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Mixed Infections of *Bacillus subtilis* Involving Bacteriophages SP82 and β22

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Progeny yields and the synthesis of nucleic acids have been investigated in two strains of *Bacillus subtilis* mixedly infected with two unrelated phages, SP82 and β22. When *B. subtilis* strain 168 was the host, the first phage added dominated the infection; when *B. subtilis* strain SB11 was the host, β22 produced progeny even when added to cells 5 min after infection with SP82. Dominance in these mixed infections could be correlated with qualitative and quantitative differences in the synthesis of phage-specific RNAs.

It has generally been observed that when two unrelated bacteriophages simultaneously or consecutively infect the same host cell only one is able to produce significant numbers of progeny. This phenomenon has been called mutual exclusion (3, 4). In previous communications (9, 11) we reported that two virulent *Bacillus subtilis* phages, SP82 and β22, suppressed the development of SPO2c$_1$, a clear-plaque mutant of the temperate phage SPO2, when either of the former phages was added to cells before, simultaneously, or shortly after infection with SPO2c$_1$. In both SP82-SPO2c$_1$ and β22-SPO2c$_1$ mixed infections, failure of SPO2c$_1$ to produce progeny was associated with a greatly reduced capacity of this subordinate phage to transcribe and replicate its DNA.

In the present study, we have examined the synthesis of nucleic acids in *B. subtilis* cells mixedly infected with β22 and SP82, the two dominant phages in mixed infections with SPO2c$_1$. These infections have been of special interest for two reasons: first, although these are both large, virulent phages, β22 and SP82 are completely unrelated. They also differ with respect to DNA base substitution: SP82 DNA contains 5-hydroxymethyluracil in place of thymine, whereas the DNA of β22 is unsubstituted. We were interested to know if the presence of the unusual base might favor survival and functioning of the SP82 genome in mixed infections. Secondly, previous observations (11) indicated that dominance, measured as the ability to produce progeny, was dependent on the bacterial host. When *B. subtilis* SB11, a derivative of the nontransformable strain W23, was mixedly infected with SP82 and β22, the latter suppressed development of the 5-hydroxymethyluracil-containing partner even when added to cells 5 min after infection with SP82. However, when the transformable strain of *B. subtilis* 168 was the host, dominance was dependent on the order of addition. When cultures were preinfected with SP82, β22 development was blocked; when the order of infection was reversed, β22 progeny were released and few SP82 were produced.

The results of the present investigation indicate that dominance in SP82-β22 mixed infections is correlated with qualitative and quantitative differences in the expression of the viral genomes.

MATERIALS AND METHODS

Bacterial strains, phages, and growth conditions. *B. subtilis* 168 (try$^-$) and *B. subtilis* SB11 (try$^-$) were obtained from E. W. Nester. Phages β22 and SP82 were supplied by R. H. Doi and C. H. Stewart, respectively. A modified M medium (16) was used for cell growth, isotopic labeling of infected cells, and preparation of phage lysates as described previously (9). Titering of phage, determination of progeny, and multiplicity of infection (MOI) were outlined earlier (11).

Nucleic acid hybridization. The method of Gillespie and Spiegelman (7) was used for RNA-DNA hybridization and that of Denhardt (5) for DNA-DNA hybridization. The procedure described earlier (9) for performing competition hybridization was modified as follows: all reaction mixtures contained 10 μg of [H^3]RNA, competitor RNAs as indicated and 40 μg of denatured DNA adsorbed to filters in a final volume of 0.3 ml of 0.3 M NaCl plus 0.03 M sodium citrate (2 × SSC). After incubation at 65 C for 15 h, the filters were removed, treated for 30 min with 10 μg of RNase A (Sigma Chemical Co., St. Louis, Mo.) per ml, washed thoroughly in 2 × SSC, and dried, and the radioactivity was determined using a toluene-based scintillant. Chloramphenicol-RNA (CM-RNA) was prepared by adding chloramphenicol (20 μg/ml final

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concentration) 5 min before adding phage; cells were harvested 15 min after phage addition. The hybridization reactions are highly specific (9). For example, addition of 1 mg of β22 RNA (RNA extracted from *B. subtilis* 5, 15, or 30 min after single infection with β22) produced a decrease of 3 to 5% in the binding of SP82-specific [H^3]RNA to SP82 DNA. The same percentage of decrease was obtained on addition of 1 mg of completely heterologous RNA (sea urchin RNA).

