BIKEN'S JOURNAL
Preliminary Report

Genetic Studies on the Non-infectious Phage Produced in the Presence of Mitomycin C*

An antibiotic mitomycin C inhibits DNA synthesis of *E. coli* B, but does not inhibit that of phage-infected cells (Sekiguchi and Takagi, 1960a). However, the phages produced in the presence of mitomycin C are non-infectious**, that is, they lose the ability to make a complete cycle of infection terminating in the release of more infective phages, although they can be adsorbed on the sensitive bacteria, kill them, and inject their DNA (Sekiguchi and Takagi, 1960b). These results suggest that the non-infectivity of the MC-phage may be due to the biological inactivity of their DNA. Thus, it seems of great interest to investigate the genetic nature of the MC-phages.

E. coli B were infected with 1 to 3 phage particles per bacterium in a synthetic growth medium containing 50 μg per ml of mitomycin C, and shaken at 37°C for 2 to 4 hr. KCN was added at a final concentration of M/500 to lyse infected cells completely. MC-phages were purified by differential centrifugation. The number of non-infectious particles in the MC-phage suspension was calculated by using the Poisson formula from measuring the reduction in the number of colony-forming bacteria. The active phage particles in the suspension were assayed by the soft agar layer method.

Experiments of multiplicity- and cross-reactivation were done in buffer. Logarithmic phase cells were prepared by diluting an overnight culture with synthetic growth medium and shaking it at 37°C for 3 hours. The bacteria were centrifuged and resuspended in buffer. To the phage suspension was added an equal volume of the bacterial suspension and the adsorption mixture was incubated for 5 to 10 minutes. In cross-reactivation experiments, unadsorbed phages were neutralized by addition of specific antiphage serum. Then appropriate dilutions were made in broth or buffer, and the infected bacteria were assayed on an indicator strain before lysis.

As shown in Table 1, the number of infective centers increased more than the number of active phage particles, with the multiplicity of infection. Bacteria singly infected with MC-phage produced no plaques, but some of the bacteria infected with 2 or more non-infectious phage particles did produce plaques. A decrease in the number of infective centers at the highest multiplicity of infection tested may be caused by "lysis from without" as pointed by Weigle and Bertani (1956).

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* The paper was reported at the Annual Meeting of the Genetic Society of Japan in 1960.
** These phages are called MC-phages.

Table 1. Multiplicity Reactivation of MC-phage

Multiplicity of infection	No. of input phage particles		No. of infective* center (C) (Exper.)	Efficiency of** multiplicity reactivation
	non-infective particles (A)	active particles (B)		
50	5.0×10^{9}	1.8×10^{7}	1.5×10^{7}	——
10	1.0×10^{9}	3.7×10^{6}	3.8×10^{7}	3.6×10^{-1}
2	2.0×10^{8}	7.5×10^{5}	5.6×10^{6}	8.0×10^{-2}
0.4	4.0×10^{7}	1.5×10^{5}	3.4×10^{5}	3.2×10^{-2}
0.08	8.0×10^{6}	3.0×10^{4}	3.2×10^{4}	$<2.0\times10^{-3}$

E. coli B ($S=1.0\times10^{8}$ per ml) were infected with various multiplicities of MC-T4r^{+} and incubated for 10 minutes. The adsorption mixtures were appropriately diluted and assayed for infective center* by the agar layer method.

** The efficiency of multiplicity reactivation is the ratio of productive complexes to multiply infected cells: $(C)-(B)/S\{e^{-B/S}\times[1-(A/S+1)e^{-A/S}]\}$.

It is clear from Table 2 that MC-T4r+ can contribute their genetic trait (r+ marker) to progeny phages in a infection together with active $T4r_{196}$. Another experiment showed that MC-phages cooperate with UV-inactivated phages to yield active phages (unpublished data).

