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Interaction between F Plasmid Partition Proteins SopA and SopB

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Two different yeast two hybrid systems were used to examine interaction between the partition proteins SopA and SopB of F plasmid as well as their self association. In one system, the yeast Gal4 protein DNA binding domain (Gal4-BD) is fused to the N-terminus of the bait protein, and the Gal4 activation domain (Gal4-AD) is fused to the N-terminus of the target protein (1). In the other system, the target hybrid remains unchanged but *E. coli* LexA protein (LexA) is fused to the C-terminus of the bait protein (2). It is found that C-terminus part of SopB is involved in interaction with itself, as an N-terminal truncation of SopB, SopB-(120–323) remains capable of self association. For interaction between SopA, deletion of the N-terminal part weakens but does not abolish the interaction. Interaction between SopB and SopA protein was also detected, but only by the use of the second system. Full length SopB [SopB-(1–323)] or SopB-(1–180) lacking the C-terminal region beyond amino acid 180 can interact with full-length SopA-(1–383) protein.

Low copy number plasmids in bacteria have their own partition system which ensure their equipartition into host cells undergoing division (reviewed in 3–4). The partition system of the *Escherichia coli* F plasmid includes two trans-acting genes *sopA* and *sopB* and a cis-acting site *sopC*. SopA is a 388-amino acid protein with a DNA-dependent ATPase activity. It is known to bind to four repeated sequence elements in the regulatory region of the *sopAB* operons and acts as an autorepressor (5–7). SopB is a 323-amino acid protein that binds specifically to *sopC*, a DNA segment comprised of 12 tandem repeats of 43 base pairs (bp) each. The SopB protein recognizes 7-bp inverted repeats in each of the 43-bp motif. SopB binds to *sopC* as a dimer (6, 8), and the SopB-*sopC* complex has been suggested to serve a centromere-like function through interaction with chromosomally encoded proteins that remain to be identified. The SopA protein is also involved in this centromere-like nucleoprotein structure (6). SopB appears to have a bipartite structure: the C-terminal half constitutes the DNA-binding part and the N-terminal half the part important for the silencing of the genes proximal to *sopC* in cells overexpressing SopB (8–9), and probably plays a role in partition as well (8). Recently, SopB protein fused to a green fluorescent protein (GFP) was localized to the quarter-cell positions, and this localization also requires the N-terminal part of SopB. Deletion of the N-terminal 71 amino acids abolished this specific localization (10).

Despite recent interests in various partition systems (11–13), there has been little information on interactions between SopA and SopB. In the present work, we used the yeast two-hybrid system (1–2) to examine self-association of SopA and SopB as well as interaction between the two. Two different two-hybrid systems were used, and the results also illustrate the importance of avoiding fusion constructs that may mask protein-protein interactions of interest.

Abbreviations used: BD, binding domain; AD, activation domain; bp, base pair; GFP, green fluorescent protein; PCR, polymerase chain reaction; SC, synthetic complete; 3-AT, 3-aminotriazole; β-gal, β-galactosidase; ONPG, o-nitrophenyl β-D-galactopyranoside.

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MATERIALS AND METHODS

Materials. *Escherichia coli* K-12 DH5α was used for plasmid propagation. Yeast HF7c [*MATa, ura3-52, his3-200, ade2-101, lys2-801, trp1-901, leu2-3, 112, gal4-542, gal80-538, LYS2::GAL1*$_{UAS}$-*GAL1*$_{TATA}$-*HIS3, URA3::GAL4*$_{17mer(x3)}$-*CyC1*$_{TATA}$-*lacZ*] (14) and L40 [*MATa, his3-200, trp1-910, leu2-3, 112, ade2, Lys2::*(4 *lexA*$_{op}$-*HIS3*), *URA3::*(8 *lexA*$_{op}$-*lacZ*) *GAL4*] (15) were used as the host strain for the yeast two hybrid screens. Plasmid pGBT9 and pGAD424 were purchased from CLONTECH. pFBL23 was kindly provided by J. Camonis, Institute Curie (Paris, France) (2). Oligodeoxynucleotide primers for polymerase chain reaction (PCR) were synthesized in an auto-

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mated nucleic acid synthesizer (PE Biosystems Model 394). Other reagents were purchased from commercial suppliers.

