Purification of the Gene 43, 44, 45, and 62 Proteins of the Bacteriophage T4 DNA Replication Apparatus*

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A procedure has been developed which allows the T4 bacteriophage proteins corresponding to the products of genes 43, 44, 45, and 62 to be purified to near-homogeneity from a single T4-infected cell lysate (>90% single species as judged by sodium dodecyl sulfate polyacrylamide electrophoresis). In these preparations, the major problem of removing all contaminating nucleases has been overcome.

Each of the above proteins is known from genetic analysis to be essential for phage DNA replication. The protein product of gene 43 is T4 DNA polymerase, and its recovery can be monitored using a standard DNA polymerase assay. The other three gene products have been designated as "polymerase accessory proteins," since they directly enhance polymerase function on both single- and double-stranded DNA templates. Their activities were monitored by an "in vitro complementation assay," which measures the stimulation of DNA synthesis observed in a concentrated lysate of T4 mutant-infected Escherichia coli cells when the missing T4 wild type protein is added. Starting from 300 g of infected cell paste, we obtained 9.8 mg of gene 43 protein, 21 mg of gene 45 protein, and 70 mg of a tight complex made up of 44 and 62 proteins; final yields were estimated at 30%, 14%, and 28%, respectively, of the initial activity present in the lysate.

When the above purified proteins are incubated with preparations of two other T4 DNA replication proteins (gene 41 and gene 32 proteins) plus deoxyribonucleoside and ribonucleoside triphosphates, extensive DNA synthesis occurs on both single- and double-stranded DNA templates. As reported elsewhere, this synthesis mimicks that catalyzed by the T4 DNA replication apparatus in vivo.

There are at least six known T4 bacteriophage genes whose products are involved with DNA chain elongation and replication fork movement (1–20). These are the T4 genes 32, 41, 43, 44, 45, and 62. When purified preparations of the proteins specified by these six genes are incubated in the presence of deoxyribonucleoside and ribonucleoside triphosphates, extensive DNA synthesis is catalyzed on both single- and double-stranded DNA templates (17–20). Detailed characterization of the activities of these proteins and the in vitro DNA synthesis reactions they catalyze will be found elsewhere (13–22). Here we report a purification procedure, developed and refined in this laboratory during the past several years, which allows reproducible preparation in high yield of the proteins made by T4 genes 43, 44, 45, and 62, starting from a single lysate. The procedures and results are outlined in the text, and are presented in detail in the miniprint supplement.2

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Nuclease Assays—The test for double strand nicking activity is based on the large electrophoretic mobility difference between circular single-stranded DNA and its linear nicked product. Details are described in the legend to Fig. 4. The test for exodeoxyribonuclease used measures the release of acid-soluble radioactivity from fragmented 'H labeled double- and single-stranded DNAs, according to a protocol described elsewhere (15).

Preparation of Norleucine-Sepharose—This procedure is based on that described by Rimerman and Hatfield (32). A 100-ml packed volume of Sepharose 2B (Pharmacia) was washed with a sintered glass funnel and resuspended with water to a total volume of 200 ml. Throughout the remaining procedures, the gel particles are maintained at a temperature of 13°C by addition of crushed ice made from distilled water and gently stirred. First the pH of the gel slurry is raised to 11 by the dropwise addition of 8 ml NaOH. In a fumehouse, the gel is then activated by the addition of finely ground CNBr, 1 g at a time, while the temperature and pH are maintained by the addition of crushed ice and 8 ml NaOH. Within 15 min, 20 g of CNBr should have been added. After the CNBr has completely dissolved and the rate of pH drop has fallen to about half of its initial rate (to about 0.8 pH unit drop/min), the reaction mixture is diluted with 200 ml of ice-cold water. This solution is immediately transferred to a cold, coarse sintered glass funnel where the gel is quickly collected and washed with 1 liter of 0.1 M NaHCO₃ (pH 10). The activated gel is then resuspended into 100 ml of 0.1 M NaCl, previously prepared to contain 0.1 M Na₂EDTA, pH 7.5, and 0.02% NaN₃. The procedure will yield about 15 ml of norleucine bound/packed ml of Sepharose 2B, which can retain as much as 30 mg of protein/packed ml.

