JOURNAL OF BACTERIOLOGY, Dec. 1984, p. 1178–1180
0021-9193/84/121178-03$02.00/0

Vol. 160, No. 3

Reorienting and Expanding the Physical Map of Temperate *Bacillus subtilis* Bacteriophage ϕ105

JAY S. LAMPEL, DANIEL M. ELLIS, AND DONALD H. DEAN*

Department of Microbiology, The Ohio State University, Columbus, Ohio 43210

Received 3 May 1984/Accepted 7 September 1984

During the course of extending the physical map of *Bacillus subtilis* temperate bacteriophage ϕ105 to include *Sst*I, *Xho*I, and *Hpa*I, we recognized that the previous physical map for *Eco*RI was incorrect. The new enzyme maps were determined by single, double, and partial enzyme digestions, redigestion of purified phage fragments, end joint analysis, and DNA-DNA hybridization. The *Eco*RI physical map was corrected by double digestion of isolated fragments, DNA hybridization, and physical mapping by partial digestion of end-labeled fragments. *Eco*RI fragment G was repositioned to give the order D-G-I-E-B-H-F-C.

The temperate *Bacillus subtilis* bacteriophage ϕ105 has several properties which are different from those of the prototype temperate phage, lambda. The ϕ105 prophage map and the mature phage map have been reported to be colinear (1, 4), implying that the ends of the DNA are very near to the attachment site (4). The phage DNA ends are single stranded and cohesive (4, 14), as are those of lambda, but they reassociate more readily after heating (14) and dissociate more readily after treatment with formamide than do the lambda *cos* sites (4). Bacteriophage ϕ105 also has an unusual induction mechanism. Rather than excising a circular intermediate by the Campbell model (3), the prophage replicates in situ in the chromosome before packaging (2). The exact mechanisms of excision are unknown.

As a cloning vector, ϕ105 has also contributed to *B. subtilis* genetics. The *spo0B* and *spo0F* genes of *B. subtilis* were cloned into ϕ105 by replacing the *Eco*RI-E-I-G and *Eco*RI-G region of the phage with chromosomal DNA (8), which resulted in defective phage particles. ϕ105 has also been used as a specialized transducing phage for the *met*B (9), *lys*, and *spo*IIIA genes (10).

A genetic map has been established for ϕ105 by two- and three-factor crosses (13) and marker rescue experiments (1). Physical maps have been also determined for ϕ105: *Eco*RI (9, 12, 15), *Kpn*I (6, 10; J. B. Perkins, Ph.D. thesis, The Ohio State University, Columbus, 1981), *Sma*I (6; Perkins, Ph.D. thesis), *Sal*I (12), *Sac*I (=*Sst*I [6]), and *Xho*I (11).

In the process of extending the physical map of ϕ105 to include *Hpa*I and confirming the maps for *Sst*I and *Xho*I, we obtained evidence that places the *Eco*RI-G restriction fragment at a new position. Since several papers report or cite the previous *Eco*RI map (8–10), we feel that it is important to report our correction and to support our new findings by several physical mapping approaches.

A physical map of *Hpa*I was constructed by double digestions with *Sal*I and *Sst*I and by isolation of *Eco*RI-B band from agarose gels and digestion, partially and completely, with *Hpa*I. Maps were also constructed with *Xho*I and *Sst*I and found to be identical with those published earlier (6, 11). Table 1 gives the molecular weights of the limit digest products; the physical maps are shown in Fig. 1.

When ϕ105 DNA was simultaneously digested with *Hpa*I and *Eco*RI, *Eco*RI-G (1.1 kilobases [kb]) was reduced to 0.84- and 0.30-kb fragments (Fig. 2). According to the current *Eco*RI map and our alignment of *Hpa*I fragments, *Eco*RI-G should not have been cleaved (see Fig. 1).

