Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 5723–5726, August 1986
Microbiology

An inhibitor of SOS induction, specified by a plasmid locus in *Escherichia coli*

(activation of RecA protein/plasmid R6-5/Psi proteins/anti-RecA activity/*sfiA* induction)

MICHAEL BAGDASARIAN*†‡, ADRIANA BAILONE§, MIRA M. BAGDASARIAN*‡, PAUL A. MANNING*¶, RUDOLF LURZ*, KENNETH N. TIMMIS*‖, AND RAYMOND DEVORET§**

*Max-Planck-Institute for Molecular Genetics, Ihnestrasse 63, D-1000 Berlin 33, Federal Republic of Germany; and §Groupe d'Etude de la Mutagenèse et de la Cancérogenèse, Laboratoire d'Enzymologie, Centre National de la Recherche Scientifique, F-91190 Gif-sur-Yvette, France

Communicated by Evelyn M. Witkin, April 3, 1986

ABSTRACT **Plasmid R6-5 contains a locus whose product inhibits induction of *sfiA* and prophage λ in a *recA441* mutant at 42°C and in a *recA*$^+$ host after treatment with nalidixic acid. This plasmidic SOS-inhibition locus (*psi*) is situated on an 8.1-kilobase DNA fragment near *oriT*, the origin of plasmid R6-5 conjugational transfer. Loss of the Psi function, resulting from the insertion of Tn*3* into *psi*$^+$, greatly reduced the synthesis of two proteins, designated PsiA (M_r 24,500) and PsiB (M_r 12,500). Using host cells in which there was an inactive LexA repressor, we found that Psi function does not act by interfering with the expression of the SOS pathway. The Psi function may affect the generation of an SOS signal. We postulate that during the course of evolution, the Psi function has been selected in some conjugative plasmids so as to permit them to transfer single-stranded DNA without generating an SOS signal.**

When chromosome replication in *Escherichia coli* is affected by DNA lesions, an "SOS" signal is produced that provokes the activation of RecA protein (for review, see ref. 1). Once activated, RecA protein promotes the cleavage of LexA and prophage repressors, resulting in the derepression of cellular SOS genes and prophages. The SOS pathway that follows the formation of an SOS signal is well-documented (for review, see refs. 2–4).

In contrast, the pre-SOS pathway (that is, the process triggered by DNA lesions that generates an SOS signal) is not as well-understood. *In vitro*, single-stranded DNA is required as well as dNTPs for activation of RecA protein (5).

To characterize the SOS signal generated *in vivo*, we have resorted to using plasmid functions that promote or inhibit SOS induction (6–10). *In vivo*, DNA unwinding produced by the RecBC enzyme may generate an SOS signal (9, 11), the long-lasting stretches of single-stranded DNA formed *in vivo* providing sites for activation of RecA protein.

We describe here a plasmid locus, designated *psi*, whose expression inhibits the induction of cellular SOS genes and prophage λ. The Psi function does not interfere with the expression of the SOS pathway but rather affects the generation of an SOS signal. We postulate that during the course of evolution, the Psi function has been selected in some conjugative plasmids so as to permit them to transfer single-stranded DNA and avoid, at the same time, the generation of an SOS signal.

MATERIALS AND METHODS

***E. coli* K-12 Strains, Phages, and Plasmids.** Prophage λ induction was tested in GC908 [*recA441* (λ)] (10), and *sfiA* induction was tested in lysogens for λ*cIind1 sfiA*::*lacZ*$^+$ (12) such as GC4473 (*recA441 sfiA85*); GY7144 (*recA*$^+$ *sfiA211 srlC300*::Tn*10*), derived from JMRP (E. Witkin, personal communication); GY7145 (*recA730 sfiA211 srlC300*::Tn*10*), derived from JM30 (E. Witkin, personal communication; ref. 13); GY7146 [*recA*$^+$ *lexA3 lexA51*(Def) *sfiA11*], derived from DM1420 (14); and GY6867 (*recA*$^+$ Δ*lac–pro*) (9). GY7139 (Δ*lac–pro*) was lysogenic for λ*cIind1 recA*::*lacZ*$^+$ (15). M2141 (*minA minB*) was used to prepare minicells (16).