Definition of buffers used in purification—Note that all per cent are w/v, and that pH values are those measured at 20°C.

| Buffer | Composition |
|--------|-------------|
| A. buffer | 12.5% glycerol, 10 mM β-mercaptoethanol, 40 mM Tris-HCl (pH 7.4), 1 mM MgCl₂ |
| A₁ buffer | Same as A. buffer except 0.2 M NaCl added |
| A₂ buffer | Same as A. buffer except 2 M NaCl added |
| B. buffer | 10% glycerol, 5 mM Na₂EDTA, 40 mM Tris-HCl (pH 8.1), 10 mM β-mercaptoethanol, 100 mM NaCl |
| B₁ buffer | Same as B. buffer except 250 mM NaCl |
| B₂ buffer | Same as B. buffer except 2 M NaCl |
| C₁ buffer | 10% glycerol, 10 mM β-mercaptoethanol, 40 mM Tris-HCl (pH 8.1), 100 mM NaCl |
| C₂ buffer | Same as C₁ buffer except 20% (NH₄)₂SO₄ |
| C₃ buffer | Same as C₁ buffer except 250 mM NaCl |
| C₄ buffer | Same as C₂ buffer except 20% (NH₄)₂SO₄ |
| D₁ buffer | 62.5% glycerol, 100 mM potassium phosphate (pH 6.5), 10 mM β-mercaptoethanol |
| D₂ buffer | Same as D₁ buffer except 12.5% glycerol |
| G₁ buffer | 19% glycerol, 0.1 M MgSO₄, 0.5 mM dithiothreitol, 20 mM potassium phosphate (pH 7.0) |
| G₂ buffer | Same as G₁ buffer except 100 mM potassium phosphate (pH 7.0) |
| H₁ buffer | 62.5% glycerol, 0.5 mM dithiothreitol, 5 mM MgSO₄, 50 mM potassium phosphate (pH 7.0) |
| I₁ buffer | 12.5% glycerol, 0.5 mM dithiothreitol, 20 mM Tris-acetate (pH 7.8), 2 mM MgSO₄ |
| M₁ buffer | 12.5% glycerol, 0.5 mM dithiothreitol, 80 mM potassium phosphate (pH 7.0) |
| N₁ buffer | Same as M₁ buffer except 150 mM potassium phosphate (pH 7.0) |
| O₁ buffer | Same as M₁ buffer except 400 mM potassium phosphate (pH 7.0) |
| P₁ buffer | Same as G₁ buffer except 400 mM potassium phosphate (pH 7.0) |
| Q₁ buffer | 62.5% glycerol, 0.5 mM dithiothreitol, 5 mM MgSO₄, 50 mM potassium phosphate (pH 7.0) |
| R₁ buffer | 12.5% glycerol, 0.5 mM dithiothreitol, 20 mM Tris-acetate (pH 7.8), 2 mM MgSO₄ |
| S₁ buffer | 12.5% glycerol, 0.5 mM dithiothreitol, 80 mM potassium phosphate (pH 7.0) |
| T₁ buffer | Same as M₁ buffer except 150 mM potassium phosphate (pH 7.0) |
| U₁ buffer | Same as M₁ buffer except 400 mM potassium phosphate (pH 7.0) |

The abbreviations used are: albumin, bovine serum albumin; SDS, sodium dodecyl sulfate; CNBr, cyanogen bromide.
RESULTS

Purification of the Gene 43, 44/62, and 45 Proteins from a Single Infected Cell Lysate—All procedures were carried out at 4°C unless otherwise indicated. Note that the two polypeptides coded for by genes 62 and 44 are isolated together as a stable complex containing multiple copies of the two gene products (14). The first purification step is DEAE-cellulose chromatography of a cleared cell lysate, prepared as described under “Materials and Methods.” All three protein species are separated by this step and are subsequently purified independently of each other.

DEAE-Cellulose Chromatography—A column of Whatman DE52 DEAE-cellulose (29 × 8.5 cm) with a packed volume of 1.7 liters was prepared as described by the manufacturer and eluted with A0 buffer (for buffer definitions, see “Materials and Methods”). All flow rates were 500 ml/h. Fraction I (575 ml prepared as described under “Materials and Methods”) was loaded onto the column followed by washing with 2 liters of A0 buffer. A 5-liter linear salt gradient from 0 to 0.2 M NaCl (A0 to A1 buffer) was run, and the protein elution profile shown in Fig. 1 was obtained. To find the 44/62 protein complex, a gene 44 complementation assay was performed on all column fractions (see “Materials and Methods”); as indicated, all of the activity was found in the fractions which did not bind to the column, slightly leading the major peak of protein in the breakthrough (Fig. 1). A DNA polymerase assay was performed to locate the gene 43 protein activity. This polymerase activity was found in fractions eluting during the early part of the gradient (60 to 90 mM NaCl), ahead of the major peak of salt-eluted protein (Fig. 1). To locate the 45 protein, a gene 45 complementation assay was performed; as indicated (Fig. 1), this activity eluted late in the salt gradient along with the major protein peak (110 to 150 mM NaCl). In each case, peak fractions estimated to contain 80% of the total recovered activity were pooled, giving rise to the three different Fraction II preparations to be described (see Tables I, II, and III, below).

