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Identification of the Gene Products Responsible for F Plasmid Partitioning

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DNA subfragments, *sop*A, *sop*B and *sop*C which help to maintain the stability of an *ori* C plasmid, were derived from a mini-F plasmid DNA (*Eco*RI restriction fragment f5) after digestion with restriction endonuclease, and cloned in the vector plasmid pBR322. The recombinant plasmids obtained were introduced into *E. coli* KY7231 and *E. coli* CSR603 strains, and proteins specified by the mini-F fragments were analysed by SDS-PAGE. Two proteins encoded by the F fragments were detected, and their molecular weights were 41,000 and 37,000 daltons. Fluorography after one and two dimensional gel electrophoresis of the lysates showed that these two proteins had been overproduced in the cells which were allowed to incorporate radioactive amino acid after plasmid amplification by chloramphenicol treatment. The isoelectric points of *sop*A and *sop*B proteins were 6.6 and 7.0, respectively.

Plasmids are present in a defined number of copies per cell. For some plasmids, such as F plasmid of *E. coli*, their copy numbers are strictly controlled at the level of one to two per host chromosome (2, 5). However, most plasmids with low copy number are completely stably inherited and plasmid-free cells are only rarely formed (Fig. 1). This has been taken as an indication of the existence of a mechanism that partitions the plasmid copies to daughter cells at cell division (12).

A mini-F plasmid which contains a 9.2 kb *Eco*RI fragment f5 of the F factor (9, 17, 18) has been shown to retain all the characteristics of an F plasmid replication, including the low copy number, stability and FI-type incompatibility.

The partitioning mechanism of F plasmid has been described by Ogura and Hiraga (14) as follows: the segment necessary for a stable partitioning is located within 3 kb segment outside of the segment essential for autonomous replication of the plasmid. This segment contains three functionally distinct regions; two of them (designated *sop*A and *sop*B) specify gene products that act in *trans*, whereas the gene products of third region (*sop*C) acts in *cis*. All three regions seem to be essential for the normal partitioning of the plasmid into daughter cells during cell division.

Proteins responsible for the plasmid partitioning has been identified and the results obtained are presented in this paper.

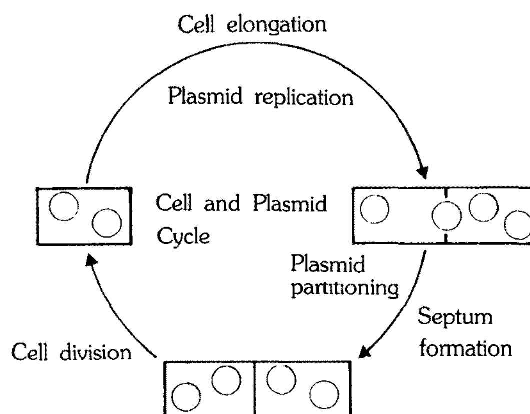

Fig. 1. Life cycles of bacteria and plasmids.

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MATERIALS AND METHODS

Strains and Media

The genotypes of bacterial strains and plasmids used in these studies are listed in Table 1. The plasmids, pXX 167, pXX288, pXX157 and pXX300 (14), which were constructed from a mini F-pBR322 composite plasmid pKP1033 (14), were provided by S. Hiraga and their constructions are shown in Fig. 2. Plasmid pXX167, which carries the whole C-A2 segment, was constructed by *Pst* I digestion from pKP1033, which consisted of a mini-F (the f5 segment of F) and a pBR322. Plasmid pXX288 was constructed by the deletion of a *Hinc* II segment within the A2 segment from pXX167. Plasmid pXX300 is *Pvu* I deletion derivative of pKP1033. Plasmid pXX157 which carries the A2 segment was constructed by *Pst* I digestion from pKP1033. △ in pXX157 represents a spontaneous deletion. L-broth containing 1% tryptone, 0.5% NaCl, 0.4% glucose in a liter of distilled water was used throughout the experiment. Tetracycline was added to give a final concentration of 15 μg/m*l*.

