THE CHEMICAL BASIS FOR A CASE OF HOST-INDUCED MODIFICATION IN PHAGE T2

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The first well-documented case of host-induced modification (Luria and Human, 1952) concerns the multiplication of phage T2 in certain strains of Escherichia coli B that can no longer adsorb the phages T3, T4 and T7. Normal T2 phage particles produce plaques with a high efficiency when plated with coli B or with Shigella dysenteriae bacteria, but produce no plaques when plated in moderate amounts with these B/3,4,7 strains. The B/3,4,7 cells do, however, adsorb T2 phages, and proceed to carry out one cycle of phage growth, yielding a burst of progeny phage particles which are still able to produce plaques when plated with Shigella, but which now possess the novel character of being unable to produce plaques when plated with coli B. This character is not inherited, since one further growth cycle in Shigella restores to all progeny phage the capacity to produce plaques with coli B.

It is clear that the strains B and B/3,4,7 differ in at least two ways. Firstly, the polysaccharide layer in the cell wall of the mutant has been altered, for it is known that it is this layer which contains the receptor sites for the phages T3, T4 and T7 (Weidel, 1958). Secondly, T2 phage DNA synthesized in the mutant differs from the normal in some manner which prevents

it from carrying through a normal cycle of infection in cells of coli B.

In considering what single chemical change could have been brought about by the mutation from B to B/3,4,7 that would account for both of these differences, we noted the recent publications which have described the characteristics of mutant bacteria which lack the capacity to synthesize uridine diphosphate glucose (UDPG) (Fukasawa et al, 1962; Sundarajan et al, 1962). It seemed that this type of mutational change could adequately explain the observed results. On the one hand UDPG is necessary for the incorporation of glucose and galactose into the polysaccharide layer of cell walls (Sundarajan et al, 1962); the absence of these sugars would certainly alter the phage receptor sites in this structure. On the other hand, UDPG is utilized for the addition of glucose to the 5-hydroxymethylcytosine found in the related phages T2, T4 and T6 (Kornberg et al, 1959). Any phages released by these UDPG mutants would therefore contain no glucose (Erikson and Szybalski, 1963; Shedlovsky and Brenner, 1963), and this lack of glucosylation in the T2 DNA could well render it more susceptible to nuclease attack in newly-infected cells of certain strains of bacteria.

If the lack of the enzyme UDPG synthetase was indeed the basic chemical difference between B and B/3,4,7 cells, it should have one further easily observed consequence: the mutant strains should be unable to ferment galactose.

We have isolated a number of independent B/3,4,7 strains and shown that all of those which were not able to plate T2 phage were galactose negative. In fact, they were all galactose sensitive, in that growth was stopped in minimal medium

by the addition of M/100 galactose, which indicates that the inability to ferment galactose was not due to the lack of galactokinase. The DNA of the T2 phage particles released by a single cycle of growth in one of these mutants has been shown by the anthrone test to contain less than 5% of the glucose of an equivalent amount of normal T2 DNA.

It would appear that a large class of B/3,4,7 mutants owe their resistance to the phages T3, T4 and T7 to their inability to synthesize UDPG. The chemical basis for the form of host-induced modification described with these mutants must therefore reside in the presence in coli B and its derivatives (but not in Shigella) of some enzymes, perhaps nucleases, which prevent T2 DNA that is unprotected by glucosylation from successfully initiating a cycle of phage multiplication.

While this manuscript was in preparation we learnt that similar conclusions have been arrived at by workers in the laboratory of Dr. Luria at the Massachusetts Institute of Technology.

References

Erikson,R.L. and Szybalski,W., Abstr.Biophys.Soc., 7th Ann.Meet., p.WA8, 1963.

Fukasawa,T., Jokura,K. and Kurahashi,K., Biochem.biophys.res.Comm., 7, 121, 1962.

Kornberg,A., Zimmerman,S.B., Kornberg,S.R. and Josse,U., Proc.nat.Acad.Sci.(Wash.), 45, 772, 1959.

Luria,S.E. and Human,Mary L., J.Bact., 64, 557, 1952.

Shedlovsky,A. and Brenner,S., Proc.nat.Acad.Sci.(Wash.), in press.

Sundarajan,T.A., Rapin,A.M.C. and Kalckar,H.M., Proc.nat.Acad. Sci.(Wash.), 48, 2187, 1962.

Weidel,W., Ann.Rev.Microbiol., 12, 27, 1958.