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# Cloning and expression of T4 DNA polymerase

**(DNA replication/expression vector/overproduced protein)**

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ABSTRACT **The structural gene coding for bacteriophage T4 DNA polymerase (gene *43*) has been cloned into inducible plasmid vectors, which provide a source for obtaining large amounts of this enzyme after induction. The T4 DNA polymerase produced in this fashion was purified by an innovative three-step procedure and was fully active.**

Studies carried out with bacteriophage T4 have provided many important insights about the enzymology and mechanism of viral DNA replication that will continue to aid in our understanding of this process in other organisms (for reviews, see refs. 1–3). One of the advantages of using T4 as a model system for studying DNA replication is that all of the proteins required for strand elongation are phage encoded. This has facilitated the construction of *in vitro* systems for primer extension that have many of the characteristics of DNA replication *in vivo*, such as fidelity, processivity, and rates of nucleotide addition (4–6). Although 11 T4-encoded proteins have been identified as participating in the formation and movement of DNA replication forks, only 5 of these are necessary to reconstitute a "core" system in which leading-, but not lagging-, strand synthesis occurs (1, 7). These 5 proteins, which form a functional complex at replication forks, are the DNA polymerase (43P), a single-stranded DNA binding protein (32P), and the DNA polymerase accessory proteins (44P/62P and 45P). The genes for 4 of these proteins are clustered in one region of the T4 phage genome (Fig. 1).

Although rapid progress has been made in defining the function of each of these proteins, certain important problems remain to be solved. These include the location of active sites in proteins with enzymatic activities, the identification of interfaces among interacting proteins, and the solution of the three-dimensional structure of the replication complex at high resolution.

In this paper we report cloning of the gene for T4 DNA polymerase (gene *43*) and a procedure that has permitted rapid purification of large quantities of the DNA polymerase in a nearly homogenous form, free of contaminating endo- and exonucleases. Since we have also constructed plasmids that produce large quantities of the other four proteins of the core system (ref. 9 and T.-C.L., J.R., and W.H.K., unpublished data), our ability to obtain large quantities of T4 DNA polymerase now allows us to reconstruct a DNA replication complex for high-resolution structural studies. This work, together with the extensive genetic and biochemical studies already completed (1), should provide the most precise description of DNA replication available in any system.

## MATERIALS AND METHODS

**Bacterial Strains.** *Escherichia coli* strain 71-18 (K-12, Δ[*lac, pro*], F′ *lacI*$^{Q}$, *Z*ΔM15 *pro*$^{+}$) was used when propagating M13 vectors and when strong repression of the *lac* or *tac* promoters was necessary for constructing certain plasmids. Strain RR1 [F$^{-}$, *hsdS20* ($r_B^-$, $m_B^-$), *ara-14, proA2, lacY1, galK2, rpsL20* (Sm$^{r}$), *xyl-5, mtl-1, supE44*, λ$^{-}$] was used for maintaining plasmid pTL43W, and strain HB101 (as RR1 except *recA13*) was used for all other plasmid constructions.

**Cloning Vectors.** Vectors M13mp8, -mp19, pUC9, and pUC18 (10) were purchased from Bethesda Research Laboratories; plasmid p*tac*12 was the kind gift of J. Brosius (Columbia University, New York) (11). Plasmid pGW7, phage λ NM761-4 (12, 13), and T4GT7 (14) were the generous gifts of G. Wilson, New England Biolabs.

**Cloning Procedures.** Restriction endonucleases were purchased from New England Biolabs, Boehringer Mannheim, or Bethesda Research Laboratories. The *Xho* I linker (5′ CCTCGAGG 3′) was obtained from Pharmacia. The *Bam*HI linker (5′ CCGGATCCGG 3′), Klenow fragment, and polynucleotide kinase were purchased from Bethesda Research Laboratories. Linkers were phosphorylated with polynucleotide kinase before use.

DNA fragments, purified by electrophoresis on agarose gels, were adsorbed to DEAE membranes (15) and eluted with buffers containing high concentrations of salt. DEAE-coupled membranes, NA-45, were purchased from Schleicher and Schuell and were used according to the instructions of the manufacturer.

