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Pre-Early Polypeptides of Bacteriophages T5 and BF23

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Nine pre-early polypeptides have been detected after infection with bacteriophage T5, and 10 pre-early polypeptides have been detected after infection with bacteriophage BF23. Only about one-half of the coding capacity of the redundant ends of the phage DNA, which code for pre-early proteins, is needed for these 9 to 10 pre-early polypeptides. The direction of transcription of pre-early genes A1 and A2 has been established from the size of N-terminal polypeptide fragments induced by amber mutants and from the known intragenic loci of the amber mutations. Some pre-early functions appear to be nonessential, because a viable deletion mutant of BF23 fails to induce three and possibly four of the detectable pre-early polypeptides.

Bacteriophage T5 induces three sets of proteins in a temporal sequence after infection of sensitive cells (17). The first set, termed "pre-early," is involved with preparing the cell for a successful infection, the second set, termed "early," is required mainly for the synthesis of phage DNA, and the last set, termed "late," is made up predominantly of structural proteins for progeny phage particles (16). In previous studies, three pre-early proteins (17) and four pre-early polypeptides (2) were detected. Only two pre-early polypeptides have been correlated with function (2). This paper is concerned with the identification and function of additional pre-early polypeptides of T5 and of the T5-related phage BF23. Nine pre-early polypeptides from phage T5 and 10 pre-early polypeptides from phage BF23 are identified.

The two polypeptides of T5 previously correlated with function have been assigned structural genes on the basis of the size of N-terminal peptides produced after infection with amber mutants in which the genetic loci of the amber mutations are known. Finally, a deletion mutant of BF23 indicates that some pre-early functions are nonessential.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. *Escherichia coli* F and $F_{su\beta}{}^{+}$, the nonpermissive and permissive strains, respectively, for amber mutants of T5, and *E. coli* 3110 and CR63, the nonpermissive and permissive strains, respectively, for amber mutants of BF23, have been described previously (12, 18), as have the amber mutants themselves (12). BF23*st*(4) is a recently isolated deletion mutant (14).

Growth and infection of bacteria and radioactive labeling of infected bacteria. To label pre-early phage proteins, *E. coli* F or W3110 was grown to about midlog phase at 2×10^8 cells/ml in morpholinopropane sulfonate (MOPS)-glucose-salts medium (21). The cells were harvested by centrifugation in an SS34 rotor at 10,000 rpm for 15 min at 4°C and resuspended at 2×10^9 cells/ml at 0°C in MOPS-salts, which has the same composition as the bacterial growth medium but lacks glucose. The cells were then irradiated for 20 min with UV light at 254 nm from a UVS-54 Mineralight placed 10 cm above the surface of the cell suspension, which was contained in an uncovered 9-cm petri dish. This dose reduces the viable cell fraction to less than 10^{-8} and reduces the level of ^{14}C incorporation in irradiated uninfected cells to about 6% of the unirradiated, uninfected cells. Infection and radioactive labeling of proteins was started by addition of 0.5 ml of the irradiated cell suspension (at 2×10^9 cells/ml) to 4.5 ml of MOPS-glucose-salts medium at 37°C containing a total of 2 μCi of ^{14}C-labeled amino acids and 7 $\times 10^9$ to 8×10^9 phage particles (input ratio, 7 to 8). Aeration was begun immediately after mixing. After 5 min at 37°C, the radioactive, infected culture was chilled to 0°C and centrifuged in an SS34 rotor at 10,000 rpm for 15 min at 4°C. The resulting pellet of infected cells was resuspended in 0.5 ml of the sample preparation buffer defined by Laemmli (13).

For pulse-labeling phage proteins throughout the course of an infection, *E. coli* was grown in MOPS-glucose-salts medium to 2×10^8 cells/ml and infected at that concentration with phage at an input ratio of 7 to 8. Five-milliliter volumes were withdrawn at various times after infection, quickly mixed with 0.5 ml of MOPS-salts containing 2 μCi of ^{14}C-labeled amino acids, and incubated with aeration at 37°C for a specified period of time, usually 2 to 5 min. At the end of each labeling period, the radioactive samples were cooled to 0°C and centrifuged at 10,000 rpm for 15 min in the SS34 rotor of a Sorvall RC2B centrifuge at 4°C. The resulting pellets were resuspended in 0.5 ml of sample preparation buffer.

