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Biochemical Analysis of the Naturally Repaired Sections of Bacteriophage T5 Deoxyribonucleic Acid. II. Conditions for Nucleotide Incorporation under Nonpermissive Conditions*

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ABSTRACT: The nucleotide incorporation into parental bacteriophage T5 DNA under nonpermissive conditions for DNA replication was characterized by means of CsCl equilibrium density gradient centrifugation and DNA–DNA hybridization. Bacteria were infected with a mutant of T5 which has temperature-sensitive T5 DNA polymerase. Such infected bacteria, when incubated at nonpermissive temperature in dBrUrd medium, synthesized small but definite amounts of DNA that band with parental T5 DNA in neutral and alkaline CsCl equilibrium density gradient centrifugation. Thus it is different from normal replication and very likely due to breakage and repair. DNA synthesis at the nonpermissive temperature occurred even in the mutants of *Escherichia coli* with negligible amounts of DNA polymerase I in their cell extracts as assayed by conventional methods. When chloramphenicol was added immediately after infection, the incorporation was completely inhibited. Thus this DNA synthesis depends on a phage-induced protein(s).

Bacteriophage T5ts53 is a mutant which has a temperature-sensitive T5 DNA polymerase (DeWaard *et al.*, 1965). When bacteria are infected with this mutant at the nonpermissive temperature, there is still some DNA synthesis. When such a synthesis occurs in the presence of dBrUrd,[1] there is no shift in the buoyant density of the newly synthesized DNA, indicating it is a repair-type synthesis (Fujimura and Volkin, 1967).

In the present paper it is confirmed that such a synthesis in the presence of dBrUrd occurs without any detectable density shift. Furthermore, it is shown that the synthesized DNA hybridize specifically to T5 DNA, that the synthesis is not affected by use of *Escherichia coli* mutants that have no detectable DNA polymerase I in their cell extracts as assayed by conventional methods (DeLucia and Cairns, 1969; Kato and Kondo, 1970), and that the synthesis is dependent on a T5-induced protein(s). The accompanying paper (Fujimura, 1971) is on the nucleotide composition of DNA synthesized under nonpermissive conditions.

Materials and Methods

For materials and methods not described here, see Fujimura and Volkin (1968). *E. coli* R15 was obtained from Dr. S. Kondo; and *E. coli* pol A$^-$, originally isolated in Dr. J. Cairns' laboratory, was obtained through Dr. J. Boyle. Bacteriophage T5ts53 labeled with ^{32}P was prepared from a medium containing 0.2–0.6 mCi of ^{32}P (H_3PO_4, New England Nuclear or Schwarz) per mg of phosphorous. Bacteriophage T7 DNA was a gift from Mrs. Ann C. Olson. Chloramphenicol was obtained from Parke Davis and Co. The dBrUrd-containing medium is MGM-CA medium containing 5 μg/ml of dFUrd, 200 μg/ml of dBrUrd, and 25 μg/ml of Urd as described by Fujimura and Volkin (1968).

Preparation of DNA from Infected Bacteria. Bacteria were grown at 37° in dBrUrd-containing medium from 1.5×10^8 to 3×10^8 per ml. They were centrifuged and resuspended in one-tenth the volume of MGM (Lanni, 1961) containing 10^{-3} M $CaCl_2$. The phage was added at a multiplicity of infection of 5 and adsorbed for 5 min. For *E. coli* F and R15, about 99% of the plaque-forming units was adsorbed within 5 min. For pol A$^-$, 94% was adsorbed. Infected bacteria were

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[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: dBrUrd, bromodeoxyuridine; TES, Tris–EDTA–saline; SDS, sodium dodecyl sulfate; SSC, standard saline citrate.

