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A Gene Encoding an SOS Inhibitor Is Present in Different Conjugative Plasmids

EFIM GOLUB,[1]† ADRIANA BAILONE,[2] AND RAYMOND DEVORET[2]*

Radiobiology Laboratories, Yale University School of Medicine, New Haven, Connecticut 06510,[1] and G. E. M. C., Enzymologie, Centre National de la Recherche Scientifique, F-91190 Gif-sur-Yvette, France[2]

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In 9 of 20 conjugative plasmids of different incompatibility groups, including F and R100 (or R6-5), coexist two sequences which are homologous, respectively, to the gene *psiB*, which encodes an inhibitor of SOS induction, and to the gene *ssb*, which encodes a single-stranded-DNA-binding protein.

Damage to the chromosomes of procaryotes such as *Escherichia coli* induces cellular SOS functions (26, 32). A novel plasmid function preventing SOS induction, called Psi (for plasmid SOS inhibition), has been found recently in plasmid R6-5 (2). The inhibition of SOS induction is caused by a 12-kilodalton polypeptide expressed by the gene *psiB* (A. Bailone, A. Bäckman, S. Sommer, J. Celerier, M. M. Bagdasarian, M. Bagdasarian, and R. Devoret, Mol. Gen. Genet., in press). The role of the *psiB* gene has not yet been elucidated. Nevertheless, we reasoned that if *psiB* was essential to the transfer of conjugative plasmids it would be found in conjugative plasmids other than R6-5.

The *psiB* gene has been located in the leading region of plasmid R6-5 near *oriT*, the origin of conjugal transfer (2; Bailone et al., in press). Although there is extensive homology between the leading region of plasmid R6-5 and that of F (14, 28), there was no SOS inhibition promoted by the F sex factor (1). We demonstrate here the presence of a *psiB* gene in the F sex factor and in other conjugative plasmids. SOS inhibition by the F sex factor *psiB* gene requires gene amplification.

Bacteria, plasmids, and measurement of SOS induction. SOS induction was monitored with two assays, phage λ induction and *sfiA* induction, by using two *recA441* derivatives of *E. coli* K-12: GY7429 (*sfiB114* [λ *cI ind1 sfiA*::*lacZ*$^+$]) (Bailone et al., in press) and GY3067(λ) (3). Induction of phage λ was monitored by cell killing at 42°C. Induction of *sfiA* fused to *lacZ* (20) was measured on the basis of β-galactosidase activity (29). GY7429 and GY3067 were grown in YM9 medium plus Casamino Acids (plus uracil for GY3067) to a density of 10^8 cells per ml at 32°C. The *sfiA* gene and phage λ were induced by shifting the cultures to adenine-containing (100 μg/ml) YM9 medium plus Casamino Acids at 42°C (4). Plasmids pKL3, pEG153, pEG302, and pEG303 and other plasmids used were described before (13, 21) (Table 1 and Fig. 1). Plasmid pMMB182 carries the R6-5 *psiB* gene (M. Dutreix, A. Bäckman, J. Célérier, M. M. Bagdasarian, S. Sommer, A. Bailone, R. Devoret, and M. Bagdasarian, submitted for publication).

Hybridization techniques. The 440-base-pair *Hin*dIII-*Mnl*I fragment of plasmid pMMB182 was used as a *psiB* probe in Southern hybridization experiments. The fragment covers most of *psiB*, starting 20 base pairs before the ATG codon and ending 13 base pairs before the termination codon (Dutreix et al., in preparation). The fragment was labeled with [α-^{32}P]dCTP (3,000 Ci/ml, NEG-013H; Dupont, NEN Research Products, Boston, Mass.) by using the Random Primed DNA Labeling Kit (no. 1004760; Boehringer GmbH, Mannheim, Federal Republic of Germany) as recommended by the manufacturer.

Plasmid digests were electrophoresed in a 0.8% agarose gel, transferred to GeneScreenPlus nylon membrane (NEF-926; Dupont, NEN) by the procedure described by P. Chromczynski and P. K. Qasba (*NEN Products News* **4**, N1, p. 2), and hybridized with ^{32}P-labeled *psiB* probe in a solution containing 1% sodium dodecyl sulfate, 1 M NaCl, 10% dextran sulfate, and 10 μg of denatured salmon DNA per ml at 65°C as recommended in the Dupont, NEN, instructions. Filter washing was also done as recommended in the Dupont, NEN, instructions.

