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The Fate of the Bacterial Chromosome in *P22*-Infected Cells of *Salmonella typhimurium*

Horst Schmieger

Institut für Mikrobiologie
der Gesellschaft für Strahlenforschung mbH, München
Abt. Mikrobengenetik, Göttingen

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Summary. Evidence is presented that in cells of *Salmonella typhimurium* infected with phage *P 22* there is no conversion of bacterial DNA material into phage DNA material. It has been shown also that infection with *P 22* does not cause measurable breakdown of the bacterial chromosome to phage-sized or smaller DNA fragments.

Introduction

In earlier publications it has been supposed that host DNA becomes available for the formation of general transducing particles by breakdown of the bacterial chromosome in smaller pieces (Zinder, 1955; Stocker, 1958). Zinder concluded even that in *Salmonella typhimurium* the major portion of bacterial nucleic acid is converted to *P 22* nucleic acid. This suggestion about the origin of transducing particles was mainly inspired by the events in *T2*-infected *E. coli* cells (Hershey *et al.*, 1953).

Ozeki and Ikeda (1968) proposed essentially the same mechanism for picking up bacterial DNA into transducing particles and called it the "wrapping choice model". This model is based on the results of Okubo *et al.* (1963) obtained from the transducing system *B. subtilis*-phage *SP10* and on Ikeda and Tomizawa's investigations of *E. coli*-phage *P1* (1965).

Since we are interested in the mechanism of the formation of generalized transducing particles in *Salmonella*-phage *P 22*, we looked for a possible breakdown and conversion of host DNA to phage DNA during vegetative growth of *P 22*.

However, the experiments to be reported below did not give any indication for such a process.

Materials and Methods

Bacteria. For preparation of radioactive bacterial or phage DNA as well as for propagation of 5-BUdR-labelled phages a thymidine-requiring mutant of *Salmonella typhimurium LT 2*, called *T4/2*, was used. Indicator strain for titration of *P 22* phages was wildtype strain *LT 2*.

Phages. For all experiments the clear-plaque mutant *H 5* of phage *P 22* was used.

Media and Solvents. M9-medium is described in Smith and Levine (1964).TCG-medium was prepared according to Kozinski and Szybalski (1959).

TCG/20 and TCG/200 means TCG supplemented with 20 μg thymidine/ml respectively 200 μg/ml.

SSC: 0.15 M NaCl + 0.015 M sodium citrate. 1/10 SSC means an 1/10 dilution of SSC.

Labelling and Extraction of Bacterial DNA. Salmonella DNA was labelled with titrium. The thymidine-requiring mutant *T4/2* was grown for 3–4 generations in M9-medium enriched with 0.05% vitamine-free casamino acids (Difco) and supplemented with 2 μg cold thymidine and 10 μC ^{3}H-thymidine per ml. For extraction of DNA the cells were lysed with the lysis mixture of Botstein (1968). This mixture contained 0.1 M Tris-buffer (pH 8.0), 0.1 M EDTA (pH 8.5), and 0.01 M NaCN. To 10 ml lysis mixture 0.1–0.2 ml lysozyme (10 mg/ml) freshly prepared in 0.25 M Tris (pH 8.0) were added. The bacterial culture was mixed with an equal volume of this lysis mixture, incubated for 10 min at 37°C and then transferred carefully to 65° C. After 3 min sodium-dodecylsulfate-solution was added down the side of the tube to give a final concentration of 2%. Incubation was continued at 65° C for 20 min. The crude lysate was extracted gently with an equal volume of phenol saturated with 0.1 M Tris-buffer pH 7.4 by rolling for 2 hours in an incubator at 25°C. After cooling and centrifugation at 5000 rpm the aqueous phase was removed carefully with a wide-bored pipet and dialyzed against Tris-buffered saline (0.1 M NaCl, 0.01 M Tris pH 7.4).