TABLE I: Specificity of DNA Hybridized in 40% Formamide 6 × SSC.[a]

DNA Hybridized	Input of [^{3}H]DNA	DNA Membrane (20 μg of Bound/ Membrane)	Temp (°C)	Fraction Annealed to Membrane	
				DNA	Blank
T5	1220 cpm/0.87 μg	T5	25	0.930	0.000
T5	1220 cpm/0.87 μg	*E. coli*	25	0.323	0.002
T5	1220 cpm/0.87 μg	T5	37	0.681	0.001
T5	1220 cpm/0.87 μg	*E. coli*	37	0.001	0.000
T5	2824 cpm/1.02 μg	T7	37	0.010	0.011
T7	1890 cpm/0.76 μg	T7	37	0.478	0.003
T7	1890 cpm/0.76 μg	T5	37	0.008	0.004
T7	1890 cpm/0.76 μg	*E. coli*	37	0.003	0.003

[a] The detailed procedure for hybridization is given in the text. The values given were the averages of two determinations under each condition.

then diluted tenfold into fresh dBrUrd-containing medium preincubated at a desired temperature. At a desired time, a culture was transferred to a centrifuge tube containing, at half the weight of the culture, crushed frozen TES buffer (0.1 M Tris-HCl–0.05 M EDTA–0.2 M NaCl, pH 8) and sodium azide at a final concentration of 0.1 M. The sample was centrifuged and the bacteria were resuspended in TES buffer of one-sixth the volume of the culture. DNA was extracted from the suspension by sequential treatment with 0.7% SDS, 37° for 10 min, 1 mg/ml of Pronase, 37° for 5–8 hr, and an equal volume of phenol at 50° for 5 min in the presence of 1 M NaCl. The phenol phase was washed twice with TES buffer of one-fourth the volume of the sample. The aqueous phases were combined and dialyzed against the buffer used for CsCl centrifugation. The volume of dialysate was at least 200 times that of the sample. The recovery of total ^{32}P activity at various steps was determined by measuring Cerenkov radiation (Clausen, 1968). Acid-precipitable fractions were assayed after they were collected on a Millipore filter.

CsCl Equilibrium Density Gradient Centrifugation. For centrifugation at neutral pH, a sample was dialyzed against 0.1 SSC (1 × SSC = 0.15 M NaCl and 0.015 M sodium citrate, pH 7), and the volume adjusted to 5.5 ml and made to 57% w/w with CsCl. For centrifugation at alkaline pH, a sample was dialyzed against 0.04 M potassium phosphate (pH 12) and the volume adjusted to 5.5 ml and made to 58.5% CsCl. The samples were centrifuged in polyallomer tubes in a Beckman No. 40 rotor at 36,000 rpm, 25°, for at least 48 hr. Fractions were collected from the bottoms of the tubes with a tube-piercing device of Büchler Instruments.

DNA–DNA Hybridization. Hybridization was done on a nitrocellulose membrane in the presence of formamide by a method similar to that of McConaughy *et al.* (1969), with two major differences: DNA membranes were not preincubated in Denhardt's preincubation medium (Denhardt, 1966), and DNA was fragmented at alkaline pH before hybridization. The procedure was as follows.

DNA was denatured just before preparation of DNA membranes by diluting to 10 μg/ml in 0.1 N NaOH. After 5 min the solution was neutralized with HCl and adjusted to 6 × SSC. The denatured DNA (20 μg) was immobilized on a nitrocellulose membrane (Schleicher & Schuell, type 7, 25 mm) as described by Gillespie and Spiegelman (1965); 6 × SSC was used throughout the procedure.

The DNA to be hybridized was denatured in 0.1 N NaOH and fragmented by a Raytheon sonicator (10-kc oscillator) at a maximum frequency for 3 min and neutralized just before hybridization. The amount hybridized was slightly higher if denatured DNA in alkali rather than native DNA was sonicated. DNA nitrocellulose membranes as well as blank membranes were presoaked in 40% formamide–6 × SSC solution just before use. Unless otherwise noted, both a blank membrane disk and a DNA nitrocellulose membrane disk were put in each vial used for the hybridization. Each sample was tested in duplicate. Usually a 2-ml sample in 40% formamide–6 × SSC was added to each vial. The hybridization was carried out at 37° with shaking in a Dubnoff shaker water bath for 18 hr unless otherwise noted. Under these conditions, the time for half of the maximum amount of T5 DNA to be hybridized was 3 hr.