Subsequent to this point, the three protein fractions of interest were purified separately. If necessary, unused fractions could be stored on ice while awaiting further purification. Since 45 and 43 protein activities appear to be reasonably stable. However, with practice, all three preparations can be completed in less than a week starting from cell lysis, with two individuals sharing the work involved. If one person handles the cell lysis and purification of 43 protein, while the other person purifies 44/62 and 45 proteins, a reasonable balance of time and effort is achieved and the proteins are recovered with activities comparable to those to be described. (A suitable time schedule is given in the miniprint supplement).

Purification of 44/62 Protein—A summary of the 44/62 purification results is presented in Table I. A total of 70 mg of highly purified protein complex was obtained from the above DEAE-cellulose elute (Fraction II) by a two-step procedure (hydroxylapatite chromatography followed by phosphocellulose chromatography). Because of the overproduction of 44/ 62 protein induced by the regA mutation (15), the purification required from the cleared lysate was only 73-fold. The final protein obtained represented about a 30% yield of the starting 44/62 activity, as judged by the in vitro complementation assay (Table I). Fig. 2 presents typical data from which such quantitative estimates of activity are made; enzyme units are defined as described in the legend. It should be noted that, for reasons not understood, these complementation activity assays show a logarithmic, rather than a linear, response to added enzyme. (This is shown in Fig. 2 for 44/62 protein, and it is true for the other complementation activities as well (15)). Although we find that estimates made in successive assays are remarkably reproducible, this logarithmic response reduces the accuracy with which the recovery of complementation activity can be quantitated. Note that it also artificially broadens the complementation activity peaks measured from column eluates (e.g. see Fig. 1 and the miniprint supplement), a

Table I

Summary of 44/62 protein purification

An enzyme unit is defined as the amount of activity sufficient to give a 2.0-fold stimulation of DNA synthesis in the standard assay with 15 µl of 44-deficient cell lysate. The activities reported were determined by assay of serial dilutions of each fraction with the same receptor lysate on a single day.

| Fraction | Step          | Volume | Units/ml × 10^6 | Total units × 10^6 | Protein | Units/mg protein | Yield | Enzyme purification % | -fold |
|----------|---------------|--------|-----------------|--------------------|---------|------------------|-------|-----------------------|-------|
| I        | Cleared lysate| 575    | 35              | 20                 | 32      | 1,000            | 100   | 1.0                   |       |
| II       | DEAE-cellulose| 730    | 40              | 29                 | 2.9     | 13,800           | 145   | 12.6                  |       |
| III      | Hydroxylapatic| 126    | 54              | 6.8                | 3.3     | 16,400           | 34    | 15.0                  |       |
| IV       | Phosphocellulose | 28.2  | 201             | 5.7                | 2.5     | 80,400           | 28    | 73.5                  |       |
fact which should be kept in mind when deciding where to truncate the pooling of such peak fractions after each fractionation step.

The 44/62 protein obtained is stored at high concentration, unfrozen at -20°C in Buffer H. Its specific activity has remained essentially unchanged under these conditions for at least 6 months. Precise details of the purification procedures used for this and the other proteins are presented in the miniprint supplement to this paper. All of these procedures have proven to be highly reproducible, and have been in use for several years in three different laboratories.

**Purification of 45 Protein**—A summary of the 45 protein purification results is presented in Table II. A total of 21 mg of highly purified protein was obtained, starting from the DEAE-cellulose eluate previously described (Fig. 1). The additional purification steps used were ammonium sulfate precipitation, hydroxyapatite chromatography, hydrophobic chromatography on norleucine-Sepharose, and a second DEAE-cellulose fractionation. The final preparation, representing 14% of the starting 45 protein complementation activity, was stored unfrozen at -20°C in Buffer O. Its specific activity has not changed noticeably over the course of a year. Precise details of the purification procedures used are presented in the miniprint supplement to this paper.

