TRANSFER TO PROGENY OF BOTH DNA STRANDS OF PHAGE 2C

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SUMMARY : The efficiency of transfer of parental DNA components to offspring phage 2C has been measured, by taking advantage of the differences in buoyant density and in affinity for polyG displayed by the 2 strands. When unlabelled cells of B. subtilis were infected with purified phages labelled in their DNA by either (^{32}P) orthophosphate or (^{3}H) uracil (2C DNA contains hydroxymethyluracil in the place of thymine), the 2 strands of progeny virus DNA were found equally labelled. Nucleotide composition of the heavy strand in virions of the first and second generation were identical, and the same held true for the light strand. Carry-over of non replicating DNA was excluded by showing the absence of heavy and hot native parental DNA in progeny particles replicated in light and non radioactive host. It can, thus, be concluded that 2C DNA is highly conserved and transferred to offspring phage, throughout the viral cycle and the DNA duplication process.

It is well established that the DNA of T-even coliphages is transferred to progeny particles with very high efficiency, and that the losses observed in different experiments can be accounted for by the limitations of the biological systems involved (1-3). Parental T4-DNA, however, does not appear in offspring virions as intact molecules, for semi-conservatively replicated DNA undergoes genetic recombination within the vegetative pool of the host cell (4-8). Hence, a study of the mode of transfer of phage DNA may help understanding the mechanisms of both duplication and exchange between viral chromosomes. Phage 2C offers obvious advantages for this type of study. Its chromosome, a molecule of double stranded DNA of 100 millions daltons, has two strands of different buoyant densities and

contains hydroxymethyluracil (HMU) in place of thymine (uracil can, thus, be used as specific marker for viral DNA) (9-11). In the present work, the efficiency of transfer of 2C-DNA strands has been investigated at molecular and submolecular levels with consistent results.

RESULTS

Virions labelled in their DNA by either ^{32}P or ^{3}H were obtained by infection of B. subtilis in the presence of either (^{32}P) orthophosphate or (^{3}H) uracil. Phage particles of first generation were concentrated by centrifugation of nuclease - treated lysates, and further purified by equilibrium centrifugation in CsCl. Aliquots of these preparations were extracted with phenol, and viral DNA was denatured in the presence of polyguanylic acid (polyG). Still other aliquots of the same phage suspensions were used to infect at low multiplicity bacteria in media containing an excess of unlabelled phosphate and uracil. From purified particles of second generation, DNA was extracted and denatured in the same way, in the presence of polyG. Preparations of denatured viral DNA of 1st and 2nd generation were centrifuged at equilibrium in CsCl gradients, and radioactivity of collected fractions was measured.

Fig. 1 shows the separation of the 2 strands of first and second generation DNA. Apparent buoyant densities of 2C-DNA components in CsCl were : 1.762 g/cm^3 for the heavy (H) strand, and 1.752 g/cm^3 for the light (L) strand. After the binding of polyG, the density of the H strand was shifted to 1.810 g/cm^3. Integration of the surfaces of radioactivity peaks in Fig. 1A (1st generation DNA) and 1B (2nd generation DNA) shows that the 2 strands of viral DNA were transferred with equal efficiency to progeny particles.