Preparation and Extraction of Density- and Radioactive-labelled Phages. For the experiments to be described it was necessary to label *P22*-T-BU-lysates (Schmieger, 1968) with isotopes. The DNA synthesized before infection by *P22* and containing thymine was labelled with ^{3}H, the DNA synthesized after infection and containing the heavy BU incorporated ^{32}P. This was accomplished by the following procedure: The cells of a log-phase culture of *T4/2*, grown in 10 ml TCG/20 to a density of 1×10^8 cells/ml were collected on a membrane filter (Schleicher & Schüll, BA 85/1) and washed with 10 ml TCG without thymidine. The filter was suspended in 100 ml TCG with 1 μg cold thymidine, 10 μC ^{3}H-thymidine and 250 μg deoxyadenosine per ml. This cell suspension was aerated in 37°C until a density of 1×10^8 cells/ml was reached. Then the cells were collected on a filter (Schleicher & Schüll, no. 1121, 14 cm ∅), resuspended in 100 ml prewarmed TCG/200 and aerated for 30 min. After this time the culture was infected with *P22* phages (moi = 5). Adsorption was allowed for 3 min, then the infected cells were again collected on a filter no. 1121, washed with 100 ml prewarmed TCG without thymidine and suspended in 100 ml TCG with 1 μg thymidine, 5 μg BUdR, 250 μg deoxyadenosine and 10 μC ^{32}P per ml. The suspension was aerated for 80 min and sterilized with chloroform. Bacterial debris were removed by centrifugation. The lysate was treated with 10 μg RNase for 30 min in 37°C. Then the phages were concentrated and purified on a ECTEOLA-column and by sedimentation through a discontinuous CsCl-gradient as described elsewhere (Schmieger, 1970). CsCl was removed by dialysis against 1/10 SSC.

DNA was extracted by adding sarcosyl NL 30 (Geigy GmbH, Germany) to a final concentration of 1% and incubation in 65°C for 20 min.

Density gradient centrifugation: CsCl-equilibrium centrifugation and sedimentation through 5–20% sucrose gradients as well as the preparation of the fractions for scintillation counting were performed as described by Schmieger (1970).

Results

Is there any Conversion of Salmonella DNA to Phage DNA?

In the proceeding papers we described experiments which suggested that after *P22* infection there is only a very limited transition—if at all—from nucleotides of the bacterial DNA synthesized before infection to the DNA of plaque-forming phage particles. Those experiments showed that infectious phages of lysates prepared on cells grown in BUdR containing medium before phage infection (so-called BU-T-lysates) exhibited exactly the same density as phages grown on thymidine-cells (Schmieger, 1968). If any bacterial DNA would have been used for phage DNA synthesis the phages should have shown some density increase.

When the density of extracted DNA was measured the results were similar (Schmieger, 1970): If one grows a *P22* lysate on bacterial cells labelled with ^{3}H-thymidine until phage infection only the DNA of transducing phages carries

the ^{3}H-label. This DNA has a higher density than the DNA of infecting particles and therefore both can be separated by density gradient centrifugation. The experiment showed (Schmieger, 1970, Fig. 4) no ^{3}H-accumulation at the position where the ^{32}P-labelled DNA of plaque-forming phages bands. However, the distribution of ^{3}H- and ^{32}P-counts are partially overlapping, so that a small utilization of ^{3}H-labelled bacterial DNA in phage genomes might have excaped detection.

Therefore we deviced a much more sensitive experiment. We prepared a T-BU-lysate in which the host DNA synthesized before infection was light due to thymidine incorporation whereas the after-infection DNA was made heavy by BUdR-incorporation. In addition both kinds of DNA were differentially labelled with radioactivity: the preinfection DNA with ^{3}H-thymidine, the postinfection-DNA by ^{32}P.

After purification and concentration of the *P22* lysate the phage DNA was extracted and centrifuged in a CsCl-gradient to equilibrium. Then fractionation was achieved by pumping a saturated CsCl-solution into the bottom of the centrifuge tube and dropping out the gradient starting at the meniscus. The result is shown in Fig. 1. The light DNA of transducing phages carrying the ^{3}H-label banded far apart of the ^{32}P- and BU-labelled infectious DNA (Fig. 1). However, there is some asymmetry of the ^{3}H-distribution towards the peak of infectious DNA, which cannot be explained by an accidential trailing of the labelled material since the distribution of ^{32}P-counts is completely symmetric. In addition, the same asymmetry was observed in a gradient which was fractionated from the bottom.

There remain two explanations for this ^{3}H-activity in the region of infectious DNA.

1. It could be due to some utilization of bacterial DNA material for synthesis of infectious phage DNA.

2. It could be due to the presence of the so called "heavy transducing particles" (Schmieger, 1968). These particles comprise only a small fraction of all transducing particles—detectable only after growth of a *P22* lysate under T-BU conditions—which contain instead of 90% bacterial and 10% phage DNA about 70–80% phage DNA and only 20–30% bacterial DNA.

