JOURNAL OF VIROLOGY, June 1981, p. 1077-1080
0022-538X/81/061077-04$02.00/0

Vol. 38, No. 3

NOTES

Restriction and Modification in *Bacillus subtilis*: Gene Coding for a *Bsu*R-Specific Modification Methyltransferase in the Temperate Bacteriophage ϕ3T

M. NOYER-WEIDNER, B. PAWLEK, S. JENTSCH, U. GÜNTHERT, AND T. A. TRAUTNER*

Max-Planck-Institut für molekulare Genetik, D-1000 Berlin 33, Germany

Received 21 November 1980/Accepted 25 February 1981

The resistance of ϕ3T DNA to degradation by the restriction enzyme *Bsu*R or its isoschizomer *Hae*III is due to obligatory modification of such DNA. Biochemical and genetical experiments indicate that ϕ3T codes for a methyltransferase, which methylates ϕ3T DNA itself or heterologous DNA at target sites 5'-GG*CC.

Analyses of restriction and modification of *Bacillus subtilis* phages showed that most phages studied are not sensitive to *Bsu*R restriction (13). This observation is surprising, in view of the high incidence with which *Bsu*R recognition sequences GGCC are expected to be present in DNA. Resistance of *B. subtilis* phage DNA to *Bsu*R restriction could be attributed to either a strong bias against this sequence (ϕ29 [9]; SPO1 [12]) or, as we have recently reported for SPβ (14), to the activity of a phage-coded modification enzyme which methylates *Bsu*R target sites in phage DNA.

Continuing our studies to understand the mechanisms and possibly the evolution of resistance of *B. subtilis* phages against *Bsu*R restriction, we report here studies with *B. subtilis* phage ϕ3T, whose DNA is also not sensitive to *Bsu*R degradation (2). ϕ3T is a temperate *B. subtilis* phage which has some similarity to SPβ on the basis of immunological cross-reaction (16). ϕ3T and SPβ, however, are distinguishable since they are heteroimmune and have different prophage attachment sites in the *B. subtilis* chromosome (17, 18) and different transduction potential (15, 18). Furthermore, the fragment patterns obtained with ϕ3T and SPβ DNAs after degradation with *Eco*RI (Fig. 1A), for example, are different. The data communicated here demonstrate that the resistance of ϕ3T to *Bsu*R restriction is caused by modification of ϕ3T by a phage-coded, GGCC-specific methyltransferase. This mechanism of obtaining *Bsu*R resistance is similar to the one observed with SPβ (14). A preliminary version of some aspects of this work has already been presented (S. Jentsch, B. Pawlek, M. Noyer-Weidner, U. Günthert, and T. A. Trautner, Proc. 5th Eur. Meet. Bacterial Transformation Transfection, in press). Cregg et al. (2a) communicated different experiments which led them independently to the same conclusion.

***Bsu*R restriction-sensitive mutant of ϕ3T.** If the resistance of phage ϕ3T to *Bsu*R restriction were due to the activity of a self-modifying, phage-coded DNA methyltransferase, we would expect to find phage mutants defective in this function. In contrast to the wild-type phage, such mutants would plate poorly on a restricting and modifying strain (TB804 r^+ m^+ [14]) but would grow normally on a nonrestricting strain (SB1207 r^- m^- [14]). On a mixed indicator composed of both bacterial strains, phage mutants would form turbid plaques and wild-type phage would form clear plaques, provided that the phage did not lysogenize. To obtain restriction-sensitive mutants, SB1207 cells were infected with a clear-plaque mutant of ϕ3T (ϕ3T*c*). The infected cells were incubated in the presence of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (250 μg/ml) for 10 min and allowed to lyse after removal of the mutagen. The lysate obtained was then used to infect SB1207 cells (multiplicity of infection $< 10^{-3}$). Infected cells were plated on a mixed indicator of SB1207 and TB804. About 0.3% of the plaques obtained were turbid. Phages, like ϕ3T11*c*, isolated from such plaques had the expected phenotype in that they were heavily restricted on r^+ m^+ cells (Table 1).