**Induction of Gene Expression.** Luria-Bertani (LB) medium supplemented with ampicillin (100 μg/ml) was inoculated with frozen stock of *E. coli* harboring plasmids pTL43W or pTL43Q, grown overnight at 30°C, and then used to inoculate larger cultures.

For analytical experiments (volumes <10 ml), cultures harboring plasmid pTL43W, grown at 30°C in the medium described above, were induced by adding an equal volume of medium warmed to 54°C, which rapidly brought the temperature to 42°C. For larger scale experiments, it was more convenient to induce by adding small amounts of boiling medium with vigorous stirring and monitoring the temperature until it reached 40 or 42°C. Cultures harboring pTL43Q were induced after growth at 30, 40, or 42°C by adding 3 μl of 0.1 M isopropyl β-D-thiogalactopyranoside per ml culture. Care was taken to insure that cultures were induced while still in the logarithmic phase of growth.

**Cell Lysis.** Cells were lysed according to the procedure of Burgess and Jendrisak (16), modified as follows. Cells were resuspended in 50 mM Tris·HCl, pH 8.0/2 mM EDTA/0.1 mM dithiothreitol/1 mM 2-mercaptoethanol at a density of 50 g of cells per liter. Lysozyme was added to 20 μg/ml, and phenylmethylsulfonyl fluoride was added to 1 mM. After 30 min of vigorous stirring at 25°C, sodium deoxycholate was added to 0.05%, and phenylmethylsulfonyl fluoride was added again to a final concentration of 1.5 mM. The lysate was stirred at 25°C for 10 min, placed in ice water, and stirred for 20 min more. The lysate was then sonicated with a Branson Heat Systems sonicator. Cellular debris was removed by centrifugation in a Beckman JA10 rotor at 8000 rpm for 20 min at 4°C.

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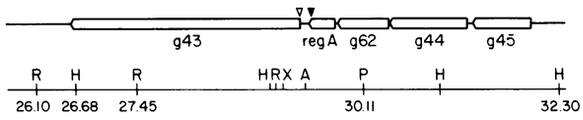


FIG. 1. Restriction map of gene *43* and genes upstream of gene *43*. The promoter (▼) and ribosome binding site (▽) of gene *43* are indicated. Restriction sites are *Eco*RI (R), *Hin*dIII (H), *Xho* I (X), *Ava* I (A), and *Pst* I (P). The numbers under certain restriction sites indicate T4 map units (8).

**Enzyme Purification and Enzyme Assays.** 43P was purified by a slight modification of the method of Nossal (17) for initial enzymatic characterization. DNA polymerase and 3′-to-5′ exonuclease activities were assayed according to the method of Nossal (17), except that at the end of the polymerase assay tritiated deoxynucleotide triphosphate substrates were separated from tritiated product DNA by adsorption to DE 81 filters, essentially as described by Brutlag and Kornberg (18). For the rapid purification procedure, single-stranded DNA-cellulose chromatography was performed as described by Nossal (17), and affinity chromatography using immobilized T4 gene *32* protein was performed as described by Formosa *et al.* (19). $NaDodSO_4$/polyacrylamide gel electrophoresis was performed according to the method of Laemmli (20).

## RESULTS

**Plasmid Construction Strategy.** Gene *43* was cloned as two separate pieces that were joined together before transfer to expression vectors. Attempts to clone gene *43* in its entirety in a single step were not successful, presumably because a putative "lethal" sequence located immediately downstream of gene *43* interfered with cloning that region of DNA. All known restriction sites downstream of gene *43* also turned out to be downstream of this lethal sequence, so that every restriction fragment containing the 3′ end of gene *43* also included this lethal site. This compelled us to revise our initial cloning strategy, which had been to try to clone full-length gene *43* in one step, and to clone gene *43* as two fragments that were later joined to produce the intact gene.