The proteins in the radioactive samples were sol-

ubilized by heating in sample preparation buffer for 5 min at 100°C and were then separated by electrophoresis in polyacrylamide slab gels prepared from solutions containing 15% acrylamide, 0.4% bisacrylamide, and 0.1% sodium dodecyl sulfate (SDS), using the buffer system of Laemmli (13). After electrophoresis, the gels were fixed in 10% trichloroacetic acid for 30 min, stained with Coomassie brilliant blue, and destained in 7.5% acetic acid. The destained gels were dried under vacuum onto Whatman 3MM paper and clamped together with Kodak DF-85 dental X-ray film in the dark for autoradiography.

Reagents. The ^{14}C-labeled amino acid mixture used for labeling infected cells was the reconstituted protein hydrolysate (no. 3122-08) supplied by Schwarz/Mann. In one experiment (Fig. 6), [^{35}S]-methionine was used for labeling. All common chemicals were reagent grade.

RESULTS

Standard curve for molecular weights of polypeptides. We have used 15% polyacrylamide gels, as described above, to resolve low-molecular-weight polypeptides (down to 2,500) and to estimate their molecular weights. A plot (Fig. 1) of effective electrophoretic mobility in such gels versus the logarithm of the molecular weight of standard polypeptides yields a slightly hyperbolic curve in the region of molecular weights above 20,000. Other workers have obtained similarly shaped curves in this range of molecular weights (22). A break occurs in the standard curve at 15,000 to 20,000 daltons, and a straight line can be drawn through the points for standard polypeptides with molecular weights from about 15,000 down to 1,400. The molecular weights of phage-specific pre-early polypeptides interpolated from this standard curve are given in Table 1 and are in very good agreement with previously published values. Molecular weights of the smaller pre-early polypeptides induced by T5 and BF23 have not been reported before, and the correctness of the values for them (Table 1) depends upon the validity of our standard curve of Fig. 1. The steepness of this curve in the region from 15,000 to 1,400 increases the error of our estimated molecular weights, because small differences in distances migrated cause relatively large differences in estimated molecular weights, and it is difficult to determine the precise point to which a given small polypeptide migrates.

Identification of pre-early polypeptides. T5 and BF23 have the advantage of delineating pre-early gene expression by initially transferring to host cells only the 8% portion of their genomes that codes for pre-early proteins. Thus, infection with a phage mutant defective in transfer of DNA past the initial 8% portion

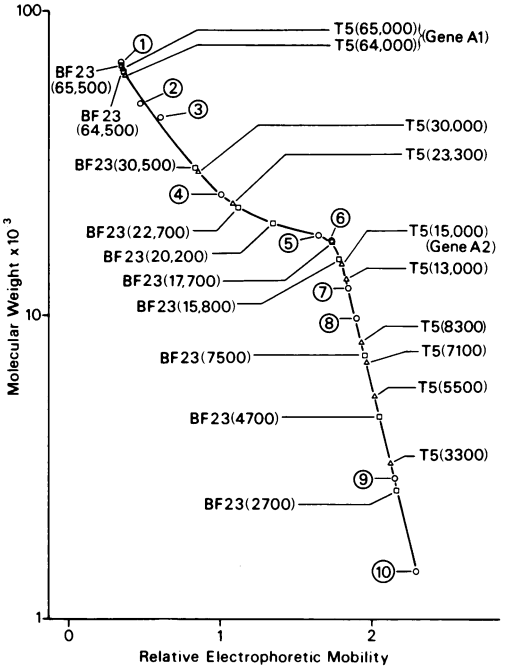

FIG. 1. *Relationship between molecular weight and relative electrophoretic mobility of denatured standard proteins in 15% polyacrylamide gels. The electrophoretic mobilities were calculated relative to that of chymotrypsinogen, which was assigned a value of 1. The open circles and the circled numbers on the curve represent the various standard proteins used. Together with the molecular weights of their subunits, they are: (1) bovine serum albumin (67,000); (2) gamma globulin (50,000); (3) ovalbumin (45,000); (4) chymotrypsinogen (25,000); (5) apoferritin (18,500); (6) myoglobin (17,700); (7) cytochrome c (12,400); (8) neurophysin (9,850); (9) synacthen (2,950); (10) bacitracin (1,450). The pre-early polypeptides induced by T5 and BF23 are indicated by open triangles and open squares, respectively, together with interpolated values for their molecular weights.*

will lead to the synthesis of pre-early, but not of early or late, proteins. Figure 2 shows the polypeptides that are synthesized at various times after infection with T5·A1·*am*H27, an amber mutant defective in gene A1 that can transfer to host cells only the initial 8% portion of its DNA that codes for pre-early proteins. A wild-type pattern is included in Fig. 2 to show that the band designated PE1 is not present after infection with an A1 amber mutant. The proteins were labeled with ^{14}C-amino acids for 5-min periods starting at different times after infection, dissociated into individual polypeptide subunits with SDS and mercaptoethanol, and separated by electrophoresis in polyacrylamide

