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NOTE

Use of the Yeast 1.5-Hybrid System to Detect DNA-Protein-Protein Interactions

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***Escherichia coli* F plasmid partition apparatus is composed of two trans-acting proteins (SopA and SopB) and one cis-acting DNA sequence (*sopC*). The SopB-*sopC* complex has been suggested to serve a centromere-like function through its interaction with chromosomally encoded proteins which remain to be identified. In this paper, we are introducing a new yeast 1.5-hybrid system which assembles the two-hybrid and one-hybrid system as a mean to find an additional component of the F plasmid partition system, interacting with DNA (*sopC*)-bound SopB protein. The result indicates that this system is a promising one, capable of selecting an interacting component.**

Key words: DNA-protein interaction, F plasmid partition apparatus, yeast 1.5-hybrid system

Many biological processes rely upon macromolecular interactions. Networks of physically interacting factors orchestrate complex patterns of gene expressions that control cell growth and differentiation. Traditionally, the tools available to identify and characterize molecular interactions have been limited to biochemistry. However, a genetic method, the two-hybrid system (1), has recently emerged as a powerful method to identify protein-protein interactions. Two interacting proteins are expressed in yeast as hybrids fused to a DNA-binding domain (BD) or an activation domain (AD). The interaction between the BD and AD reconstitutes a functional transcriptional factor that activates a reporter gene driven by a promoter containing BD recognition sites.

The one-hybrid system allows the identification of DNA-binding proteins (DBP). When the DBP is expressed as a hybrid with an AD, the interaction between DBP fused with AD and its binding site activates reporter genes. In fact ORC6, a component of the yeast replication origin recognition complex, was isolated by this system (6).

Low copy number plasmids in bacteria such as F and P1 have their own partition systems which ensure their equipartition in the host cells undergoing division (15, 18). The partition system of the *Escherichia coli* F plasmid includes two trans-acting genes, *sopA* and *sopB,* and a cis-acting site *sopC* (Fig. 1) (9, 12). SopA is a 388-amino acid protein with DNA-dependent ATPase activity and is known to bind to four repeated sequences in the regulatory region of the *sopAB* operon for which it acts as an autorepressor (17). Recently, the *sopC* was required for full repression of the *sop* operon as well as SopA (19). SopB is a 323-amino acid protein that binds specifically to *sopC*, a DNA segment comprised of 12 tandem repeats of 43 base pair (13, 16). The SopB-*sopC* complex has been suggested to serve a centromere-like function through its interaction with chromosomally encoded proteins which remain to be identified. SopA is also involved in this centromere-like nucleoprotein structure (5, 14). SopB appears to have a bipartite structure: the amino-terminal half constitutes the DNA-binding part and the carboxy-

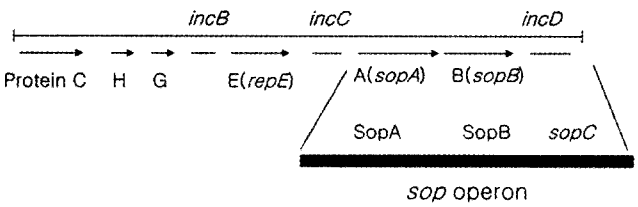

Fig. 1. The *sop* operon region of *Escherichia coli* F plasmid. The genetic map of the *sopA* and *sopB* genes and the *cis*-acting *sopC/incD* region. The arrows indicate 12 tandem direct repeats of the 43 bp in the *sopC* region. *inc,* incompatibility; Protein H and G, *ccdA* and *ccdB* (coupled cell division); Protein C, replication protein (*oriV*); Protein E, replication protein (*oriS*).

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terminal half is important in silencing of the genes proximal to *sopC* in cells overexpressing SopB and may play a role in partition as well (3, 10).

In this paper, we demonstrated the utility of the yeast 1.5-hybrid system which assembles the two-hybrid and one-hybrid system to find the interacting component of the nucleoprotein complexes. Our test case here was the *E. coli* F plasmid partition system.