In addition, the promoter of gene *43* was removed before joining the separately cloned segments of gene *43*. This was done for two reasons. First, the promoter was removed as a precaution to prevent uncontrolled expression of gene *43*, because synthesis of T4 DNA polymerase above a certain level is likely to be detrimental to the host cell. Our suspicion that gene *43* expression would be detrimental to the host was confirmed in later experiments, as all attempts to replace the promoter upstream of intact, reassembled gene *43* failed to produce stable recombinants. Second, the promoter was removed to prevent 43P from repressing its own synthesis from expression vectors. Genetic studies have shown that 43P regulates the transcription of its gene (21, 22), although the requirements of this autoregulation have not been precisely defined. For these reasons, we decided to remove the gene *43* promoter, leaving its ribosome binding site intact, as an early step in the construction of a full-length gene *43* clone.

**Subcloning and Manipulation of the 5′ End of Gene *43*.** The 5′ region of gene *43* was subcloned from a λ-T4 recombinant, λ NM761-4 (shown at the top of Fig. 2), originally constructed from a partial *Hin*dIII digest of cytosine-containing T4 DNA by Wilson and Murray (12, 13). This recombinant phage contained a 5.6-kilobase-pair (kbp) fragment of T4 DNA (26.68 to 32.30 map units, Fig. 1) and included the coding regions for genes *45*, *44*, *62*, and *regA*, as well as the largest segment of gene *43* that had been cloned from T4 DNA as a restriction fragment. The cloning strategy was to transfer a restriction fragment containing gene *43* from λ NM761-4 into a plasmid vector, to remove the genes upstream of gene *43* along with the gene *43* promoter, and then to reassemble intact gene *43* by adding the separately cloned 3′ end. Towards that end, a 3.9-kbp *Pst* I fragment, containing the 3′ end of gene *62*, all of *regA*, and the 5′ end of gene *43*, was subcloned into pUC9, generating the plasmid p43NR (Fig. 2,

