JOURNAL OF BACTERIOLOGY, Apr. 1988, p. 1461–1466
0021-9193/88/041461-06$02.00/0

Vol. 170, No. 4

Two Genes, *pemK* and *pemI*, Responsible for Stable Maintenance of Resistance Plasmid R100

SUGURU TSUCHIMOTO, HISAKO OHTSUBO, AND EIICHI OHTSUBO*

Institute of Applied Microbiology, University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan

Received 14 September 1987/Accepted 30 December 1987

Plasmid R100 was found to have two genes, designated *pemK* and *pemI*, that were responsible for its stable inheritance during cell division. They are located near the region that is essential for autonomous replication. Under conditions that inhibit replication of R100 derivatives, the plasmid containing these *pem* genes gave only a few segregants in viable cells and increased the number of nonviable cells in the population, suggesting that a product from the *pem* region stabilized the plasmid by killing plasmid-free segregants. Inactivation of one of the two translational open reading frames in the *pem* region caused the loss of the killing function, and thus, the open reading frame is a gene designated *pemK*, which encodes the killing factor. The coexistence of the *pem*$^+$ plasmid with a high-copy-number plasmid carrying the other open reading frame inhibited stabilization, and thus, the second open reading frame is a gene designated *pemI*, which encodes the inhibitor which might control the killing function of *pemK*. It is likely that the two open reading frames were transcribed from a promoter. There were no significant homologies in DNA sequences between the *pem* gene of R100 and the genes previously shown to be responsible for the stable inheritance of the other plasmids.

Many natural low-copy-number plasmids, such as R100, R1, P1, pSC101, and F, are stably maintained in dividing cells. They are thought to have mechanisms that are responsible for stable maintenance (3, 12, 15, 17–19). The stable maintenance of some of these plasmids has been studied in detail. Plasmid F has two different mechanisms for stable maintenance. One mechanism, which depends on *sopA*, *sopB*, and *sopC* genes, acts to equally partition plasmid DNA molecules into daughter cells (18). The other mechanism, which depends on *ccdA* and *ccdB* genes (also named *letA* and *letD*, respectively) (14, 19), guarantees that plasmid-carrying cells grow preferentially in the population by killing plasmid-free segregants (9, 10). Plasmid R1, which is closely related to plasmid R100, also encodes two different stability loci; these are designated *parA* and *parB* (6). The *parA* region has been shown to code for the equal partition system (7), and the *parB* region containing the *hok* and *sok* genes confers genetic stability to the plasmid by killing plasmid-free segregants (8).

In this report we describe the existence of novel genes designated *pemK* and *pemI* (*pem* for plasmid emergency maintenance) that are used for the stable maintenance of R100. These genes are located near the replication origin of R100, far from the *stb* locus that has been reported previously (15) as the gene responsible for the stable maintenance of R100. We show here that *pemK* encodes a factor which stabilizes the plasmid by killing the host cells and that *pemI* inhibits the stabilization. The nucleotide sequence in the *pem* region shows two open reading frames (ORF2 and ORF3) that have been shown to be organized into an operon (2, 21). We assume that ORF3 corresponds to *pemK* and that ORF2 corresponds to *pemI*, which might control the killing function of *pemK*.

MATERIALS AND METHODS

Bacterial strains and plasmids. *Escherichia coli* K-12 strains km1213 [F$^-$ *polA*(Ts) *his argG metB leu rpsL xyl lacY thy*] (26) and P3478 (F$^-$ *polA thyA36*) (4) were used in this study.

Plasmid pHO100 was a composite plasmid between plasmids pSM1 and pMB8::Tn*3* (20). pSM1 was derived from R12, a high-copy-number mutant of R100 (12). The mutants of pHO100 used were pHO100-B4, pHO100-B5, pHO100-B24, pHO100-B27, pHO100-B37, pHO100-B46, and pHO100-E217. They have been constructed previously by the linker mutagenesis technique (21). pYK2, another pHO100 derivative, was constructed by combining pHO100-B24 and pHO100-B4 to eliminate the *ori* region (Y. Kawai and E. Ohtsubo, unpublished data). The other pHO100 derivatives constructed for this study are described below.

Plasmid pDOM11 was constructed by cloning a fragment containing *pem* into plasmid pHS12, which is a high-copy-number mutant of the tetracycline resistance plasmid pSC101 (1) (see below). In the process of this construction, we used plasmid pINJ1622, which is a pUC derivative with the chloramphenicol resistance gene *cat* that was flanked by the two identical multiple cloning sites in opposite directions of each other (I.-N. Jin, Ph.D. thesis, Tokyo University, Tokyo, Japan, 1986).

