INDUCTION OF PURE MUTANT CLONES BY REPAIR OF INACTIVATING DNA ALTERATIONS IN PHAGE T4

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CHEMICAL alterations of DNA, which can eventually produce hereditary DNA changes (mutations), have been subdivided into "mutagenic" and "inactivating" alterations (FREESE 1966; FREESE and FREESE 1965b). "Mutagenic alterations" involve minor chemical changes of DNA, usually of one DNA base, which do not prevent DNA replication but which induce a heritable base change (point mutation) in some of the progeny DNA. "Inactivating alterations," in contrast, are more drastic changes of DNA that do block nucleic acid replication, except when they are repaired or occasionally overcome otherwise; they induce most frequently the loss or large alteration of chromosomes. Most agents that induce mutagenic DNA alterations also induce inactivating alterations, with a relative frequency which depends on the agent and the conditions employed. The strongest mutagenic effect per lethal hit has been observed for high concentrations ($\geq$ 1 M) of hydroxylamine (HA)[1] (FREESE, FREESE and BAUTZ 1961; FREESE and FREESE 1964), especially when the production of hydrogen peroxide, by oxidation of HA was inhibited (FREESE and FREESE 1965a).

The mutagenic alteration of a single base in double stranded DNA should produce a mixed clone of mutant and nonmutant offspring, if each of the two DNA strands is copied during replication and if both new double strands end up in viable progeny. The induction of such mixed mutant clones has often been observed and has been most clearly related to the double stranded structure of DNA for the production of mottled *r*-plaques by phage T4 (for nitrous acid: TESSMAN 1959, and VIELMETTER and WIEDER 1959; for 5-bromouracil: PRATT and STENT 1959; for hydroxylamine: FREESE, FREESE and BAUTZ 1961, and SCHUSTER and VIELMETTER 1961).

After phage treatment with an agent such as 1 M HA, which predominantly induces mutagenic DNA alterations, pure mutant clones should be induced only rarely. Except for the occasional loss of some progeny DNA, they should arise almost only when both DNA strands harbor a mutagenic alteration within the same genetic *r*-region. If the two mutagenic alterations would occur in distant DNA regions, pure *r*-clones would appear only rarely, because the unmutated (r^+) information of one gene should often join by recombination the unmutated

[1] Definitions: HA = hydroxylamine; P = titer of phages on bacteria B at time t; P_0 = same at time zero; lethal hits $n = ln(P_0/P)$; r = frequency of pure *r*-plaques per viable phage; m = frequency of mottled *r*-plaques per viable phage; r^+ = standard type strain of phage T4, v = T4v_1 = ultraviolet (UV) sensitive mutant.

information of the distant gene, producing viable r^+ phages in addition to r^- phages. (There are five rounds of mating in one phage cycle: VISCONTI and DELBRÜCK 1953). This expectation is borne out by the experimental results reported below.

It is not possible, however, to predict from previous knowledge what effect inactivating DNA alterations would have on the expression of mutagenized phage DNA. If the inactivating alteration would prevent the replication of both DNA strands, mutagenized phages would be inactivated and the remaining ones would still produce mixed mutant clones. But phages, carrying both a mutagenic and an inactivating DNA alteration, might produce pure mutant clones if either one of two mechanisms would operate: (1) DNA might replicate, at least occasionally, across the inactivating alteration by copying the non-inactivated strand alone; if that strand carried the mutagenic alteration a pure mutant clone would be produced. (2) The inactivating DNA alteration might be excised and the subsequent repair mechanism copy the complementary strand containing the mutagenic alteration. This paper demonstrates the production of pure mutant clones and establishes as its cause the repair of inactivating DNA alterations.

MATERIALS AND METHODS

Phages and bacteria: Standard phage T4v+ (BENZER) and the ultraviolet (UV)-sensitive mutant $T4v_1$ of W. HARM (1963a) have been used. Phage stocks were grown in M-9 with *Escherichia coli* BB (Berkeley), while the phage plating was done on *E. coli* BA (American).

