THE TRANSFER OF RADIOACTIVE PHOSPHORUS FROM PARENTAL TO PROGENY PHAGE

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Communicated by M. Delbrück, June 25, 1951

Introduction.—Reproduction is perhaps the most basic and characteristic feature of life. From the chemical point of view it is also the most obscure feature: atoms do not reproduce. When a living organism reproduces, there are now two atoms in the system for each one of the parent system. The additional atoms, of course, have not been "generated" by reproduction of the parent's atoms, but have been assimilated from the environment. Although the two progeny organisms may be biologically identical we should consider that their atoms can be classified into two classes: parental atoms and assimilated atoms. How are these atoms distributed between the two progeny organisms? Is one of the progeny all parental, the other all assimilated, or each half and half? Or perhaps both assimilated and the parental atoms dissimilated and passed into the environment? Are there specific macromolecular structures (genes?) that are preserved and passed on intact to the progeny? To answer questions of this kind we must be able to distinguish between parental and assimilated atoms and, in principle, this can be accomplished by the use of tracers.

Bacteriophages are especially suited for such studies because the division of their life cycles into inter- and intra-cellular phases enables one to label the virus particles without labeling the cellular environment within which they multiply. This was first done by Putnam and Kozloff[1] using phage T6r$^+$ active on *Escherichia coli* strain B, labeled with P^{32}. These authors infected unlabeled bacteria in unlabeled medium with labeled phage and found that 30% of the label of the infecting particles was transmitted to material characterized as phage by means of differential centrifugation. Similar results have been obtained with the phages T2r$^+$ and T4r$^+$ by Lesley, French and Graham.[2] This finding gives rise to numerous questions regarding the nature of this transmitted phosphorus: (1) Its origin: does it come from a specific part of the infecting particle; (2) its mode of transmission: is it transmitted in the form of large blocks of DNA or after complete breakdown and reassimilation; (3) its distribution: is it in one particle of the progeny, or distributed over all of them; (4) its location within the progeny particles: in specific parts of them, or distributed at random?

In consideration of recent work showing a genetic system in phage, Putnam and Kozloff suggested that the 30% transfer might be due to a bipartite structure of the phage, a genetic and a non-genetic part, and that

only the genetic part is transmitted. The P^{32} which is lost could then be considered as belonging to dispensable portions of the phage which are always lost during reproduction. At a phage meeting in Cold Spring Harbor in August, 1950, Dr. S. S. Cohen suggested that this hypothesis might be tested by allowing the progeny phage particles, which contain 30% of the parental P^{32}, to go through another cycle of reproduction in unlabeled bacteria. This experiment will be referred to as the "second generation experiment." If the notion of Putnam and Kozloff is correct, the "second generation experiment" should give 100% transmission, since the parental particles of this experiment would be specifically labeled in the transmissible part.

We have carried out experiments of this type by growing phage in labeled bacteria in labeled medium. These phages were allowed to infect unlabeled bacteria to produce progeny phage containing 30% of the radioactivity of the parental phage (first generation experiment). When the progeny phage were used to infect unlabeled bacteria, it was found that again 30% of the label is transferred to the progeny (second generation experiment).

This finding says, in essence, that the progeny particles are similar to the parental particle with regard to the localization of the phosphorus label. Since the parental particles of the first generation experiment were labeled uniformly throughout the particles, the same must be said about the progeny particles. Our experiment thus rules out Putnam and Kozloff's hypothesis—and answers the fourth of the questions listed above. It answers this fourth question only with respect to phosphorus. A different answer might be obtained with a label like sulphur, that would label specifically the protein moiety of the phage. The other three questions listed above remain unanswered by our experiment.

Material and Methods.—$T2r^+$ bacteriophage and the mutant B/1 of *Escherichia coli* strain B were used; the latter was chosen because contamination of plates with T1 bacteriophage occurs in our laboratory. The broth used in the transfer experiments is a watery meat extract to which is added 1% peptone, 0.02% Tween 80 and 0.5% NaCl; the pH is adjusted to 7.4. The latent period of $T2r^+$ in this medium is 22 minutes at 37° C. For preparation of radioactive stocks, a synthetic medium, g, of low phosphate concentration was used. It has the following composition:

NaCl 5 g.; NH_4Cl 1 g.; $MgSO_4(7\ H_2O)$ 0.1 g.; $CaCl_2$ 0.1 g.; $FeSO_4$ $(7\ H_2O)$ 0.01 g.; *dl*-tryptophane 0.01 g.; glycerol 2 g.; gelatin 0.01 g.; Tween 80 0.2 g.; casamino acids (Difco) 1 g.; KH_2PO_4 0.005 g.; Na_2HPO_4 0.01 g. To these substances were added 1000 cc. of distilled water and the pH adjusted to 7.1 with NaOH.