Table 1. Bacterial strains and plasmids

Strains & plasmids	Characteristics	References
E. coli KY7231	F^-, *trp*B9578, *tna*$^-$2, str, rec Al	(15)
E. coli CSR603	F^-, *thr*$^-$1, *leu* B6, *pro* A2, *phr*$^-$1, *rec* Al, *arg* E3, *thi*$^-$1, *uvr* A6, *ara*$^-$14, *lac* Y1, *gal* K2, *xyl*$^-$5, *mtl*$^-$1, *rps* L31, *tsx*$^-$33, λ^-, *sup* E44	(16)
pKP1033	Tc^r, Ap^r	(14)
pXX167	Tc^r, sopA, B, C	*ibid*
pXX288	Tc^r, sopA	*ibid*
pXX157	Tc^r, sopB	*ibid*
pXX300	Tc^r, sopC	*ibid*

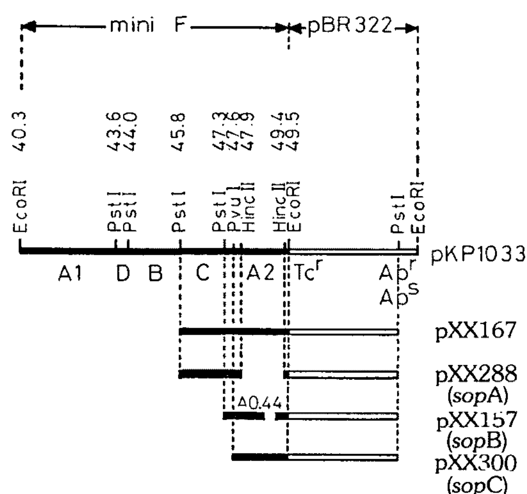


Fig. 2. Construction of deletion derivatives of pKP1033.
*△ in pXX157 represents a spontaneous deletion.

Isolation of Plasmid and Transformation

Plasmid DNA was isolated by a rapid cleared lysate technique (3). Transformation of *E. coli* CSR603 was achieved using the procedure described by Mandel (10). Plasmid DNA was analysed by electrophoresis using a 0.7% agarose gel.

Preparation and Fractionation of Cell Lysate

The cells were grown in L-broth overnight and harvested by centrifugation at 8,000×g for 5 min at 4℃. The cells were suspended in 0.3 m*l* of sonication buffer (0.01 M Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 50 μg/m*l* Pancreatic RNase A) and broken by two 30 second sonication cycles with 30 second intervals in an ice bath with an Ohtake sonicator. Unbroken cells were removed by centrifugation at 8,000×g for 5 min. A hundred μ*l* of the lysate was taken out and stored at −20℃. The remaining cell lysate was spun at 189,000×g for 45 min by a Hitach preparative ultracentrifuge in a RP 65 rotor and fractionated into cytoplasm and crude membrane.

Sodium Dodecyl Sulfate (SDS) Gel Electrophoresis and Determination of Molecular Weight of Protein

SDS-polyacrylamide gel was prepared as described by Laemli and Favre (7). Samples were loaded on the 12.5% acrylamide gel. The molecular weight standards consisted of ovalbumin (45 K), pepsin (35 K), trypsinogen (24 K). The gel was run at 20 mA, then 30 mA until the dye front reached the bottom of the gel. Then the gel was stained by Coomassie brilliant blue and destained by the method of Fairbanks (4).

Two Dimensional Gel Electrophoresis of Protein and Determination of Isoelectric Point

Cell proteins were fractionated according to the method of O'Farell (13) with a slight modification. Isoelectric focusing gel (IF gel) for the first dimension was made in a glass tubing (100×2.5 mm inside diameter) sealed at the bottom with parafilm. To make 10 m*l* of gel mixture, 5.5 g of urea was added to a side arm flask, then 1.33 m*l* of 30% acrylamide stock, 2 m*l* of 10% Triton X-100, 1.97 m*l* of water, 0.4 m*l* of Ampholines (pH range between 5 and 7), 0.1 m*l* of Ampholines (pH range between 3.5 and 10) and 10 μ*l* of ammonium persulfate were added. The solution was degassed under

