

Identification of P48 and P54 as Components of Bacteriophage T4 Baseplates¹

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The involvement of two bacteriophage T4 gene products in the initiation of T4 tail tube and sheath polymerization on mature baseplates has been studied by radioautography of acrylamide gels of various partially completed tail structures. The products of genes 48 and 54 (P48 [the nomenclature P48 refers to the protein product of bacteriophage T4 gene 48] and P54), which are known to be required for the synthesis of mature baseplates, have been shown to be structural components of the baseplate. These gene products have molecular weights of 42,000 and 33,000, respectively. The addition of P54 to the baseplate not only permits the polymerization of the core protein, P19, onto the baseplate, but also causes the disappearance of a polypeptide of molecular weight about 15,000 from the supernatant fraction of infected cells. Another gene product, P27, has been identified in the crude extracts of infected cells. This gene product, which is required for the synthesis of baseplate structures, has the same mobility as one of the unidentified structural polypeptides of the baseplate and is therefore probably also a baseplate component.

The tail of bacteriophage T4 is a complex structure which requires the action of at least 22 gene products for its synthesis (5). The basic outline and many of the details of tail assembly have been described by King and co-workers (10-13). These results are summarized in Fig. 1. The completed tail consists of stabilized sheath protein (P18) and core protein (P19) polymerized on a hexagonal baseplate structure. Proteins P6 through P12 and P29 have been identified as components of the baseplate of phage T4 and are required for its synthesis. The products of genes 5, 25 through 28, 51, and 53 are also required for baseplate assembly but have not yet been identified as baseplate components (13). The results of Snustad (18) suggest that only P26, P28, and P51 are required catalytically during tail assembly, so some of the other unassigned proteins (P5, P25, P27, P53) may also be components of the baseplate.

Proteins P48 and P54 are not required for the synthesis of a "baseplate-like" structure but must be present for the synthesis of mature baseplates on which P19 can subsequently polymerize to form the core structure (10, 11). Neither P48 nor P54 has been identified as a baseplate component, but both are required sto-

ichiometrically for phage synthesis according to the gene dosage experiments of Snustad (18). The steps governed by genes 48 and 54 are somewhat unique in that the modification of the baseplate structure during these steps provides the first site, i.e., the initiation site, for P19 polymerization. The identification of the nature of this modification may provide insights into controlled polymerization reactions in other biological systems.

This report describes experiments designed to determine the nature of the gene 48 and 54 modifications to the baseplate structure. We began these experiments with the assumption that P48 and P54 had not been identified as baseplate components in earlier experiments (13) either because they are present in baseplates in small quantities, or because they were obscured by other more abundant structural proteins in the analytical systems used, or because they are not structural components of the baseplate and the modifications to the baseplate structures were unidentifiable.

MATERIALS AND METHODS

Chemicals and equipment. Sucrose for gradient centrifugation was ultrapure grade from Schwarz/Mann. Sodium dodecyl sulfate (SDS) was specially pure grade from BDH purchased from Gallard-Schlesinger Chemical Manufacturing Corp. Bovine serum albumin and ovalbumin were purchased

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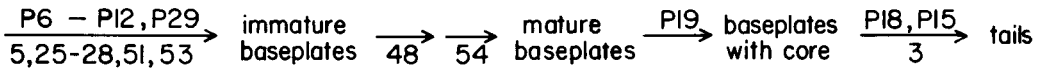


FIG. 1. Morphogenesis of T4 tails. Polypeptides known to be tail components are listed above the arrows. Gene products which have not as yet been identified as tail components are listed below the arrows.

from Sigma Chemical Co. RNase A and DNase I were purchased from Worthington Biochemical Corp. *N,N,N',N'*-tetramethylethylenediamine (TEMED), acrylamide (electrophoresis grade), *N,N'*-methylenebisacrylamide (bisacrylamide) and X-ray film (RP Royal X-omat) were purchased from Eastman-Kodak. [³⁵S]methionine, with a maximum specific activity of 20 Ci/mmol, was prepared and stored according to the methods of Bretscher and Smith (2) from 10-mCi lots of ³⁵SO₄²⁻ purchased from Amersham/Searle. Radioactivity was measured in a Beckman LS-235 scintillation counter using a cocktail composed of 6 g of 2,5-diphenyloxazole per liter of toluene-Triton X-100 (2:1).