Construction of other pHO100 derivatives. pHO100-B2405 was constructed by ligating the *Eco*RV-*Bam*HI fragment (positions 1093 to 1524) of pYK2 with the *Bam*HI-*Eco*RV fragment (positions 2835 to 1092) of pHO100-B5. pHO100-B2437 contained the *Eco*RV-*Bam*HI fragment (positions 1093 to 1524) of pYK2 and the *Bam*HI-*Eco*RV fragment (positions 4102 to 1092) of pHO100-B37. pHO100-B2446 contained the *Eco*RV-*Bam*HI fragment (positions 1093 to 1524) of pYK2 and the *Bam*HI-*Eco*RV fragment (positions 3488 to 1092) of pHO100-B46. pHO100-B2427 contained the *Eco*RI-*Bam*HI fragment (positions 3918 to 1524) of pYK2 and the *Bam*HI-*Eco*RI fragment (positions 2221 to 3917) of pHO100-B27. pHO100-B546 contained the *Eco*RI-*Nde*I fragment (positions 3918 to 3065) of pHO100-B2405 and the *Nde*I-*Eco*RI fragment (positions 3066 to 3917) of pHO100-B46.

Media and buffer. The growth medium used was Luria broth (1.0% tryptone [Difco Laboratories, Detroit, Mich.],

* Corresponding author.

0.5% yeast extract [Difco], 0.5% NaCl, 0.2% glucose [pH 7.0]) supplemented with 50 μg of thymine (Sigma Chemical Co., St. Louis, Mo.) per ml. Agar plates contained 1.5% Bacto-Agar (Difco). Ampicillin (Sigma) was added to the medium at a concentration of 50 μg/ml, and tetracycline (Sigma) was added at a concentration of 10 μg/ml, to select for cells with antibiotic resistance. Peptone dilution buffer (0.1% tryptone, 0.3% NaCl [pH 7.0]) was used to dilute the cell culture.

Enzymes. Restriction endonucleases (*Eco*RI, *Bam*HI, and *Eco*RV) and T4 DNA ligase were obtained from Takara Shuzo Co. (Kyoto, Japan). The restriction enzyme *Nde*I was obtained from New England BioLabs, Inc. (Beverly, Mass.). RNase A was purchased from Sigma. The enzymes were used as recommended by the manufacturers.

DNA preparation. Plasmid DNA was isolated by the method of Ohtsubo et al. (20). The crude lysis method of Machida et al. (11) was used for the small-scale preparation of plasmid DNA. We used the method of Yoshioka et al. (27) for the transformation of bacterial cells with plasmid DNA.

Stability test. The culture was grown at 30°C to the stationary phase and was diluted 10^{-4}-fold; it was then incubated at 30 or 42°C for 24 h. It was appropriately diluted, plated onto agar plates, and incubated at 30°C. The stability was measured by determining the fraction of plasmid-containing cells by replica plating from plates containing no antibiotics to the plates containing 100 μg of ampicillin per ml. The fraction with over 90% plasmid-containing cells was shown as stable, and that with less than 10% plasmid-containing cells was shown as unstable. There were no fractions containing between 90 and 10% plasmid-containing cells.

Cell growth. The turbidity of the culture was measured at 590 nm with a spectrophotometer (Spectronic 20A; Shimadzu Corp.). The total number of cells was counted on a counting chamber (Thoma; Atago) with a phase-contrast microscope (S-Ke; Nikon). The number of viable cells was determined as the number of CFU on the plates containing no antibiotics.

RESULTS

Existence and mapping of the gene responsible for the stability of R100. Plasmid pHO100 (Fig. 1), which is 7,052 base pairs in length, is a hybrid between R100 miniplasmids pSM1 and pMB8 (21). The replication system of pMB8 is related to that of ColE1, which cannot replicate in the *polA* mutant strain. pHO100 derivatives with deletions in various portions of the R100 sequence in pHO100 have been constructed previously. Some of these derivatives, which are shown in Fig. 1, were used for this study.

