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Localization of Sop Proteins and Interaction of Plasmid DNA with the Cell Membrane of Host Bacteria in Partitioning

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A sopA protein (41K) encoded by plasmid pXX288 was observed in the cytoplasm, whereas a sopB protein (37K) encoded by plasmid pXX157 was observed in the membrane fraction. Most of the sopB protein was solubilized from the crude membrane by treatment with Sarkosyl, which suggested that the protein may be located in the inner membrane. The sopA protein was precipitated at the concentration of 30 to 60% ammonium sulfate. The sedimentation profile of the crude membrane fraction showed a little difference according to culture media used, and the sopB protein existed in all fractions of inner membrane. The DNA of plasmids, pXX157, pXX300, and pXX167 co-sedimented with inner membrane fraction.

The presence on bacterial plasmids of a function that ensures their stable inheritance in the growing population of host cells recently has been demonstrated in several laboratories. Termed *par* or *stb*, the loci specifying this function have been identified on plasmid pSC101 (8), NR1 (9), R1 (10), and the F plasmid (13). In these plasmids a region (*par or stb*) has been found to be responsible for controlled partitioning. Deletion of this region results in the loss of plasmid stability and the quantitative properties of this instability have been found to be in agreement with a random distribution of plasmid copies in the daughter cells at cell division (10).

Very little is known about the mechanism that controls partitioning. However, there seems to be a randomization step involved (5) resulting in a weak incompatibility between two (compatible) plasmids that have the same *par* function (11). This could indicate competition for a site. This site could be connected with the folded chromosome, since many plasmids co-sediment with the folded chromosome (6). However, a more likely correlation might be the involvement of membrane attachment (4). Recent results indicate that the *E. coli* chromosome shows a specific binding to the outer membrane and that this binding is located close to the origin of replication of the chromosome (16).

To provide the basis for the control mechanism of F plasmid partitioning, we have conducted experiments for the localization of sopA and sopB proteins identified in the preceding paper (5) and the interaction of plasmid DNA with the cell membrane of the host bacteria in partitioning.

MATERIALS AND METHODS

Strains and Media

The strains and plasmids described in the preceding paper (5) were used in this experiment. *E. coli* transformants with plasmid were cultured at 37℃ in L-broth, M9 minimal medium (Na_2HPO_4 6 g, NaCl 0.5 g, NH_4Cl 1 g, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, glucose 4 g, leucin 0.1 g, arginine 0.2 g, proline 0.2 g, threonine 0.1 g, thiamine 1 mg per liter), M9 enriched medium (Na_2HPO_4 6 g, KH_2PO_4 3 g, NaCl 0.5 g, NH_4Cl 1 g, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, glucose 4 g, vitamine free casamino acid 2 g, thiamine 1 mg per liter), and K medium (same as M9 enriched medium except that vitamin-free casa-

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mino acid 10 g per liter is used here).

Sodium Dodecyl Sulfate (SDS) Gel Electrophoresis

SDS-polyacrylamide gel was performed as described in the preceding paper (5).

Isolation of Cytoplasmic and Outer Membrane

Cells were harvested by centrifugation, resuspended at 4℃ in buffer D (50 mM Tris-HCl, pH 7.9, 0.3 M KCl, 0.1.mM EDTA, 12 mM β-mercaptoethanol). The suspension was broken by being passed through a French Press Cell at 10,000 lbs/in^2 at 4℃. Unbroken cells were removed by centrifugation at 8,000×g for 10 min. The supernatant was then centrifuged at 189,000×g for 45 min. The pelleted crude membrane was resuspended in buffer D, layered on a 10 ml, 30~50%(w/w) sucrose linear gradient in buffer D over a 2 ml, 55%(w/w) sucrose/buffer D shelf, and centrifuged at 152,000×g for 16 hr at 4℃.

Isolation of Membrane-bound DNA for Hybridization

Isolation of membrane-bound DNA was performed as described by Gustafsson (3), with a slight modification. Cells were grown in L-broth and harvested in mid-logarithmic growth phase. Outer and cytoplasmic membranes were separated from these cells as described above. The membrane fractions were collected into small tubes in 0.6 ml aliquots using a peristaltic pump and the absorbance of each fraction was measured by Lowry method (7). The aliquots were then pooled into three different groups (bottom, middle, and top) and dialysed overnight at 4℃. They were concentrated with polyethylene glycol 20,000 until the volume reached 0.3~0.5 ml, and treated with RNase for 30 min at 37℃. The aliquots were then added with 0.1% sodium dodecyl sulfate, and heated for 5 min at 65℃. Sodium chloride was added to give the aliquots the final concentration of 1 M, and the aliquots were allowed to stand for 30 min at 0℃. The aliquots were centrifuged for 5 min. Ethanol was then added to the supernatant and the DNA was precipitated overnight at −20℃. The DNA was washed once with ethanol and the pellet was dried. The resulting pellet was dissolved in 20 μl of TEN buffer and used for hybridization.