vacuum for 5 min. Immediately after the addition of 7 μl of TEMED, the solution was loaded into the gel tubes. The gels were overlayed with water and allowed to set for 4 hrs. Then the water was removed and the gels were placed in an electrophoresis chamber. Twenty μl of lysis buffer (9.5 M urea, 2% Triton X-100, 1.6% Ampholines, pH range 5 to 7, 0.4% Ampholines, pH range 3.5 to 10) were loaded on the gel and the upper reservoir was filled with 0.02 M of degassed NaOH. The lower reservoir was filled with 0.01 M of H_3PO_4. The gels were then prerun according to the following schedule: 200 V for 15 min, 300 V for 30 min, and 400 V for 30 min. Then the lysis buffer and NaOH were removed and the samples were loaded. The gels were run at 400 V for about 12 hrs and then 800 V for 60 min. The gels were removed from tubes into an SDS sample buffer and equilibrated fro 2 hrs at room temperature. SDS-polyacrylamide gel for the second dimension was prepared as described above. Melted agarose solution was put in the notch, and the IF gel was put into the solution. The gels were run as described above. The isoelectric point was determined according to the method of O'Farell (13).

Incorporation of Radioactive Methionine into Proteins

One tenth percent of overnight culture broth was inoculated in fresh L-broth. At an A_{550}: 0.4, 150 μg/ml of chloramphenicol was added to the culture broth, and incubated at 37℃ for 12 to 16 hrs. After cultivation, the cells were harvested, washed twice with M9 minimal salts, and resuspended in a medium of M9 glucose-casamino acids (minus methionine and cysteine). To the cell suspension 50 μci/ml of ^{35}S-methionine was added, then the cells were further cultivated at 37℃ for 60 min. The cell suspension was centrifuged, washed once with M9 salts-casamino acids (minus methione and cysteine), and resuspended in 400 μl of SDS-sample buffer (10% glycerol, 5% β-mercaptoethanol, 2.3% SDS, 0.0625 M Tris-HCl, pH 6.8). The samples were stored at −20℃ after a 2 min heat treatment in boiling water.

Fluorography of the Gels

After electrophoresis of the samples prepared from cells labelled with radioactive methionine, the gels were treated as described by Bonner and Laskey (1). The gels were put into about 20 fold volume of dimethyl sulfoxide (DMSO) for 30 min, followed by a second immersion in fresh DMSO for 30 min, 4 volumes of 20% diphenyloxazole (PPO) dissolved in DMSO (22.2%) for 3 hr, and then in 20 volumes of water for 1 hr. After precipitation of PPO in tap water, the gels were dried and exposed to a preexposed X-ray film (Kodak X-Omat) at −70℃ for a proper period (8).

RESULTS

Transformation and Agarose Gel Electrophoresis of Cleared Lysate of Transformants

Plasmid DNA from *E. coli* KY7231 transformants was prepared from the cleared lysate. *E. coli* CSR603 also was transformed with the plasmid, and colonies were selected on L-broth plate containing 15 μg/ml of tetracycline. After the analysis of cleared lysates of tetracycline resistant colonies by agarose gel electrophoresis, we found a plasmid band at the same position as that of *E. coli* KY7231 carrying the plasmids except for pXX288 (Fig. 3). In the case of transformation with pXX288, all of the tetracycline-resistant colonies derived from the *E. coli* CSR603 examined, carried only the vector plasmid pBR322 fragment, and the DNA fragment corresponding to sopA was deleted. *E. coli* CSR603 transformants with pXX167, pXX157 and pXX300, and *E. coli* KY7231 carrying pXX288 were selected for further experiments.