After hybridization, the membranes were washed directly in the vials by successively filling the vials with 40% formamide–6 × SSC, and then 3 × 10^{-3} M Tris-HCl (pH 9.3). The membranes were left in the Tris-HCl buffer, if it was not possible to proceed to the next step immediately. The membranes were washed individually by filtering 25 ml of the Tris-HCl buffer, and then dried in air. When the membranes were washed with 6 × SSC or 1 × SSC instead of the Tris-HCl buffer, the same results were obtained.

The amount of DNA hybridized to the filters was expressed as a fraction of input DNA. There was no significant amount of quenching of [^{3}H]DNA on a hybridized membrane washed in the Tris-HCl buffer. The fraction that remained unhybridized was precipitated with 5% trichloroacetic acid and collected on a Millipore filter; it was then successively washed with 5 ml of 5% trichloroacetic acid and 70% ethanol and air-dried. Radioactivity on all the membranes was counted in a Packard counter with BBOT–toluene scintillator (4 g of 2,5-bis[2-(5-*tert*-butylbenzoazol)]thiophene/l. of toluene). The sum of ^{3}H activity hybridized on the membrane and that which remained unhybridized in the solution was about 100%.

By the method described, it is unnecessary to pretreat a DNA membrane with Denhardt's preincubation medium (Denhardt, 1966). As shown in Table I, the fraction of DNA on the blank membranes was negligible. In fact the pretreatment of a DNA membrane before hybridization in the formamide solution lowered the efficiency of hybridization without further lowering the background. The shaking of the vials during hybridization improved the efficiency almost twofold. Table I shows that at 37° but not at 25° the hybridization was highly specific, in agreement with McConaughy *et al.* (1969). Hybridization to a heterologous DNA membrane was about the same as that to a blank. The dependence of efficiency of hybridization on the amount of input is shown in Figure 1. The efficiency decreased rapidly with increasing input up to about 2 μg. At the higher input (tested to about 10 μg), the fraction hybridized was almost independent of concentration. There was no significant difference in curves obtained with membranes to which 5–20 μg of DNA was bound. Nor was there a significant difference between T5st and T5ts53 DNA. Since the smaller amount of material is more efficiently hybridized, this procedure is a good way of testing for the presence of contaminant. To test for the purity of a preparation, usually about 1 μg of DNA was put in. For a quantitative comparison between different prepara-

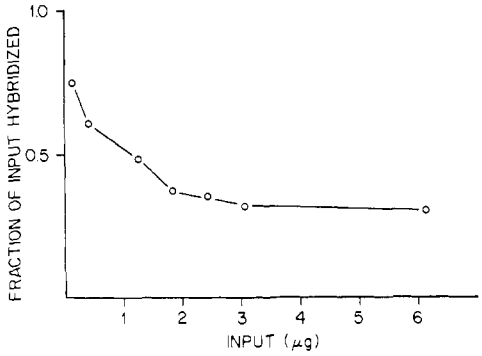

FIGURE 1: Dependence of amount of DNA hybridized on DNA input. Various amounts of [^{3}H]T5st DNA (3.9×10^3 cpm/μg) were put in with T5st DNA membranes, 20 μg bound per membrane, and hybridized as described in Methods. The amount bound to a blank disk was about 0.002 of the input throughout the range shown.

tions, it is important that a test sample be free of RNA and protein; otherwise, there is a great variation in the efficiency of hybridization, and the radioactive counts of the blank disks are higher. The interfering substance may be removed before hybridization by filtering samples through nitrocellulose membranes three times in 6 × SSC as mentioned by Gillespie (1968).

Results

DNA Synthesis under Permissive and Nonpermissive Conditions. DNA synthesis at 30 and 43° of T5ts53 in *E. coli* F and R15 was compared by means of the density shift of parental DNA and newly labeled DNA in neutral and alkaline CsCl centrifugation. Since Kornberg's DNA polymerase or DNA polymerase I is believed to be a repair enzyme (Kelly *et al.*, 1969), the synthesis of phage DNA was investigated with R15, a mutant with no detectable activity of the enzyme in its cell extract (Kato and Kondo, 1970).