Plasmid construction. Full-length *sopA* and *sopB* open reading frames were generated by PCR and subcloned in-frame into the yeast vectors pGBT9 and pGAD424 (CLONTECH Matchmaker) to make Gal4-BD fused bait and Gal4-AD fused target protein, respectively. In another two hybrid screen, pFBL23 was used to fuse the DNA binding domain of LexA to the C-terminus of F plasmid partition proteins (SopA and SopB). Deletions of SopA and SopB codons were done by PCR using appropriate pairs of oligodeoxynucleotide primers, and these fragment were fused in-frame into pFBL23 and pGAD424. All subcloned PCR products were verified by sequencing.

Yeast transformation and two-hybrid assays. Yeast transformation was performed using the PEG/LiAcetate method as described (16). Yeast strain HF7c was transformed with pGBT9-SopA (or B) and pGAD424-SopA (or B). Yeast strain L40 was transformed with both pFBL23-derived plasmids and pGAD424-derived plasmids simultaneously. Transformed cells were plated on synthetic complete medium (SC) lacking tryptophan, leucine and histidine and containing 25 mM 3-aminotriazole (3-AT) (Sigma). Plates were incubated at 30° for 2–3 days. 3-AT was used to repress the basal activity of the His3 reporter gene, which otherwise results in nonspecific background growth in the absence of exogenous histidine (17). His^+ colonies were assayed for β-galactosidase (β-gal) activity by the filter-lift assay (18) and quantitative liquid culture assay using o-nitrophenyl β-D-galactopyranoside (ONPG) as a substrate (19).

RESULTS AND DISCUSSION

The difference between two different two-hybrid systems. To identify the interaction between SopA and SopB protein, we constructed 6 plasmids depicted in Figure 1. pSK200 and pSK201 are derivatives of pGAD424 in which codons for the Gal4 activation domain (AD) is fused to open reading frames encoding SopA and SopB protein, respectively. pSK300 and pSK301 were constructed to express proteins with the Gal4 DNA-binding domain fused to SopA and SopB, respectively. pSK400 and pSK401 are derivatives of pFBL23 in which the DNA binding domain of LexA was fused to the C-terminus of SopA and SopB, respectively, to generate bait proteins without fused polypeptides at their N-termini. After transformation with each pair of two plasmids, the His3 reporter activity was assayed by testing the growth of the transformants on SC medium lacking histidine and containing 3-AT (Figure 2B). LacZ reporter gene expression was monitored by the filter-lift assay and quantitative enzymatic assays of β-gal is summarized in Figure 2C.

Using the pGBT9-based Gal4 two-hybrid system (CLONTECH), no interaction between SopA and SopB protein was detectable. pFBL23-based LexA two-hybrid system, on the other hand, showed interactions between SopA and SopB protein as well as self-association of each protein. In case of interaction between SopA and SopB protein (L2 and L3 of Figure 2C), the SopB-LexA bait hybrid with unmodified SopB

