Regulation of the Temporal Synthesis of Proteins in Bacteriophage BF23-Infected Cells

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Regulation of temporal synthesis of pre-early, early, and late proteins in bacteriophage BF23-infected cells has been studied by using five amber mutants defective in genes 1, 2, 10, 14, and 19. The synthesis of pre-early proteins is negatively regulated by the actions of gene 1, a pre-early gene. The switch from pre-early to early protein synthesis is mainly regulated by the second-step DNA transfer reaction, which is controlled by at least genes 1 and 2. Early proteins can be kinetically and genetically divided into two regulatory classes, designated Ea and Eb. The shut-off of Eb-pre-early protein synthesis is associated with the turn-on of late protein synthesis. This step is controlled by genes 10, 14, and 19. Gene 10 also regulates negatively the synthesis of Ea-early proteins, indicating that this gene has a dual function in the regulation of early protein synthesis. The temporal synthesis of phage-encoded proteins is regulated mainly at the transcriptional level. Evidence is presented indicating that the host RNA polymerase is modified by the interaction with the gene products of genes 2, 10, and 14 (gp2, gp10, and gp14, respectively), gp2 interacts with the enzyme in the earlier stage of infection but is replaced by gp10 in the later stage. This exchange reaction depends on the presence of gp14 and gp19 and is related to the switch from Eb to late protein synthesis. Thus, the regulation of BF23 gene expression occurs in a coordinated manner throughout the development of this phage.

Temporal control of gene expression is one of the characteristics commonly observed during the development of various phages. It is mainly regulated at the transcriptional level. A large amount of accumulated evidence has shown that different phages have different strategies for their temporal control of gene expression. For example, phage lambda synthesizes N and Q proteins, both of which function for the host RNA polymerase (RNAP) as antiterminators in the transcription of early and late genes, respectively (for a review, see reference 8). T7 utilizes the host RNAP early in infection for the transcription of certain specific genes. One of them is gene 1, which encodes the T7-specific RNAP that transcribes selectively the middle and late genes (3, 23). T4 synthesizes several phage-encoded proteins that modify the host RNAP to change its specificity in several steps of their propagation (20).

Yet in both BF23 and T5, a two-step mechanism of DNA transfer has been shown to be involved in the time-ordered synthesis of pre-early and early proteins (for a review, see reference 12). After adsorption of phages, about 8% of the phage DNA, termed the first-step transfer segment, is initially transferred into the host cells, and pre-early genes are expressed. Then the rest of the DNA corresponding to early and late genes is injected into the cells as the second-step DNA transfer, which depends on the functions of some pre-early proteins, including PE1 (encoded by BF23 gene 1 and its equivalent T5 gene A1) (11, 14, 16). Furthermore, it has been reported for T5 that at least three genes, C2, D5, and D15, are responsible for the regulation of the synthesis of early and late proteins (4–6).

In an accompanying report, we show the considerable alteration of the patterns of protein synthesis in the cells infected with amber mutants defective in genes 1, 2, 10, 14, and 19 (19). These mutants were classified as type Iib by genetic complementation tests (17). To understand the temporal control of gene expression of BF23, we further characterized these mutants. In this report, we describe first the point(s) affected by each mutation in the temporal synthesis of phage-encoded proteins. Analysis of the regulatory gene products by anti-host RNAP serum indicated that the products of genes 2, 10, and 14 interacted with the host RNAP. On the basis of these results and others, mechanisms of the temporal control of gene expression in BF23 are discussed.

MATERIALS AND METHODS

Media. The synthetic media for growth of uninfected and infected bacteria and buffer for suspension and adsorption of phages were as described previously (15). Rifampin (Dai-ichi Seiyaku Co.) was added to a concentration of 120 μg/ml whenever necessary.

Bacterial and phage strains. The bacterial strains used were Escherichia coli K12W3110 supR rif and supR RifR. The mutant phages used were am57 (gene 1), amh30 (gene 2), amhH11 (gene 10), amhH85 (gene 14), and amh182 (gene 19) (17, 19).

Pulse-labeling of phage-encoded proteins. Proteins synthesized in the cells infected with the wild-type and mutant phages were labeled with [35S]methionine (1,100 Ci/mmole; Amersham Corp.) and analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis followed by autoradiography as described previously (19).