Media: M-9 (ADAMS 1959) + tryptophan 25μg/ml. T-broth = Bacto-tryptone (Difco) 1% plus 0.5% NaCl. Soft agar = T-broth + 0.7% agar. Plate agar = T-broth + 1.5% agar; 45–50 ml per plastic petri dish poured at 50°C. Stopping mixture = 0.05 M Tris, pH 7.5, + 1 M NaCl + 10% acetone. (Acetone added just before use.)

Phage-plating: Plates: T-broth plus 1.5% Bacto-agar (Difco). Top-layer: 2 ml soft agar plus 0.5 ml bacteria grown to 0.5 to 1×10^9/ml in T-broth. The high concentration of bacteria insured that the total multiplicity of plating, with active plus dead phage particles was always less than 1/500. Plates and soft agar were never older than 1 day (stored in the dark at room temperature). Phages were plated in yellow light and incubated for 12 hours at 37°C in the dark.

For the scoring of mutant plaques the phage suspensions were diluted to yield 60 to 100 plaques per plate; 20 to 40 plates were used for each experimental point. For all points of an individual inactivation curve the same tube of bacteria and batch of plates were used for plating.

Mutation induction by hydroxylamine (HA): The reaction mixture contained: $NH_2OH \times HCl$ (Fluka, Zurich, Switzerland), concentration as indicated, adjusted to pH 6.2 by NaOH; 0.05 M sodium phosphate 10^{-3} M $MgCl_2$. Phages ($\sim 1 \times 10^{11}$/ml) were diluted tenfold into ice-cold reaction mixture, mixed well, and a control sample diluted 50-fold into ice-cold stopping mixture. The reaction tube was then placed in a water bath at 37°C and aliquots were taken after different times.

Inactivation by ultraviolet irradiation: After treatment of the phages by HA, 2.5 ml of the mixture were placed onto a sterile watchglass (on ice), which rotated during the UV irradiation. The distance between the 15-watt General Electric "Germicidal" tube and the watchglass was 100 cm. Aliquots were diluted 50-fold into the ice-cold stopping mixture.

RESULTS

Mutation induction at different concentrations of hydroxylamine: Hydroxylamine (HA) exerts two separable effects on phage T4: it induces mutations and

it inactivates (FREESE, FREESE and BAUTZ 1961). The frequency of mutations per viable phage [r + mottled (m) plaques] increases linearly with both the time of treatment and the concentration of HA. The mutations result from the direct reaction of HA with cytosine. The inactivation rates, however, are low at high (1 M) and maximal at intermediate (2×10^{-2} M) HA concentrations. A similar unusual concentration dependence has been observed for the inactivation of transforming DNA (FREESE and FREESE 1964) and has been explained by the oxygen dependence of radical production which is responsible for the inactivating reaction (FREESE and FREESE 1965a). Most phages are inactivated by this reaction with DNA (as we have established by functional rescue experiments), although some may be inactivated by a reaction with the phage protein, preventing bacterial infection.

As expected from these earlier observations, the frequency of mutations [per cent ($r+m$)] per lethal hit was much lower when standard type phages were treated by 0.2 M HA than when they were treated by 1 M HA (Figure 1). But, although the inactivating DNA alterations inflicted by low HA concentrations did not induce mutations, they nevertheless increased the frequency at which induced mutations appeared as pure clones. This is apparent from Figure 2 which shows that the fraction $m/(r+m)$ of mottled clones among all mutant clones decreased, with increasing frequency of mutants [per cent ($r+m$], much faster at 0.2 M HA than at 1 M HA. (The initial increase of $m/(r+m)$ resulted from the background in the phage stock of 0.08% spontaneous r-mutants, which produced pure r-clones; only after some time of treatment could the newly induced mutations dominate the mutant character of the phage preparation.)