The correct positioning of *Eco*RI-G was achieved by purifying individual *Eco*RI fragments, nick translating them, and probing them to a complete *Hpa*I digest. Purified *Eco*RI-B hybridized to *Hpa*I-A, -C, -G, and -H, confirming that a portion of *Hpa*I-C was in fact contained within *Eco*RI-B (Fig. 3). *Eco*RI-C hybridized to *Hpa*I-B, -E, and -F. There was no detectable hybridization to *Hpa*I-J because either *Hpa*I-J was not dissociated from *Hpa*I-D or *Hpa*I-J was not present in sufficient quantity, owing to gel drying or denaturation steps. The *Eco*RI-D probe hybridized to *Hpa*I-B and *Hpa*I-D. *Eco*RI-E hybridized specifically to *Hpa*I-C, whereas *Eco*RI-F hybridized only to *Hpa*I-A. Finally, *Eco*RI-G hybridized to *Hpa*I-C and *Hpa*I-I.

Further evidence supporting the realignment of *Eco*RI-G was provided by hybridizing the labeled *Eco*RI-G probe to a complete *Kpn*I digest of ϕ105 (Fig. 4). According to the *Kpn*I map (6, 10; Perkins, Ph.D. thesis), *Eco*RI-G should be contained within *Kpn*I-D. Hybridization, however, was to *Kpn*I-A (the end join fragment) and to *Kpn*I-C. Also, when *Kpn*I-D was purified and redigested with *Eco*RI, the largest

TABLE 1. Molecular sized of ϕ105-generated restriction fragments

Fragment	Mean ± SD molecular size (kb) of fragment from[a]:			
	*Sst*I	*Xho*I	*Hpa*I	*Eco*RI[b]
A	32.0[c]	25.5 ± 0.80	9.5 ± 0.30	16.94[c]
B	26.5[d]	14.5 ± 0.35[c]	8.0 ± 0.19[c]	11.04
C	6.80 ± 0.16[d]	9.1 ± 0.20[d]	7.5 ± 0.18	8.80[d]
D	3.10 ± 0.08	3.80 ± 0.14[d]	7.35 ± 0.18[d]	7.50[d]
E	1.95 ± 0.06		4.95 ± 0.10	5.36
F			3.30 ± 0.15	3.00
G			2.30 ± 0.10	1.09
H			1.95 ± 0.10	0.92
I			1.00 ± 0.05	0.39
J			0.78 ± 0.06[d]	
Total	38.35	38.40	38.63	38.04

[a] Fragments expressed to 0.1 kb are from nonlinear portions of standard curve; fragments expressed to 0.01 kb are from linear portions of standard curve. Standard deviations are from the averages of five determinations.
[b] Determined by Perkins et al. (12).
[c] End join fragment.
[d] Terminal fragment.

* Corresponding author.

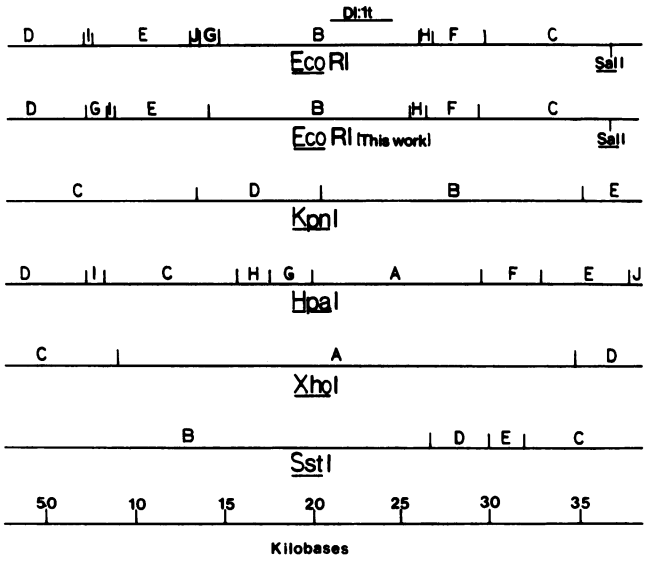

FIG. 1. Physical maps of φ105. The top line is the *Eco*RI map of Perkins et al. (12). The second line is the reoriented *Eco*RI map based on data in this paper. The third, fourth, fifth, and sixth lines are maps of *Kpn*I, *Hpa*I, *Xho*I, and *Sst*I, respectively, determined during the course of these studies. The lowest line is a scale in kilobases. The bar near the top of the figure, marked DI:1t, is the region of the phage genome deleted by a deletion mutant described by Flock (7).