E. coli F and R15 were infected with [^{32}P]T5ts53 at a multiplicity of infection of 5 in a dBrUrd-containing medium as described in Methods. Each of the infected bacterial cultures was split into two equal fractions; then one was incubated at 30° and the other at 43°. All the samples had 2.3 μCi/ml of [^{3}H]dUrd from the beginning of incubation of the infected cells. After 2-hr incubation (just before the beginning of lysis), the infected cells were harvested. This long period of incubation was used so that residual replication at 43° would be detected. The DNAs were extracted and centrifuged to equilibrium in neutral CsCl gradient. As expected, samples incubated at 30° formed progeny DNA of heavier density, indicating replication (Figure 2a,c). However, only some of the parental DNA increased in density, indicating that only a small fraction of the parental DNA had replicated. In samples incubated at 43°, both R15 and F showed only the synthesis without shift in density, as indicated by labels only in light parental DNA (Figure 2b,d). Fractions containing newly labeled DNA were pooled and recentrifuged to equilibrium in alkaline CsCl gradients. The samples incubated at 43° showed practically no heavier progeny DNA (Figure 3b,d). Thus almost all the ^{3}H label must have been incorporated by repair synthesis. The sample from *E. coli* F incubated at 30° had denser progeny DNA that contained some parental DNA (Figure 3a,c). The density shift of progeny DNA in 30° samples of R15 was less than that of *E. coli* F both in neutral and

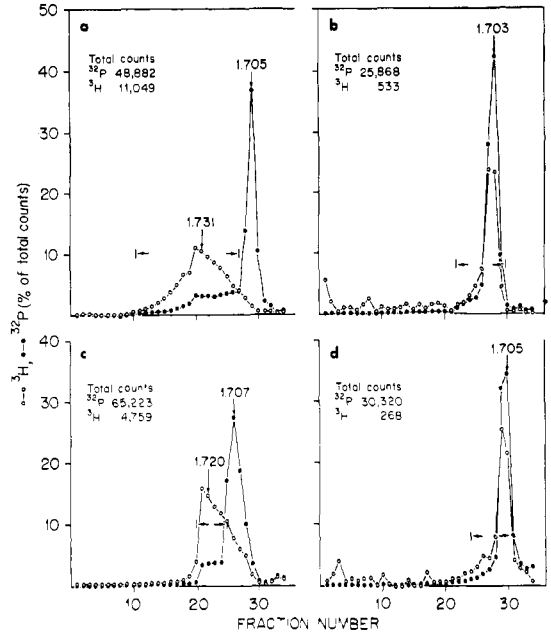

FIGURE 2: Neutral CsCl centrifugation of phage DNA extracted from infected cells. Bacteria were infected with [^{32}P]T5ts53 at a multiplicity of infection of 5, incubated in the presence of 2.3 μCi/ml of [^{3}H]dUrd in dBrUrd-containing medium, and harvested 2 hr after the infection. Infected *E. coli* F was incubated at 30° (a) and 43° (b). Infected *E. coli* R15 was incubated at 30° (c) and at 43° (d). From each fraction, alkaline-resistant, acid-insoluble activities were assayed as described previously (Fujimura and Volkin, 1968). Fractions were numbered from the bottom. ^{32}P (●) and ^{3}H (○).

alkaline CsCl centrifugation. Similar results were obtained with pol A$^-$ and W3110, a parent strain of pol A$^-$, even though both are *thy*$^-$, and thus greater substitution with dBrUrd was expected. The total amount of ^{3}H incorporated into phage DNA in R15 was less than that into the phage DNA in *E. coli* F. However, in both strains total amounts of ^{3}H incorporated at 43° into parental phage DNA were only about 2% of those in the replicated DNA at 30°, as calculated from the ratio of ^{3}H to ^{32}P at corresponding peak regions in neutral CsCl centrifugation. The same value was obtained for all three different sets of preparations. Thus, the amount of synthesis into light DNA was about the same in both strains of *E. coli*, and the observed synthesis is presumably not due to DNA polymerase.

