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# Order of the Two Major Head Protein Genes of Bacteriophage φ29 of *Bacillus subtilis*

RAFAEL P. MELLADO,* ENRIQUE MÉNDEZ, ELADIO VIÑUELA, AND MARGARITA SALAS

*Centro de Biología Molecular (C.S.I.C.-U.A.M.), Madrid-6, Spain*

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Bacteriophage φ29 mutation *sus*8(22) has been mapped by two-factor crosses between markers *sus*8(769) and *ts*8(93). When *sus*8(22) infects *Bacillus subtilis* $su^-$ proteins, HP1 (major head protein) and HP3 (fiber protein) are not synthesized; instead, a fragment with a molecular weight of 25,000 is produced. The tryptic peptides of the fragment overlap with corresponding peptides in protein HP1, but not with the peptides of protein HP3, showing that cistron 8 codes for the major head protein HP1.

*Bacillus subtilis* phage φ29 contains a double-stranded DNA with a molecular weight of $12 \times 10^6$ (1; A. Talavera, personal communication) that can be isolated from the viral particle as a circle closed noncovalently by a protein (13).

A total of 17 genes, arranged in a linear genetic map, have been identified (9), and the protein products of most of the genes have been characterized (2, 4, 5, 8, 15). None of the *sus* mutants assigned to cistron 8 produces detectable amounts of protein HP1 (major head protein) or HP3 (fiber protein) (2, 5, 10). Since the direction of transcription of late genes coding for proteins HP1 and HP3 is from left to right (5, 15), the above results could be explained by assuming that proteins HP1 and HP3 are synthesized from a polycistronic mRNA and by the existence of a polar effect of the mutation in cistron 8, coding for one of these two proteins on the expression of the next cistron, coding for the other protein (Fig. 1).

In this paper we show that cistron 8 codes for protein HP1 and that the absence of protein HP3 after infection with *sus* mutants in this cistron is due to a polar effect.

## MATERIALS AND METHODS

**Bacteria, phage, and media.** The nonpermissive host *B. subtilis* 110NA *try*$^-$ *spoA*$^-$ *su*$^-$ and the permissive bacterium *B. subtilis* MO-101-P *thr*$^-$ [*met*$^-$]$^+$ *spoA*$^-$ *su*$^{+44}$ were obtained as described (10).

Mutants *sus*8(22) and *sus*8(207) were produced by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and scored on strain *B. subtilis* MO-101-P *su*$^{+44}$ (10). Mutant *sus*8(769) was obtained from D. L. Anderson and B. E. Reilly. Mutant *ts*8(93) is from the collection of Talavera et al. (17). Two revertants of mutant *sus*8(22), r1 and r2, were isolated by plating the mutant on *B. subtilis* 110NA. The frequency of reversion was $10^{-6}$.

Preparations of phage stocks and phage assays were as described (17).

Minimal medium (6), broth (12), and phage buffer (11) were as described.

**Recombination.** Two-factor crosses were carried out as described (12), except *B. subtilis* *su*$^{+44}$ was used since mutant *sus*8(22) does not grow in *B. subtilis* *su*$^{+3}$.

**Labeling of viral proteins.** *B. subtilis* 110NA *su*$^-$ was grown and irradiated with UV light as described (6). The irradiated bacteria were resuspended at $5 \times 10^8$ cells/ml in minimal medium containing either 0.1 mM amino acids when the label was a $^{14}$C-labeled protein hydrolysate or 0.5 mM amino acids, except for leucine which was 0.01 mM, when the label was [$^{14}$C]leucine. The cells were infected with phage φ29, mutant *sus*8(22), or the revertant r1 or r2 at a multiplicity of 20 and shaken at 37°C. A control was kept uninfected. At 28 min postinfection, samples were removed and labeled with [$^{14}$C]leucine (7.5 μCi/ml; 0.02 mM), and 7 min later a 100-fold excess of unlabeled leucine was added and the incubation was continued for 2 min. For the preparative isolation of the protein fragment produced by mutant *sus*8(22), the infected cells were labeled at 35 min postinfection with a $^{14}$C-labeled protein hydrolysate (5 μCi/ml; 58 Ci/atom of carbon), and at 50 min a 10-fold excess of unlabeled amino acids was added and the incubation was continued for 2 min. The bacteria were lysed, and the proteins were prepared for slab gel electrophoresis as indicated (10).

