JOURNAL OF VIROLOGY, June 1981, p. 1081-1083
0022-538X/81/061081-03$02.00/0

Vol. 38, No. 3

Predominance of Bacteriophage SP82 over Bacteriophage SP01 in Mixed Infections of *Bacillus subtilis*

CHARLES R. STEWART* AND MICHELLE FRANCK

Department of Biology, Rice University, Houston, Texas 77001

Received 5 December 1980/Accepted 17 March 1981

In mixed infections with *Bacillus subtilis* phages SP82 and SP01, the SP82 genotype is predominant among the progeny. This predominance is determined by a specific region of the genome, the *pos* region, which apparently is located near genes 29 to 32 (by the SP01 numbering system). Recombination between SP82 and SP01 yields phage which have both the SP82 *pos* region and an SP01 mutation. This mutation then behaves in mixed infection as if it were part of an SP82 genome.

Bacillus subtilis bacteriophages SP01 and SP82 are closely related (7, 16, 21), having similar restriction maps (9, 15), genetic maps (6, 11), and patterns of RNA and protein synthesis (2, 9, 17, 19). Their gene products are freely interchangeable, since each of the known DNA replication-deficient mutants of either phage is complemented by mutants of the other phage (our unpublished data). Here we show that SP82 predominates over SP01 in mixed infection. A specific region of the genome is responsible for the SP82 advantage. By recombination, this region of the SP82 genome can be incorporated into the SP01 genome, giving the recombinant the same advantage usually enjoyed by SP82.

The basic procedures, media, and SP01 strains were described by Glassberg et al. (3–5). SP82 strains were generously provided by E. Kahan and D. Green. *B. subtilis* strains CB-312 and CB-314 were suppressor minus (Su^-) and suppressor plus (Su^+), respectively. In our standardized designation of phage strains, the first term indicates the phenotype, the second term the cistron number, and the third term the specific mutation within that cistron. Thus, *sus* 32-1 refers to a suppressor-sensitive mutant, affected in cistron 32, designated number 1. The strain 5-1 is a suppressor-sensitive (*sus*) mutant produced by recombination between SP82 wild type and SP01 mutant *sus* 32-1. Abbreviations include the following: gp, gene product; *sus*, suppressor-sensitive; and *pos*, predominance over SP01.

The latent period for SP01 is slightly shorter than that for SP82 (20). Cultures infected either with SP01, with SP82, or with both phages produce similar burst sizes. However, in mixed infection, the SP82 genotype predominates among the phage in the burst. Table 1 shows that, in mixed infection between SP82 wild type and SP01 mutant *sus* 32-1, the burst was predominantly wild type. The control experiment, a mixed infection between SP01 wild type and *sus* 32-1, yielded equal numbers of mutants and wild type. The same results were obtained whether the mutant or the wild type had the larger multiplicity of infection. The same results were also obtained whether the conditions were permissive or restrictive for mutant *sus* 32-1, showing that the deficiency of SP01 genotypes was not due to the absence of SP01 gp32. (*sus* 32-1 produces a burst size equal to that of the wild type, when grown on the Su^+ strain.)

Some of the progeny of the mixed infection with SP82 wild type and *sus* 32-1 were recombinants. For instance, one of the rare *sus* mutants that emerged from such an infection was labeled 5-1 and was tested in mixed infection with SP01 wild type and with SP82 wild type. The results, presented in the lower half of Table 1, show that this strain predominated over SP01 wild type to the same extent that the SP82 wild type predominated over the SP01 mutant and that mixed infection between this strain and SP82 wild type produced an approximately equal mixture of wild type and mutant progeny. Thus, the 5-1 was apparently produced by a recombination which linked the *sus* 32-1 mutation to the region of the SP82 genome which causes its predominance over SP01. We will call this region the pos^+ region, and the corresponding region of the SP01 genome will be designated pos^-. The apparent genotype of 5-1 is *sus* 32-1, pos^+.

