SHORT PAPER

Uneven distribution of nitrosoguanidine- and hydroxylamine-induced mutants of three coliphages

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ABSTRACT

166 suppressor-sensitive (*sus*) mutants belonging to phages HK243, HK022, and λ were analysed. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)-induced mutants of all three phages showed a restricted distribution; a majority being of one or the other of two cistrons. Hydroxylamine (HA)-induced mutants of phage HK022 were found to be randomly distributed over a number of cistrons but in the case of phage λ , out of 21 HA-induced mutants 10 were of cistron *P* and 6 of cistron *R*.

In connection with genetical investigations of locally isolated bacteriophages, a number of suppressor-sensitive (*sus*) mutants have been isolated. Complementation tests have revealed that the frequencies of the *sus* mutants of various cistrons are far from random, especially if the mutagenic agent used is *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). Experiments were extended to bacteriophage λ hoping that study of this genetically well understood phage may provide some explanation of the non-random frequencies of *sus* mutants of the local phages. A total of 166 *sus* mutants were studied. We describe below the distribution pattern of the MNNG-induced *sus* mutants in various cistrons of the three phages and compare it with the distribution pattern of the hydroxylamine (HA)-induced mutants.

The following clear plaque mutants of the three coliphages were used; λ cI857 (Sussman and Jacob 1962; henceforth λ) was used for HA- or MNNG-mutagenesis; HK022cI12 was subjected to HA treatment and HK022v-1 (Dhillon and Dhillon 1976) was subjected to MNNG treatment whereas HK243r-1 (Dhillon *et al.* 1980) was subjected to MNNG treatment only. MNNG treatment was *in vivo*, essentially as described before (Dhillon and Dhillon 1974). HA treatment was as described by Tessman (1968) with minor modifications. For example, phage λ was treated in 1.0 M aqueous solution of HA containing 1 mM of di-

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sodium salt of ethylenediamine tetracetic acid (EDTA) for six hours at room temperature. HK022*cI12* was treated in a solution of 20 mM sodium phosphate, 1 mM EDTA, pH 5.6 with the concentration of HA varying from 0.4 to 1.0 M in different experiments. Most of the mutants described here were isolated after 24 h of HA treatment. Suppressor-sensitive (*sus*) mutants were detected by using mixed indicator containing equal volumes of Su^+ and Su^- cultures. Complementation tests were used to assign mutants to their respective cistrons as described (Dhillon and Dhillon 1976).

The defective cistrons of *sus* mutants of λ were identified by complementation tests using canonical *sus* mutants of cistrons *N*, *O*, *P*, *Q*, *S*, *R*, *A*, *L*, and *J*. The defective cistrons of mutants belonging to cistrons other than these could not be identified but their map locations were determined by using prophage deletion mutants isolated from a Su^- strain lysogenic for $\lambda cI857$ *xis-1* (Guarnero and Echols 1970). Fig. 1a shows seven prophage segments defined by six prophage deletion mutants, the location of cistrons in the prophage segments and the distribution of *sus* mutants.

An uneven distribution of MNNG-induced *sus* mutants was originally observed in the case of phage HK243. As can be seen from Table 1, 9 mutants of cistron *D* and 5 mutants of cistron *H* when added together represent 44% of the 32 MNNG-induced *sus* mutants.

A similar clustering was observed when MNNG-induced *sus* mutants of phage HK022 were analysed (Table 1). Again, two cistrons, viz., *B* and *G* accounted for 56% of the MNNG-induced *sus* mutants. This uneven distribution explains why 50 *sus* mutants were confined to either one of a total of 13 cistrons giving an average of 3.8 mutants per cistron. In contrast, 32 of the HA-induced *sus* mutants were found to be distributed over 16 cistrons giving an average of 2 mutants per cistron. Furthermore, HA-induced mutants did not show any evidence of preferential localization to any single cistron.

Only 4 out of 25 established cistrons of phage HK022 were seen to have been mutated by both mutagens. Nine cistrons had been mutated only by MNNG and 12 cistrons only by HA. Thus, we conclude that HA- and MNNG-induced mutants of phage HK022 do not show any significant overlap of the mutated cistrons.

We felt that an understanding of the mechanisms underlying the distribution patterns of *sus* mutants induced by various mutagens might be forthcoming if the sequences of the cistrons, the functional nature of various cistrons, and the pattern of DNA synthesis were known. Information on these aspects for phages HK022 and HK243 is not available. For this reason, MNNG and HA were used to induce *sus* mutants of bacteriophage λ because of the availability of vast amount of genetic data on this phage (Hershey 1971).

As in the case of phages HK022 and HK243, MNNG-induced *sus* mutants of phage λ also showed a clustered distribution (Table 1b and Fig. 1a). 50%

Fig. 1a. Deletion-defined segments of the genetic map of prophage *lambda* and the frequencies of MNNG- and HA-induced mutants belonging to different prophage segments.

Fig. 1b. Genetic distances (recombination frequencies) between various mutants of the *P* cistron of phage *lambda*. Frequencies of *sus*⁺ progeny in control cultures were 0.001 percent or less.