TABLE 1. *Molecular weights and designations of pre-early polypeptides induced by T5 and BF23*

Pre-early polypeptide	Mol wt					
	T5			BF23		
	This paper	Previously reported	Reference	This paper	Previously reported	Reference
PE1 (gp A1)	65,000	57,000[a]	2	65,500	55,500	3
	64,000	58,000[a]	9	64,500		
PE2	30,000	–[b]		30,500	31,000[c]	3
PE3	23,300	–		22,700	–	
PE4 (gp A2)	15,000	15,000	2	17,700	–	
PE5	13,000	–		15,800	–	
PE6	8,300	–		7,500	–	
PE7	7,100	–				
PE8	5,500	–		5,000	–	
PE9	3,300	–		2,700	–	
PE10	–	–		20,200	–	

[a] Average of the doublet.
[b] –, Not previously reported.
[c] Presumed to be the same polypeptide.

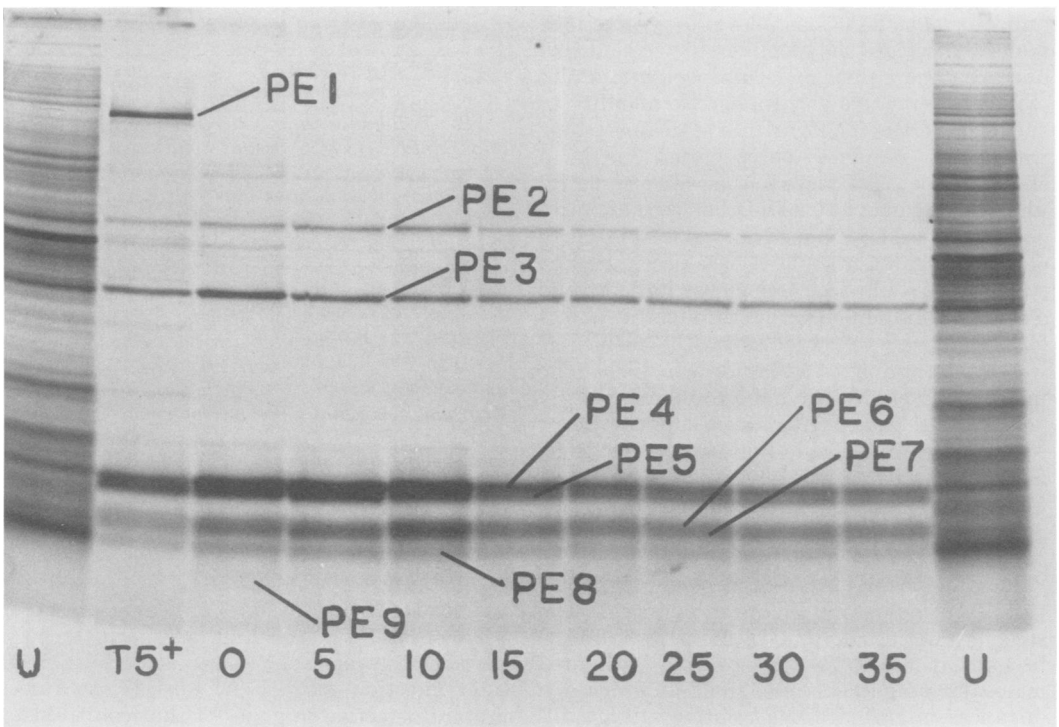

FIG. 2. *Pattern of protein synthesis after infection of E. coli F with a mutant, T5amH27, carrying an amber mutation in gene A1. A single wild-type pattern is shown for comparison. T5amH27 induces the syntheses of all pre-early polypeptides except PE1, which can be seen in the wild-type pattern. No confusion of early gene products with pre-early gene products occurs in this pattern because T5amH27 cannot induce the synthesis of early polypeptides. The number below each pattern specifies the time at which a 5-min labeling period was begun. T5+ designates the wild-type pattern and U designates the pattern from uninfected cells.*

gels containing SDS. Nine bands, designated PE1 to PE9, that correspond to nine pre-early polypeptides are identified in Fig. 2, which represents an autoradiogram of the dried gel. Since PE9 is a faint band, a microdensitometer trace of the wild-type pattern shown in Fig. 6 is