**Purification of 43 Protein (T4 DNA Polymerase)**—A summary of the 43 protein purification is presented in Table III. A total of 9.3 mg of highly purified protein was obtained, starting from the DEAE-cellulose eluate in Fig. 1. The additional purification steps used were DNA-cellulose chromatography, hydroxyapatite chromatography, hydrophobic chromatography on norleucine-Sepharose, and a second DEAE-cellulose fractionation. The final preparation is stored as a liquid in Buffer D3 at -20°C at a concentration of 0.5 mg/ml or higher. Under these conditions, it appears to be quite stable.

For this particular preparation, on overall yield of about 30% of the original activity was obtained in the most purified fractions (Table III), as judged by the standard DNA polymerase assay described under “Materials and Methods.” When this assay and the gene 43 complementation assay were compared side by side during a similar purification run, compa-

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**Table II**

Summary of 45 protein purification

| Fraction | Step                  | Volume | Units/ml \(\times10^{-3}\) | Protein | Units/mg protein | Yield | Enzyme purification |
|----------|-----------------------|--------|----------------------------|---------|-----------------|-------|---------------------|
| I        | Cleared lysate        | 575    | 7.35                       | 4.23    | 32              | 100   | 1.0                 |
| II       | DEAE-cellulose        | 1,320  | 21.4                       | 2.83    | 7.0             | 306   | 67                  |
| III      | (NH₄)₂SO₄ precipitation| 100    | 17.4                       | 1.74    | 15              | 1,100 | 41                  |
| IV       | Hydroxyapatite        | 180    | 5.49                       | 0.989   | 0.90            | 6,100 | 23                  |
| V        | Norleucine-Sepharose  | 42     | 16.1                       | 0.675   | 0.75            | 21,400| 16                  |
| VI       | DEAE-cellulose after concentration | 7 | 87.1 | 0.610 | 3.0 | 29,000 | 14 | 126 |

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**Table III**

Summary of 43 protein purification (DNA polymerase)

An enzyme unit is defined as the amount of enzyme which will polymerize 1 nmol of total nucleotide in 5 min at 37°C in a standard assay.

| Fraction | Step                  | Volume | Units/ml | Total units \(\times10^{-3}\) | Protein | Units/mg protein | Yield | Enzyme purification |
|----------|-----------------------|--------|----------|----------------------------|---------|-----------------|-------|---------------------|
| I        | Cleared lysate        | 575    | 840      | 483                       | 39      | 96              | 100   | 1.0                 |
| II       | DEAE-cellulose        | 1,100  | 250      | 275                       | 1.6     | 155             | 57    | 6.0                 |
| III      | DNA-cellulose         | 22     | 7,700    | 169                       | 1.8     | 4,180           | 35    | 161                 |
| IV       | Hydroxyapatite        | 9.5    | 21,500   | 304                       | 2.3     | 9,270           | 42    | 356                 |
| V        | Norleucine-Sepharose  | 7      | 39,000   | 273                       | 2.9     | 13,400          | 57    | 517                 |
| VI       | DEAE-cellulose        | 3.0²   | 48,100   | 144³                      | 3.1     | 15,600          | 30    | 599                 |

² Only one-half of Fraction V was loaded onto the DEAE-cellulose column. Therefore, for normalization purposes, the volume and total unit columns in Table III have been doubled.
Purification of T4 Gene 43, 44, 45, and 62 Proteins

TABLE IV

| Designation and T4 gene number | DNA polymerase | Polymerase accessory proteins |
|-------------------------------|---------------|-------------------------------|
| Activities                    | 5' → 3' polymerase | Single-stranded DNA-dependent rATPase, dATPase |
|                               | 3' → 5' exonuclease | Stimulator of 44/62 ATPase. Required for ATP-dependent polymerase stimulation by 44/62 protein |
| Approximate $M_\text{r} \times 10^3$ | 110 | 4 × 34, 2 × 20 |