To distinguish between these two possibilities we recentrifuged fraction numbers 48 to 70. In case (1), i.e. conversion of bacterial DNA to phage DNA the heavy ^{3}H-DNA should exhibit exactly the same density as the ^{32}P-DNA of the infectious phages. In case (2) however, i.e. if ^{3}H is localized within the DNA of "heavy" transducing particles, the ^{3}H-peak should correspond to a smaller density than the ^{32}P-DNA of fully BU-labelled infectious phages. The result of the recentrifugation is shown in Fig. 2. There are two ^{3}H-peaks. The lighter peak corresponds to the DNA of "normal" transducing particles. Since the heavier ^{3}H-peak is still lighter then the ^{32}P-DNA of infectious particles the prediction of case (2) is fulfilled. This bimodal distribution was observed in three independent experiments with different phage lysates and DNA preparations. If the density difference between the normal ^{3}H-peak and the ^{32}P-peak of fully BU-labelled DNA is taken as 100% the distance between the normal and the heavy ^{3}H-DNA amounts to about 70%. We conclude therefore that the heavy ^{3}H-DNA is composed of about 70% BU-DNA and 30% light DNA. This value is in good agreement with the results obtained with intact phages.

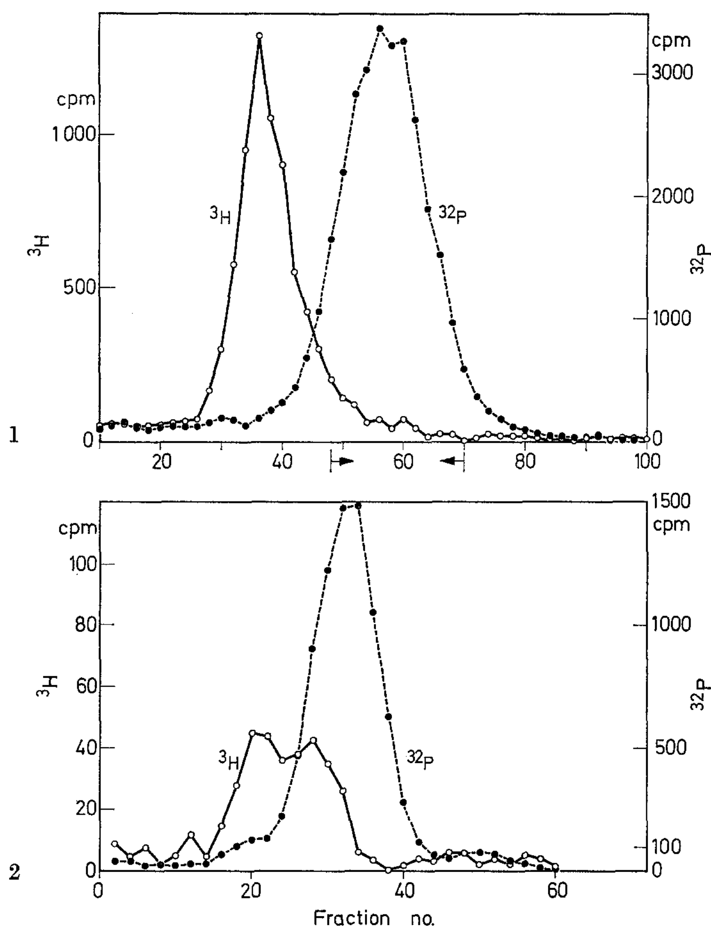

Fig. 1. Cells labelled with ^{3}H-thymidine were infected with *P22*. Phage growth occurred in ^{32}P-containing BUdR-medium. DNA of the purified lysate was centrifuged for 40 hours in CsCl (starting density $\varrho = 1.69\ \mathrm{g} \times \mathrm{cm}^{-3}$) at 32000 rpm ($= 90000 \times g$) in a 8×12 ml fixed angle-rotor. 10-drop fractions were collected starting at the meniscus. Of the even numbered fractions 0.01 ml were soaked into a glass-fiber-filter, dried, precipitated in 10% cold TCA, washed with acetone, dried and counted in a Tricarb scintillation counter. The arrows show the fractions which were recentrifuged. —o—o— ^{3}H; --●---●-- ^{32}P

Fig. 2. Recentrifugation profile of the united fractions indicated in Fig. 1. Technical data are the same as for Fig. 1. —o—o— ^{3}H; --●---●-- ^{32}P

Is there a Breakdown of Bacterial DNA to Larger Pieces?