Growth of bacteria and phage. *Escherichia coli* CR63 (*su*⁺) was used as the permissive strain on which amber mutant phage were propagated and titered. *E. coli* B/5 and S/6/5 (both *su*⁻) were used as a nonpermissive strain for phage infections and as an indicator strain, respectively. Phage were grown either by single- or multiple-cycle infections in the medium described by Fraser and Jerrel (7). Chloroformed phage suspensions were incubated with DNase (10 μg/ml) for 30 min at 30 C before removing debris by centrifugation at 10,000 × *g* for 10 min. Phage were pelleted by centrifugation at 16,000 × *g* for 90 min and gently resuspended in one-tenth to one-fifth their original volumes of M9 salts (0.7% Na₂HPO₄, 0.3% KH₂PO₄, 0.1% NH₄Cl, 0.05% NaCl) containing 10⁻³ M MgSO₄. Amber mutants (6) of T4 bacteriophage used in this investigation were provided by D. P. Snustad and J. King and are listed in Table 1. The amber mutant tA₃ in gene t is described by Josslin (8) and was also provided by J. King. Phage titers were determined using techniques described by Adams (1). Multiple amber mutants were constructed using techniques described by Edgar (3). Occasionally unequal inputs of parental phages were used in crosses to protect the genotype of one parent.

Sucrose gradient centrifugation. Thirteen-milliliter 10 to 30% linear sucrose gradients were made using TED buffer (0.01 M Tris-chloride, pH 7.5, 0.02 M dithiothreitol, and 10⁻⁴ M EDTA) containing 10⁻⁴ M MgSO₄. Samples (100 to 200 μl) were layered onto these gradients and centrifuged for 4.5 h at 40,000 rpm in an SW40 rotor at 5 C in a Beckman L2-75 centrifuge. Fractions comprising approximately 3% of the total gradient volume were collected by piercing the bottom of the gradient tube.

Preparation of labeled extracts. A 20-ml amount of *E. coli* B/5 grown to 2 × 10⁸ cells/ml in M9 medium (M9 salts containing 1 mM MgSO₄ and 0.4% glucose) were infected at a multiplicity of five to seven phage per cell and vigorously aerated at 30 C. Eleven minutes after the initial infection, cultures infected with phage carrying the wild-type t allele were superinfected at the same multiplicity to in-

TABLE 1. Bacteriophage T4 mutants used

Gene	Mutant
5	amN135
6	amN102
7	amB16
8	amN132
9	amE17
10	amB255
11	amN93
12	amN69
15	amN133
18	amE18
19	amNG524
23	amH11
24	amN65
25	amN67
26	amN131
27	amN120
28	amA452
29	amB7
48	amN022x
51	amS29
53	amH28
54	amH21
t	amtA3

sure lysis inhibition. Cultures to be used as a source for gradient extracts were labeled with three aliquots (100 μCi each) of [³⁵S]methionine at 20, 25, and 30 min after infection. Cultures used to make crude extracts were labeled at 25 min after infection with 50 μCi of [³⁵S]methionine. Casamino Acids (0.1% final concentration; Difco) were added to these cultures 45 min after infection. Cells were harvested after 60 min by centrifugation and were resuspended to 0.5 × 10¹¹ to 2 × 10¹¹ cells/ml in 0.1 × M9 salts containing 10⁻⁴ M MgSO₄ and 500 μg of DNase per ml. These suspensions were frozen and thawed twice and centrifuged at 10,000 × *g* for 10 min to remove debris. Extracts for gradient analysis were further centrifuged at 30,000 × *g* for 30 min.