Construction of the reporter strain and plasmids

In order to construct the yeast reporter strain, plasmid pRY171-*sopC* was generated from pRY171 (which was

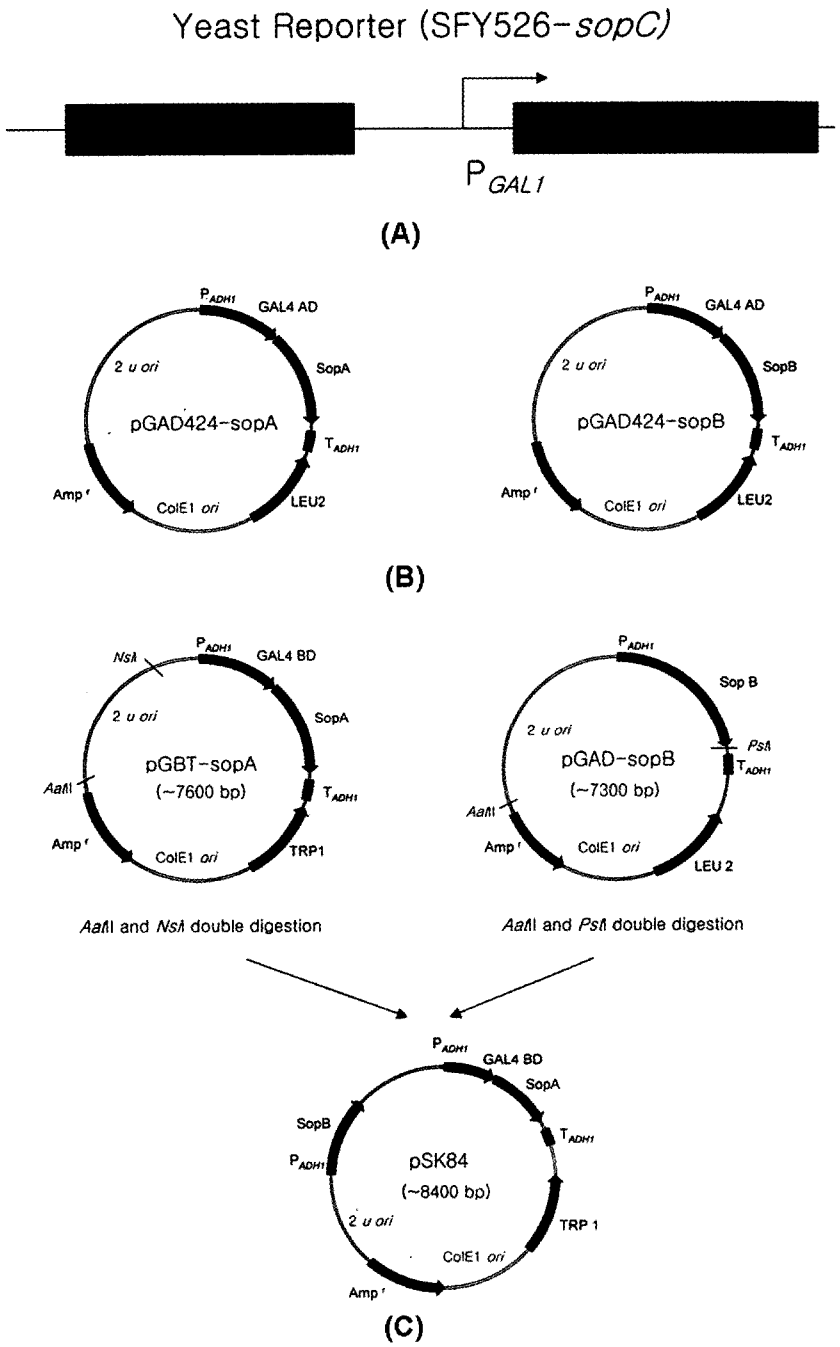

Fig. 2. Construction of yeast SFY526-*sopC* reporter strain and hybrid plasmids. (A) The genetic organization of the yeast SFY526-*sopC* strain. The *sopC* locus is located in the upstream of the *lacZ* gene which is driven by the *GAL1* promoter. (B) The genetic map of the plasmids pGAD424-sopA and pGAD424-sopB. (C) Construction of plasmid pSK84. The abbreviations are : Amp^r, ampicillin resistance gene; ColE1 *ori*, origin of replication for *E. coli*; 2 *ori*, origin of replication for yeast; *TRP1*, yeast tryptophan synthetic gene; P_{ADH1}, truncated *ADH1* promoter; T_{ADH1}, transcriptional terminator.

kindly provided by M. Ptashne, (20)) by replacing four upstream activation sequences $(UAS)_4$ with the *sopC* sequences. The resulting vector has a unique *Stu* I site for targeted integration of the plasmid pRY171-*sopC* at the *URA3* locus of *Saccharomyces cerevisiae* SFY526 [*MATa*, *ura*3-52, *his*3-200, *ade*2-101. *lys*2-801. *trp*1-901, *leu*2-3, 112, *can*^{r}, *gal*4-542, *gal*80-538, URA3::$GAL1_{UAS}$-$GAL1_{TATA}$-*lacZ*] (4). The diagram of the yeast SFY526-*sopC* is shown in Fig. 2(A). The integration of *sopC* sites into the SFY526 was identified by Southern hybridization (data not shown) using *sopC* DNA fragment as a probe.

