Development of Bacteriophage α : Requirement for the Integrity of One Strand of Parental Deoxyribonucleic Acid

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Bacteriophage α -*Bacillus megaterium* complexes are as sensitive as free phage to the decay of ³²P incorporated in parental phage deoxyribonucleic acid during the eclipse period.

Bacteriophage α is unusually sensitive to X-irradiation and to the decay of incorporated ³²P atoms (1). The efficiency of inactivation for X or γ rays (4) or ³²P decay is 0.5 for free phage, whereas for most double-stranded deoxyribonucleic acid (DNA) phages, it is 0.05 to 0.1 (7, 10). In order to explain this unusual radiosensitivity, developing complexes of α -infected *Bacillus megaterium* (tiberius), a sensitive host, were examined by Donini and Epstein (2) for their sensitivity to X rays and ultraviolet (UV) light. Two distinct responses were found; for X rays the sensitivity of the complex decreased gradually as a function of time of development, whereas for UV irradiation the sensitivity remained that of free phage for 20 min, although a multitarget shoulder developed after 10 min. Neither multiplicity reactivation nor DNA pool size could explain the UV results and a repair system was suggested as one explanation of the X ray results. In an attempt to discriminate between these possibilities, we investigated the effect of ³²P decay on developing complexes and found an unusual pattern of sensitivity.

B. megaterium and bacteriophage α_{cs} , a clear plaque-forming mutant, were kindly supplied by P. F. Davison, Biology Department, Massachusetts Institute of Technology. α_{cs} Phage were labeled with ³²P by infecting an aerated culture of *B. megaterium* at 5×10^7 cells/ml, in a tris-(hydroxymethyl)aminomethane minimal medium supplemented with 0.1% tryptone broth and containing ³²P at a specific activity of either 490 mCi/mmole or 920 mCi/mmole [³¹P in medium determined by a colorimetric method based on the reduction of phosphomolybdate to molybdenum blue (3)] at a multiplicity of infection (MOI) of 0.1. After lysis, the phage were purified

by differential centrifugation. Complexes were prepared by infecting *B. megaterium* in broth medium with purified labeled phage at an MOI of 0.001 to 0.01. Samples were removed from the mixture at various times after infection, immediately arrested by freezing in a dry ice-acetone bath, and stored at -65 C to accumulate decays. At various time intervals up to 25 days, both ³²P-labeled complexes and corresponding controls were thawed and immediately assayed for plaque-forming ability.

The results of this experiment are given in Fig. 1 and 2 for the two specific activities. The age of the complex together with the calculated value for *P*, the probability of inactivation per ³²P decay, which is determined from the slope of the line, are indicated. Recovery of unlabeled complexes stored at -65 C was greater than 90% for the duration of the experiment. In Fig. 3, *P* is plotted against the development time; the sensitivity of the complex remains at the value for free phage (0.5) for 20 to 25 min which is approximately the length of the eclipse period (2). After this time the value of *P* decreases markedly until it has reached a value of essentially 0 at 33 min after phage infection.

Considering that the decay of ³²P causes single-strand breaks with an efficiency of one (9), two conclusions can be drawn immediately from our data: no repair system is induced by phage α for ³²P lesions, and the integrity of the parental phage DNA is indispensable for the first 20 to 25 min of phage development. Since the killing efficiency is 0.5 and since it has been shown that transcription takes place from one strand of α DNA both in vivo (11) and in vitro (6), it is tempting to suggest that the value of 0.5 for the efficiency of inactivation arises from the importance of this strand.

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FIG. 1. Inactivation of α phage-*B. megaterium* complexes by the decay of ^{32}P incorporated into parental phage. The number of ^{32}P -atoms per phage is 4.5. The age of the complex and P , the calculated probability of inactivation per ^{32}P -decay, are shown in the figure. The experimental error bars represent $\pm$ one standard deviation.

FIG. 2. Inactivation of α phage-*B. megaterium* complexes by the decay of ^{32}P incorporated into parental phage. The number of ^{32}P -atoms per phage is 8.5. The age of the complex and the probability of inactivation per ^{32}P -decay, P , are shown.

FIG. 3. Probability of inactivation per ^{32}P -decay for α phage-*B. megaterium* complexes as a function of time in development. N^* is the number of ^{32}P -atoms per phage.

If one strand of α controls development, either ribonucleic acid (RNA) or DNA synthesis must take place from this strand rather than from progeny DNA. We have not yet tested this directly, but three observations provide indirect evidence. (i) It has been shown that there exists a quantitative relationship between X-ray-induced breaks and the reduction of *in vitro* RNA synthesis (8); (ii) we observed that phage development is arrested by rifampin, which inhibits RNA polymerase (5), during the eclipse period; and (iii) our preliminary experiments show that normally DNA synthesis starts within 2 min of

infection and continues approximately linearly during eclipse.

We conclude, therefore, that, for α phage development, the molecular integrity of one strand of the parental phage genome must be maintained, and that ^{32}P decay in this strand blocks either RNA synthesis or DNA synthesis which is a control of late transcription.

A relationship between the transcription process and the Luria-Latarjet effect for X rays will be discussed in a future paper.

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