SDS-gel electrophoresis. SDS-gel electrophoresis was performed using the discontinuous buffer system of Laemmli (14). Slab gels were run using the methods of Studier (20) and Maizel (15). The conditions of the gradient gel system used were suggested by Dwight Anderson. The separating gel consisted of 0.375 M Tris-chloride, pH 8.8, 0.1% (wt/vol) SDS, 0.025% (vol/vol) TEMED, 0.03% (wt/vol) ammonium persulfate, and acrylamide-bisacrylamide in a ratio of 30:0.8 to make the desired weight/volume percentage of acrylamide. The stacking gel consisted of 0.125 M Tris-chloride, pH 6.8, 0.1% SDS, 0.1% TEMED, 0.03% ammonium persulfate, and the same acrylamide-bisacrylamide mixture to give

3.0% acrylamide. The electrode buffer consisted of 0.025 M Tris, 0.192 M glycine, 0.1% SDS, pH 8.3. An 8 (top) to 20% (bottom) separating gradient gel was formed using a standard two-chambered gradient-forming device. The polymerizing mixture was pumped between glass plates and layered with water to give a 12 by 15 by 0.15 cm slab. Three hours later the stacking gel was poured around the well-forming comb. Samples were prepared by immersing 20 μ l of sample plus 10 μ l of 3 \times sample preparation buffer (0.1875 M Tris-chloride, pH 6.8, 30% [vol/vol] glycerol, 15% [vol/vol] 2-mercaptoethanol, 6.9% [wt/vol] SDS, 0.007% [wt/vol] bromophenol blue) into boiling water for 2.5 min, and these samples were layered into the sample wells 40 min after pouring the stacking gel. Electrophoresis was carried out for 17 h at 10 mA constant current. The gel was fixed for 24 h in two changes of 3 volumes of 50% trichloroacetic acid. The gel was then stained in 0.1% Coomassie brilliant blue in 50% trichloroacetic acid and destained by diffusion in 7% acetic acid. These gels were then dried onto Whatman 3MM paper and exposed to X-ray film for 1 to 3 weeks.

RESULTS

Construction of multiple mutants. Of prime importance during the isolation of baseplates from crude extracts of infected cells is the separation of baseplates from all other phage-induced proteins. Since the baseplate is a large, supermolecular structure of about 80S, purification away from small proteins should be fairly straightforward. Structures of larger or comparable size such as ribosomes, phage heads, and head-related structures could present some difficulties during isolation of pure baseplates. To facilitate isolation of baseplates, we constructed multiple amber mutants. To eliminate head formation we included amber mutations in head genes (*amH11* and *amN65* in genes 23 and 24, respectively) in all phage strains used. The *amE18* mutation in gene 18, which blocks tail maturation and prevents formation of poly-sheath (9), and the *amtA3* mutation in gene t, which prevents lysis and metabolic shutdown of the infected cells at late times (8), were also included in all phage strains used. The common

genetic background *amE18/amH11/amN65/amtA3* will be referred to as X in the genetic descriptions of the phages used.

To block baseplate maturation at the various steps of interest, amber mutations NG524, H21, and NO22x in genes 19, 54, and 48, respectively, were added to the X background in a stepwise manner. This insured that, even though the modification steps controlled by genes 48 and 54 are strictly sequential (11, 16), possible interference by the unreacted gene products would be eliminated. Phage strains unable to make baseplates were used as controls and were made by crossing the amber mutation B255 in gene 10 into each of the multiple mutants listed above. The resulting strains with their genotypes and tail structure phenotypes are listed in Table 2.

Purification of baseplates. Since 70S ribosomes isolated from infected cells also contain newly synthesized proteins (17), it is necessary to separate baseplates from ribosomes before analysis of the baseplate proteins on the SDS-acrylamide gels. This was accomplished by sedimenting crude extracts of infected cells through sucrose gradients in the presence of 10^{-4} M $MgSO_4$. The low magnesium ion concentration causes the 70S ribosomes to dissociate into 30S and 50S ribosomal subunits, which are easily separated from baseplates in these gradients (Fig. 2). A peak of radioactivity sediments at 80S only when baseplate synthesis occurs; the presence of the amber mutation in gene 10 in the infecting phage reduces the amount of radioactivity in the 80S region of gradients run from the infected cell extracts to less than 5% that found in gradients run from gene 10⁺-infected cell extracts. We assume that the material sedimenting at this position is baseplates in a homogeneous form with regard to newly synthesized phage proteins.