Among the derivatives, pYK2 lacked the replication origin region (positions 1641 to 1842) (*ori* in Fig. 1) that is essential for R100 replication. Another mutant, pHO100-E217, lacked *ori* and a nonessential region (positions 2177 to 4101) for R100 replication downstream of *ori* (Fig. 1). We tested the stability of these mutants in a *polA*(Ts) strain km1213 at 42°C. Both pYK2 and pHO100-E217 have been shown to be unable to transform a *polA* mutant strain (P3478) (21); thus, both of the mutants are expected to segregate from the cells of km1213 under the restrictive conditions for their replication at 42°C. pHO100-E217, in fact, gave segregants at frequencies of over 90% of the viable cells after a 24-h incubation at 42°C. Unexpectedly, however, pYK2 gave segregants only at frequencies of less than 10% of the viable cells. This result suggests that pYK2 has some mechanism to maintain itself stably within cells. The region at positions 2177 to 4101, from which pHO100-E217 was deleted, is responsible for the stable maintenance of pYK2 and must contain the gene(s) responsible for the stable maintenance of the plasmid. The locus of the gene(s) was designated *pem* (plasmid emergency maintenance).

To map *pem* more precisely, we constructed five pHO100 derivatives with deletions in *ori* as well as in the various regions downstream of it (Fig. 1), and we tested the stability of these plasmids in km1213 under restrictive conditions for their replication at 42°C. The two pHO100 derivatives pHO100-B2427 and pHO100-B2405, which had deletions within the region from positions 1525 to 2834 of the R100 sequence, were maintained stably in the cell. However, the other two derivatives, pHO100-B2446 and pHO100-B2437, which had deletions within the region from positions 2835 to 3917 of R100, were not (Fig. 1). This suggests that *pem* is located within the region from positions 2835 to 3917. The *tir* gene (Fig. 1), which is responsible for the inhibition of transfer of plasmid RP4 (25), was not essential for the

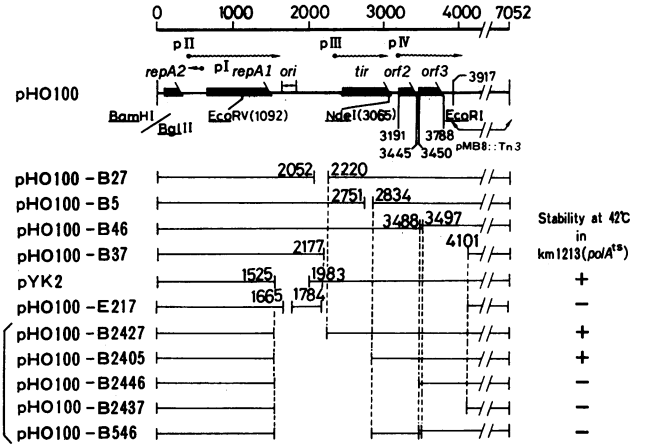

FIG. 1. Structures of pHO100 derivatives and results of the stability test for the derivatives deleted for the *ori* region of R100. A linear map of pHO100 shown with the nucleotide sequence coordinate numbers at the top contains the sequence (positions 1 to 3917) of R100 and the sequence (positions 3918 to 7052) of pMB8::Tn*3* (21). The closed thick bars on the R100 sequence are genes (*repA2*, *repA1*, and *tir*) and ORFs (ORF2 and ORF3) and indicate their translational orientations. The locations of four transcripts (RNAI to RNAIV) and their promoters (pI to pIV) (23) are shown. *ori* is the replication origin region (22). The restriction sites used for the construction of the five pHO100 derivatives (see below) are shown. The pMB8 sequence contains the *bla* gene, which encodes β-lactamase (data not shown). Below the pHO100 map, the pHO100 derivatives used for the present study are listed. Each pHO100 derivative has a deletion(s), a duplication, or both. Such mutations are shown by an interruption of the solid straight lines. The derivatives with a B number, except pHO100-B46, have one or two *Bam*HI linkers which are substituted to the deletions in them. pHO100-B46 has a duplication, as shown. The junction of the duplicated sequence contains a *Bam*HI linker. pHO100-E217, a derivative of pHO100-B37 with a deletion, has a second deletion from positions 1665 to 1784, where a *Eco*RI linker is substituted. The exact junctions of the mutations are indicated by numbers. The five plasmids shown at the bottom are those that were newly constructed for this study. Results of stability of each of *ori* mutant plasmids in km1213 [*polA*(Ts)] at 42°C are shown to the right of the map. Stability was indicated by the fraction of plasmid-containing viable cells, as described in the text. Symbols: +, greater than 90% plasmid-containing viable cells; −, less than 10% plasmid-containing viable cells.

function of *pem*, since pHO100-B2405, in which the N-terminal region of the *tir* gene was deleted, was maintained stably (Fig. 1).