Fig. 3. Monitoring the purification of each protein by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After slab gel electrophoresis as described under "Materials and Methods," the gel was stained with 0.25% Coomassie blue for 30 min, and then destained in a solution consisting of 5 parts of methanol, 5 parts of H$_2$O, and 1 part of glacial acetic acid. Gel A: Slots A to D follow the purification of 44/62 protein, and show the major protein species present in Fractions I to IV, respectively (Table I). Slots E to I follow the purification of 45 protein, and show the major protein species present in Fractions I, II, III, IV, and VI, respectively (Table II). Slots J to N show the six purified protein fractions described here and elsewhere, and utilized in Figs. 4 and 5 (27, 28); Slot J, 44 protein (34,000 daltons) and 62 protein (20,000 daltons); Slot K, 45 protein (27,000 daltons); Slot L, 43 protein (110,000 daltons); Slot M, 32 protein (35,000 daltons); Slot N, 41 protein (58,000 daltons). At the extreme right-hand side of the figure, a molecular weight ($\times 10^3$) scale is indicated. Gel B, Slots A to E follow the purification of 43 protein, and show the major protein species present in Fractions I, II, III, IV, and VI, respectively (Table III). On the far right-hand side of the figure, the position expected for the 110,000-dalton polymerase is indicated. Note that the 32 protein shown here (Slot M) and used in Figs. 4 and 5 below contains an independent activity recently identified as a seventh T4-induced protein. This protein functions in such small amounts as not to be detectable as a band even on strongly overloaded gels. Nevertheless, its activity is required for the in vitro replication seen in Fig. 5 (Refs. 20, 37).

Catalytic Properties of the Purified Proteins—The most striking in vitro activities of the T4 replication proteins require

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D. Mace, unpublished results.

C. Liu and R. Burke, unpublished results.
that all of them be present simultaneously, and there is physical evidence that they form a multienzyme complex (see Refs. 19 and 20). Since analysis of individual functions in such a multicomponent system is difficult, it is instructive to study in detail as many “partial reactions” as possible. Thus, Table IV lists the well known DNA polymerase and proof-reading exonuclease activities of 43 protein, both of which strongly prefer single-stranded DNAs as substrates. Also, included in Table IV is the fact that the 44/62 protein complex is an ATPase which can hydrolyze either ribo- or deoxyribo-ATP to the corresponding diphosphate and inorganic phosphate. This ATPase reaction is strongly stimulated by single-stranded DNA ends and by addition of 45 protein. Moreover, the 45 and 44/62 proteins synergistically facilitate T4 DNA polymerase (43 protein) utilization of long single-stranded DNA templates, in a reaction requiring ATP (16, 21, 22). In this situation, these proteins can be shown (15) to increase both the rate at which an individual polymerase molecule moves along the DNA template, and its “sticking distance” (or processiveness). Thus, the 44/62 and 45 proteins may be considered to be polymerase “accessory proteins,” which increase the efficiency of the DNA polymerase reaction by an ATP-dependent mechanism whose details are not yet known.

In addition to its crucial role as an accessory protein in T4 DNA replication, the gene 45 protein is essential for late T4 RNA transcription (33, 34). For this second process, it is presumably relevant that the 45 protein has been observed to bind specifically to the T4-modified, host RNA polymerase (35). The 45 protein thus has two different essential roles during T4 infections, and in each role it interacts with a different polymerase.

**Purity**—Analysis of the purified proteins by polyacrylamide electrophoresis in the presence of SDS reveals only one very predominant protein band for each gene product, as illustrated in Fig. 3. Moreover, the mobility (and thus the molecular weight) of each of these bands corresponds to the value expected from previous studies, in which a single altered polypeptide was found after infection with T4 amber mutants in each respective gene (5, 14, 15, 36). As determined by tracing the Coomassie blue-stained gel in Fig. 3 on a Joyce-Loebl densitometer, each protein preparation obtained by our methods is at least 90% homogeneous.

**Absence of Nuclease**—The original T4-infected cell lysate is very rich in nuclease. The purification steps listed in Tables I, II, and III were designed to remove these activities, which otherwise severely distort the *in vitro* replication process. It is crucial that each batch of purified proteins be tested for endodeoxyribonuclease activity with both single-stranded and double-stranded DNA as substrates. This is most conveniently done with the electrophoresis assay shown in Fig. 4. With 20 min of incubation at 37°C under the salt conditions used for the *in vitro* DNA synthesis assay, none of the proteins caused detectable nuclease activity, whether present singly or in the complete replication protein mixture (Fig. 4). The lower limit of visual detection with this assay is estimated to be 10% of the double-stranded or single-stranded template being nicked; thus, with all proteins present, less than one nick should be introduced per 60,000 DNA nucleotides in a 20-min *DNA* replication synthesis reaction.