Gene *psiB* is present on many conjugative plasmids. We found that the plasmids F, R100, R124, pGL611A, R387, pIP231, R483, pIP71a, and R16 carry DNA sequences homologous to the plasmid R6-5 *psiB* gene (Table 1, Fig. 2, and data not shown). The leading regions of all these plasmids are homologous and carry an *ssb*-like gene (13, 14).

The 10 other conjugative plasmids tested (Table 1), which have no extensive homology to the leading region of the F sex factor (14), did not hybridize with the *psiB* probe (data not shown).

***psiB* gene of the F sex factor.** We found that the R6-5 *psiB* probe hybridized to two subfragments produced by the *Eco*RV digest of the 3.1-kilobase (kb) *Sma*I fragment (coordinates 61.1 to 64.2) of the F sex factor (Fig. 1). The R6-5 *psiB* probe hybridized mainly to the 0.5-kb *Eco*RV fragment (coordinates 62.3 to 62.8) and much less to the 1.2-kb *Sma*I-*Eco*RV fragment (coordinates 61.1 to 62.3) (data not shown). We conclude that the *Eco*RV site (coordinate 62.3) is within the *psiB* gene but close to the beginning of the coding sequence (Fig. 1).

We tested whether SOS induction would be prevented if F *psiB* was amplified. We found that plasmid pKL3, a derivative of pBR322 carrying the F *psiB* gene, inhibited phage λ and *sfiA* induction. In contrast, plasmid pEG153 from which the 3.1-kb *Sma*I fragment that encodes the *psiB* gene had been deleted failed to inhibit SOS induction (Table 2). Evidently, plasmid F carries an analog of R6-5 *psiB*.

Plasmid R6-5 prevents induction of prophage λ in a *recA441* mutant at 42°C (2), while the F sex factor does not (1) (Table 2). Since F and R6-5 have approximately the same

* Corresponding author.
† Present address: Molecular Virology Laboratory, Antenucci 709, St. Luke's/Roosevelt Hospital, New York, NY 10019.

TABLE 1. Classification of conjugative plasmids on the basis of their ability to hybridize to the R6-5 *psiB* gene

Plasmid type	Plasmid name	Reference(s)	Incompatibility group	*ssb*-like gene presence
psiB$^+$	F	9	FI	+
	R100	6	FII	+
	R124	17	FIV	+
	pGL611A	—[a]	FVI	+
	R387	16	K	+
	R483	7, 18	I_α	+
	pIP231	—	Y	+
	pIP71a	25	9	+
	R16 (=pSF6)	12	B = 0	+
Δ*psiB*	pIP55 (=R55)	23	A = C = E	−
	R7116	—	D	−
	TP114	15	I_2	−
	R446b	19	L = M	−
	N3 (=RN3)	8	N	−
	RP4, RK2	10	P	−[b]
	R1460	—	U	−
	S-a	16, 30	W	−
	R6K	22	X	−[b]
	R27 (=TP117)	11, 15	H	−
	pHH1508a	—	H	−

[a] —, E. M. Lederberg, Plasmid Reference Center, Stanford University School of Medicine, Stanford, Calif.

[b] Plasmids RP4 and R6K were mistakenly classified before as *ssb*$^+$ (13). These plasmids do not carry sequences homologous to the leading region of the F sex factor (14).

copy number (24), it means that the F *psiB* gene product is either less active or expressed poorly.

F sex factor SSB protein enhances SOS inhibition by F *psiB*. Since the plasmids that encode *psiB* also carry an *ssb* sequence, we checked whether the F *ssb* gene product had an effect on SOS inhibition caused by polypeptide PsiB. We compared two F *psiB*$^+$ plasmids differing in the expression of the *ssb* gene. We found that plasmid pEG302, encoding an active F SSB protein, inhibited SOS more efficiently than plasmid pEG303, encoding a mutant SSB protein (Table 2).