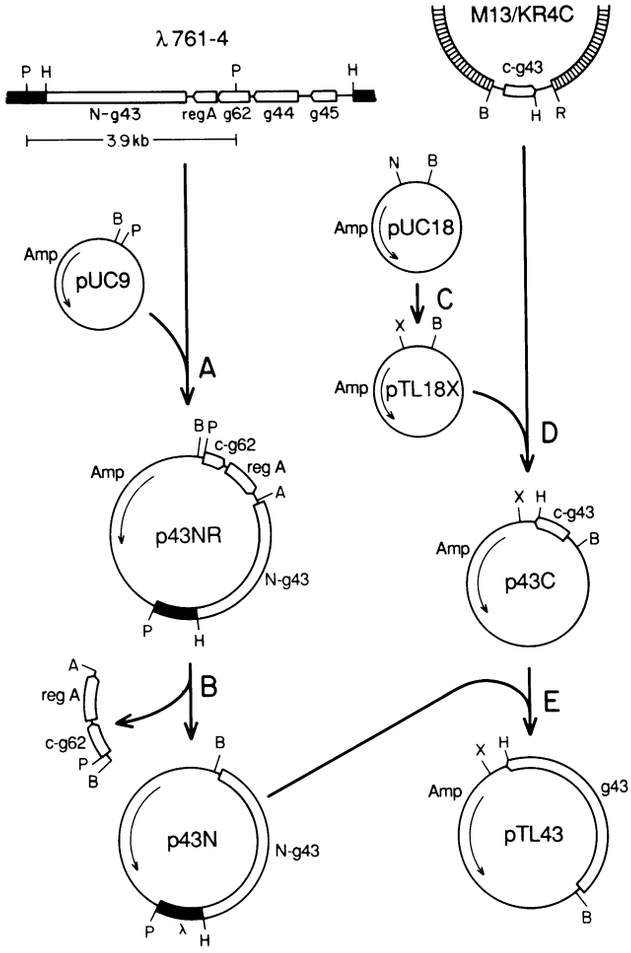


FIG. 2. Separate cloning of the 5′ and 3′ ends of gene *43* and assembly of intact gene *43*. Cloning the 5′ end of gene *43* into pUC9 (arrow A). A 3.9-kbp *Pst* I fragment from λ NM761-4, containing the 5′ end of gene *43*, was cloned into the *Pst* I site of pUC9 to give the plasmid p43NR. Removal of the gene *43* promoter and upstream T4 genes (arrow B). The gene *43* promoter was removed from p43NR by digestion with *Bam*HI, followed by a partial *Ava* I digestion that also removed the 3′ end of gene *62* and the *regA* gene. The 5′ overhang made by *Ava* I treatment was filled in with Klenow fragment. *Bam*HI linkers were attached to the resulting blunt ends. After digestion with *Bam*HI, the plasmid was recircularized with DNA ligase to give the plasmid p43N. Construction of the vector pTL18X (arrow C). The *Nde* I site of pUC18 was changed to an *Xho* I site. pUC18 was digested with *Nde* I, and the resulting 5′ overhang was filled in with Klenow fragment. *Xho* I linkers were attached. After digestion with *Xho* I, ligation and recircularization gave the plasmid pTL18X. Cloning the 3′ end of gene *43* into pTL18X (arrow D). The replicative form of M13/KR4C was digested with *Eco*RI, the *Eco*RI overhang was filled in with Klenow fragment, and *Xho* I linkers were attached. The M13 recombinant was then treated with *Xho* I and *Bam*HI, and the fragment containing the 3′ end of gene *43* was isolated. pTL18X was also treated with *Xho* I and *Bam*HI and ligated with the purified M13/KR4C fragment to give p43C. Assembly of gene *43* from the separately cloned 5′ and 3′ ends (arrow E). p43N and p43C were treated with *Bam*HI and *Hin*dIII, but a partial *Hin*dIII digest was performed on p43N to prevent cleavage at *Hin*dIII sites within gene *43*. Replacement of the smaller *Bam*HI–*Hin*dIII fragment in p43C, which contains sequences already present in the fragment from p43N, with the *Bam*HI–*Hin*dIII (partial) gene *43*-containing fragment from p43N gave pTL43, the full-length clone. Restriction sites are *Ava* I (A), *Bam*HI (B), *Hin*dIII (H), *Nde* I (N), and *Xho* I (X).

arrow A). Further manipulation of p43NR to remove the T4 genes upstream of gene *43* and the gene *43* promoter gave the plasmid p43N (Fig. 2, arrow B). It was estimated that this plasmid contained at least 95% of gene *43*, based on the length of the insert and the size of T4 DNA polymerase determined by $NaDodSO_4$/PAGE. Cloning the 3′ end of gene *43* allowed us to deduce the precise size of gene *43* and showed that p43N lacked only 21 nucleotides from the 3′ end of gene *43*.

**Cloning the 3′ End of Gene *43*.** In our first attempt to clone the segment of gene *43*, which was not present in λ NM761-4, a *Hin*dIII–*Eco*RI fragment was isolated from cytosine-containing T4GT7 DNA (14). This fragment spanned T4 map units 26.68 to 26.10, a region of the T4 genome that contains the 3′ end of gene *43* (Fig. 1). The *Hin*dIII site at map unit 26.68 is the site where truncated gene *43* is joined to λ sequences in λ NM761-4. Repeated attempts to clone this *Hin*dIII–*Eco*RI fragment into a variety of vectors, e.g., pUC8 and M13mp8, failed, although other T4 DNA fragments derived from the same digest were readily cloned. We assumed that the fragment containing the 3′ end of gene *43* could not be readily cloned into these vectors because it contained a nucleotide sequence that interfered with replication of the plasmid or with survival of the *E. coli* host. Another attempt to clone the 3′ end of gene *43* from a restriction fragment was made by digesting the *Hin*dIII–*Eco*RI fragment with *Msp* I to produce a 280-bp *Hin*dIII–*Msp* I fragment. This smaller fragment also could not be cloned. Thus, it appears that a lethal sequence exists within a 280-bp region downstream of the *Hin*dIII site at 26.68 map units.

