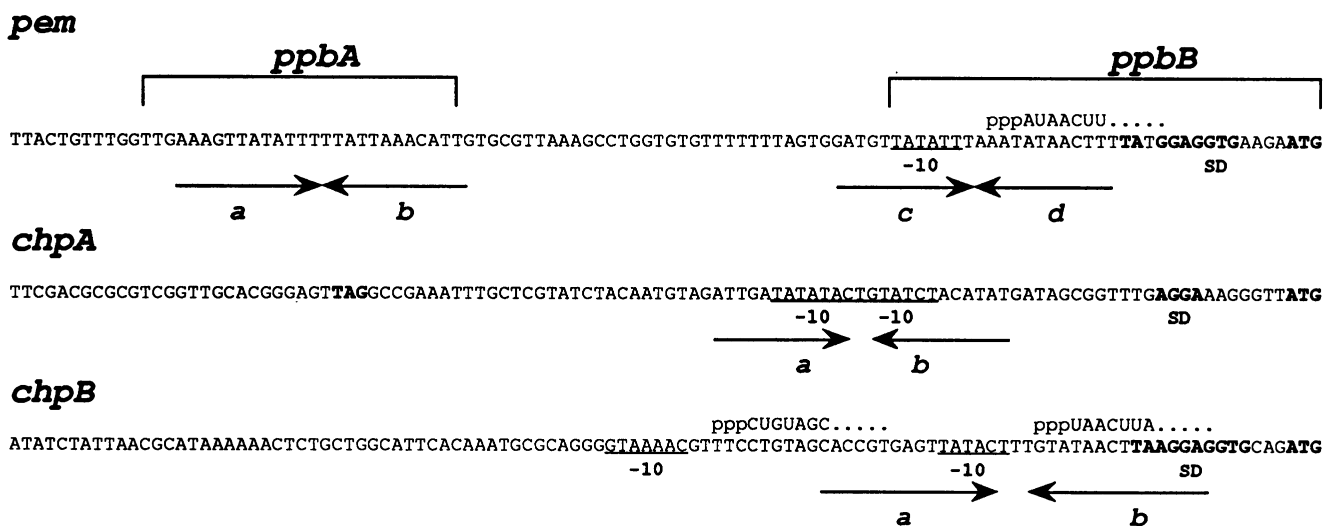
Analysis of functions of the two genes in *chpB*. To determine whether the *chpBK* gene encodes a growth inhibitor like *pemK*, we constructed plasmid pYOU95 carrying a heat-inducible *chpBK* gene (Table 2) by using expression vector pJG200. The plasmid carries fusion gene *chpBK**, which encodes a tripartite protein, ChpBK–collagen–β-galactosidase, ChpBK*. Protein ChpBK* produced in cells harboring pYOU95(*chpBK**) inhibited the growth of NM554 host cells (Fig. 4C), as did the PemK* protein when expressed under similar conditions (Fig. 4B). This indicates that protein ChpBK is a growth inhibitor like PemK.

We next investigated whether protein ChpBI could suppress the inhibitory function of ChpBK like the PemI protein suppresses that of PemK. We introduced plasmid pYOU85 (*chpBI*) into strain NM554 harboring plasmid pYOU95(*chpBK**). Growth of cells harboring both plasmids was no longer inhibited upon expression of the ChpBK* protein, while growth of cells harboring both pYOU95(*chpBK**) and pHS12 with no *chpBI* gene was (Fig. 5C). This indicates that protein ChpBI suppresses the inhibitory function of ChpBK.

Both PemI and ChpAI share extensive homology with ChpBI (Fig. 2); thus, either PemI or ChpAI might suppress the inhibitory function of ChpBK. To test this possibility, we introduced plasmid pDOM11(*pemI*) or pYOU84(*chpAI*) into strain NM554 harboring pYOU95(*chpBK**). The growth of cells harboring both pYOU95(*chpBK**) and pDOM11(*pemI*) was not inhibited, but the growth of cells harboring both pYOU95(*chpBK**) and pYOU84(*chpAI*) was (Fig. 5C). This shows that protein PemI can suppress the inhibitory function of ChpBK*, whereas the ChpAI protein cannot. Interestingly, although protein PemI suppressed the inhibitory function of ChpBK*, protein ChpBI did not suppress the inhibitory function of PemK, since the growth of cells harboring pDOM106 (*pemK**) was still inhibited in the presence of pYOU85(*chpBI*) (Fig. 5B).