Separate production of mutagenic and inactivating DNA alterations: The treatment by low HA concentrations had induced both mutagenic and inactivating DNA alterations simultaneously. In order to analyze the effects of the

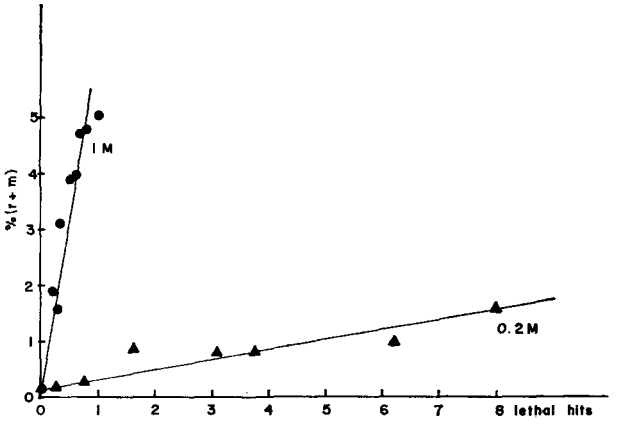


FIGURE 1.—Induction of r-type mutations at two different HA concentrations. pH 6.2, 37°C. (Unless especially explained, different symbols in this and later figures indicate different experiments run under the same conditions.)

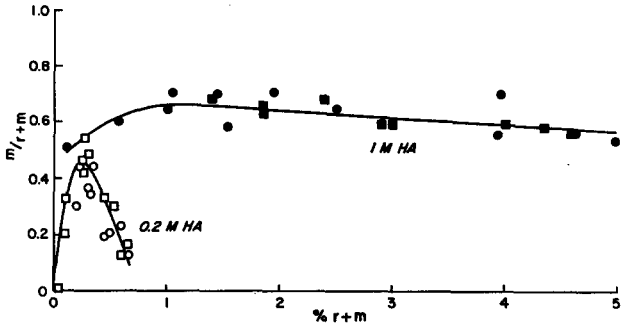


FIGURE 2.—Change of mottled fraction $m/(r+m)$ with the frequency of total mutant plaques, $\%(r+m)$. Solid points: 1 M HA; ● = T4v+; ■ = $T4v_1$. Hollow points: 0.2 M HA; ○ = T4v+; □ = $T4v_1$.

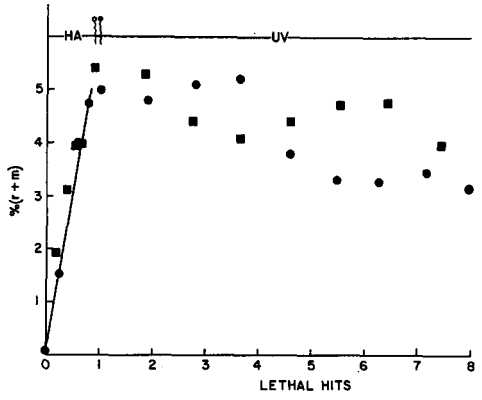
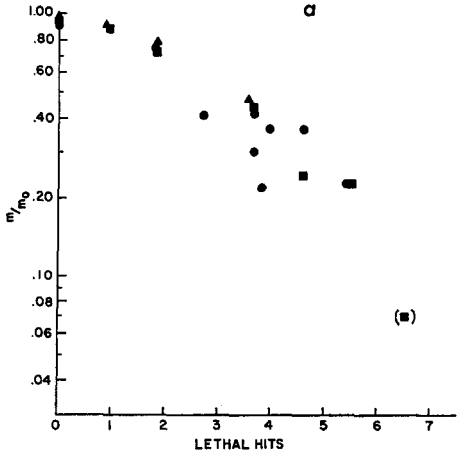


FIGURE 3.—Change of the mutant frequency $[\%(r+m)]$ of T4v+ during the treatment with 1 M HA and the subsequent treatment with UV.



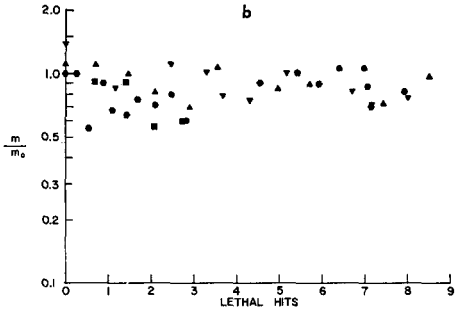


FIGURE 4.—Change of the frequency of mottled plaques during treatment of mutagenized phages by UV: (a) T4v+; (b) $T4v_1$.

two alterations separately, we employed 1 M HA to induce (mainly) mutagenic alterations and subsequently UV to superimpose inactivating DNA alterations. The mutagenic effect of UV on free phages is negligible [about 2.5×10^{-4} *r*-mutants/lethal hit (FOLSOME 1962)] compared to that of HA. Standard type T4 phages (r^+v^+) were treated by 1 M HA, pH 6.2, at 37°, chilled and then exposed to UV light for different times. Aliquots were diluted in ice-cold stopping mixture and plated on bacteria B for the determination of both survival and the frequency of *r*- and *m*-plaques.

