Vol. 86, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 14, 1979 Pages 915-919

TRANSDUCING ACTIVITY OF BACTERIOPHAGE SPP1

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Received December 11, 1978

Summary. The generalized transduction system mediated by SPP1 lysates was analyzed. After removal of contaminating PBS particles, SPP1 lysates retain the same levels of transducing activity. The SPP1 particles of normal size, shape and buoyant density carrying bacterial DNA, are vectors in a true transduction process.

INTRODUCTION

Yasbin and Young have shown (1) that Bacillus subtilis lysates of the virulent bacteriophage SPP1 have generalized transducing activity. These authors demonstrated by linkage data, that SPP1 is capable of transducing approximately 1% of the bacterial chromosome. Therefore it appears that this homologous transducing system can be used for fine structure analysis in the mapping of the B. subtilis chromosome (2). More recently, however, it was established that infection of B. subtilis by SPP1, induces the lysogenic defective bacteriophage PBSX (3). Moreover PBSX (also called PBSH) has also the capability of mediating gene transfer in B. subtilis probably by cell penetration of released DNA (4).

Although the evidence presented by Yasbin and Young (1) strongly suggests that the observed transducing activity was mediated by SPP1, it was appropriate and necessary to explore this process further. The evidence to be presented establishes that SPP1 particles are the vectors in this generalized transducing system.

MATERIALS AND METHODS

Growth of the cultures

Cultures were grown at 37° C with vigorous aeration.

0006-291X/79/030915-05$01.00/0

Preparation of the lysates

Bacillus subtilis 17 [a thyA thyB leu2 transformant from the strain described by Farmer and Rotham (5)] was grown overnight in MIIIM medium (6) supplemented with thymine (5 µg/ml) and leucine (50 µg/ml). The culture was then diluted to a cell density of 10^7/ml in the same medium to which 10 µCi methyl-[^{3}H] thymidine/ml (23 Ci/mmole) were added. Incubation followed up to a concentration of 2 x 10^8 cells/ml. A large excess of cold thymine (200 µg/ml) was then added and the culture was further incubated for 15 min. SPP1 wt phages were then given at an input multiplicity of 5. Nine minutes thereafter NaCN (2.5 x 10^{-3} M) was added and adsorption allowed to continue for 6 min. Cells collected by centrifugation were resuspended in three volumes of pre-warmed MIIIM medium containing 200 µg/ml of thymine. After 6 hr chloroform was added. Following purification of phages by low speed centrifugation, the lysates were concentrated by high speed centrifugation, treated with DNAse (100 µg/ml) RNAse A (60 µg/ml) and RNAse T_1 (40 µg/ml), purified by discontinuous cesium chloride gradients, and titrated on B. subtilis BR151 (lys-3, trpC2 metB10) as described (1).

Bacillus subtilis VVB112 (thyA thyB trpC2, a bromouracil tolerant strain obtained from N. Harford) was used, as the donor strain, when the cells were labelled with 5-BU (5-bromo-2'-deoxyuridine) prior to infection by SPP1. In these experiments the overnight culture was diluted in MIIM medium supplemented with thymine (5 µg/ml), 5-BU (25 µg/ml), [^{3}H]-thymidine (10 µCi/ml), tryptophan (60 µg/ml), casein (500 µg/ml), histidine (100 µg/ml), yeast extract (200 µg/ml) and alanine (100 µg/ml).

Cesium chloride density-gradient centrifugation

Cesium chloride gradients for phage particles were prepared by adding cesium chloride to purified phage particles to a final density of 1.544 g/cc. The gradients were centrifuged at 16° C and 70,000 x g for 24 hours.

Analysis of the fractions from the gradient

B. subtilis BR151 was used as the recipient for transduction. The cells were grown overnight in supplemented MIIIM medium, centrifuged and resuspended in the same medium (twice the original volume) and incubated for 1 hr. The culture was then washed twice and resuspended (half the original volume) in unsupplemented MIIIM medium). Samples were added, in the presence of DNAse (100 µg/ml), to appropriate aliquots of each fraction from the gradient. Incubation followed for 20-30 min. Transductions were selected and plaque-forming units were scored (1). Samples for radioactivity were deposited on Whatmann GF/C glass fibre discs, dried, and treated by cold 5% trichloroacetic acid and cold 95% ethanol. The scintillation fluid contained 4 gm of PPO and 0.25 gm of dymethyl POPOP per litre of toluene.

RESULTS AND DISCUSSION

The presence of induced PBSX bacteriophage in SPP1 lysates (3) could complicate the interpretation of the gene transfer activity of such lysates. Because the density of PBSX particles is much lower (1.375 g/cc; 7) than that of the SPP1 phages (around 1.544 g/cc as indicated above), SPP1 lysates were purified from these defective phages by centrifugation through a discontinuous cesium chloride gradient, and thereafter analyzed in cesium chloride equilibrium