From plasmid R6-5 (17), DNA fragments bearing the *psi*$^+$ locus were cloned into plasmids pACYC184 (18), pBR322 (19), and pKT043 (20) to give plasmids pKT217, pKT200, and pKT246 (Fig. 4), which prevented λ induction and restored the viability of GC908 [*recA441* (λ)] on EMMA medium at 42°C.

Transposition of Tn*3* into pKT217 was obtained following cohabitation of plasmid R1::Tn*3* with pKT217 for about 100 generations at 30°C. After transformation of GC908 by pKT217::Tn*3* DNA, the Tet^r Amp^r plasmids pKT221, pKT223, and pKT224 were tested for allowing prophage λ induction to occur.

Purification of plasmids, digestion with restriction endonucleases, and ligation procedures were as in ref. 17.

Media, Growth of Cultures, and Measurements of *sfiA* and *recA* Expression. Cultures were at 37°C in LBT (21) except for *recA441* derivatives, which were at 32°C in enriched M9 medium EMM (10). EMMA was EMM plus 0.5 mM adenine. Growth of cultures was monitored at OD_{650}. Concentrations of ampicillin (Amp), tetracycline (Tet), and nalidixic acid were 100 μg/ml, 10 μg/ml, and 40 μg/ml in liquid and solid media. Expression of *sfiA* and *recA* was monitored by measuring the amount of β-galactosidase [units (u)/mg; ref. 8] produced by *lacZ* fused to the gene studied.

Detection of Plasmid-Encoded Proteins in Minicells. Minicells, purified from M2141 carrying plasmids (16) by sedimentation through sucrose gradients, were incubated in Difco methionine-assay medium supplemented with L-[^{35}S]methionine. Proteins were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis followed by autoradiography (16). Proteins encoded by pRS27 (*tra*$^+$) (22) served as molecular weight standards.

RESULTS

Inhibition of *recA441*-Promoted Induction of Prophage λ by Plasmids Carrying the *psi*$^+$ Locus. In a *recA441* mutant, SOS

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: u, unit(s); kb, kilobase(s).

†To whom reprint requests should be addressed.
‡Present address: Institute for Applied Cell and Molecular Biology, Umeå University, S-90187 Umeå, Sweden.
¶Present address: Department of Microbiology and Immunology, University of Adelaide, Adelaide SA 5001, Australia.
‖Present address: Département de Biochimie, Faculté de Médecine, Université de Genève, Geneva, Switzerland.
**To whom correspondence should be sent.

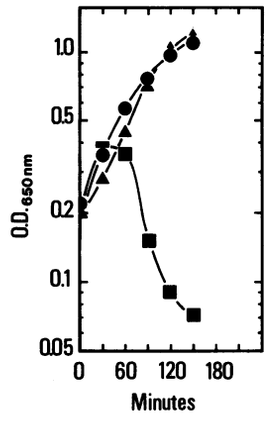

FIG. 1. Inhibition of prophage λ induction in a *recA441* host carrying plasmid R6-5. Cultures of GC908 (■), GC908 (R6-5) (●), and GC908 carrying pKT217 (*psi*$^+$) (▲) growing at 30°C in EMM were shifted to 42°C, and adenine was added to the medium (time 0).

functions are fully expressed at 42°C (23). Plasmid R100 was reported to inhibit prophage λ induction in a *recA441* λ lysogen (10). Plasmid R6-5, akin to R100, also prevented prophage induction in GC908 [*recA441* (λ)] and restored bacterial growth (Fig. 1). We call Psi (*p*lasmid-mediated *S*OS *i*nterference) the plasmid function that prevents SOS induction as shown below.