Figure 3 shows how the frequency of all *r*-type mutant plaques [per cent $(r+m)$] increased during the treatment by HA, whereas it slowly decreased during the UV treatment. The components of this mutant composite, however, responded in opposite fashion: The frequency *m* of mottled *r*-plaques rapidly decreased (Figure 4a) whereas that of pure *r*-plaques slightly increased (Figure 5a) during the UV treatment of mutagenized v^+ phages. The decrease in the frequency of mottled plaques relative to that of all mutant plaques can be seen by plotting the ratio $m/(2r+m)$ either against lethal hits (Figure 6a) or against time (Figure 6b). (The reason for plotting $m/(2r+m)$ instead of $m/(r+m)$ will become apparent in the discussion. The values in Figures 4, 5 and 6 have been normalized to the extrapolated value of zero time of UV treatment, obtained by drawing the approximately best straight line through the initial points of an individual experiment. In this way different experiments could be plotted in the same graph although their initial mutant frequencies differed slightly.)

After extensive treatment of phages by UV, plaques frequently are smaller than normal. When phages from such small plaques are replated they mostly produce again plaques of normal size. UV treated phages apparently suffer sublethal and non-hereditary damages. (A few altered plaque types are inherited, indicating a mutagenic effect of UV.) Since different mutant plaque types are harder to distinguish in small than in normal sized plaques, there is a distinct

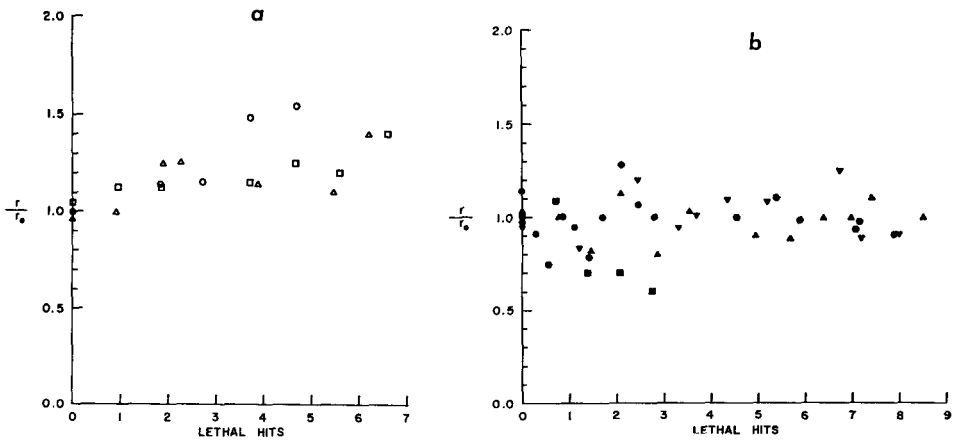


FIGURE 5.—Change of the frequency of pure *r*-plaques during treatment of mutagenized phages by UV: (a) T4v$^+$; (b) T4v$_1$.