Further insight into the transfer process was gathered by

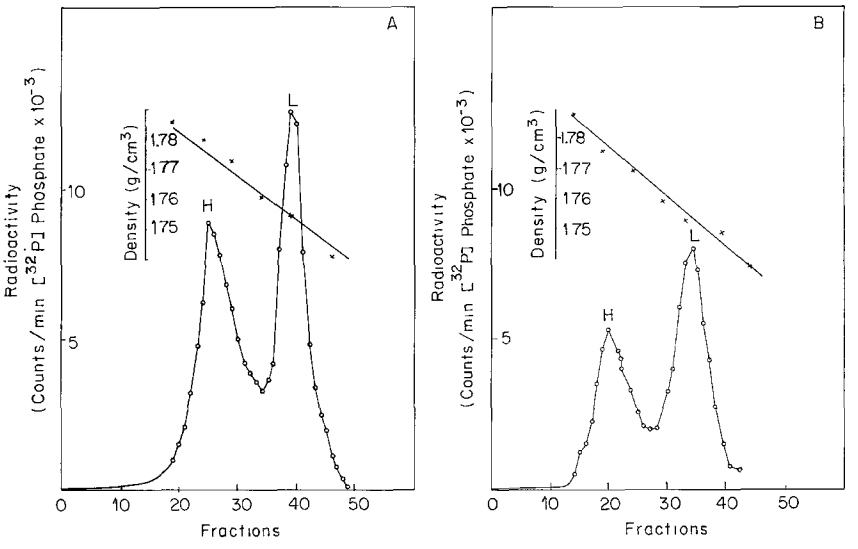

Figure 1. Ultracentrifugal analysis of parental and progeny DNA, denatured in the presence of PolyG.
Labelled "parental" virus 2C was prepared by infecting (MOI = 20) B. subtilis 168/2 previously grown for 1 generation in YS-medium (11) containing 0.25 mCi (^{32}P) orthophosphate/1.25 mM (^{31}P) orthophosphate/2.5 x 10^7 bacteria/ml. Virus particles from the lysate were treated with nucleases, concentrated by ultracentrifugation (20,000 rpm, 30 min, 4°C), and purified in CsCl density gradients ($Q_{23°}$ = 1.52 g/cm^3).
Unlabelled B. subtilis 168/2 (5 x 10^7 bacteria/ml) in high-phosphate medium were infected with (^{32}P)-labelled phages (MOI = 3.8; input = 1.0x10^6CPM/ml), and viral particles were purified in the same way. DNA from first (Fig. 1A) and second generation (Fig. 1B) virions was extracted with phenol and 5 % sodium lauryl sulfate, and denatured for 4 min at 96°C in the presence of polyguanylic acid (25 µg/l 0.D_{260nm} unit DNA). The mixture was rapidly cooled, and centrifuged in a CsCl density gradient ($Q_{23°}$ = 1.75 g/cm^3 ; 72 hr; 33,000 rpm; 18°C; S.W. 50.1 Spinco rotor). Radioactivity of fractions collected on 3 MM Whatman filters was measured by scintillation spectrometry.

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studying the nucleotide composition of ^{32}P-labelled DNA strands within virions of first and second generation. For this purpose, the DNA extracted from purified particles of ^{32}P-labelled phage was denatured, and separated strands were hydrolysed with DNAase and snake venom phosphodiesterase. Resulting mononucleotides were then fractionated either by electrophoresis (12) or by column chromatography (13). Aliquots of labelled virions were also used

to infect cells in unlabelled medium, and DNA strands from progeny particles were submitted to the same procedure.

Data reported in Table 1 indicate that the H strands of virions of 1st and 2d generation had very close compositions. Nucleotide ratios of parental and progeny L strands were also similar.

TABLE 1

Percent composition of DNA strands of parental and progeny 2C phages.

		CMP	HMU MP	AMP	GMP	GMP + CMP
L-STRAND	parental	19.9	27.1	31.6	21.2	41.1
	progeny	20.2	26.7	33.3	19.7	39.9
H-STRAND	parental	23.3	32.0	27.8	16.8	40.1
	progeny	21.8	32.2	28.9	17.1	38.9
NATIVE DNA		19.8	30.7	31.2	18.1	38.0

Table 1. Complementary strands of parental and progeny DNA were separated as described in the legend for Fig. 1. PolyG was removed by incubating the pooled fractions with 0.3 M KOH for 4 hours at 37°C, followed by extensive dialysis against water. Single stranded DNA was hydrolysed with DNAase in 12 mM Tris-HCl buffer pH 7.4 containing 6 mM MgCl (20 µg DNAase/1 $O.D._{260nm}$ unit DNA/30,000 CPM TCA-insoluble (^{32}P) orthophosphate; incubation : 1 hr, 37°C). The pH of the solution was brought to 8.5 with KOH, and hydrolysis of DNA was pursued with snake venom phosphodiesterase (20 µg enzyme/ 1 $O.D._{260nm}$ unit DNA, 2 hr, 37°C). Nucleotide composition was analysed by low-voltage electrophoresis (2.5 hours, 300 V) (12). Results presented are the average of four experiments.

Such conclusion, however, cannot be warranted without excluding a passive carry-over of parental DNA to offspring particles. The appropriate control was as follows. Double-labelled phages were prepared by infection of B. subtilis in medium containing 6-(^{3}H)-uracil and deuterium oxide replacing H_2O. One aliquot of D_2-(^{3}H)-particles was extracted with phenol, and native viral DNA was analysed in CsCl density gradients.