RNA synthesis. A portion of the infected cells was taken at the indicated time, transferred to a tube containing [5-3H]uridine (30 Ci/mmole; Amersham) at a final concentration of 20 μCi/ml, and cultured for 1 min with shaking. At the end of the labeling, NaNO3 was added to the culture to a concentration of 2.3 mM, and the cells were chilled in an ice bath. RNA was extracted from the infected cells with hot phenol by the method of Zivin and Zehring (26), precipitated with ethanol, dried in vacuo, and finally suspended in TE buffer (10 mM Tris hydrochloride, pH 7.5, and 1 mM disodium EDTA). To analyze the phage RNA, BF23 DNA was prepared from CsCl-purified wild-type phages. After denaturation of phages with 0.5% SDS for 5 min at room tempera-

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ture, DNA was extracted twice with phenol saturated with TE buffer and dialyzed against the same buffer. After digestion of DNA with restriction enzymes EcoRI (Takara Shuzo Co.) and HindIII (Takara Shuzo Co.) by the procedures recommended by the manufacturers, the fragments were separated by electrophoresis on 0.1% agarose gel in buffer (30 mM Tris hydrochloride, pH 7.6, 30 mM NaH₂PO₄, and 1 mM disodium EDTA) and transferred to a nitrocellulose filter as described previously (22). DNA-RNA hybridization was performed as described previously (26) and visualized by fluorography (2).

Immunoprecipitation of phage-encoded proteins. Five milliliters of the infected cells (5 x 10⁹/ml) grown in TGAP medium lacking methionine was labeled with [³⁵S]methionine (10 μCi/0.75 μg of methionine per ml) from 1 min to the indicated time of infection. The labeled cells were sedimented by centrifugation at 7,000 rpm in a Hitachi RPR 20-2 rotor for 15 min, washed once with the buffer, and suspended in 0.4 ml of 10 mM Tris hydrochloride buffer, pH 8.1, containing 25% sucrose. After treatment of the cells with lysozyme (0.5 mg/ml) and 0.1 mM disodium EDTA at 0°C for 10 min, 0.5% Brij 58, 0.2 M KCl, and 10 mM MgCl₂ were added to the cell suspensions. The cells were sonicated four times for 15 s each at 0°C. After treatment with pancreatic RNase (0.1 mg/ml; Worthington Diagnostics) for 15 min at 30°C, the lysates were centrifuged at 16,000 rpm for 1 h and the supernatant fraction was collected. To this fraction, 0.01 ml of rabbit antiserum against E. coli RNA polymerase enzyme was added, and the mixture was incubated at 4°C for 38 h. Then, 0.1 ml of a 10% suspension of lyophilized Staphylococcus aureus Cowan I cells (Immunoconsult; Wako Chemical Co.) in 10 mM sodium phosphate buffer, pH 7.2, 150 mM NaCl, and 0.1% NaN₃ was added and the mixture was incubated at 23°C for 10 min, being centrifuged at 13,000 rpm for 10 min. The precipitated fraction was collected, washed three times with buffer (0.5% Nonidet P-40, 50 mM Tris hydrochloride, pH 7.4, 150 mM disodium EDTA, and 0.02% NaN₃), and suspended in a small volume of the SDS-sample buffer of Laemmli (9). After treatment at 98°C for 5 min, the sample was centrifuged at 13,000 rpm for 10 min and the supernatant fraction was used for the analysis of proteins by SDS-polyacrylamide gel electrophoresis followed by autoradiography.

RESULTS

Pulse-labeling pattern of protein synthesis. Five amber mutants, am57 (gene 1), amH30 (gene 2), amHII1 (gene 10), amH85 (gene 14), and am182 (gene 19), were classified as type IIb mutants and shown to alter considerably their patterns of protein synthesis in the infected cells (17, 19). To further characterize these mutants, pulse-labeling patterns of protein synthesis in cells infected with each mutant were compared with that in the wild-type-infected cells presented in Fig. 1 of the accompanying report (19).

Infection of nonpermissive cells with amH30 (gene 2) showed that the synthesis of pre-early proteins proceeded normally, but neither early nor late proteins were synthesized (Fig. 1a). The failure of the synthesis of early and late proteins is due to the apparent defect in the second-step DNA transfer (data not shown). On the other hand, the am57 (gene 1)-infected cells showed neither shutoff of pre-early protein synthesis nor turn-on of both early and late protein syntheses (Fig. 1b), as reported previously (16). The lack of early and late protein syntheses in the am57-infected cells has already been shown to be due to the defect in the second-step DNA transfer (15, 16).