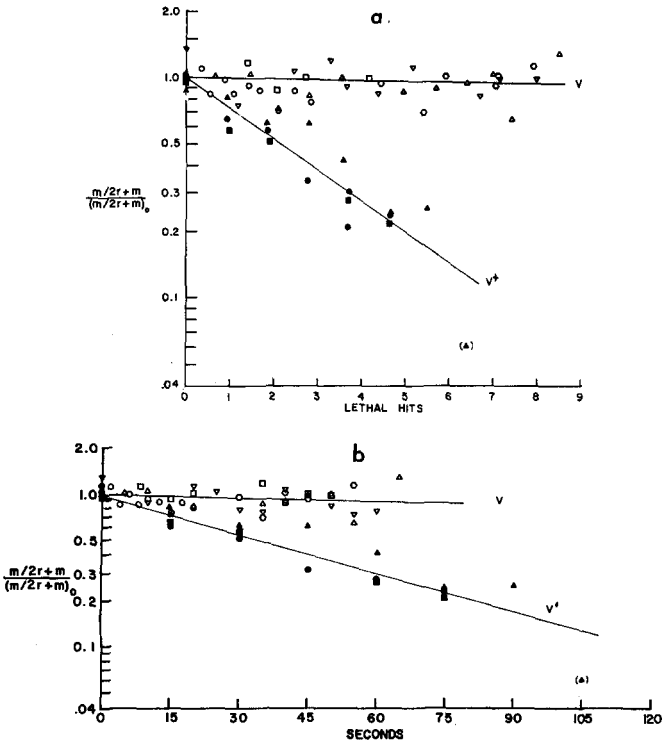


FIGURE 6.—Change of the fraction $m/(2r+m)$ of mottled plaques during the treatment of mutagenized phages by UV: Solid points = T4v+; hollow points = T4v_1. Abscissa: (a) Lethal hits, (b) time of UV treatment. At zero time of UV treatment $m/(2r+m)$ was about 0.4.

danger to classify them incorrectly. The tendency of misclassification would be especially serious after extensive UV treatment. In order to evaluate the magnitude of this possible error we have picked, from the plates corresponding to the value at 5.5 lethal hits, over 1000 plaques of different types and replated the phages. The result of this reclassification is shown in Table 1. One can calculate from these data that the error for the determination of both m and $m/(2r+m)$ was about 40%; i.e. the ratio $r/(2r+m)$ would be 0.31 instead of the value 0.22

TABLE 1

Actual mutant composition of plaques

Plaques	Observed as	Reclassified as r^+	Reclassified as m	Reclassified as r
938	$r+$	**937**	1	0
25	m	1	**23**	4
50	r	0	1	**46**

v+r+ phages were mutagenized by 1 M HA and then treated for 90 seconds by UV. Plaques of r^+, r, and m type were picked and replated on bacteria B. Plaques containing less than 1/30 r-type but at least 1/30 r^+ type were scored m (the ratio of the two types was usually smaller than 5:1), and plaques containing less than 1/30 r^+ type were scored r.

that had been determined by inspection of plaques. For small numbers of lethal hits a smaller error can be expected. Since the experimental values are evaluated logarithmically, the error does not seem to alter the conclusions reached: the slope of the straight line in Figure 6 seems to deviate from the correct value by not more than 30%.

Pure clone formation in a UV-sensitive strain: Phage T4 DNA is not subject to the host cell reactivation (HCR) observed for other phages (such as T1), because T4v$^+$ exhibits the same UV inactivation curves in different HCR types of bacteria (HARM 1963b). Nevertheless, phage T4 can repair UV damage, apparently by a phage induced enzyme, because UV-sensitive strains are known (STREISINGER 1956; HARM 1958, 1959, 1963a). We have repeated the previous experiment with one of these mutants, T4v$_1$(= v) (HARM 1963a) which has the standard phenotype and permits the induction of *r*-type mutants. The UV inactivation curves of v$^+$- and v-phages are displayed in Figure 7.

When v-phages were treated by 1 M HA, the frequency of $r + m$ plaques increased and during a subsequent UV treatment it slowly decreased, similar to the result for the standard type phage v$^+$. In contrast to the previous results, however, the frequency of mottled plaques did not rapidly decrease during the UV treatment, as can be seen from Figure 4b. Since the frequency of *r*-plaques also did not change during the UV treatment (Figure 5b), $m/(2r + m)$ barely decreased (Figure 6: v).

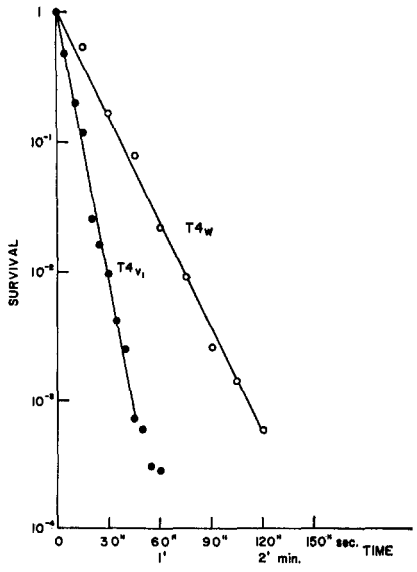


FIGURE 7.—UV inactivation curves of T4v$^+$ and T4v$_1$.