The nucleotide sequence of the region from positions 2835 to 3917 is shown in Fig. 2. This region is located downstream from *tir* and contains two ORFs (ORF2 and ORF3), which have been shown to encode polypeptides with sizes close to 9.3 kilodaltons (85 amino acids) and 11.9 kilodaltons (110 amino acids), respectively (2). The two ORFs have been shown to be transcribed from a promoter to initiate synthesis of RNAIV (21) (Fig. 1 and 2). pHO100-B546, another pHO100 mutant that was constructed for this study, had the same deletion in pHO100-B2405, but in addition, it had a 10-base-pair duplication near the N-terminal end in ORF3, causing a frameshift in ORF3 (Fig. 1). pHO100-B546 was segregated frequently at 42°C, unlike pHO100-B2405 (Fig. 1), indicating that the duplicated region is essential for the stable maintenance of the plasmid and, thus, that the product made from ORF3 is essential for the *pem* function.

Plasmid-free segregants are killed postsegregationally by the product from the *pem* region. During the course of the stability tests described above, we found that cells carrying the *pem*$^+$ plasmid grew more slower than did those carrying the *pem* mutant plasmid at 42°C. We presumed that *pem* might maintain the plasmid by killing host cells that lost the plasmid, like the other stability genes such as *ccdB* of plasmid F (10) and *hok* of plasmid R1 (8). To test this possibility, we analyzed the kinetics of stability of pHO100-B2405 (*pem*$^+$) or pHO100-B2446 (*pem* mutant) in km1213 [*polA*(Ts)] after it was transferred from 30 to 42°C. The fraction of plasmid-containing cells after the temperature shift to 42°C is shown in Fig. 3. In the case of pHO100-B2446 (*pem* mutant), plasmid-free segregants appeared about 4 h after the shift. In contrast, pHO100-B2405 (*pem*$^+$) was maintained in most of colony formers (96%), even 8 h after the shift.

The results of analyses of the number of viable cells, the number of total cells, and the turbidity after the temperature transfer are shown in Fig. 3. In the case of pHO100-B2446 (*pem* mutant), the number of viable cells, the number of total cells, and the turbidity increased at the same rate for 8 h after the temperature shift. However, in the case of pHO100-B2405 (*pem*$^+$), the number of viable cells increased up to 4 h, but after that the rate of increase was markedly reduced. The number of total cells and turbidity continued to increase at the same rate for another 2 h, or about two generations, before their rates were reduced.

Considering the copy number of pHO100-B2405 and pHO100-B2446 (ca. 20 to 30 copies per cell), it took 4 h, or four to five generations, to dilute the copy number of these plasmids to one per cell under the restrictive condition for replication of these plasmids. In fact, pHO100-B2446, which is a *pem* mutant, began to segregate after 4 h. In contrast, pHO100-B2405, which is *pem*$^+$, did not segregate, but in turn, it reduced the fraction of viable cells (Fig. 3). In this case, the difference between the total number of cells and the number of viable cells increased, even after a reduction in the rate of increase of total cells. This indicates that the number of nonviable cells increased. Also, most of the viable cells were those that carried plasmid pHO100-B2405 (*pem*$^+$), suggesting that nonviable cells are plasmid-free cells, since if all cells were viable, many plasmid-free segregants would have appeared.

These observations indicate that in the case of *pem*$^+$ plasmids, plasmid-free cells are killed postsegregationally, causing a high fraction of the plasmid-containing cells in the viable population. Killing of the cells that lost plasmid pHO100-B2405 (*pem*$^+$) is the reason that the *pem*$^+$ plasmid was maintained in the population. The *pem* region thus encodes a killing factor, the gene of which was designated *pemK*. *pemK* presumably corresponds to ORF3.

The *pem* region also encodes an inhibitor of stabilization.