Considerable amounts of P were introduced with the casamino acids, bringing the total phosphate concentration up to 15.5 γ/cc. All experi-

ments were conducted at 37° C. The general methodology used was similar to that described by Adams.[3] All centrifugations were done in a Servall Angle centrifuge at 10° C. In some experiments ultra-violet irradiated T2r+ was used to inhibit lysis or to lyse from without; a heavy dose of irradiation giving about 50 hits per particle[4] was administered just before the phage was to be used. Measurements of radioactivity were made on 0.15-cc. liquid samples by means of an end-window G.M.-tube. Under these conditions 1 μC of P^{32} gave 4×10^5 counts/min., an efficiency of 20%.

Experimental.—Preparation of P^{32} Labeled Bacteriophage: T2r+ was labeled by growth on B/1 in *g* medium containing 5 μC P^{32} per cc. After incubating several days at 37° C. the phage was purified by differential low- and high-speed centrifugation. The phage was first resuspended in 0.05% NaCl since at this low salt concentration complexes consisting of phage plus bacterial debris dissociate so that the debris can be removed by centrifugation.[5] The phage was finally resuspended in *g* medium. For subsequent experiments it was important to make sure that, apart from phage, the preparation contained no P^{32}-labeled material which would adsorb on bacteria; to remove any such material heat killed B/2 was added, left in for 30 minutes and then removed by low-speed centrifugation. Less than 10% of the P^{32} of the preparation were removed in this way; it should be noted, however, that dead phage particles are not removed by this treatment. The final preparation contained 1.5×10^{11} particles/cc. with a radioactivity of 5×10^5 counts/min./cc. In phage prepared similarly by Lesley, French and Graham over 95% of the P^{32} was located in DNA.[6] Our stock adsorbed well on exponentially growing B/1, consistently giving over 97% adsorption in 4 minutes when the B/1 concentration was 5×10^8 cells/cc. This phage was used to infect bacteria.

Experiment I. Transfer of P^{32} During the First Generation of Phage Growth.—Exponentially growing bacteria from an aerated culture in unlabeled broth were concentrated in the centrifuge at 10° C. and resuspended in unlabeled broth at 37° C. to give a final concentration of 5×10^8 cells per cc. Labeled T2r+ was then added at a ratio of 2 phage particles per bacterium. Under these conditions approximately 90% of the phage was adsorbed in 1 minute. Two minutes after infection, the culture was chilled, and centrifuged at 5000 g. for 4 minutes. The supernatant containing unadsorbed radioactive material was discarded and the pellet resuspended in broth at 37° C. to give a final concentration of infected cells of approximately 10^8/cc. A phage assay was then made to determine the number of infected cells, and aeration was started. Five minutes after resuspension, approximately 5 particles per bacterium of unlabeled, ultra-violet (UV) irradiated T2r+ were added to inhibit lysis uniformly in the whole culture.[7] On incubation overnight the culture cleared and was centrifuged twice at

5000 g. for 5 minutes to remove bacterial debris and then at 12,000 g. for 1 hour to sediment the phage. The various pellets were resuspended in broth and assayed for P^{32} and viable phage. When assaying for phage, intermediate dilutions were made into distilled water and were kept for 10 hours to remove bacterial debris which temporarily inactivates phage by blocking its ability to adsorb.[5] The number of active particles often rises by a factor of 2 to 3 after distilled water treatment.

