Energy-Dependent Activation of the Temperature-Sensitive DNA Polymerases Induced by Bacteriophage T4 Gene 43 Mutants

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Temperature shift experiments with temperature-sensitive mutants in T4 gene 43 indicate that the production of a functional T4 DNA polymerase involves the energy-dependent conversion of an inactive precursor to the active enzyme. Comparisons of the inactive precursor with the active polymerase have demonstrated that the energy-dependent activation affects both the polymerase and 3' -> 5' exonuclease activities of the enzyme. Despite evidence suggesting that the energy source for the activation process is a high-energy phosphate compound, the energy-dependent step does not appear to involve covalent modification of the enzyme.

The DNA polymerase synthesized after infection of Escherichia coli with bacteriophage T4 is absolutely essential for the initiation and maintenance of viral DNA replication (Epstein et al., 1963; Warner and Hobbs, 1967). As an approach toward understanding more precisely the role of T4 DNA polymerase in the replication of T4 DNA, we examined the DNA polymerase induced after infection of E. coli with the T1 mutant, ts L56, which bears a temperature-sensitive mutation in gene 43, the structural gene for T4 DNA polymerase (de Waard et al., 1965). In the course of these studies, a novel phenomenon was encountered. It was found that at the nonpermissive temperature, an inactive form of the polymerase was synthesized, which could be converted to the functional enzyme upon lowering the temperature. The appearance of active enzyme after temperature stepdown was not prevented by inhibitors of protein synthesis, but was blocked by anaerobiosis or by inhibitors of energy-yielding metabolism (Swartz et al., 1972). The fact that the appearance of active enzyme was energy-dependent clearly distinguished the effect from facile renaturation of a thermolabile protein. Thus, the behavior of the ts L56 mutant suggested the involvement of an activation process in the formation of functional T4 DNA polymerase that had previously gone undetected.

In this report, we present experiments that support and extend our initial observations and that attempt to define the molecular basis of the activation process.

MATERIALS AND METHODS

Chemicals and enzymes. Reagent chemicals were obtained from the following sources: ribo- and deoxyribonucleoside triphosphates from Pabst Laboratories; triethanolamine from Fisher; Tris from Sigma; chloramphenicol from Parke-Davis.

Abbreviations: Tris, tris (hydroxymethyl) aminomethane; Brij 58, polyoxyethylene-20-cetyl ether; BSA, bovine serum albumin; dTTP, deoxythymidine 5'-triphosphate; and m.o.i., multiplicity of infection.
vis; Brij 58 from Atlas Chemicals; salt-free lysozyme from Worthington; BSA (fatty acid-free) from Pentex; salmon sperm DNA from Calbiochem; sodium dodecyl sulfate from BDH Chemicals; and media constituents from Difco. Radiochemicals were purchased from the following suppliers: [3H]dTTP from Schwarz/Mann; [35S]H2SO4, [32P]H3PO4, and [125I]NaI from New England Nuclear. All other chemicals were reagent grade. Alkaline-denatured salmon sperm DNA and nicked heat-denatured [3H]DNA from E. coli were prepared as previously described (Huang and Lehman, 1972a). Antiserum directed against T4 DNA polymerase was kindly prepared for us by Dr. L. Levine. Antisera directed against E. coli exonuclease I was obtained as described by Aposhian and Kornberg (1962). Antisera were diluted into 0.15 M NaCl and heated at 70° for 30 min prior to use in order to inactivate nuclease activity. Wild-type T4 DNA polymerase was kindly prepared for us by Dr. L. Levine. Antisera directed against E. coli exonuclease I was obtained as described by Aposhian and Kornberg (1962). Antisera were diluted into 0.15 M NaCl and heated at 70° for 30 min prior to use in order to inactivate nuclease activity. Wild-type T4 DNA polymerase was kindly prepared for us by Dr. L. Levine. Antisera directed against E. coli exonuclease I was obtained as described by Aposhian and Kornberg (1962).

