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Roles of Genes 44, 50, and 51 in Regulating Gene Expression and Host Takeover during Infection of *Bacillus subtilis* by Bacteriophage SPO1

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We show that the products of SPO1 genes 44, 50, and 51 are required for the normal transition from early to middle gene expression during infection of *Bacillus subtilis* by bacteriophage SPO1; that they are also required for control of the shutoff of host DNA, RNA, and protein synthesis; and that their effects on host shutoff could be accounted for by their effects on the regulation of gene expression. These three gene products had four distinguishable effects in regulating SPO1 gene expression: (i) gp44-50-51 acted to restrain expression of all SPO1 genes tested, (ii) gp44 and/or gp50-51 caused additional specific repression of immediate-early genes, (iii) gp44 and/or gp50-51 stimulated expression of middle genes, and (iv) gp44 and/or gp50-51 stimulated expression of some delayed-early genes. Shutoff of immediate-early gene expression also required the activity of gp28, the middle-gene-specific sigma factor. Shutoff of host RNA and protein synthesis was accelerated by either the 44^- single mutant or the 50^-51^- double mutant and more so by the $44^-50^-51^-$ triple mutant. Shutoff of host DNA synthesis was accelerated by the mutants early in infection but delayed by the $44^-50^-51^-$ triple mutant at later times. Although gp50 is a very small protein, consisting almost entirely of an apparent membrane-spanning domain, it contributed significantly to each activity tested. We identify SPO1 genes 41 to 51 and 53 to 60 as immediate-early genes; genes 27, 28, and 37 to 40 as delayed-early genes; and gene 52 as a middle gene.

During infection of *Bacillus subtilis* by bacteriophage SPO1, there is a complex program of gene actions. Early genes are transcribed by the unmodified host RNA polymerase (RNAP), from promoters recognized by *B. subtilis* σA. Early gene 28 specifies a new sigma factor, gp28, which substitutes for σA and directs transcription of the middle genes. Middle genes 33 and 34 specify a new sigma factor and accessory protein, which direct transcription of the late genes (4, 5, 10, 18). Each of the major groups of genes can be divided into subsets showing different regulatory patterns, which cannot be accounted for by the known regulatory mechanisms. For the most part, middle genes specify the phage DNA replication machinery, while the late genes specify the structural and morphogenetic proteins (16).

The early genes appear to be involved primarily in host takeover, in which the host's biosynthetic machinery is subverted to the purposes of the infecting bacteriophage (17). Host mRNA, protein, and DNA syntheses are shut off rapidly and are replaced by synthesis of the corresponding phage-specific macromolecules (5, 9, 12, 15).

SPO1 has 26 known early genes, arranged in 13 operons, whose transcription is directed by early promoters P_E1 to P_E13, each recognized by the host RNAP with σA (1, 13, 16, 17). (Two other early promoters are known [1], but the downstream genes have not been identified.) Operons 1 to 12 form a continuous cluster of 24 genes, genes 37 to 60, which we have called the "host takeover module," located within an 11.5-kb segment that constitutes most of the SPO1 terminal redundancy (17) (accession number AF031901). In operon 13, genes 28 and 27 are transcribed from P_E13, and gene 27 is also transcribed from a middle promoter that lies between 28 and 27 (2).

Our initial studies of the roles of individual genes in host takeover have focused on genes 44, 50, and 51. When gene 44 was expressed in uninfected cells of either *B. subtilis* or *Escherichia coli*, it caused the shutoff of bacterial RNA synthesis and cell death (20, 21). DNA and protein synthesis were also shut off, presumably as indirect results of the effect on RNA synthesis. An *E. coli* mutation substituting valine for glutamate at position 1272 of the B subunit of the RNAP provided resistance to the lethal effects of gene 44 (21; A. Sampath, unpublished results). Genes 51 and 50 constitute a two-gene operon which, when expressed in uninfected cells, also caused the shutoff of RNA, DNA, and protein synthesis and cell death (our unpublished results). The products of both gene 44 and gene 51 include acidic-hydrophobic domains similar to the domain in *E. coli* σ54 that is required for binding to RNAP (19), and one segment of gp51 shows substantial similarity to the product of SPO1 gene 27, which is required for normal transcription of late genes (7, 17). Thus, it seemed likely that the roles of gp44 and gp51 in infection were to interact with the host RNAP, presumably for the purpose of changing its specificity from host to phage transcription, and that one consequence of this interaction with either gp44 or gp50-51 was inactivation of the RNAP with respect to transcription of host genes.