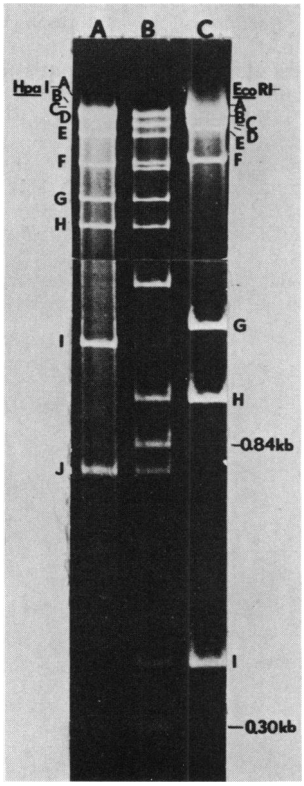

FIG. 2. Double digestion of φ105 with *Eco*RI and *Hpa*I electrophoretically separated on 3.5% polyacrylamide. Lane A, φ105 digested with *Hpa*I; lane B, φ105 digested with *Hpa*I and *Eco*RI, showing that *Eco*RI-G is cleaved by *Hpa*I and the generation of 0.84- and 0.3-kb fragments; lane C, φ105 digested with *Eco*RI.

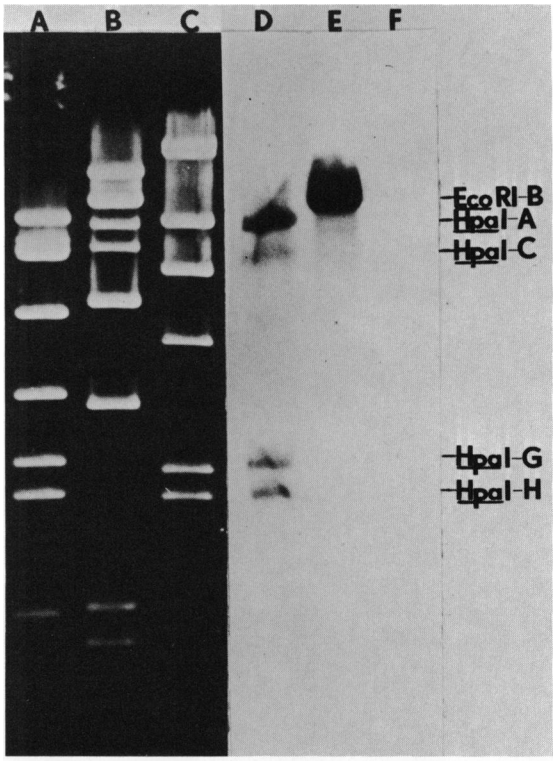

FIG. 3. Hybridization of φ105 [^{32}P]*Eco*RI-B to an *Hpa*I digest of φ105. Lanes A through C are ethidium bromide-stained gels, and lanes D through F are the corresponding autoradiograms after hybridization of [^{32}P]*Eco*RI-B to the gel. Lane A, φ105 digested with *Hpa*I; lane B, φ105 digested with *Eco*RI; lane C, λ digested with *Hin*dIII; lane D, [^{32}P]*Eco*RI-B probed to an *Hpa*I digest of φ105, showing hybridization to *Hpa*I-A, -C, -G, and -H; lane E, [^{32}P]*Eco*RI-B probed to an *Eco*RI digest of φ105; lane F, [^{32}P]*Eco*RI-B probed to λ digested with *Hin*dIII.