Since no other restriction sites were known to exist between the *Msp* I and *Hin*dIII sites, we separated the 3′ end of gene *43* from the lethal sequence by sonicating cytosine-containing T4 DNA. Fragments with an average length of 400 bp were used to produce a T4–M13mp8 library that was screened with a $^{32}$P-labeled *Eco*RI–*Hin*dIII restriction fragment (map units 27.45 to 26.68) isolated from p43N. DNAs from plaques that hybridized with the probe were sequenced to confirm that they contained the probe site. One of the positive phage isolates, M13/KR4C, contained the probe sequence upstream of the *Hin*dIII site and 102 bp downstream from the *Hin*dIII site (E.K.S., J.R., C. Fung, J. D. Karam, and W.H.K., unpublished data). The downstream sequence had a 21-bp open reading frame followed by two tandem in-frame translation termination codons. This 27-bp region, corresponding to the 3′ end of gene *43* with its tandem termination codons, was cloned into a derivative of pUC18, as described below.

**Assembly of Gene *43* and Cloning into Expression Vectors.** To join the two portions of gene *43*, it was necessary to create a convenient restriction site by inserting a linker downstream of the 3′ end of gene *43*. We chose an *Xho* I linker, anticipating the transfer of gene *43* into the compatible *Sal* I site of our overproduction vectors in a later step. *Xho* I linkers were also inserted in pUC18 to form the vector pTL18X (Fig. 2, arrow C). The 3′ end of gene *43* was moved from M13/KR4C into pTL18X to produce the plasmid p43C (Fig. 2, arrow D). The 5′ end of gene *43* was then transferred from p43N to p43C, joining the two segments of gene *43* at the *Hin*dIII site, to give pTL43, containing the entire gene *43* (Fig. 2, arrow E).

Gene *43*, reassembled in pTL43, was then cloned into two different expression vectors. One vector, pTL9W, contains the strong λ $P_L$ promoter, the λ gene for the temperature sensitive $cI^{857}$ repressor of the $P_L$ promoter, the λ gene for the antitermination protein N, and the λ *rexA* and *rexB* genes (Fig. 3, arrow A). Transfer of gene *43* from pTL43 to pTL9W gave pTL43W, where gene *43* is under the control of the $P_L$ promoter and is expressed after inactivating the temperature sensitive $cI^{857}$ repressor by elevating the culture temperature (Fig. 3, arrow B). A second expression vector, pTL19Q,

