SP62, a Viable Mutant of Bacteriophage T4D Defective in Regulation of Phage Enzyme Synthesis

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SP62 is a mutant of bacteriophage T4D that was discovered because it produces fewer phage than the wild type in the presence of 5-fluorodeoxyuridine. In the absence of phage DNA synthesis, SP62 solubilizes host DNA slower than normal; this may explain the sensitivity to 5-fluorodeoxyuridine. In *Escherichia coli* B at 37°C in the absence of drugs, SP62 makes DNA at a normal rate and the kinetics of appearance of phage are nearly normal. Under the same conditions, SP62 produces T4 lysozyme (gene *e*) at a normal rate until 20 min, but then produces it at twice the normal rate until at least 60 min. It has long been known that, when T4 DNA synthesis is blocked (DNA⁻ state) in an otherwise normal infection, the synthesis of a number of early enzymes continues beyond the shutoff time of about 12 min seen in the DNA⁺ state, but still stops at about 20 min. We have termed the 12-min shutoff event S1 and the 20-min shutoff event S2. We show here that, in the DNA⁺ state, SP62 makes four early enzymes normally, i.e., S1 occurs. However, in the DNA⁻ state (where S1 is missing), SP62 continues to make dCTPase (gene 56), dCMP hydroxymethylase (gene 42), and deoxynucleotide kinase (gene 1) for at least an hour; this results in production of up to 13 times the normal level of dCTPase at 60 min after infection, or 6 times the DNA⁻ level. We conclude that SP62 is defective in the second shutoff mechanism, S2, for these three enzymes. In contrast, SP62 causes premature cessation of dTMP synthetase production in the DNA⁻ state; the result is a twofold underproduction of dTMP synthetase. Autoradiograms of pulse-labeled proteins separated by slab-gel electrophoresis in the presence of sodium dodecyl sulfate show that a number of other T4 early proteins, including the products of genes 45, 46, and rIIA, are synthesized longer than normal by SP62 in the DNA⁻ state. Few late proteins are made in the DNA⁻ state, but in autoradiograms examining the DNA⁺ state there is little or no effect of the SP62 mutation on the synthesis of T4 late or early proteins. Circumstantial evidence is presented favoring a role for the gene of SP62 in translation of certain mRNAs. At very high temperatures (above 43°C) in the absence of drugs, phage production, but not DNA synthesis, is much reduced in SP62 infections relative to wild-type T4 infections; this temperature sensitivity is greater on *E. coli* CR63 than on *E. coli* B. This property has facilitated recognition of the SP62 genotype and aided in complementation testing and genetic mapping. A later publication will provide evidence that SP62 defines a new T4 gene named *regA*, which maps between genes 43 and 62.

The regulation of RNA and protein synthesis in bacteriophage T4-infected cells of *Esche-*

richia coli has been reviewed recently (20, 34, 36, 38, 60); regulatory phenomena have been shown to operate at the levels of both transcription and translation. Epstein et al. (14) showed that, when phage DNA synthesis was blocked

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genetically, a large class of "late," or maturation, proteins did not appear. Wiberg et al. (66) showed that another result of genetically blocking phage DNA synthesis was the failure of a regulatory process that normally shuts off the synthesis of a large class of "early," or DNA synthetic, enzymes at about 12 min after infection at 37 C. However, in the absence of DNA synthesis, the production of these enzymes continues only until about 20 min, suggesting that a second shutoff mechanism becomes operative then. Mutants specifically defective in either of these shutoff mechanisms would be of great value in attempting to understand the mechanisms, but none have been described. This report describes the isolation and some properties of a T4 mutant, SP62, which appears to lack the second shutoff mechanism for the synthesis of some early proteins. We will often refer to SP62 as [*regA*] (see Materials and Methods). The mutation in [*regA*] also affects the synthesis of phage lysozyme, a type of late protein. We present circumstantial evidence that [*regA*] is defective in a translational control.

MATERIALS AND METHODS

Bacteriophage and bacterial strains. Both *E. coli* B, nonpermissive for T4 amber (*am*) mutants, and *E. coli* CR63, permissive for T4 *am* mutants, have been described (63). *E. coli* B₈₋₁ has been described (23). *E. coli* B/6 was obtained from R. Christensen. Wild-type T4D (T4⁺), *amC64*, *amN55*, *amH26*, and *tsL13* were obtained from R. Edgar; *amE4332* was from J. Drake. *denA* mutant S112x5 has been described (22). The suffix x3 or x5 indicates that a mutant was purified genetically in this laboratory (63). Unless otherwise specified, all studies on SP62 described in this paper were done with a genetically purified version (i.e., SP62x3) but for brevity it is referred to as SP62. As before (22, 29), specific phage mutants are referred to by the gene number or symbol enclosed in brackets: *amN55x5* (deoxycytidylate hydroxymethylase) = [42]; *amE4332* (DNA polymerase) = [43]; *amC64x5* (late protein synthesis) = [55]; S112x5 (T4 endonuclease II) = [*denA*]; SP62x3 (regulation) = [*regA*]; *amH26x5* (lysozyme) = [*e*]. We note here, for the benefit of those who use this lysozyme mutant, that the original *amH26* contains a temperature-sensitive (*ts*) mutation in gene 30 (DNA ligase) and that this *ts* mutation is not present in our *amH26x5* (Swift and Wiberg, unpublished data).

Double mutants containing SP62 and various *ts* or *am* mutations were constructed as described previously (22), except that the SP62 component was identified by virtue of its sensitivity to 5-fluorodeoxyuridine (FdUrd) on spot plates and in liquid-culture complementation tests.

Growth medium. All bacteria were grown and infected in glycerol-Casamino Acids (GCA) medium

(16). The preparation of phage stocks and plating media and the assay procedures for viable phage and bacteria were noted previously (22). Stocks of T4⁺ were grown on *E. coli* B to suppress accumulation of spontaneous *am* mutants, and at 37 C to decrease the accumulation of spontaneous *ts* mutants. These are not trivial points, since we use this stock to genetically purify most mutants we use.

Chemicals. FdUrd and chloramphenicol were generous gifts from Hoffman-LaRoche, Inc., and Parke, Davis & Co., respectively. Uracil and deoxyadenosine (dAdo) were purchased from Calbiochem or Sigma Chemical Co. Hydroxyurea (HU) and d,1-L-tetrahydrofolic acid were purchased from Nutritional Biochemicals Corp. [*Methyl-³H*]thymidine (3 Ci/mmol) was purchased from Schwarz BioResearch, Inc. [*5-³H*]dCMP (15 Ci/mmol) was purchased from Schwarz/Mann. [*5-³H*]dUMP (0.56 Ci/mmol) was purchased from Calotomic. Charcoal (activated, U.S.P. powder) was purchased from Merck & Co., Inc. Sodium dodecyl sulfate (SDS) was purchased from BioRad. Acrylamide and *N,N'*-methylene-bis-acrylamide were purchased from Eastman.

Spot-plate identification of SP62 genotype: procedure 1. Procedure 1 was used in the original discovery of SP62 and in the identification of some multiple mutants containing SP62. To 3 ml of top agar was added 0.15 ml of *E. coli* B culture (grown to 10⁹ to 2 × 10⁹ cells/ml in GCA), 0.1 ml of FdUrd (1 mM), and 0.03 ml of uracil (10 mM). This is mixed and poured onto bottom agar (20 ml in a standard 100-by 15-mm plastic petri dish). After a few minutes, plaque suspensions in GCA, or nutrient broth (0.8%) containing 0.5% NaCl, were printed onto the plate (22), which was then placed at 37 or 42 C. If *E. coli* CR63 or *E. coli* W3110 is substituted for *E. coli* B, 0.2 ml (50 mM) or 0.1 ml (10 mM) of uracil must be used, respectively. *denA* mutants are reproducibly FdUrd sensitive on such plates, but the FdUrd sensitivity of SP62 varies considerably from day to day. Presumably this difficulty results from the incompleteness of the block of SP62 to host DNA degradation (see Fig. 1).

Since FdUrd inhibits de novo synthesis of one deoxyribonucleotide, dTMP (10), the rationale for its use is analogous to that for HU, which inhibits de novo synthesis of all four deoxyribonucleotides. Uracil is added to minimize the incorporation into RNA of fluorouracil, which may derive from FdUrd (10). We substituted FdUrd for HU because we feared that HU might cause the introduction of nicks into the host DNA upon infection by T4; Rosenkranz and Rosenkranz (43) observed that an oxidation product of HU has such an effect in vitro. One of us (J. S. Wiberg, unpublished data) since has shown that HU causes extensive conversion of host DNA to acid-soluble products (hereafter termed "solubilization") after infection by a T4 mutant defective in genes 42, 46, and 47; this mutant normally causes negligible solubilization. Such an effect of HU might mimic the action of a particular T4 DNase (thus masking a mutational lack of the nuclease) and prevent detection of the mutant.

Procedure 2. Procedure 2 relies on the temperature sensitivity of SP62 (see Table 2) and was also used to identify some multiple mutants containing SP62. SP62 (5×10^7 total) is added as "helper" to the top agar, along with 10^8 to 2×10^8 *E. coli* B. Plaque suspensions are then printed onto the plate (22). The plate is incubated at about 45°C to suppress growth of the helper, while growth of wild-type recombinants produces a zone of clearing. Close control of the incubator temperature is critical: a temperature slightly too low permits enough "leakiness" of the helper SP62 to abolish the entire lawn of bacteria; a temperature slightly too high may suppress growth of T4⁺ or even prevent growth of the bacteria.