Full-length *sopA* and *sopB* gene fragments were generated by the polymerase chain reaction (PCR) and were subcloned in-frame into the yeast vector pGAD424 (purchased from Clontech) to produce GAL4-AD fused target proteins. The resulting plasmids, pGAD424-sopA and pGAD424-sopB, were used (Fig. 2(B)) as AD plasmids.

To construct the expression vector which expresses SopA and SopB proteins simultaneously, the *Aat*II-*Nsi*I (5,476 bp) fragment of pGBT-sopA and the *Aat*II-*Pst*I (2,890 bp) fragment of pGAD-sopB were ligated. This recombinant plasmid was designated as pSK84 and the scheme of its construction was shown in Fig. 2(C).

Verification of the yeast 1.5-hybrid system

Yeast transformation was performed using the PEG/Li-acetate method as previously described (2). The transformants were plated on synthetic complete (SC) medium lacking tryptophan and leucine, and containing 5-bromo-4-chlolo-3-indolyl-β-D-galactopyranoside (X-gal, 80 μg/ml) and 1 mM of isopropyl 1-thio-β-D-galactopyranoside (IPTG). Plates were incubated at 30°C for 2 to 3 days. Blue colonies were assayed for β-galactosidase (β-gal) activity by quantitative liquid culture using o-nitrophenyl β-D-galactopyranoside (ONPG) as a substrate (11).