Protein Products Directed by the Plasmid

The protein distribution of cell lysates prepared from the cells which had been disrupted by sonication were examined by Coomassie brilliant blue staining after SDS-PAGE. As shown in Fig. 4, there were novel protein bands in the lysates of transformants with pXX288 and

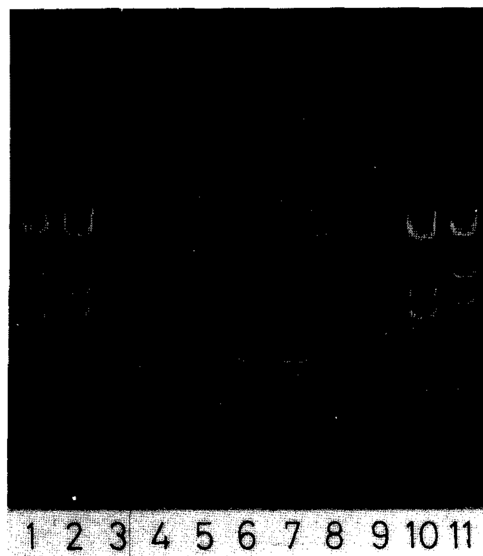
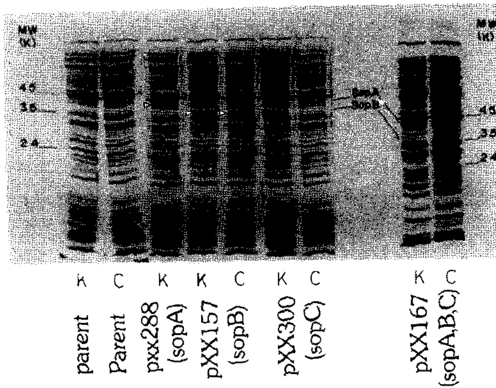


Fig. 3. Agarose gel electrophoresis of cleared lysates of transformed cells.
1,11: λ DNA digested with *Hind*III, 2,10: λ DNA digested with *Eco*RI, 3: pXX167 (*E. coli* KY7231), 4: pXX167 (*E. coli* CSR 603), 5: pXX288 (*E. coli* KY7231), 6: pXX157 (*E. coli* KY 7231), 7: pXX157 (*E. coli* CSR603), 8: pXX300 (*E. coli* KY 7231), 9: pXX300 (*E. coli* CSR603).



K: *E. coli* KY7231, C: *E. coli* CSR603.

Fig. 4. SDS-polyacrylamide gel electrophoresis of cell lysates of *E. coli* with or without plasmids.

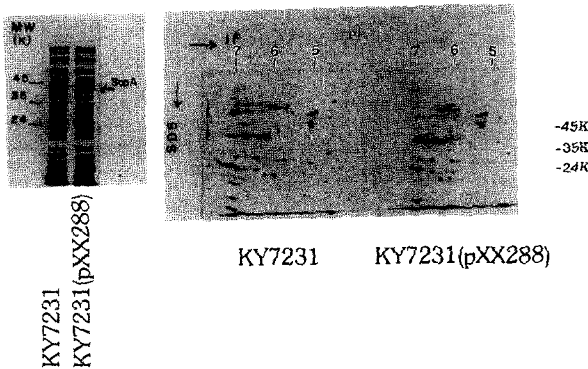


Fig. 5. Fluorography of one and two dimensional gel electrophoresis of *E. coli* KY7231 (pXX288) cell lysate.

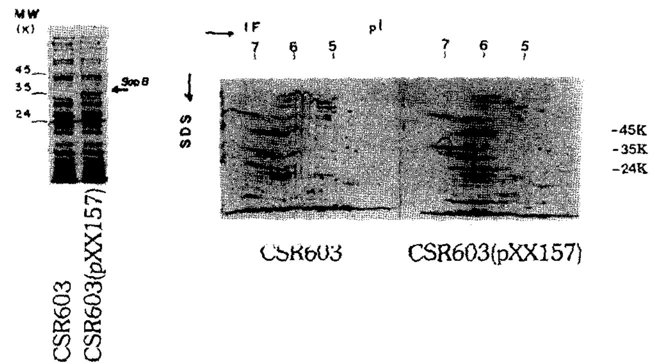


Fig. 6. Fluorography of one and two dimensional gel electrophoresis of *E. coli* CSR603 (pXX157) cell lysate.

