SHORT COMMUNICATION

Molecular Origin of Transducing DNA in Bacteriophage SPPI

By HERMÍNIA DE LENCASTRE* AND LUÍS J. ARCHER
Grupo de Genética Molecular, Instituto Gulbenkian de Ciência, 2781 Oeiras, Portugal, and Departamento de Biologia e Bioengenharia, Universidade Nova de Lisboa, Portugal

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Transducing particles produced by bacteriophage SPPI infection of Bacillus subtilis were separated from plaque-forming units by CsCl density-gradient centrifugation. The density in CsCl of DNA isolated from such purified transducing particles was similar to that of bacterial DNA, indicating that the transducing particles probably contain DNA of exclusively bacterial origin. Bacterial DNA synthesized after infection of the donor culture was also encapsulated in the transducing particles. The number of transducing particles was at least 10 times higher than that of the transductants.

INTRODUCTION

Bacillus subtilis lysates of the virulent bacteriophage SPPI have been shown to mediate generalized transduction (Yasbin & Young, 1974). When such lysates were purified, in CsCl, from bacteriophage PBSX particles induced by SPPI infection, the transducing activity was retained (Lencastre & Archer, 1979). Furthermore, by buoyant density, sedimentation coefficient and anti-SPPI serum inactivation of the transducing activity, SPPI units were identified as vectors in transduction (Lencastre & Archer, 1980). When donor cells were pre-treated with density and radioactive labels and then infected with SPPI phage in light, non-radioactive medium, the transducing particles could be separated, by density, from the plaque-forming units (Lencastre & Archer, 1979).

Here we further analyse the purified transducing particles with respect to the amount and origin of their DNA, as well as to their number. The results show that SPPI transducing particles have a DNA content indistinguishable from that of the SPPI phage, but that the DNA is of bacterial origin, synthesized either before or after infection of the culture, and no detectable amounts of phage DNA are present. The number of transducing particles vastly exceeds the number of transductants.

METHODS

Bacterial and bacteriophage strains. These were as described previously (Lencastre & Archer, 1980).

Chemicals and media. 5-Bromo-2'-deoxyuridine (5-BU) was obtained from Sigma and $^{32}$Porthophosphate in HCl solution, pH 2.3: 156 Ci (mg P)$^{-1}$, 5.77 TBq (mg P)$^{-1}$; 1 mCi ml$^{-1}$, 37 MBq ml$^{-1}$ from The Radiochemical Centre, Amersham. When $^{32}$P was used, 10 μCi $^{32}$Porthophosphate was added per ml III/H medium buffered with 20 μg P (as phosphate) per ml. All other chemicals, media and procedures were as previously described (Lencastre & Archer, 1979, 1980).

RESULTS

Density-shift of transducing particles. Bacillus subtilis VUB112 (5-BU tolerant) donor cells, previously labelled with 5-BU and $^{3}$Hthymidine, were infected in light, non-radioactive
medium with SPPl phage for 2.5 to 18 h. The concentrated and purified phage were analysed by CsCl equilibrium density-gradient centrifugation. The results for one such gradient were presented previously [Fig. 2 of Lencastre & Archer (1979)]; data from further experiments are summarized in Table 1. Under these conditions the transducing particles were shifted to a density higher than that of the plaque-forming units, due presumably to the presence of bacterial DNA replicated in the presence of 5-BU before infection (Lencastre & Archer, 1979). The heavy peaks were broad and seemed to show some asymmetry. Furthermore, when lysates were prepared by incubation with phage for longer periods, an increasing fraction of the transducing activity banded at the light peak (ratio D/C in Table 1). This indicated that transducing particles also encapsulate bacterial DNA replicated after infection of the culture.

It was surprising to find considerable radioactivity banding with the light phage particles (column B, Table 1). However, as many more plaque-forming units than transducing particles were present in the gradient, the radioactivity incorporated per phage was many times lower than that incorporated per transducing particle (see Discussion for calculations).