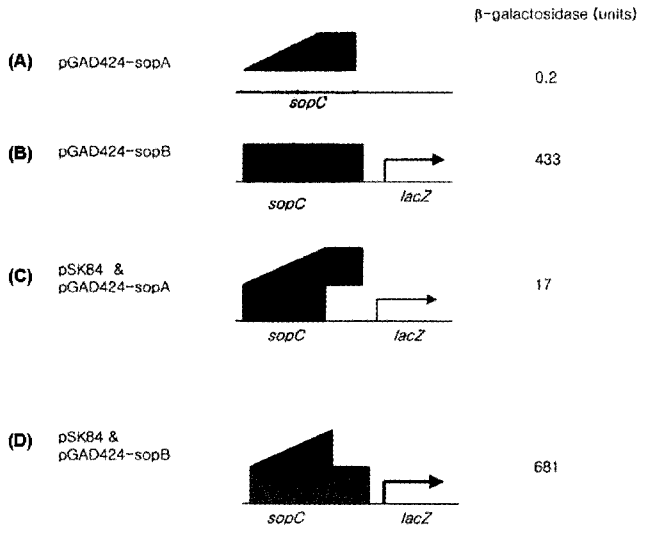

Fig. 3. Yeast 1.5-hybrid assays using several plasmids : (A) pGAD424-sopA, (B) pGAD424-sopB, (C) pSK84 and pGAD424-sopA, (D) pSK94 and pGAD424-sopB. The b-gal activity was quantified enzymatically (see in the text). The enzyme activities are the average of independent measurements of β-gal activity from three yeast colonies.

To confirm whether the yeast 1.5-hybrid system functions, yeast SFY526-*sopC* was transformed with pGAD424-sopA and pGAD424-sopB, respectively. The results are shown in Fig. 3 (A and B). Because SopB-AD (SopB fused with AD) was able to bind to the *sopC* site specifically, it showed about 433 units of β-galactosidase (β-gal) activity, which meant it could be a transcriptional activator by itself. On the other hand, as SopA-AD was not able to bind to the *sopC* site directly, it showed only a basal level of β-gal activity (0.2 units). When pSK84 was transformed alone, the β-gal activity was about 0.1 units.

Meanwhile when pGAD424-sopA and pSK84 were transformed simultaneously, the activity was 17 units (Fig. 3(C)). This was about 80 times higher than the pGAD424-sopA alone suggesting that SopA maybe involved in the SopB-*sopC* complex in certain ways. When pGAD424-sopB and pSK84 were transformed simultaneously (Fig. 3 (D)), the β-gal activity was the highest, 50% higher than being transformed with pGAD424-sopB alone (681 units). The result implies that SopA may support SopB-SopB dimerization activity or the formation of the SopB-*sopC* partition complex. It may also imply that SopA may be involved in the complex directly or indirectly.

Screening of an AD-library using yeast 1.5-hybrid system

In order to find additional proteins which might be involved in the F plasmid partition apparatus, we screened the *E. coli* Matchmaker genomic library (purchased from Clontech) using this newly designed yeast 1.5-hybrid system. First, yeast SFY526-*sopC* was transformed with a pSK84 plasmid. Then, SFY526-*sopC* (pSK84) was transformed further with the library. A candidate yeast transformant which showed a blue color was identified through this screening. The β-gal activity of this candidate by quantitative liquid culture using ONPG as a substrate was estimated to be about 270 units. From this selected clone, plasmids were extracted. We transformed these plasmids into *E. coli* KC-8 to specifically distinguish the AD-plasmid. We then extracted the AD-plasmid from *E. coli* KC-8, and then sequenced it. The sequencing result showed that this candidate plasmid contained the amino-terminal part of SopB (12-119 amino acids). However, this result failed to reveal a new component of the F plasmid partition system which was yet to be identified. This result reaffirmed the fact that the amino-terminal part of SopB was responsible for the SopB dimerization and the partition of the F plasmid which was already known (7, 8) One of the reasons that we failed to find the other component may be that the library we used was not big enough to have a chromosomally encoded partition protein.

There are many intracellular complexes other than the F

plasmid partition system, which involve interactions between DNA and multitle proteins. Thus, we expect that the yeast 1.5-hybrid system works as a search engine to find either the binding component or the degradation factor of any nucleoprotein complexes.