To test that hypothesis, we inactivated each of genes 44, 50, and 51 by mutation and tested the effects of these mutations on

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TABLE 1. Effect of triple mutant on stability of specific RNAs[a]

Gene(s)	$44^{-}50^{-}51^{-}$			28^{-}			Wild type		
	Initial activity at 15 min postinfection	Activity remaining after 10 min of rifampin	Fraction surviving	Initial activity at 15 min postinfection	Activity remaining after 10 min of rifampin	Fraction surviving	Initial activity at 15 min postinfection	Activity remaining after 10 min of rifampin	Fraction surviving
28	48	18	0.38	350	113	0.32	83	35	0.42
37–39	736	338	0.46	1,112	404	0.36	349	166	0.48
41	608	37	0.06	45	0		0	0	
42	399	22	0.06	51	0		0	0	
43	563	28	0.05	60	0		0	0	
44	3,847	1,966	0.51	407	41	0.1	70	7	0.1
45–46	1,546	168	0.11	276	40	0.14	32	21	0.66
56–58	2,654	386	0.15	679	45	0.07	32	12	0.38
59–60	2,816	218	0.08	611	78	0.13	0	0	

[a] In cultures parallel to those analyzed for Fig. 1, rifampin was added 15 min after infection, and the RNA was extracted 10 min later. The proportion of RNA surviving rifampin treatment was determined by densitometry, as described in Materials and Methods.

host shutoff during infection. (Gene 50 was included because the last 8 nucleotides of gene 51 overlap the first 8 nucleotides of gene 50, suggesting the possibility of translational coupling and joint activity. Its product includes only 23 amino acids and consists almost entirely of an apparent membrane-spanning domain, with charged amino acids at each end.) We show that, contrary to our expectations, the mutations accelerated shutoff, suggesting that the role of these gene products in host shutoff may be regulatory, rather than directly causative. In confirmation of that suggestion, we show that the mutations do affect the expression of all known SPO1 early genes and of all of the middle genes tested and that these effects on gene expression are sufficient to account for the observed effects on host shutoff.

In the course of assaying their expression, we have been able to identify each of the known early genes as immediate-early genes, delayed-early genes, or, in one case, actually a middle gene.

MATERIALS AND METHODS

Growth conditions. Bacterial strains, growth media, bacterial growth, and phage infection were as described previously (20). Routine propagation of phage cultures was at 37°C in veal-yeast extract (VY) medium on CB313, a nonsense-suppressor strain. Experiments testing the effects of the mutations were done at 30°C on CB10, the nonsuppressing strain, in the medium designated for each type of experiment. The generation time and the latent period were about twice as great at 30°C as at 37°C, providing greater resolution of events occurring at different times and also, serendipitously, increasing observed differences between mutants and wild type.

SPO1 strains. The formation of the gene 44 mutation was described previously (20), where it was called e3m3. For genes 50 and 51, cloned genes were mutagenized using the Stratagene QuikChange kit. In each case, a nonsense codon was substituted for the first lysine codon, which was codon 3 for each of the three genes. Each mutagenized plasmid was allowed to recombine with superinfecting SPO1. Recombinant progeny were identified by plaque-lift hybridization, using the Amersham ECL 3′-oligolabeling and detection kit, and allowed to segregate pure mutant strains as described previously (20). Multiple mutants were produced by successive recombination with the appropriate mutagenized plasmids, and all genotypes were confirmed by DNA sequencing. The mutants were propagated on a suppressor strain, CB313, which inserts lysine at nonsense codons. The first 8 nucleotides of gene 50 overlap the last 8 nucleotides of gene 51, and the gene 50 mutation destroys the termination codon of gene 51, apparently permitting translation of gene 51 to run on for about 30 more amino acids, beyond its normal 151. Thus, definitive conclusions about the role of gp50 could not be made from single mutants in which only gene 50 was mutated. The gene 28 mutant was derived from mutant F21, initially described by Okubo et al. (11), by five successive backcrosses to wild-type SPO1. We have previously called it sus28-1 (3), and our lab bookkeeping lists it as F21 (5).

RNA preparation. Cultures in VY medium were harvested at the indicated times after infection, as described previously (20). The RNA was purified using the Qiagen RNeasy Mini kit, following the manufacturer's protocol.

Dot blots. PCR products carrying a particular gene or genes were denatured by incubating them for 15 min at 37°C in 0.2 M NaOH. Ten nanograms of DNA in 2 μl was spotted onto positively charged nylon membranes, Amersham Hybond N^{+}, and bound by UV cross-linking. Each 11- by 5-cm membrane held an array of about 23 spots. The PCR fragments included all, or nearly all, of the specified gene or genes, except that the one for gene 31 included only the first exon.

Hybridization. For each RNA preparation, a 1.0-μg aliquot was labeled with alkaline phosphatase, using the Amersham AlkPhos direct labeling and detection kit. The entire labeled preparation was hybridized to one membrane carrying a dot blot array, with use of the hybridization buffer defined in the kit. Hybridization was at 60°C for 2 h in a Robbins Model 400 hybridization incubator rotating at about 4 rpm. Wash buffers were as defined in the kit, with the primary wash at 65°C and the secondary wash at room temperature. Detection of hybridized RNA used the Amersham CDP Star chemiluminescent detection reagent, following procedures from the kit. The alkaline phosphatase catalyzes decomposition of dioxetane with emission of light, which is detected on film, with the use of Hyperfilm ECL from Amersham. All hybridizations were done under the same conditions, without attempting to identify optimal conditions for each gene, and we have not made independent measurements of the concentrations of the various transcripts or of their intrinsic hybridization efficiencies. Thus, gene-to-gene differences in the absolute amount of signal detected could be due to differences in intrinsic hybridization or labeling efficiency. Conclusions were drawn only from comparisons between signal intensities for the same gene under different conditions.