Bacterial and bacteriophage strains and growth conditions. E. coli strains B (wild-type) and CR63 (sup D+) were originally obtained from Drs. R. S. Edgar and J. Drake. Strain JT1100 is a polA− derivative of E. coli B constructed in the following way. A spontaneous spc+ clone of E. coli B was lightly mutagenized with ethylmethane sulfonate as described by Miller (1972), and a rha+ derivative was picked from a rhamnose-tetrazolium indicator plate. A spontaneous methyl-methane sulfonate-resistant (MMS+) clone of the mutagenized strain was then selected and presumably represents a revertant to ion+. (The uv- and MMS-sensitivity of wild-type E. coli B are manifestations of its ion− character.)6 This MMS+ derivative was then mated with E. coli K12 strain JG78 (Hfr KL-25, met E−, pol A1, rha+, spc+) obtained from Dr. I. Herskowitz. The rha+ recombinants were selected on rhamnose-minimal salt plates containing spectino- mycin and methionine, and then were scored for their methionine requirement, and for the presence of the pol A1 mutation by their uv- and MMS-sensitivity. Finally, sonic extracts were prepared from the recombinants with the expected phenotype and were assayed for DNA polymerase I activity as previously described (Lehman and Chien, 1972). JT1100 (met E−, pol A1, rha+, spc+) had less than 0.3% of the DNA polymerase I activity of either its immediate parent or E. coli B. It grew in broth with essentially the same doubling time and to the same cell density as either its immediate parent or E. coli B. E. coli K12 strain AN120 (unc A−) and its isogenic parent AN180 have been described (Butlin et al., 1971) and were obtained from Dr. F. Gibson.

Temperature-sensitive mutants of bacteriophage T4: L107, L97, L56, S9, L88, L141, and L91 (all in gene 43); and amber mutants: N82 (gene 44) and E315 (gene 32), were originally obtained from Drs. R. S. Edgar and J. Drake. Double mutants were constructed by standard crossing procedures (Hayes, 1968).

Media (H-broth and EHA-agar plates) used for the growth of cells and for the propagation and assay of T4 phages were those described by Steinberg and Edgar (1967). In some experiments, HT-broth or HP-broth (H-broth buffered at pH 7.0 with 50 mM triethanolamine hydrochloride or 50 mM potassium phosphate, respectively) were used. Media were supplemented with 0.2 mM arginine and 0.2 μM thiamine for experiments with E. coli K-12 strains AN190 and AN180.

Preparation of cell extracts and enzyme assays. Cultures (150–300 ml) were grown in 1-liter flasks with vigorous aeration in a gyratory water bath at the appropriate temperature. At various times after phage infection, portions (25 ml) of the infected culture containing about 5 x 108 cells/ml were removed from the growth flask as rapidly as possible and immediately added to an approximately equal volume of chilled ice containing 1.5 ml of 1.0 M NaN3. These precautions prevented spontaneous activation of T4 DNA polymerase during preparation of the extract. After all
samples were collected, they were centrifuged at 8000 g for 10 min and the cell pellets were resuspended in 2.0 ml of 50 mM Tris–HCl, pH 7.5, containing 10 mM 2-mercaptoethanol. The cells in suspension were then ruptured at 0° by two 30-sec bursts of sonic irradiation from a Mullard sonicator. After the extracts were clarified by centrifugation for 20 min at 12,000 g, the supernatant fluid was removed and was assayed for T4 DNA polymerase activity at 30° essentially as described by DeWaard et al. (1965) or by the method of Brutlag and Kornberg (1972). Under either assay condition, activity due to E. coli DNA polymerases II and III is negligible (Kornberg and Gefter, 1971, 1972). The 3'→5' exonuclease activity of T4 DNA polymerase was assayed as reported previously (Huang and Lehman, 1972a). Brij lysates were prepared with only minor modifications of the procedure described before (Swartz et al., 1972).

Other methods. Protein concentration was determined by the technique of Lowry et al. (1951), using BSA as a standard. Preparation of 35S labeled phage-infected cells, electrophoresis in slabs of polyacrylamide containing sodium dodecyl sulfate, and radioimmune assay of T4 DNA polymerase protein were all performed essentially as described previously (Huang and Lehman, 1972b). Microdetermination of phosphate was performed by minor modification of the method of Chen et al. (1956).

