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Transcriptional Regulation of Bacteriophage SPO1 Protein Synthesis In Vivo and In Vitro

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There are six classes of SPO1 transcripts which are, at least partially, regulated independently of each other. Analysis of proteins made in infections by phage mutants defective in DNA synthesis, or in genes which positively control transcription, permitted each protein to be assigned to one transcription class. Most of the late proteins belong to transcription class m_2l. There seem to be few, if any, phage proteins in the *l* class whose mRNA synthesis depends absolutely on phage DNA synthesis. UV irradiation of host cells allowed the detection of many additional early proteins. The early proteins detected in vivo were compared with proteins synthesized in vitro, using bacterial or gp28 phage-modified RNA polymerase in an *Escherichia coli* cell-free system. Proteins characterized in vivo as belonging to the *e* transcription class could be made efficiently in vitro only when transcription was performed by bacterial RNA polymerase. *em* proteins could be elicited through the use of either bacterial or gp28 polymerase, indicating that their genes can be transcribed in either the early or the middle mode.

Upon infection of *Bacillus subtilis*, bacteriophage SPO1 initiates a complex program of transcription involving six distinct classes of RNA transcripts (9). Three transcriptional control genes have been identified (8) whose products regulate the sequential expression of these transcripts. These genes encode polypeptides which bind tightly to the RNA polymerase and change its recognition specificity (5, 22, 26). Table 1 summarizes the time of appearance and genetic control over these classes of transcripts.

Several studies have used DNA-dependent, transcription-translation-coupled, cell-free systems to demonstrate the altered transcription specificity of phage-modified RNA polymerases derived from cells infected by SPO1 or its close relative SP82 (13, 25). In such an assay one can take advantage of the high resolving power of polyacrylamide gels for polypeptides to individually measure the ability of a purified RNA polymerase to transcribe a large number of phage genes in a single reaction. The utility of this approach has been limited, however, by the lack of the ability to identify the proteins made in these in vitro reactions with the authentic SPO1 polypeptides made during infection. In addition, transcriptional control of the temporal order of appearance of SPO1 proteins has never been adequately described.

One of the difficulties in assigning SPO1 proteins to the known transcription classes has been the difficulty in identifying the SPO1-specific proteins during the first few minutes of infection. The first transcription switch event in SPO1 infection occurs at 4 min at 37°C. Yet the shutoff of host protein synthesis in such infections is sufficiently gradual that at this critical time most of the SPO1 proteins displayed on an acrylamide gel are surely masked by the residual synthesis of host cell proteins (14). An attempt to deal with this problem through the study of infections of non-nucleated minicells (19) suffered from the fact that the temporal appearance of SPO1 proteins in these cells is not the same as in intact cells in all instances.

In this report, we have assigned individual SPO1 polypeptides to a given transcription class on the basis of their programs of synthesis in UV-irradiated and unirradiated cells infected with either wild-type SPO1 or the known transcriptional regulatory mutants. In addition, we have been able to correlate the proteins synthesized from SPO1 DNA in an *Escherichia coli*-coupled transcription-translation system (11, 21), using either bacterial or SPO1-modified RNA polymerase, with those polypeptides synthesized in vivo.

MATERIALS AND METHODS

Bacteria and phage. *B. subtilis* 168 (trp^- Su^-) was used for in vivo studies. RNA polymerase was prepared from *B. subtilis* 1005 (Rif^r). *E. coli* 514 (F^- Δ*lac trp tsx* Str^r) was used in the preparation of the coupled transcription-translation system (11).

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TABLE 1. Transcription classes of SPO1[a]

Class	Time after infection (min)		SPO1 functions necessary for synthesis
	First detected	Synthesis stops	
e	1	4	
em	1	12	
m	4	12	gp28
m_1l	4		gp28
m_2l	8–10		gp28, 33, 34
l	13		gp28, 33, 34 and DNA synthesis

[a] Data from references 8 and 9.

Wild-type and mutant SPO1 were grown and purified by cesium chloride centrifugation as previously described (21). Mutant phage were provided by E. P. Geiduschek and S. Okubo.

Preparation of SPO1 DNA. Wild-type SPO1 were diluted to an optical density at 260 nm of 30 in 0.1 M Tris-hydrochloride (pH 7.5)–5 × 10^{-3} M $MgCl_2$–0.1 M NaCl. The phage were extracted twice with an equal volume of phenol saturated with 0.1 M Tris-hydrochloride, pH. 7.5, and the aqueous phase was dialyzed at 4°C overnight into 0.1 M Tris-hydrochloride (pH. 7.9)–0.5 M NaCl–0.1 mM EDTA.

Preparation of SPO1-infected extracts. *B. subtilis* 168 was grown at 37°C to 2 × 10^8 cells per ml in M9 medium plus 2 × 10^{-5} M $MnCl_2$ and 20 μg of tryptophan per ml (21). The cells were infected with wild-type or mutant SPO1 at a multiplicity of about 5, and aliquots were pulse-labeled at the times indicated, using ^{14}C-amino acids (Schwarz/Mann). Unirradiated cells were labeled with 0.25 μCi/ml, and irradiated cells were labeled with 2.0 μCi/ml. The pulse was terminated by pouring the samples over ice, and the cells were concentrated by centrifugation and lysed by addition of 20 μl of resuspension buffer (10 mM Tris-hydrochloride, pH 7.5, 10 mM $MgSO_4$, 200 μg of chloramphenicol per ml, 100 μg of egg white lysozyme per ml, 5 μg of RNase per ml, 5 μg of DNase per ml) per ml of original volume, incubated at 37°C for 2 min, and prepared immediately for gel electrophoresis.

Irradiation of bacteria. All irradiations were performed with a George K. Gates MR-4 UV lamp placed 19 cm above the dish. At this height the incident radiation is 40 ergs/mm^2 per s.