fragment measured 0.78 kb, not the expected 1.09 kb of *Eco*RI-G (data not shown). Therefore, the confirmed order of *Hpa*I fragments is D-I-C-H-G-A-F-E-J, and the *Eco*RI fragment order is D-G-I-E-B-H-F-C. We have no evidence of the *Eco*RI-J fragment reported by Scher et al. (15), and we conclude that our previous inclusion of *Eco*RI-J, based on partial digests, was incorrect. In retrospect, the molecular weights of several partial digestion fragments (12) would support arguments for other arrangements of the left subterminal fragments.

Confirming evidence of *Eco*RI fragment order was sought through a modification of the method of Smith and Birnstiel (16) by labeling with [^{32}P]cordycepin-5′-triphosphate and terminal transferase. Under conditions where only the left end was labeled (by removing the right end with *Sal*I), partial digests with *Eco*RI were generated. The results were a series of molecules with a common labeled end: D, D-G, and D-G-I (data not shown). No molecules with molecular weights corresponding to D-I or D-E were observed. We should perhaps emphasize that the difference between the current order of *Eco*RI and the previous ones we published (6, 12) is due to an error in placement of *Eco*RI-G, not in an alteration in the phage genome. The stocks used earlier are the same as those used in this study, and all restrictions patterns and fragment sizes are the same as those previously published. All strains were obtained from the *Bacillus* Genetic Stock Center, The Ohio State University, Columbus.

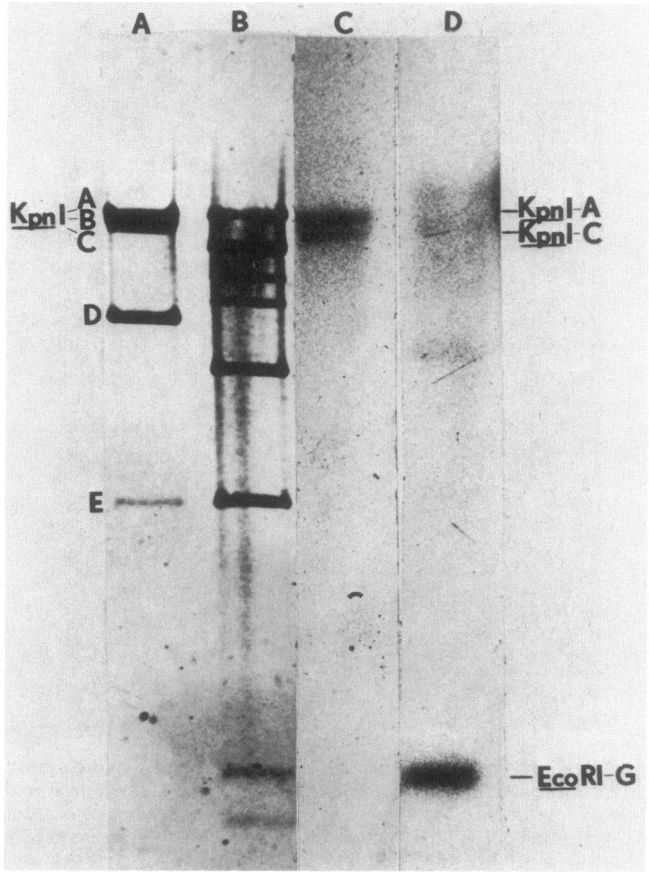

FIG. 4. Hybridization of ϕ105 [^{32}P]*Eco*RI-G to a *Kpn*I digest of ϕ105. Lane A, ϕ105 digested with *Kpn*I; lane B, ϕ105 digested with *Eco*RI; lane C, autoradiogram of [^{32}P]*Eco*RI-G to a *Kpn*I digest of ϕ105, showing hybridization to *Kpn*I-A (end joint containing *Kpn*I-C) and *Kpn*I-C; lane D; autoradiogram of [^{32}P]*Eco*RI-G to an *Eco*RI digest of ϕ105. Lanes A and B are reverse-contrast pictures of ethidium bromide-stained gels, and lanes C and D are corresponding autoradiograms after hybridization of [^{32}P]*Eco*RI-G to the gel.