**Labeling and purification of phage φ29.** Preparation of φ29 was carried out as described (3) in the presence of a $^3$H-labeled amino acid mixture (20 μCi/ml). All labeled amino acids were obtained from Radiochemical Centre, Amersham, England.

**Polyacrylamide gel electrophoresis.** (i) Analytical or preparative slab gel electrophoresis was performed on linear 10 to 20% acrylamide gradients as described (5). After preparative electrophoresis, used for the isolation of the protein fragment produced in the mutant *sus*8(22) infection, the slab was cut in slices, and the protein was eluted from the gel

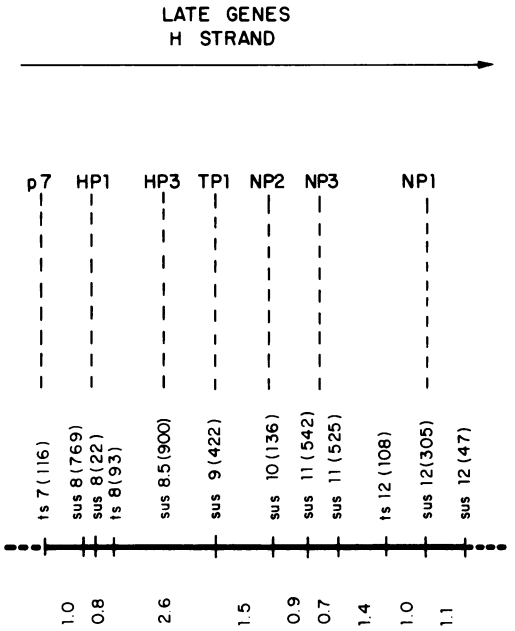


FIG. 1. *Central region, coding for late proteins, of the genetic map of bacteriophage $\phi$29, adapted from that of Mellado et al. (9). The recombination units are given on the bottom. Mutants sus8(769) and ts8(93) gave 0.8 recombination unit; from this value and those presented in this paper (Table 1), mutant sus8(22) has been located between mutants sus8(769) and ts8(93), at 0.3 and 0.5 unit, respectively. Cistron 8.5 codes for protein HP3 (Reilly et al., submitted for publication). Its precise genetic location has not been determined. p7 refers to the identified polypeptide chain of gene 7. HP1, HP3, TP1, NP2, NP3, and NP1 refer to the phage structural proteins (14, 16). The arrow indicates the direction of transcription.*

by incubation for 24 h at 37°C in 0.5 ml of a buffer containing 10 mM sodium phosphate (pH 7.1), 0.1% sodium dodecyl sulfate, and 0.58 mM phenylmethylsulfonyl fluoride to inhibit protease activity. Electrophoresis of uninfected cells, labeled under the same conditions as the infected cells, showed that the radioactivity present at the position of the protein fragment was negligible.

(ii) Gel electrophoresis in 24-cm-long tubes containing 10 to 20% acrylamide gradients (5) was performed to determine the purity of the fragment of mutant *sus*8(22). By this criterion, the fragment was 97% pure.

For the isolation of the major head (HP1) and fiber (HP3) proteins from $\phi$29 particles, electrophoresis was carried out in 10-cm-long gels containing 10% acrylamide (6). After electrophoresis, the gels were cut in 0.8-mm slices, and the protein was eluted from each slice by incubation in 0.25 ml of the elution buffer described above.