Restricted growth of ϕ3T11*c* on r^+ m^+ cells is correlated with the accessibility of ϕ3T11*c* DNA to *Hae*III (isoschizomer of *Bsu*R) restriction in vitro. The DNA of ϕ3T*c* is insensitive to *Hae*III degradation, whereas ϕ3T11*c* DNA is frag-

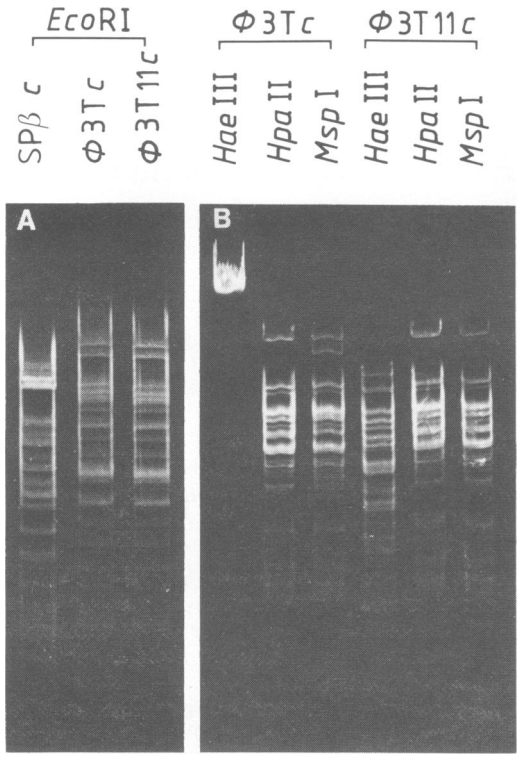

FIG. 1. *Gel electrophoresis of phage DNA after degradation with restriction enzymes. (A) Comparison of EcoRI-restricted SPβc, φ3Tc, and φ3T11c DNAs; (B) comparison of φ3Tc and φ3T11c DNAs after degradation with HaeIII (BsuR), HpaII, and MspI. The restriction enzymes were purchased from Boehringer Mannheim Corp. and used as described by the supplier. Vertical gel electrophoresis was performed as described by Bron and Hörz (1).*

mented into more than 50 fragments (Fig. 1B). Restriction patterns obtained with *Eco*RI and *Hpa*II (Fig. 1) were identical for φ3T11*c* and φ3Tc. Slight differences were observed in the *Msp*I patterns. These can be accounted for by the consideration that *Bsu*R modification in an overlapping *Bsu*R/*Msp*I sequence

*Bsu*R
*
GGCCGG
*Msp*I

causes simultaneous *Msp*I resistance in this sequence, implying that the methylation of the outer cytosine in the *Msp*I target sequence CCGG provides *Msp*I modification.

φ3Tc and φ3T11*c* DNAs differ in their extent of methylation. The sensitivity of mutant phage to *Bsu*R restriction is indeed caused by the absence of *Bsu*R-specific methylation in φ3T11*c* DNA. We have demonstrated this in two ways. (i) In vitro methylation of φ3T11*c* DNA with the *Bsu*R-specific modification enzyme before exposure of the DNA to *Hae*III rendered the DNA insensitive to *Hae*III restriction (Fig. 2). (ii) The methylated cytosine (5-mCyt) content of φ3T*c* DNA was much higher than that of φ3T11*c* DNA (Table 1). The residual methylation of φ3T11*c* phage DNA is as yet unaccounted for.

TABLE 1. *Phage restriction and content of 5-mCyt*[a]

Phage	Restriction ratio ($r^+ m^+/r^- m^-$ plating efficiency)	Content (cpm) of:		5-mCyt/(Cyt + 5-mCyt) (%)
		Cyt	5-mCyt	
φ3T*c*	9.3×10^{-1}	632,441	11,045	1.72
φ3T11*c*	3.3×10^{-7}	694,672	4,187	0.60
SPP1.R	7.1×10^{-1}			1.26[b]
SPP1.O	6.1×10^{-6}			0.08[b]

[a] For growth of bacteria, preparation of φ3T lysates, and plating, modified M medium (11) was used. φ3Tc, φ3T11*c*, and SPP1.O were grown on strain SB1207; SPP1.R was grown on strain TB804. The 5-mCyt content was determined as described by Günthert et al. (6).

[b] Reference 8.