LITERATURE CITED

1. **Armentrout, R. W., and L. Rutberg.** 1970. Mapping of prophage and mature deoxyribonucleic acid from temperate *Bacillus* bacteriophage ϕ105 by marker rescue. J. Virol. **6**:760–767.
2. **Armentrout, R. W., and L. Rutberg.** 1971. Heat induction of prophage ϕ105 in *Bacillus subtilis*: replication of the bacterial and bacteriophage genomes. J. Virol. **8**:455–468.
3. **Campbell, A.** 1962. Episomes. Adv. Genet. **11**:101–145.
4. **Chow, L. T., L. B. Boice, and N. Davidson.** 1972. Map of the partial sequence homology between DNA molecules of *Bacillus subtilis* bacteriophages SPO2 and ϕ105. J. Mol. Biol. **68**:391–400.
5. **Chow, L. T., and N. Davidson.** 1973. Electron microscope study of the structures of the *Bacillus subtilis* prophages SPO2 and ϕ105. J. Mol. Biol. **75**:257–264.
6. **Dean, D. H.** 1980. Cloning in and of *Bacillus*, p. 107–122. *In* D. H. Dean, L. F. Johnson, P. C. Kimball, and P. S. Perlman (ed.), Gene structure and expression. The Ohio State University Press, Columbus.
7. **Flock, J. I.** 1977. Deletion mutants of temperate *Bacillus subtilis* bacteriophage ϕ105. Mol. Gen. Genet. **155**:241–247.
8. **Hirochika, H., Y. Koybayashi, F. Kawamura, and H. Saito.** 1982. Construction and characterization of ϕ105 specialized transducing phages carrying sporulation genes *spo*0B and *spo*0F of *Bacillus subtilis*. J. Gen. Appl. Microbiol. **28**:225–229.
9. **Iijima, T. F., F. Kawamura, H. Saito, and Y. Ikeda.** 1980. A specialized transducing phage constructed from *Bacillus subtilis* phage ϕ105. Gene **9**:115–126.
10. **Jenkinson, H. F., and J. Mandelstam.** 1983. Cloning of the *Bacillus subtilis lys* and *spo*IIIB genes in phage ϕ105. J. Gen. Microbiol. **129**:2229–2240.
11. **Jentsch, S.** 1983. Restriction and modification in *Bacillus subtilis*: sequence specificities of restriction/modification systems *Bsu*M, *Bsu*E, and *Bsu*F. J. Bacteriol. **156**:800–808.
12. **Perkins, J. B., C. D. Zarley, and D. H. Dean.** 1978. Restriction endonuclease mapping of bacteriophage ϕ105 and closely related temperate *Bacillus subtilis* bacteriophages ρ10 and ρ14. J. Virol. **28**:403–407.
13. **Rutberg, L.** 1969. Mapping of a temperate bacteriophage active on *Bacillus subtilis*. J. Virol. **3**:38–44.
14. **Scher, B. M., D. H. Dean, and A. J. Garro.** 1977. Fragmentation of *Bacillus* bacteriophage ϕ105 DNA by restriction endonuclease *Eco*RI: evidence for complementary single-stranded DNA in the cohesive ends of the molecule. J. Virol. **23**:377–383.
15. **Scher, B. M., M. F. Law, and A. J. Garro.** 1978. Correlated genetic and *Eco*RI cleavage map of *Bacillus subtilis* bacteriophage ϕ105 DNA. J. Virol. **28**:395–402.
16. **Smith, H. O., and M. L. Birnstiel.** 1976. A simple method for DNA restriction site mapping. Nucleic Acids. Res. **3**:2387–2399.