Cells infected with amH111 (gene 10) showed an extended synthesis of early proteins and a substantially diminished synthesis of late proteins (Fig. 2a). Infection with either amH85 (gene 14) or am182 (gene 19) showed similar gel patterns to that of amH111-infected cells, except that some early proteins such as E1 and E8 were shut off normally as observed in the wild-type-infected cells (Fig. 2b and c). It should be noted that these proteins were kinetically distinguishable from the other proteins such as E4 and E9 (19). These results strongly indicate that early proteins can be divided into at least two regulatory classes. We therefore designated these classes Ea and Eb, as indicated in Fig. 2. The synthesis of Ea-early proteins is not affected by the actions of genes 14 and 19, while that of Eb-early proteins is under the control of these genes. In regard to this, the products of genes 14 (E18) and 19 (E14) were not shut off in the cells infected with am182 (gene 19) and amH85 (gene 14), respectively, indicating that these are Eb proteins.

Kinetic analyses of the protein synthesis with type IIb mutants argues for some unique features of temporal control of BF23 gene expression. Here, three things should be noted. First, the synthesis of pre-early proteins is negatively regulated by the action of gene 1. Since the product of gene 1 is PE1 (19), it can be said that the synthesis of pre-early proteins is autoregulated by pre-early protein. Second, the failure of shut off of Eb-early protein synthesis results in the diminishment of late protein synthesis, indicating that these two phenomena are associated. Third, gene 10 regulates the synthesis of not only Ea-early proteins but also Eb-early proteins.
ones. The latter regulation also requires the actions of both genes 14 and 19, while the former does not. This information indicates that gene 10 has a dual function in the control of early protein synthesis.

RNA synthesis. To determine at which level (transcriptional or translational) the gene expression was regulated, Southern blot analysis of phage RNA was performed. When $^3$H-labeled RNA was extracted at various times from the wild-type-infected cells and then hybridized with phage DNA fragments, three distinct classes of RNA were detected (Fig. 3a). The first class of RNA was synthesized immediately after infection and hybridized exclusively with fragments C and D, which contain the terminal redundant sequences of BF23 DNA, corresponding roughly to the first-step transfer DNA segment. The second class of RNA was synthesized at the middle stage of infection and hybridized specifically with fragments A, B, F, G, I, L, N, and O, which are located in a cluster at approximately the 10 to 65% region of the restriction map. The third class of RNA was only detected at the late stage of infection and hybridized with fragments H, J, K, and M.

Figure 4 shows the RNA synthesis after infection with each mutant. In am57 (gene 1)- and amH30 (gene 2)-infected cells, only the first class of RNA was synthesized. On infection with amH111 (gene 10), amH85 (gene 14), or am182 (gene 19), the first and second classes (but not the third class) of RNA were synthesized. These results, together with the patterns of protein synthesis shown in Fig. 1 and 2, indicate that the first, second, and third classes of RNA correspond to pre-early, early, and late RNA, respectively, and that the temporal sequence of the BF23 gene expression is mainly regulated at the transcriptional level.

Interaction of the host RNAP with phage-encoded proteins. It has been shown that the transcription of T5 genes is inhibited by rifampin, an inhibitor of the host RNAP (1). Previous studies also reported that some T5-encoded proteins interacted to form a complex with the host RNAP (7, 13, 24, 25). We have observed that rifampin inhibits the synthesis of RNA in BF23-infected cells (data not shown). Figure 5 shows the autoradiograms of proteins synthesized after addition of the drug. When the drug was added at 2 min after infection, only residual synthesis of pre-early proteins was observed (Fig. 5a). Addition of the drug at 10 min after infection also resulted in residual synthesis of early proteins (Fig. 5b). It is noted that the accumulation of pre-early and early proteins synthesized in the presence of the drug did not induce the synthesis of early and late proteins, respectively. On the other hand, when rifampin-resistant cells were infected with wild-type phages even in the presence of the drug, synthesis of phage proteins proceeded normally (Fig. 5c). These results demonstrate that the propagation of BF23 requires the host RNAP throughout the life cycle.

On the basis of these observations, we examined the effect of antiserum against E. coli RNAP holoenzyme on the immunoprecipitation of phage-encoded proteins. Figure 6 presents the results of experiments in which the extracts were prepared from the wild-type-infected cells at 35 min after infection. When the gels were stained with Coomassie brilliant blue, the amounts of host RNAP precipitated were proportional to the amounts of the antiserum added (Fig. 6a). Under these conditions, at least seven phage-specific proteins (three major and four minor) were immunoprecipitated, with an increase in the amount of the antiserum (Fig. 6b). When the antiserum was replaced by rabbit serum, no immunoprecipitates were detected (data not shown).