Table 1. *Frequency of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)- and hydroxylamine (HA)-induced mutants of different cistrons of three coliphages*

Phage, Cistron, Mutagen and Frequency of Mutants*								
HK243		HK022			Lambda			
Cistron**	MNNG	Cistron**	MNNG	HA	Prophage Segment***	Cistron**	MNNG	HA
A	1	A	2	0	2	P	5	10
B	1	B	15	2				
C	1	C	4	0	3	Q	0	1
D	9	D	2	0		S	0	1
E	2	E	1	3		R	10	6
F	1	F	2	0				
G	1	G	13	0	4	A	2	0
H	5	H	1	0		NG18	2	0
J	2	J	4	0				
K	3	K	2	0	5	NG3	3	1
L	1	L	2	0				
M	1	M	1	1		NG8	2	0
N	1	N	0	1		NG19	1	0
P	1	O	0	2	6	NG25	1	0
Q	1	P	0	1		L	0	0
R	1	Q	1	3				
		R	0	4				
		S	0	4		HA7	0	2
		T	0	2		J	4	0
		U	0	3				
		V	0	1				
		W	0	1				
		X	0	2				
		Y	0	1	7			
		Z	0	1				
Total	32		50	32			31	21

* Figures under columns MNNG and HA denote the number of mutants belonging to different cistrons recovered during this study.

** Cistrons of phages HK243 and HK022 were assigned capital letters in the order of recovery of mutants. The genetic maps of these two phages have not yet been prepared and therefore the sequences of cistrons are not known.

Mutants of nine cistrons of phage *lambda* were identifiable by the availability of their respective alleles. The remaining cistrons are designated by one of their alleles recovered during this study. The identifying alleles bearing the prefix NG were obtained after MNNG treatment whilst those bearing the prefix HA were obtained after HA treatment.

The exact sequence of cistrons in prophage segment 6 is not known.

*** See Fig. 1a.

of the *sus* mutants were confined to either one or the other of two cistrons; viz., cistrons *P* and *R*. However, in contrast to phage HK022 in which HA-induced mutants were more evenly scattered over various cistrons, a clustering of HA-induced mutants of λ was observed. Over 76% of the HA-induced *sus* mutants were found to be confined to cistrons *P*, or *R* i.e., the same two cistrons which had been observed to be highly responsive to MNNG. The confinement of large numbers of HA- or MNNG-induced mutants to cistrons *P* and *R* leaves very few mutants belonging to other cistrons. This paucity of numbers precludes making any judgement as to the overlap of MNNG- and HA-induced mutants for the rest of the *lambda* phage genome.

Appearances of large numbers of *P* cistron mutants after HA treatment raised the possibility of inadvertant selection of clonal isolates preexistent in the lysates used for mutagenesis. To eliminate this possibility four of the HA-induced mutants of the *P* cistron were crossed with one another. In order to determine their sequence relative to gene *cI*, they were also crossed with the *lambda* strain *cI*⁺*Pam*80. Pertinent data are presented in Fig. 1b. It is quite clear that neither of the four HA-induced mutants tested were mutated at the same site. The four mutants were found to be clearly separable into two pairs lying on either side of *am*80. Members of each pair, however, were genetically distinguishable by virtue of their ability to produce wild type recombinants when crossed with one another. These data, therefore, exclude the possibility of inadvertant clonal selection.

Since we do not as yet know either the functions of the gene products of phages HK022 and HK243 or the spatial relationships of the various cistrons, it is not worthwhile to speculate on the mechanisms underlying the non-random frequencies of *sus* mutants. In the case of phage λ it is to be noted that the two cistrons, namely *P* and *R* which were most responsive to both HA and MNNG are located on the right-hand arm of the vegetative map of this phage (Campbell 1971). These two cistrons control unrelated functions, product of gene *P* being essential for phage-DNA synthesis (Kaiser 1971) and the product of gene *R* being an endopeptidase (Taylor 1971). Referring to the physical and the genetical co-ordinate map of λ (Gottesman and Adhya 1977), it can be seen that genes *P* and *R* are within a DNA segment which is not more than 20% of the total DNA of λ -phage. 50% of the MNNG-induced mutants and 85% of the HA-induced mutants were mappable to the 20% DNA of the right-hand end of the λ map. However, this region also contains three cistrons (*O*, *Q* and *S*) which proved to be non-responsive to MNNG and two of these were only weakly responsive to HA (*Q* and *S*). Thus the causal mechanism leading to non-random frequencies of the *sus* mutants may not be active on the entire genetic region between cistrons *P* and *R*. We are therefore compelled to conclude that whatever mechanism is responsible for the non-random distribution of *sus* mutants, it is likely to be due to some unknown structural features of

these two cistrons.

Although prophage HK022 does not integrate either at the λ - or the ϕ 80-attachment site (Manuscript in preparation), this phage has recently been shown to be a member of the λ -family of temperate coliphages (Dhillon *et al.* 1981). If HK022 has a genetic map orientation of its cistrons similar to that of λ and if identical mechanisms have been involved in giving high frequencies of *sus* mutants of certain cistrons in these two phages, it can be predicted that cistrons *B* and *A* of phage HK022 may be analogous to cistrons *P/R* of phage λ . Experiments to test this possibility are in progress.

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