**Density-shift of DNA from transducing particles.** The question of how much of the DNA present in the purified transducing particles was of bacterial origin could be answered by the analysis of its density, as, in our experiments, only bacterial DNA incorporated appreciable amounts of 5-BU. DNA isolated from purified transducing particles, collected from the heavier band of experiment 2 (Table 1), was co-centrifuged in a CsCl equilibrium density gradient with previously denatured 14C-labelled SPPl DNA as reference. DNA isolated from the donor culture of that experiment immediately before phage infection was co-centrifuged with the same reference DNA in a similar gradient, of the same centrifugation. The results (Fig. 1) show that the density of most of the transducing DNA was similar to that of the DNA from the donor culture. The fact that some DNA from the transducing particles accumulated towards the lighter side of the gradient was consistent with the possibility, indicated above, that bacterial DNA replicated after phage infection was also accepted by transducing particles.

**Table 1. Summary of data calculated from analysis of CsCl equilibrium density gradients of different lysates**

*Bacillus subtilis* VUB112 was density-labelled with 5-bromo-2'-deoxyuridine and radioactively labelled with [3H]thymidine (experiments 1 to 5) or [32P]orthophosphate (experiments 6 to 8). Cells were then incubated with SPPl phage in light, non-radioactive medium for different periods. Phage were concentrated and purified from the resulting lysates and analysed by CsCl equilibrium density-gradient centrifugation. The trichloroacetic acid (TCA)-precipitable radioactive material in the light and heavy bands of the gradient, the total number of transductants for one marker obtained with the light and heavy bands and the total number of plaque-forming units (p.f.u.) in the gradient were determined.

<table>
<thead>
<tr>
<th>Expt</th>
<th>Incubation time (h)</th>
<th>10^{-4} \times TCA-precipitable radioactivity (c.p.m.)</th>
<th>10^{-3} \times No. of transductants</th>
<th>10^{-11} \times Total p.f.u.</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Heavy (A)</td>
<td>Light (B)</td>
<td>B/A</td>
</tr>
<tr>
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<tr>
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<td>0</td>
<td>110</td>
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</tr>
<tr>
<td>8</td>
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<td>691</td>
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Fig. 1. CsCl equilibrium density-gradient analysis of DNA from purified transducing particles (a) and from the donor culture immediately before phage infection (b). (a) DNA from purified transducing particles (experiment 2, Table 1) was extracted with phenol and centrifuged in a 6 × 4 ml swinging-bucket rotor at 130000 g using, as reference, alkali-denatured (Riva et al., 1968) 14C-labelled SPP1 DNA (indicated by arrows). Four-drop fractions were collected from the bottom of the tube on glass-fibre discs. (b) DNA from the donor culture used in experiment 2 (Table 1) was extracted with phenol immediately before phage infection and centrifuged as in (a).

**DISCUSSION**

Amount and origin of the DNA from transducing particles. The DNA content of transducing particles is not always equal to that of the corresponding phage particles (Weigle et al., 1959; Ting, 1962; Porter et al., 1979). For SPP1, however, we have shown (Lencastre & Archer, 1980) that purified transducing particles and SPP1 bacteriophages have indistinguishable sedimentation coefficients. Furthermore, while a 5% deletion in \( \phi105 \) bacteriophage was easily detected in CsCl equilibrium density-gradient centrifugation (Flock, 1977), no difference in density was found between SPP1 transducing particles and plaque-forming units, even upon refractionation (Lencastre & Archer, 1980). This strongly suggests that the amount of DNA present in SPP1 transducing particles was the same as that of the infectious particles.