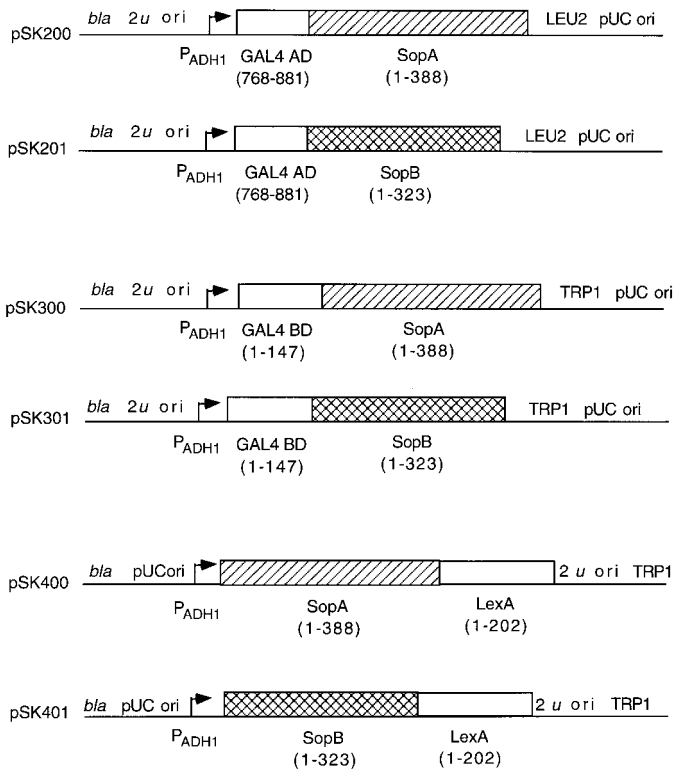
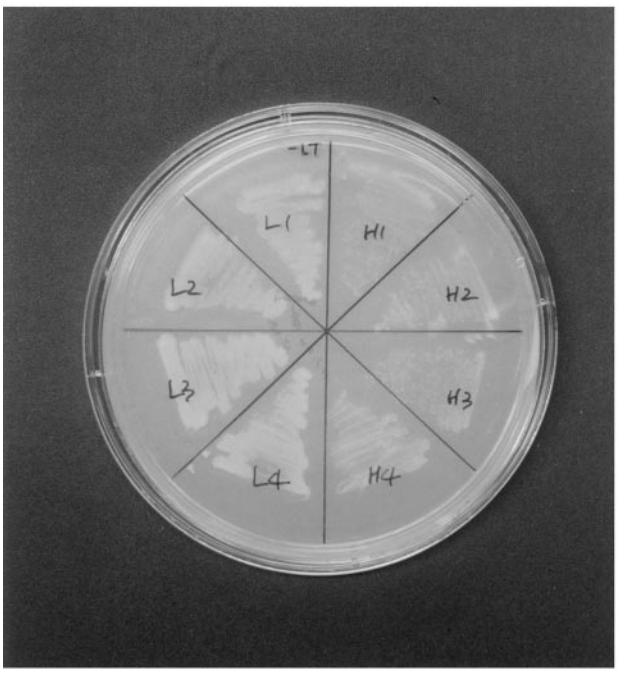


FIG. 1. Illustrations of full-length SopA and SopB protein fused to the binding domain (BD) or activation domain (AD). Shown are the genetic maps of the resulting plasmids. The plasmids are *Escherichia coli*-yeast shuttle vectors and the fusion genes are transcribed from the yeast ADH1 promoter.

N-terminus showed interaction with Gal4-AD-SopA target hybrid. Thus fusion of Gal4-BD to the N-terminus of SopB protein apparently masks the domain involved in interaction with SopA protein. The detection of SopA-SopB interaction by the use of the second yeast two-hybrid system is consistent with previous *in vivo* studies, which indicate that the expression of SopB enhances the repression of the SopAB operon by SopA (6, 20).

Domain mappings. To test which domains are important for various interactions, we constructed two sets of fusion proteins of SopA and SopB for two-hybrid assays. These fusion proteins are tabulated in the first two columns of Table 1. L40 was transformed with each pair of bait and target protein. Figure 3A and 3B illustrates the His3 and LacZ reporter gene activities of the transformants, and the results are summarized in the last two columns of Table 1. These results show that the C-terminal part of SopB protein is important SopB dimerization. Full-length SopB (1–323) protein does not form a heterodimer with SopB-(1–240). However, lacking the N-terminal domain of SopB, SopB-(120–323) can form a dimer with full-length SopB or

A



B

C

Yeast strain		Bait hybrids	Target hybrids	His3 gene	LacZ reporter (β-gal units)
L1	L40	pSK400 (SopA-LexA)	pSK200 (Gal4AD-SopA)	+++	10.6
L2	L40	pSK400 (SopA-LexA)	pSK201 (Gal4AD-SopB)	-	0.2
L3	L40	pSK401 (SopB-LexA)	pSK200 (Gal4AD-SopA)	+++	60.3
L4	L40	pSK401 (SopB-LexA)	pSK201 (Gal4AD-SopB)	+++	242.4
H1	HF7c	pSK300 (Gal4BD-SopA)	pSK200 (Gal4AD-SopA)	-	0.1
H2	HF7c	pSK300 (Gal4BD-SopA)	pSK201 (Gal4AD-SopB)	-	0.2
H3	HF7c	pSK301 (Gal4BD-SopB)	pSK200 (Gal4AD-SopA)	-	0.1
H4	HF7c	pSK301 (Gal4BD-SopB)	pSK201 (Gal4AD-SopB)	-	0.3