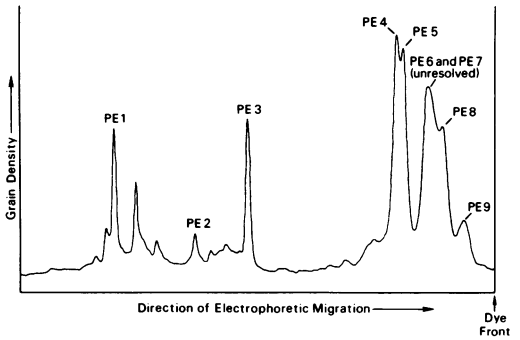

FIG. 3. *Densitometer scan of electrophoretically separated polypeptides synthesized during the pre-early period after infection with T5⁺. The scan is of the T5⁺ pattern shown in Fig. 6. Densitometry was carried out with an Ortec densitometer.*

provided in Fig. 3, which shows PE9 as a small peak near the dye front. Our minimal criteria for differentiating between a phage-specified pre-early polypeptide and a host polypeptide that continues to be synthesized after infection are (i) that the synthesis of phage-specified polypeptides begins within the first 2 min after infection, and (ii) that the amount of ^{14}C incorporated into it per labeling period after infection is more than that of any polypeptide of the host that happens to migrate to the same position as the phage-specific, pre-early polypeptide. An alternative to the second criterion is that if the time at which the synthesis of a putative pre-early polypeptide ceases agrees more with the time that the synthesis of other pre-early polypeptides ceases than with the time that the synthesis of host polypeptides ceases, it would be designated as a phage-specified pre-early polypeptide. However, our main criterion for identifying a phage-specific polypeptide is the electrophoretic migration of a radioactive polypeptide to a position in the polyacrylamide gel where no host-specific polypeptide migrates. Confirmation that each polypeptide designated is indeed phage specific and pre-early needs to come from studies on its function or its genetics.

PE1 meets all the above criteria for a phage-specified, pre-early polypeptide. In addition, amber mutations at four loci in gene A1 eliminate the complete synthesis of PE1, and one of these mutations leads to the synthesis of an N-terminal fragment of PE1 with a size (Fig. 4) compatible to the genetic locus of this mutation (Fig. 5). These data confirm that PE1 is a phage-specified polypeptide and that its structural gene is A1. In addition to these data, we have isolated a temperature-sensitive revertant of T5·A1·*am*H27 that displays the same phenotype at a nonpermissive temperature as does an amber mutant of gene A1 in a nonpermissive host. This behavior of a temperature-sensitive mutant of gene A1 has been reported previously (15).

The intragenic mapping data in Fig. 5 coupled to the data on the size of the amber fragments in Fig. 4 establish the direction of transcription of gene A1, which was suggested previously (1). In addition, the direction of transcription of gene A2 is indicated to be the same direction as for gene A1, since T5·A2·*am*231 induces the synthesis of an amber fragment whereas T4·A2·*am*276 does not (Fig. 6). Assuming that the amber fragment induced by T5·A2·*am*276 is too small to be detected by our gel system, the direction of transcription of gene A2 is from *am*276 to *am*231 and then on toward gene A1, since *am*231 is closer to gene A1 than is *am*276 (Fig. 5).

The polypeptide product of gene A1 appears as a double band after electrophoresis in SDS-polyacrylamide gels. It has been proposed that one of these bands represents a modified form of the other (1). The present data indicate that the putative modified form is present in about the same amount as the presumably unmodified form, even when only an N-terminal fragment is made due to the presence of an amber codon (Fig. 4 and 7). The polypeptide product of gene A1 is involved in at least three functions, namely, the degradation of host DNA, the shut-off of pre-early transcription, and, together with the product of gene A2, the completion of phage DNA transfer (16). The putative modified and unmodified forms of the polypeptide product of gene A1 may organize into different oligomeric proteins, which in turn may account for more than one function that depend upon the product of one gene.