Preparation of infected extracts from irradiated cells was essentially as described above, expect that when the cells reached 2 × 10^8 cells per ml a 10-ml aliquot was withdrawn, placed in a 9-cm-diameter petri dish, and irradiated for 2 min with constant agitation (except for Fig. 2, where other times of irradiation were used). The cells were then returned to 37°C for 10 min. At this time they were infected and pulse-labeled as described above.

Preparation of RNA polymerase. RNA polymerase was prepared essentially according to Burgess and Jendrisak (2) as previously described (22). A Sephacryl S-200 column (3 by 30 cm) was included between the Polymin P (BASF Wyandotte Corp.) precipitation and DNA-cellulose chromatography as a sizing and desalting step.

In vitro coupled transcription-translation. The *E. coli*-coupled system was prepared and incubations were performed as previously described (22). RNA synthesis was made dependent on added exogenous Rif^r RNA polymerase by including rifampin in the reaction mixture. To obtain radioactive proteins, a ^{14}C-labeled amino acid mixture (8.0 μCi; Schwarz/Mann, no. 3122-09) was included in the reaction.

Gel electrophoresis. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of proteins was done according to Blattler et al. (1). Samples were prepared by addition of an equal volume of 0.08 M Tris-hydrochloride (pH 6.8)–0.1 M dithiothreitol–2.0% SDS–10% glycerol and heating for 3 min at 100°C.

RESULTS

Protein synthesis in SPO1-infected *B. subtilis*. Host protein synthesis is not efficiently shut off immediately after SPO1 infection of *B. subtilis* (14). It is difficult, therefore, to detect synthesis of phage-specific proteins at very early times after the onset of infection. To study SPO1-specific protein synthesis at these early times, we have used UV light to eliminate host RNA and protein syntheses. However, it is necessary to examine the proteins synthesized in unirradiated and UV-irradiated infected cells to establish the entire temporal sequence of expression of the SPO1 gene products.

Figure 1 shows the proteins synthesized in 2-min intervals during infection of unirradiated *B. subtilis* with wild-type SPO1 or DNA synthesis (D0) mutants in cistrons 23a (*sus*F20) and 31 (*sus*F39) (15). A sample from uninfected cells is not included because no new proteins appear during the initial 0- to 2-minute pulse-labeling interval. There are several important features of SPO1 gene expression which are evident from this figure. Host protein synthesis is gradually shut off during the first 6 to 8 min of infection. Two prominent host bands (h1 and h2) are indicated, and trace amounts of these proteins continue to be synthesized during the 6- to 8-min pulse interval. Before 6 min, there are so many host proteins being synthesized that only a few phage-specific proteins can be detected. However, it is apparent that the SPO1 proteins are synthesized according to a temporal sequence which correlates closely with the transcription classes described by Gage and Geiduschek (9). Representative proteins whose temporal expression corresponds to these classes are indicated in Fig. 1, using a numbering system presented in Table 2.

SPO1 protein e3 is easily detected during the 2- to 4-min pulse interval, and its synthesis is shut off before the 6- to 8-minute pulse. Other SPO1 proteins can also be detected very early after infection (e5 and e14, which comigrates with a host band) and continue to be synthesized until at least 14 min, but are not synthesized very late during infection (24 to 26 min). These

TABLE 2. Proteins synthesized during SPO1 infection[a]

Proteins				In vitro synthesis	
Name	Mol wt ($\times 10^3$)	Name	Mol wt ($\times 10^3$)	*B. subtilis*	gp28
Early					
	e	*em*			
		e1	63.2	−	−
		e2	51.9	−	−
e3	44.0			+	−
		e4	42.0	(?)	+
		e5	34.5	+	+
e6	30.5			+	(?)
e7	29.5			+	−
		e8 (gp28)	26.0	+	+
e9	24.5			+	−
		e10	23.5	+	+
		e11	21.8	+	+
		e12	19.6	+	+
e13	19.0			+	−
		e14	17.5	+	+
e15	16.3			+	−
		e16	14.9	+	+
e17	12.7			+	−
e18	12.2			+	−
e19	11.2			+	−
e20	10.8			+	(?)
e21	10.0			+	(?)
Middle					
	m	*m*$_1$*l*			
m1	80.0			−	−
		m2	71.5	−	−
m3	54.3			−	+
		m4	42.0	−	(?)
		m5	41.0	−	−
		m6	39.5	(?)	+
m7	37.5			−	+
m8	17.5			−	+
		m9	12.5	(?)	+
Late					
*l*1	>100				
*l*2	>100				
*l*3	61.0				
*l*4	57.0				
*l*5	55.5				
*l*6	46.0				
*l*6a	44.5				
*l*7	37.0				
*l*8	34.0				
*l*9	33.0				
*l*10	25.5				
*l*11	25.0				
*l*12	24.3				
*l*13	22.1				
*l*14	18.0				
*l*15	15.2				
*l*16	13.2				

[a] The molecular weight estimates were obtained by comparing the electrophoretic migration of SPO1-specific proteins with standards of known molecular weight: *B. subtilis* RNA polymerase, chymotrypsinogen, cytochrome *c*, bovine serum albumin.