Another aliquot of the virus suspension was used to infect bacteria in H_2O - unlabelled medium. Comparison of pattern in Fig. 2A and 2B indicates that, indeed, 2nd generation virions did

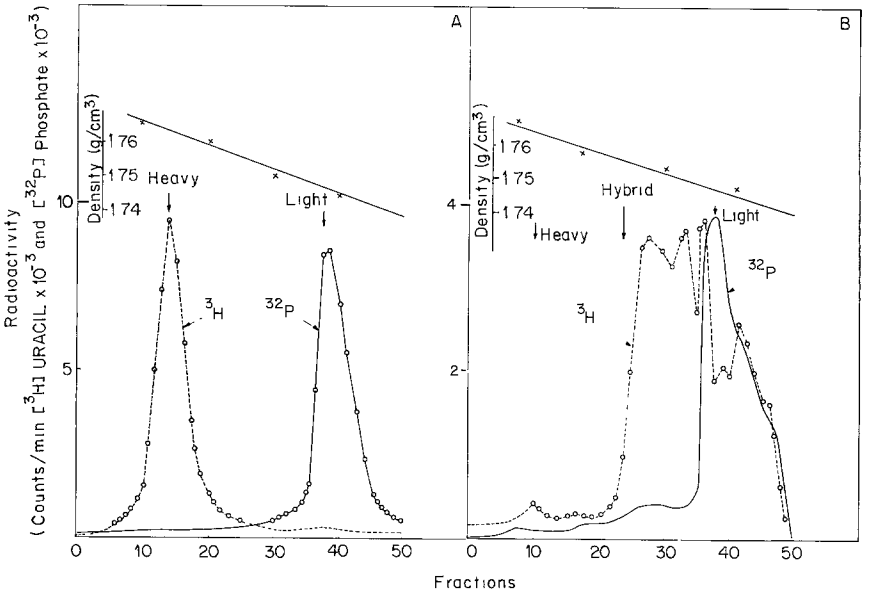

Figure 2. Equilibrium centrifugation of native density labelled phage DNA isolated from virions of 1st and 2nd generation. D_2-(^{3}H)-parental phage particles were prepared by infecting overnight cultures of B. subtilis 168/2 in YS medium containing 0.1 mCi (^{3}H)uracil/5 x 10 cells/ml with D_2O (99.75 %) replacing completely H_2O. Virions were purified as outlined in the legend to Fig. 1, and used to infect host cells (0.25 x 10^6 CPM/4 x 10^8 PFU/ml; MOI = 8) in H_2O-unlabelled medium. After adsorption, infected cells were washed, and the lytic cycle was allowed to go to completion. DNA extracted from parental (Fig. 2A) and progeny (Fig. 2B) particles was analysed by centrifugation in CsCl density gradients, after addition of ^{32}P-H_2O DNA as reference.

not contain any appreciable amount of labelled DNA binding at the density of parental DNA. Instead, progeny DNA was quite heterogeneous in density, and included a population of molecules of densities ranging between those of fully light and those of hybrid species. This finding excludes the passive incorporation of parental molecules into offspring particles.

DISCUSSION

Incorporation of parental chromosome into progeny particles is a highly unpredictable event for every kind of viral nucleic acids, for complete and partial transfer, as well as lack of transfer, have been observed with different virus-host systems (14-18). Also, the fact that double-stranded viral DNA is replicated according to a semi-conservative model does not imply that both strands are equally conserved and transferred. Moreover, since the 2 strands of 2C-DNA are involved to a different extent in viral message transcription (19) it is not unconceivable that they might also be incorporated with different efficiency into progeny particles. Data in Fig. 1 show, however, that in spite of an asymmetrical transcription, the transfer of parental strands to the offspring is symmetrical, in the case of phage 2C.

Although most of the reports agree that the single-stranded DNA (15) and RNA (16-17) genomes of bacterial viruses are not transferred at all to progeny virions, an apparent transfer of single stranded RNA (18) and DNA (19) were observed with particular virus-host systems. Three mechanisms were indicated as responsible for such unexpected findings : a) degradation and highly efficient reincorporation of DNA fragments; b) sloughing of abortively attached particles; and c) encapsulation of non-replicated viral chromosomes in multiply-infected host cells. A possible involvement of these 3 mechanisms in our experimental system was excluded by

the experiments reported in the present paper. In fact, hydrolysis plus resynthesis was ruled out by the nucleotide analysis of single strands in 1st and 2d generation particles, whereas sloughing of virions and carry-over of intact chromosomes were excluded by the double-labelling experiment reported in Fig. 2.

Pattern in Fig. 2 points to the uneven distribution of parental DNA material among progeny. This observation can be explained by efficient recombination between viral chromosomes within the vegetative precursor pool. Extensive exchange of genetic material between replicating T-even phages has been previously reported (3-4). Recombination of viral DNA in 2C infected B. subtilis is now extensively studied in our laboratory.

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