Identification of baseplate polypeptides. The baseplate-containing peak fractions from these gradients were treated with SDS and mercaptoethanol at high temperatures and fractionated on SDS-acrylamide gels. The resulting autoradiogram is shown in Fig. 3. The presence of a polypeptide with a slightly smaller mobility than that of P9 in the fractions from cells infected with X19 phage, which is missing in the fractions from cells infected with X54 phage, strongly suggests that this polypeptide is P54 and that P54 is a component of the baseplate. Only one significant polypeptide band is present in the comparable 10⁻ fractions, but this polypeptide does not interfere with the identification of the baseplate polypeptides previously identified (12, 13, 21). This band is probably

TABLE 2. Multiple mutant phage strains constructed

Genetic designation	Genes mutated	Tail structure synthesized
X18	18, 23, 24, t	Baseplate with core
X19	18, 19, 23, 24, t	Mature baseplates
X54	18, 19, 23, 24, 54, t	54 ⁻ baseplates
X48	18, 19, 23, 24, 54, 48, t	48 ⁻ baseplates
X1810	18, 23, 24, t, 10	None
X1910	18, 19, 23, 24, t, 10	None
X5410	18, 19, 23, 24, 54, t, 10	None

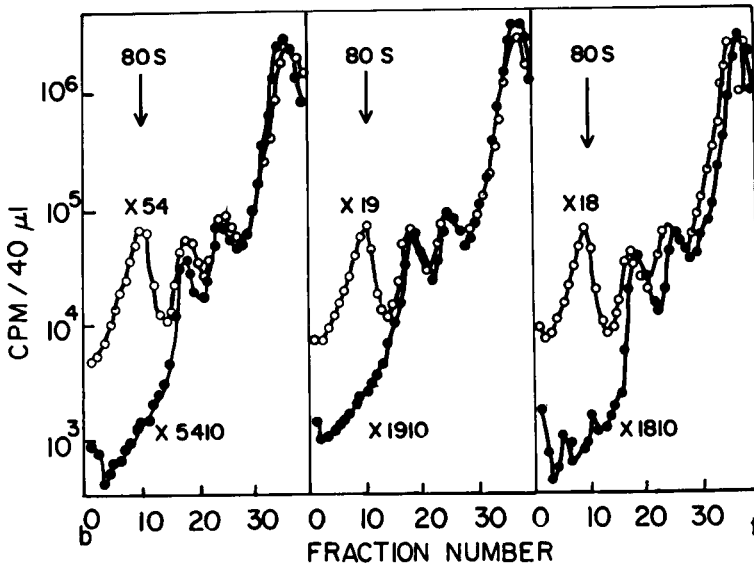


FIG. 2. Sucrose gradient sedimentation of baseplates. Gradients were run using $30,000 \times g$ supernatant fractions from baseplate deficient extracts (●) and baseplate-containing extracts (○).

rapidly sedimenting, aggregated tail fiber subunit P37 (12).

A similar experiment was done including an extract from cells infected with X48 phage (Fig. 4). The results suggest that a polypeptide migrating closely with three others near the center of the pattern is P48 and that P48 is also a component of the baseplate. A polypeptide with a slightly smaller mobility than that of P54 is observed in the X48 fractions, and this may be the P48 amber fragment which still has limited affinity for the baseplate structure.

Tail mutant survey. In an attempt to identify other polypeptides coded for by genes known to be required for baseplate formation, labeled extracts from cells infected with phage carrying single amber mutations were also analyzed on SDS-acrylamide gels (Fig. 5). The polypeptides P7 through P12, P15, P18, and P19 were readily identified and migrated in the order expected from their previously reported molecular weights (13, 21). Close examination of the banding pattern near the center of the gel also revealed the absence of a polypeptide in the gene 27⁻ extract (Fig. 6). We assume that this polypeptide represents the product of gene 27, P27. Additional support for the assignment of this polypeptide as P27 comes from the physical evidence of the polarity of the gene 51 amber mutation used on the synthesis of this polypeptide. Comparison of the P27 band from the gene 51⁻ extract with other gene 27⁺ extracts reveals a notable decrease in the intensity of the P27

band in the gene 51⁻ extract (Fig. 6). This observation, as well as the obvious polarity effect of the amber mutation in gene 9 on the synthesis of P10 (Fig. 5), is in complete agreement with the relative map positions of these pairs of genes and previous co-transcriptional analysis (19).