A note on the expression of *pemK* homologs. We measured β-galactosidase activity after heat induction of each of the three fusion genes *chpAK**, *chpBK**, and *pemK** in the absence or presence of each *pemI* homolog or *pemI*. The β-galactosidase activity in strain YM1000 harboring pYOU78(*chpAK**) did not increase at 30 min after induction of ChpAK* (Fig. 6A), as the cells ceased to grow (Fig. 4A and 5A); the β-galactosidase activity in cells harboring both pYOU78 (*chpAK**) and pYOU84(*chpAI*) increased only slightly (Fig. 6A), as the cells continued to grow (Fig. 5A). Similarly to the *chpA* system, the β-galactosidase activity in strain NM554 harboring pDOM106 (*pemK**) did not increase much (Fig. 6B), even when the cells continued to grow in the presence of pDOM11(*pemI*) (Fig. 5B). Whereas the β-galactosidase activity in NM554 harboring pYOU95(*chpBK**) did not increase at 30 min after induction of ChpBK* (Fig. 6C) when the cells ceased to grow (Fig. 4C and 5C), the β-galactosidase activity in NM554 harboring both pYOU95(*chpBK**) and pYOU85(*chpBI*) continued to increase (Fig. 6C), as the cells continued to grow (Fig. 5C). These differences in the expression of the *pemK* homologs in the absence or presence of the corresponding suppressor gene may be due to the presence of regulatory elements at the proximal region of *pemK* homologs, as discussed below.

A



B

Palindromic sequences

pem	*a*	......**AAAGTTATATTT**
	b	......**AATGTT**t**a**a**AT**aa
	c	......g**ATGTTATATTT**
	d	......**AAAGTTATATTT**
Consensus		**AA$^{A}_{T}$GTTATATTT**
chpA	*a*	......**A**t**TG**a**TATAT**ac
	b	......**A**t**A**tg**TA**g**AT**ac
chpB	*a*	.caccgtg**AGTTATA**c**TT**
	b	.ctcctt**AAGTTATA**caa

FIG. 3. Promoter-proximal regions of *pem*, *chpA*, and *chpB*. (A) The *ppbA* and *ppbB* sequences (brackets) are the regions bound by a complex between the PemI and PemK proteins (27). The −10 regions of putative promoters are underlined. The 5′ sequences of transcripts (18, 30) are shown above the DNA sequences. Boldface letters represent putative ribosomal binding sites (SD) and the initiation codons. A stop codon, TAG, of the *relA* gene is shown in boldface in the region upstream of *chpA*. Palindromic sequences are shown by pairs of arrows. (B) Sequences a to d of *pem*, a and b of *chpA*, and a and b of *chpB* are shown together with the consensus sequence. Boldface letters indicate bases matched to those in the consensus sequence in *pem*; small letters indicate unmatched bases.

DISCUSSION

In this study, we identified two *pem* locus homologs on the *E. coli* chromosome, *chpA* and *chpB*, each containing genes homologous to *pemI* and *pemK*. We then characterized the functions of the genes in each locus and found them to be similar to those in *pem*: The *pemK* homolog encodes a growth inhibitor, and the *pemI* homolog encodes a suppressor for that inhibitory function. This indicates that proteins PemK, ChpAK, and ChpBK may attack a common target(s) to inhibit the growth of the host. We have shown here that the inhibitory function of protein PemK encoded by plasmid R100 was not suppressed by the products from the *pemI* homologs on the host chromosome but that protein PemI encoded by R100 can suppress the inhibitory function of protein ChpBK encoded by the host. These findings explain how the *pem* system can control the growth of host cells and stably maintain the *pem* plasmid, even though the host encodes its own *pem* locus homologs.

The regions preceding *chpA* and *chpB* both contained sequences homologous to the *ppb* consensus sequence as found in the *pem* locus. Expression of *pem* is repressed by the action of a complex formed between proteins PemI and PemK at these *ppb* sequences (27). This implies that expression of the *chpA* and *chpB* genes is also autoregulated by a complex formed between ChpAI and ChpAK and between ChpBI and ChpBK, respectively.