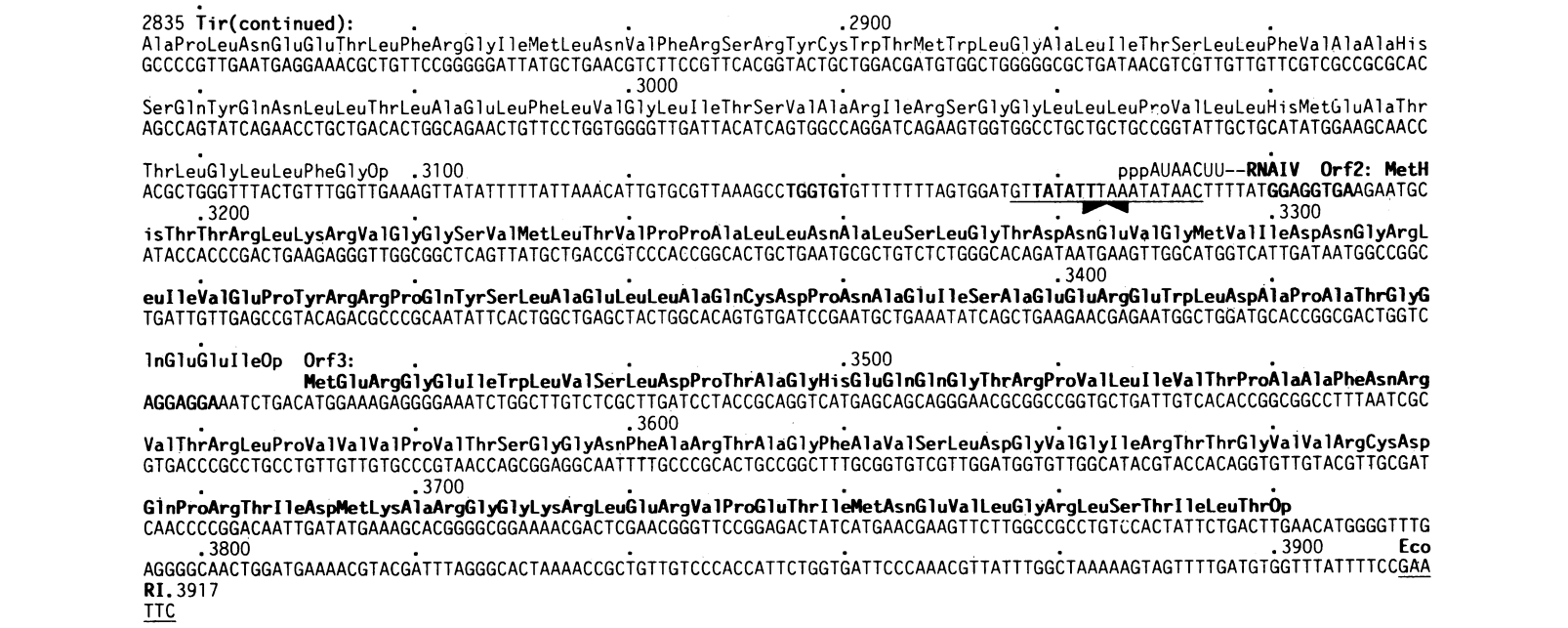

FIG. 2. Nucleotide sequence of the region from positions 2835 to 3917 of R100 in pHO100. The nucleotide sequence shown has been determined previously (21). The putative amino acid sequences of ORF2 and ORF3 and the C-terminal region of *tir* are shown. In the nucleotide sequence, boldface letters indicate particular sequences, such as possible −10 and −35 regions of the promoter and ribosome-binding sequences. The RNA transcript (RNAIV) that has pppA at its 5′ end is shown (21). A major dyad symmetry is indicated by the arrows.

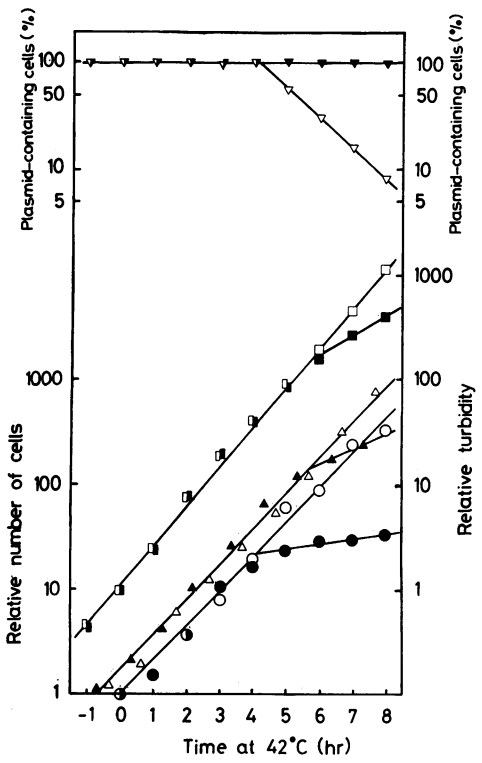

FIG. 3. Kinetic analysis of plasmid stability and cell growth. Strain km1213 harbored the *pem*$^+$ plasmid pHO100-B2405 (closed symbols) or the *pem* mutant plasmid pHO100-B2446 (open symbols) and was grown exponentially at 30°C; at time zero the culture was shifted to 42°C. The culture was diluted at intervals with fresh, prewarmed medium to maintain cells in the exponential phase. Samples were analyzed for the fraction of plasmid-containing cells among viable cells (percent) (▼, ▽), for turbidity at 590 nm (■, □), for the number of total cells (▲, △), and for the number of viable cells (●, ○), as described in the text.