None of the other unassigned gene products could be unequivocally identified by this procedure. An interesting observation, however, is the absence of a small polypeptide, designated G, from only those extracts containing mature P54⁺ baseplates (i.e., extracts infected with 11⁻, 12⁻, 15⁻, 18⁻, or 19⁻ phage). All of the extracts that cannot synthesize mature 54⁺ baseplates contain this polypeptide. Although P11 and P12 are shown in Fig. 1 to be required for baseplate synthesis, these gene products can actually add at any time during assembly (P11 must add first) and therefore do not have an obligate role in the assembly order (4). Possible explanations for the behavior of polypeptide G are that, when P54⁺ baseplates are made, G becomes membrane bound, is cleaved to smaller fragments which are unobservable on the gel, or is covalently fused with some polypeptide to form an undetected larger polypeptide. It is not clear from the results presented which, if any, of these explanations is valid.

Molecular weight assignments. The molecular weights of the newly identified gene products were determined by plotting mobility relative to P7 versus both the molecular weights of

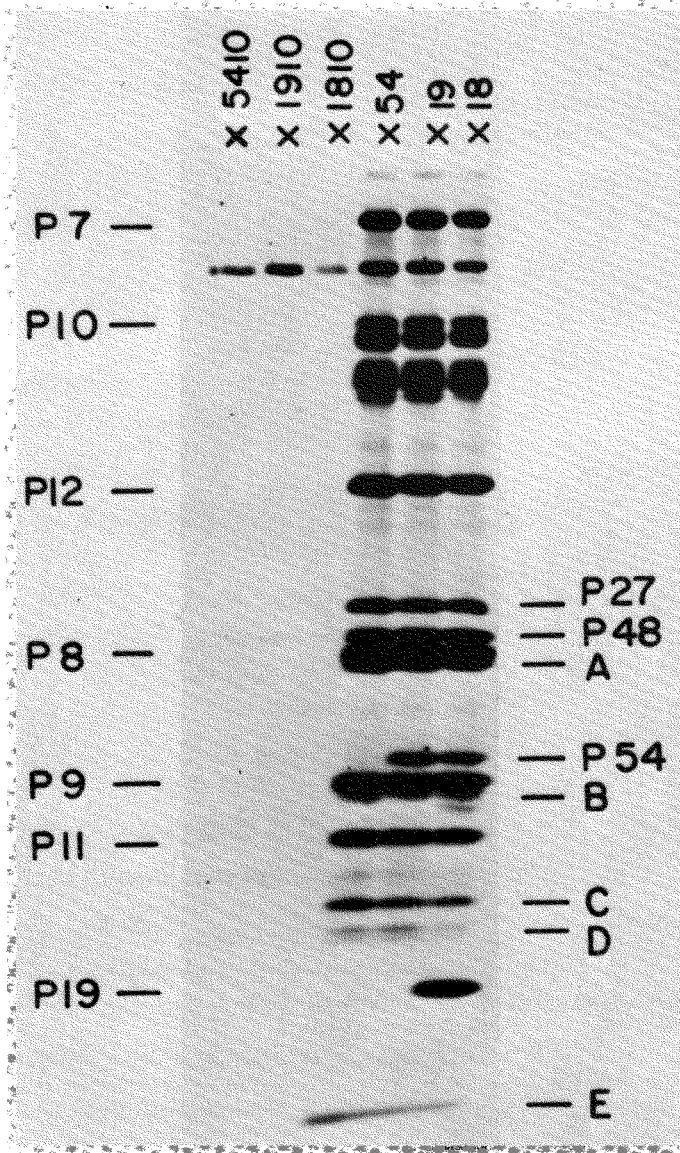


FIG. 3. SDS-polyacrylamide gel electrophoresis autoradiogram of baseplate polypeptides. Portions (20 μ l) of the baseplate-containing peak fractions from sucrose gradients were treated as described in Materials and Methods and electrophoresed on SDS-acrylamide gradient gels. The designations on the left indicate those polypeptides identified in Fig. 5. The designations on the right indicate polypeptides not previously identified in baseplate preparations. The distinction between major and minor bands is blurred somewhat by overexposure of the gel.

standard proteins and the known molecular weights of baseplate polypeptides (21) identified by comparing Fig. 4 and 5 (Fig. 7). From these data we conclude that P27, P48, and P54 have approximate molecular weights of 46,000, 42,000, and 33,000, respectively. Band G migrates slightly slower than RNase in the survey gel

and therefore has a molecular weight of approximately 15,000 (Fig. 5).