Levels of methyltransferase activity in φ3T lysogenic cells. The capacity of φ3T to express a *Bsu*R-specific methyltransferase was further determined in cells lysogenized either with φ3T or with a temperate derivative of φ3T11*c* both with and without mitomycin C induction of the prophage (Table 2). These measurements included direct determinations of methyltransferase activity of crude cell lysates and an estimate of the cells' potential to modify superinfecting, heterologous SPP1.O DNA. It is obvious that the basal level of methyltransferase activity was enhanced in φ3T lysogenic cells in comparison with the nonlysogenic control or with cells with φ3T11 as prophage (Table 2). This high activity level in the φ3T lysogen, which we attribute to expression of spontaneously induced φ3T, is further augmented when the prophage is induced with mitomycin C. This general pattern was also obtained when the restriction ratio of SPP1.O grown on various strains was determined: the highest restriction ratio was observed with phages grown on strain TB300. Whether unidentified methyltransferases (Table 1) are responsible for the difference in restriction ratios between phages grown on SB1207 or TB311 remains to be clarified.

The potential for self-methylation has been identified as the mechanism underlying the resistance of φ3T to *Bsu*R restriction. This is the same mechanism by which phage SPβ attains

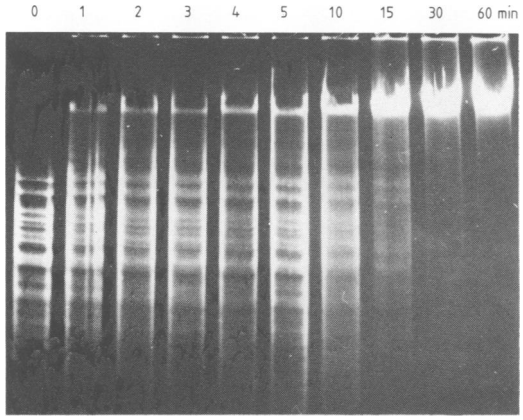

FIG. 2. *Development of resistance of φ3T11c DNA against HaeIII restriction. φ3T11c DNA was preincubated for the times indicated with the bacterial BsuR-specific methyltransferase (5; U. Günthert, M. Freund, and T. A. Trautner, J. Biol. Chem., in press) in the presence of S-adenosylmethionine, resulting in HaeIII-resistant recognition sites (8, 10), and were then degraded to completion with HaeIII. Agarose gel electrophoresis was performed as described by Bron and Hörz (1).*

TABLE 2. *Inducibility of methyltransferase*

Bacterial strain and relevant genotype	Mitomycin C treatment[a]	Methyltransferase activity (U of enzyme per mg of protein)[b]	Restriction ratio of SPP1.0 phage grown on strain[c]
SB1207	−	31	1.5×10^{-6}
$r^- m^-$ $(\phi 3T)^-$	+	25	1.2×10^{-6}
TB300	−	68	1.0×10^{-2}
$r^- m^-$ $(\phi 3T)^+$	+	240	5.8×10^{-2}
TB311	−	19	1.5×10^{-4}
$r^- m^-$ $(\phi 3T11)^+$	+	18	2.9×10^{-4}

[a] Mitomycin C treatment was performed as described by Günthert et al. (4).

[b] Cell-free extracts were assayed for their capacity to incorporate [^{3}H]methyl groups from *S*-adenosyl-[*methyl*-^{3}H]methionine into unmodified DNA as described by Trautner et al. (14).

[c] After growth of unmodified SPP1 phage on mitomycin C-treated and untreated cells for one cycle of growth, the restriction ratios of the released phage were determined.

its insensitivity to *Bsu*R action (14). We consider the identification of a phage-coded methyltransferase in φ3T of particular interest since φ3T carries a gene specifying a thymidylate synthetase which is homologous to a corresponding bacterial gene (3, 15). In analogy to this situation, we wonder whether the φ3T-borne methyltransferase gene is related to the bacterial gene specifying *Bsu*R methylation. We would also like to understand whether the *Bsu*R methyltransferase gene in phage φ3T is related to the corresponding SPβ gene, in view of differences in the potentials of these phages to methylate cytosine-containing target sequences other than the *Bsu*R site (S. Jentsch et al., in press, and unpublished data). Cloning of the methyltransferase genes identified, together with DNA/DNA hybridizations, and purifications of the enzymes involved are being undertaken to answer some of the evolutionary questions raised.

We thank D. Ehrlich for sending us the φ3T lysogenic *B. subtilis* strain and J. Ito for communicating unpublished results.

This work was in part supported by Deutsche Forschungsgeminschaft (Tr 25/8).