Like the *ccd* genes of plasmid F, the *pem* region might also encode an inhibitor to control the killing of the host cells, in addition to the killing factor PemK. To prove this, we cloned the region from positions 2835 to 3497, which contains the promoter for RNAIV and ORF2, into plasmid pHS12, a high-copy-number mutant of the tetracycline resistance plasmid pSC101 (Fig. 4). The resulting plasmid pDOM11, as well as its parent pHS12, were introduced into km1213 harboring pHO100-B2405 (*pem*$^+$); and the stability of pHO100-B2405 at 42°C was then examined. The fraction of ampicillin-resistant colonies among the tetracycline-resistant colonies was found to be less than 1% in the presence of pDOM11 and 98% in the presence of pHS12 after incubation for 24 h at 42°C. At 30°C, the fraction was over 98% in the presence of either pDOM11 or pHS12. These results suggest that pHO100-B2405 (*pem*$^+$) cannot be maintained stably when pDOM11 coexists with it, and that there exists a *trans*-acting inhibitor for killing in the *pem* region that is carried by pDOM11. We designated the gene encoding the inhibitor *pemI*. Presumably, ORF2 in the region from positions 2835 to 3497 corresponds to *pemI*.

DISCUSSION

We have found and characterized *pem*, which is responsible for the stabilization of inheritance of an R100 derivative. *ccd* (*let*), which is responsible for stable maintenance of plasmid F, is known to have two genes, *ccdA* (*letA*) and *ccdB* (*letD*) (14, 19). *ccdB* is essential for the stable maintenance of the plasmid because it kills the host cells, while *ccdA* encodes an inhibitor which inhibits the killing function by *ccdB*. As shown above, the *pem* system is like the *ccd* (*let*) system and contains not only a gene (*pemK*) that encodes a factor for killing the host cells but also a gene (*pemI*) that inhibits the killing function by *pemK*. This suggests that the *pem* system kills the cells and controls the killing function in a manner similar to that done by *ccd*. However, there is no significant homology between *pem* and *ccd* at the nucleotide sequence level or at the amino acid sequence level, although polypeptides encoded by *pem* are similar in size to those encoded by *ccd* (16). The *parB* region contains the genes *hok* and *sok* which also stabilize the inheritance of plasmid R1 by killing host cells (5, 8). *parB* has been shown to have a different mechanism for killing host cells from that of *ccd* of plasmid F and thus, perhaps, also from that of *pem*. Note that no nucleotide or amino acid sequence homology was found between *pem* and *parB*.

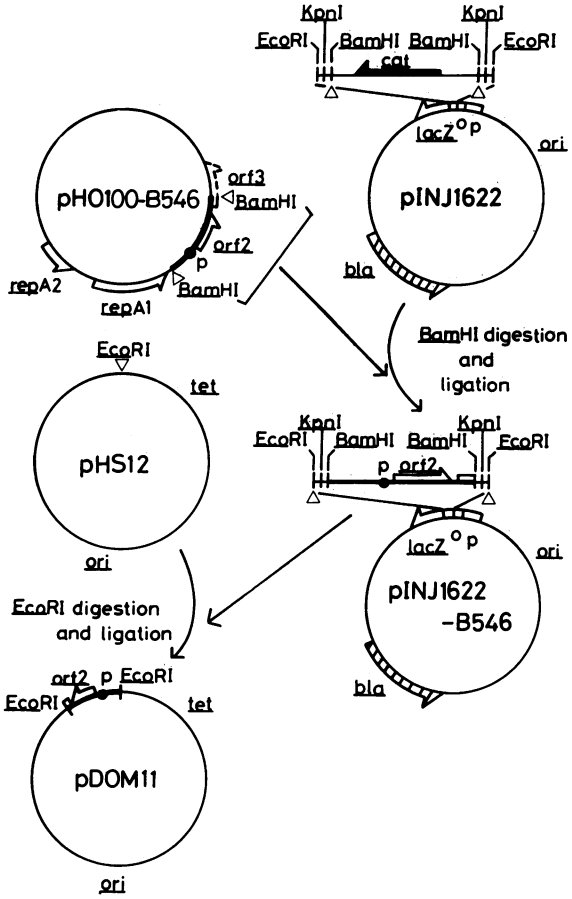

FIG. 4. Construction of plasmid pDOM11, a pSC101 derivative carrying the ORF2 region of R100. To construct pDOM11, a plasmid which contains *pem* was first prepared by cloning the *Bam*HI fragment (positions 2835 to 3497) of pHO100-B546 into *Bam*HI-digested pINJ1622, a pUC derivative which has the chloramphenicol resistance gene *cat* that was flanked by the two identical multiple cloning sites in opposite directions. The plasmid was then digested with *Eco*RI to remove the fragment that had the region of pHO100-B546 from positions 2835 to 3497 with *Eco*RI cohesive ends. The *Eco*RI fragment was cloned into an *Eco*RI site of pHS12, which is a high-copy-number mutant of the tetracycline resistance plasmid pSC101, to finally give pDOM11.