At least five other polypeptides in X19 baseplates, which have not been previously reported, are observed in the gels in Fig. 3 and 4. These are designated A through E and are presumably baseplate components also. Their ap-

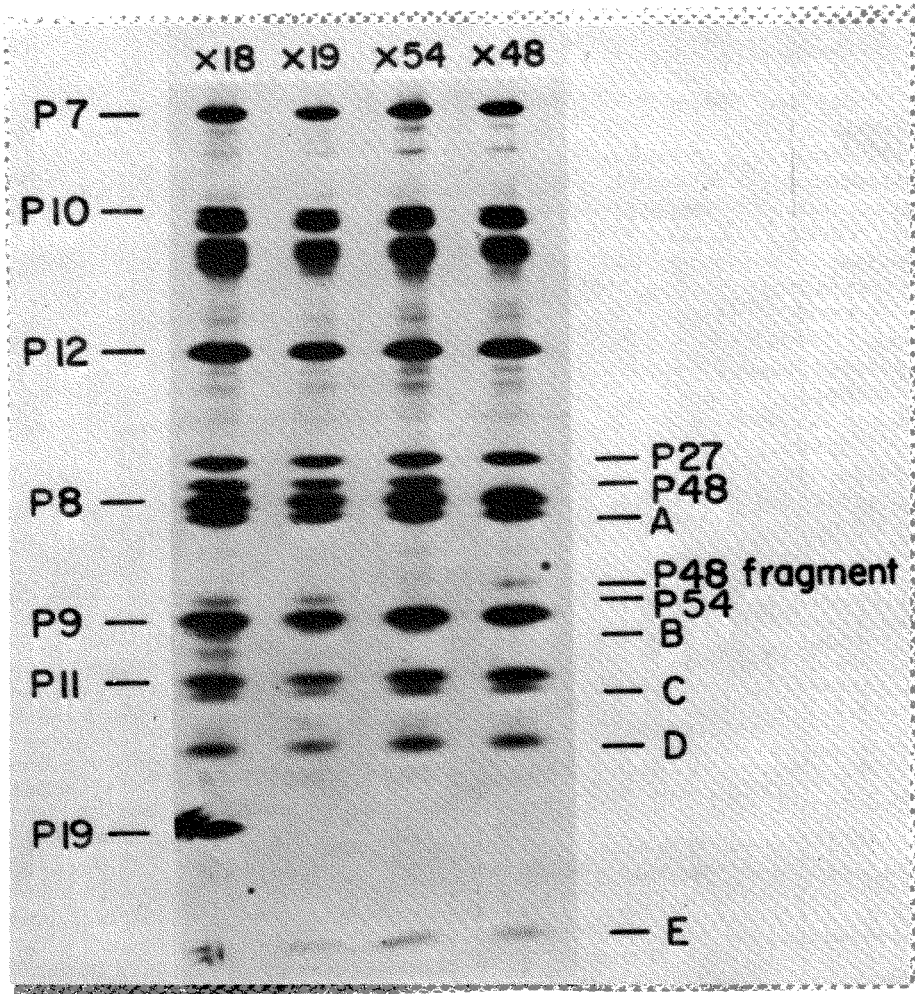


FIG. 4. SDS-polyacrylamide gel electrophoresis autoradiogram of baseplate polypeptides. Samples from sucrose gradients were treated and electrophoresed as described in Fig. 3.

proximate molecular weights are 39,000, 29,000, 26,000, 24,000, and 11,000, respectively. Polypeptide A probably corresponds to that earlier designated X (13), but polypeptides corresponding to B through E have not been previously reported. The identification of these polypeptides as specific gene products is forthcoming (Kikuchi and King, personal communication).