Densitometry. The intensities of the dot blot signals were measured with a Molecular Dynamics computing densitometer. Positions at which no spot was visible on the film gave intensity values ranging from −20 to +20. Some spots that were faintly visible gave intensity readings less than 20, which are reported as measured, with the recognition that they represent only approximations of the actual value. Spots not visible on film are reported as 0. Intensity values as high as 1,700 were within the linear dose-response range. Where undiluted samples gave values beyond the linear range, we measured hybridization by diluted samples and corrected for the dilution factor and the experiment-to-experiment difference in hybridization efficiency (see details for Table 1 in next section).

Measurements of RNA stability, as reported in Table 1. Rifampin was obtained from Sigma and was used at a final concentration of 4 μg/ml, which is four times the concentration of rifamycin that was sufficient to prevent SPO1 RNA synthesis (6). Pilot experiments showed that that concentration was sufficient to prevent synthesis of SPO1 RNA detectable by our assay system. In cultures parallel to those analyzed in Fig. 1, rifampin was added at 15 min after infection, and the RNA was extracted 10 min later. Hybridization and detection of this RNA were performed as described above. For two delayed-early and seven immediate-early genes (ranging from the lowest to the highest signal intensities), Table 1 shows the intensity at 15 min, the intensity remaining after 10 min of further incubation with rifampin, and the proportion of the activity present at 15