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References

1. Fields, S. and O. Song. 1989. A novel genetic system to detect protein-protein interaction *Nature* (London) 340, 245-246.
2. Gietz, D., A. Jean, R.A. Woods, and R. H. Schiesh. 1992. Improved method for high efficiency transformation of intact yeast cells. *Nucleic Acids Res.* 20, 1425.
3. Hanai, R., R. Liu, P. Benedetti, P.R. Caron, A.S. Lynch, and J.C. Wang. 1996. Molecular dissection of a protein SopB essential for *Escherichia coli* F plasmid partition. *J. Biol. Chem.* 271, 17469-17475.
4. Harper, J.W., G.R. Adami, N. Wei, K. Keyomarsi, and S.J. Elledge. 1993. The p21 Cdk-interacting protein Cip 1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* 75, 805-816.
5. Hirano, M., M. Mori, T. Onogi, M. Yamazoe, H. Niki, T. Ogura, and S. Hiraga. 1998. Autoregulation of the partition genes of the mini-F plasmid and the intracellular localization of their products in *Escherichia coli*. *Mol. Gen. Genet.* 257, 392-403.
6. Joachim, J.L. and H. Ira. 1993. Isolation of ORC6, a component of the yeast origin recognition complex by one-hybrid system. *Science* 262, 1870-1873.
7. Kim, S-K. and J.C. Wang. 1998. Localization of F plasmid SopB protein to positions near the poles of *Escherichia coli* cells. *Proc. Natl. Acad. Sci. USA*. 95, 1523-1527.
8. Kim, S-K. and J. Shim. 1999. Interaction between F plasmid partition proteins SopA and SopB. *Biochem. Biophys. Res. Commun.* 263, 113-117.
9. Kusugawa, N., H. Mori, A. Kondo, and S. Hiraga. 1987. Partitioning of the F plasmid: overproduction of an essential protein for partition inhibits plasmid maintenance. *Mol. Gen. Genet.* 208, 365-372.
10. Lynch, A.S. and J.C. Wang. 1995. SopB protein-mediated silencing of genes linked to the *sopC* locus of *Escherichia coli* F plasmid. *Proc. Natl. Acad. Sci. USA*. 92, 1896-1900.
11. Miller, J.H. 1972. Experiments in Molecular Genetics. *Cold Spring Harbor Lab.*, Cold Spring Harbor, NY.
12. Mori, H., A. Kondo, A. Ohshima, T. Ogura, and S. Hiraga. 1986. Structure and function of the F plasmid genes essential for partitioning. *J. Mol. Biol.* 192, 1-15.
13. Mori, H., Y. Mori, C. Ichinose, H. Niki, T. Ogura, A. Kato, and S. Hiraga. 1989. Purification and characterization of SopA and SopB proteins essential for F plasmid partitioning. *J. Biol. Chem.* 264, 15535-15541.
14. Niki, H. and S. Hiraga. 1999. Subcellular localization of plasmids containing the *oriC* region of the *Escherichia coli* chromosome with or without the *sopABC* partitioning. *Mol. Microbiol.* 34, 498-503.
15. Ogura, T. and S. Hiraga. 1983. Partition mechanism of F plasmid: two plasmid gene-encoded products and cis-acting region are involved in partition. *Cell* 32, 351-360.
16. Watanabe, E., S. Inamoto, M.H. Lee, S.U. Kim, T. Ogua, H. Mori, and S. Hiraga. 1989. Purification and characterization of the *sopB* gene product which is responsible for stable maintenance of mini-F plasmid. *Mol. Gen. Genet.* 218, 431-436.
17. Watanabe, E., M. Wachi, M. Yamasaki, and K. Nagai. 1992. ATPase activity of SopA, a protein essential for active partitioning of F plasmid. *Mol. Gen. Genet.* 234, 346-352.
18. Williams, D.R. and C.M. Thomas. 1992. Active partitioning of bacterial plasmids. *J. Gen. Microbiol.* 138, 1-16.
19. Yates, P., D. Lane, and D.P. Biek. 1999. The F plasmid centromere, *sopC*, is required for full repression of the *sop* operon. *J. Mol. Biol.* 290, 627-638.
20. Yocum, R.R., S. Hanley, R. Jr. West, and M. Ptashne. 1984. Use of *lacZ* fusions to delimit regulatory elements of the inducible divergent GAL1-GAL10 promoter in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 4, 1985-1998.