Contaminating endodeoxyribonuclease activities have been less of a problem in these preparations. With the exception of the T4 DNA polymerase which has an inherent 3’-to-5’-exonuclease activity (7–10), the replication proteins prepared according to the above methods contain no detectable activity in standard exonuclease assays (less than 1% of the DNA rendered acid soluble in a 45-min incubation at 37°C with 20 μg/ml of each protein; for details, see “Materials and Methods” and Footnote 3).

**Stimulation of the *in Vitro* T4 DNA Replication Reaction**—The protein products corresponding to genes 43, 44, 45, and 62 are each an essential component of the T4 DNA replication apparatus, as judged both by *in vivo* (1–4) and *in vitro* studies (17–20). In Fig. 5, we present the rate of DNA synthesis observed as an increasing amount of each purified protein is added to a complete reaction mixture missing only that particular component. (In addition to the above proteins, this reaction requires the gene 41 and 32 proteins, prepared as described in Ref. 27 and Footnote 3). The optimal concentration appears to be around 15 to 20 μg/ml for 44/62 or 45 protein and around 10 to 15 μg/ml for 43 protein.

Several characteristics of this reaction, using as template either a single-stranded fd bacteriophage DNA (17, 18) or a
variety of double-stranded DNAs \( (17, 19, 20) \) have been published. The reaction is a very efficient one, with many times as much DNA made as was originally added as template. Further detailed manuscripts, describing a variety of partial reactions, and the fidelity and the rate of the complete reaction, are in preparation and will be published elsewhere. In addition, many results comparable to ours have been obtained recently in the Nossal laboratory \((37)\).

**DISCUSSION**

Using an *in vitro* complementation assay and a standard DNA polymerase assay, it has been possible to purify the T4 proteins corresponding to genes 43, 44, 45, and 62 to 90% or greater homogeneity, with no detectable exo- or endodeoxyribonuclease contamination. All the proteins are purified from a single T4-infected cell lysate in less than one week's time. In this particular preparation, 70 mg of 44/62 protein, 21 mg of 45 protein, and 9 mg of 43 protein were isolated from 300 g of T4-infected cell pellet. The 44/62 and 43 protein quantities obtained here are about usual, but the 45 protein was obtained in less than half of the expected amount. The 45 protein yield can be improved by increasing the time allowed for growth of the T4-infected bacterial culture used as the source of the purified proteins (to at least 40 min).

It is hoped that this paper and the adjoining paper in this journal \((27)\) will provide a convenient purification scheme which will allow others to purify readily these T4 DNA replication proteins, and thus encourage a wide variety of studies on their physical and chemical properties in other laboratories.

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**REFERENCES**

1. Wood, W. B., and Revel, H. R. (1976) *Bacteriol. Rev.* 40, 847–868
2. Epstein, R. H., Bolle, A., Steinberg, C. M., Kellenberger, K., Boy de la Tour, E., Chevalley, R., Edgar, R. S., Susman, M., Denhardt, G. H., and Lielausis, A. (1963) *Cold Spring Harbor Symp. Quant. Biol.* 28, 379–394
3. Warner, H. R., and Hobbs, M. D. (1967) *Virology* 33, 376–384
4. Riva, S., Casciano, A., and Geiduschek, E. P. (1970) *J. Mol. Biol.* 54, 55–102
5. DeWaar, A., Paul, A. V., and Lehman, I. R. (1965) *Proc. Natl. Acad. Sci. U. S. A.* 54, 1241–1248
6. Warner, H. R., and Barnes, J. (1966) *Virology* 28, 100–107
7. Englund, P. T. (1971) *J. Biol. Chem.* 246, 5684–5687
8. Goulian, M., Lucas, Z. J., and Kornberg, A. (1968) *J. Biol. Chem.* 243, 627–638
9. Nossal, N. G., and Hershfield, M. S. (1971) *J. Biol. Chem.* 246, 5414–5426
10. Lehman, I. R. (1974) *Methods Enzymol.* 29, 46–53
11. Alberts, B., and Frey, L. (1970) *Nature* 227, 1313–1318
12. Dolin, H., Mantell, N. J., and Alberts, B. (1972) *J. Mol. Biol.* 67, 341–350
13. Huberman, J. A., Kornberg, A., and Alberts, B. M. (1971) *J. Mol. Biol.* 62, 39–52
14. Barry, J., and Alberts, B. (1972) *Proc. Natl. Acad. Sci. U. S. A.* 69, 2717–2721
15. Barry, J., Homa Inaba, H., Moran, L., Alberts, B., and Wiberg, J. (1973) in *DNA Synthesis in Vitro* (Wells, R. D., and Inman, R. B., eds) pp. 195–214, University Park Press, Baltimore
16. Mace, D. (1975) Ph.D. thesis, Princeton University, Princeton, N. J.
17. Alberts, B., Morris, C. F., Mace, D., Sinha, N., Bittner, M., and