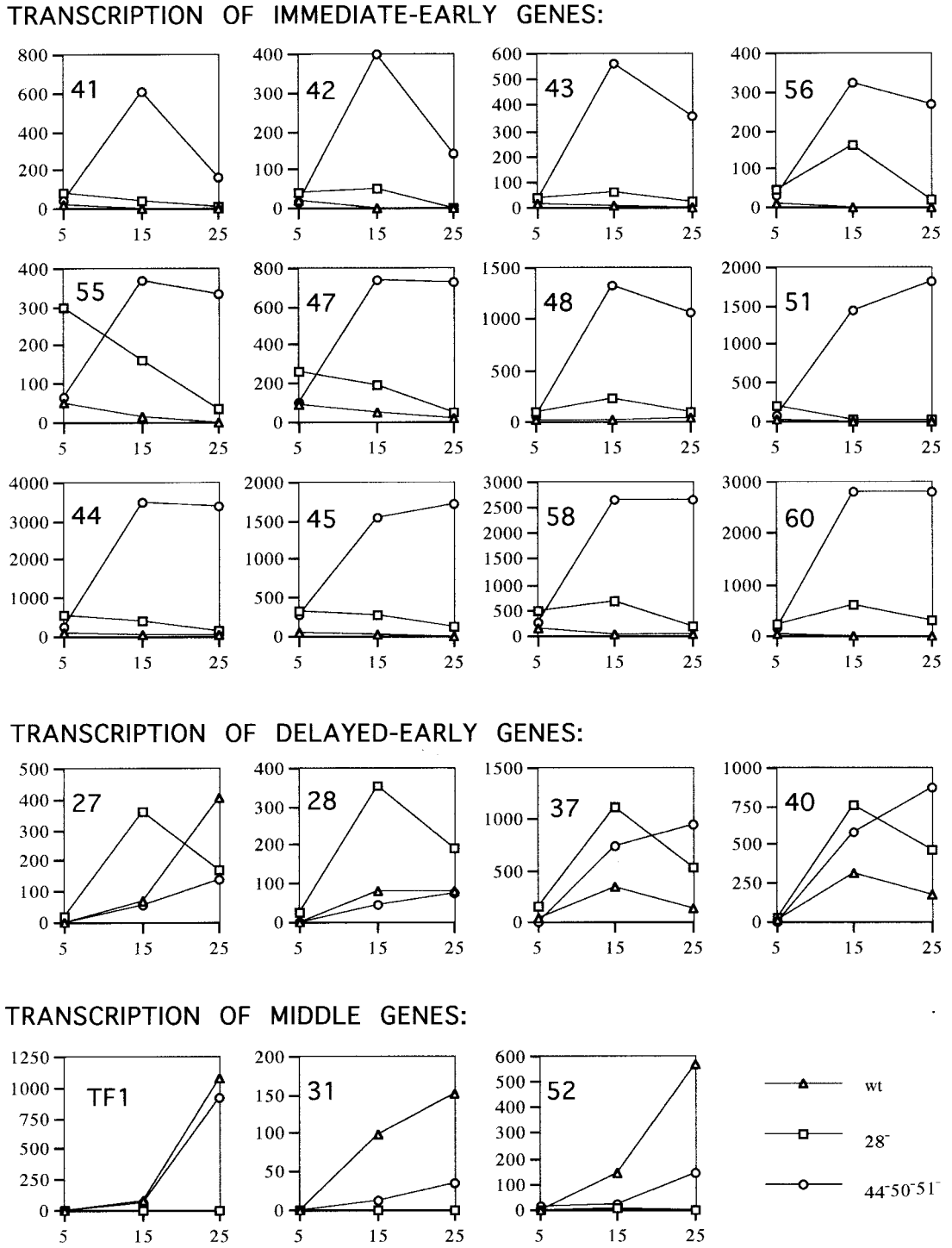

FIG. 1. Gene-specific expression patterns. Cultures infected with wild-type (wt) SPO1, mutant 28^-, or triple mutant $44^-50^-51^-$ were harvested at 5, 15, or 25 min (*x* axis) after infection at 30°C. RNA was prepared, labeled, hybridized to dot blots, and quantitated by densitometry as described in Materials and Methods. The densitometric values for each gene (*y* axis) are plotted as a function of time after infection. In each graph, the number in the upper left corner indicates the gene whose activity is plotted. Where an operon of more than one gene was assayed, the graph is labeled with the number of the first gene in the operon. This applies to operons 37-39, 45-46, 48-49, 51-50, 55-53, 58-56, and 60-59. Note that the scales are individualized for each gene and that the maximum levels of activity vary by as much as 20-fold. To a first approximation, the immediate-early genes have been arranged from top to bottom in order of increasing activity. Gene 27 could have been displayed with either the delayed-early or the middle genes..

min that survived the 10-min incubation with rifampin (the ratio of the first two numbers). For genes 44, 45-46, 56-58, and 59-60, hybridization to the undiluted RNA from the triple mutant at 15 min gave a signal beyond the linear range. Therefore, the data for those genes from the triple mutant were taken from spots made by hybridization to 1:4 dilutions of the 15-min RNA preparations. Since those values were compared with the values for the same genes after 10 more min of incubation with rifampin, the latter values for the same four genes were also taken from 1:4 dilutions. Each value thus obtained was multiplied by the dilution factor and by a normalization factor, which corrects for the experiment-to-experiment difference in hybridization efficiency. The normalization factor was the ratio of the average signal given by hybridization of undiluted RNAs in the original experiment to the average signal given by the same undiluted RNAs in a control hybridization done simultaneously with the diluted samples, making use only of signals that were in the linear range. Since both the dilution factor and the normalization factor were the same for the two sets of samples, they did not affect the calculation of the fraction surviving. These measurements of stability are imprecise, because of the low signal-to-noise ratio at low RNA concentrations (particularly significant for wild type, where most starting values were already low) and because the activity that survived rifampin treatment included RNA that was synthesized after the rifampin was added but before it could completely stop RNA synthesis. Thus, the calculated ratios probably overestimate the proportion of RNA existing at 15 min that survived until 25 min, so the triple mutant RNA probably was even less stable than indicated by the calculated ratios.

Pulse-labeling. Rates of synthesis of host and phage RNA or protein were measured in C4 medium as described previously (20), by pulse-labeling for 5 min with [5-^{3}H]uridine or [4,5-^{3}H]leucine, precipitating with trichloroacetic acid, and counting the precipitate. Since SPO1 DNA contains hmUra in place of thymine, host DNA synthesis could be measured independently by pulsing with [methyl-^{3}H]thymidine. Phage DNA synthesis was measured by pulsing with [2,8-^{3}H]adenine and measuring alkali-stable, trichloroacetic acid-precipitable counts. That measures host DNA synthesis as well, but most host DNA synthesis has been shut off by the time that phage replication begins, even in mutant infections, so incorporation of label after 12 min is overwhelmingly into phage DNA. The time given for each pulse is the time at which the pulse began.

RESULTS

Identification of immediate-early, delayed-early, and middle genes. Expression of specific genes was measured by dot blot hybridization and densitometry, as described in Materials and Methods. Figure 1 shows the expression patterns of all of the known early genes and several representative middle genes, in the wild type or when altered by either the 28$^-$ mutation or the 44$^-$50$^-$51$^-$ triple mutant. The 28$^-$ mutation inactivates the middle-gene-specific sigma factor, permitting identification of middle gene activity. Note that different genes are expressed on different scales, as the absolute level of expression varies widely from gene to gene.

Genes 31 and TF1 were previously known to be middle genes (8, 14). The others are now identified as immediate-early (genes 41 to 51 and 53 to 60), delayed-early (genes 27, 28, and 37 to 40), or middle (gene 52), on the basis of time of expression in wild-type or 28$^-$ infection and of gp28 dependence of their expression. Gene 27 had characteristics of both delayed-early and middle genes. Genes 48 and 49 also showed substantial gp28-dependent expression at late times (data not shown).

Roles of genes 44, 50, and 51 in regulating early and middle gene expression. The top three rows of Fig. 1 show that expression of every immediate-early gene was dramatically increased by the triple mutant, in comparison with its expression in wild-type infection. In contrast, the triple mutant decreased expression of the middle genes, slightly for TF1 and substantially for genes 31 and 52. Thus, the mutation of genes 44, 50, and 51 inhibited the transition from immediate-early to middle gene expression, suggesting that those gene products are required for normal progress of that transition. (The delayed-early genes are more complicated and will be discussed with Fig. 2.)