RESULTS

Increase in DNA polymerase activity after downshift is due to the phage-induced enzyme. It was possible that in our earlier experiments (Swartz et al., 1972) the presence of host DNA polymerase I obscured events related to the production of T4 DNA polymerase after infection. To avoid this problem, a polA1 derivative of E. coli B was constructed. Infection of this strain with T4 ts L56 confirmed our earlier observations. As shown in Fig. 1, only a small amount of T4 DNA polymerase activity appeared after infection at 43°. However, upon lowering the temperature to 30°, a marked and sustained increase in T4 DNA polymerase activity occurred almost immediately. This increase cannot be due to the synthesis of new T4 DNA polymerase molecules since it was shown earlier that such synthesis is completely prevented by the addition of chloramphenicol (Swartz et al., 1972). That the increase in DNA polymerase activity is indeed due to the T4-induced enzyme was demonstrated by the fact that there was essentially no activity if the extracts were assayed in the presence of antiserum directed against purified T4 DNA polymerase.

Appearance of T4 DNA polymerase activity after downshift requires unperturbed energy-yielding metabolism. What distinguishes the production of active enzyme after temperature stepdown from a simple rapid renaturation of the thermolabile protein is its sensitivity to 2,4-dinitrophenol.
an uncoupler of oxidative phosphorylation. Half maximal inhibition of the appearance of T4 DNA polymerase activity occurred at 55 μM and essentially complete inhibition was achieved at 0.3 mM when E. coli B was used as the host (Fig. 2). About tenfold higher concentrations of the uncoupler were needed to prevent completely the appearance of active enzyme when JT1200 or an E. coli K12 strain was used, probably because of the lower permeability to the drug in the latter strains. The appearance of active enzyme could also be blocked by shifting the infected cells from 43 to 4°C. The cells could be maintained at this low temperature for considerable periods of time, with active enzyme appearing only when the culture was returned to 30°C (Fig. 3). Earlier studies showed that anaerobiosis or inhibitors of cytochrome oxidase (CN− and N3−) also prevented appearance of active enzyme (Swartz et al., 1972). The appearance of functional T4 DNA polymerase activity after temperature-downshift has been termed "activation."

Activation of T4 DNA polymerase activity is not an allele-specific effect. Six other temperature-sensitive mutations of T4 DNA polymerase that are located throughout gene 43 (Fig. 4) were tested for activation. As shown in Fig. 5, mutants L107, L97, and S9 behaved like L56: T4 DNA polymerase activity increased ten- to thirty-fold after temperature stepdown. Furthermore, the increase in activity for each of these mutants was completely prevented by 2,4-dinitrophenol. Mutants ts L88 showed a slight increase in T4 DNA polymerase activity over that present immediately before downshift, which was blocked by 2,4-dinitrophenol. Mutants ts L91 and ts L141 showed no significant increase in T4 DNA polymerase activity upon temperature shiftdown.

Activation does not require a functional "replication complex." Purified T4 DNA polymerase has been shown to interact in vitro with purified preparations of the single-stranded DNA binding protein that is the product of T4 gene 32 (Huberman et al., 1971). Moreover, recent experiments indicate that the absence of T4 DNA polymerase prevents the binding of several other T4 proteins to DNA-cellulose columns, suggesting that the interaction with...
the polymerase is necessary for their binding to DNA (Huang and Buchanan, 1974). It also has been demonstrated both in vivo and in vitro that at least six T4 gene products are required for T4 DNA replication and two of these proteins, the products of genes 44 and 62, have been purified and shown to exist as a complex (Epstein et al., 1963; Barry and Alberts, 1972). However, with double mutants ts L56 am N81 and ts L56 am E315 in which DNA synthesis was completely blocked by amber mutations in gene 44 or in gene 32, respectively, the formation of active T4 DNA polymerase upon temperature stepdown occurred identically to that found for ts L56 alone. These observations suggest that activation can occur even when the polymerase is not part of a functional replication complex.

An inactive precursor is synthesized at the nonpermissive temperature. The finding that the increase in T4 DNA polymerase activity that occurs upon temperature shift down does not depend on protein synthesis suggests that the molecules that are activated subsequently to form the functional enzyme have already been made at the restrictive temperature. That this is the case is demonstrated by a radioimmune assay that detects material that cross-reacts with antibody directed against purified T4 DNA polymerase. Despite the fact that little or no T4 DNA polymerase activity appeared at 43°C, cross-reacting material (CRM) for the antipolymerase antibody could be detected with the radioimmune assay (Table 1). In fact, as much or more CRM was made at the nonpermissive temperature as active enzyme made at the permissive temperature. It was also found (Table 2) that the level of CRM increased after infection, reaching a maximum at about 20 min postinfection. This direct measurement correlates well with the kinetics of formation of the precursor inferred from the T4 DNA polymerase activity ultimately achieved upon step down after various periods at 43°C (Swartz et al., 1972). Even those temperature-sensitive mutants, L91 and L141, that did not form active polymerase, nevertheless did produce substantial amounts of CRM (data not shown).