Procedure 3. Procedure 3 is a more reliable version of procedure 2, in that it does not require such critical temperature regulation; as helper we use an *am*, SP62 double mutant instead of SP62. The lethal *am* mutation is in a gene far removed from any in the multiple mutants being characterized. The *am* mutation completely prevents leakiness of the helper (at any temperature), yet permits efficient formation of *am*⁺ recombinants so that only the presence or absence of the SP62 mutation in the applied spot is at issue: a mutant *regA* gene in the spot gives a weak, hazy zone (*am*⁺, SP62), whereas a wild-type *regA* gene gives a readily distinguishable clear zone (T4⁺) at 44.8°C on *E. coli* B.

Radioactive labeling of DNA. Bacterial DNA was labeled (see Fig. 1, 2) as previously described (22) except that (i) no unlabeled thymidine (dThd) was added just before chilling and resuspending the cells in unlabeled medium, and (ii) the labeled cells were collected by centrifugation (15 min at $3,000 \times g$) rather than by filtration. [*Methyl-³H*]dThd was present at 0.7 μ Ci/ml (Fig. 1) or 1.4 μ Ci/ml (Fig. 2). Phage DNA was labeled (Fig. 4) as follows. *E. coli* B at 5×10^8 cells/ml was infected at 37°C at zero time with phage at a multiplicity of infection (MOI) of 5 and again at 3 min. At 4 min, a mixture of [*3*H]dThd and dAdo was added, giving a final concentration in the culture of 2 μ Ci/ml and 1 mM, respectively. Samples of 50 μ liters were taken at various times onto filter paper disks as previously described (22).

Transfer of radioactive label from host DNA to phage. The procedure for transferring radioactive label from host DNA to phage is a modification of that used previously (22; see Screening procedure I). *E. coli* B at 4.2×10^8 cells/ml, labeled with [*3*H]dThd as described above, was infected at 37°C at an MOI of 6 at zero time and again at 3 min. At various times, 0.7 ml was taken into a tube containing 0.1 ml of CHCl₃, mixed, and placed on ice. After at least 5 min, 0.5 ml was transferred (avoiding the CHCl₃) to another tube on ice. Micrococcal nuclease (Worthington, NFCP, 2FA; 12,776 U/mg) was added (0.1 ml of 0.2 mg/ml), and the mixture was incubated at 37°C for 3 h. Previously (22), extra CaCl₂ was added, but we have omitted it here since we noticed that this frequently caused clumping of the cells; sufficient Ca²⁺ to activate the nuclease is present in the growth medium, although the reaction is demonstrably slower. Samples (50 μ liters) were then taken onto paper filter

disks for determination of acid-insoluble radioactivity.

Preparation of extracts for enzyme assays. Extracts used for Fig. 5, 7, 8, 9, and 12 were prepared by sonic disruption of cells in the whole, chilled cultures (63). Extracts used for Fig. 6 and 13 were prepared as follows. To 2-ml portions of chilled culture (4°C) were added 50 μ liters of sodium EDTA (0.4 M, pH 8) and 50 μ liters of lysozyme (Sigma Chemical Co.; grade 1, no. L-6876) (10 mg/ml). The samples were mixed and left on ice until lysis (clearing) was complete (at least 2 h). All extracts except those for Fig. 13 were centrifuged at 4°C for 15 min at $4,000 \times g$ to remove cell debris. Control experiments showed that the two lysis procedures are equivalent for dCTPase and dCMP hydroxymethylase.

Enzyme assays. dCTPase was assayed as before (64), except that the ion-exchange resin used was Bio-Rad AG1-X8-formate (200–400 mesh), and the final column eluant was 5 ml of NH₄ formate (0.06 M, pH 3) instead of 3 ml. The absorbance of this eluate at 280 nm (OD₂₈₀) was read in a 1-cm light path.

dCMP hydroxymethylase. This enzyme was assayed by using a modification of the procedure of Yeh and Greenberg (70). The reaction measured is the release (as tritiated H₂O) of the ³H atom of [*5-³H*]dCMP. The reaction mixture contained in 0.55 ml: 25 μ mol of Tris acetate, pH 7.5; 36 nmol of [*5-³H*]dCMP (0.5 μ Ci); 50 μ mol of 2-mercaptoethanol; 1 μ mol of sodium EDTA, pH 8; 0.5 μ mol of HCHO; and 1 μ mol of d,1-L-tetrahydrofolate. The reaction mixture was incubated for 5 min at 37°C before adding extract (65) and then with extract for 45 min at 37°C. The reaction was stopped by adding about 8 mg of charcoal and shaking the tube (almost horizontal) at room temperature to keep the charcoal suspended. After 6 min the tube was centrifuged for 5 min at $2,000 \times g$. A volume of 0.3 ml of the supernatant fluid was transferred to another tube containing 1 ml of a suspension of anion-exchange resin (Bio-Rad AG1 $\times$ 8 formate, 200–400 mesh; 2 vol of suspension contain 1 vol of packed resin). The tube was shaken and centrifuged again. A volume of 0.6 ml of the supernatant fluid was mixed with 5 ml of scintillation fluid (Aquasol, New England Nuclear) and the radioactivity was measured.

In order to obtain acceptably low radioactivity in the reagent blanks, the [*5-³H*]dCMP had to be freed of tritiated water and other forms of ³H not adsorbable to charcoal and resin. This was done at 4°C by adsorption of the [*3*H]dCMP (250 μ Ci total) in 6 ml of water onto a 5- by 0.3-cm column of Bio-Rad AG1 formate, 200 to 400 mesh. The column was washed with 2 ml of water. Then the dCMP was eluted with 5 ml of 0.05 M formic acid; water and formic acid were removed by evaporation under vacuum. The residue was dissolved in 4 ml of water and stored at –15°C. The amount of radioactivity that did not adsorb to the resin constituted 6.4% of that applied to the column. Seven months later, repurification of this purified sample was necessary, as 2% of the ³H was in the form of [*3*H]H₂O and the reagent blank in the assay had

risen from 200 to 3,500 counts/min. Since then we have been able to minimize such deterioration (probably ^3H exchange) by storing the [^3H]dCMP in PO₄ buffer at pH 7 and at far lower specific activity.

Another important requirement for low reagent blanks is the use of the anion-exchange resin in addition to charcoal. Controls in which only resin, only charcoal, or two treatments with either were used gave reagent blank values that were 4 to 10 times higher.

dTMP synthetase. This enzyme was assayed by using a modification of the procedure of Lomax and Greenberg (33). The reaction mixture contained, in 0.5 ml: 50 μmol of Tris acetate, pH 8; 36 nmol of [^3H]dUMP (0.5 μCi); 50 μmol of 2-mercaptoethanol; 1 μmol of sodium EDTA, pH 8; 0.5 μmol of HCHO; and 0.5 μmol of d, l-L-tetrahydrofolate. The reaction mixture was heated for 5 min at 37 C before adding extract (65). Incubation with extract was for 60 min at 37 C. The procedures then followed were exactly the same as for dCMP hydroxymethylase (above). As with [^3H]dCMP, purification of [^3H]dUMP was necessary for an acceptably low reagent blank; in this case sufficient purification was obtained simply by removing water under vacuum. Nonetheless, the use of both charcoal and ion-exchange resin in the assay was as necessary as for hydroxymethylase.

HMdCMP kinase. The assay for hydroxymethyl dCMP (HMdCMP) kinase described previously (66) was used, with these exceptions: (i) total radioactivity per assay in [^{14}C -hydroxymethyl]HMdCMP was increased to 32,600 counts/min (normalized to the counting efficiency obtained for the values plotted in Fig. 8); (ii) 2 ml (instead of 1.5 ml) of 0.01 M formic acid was used to rinse product from the column, and this was mixed with the 1 ml of original column effluent. A 1.2-ml portion of this mixture was added to 10 ml of Aquasol and mixed, and the radioactivity was measured.

Lysozyme. This enzyme was assayed by the turbidometric technique of Sekiguchi and Cohen (52) as modified by Kutter and Wiberg (29). Although the unit of activity is that defined by Sekiguchi and Cohen, namely a decrease in optical density (OD) of 1 per min in a 1-cm path, our assay temperature was 30 C (theirs was 25 C), we used a 2-ml reaction volume (they used 3), and the OD was read at 490 nm instead of 450. A volume of 0.1 ml of extract, or extract diluted with GCA, was used in each assay.

Labeling and SDS slab-gel electrophoresis of proteins. *E. coli* B_a-1 grown in M9 medium (3.0 g of KH₂PO₄, 6.6 g of Na₂HPO₄, and 1.0 g of NH₄Cl per liter with 0.4% glucose and 10⁻³ M MgSO₄) was infected at an MOI of 10 at 37 C. Samples of 1 ml were labeled, at various times after infection, with 2 μCi of ^{14}C -amino acids (reconstituted protein hydrolysate, algal profile, 57 mCi/matom of carbon, Schwarz/Mann). Three minutes later, 0.1 ml of 10% Casamino Acids (Difco) was added to stop further incorporation of radioactivity; after an additional 3 min the cells were chilled on ice and 5 ml of ice-cold 0.1 M Tris-chloride (pH 6.8) was added. The cells were sedimented and suspended in 0.1 ml of sample buffer

(0.05 M Tris-chloride, pH 6.8; 1% SDS; 1% mercaptoethanol; 15% glycerol; and 0.1% bromophenol blue) and heated for 2 min in a boiling water bath. A 0.01-ml sample was loaded per well on the gel. The method used for vertical slab-gel electrophoresis is that described by Studier (55) and includes an electrophoresis apparatus modified from the design of Reid and Bielecki (42); electrophoresis was carried out in the discontinuous SDS-buffer system described by Laemmli (30), on slabs of 10% polyacrylamide gel, at 28 V, 3 to 6 mA, for 13.5 h. The gels were then dried for autoradiography by a modification of the procedure of Fairbanks, Levinthal, and Reeder (15). The patterns obtained (Fig. 10 and 11) were quite reproducible, and only 1 to 5% of the acid-precipitable radioactivity applied remained at the origin.