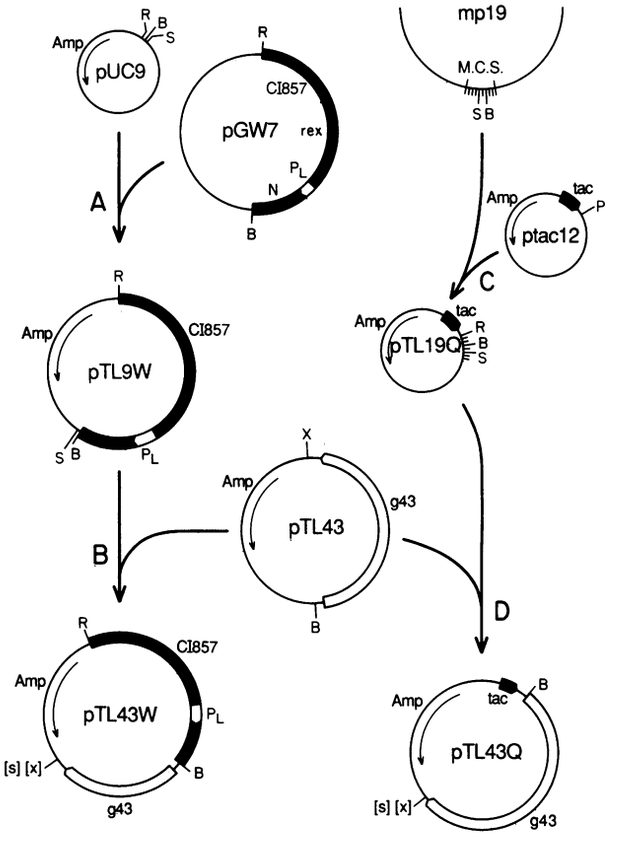


FIG. 3. Cloning gene *43* into expression vectors. Construction of the vector pTL9W (arrow A). The plasmid pGW7 is a pBR322–λ recombinant constructed by G. Wilson. The λ region of pGW7 (shown in bold lines) was removed by digestion with *Bam*HI and *Eco*RI and inserted between the *Bam*HI and *Eco*RI sites of pUC9 to give pTL9W. Cloning gene *43* downstream of the λ $P_L$ promoter (arrow B). pTL43 was digested with *Xho* I (partial) and *Bam*HI, and the purified gene *43*-containing fragment was ligated into the *Sal* I and *Bam*HI sites of pTL9W to give pTL43W. Construction of the vector pTL19Q (arrow C). The multiple cloning site (M.C.S.) was removed from mp19 by digestion with *Eco*RI and *Hin*dIII. The 5′ overhangs were filled in with Klenow fragment, and the fragment was cloned into the *Pvu* II site of p*tac*12 to give pTL19Q. All $P_{tac}$-containing plasmids were transformed into *E. coli* strain 71-18 ($lacI^Q$) to repress transcription from the $P_{tac}$ promoter until induction. Cloning gene *43* downstream of the $P_{tac}$ promoter (arrow D). pTL43 was digested with *Xho* I (partial) and *Bam*HI and inserted between the *Sal* I and *Bam*HI sites within the multiple cloning region of pTL19Q to give pTL43Q. Restriction sites are *Eco*RI (R), *Bam*HI (B), *Sal* I (S), *Xho* I (X), and *Pvu* II (P).

contains the strong *tac* (*trp–lac*) fusion promoter (23) (Fig. 3, arrow C). Transfer of gene *43* from pTL43 into this vector gave pTL43Q (Fig. 3, arrow D). In this plasmid, gene *43* expression is controlled by the $P_{tac}$ promoter, which is derepressed by treating cultures with isopropyl β-D-thiogalactopyranoside.

**Attempts to Restore the Resident Gene *43* Promoter into a Plasmid Containing Full-Length Gene *43*.** To find out whether the presence of the resident gene *43* promoter with its structural gene was detrimental to the propagation of plasmids or lethal to the host, we attempted to place a 700-bp fragment that contained the gene *43* promoter in front of gene *43*. The experiment was designed so that the location of the promoter with respect to the start of gene *43* would be the same in the plasmid as in wild-type T4 phage. This 700-bp *Bam*HI–*Xho* I fragment contains the 3′ end of gene *62* and the entire *regA* gene as well as ≈220 bp of the 5′ end of gene *43*. This fragment was mixed with plasmid pTL43W that had

been digested with *Bam*HI and *Xho* I, then ligated successfully as judged by the banding pattern on agarose gels. Transformation was attempted with this ligation mixture, but no colonies harboring plasmids with the desired construction were obtained. To show that technical problems were not responsible for this negative result, we demonstrated that a similar-size *Bam*HI–*Xho* I control fragment, when mixed with the *Bam*HI–*Xho* I promoter fragment in a ligation reaction, produced transformants containing only the control fragment. The presence of *regA* under $P_L$ control in this construction was probably not responsible for the lack of desired transformants, since other plasmids containing *regA* and parts of gene *43* under $P_L$ control have been constructed (24). For these reasons, we think the most likely cause for the failure to obtain plasmids with intact gene *43* and its promoter is that the gene *43* promoter is recognized by the host RNA polymerase and that 43P is produced in sufficient amounts to be harmful to the cell.

**Production and Purification of Large Amounts of Soluble 43P.** When cells harboring the plasmid pTL43W were induced by shifting the culture temperature from 30°C to 42°C, large amounts of 43P were produced as insoluble aggregates (Fig. 4*A*). Only high concentrations of protein-denaturing reagents were effective in releasing 43P from the aggregates (data not shown). However, when the induction temperature was reduced to 40°C, large amounts of soluble 43P were produced (Fig. 4*B*). When the induction temperature was lowered further, to 38°C, very little if any 43P was produced (data not shown). Induction of cells harboring pTL43Q with isopropyl β-D-thiogalactopyranoside at 40°C and 42°C showed a similar dependence of 43P solubility on culture temperature (Fig. 5). However, in contrast to pTL43W, induction of pTL43Q produced a large quantity of soluble 43P at temperatures as low as 30°C.