When the extracts prepared from cells at 8 min after infection were examined, one major and several minor proteins were immunoprecipitated (data not shown; see Fig. 8a). Note that this major protein was not observed in the 35-min extracts as shown later.

Comparison of the gel patterns of the immunoprecipitated proteins with those of proteins synthesized in the wild-type-and mutant-infected cells revealed that three major proteins observed in the 35-min extracts were E10 (gp16), E18 (gp14),
and E27 (gp10), and that one major protein observed in the 8-min extracts was PE5 (gp2) (gp2 denotes that a protein is a product of gene 2) (Fig. 6b and 7). Minor proteins identified were PE1 (gp1), E2 (gp15), E19, and probably E25 (Fig. 6b).

Since the relationships between some immunoprecipitated proteins and gene products were established, we use hereafter gene products instead of protein bands in nomenclature, when necessary.

Figure 7 also shows some unusual patterns of immunoprecipitates in some mutant extracts. First, gp10, gp14, and gp16 were not observed when the amH30 (gene 2)-infected cell extracts were treated with the antiserum. This is due to the apparent lack of the synthesis of early proteins as shown in Fig. 1a. Second, significant amounts of gp2 but not of gp10 were detected in the immunoprecipitates of the amH85 (gene 14)- and amH111 (gene 10)-infected cell extracts.

To clarify these phenomena, the kinetics of the appearance of immunoprecipitates were examined. Figure 8a shows the results of experiments in which extracts were prepared from cells infected with wild-type phages. It can be seen that gp2, which immunoprecipitated from the pre-early to early period of infection disappeared, with the concomitant appearance of gp10, gp14, and gp16 in the early-to-late period of infection. The disappearance of gp2 was not due to the degradation of gp2 per se, because gp2 synthesized immediately after infection was still observed without a change in amount even in the late stage of infection (Fig. 9).

When the same type of experiment was performed with extracts prepared from cells infected with amH85 (gene 14), significant amounts of gp2 were immunoprecipitated even in the late stage of infection, and the immunoprecipitation of gp10 was severely limited (Fig. 8b). Of interest was the finding that a similar phenomenon also occurred even in the am182 (gene 19)-infected cells, although the product of gene 19 was not immunoprecipitated (Fig. 8c). Note that immunoprecipitation of gp14 was not affected by mutation of gene 19, however. Furthermore, the lack of gp10 in the amH111 (gene 10) extract prevented the disappearance of gp2 from the immunoprecipitates (Fig. 7). The above results together indicate that the disappearance of gp2 from the immunoprecipitates is associated with the appearance of gp10, and that this phenomenon was controlled by the actions of at least both genes 14 and 19.

gp1 (PE1) and two unidentified pre-early gene products, PE2 and PE3, were significantly immunoprecipitated, as was gp2 (PE5) in the amH85 (gene 14)- and am182 (gene 19)-infected cells (Fig. 8b and c). However, amounts of immunoprecipitation of these three proteins varied from experiment to experiment. Therefore, these are not dealt with in this report.

Conversely, immunoprecipitation of gp16 seemed to occur independently of the actions of gp2, gp10, gp14, and gp19. The kinetics of the pattern of protein synthesis after infec-
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FIG. 5. Effect of rifampin on the synthesis of BF32-encoded proteins. E. coli K12W3110 supB RifB cells were infected with wild-type phages. At 2 min (a) or 10 min (b) after infection, 120 μg of rifampin per ml was added to the cultures. A portion of the infected cells was taken at the time indicated under the autoradiograms and pulse-labeled with [35S]methionine (4 μCi/0.1 μg of methionine per ml) for 2 min. Labeled proteins were analyzed by electrophoresis on 7.5% SDS-polyacrylamide gel. (c) Pulse-labeling patterns of protein synthesis in E. coli K12W3110 supB RifB infected with wild-type phages. Rifampin was added at 0 min. Pre-early (PE), early (E), and late (L) proteins are indicated.