How much of the DNA in the transducing particles is of bacterial origin? It has been demonstrated that SP10 transducing particles contain no phage DNA (Okubo et al., 1963). This also seems to be the case with P1 (Ikeda & Tomizawa, 1965) and PBS1 (Yagamashi & Takahashi, 1968). However, in P22 transducing particles the presence of 10% phage DNA is disputed (Schmieder, 1970; Ebel-Tsipis et al., 1972). SPP1 particles transduce about 1% of the \( B. subtilis \) chromosome (Yasbin & Young, 1974). Since the molecular weight of the \( B. subtilis \) chromosome was calculated as \( 2.0 \times 10^8 \) (Klotz & Zimm, 1972) and that of SPP1 DNA as \( 2.5 \times 10^7 \) (Riva et al., 1968), it might be concluded that at least the majority of the DNA from the SPP1 transducing particles is of bacterial origin. The DNA from SPP1 transducing particles equilibrated in CsCl at, or very close to, the density of donor DNA
isolated immediately before infection (Fig. 1). Transducing particles also encapsulate light DNA synthesized after infection (Table 1) which makes it likely that SPP1 transducing particles contain no phage DNA. This conclusion suggests that each SPP1 transducing particle contains about $2.5 \times 10^7$ dalton bacterial DNA.

**Number of transducing particles.** We tried to assess the number of transducing particles present in the heavier peak of experiment 2 (Table 1) using the value of the specific activity of the bacterial donor DNA determined in the same experiment immediately before infection, viz. $4.6 \times 10^4$ c.p.m. (µg DNA)$^{-1}$. Comparing this value with the $2.29 \times 10^4$ c.p.m. of the heavier band from the gradient, it could be estimated that a total of more than $0.5$ µg DNA was present in the heavy band of that gradient. Assuming (see preceding section) that each of the transducing particles contains about $2.5 \times 10^7$ dalton DNA, the heavy band of that gradient would contain $1.2 \times 10^{10}$ transducing particles. This number seems to be exceedingly high when compared with the actual number of transductants ($2.91 \times 10^3$). However, the plating efficiency of SPP1 is extremely low (Riva et al., 1968), sometimes below 1% (T. A. Trautner, personal communication). In our conditions, preliminary results (not shown) suggest a plating efficiency in the order of 0.3%. In addition, we could expect (see preceding section) that only about 1% of the transducing particles would contain the transducing marker. Taking these two factors into account, we could expect (see preceding section) that only about 1% of the transducing particles would contain the transducing marker. Taking these two factors into account, we could expect that the calculated $1.2 \times 10^{10}$ transducing particles would produce $3.6 \times 10^5$ transductants. This number is still high when compared with the actual value obtained ($2.91 \times 10^4$). This discrepancy might suggest that no more than about 10% of the transducing DNA injected into the recipient cells becomes integrated. A similar phenomenon has been observed in other systems (Ebel-Tsipis et al., 1972).

**Relative incorporation of radioactivity by phages and transducing particles.** A considerable and unexpected amount of radioactivity banded together with the light phage particles (Table 1). The probable explanation of this result is that bacterial DNA is degraded to nucleotides and re-utilized for phage DNA synthesis. The fraction of radioactivity banding at the light phage position is unlikely to result from deficient removal of the label when transferring cells from radioactive to non-radioactive medium. If this were the explanation, the ratio of light to heavy radioactivity should remain constant whatever the incubation time for lysate preparation. On the contrary, it steadily increases with time (ratio B/A in Table 1).

If we assume a plating efficiency of 0.3%, the 1-47 $\times 10^4$ c.p.m. of the light band (experiment 2 in Table 1) would correspond to $5 \times 10^{13}$ phage particles, representing an incorporation of $2.9 \times 10^{-10}$ c.p.m. per phage particle. On the other hand, the value of $2.29 \times 10^4$ c.p.m. found in the heavier band in the same experiment would correspond to the value of $1.2 \times 10^{10}$ transducing particles (see calculations above), giving an incorporation of about $2 \times 10^{-6}$ c.p.m. per transducing particle. We estimate, therefore, that phage particles incorporate about 7000 times less radioactivity than transducing particles.

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**References**