A simplified purification procedure allowed rapid processing of large quantities of extracts containing 43P (J.R. and W.H.K., unpublished data). Briefly, 43P was precipitated from the cell lysate with 0.2–0.3% polyethyleneimine and extracted from the polyethyleneimine pellet with 0.5 M NaCl. 43P was purified further by addition of ammonium sulfate to 55% saturation at 4°C. Affinity chromatography on single-stranded DNA-cellulose gave a product that was at least 95% pure. Further chromatography using immobilized T4 gene *32* protein produced nearly homogenous 43P, free of contaminating endonuclease and 5′-to-3′ exonuclease activities.

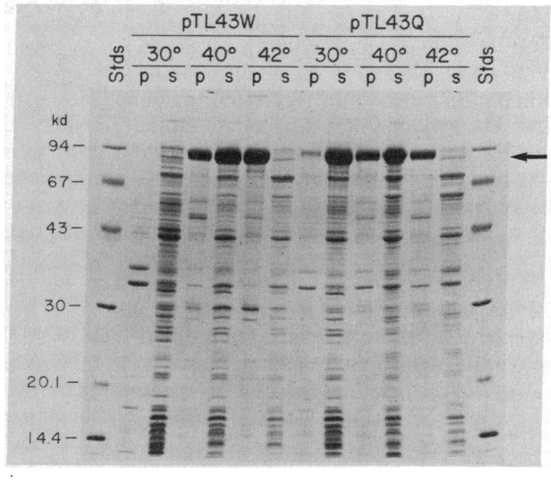


FIG. 5. $NaDodSO_4$/polyacrylamide gel analysis of cell lysate pellets (p) and supernatants (s) 2 hr after induction at 30, 40, or 42°C. Cells harboring pTL43W or pTL43Q were induced. The arrow indicates the position of 43P. kd, kDa; stds, standard molecular size markers.

**Characterization of 43P.** 43P purified from induced cells harboring pTL43W and 43P from T4 infected cells were assayed for DNA polymerase and 3′-to-5′ exonuclease activities (J.R. and W.H.K., unpublished data). We found that cloned 43P had about twice the specific activity for DNA polymerization as the 43P obtained from T4-infected cells that was used as a standard for wild-type levels of enzymatic activity. The 3′-to-5′ exonuclease specific activities of both proteins were identical.

## DISCUSSION

A number of reasons can be advanced to account for situations where attempts to clone structural genes have failed. Among the most likely explanations are that the gene product may interfere with the viability of the host cell or with replication of the plasmid or that a nucleotide sequence itself interferes with propagation of the plasmid. In our attempts to clone gene *43*, both of these explanations appeared to be valid. First, it was necessary to remove the resident promoter of gene *43* and to replace it with one that could be tightly controlled. Subsequent attempts to insert the gene *43* pro-