We have previously shown that in the *pem* system, degradation of protein PemI in the complex formed with PemK causes activation of the inhibitory function of PemK (25). It is likely, therefore, that degradation of proteins ChpAI and ChpBI also leads to activation of the function of ChpAK and ChpBK, respectively. We are currently investigating this possibility.

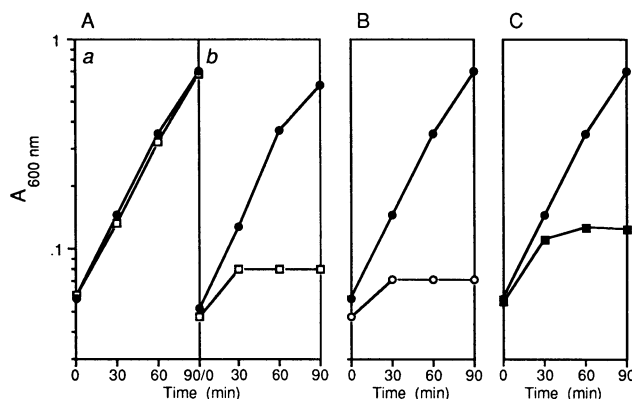


FIG. 4. Growth inhibition by the products of the *pemK* homologs. (Aa) Growth of NM554 harboring pYOU78(*chpAK**) (□) or its parent, pJG200 (●). (Ab) Growth of YM1000 harboring pYOU78(*chpAK**) (□) or pJG200 (●). (B) Growth of NM554 harboring pDOM106(*pemK**) (○) or its parent, pJG200 (●). (C) Growth of NM554 harboring pYOU95(*chpBK**) (■) or its parent, pJG200 (●). A sample of a log-phase culture incubated at 30°C was diluted 10-fold at time zero, and further incubated at 42°C in fresh medium prewarmed to 42°C.

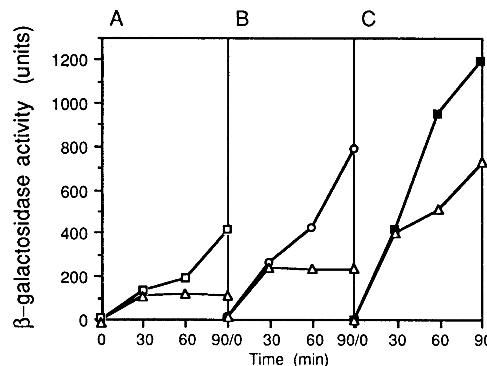


FIG. 6. Expression of the *pemK* homologs in the absence or presence of the *pemI* homologs. (A) β-Galactosidase activity in YM1000 harboring both pYOU78(*chpAK**) and pYOU84(*chpAI*) (□) or both pYOU78(*chpAK**) and pHS12 (△). (B) β-Galactosidase activity in NM554 harboring both pDOM106(*pemK**) and pDOM11 (*pemI*) (○) or both pDOM106(*pemK**) and pHS12 (△). (C) β-Galactosidase activity in NM554 harboring both pYOU95(*chpBK**) and pYOU85(*chpBI*) (■) or both pYOU95(*chpBK**) and pHS12 (△). For the culture conditions, see the legend to Fig. 4.

We have previously shown that protein PemK produced in a small amount can inhibit the growth of host cells and also that protein PemK is not produced in large amounts in the presence of the suppressor PemI (25). Moreover, mutant PemK* proteins which have lost the function to inhibit cell growth cannot be produced in much larger amounts than the active PemK* protein (12). Taken together, these findings suggest that the *pemK** gene is not efficiently expressed, probably because of the presence of regulatory elements in the region around the initiation codon of the *pemK* gene. As in the *pem* system, protein ChpAK was produced in a small amount, causing inhibition of the growth of the host, YM1000 with an insertion in *chpAI*, and was not produced in a much larger amount in the presence of the suppressor ChpAI. Protein ChpBK was, however, produced in a larger amount in the presence of the suppressor ChpBI than was either ChpAK or PemK. Possibly, the *pemK* homologs differ from each other in the structure around the initiation codon of each homolog, causing different expression of each *pemK* homolog. This may explain why ChpBK could function even in the presence of the *chpB* locus in the host chromosome, while ChpAK functioned only in the absence of *chpA* on the host chromosome.