A locus governing the Psi function (*psi*) was identified by subcloning various *Eco*RI restriction fragments of plasmid R6-5 into a ColE1-type vector (17). Recombinant plasmid pKT217, carrying restriction fragment E-4, prevented λ induction in a *recA441* host (Fig. 1). Further subcloning positioned the *psi* locus within an 8.1-kilobase (kb) fragment delimited by *Bam*HI and *Bgl* II sites (Fig. 2; ref. 17). The *psi* locus was more precisely located by the insertion of a Tn*3* transposon that inactivated the Psi function and provided a molecular marker. Out of 600 insertions in plasmid pKT217,

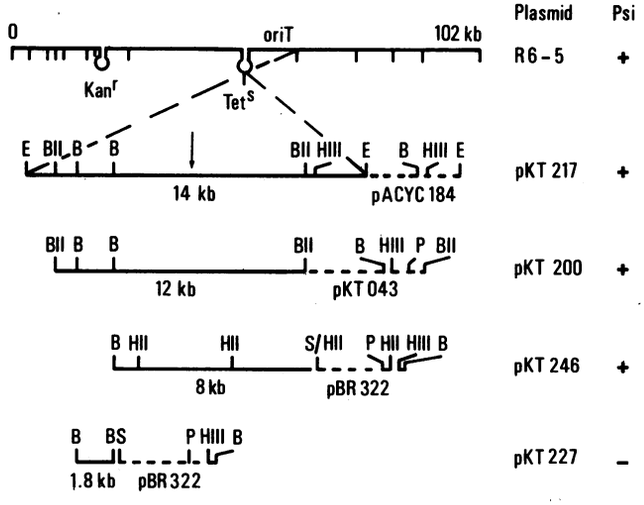

FIG. 2. Restriction map of the recombinant plasmids carrying the *psi* locus. On plasmid R6-5 (top line) are indicated *Eco*RI sites and the two stem–loops formed by Tn*903* and Tn*10* (not drawn to scale). Vector plasmids are represented by broken horizontal lines. Physical maps were determined as in ref. 17. The site of the Tn*3* insertions that inactivate the Psi function is indicated on the plasmid pKT217 map by an arrow. The position of *oriT* was deduced from the fact that plasmid pKT227, carrying the 1.6-kb *Bam*HI fragment of the E-4 region, was mobilized by the conjugative plasmid R100-1 at high frequency (data not shown). Sites of restriction enzymes are abbreviated as follows: B, *Bam*HI; BII, *Bgl* II; E, *Eco*RI; HII, *Hinc*II; HIII, *Hin*dIII; P, *Pst* I; S, *Sal* I.

3 were found to be *psi*$^-$. The three Tn*3* insertions mapped at about 7.2 kb from the left *Eco*RI site (Fig. 2). We have not ruled out the possibility that the three Tn*3* insertion mutants may be siblings.

Proteins Expressed by the *psi* Locus. Proteins produced in minicells by plasmid pKT217 and its Tn*3* *psi*$^-$ derivatives pKT221, pKT223, and pKT224 were analyzed by electrophoresis in $NaDodSO_4$/polyacrylamide gels. Tn*3* insertion reduced drastically the labeling of two polypeptides (M_r 24,500 and 12,500) designated PsiA and PsiB (Fig. 3). We have not yet determined what role is played by each of these proteins in the Psi function.

Inhibition of *sfiA* Induction by Plasmids Carrying the *psi*$^+$ Locus. The Psi function might inhibit λ induction by limiting the extent of λ repressor inactivation (subinduction; ref. 25). We tested, therefore, the induction of *sfiA*, an SOS gene under the control of the LexA repressor, which is inactivated 10 times more rapidly than λ repressor (26).

We introduced pKT217 (*psi*$^+$) and pKT246 (*psi*$^+$) into a *recA441 sfiA*::*lacZ*$^+$ host, and each plasmid reduced *sfiA* induction at 42°C by more than a factor of 10 (Fig. 4). In contrast, when pKT221 (*psi*::Tn*3*) was introduced into the same host, *sfiA* was induced normally (Fig. 4). Plasmid pKT246 also reduced *sfiA* expression in a *recA730 sfiA*::*lacZ*$^+$ host, from 4100 u/mg to 300 u/mg, the basal level of a *recA*$^+$ *sfiA*::*lacZ*$^+$ strain being 250 u/mg. This indicates that the Psi function was as efficient in the *recA730* as in the

