SHORT COMMUNICATION

Molecular Origin of Transducing DNA in Bacteriophage SPP1

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*

(Received 4 July 1980)

Transducing particles produced by bacteriophage SPP1 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 SPP1 have been shown to mediate generalized transduction (Yasbin & Young, 1974). When such lysates were purified, in CsCl, from bacteriophage PBSX particles induced by SPP1 infection, the transducing activity was retained (Lencastre & Archer, 1979). Furthermore, by buoyant density, sedimentation coefficient and anti-SPP1 serum inactivation of the transducing activity, SPP1 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 SPP1 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 SPP1 transducing particles have a DNA content indistinguishable from that of the SPP1 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 [³²P]orthophosphate [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 ³²P was used, 10 µCi [³²P]orthophosphate 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 [³H]thymidine, were infected in light, non-radioactive

medium with SPP1 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 ^{14}C -labelled SPP1 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 [^{32}P]orthophosphate (experiments 6 to 8). Cells were then incubated with SPP1 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.

Expt no.	Incubation time (h)	$10^{-4} \times \text{TCA-precipitable radioactivity (c.p.m.)}$		B/A	$10^{-3} \times \text{No. of transductants}$		D/C	$10^{-11} \times \text{Total p.f.u.}$
		Heavy (A)	Light (B)		Heavy (C)	Light (D)		
1	2.5	2.50	0.19	0.08	—	—	—	1.47
2	3.5	2.29	1.47	0.64	29.1	2.5	0.09	1.50
3	4	8.70	7.40	0.85	230	70	0.30	12
4	6	0.36	0.63	1.75	10.6	5.26	0.50	1.58
5	6	17.2	41.4	2.41	—	—	—	3.11
6	4	22.9	104	4.54	3.16	1.99	0.63	1.15
7	18	0	110	∞	—	—	—	3.96
8	18	0	691	∞	—	—	—	590

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 130 000 g using, as reference, alkali-denatured (Riva *et al.*, 1968) ^{14}C -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 $\phi 105$ 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 (Schmieger, 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 2.0×10^9 (Klotz & Zimm, 1972) and that of SPP1 DNA as 2.5×10^7 (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 2.5×10^7 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. 4.6×10^4 c.p.m. $(\mu\text{g DNA})^{-1}$. Comparing this value with the 2.29×10^4 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 2.5×10^7 dalton DNA, the heavy band of that gradient would contain 1.2×10^{10} transducing particles. This number seems to be exceedingly high when compared with the actual number of transductants (2.91×10^4). 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 that the calculated 1.2×10^{10} transducing particles would produce 3.6×10^5 transductants. This number is still high when compared with the actual value obtained (2.91×10^4). 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 1.47×10^4 c.p.m. of the light band (experiment 2 in Table 1) would correspond to 5×10^{13} phage particles, representing an incorporation of 2.9×10^{-10} c.p.m. per phage particle. On the other hand, the value of 2.29×10^4 c.p.m. found in the heavier band in the same experiment would correspond to the value of 1.2×10^{10} transducing particles (see calculations above), giving an incorporation of about 2×10^{-6} c.p.m. per transducing particle. We estimate, therefore, that phage particles incorporate about 7000 times less radioactivity than transducing particles.

We wish to thank Drs F. E. Young, G. A. Wilson and T. A. Trautner for helpful discussions, Drs M. S. Fox, P. J. Piggot and F. E. Young for constructive criticism of the manuscript, Mrs M. C. Lopes for excellent technical assistance and Mr M. Guimarães for drawing the figure.

REFERENCES

- EBEL-TSIPIS, J., BOTSTEIN, D. & FOX, M. S. (1972). Generalized transduction by bacteriophage P22 in *Salmonella typhimurium*. I. Molecular origin of transducing DNA. *Journal of Molecular Biology* **71**, 433–448.
- FLOCK, J. I. (1977). Deletion mutants of temperate *Bacillus subtilis* bacteriophage $\phi 105$. *Molecular and General Genetics* **155**, 241–247.
- IKEDA, H. & TOMIZAWA, J. (1965). Transducing fragments in generalized transduction by phage P1. I. Molecular origin of the fragments. *Journal of Molecular Biology* **14**, 85–109.
- KLOTZ, L. C. & ZIMM, B. H. (1972). Size of DNA determined by viscoelastic measurements: results on bacteriophages, *Bacillus subtilis* and *Escherichia coli*. *Journal of Molecular Biology* **72**, 779–800.
- LENCASTRE, H. & ARCHER, L. J. (1979). Transducing activity of bacteriophage SPP1. *Biochemical and Biophysical Research Communications* **86**, 915–919.

- LENCASTRE, H. & ARCHER, L. J. (1980). Characterization of bacteriophage SPP1 transducing particles. *Journal of General Microbiology* **117**, 347–355.
- OKUBO, S., STODOLSKY, M., BOTT, K. & STRAUSS, B. (1963). Separation of the transforming and viral deoxyribonucleic acids of a transducing bacteriophage of *Bacillus subtilis*. *Proceedings of the National Academy of Sciences of the United States of America* **50**, 679–686.
- PORTER, R. D., SHOEMAKER, N. B., RAMPE, G. & GUILD, W. R. (1979). Bacteriophage-associated gene transfer in pneumococcus: transduction or pseudotransduction? *Journal of Bacteriology* **137**, 556–567.
- RIVA, S., POLSINELLI, M. & FALASCHI, A. (1968). A new phage of *Bacillus subtilis* with infectious DNA having separable strands. *Journal of Molecular Biology* **35**, 347–356.
- SCHMIEGER, H. (1970). The molecular structure of the transducing particles of *Salmonella* phage P22. II. Density gradient analysis of DNA. *Molecular and General Genetics* **109**, 323–337.
- TING, R. C. (1962). The specific gravity of transducing particles of bacteriophage P1. *Virology* **16**, 115–121.
- WEIGLE, J., MESELSON, M. & PAIGEN, K. (1959). Density alterations associated with transducing ability in the bacteriophage lambda. *Journal of Molecular Biology* **1**, 379–386.
- YAGAMASHI, H. & TAKAHASHI, I. (1968). Transducing particles of PBS1. *Virology* **36**, 639–645.
- YASBIN, R. E. & YOUNG, F. E. (1974). Transduction in *Bacillus subtilis* by bacteriophage SPP1. *Journal of Virology* **14**, 1343–1348.