Notice that, in the mixed infection of Su^- bacteria by SP01 wild type and 5-1, the only gp32 present is that from SP01. In spite of this, the SP01 wild type has the disadvantage. Thus,

TABLE 1. *Mixed infection involving SP01 and SP82*[a]

Host	Wild type	*sus* strain	Proportion of *sus* mutants in infection	Proportion of *sus* mutants in burst
Su^+	SP82	*sus* 32-1	0.36	0.08
Su^+	SP01	*sus* 32-1	0.38	0.49
Su^-	SP82	*sus* 32-1	0.36	0.05
Su^-	SP01	*sus* 32-1	0.38	0.43
Su^-	SP82	*sus* 32-1	0.65	0.11
Su^-	SP01	*sus* 32-1	0.57	0.52
Su^+	SP82	5-1	0.50	0.61
Su^+	SP01	5-1	0.50	0.88
Su^-	SP82	5-1	0.50	0.59
Su^-	SP01	5-1	0.50	0.97

[a] Cultures of CB-312 (Su^-) or CB-314 (Su^+) were infected simultaneously with the indicated phage strains with a multiplicity of infection of about 4 for each phage. The infections were done at 30°C, and unadsorbed phage were inactivated by diluting through anti-SP01 antibody (which inactivates both SP01 and SP82). The resulting bursts were plated on a lawn of CB-314 (Su^+). From 90 to 100 plaques from each infection were streaked onto Su^+ and Su^- lawns to determine which had the mutant (*sus*) phenotype. The multiplicity of infection for each phage was determined by singly infecting aliquots of the same bacterial culture with the same amounts of the individual phage lysates and measuring the proportion of cells surviving the infection. The proportion of *sus* mutants in the infection was calculated as the ratio: multiplicity of infection of *sus* mutant/multiplicity of infection of *sus* mutant + multiplicity of infection of wild type.

it is clear that the advantage of pos^+ strains is not caused by any specificity of gp32.

When mixed infection with SP82 wild type was repeated with other SP01 mutants instead of *sus* 32-1, the extent to which the SP82 genotype dominated the burst varied from mutant to mutant. This is shown in Table 2. The mutations that are most strongly excluded by SP82 coinfection are clustered in a small group in the region of genes 29 to 32 (the SP01 genes appear in numerical order on the map). Mutations from cistrons elsewhere on the map appear in the burst with frequencies much closer to those of the wild type. The simplest explanation is that any SP01 genetic locus is more likely to appear in the burst if it has been linked, by recombination, to the SP82 pos^+ region and that this region is located in the vicinity of genes 29 to 32.

There are alternative explanations for these data, involving, for instance, sites on the SP01 genome which cause inviability when present on the same genome as the SP82 pos^+ region. We have not, however, been able to devise any such explanation which is consistent with all of the data and which is not extremely improbable.

On the basis of the data presented thus far, it was possible that the burst of the SP82:SP01 mixed infection would contain only pos^+ phage, so that the only way that an SP01 mutation could be a part of the burst would be if it had recombined with the pos^+ region. However, it appears that, although pos^+ phage dominate the burst, the domination is not absolute. Twenty-one *sus* progeny from mixed infections involving SP82 wild type and either *sus* 31-1, *sus* 32-1, or *sus* 34-1 were tested for the ability to dominate

TABLE 2. *Mixed infections with various SP01 sus mutants*[a]

Wild type strain	Mutant strain[b]	Proportion of mutants in burst	Ratio
SP82	*sus* 34-1	0.25	1.7
SP01	*sus* 34-1	0.43	
SP82	*sus* 32-1	0.11	4.7
SP01	*sus* 32-1	0.52	
SP82	*sus* 31-1	0.09	5.2
SP01	*sus* 31-1	0.47	
SP82	*sus* 29-1	0.06	4.2
SP01	*sus* 29-1	0.25	
SP82	*sus* 28-1	0.20	1.8
SP01	*sus* 28-1	0.35	
SP82	*sus* 23-1	0.23	1.9
SP01	*sus* 23-1	0.43	
SP82	*sus* 14-1	0.59	0.9
SP01	*sus* 14-1	0.54	
SP82	*sus* 11-1	0.20	1.7
SP01	*sus* 11-1	0.34	
SP82	*sus* 2-1	0.15	1.1
SP01	*sus* 2-1	0.16[c]	
SP82	*sus* 1-1	0.40	1.3
SP01	*sus* 1-1	0.50	