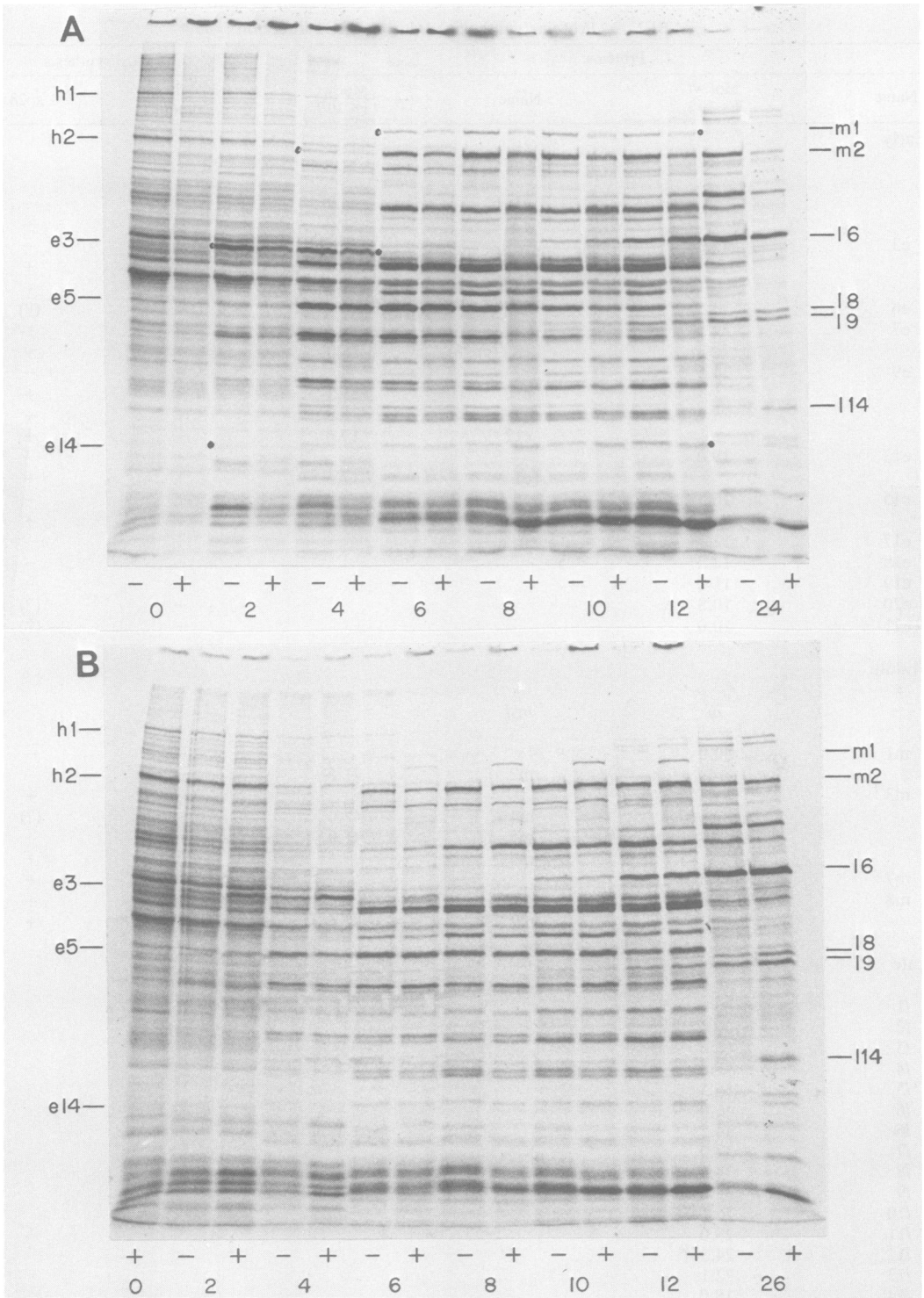

FIG. 1. Time course of proteins synthesized during SPO1 infection. Two-minute pulses with ^{14}C-labeled amino acids starting at the times after infection indicated under each sample were subjected to gel electrophoresis and autoradiography. (+) represents extracts prepared from wild-type infection; (−) represents extracts from mutant infections. (A) *sus*F20, cistron 23a; (B) *sus*F39, cistron 31. Representative proteins are labeled as described in the text. Dots indicate times of onset and cessation of synthesis.

two temporally distinct classes of SPO1 early proteins represent the *e* and *em* transcription classes, respectively.

Other SPO1 proteins do not appear until about 6 min. Some of these (e.g., m1) are shut off before 24 min and fit the *m* transcription class. Others (e.g., m2) correspond to the m_1l transcription class because they continue to be synthesized very late during infection (24 to 26 min).

There is another group of SPO1 proteins which begin to be synthesized at about 10 min after infection and are synthesized at maximal rates late in infection (e.g., *l*6 and *l*9). Since these proteins are produced in undiminished amounts in the D0 mutants *sus*F20 (Fig. 1A) and *sus*F39 (Fig. 1B), they behave like m_2l transcription products rather than *l*. There is a small group of very late-appearing proteins whose synthesis is variably reduced in the D0 mutants (e.g., *l*8) and which may correspond to the *l* transcription class.

Figure 1 includes a comparison between the two mutants and the wild-type to demonstrate the reproducibility of the technique. In the case of *sus*F39 (Fig. 1B), it is possible to identify the product of cistron 31 by locating the missing band (m1). In all cases in which the time of synthesis of a protein is used to determine its transcription class, these determinations have been confirmed and extended by analysis of the proteins synthesized during infections with the known transcription control mutants (see Fig. 4).

Protein synthesis in SPO1-infected UV-irradiated *B. subtilis*. Although it is possible to detect individual SPO1 early proteins in Fig. 1, it is apparent that many of the SPO1 early proteins must be masked by host protein synthesis. To identify these hidden SPO1 early proteins, we have used UV light to destroy host RNA and protein syntheses.

Figure 2 shows the effect of UV irradiation on host protein synthesis. Cells were irradiated for the times indicated and then returned to 37°C and incubated for 10 min to allow any preexisting mRNA to decay. They were then infected or mock infected with SPO1 and pulse-labeled from 2 to 5 min after infection. It is clear from Fig. 2 that, as the UV dose is gradually increased, host protein synthesis is reduced to a barely detectable minimum, whereas SPO1 RNA and protein syntheses are less dramatically affected. Almost all of the major SPO1 proteins can still be detected after high UV doses; however, synthesis of one early protein, e16, is extremely sensitive to host irradiation (M. Perkus, personal communication). On the basis of these data, we have chosen 2 min as the irradiation period for detection of the SPO1 early proteins.