\(^{7}\) N. Sinha, C. F. Morris, and B. M. Alberts, in preparation.
Moran, L. (1975) in DNA Synthesis and Its Regulation (Goul- lian, M., Hansawalt, P., and Fox, C. F., eds) Vol. 3, pp. 241-269, W. H. Benjamin, Menlo Park

18. Morris, C., Sinha, N., and Alberts, B. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 4800-4804

19. Alberts, B., Barry, J., Bittner, M., Davies, M., Hama-Inaba, H., Liu, C., Mace, D., Moran, L., Morris, C., Piperno, J., and Sinha, N. (1977) in Nucleic Acid-Protein Recognition (Vogel, H. J., ed) pp. 31-63, Academic Press, New York

20. Liu, C. C., Burke, R. L., Hihner, H., Barry, J., and Alberts, R. (1979) Cold Spring Harbor Symp. Quant. Biol. 43, 469-487

21. Piperno, J. R., and Alberts, B. M. (1978) J. Biol. Chem. 253, 5174-5179

22. Piperno, J. R., Kallen, R. G., and Alberts, B. M. (1978) J. Biol. Chem. 253, 5180-5185

23. Moses, R. E., and Richardson, C. C. (1970) Proc. Natl. Acad. Sci. U. S. A. 67, 674-681

24. Wiberg, J. S., Mendelsohn, S., Warner, V., Aldrich, C., and Munro, J. L. (1973) J. Virol. 13, 778-792

25. Wiberg, J. S., Mendelsohn, S. L., Warner, V., Aldrich, C., and Cardillo, T. S. (1977) J. Virol. 22, 742-749

26. Kamin, J., McCulley, C., and Leach, M. (1977) Virology 76, 865-700

27. Morris, C. F., Moran, L. A., and Alberts, B. M. (1979) J. Biol. Chem. 254, 6797-6802

28. Moran, L. (1974) Ph.D. thesis, Princeton University, Princeton, N. J.

29. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275

30. Lammli, U. K. (1970) Nature 227, 680-685

31. Studier, F. W. (1973) J. Mol. Biol. 79, 237-248

32. Rimmman, R. A., and Hatfield, G. W. (1973) Science 182, 1268-1270

33. Wu, R., Geiduschek, E. P., and Cascino, A. (1975) J. Mol. Biol. 96, 539-562

34. Coppo, A., Manzi, A., Pulitizer, J. F., and Takahashi, H. (1975) J. Mol. Biol. 96, 601-624

35. Ratner, D. (1974) J. Mol. Biol. 89, 803-807

36. O’Farrell, P. Z., Huang, W. M., and Gold, L. M. (1973) J. Biol. Chem. 248, 5499-5501

37. Silver, L. L., and Noesal, N. G. (1970) Cold Spring Harbor Symp. Quant. Biol. 43, 489-494

Additional references appear on p. 6796.
Purification of T4 Gene 43, 44, 45, and 62 Proteins

**Purification of Gene 43 Proteins**

The pooled DNA-cellulose fractions from Fig. 1 are further fractionated as described below. For purposes, see text, Table II.

Preparative Chromatography - The DNA-cellulose column breakthrough fractions from Fig. 1 were pooled and gene 43 complementation activity was assayed using 730 ml of buffer. The fractions containing gene 43 activity were pooled and dialyzed against 2 liters of buffer. The pooled fractions were then loaded onto a DEAE-cellulose column, eluted with a linear NaCl gradient (0-0.25 M), and the fractions containing gene 43 activity were pooled and saved.

**Purification of Gene 44 Proteins**

Preparative Chromatography - A column of DEAE-cellulose (100 x 2 cm) was equilibrated with 200 ml of buffer containing 0.05 M NaCl. The fractions containing gene 44 activity were pooled and loaded onto a hydroxylapatite column, eluted with a linear NaF gradient (0-0.5 M), and the fractions containing gene 44 activity were pooled and saved.