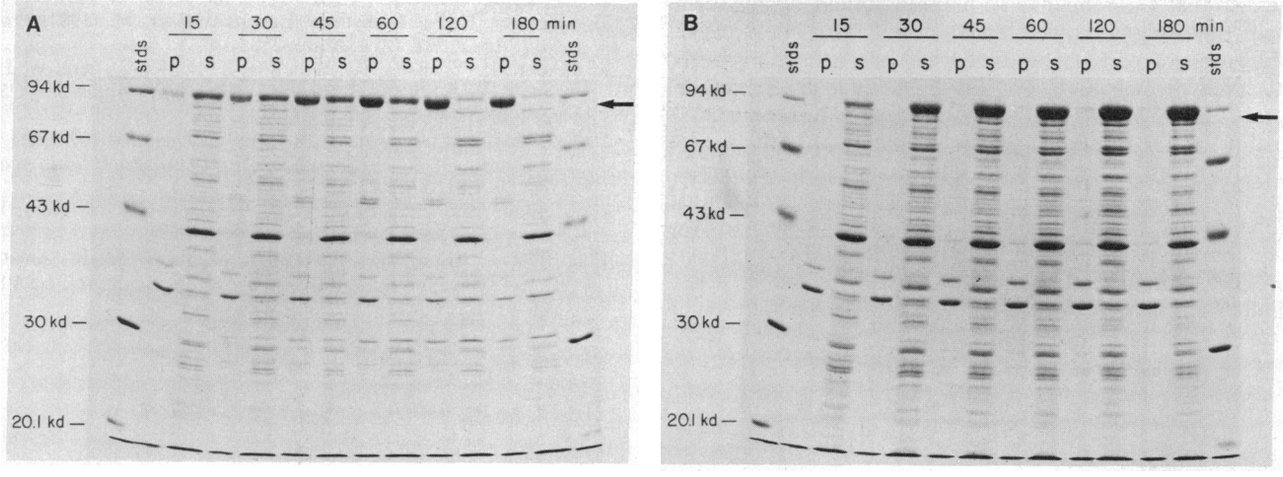


FIG. 4. $NaDodSO_4$/polyacrylamide gel analysis of pTL43W cell lysate pellets (p) and supernatants (s) at various times after induction at 42°C (*A*) or at 40°C (*B*). Two large cultures were made from a culture of cells harboring pTL43W grown at 30°C. The cultures were brought to 40°C or to 42°C. At the times indicated, aliquots were withdrawn to tubes containing chloramphenicol (at a final concentration of 100 μg/ml), stored on ice until all time points were sampled, and then lysed after correcting for differences in cell density. The arrow indicates the position of 43P. kd, kDa; stds, standard molecular size markers.

moter in the position occupied in wild-type T4 failed to give any stable recombinants containing the gene *43* promoter insert. Second, we had to revise our cloning strategy, so that we could remove a putative lethal sequence downstream from the 3′ end of gene *43*. This lethal sequence resides within 280 bp of the gene *43* translation termination codons and may be a strong early promoter, as indicated by the studies of Kutter and Rüger (8).

Our evidence for having obtained clones containing the correct 3′ end of gene *43* is based on three findings: (*i*) the clones containing the 3′ end of gene *43* and the clones containing nucleotides upstream from the most distal *Hin*dIII site in gene *43* share a large, overlapping region of DNA sequence; (*ii*) we isolated two independent clones containing the 3′ end of gene *43*, and the DNA sequences of both matched the DNA sequence that was obtained independently for this region of the T4 genome by W. Rüger (personal communication), and (*iii*) carboxypeptidase Y releases the same amino acid residues from cloned 43P and from 43P obtained from T4-infected cells.

Cells harboring pTL43W or pTL43Q produce 43P to ≈10% of the total cellular protein, which is ≈1000-fold greater than the amounts produced by cells infected with wild-type T4 phage. This increased level of expression simplifies the purification scheme enormously. The cloned 43P had the same specific activity for the 3′-to-5′ exonuclease function as wild-type 43P but had twice the specific activity for DNA polymerization. We also found that the cloned 43P had an asparagine at position 214, whereas the 43P from T4-infected cells contained serine at this position (J.R. and W.H.K., unpublished data). This alteration may account for the difference in specific polymerizing activity. After examining a number of T4 DNA polymerases from wild-type strains by peptide mapping, we found that some had asparagine at position 214, whereas others had serine at this position (J.R. and W.H.K., unpublished data). For this reason, we believe we have actually cloned one of the forms of gene *43* present in existing wild-type T4 strains.

It has been observed that some proteins that are normally soluble form insoluble aggregates when they are produced in large amounts in *E. coli* (25–28). This situation occurs with 43P when it is synthesized in cultures at temperatures of 42°C using either pTL43W or pTL43Q. One explanation for the insolubility of overexpressed 43P at elevated temperatures is that there is a temperature-dependent step in the normal folding pathway that becomes rate-limiting at high temperatures, so that 43P remains in a partially unfolded state for a longer time than at lower temperatures. This could cause aggregation of partially unfolded 43P through interactions between hydrophobic regions not yet buried in the core of the protein. By lowering the induction temperature only 2°C, most of the 43P produced remained soluble.

As described above, we have cloned authentic, full-length gene *43* and have overproduced and purified a large quantity of enzymatically active 43P. Together with the plasmids we have constructed to overproduce the other T4 DNA replication core proteins (ref. 9 and T.-C.L., J.R., and W.H.K., unpublished data), it will now be possible to initiate high-resolution structural studies on these proteins and their complexes.

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