Chloramphenicol Effects. The effects of chloramphenicol on incorporation of nucleotides into parental DNA at 43° were investigated to see if the synthesis is catalyzed at least in part by phage-induced enzyme(s). R15 was infected with [^{32}P]T5ts53 in the presence and absence of 100 μg/ml of chloramphenicol. Since phage DNA replication is inhibited by chloramphenicol (Crawford, 1959), infected cells treated with this antibiotic at 30° were used as indicators of an inhibitory effect. As in previous experiments, the infected cells were incubated in the presence of [^{3}H]dUrd and harvested 2 hr after infection. The extracted DNAs were analyzed by alkaline CsCl centrifugation. The incorporation of ^{3}H-labeled material into the parental phage DNA at 43° was almost completely inhibited by chloramphenicol (Figure 4b), as was the replication of progeny DNA at 30° (Figure 4c). In sample a, which was incubated at 43° in the absence of chloramphenicol, the ^{3}H activity formed a band at the same position as the parental [^{32}P]DNA, with a ^{3}H to ^{32}P ratio of 0.23 around the peak. In samples b and c, which were incubated

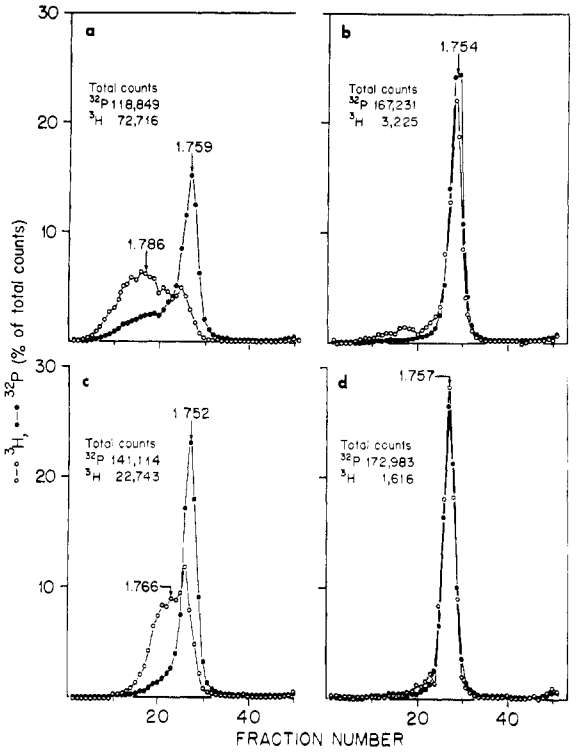

FIGURE 3: Alkaline CsCl centrifugation of phage DNA extracted from infected cells. The samples from the regions indicated in Figure 2 were pooled and recentrifuged at pH 12. Sample numbers correspond to the ones in Figure 2. Fractions were assayed for acid-insoluble activity. Fractions were numbered from the bottom. ^{32}P (●) and ^{3}H (○).

at 43 and 30°, respectively, in the presence of chloramphenicol, ^{3}H-labeled materials were apparently too small to band and were distributed throughout the centrifuge tubes. In sample b, the ^{3}H to ^{32}P ratio around the peak was only 0.017. However, the total ^{3}H activities in samples b and c were not negligible when compared to that of sample a, although in all three samples they were low if compared to the incorporation by normal replication. Thus, these materials were analyzed by hybridization to DNA membranes.

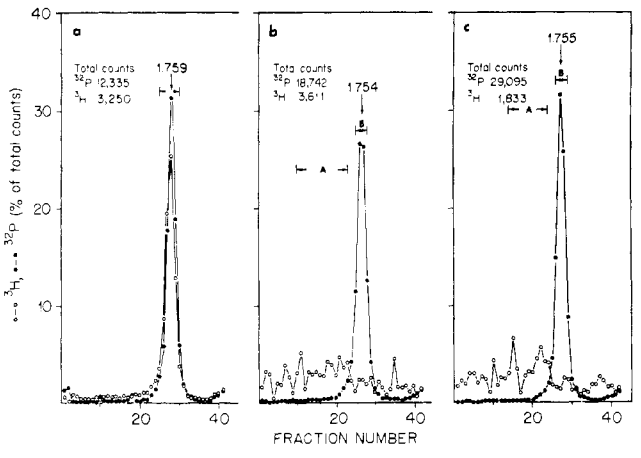

FIGURE 4: Chloramphenicol effects on incorporation of nucleotides. R15 was infected with [^{32}P]T5ts53 at a multiplicity of infection of 5, and incubated in the presence of 3 μCi/ml of [^{3}H]dUrd in dBrUrd-containing medium, and harvested 2 hr after the infection. Infected R15 was incubated at 43° (a) and 43° in 100 μg/ml of chloramphenicol (b), and at 30° in 100 μg/ml of chloramphenicol (c). Fractions were assayed for acid-insoluble activity. Fractions were numbered from the bottom. ^{32}P (●) and ^{3}H (○).