RESULTS

Isolation of SP62. Hercules et al. (22) described procedures designed to enrich for and identify spontaneous, nonlethal mutations in bacteriophage T4D that cause defects in the phage's ability to degrade host DNA. The enrichment scheme entailed heavy labeling of the DNA of *E. coli* B with [^3H]thymidine, infecting with unmutagenized T4D⁺ at an MOI of less than one, and harvesting the progeny phage at 25 min after infection at 37 C. Mutants that convert less than normal amounts of host DNA to mononucleotides incorporate less label into phage DNA; the less label in the progeny phage, the less radioactive suicide they undergo during prolonged storage. Mutants were identified by virtue of their sensitivity to HU; HU inhibits the reduction of ribonucleotides to deoxyribonucleotides, thus imposing on phage DNA synthesis an absolute requirement for degradation of host DNA (61). The procedures yielded 8 *denA* mutants defective in the ability to induce T4 endonuclease II (44) and one leaky mutant in gene 46.

Continued screening of such "enriched" phage populations for more mutants has revealed a new type of mutant, SP62, whose properties differ from those of any previously described T4 mutants. This more recent screening program involved a spot-test procedure similar to that described previously (22) except that FdUrd plus uracil was substituted for HU in the top agar of the spot plate (see Materials and Methods).

Sensitivity to FdUrd and HU. SP62 was detected because of its sensitivity to FdUrd on spot plates, specifically on *E. coli* B at 37 and 42 C; we subsequently found that SP62 is as sensitive to HU on spot plates as is [*denA*] on *E. coli* W3110 at 37 C (22). Table 1 shows a comparison of SP62 with T4⁺ and a *denA*

TABLE 1. *Effect of HU or FdUrd on phage production^a*

Incubation		<i>E. coli</i> B						<i>E. coli</i> CR63		
		HU			FdUrd			FdUrd		
Temp (C)	Time (min)	T4 ⁺	SP62	[<i>denA</i>]	T4 ⁺	SP62	[<i>denA</i>]	T4 ⁺	SP62	[<i>denA</i>]
30	60	11.8	9.6	0.12	10.5	3.0	0.11	9.6	5.1	0.64
37	30	13.9	8.9	0.16	8.1	3.7	0.10	5.9	0.8	0.30
42	25	19.3	9.4	0.20	6.3	1.9	0.12	—	—	—

^a *E. coli* B was grown at 37 C to 6×10^8 cells/ml in GCA medium and chilled. HU (1.3 M) was added to one portion so that the final concentration after phage infection was 0.02 M. FdUrd (10 mM) and dAdo (40 mM) were added to another portion so that the final concentrations after phage infection were 17.4 μ M and 860 μ M, respectively. (dAdo was used to minimize toxic interference of FdUrd in RNA metabolism. Although uracil competes directly with FU for entry into RNA [see Results], dAdo prevents formation of FU by inhibiting thymidine phosphorylase [69]. dAdo was less toxic than uracil in liquid-culture experiments.) Portions of 1 ml of these druged cultures were warmed at the respective temperatures for 3 min and then infected with phage at an MOI of 4.2 at zero time and again at 3.5 min. Samples were taken at the times indicated. *E. coli* CR63 was grown at 37 C to 5.1×10^8 cells/ml in GCA medium and chilled. FdUrd and dAdo were added so that the final concentrations after phage infection were 20.4 μ M and 815 μ M, respectively. Infection was the same as for *E. coli* B except that the MOI was 9.8 at zero time only. Values are expressed as phage per cell.

mutant, with respect to phage production in *E. coli* B and *E. coli* CR63, in the presence of FdUrd or HU at several temperatures. As shown before with HU (22), the *denA* mutant, which is unable to solubilize host DNA, produces no phage in HU or FdUrd. SP62, however, produces from 14 to 53% as many phage in FdUrd as T4⁺ produces, depending on conditions, and seems somewhat less sensitive to HU. These results confirm the spot-test indication of FdUrd and HU sensitivity and argue further that solubilization of host DNA is not completely blocked by the SP62 mutation.

Solubilization of host DNA. The indication that SP62 at least partially solubilizes host DNA was tested directly. To block incorporation of solubilized nucleotides into phage DNA, an amber mutation in gene 42 (dCMP hydroxymethylase) was bred into SP62. Fig. 1 shows that the solubilization of the DNA of *E. coli* B at 37 C by this double mutant, [*regA*, 42], is slower and less extensive than that by [42] alone. A similar effect was seen when the infection was carried out at either 30 or 42.1 C (data not shown). Since phage production by SP62 is more temperature sensitive on *E. coli* CR63 than on *E. coli* B (Table 2), solubilization of host DNA was also examined on CR63 at high temperature. In this case DNA synthesis was blocked with a *ts* mutation in gene 42, *tsL13x5*. Solubilization by SP62, *tsL13x5* occurred at about 50 and 20% the rate seen with *tsL13x5* at 43 and 44 C, respectively (data not shown).

SP62 defines a new gene. Mutations in only three genes of T4 have been shown previously to impair solubilization of host DNA, and they

essentially completely block it; these genes are 46, 47 (63), and *denA* (22, 62). Therefore the incomplete defect in solubilization exhibited by SP62 suggested that either it affected a new gene or it was a leaky mutant in one of these three genes. In work to be published separately (J. S. Wiberg, S. Mendelsohn, V. Warner, and C. Aldrich), complementation tests and genetic mapping experiments indicate that SP62 defines a new gene (*regA*) between genes 43 and 62. Preliminary experiments earlier misled us into stating that the map position was between genes 43 and 44 (J. S. Wiberg, S. Mendelsohn, V. Warner, K. Hercules, and J. Munro, Fed. Proc. 30:1263, 1971). See Wood (68) for a genetic map of T4.

SP62 is indeed a mutant of T4. In other experiments not shown here, we have obtained evidence which argues that the original SP62 (genetically unpurified) is not a chance, contaminating T2 or T6 phage, but is indeed a mutant of T4. These experiments show that (i) SP62 does not grow at 37 C on our *E. coli* B/4 strain, on which T4D⁺ does not grow, but on which T2H, T2L, and T6 do grow weakly; (ii) the original SP62 grows at 37 C on an *E. coli* B/6 strain on which T6 does not grow but on which T4D⁺ does; (iii) both the original SP62 and the x3 version have the same sensitivity to UV light (254 nm) as does T4D⁺, whereas both T2 and T6 are about twice as sensitive; and (iv) SP62 irreversibly adsorbs at 37 C to both *E. coli* B and *E. coli* CR63 at the same rate as does T4D⁺.

Transfer of label from host DNA to progeny phage. The indication from HU and FdUrd sensitivity that host DNA breakdown is im-

paired by the SP62 mutation only applies in the presence of the drugs, which largely inhibit DNA synthesis. Similarly, the fact that solubili-

FIG. 1. Effect of SP62 mutation on degradation of host DNA. *E. coli B* was labeled in DNA with [³H]thymidine as described in Materials and Methods. Portions of 1 ml were warmed at 37 C for 3 min, and then infected at an MOI of 10 at 0 min, and at an MOI of 5 at 3.5 min. Samples (50 μ liters) were taken at the indicated times for determination of acid-insoluble ³H. Symbols: Δ , [42]; O, [42, *regA*], $\times$, uninfected.

zation of host DNA by SP62 is impaired when phage DNA synthesis is blocked (DNA⁻ stage) (Fig. 1) does not necessarily mean that it is impaired when DNA synthesis is permitted (DNA⁺ state). It will be seen below that the SP62 mutation has a very different effect on enzyme synthesis in the DNA⁻ state than in the DNA⁺ state. Thus we asked whether SP62 exhibits impaired breakdown of host DNA in the DNA⁺ state. Since in the DNA⁺ state the acid-soluble products of host DNA degradation are incorporated into (acid-insoluble) phage DNA without lag, an indirect method must be used to monitor the solubilization. We used a method that follows the transfer of labeled dThd from host DNA into the DNA of phage particles; the latter is operationally defined as all label rendered resistant to DNase after lysis of infected cells (22). At 37 C, SP62 transferred only about 70 and 84% as much label from *E. coli B* into phage particles as did T4⁺ at 20 and 25 min, respectively (Fig. 2A). By 30 min the transfer was essentially normal. This relatively efficient transfer of label by SP62 suggests that *regA* mutants undergo little or no enrichment by the ³H-suicide scheme; thus they probably are present at a high frequency in wild-type stocks because their ability to produce phage is virtually normal.

Several controls were included in the same experiment (Fig. 2A, 35 min). (i) A *denA* mutant, which does not solubilize significant amounts of host DNA, exhibited less than 7% as much transfer as T4⁺. (ii) An amber mutant in gene 55 exhibited less than 3% transfer; such mutants degrade host DNA normally in the DNA⁻ state (63) and presumably do so in the DNA⁺ state also. Thus, by 35 min after infection, about 80% of the label from host DNA should be in phage DNA, but not in phage particles, as the gene 55 mutation blocks maturation (14, 24). We conclude from the negligible

TABLE 2. Phage production by SP62 and T4⁺ at 43, 44, and 45 C^a

Strain	Incubation time (min)	43 C			44 C			45 C		
		T4 ⁺ ^b	SP62 ^b	SP62/T4	T4 ⁺ ^b	SP62 ^b	SP62/T4 ⁺	T4 ⁺ ^b	SP62 ^b	SP62/T4 ⁺
<i>E. coli B</i>	25	93	71	0.77	83	43	0.52	30	6.8	0.23
<i>E. coli B</i>	50	308	259	0.84	241	127	0.53	92	36	0.39
<i>E. coli CR63</i>	25	50	9.5	0.19	23	<0.6	<0.03			
<i>E. coli CR63</i>	50	164	43	0.26	82	9.4	0.11			

^a Bacteria were grown at 37 C to a concentration of 4.6×10^8 cells/ml (*E. coli B*) and 4.0×10^8 cells/ml (CR63) and then chilled. Portions of 1 ml were warmed for 3 min at the temperatures indicated. Phage then were added at an MOI of 5.5 to 6.5 and again 3.5 min later. At 25 and 50 min after infection, samples were taken for determination of phage production.