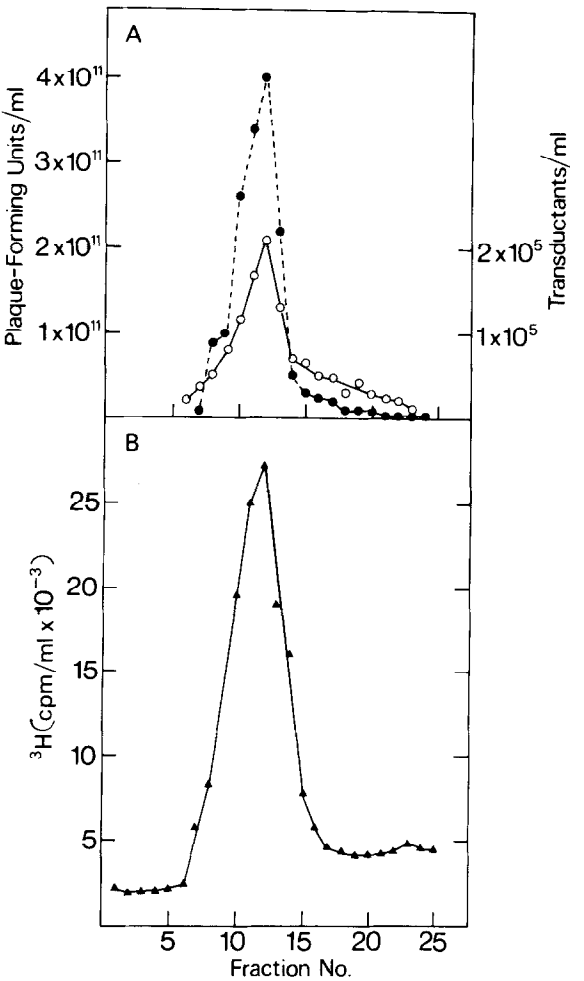

Figure 1. Cesium chloride equilibrium density-gradient analysis of a SPP1 lysate prepared in [^{3}H] thymidine labelled cells. A total of 28 fractions, 3 drops each, were collected from the bottom of the tube. (A) Fractions indicated were assayed for both plaque-forming units (+-----+) and trp$^+$ (o———o) transducing activity. (B) A 10 µl sample from each fraction was counted for radioactivity (o———o).

density gradients. Upon purification the lysates retain the same levels of transducing activity, showing that PBSX particles were not vectors in this transducing system.

In order to know the relative densities of transducing particles and SPP1 plaque-forming units, a purified SPP1 lysate, prepared in [^{3}H]-thymidine labelled bacteria was analyzed in a cesium chloride equilibrium density

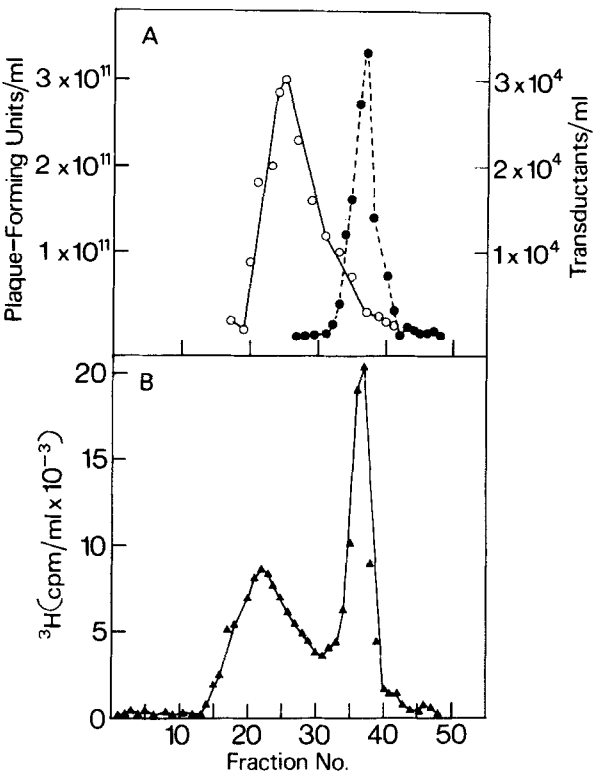

Figure 2. Cesium chloride equilibrium density-gradient analysis of a SPP1 lysate prepared in 5-BU and [^{3}H] thymidine labelled cells. A total of 50 fractions, 3 drops each, were collected from the bottom of the tube. (A) Fractions indicated were assayed for both plaque-forming units (+------+) and lys$^+$ (o———o) transducing activity. (B) A 50 µl sample from each fraction was counted for radioactivity (o———o).

gradient (Figure 1). Only one radioactive peak corresponded to SPP1 plaque-forming units and transducing activity. The same results were obtained in other experiments of the same type, including those in which a few fractions around the peak were pooled and refractionated (unpublished observations). Therefore it was concluded that transducing particles and SPP1 plaque-forming units have the same density.

In contrast, when the donor cells were prelabelled with 5-BU and [^{3}H]-thymidine, and infected with SPP1 phages in light and non-radioactive medium, the transducing activity can be separated from the plaque-forming units (Figure 2). The presence of 5-BU in the bacterial DNA synthesized before infection caused a

shift of the transducing particles to a density higher than that of the light plaque-forming units. This demonstrates that SPP1 transducing particles contain the more dense bacterial DNA replicated in the presence of 5-BU before SPP1 infection.

Since it is known and we have reconfirmed (unpublished observations), that DNA from SPP1 and B. subtilis have the same density in CsCl, and we have established that the two types of particles have the same density, it is possible to conclude that the DNA to protein ratio is the same in the transducing vectors and in the SPP1 phages. In addition, purified transducing particles and SPP1 phages show the same sedimentation behavior in sucrose gradients and the same inactivation rate by anti-SPP1 serum (unpublished observations).

We conclude, therefore, that transducing activity is carried in complete SPP1 particles of normal size, shape and density.

ACKNOWLEDGEMENTS

We wish to thank Dr. Frank E. Young and Dr. Gary A. Wilson for introducing us into technical details of the system and for constant encouragement and Mrs. Maria Candida Lopes for excellent technical assistance.

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