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SHORT NOTES

The control of host-induced modification by phage P1

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The phenomenon of host-induced modification has two facets, restriction and modification. Recently these two processes have been clarified by Arber and Dussoix in the system where phage λ multiplies either in *Escherichia coli* K12, or in K12 cells lysogenic for the phage P1. The phage λ·K† is accepted only by 1 in 10^4 recipient K(P1) cells; these produce modified λ·P1 phage particles, which then are able to infect either K or K(P1) cells with an efficiency of one. In a beautiful series of experiments Arber and Dussoix showed that modification must be a process which acts directly on the DNA of phage λ, and that when unmodified λ-DNA enters K(P1) cells it is rapidly degraded into small molecular weight fragments.

These findings led Arber to the generalization that modification should affect all forms of DNA which are synthesized in K(P1) cells, and that restriction would apply to all DNA that can be transferred from K to K(P1). He was able to demonstrate that restriction and modification did occur during the transfer of the bacterial genome in conjugation, and also during the transfer of DNA by transforming principle and by transduction (Arber & Dussoix, 1962; Dussoix & Arber, 1962; Arber, 1962).

In this note we shall first present some data extending Arber's observations to the transfer of a variety of episomes, and then show how these results can be utilized to demonstrate that the roles of restriction and modification imposed by P1 are under independent genetic control.

THE RESTRICTION AND MODIFICATION OF DIFFERENT TYPES OF DNA BY PHAGE P1

In Table 1 are collected some previous results obtained by Lederberg (1957), and by Arber & Dussoix (1962), together with a number of new observations. It can be seen that the degree of restriction imposed by P1 varies between 0 and 10^4. The virulent phages T2, T4, T5, and T6 show no restriction, while the temperate phages λ and P2, and the semi-temperate phages T1 and T3 are all restricted by approximately 10^{-4}. The episome F-lac, which after conjugation between male and female cells transfers the gene for lactose fermentation (Jacob & Adelberg, 1959), undergoes 100 times less restriction than does phage λ, and the behaviour of the colicin factor I (Monk, personal communication) is

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† Following the notation of Arber & Dussoix (1962) the host-specificity of a phage will be represented by the name of the phage followed by the name of the host strain in which it was last grown. For example λ·K means phage lambda which has been grown in K12 bacteria, while λ·P1 means phage lambda which has been grown in K12(P1) cells.

similar. The gene for galactose fermentation is also restricted 100 times less than phage λ, independently of whether it is transferred by the episome F-gal (Hirota & Sneath, 1961) or the transducing phage λdg (Morse, Lederberg & Lederberg, 1956; Arber, 1958).

Apart from the cases of F-gal and col-I transfer, which have not been tested, and phage T3, which will be discussed below, the DNA which is accepted in the minority of recipient cells is modified during growth in K(P1), and can subsequently be transferred to K and K(P1) with equal efficiency.

Table 1. *Restriction of different kinds of DNA in K(P1)*

DNA from K	Fraction of K(P1) which accepts compared with K
T1,* T3, P2,* λ†	10^{-4}
F-lac, F-gal, λdg, col-I	10^{-2}
T2,* T4,* T5,* T6*	1·0

* Data from Lederberg (1957).
† Data from Arber & Dussoix (1962).

The case of phage T3 is interesting as it undergoes no modification during growth in K(P1). The fraction of 1 in 10^4 cells which accept T3·K phages liberates only phage particles which can plate on K, but none that can plate on K(P1), so that the plating efficiency of T3 on K(P1) indicator is in fact zero. If large numbers ($> 10^8$) of T3 phage are spotted on plates seeded with K(P1) areas of lysis are observed, but these are due to killing of the K(P1) cells by the phage. This result is in contradiction to that of Lederberg (1957), who found modification of T3 by the prophage P1, but we have checked this result with T3 phage obtained from three different sources, always with the same result.

THE INDEPENDENT CONTROL OF RESTRICTION AND MODIFICATION BY PHAGE P1

After infection by phage particles it is not possible to isolate the small fraction of K(P1) cells which accept unmodified phage, as they are killed by the lytic multiplication of the phage. The finding that the episomes F-lac and F-gal, and also transduction of gal by phage λdg, are restricted in K(P1), however, made it a simple matter to isolate these rare cells and investigate their properties. After infection with F-lac it was found that about one-third of the K(P1) lac^+ cells recovered differed from normal in their ability to cause restriction and modification. By isolating the P1 phage carried in these altered cells, and using it to lysogenize K bacteria, it was possible to demonstrate that these changes in behaviour were due to alterations in the prophage, and not due to any other alterations in the recipient bacteria.

These P1 phages with novel properties were of two distinct kinds, whose behaviour with regard to restriction and modification are summarized in Tables 2 and 3.

It can be seen that, in contrast to normal P1, which causes both restriction and modification, the first type of P1 variant causes no restriction but induces the usual type of modification, while the second type causes neither restriction nor modification. If normal P1 is arbitrarily given the genetic structure r^+m^+, then these two mutants can be designated r^-m^+ and r^-m^-.

These results show that the control over the processes of restriction and modification induced by P1 are each determined by a different gene. This finding is in agreement with the demonstration of Arber & Dussoix (1962) that after the infection of K cells by P1 phage

particles modification is rapidly initiated, but that the onset of restriction does not commence for a considerable period.

When similar experiments to those performed with F-lac are performed either with F-gal or with λdg, then quite different results are obtained. Out of 100 K(P1) recipients

Table 2. *The restricting capacities of P1 prophages isolated from K(P1) cells which have accepted F-lac·K*

	Relative plating efficiencies			Relative efficiency of transfer of		
Prophage	λ	λ·P1	T3	F-lac	F-lac·P1	P1 immunity
None	1·0	1·0	1·0	1·0	1·0	−
Wild type P1 r^+m^+	10^{-4}	1·0	$<10^{-8}$	10^{-2}	1·0	+
Type I P1 r^-m^+	1·0	1·0	1·0	1·0	1·0	+
Type II P1 r^-m^-	1·0	1·0	1·0	1·0	1·0	+

Table 3. *The modifying capacities of P1 prophages isolated from K(P1) cells which have accepted F-lac·K*

	Relative plating efficiencies		Relative efficiency of transfer of	
Prophage	λ·P1 r^-m^+	λ·P1 r^-m^-	F-lac·P1 r^-m^+	F-lac·P1 r^-m^-
None	1·0	1·0	1·0	1·0
Wild type P1 r^+m^+	1·0	10^{-4}	1·0	10^{-2}

tested in each case which accepted the donor DNA, only one P1 variant was obtained, that being a P1(r^-m^-) mutant from the F-gal transfer. This demonstrates clearly that the P1 variants obtained from the F-lac experiment are not a fraction pre-existing in the K(P1) population, but rather are formed in some way after F-lac infection. The manner in which this occurs is at the moment under investigation.

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