DISCUSSION

The modification of the T4 baseplate under the control of genes 48 and 54 has been shown to be protein addition. That is, the products of genes 48 and 54 seem to be incorporated into the baseplate structure and prepare it as a site for P19 polymerization. The molecular weights of P48 and P54 are about 42,000 and 33,000, respec-

tively, but neither the exact molecular function nor the number of copies per baseplate of these added proteins is known. However, studies by Yamamoto and Uchida (22) suggest that P48 may function in the connection of the baseplate structure to the contractile sheath. Furthermore, due to the obligate order of P48 and P54 action, P48 may also influence the site of reaction of P54 with the baseplate. P54 may then act as the initiation protein for the core protein polymerization reaction. It is also possible that P54 may act as a template for P19 polymerization and thus be involved in the determination of the length of the T4 tail. A solution to the mechanism of this length determination has not been found.

The demonstrated pleiotropic effect of the con-

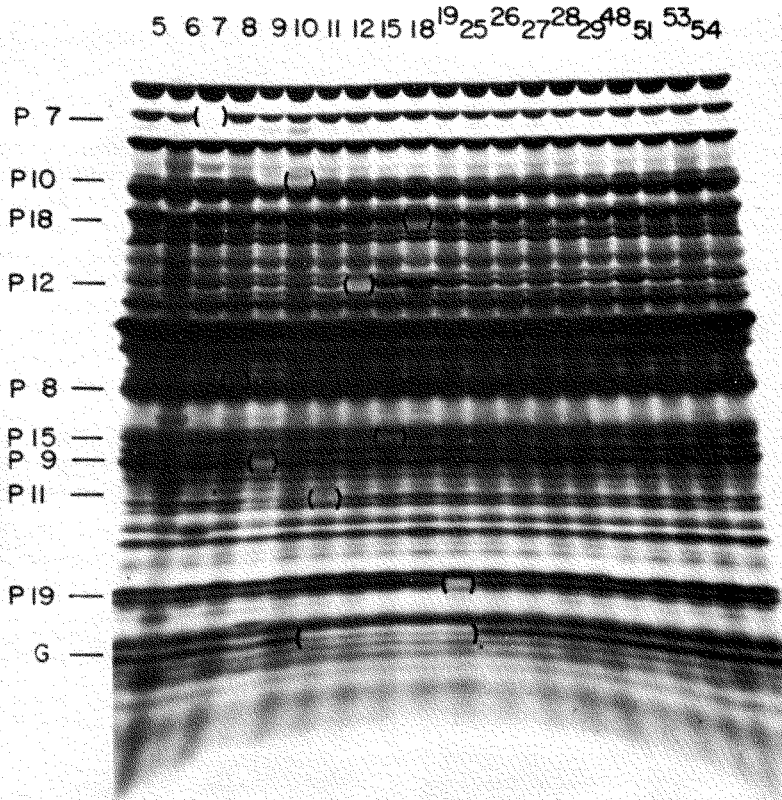


FIG. 5. SDS-polyacrylamide gel electrophoresis autoradiograms of 10,000 × g supernatant fractions of single mutant infected cells. Numbers at the top refer to the defective gene in the infecting phage. Missing polypeptides are marked by parentheses and designated by their gene number.