^b Measured in phage per cell.

FIG. 2. A, Effect of SP62 mutation on transfer of [3 H]dThd from host DNA to progeny phage. Each point is the average of duplicate samples from the same flask. 100% = 4870 counts/min. Symbols: ● and ○, T4⁺, two separate infections; ■ and □, [regA], two separate infections; ×, [denA]; ▽, [55]; ▼, uninfected bacteria. B, Phage production in experiment of part A. Same symbols as in part A.

transfer by [55] that phage DNA is also susceptible to micrococcal nuclease under the assay conditions; this eliminates the possibility that some of the label apparently transferred by T4⁺ or SP62 is in phage DNA that is not in phage particles. (iii) The DNA of uninfected cells is totally degraded by the micrococcal nuclease. This shows that the treatment with CHCl₃ (Materials and Methods) sufficiently "lyses" the cells to render the DNA accessible to the nuclease.

In this same experiment phage production was measured. The results (Fig. 2B) indicate that phage production by SP62 is somewhat delayed, and catches up with that of T4⁺ only after 30 to 35 min. Thus, the delay in label transfer argues that either SP62 does solubilize host DNA slower than normal in the DNA⁺ state, or it is slow in packaging the phage DNA into nuclease-resistant particles.

Phage production is temperature sensitive. Figure 3 shows a more extensive comparison of phage production by SP62 and T4⁺ than was shown in Fig. 2B. A further difference between the experiments is that the cells used for Fig. 2B had necessarily been grown in dAdo (to facilitate [3 H]dThd incorporation). Although most of the dAdo was washed out before infection, there

might be a residual inhibitory effect on phage production; Kutter and Wiberg reported that phage production is inhibited as much as 50% by dAdo present during infection (p. 449 of reference 29). Figure 3 also shows that SP62 exhibits a small (but reproducible) lag in phage production at 37°C on *E. coli* B, but phage production by SP62 is indistinguishable from that of T4⁺ by 40 min.

The data of Table 2 shows that phage production by SP62 is more temperature sensitive than that by T4⁺. Note that this temperature sensitivity is more marked on *E. coli* CR63 than on *E. coli* B. On both bacteria, the defect in phage production by SP62 is usually relatively greater at 25 min than at 50 min. This could be interpreted to mean that high temperatures exaggerate the small delay in phage production seen at 37°C (Fig. 3).

DNA synthesis. Figure 4 shows that the rate of DNA synthesis at 37°C in cells of *E. coli* B infected by SP62 is essentially identical to that by T4⁺. The relatively slower phage production before 40 min (Fig. 3) suggests a small delay in maturation of the DNA into plaque-forming particles. The rate of DNA synthesis of SP62 was also completely normal at 42.6°C on *E. coli* B and at 39, 43, and 44°C on *E. coli* CR63 (data

FIG. 3. Phage production by SP62 and $T4^+$ at 37 C on *E. coli* B. Bacteria were grown to 6.6×10^8 cells/ml, chilled, and centrifuged for 15 min at $3,000 \times g$. The pellet was resuspended to the original volume in fresh GCA medium. Samples of 5 ml were placed at 37 C for 3 min, and then infected with phage at an MOI of 3.8 at zero time and again at 3.5 min. Samples were taken at the times indicated, diluted into $CHCl_3$ -saturated dilution broth and assayed on *E. coli* CR63 for plaque-forming phage. Each point represents the average value for two parallel infections. Symbols: $\square$, $T4D^+$; $\circ$, [regA].

not shown). Phage production by SP62 compared to $T4^+$ at 50 min in these infections was 69, 94, 41, and 6.2%, respectively. These data agree in general with those shown in Table 2 and suggest a defect in maturation of SP62 at high temperatures.

Genetic recombination. The following experiment was performed to test whether the SP62 mutation has an effect on genetic recombination. *E. coli* B at 5×10^8 cells/ml was infected at 37 C with various mixtures of two complementing *am* mutants, each mutant at an MOI of 5. At 30 min, samples were taken into chloroformed broth, and total phage and wild-type recombinants were measured. The first value given for each cross is percent of wild-type, and the second value (in parentheses) is total phage per cell: *amE51x5* (gene 56) + *amB14x5* (gene 46), 13.7% (87); *amN55x5* (gene 42) + *amB14x5*, 7.0% (90); *amN55x5* + *amE51x5*, 10% (124). The values for the corresponding mixed infec-

tions in which each *am* mutant also contained the original SP62 mutation are: 14.4% (103); 8.3% (105) and 10.6% (113), respectively. We conclude that SP62 has no significant effect on recombination frequencies at these relatively large map distances. It is conceivable that it has an effect at much smaller map distances, but this has not been tested.

Synthesis of dCTPase. The synthesis by SP62 of several phage-induced enzymes was examined initially as a control to test whether the infection was "normal." It is seen in Fig. 5 that dCTPase (*E. C.* 3.6.1.12, dCTP nucleotidohydrolase) appears at the normal time and at the normal rate after infection by SP62, and shutoff of synthesis occurs almost as sharply at 12 min as with $T4^+$. Shutoff is delayed until about 18 min with the DNA⁻ mutant [42] as expected (29, 66). However, shutoff fails to occur at all after infection by [regA, 42]. The level of dCTPase at 60 min with [regA, 42] is 9.5 times that with $T4^+$ and 4.8 times that with [42]. We have seen these same relative dCTPase patterns with these four phage in infections of *E. coli* B at 30, 33.5, 38.5, and 40 C (data not shown). Also [regA, 43] gives

FIG. 4. DNA synthesis by SP62 and $T4^+$ at 37 C on *E. coli* B. The arrow indicates the time (4 min) of addition of $[^3H]dThd$ plus *dAdo*. Phage production at 31 min was 62 and 64 phage per cell for $T4^+$ and SP62, respectively. These values are lower than normal (see Fig. 3) because of the presence of *dAdo* (10). Symbols: $\circ$, $T4^+$; Δ , [regA].

FIG. 5. dCTPase production. *E. coli* B was grown to 6×10^8 cells/ml and chilled. The cells were sedimented ($2,000 \times g$ for 10 min) and suspended in the original volume of fresh GCA medium. Portions were placed at 37 C for 3 min. At zero time and again at 3 min, they were infected at an MOI of 4.2. Samples of 2 ml were chilled at the times indicated and frozen until extracts were made. Values for phage production at 60 min for T4⁺ and SP62 were 376 and 346 phage per cell, respectively. A volume of 50 μ liters of extract was assayed except as noted below. An OD₂₈₀ value of 1 corresponds to 214 μ mol of dCMP per 10^{12} cells per min. An OD₂₈₀ value of 1.4 represents complete exhaustion of substrate; therefore values above 1.0 were obtained by assaying less than 50 μ liters and multiplying the result by the appropriate correction factor. Assay values varied linearly with amounts of extract below those giving an OD₂₈₀ value of 1.0. Uninfected cell extract, or an extract of cells infected with a gene 56 am mutant (dCTPase⁻), gave values of OD₂₈₀ from 0.03 to 0.05; 0.04 was subtracted from each value before plotting. Symbols: ○, T4⁺; △, [regA]; ▽, [42]; □, [42, regA]; ●, average of points shown in larger scale in inset.

the same overproduction of dCTPase that is seen with [regA, 42] (data not shown). Thus, the *regA* gene appears to control a mechanism needed in the DNA⁻ state (but not in the DNA⁺ state) to shut off synthesis of dCTPase.

Is protein synthesis required for the extended "synthesis" of dCTPase? It is possible that the apparent shutoff of dCTPase synthesis in either the DNA⁻ or DNA⁺ states, or both, is due to a block in activation of an enzyme precursor, rather than to cessation of protein synthesis. In Fig. 6, it can be seen that (i) addition of chloramphenicol (CM) at 12 min to the [42] infection stops dCTPase synthesis immediately, while in the parallel infection without CM, enzyme accumulates to almost twice this level; (ii) addition of CM at 12 min to the [regA, 42] infection stops dCTPase synthesis within about 1 min, while in the parallel infection in which CM addition was delayed until 25 min, enzyme accumulates to about four times this level; (iii) addition of CM at 25 min to the [regA, 42] infection drastically slowed enzyme synthesis, as compared with a comparable in-

FIG. 6. Arrest of dCTPase synthesis by chloramphenicol (CM). *E. coli* B was grown to 4.2×10^8 cells/ml and chilled. Portions were warmed for 3 min at 37 C and infected with phage at an MOI of 6 at 0 min and again at 3 min. Where used, CM (2100 μ g/ml) was added to a final concentration of 93 μ g/ml at the times indicated. T4⁺ produced 395 phage/cell at 60 min. The enzyme activities have been corrected for the small dilution by CM solution. Portions (20 μ liters) of extract were assayed. Symbols: △, T4⁺; ●, [42]; ▲, [42] + CM at 12 min; □, [42, regA], CM at 12 min; ○, [42, regA], CM at 25 min.

fection lacking CM (Fig. 5). These results argue strongly that protein synthesis is required for the overproduction of dCTPase caused both by a DNA⁻ mutation and by SP62 in the DNA⁻ state. The same conclusion, with respect to overproduction of other T4 early proteins, is supported by the gel electrophoresis studies presented below (Fig. 10 and 11) in which protein synthesis is visualized directly, not indirectly as an enzyme activity.