PE2 of T5 is not labeled as heavily as the analogous PE2 of BF23 (Fig. 6). Nevertheless, we do detect it in T5 infections, and it has a molecular weight of about 30,000 as determined by SDS gel electrophoresis (Fig. 1, Table 1). Billmire and Duckworth (3) have reported a polypeptide with this same molecular weight in the inner membrane fractions from both T5- and BF23-infected cells. Thus, PE2 appears to be a phage-specified membrane protein. The low level of radioactive labeling of PE2 after T5 infections had previously obscured not only its detection, but also the present finding that it is missing after infections with an A2 amber mutant of T5 (Fig. 6). Previously, PE4 (Fig. 6) was considered to be the only polypeptide missing after infection with an A2 mutant (2). However, the predictions based upon this previous conclusion are probably still correct, because it

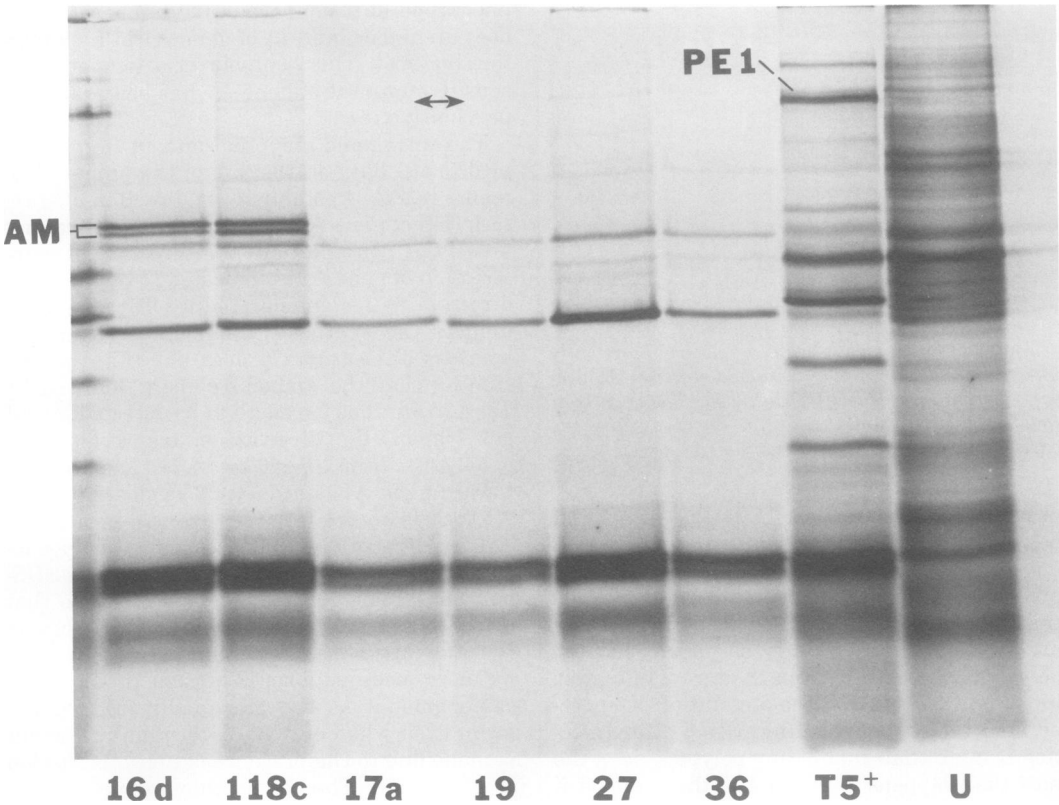

FIG. 4. *Patterns of protein synthesis after infection of E. coli F with T5+ and several amber mutants of gene A1. Polypeptide fragments of identical size can be seen after infection with am118c and am16d, whereas no fragments can be detected after infection with the other amber mutants. The numbers below each pattern define the particular amber mutant used. T5+ identifies the wild-type pattern and U identifies the polypeptide pattern obtained from uninfected cells. AM identifies a polypeptide fragment of PE1 that appears in su⁻ cells after infection with am16d or am118c. The arrows show the normal position of PE1, which is absent in all the amber patterns.*

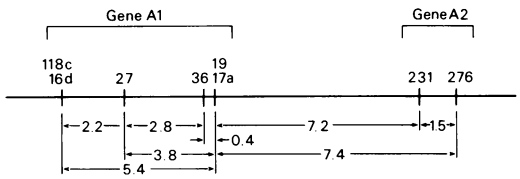

FIG. 5. *Intragenic and intergenic map of amber mutations in genes A1 and A2. The numbers above the uninterrupted solid line designate the amber mutants used in the genetic crosses. The numbers between the arrows below the line represent recombination percentages. The order of am231 and am276 was established by a three-factor cross between a double mutant, amHD-4, derived from a cross between am27 and am231 and a single mutant, am276 or am19. The percentage of doubly recombinant progeny arising from such three-factor crosses were: amHD-4 × am276 = 1.0 (expected value if am276 were outside the markers in HD-4, 1.5; expected value if inside, 0.1); amHD-4 × am19 = 0.9 (expected value, 0.3).*