The 3' → 5' exonuclease activity of T4 DNA polymerase undergoes activation. Despite the lack of polymerase activity at the restrictive temperature, it was possible that the CRM made at 43°C might retain the 3' → 5' exonuclease activity of T4 DNA polymerase. However, as shown in Fig. 6, the 3' → 5' exonuclease activity was absent at 43°C and only appeared when the infected
ACTIVATION OF T4 DNA POLYMERASE

6 MINUTES AFTER INFECTION

FIG. 5. Time course of T4 DNA polymerase appearance after infection of JT1200 by various temperature-sensitive mutants in gene 43. Separate cultures (200 ml) of JT1200 growing at 43° in HT-broth were infected at an A₅₉₀ of 0.8 with the mutant T4 phages indicated at an m.o.i. of 5 each. The experiment was performed as described for Fig. 1. The triangles (ts L97, ts S9 and ts L141) show the level of activation achieved in separate experiments with a less detailed time course. The closed circles (ts L88 and ts L141) represent experiments in which the initial temperature was 45°.

TABLE 1

<table>
<thead>
<tr>
<th>T4 phage</th>
<th>Gene</th>
<th>Temperature of infection (degrees)</th>
<th>T4 DNA polymerase Activity CRM (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tsL56</td>
<td>amN82 43, 44</td>
<td>43, 44</td>
<td>0.5</td>
</tr>
<tr>
<td>tsL66</td>
<td>amN82 43, 44</td>
<td>43, 44</td>
<td>6.3</td>
</tr>
<tr>
<td>amN82</td>
<td>44</td>
<td>37</td>
<td>6.9</td>
</tr>
<tr>
<td>am4309</td>
<td></td>
<td>43</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Cultures of E. coli B growing at the temperatures indicated were infected at an A₅₉₀ of 0.8 with the T4 phages listed at an m.o.i. of 5. After 15 min, T4 DNA polymerase and CRM activities were assayed at 30° in Brij lysates of the infected cultures as described under Materials and Methods.

TABLE 2

<table>
<thead>
<tr>
<th>Time after infection (min)</th>
<th>CRM (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;1</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
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<tr>
<td>20</td>
<td>13.4</td>
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<tr>
<td>40</td>
<td>10.5</td>
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<tr>
<td>60</td>
<td>8.7</td>
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</table>

* A culture of E. coli B growing at 43° was infected at an A₅₉₀ of 0.8 with T4 ts L56 amN82 at an m.o.i. of 5. At the times indicated, portions of the culture were withdrawn and assayed for CRM as described under Materials and Methods.

...cells were shifted to 30°, exactly as is found for the polymerase activity.

Energy for activation is derived from "high energy" phosphate. Several experiments suggest that the energy necessary for activation can be derived from either oxidative or substrate-level phosphorylations, and thus, it probably represents the need for some high energy phosphate compound (or compounds). First, as demonstrated in Fig. 7, at no time after lowering the temperature did activation escape the need for metabolic energy. Apparently, energy-yielding metabolism must operate continuously to provide a large enough pool of the required component for the activation process to go to completion. Second, arsenate was an effective inhibitor of the...
Fig. 6. Time course of appearance of polymerase and 3'→5' exonuclease activities of T4 DNA polymerase. A culture (200 ml) of JT1200 growing at 43°C in HT-broth was infected at an A₉₀₀ of 0.8 with T4 ts L56 at an m.o.i. of 5. The experiment was performed as described for Fig. 1, except that assays for exonuclease activity were carried out in the presence of 100 µl of anti-E. coli exonuclease 1 serum. The 3'→5' exonuclease activity of T4 DNA polymerase is that exonuclease activity present in each of the extracts that was inhibited by 25 µl of anti-T4 DNA polymerase serum.

