THE MOLECULAR FATE OF DEOXYRIBONUCLEIC ACID OF BACTERIOPHAGE T_4 AFTER INFECTION OF ESCHERICHIA COLI B

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In order to gain further information on the mechanism of transfer of genetic information during bacteriophage infection the fate of bacteriophage T_4 DNA during its sojourn in the bacterial cell was studied.

^{32}P-labeled purified bacteriophage DNA may be separated from E. coli DNA and RNA by chromatography on an esterified albumin celite column (Mandell and Hershey, 1960) as shown in Fig. 1.

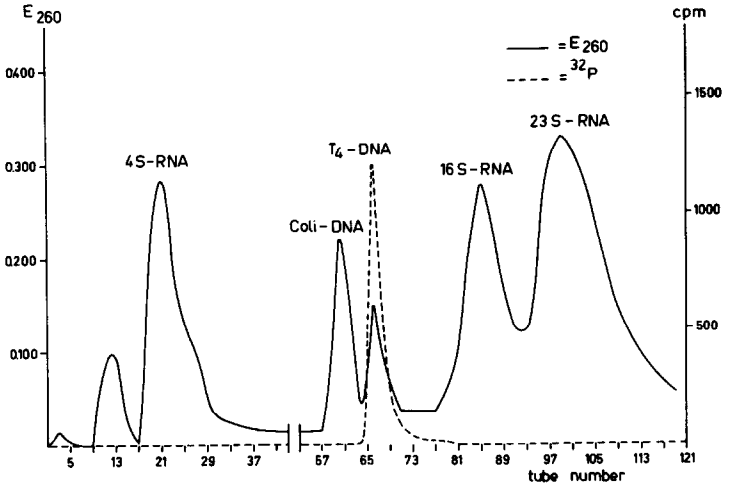

Fig. 1. Chromatography of the nucleic acids isolated from uninfected E. coli B. ^{32}P-labeled purified bacteriophage T_4 DNA was added as a "marker". The column was eluted with a salt solution using a concentration gradient from 0.4 to 1.0 M NaCl in 0.05 M phosphate buffer pH 6.7.

The recovery of both phage DNA and bacterial DNA was approximately 70%. Transfer RNA, E. coli DNA, bacteriophage T_4 DNA, 16 S and 23 S microsomal RNA are eluted at 0.40, 0.60, 0.64, 0.75 and 0.85 M NaCl respectively.

The synthesis of intracellular phage DNA was studied as follows. E. coli B was infected with 5 phages per bacterium and ^{32}P-labeled inorganic phosphate was added to the culture 3 min. after infection; 8 min. after infection chloramphenicol (40 μg/ml) was added to stop further protein synthesis. Samples were removed from the mixture at 0, 3, 6, 10 and 20 min. after infection and phage development was arrested by adding NaCN to a final concentration of 0.01 M. Cells were harvested by centrifugation and unadsorbed phages were removed by repeated washings with 0.05 M phosphate buffer pH 7.0 containing 0.01 M NaCN.

Part of the samples was subjected to the Schmidt Thannhauser (1945) procedure in order to determine the amount of ^{32}P incorporated into DNA. The results are given in Fig. 2A.

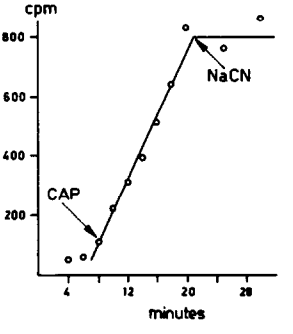

Fig. 2A. The amount of ^{32}P incorporated into bacteriophage precursor DNA versus time after infection. For details see text.

It may be seen that incorporation of ^{32}P into DNA is first observed at 6-8 min. after infection, in accordance with data from Hershey (1953);

the addition of chloramphenicol 8 min. after infection did not inhibit DNA synthesis in perfect agreement with results of Tomizawa and Sunakawa (1956).