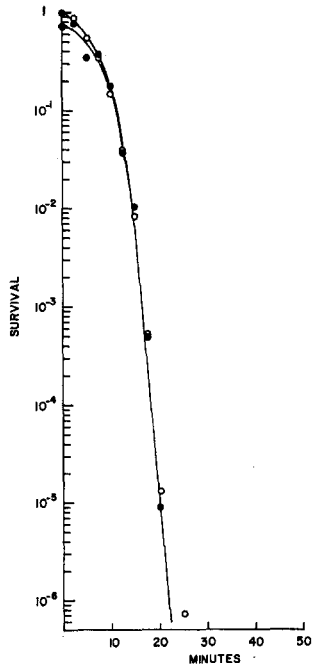


FIGURE 8.—Inactivation curves of T4v$^+$ (●) and T4v$_1$ (○) in 0.2 M HA. The curves have been shifted so as to show the agreement in the ultimate inactivation rates.

Whereas the UV-resistant strain v^+ and the UV-sensitive mutant v responded very differently to UV, they responded similarly to the inactivating DNA alterations induced by low concentrations of HA. Figure 8 shows that the inactivation rates in 0.2 M HA were similar for both phages. Both curves were obtained simultaneously, using the same HA solution. Their rates initially increased, apparently because the oxidation products of HA had to accumulate (FREESE and FREESE 1965a). Furthermore, the inactivating DNA alterations, induced by 0.2 M HA, produced pure mutant clones in both phages v^+ and v (see Figure 2) in contrast to the inactivating alterations induced by UV.

DISCUSSION

When mutagenized standard type phage T4 suffer additional inactivating DNA alterations, the fraction of mixed clones, among the r-type mutant clones, decreases. This effect has been observed for inactivating alterations that were induced either by UV or by low concentrations of HA.

This finding explains an apparent discrepancy, concerning the production of mottled *r*-mutant clones, between our results (FREESE, FREESE and BAUTZ 1961) and those of SCHUSTER and VIELMETTER (1961). Believing that HA reacted with DNA by only one mechanism (reaction with cytosine) we had plotted in one graph the values of $m/(r+m)$ obtained from experiments run at various HA concentrations (between 0.1 and 1 M). Although our points scattered much, they indicated a relatively rapid decrease of the mottled fraction of *r*-mutant plaques $[m/(r+m)]$ with increasing frequency of total *r*-mutants, percent $(r+m)$. It is now clear that we have mainly reported the results of a combination of mutagenic and inactivating DNA alterations. When SCHUSTER and VIELMETTER (1961) repeated our experiments, they apparently used conditions (1 M HA at pH 6.8) which we had found to be predominantly mutagenic. Thus minimizing the inactivating effect of HA, they observed a slow decrease of $m/(r+m)$. Both types of curves can now be reproduced using different HA concentrations (see Figure 2).

Treatment of T4 phages by UV induces not only lethal but also sublethal damage which leads to the production of small plaques. Most of the latter changes are not inherited because phages from small plaques produce normal size plaques on replating. But the sublethal effect possibly could cause the decrease of the fraction of mottled plaques, as an artifact: (1) Since it is more difficult to distinguish different plaque types in small plaques, the classification of plaques into r^+, *r*, and *m* might contain, after extensive UV treatment, a large error. The actual error has been determined for mutagenized v^+ phages, treated by UV to give 5.5 lethal hits (Table 1): the slope of the straight lines seems to deviate from the correct value by not more than 30%. For the UV-sensitive (v) phages, the effect of sublethal damages should be even smaller, because less UV irradiation is needed to induce the same number of lethal hits. The error seems therefore too small to explain the difference in v and v^+ phages with respect to the elimination of mottled plaques by UV. (2) Sublethal UV damage might reduce the burst size of infected bacteria and eliminate mixed bursts by fluctuation in the sampling of the phage pool. This effect, which would be pronounced only for extensive UV treatment, does not seem to be the major cause of the decrease in the frequency of mottled plaques, because if it were, the v phages should show

at least the same decrease, for a given UV dose, as the v^+ phages. In fact, however, the decrease of mottled plaques is more rapid in v^+ than in v phages even when $m/(2r + m)$ (or m itself) is plotted against time (i.e., dose) of UV treatment (instead of lethal hits).