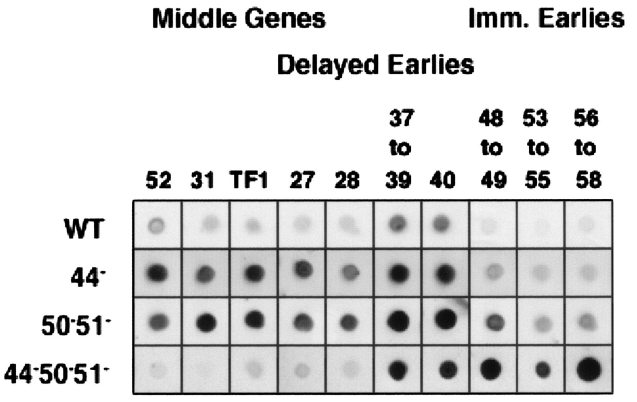

FIG. 2. Differential effects of mutations on immediate-early, delayed-early, and middle transcription. Cultures infected with wild-type (WT) SPO1, mutant 44$^-$, double mutant 50$^-$51$^-$, or triple mutant 44$^-$50$^-$51$^-$ were harvested 15 min after infection at 30°C. RNA was prepared, labeled, hybridized to dot blots, and detected on film as described in Materials and Methods. The films were scanned using the UMAX Vista Scan program with a UMAX Astra 1220S scanner. A 25- by 25-pixel square surrounding each spot was copied and pasted into the grid displayed. The numbers at the top represent the gene or genes present in that spot on the dot blot. All of the middle and delayed-early genes assayed are presented, along with three representative immediate-early operons. The gene categories are indicated across the top. Gene 27 falls under both middle and delayed-early, since it is expressed from both an early and a middle promoter.

Since the dot blot hybridizations measure the total amount of specific RNAs present at a particular time, rather than the rate of synthesis, it was possible that the increased quantities of specific RNAs obtained from cells infected by the triple mutant were due to increased stability rather than to increased synthesis. We tested this by using rifampin to inhibit new RNA synthesis and observing the persistence of RNA already present. In cultures parallel to those analyzed for Fig. 1, rifampin was added at 15 min after infection, and the RNA was extracted at 25 min. Table 1 shows that, except for gene 44 RNA, the RNAs made by the triple mutant were not substantially more stable than those made by the wild type or the 28$^-$ mutant.

Figure 2 shows the effects of the separate components of the triple mutant, the single mutant 44$^-$ and the double mutant 50$^-$51$^-$. It shows that the products of genes 44, 50, and 51 had four distinguishable effects, one requiring all of gp44-50-51 and the others requiring either gp44 and/or gp50-51.

Effect 1. gp44-50-51 acted to repress every gene's expression, and the full effect of this repression required the participation of both gp44 and gp50-51. This can be seen by comparing the data in the wild-type row with those in the next two rows. The expression of every gene was increased when either gene 44 or genes 50 and 51 were inactivated by mutation.

Effect 2. Either gp44 or gp50-51, even in the absence of the other one, caused residual repression of immediate-early genes. This can be seen by comparing the two middle rows with the bottom row, looking specifically at the immediate-early genes. Although the repressive activity of effect 1 had been inactivated by either the 44$^-$ or 50$^-$51$^-$ mutation, those strains

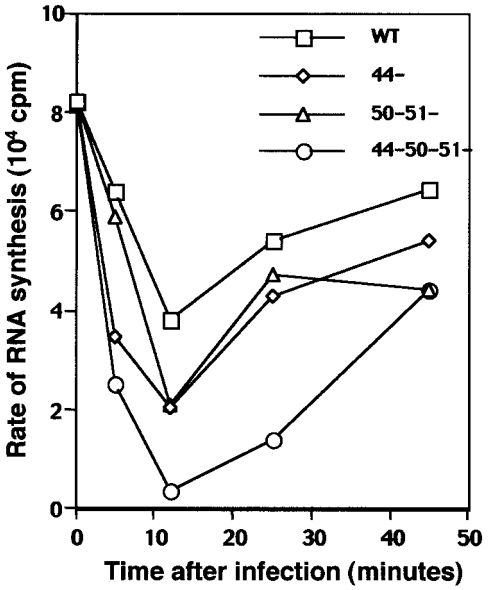

FIG. 3. Shutoff of host RNA synthesis. Rates of RNA synthesis were measured by pulse-labeling with [5-^{3}H]uridine as described in Materials and Methods, as a function of time after infection by the indicated strains.

retained substantial repressive activity, which was lost when the remaining wild-type gene(s) was inactivated to form the triple mutant.

Effect 3. Either gp44 or gp50-51 stimulated middle gene activity. This can be seen by comparing the two middle rows with the bottom row, looking specifically at the middle genes. When the repressive activity of effect 1 had been inactivated by either the 44^- or the 50^-51^- mutation, those strains had active middle gene expression, as seen in the two middle rows. Much of this expression depended upon the remaining active gp44 (in the 50^-51^- mutant) or gp50-51 (in the 44^- mutant), since it was lost when those genes were inactivated to form the triple mutant.

Effect 4. Either gp44 or gp50-51 stimulated the expression of certain delayed-early genes. This can be seen by comparing the two middle rows with the bottom row, looking specifically at the delayed-early genes. When the repressive activity of effect 1 had been inactivated by either the 44^- or the 50^-51^- mutation, those strains had active delayed-early gene expression, as seen in the two middle rows. For genes 27 and 28, much of this expression depended upon the remaining active gp44 (in the 50^-51^- mutant) or gp50-51 (in the 44^- mutant), since it was lost when those genes were inactivated to form the triple mutant. For genes 37 to 40, the same activities appear to be present, but the effect of gp50-51 was so slight that it could not be documented conclusively.