Southern Hybridization

After agarose gel electrophoresis of the isolated membrane-bound plasmid DNA, the gel was transferred to a nitrocellulose filter according to the method of Southern (15). The plasmid pBR322 probe was labelled using a nick translation kit from Amersham.

RESULTS AND DISCUSSION

Localization of SopA Product in Cell, and Fractionation by Ammonium Sulfate Precipitation

The lysate prepared from the cells bearing pXX288 was fractionated into cytoplasm and crude membrane by differential centrifugation (Fig. 1). Most of the sopA protein was observed in the cytoplasm fraction. It was also shown by SDS-polyacrylamide gel electrophoresis that the sopA protein can be precipitated by adding 30~60% ammonium sulfate to the cytoplasm. The results are presented in Fig. 2.

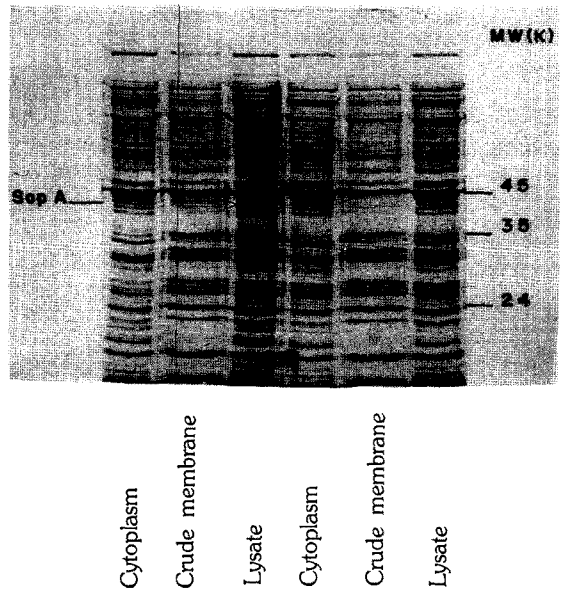
Localization of SopB Product in Cell and Extraction from the Membrane

As shown in Fig. 3, sopB protein was exclusively recovered in the crude membrane fraction. The sopB protein was effectively extracted from the membrane with 2 M urea but a significant amount of the protein was not solubilized even after treatment of the membrane with 4 M urea (Fig. 4). It has been reported that the proteins of cytoplasmic membrane in the crude membrane fraction can be specifically solubilized by 2% Triton X-100 with Mg^{++} (14) or 0.5% Sarkosyl (2). The sopB protein was extracted considerably by TritonX-100 and completely by Sarkosyl from the crude membrane (Fig. 5).

Effect of Culture Media on the Isolation of SopB Protein

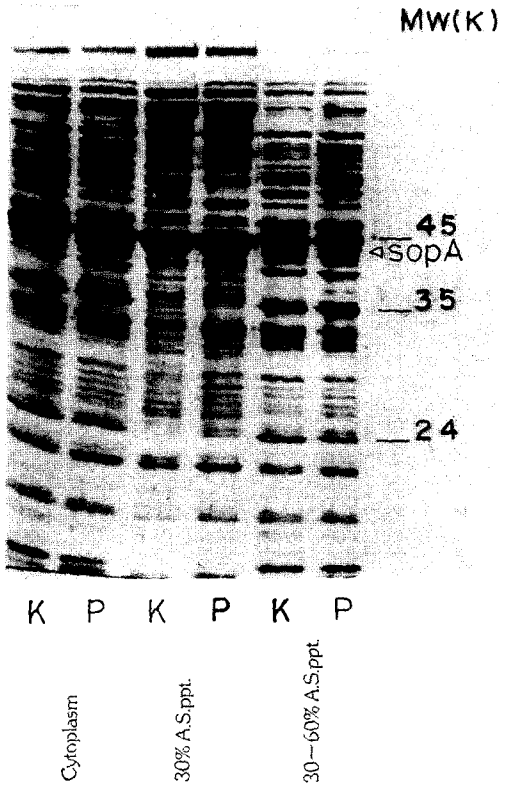
The crude membrane prepared from cells with or without plasmid were fractionated into outer and inner membranes by sucrose density gradient centrifugation.