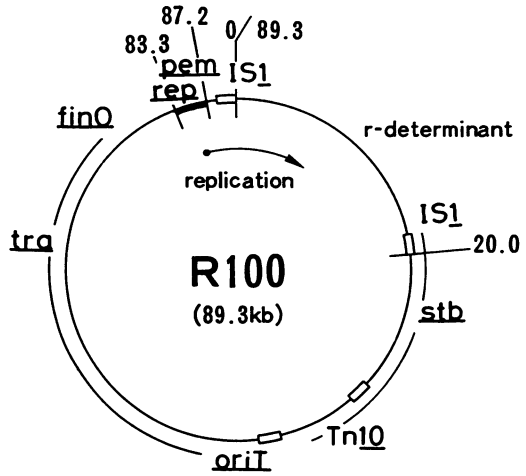

FIG. 5. Circular map of R100. Numbers are coordinates to R100 in kilobase pairs (kb) from a selected origin at the end of insertion sequence IS*1*, which is present in R100 (13, 15, 20, 24). Transposon Tn*10* is denoted. The r determinant is the region that includes the genes for resistance to mercuric ions, sulfonamide, streptomycin, fusidic acid, and chloramphenicol. *oriT tra finO* denotes the region responsible for the conjugative transfer of R100. *rep* denotes the region required for the replication of R100. *pem* and *stb* are the genes responsible for the stable maintenance of R100 (see text). The thick line represents the region of R100 in pHO100.

In R100, another gene that is responsible for its stable inheritance, designated *stb*, has been reported (15) in the region which is located at least 21 kilobase pairs apart from *pem* on the opposite side of the r-determinant region (Fig. 5). In this study we used a derivative of an R100 high-copy-number mutant which was able to replicate and was maintained stably without *stb* or even *pem*. However, we assume that under the critical circumstances, for example, reduction of the copy number of R100, both *pem* and *stb* could function so that they would not disappear from the cells. *pem*, which is carried by the low-copy-number plasmid pHS1, a temperature-sensitive replication mutant of pSC101, is in fact expressed to stabilize the plasmid, when the replication of the pHS1 derivative in *E. coli* C600, which is different than the strain km1213 used in the present study, is inhibited at 42°C (unpublished data).

There is an extensive homology by the heteroduplex criterion between plasmids R100 and R1, which is closely related to R100 (24). There seems to be a strong homology between R100 and R1 in the region around *pem*, which is located close to the origin of replication of R100. This suggests that R1 may also encode the *pem* gene. There also seems to be a strong homology between the region around *stb* of R100, apart from the replication origin, and the region of R1 where *parA* and *parB* are located. Thus, it is interesting that one plasmid has several genes that are responsible for its stable maintenance to ensure its stable inheritance.

ACKNOWLEDGMENTS

We thank S. Hiraga for sending us strain km1213, for generous suggestions, and for critical reading of the manuscript.

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

ADDENDUM IN PROOF

After this paper was submitted, Bravo et al. (Mol. Gen. Genet. **179**:241–252, 1987) reported a stable maintenance system, ParD, of R1 which was found to be identical to *pem* of R100 described in this paper.