TABLE II: Hybridization of Samples from CsCl Centrifugation (Figure 4) to T5 and *E. coli* DNA Membranes.[a]

Sample		Input (cpm/Vial)	Fraction Hybridized: T5 DNA	Fraction Hybridized: *E. coli* DNA
a	^{32}P	3000	0.33 ± 0.05	0.01 ± 0.03
	^{3}H	746	0.34 ± 0.02	0.01 ± 0.02
b-A	^{32}P	402	0.60 ± 0.07	0.04 ± 0.02
	^{3}H	578	−0.02 ± 0.01	0.04 ± 0.03
b-B	^{32}P	3710	0.79 ± 0.09	0.00 ± 0.00
	^{3}H	117	0.26 ± 0.15	−0.01 ± 0.02
c-A	^{32}P	408	0.55 ± 0.11	0.03 ± 0.01
	^{3}H	208	0.01 ± 0.03	0.04 ± 0.00
c-B	^{32}P	5730	0.55 ± 0.19	−0.03 ± 0.02
	^{3}H	66	0.11 ± 0.05	−0.03 ± 0.03

[a] Details of the hybridization are given in Methods. From the average of the radioactive materials hybridized to each kind of DNA membrane, the average amount on the corresponding blank membrane was subtracted. The fraction hybridized was calculated from the net average divided by the input. The uncertainty value was the average deviation from the mean divided by the input. The specific activity of the ^{32}P-labeled parental DNA was 4.5×10^{3} cpm/μg.

DNA–DNA Hybridization. To make certain that the incorporation of ^{3}H label at 43° was into T5 DNA, the regions from alkaline CsCl gradients were pooled as indicated in Figure 4, and hybridized against T5 and *E. coli* DNA disks by the procedure described in Methods. As shown in Table II, the newly incorporated ^{3}H label in a sample which had been incubated at 43° hybridized to T5 DNA disks just as well as parental DNA labeled with ^{32}P, but did not hybridize to *E. coli* disks (sample a). When the infected cells were incubated at either 43 or 30° in the presence of chloramphenicol, the residual ^{3}H label in the region of replicated DNA showed little or no indication of T5 specificity (b-A and c-A), although there was some evidence for ^{3}H-labeled T5 DNA in parental DNA regions (b-B and c-B). The identity of the residual ^{3}H-labeled material in samples b and c is not know, since it did not hybridize to either T5 or *E. coli* DNA membranes. For many of the samples of Table II there was considerable variation in the amount hybridized to DNA membranes and in the amount that remained on blank membranes. However, the results show clearly that the ^{3}H-labeled material banded with parental DNA is T5 DNA and that there was hardly any detectable ^{3}H-labeled T5 DNA made in the presence of chloramphenicol. In these experiments, there was little labeled *E. coli* DNA, but in one group of experiments (not shown), there was some ^{3}H-labeled *E. coli* DNA produced in the presence of chloramphenicol, presumably because of the presence of a small number of uninfected *E. coli* cells.

Recovery of Parental Phage DNA. The DNA extraction procedure used in the current experiments was considerably simpler than the previous procedure (Fujimura and Volkin, 1968) with no differences detected in the recovery of parental phage DNA during the extraction. The recovery from the

TABLE III: Retention of ^{32}P-Labeled T5 DNA in Infected Cells of *E. coli* (Strains R15 and F).