The sedimentation profile of the crude membrane fraction showed a little difference according to the culture media used, and the sopB protein existed in all fractions



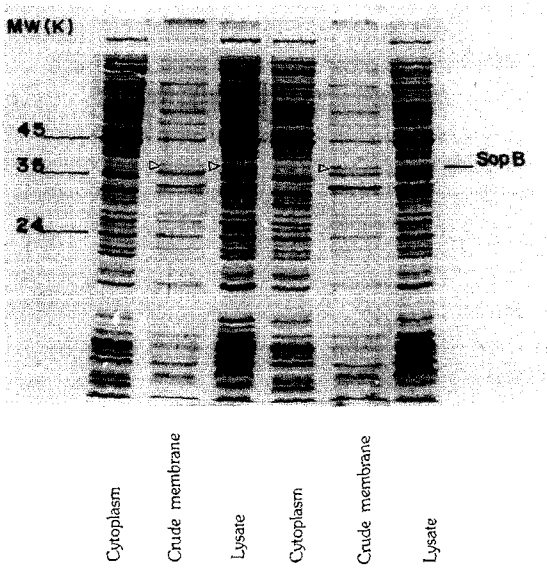
E. coli KY7231(pXX288), *E. coli* CSR603

Fig. 1. Localization of sopA protein.



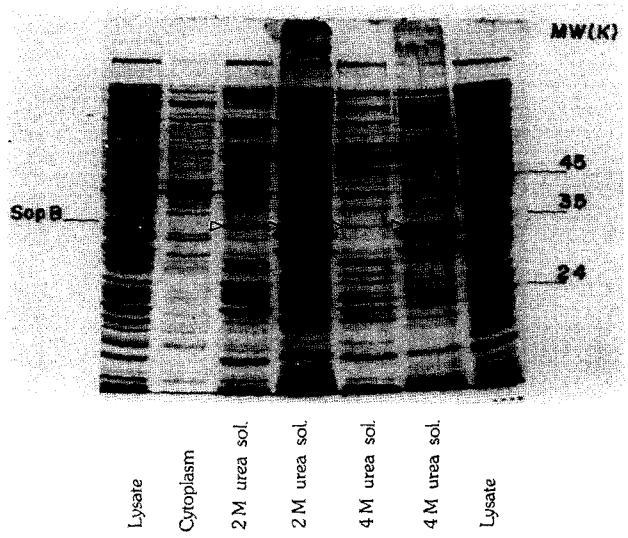
K: *E. coli* KY7231, P: *E. coli* KY7231 (pXX288).

Fig. 2. Precipitation of sopA protein by ammonium sulfate.



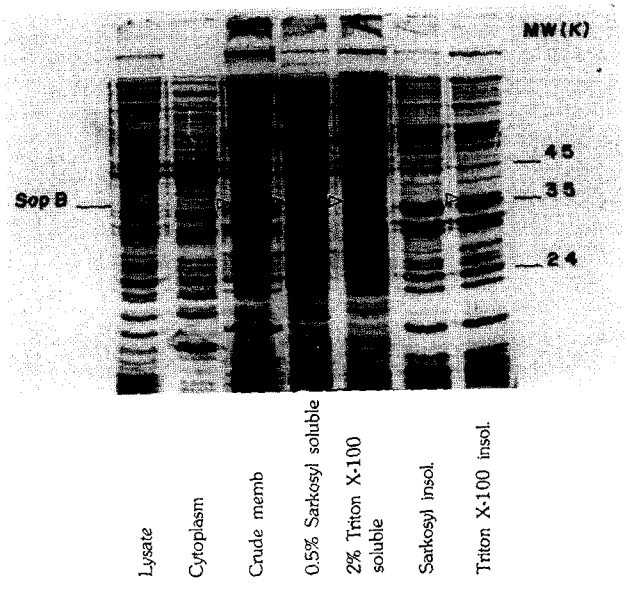
E. coli KY7231(pXX157). *E. coli*(pXX157)

Fig. 3. Localization of sopB protein.



E. coli CSR603(pXX157), *E. coli* KY7231(pXX167)

Fig. 4. Extraction of sopB protein with urea from the crude membrane.



E. coli CSR603(pXX157)

Fig. 5. Solubilization of sopB protein by Triton X-100 and Sarkosyl from the crude membrane fraction.

of the inner membrane (Fig. 6). These results were consistent with the results from urea analysis, which suggested that sopB protein may be tightly bound to the cell membrane.