In effects 2 and 3, both gp44 and gp50-51 had activities that repressed immediate-early gene expression and stimulated middle gene expression, thus facilitating the transition from immediate-early to middle gene expression.

Roles of genes 44, 50, and 51 in shutoff of host-specific biosyntheses. (i) Shutoff of host gene expression. The triple mutant caused host RNA synthesis to be shut off much more rapidly and completely than in wild-type infection. Figure 3 shows the rate of RNA synthesis as a function of time after infection. These assays measure the sum of host and phage RNA synthesis, so the decrease in total RNA synthesis by 12 min after infection provides a minimum estimate of the extent to which host RNA synthesis had been shut off by that time. By 12 min after infection, the rate of RNA synthesis in triple mutant infection was decreased to less than 10% of the rate in wild-type infection and to less than 5% of the rate in uninfected cells.

Each of the three mutations contributed to this effect. 44^- and 50^-51^- each shut off host RNA synthesis more completely

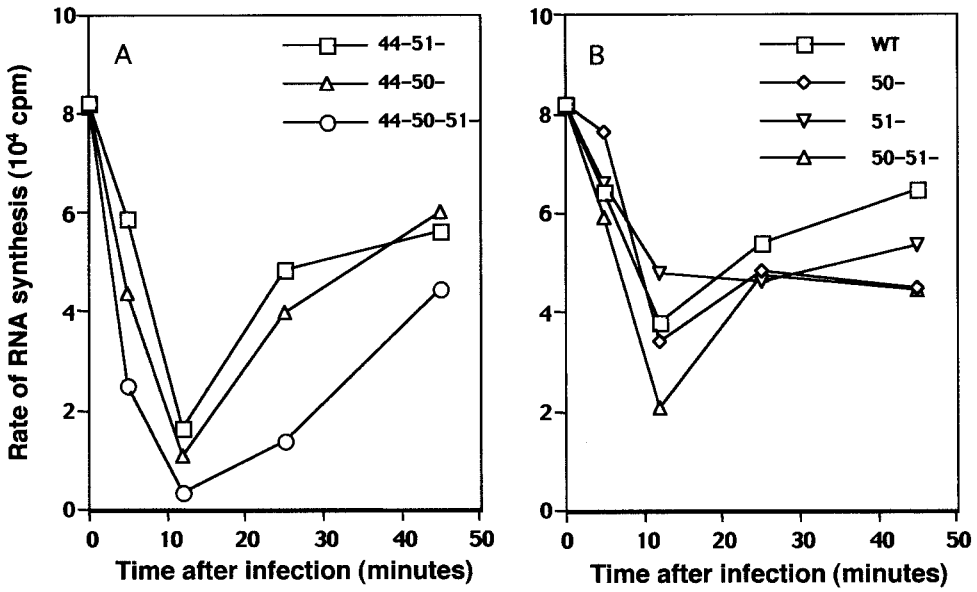

FIG. 4. Distinguishable effects of genes 50 and 51. Rates of RNA synthesis were measured by pulse-labeling with [5-^{3}H]uridine as described in Materials and Methods, as a function of time after infection by the indicated strains.

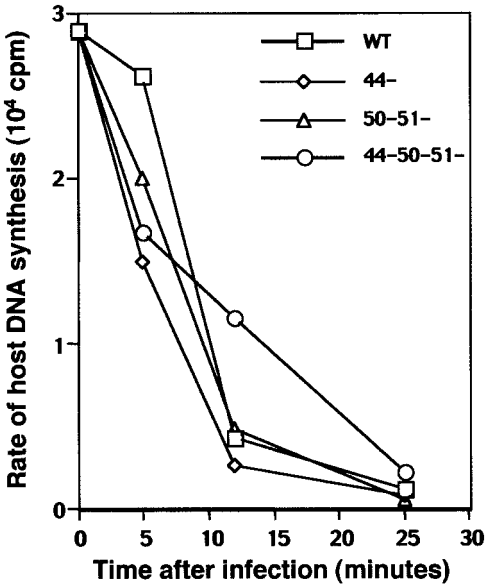

FIG. 5. Shutoff of host DNA synthesis. Rates of host DNA synthesis were measured by pulse-labeling with [methyl-^{3}H]thymidine as described in Materials and Methods, as a function of time after infection by the indicated strains.

than wild type did but less completely than the triple mutant did. Figure 4 shows that, while each of the 50^- and 51^- mutations had little effect by itself, each was necessary for the full effect of the triple mutant, $44^-50^-51^-$, and the double mutant, 50^-51^-.

Similar results were seen when shutoff of host protein synthesis was measured by pulse-labeling with [^{3}H]leucine or by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins pulse-labeled with ^{35}S-labeled amino acids (data not shown). Thus, gp44-50-51 restrained the normal shutoff of host gene expression, and each of genes 44, 50, and 51 contributed significantly to that activity.