Conclusions. The SOS inhibition caused by polypeptide PsiB was originally observed with plasmid R100. It was studied further by using plasmid R6-5. In contrast, the F sex factor did not notably inhibit SOS induction (1). We have now proven that (i) the F sex factor has a sequence homologous to that of R6-5 *psiB* and that (ii) the F *psiB* gene

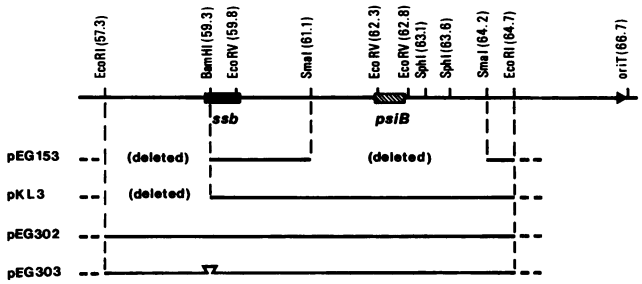

FIG. 1. Location of the *psiB* gene on the map of the F sex factor. Coordinates of *oriT* and restriction sites were taken from references 27 and 5 and from our data. Coordinates are based on the accepted 100-kb map of the F sex factor (31). The DNA fragments present in pEG153, pKL3, pEG302, and pEG303 are indicated by a solid line. Dotted lines represent the DNA of the pBR322 vector. Note the small insertion in the *ssb* gene of pEG303.

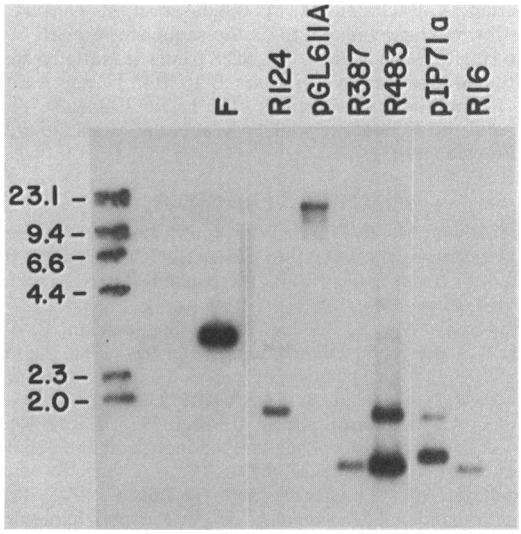

FIG. 2. Southern hybridization of the R6-5 *psiB* gene to DNA of different conjugative plasmids. The DNA tested was a *Pst*I digest of plasmids indicated at the top or a purified 3.1-kb *Sma*I fragment of plasmid F (coordinates, 61.1 to 64.2). The sizes (in kilobases) of the fragments obtained by digestion of λ phage DNA with *Hin*dIII are indicated to the left of the figure.

inhibits SOS induction when carried on a multicopy plasmid. Recent data point to the homology of the two *psiB* genes, except for the regulatory sequences (Dutreix et al., in preparation).

The simultaneous presence of *ssb* and *psiB* on almost one-half of the different conjugative plasmids raises two questions. (i) Are there at least two types of leading regions in conjugal plasmids? (Knowing that one leading region is common to a few conjugal plasmids, is the other type of leading region homogeneous and involving a common series of genes?) (ii) Are genes *ssb* and *psiB* required for conjugal replication in some hosts? These questions must be answered in the future.

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TABLE 2. Inhibition of phage λ and *sfiA* induction by F *psiB* gene product

Plasmid	Plasmid genotype	β-Galactosidase production[a] at:		% of cells surviving[b] at 42°C
		32°C	42°C	
None		60	3,000	0.010
F	*psiB*$^+$ *ssb*$^+$	50	2,600	0.003
R6-5	*psiB*$^+$ *ssb*$^+$	50	50	80
pKL3	*psiB*$^+$ Δ*ssb*[c]	60	60	70
pEG153	Δ*psiB* Δ*ssb*[c]	90	3,100	0.003
pEG302	*psiB*$^+$ *ssb*$^+$	10	40	90
pEG303	*psiB*$^+$ *ssb*	150	550	30

[a] Plasmids (column 1) were in *E. coli* GY7429 cells (*recA441 sfiA*::*lacZ*$^+$). As indicative of *sfiA* induction, β-galactosidase (in units per milligram) was measured in cultures incubated at 32°C or at 42°C for 2 h.

[b] Plasmids (column 1) were in *E. coli* GY3067 cells (*recA441* [λ]). Cell survival of lysogens was measured by plating bacteria at 42°C with adenine while control plates were incubated at 32°C.

[c] The deletion removes the promoter and a few amino acids of the SSB protein (5).

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