**Tryptic peptide analysis.** A mixture of $^{14}$C- and $^{3}$H-labeled proteins was reduced, carboxymethylated, and digested with trypsin as described (7). The tryptic digest was dissolved in 1 ml of 0.2 M pyridine-acetate buffer (pH 3.0), adjusted to pH 1.9 with glacial acetic acid, and applied to a column (9 by 23 cm) of Beckman PA-35 ion-exchange resin equilibrated with 0.2 M pyridine-acetate buffer (pH 3.0) maintained at 45°C. The column was washed under pressure with 60 ml of the above buffer followed by a linear gradient of pyridine-acetate buffer from 0.2 M (pH 3.0) to 2.0 M (pH 5.0) in a total volume of 600 ml at a flow rate of 20 ml/h. Fractions of 2 ml were collected and counted (11).

## RESULTS

**Mapping of mutant *sus*8(22) by two-factor crosses.** Mutant *sus*8(22) was assigned to cistron 8 since (i) it does not show spot-test complementation with other mutants in this cistron and (ii) under restrictive conditions, it does not induce the synthesis of proteins HP1 and HP3, but produces a fragment of lower molecular weight (10). Since mutant *sus*8(22) does not grow on the suppressor strain *B. subtilis su*$^{+3}$, on which the previous mapping of $\phi$29 mutants had been carried out (9, 12), the location of this mutant by two-factor crosses was done on *B. subtilis su*$^{+44}$ (10). Table 1 gives the recombination percentages obtained in the different crosses. It can be seen that mutant *sus*8(22) is approximately halfway between mutants *sus*8(769) and *ts*8(93).

**Proteins induced after restrictive infection with mutant *sus*8(22) and revertants r1 and r2.** As previously reported (10) and shown in Fig. 2, mutant *sus*8(22) does not induce the synthesis of proteins HP1 and HP3 when it infects *B. subtilis su*$^{-}$, giving rise instead to a fragment with a molecular weight of 25,000. To test the possibility that the lack of the two proteins was due to a polar effect or to the existence of a double mutation, two revertants, r1 and r2, of mutant *sus*8(22) were isolated, and the proteins induced after infection of *B. subtilis* 110NA *su*$^{-}$ were analyzed by slab gel electrophoresis. As shown in Fig. 2, the two revertants have normal HP1 and HP3 proteins. This result makes the second possibility unlikely.

**Tryptic peptide analysis of the fragment relative to HP1 and HP3.** To determine which

TABLE 1. *Recombination percentages between mutants in cistron 8*

| Cross | % of recombination |
|---|---|
| *sus*8(769) × *ts*8(93) | 0.40 |
| *sus*8(207) × *ts*8(93) | 0.41 |
| *sus*8(769) × *sus*8(207) | <0.01 |
| *sus*8(22) × *sus*8(207) | 0.14 |
| *sus*8(22) × *sus*8(769) | 0.15 |
| *sus*8(22) × *ts*8(93) | 0.20 |

of the two proteins, HP1 or HP3, is the product of cistron 8, we compared the tryptic peptides of the fragment produced by mutant *sus*8(22) (see Fig. 1 for the genetic location of the mutation) and those of proteins HP1 and HP3. Figure 3A shows that most of the peptides present in the fragment overlap with corresponding peptides in protein HP1, in contrast to the result obtained with protein HP3 (Fig. 3B).

## DISCUSSION

*sus* mutants in cistron 8 do not induce the synthesis of proteins HP1 and HP3 (2, 5, 10). The fact that the two proteins are missing in these mutants could be due either to a polar effect because of the *sus* mutation in cistron 8 or to the existence of a double mutation. The second possibility is unlikely since 13 different *sus* mutants in cistron 8 analyzed lack the two proteins (2, 5, 10; unpublished data). Moreover, two revertants of mutant *sus*8(22) regain both proteins, HP1 and HP3, in a one-step mutation.