FIG. 2. Yeast two-hybrid assays using twodifferent systems. (A) Growth of yeasts L40 (L1–L4) and HF7c (H1–H4) on SC medium lacking leucine and tryptophan. (B) Growth of yeasts on SC medium lacking leucine, tryptophan, and histidine and containing 25 mM 3-AT. (C) A summary of the relative growth and β-gal activity. Growth on SC plates was scored as follows: +++, strong growth; −, no growth. The β-gal activity was quantified enzymatically (see Materials and Methods). The enzyme activities are the average of three independent yeast colonies.

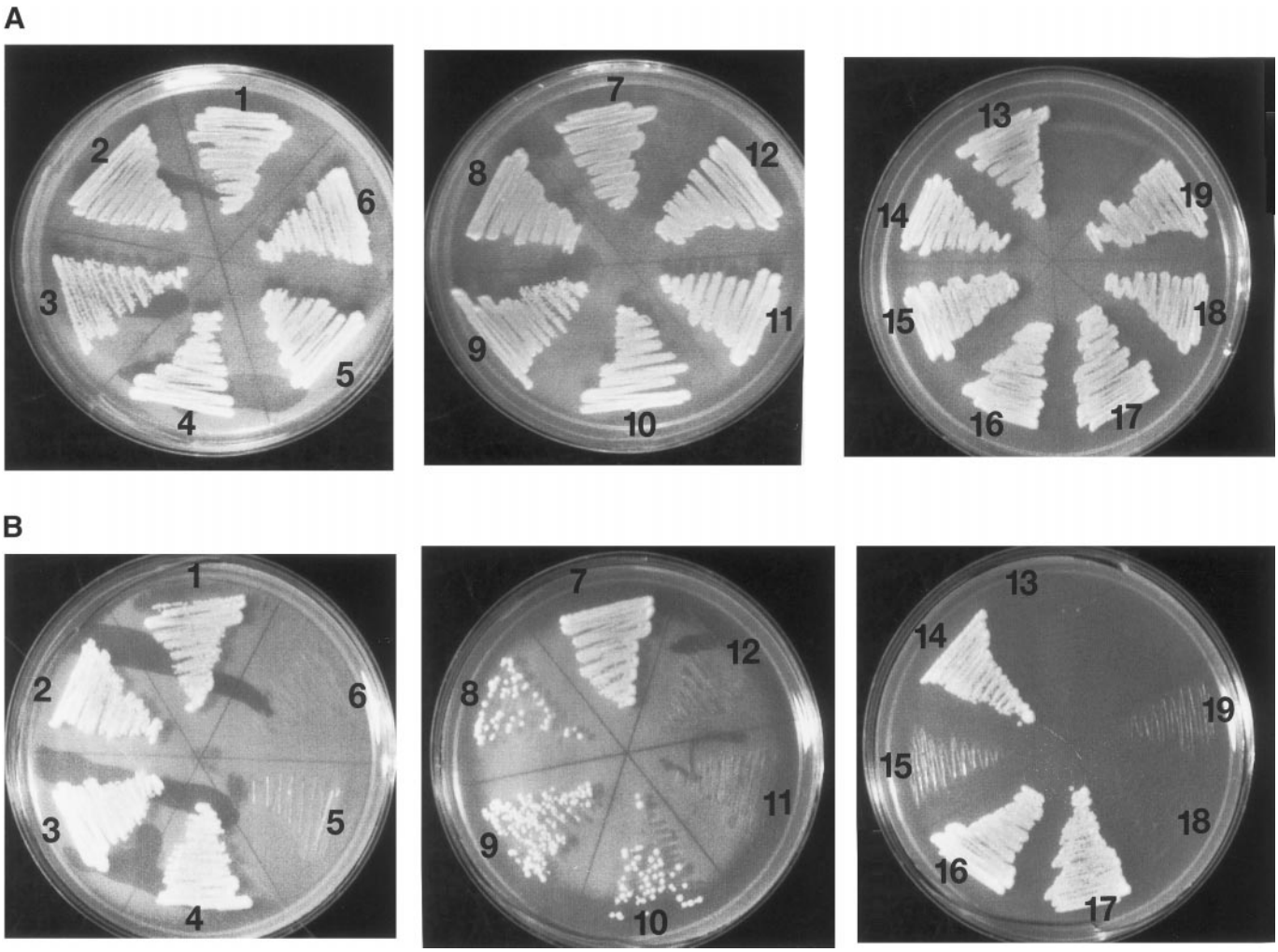


FIG. 3. Examples of the growth yeast cells harboring various pairs of plasmids. (A) Growth of L40 transformants on SC media lacking leucine and tryptophan. (B) Growth of L40 yeasts on SC media lacking leucine, tryptophan, and histidine and containing 25 mM 3-AT.

with itself. Moreover, SopB-(80–323) dimerization appears to be more prominent than full-length SopB protein dimerization. It has been previously observed that the yeast two hybrid generally works better when fragments rather than full-length large protein were used as baits and targets (21–22).

Self-association of SopA protein is much weaker (by about 20-fold) than SopB protein dimerization. This association does not occur without the C-terminal 88 amino acids (301–388) of SopA protein. SopA protein lacking 99 N-terminal residues also shows weak association with each other. In previous studies of the partition system of plasmid R1, interaction between the molecules of the ParM protein a homologue of SopA, was not observed (23). This weak but discernible interaction between SopA molecules reported here suggests that ParM is also likely to associate with itself. Interaction between SopA and SopB protein is evident when SopB-LexA bait hybrids were used, in which the N-terminal domain of SopB is not masked. Full-length SopB or