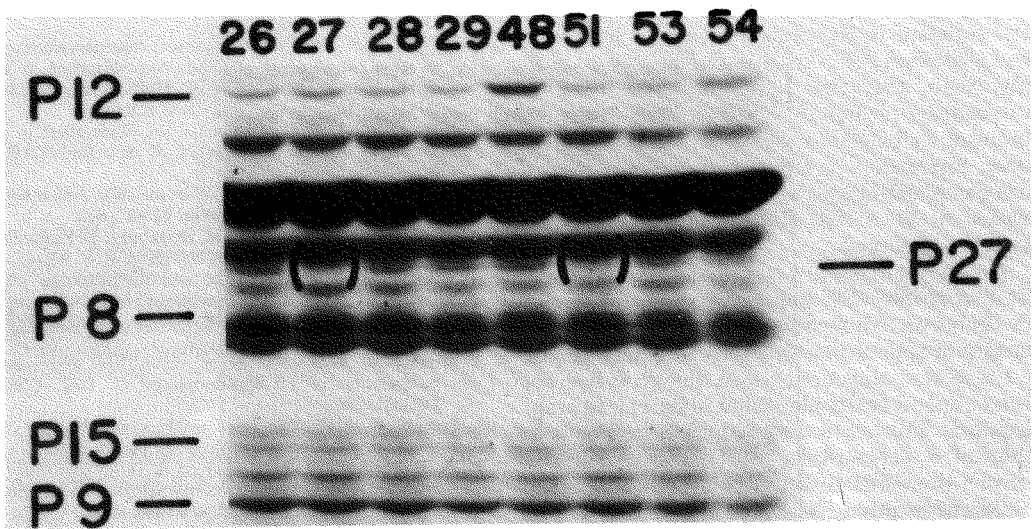


FIG. 6. Photographic enlargement of the P27 region. The gel shown in Fig. 5 was exposed for a shorter time to increase the resolution of minor bands near major bands.

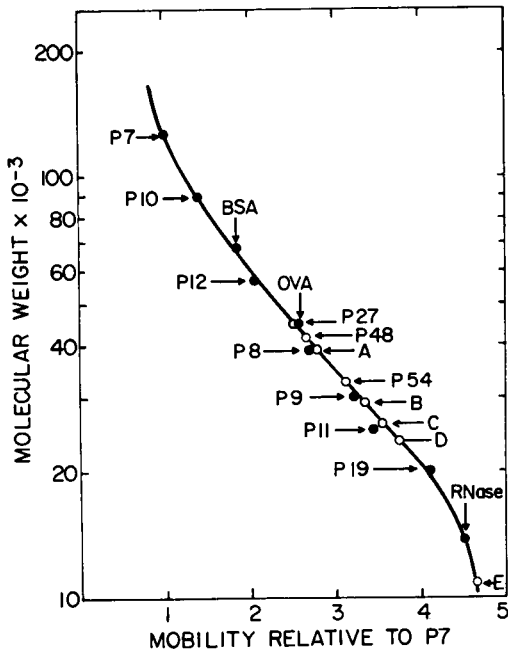


FIG. 7. Determination of molecular weights of polypeptides in SDS-polyacrylamide gradient gels. Log molecular weights of standard proteins and baseplate polypeptides (21) identified in Fig. 5 (●) are plotted as a function of their relative mobilities to P7 in the gel in Fig. 4. The molecular weights of polypeptides A-E, P27, P48, and P54 (○) are determined from their relative mobilities.

version of P54⁻ baseplates to P54⁺ baseplates is unusual. It is difficult to imagine that the gene coding for the polypeptide G, if it has an essential function in the phage baseplate assembly, has remained genetically undefined. It is possible, then, that this band may represent an unessential protein involved in some unknown manner in baseplate assembly. It may, however, represent a protein involved in a step in the complicated process of assembling the core structure which has eluded both physical and genetic detection.

Another polypeptide required for synthesis of T4 baseplates, P27, has also been identified in the gels run. This polypeptide has a molecular weight of approximately 46,000 and may also be a baseplate component, even though it has not been unequivocally identified in baseplate preparations. This molecular weight corresponds to that of a polypeptide, which moves slightly slower than P48 in these gels, and this baseplate polypeptide has accordingly been tentatively designated P27 in Fig. 3 and 4.

Five other polypeptides have been identified as structural components of the baseplate and

have molecular weights of 39,000, 29,000, 26,000, 24,000, and 11,000, respectively. However, the survey gel results did not provide any information about the molecular weights of the products of genes 5, 25, or 53, so these can not be correlated with any of the above baseplate components. Apparently there are too few copies of these polypeptides in the crude extracts to distinguish between their presence and absence, or they comigrate with other polypeptides.

It should be noted that the migration order for P8 and P48 in our gradient gel system is different than that of Vanderslice and Yegian (21) and Kikuchi and King (personal communication). Also, the rate of migration of polypeptides migrating between P11 and P19 varied somewhat from gel to gel (Fig. 3 and 4). The molecular weights for the polypeptides reported here should therefore be viewed in light of this variation.

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