Synthesis of dCMP hydroxymethylase. The effect of SP62 on the synthesis of another early enzyme, dCMP hydroxymethylase, was examined. It can be seen in Fig. 7 that the patterns are very similar to those seen with dCTPase (Fig. 5). Note, however, that the DNA⁻ mutant [43] produces about three times the T4⁺ level as contrasted with only a doubling in the case of dCTPase (Fig. 5); this effect has been seen before (66). The level of hydroxymethylase at 60 min with [regA, 43] is seven times that with T4⁺.

FIG. 7. dCMP hydroxymethylase production. *E. coli* B was grown at 37°C to 6.2×10^8 cells/ml and chilled. Portions were warmed at 37°C for 3 min. Phage were added then (zero time) at an MOI of 4 and again at 3.5 min. Samples (2 ml) were chilled at various times and frozen until extracts were made. T4⁺ and SP62 produced 390 and 346 phage per cell, respectively, at 60 min. A background value of 210 counts per min (seen with both reagent blank and uninfected cell extract) has been subtracted. Portions (50 μ liters) of extract were assayed. Symbols: ○, T4⁺; □, [regA]; △, [43]; ●, [43, regA].

Synthesis of HMdCMP kinase. Figure 8 shows that the patterns are very similar to those seen with dCTPase (Fig. 5). The level of kinase at 60 min with [regA, 42] is 4.2 times that with T4⁺.

Synthesis of dTMP synthetase. In contrast to the results with the other three early enzymes (Fig. 5, 7, and 8), SP62 appears to shut off synthesis of dTMP synthetase a little sooner than normal in the DNA⁺ state and considerably sooner than normal in the DNA⁻ state (Fig. 9). Thus [regA, 42] produces about half as much dTMP synthetase as does [42].

SDS polyacrylamide gel electrophoresis of proteins synthesized after infection by SP62 mutants. We have examined the SDS polyacrylamide gel electrophoresis patterns of pro-

FIG. 8. HMdCMP kinase production. Portions (10 μ liters) of the same extracts used for Fig. 7 (dCMP hydroxymethylase) were assayed. A blank (uninfected bacteria) value of 210 counts/min has been subtracted. A value of 2,000 counts/min corresponds to 61 μ mol of HMdCMP phosphorylated per 10^{12} cells per min. 13,000 counts/min represents complete exhaustion of substrate, and values above 7,000 counts/min are not linear with respect to amount of extract; therefore the two values above 7,000 counts/min were obtained by assaying 5 μ liters and multiplying the results by 2. Symbols: ○, T4⁺; □, [regA]; △, [43]; ●, [43, regA].

FIG. 9. dTMP synthetase production. Portions (100 μ liters) of the same extracts used for dCTPase (Fig. 5) and lysozyme (Fig. 12) were assayed. The value obtained with uninfected cell extract (425 counts/min) has been subtracted; this value includes a reagent blank of 285 counts/min. The symbol designations are the same as in Fig. 5.

teins synthesized at various times after infection by mutants carrying the SP62 mutation. The autoradiograms shown in Fig. 10 indicate that at late times after infection by [42] the rate of synthesis of many proteins is drastically reduced (see especially bands 46, a, b, c1, c2, d, 45, f), whereas after infection [regA, 42] the rate of synthesis of most of these proteins is maintained or even increases until the end of the experiment, at 36 min. Thus the SP62 mutation appears to have a generalized effect in allowing continuation of synthesis of most of the early proteins whose synthesis is normally reduced at late times after infection in the DNA⁻ state. SP62 in the DNA⁻ state appears to cause great overproduction of the rIIA protein (Fig. 10), although in the normal DNA⁻ state the synthesis of this protein does not exhibit the usual shutoff pattern; there appears to be a premature slowdown followed by a second burst of synthesis between 21 and 30 min (Fig. 10). It is conceivable that both this "second burst" by [42] and at least some of the "overproduction of the rIIA protein" by [regA, 42] may be due instead to synthesis of gene 18 product, a late pro-

tein that migrates with the rIIA protein (Fig. 11). However, late gene expression is generally minimal in DNA⁻ infections (20).

The prolongation of synthesis of the gene 45 protein by SP62 in the DNA⁻ state (Fig. 10) confirms work in Alberts' laboratory (3; B. Alberts, personal communication; L. A. Moran, J. E. Barry, H. Hama-Inaba, D. C. Mace, J. Wiberg, and B. Alberts, Abstr. Cold Spring Harbor Bacteriophage Meet., p. 42, 1972). Although the data of Fig. 10 suggests a small prolongation of synthesis of the gene 43 protein (DNA polymerase) by SP62 in the DNA⁻ state, we are not confident that this small difference is significant. Furthermore, Nossal (personal communication) (measuring enzyme activity after partial purification) concludes that SP62 has little or no effect on synthesis of the gene 43 product in the DNA⁻ state. Note that the synthesis of early protein g is not prolonged by SP62 either in the DNA⁺ or DNA⁻ state (Fig. 10 and 11).

FIG. 10. Autoradiogram of SDS slab-gel electrophoresis of proteins pulse-labeled with ¹⁴C-amino acids at the times indicated after *E. coli* B₂₁ was infected by [42] or [regA, 42]. Samples were prepared and subjected to electrophoresis as described in Materials and Methods. Some of the genes whose protein products have been identified (as bands missing in extracts from cells infected by phage amber mutants) are indicated by gene number or name adjacent to the corresponding band; bands a to g are not identified by gene.

Little if any extended synthesis by SP62 of the early enzymes dCMP hydroxymethylase, dCTPase, and HMdCMP kinase was detected in the DNA⁺ state (Fig. 5, 7, 8). This same virtual lack of effect is seen with the early proteins exhibited in the gel patterns (Fig. 11). No obvious effect of the SP62 mutation on synthesis of late proteins is apparent (Fig. 11). The slightly earlier than normal appearance of some late proteins in the SP62 infection is probably caused by procedural variations, since the two autoradiograms represent two independent experiments. Also, late in infection, small changes in the rate of labeling that may be caused by the SP62 mutation are hard to detect because these changes occur in a high "background" (i.e., the T4⁺ control) of labeling already underway (Fig. 11), in contrast to late effects on labeling of early proteins, where this background is almost negligible (Fig. 10).

Synthesis of lysozyme. Lysozyme normally appears much later after infection than do the early enzymes examined above, and very little is made in the DNA⁻ state (28, 29, 35, 37). In Fig. 12A it can be seen that SP62 produces lysozyme at a completely normal rate in the DNA⁺ state until about 20 min; at that time the rate doubles. Even in the DNA⁻ state, where lysozyme synthesis is extremely low, the SP62 mutation causes overproduction beginning at about 20 min (Fig. 12B).

Emrich and Streisinger (13) have shown that a second, much less active, lytic activity is produced in T4 infections, in addition to the *e* gene product (54) that we are presumably measuring here. However, we find that [e, SP62] makes no lysozyme detectable by our assay as late as 70 min after infection (data not shown). Thus the "lysozyme" overproduced by SP62 is indeed the *e* gene product.

Pryme, Joner, and Jensen (41) have described a polysaccharide inhibitor of T4 lysozyme in extracts of *E. coli*. This suggested to us the possibility that the "overproduction" of lysozyme by SP62 might be due to removal of some of this inhibitor. However, when the lysozyme is treated to remove the inhibitor (27), 91 to 98% of the lysozyme is recovered, and about twice as much activity is recovered with SP62 as with T4⁺ (Table 3), thus matching the ratio of activities in the extracts applied. Furthermore, the data provide no evidence for any inhibitor in our extracts; also, direct tests of fractions 1 revealed no significant inhibitory capacity (data not shown). Thus a lysozyme inhibitor probably is not involved in the abnormal pattern of lysozyme "synthesis" by SP62.

Test for dominance of SP62 in mixed infections with T4⁺. To test whether the wild-type or the mutant *regA* gene is dominant with respect to shutoff of dCTPase synthesis, the experiment shown in Fig. 13 was performed. It can be seen that infection with a mixture of equal parts of [42] and [*regA*, 42] results in a 3- to 4-min delay in the shutoff; when the ratio is one part [42] and three parts [*regA*, 42], shutoff is delayed by about 7 min. Thus the degree of dominance is clearly dependent on gene dosage. The results suggest that the SP62 gene product must accumulate to a certain level for shutoff of enzyme synthesis to occur.

Another mutant in the *regA* gene. This mutant (R9) has been isolated independently by J. D. Karam (personal communication and Abstr. Cold Spring Harbor Bacteriophage Meet., p. 12, 1972). He detected his mutant as a weak suppressor of a leaky, DNA⁻ amber mutant (amE1140) in T4 gene 62; this leakiness is apparently the result of ribosomal ambiguity (18). The R9 mutation apparently suppresses by overproducing the small amount of gene 62 product made by ribosomal ambiguity. We have shown that R9 and SP62 fail to complement each other with respect to phage production in FdUrd (*E. coli* B at 42 C), and that R9, amN55x5

FIG. 11. Autoradiogram of SDS slab-gel electrophoresis of proteins pulse-labeled with ¹⁴C-amino acids at the times indicated after infection by T4⁺ or [*regA*]. Procedures are the same as described in Fig. 10. Band 23 (★) (head protein) is a cleavage product of band 23. Note that bands rIIA and 18 superimpose; presumably the gene 18 protein is not made in the DNA⁻ state (Fig. 10). Note the difference in time scale compared to Fig. 10.