The rest of the samples was subjected to sodiumlaurylsulphate treatment followed by phenol extraction in order to isolate the nucleic acids. Phenol was removed from the water layer by repeated extractions with peroxide free ether. RNA was degraded by short treatment with RNase and the whole mixture was chromatographed on an esterified albumin celite column. The results are presented in Fig. 2B.

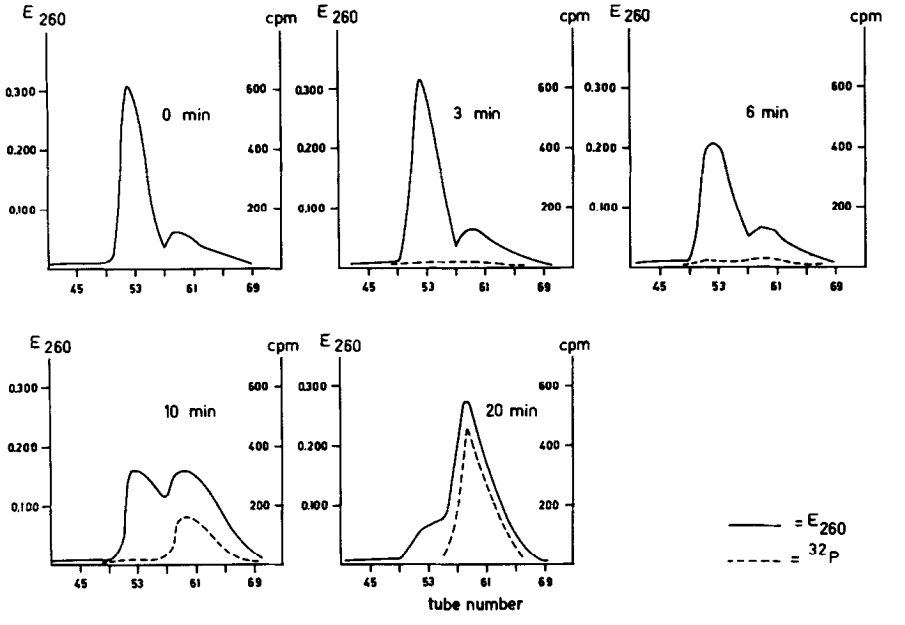

Fig. 2B. Chromatography of the nucleic acids isolated from E. coli B, infected with bacteriophage T_4. For details see text.

E. coli DNA is found roughly at tube number 53 and radioactive material at tube number 59. The position of radioactive material in Fig. 2B (and of intracellular T_4 phage DNA in Fig. 3) corresponds to the position of shear degraded purified T_4 phage DNA. In these experiments no precautions were taken to protect the phage DNA against hydrodynamic shearing forces. The bacterial DNA is gradually degraded to oligonucleotides which are eluted from the columns with a lower salt concentration (0.2 M NaCl). The

radioactive material after column chromatography was collected and characterized as follows:

The material is DNase sensitive but RNase resistant. The relation O.D. at 260 mμ against temperature was determined. The curve virtually coincided with a meltingcurve of purified bacteriophage DNA. It is therefore concluded that under these circumstances double stranded phage precursor DNA is synthesized.

In another experiment the fate of the infecting phage DNA was studied. E.coli B was infected with ^{32}P labeled bacteriophage T_4 using conditions of single infection (1 phage per 10 bacteria). Isolation of the nucleic acids and chromatography were performed exactly as described in the previous experiment. The results are given in Fig. 3.