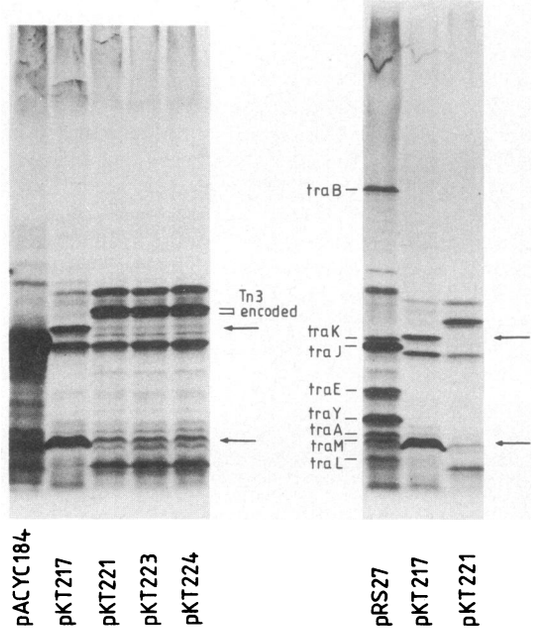

FIG. 3. (*Left*) Proteins expressed by pKT217 (*psi*$^+$) and by *psi*::Tn*3* derivatives pKT221, pKT223, and pKT224. Labeled proteins from minicells carrying plasmids were separated by electrophoresis in 11–20% gradient $NaDodSO_4$/polyacrylamide gels (16). The amount of radioactive material from minicells containing pACYC184 was higher than in the other minicell preparations. The band at highest molecular weight (lane 1) corresponds to chloramphenicol acetyltransferase expressed by pACYC184. This band disappears when a piece of DNA is cloned in its *Eco*RI site (lanes 2–5). Note that the Tn*3* insertions in pKT221, pKT223, and pKT224 reduce the intensity of PsiA and PsiB protein bands (arrows). Tn*3* insertion also leads to the overproduction of Tn*3*- and pR6-5-encoded proteins, the latter likely being transcribed from the β-lactamase promoter (24). (*Right*) Estimation of molecular weights of PsiA and PsiB. The following F-plasmid Tra proteins, expressed by the recombinant plasmid pRS27 (22), were used as molecular weight markers: TraB (M_r 29,000), TraJ (M_r 23,500), TraE (M_r 19,000), TraA (M_r 13,500), TraM (M_r 13,000), and TraL (M_r 11,000).

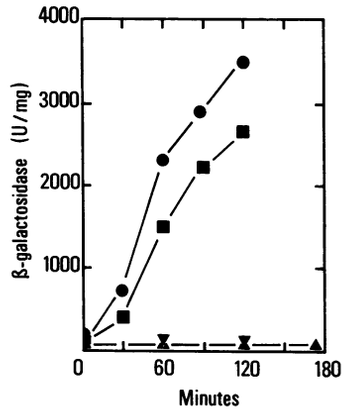

FIG. 4. Inhibition of *sfiA* induction in a *recA441* host carrying the cloned *psi*$^+$ locus. Cultures of GC4473 (●) and of derivatives carrying plasmids pKT246 (*psi*$^+$, ▼), pKT217 (*psi*$^+$, ▲), or pKT221 (*psi*::Tn*3*, ■) were grown in EMM at 30°C, shifted to 42°C, and diluted 1:4 into prewarmed EMMA. Samples were assayed for β-galactosidase activity (8).

recA441 mutant, the former being SOS-constitutive at all temperatures (13), whereas the latter is thermally inducible.

Moreover, plasmid pKT246 inhibited *sfiA* induction by nalidixic acid in a *recA*$^+$ host, indicating that the Psi function does prevent activation of wild-type RecA protein (Fig. 5).

Psi Function Does Not Act in Reducing Expression of *sfiA* and *recA*. Does the Psi protein prevent *sfiA* transcription by interacting with the *sfiA* operator as an analog of LexA protein? This possibility was ruled out by the fact that the presence of pKT246 (*psi*$^+$) failed to reduce the high level of *sfiA* expression in a host devoid of active LexA protein.