FIG. 12. Lysozyme production. Extracts were those used for dCTPase (Fig. 5) and dTMP synthetase (Fig. 9). Symbols for A: ○, T4⁺; □, [regA]. Inset shows early time points on expanded scale. Symbols for B: ●, [42]; △, [42, regA]. Note difference in scale of the ordinates between A and B.

TABLE 3. Removal of possible lysozyme inhibitor with deaminated chitin columns^a

Solution applied to column	Amt applied (ml)	Effluent assayed (ml)	T4 ⁺			SP62		
			Activity observed (U)	Total recovered		Activity observed (U) ^c	Total recovered	
				(U) ^b	% of U applied ^c		(U) ^b	% of U applied ^c
1) Extract diluted 9× with H ₂ O	4.0	0.10	0.26	10.4	18	0.51	20.4	21
2) 0.0125 M PO ₄ buffer	3.0	0.10	0.052	1.6	2.8	0.088	2.6	2.6
3) 0.05 M Na acetate, pH 4.6 in 0.25 M KCl	2.0	0.025	0.41	33	57	0.75	60	61
	2.0	0.10	0.26	5.2	9.0	0.45	9.1	9.3
	2.0	0.10	0.12	2.4	4.1	0.21	4.2	4.3

^a Extracts were prepared in the same manner as for Fig. 5; cells were harvested at 70 min after infection at 37 C. Columns of deaminated chitin (5 by 0.3 cm) were washed, before use, with 2 ml of 0.0125 M PO₄ buffer (pH 7, 0.0041 M in K⁺, 0.019 M in Na⁺). Extracts were diluted nine times in water to lower the ionic strength for efficient adsorption of lysozyme. All column procedures were carried out at 4 C. Control experiments showed that these amounts of PO₄ buffer or Na acetate-KCl solution did not affect lysozyme activity in the assay. Lysozyme in the diluted extracts and in the various column effluents was unstable at 4 C; about half the activity was lost in 18 to 20 h. This instability can be prevented by addition of bovine serum albumin (BSA) to a concentration of 0.05%. No BSA was added in the present experiment, however, since the assays were performed within 1 h of collecting each fraction.

^b Units (U) applied were: for T4, 58 (0.025 ml gave 0.36 units); for SP62, 98 (0.025 ml gave 0.61 units).

^c Equivalent to 100× (activity observed) (milliliters applied)/(milliliters assayed).

overproduces dCTPase to exactly the same extent as does SP62, amN55x5 (*E. coli* B, 37 C). Both Karam and we have shown that SP62 and R9 map at different sites about 0.1 map unit apart.

DISCUSSION

We have shown that, under appropriate conditions, the SP62 mutation results in overproduction of the products of T4 genes 1, 42, 45, 46,

FIG. 13. dCTPase production in mixed infection by [42, *regA*] plus [42]: test for dominance by wild *regA* gene. *E. coli* B at 4.6×10^8 cells/ml was infected at 37°C at zero time at a total MOI of 11. Symbols: ○, [42]; Δ, [42, *regA*]; □, 1:1 ratio of [42] and [42, *regA*]; ▽, 1.3 ratio of [42] and [42, *regA*]; ●, composite of all four values, which superimpose. Values for [42] at 90, 120, and 180 min were 0.32, 0.38 and 0.40, respectively. Corresponding values for [42, *regA*] were 2.6, 3.0 and 3.3. The values plotted correspond to 20 μ liters of extract; smaller portions of very active extract were assayed as described in legend to Fig. 5.

56, *rIIA* and *e*; work in Alberts' laboratory showed the same overproduction of the gene 45 product and further showed that the products of genes 44 and 62 are also overproduced (3; Moran et al., Abstr. Cold Spring Harbor Bacteriophage Meet., p. 42, 1972). The extent of overproduction varies from gene to gene, with dCTPase exhibiting the greatest effect among the enzymes for which data are presented in this paper. These genes all lie on a segment of the genetic map that constitutes about $\frac{1}{3}$ of the T4 "chromosome" (68). On the other hand, dTMP synthetase (gene *td*) (Fig. 9) and polynucleotide ligase (gene 30) (R. Swift and J. Wiberg, unpublished experiments) are not overproduced by SP62; these genes lie roughly opposite the $\frac{1}{3}$ -chromosome segment.

Two classes of early enzymes have been distinguished: the mRNA for immediate-early (IE) enzymes appears when chloramphenicol is added before infection, while that for delayed-early (DE) enzymes does not (1, 11, 19, 47). This result has been interpreted to mean that the expression of DE genes requires the prior expression of IE genes, but there is no consensus on what the basis of this distinction is (4, 5, 7, 39, 50, 51, 56, 57). In any case, SP62 overproduces enzymes in both classes: HMdCMP kinase (32, 45, 46, 58) and dCTPase (32) are DE, whereas dCMP hydroxymethylase is IE (25, 32, 58). Similarly, SP62 fails to overproduce at least one gene product in each class: DNA ligase is IE (25), whereas dTMP synthetase is DE (32, 58).

It was shown in 1962 that the shutoff of synthesis of a number of early T4 enzymes at about 12 min at 37°C could be delayed to about 20 min by blocking phage DNA synthesis genetically (66). We shall assign the term S1 (first shutoff) to this 12-min event that fails when DNA synthesis is blocked. A late gene product probably is involved in the S1 event, since S1 does not occur when late mRNA is not produced, e.g., with DNA⁻ (6, 20, 47) and maturation-defective (genes 33 and 55) mutants (24). Similarly, we shall assign the term S2 to the second shutoff event, that occurring at about 20 min. Our results argue that S2 is controlled (at least for some enzymes) by the *regA* gene. *regA* cannot be a true late gene since it functions in the DNA⁻ state; furthermore, it maps genetically among many early genes. We conclude that *regA* is probably an IE, DE, or quasi-late gene (20, 47).

Does S1 control transcription? A number of workers have suggested that S1 may be a transcriptional control, based on their demonstrations that transcription of early genes decreases at late times after infection (47, 53, 71). This argument is not convincing, however, because the mere cessation of synthesis of a given mRNA would not necessarily dictate the cessation of synthesis of the corresponding enzyme; as long as any of the mRNA persists, one would expect some enzyme synthesis unless translation of the mRNA is inhibited. This inhibition could take at least two forms: (i) active inhibition, e.g., the type involving specific initiation factors, or (ii) passive inhibition, in which, e.g., all mRNAs compete with each other simply by mass action. In view of this, and considering the evidence for translational regulation of T-even, early gene expression (2, 8, 9, 17, 21, 31, 32, 40), it appears premature to judge whether S1 is a transcriptional or a translational control.

What is the *regA* gene product? Since SP62

affects regulation, the following candidates for its gene product come to mind: (i) an RNase controlling mRNA longevity; (ii) a factor controlling the intracellular level of cations; (iii) a sigma-like transcription factor or a modifier of RNA polymerase; (iv) a new tRNA; (v) a translation factor or a modifier of translation factors such as IF3 (see below); (vi) an enzyme that modifies (e.g., methylates) DNA or RNA (mRNA, tRNA), thus affecting specificity of transcription or translation. We are testing some of these possibilities.

The *regA* gene probably controls translation. Although we have no direct evidence for it, two circumstantial arguments suggest a role in translation for the *regA* gene. (i) Sakiyama and Buchanan (46) offered evidence that functional mRNA for deoxynucleotide kinase overaccumulated, and decayed much slower, in a DNA⁻ infection compared to a DNA⁺ infection; at 30 min (37 C) in the DNA⁻ infection, as much mRNA remained as was ever present in the DNA⁺ infection (which yielded a maximum level at 10 min). Both under their conditions (S. Sakiyama, personal communication) and ours (Fig. 8 and ref. 66), the S2 event for HMdCMP kinase occurs at 20 to 25 min at 37 C in the DNA⁻ state; for kinase, S2 represents a drastic slowdown rather than complete shutoff. Since this S2 event occurs despite the presence of large amounts of kinase mRNA, we must conclude that S2, for kinase at least, represents a translational control. Since SP62 controls this S2 event, we consider this a strong indication of a translational role for SP62. Furthermore, Sakiyama (personal communication) has found that the SP62 mutation has little or no effect on the pattern of accumulation of kinase mRNA in the DNA⁻ state, which argues against a transcriptional role for SP62. (ii) Until now, the only regulatory "correlation" between lysozyme synthesis and early enzyme synthesis has been an inverse one: the DNA⁻ state has an opposite effect on synthesis of early enzymes (overproduction) (24, 66) and lysozyme (underproduction) (28, 29, 35, 37). However, the SP62 mutation affects both classes of enzyme in the same direction, causing overproduction; a T4-controlled effect on translation in vitro has been described that appears to exhibit this same regulatory duality. Pollack, Groner, Aviv, and Revel (40) reported that T4 infection causes a change in initiation factor 3 (IF3), whereby the ability of their system to translate both T4 early mRNA and T4 lysozyme mRNA decreased relative to other mRNAs including T4 late mRNA. This suggests to us that the *regA* gene may control this change in

IF3. Thus in the presence of the SP62 mutation, the change in IF3 would not occur and early mRNA and lysozyme mRNAs would be translated more efficiently.

Why is lysozyme overproduced? The fact that the SP62 mutation results in a sudden doubling of lysozyme synthesis at 20 min argues that the wild-type *regA* gene product partially inhibits lysozyme synthesis starting at 20 min. As we implied above, this effect is reasonable if the *regA* gene controls a known, T4-induced change in IF3, an initiation factor affecting the mRNA specificity of translation (40). Several workers have offered evidence that, after infection by T4⁺, not all "late" lysozyme mRNA is translated in vivo (49, 67). Alternatively, the SP62 mutation might permit the translation of "early" lysozyme mRNA (26, 28) which normally is not translated in vivo at early times (28) and which is not translated at all in vitro, using ribosomes from uninfected *E. coli* (48). Thirdly, SP62 may affect transcription of the lysozyme gene.