RESULTS

Progeny release and time of lysis in SP82-β22 mixed infections. Table 1 summarizes the results of studies of progeny released in the mixed infections discussed in the present paper. These data show that dominance was strain dependent, with β22 always blocking development of SP82 in mixed infections in *B. subtilis* SB11. In contrast, considerable numbers of SP82 progeny were released when SP82 was added to *B. subtilis* 168 cultures before or simultaneously with β22. It should be noted, however, that the dominant phage in either infection never produced the same yield of progeny in mixed infections as in single infections. Our previous studies (11) have shown that the progeny yields in these mixed infections are largely independent of MOI and that both phages adsorb with high efficiency to cells which have been infected simultaneously or consecutively with both phages. The time of lysis after infection with SP82 was 35 to 40 min for strain 168 and 40 to 50 min for strain SB11; there were no significant differences in the time of lysis (50 to 55 min) of the two host strains by β22. The time of lysis of mixed infections in either strain was 40 to 50 min; hence, it was not apparent whether lysis was controlled by one of the phages. However, the plaque-forming efficiency of SP82 was significantly lower on strain SB11 than on strain 168, and the progeny yield at low MOI (0.2) was markedly less in infections of SB11 than 168 although equally high progeny yields were obtained from infections at MOI of 5 to 10 (11). Comparisons of the two strains infected with β22 showed no significant differences either in the plaque-forming efficiency or the progeny yield at low MOI.

DNA synthesis in SP82-β22 mixed infections. Replication of DNA in bacteria singly or mixedly infected with β22 and/or SP82 was studied by pulse-labeling cells with [H^3]adenosine, isolating the DNA and hybridizing it to phage or host DNA immobilized on nitrocellulose membrane filters. The results (Table 2) show that very little host DNA was made in either single or mixed infections 20 to 30 min into the latent period whereas large amounts of β22 and SP82 DNA were synthesized in single infections of both host strains.

Control of DNA synthesis in mixedly infected cells corresponded closely to observations made in progeny studies. In *B. subtilis* 168, the first phage entering the cell was able to replicate its DNA whereas replication of the second phage

TABLE 1. *Relative number of progeny released in SP82-β22 mixed infections in Bacillus subtilis strains 168 and SB11*[a]

Host strain of *B. subtilis*	Time of addition of SP82 (min)	Time of addition of β22 (min)	SP82 progeny	β22 progeny
	0		100	
		0		100
168	0	0	30	4
	0	5	59	1
	5	0	8	20
	0		100	
		0		100
SB11	0	0	<1	78
	0	5	<1	50
	5	0	<1	21

[a] A value of 100 = 125 × 10^8 PFU per ml for β22 in *B. subtilis* 168 and 46 × 10^8 PFU per ml in strain SB11. The corresponding values for SP82 were 93 × 10^8 and 214 × 10^8 PFU per ml. The MOI was 6 for each phage.

TABLE 2. *Host and phage DNA synthesis in SP82-β22 mixed infections*[a]

Strain	Time of addition of phage (min)		Time of pulse[b]	Radioactivity (%) hybridized to DNA from:[c]		
	SP82	β22		SP82	β22	Host
168	0		20–30	58.7		0.4
		0	20–30		38.0	0.1
	0	5	25–30	35.8	5.4	0.14
	5	0	25–30	2.3	26.0	0
SB11	0		20–25	53.0		0.23
		0	20–25		44.5	0.11
	0	5	25–30	9.3	25.6	0.22
	5	0	25–30	0.36	27.0	0

[a] The experiments were performed at an MOI of 10 for each phage.

[b] Minutes after addition of the first phage.