In *lexA3 lexA51*(Def) *sfiA*::*lacZ*$^+$ cells, the level of β-galactosidase produced from the derepressed *sfiA* promoter was 3700 u/mg, and this level did not decrease when pKT246 was introduced into the cells (4700 u/mg).

SOS induction is abolished when the normal level of RecA protein is reduced by a factor of at least 5 (27–29). Does the Psi function prevent the induction of SOS by reducing *recA* gene expression at the promoter level? It was found that, in a host containing a *recA*::*lacZ*$^+$ operon fusion (15), plasmid pKT246 failed to reduce the expression of the *recA* promoter (data not shown).

DISCUSSION

We have demonstrated here the existence of an inhibitor of SOS induction expressed by a plasmid locus we have designated *psi*. The two SOS functions tested were prophage λ and *sfiA* induction, respectively controlled by λ cI and LexA repressor. SOS induction was reduced as efficiently in *recA441* at 42°C as in *recA*$^+$ cells treated by nalidixic acid.

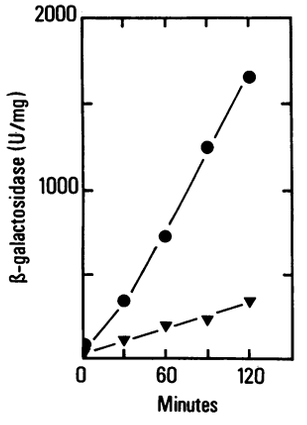

FIG. 5. Inhibition of *sfiA* induction in a *recA*$^+$ host carrying plasmid pKT246 (*psi*$^+$). Cultures of GY6867 (*sfiA*::*lacZ*$^+$, ●) and its pKT246 (*psi*$^+$) derivative (▼) growing in LBT at 37°C were treated with nalidixic acid. Samples were assayed for β-galactosidase activity.

The position of the *psi* locus on the R6-5 map has been delimited by subcloning and by insertion of a Tn*3* transposon that reduced the synthesis of two proteins (PsiA and PsiB). It is not known whether Tn*3* has been inserted into a structural gene or a regulatory site. We have not characterized biochemically the action of the Psi proteins, nor have we excluded that the smaller protein might be derived from the larger one.

The time-course of SOS induction can be divided into two periods. During the first, the SOS signal is generated and RecA protein is activated; during the second period (that is, the SOS pathway itself), inactivation of LexA or λ repressor takes place, leading to the induction of SOS genes.

Does the Psi function interfere with the SOS pathway itself? It might do so by (*i*) repressing SOS genes in a way analogous to that of LexA protein or (*ii*) reducing *recA* transcription. These two possible mechanisms have been ruled out by the data reported in *Results*. We must conclude that the Psi protein(s) interferes with the activation of the *recA* gene product.

The anti-RecA activity of Psi proteins may result from two types of interaction. Psi protein(s) (*a*) may interact directly with RecA protein or (*b*) may interfere or compete with RecA protein binding to single-stranded DNA and therefore inhibit RecA protein activation (30). Overproduction of SSB, the single-strand binding protein of *E. coli*, does not reduce induction of prophage λ in *recA441* cells at 42°C (31). Although the activities of Psi and SSB proteins might share some similarities, they are nevertheless different in their efficiency.

In *E. coli*, single-stranded DNA is transmitted upon conjugation from donor to recipient cell, in which the second strand is synthesized (for review, see ref. 32). If persisting, long stretches of single-stranded DNA generate a potent SOS signal in bacteria (1), then, during the course of evolution a mechanism was selected that permits conjugative plasmids to transfer single-stranded DNA and avoid, at the same time, the generation of an SOS signal. This may precisely be the function of Psi (or Psi-like) proteins in some conjugative plasmids.

This paper is dedicated to the memory of Ernest Borek. We are grateful to D. Bouillon and M. Pierre for their expert technical assistance. We thank M. Achtman, J. W. Little, E. Witkin, S. Sommer, and G. Weinstock for helpful discussions and gifts of strains. Grants from Euratom, Association pour la Recherche sur le Cancer, Ligue Francaise contre le Cancer, Fondation pour la Recherche Medicale, Deutsche Forschungsgemeinschaft, the Swedish National Research Council, and Collaboration Franco-Suedoise are gratefully acknowledged.