Initial experiments conducted to determine the effect of UV irradiation on the temporal sequence of SPO1 gene expression indicated

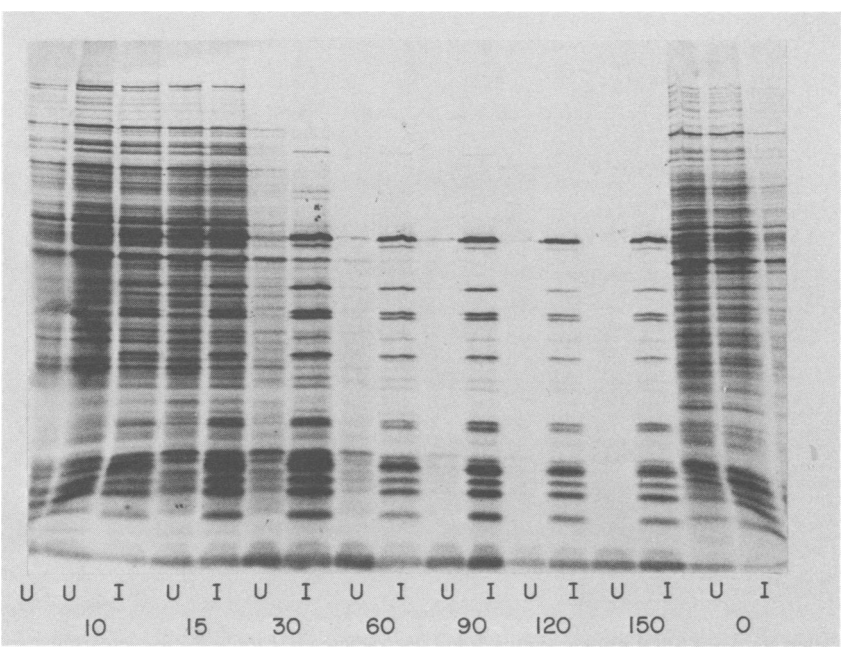

FIG. 2. Effect of UV irradiation on host and phage protein synthesis in SPO1-infected *B. subtilis*. Cells were irradiated for the times indicated in seconds, mock infected (U) or infected with SPO1 (I), and pulse-labeled 2 to 5 min after infection, using ^{14}C-amino acids. Extracts were separated by gel electrophoresis, and radioactivity was detected by autoradiography.

that the course of infection in irradiated cells might be somewhat elongated. For this reason, we have examined proteins synthesized during successive 4-min intervals from 0 to 32 min of infection. Pulses done after this time reveal only quantitative differences in the rate of synthesis of a few proteins.

Figure 3 shows a pulse series comparing *sus*F39 and wild-type SPO1 infections of irradiated cells. The proteins chosen to represent the transcription classes in Fig. 1 are again indicated in this experiment. The most obvious point to be made from this experiment is that UV irradiation of host cells before infection results in the ability to detect many SPO1 proteins, during the 0- to 4-min pulse interval, which were not apparent in the corresponding samples from unirradiated cells. In addition, the time of first synthesis of these proteins corresponds quite well with the onset of their synthesis in unirradiated cells. This is easily seen if one examines the proteins chosen to represent each of the transcription classes (Fig. 1 and 3). Even m_2l proteins are synthesized with a delay of only a few minutes, beginning at about 10 min in unirradiated cells and at about 12 or 16 min in irradiated cells. However, the category of proteins whose synthesis seems to be coupled to DNA synthesis (e.g., *l*8 and *l*14) are never synthesized at a high rate in UV-irradiated cells. Infection of irradiated cells does not result in SPO1 DNA accumulation or phage production (Perkus, personal communication).

On the other hand, the shutoff of the synthesis of the various classes of SPO1 proteins is not maintained in UV-irradiated cells. Those early proteins which are normally turned off at about 4 to 6 min after infection (e.g., e3) continue to be synthesized at a reduced rate until about 20 min after infection of UV-irradiated cells. Proteins belonging to the em (e5) or *m* (m1) transcription classes are synthesized at reduced rates late in infection in irradiated cells, but their synthesis is never terminated. The reason for this faulty regulation is not known, although it is significant that the regulation of initiation appears to be uncoupled from the mechanism of shutoff. It has recently been reported (20) that, when DNA synthesis in SPO1-infected cells is inhibited by novobiocin, a DNA gyrase inhibitor, repression of early transcription does not occur.

The "missing band" identifying the *sus*F39 mutation is also readily apparent in this gel, as well as the "nonsense fragment" of this gene