[c] One-hundred percent radioactivity in these experiments ranged from 1,200 to 5,000 counts/min. The values given in the table were determined by plotting the counts per minute hybridized under conditions described in Materials and Methods when three concentrations of each labeled nucleic acid were incubated with 40 μg of each filter-bound DNA.

genome was greatly reduced. Quantitatively, the proportions of the two phage DNAs corresponded to the yields of progeny (Table 1). Viral genome replication in mixed infections conducted in *B. subtilis* SB11, on the other hand, was dominated by β22 regardless of the order of addition of the phages, although both SP82 and β22 grew in this host in single infections. However, the amounts of SP82 DNA synthesized in strain SB11 when this phage preceded β22 (ca. 9% of the input radioactivity hybridized) was large in relation to the negligible (< 1%) progeny yield. Thus, it seems unlikely that replication of SP82 DNA can be attributed to single infection of a small proportion of the cells and suggests that some replication of SP82 DNA occurred in cells infected with β22.

RNA synthesis in SP82-β22 mixed infections. Transcription in mixed infections was investigated by pulse-labeling cultures with [H^3]uridine at an early and a late time during the latent period, extracting the RNA and hybridizing it to filter-bound denatured SP82, β22, or host DNA. The data in Table 3 show that in mixed infections in *B. subtilis* 168 the first phage added to the culture largely controlled transcription with relatively little synthesis of RNA specific to the subordinate phage. Conversely, when *B. subtilis* SB11 served as host (Table 4), the order of addition had no effect: RNA specific for β22 was produced preferentially whether β22 was added before or after SP82. However, as in the case of DNA synthesis, a greater proportion of SP82-specific RNA was detected relative to the progeny yield (< 1%) when SP82 was added before β22. Again, these results probably cannot be attributed to synthesis of SP82 RNA by a small population of cells singly infected with this phage.

Analysis of SP82-specific RNA synthesized in mixed infections. The results of the preceding experiments indicate that dominance in SP82-β22 mixed infections was correlated with the ability of the successful phage, and an inability of the subordinate partner, to direct the synthesis of near normal amounts of nucleic acids. These results do not, however, provide any information concerning the nature of the phage-specific RNAs synthesized in the mixed infections. In several such infections, for example, the subordinate phage was able to produce small but significant amounts of RNA and it would be of interest to know whether the temporal sequence of transcription was the same as in single infections. Moreover, in all mixed infections, the yield of the dominant phage was less than that observed in single

TABLE 3. *Host and phage RNA synthesized in SP82-β22 mixed infections in B. subtilis 168*[a]

Time of addition of phage (min)		Time of pulse[b]	Radioactivity (%) hybridized to DNA from:[c]		
SP82	β22		SP82	β22	Host
0		5–10	9.1		5.5
0		20–25	26		4.2
	0	5–10		15	2.0
	0	20–25		22	2.8
0	5	10–15	22	2.4	5.0
0	5	25–30	38	3.6	1.9
5	0	10–15	8	31	4.2
5	0	25–30	10	35	2.8

[a] The experiments were performed at an MOI of 10 for each phage.
[b] Minutes after addition of the first phage.
[c] One-hundred percent radioactivity in these experiments ranged from 2,000 to 40,000 counts/min; percent hybridized to each DNA was calculated as described for Table 2.

TABLE 4. *Host and phage RNA synthesized in SP82-β22 mixed infections in B. subtilis SB11*[a]

Time of addition of phage (min)		Time of pulse[b]	Radioactivity (%) hybridized to DNA from:[c]		
SP82	β22		SP82	β22	Host
0		5–10	14.2		4.1
0		20–25	31.0		0.01
	0	5–10		25.4	3.0
	0	20–25		30.0	4.5
0	5	10–15	4.3	34.0	6.8
0	5	25–30	12.5	26.0	3.2
5	0	10–15	2.9	27.0	4.9
5	0	25–30	2.0	37.0	5.7

[a] The experiments were performed at an MOI of 10 for each phage.
[b] Minutes after addition of the first phage.
[c] One-hundred percent radioactivity and calculation of percent hybridized to each DNA was as described for Table 3.

infections of the same phage. It is possible that this "depressor effect" reflected qualitative and/or quantitative changes in the transcription of the dominant phage resulting from the presence of the subordinate phage.