LITERATURE CITED

1. **Bron, S., and W. Hörz.** 1980. Purification and properties of the *Bsu* endonuclease. Methods Enzymol. **65**:112–132.
2a. **Cregg, J. M., A. H. Nguyen, and J. Ito.** 1980. DNA modification induced during infection of *Bacillus subtilis* by phage φ3T. Gene **12**:17–24.
2. **Cregg, J. M., and J. Ito.** 1979. A physical map of the genome of temperate phage φ3T. Gene **6**:199–219.
3. **Duncan, C. H., G. A. Wilson, and F. E. Young.** 1978. Mechanism of integrating foreign DNA during transformation of *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. **75**:3664–3668.
4. **Günthert, U., B. Pawlek, J. Stutz, and T. A. Trautner.** 1976. Restriction and modification in *Bacillus subtilis*: inducibility of a DNA methylating activity in nonmodifying cells. J. Virol. **20**:188–195.
5. **Günthert, U., B. Pawlek, and T. A. Trautner.** 1977. Transfection as a tool in the study of modification. Proc. 3rd Eur. Meet. Bacterial Transformation Transfection, p. 249–256.
6. **Günthert, U., M. Schweiger, M. Stupp, and W. Doerfler.** 1976. DNA methylation in adenovirus, adenovirus-transformed cells and host cells. Proc. Natl. Acad. Sci. U.S.A. **73**:3923–3927.
7. **Günthert, U., K. Storm, and R. Bald.** 1978. Restriction and modification in *Bacillus subtilis*. Localization of the methylated nucleotide in the *Bsu*RI recognition sequence. Eur. J. Biochem. **90**:581–583.
8. **Günthert, U., J. Stutz, and G. Klotz.** 1975. Restriction and modification in *B. subtilis*. The biochemical basis of modification against endo R·*Bsu*R restriction. Mol. Gen. Genet. **142**:185–191.
9. **Ito, J., and R. J. Roberts.** 1979. Unusual base sequence arrangement in phage φ29 DNA. Gene **5**:1–7.
10. **Mann, M. B., and H. O. Smith.** 1977. Specificity of *Hpa*II and *Hae*III DNA methylases. Nucl. Acids Res. **4**:4211–4221.
11. **Palefski, S., H. E. Hemphill, P. E. Kolenbrander, and H. R. Whiteley.** 1972. Dominance relationships in mixedly infected *Bacillus subtilis*. J. Virol. **9**:594–601.
12. **Reeve, J. N., E. Amann, R. Tailor, U. Günthert, K. Scholz, and T. A. Trautner.** 1980. Unusual behaviour of SPO1 DNA with respect to restriction and modification enzymes recognizing the sequence 5'GGCC. Mol. Gen. Genet. **178**:229–231.
13. **Trautner, T. A., B. Pawlek, S. Bron, and C. Anagnostopoulos.** 1974. Restriction and modification in *B. subtilis*: biological aspects. Mol. Gen. Genet. **131**:181–191.

14. **Trautner, T. A., B. Pawlek, U. Günthert, U. Canosi, S. Jentsch, and M. Freund.** 1980. Restriction and modification in *Bacillus subtilis*: identification of a gene in the temperate phage SPβ coding for a *Bsu*R specific modification methyltransferase. Mol. Gen. Genet. **180:** 361–367.
15. **Tucker, R. G.** 1969. Acquisition of a thymidylate synthetase activity by a thymine-requiring mutant of *Bacillus subtilis* following infection by the temperate phage ϕ3. J. Gen. Virol. **4:**489–504.
16. **Warner, F. D., G. A. Kitos, M. P. Ramano, and M. E. Hemphill.** 1977. Characterization of SPβ: a temperate bacteriophage from *Bacillus subtilis* 168 M. Can. J. Microbiol. **23:**45–51.
17. **Williams, M. T., and F. E. Young.** 1977. Temperate *Bacillus subtilis* bacteriophage ϕ3T: chromosomal attachment site and comparison with temperate bacteriophages ϕ105 and SPO2. J. Virol. **21:**522–529.
18. **Zahler, S. R., R. Z. Korman, R. Rosenthal, and E. H. Hemphill.** 1977. *Bacillus subtilis* bacteriophage SPβ: localization of the prophage attachment site, and specialized transduction. J. Bacteriol. **129:**556–558.