appears that PE2 is a nonessential polypeptide. Its non-essentiality is revealed, however, by a deletion mutant of BF23, and hence its non-essentiality is argued by analogy. BF23*st*(4) does not induce PE2, a finding that supports the conclusion that BF23*st*(4) has a portion of DNA deleted from its terminally redundant ends (A. R. Shaw and D. J. McCorquodale, manuscript in preparation).

PE3 is a heavily labeled polypeptide in both T5- and BF23-infected cells (Fig. 6) and has a molecular weight of about 23,000 (Fig. 1, Table 1). The function of PE3 is not known, but since its molecular weight (23,000) is close to that of the pre-early 5′-nucleotidase-endonuclease (H. Warner, personal communication) found by Warner et al. (27), it is a possible candidate for this enzyme.

The remaining pre-early polypeptides have molecular weights below 15,000, and they are neither easily nor consistently resolvable. We

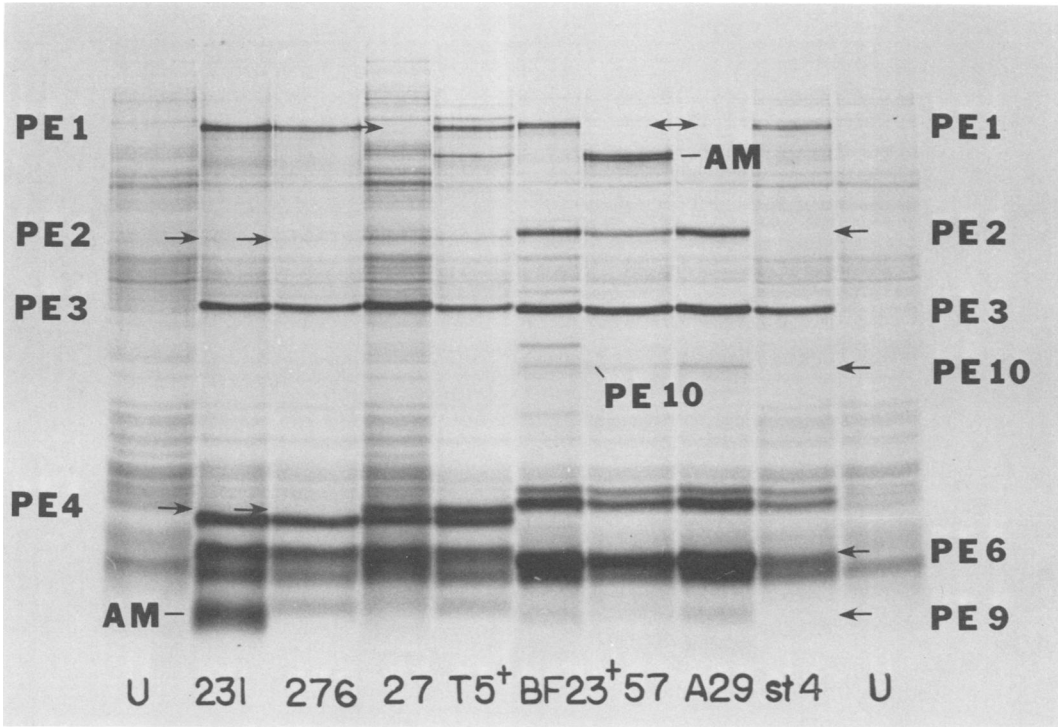

FIG. 6. *Pre-early polypeptides synthesized after infection of E. coli F with T5+, BF23+, representative amber mutants of each phage (designated below their respective patterns), or a deletion mutant, BF23st(4). Wild-type patterns are designated by T5+ and BF23+, whereas uninfected patterns are designated by U. T5am231 and T5am276 carry amber mutations in gene A2, whereas T5am27 carries an amber mutation in gene A1. BF23am57 and BF23amA29 carry amber mutations in gene A1. [35S]methionine was used to label the polypeptides in this experiment. The T5+ and A1 amber mutant patterns are as seen in Fig. 4, but can be compared more easily in this figure with the BF23 patterns. A polypeptide fragment (AM) can be seen after infection with T5am231, but not with T5am276. Another polypeptide fragment can be seen after infection with BF23am57. BF23st(4) does not induce the synthesis of PE2, PE9, PE10, and possibly PE6. PE10 is the only pre-early polypeptide of BF23 that does not seem to have a counterpart among the pre-early polypeptides of T5. The arrows indicate the normal position of polypeptides that are missing after infection with the mutants.*