The experiments described above exclude clearly a degradation of host DNA by the infecting and replicating phages to nucleotides as it was found in the system *E. coli*-phage *T2*. But it is not excluded that degradation results in formation of larger pieces, possibly of the size of phage chromosomes. These breakdown products would be together with the replicated phage genomes in the bacterial cell. During the unknown process of packing the DNA into phage heads these

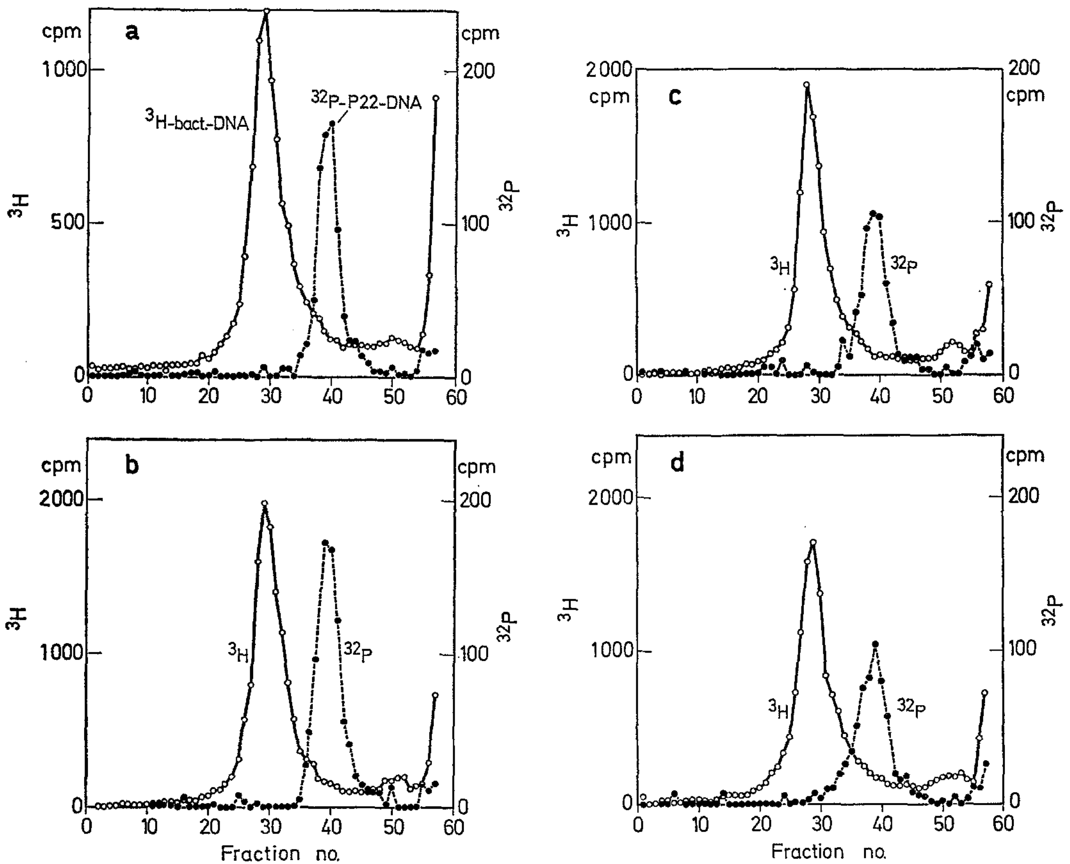

Fig. 3a–d. ³H-labelled bacteria were infected with *P22*. At different times after infection samples were lysed and DNA gently extracted. The ³H-DNA was layered on top of a 5–20% sucrose gradient and ³²P-labelled DNA of phage *P22* added as a reference. This sample was centrifuged for 120 min at 35000 rpm (=140000×g) in a 3×5 ml swinging bucket rotor at 22,5°C. 6-drop fractions were collected on glass-fiber filters starting from the bottom and prepared for scintillation counting as described in the legend of Fig. 1. a Uninfected cells as control. b–d Cells were lysed 10 min, 25 min and 35 min, respectively, after phage infection. —o—o— ³H; --•---•-- ³²P

pieces could then be picked up at random, as it was proposed in the models of Zinder (1955), Stocker (1958) and Ozeki and Ikeda (1968).