Why is dTMP synthetase underproduced? We can suggest two models to explain the underproduction of dTMP synthetase by SP62. (i) The wild-type *regA* product may exert a direct, positive effect on expression of the *td* gene at the same level of regulation (efficiency of translation of mRNA?) at which it exerts a direct negative effect on expression of other genes. (ii) The second shutoff (S2) for dTMP synthetase may be controlled by a gene other than *regA*, and SP62 simply overproduces this gene product so that the S2 event occurs earlier than normal. Trimble, Galivan, and Maley (58) offered evidence that the mRNA for dTMP synthetase rapidly decays to an undetectable level, in contrast to the mRNAs for several other T2 early enzymes. Thus we would suggest that the S2 event for dTMP synthetase may simply reflect total decay of its mRNA, whereas for those early genes whose mRNA persists at some finite level for a long time after infection, S2 may reflect specific inhibition of translation, as discussed above.

Why is breakdown of host DNA slow after infection by [42, *regA*]? The overproduction of early enzymes is seen only in the DNA⁻ state and we have demonstrated slow breakdown of host DNA unambiguously only in the DNA⁻ state. Presumably the sensitivity of SP62 to FdUrd and HU is a direct reflection of slow breakdown in the nearly DNA⁻ state imposed by the drugs. Slow breakdown is not due to overproduction of polynucleotide ligase since we find that ligase is not overproduced by SP62 in either the DNA⁺ or the DNA⁻ state (R. Swift

and J. Wiberg, unpublished experiments). It is possible that SP62 underproduces one or more nucleases, perhaps by analogy with its effect on dTMP synthetase (Fig. 9). It is also possible that the *regA* product is a nuclease, perhaps one with both DNase and RNase activity; preliminary evidence (J. S. Wiberg, unpublished experiments) indicates that the SP62 mutation extends the life of some early mRNAs.

Label transfer experiments. As a control for the label transfer experiments it was shown that [*denA*] transfers very little label from host DNA into (nuclease resistant) phage particles. However, about 4% of the host DNA in the extract of cells infected by [*denA*] for 35 min remains inaccessible to micrococcal nuclease (Fig. 2A); a similar result was observed previously at 25 min after infection (22). This nuclease-resistant fraction could represent a slight leakiness of [*denA*] whereby a little solubilization of host DNA occurs, followed by reincorporation of the labeled nucleotides into phage DNA. However, a more interesting possibility is that some phage-size segments of host DNA are packaged into phage-like particles, which thus might be transducing particles; Drexler has shown that T1, another virulent phage, can transduce (12). Either explanation is supported by the observation of Hercules et al. (22) that, in both neutral and alkaline sucrose gradients, [*denA*] left roughly this same small fraction of label from host DNA in fragments the size of intact T4 DNA, whereas the rest of the label from host DNA was in much smaller fragments.

Why is SP62 temperature sensitive? It is not clear why SP62 is temperature sensitive; we find that Karam's mutant R9 is about equally temperature sensitive. We have no evidence to indicate whether the mutation is missense, nonsense, etc. It is possible that the SP62 mutation permits partial activity of the *regA* gene at 37 C, and that very high temperatures are required to inactivate the gene product totally. However, we think it more likely that the mutant gene is totally inactive at all temperatures examined (30 C and higher), and that the temperature sensitivity of phage production suggests some role for the *regA* gene that is obligatory only at high temperature.

The SP62 mutation is proving to be of considerable practical use, to increase the yields of those enzymes and proteins that respond to the *regA* control. Alberts and co-workers have used it to produce large amounts of the products of genes 44, 45, and 62, with which they have demonstrated stimulation of *in vitro* DNA synthesis (3; Moran et al., Cold Spring Harbor Bacteriophage Meet., p. 42, 1972).

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ADDENDUM

Preliminary reports of parts of this work have been presented (Fed. Proc. Vol. 30, p. 1263, 1971; Abstr. Cold Spring Harbor Lab. Bacteriophage Meet., p. 80, 1971). In the latter abstract we stated erroneously that HMdCMP kinase synthesis was unaffected by SP62; the trouble later was traced to exhaustion of substrate in the very active extracts.

LITERATURE CITED

- Adesnik, M., and C. Levinthal. 1970. RNA metabolism in T4-infected *Escherichia coli*. *J. Mol. Biol.* **48**:187-208.
- Baldi, M. I., and R. Haselkorn. 1967. Ribosome-bound messenger RNA in T4-infected bacteria. *J. Mol. Biol.* **27**:193-195.
- Barry, J., and B. Alberts. 1972. *In vitro* complementation as an assay for new proteins required for T4 bacteriophage DNA replication: purification of the complex specified by T4 genes 44 and 62. *Proc. Nat. Acad. Sci. U.S.A.* **69**:2717-2721.
- Bautz, E. K. F., and J. J. Dunn. 1969. DNA-dependent RNA polymerase from phage T4 infected *E. coli*: an enzyme missing a factor required for transcription of T4 DNA. *Biochem. Biophys. Res. Commun.* **34**:230-237.
- Black, L. W., and L. M. Gold. 1971. Pre-replicative development of the bacteriophage T4: RNA and protein synthesis *in vivo* and *in vitro*. *J. Mol. Biol.* **60**:365-388.
- Bolle, A., R. H. Epstein, W. Salser, and E. P. Geiduschek. 1968. Transcription during bacteriophage T4 development: requirements for late messenger synthesis. *J. Mol. Biol.* **33**:339-362.
- Brody, E., R. Sederoff, A. Bolle, and R. H. Epstein. 1970. Early transcription in T4-infected cells. *Cold Spring Harbor Symp. Quant. Biol.* **35**:203-211.
- Cohen, P. S. 1972. Translational regulation of deoxycytidylate hydroxymethylase and deoxynucleotide kinase synthesis in T4-infected *Escherichia coli*. *Virology* **47**:780-786.
- Cohen, P. S., B. R. Zetter, and M. L. Walsh. 1972. Evidence that more deoxynucleotide kinase mRNA is transcribed than translated during T4 infection of *Escherichia coli*. *Virology* **49**:808-810.
- Cohen, S. S., J. G. Flaks, H. D. Barner, M. R. Loeb, and J. Lichtenstein. 1958. The mode of action of 5-fluorouracil and its derivatives. *Proc. Nat. Acad. Sci. U.S.A.* **44**:1004-1012.
- Colvill, A. J. E. 1970. Early *in vitro* and *in vivo* transcription of T4 DNA. I. Effect of phage T4 infection on RNA production in *Escherichia coli*. *Biochim. Biophys. Acta* **209**:97-105.
- Drexler, H. 1970. Transduction by bacteriophage T4. *Proc. Nat. Acad. Sci. U.S.A.* **66**:1083-1088.
- Emrich, J., and G. Streisinger. 1968. The role of phage

