PERSISTENCE OF P1 TRANSDUCING DNA IN RECIPIENT CELLS

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SUMMARY: Studies have been pursued to determine the fate of P1 transducing DNA after it is injected into the recipient cells. Cesium chloride density gradient analysis reveals that the major part of the transducing DNA persist in the recipient cells as double stranded DNA for at least 40 minutes of incubation without degradation.

Transformation, conjugation and transduction are the three known systems in which a segment of bacterial DNA is transferred from a donor to a recipient cell wherein a recombination event between the recipient chromosome and the incoming segment may ultimately take place. In the case of transformation, evidence has accumulated to show that the incoming double-stranded donor DNA is reduced to the single stranded state rather rapidly, before it becomes integrated into the recipient cell chromosome (4, 6, 10). In the case of conjugation the evidence also favors a single stranded state of the incoming DNA (1).

On the other hand, little study has been given to the fate of transducing DNA after it enters the recipient cells. Guild et al. (4) mentioned that transducing DNA would be expected to be injected as double-stranded DNA, because the transducing particles do possess the whole phage injecting apparatus. The present study was undertaken to investigate the events that follow the injection of transducing DNA by bacteriophage P1.

MATERIALS AND METHODS

Bacterial and Phage Strains: Two auxotrophic strains of *Escherichia coli* K12 were used in the experiments: UTH 3291, which requires thymine and methionine for growth, was chosen as donor; UTH 444 which requires trypto-

phan for growth served as recipient. The phage P1 used is a virulent mutant originally obtained from Dr. Mitsuhashi and described by Ikeda and Tomizawa (5).

Media: (1) TGB contains 1 g $(NH_4)_2SO_4$, 0.011 g $CaCl_2$, 0.095 g $MgCl_2$, 0.008 g $FeCl_3$, 0.0227 g Na_2SO_4, 0.0436 g KH_2PO_4 and 6.05 g tris per liter (pH 7.2). After sterilization 4 ml 50% glucose and 5 ml 0.1% thiamine are added. (2) L broth, L agar and soft agar have been described (7). $CaCl_2$ is added to 2.5×10^{-3} M whenever needed. (3) Minimal agar is minimal medium (2) with 1.5% agar (for plates) or 0.65% agar (for soft agar).

Labeling and Separation of Transducing Particles: An overnight culture of UTH 3291 in TGB supplemented with 2 µg/ml thymine and 20 µg/ml methionine was diluted into TGB with the same supplements, then aerated at 37C for 2 hours. The cells were collected by filtration and resuspended in the same medium to a final concentration of 5×10^7 cells/ml, $^{32}PO_4$ was added to 133 µc/ml, and the culture was further aerated at 37C for 2.5 hours. The thymine was then washed out by filtration and the cells were resuspended in two volumes of TGB supplemented with methionine, $^{32}PO_4$ as before, and 5-bromouracil (5 µg/ml), and aerated at 37C for another hour. The culture was then filtered and resuspended in L broth plus Ca^{++} at one-tenth its original volume and P1 was added at a multiplicity of infection of 5. After 30 min adsorption at 37C, the mixture was brought to the original volume by addition of L broth, and shaken at 37C until lysis was obvious. The lysate was sterilized with chloroform and the cell debris was removed by low speed centrifugation. The particles were then sedimented by centrifugation at 20,000 rpm in a Beckman Spinco Type 30 rotor for 2 hours. The pellet was resuspended in a small volume of L broth plus Ca^{++} and mixed with cesium chloride to give a final density of 1.45 g/cc. The mixture was centrifuged at 40,000 rpm in a Beckman Spinco 50 Ti rotor for 45 hours. Ten drop-fractions were collected into 0.5 ml L broth plus Ca^{++}. For plating of the infective particles, 0.1 ml of each fraction was mixed with 2.5 ml of soft L broth agar

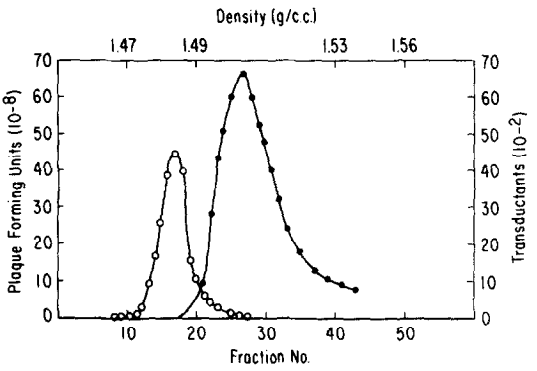

Figure 1. Separation of transducing particles from infective particles in a CsCl density gradient.

seeded with 0.1 ml overnight UTH 444 culture and overlaid on L broth agar. Plaques were counted after overnight incubation at 37C. For assay of transductants, UTH 444 cells were grown in L broth plus Ca^{++} and concentrated to give 2 x 10^9 cells/ml. Two-tenth ml of the cells were then mixed with 0.1 ml of each CsCl fraction and incubated at 37C for 30 min. Finally 0.1 ml of the mixture was mixed with 2.5 ml soft minimal agar, overlaid on a minimal agar plate, and incubated at 37C for 48 hr. Colonies that appeared after 48 hr incubation were counted as transductants. Uninfected UTH 444 cells were plated as controls.