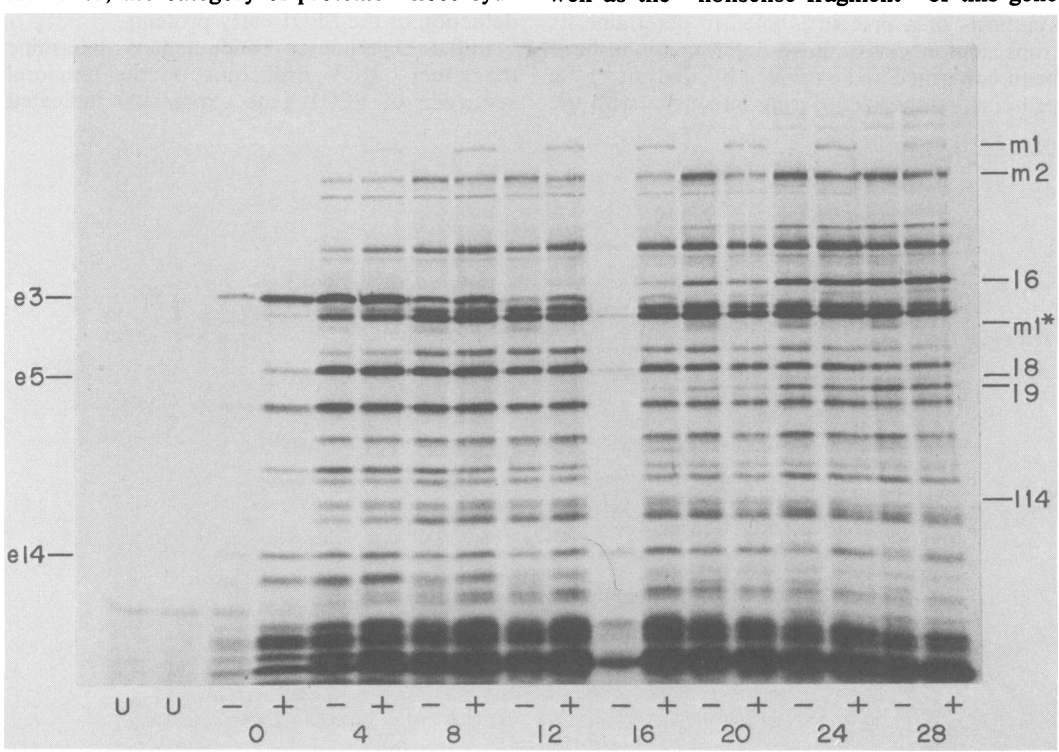

FIG. 3. Time course of SPO1 protein synthesis in UV-irradiated *B. subtilis*. Cells were irradiated and infected by mutant *sus*F39 (−) or wild-type (+) SPO1. Aliquots were removed and pulse-labeled with ^{14}C-amino acids for 4 min, starting at the times after infection indicated under each sample. Uninfected irradiated cells (U) were also labeled with ^{14}C-amino acids for 4 min. Extracts were separated by gel electrophoresis, and radioactivity in proteins was detected by autoradiography.

product (m1*). The information obtained from studies on the temporal expression of individual SPO1 proteins can be supplemented with information obtained from analysis of proteins synthesized by phage which are mutant in the pleiotropically acting genes 28, 33, and 34 to define the SPO1 early, middle, and late gene products. In addition, proteins specified in vitro from the SPO1 genome, using bacterial or SPO1-modified RNA polymerase, can be compared with these proteins to assess the specificity and fidelity of SPO1 gene expression in vitro. To make these correlations, cells infected under various conditions were pulse-labeled at appropriate times, and in vitro reactions were done with SPO1 DNA and bacterial or gp28-modified RNA polymerase. The resulting in vivo and in vitro samples were prepared as described here, except that they were dialyzed at 4°C for 24 h against a common buffer. The samples were then run on a 17.5% polyacrylamide-SDS gel and autoradiographed (Fig. 4).

Protein synthesis in transcriptional control mutants. Fujita et al. (8) have examined RNA synthesis in cells infected with the transcription control mutants and report that mutants in gene 28 (e.g., *sus*F21) synthesize only *e* and *em* RNA and that mutants in gene 33 or 34 (e.g., *sus*F14 and *sus*F4) can synthesize *e*, *em*, *m*, and m_1l RNA. Therefore, we have analyzed proteins synthesized during infection with these mutants and wild-type SPO1 (Fig. 4) and defined each protein as a member of a particular transcription class according to the following criteria.

(i) Early proteins. Early proteins are proteins synthesized during a 0- to 10-min pulse in UV-irradiated cells infected with *sus*F21 (lane C or M).

(a) *e*. *e* proteins are early proteins which are synthesized at a greatly reduced rate in a 10- to 14-min pulse of *sus*F14-infected unirradiated cells (lane F).

(b) *em*. *em* proteins are early proteins synthesized at the same or a higher rate in a 10- to 14-min pulse of *sus*F14-infected unirradiated cells (lane F).

(ii) Middle proteins. Middle proteins are those synthesized in a 10- to 14-min pulse of *sus*F14-

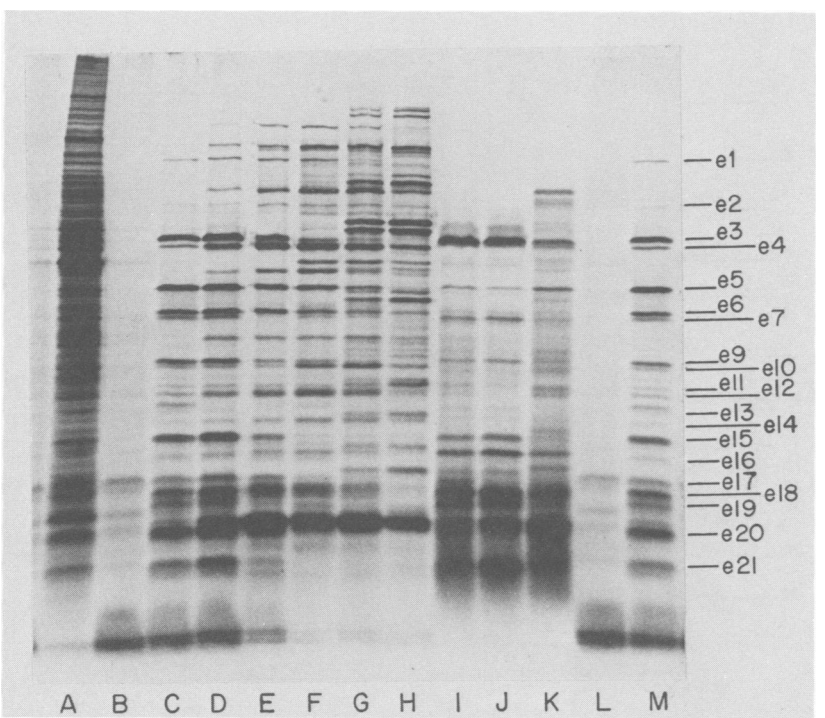

FIG. 4. Comparison of proteins in vivo during SPO1 infection and in vitro, using SPO1 DNA. The proteins were separated electrophoretically (17.5% acrylamide gel) and detected by autoradiography. The phage used for infection in vivo, the labeling interval, and the state of the host cells were: (A) uninfected, 4 min, unirradiated; (B and L) uninfected, 10 min, irradiated; (C and M) *sus*F21, 0 to 10 min, irradiated; (D) *sus*F14, 0 to 10 min, irradiated; (E) *sus*F14, 10 to 30 min, irradiated; (F) *sus*F14, 10 to 14 min, unirradiated; (G) wild type, 14 to 20 min, unirradiated; (H) wild type, 20 to 24 min, unirradiated. The RNA polymerase used for in vitro reactions were: (I) *B. subtilis* RNA polymerase; (J) *E. coli* RNA polymerase (endogenous in cell-free system); (K) gp28-modified *B. subtilis* RNA polymerase (prepared from *sus*F14 infection).

infected cells (lane F) which cannot be synthesized in a 0- to 10- min pulse of UV-irradiated cells infected with *sus*F21 (lane C or M).