FIG. 6. Effect of concentration of antiserum against E. coli RNA L holoenzyme on the immunoprecipitation of BF32-encoded proteins. Cells were infected with wild-type phages and labeled with [35S]methionine (10 μCi/0.75 μg of methionine per ml) for 34 min from 1 min after infection. The lysates were prepared as described in Materials and Methods. To 0.4 ml of lysates, 0 (lane 1), 1 (lane 2), 2 (lane 3), 5 (lane 4), or 10 (lane 5 μl of the anti-host RNAP serum was added. The immunoprecipitated proteins were collected by centrifugation and dissolved in 50 μl of SDS-sample buffer. Ten microliters of each sample was used for the analysis of proteins by electrophoresis on a 12.5% polyacrylamide gel in SDS. (a) Staining patterns of proteins before autoradiography. (b) Autoradiograms of the labeled proteins immunoprecipitated. Lane 6, autoradiogram of the labeled proteins before treatment with the antiserum. Positions of pre-early (PE) and early (E) proteins are indicated.

The use of expressions with wild-type phages in these experiments showed that synthesis of pre-early, early, and late proteins proceeded almost normally except that late proteins synthesized were low in amount when compared with those of wild-type phages. However, this unusual synthesis was not observed when cells were infected with the am151am141 (gene 16 and gene 4) double mutant, and we do not state why this is (data not shown). These results suggest that gp16 is not related directly to the temporal control of the BF32 gene expression. Because of this, we will not consider this problem in this report.

Immunoprecipitation of phage-encoded proteins by the anti-host RNAP serum has been interpreted as suggesting that the phage-encoded proteins interact with the host RNAP to result in some modification of the enzyme. Such modification was considered to be important for the temporal control of gene expression in T5 (24). Our data may also be interpreted as suggesting that the host RNAP is modified with the BF32-encoded proteins. If that is the case, at least three types of modification take place during the developmental cycle of this phage. The first type of modification occurs in the pre-early period, in which gp2 interacts with the host RNAP. This interaction was observed even in the am57 (gene 1)-infected cells (Fig. 7), in which only pre-early proteins were synthesized. This fact implies that the modification of the host RNAP by gp2 does not alter the specificity of the enzyme. The second type of modification is due to the interaction of gp14 in the early period of infection. This modification is independent of the function of gene 19. The third type of modification occurs through the interaction of gp10 with the concomitant disappearance of gp2 and is controlled by the functions of genes 14 and 19. The second and third types of modification correlate not only with the shut off of Eb-early gene expression but also with the turn-on of late gene expression. Thus, these modifications are pre-
sumably important for the switch of protein synthesis from Eb-early to late. At present, the biological significance of the first type of modification is unclear. However, the facts that the disappearance of gp2 and appearance of gp10 are associated with each other and that mutation in gene 2 causes the failure of the second-step DNA transfer would argue that the gene regulation in one step is tightly coordinated with those in subsequent steps throughout the whole cycle of BF23 development.

### DISCUSSION

Infection of *E. coli* cells with BF23 induces pre-early, early, and late proteins in a well-defined temporal sequence. The kinetics of synthesis of phage-encoded proteins indicated that early proteins were further divided into at least two regulatory classes, Ea and Eb. This was supported by the observation that *amH*85 (gene 14)- or *am182* (gene 19)-infected cells exhibited shutoff of the synthesis of Ea-early but not Eb-early proteins. Thus, at least four classes of phage-encoded proteins were distinguishable during the development of BF23. The synthesis of each class of proteins is regulated at the transcriptional level, and the host RNAP is required for the transcription of phage genes throughout the entire development of this phage.

A point of interest is the observation that at least three regulatory proteins, gp2, gp10, and gp14, are coprecipitated with anti-host RNAP serum. Although this type of experiment does not necessarily tell us the nature of the interaction between phage proteins and the host RNAP, it is reasonable to assume that such interaction results from the formation of some complexes of the host RNAP and phage proteins and, consequently, that the host RNAP is modified as summarized in Fig. 10.

After gp2 is synthesized in the pre-early period of infection, it interacts with the host RNAP to result in the formation of a putative complex of RNAP-gp2 (step I). This complex then reacts with gp14 to form a RNAP-gp2-gp14 complex, because the interaction of gp14 with RNAP is not affected by either gp10 or gp19 (step II). The final step (step III) is the substitution of gp10 for gp2 to give rise to a RNAP-gp10-gp14 complex. The last step is dependent on the
function of gp19. Mutations in either gene 14 or gene 19 prevent the interaction of gp10 with the RNAP and, consequently, gp2 is not released from the enzyme even in the late period of infection. Thus, this proposed mechanism satisfies all the data of the immunoprecipitation experiments.