Table 1 shows the results of this experiment in which 37% of the radioactivity was found in the high-speed pellet. This pellet also contained the large majority of the progeny phage. To determine what fraction of the radioactivity of the high-speed pellet was actually in progeny phage, anti-T2 rabbit serum, which had been absorbed previously with a great amount of live B/1 to remove possible antibacterial antibodies, was added to the resuspended pellet at a final dilution of 1 to 10. The mixture was incubated at 45° C. for 2 hours and left at 4° C. overnight. The precipitate of phage and antibody which formed was collected by low-speed centrifuga-

TABLE 1

DISTRIBUTION OF RADIOACTIVITY AFTER INFECTION WITH P^{32} LABELED $T2r^+$

Bacterial concentration = 1.05×10^8/cc. Adsorbed phage particles/bacterium = 2.1. Burst size = 465.

MATERIAL	PHAGE TITERS/CC.	RADIOACTIVITY IN 20 CC. COUNTS/MINUTE		%
Lysate	4.9×10^{10}	14,140		...
Low-speed pellets	0.10×10^{10}	940		6.2
High-speed pellet	4.2×10^{10}	5,590	15,080	37.0
High-speed supernatant	0.15×10^{10}	8,550		56.8

tion and was found to contain over 90% of the radioactivity originally present in the high-speed pellet. It is thus highly probable that the P^{32} in the high-speed pellet was largely present as phage material.

The possibility remains, however, that the radioactivity is not associated with the progeny phage but with slightly altered infecting particles that sediment at high speed. The question might be decided by examining the infected bacteria midway in the latent period just before the first infective particles begin to appear.[8] If after artificial lysis at this time the radioactivity is in sedimentable fragments of the infecting phages we might expect to be able to isolate them. On the contrary if this radioactivity is associated with progeny particles, that are not yet large enough to sediment, then we might not find much radioactivity in the high-speed pellet.

Experiment II. Distribution of Parental P^{32} Before the First Progeny Particle Appears in an Infected Bacteria.—Bacteria in the logarithmic phase of growth were concentrated to 5×10^8 cells/cc. and infected with an average of 6 labeled $T2r^+$ particles per bacterium. Two minutes after infec-

tion, the culture was chilled and centrifuged at 5000 g. for 4 minutes to remove unadsorbed radioactive material. The pellet was resuspended in broth at 37° C. at a bacterial concentration of 10^8 cell per cc. Aeration was started and an assay made of the number of infected bacteria. The cooling and centrifugation retards the progress of phage growth by about 8 minutes.

Thirteen minutes after infection 5 UV-inactivated T2r+ particles per bacterium were added to inhibit lysis. At 19 minutes, corresponding to about time 11 minutes in the experiments of Doermann, the culture was divided into two tubes, A and B. To tube A was added sufficient KCN to make a final concentration of $^1/_{1000}$ M and approximately 2000 UV-treated T2r+ particles per bacterium. By this means, the infected bacteria are broken open and any newly formed particles released into the medium.[8] Tube B was aerated until 42 minutes when it was similarly treated. The large excess of UV-treated T2r+ served both to break open the bacteria and to saturate the bacterial surface.[9] Readsorp-

TABLE 2

DISTRIBUTION OF RADIOACTIVITY AFTER PREMATURE LYSIS OF BACTERIA INFECTED WITH LABELLED T2r+

Bacterial concentration = 1.12×10^8/cc. Adsorbed phage particles/bacterium = 6.7.

TUBE	TIME OF ADDITION OF KCN + UV T2r+	PROGENY PHAGE PER BACTERIUM	P^{32} DISTRIBUTION, %		
			LOW-SPEED PELLET	HIGH-SPEED PELLET	HIGH-SPEED SUPERNATANT
A	19 minutes	0	12	1.6	86.5
B	42 minutes	248	13	29	58

tion of the newly formed particles onto unlysed bacteria or bacterial debris is thus effectively prevented.[5]

The cultures were then incubated for 2 hours, by which time complete clearing (lysis) had occurred. They were then centrifuged twice at 5000 g. for 5 minutes to remove bacterial debris and finally at 12,000 g. for 1 hour to sediment phage. The excess of UV-treated T2r+ served as carrier for any possible compounds which might be sedimented at high speed in tube A. The various pellets were resuspended in broth and assayed for P^{32} and active phage.