The activation process, with half-maximal effect at 2–3 mM (Fig. 8). This concentration of arsenate had no effect on the activity of the purified polymerase. Addition of arsenate has been shown to lower drastically the intracellular pools of ATP, phosphoenolpyruvate, and other "energy-rich" phosphate compounds (Klein and Boyer, 1972; Berger, 1973). Third, activation occurred normally in a mutant of E. coli, designated uncA⁻, in which the Ca²⁺, Mg²⁺-stimulated ATPase associated with oxidative phosphorylation is defective and which therefore must obtain its ATP from the substrate-level phosphorylations of glycolysis (Fig. 9). In addition, activation in the uncA⁻ strain proceeded essentially normally in the presence of 2,4-dinitrophenol, but was effectively inhibited by arsenate (data not shown). Fourth, de novo deoxyribonucleotide biosynthesis was not required for activation since the addition of 50 mM hydroxyurea, an inhibitor of both the cellular and the phage-induced ribonucleotide reductase (Neuhard, 1967; Warner and Hobbs, 1969), either at 5 min after infection or at the time of temperature downshift, did not block subsequent activation (data not shown).

Covalent modification of the protein does not occur upon activation. Because of the evidence in vivo for the participation of high-energy phosphate compounds in the activation process, the possible involvement of covalent modifications that have been described in other systems (Walsh et al., 1968; Kingdon et al., 1967; Honjo et al., 1968) was investigated. Our results suggest that activation does not involve phosphorylation, adenylylation, or adenosine diphosphoribosylation of the enzyme. Purified preparations of active T4 DNA polymerase contained no detectable phosphorus by direct chemical analysis under conditions where the requisite number of phosphate residues were found for a known phosphoprotein (pepsinogen). Furthermore, there is no shoulder at 260 nm in the ultraviolet adsorption spectrum of purified T4 DNA polymerase (Goulian et al., 1968).

In addition, when infected cells prelabeled

Fig. 7. Effect of 2,4-dinitrophenol on the appearance of T4 DNA polymerase. A 350-ml culture of E. coli B growing at 43°C in H-broth was infected at an A₉₀₀ of 0.8 with T4 ts L56 at an m.o.i. of 5. The experiment was performed as described in Fig. 1 except that at the times indicated, 30-ml samples were withdrawn and their incubation continued in the presence of a final concentration of 1 mM 2,4-dinitrophenol, while the remainder of the culture was incubated at 30°C in the absence of 2,4-dinitrophenol.
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Fig. 8. Effect of various concentrations of arsenate on the time course of T4 DNA polymerase appearance. A culture (400 ml) of E. coli B growing at 43° in HT-broth was infected at an A₅₅₀ of 0.8 with T₄ ts L56 at an m.o.i. of 5. The experiment was performed as described for Fig. 2, except that at the time of temperature shift, arsenate was added to different portions of the culture. ■, 100 mM arsenate; ◇, 50 mM arsenate; □, 20 mM arsenate; ▲, 10 mM arsenate; ○, 5 mM arsenate; △, 2 mM arsenate; ○, 1 mM arsenate; ●, no arsenate.

with [³²P]PO₄³⁻ were used in a typical activation experiment, and the proteins extracted from the cells at various times after infection were radioautographed after sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis, no radioactivity was found associated with the band corresponding to T4 DNA polymerase. Thus, it must be concluded that the enzyme is neither phosphorylated, adenylylated, nor adenosine diphosphoribosylated, either transiently or permanently, during the process of activation. Also, unlike several structural proteins of the phage (Celis et al., 1973; Goldstein and Champe, 1974; Bachrach and Benchetrit, 1974; Poglazov and Levshenko, 1974; Georgopoulos et al., 1972), T4 DNA polymerase does not appear to be generated from a larger molecular weight precursor polypeptide. When cells were pulse-labeled with [³⁵S]-SO₄²⁻ at various times after infection in a typical activation experiment, a labeled species that comigrated on dodecyl sulfate-polyacrylamide slab gels with authentic T4 DNA polymerase appeared at about 5 min after infection. This band was the largest molecular weight species present and at no time before or after temperature downshift was its mobility different from that of the purified enzyme within the resolution of this technique. In fact, when the CRM for anti-T4 DNA polymerase was precipitated from extracts of cells infected at the restrictive temperature, a discrete band was found with a mobility indistinguishable from that of ³²P-labeled purified T4 DNA polymerase marker.