[a] The procedure was the same as described in Table 1. The host strain was CB-312. The "ratio" listed in the last column is calculated as: proportion of mutants in burst of mixed infection with SP01/proportion of mutants in burst of mixed infection with SP82. The numbers for *sus* 28-1 and *sus* 11-1 are each from a single experiment. All other numbers are representative of the results of repeated experiments.

[b] The mutations on the SP01 map are arranged in numerical order, with *sus* 1-1 at the left and *sus* 34-1 at the right. These markers span all of the known genetic map, except for the markers in the terminal redundancy (1, 11).

[c] Mixed infection of *sus* 2-1 and SP01 wild type consistently yielded bursts with low proportions of mutants.

SP01 wild type in mixed infection. Seventeen of these had this ability, and four did not. It is conceivable that the latter four did not infect a cell which was also infected with SP82. This is unlikely, however, since unadsorbed phage were inactivated by antibody and since the Su^- host should not permit a burst from any cells which were infected only with *sus* phage.

There are two components of the pos^+ phenotype. Pos^+ strains predominate over SP01 strains in mixed infection, and pos^+ strains are not dominated by other SP82 strains. Although it is reasonable to expect that the same event causes both effects, it is conceivable that they require two different events. If so, they might be specified by two different loci. To the extent that we have been able to test, both traits are determined by the same locus, but we have not tested enough possible recombinants to draw a definite conclusion. We have tested four of the *sus* phage produced by mixed infection between SP01 mutant *sus* 32-1 and SP82 wild type. Three of them, including 5-1, have both of the pos^+ traits. The fourth has neither. In addition, a sus^+ strain, produced by recombination between 5-1 and SP01 wild type, has both traits. Thus, the two pos^+ traits were not separated by any of four recombination events which occurred in or near the pos^+ region.

At what stage in the infection does the *pos*-mediated event take place? Because of the presence of a replication origin in or near the *pos* region (5), it is tempting to guess that the event is initiation of replication, with pos^+ strains having a competitive advantage in attachment of the DNA to the cell membrane or some more specific replication complex. The concatemeric replication of SP01 DNA (1, 10) complicates, but does not invalidate, this interpretation. Alternatively, the competition could be at the level of DNA packaging. However, this seems likely to produce reduced burst sizes in mixed infection, unless a substantial excess of unpackaged DNA is produced, and we did not observe such reduced burst sizes. A third alternative is a restriction mechanism, which would require two sites in the *pos* region, the target site and the gene specifying the restriction enzyme.

Dominance relationships have been described for other phages, including *B. subtilis* phages SP02, β22, and SP82 (8, 12, 22) and including *Escherichia coli* phages T2 and T4 (13, 14, 18). In the latter case, the effect is also specific to certain regions of the chromosome, and it is possible that the mechanism is similar to that for SP82:SP01.

This work was supported by research grant PCM77-24086 from the National Science Foundation and by a research grant from the Hamman Foundation.