(ii) Shutoff of host DNA synthesis. Figure 5 shows that gp44-50-51 also affected the shutoff of host DNA synthesis. The 44^- mutant caused hyperfast shutoff. The triple mutant caused hyperfast shutoff early in infection but delayed the completion of the shutoff that occurred later in infection. Thus, there appear to be at least two components to the shutoff, an early component which is restrained by gp44-50-51 and a later component which requires the activity of either gp44 or gp50-51.

It is possible that the phage-induced decrease in total RNA synthesis could indirectly increase the pool size of deoxyribonucleoside triphosphates and thereby cause a spurious decrease in measured rates of DNA synthesis. However, such an effect could not account for the actual differences observed. Such an effect would cause the measured rate of host DNA synthesis to be decreased more in the $44^-50^-51^-$ infection than in the 44^- infection, whereas the opposite is true. Such an effect would cause the measured rate of host DNA synthesis to be decreased more in $44^-50^-51^-$ than in wild type at all times during infection, whereas the observed difference is in one direction at 5 min and in the opposite direction at 12 and 25 min. The amount of the difference between wild type and the triple mutant caused by any such effect must be substantially less than twofold, since even wild-type infection decreases total RNA synthesis by more than 50%; thus, such an effect could not account for the eightfold difference in phage DNA synthesis between wild type and the triple mutant, which is seen below in Fig. 6.

Roles of genes 44, 50, and 51 in phage DNA synthesis and growth. Figure 6 shows that phage DNA synthesis was substantially deficient in infection by the triple mutant. Presumably this was due to the deficiency in expression of the middle genes, which specify most of the enzymes required for phage DNA synthesis. The 44^- and 50^-51^- mutations alone caused slight inhibitory or stimulatory effects, respectively. Presumably because of the delay in middle gene expression and DNA synthesis, the triple mutation caused a modest extension of the latent period and a modest decrease in burst size (Fig. 7). Plating efficiency and plaque size on a Sup^- lawn were less than half of those of wild-type phage, and the plaque size ranged down to barely visible. Neither 44^- nor 50^-51^- had any consistently observable effect on phage growth.

DISCUSSION

Delayed-early genes. Since genes 27, 28, and 37 to 40 were expressed primarily at middle time but independently of gp28, we are calling them delayed-early genes. There is nothing known about the early promoters from which these genes are transcribed that would account for such a delay in their expression, given the high level ultimately achieved (1, 2, 13, 17). Thus, there must be a heretofore-unrecognized activity that stimulates transcription of these two early operons at middle times, independently of gp28 and without similarly affecting transcription of the immediate-early genes. That activity could be provided by gp44 (with or without gp50-51), since it stim-

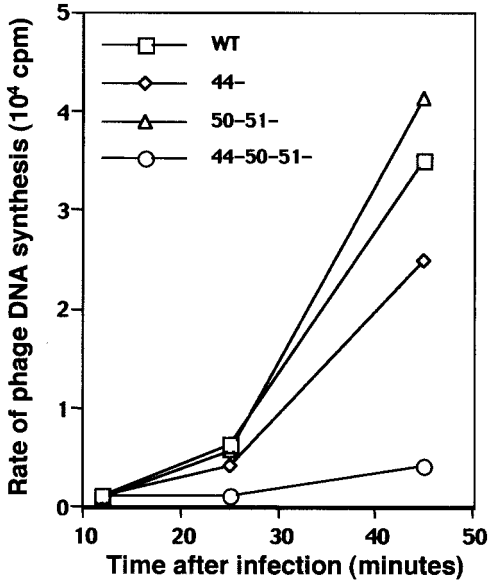

FIG. 6. Phage DNA synthesis. Rates of phage DNA synthesis were measured by pulse-labeling with [2,8-^{3}H]adenine as described in Materials and Methods, as a function of time after infection by the indicated strains.

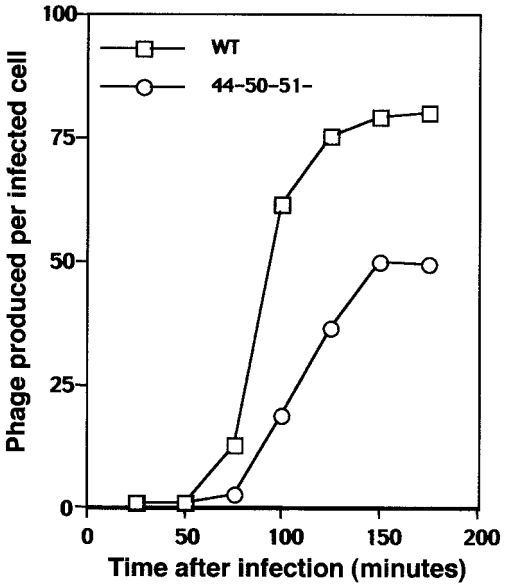

FIG. 7. Single-step growth curves. Cultures of CB10, growing in VY medium at 30°C, were infected with SPO1 wild type (WT) or $44^-50^-51^-$ at a multiplicity of infection of about 0.1. After 10 min the cultures were diluted 1:2 into VY medium containing anti-SPO1 antibody. After being shaken for 10 more min, the cultures were diluted $1:2 \times 10^4$ into fresh VY medium. They continued to be shaken at 30°C and were assayed for PFU at 25-min intervals. The value plotted for each time point is the ratio of the PFU at that time point to the PFU for the same culture at 25 min.

ulates expression of the delayed-early genes. If so, the delay in expression of the delayed-early genes could be the time required for synthesis of sufficient quantities of gp44.