(a) *m*. *m* proteins are middle proteins whose rate of synthesis is greatly reduced in a 20- to 24-min pulse of cells infected with wild-type SPO1 (lane H).

(b) m_1l. m_1l proteins are middle proteins synthesized at an equal or a higher rate during a 20- to 24-min pulse with wild-type SPO1 (lane H).

(iii) Late proteins. Late proteins are those synthesized in a 20- to 24-min pulse of SPO1-infected cells (lane H) which are not synthesized during a 10- to 14-min pulse in *sus*F14-infected cells (lane F). The temporal interval over which these proteins are produced, and the lack of coupling with DNA synthesis (Fig. 1), defines most of these polypeptides as m_2l. As was noted in the presentation of Fig. 1, a few of the late proteins (most notably *l*14) appear later than the other late ones and are severely reduced in D0 infection. These may represent proteins whose messages belong to the *l* class of SPO1 RNA.

The positive regulatory subunit of RNA polymerase, gp28 (product of gene 28), has a molecular weight of 26,000 and is responsible for initiating the synthesis of middle RNAs (5, 7). The mRNA for this protein must be initially synthesized from early promoters. A protein of this size, which is present in infections by both *sus*F39 (Fig. 3) and *sus*F14 (Fig. 5), is absent from the proteins produced by *sus*F21. We have, therefore, assigned this protein to the early class (e8) as the product of gene 28. A protein which is unique to *sus*F21 infections (e8*), with a molecular weight of about 19,000, is probably the prematurely terminated nonsense fragment.

Relevent lanes of Fig. 4 are reproduced in Fig. 5 so that the early, middle, and late proteins can be enumerated. According to this definition, we

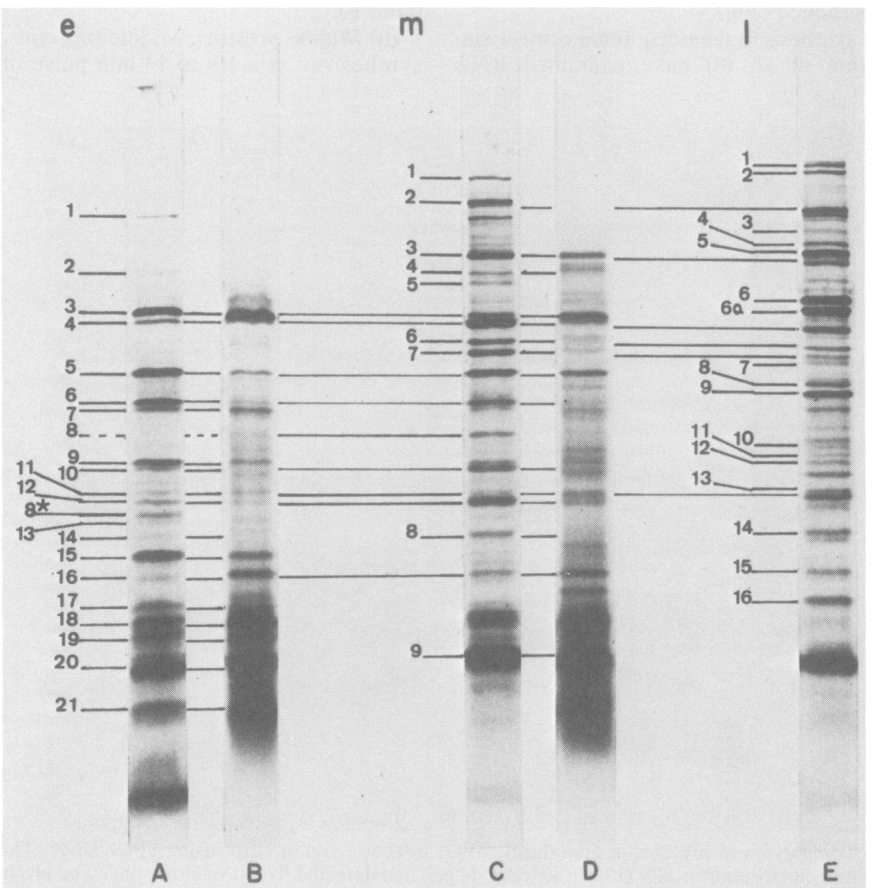

FIG. 5. Composite picture showing each SPO1 polypeptide identified and defined as early (*e*), middle (*m*), or late (*l*) according to the criteria indicated in the text. The autoradiograms shown are taken from Fig. 4 and rearranged to illustrate the band assignments referred to in the text: (A) Fig. 4C, 0 to 10 min, *sus*F21, irradiated cells; (B) Fig. 4I, *B. subtilis* RNA polymerase in vitro; (C) Fig. 4F, 10 to 14 min, *sus*F14, unirradiated cells; (D) Fig. 4K, gp28-modified RNA polymerase in vitro; (E) Fig. 4H, 20 to 24 min, wild-type SPO1, unirradiated cells.

are able to identify 21 distinct SPO1 early proteins. The large differences in the rates of synthesis of these proteins are consistent with the fact that only those synthesized at a very high rate are detectable in unirradiated *B. subtilis*. There are 9 major middle proteins according to this definition and 16 major late proteins. It is possible to detect additional faint bands in these samples, and there are undoubtedly many proteins of similar molecular weight which we cannot detect by migration in one dimension when electrophoresed in an SDS-polyacrylamide gel. Therefore, the 46 proteins listed in Table 2 are a lower limit to the number of proteins synthesized during SPO1 infection.