Figure 1 shows the separation of the peaks of transducing and infective particles that is achieved by this method. The fractions between the densities of 1.49 g/cc and 1.54 g/cc are pooled and used for further experiments. In this way, over 90% of the infective particles can be eliminated with little sacrifice of real transducing particles.

Extraction and CsCl Density Gradient Analysis of Transducing DNA: UTH 444 cells were grown to 3.8 x 10^8 cells/ml in L broth plus Ca^{++}. The cells were filtered and resuspended in one-tenth the original volume and mixed with labelled, concentrated transducing particles prepared as described in the previous section. After 30 min adsorption, the mixture was separated into 3 parts. One part of the mixture was chilled quickly in cold 0.8% saline

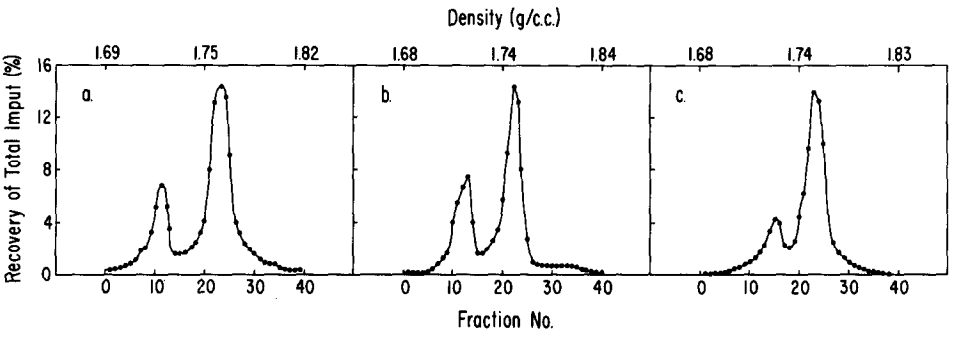

Figure 2. CsCl density gradient analysis of transducing DNA. a) non-adsorbed transducing DNA. b) 0 time transducing DNA. c) 20 min or 40 min transducing DNA.

solution and the unadsorbed particles were removed by three cycles of low speed centrifugation (0 time cells). L broth was added to each of the other two portions and they were incubated at 37° for 20 and 40 min, respectively. Each was then chilled and centrifuged as in the case of the 0 time cells. The DNA from each of these samples was extracted separately by phenol (8). CsCl was added to 1.77 g/cc (pH 8.0) and the mixture was centrifuged at 33,000 rpm in the SW39 rotor of a Spinco preparative ultracentrifuge for 48 hr. Fractions were collected onto paper discs and counted in a Tri-Carb Liquid Scintillation Counter.

RESULTS AND DISCUSSION

Figure 2a shows DNA extracted from the phage particles before adsorption. The heavier DNA is that from the transducing particles; the lighter peak represents the DNA from those infective particles that remain in the pool of transducing particles. The heavier 1.77 g/cc DNA is double stranded DNA containing principally BU in one strand, for when this material was heat denatured and recentrifuged two peaks were observed, one at a density of 1.76 g/cc, one at 1.82 g/cc.

Figure 2b and c show that the peak position of the DNA injected into the recipient cells did not change significantly, with time, either in size or position. Thus, the incoming DNA is very stable after it enters the

recipient cells, even after 40 min of incubation. The DNA shown here was actually inside the recipient cells as shown in the following control experiments. (a) Recipient cells were mixed with transducing particles, the mixture was chilled rapidly, washed three times by low speed centrifugation, and the DNA was extracted and counted; the counts found were almost at the background level indicating that the unadsorbed particles were well washed out. (b) A fraction each of the 0 time and of the 40 min cells from the experiment shown in Fig. 2 was converted to spheroplasts in accordance to the procedure of Neu and Heppel (9); the spheroplasts were collected by centrifugation and counted; over 95% of the counts were found in association with the spheroplasts and less than 5% remained in the supernatant.

The data presented in this report correlates well with the fact that the majority of the transductants in this system were found to be abortive (3; Lee and Suit unpublished results). For some reason, as yet unexplained, once the DNA is in the recipient cell, it is well protected from degradation and integration. Evidently transcription of the persisting DNA takes place, but replication does not, since it is not a complete replicon. The integration of the transducing DNA might have been too infrequent to be detected if actual recombination occurs by single-stranded intermediates as in the case of transformation. Alternatively, our data might suggest that the true transductants are the result of double-stranded recombination so that they are indistinguishable from the abortive ones in the CsCl gradient.

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