Condition	% of Input of Labeled Phage[a]			
	R15		F	
	Total	Acid Insoluble	Total	Acid Insoluble
After 5-min adsorption[b]	62 ± 2	68 ± 1	59 ± 5	64 ± 6
After 2-hr incubation at 30°	35 ± 7	27 ± 6	38 ± 3	29 ± 2
With chloramphenicol	60 ± 8	48 ± 8		
After 2-hr incubation at 43°	38 ± 6	19 ± 4	28 ± 0	17 ± 1
With chloramphenicol	46 ± 4	33 + 3		

[a] Average deviations were among the different experiments. [b] 99% of the plaque-forming units was adsorbed.

infected cells incubated at various conditions is shown in Table III. When [^{32}P]T5ts53 phage was used, about 60% of input ^{32}P activity was adsorbed to both *E. coli* F and R15, even though 99% of the plaque-forming units was adsorbed. The rest of the ^{32}P activity was presumably with defective phage particles, since it was almost all acid insoluble. This is not surprising since the number of plaque-forming units per A_{260} is usually only 2×10^{10} to 3×10^{10}, or one-third to one-fifth that of the normal strain. An additional 25% of the input label was lost into the medium during 2-hr incubation from both R15 and F. The time course of parental DNA degradation showed that most of the label was lost early after infection. Also, there was no obvious sign of lysis in dBrUrd-containing medium by 2 hr even at 30°. Of the ^{32}P activity remaining in the bacteria after 2-hr incubation, the fraction that was still acid insoluble varied with the incubation temperature. At 30°, about 77% of the ^{32}P activity was acid insoluble in both R15 and F, but at 43° only 60% in F and 50% in R15 were acid insoluble. Thus a greater fraction of parental DNA was recovered from a 30° culture than from a 43° one. In the presence of chloramphenicol, 60 and 46% of the input ^{32}P activity was still in the bacteria in the 30° and the 43° cultures, respectively. Thus chloramphenicol prevented the loss of ^{32}P activity into the medium from the infected bacteria. In the presence of chloramphenicol, the acid-insoluble fractions in 30 and 43° cultures were 80 and 72%, respectively, of total activity present. Thus they were about the same as that from 30° cultures in the absence of chloramphenicol, indicating that degradation at 43° was inhibited considerably by chloramphenicol. The recovery of the DNA at the end of dialysis was about 89% of the acid-insoluble activity present at the time of harvest for 30° cultures and 76% for 43° cultures, both in the presence and absence of chloramphenicol. There was no detectable loss during Pronase digestion at 37°, which is in agreement with Kelly and Thomas (1969). Most of the loss after harvest was at the phenol extraction step.

Discussions and Conclusions

We have proposed a working hypothesis (Fujimura and Volkin, 1968) that intracellular T5 phage DNA undergoes continuous breakage and repair by a process involving the formation of "gaps" and their subsequent repair by the synthesis of oligonucleotides. DNA synthesis under nonpermissive conditions for T5 DNA polymerase was studied with the hope that under these conditions most of the nucleotide incorporation occurs by this proposed type of repair. In the present report, conditions for the DNA synthesis under nonpermissive temperature were studied by using [^{3}H]dUrd as a labeled precursor in the presence of dBrUrd. The incorporation of labeled nucleoside occurred without a noticeable shift in the buoyant density of ^{3}H-labeled DNA from the parental DNA even though the incubation was done in the presence of dBrUrd for 2 hr. This length of incubation was just about the longest possible without the lysis of the cells. The amount of nucleotides incorporated per parental T5 DNA was small enough to be due to repair.

Under the permissive temperature for replication, the density shift of the progeny DNA was observable within 20 min after the phage infection (R. K. Fujimura, unpublished observation). With a longer period of incubation, the denser DNA forms a broader band, indicating increasing density heterogeneity. This is due to parent–progeny recombination and/or to an increasing amount of synthesis without dBrUrd incorporation as reported in the accompanying paper (Fujimura, 1971). A possibility of preferential reutilization of degraded parental DNA during replication is eliminated by the experiments done in the accompanying paper.

Thus the comparison of DNA syntheses under nonpermissive and permissive conditions indicates that they are different from each other. However, the DNA synthesis under the nonpermissive temperature is still dependent on phage-induced protein(s), because the incorporation is inhibited by chloramphenicol added almost immediately after infection. Also it is probably not dependent on DNA polymerase I.