In vitro synthesis of SPO1 proteins. It has been demonstrated that the *E. coli*-coupled transcription-translation system used in this study results in the production of specific phage proteins from SPO1 DNA (21, 25). The endogenous *E. coli* RNA polymerase and the purified *B. subtilis* RNA polymerase specify the same SPO1 early proteins when SPO1 DNA is used as a template in this system (22) (Fig. 4, lanes I and J) and compete for initiation sites on the template DNA (22). The gp28-modified RNA polymerase, on the other hand, initiates at different sites on SPO1 DNA and specifies a set of proteins distinct from those produced with bacterial enzymes (5, 22, 26).

In this study, a correlation between those proteins synthesized in vitro, using SPO1 DNA and the *B. subtilis* or gp28-modified RNA polymerase, is made on the basis of migration in an SDS-polyacrylamide gel. Of course, comigration of two bands in these gels does not establish identity. However, examination of many gels and the overall similarity in the appearance of the in vivo and in vitro proteins synthesized make one reasonably confident that, in regions of the gel where proteins are well resolved, one can make these correlations. We have shown that the SPO1 early protein e3 is indistinguishable from the in vitro protein comigrating with it, using the peptide mapping technique of Cleveland et al. (3; unpublished data).

Comparison of the proteins synthesized from SPO1 DNA in vitro, using *B. subtilis* RNA polymerase (Fig. 4I and 5B), and the SPO1 early proteins (Fig. 4C and 5A) indicates that most of the SPO1 early proteins are synthesized in vitro. Proteins e1 and e2 are not produced in the coupled system, probably due to a combination of their large size and low rate of synthesis in vivo.

There are several differences in the rates of synthesis of some of these proteins in vivo and in vitro. For example, protein e6 is produced at a higher rate than e7 in vivo, but this situation is reversed in vitro. The reasons for these quantitative differences in the production of specific SPO1 proteins in vitro are not known. It is significant that all but two of the SPO1 early proteins are synthesized in the *E. coli*-coupled transcription-translation system.

The proteins synthesized in vitro, using gp28-modified RNA polymerase (Fig. 4K), include many of the major SPO1 middle proteins (e.g., m3, m6, m7, and m9) as well as some of the SPO1 early proteins. It is important to consider the differences in the quantity of a given protein synthesized by *B. subtilis* or gp28-modified enzyme in vitro to assess the specificity of these enzymes. The early protein e3 is produced by both enzymes in the reactions shown (Fig. 4I and K and 5B and D); however, it is synthesized at a much higher rate by *B. subtilis* RNA polymerase than by gp28-modified enzyme. In other experiments this difference in the rate of synthesis of e3 is even greater; in fact, it cannot be detected in many reactions stimulated by gp28-modified RNA polymerase (22). We know that apparently unmodified *B. subtilis* holoenzyme is present in large amounts in RNA polymerase obtained from SPO1-infected cells (4; our own observations) and that this enzyme is highly active in transcription from early promoters. Holoenzyme is separated from gp28-modified forms during the purification. Although the RNA polymerase purified from *sus*F14-infected cells, which was used in this experiment, contained gp28 and no detectable gp33 or gp34 as expected (6), it still had trace amounts of sigma polypeptide. This accounts for the reduced synthesis of e3 and other early proteins in the reaction displayed in Fig. 4K and 5D. Therefore, the assessment of the ability of a particular SPO1 protein to be expressed in vitro, using the purified RNA polymerases (Table 2), relied on a comparison of the amount of protein synthesized with *B. subtilis* versus gp28-modified RNA polymerase.

Most proteins synthesized according to the *e* transcription mode in vivo (e3, e6, e7, e9, e15, e17, e18, e19, e20, and e21) are synthesized in high amounts with *B. subtilis* RNA polymerase in the coupled transcription-translation system but are produced in greatly reduced quantities or not at all with gp28-modified enzyme. Bands e6 and e20 appear to be exceptions, since they are made in significant amounts under control of gp28-modified polymerase. In some of our gels, the e6–e7 region can be resolved into three bands. It is possible that band e6, on this gel, consists of two proteins, one under *e* transcription control and a minor component under *em* control. Similarly, it can be seen (Fig. 4) that there are two barely resolved bands in the region labeled e20. In other experiments in which better resolution has been obtained in this area, we

have observed in vitro synthesis of one of these proteins only when unmodified bacterial polymerase was used.

The proteins belonging to the *em* transcription class, on the other hand, are made in equivalent amounts with bacterial or phage-modified enzyme (e8, e14, and e16) or in greater amounts with gp28-modified enzyme (e5, e11, and e12). Since it has been demonstrated (22) that *E. coli* and *B. subtilis* RNA polymerases compete for sites on the template DNA, whereas gp28-modified enzyme recognizes different sites, synthesis of these *em* proteins may be accomplished from two different RNA polymerase initiation sites on the SPO1 genome. Furthermore, the sites for initiation of gp28-modified enzyme may be closer to the *em* structural gene because rifampin-poisoned *E. coli* RNA polymerase does not block their synthesis (22).

DISCUSSION

SPO1 protein synthesis in vivo. In this study, we have classified many of the polypeptides synthesized during SPO1 infection according to criteria roughly equivalent to those originally used to define the six classes of SPO1 RNA transcripts (8, 9). To generate a complete description of SPO1 protein synthesis, it has been necessary to correlate results obtained during infection under a variety of conditions.