To look for such a cutting of the bacterial chromosome, cells with ³H-thymidine labelled DNA were infected with *P22*. At different times after infection samples were taken and the cells lysed. Then DNA was extracted and sedimented through a 5–20% sucrose gradient. As a reference ³²P-labelled *P22*-DNA was added before centrifugation. As a control the same centrifugation experiment was made with DNA of uninfected cells. Fig. 3b–d show the profiles of samples taken 10 min, 25 min and 35 min after infection of the ³H-labelled cells. These times were chosen for certain reasons: At 10 min after infection there is an UV-sensi-

tive phase in the production of transducing particles (Schmieger, to be published) and at the same time DNA synthesis induced by phage infection is still continuing (Smith and Levine, 1964; Dopatka, to be published). The 25 min sample represents the time when the first mature phages appear in the cells which has been measured to occur at about 22 min (Zinder, 1955; Schmieger, to be published). At 35 min the lysis of the cells has just started under these experimental conditions.

Table

Time	Total ^{3}H-counts
10 min	18.320
25 min	16.977
35 min	17.742

As can be seen, there is no difference in DNA sedimentation rates between the uninfected and the infected cells. If there would be a measurable breakdown of bacterial DNA under the influence of the infecting phage one should expect an accumulation of ^{3}H in the regions nearer to the meniscus of the centrifuge tube, probably at the position of the ^{32}P-peak which resembles the molecule size of mature phage DNA. There is also evidence that DNA was not degraded to acid soluble material, and therefore not detectable. The recovery of radioactivity from all three gradients is nearly the same, as shown in the table. This should not be expected, if degradation of bacterial DNA to acid soluble material had occurred. This confirms the conclusion drawn already in the first part of this paper that no conversion of bacterial to phage DNA occurs at the nucleotide level.

Discussion

The described experiments show clearly that after infection of *Salmonella typhimurium* with phage *P22* there is no conversion of bacterial nucleic acid to phage DNA as measured by transition of thymidine from bacterial to phage DNA. Therefore *P22* behaves differently to the virulent *E. coli*-phage *T2*. The latter phage does not only stop the bacterial DNA synthesis after infection (Hershey *et al.*, 1953) but also breaks it down to the oligonucleotide level. The low molecular weight fragments are subsequently repolymerized into phage-specific polynucleotides. It seemed very appealing to suppose that during the lytic cycle of *P22* a similar, perhaps less radical process resulted in the formation of bacterial DNA fragments, which could be packed by phage protein to transducing particles.

In contrast to *T2*, with *P22* we could not show any breakdown of bacterial DNA, even not into larger pieces of about the size of phage DNA. Consequently, in agreement with our earlier findings (Schmieger, 1968, 1970) there is no reason for believing that the formation of generalized transducing particles is the result of a process by which bacterial DNA fragments of the size of phage DNA are packed at random in a *P22* protein coat.

Because the DNA molecule in a transducing particle consists of two distinct parts, about 90% of bacterial origin and 10% phage DNA, one is tempted to

conclude that a generally transducing DNA molecule is formed by a recombination process (Schmieger, 1970).

In the DNA preparation of T-BU-lysates it was possible to demonstrate the presence of DNA-molecules of the "heavy" transducing particles. This shows, that these particles probably can carry any part of the bacterial chromosome and not only his^+ transducing DNA as one might have argued according to their first identification in T-BU-lysates of *P22* (Schmieger, 1968). The "heavy" his^+ transducing particles constituted about 2% of all his^+ transducing particles. The sum of the "heavy" ^{3}H-counts in the present experiments makes up about 3% of all ^{3}H-counts. Because the ^{3}H-counts in our preparations represent the DNA of *all* transducing particles, the heavy particles are evidently a general phenomenon. These particles carry a much larger proportion of newly synthesized DNA and only 30% of original bacterial DNA synthesized before phage infection. Again these heavy transducing particles must have been formed by a recombination process.

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Dr. Horst Schmieger
Institut für Mikrobiologie
Gesellschaft für Strahlenforschung mbH
München
Abteilung Mikrobengenetik
BRD-3400 Göttingen, Grisebachstr. 8
Deutschland