Although it is more interesting for me to think that the phage-induced protein is a DNA-polymerizing enzyme, it is possible that it is a phage-induced nuclease(s). Analysis of the recovery of parental T5 DNA through the infection and extraction processes revealed that parental phage DNA is degraded to a greater extent at 43° than at 30°, and that this degradation is inhibited by chloramphenicol. Since the difference in recovery between R15 and F was small, this degradation is apparently not due to bacterial nucleases. Otherwise, greater amounts of degradation would be expected in R15, because there are high levels of nuclease in R15 (Kato and Kondo, 1970). Thus the degradation may be caused by one or more phage-induced enzymes, and it appeared to be more active in the absence of DNA replication.

Acknowledgment

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Biochemical Analysis of the Naturally Repaired Sections of Bacteriophage T5 Deoxyribonucleic Acid. III. Nucleotide Analysis of Deoxyribonucleic Acid Synthesized under Nonpermissive Conditions*

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ABSTRACT: The DNA synthesized at a nonpermissive temperature by *Escherichia coli* infected by a phage T5 mutant that induces a temperature-sensitive DNA polymerase was examined to establish whether it is the product of residual replication or of a "repair" synthesis. *E. coli* was grown in a medium containing bromodeoxyridine (dBrUrd) and [^{32}P]-P_i, infected with ^{3}H-labeled T5 phage, and incubated at the nonpermissive temperature. The ^{32}P-labeled DNA, extracted and purified until it hybridized specifically to T5 DNA and not to *E. coli* DNA, had the same buoyant density as the parental DNA in a CsCl gradient centrifuged to equilibrium. Nucleotide analysis showed no detectable amount of dBrUrd incorporation. The amount of [^{32}P]nucleotide incorporated was about 1–5% of parental DNA, with not much dependence on the length of incubation of the infected cells. When bacteria were grown in a normal medium with [^{32}P]P_i infected with ^{3}H-labeled T5 containing dBrUrd, and incubated at the nonpermissive temperature, the isolated and purified DNA yielded no 5-[^{32}P]dBrUMP on digestion to 5′-nucleotides, but a small amount of 3′-[^{32}P]dBrUMP on digestion to 3′-nucleotides. If it is assumed that all the ^{32}P incorporation under nonpermissive conditions is due to repair, it can be calculated from the amount of 3′-[^{32}P]dBrUMP that the average chain length is very short (10–30 nucleotides). From the nucleotide analyses it is concluded also that the absence of dBrUrd incorporation from the medium (in the first experiment above) is not due to preferential reutilization of precursors coming from degraded parental T5 DNA or *E. coli* DNA. DNA synthesis was also studied in a medium containing ^{15}N and ^{2}H under nonpermissive conditions. There was a slight density shift of the isolated [^{32}P]DNA but not as much as at the permissive temperature, and the amount of DNA synthesized was less than the amount of parental DNA. It is concluded that DNA synthesis at the nonpermissive temperature is distinctly different from the bulk of synthesis under permissive conditions and is very likely a "repair" synthesis, although it may involve more than one kind of repair.

The preceding report (Fujimura, 1971) showed that in bacteria infected with T5 phage in the absence of a functional T5 DNA polymerase there is still a phage-controlled DNA synthesis that, in the presence of dBrUrd,[1] occurs without a shift in the buoyant density of the DNA synthesized. One explanation is that this is a type of repair, and that the repaired sections are too small a fraction of the total DNA molecule to affect the buoyant density. Another possibility is that there is no incorporation of the analog under nonpermissive conditions. However, Fujimura and Volkin (1968) showed that dBrUrd is incorporated covalently into DNA in positions proximal to parental DNA under nonpermissive conditions. If the dBrUrd incorporation observed is the same as the ^{32}P incorporation that occurs in the bulk of synthesis under nonpermissive conditions, there should be enough incorporation of the analog to be detected easily.

To clarify these points, nucleotide analysis of the DNA synthesized under nonpermissive conditions was carried out.

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[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: dBrUrd, bromodeoxyuridine; dFUrd, fluorodeoxyuridine; dBrUMP, bromodeoxyuridylic acid; SSC, standard saline citrate, *i.e.*, 0.15 M NaCl–0.015 M sodium citrate (pH 7).