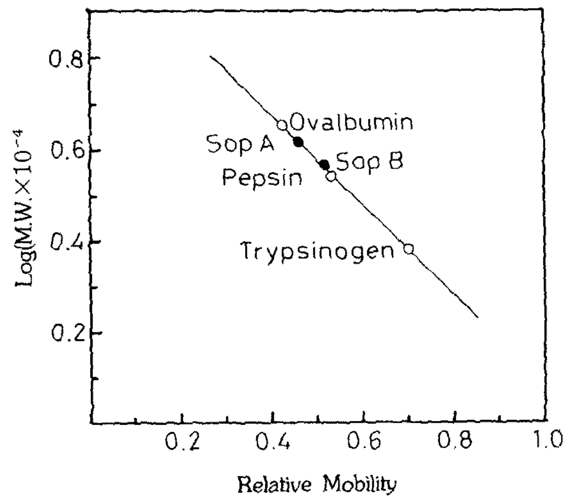


Fig. 7. Molecular weight determination of the sopA and sopB proteins by SDS-polyacrylamide gel electrophoresis.

pXX157 which bore F plasmid genes sopA and sopB, respectively. More sopB protein was produced in *E. coli* CSR603 transformant as compared with that produced in *E. coli* KY7231 transformant.

On the other hand, there was no novel protein band detected in the pXX300 transformant which bore sopC locus of F plasmid. Gene products of plasmid pXX167 which is comprised of sopA, sopB, and sopC regions, were not detectable by this method in the lysates of the transformants.

SopA and SopB Proteins Analysed by Fluorography

As the replication of vector plasmid pBR322 was derived from Col El, the copy number of the plasmid could be amplified by the treatment of the cells with chloramphenicol (11). The cells of *E. coli* KY7231 (pXX288) and *E. coli* CSR603 (pXX157) were allowed to incorporate radioactive methionine after chloramphenicol treatment as described in Methods. Cell lysates were subjected to one and two dimensional gel electrophoresis, followed by fluorography. An extensive incorporation of radioactivity was detected in the bands corresponding to sopA and sopB proteins. This suggested that sopA and sopB proteins may have been overproduced after gene amplification. Two dimensional gel electrophoresis showed that the isoelectric points of sopA and sopB proteins were 6.6 and 7.0, respectively. The results are shown in Fig. 5, 6.

Determination of Molecular Weight of Sop Proteins

The cell lysates of transformants with plasmid pXX288 and pXX157 were examined by SDS-PAGE and their molecular weights were determined by comparing with those of the standard proteins.

As shown in Fig. 7, the molecular weights of sopA and sopB proteins were evaluated to be 41,000 and 37,000 daltons, respectively.

DISCUSSION

In this paper we described the identification of proteins encoded by the mini-F responsible for the partitioning of an *ori* C plasmid (14). Deleted derivatives of *Eco* RI fragment (f5: 40.3-49.5 kb) of the plasmid cloned in pBR322, allowed us to detect two proteins which are encoded by *sop*A and *sop*B genes. The molecular weights of these two products were 41,000 and 37,000 daltons, respectively.

We observed that these two proteins were overproduced in the lysates prepared from cells carrying *sop*A and *sop*B fragments. The overproduction was more evident when the cells were labelled with radioactive methionine after the amplification of plasmid with chloramphenicol treatment. The gene products of pXX167, which is comprised of *sop*A, *sop*B and *sop*C regions, were not detectable through the method used, though the copy of the plasmid was similar to others. These results suggested that overproduction of the products of pXX167 were suppressed by a certain regulatory mechanism within the cell.

On the other hand, the *sop*A and *sop*B genes seem to correspond to the region encoding proteins with molecular weights of 41 to 44 kd and 36 to 37 kd, respectively (6). Therefore, we concluded that 41 K and 37 K proteins are the *sop*A and *sop*B products respectively. It was proposed by Ogura and Hiraga (14) that the proteins coded by sopA and sopB act in *trans* to partition a plasmid, while the *sop*C region acts in *cis* to stabilize it. This observation is consistent with our findings.

As mentioned above, it would be interesting to investigate further the function of the proteins necessary for the partitioning of a F plasmid because much remains yet to be elucidated.

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