- lysozyme in the life cycle of phage T4. *Virology* **36**:387-391.
14. Epstein, R. H., A. Bolle, C. M. Steinberg, E. Kellenberger, E. Boy de la Tour, R. Chevalley, R. S. Edgar, M. Susman, G. H. Denhardt, and A. Lielausis. 1963. Physiological studies of conditional lethal mutants of bacteriophage T4D. Cold Spring Harbor Symp. Quant. Biol. **28**:375-394.
 15. Fairbanks, G., C. Levinthal, and R. H. Reeder. 1965. Analysis of C¹⁴ labeled proteins by disc electrophoresis. *Biochem. Biophys. Res. Commun.* **20**:393-399.
 16. Fraser, D., and E. A. Jerrel. 1953. Amino acid composition of T3 bacteriophage. *J. Biol. Chem.* **205**:291-295.
 17. Friesen, J. D., B. Dale, and W. Bode. 1967. Presence of T4 "early" messenger RNA on polysomes late in infection. *J. Mol. Biol.* **28**:413-422.
 18. Gorini, Luigi. 1969. The contrasting role of *strA* and *ram* gene products in ribosomal functioning. Cold Spring Harbor Symp. Quant. Biol. **34**:101-111.
 19. Grasso, R. J., and J. M. Buchanan. 1969. Synthesis of early RNA in bacteriophage T4-infected *Escherichia coli* B. *Nature (London)* **224**:882-885.
 20. Guha, A., W. Szybalski, W. Salser, A. Bolle, E. P. Geiduschek, and J. F. Pulitzer. 1971. Controls and polarity of transcription during bacteriophage T4 development. *J. Mol. Biol.* **59**:329-349.
 21. Hall, B. D., A. P. Nygaard, and M. H. Green. 1964. Control of T2-specific RNA synthesis. *J. Mol. Biol.* **9**:143-153.
 22. Hercules, K., J. L. Munro, S. Mendelsohn, and J. S. Wiberg. 1971. Mutants in a nonessential gene of bacteriophage T4 which are defective in the degradation of *Escherichia coli* deoxyribonucleic acid. *J. Virol.* **7**:95-105.
 23. Hill, R. F. 1960. A radiation sensitive mutant of *Escherichia coli*. *Biochim. Biophys. Acta* **30**:636-637.
 24. Hosoda, J., and C. Levinthal. 1968. Protein synthesis by *Escherichia coli* infected with bacteriophage T4D. *Virology* **34**:709-727.
 25. Jayaraman, R. 1972. Transcription of bacteriophage T4 DNA by *Escherichia coli* RNA polymerase *in vitro*: identification of some immediate-early and delayed-early genes. *J. Mol. Biol.* **70**:253-263.
 26. Jayaraman, R., and E. B. Goldberg. 1970. Transcription of bacteriophage T4 genome *in vivo*. Cold Spring Harbor Symp. Quant. Biol. **35**:197-201.
 27. Jensen, H. B., and K. Kleppe. 1972. Studies on T4 lysozyme affinity for chitin and the use of chitin in the purification of the enzyme. *Eur. J. Biochem.* **26**:305-312.
 28. Kasai, T., and E. K. F. Bautz. 1967. Interdependence of translation and transcription in T4-infected *Escherichia coli*. p. 111-126. In H. J. Vogel, J. O. Lampen, and V. Bryson (ed.), *Organizational biosynthesis*. Academic Press Inc., New York.
 29. Kutter, E. M., and J. S. Wiberg. 1969. Biological effects of substituting cytosine for 5-hydroxymethylcytosine in the deoxyribonucleic acid of bacteriophage T4. *J. Virol.* **4**:439-453.
 30. Laemmli, U. K. 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. *Nature (London)* **227**:680-685.
 31. Lee-Huang, S., and S. Ochoa. 1971. Messenger discriminating species of initiation factor F₁. *Nature N. Biol.* **234**:236-239.
 32. Lembach, K. J., and J. M. Buchanan. 1970. The relationship of protein synthesis to early transcriptive events in bacteriophage T4-infected *Escherichia coli* B. *J. Biol. Chem.* **245**:1575-1587.
 33. Lomax, M. I. S., and G. R. Greenberg. 1967. A new assay of thymidylate synthetase activity based on the release of tritium from deoxyuridylate-5-³H. *J. Biol. Chem.* **242**:109-113.
 34. Losick, R. 1972. *In vitro* transcription. *Annu. Rev. Biochem.* **41**:409-446.
 35. Mark, K-K. 1970. The relationship between the synthesis of DNA and the synthesis of phage lysozyme in *Escherichia coli* infected by bacteriophage T4. *Virology* **42**:20-27.
 36. Mathews, C. K. 1971. Bacteriophage biochemistry, p. 157-187. Van Nostrand Reinhold Co., New York.
 37. Mathews, C. K., and R. H. Kessin. 1967. Control of bacteriophage-induced enzyme synthesis in cells infected with a temperature-sensitive mutant. *J. Virol.* **1**:92-96.
 38. Notani, G. W. 1973. Regulation of bacteriophage T4 gene expression. *J. Mol. Biol.* **73**:231-249.
 39. Peterson, R. F., P. S. Cohen, and H. L. Ennis. 1972. Properties of phage T4 messenger RNA synthesized in the absence of protein synthesis. *Virology* **48**:201-206.
 40. Pollack, Y., Y. Groner, H. Aviv (Greenspan), and M. Revel. 1970. Role of initiation factor B (F3) in the preferential translation of T4 late messenger RNA in T4 infected *E. coli*. *FEBS Lett.* **9**:218-221.
 41. Pryme, I. F., P. E. Joner, and H. B. Jensen. 1969. The isolation of an inhibitor of T-even phage lysozyme from *E. coli* B cells. *FEBS Lett.* **4**:50-51.
 42. Reid, M. S., and R. L. Bielecki. 1968. A simple apparatus for vertical flat-sheet polyacrylamide gel electrophoresis. *Anal. Biochem.* **22**:374-381.
 43. Rosenkranz, H. S., and S. Rosenkranz. 1969. Degradation of DNA by carbamoyloxurea—an oxidation product of hydroxyurea. *Biochim. Biophys. Acta* **195**:266-267.
 44. Sadowski, P. D., H. R. Warner, K. Hercules, J. L. Munro, S. Mendelsohn, and J. S. Wiberg. 1971. Mutants of bacteriophage T4 defective in the induction of T4 endonuclease II. *J. Biol. Chem.* **246**:3431-3433.
 45. Sakiyama, S., and J. M. Buchanan. 1971. *In vitro* synthesis of deoxynucleotide kinase programmed by bacteriophage T4-RNA. *Proc. Nat. Acad. Sci. U.S.A.* **68**:1376-1380.
 46. Sakiyama, S., and J. M. Buchanan. 1972. Control of the synthesis of T4 phage deoxynucleotide kinase messenger ribonucleic acid *in vivo*. *J. Biol. Chem.* **247**:7806-7814.
 47. Salser, W., A. Bolle, and R. Epstein. 1970. Transcription during bacteriophage T4 development: a demonstration that distinct subclasses of the "early" RNA appear at different times and that some are "turned off" at late times. *J. Mol. Biol.* **49**:271-295.
 48. Salser, W., R. F. Gesteland, and A. Bolle. 1967. *In vitro* synthesis of bacteriophage lysozyme. *Nature (London)* **215**:588-591.
 49. Salser, W., R. F. Gesteland, and B. Ricard. 1969. Characterization of lysozyme messenger and lysozyme synthesized *in vitro*. Cold Spring Harbor Symp. Quant. Biol. **34**:771-780.
 50. Schachner, M., W. Seifert, and W. Zillig. 1971. A correlation of changes in host and T₄ bacteriophage specific RNA synthesis with changes of DNA-dependent RNA polymerase in *Escherichia coli* infected with bacteriophage T₄. *Eur. J. Biochem.* **22**:520-528.
 51. Schmidt, D. A., A. J. Mazaitis, T. Kasai, and E. K. F. Bautz. 1970. Involvement of a phage T4 σ factor and an anti-terminator protein in the transcription of early T4 genes *in vivo*. *Nature (London)* **225**:1012-1016.
 52. Sekiguchi, M., and S. S. Cohen. 1964. The synthesis of messenger RNA without protein synthesis. II. Synthesis of phage-induced RNA and sequential enzyme production. *J. Mol. Biol.* **8**:638-659.
 53. Sköld, O. 1970. Regulation of early RNA synthesis in bacteriophage T4-infected *Escherichia coli* cells. *J. Mol. Biol.* **53**:339-356.
 54. Streisinger, G., F. Mukai, W. J. Dreyer, B. Miller, and S. Horiuchi. 1961. Mutations affecting the lysozyme of phage T4. Cold Spring Harbor Symp. Quant. Biol.

- 26:25-30.
55. Studier, F. W. 1972. Bacteriophage T7. *Science* **176**:367-376.
56. Travers, A. 1970. RNA polymerase and T4 development. Cold Spring Harbor Symp. Quant. Biol. **35**:241-251.
57. Travers, A. A. 1970. Positive control of transcription by a bacteriophage sigma factor. *Nature* (London) **225**:1009-1012.
58. Trimble, R. B., J. Galivan, and F. Maley. 1972. The temporal expression of T2r+ bacteriophage genes *in vivo* and *in vitro*. *Proc. Nat. Acad. Sci. U.S.A.* **69**:1659-1663.
59. Trimble, R. B., G. F. Maley, and F. Maley. 1972. The *in vitro* synthesis of T4 bacteriophage-induced deoxycytidylate deaminase and its regulation by allosteric effectors. *Arch. Biochem. Biophys.* **153**:515-525.
60. Wainwright, S. D. 1972. Control mechanisms and protein synthesis. Columbia University Press, New York.
61. Warner, H. R., and M. D. Hobbs. 1969. Effect of hydroxyurea on replication of bacteriophage T4 in *Escherichia coli*. *J. Virol.* **3**:331-336.
62. Warner, H. R., D. P. Snustad, S. E. Jorgensen, and J. F. Koerner. 1970. Isolation of bacteriophage T4 mutants defective in the ability to degrade host deoxyribonucleic acid. *J. Virol.* **5**:700-708.
63. Wiberg, J. S. 1966. Mutants of bacteriophage T4 unable to cause breakdown of host DNA. *Proc. Nat. Acad. Sci. U.S.A.* **55**:614-621.
64. Wiberg, J. S. 1967. Amber mutants of bacteriophage T4 defective in deoxycytidine diphosphatase and deoxycytidine triphosphatase. On the role of 5-hydroxymethylcytosine in bacteriophage deoxyribonucleic acid. *J. Biol. Chem.* **242**:5824-5829.
65. Wiberg, J. S., and J. M. Buchanan. 1964. Studies on labile deoxycytidylate hydroxymethylases from *Escherichia coli* B infected with temperature-sensitive mutants of bacteriophage T4. *Proc. Nat. Acad. Sci. U.S.A.* **51**:421-428.
66. Wiberg, J. S., M. L. Dirksen, R. H. Epstein, S. E. Luria, and J. M. Buchanan. 1962. Early enzyme synthesis and its control in *E. coli* infected with some amber mutants of bacteriophage T4. *Proc. Nat. Acad. Sci. U.S.A.* **48**:293-302.
67. Wilhelm, J. M., and R. Haselkorn. 1969. *In vitro* synthesis of T4 proteins: lysozyme and the products of genes 22 and 57. Cold Spring Harbor Symp. Quant. Biol. **34**:793-798.
68. Wood, W. B. 1973. The genetic map of bacteriophage T4. In R. C. King (ed.), *Handbook of genetics*. Van Nostrand-Reinhold Co., New York.
69. Yagil, E., and A. Rosner. 1971. Phosphorolysis of 5-fluoro-2'-deoxyuridine in *Escherichia coli* and its inhibition by nucleoside. *J. Bacteriol.* **108**:760-764.
70. Yeh, Y.-C., and G. R. Greenberg. 1967. Tetrahydrofolate-dependent labilization of the hydrogen atom of carbon 5 of 5'-deoxycytidylate, a step in the deoxycytidylate hydroxymethylase reaction. *J. Biol. Chem.* **242**:1307-1313.
71. Young, E. T., II, and G. Van Houwe. 1970. Control of synthesis of glucosyl transferase and lysozyme messengers after T4 infection. *J. Mol. Biol.* **51**:605-619.