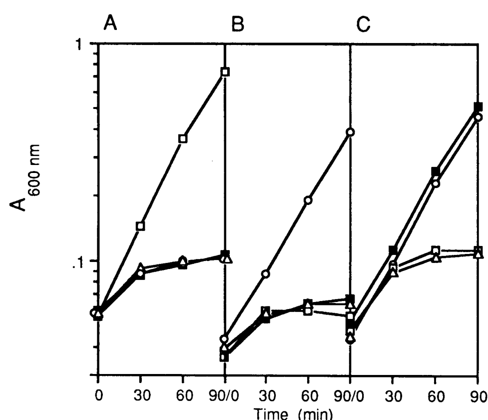


FIG. 5. Suppressor activity of the products of the *pemI* homologs. (A) Growth of YM1000 harboring pYOU78(*chpAK**) and the second plasmid pYOU84(*chpAI*) (□), pYOU85(*chpBI*) (■), pDOM11(*pemI*) (○), or their parent, pHS12 (△). (B) Growth of NM554 harboring pDOM106(*pemK**) and the same second plasmid as above. (C) Growth of NM554 harboring pYOU95(*chpBK**) and the same second plasmid as above. For the culture conditions, see the legend to Fig. 4.

Other chromosomal genes have been identified which are homologous to plasmid-encoded genes responsible for plasmid maintenance. For example, a family of ParA proteins, required for partitioning of several plasmids, are homologous to the cell division inhibitor MinD encoded by a gene on the *E. coli* chromosome (16). The Hok protein, which is responsible for postsegregational killing to stably maintain plasmid R1, is homologous to the product of the *relF* gene, which is in the *relB* operon containing genes required for the stringent response (8).

What are the roles of *pem* homologs *chpA* and *chpB*? We know that *chpA* is within the *relA* operon, which is responsible for the stringent response (5), and that *chpB* is located in the region next to *ppa* that encodes pyrophosphatase, which seems to be involved in the metabolism of ppGpp, which is responsible for the stringent response (5). This suggests that *chpA* and *chpB* are also involved in the stringent response. Bacteria in natural environments often suffer from heat shock, cold shock, and limiting amount of nutrients etc., and have evolved genetic systems to respond to these assaults. The *chp* systems in *E. coli* may function under environmental conditions in which rapid growth is harmful.

ACKNOWLEDGMENTS

We thank D. Bastia for kindly sending us plasmid pJG200 and A. Nishimura for the strain harboring pLC10-47. We also thank G. C. Allen, Jr., for critical reading of the manuscript.

This research was supported by a grant-in-aid for science research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. **Armstrong, K. A., R. Acosta, E. Ledner, Y. Machida, M. Pancotto, M. McCormick, H. Ohtsubo, and E. Ohtsubo.** 1984. A 37 × 10^3 molecular weight plasmid-encoded protein is required for replica-