The genetic location of mutant *sus*8(22) was determined by two-factor crosses on *B. subtilis* $su^{+44}$. *B. subtilis* $su^{+3}$ had been used to construct the genetic map of phage $\phi$29 (9, 12). Despite the fact that the recombination units obtained in the cross *sus*8(769) and *ts*8(93) in strain $su^{+44}$ are half those obtained in strain $su^{+3}$ (Table 1 and Fig. 1), mutant *sus*8(22) can be located approximately midway between mutants *sus*8(769) and *ts*8(93).

Since mutant *sus*8(22) produces a protein fragment with a molecular weight of 25,000, we analyzed the tryptic peptides of this fragment in comparison with those of HP1 or HP3 to determine which of the two missing proteins is the product of cistron 8. The tryptic peptides of the fragment coincide with those of HP1; therefore, protein HP1 is the product of cistron 8. Protein HP3 would be coded by a new cistron named cistron 8.5 (14a) located to the right of cistron 8 and to the left of cistron 9.

The results in this paper are in agreement with those of Reilly et al. (14a). They have isolated a *sus* mutant in the fiber protein (HP3) that induces the synthesis of normal protein HP1, indicating that no polar effect exists in this case, a finding consistent with the order HP1-HP3. Moreover, by three-factor crosses,

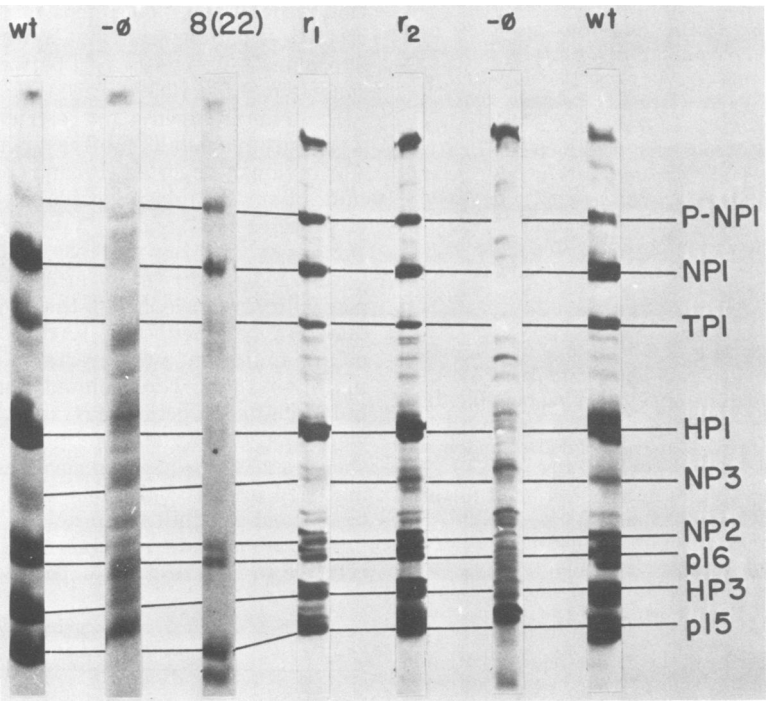


FIG. 2. *Autoradiograph of the viral proteins labeled with a 28- to 35-min pulse in B. subtilis* $su^-$ *infected with mutant sus8(22) or with revertants r1 and r2 separated by slab gel electrophoresis. The proteins labeled with [*$^{14}$*C]leucine were subjected to slab gel electrophoresis in 10 to 20% acrylamide gradients, as described in the text. wt, Wild-type-infected cells;* $-\phi$*, uninfected cells; 8(22), infection with mutant sus8(22); r1, infection with revertant r1; r2, infection with revertant r2.*