Studies of the populations of phage-specific RNAs synthesized in mixed infections centered on SP82 because the transcriptional program of this phage has been studied more thoroughly than that of β22. Moreover, it seemed reasonable to assume that transcription of β22 in mixed infections in strain SB11 would parallel that in single infections since β22 dominated the infection with regard to synthesis of nucleic acids

and progeny yield. Previous investigations showed that six temporal classes of SP82-specific RNA can be distinguished during infection of *B. subtilis* 168 (13) and SB11 (Whiteley, unpublished data); these classes are defined on the basis of the onset and cessation of synthesis as measured by competition hybridization. A similar program of transcription has also been established for the related phage SPO1 (6). It should be noted, however, that competition hybridization using total phage DNA is a relatively insensitive method especially when applied to transcription of large phages such as SP82 and β22, which undoubtedly have highly complex transcriptional programs. However, direct comparisons of RNA populations produced in single and mixed infections should permit detection of large changes in the transcriptional program or marked quantitative differences in the RNA classes.

The analysis of SP82 transcription in mixed infections utilized three competitor unlabeled RNA preparations obtained from two strains of singly infected *B. subtilis*: CM-RNA, 15-min RNA, and 30-min RNA. CM-RNA was isolated from either strain 168 or strain SB11 15 min after infection with SP82 in the presence of chloramphenicol; such RNA is known to contain only early transcripts synthesized by unmodified host RNA polymerase (13, 14). The other two competitor RNAs were extracted from SP82-infected cells harvested at the indicated times during the latent period; these are expected to contain middle and late RNAs, respectively.

The data in Fig. 1 come from an analysis of radioactive RNAs isolated at two intervals from *B. subtilis* 168 cells infected first with SP82 and then with β22. The competition curves for this mixed infection show that CM-RNA was a moderately good competitor for the 10- to 15-min pulse-labeled RNA but only slightly reduced the binding of the 27- to 30-min pulse-labeled preparation. The 30-min unlabeled RNA, on the other hand, was an excellent competitor for the latter transcripts, but was a poor competitor for the 10- to 15-min radioactive RNA. As expected, 15-min RNA was a better competitor when tested with the 10- to 15-min labeled RNA than were either CM-RNA or 30-min RNA, indicating that a distinctive population of transcripts was present in the 10- to 15-min interval of the latent period. These data are consistent with the view that, as in single infections, early, middle, and late classes of SP82 RNA were made in this mixed infection in which SP82 was dominant and successful in producing progeny.

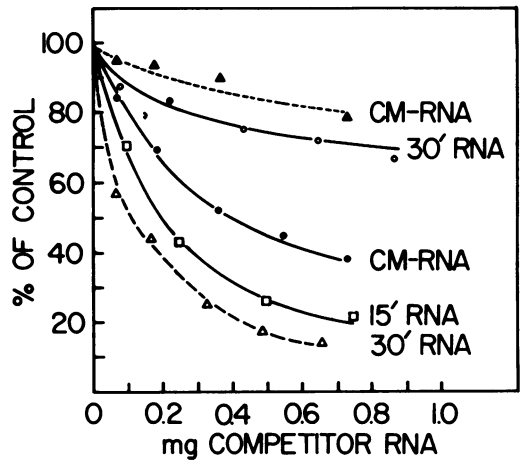

FIG. 1. *Competition between unlabeled RNA isolated from B. subtilis 168 singly infected with SP82 and radioactive RNA from B. subtilis 168 infected with SP82 5 min before addition of β22. Hybridization reactions were performed as described in Materials and Methods. The specific activities and percent hybridization in the absence of competitor RNA were 400 counts/min per μg and 18% for the 10- to 15-min [H³]RNA (———) and 1,418 counts/min per μg and 34% for the 27- to 30-min [H³]RNA (-----).*

Even though only a small amount of SP82-specific RNA was synthesized in the reciprocal experiment in which β22 was added 5 min before SP82, the classes of transcripts produced by the latter were qualitatively similar to those obtained in the preceding experiment (Fig. 2). Once again, a population of RNA molecules containing less late RNA and more early classes of RNA was found in the 10- to 15-min pulse-labeled RNA and the reverse was observed in the 25- to 30-min labeled preparation. It should be noted that the times of labeling do not correspond precisely to those in the previous experiment because SP82 was added 5 min after β22, but the data show that the most easily distinguished possible changes in transcription (i.e., continuation of early RNA synthesis into late times or production of late RNA at an earlier time in the latent period) did not occur. The above observations imply that any modification of the RNA polymerase or transcription factors by the dominant β22 phage (i.e., the first added phage) did not interfere, at least qualitatively, with the transcription of the second phage genome.