In table 2 the results are presented. It can be seen that the bacteria in tube A were broken open before any progeny particles were present, while in tube B approximately 250 particles per cell were present. The amount of P^{32} in the low-speed pellets was approximately the same in both tubes, 12% of the input. In striking contrast to this similarity between the low-speed pellets, the high-speed pellets from tubes A and B contained 1.6 and 29% of the input P^{32}, respectively. The P^{32} in the high-speed pellet taken

after lysis thus cannot be due to slightly altered infecting particles but represents the incorporation of the P^{32} into the progeny phage. Furthermore, this experiment can be considered as additional evidence of the breakdown of the infecting particle, first postulated by Doermann on the basis of premature lysis experiments.[8] In experiments of this kind virtually all the P^{32} left in the supernatant after high-speed centrifugation can be precipitated by addition of 5% trichloroacetic acid.

The above transfer experiment has been repeated with ratios of adsorbed phage to bacteria ranging from 1 to 6. The transfer of radioactivity to the progeny particles ranged from 20 to 40%, but was not correlated with the number of adsorbed particles per bacterium. There furthermore does not seem to be a noticeable correlation between the burst size and the degree of transfer. While the burst sizes varied from 150 to 600, the transfer percentage in most experiments ranged between 28 and 33%.

TABLE 3

DISTRIBUTION OF RADIOACTIVITY AFTER INFECTION WITH T2r$^+$ LABELED BY ONE GENERATION OF GROWTH IN NON-LABELED MEDIUM AND BACTERIA

Bacterial concentration = 1.32×10^8/cc. Adsorbed phage particles/bacterium = 5.2. Burst size = 316.

MATERIAL	PHAGE TITERS/CC.	RADIOACTIVITY IN 150 CC. COUNTS/MINUTE	RADIOACTIVITY IN 150 CC. %
Lysate	4.17×10^{10}	390	100
Low-speed pellets	0.08×10^{10}	57.5	14.7
High-speed pellet	3.84×10^{10}	115	30
High-speed supernatant	0.38×10^{10}	[a]	

[a] Due to large volume, this could not be counted.

It has been shown by French, Lesley, Graham and Van Rooyen that late adsorbed phage is broken down rapidly.[10] The transfer percentage found in experiments like our No. I, would be too low if some of the progeny had been lost in this way through readsorption unto unlysed bacteria; however, in experiments like our No. II, when readsorption has been prevented, we find similar transfer values and therefore conclude that in our experiments readsorption is not an important factor.[5]

Experiment III. Transfer of P^{32} from Labeled Phage Which Has Gone Through One Cycle of Reproduction in Unlabeled Bacteria: The radioactive phage obtained in the high-speed pellet of experiment I was used to infect non-labeled bacteria in non-labeled broth. Bacteria in the logarithmic phase of growth and at a concentration of 1.3×10^8 cells/cc. were infected with an average of 5 phage particles per bacterium. Following an adsorption period of 5 minutes, the infected bacteria were centrifuged at 5000 g. for 5 minutes to remove unadsorbed materials and were resuspended in the original volume of broth. Aeration was started and 5 minutes later, 5 UV-

treated, unlabeled T2r+ particles per bacterium were added to inhibit lysis. Following overnight incubation, the phage was isolated by differential centrifugation and assays made of the radioactivity and phage in the various fractions.

The results, shown in table 3, indicate a transfer to the progeny of 32%—that is a value similar to that obtained upon reproduction of phage labeled by growth on labeled bacteria in labeled medium.

Conclusion and Summary.—Bacteriophage T2r+ has been labeled with P^{32} by growth in labeled bacteria in labeled medium. This phage has been carried through two successive cycles of reproduction in unlabeled bacteria. In both cycles it was found that the progeny phage had received about 30% of the P^{32} of the infecting phage particles. It is concluded that the phosphorus which is transferred to the progeny is not located, after transfer, in specific parts of the DNA of some or all of the particles of the progeny.

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*A SINGLE-CELL ANALYSIS OF THE TRANSMISSION OF ENZYME-FORMING CAPACITY IN YEAST**

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Communicated by M. M. Rhoades, June 13, 1950

Introduction.—Winge and Roberts[1] reported that certain yeast strains can be distinguished by the fact that they take 5 to 6 days longer than normal varieties to adapt to galactose. They named this phenomenon "long-term adaptation" and showed that the character was determined by a single recessive allele (g_s) which segregated normally in crosses to the fast adapting wild type, bearing the dominant gene (G).