have found that 15% polyacrylamide gels give a reasonable but somewhat variable separation from day to day. In such gels, six low-molecular-weight, pre-early polypeptides can be discerned (Fig. 2). PE4, which we estimate has a molecular weight of 15,000, is clearly identifiable because it is the product of gene A2 and, hence, is absent after infection with amber mutants in this gene (Fig. 6). It is recalled that the product of gene A2 is required along with the product of gene A1 for complete transfer of phage DNA to host cells (15). Just below PE4 is PE5, which is followed in turn by a doublet, PE6 and PE7, and finally by PE8 and PE9. No functions for PE5, -6, -7, -8 or -9 are known, although it is possible that one of them binds to the cellular membranes of infected cells (9, 10) and corresponds to the product of gene A3 (19). Appropriate mutations in gene A3 allow T5 or BF23 to grow in host cells harboring a ColIb plasmid (18).

PE2, PE9, and an additional polypeptide, PE10, are missing after infection with BF23*st*(4) (Fig. 6), which has the left half of its redundant ends deleted. These circumstances allow tests to determine what pre-early functions are missing after infection with BF23*st*(4) and to assign, thereby, certain functions to certain polypeptides. Pre-early functions that might be absent after infection with BF23*st*(4) include the inactivation of the host cell reactivation system (7, 8) and probably the inactivation of the host's *recBC* nuclease (23).

DISCUSSION

The data in this paper bring together a number of facets concerning pre-early gene products of T5 and BF23. First, we detected 9 pre-early

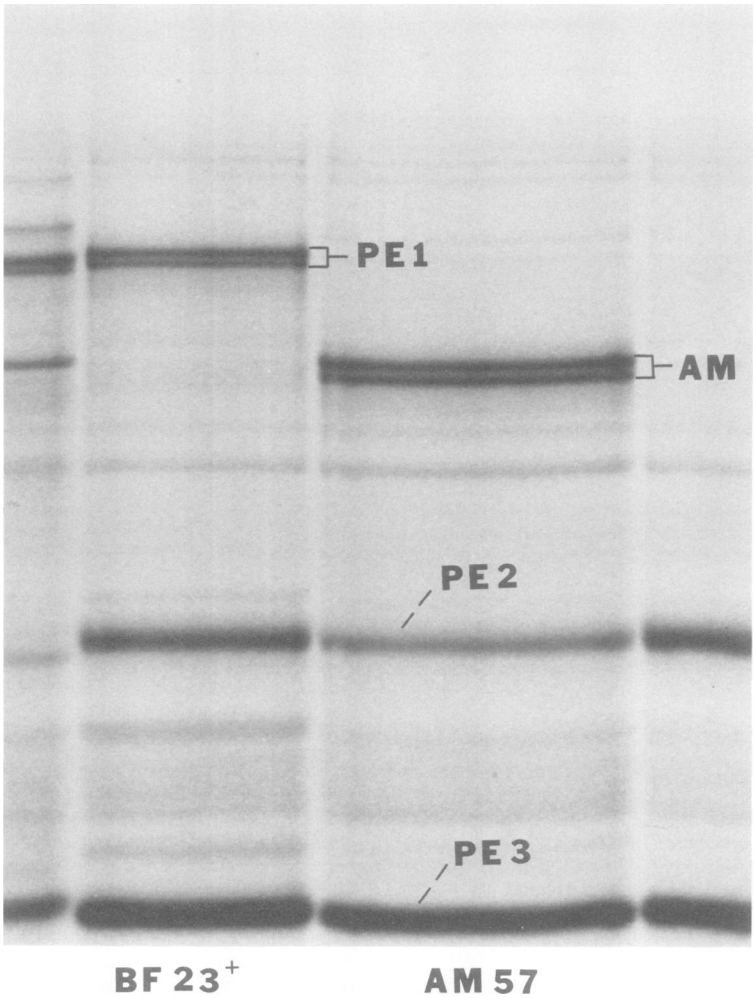

FIG. 7. *Enlarged view from Fig. 6 of PE1 induced by BF23⁺ and of a fragment of PE1 induced by BF23am57, which carries an amber mutation in gene A1. Both the intact PE1 and the amber fragment of PE1 display a double band.*