We have shown that UV irradiation of host cells before infection removes background host protein synthesis and allows one to study events occurring early after infection. However, the temporal expression of the SPO1 polypeptides is abnormal in these cells. Shutoff of the SPO1 early and middle gene products is delayed, and at least one polypeptide (*l*14) is never synthesized in irradiated cells. It is, therefore, necessary to correlate results from irradiated cells with those obtained during infection of unirradiated cells to study the temporal sequence of events occurring throughout SPO1 infection. Since host protein synthesis declines to a low background level at about 6 min of infection in unirradiated cells, we have been able to make these correlations and obtain an accurate picture of the temporal expression of many of the SPO1 proteins. It has not been possible to identify all of the SPO1 gene products by electrophoresis in one dimension, as indicated by our inability to locate a "missing band" during infection with many *sus* mutants (e.g., *sus*F20; Fig. 1). Indeed, the combined molecular weights of all of the proteins in Table 2 account for only about 35% of the coding capacity of the unique sequences of SPO1 DNA.

The information obtained concerning the temporal expression of each of the SPO1 polypeptides must be supplemented with their ability to be synthesized in cells infected with the known transcriptional regulatory mutants because the times of synthesis of different proteins belonging to a given class are not always synchronously initiated and terminated. In cases in which this is true, we have classified a given protein on the basis of its ability to be synthesized during *sus*F21 and *sus*F14 infections. The results reported here demonstrate that five of the six classes of SPO1 transcripts described by Gage and Geiduschek (9), *e*, *em*, *m*, m_1l, and m_2l, are the mRNAs for groups of coordinately regulated proteins. The role of the *l* class of SPO1 RNA remains problematical. Stewart et al. (24) showed that for SP82, a phage closely related to SPO1, the late protein(s) which blocks the inactivating effect of anti-phage serum (serum blocking power, SBP) is made at the normal time (but in reduced amount) in the absence of phage DNA synthesis. A SPO1 D0 mutant showed delayed onset as well as reduced amount of synthesis of SBP. They concluded that DNA synthesis is not absolutely required for expression of late genes in these phages. Our results are in general agreement with this conclusion, although they also support the notion that DNA synthesis is required for synthesis of a small subset of late SPO1 proteins. There does not seem to be a large class of SPO1 polypeptides whose synthesis absolutely requires DNA replication (Fig. 1). Rather, most of the proteins which are not produced in infections by the transcription control mutant *sus*F14, including the precursor of the major phage capsid protein *l*6 (our unpublished data), are made in normal relative amounts in infections of Su^- cells by D0 mutants and are therefore translated from m_2l transcripts. A small number of proteins (*l*8 and *l*14) appear much later than the other late ones and in D0 infections are reduced in amount (*l*8) or are undetectable (*l*14). This is also true (our unpublished data) for other D0 mutants, including *sus*F30 (gene 22), which was used to establish the existence of the *l* (or DNA synthesis-dependent) class of SPO1 transcripts (8). However, the D0 mutant *sus*HA20 (gene 27) is deficient in production of all late proteins (our unpublished data). Greene et al. (12) have independently observed that *sus*HA20 is defective in late transcription. We suggest that most of the late genes can be transcribed in normal amounts from the input infecting DNA (multiplicities of infection of 5 used in our experiments), whereas a high level of synthesis of a few proteins requires the accumulation of a large pool of replicating DNA. On the other hand, the product of gene 27 may be directly involved in both DNA replication and late transcription. Glassberg et al. (10) described two temperature-sensitive iso-

lates of SPO1 whose mutations were mapped to gene 27, where the lethal phenotype was uncoupled from a defect in DNA synthesis. Interestingly, the product of phage T4 and 45 seems to play just a dual role (27), and evidence has been presented that it interacts with RNA polymerase (18, 23). The only protein we have described which fits the criteria for being transcribed exclusively from *l* RNA is *l*14. It is possible that there are other proteins in this category which we have not detected. Alternatively, *l* RNA may be primarily composed of non-mRNA transcripts.

The results we have presented here are difficult to compare with the previous report of protein synthesis in SPO1-infected minicells (19). The general program of infection in minicells or UV-irradiated cells is similar, although irradiated cells are able to synthesize SPO1 late proteins whereas minicells do not. The specific differences between these two systems are difficult to assess and are not relevant to the present discussion because it is not possible to describe the entire program of SPO1 protein synthesis with either system.

SPO1 protein synthesis in vitro. We have also demonstrated in this study that it is possible to correlate the proteins synthesized in an *E. coli*-coupled transcription-translation system from SPO1 DNA, using bacterial or gp28-modified RNA polymerase, with the SPO1 proteins synthesized in vivo. These correlations have been made on the basis of migration in an SDS-polyacrylamide gel, and we believe that, in most cases, they are correct because of the overall similarity in the pattern of proteins synthesized in vivo or in vitro under the appropriate conditions.

RNA polymerase purified after SPO1 infection, lacking sigma polypeptide and containing gp33 and gp34, has been shown to synthesize m_2l RNA in vitro (17). In experiments not reported here, we have examined the proteins produced from SPO1 DNA when the coupled cell-free system was stimulated by RNA polymerase purified from *sus*F30 (D0)-infected cells at 30 min after infection. This enzyme preparation lacked detectable sigma polypeptide, but contained the host subunit delta (16) as well as gp28, gp33, and gp34. This enzyme was unable to stimulate the synthesis of any polypeptide besides those specified by phage polymerase containing gp28 alone. Reactions carried out over a variety of salt concentrations failed to elicit the production of the late proteins (K. Walsh and D. Shub, unpublished data). It is tempting to speculate that gp28 interferes in some way with transcription of late genes, but the relevant experiments have not been done.

The most significant results reported in this study deal with the ability of the SPO1 early proteins to be synthesized in the coupled system, using bacterial or gp28-modified RNA polymerase. The SPO1 early proteins belonging to the *e* class are synthesized only by bacterial RNA polymerase, whereas the SPO1 *em* proteins can be made in vitro, using either bacterial or gp28-modified enzyme. All promoters recognized by *B. subtilis* RNA polymerase in vitro can be blocked by rifampin-poisoned *E. coli* RNA polymerase, whereas the transcripts for those early proteins now identified as *em* can be made by gp28-modified polymerase under those conditions (22). These results suggest that the *em* transcripts are synthesized from two different RNA polymerase promoter sites, in both the early and the middle transcription modes.

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