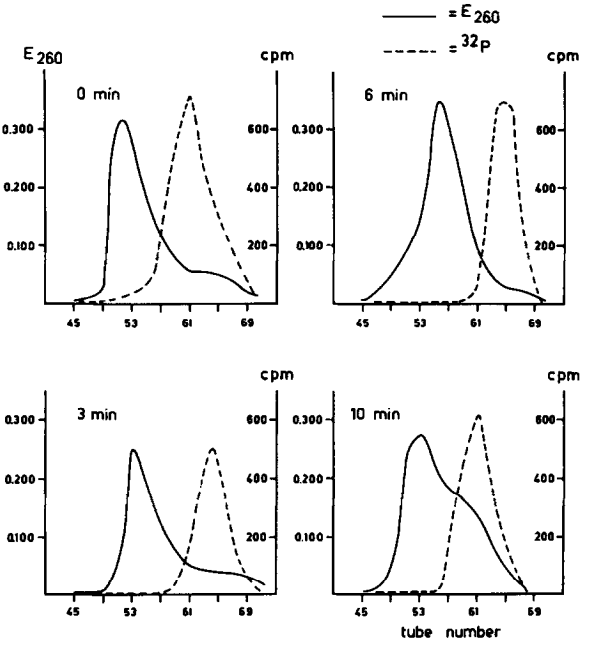

Fig. 3. Chromatography of the nucleic acids isolated from E.coli B, infected with ^{32}P-labeled bacteriophage T_4. For details see text.

Because 90% of the cells are uninfected no degradation of bacterial DNA is observed. The recovery of ^{32}P-labeled intracellular bacteriophage DNA was at all periods after infection approximately 70%, in perfect

agreement with the results obtained for purified bacteriophage DNA. ^{32}P-labeled phage DNA obtained after chromatography was incubated with phosphodiesterase from E. coli, which is known to digest only single stranded DNA (Lehman, 1960). The results are given in Table I.

Table I

Hydrolysis of intracellular bacteriophage T_4 DNA by phosphodiesterase from Escherichia coli

DNA samples	Percentage hydrolyzed after 30 min.
0 min. DNA	1.6
3 min. DNA	0.25
6 min. DNA	0
10 min. DNA	0
20 min. DNA	0.2
Purified T_4 DNA	0.25
Purified T_4 DNA x)	44.0

Intracellular bacteriophage T_4 DNA isolated 0 min., 3 min. etc. after infection. The reaction mixture contained: 10 μg DNA; 1 μMole $MgCl_2$; 2 μMoles Glycine buffer pH 9.0; 40 units phosphodiesterase per ml. Incubation for 30 min. at 37°C.

x) Heated at 100°C for 5 min.

From the data in Table I it can be seen that under circumstances where the enzyme is fully active, intracellular phage DNA was completely resistant toward attack by the enzyme, suggesting a double stranded structure for intracellular phage DNA at all periods after infection.

The biological activity of the intracellular phage DNA was determined using the phage transformation test (Veldhuisen et al.,

1962). It has been reported that purified DNA obtained from bacteriophage T4rII+ (wild type) when added concomitantly with urea-disrupted T4rII mutant to E. coli B spheroplasts induces the formation of a small number of T_4 wild type particles among the vast majority of T4rII mutant particles (phage transformation). The results are given as transformation frequencies (T.F.), c.q. the number of wild type particles divided by the number of mutant particles (Table II).

Table II

Biological activity of intracellular bacteriophage T_4 DNA

DNA samples	T.F. x 10^8
0 min. DNA	4.0
3 min. DNA	6.5
6 min. DNA	4.8
10 min. DNA	26
20 min. DNA	93
Purified T_4 phage DNA	7.5

Each preparation of intracellular phage DNA was obtained from 5 x 10^{11} E. coli B cells infected with 5 x 10^{10} ^{32}P-labeled T_4 phages. DNA preparations were isolated at 0 min., 3 min. etc. after infection using conditions of extraction and chromatography as described in the text. The recovery of parental DNA on a basis of its ^{32}P activity amounted to 30-50%. One tenth of the total amount of intracellular phage DNA roughly corresponding to 2 x 10^9 parental phage equivalents DNA was put to the transformation test. The last line of the Table represents the T.F. of an amount of purified phage DNA corresponding to 5 x 10^9 phage equivalents DNA.

The data from Table II indicate that during a period of 0 to 6 min. after infection the biological activity of intracellular phage DNA per

weight unit of DNA is about equal to that of purified phage DNA. An increase of the biological activity at later periods after infection is observed because of the replication of the phage DNA. These results suggest that parental DNA accounts for the preservation of genetic information during the early eclipse period.

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