polypeptides of T5 and 10 pre-early polypeptides of BF23 with combined molecular weights of about 170,000 and 190,000, respectively. The amount of DNA required to code for this amount of polypeptide is about 3.2×10^6 daltons of double-stranded DNA. Since the initially injected 8% segment of DNA that codes for pre-early proteins has a molecular weight of about 6.4×10^6, we are apparently detecting only about one-half of the polypeptides for which it could code. Either the other polypeptides are produced in amounts insufficient to be detected by the procedures used in this report or about one-half of the initial 8% DNA segment does not code for proteins. However, since a set of pre-early RNAs with a combined molecular weight of about 2.3×10^6 has been detected (20, 24, 25), we are inclined to the view that most, but not all, of the initial 8% DNA segment codes for polypeptides. This view presumes that each of the previously detected pre-early RNAs (20, 24, 25) is a specific mRNA rather than some precursor or processed product of another. This specificity could be determined by testing each pre-early RNA in an in vitro translation system. If all nine species of pre-early RNA are unique, either about 70% only of the terminally redundant DNA is transcribed, or the remaining 30% is transcribed to such a small extent that it is undetectable by the techniques used (20, 24, 25).

Some of the polypeptides coded by the initial 8% DNA segment are apparently nonessential, at least in the common laboratory hosts we used

for our infections. This conclusion arises from the behavior of the deletion mutant BF23*st*(4), which has about one-half (the left half) of the initial 8% DNA segment deleted at each end (Shaw and McCorquodale, in preparation). Three and possibly four polypeptides (PE2, PE9, PE10, and possibly PE6) are missing after infection with this deletion mutant, which indicates that 6 of the 10 pre-early polypeptides that we have detected are coded within the right half of the initial 8% DNA. Right and left halves of the initial 8% DNA are defined according to the orientation of T5 and BF23 DNA molecules depicted by McCorquodale (16). Thus, the right half codes for essential pre-early proteins produced in relatively large amounts, and it should therefore be more active in the production of mRNA's than the left half. This conclusion is compatible with the results of Chen and Bremer (6), who found that 70% of pre-early RNA hybridizes to the unnicked strand of T5 DNA and that the sequences corresponding to this pre-early RNA are in the right half of the initial 8% DNA. Furthermore, the six polypeptides coded within the right half have an additive molecular weight of 135,000, which corresponds to about 2.4×10^6 daltons of DNA or about 70% of the right half of the initial 8% DNA. If the remaining 30% of the right half is not transcribed, it might correspond to the nontranscribed region proposed by Chen and Bremer (5, 6).

We have presented a genetic map of genes A1 and A2 that includes all the mutants we have in these two genes. Since the sizes of amber fragments found in cells infected with amber mutants in genes A1 and A2 correspond to the genetic location of the amber mutation within these two genes, we conclude that gene A1 is the structural gene for polypeptide PE1 and that gene A2 is the structural gene for polypeptide PE4, and that neither gene A1 nor A2 is a regulatory gene for synthesis of either of these two polypeptides. We expected to find an amber fragment in cells infected with T5*am*H27, since it maps more than halfway from T5*am*H19 to T5*am*H16d and should therefore produce an amber fragment of reasonable size. Since we did not find such an amber fragment, it may be that incomplete polypeptides coded by gene A1 must reach a certain minimum size before they can enter into a conformation that is resistant to degradative enzymes in the infected cell. However, we have used the *deg*$^-$ strains of Bukhari and Zipser (4) as hosts to see if this interpretation is indicated, but we still did not detect an amber fragment after infection of such cells with T5*am*H27.

The present data confirm the direction of transcription of gene A1, establish the direction of transcription of gene A2, and more firmly establish the physical order of genes A1, A2, and A3 along the T5 and BF23 molecules. Since the deletion mutant BF23*st*(4) induces the synthesis of the polypeptide coded by genes A1 and A2 and is able to mutate so that it can grow in hosts harboring a ColIb plasmid (unpublished data from this laboratory), it must contain the A1, A2, and A3 genes. BF23*st*(4) retains only the right half of the initial 8% DNA, and since the sense strand in this region is exclusively the intact strand (5, 6), the physical order of genes A1, A2, and A3 must be as previously reported (1). It should be pointed out that the data in this paper provide evidence that genes A1, A2, and A3 are transcribed from the intact strand, although analogy is part of the argument. This point was only surmised before (1, 11).

ACKNOWLEDGMENTS

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