The analyses of SP82-specific RNA synthesized in mixed infections in *B. subtilis* SB11 were limited to the infection in which SP82 was added 5 min before β22 since only negligible amounts of SP82 RNA were found in the reciprocal experiment. The results presented in

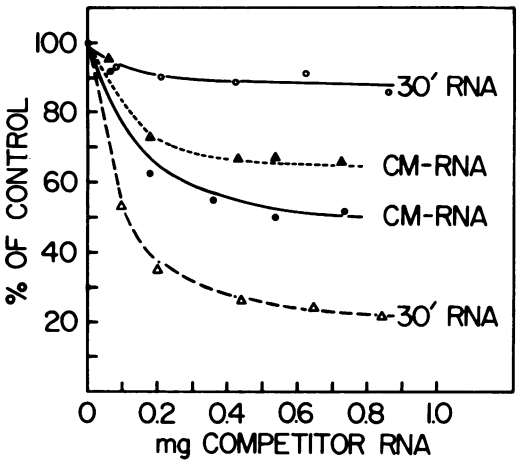

FIG. 2. *Competition between unlabeled RNA isolated from B. subtilis 168 singly infected with SP82 and radioactive RNA from B. subtilis 168 infected with β22 5 min before addition of SP82. Hybridization reactions were performed as described in Materials and Methods. The specific activities and percent hybridization in the absence of competitor RNA were 300 counts/min per μg and 6% for the 10- to 15-min [H³]RNA (——) and 550 counts/min per μg and 10% for the 25- to 30-min [H³]RNA (-----).*

Fig. 3 show some qualitative and quantitative differences from the competition curves obtained in the analogous experiment in *B. subtilis* 168 (Fig. 1). First, it may be noted that the 10- to 15-min pulse-labeled RNA from the infection in strain SB11 was almost completely competed by CM-RNA whereas its binding to DNA was only slightly affected by 30-min RNA. Although similar to the results with the corresponding radioactive RNA from mixedly infected *B. subtilis* strain 168, the enhanced effectiveness of CM-RNA in the present experiment suggests that RNA synthesized 10 to 15 min after infection in SB11 contained mostly early transcripts rather than middle RNAs. This conclusion was also supported by the competition with 15-min RNA. In the SP82-β22 mixed infection in strain 168 (Fig. 1), the latter RNA was the most efficient competitor of 10- to 15-min pulse-labeled RNA, whereas in the corresponding experiment in strain SB11 15-min RNA was not as effective as CM-RNA. Together, these experiments suggest a delay in the synthesis of middle classes of RNA in the mixed infections in SB11.

Competition experiments with 25- to 30-min pulse-labeled RNA obtained from mixedly infected SB11 cells demonstrate, however, that at least some classes of late SP82 RNAs were synthesized by this subordinate phage. However, CM-RNA remained a more effective competitor of 25- to 30-min pulse-labeled RNA than of the corresponding RNA from the identical infection in *B. subtilis* 168 in which SP82 was dominant.