LITERATURE CITED

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 replication and copy number control in the plasmid pSC101 and its temperature-sensitive derivative pHS1. J. Mol. Biol. **175**:331–347.
2. **Armstrong, K. A., H. Ohtsubo, W. R. Bauer, Y. Yoshioka, C. Miyazaki, Y. Maeda, and E. Ohtsubo.** 1986. Characterization of the gene products produced in minicells by pSM1, a derivative of R100. Mol. Gen. Genet. **205**:56–65.
3. **Austin, S., F. Hart, A. Abeles, and N. Sternberg.** 1982. Genetic and physical map of a P1 miniplasmid. J. Bacteriol. **152**:63–71.
4. **De Lucia, P., and J. Cairns.** 1969. Isolation of an *E. coli* strain with a mutation affecting DNA polymerase. Nature (London) **224**:1164–1166.
5. **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.
6. **Gerdes, K., J. E. L. Larsen, and S. Molin.** 1985. Stable inheritance of plasmid R1 requires two different loci. J. Bacteriol. **161**: 292–298.
7. **Gerdes, K., and S. Molin.** 1986. Partitioning of plasmid R1. Structural and functional analysis of the *parA* locus. J. Mol. Biol. **190**:269–279.
8. **Gerdes, K., P. B. Rasmussen, and S. Molin.** 1986. Unique type of plasmid maintenance function: postsegregational killing of plasmid-free cells. Proc. Natl. Acad. Sci. USA **83**:3116–3120.
9. **Hiraga, S., A. Jaffé, T. Ogawa, H. Mori, and H. Takahashi.** 1986. F plasmid *ccd* mechanism in *Escherichia coli*. J. Bacteriol. **166**:100–104.
10. **Jaffé, A., T. Ogura, and S. Hiraga.** 1985. Effects of the *ccd* function of the F plasmid on bacterial growth. J. Bacteriol. **163**: 841–849.
11. **Machida, Y., C. Machida, and E. Ohtsubo.** 1982. A novel type of transposon generated by insertion element IS*102* present in a pSC101 derivative. Cell **30**:29–36.
12. **Meacock, P. A., and S. N. Cohen.** 1980. Partitioning of bacterial plasmids during cell division: a cis-acting locus that accomplishes stable plasmid inheritance. Cell **20**:529–542.
13. **Mickel, S., E. Ohtsubo, and W. Bauer.** 1977. Heteroduplex mapping of small plasmids derived from R-factor R12: in vivo recombination occurs at IS*1* insertion sequences. Gene **2**:193–210.
14. **Miki, T., Z.-T. Chang, and T. Horiuchi.** 1984. Control of cell division by sex factor F in *Escherichia coli*. II. Identification of genes for inhibitor protein and trigger protein on the 42.84-43.6 F segment. J. Mol. Biol. **174**:627–646.
15. **Miki, T., A. M. Easton, and R. H. Rownd.** 1980. Cloning of replication, incompatibility, and stability functions of R plasmid NR1. J. Bacteriol. **141**:87–99.
16. **Miki, T., K. Yoshioka, and T. Horiuchi.** 1984. Control of cell division by sex factor F in *Escherichia coli*. I. The 42.84-43.6 F segment couples cell division of the host bacteria with replication of plasmid DNA. J. Mol. Biol. **174**:605–625.
17. **Nordström, K., S. Molin, and H. Aagaard-Hansen.** 1980. Partitioning of plasmid R1 in *Escherichia coli*. I. Kinetics of loss of plasmid derivatives deleted of the *par* region. Plasmid **4**:215–227.
18. **Ogura, T., and S. Hiraga.** 1983. Partition mechanism of F plasmid: two plasmid gene-encoded products and a cis-acting region are involved in partition. Cell **32**:351–360.
19. **Ogura, T., and S. Hiraga.** 1983. Mini-F plasmid genes that couple host cell division to plasmid proliferation. Proc. Natl. Acad. Sci. USA **80**:4784–4788.
20. **Ohtsubo, E., M. Rosenbloom, H. Schrempf, W. Goebel, and J.**

Rosen. 1978. Site specific recombination involved in the generation of small plasmids. Mol. Gen. Genet. **159**:131–141.
21. **Ohtsubo, H., T. B. Ryder, Y. Maeda, K. Armstrong, and E. Ohtsubo.** 1986. DNA replication of the resistance plasmid R100 and its control. Adv. Biophys. **21**:115–133.
22. **Rosen, J., H. Ohtsubo, and E. Ohtsubo.** 1979. The nucleotide sequence of the region surrounding the replication origin of an R100 resistance factor derivative. Mol. Gen. Genet. **171**:287–293.
23. **Rosen, J., T. Ryder, H. Ohtsubo, and E. Ohtsubo.** 1981. Role of RNA transcripts in replication incompatibility and copy number control in antibiotic resistance plasmid derivatives. Nature (London) **290**:794–797.
24. **Sharp, P. A., S. N. Cohen, and N. Davidson.** 1973. Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli*. II. Structure of drug resistance (R) factors and F factors. J. Mol. Biol. **75**:235–255.
25. **Tanimoto, K., T. Iino, H. Ohtsubo, and E. Ohtsubo.** 1985. Identification of a gene, *tir* of R100, functionally homologous to the *F3* gene of F in the inhibition of RP4 transfer. Mol. Gen. Genet. **198**:356–357.
26. **Tsutsui, H., and K. Matsubara.** 1981. Replication control and switch-off function as observed with a mini-F factor plasmid. J. Bacteriol. **147**:509–516.
27. **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 of IS*3* into F *finO*. J. Bacteriol. **169**:619–623.