1. Roberts, J. W. & Devoret, R. (1983) in *Lambda II*, eds. Hendrix, R. W., Roberts, J. W., Stahl, F. W. & Weisberg, R. A. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Chapt. 7, pp. 123–144.
2. Witkin, E. M. (1982) *Biochimie* **64**, 549–555.
3. Little, J. W. & Mount, D. W. (1982) *Cell* **29**, 11–22.
4. Walker, G. C. (1984) *Microbiol. Rev.* **48**, 60–93.
5. Craig, N. L. & Roberts, J. W. (1980) *Nature (London)* **283**, 26–30.
6. Bailone, A., Brandenburger, A., Levine, A., Pierre, M., Dutreix, M. & Devoret, R. (1984) *J. Mol. Biol.* **179**, 367–390.
7. Brandenburger, A., Bailone, A., Levine, A. & Devoret, R. (1984) *J. Mol. Biol.* **179**, 571–576.
8. Sommer, S., Bailone, A. & Devoret, R. (1985) *Mol. Gen. Genet.* **198**, 456–464.
9. Bailone, A., Sommer, S. & Devoret, R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5973–5977.
10. Bagdasarian, M., D'Ari, R., Filipowicz, W. & George, J.

(1980) *J. Bacteriol.* **141,** 464–469.
11. Chaudhury, A. M. & Smith, G. R. (1985) *Mol. Gen. Genet.* **201,** 525–528.
12. Huisman, O. & D'Ari, R. (1983) *J. Bacteriol.* **153,** 169–175.
13. Witkin, E. M. & Kogoma, T. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7539–7543.
14. Mount, D. W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 300–304.
15. Weisemann, J. M., Funk, C. & Weinstock, G. M. (1984) *J. Bacteriol.* **160,** 112–121.
16. Thompson, R. & Achtman, M. (1978) *Mol. Gen. Genet.* **165,** 295–304.
17. Timmis, K. N., Cabello, F. & Cohen, S. N. (1978) *Mol. Gen. Genet.* **162,** 121–137.
18. Chang, A. C. Y. & Cohen, S. N. (1978) *J. Bacteriol.* **134,** 1141–1156.
19. Bolivar, F., Rodriguez, R. L., Greene, P. J., Betlach, M. C., Heyneker, H. L., Boyer, H. W., Crosa, J. & Falkow, S. (1977) *Gene* **2,** 95–113.
20. Timmis, K. N., Andres, I. & Slocombe, P. M. (1978) *Nature (London)* **273,** 27–32.
21. Devoret, R., Pierre, M. & Moreau, P. L. (1983) *Mol. Gen. Genet.* **189,** 199–206.
22. Kennedy, N., Beutin, L., Achtman, M., Skurray, R. A., Rahmsdorf, U. & Herrlich, P. (1977) *Nature (London)* **270,** 580–585.
23. Castellazzi, M., George, J. & Buttin, G. (1972) *Mol. Gen. Genet.* **119,** 139–152.
24. Heffron, F., McCarthy, B. J., Ohtsubo, H. & Ohtsubo, E. (1979) *Cell* **18,** 1153–1163.
25. Bailone, A., Levine, A. & Devoret, R. (1979) *J. Mol. Biol.* **131,** 553–572.
26. Little, J. W. (1983) *J. Mol. Biol.* **167,** 791–808.
27. Rebollo, J. E., Moreau, P. L., Blanco, M. & Devoret, R. (1984) *Mol. Gen. Genet.* **195,** 83–89.
28. Castellazzi, M., George, J. & Buttin, G. (1972) *Mol. Gen. Genet.* **119,** 153–174.
29. Dutreix, M., Bailone, A. & Devoret, R. (1985) *J. Bacteriol.* **161,** 1080–1085.
30. Resnick, J. & Sussman, R. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2832–2835.
31. Moreau, P. L. & Roberts, J. W. (1984) *Mol. Gen. Genet.* **198,** 25–34.
32. Willetts, N. & Wilkins, B. (1984) *Microbiol. Rev.* **48,** 24–41.