Time of synthesis of SP82 RNA in the two host strains. The above finding that CM-RNA was a significantly more effective competitor in the binding of early [H³]RNA synthesized in strain SB11 (Fig. 3) than in the same experiments performed with [H³]RNA produced after infection of strain 168 (Fig. 1) suggests that the temporal program of SP82 RNA synthesis in the former strain must be altered in some way. To determine whether this was a feature of mixed infections or reflected some inherent aspect of the infection of each host by SP82, a comparison was made of the extent of synthesis of SP82-specific RNA in single infections of the two host strains. There was markedly less SP82 RNA detected in [H³]RNA extracted early in the infection when strain SB11 was the host, suggesting that an early step in the phage infection was affected (Table 5). In contrast, synthesis of SP82 RNA began promptly after infection of strain 168. However, approximately equal levels of SP82 RNA were found in RNAs extracted from both strains later in the infection. No significant differences could be found in the over-all incorporation of [H³]uridine into RNA or in the specific activities of host RNA polymerases either in crude extracts or in purified fractions obtained from uninfected cells of the two *B. subtilis* strains (Spiegelman and

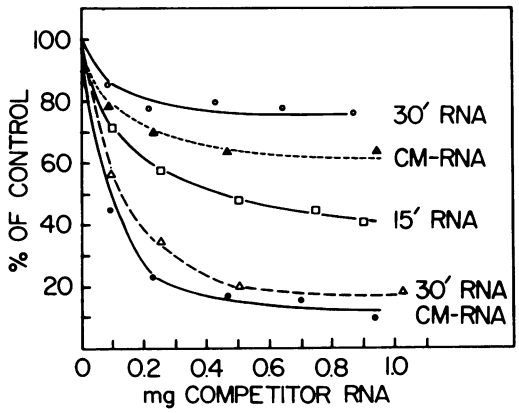

FIG. 3. *Competition between unlabeled RNA isolated from B. subtilis SB11 singly infected with SP82 and radioactive RNA from B. subtilis SB11 infected with SP82 5 min before addition of β22. Hybridization reactions were performed as described in Materials and Methods. The specific activities and percent hybridization in the absence of competitor RNA were 1,040 counts/min per μg and 4% for the 10- to 15-min [H³]RNA (——) and 810 counts/min per μg and 12.5% for the 25- to 30-min [H³]RNA (-----).*

TABLE 5. *Synthesis of SP82 RNA in two strains of B. subtilis*

Time of pulse[b]	Radioactivity (%) hybridized to SP82 DNA when pulse-labeled in:[c]	
	B. subtilis 168	*B. subtilis* SB11
-2-1.5	1.37	0.25
-2-3	2.4	0.25
0-5	8.2	1.5
5-10	9.1	14.2
10-15	12.4	17.8
20-25	26.0	31.0

[a] The experiments were performed at an MOI of 10 for each phage.

[b] Minutes, relative to addition of phage.

[c] One-hundred percent radioactivity in these experiments ranged from 17,300 to 80,000 counts/min; percent hybridized to SP82 DNA was calculated as described for Table 2.

Whiteley, unpublished data). Thus, it does not seem likely that the decreased level of SP82 transcription in strain SB11 can be attributed to the presence of a less active host RNA polymerase. It will be recalled that the synthesis of early RNA classes in this infection, as in other phage infections (1, 2), is catalyzed by the unmodified host polymerase (13).

DISCUSSION

From the results of the present study of SP82-β22 mixed infections and previous investigations of β22-SPO2c$_1$ and SP82-SPO2c$_1$ infections (9), it can be concluded that the subordinate phage is unsuccessful because it cannot efficiently replicate and transcribe its DNA. It is also possible, of course, that other steps in development of this phage are inhibited, but the reduced capacity to synthesize nucleic acids would, in itself, be sufficient to explain subordination. The dominant phage, on the other hand, carries out a program of DNA replication and transcription which appears to be qualitatively similar over-all to that occurring in single infections but which may be quantitatively less and thus correlated with the depressor effect.

In β22-SP82 mixed infections in which *B. subtilis* 168 was the host bacterium, dominance was dependent on the order of addition and it is tempting to speculate that the first phage, perhaps reflecting a temporal advantage in synthesizing the first proteins, interfered with expression of the second phage. Such interference could occur at several points. We have previously considered the possibility that the first phage saturates all receptor sites, thus preventing attachment of the second phage, but have found that the super-infecting phage binds with high efficiency (11).