Interaction of Plasmid DNA with the Cell Membrane of Host Bacteria in Partitioning

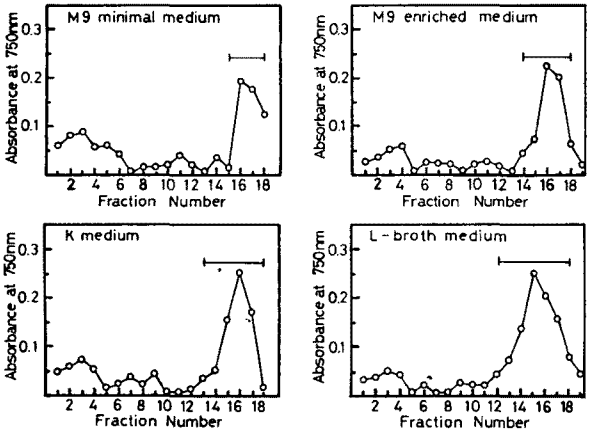


Fig. 6. Effect of culture media on isolation of the sopB protein.
*Bars indicate the fractions containing the sopB protein.

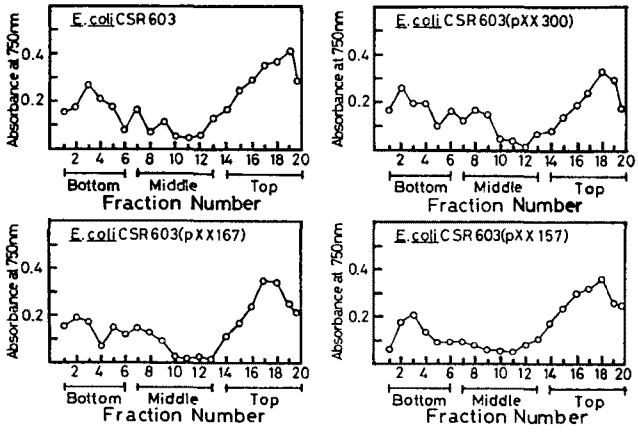


Fig. 7. Distribution of crude membrane fractions on sucrose density gradient centrifugation for isolating the membrane-bound DNA.

The membrane fractions obtained by differential centrifugation (Fig. 7) were treated as described in Materials & Methods, and the isolated membrane-bound DNA was hybridized with radioactive labelled probe DNA.

As shown in Fig. 8, our present finding that a plasmid DNA cosedimented with inner membrane, corresponds to the results of Archibold *et al* (1) in which they suggested a binding between plasmid DNA and inner membrane. However, since there was no difference among the plasmids with (pXX167) or without (pXX157, pXX 300) *par* region, the interaction will not be specific enough for stable partitioning. Those plasmids recovered in the membrane fraction may be on the way of replication, or the interaction may contribute to the random partitioning of the plasmids. Specific binding between

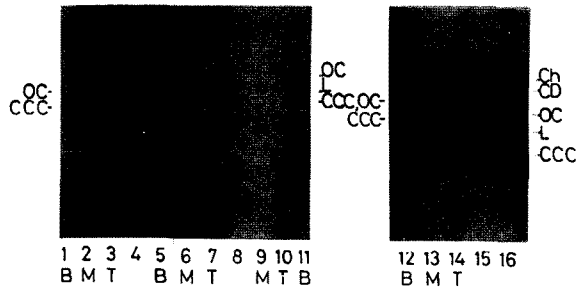


Fig. 8. Southern hydridization analysis of isolated membrane-bound DNA.
1,2,3: *E. coli* CSR603, 5,6,7: *E. coli* CSR603 (pXX300), 9,10,11: *E. coli* CSR603 (pXX167), 12,13,14: *E. coli* CSR603 (pXX157), 16: Probe DNA (pBR322), 4,8,15: No loading.
*Ch: Chromosomal DNA, CCC: Covalently closed circular DNA, OC: Open circular DNA, CD: CCC dimers, L: Linear DNA.
*B: Bottom, M: Middle, T: Top.

the plasmid with *par* region and outer membrane has been reported by Gustafsson *et al* (3).

Meanwhile, Ogura and Hiraga (13) postulated that the *sop*C region on the plasmid molecule seems to interact with the cellular component consisting of the *sop*B protein (37K) and at least two host proteins (75 K and 33 K). The *sop*B protein may act to associate plasmid DNA molecules with the cellular components through its binding to the *sop*C region, or alternatively, the *sop*B protein may be a member of the active cellular component, and only the component including the sopB protein may be able to interact with the *sop*C region. They also suggested that the *sop*A product may also interact with the *sop*C region or indirectly with the cellular component associated with the *sop*C region, or the *sop*A gene product may regulate the expression of the *sop*B gene. However, the function of *sop*A, *sop*B and *sop*C of F plasmid, in relation to the membrane, remains yet to be elucidated.

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