DISCUSSION

It is well established that the primary sequence of a protein determines its tertiary structure (Epstein et al., 1963), however, it has been stressed by many investigators (Alpers and Paulus, 1971; Teipel

Fig. 9. Time course of T4 DNA polymerase appearance in a mutant defective in oxidative phosphorylation. Separate cultures (600 ml) of E. coli AN180 and AN120 were grown at 43° in HT-broth to an A₅₅₀ of 0.4. The cells were chilled and collected by brief centrifugation at 8000 rpm. They were then resuspended in one-twentieth their original volume of phage absorption buffer (0.1 M NaCl, 2 mM MgCl₂, 10 μg/ml tryptophan, and 20 mM triethanolamine hydrochloride, pH 7.5) and were infected at 0° with T₄ ts L56 at an m.o.i. of 5. After allowing adsorption to occur for 10 min, the cells were rapidly diluted into 110 ml of HT-broth prewarmed to 43°. The rest of the experiment was as described in Fig. 1.
and Koshland, 1971; Frieden, 1970; Hatfield and Burns, 1970) that other factors may influence the ultimate conformation that a protein assumes and may dictate whether or not that state of the protein will be functional. Therefore, it might be expected that mutations will exist that will affect not only the activity of enzymes, but also their formation.

We have previously found that after infection of E. coli at 43° with the temperature-sensitive T4 mutant tsL56, an inactive polymerase was synthesized that was rapidly converted to active enzyme on shift to a permissive temperature (30°). Although the conversion occurred in the absence of protein synthesis, it was prevented by inhibitors of energy-yielding metabolism, such as sodium azide, sodium cyanide, or 2,4 dinitrophenol.

In the present study, we have demonstrated that activation of T4 DNA polymerase is not an allele-specific effect; a variety of mutations in gene 43, which confer temperature-sensitivity on the activity of T4 DNA polymerase, also affect the formation of a functional enzyme. Furthermore, at least in the case of tsL56, the activation affects both polymerase and exonuclease activities.

We have also shown that during infection at the nonpermissive temperature, cross-reacting material for T4 DNA polymerase-specific antibody is made. Two to three times as much CRM accumulates at the nonpermissive temperature as active enzyme formed at the permissive temperature (Tables 1 and 2). These findings can be explained most simply as an increased accessibility of the antigenic sites on the unfolded precursor.

Our in vivo experiments strongly suggest the participation of “high energy” phosphate in the activation process. On the other hand, direct comparisons of the inactive precursor and the functional enzyme indicate that the energy-dependent activation does not involve phosphorylation, adenyllylation, or adenosine diphosphoribosylation of the protein, although such covalent modifications have been detected in other phage systems (Rahmsdorf et al., 1974; Horvitz, 1974a, 1974b; Goff, 1974; Seifert et al., 1971). Also, despite the existence in E. coli of an energy-dependent system responsible for the degradation of “missense” proteins (Prouty and Goldberg, 1972; Goldberg, 1972), activation does not appear to involve proteolytic cleavage of the inactive precursor. Of course, covalent modifications of the protein during activation can be ruled out definitely only by purification of the inactive precursor and the active enzyme, followed by peptide fingerprinting and sequencing of the two proteins.

Although it seemed reasonable to suppose that the energy-dependent activation may be related to insertion of the protein into a replication complex and that mutations that affect interaction of T4 DNA polymerase with other T4 proteins may interfere with its ability to form the complex, mutations that should have a drastic effect on its ability to form the replication complex did not prevent the appearance of active T4 DNA polymerase.

The gene 43 amber mutant B22 (Fig. 4), which lacks 20% of the carboxyl-terminal portion of the protein, retains its 3' → 5' exonuclease despite complete loss of polymerase activity (Nossal and Hershfield, 1972). However, temperature-sensitive mutations in the carboxyl-terminal region of the protein (tsL88, L91, and L141) prevent activation of both nuclease and polymerase activities. Taken together, these results suggest that an intact carboxyl region of the protein is somehow necessary for activation, and that its absence is preferable to a defect in this region for the production of the enzymatically active molecule.

Our first attempts to prepare an in vitro system capable of generating active T4 DNA polymerase met with only limited success (Swartz et al., 1972). The involvement of transient intermediates or labile components may have been responsible, in part, for the difficulty, especially since the assay for activation requires the completion of a multistep process. Indeed, the extent of activation that can be achieved in the various cell-free systems that we have attempted to date is only 10% or so of the levels obtainable in vivo. Moreover, while
this limited in vitro activation occurs most rapidly at 30°, nevertheless, it will proceed upon storage of extracts at 4°. Despite these problems, preparation and fractionation of an in vitro system seems the best approach toward elucidating the mechanism of the activation process.

REFERENCES