The proposed mechanism also explains the switch from Eb-early to late gene expression. It has been shown that the shut off of Eb-early protein synthesis is associated with the turn-on of late protein synthesis. This reaction corresponds to the conversion of step II to step III and, as a result of it, RNAP-gp10-gp14 somehow directs the specificity of the enzyme from Eb-early to late transcription. Thus, it can be said that gp10, gp14, and gp19 are negative regulators for Eb-early gene expression but positive regulators for late gene expression. In this context, it becomes important to know the nature of early and late gene promoters for an understanding of the roles of gp10 and gp14 in the transcription of early and late genes.

One question is what is the role of gp19 in the interaction of gp10 with the host RNAP? Two possibilities among several could be considered. One possibility is that gp19 catalyzes in some way the modification of some components in the formation of the complexes. As a result of this, gp10 can interact with the RNAP-gp2-gp14 complex. A simpler explanation is that gp19 acts as a carrier of gp10. If this were the case, the formation of the RNAP-gp10-gp14 complex could be rationalized in two ways. First, gp19 reacts with gp10 to form a putative gp10-gp19 complex prior to the interaction of gp10 with the RNAP. This complex then serves as a donor of gp10 to the RNAP-gp2-gp14 complex. After formation of the RNAP-gp10-gp14 complex, gp19 becomes free and is reutilized. Second, gp19 also reacts with the RNAP-gp2-gp14 complex to form a RNAP-gp2-gp14-gp19 complex. Then it reacts with gp10. In this case, interaction of gp19 might be too weak to be detected by our immunoprecipitation method. These possibilities should be clarified in the future.

Another point of interest is the observation that gp10 is required for shut off of not only the Eb-early gene expression but also the Ea-early gene expression. This implies that the regulation of Ea-early gene expression is not independent of that of Eb-early gene expression. Thus, it can be said that both early and late gene expression are well regulated through a dual function of gp10.

The identification and characterization of the BF23 regulatory genes permitted to some extent the comparison of the T5 regulatory genes, of which at least four genes (A1, C2, D5, and D15) have been identified (4-6, 14). In addition, modification of the host RNAP by two pre-early proteins (T5gpA1 and 11-kilodalton [kDa] species) (13, 25) and at least two early proteins, including T5gpC2 and 15-kDa species (24, 25) were reported by two groups, although their data are not completely consistent.

Genetic complementation tests showed that T5 genes A1 and D5 are identical to BF23 genes 1 and 14, respectively (12). Biochemical analyses of the mutations in these genes from both phages support these results.

The cells infected with a mutant of T5 gene D15 failed to turn on the late protein synthesis, but most early proteins except T5E3, T5E10, and T5E12 were synthesized normally (4). Inspection of the gel patterns indicates to us that they are very similar to that of amrB12 (gene 19)-infected cells. Therefore, we suspect that T5 gene D15 is similar to BF23 gene 19 in function. The authors interpreted their results as suggesting that the turn-on of late protein synthesis requires the presence of nicked in the phage DNA that are introduced by the action of the gene D15 product. However, this may not be the case, because the nickless mutants of T5 do propagate normally as wild-type T5 does (21). Also, Ficht and Moyer (7) reported that gpD15 has a molecular size of 35 kilodaltons (kDa) and interacts with the host RNAP. This does not agree with our results. The 35-kDa species that interacted with the host RNAP in BF23 gp16, equivalent to the product of T5 gene D11. This should be examined by use of extracts prepared from cells infected with the corresponding mutants.

The product of T5 gene C2 with a molecular size of 90-kDa was shown to be bound to the host RNAP (25). Although at present a similar mutant is not available in BF23, we could not detect significant amounts of immunoprecipitates around 90 kDa by our method. This problem should be clarified in the future.

Finally, T5 gene A3 was identified by isolation of the mutants that can propagate on cells harboring plasmid ColIb (12, 18, 24). This gene is identical to BF23 gene 2. Although conditional lethal mutants of T5 gene A3 have not been reported yet, one pre-early protein that interacts with the host RNAP may correspond to the product of T5 gene A3. Likewise, the gene corresponding to BF23 gene 10 is not available in T5. One unidentified protein of T5 with a molecular size of 15 kDa which coprecipitated with the host RNAP (24) may correspond to the gene product equivalent of BF23 gene 10.

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