tion and copy number control in the plasmid pSC101 and its temperature-sensitive derivative pHS1. J. Mol. Biol. **175**:331–347.
2. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heynecker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
3. **Bravo, A., G. de Torrontegui, and R. Diaz.** 1987. Identification of components of a new stability system of plasmid R1, ParD, that is close to the origin of replication of this plasmid. Mol. Gen. Genet. **210**:101–110.
4. **Casadaban, M. J., and S. N. Cohen.** 1980. Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. J. Mol. Biol. **138**:179–207.
5. **Cashel, M., and K. E. Rudd.** 1987. The stringent response, p. 1410–1438. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
6. **Clarke, L., and J. Carbon.** 1976. A colony bank containing synthetic ColE1 hybrid plasmids representative of the entire *E. coli* genome. Cell **9**:91–99.
7. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12**:387–395.
8. **Gerdes, K., F. W. Bech, S. T. Jørgensen, A. Løbner-Olesen, P. B. Rasmussen, T. Atlung, L. Boe, O. Karlstrom, S. Molin, and K. von Meyenburg.** 1986. Mechanism of postsegregational killing by the *hok* gene product of the *parB* system of plasmid R1 and its homology with the *relF* gene product of the *E. coli relB* operon. EMBO J. **5**:2023–2029.
9. **Germino, J., and D. Bastia.** 1984. Rapid purification of a cloned gene product by genetic fusion and site-specific proteolysis. Proc. Natl. Acad. Sci. USA **81**:4692–4696.
10. **He, M., A. Wilde, and M. A. Kaderbhai.** 1990. A simple single-step procedure for small-scale preparation of *Escherichia coli* plasmids. Nucleic Acids Res. **18**:1660.
11. **Lahti, R., T. Pitkäranta, E. Valve, I. Ilta, E. Kukko-Kalske, and J. Heinonen.** 1988. Cloning and characterization of the gene encoding inorganic pyrophosphatase of *Escherichia coli* K-12. J. Bacteriol. **170**:5901–5907.
12. **Masuda, Y., and E. Ohtsubo.** Unpublished data.
13. **Metzger, S., I. B. Dror, E. Aizenman, G. Schreiber, M. Toone, J. D. Friesen, M. Cashel, and G. Glaser.** 1988. The nucleotide sequence and characterization of the *relA* gene of *Escherichia coli*. J. Biol. Chem. **263**:15699–15704.
14. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Miura, K.** 1967. Preparation of bacterial DNA by the phenol-pH 9-RNase method. Methods Enzymol. **68**:543–545.
16. **Motallebi-Veshareh, M., D. A. Rouch, and C. M. Thomas.** 1990. A family of ATPases involved in active partitioning of diverse bacterial plasmids. Mol. Microbiol. **4**:1455–1463.
17. **Nishimura, A., K. Akiyama, Y. Kohara, and K. Horiuchi.** 1992. Correlation of a subset of the pLC plasmids to the physical map of *Escherichia coli* K-12. Microbiol. Rev. **56**:137–151.
18. **Ohtsubo, H., T. B. Ryder, Y. Maeda, K. Armstrong, and E. Ohtsubo.** 1986. DNA replication of the resistant plasmid R100 and its control. Adv. Biophys. **21**:115–133.
19. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85**:2444–2448.
20. **Raleigh, E. A., N. E. Murray, H. Revel, R. M. Blumenthal, D. Westaway, A. D. Reith, P. W. J. Rigby, J. Elhai, and D. Hanahan.** 1988. McrA and McrB restriction phenotypes of some *E. coli* strains and implications for gene cloning. Nucleic Acids Res. **16**:1563–1575.
21. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239**:487–491.
22. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
24. **Stahl, F. W., I. Kobayashi, D. Thaler, and M. M. Stahl.** 1986. Direction of travel of RecBC recombinase through bacteriophage lambda DNA. Genetics **113**:215–227.
25. **Tsuchimoto, S., Y. Nishimura, and E. Ohtsubo.** 1992. The stable maintenance system *pem* of plasmid R100: degradation of PemI protein may allow PemK protein to inhibit cell growth. J. Bacteriol. **174**:4205–4211.
26. **Tsuchimoto, S., and E. Ohtsubo.** 1989. Effect of the *pem* system on stable maintenance of plasmid R100 in various *Escherichia coli* hosts. Mol. Gen. Genet. **215**:463–468.
27. **Tsuchimoto, S., and E. Ohtsubo.** 1993. Autoregulation by cooperative binding of the PemI and PemK proteins to the promoter region of the *pem* operon. Mol. Gen. Genet. **237**:81–88.
28. **Tsuchimoto, S., H. Ohtsubo, and E. Ohtsubo.** 1988. Two genes, *pemK* and *pemI*, responsible for stable maintenance of resistance plasmid R100. J. Bacteriol. **170**:1461–1466.
29. **Vieira, J., and J. Messing.** 1987. Production of single-stranded plasmid DNA. Methods Enzymol. **153**:3–11.
30. **Weiss, D. L., D. I. Johnson, H. L. Weith, and R. L. Somerville.** 1986. Structural analysis of the *ileR* locus of *Escherichia coli* K12. J. Biol. Chem. **261**:9966–9971.
31. **Yoshioka, Y., H. Ohtsubo, and E. Ohtsubo.** 1987. Repressor gene *finO* in plasmids R100 and F: constitutive transfer of plasmid F is caused by insertion IS*3* into F *finO*. J. Bacteriol. **169**:619–623.