**Purification of Gene 45 Proteins**

Preparative Chromatography - A column of hydroxylapatite (100 x 2 cm) was equilibrated with 200 ml of buffer containing 0.05 M NaCl. The fractions containing gene 45 activity were pooled and loaded onto a DEAE-cellulose column, eluted with a linear NaCl gradient (0-0.25 M), and the fractions containing gene 45 activity were pooled and saved.

**Purification of Gene 62 Proteins**

Preparative Chromatography - A column of hydroxylapatite (100 x 2 cm) was equilibrated with 200 ml of buffer containing 0.05 M NaCl. The fractions containing gene 62 activity were pooled and loaded onto a DEAE-cellulose column, eluted with a linear NaCl gradient (0-0.25 M), and the fractions containing gene 62 activity were pooled and saved.

**Further Details of the Purification Procedures**

The pooled DNA-cellulose fractions from Fig. 1 are further fractionated as described below. For purposes, see text, Table II.

**DEAE Cellulose Chromatography** - A column of DEAE-cellulose (100 x 2 cm) was equilibrated with 200 ml of buffer containing 0.05 M NaCl. The fractions containing gene 44 activity were pooled and loaded onto a hydroxylapatite column, eluted with a linear NaF gradient (0-0.5 M), and the fractions containing gene 44 activity were pooled and saved.

**Hydroxylapatite Chromatography** - A column of hydroxylapatite (100 x 2 cm) was equilibrated with 200 ml of buffer containing 0.05 M NaCl. The fractions containing gene 45 activity were pooled and loaded onto a DEAE-cellulose column, eluted with a linear NaCl gradient (0-0.25 M), and the fractions containing gene 45 activity were pooled and saved.

**DEAE Cellulose Chromatography** - A column of DNA-cellulose (100 x 2 cm) was equilibrated with 200 ml of buffer containing 0.05 M NaCl. The fractions containing gene 62 activity were pooled and loaded onto a hydroxylapatite column, eluted with a linear NaF gradient (0-0.5 M), and the fractions containing gene 62 activity were pooled and saved.
PURIFICATION OF T4 GENE 43, 44, 45, AND 62 PROTEINS

PRELIMINARY NOTES ON THE PURIFICATIONS

CONVENTIONAL TIME SCHEDULE FOR PURIFYING THE T4 GENE 43, 44, 45, AND 62 PROTEINS

Day 1: Cell lysis, collect Fraction I in dialysis, change dialysis of Fraction I at 24 h.

Day 2: Start DEAE cellulose chromatography, count DEAE cellulose breakthrough of Fraction II, and start hydrophobic chromatography with 45/47 Fraction II, wash and place 45/47 Fraction II in dialysis.

Day 3: Assay DEAE cellulose for 45 and 47 activity, place 45 Fraction II in dialysis, start DEAE cellulose chromatography with 45 Fraction II, wash and place 45 Fraction III in dialysis, start phenyl-Sepharose chromatography with 45/47 Fraction III, wash and place 45/47 Fraction III in dialysis.

Day 4: Start hydrophobic chromatography with 45 Fraction III, wash hydrophobic chromatography with 45 Fraction IV, place dialyzed 45/47 Fraction IV in 20% storage, precipitate 45 Fraction IV with 10% TCA, wash and place 45 Fraction V in dialysis.

Day 5: Place dialyzed 45 Fraction V in 20% storage, start hydrophobic chromatography with 45 Fraction V, wash and place 45 Fraction VI in dialysis.

Technical Considerations: In the prep reported above, the first DEAE cellulose chromatography may differ with the different protein purification schemes, but may be important. Although all proteins to be purified should normally be purified, the purification may not be as great as usual, necessitating the addition of a second, smaller dose of DEAE cellulose to the second preparation. For this purpose, the second DEAE cellulose is added to the first preparation.

REFERENCES
1. Alberts, B., and Herrick, G. (1971) Methods in Enzymology, 20: 198-207.
2. Bittner, M., Burke, F., and Alberts, B. (1973) Cell, in press.
Purification of the gene 43, 44, 45, and 62 proteins of the bacteriophage T4 DNA replication apparatus.
C F Morris, H Hama-Inaba, D Mace, N K Sinha and B Alberts

J. Biol. Chem. 1979, 254:6787-6796.

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