SopB-(1–180) lacking the C-terminal region beyond amino acid 180, can interact with full-length SopA protein. It appears that SopB protein has a bipartite structure in its interaction with SopA and SopB: the N-terminal half of the SopB protein (1–180) has a domain which can interact with SopA protein, and the C-terminal half of the SopB-(120–323) is important for SopB dimerization. Finally, the results reported here underscores the importance of use appropriate fusion protein constructs in the use of the yeast two hybrid screens. Otherwise an important domain required for interaction may be masked.

TABLE 1

A Summary of Results for Various Truncation Derivatives of SopA and SopB

Bait hybrids	Target hybrids	His3 gene	LacZ reporter (β-gal units)
1. SopB-(1–323) BD	AD SopB-(1–323)	+++	242.4
2. SopB-(120–323) BD	AD SopB-(1–323)	+++	112.3
3. SopB-(80–323) BD	AD SopB-(80–323)	+++	290.2
4. SopB-(120–323) BD	AD SopB-(120–323)	+++	22.7
5. SopB-(1–323) BD	AD SopB-(1–240)	−	0.3
6. SopB-(1–240) BD	AD SopB-(1–323)	−	0.1
7. SopA-(1–388) BD	AD SopA-(1–388)	+++	10.6
8. SopA-(100–388) BD	AD SopA-(1–388)	+	2.1
9. SopA-(1–388) BD	AD SopA-(100–388)	+	2.4
10. SopA-(100–388) BD	AD SopA-(100–388)	+	1.7
11. SopA-(1–300) BD	AD SopA-(1–388)	−	0.1
12. SopA-(1–388) BD	AD SopA-(1–300)	−	0.2
13. SopA-(1–388) BD	AD SopB-(1–323)	−	0.2
14. SopB-(1–323) BD	AD SopA-(1–388)	+++	60.3
15. SopB-(80–323) BD	AD SopA-(1–388)	−	0.3
16. SopB-(1–240) BD	AD SopA-(1–388)	+++	6.6
17. SopB-(1–180) BD	AD SopA-(1–388)	+++	6.1
18. SopB-(1–323) BD	AD SopA-(100–388)	−	0.1
19. SopB-(1–323) BD	AD SopA-(1–300)	−	0.3

Note. Growth on SC plates was scored as follows: +++, strong growth; +, poor growth, −, no growth. The β-gal activity was quantified enzymatically (see Materials and Methods). The enzyme activities are the average of three independent yeast colonies.

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