Effects of the products of genes 44, 50, and 51 on regulation of SPO1 gene expression. Table 2 summarizes the effects of wild-type SPO1 and of mutants 44^-, 50^-51^-, and $44^-50^-51^-$ on each of the processes studied during SPO1 infection. Pluses and minuses show the direction and relative amount of the effect of each SPO1 strain on each of the processes listed. The first three columns of Table 2 summarize the data in Fig. 2 and show that these gene products had four distinguishable effects on expression of SPO1 genes.

Effect 1. In comparison with wild type, either the 44^- mutation or the 50^-51^- double mutant caused increased expression of all genes tested, including immediate-early, delayed-early, and middle genes. Therefore, gp44-50-51 repressed expression of all of the SPO1 genes tested, and the full effect of this repression required the activity of both gp44 and gp50-51.

Effect 2. In comparison with 44^- or 50^-51^-, the triple mutant $44^-50^-51^-$ caused a further increase in expression of immediate-early genes. Therefore, there must be residual repressive activity present in the 44^- or 50^-51^- strains, which is no longer there in $44^-50^-51^-$ and which therefore must be caused by gp50-51 in the 44^- strain and by gp44 in the 50^-51^- strain.

Effect 3. In comparison with 44^- or 50^-51^-, the triple mutant caused a decrease in expression of middle genes. Therefore, there must be an activity present in the 44^- or 50^-51^- strains which stimulates middle gene expression. Since that activity is no longer there in $44^-50^-51^-$, it must be caused by gp50-51 in the 44^- strain and by gp44 in the 50^-51^- strain.

Effect 4. In comparison with 44^- or 50^-51^-, the triple mutant caused a decrease in expression of delayed-early genes 27 and 28. Therefore, there must be an activity present in the 44^- or 50^-51^- strains which stimulates delayed-early gene expression. Since that activity is no longer there in $44^-50^-51^-$, it must be caused by gp50-51 in the 44^- strain and by gp44 in the 50^-51^- strain. With respect to delayed-early genes 37 to 40, such an activity can be seen for gp44 in the 50^-51^- strain but not conclusively for gp50-51 in the 44^- strain.

Note that, in the wild type, both activities 1 and 3 or 1 and 4 would be active and would cause effects in opposite directions. To varying extents they would cancel each other out, sometimes obscuring the difference between wild type (which has both activities) and the triple mutant (which has neither).

Thus, the four activities are (i) repression of all SPO1 genes, requiring the activity of both gp44 and gp50-51; (ii) residual repression of immediate-early genes by either gp44 or gp50-51, even in the absence of the other; (iii) stimulation of expression of middle genes by either gp44 or gp50-51; and (iv) stimulation of expression of certain delayed-early genes by either gp44 or gp50-51.

Effects of the products of genes 44, 50, and 51 on host shutoff. These gene products also played significant roles in the shutoff of host DNA, RNA, and protein synthesis. gp44-50-51 restrained the rate of shutoff of host gene expression, and each of genes 44, 50, and 51 contributed significantly to that activity. gp44-50-51 restrained the early component of the shutoff of host DNA synthesis, while a later component required the activity of gp44 and/or gp50-51. These effects might have been accomplished by additional direct activities of gp44-50-51. However, we prefer the simpler explanation that the effects on host shutoff resulted from the already-documented effects of gp44-50-51 on phage gene expression. Table 2 summarizes the effects of the mutations on host shutoff and suggests a plausible model. Note that the increase from strain to strain in the expression of immediate-early genes parallels the increase in the efficiency with which host RNA synthesis is shut off. This can be explained if one or more of the immediate-early genes, regulated by gp44-50-51, specifies a product that causes shutoff of host RNA synthesis. The overexpression of such genes, caused by the triple mutant, could cause the accelerated shutoff of host RNA synthesis that was observed in triple mutant infection. The intermediate levels of overexpression caused by

TABLE 2. Summary of effects of SPO1 strains[a]

SPO1 strain	Transcription			Host synthesis		
	Immediate-early genes	Delayed-early genes	Middle genes	RNA	DNA	
					At early times	At middle times
WT	+	++	++	−	−	−−−
44^-	++	+++	+++	−−	−−	−−−
50^-51^-	++	+++	+++	−−	−−	−−−
$44^-50^-51^-$	++++	++	+	−−−	−−	−−

[a] Pluses and minuses show the direction and relative amount of the effect of each SPO1 strain on each of the processes listed. They represent crude approximations, since there is considerable variation within each class of genes. WT, wild type.

mutants 44^- and 50^-51^- could account for their also causing intermediate levels of accelerated shutoff of host RNA synthesis. Similarly, the deficient shutoff of host DNA synthesis by the triple mutant at middle times could be explained if that shutoff required one or more of the middle gene products whose expression is deficient in the triple mutant.

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