A more likely locus of competition may be the membrane replication positions or transcription sites. Snustad (15) has shown that in T4 infections of *E. coli* only a limited number of viral genomes can be replicated or phenotypically expressed in multiply infected cells. One interpretation of this finding is that each cell contains a finite number of compartments, perhaps membrane sites, one of which must be occupied by a phage genome before viral-directed macromolecular synthesis can occur. Evidence for an exclusion from genetic activity, suggesting similar compartmentalization, has been obtained in studies of sequential infections of *B. subtilis* by mutants of phage ϕ1 (C. W. Rettenmier and H. E. Hemphill, unpublished data). It is not known, however, whether unrelated phages such as SP82 and β22 compete for the same sites.

Another early step in phage development which might affect the yield of the second phage are viral-directed changes in the host DNA-dependent RNA polymerase. Infection of *B. subtilis* 168 with SP82 results in the appearance of a modified RNA polymerase as early as 5 to 10 min after infection (14; unpublished data). This altered enzyme has a higher specific activity than the host enzyme and has a different subunit composition: it contains the β, β', and α subunits found in the host enzyme but has a greatly diminished quantity of σ subunit and contains small polypeptides not found in the host enzyme. The modified polymerase synthesizes early and middle classes of SP82 RNA in vitro, whereas the host polymerase produces only early RNA in vitro (13). Although the polymerase isolated from β22-infected cells has not been examined in similar detail, it is known that the subunit composition is also different from that of the host enzyme: it has a lower content of the σ subunit and has small polypeptides whose molecular weights are different from the small polypeptides found in SP82-modified polymerase (Whiteley et al., unpublished data). Preliminary investigations of enzyme specificity in vitro indicate that purified SP82-modified RNA polymerase transcribes only early RNAs from β22 DNA. If the modified β22 polymerase has the same type of specificity, dominance in *B. subtilis* 168 could result from the inability of the second phage to produce a sufficient quantity of modified polymerase. A small amount of the latter enzyme could be synthesized, however, since host protein synthesis, including the production of core polymerase

subunits, continues until at least the mid-point of the latent period (Spiegelman and Whiteley, unpublished data). Such synthesis could account for the observed production of small amounts of SP82-specific middle and late RNA populations in infections where this phage was the subordinate partner.

Perhaps the most puzzling aspect of the β22-SP82 mixed infections was the ability of β22 to block development of SP82 when *B. subtilis* SB11 was the host. In single infections of SB11, at the high MOI used in the present study, SP82 yielded progeny, modified the polymerase (Spiegelman and Whiteley, unpublished data), and carried out a program of transcription which was similar to that found in strain 168. One might, therefore, expect that SP82 would produce progeny under the same conditions in which it expressed dominance in mixed infections of strain 168 (i.e., when SP82 was the first phage infecting the cell). This would be especially true if dominance depends on a competition for sites of genetic expression. Interference in the production of SP82 progeny could also occur at the translational level or in the final stages of phage assembly. These possibilities receive some support from the finding that an appreciable amount of SP82-specific RNA (ca. one-third the amount synthesized in single infections) is produced at a late time in the mixed infection even though very few intact phages are released.

It is also known that *B. subtilis* SB11 and other derivatives of W23 possess an interference mechanism which causes abortive infection of several 5-hydroxymethyluracil-containing phages at low MOIs (12). For example, the plating efficiency of SP82 is 10,000-fold lower in SB11 than in 168. This interference phenomenon is not seen at high MOI in liquid media and the development of the phage appears normal by the criteria studied in the present investigation. However, the requirements for high MOI for productive infection in SB11 and the present finding that the transcription of the SP82 genome is reduced early in infection suggests that some cooperative effect between several SP82 genomes may be needed for the infection to succeed. A similar type of phenomenon has been noted previously for transfection of *B. subtilis* by SP82 DNA (8). Several molecules of DNA are required for transfection apparently because the entering DNA is inactivated by host nuclease(s). McAllister and Green (10) reported that this inactivation process is inhibited in phage infection of a transformable strain and that maximal inhibition occurs at approximately 6 min after addition of the phage. Thus, in β22-SP82 mixed infections in strain SB11, β22 could either interfere with some cooperative process required for successful infection of SB11 by SP82 or the temporal delay required to organize the cooperative process could provide a sufficient advantage so that β22 can dominate the infection.

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