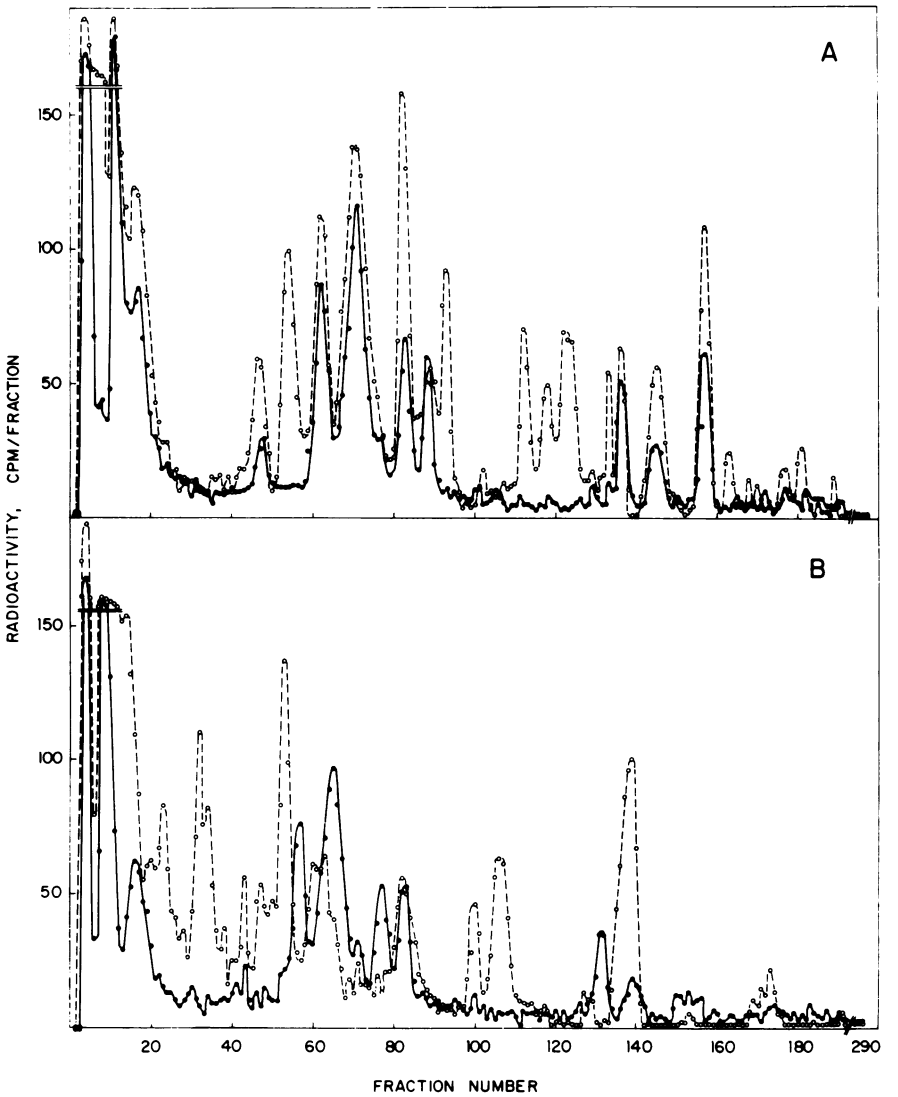


FIG. 3. *Tryptic peptide analysis of the fragment produced after infection with mutant sus8(22) and of proteins HP1 and HP3. The fragment of mutant sus8(22), labeled with $^{14}C$-labeled protein hydrolysate, and proteins HP1 and HP3, labeled with a $^{3}H$-amino acid mixture, were isolated as described in the text. A mixture of the fragment (8,000 cpm) and HP1 (20,000 cpm) (A) or HP3 (21,000 cpm) (B) was reduced, carboxymethylated, treated with trypsin, and chromatographed through a column of Beckman PA-35 ion-exchange resin as indicated. Symbols: ●, $^{14}C$ radioactivity; ○, $^{3}H$ radioactivity.*

they have been able to locate the cistron coding for the fiber protein to the right of that coding for the major head protein.

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