LITERATURE CITED

1. **Cregg, J. M., and C. R. Stewart.** 1978. Terminal redundancy of "high frequency of recombination" markers of *Bacillus subtilis* phage SP01. Virology **86**:530–541.
2. **Gage, L. P., and E. P. Geiduschek.** 1971. RNA synthesis during bacteriophage SP01 development: Six classes of SP01 RNA. J. Mol. Biol. **57**:279–300.
3. **Glassberg, J., R. A. Slomiany, and C. R. Stewart.** 1977. Selective screening procedure for the isolation of heat and cold-sensitive, DNA replication-deficient mutants of bacteriophage SP01 and preliminary characterization of the mutants isolated. J. Virol. **21**:54–60.
4. **Glassberg, J., M. Franck, and C. R. Stewart.** 1977. Initiation and termination mutants of *Bacillus subtilis* bacteriophage SP01. J. Virol. **21**:147–152.
5. **Glassberg, J., M. Franck, and C. R. Stewart.** 1977. Multiple origins of replication for bacteriophage SP01. Virology **78**:433–441.
6. **Green, D., and D. Laman.** 1972. Organization of gene function in *Bacillus subtilis* bacteriophage SP82G. Virology **9**:1033–1046.
7. **Hemphill, H. E., and H. R. Whiteley.** 1975. Bacteriophage of *Bacillus subtilis*. Bacteriol. Rev. **39**:257–315.
8. **Kolenbrander, P. E., H. E. Hemphill, and H. R. Whiteley.** 1973. Mixed infections of *Bacillus subtilis* involving bacteriophage SP02C. J. Virol. **11**:25–34.
9. **Lawrie, J. M., J. S. Downard, and H. R. Whiteley.** 1978. *Bacillus subtilis* bacteriophages SP82, SP01, and ϕe: a comparison of DNAs and of peptides synthesized during infection. J. Virol. **27**:725–737.
10. **Levner, M. H., and N. R. Cozzarelli.** 1972. Replication of viral DNA in SP01-infected *Bacillus subtilis*. I. Replicative intermediates. Virology **48**:402–416.
11. **Okubo, S., T. Yanagida, D. J. Fujita, and B. M. Ohlsson-Wilhelm.** 1972. The genetics of bacteriophage SP01. Biken J. **15**:81–97.
12. **Palefski, S. H., H. E. Hemphill, and H. R. Whiteley.** 1972. Dominance relationships in mixedly infected *Bacillus subtilis*. J. Virol. **9**:594–601.
13. **Pees, E.** 1970. Bacteriophage T4 mutants unable to exclude gene 56 of T2 from the progeny of crosses. Mut. Res. **9**:345–348.
14. **Pees, E., and B. de Groot.** 1970. Partial exclusion of genes of bacteriophage T2 with T4 glucosylated DNA in crosses with bacteriophage T4. Genetica **41**:541–550.
15. **Pero, J., N. M. Hannett, and C. Talkington.** 1979. Restriction cleavage map of SP01 DNA: General location of early, middle, and late genes. J. Virol. **31**:156–171.
16. **Rabussay, R., and E. P. Geiduschek.** 1977. Regulation of gene action in the development of lytic bacteriophages. Compr. Virol. **8**:1–196.
17. **Reeve, J. N., G. Mertens, and E. Amann.** 1978. Early development of bacteriophages SP01 and SP82G in minicells of *Bacillus subtilis*. J. Mol. Biol. **120**:183–207.
18. **Russell, R. L., and R. J. Huskey.** 1974. Partial exclusion between T-even bacteriophages: an incipient genetic isolation mechanism. Genetics **78**:989–1014.
19. **Spiegelman, G. B., and H. R. Whiteley.** 1974. *In vivo* and *in vitro* transcription by ribonucleic acid polymerase from SP82-infected *Bacillus subtilis*. J. Biol. Chem. **249**:1483–1489.
20. **Stewart, C. R., B. Click, and M. F. Tole.** 1972. DNA replication and late protein synthesis during SP82 infection of *Bacillus subtilis*. Virology **50**:653–663.
21. **Truffaut, N., B. Revet, and M. Soulie.** 1970. Etude comparative des DNA de phages 2C, SP8*, SP82, ϕe, SP01 et SP50. J. Biochem. **15**:391–400.
22. **Whiteley, H. R., P. E. Kolenbrander, and H. E. Hemphill.** 1974. Mixed infection of *Bacillus subtilis* involving bacteriophages SP82 and *B*22. J. Virol. **14**:1463–1469.