Table 2. Cross Reactivation of MC-T4r^{-} by $T4r_{196}$

Host cell	Parent phages	Cells which liberate r^{+} phages per ml. (plaques on K-12)	Efficiency of** cross reactivation
B	MC-T4r^{+}	6.3×10^{5} (B)	
	MC-T4r$^{+}\times T4r_{196}$	1.9×10^{6} (A)	4.2×10^{-2}
	$T4r_{196}$	$<10^{4}$	
K-12	MC-T4r^{+}	2.7×10^{5} (B)	
	MC-T4r$^{+}\times T4r_{196}$	1.3×10^{6} (A)	3.3×10^{-2}
	$T4r_{196}$	$<10^{4}$	

E. coli B ($S=8.0\times10^{7}$) and K-12 ($S=6.0\times10^{7}$) were infected with 0.5 and 0.67 multiplicities (n_1) of the MC-phage and with 5.2 and 8.3 (n_2) of $T4r_{196}$, respectively. The infected bacteria were plated on K-12.

** The efficiency of cross reactivation is the ratio of the number of plaques on K-12 to that of bacteria with mixed infection: $(B)-(A)/S(1-e^{-n1})(1-e^{-n2})$.

Delbrück and Luria (1942) found that, if a bacterium is infected simultaneously by T1 and T2, only T2 grows and T1 multiplication is completely suppressed. Furthermore, they observed that UV-inactivated T2 was still able to exclude T1 completely (Luria and Delbrück, 1942). The excluding ability of MC-phages was examined. There was no decrease in turbidity of a culture of *E. coli* B, when infected simultaneously with MC-T2r and active T3 (Fig. 3).

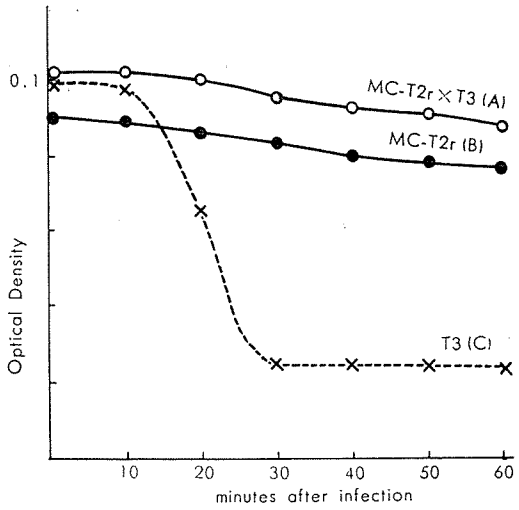

Fig. 1. Exclusion Effect of T3 by MC-T2r

Change in turbidity of a culture of *E. coli* B infected with T3 (m. o. i.=5.0) and MC-T2r (m. o. i.=3.8). T3-productive complexes were assayed on B/2 7 minutes after infection and the following values were obtained.

A=4.1×10^6/ml.

C=8.6×10^7/ml.

Three phenomena, known to occur after UV irradiation (Stahl, 1959), namely, multiplicity reactivation, cross reactivation, and the mutual exclusion effect, also occur with the MC-phage. Mitomycin C does not inactivate free phage particles, but acts on phage-infected bacteria to produce non-infective phage particles. The MC-phages used were produced in the presence of mitomycin C during all stages of infection from adsorption to lysis. The above experiments showed that there are some healthy regions in the phage DNA produced in the presence of mitomycin C, although the above condition is different from that of UV-inactivation. The number of phage particles in the MC-phage suspension calculated from the DNA content was nearly equal to the bacteria killing titer. The MC-phage may carry a complete set of phage genomes in which inactive regions are present. It is of interest to investigate how such a MC-phage is produced, and this investigation will clarify the mechanism of the action of mitomycin C.

ACKNOWLEDGEMENTS

The author wishes to express his thanks to Prof. H. Kikkawa and Dr. M. Sekiguchi for their advice and discussion.

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Received on February 17, 1962