Another possibility, which has to be considered as an explanation for the reduction in mixed mutant clones by UV, would be the preferential inactivation of those phages which contain mutagenic DNA alterations. Cytosine bases that have been altered by HA might be more sensitive to UV irradiation than normal bases. This possibility appears to be ruled out for two reasons. (1) The frequency of HA induced r-type mutants, percent $(r + m)$, decreased during the inactivating treatment by UV much more slowly than the fraction of mottled plaques. (The observed decrease may result on the one hand from the conversion of some phages producing m-plaques into phages producing r^+ plaques, by an inactivation alteration plus repair in the mutagenized DNA strand, and on the other hand by the difficulty of distinguishing different mutant types in small plaques found after UV treatment.) (2) After treatment by 1 M HA, not only the phages producing mottled but also most of those producing r-plaques carry their mutation as a result of the mutagenic alteration of cytosine bases by HA. Nevertheless, the subsequent treatment by UV did not reduce but rather increased their frequency per viable phage. (These phages may produce r-plaques either because they received both a mutagenic and an inactivating hit already during the HA treatment or because one of the two DNA strands occasionally is lost during normal phage DNA replication.)

It appears necessary to conclude that mutagenized DNA which produces mixed mutant clones can be converted, by the random insertion of inactivating DNA alterations, into DNA which produces pure mutant clones. This conversion seems to depend on the ability of the phages to excise and repair their own UV lesions, because it was not observed for the UV sensitive strain v. The standard type strain v^+ actually can excise thymine dimers, whereas the mutant strain v has lost this ability (SETLOW and CARRIER 1966). Since the conversion to pure clone production is absent in the v strain, the basic replication mechanism (without repair) does not seem to permit the copying of one DNA strand alone but usually seems to stop replication of both strands at the inactivated site.

In bacteria, not only the thymine dimer but a longer DNA segment seems to be excised during repair (SETLOW and CARRIER 1964; BOYCE and HOWARD-FLANDERS 1964). If the same process occurred in phage v^+ the excised region would occasionally overlap the original mutagenic DNA alteration. During the subsequent repair, which would involve copying of the unrepaired DNA strand, the mutagenized base would be copied as mutation; in this way DNA would be produced in which both DNA strands would now behave as mutant strands. This is illustrated in Figure 9. The observed induction of pure mutant clones indicates that this interpretation of the repair process is correct. (If a mutagenized base would only sometimes pair with the wrong complementary base, a pure mutant clone would arise only occasionally. In the case of HA, however, we assume that a mutagenized cytosine behaves as thymine in most of the cases, because only

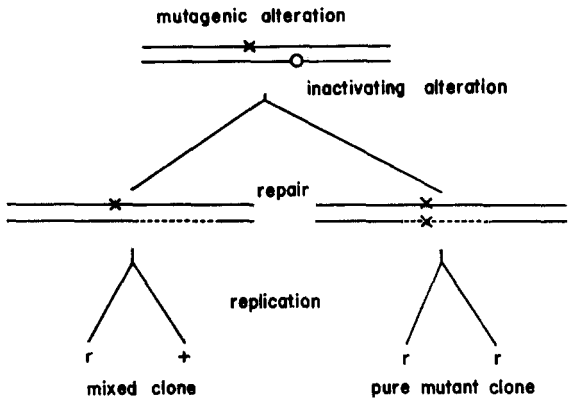


FIGURE 9.—Model explaining production of pure clones by excision and repair of one DNA strand, by copying the other strand.

pure mutant clones have been found after induction by HA of linked mutations in transforming DNA [FREESE and STRACK 1962].)

One can obtain an upper limit for the average size of the excised DNA piece by using the slopes in Figures 6 and 7:

The frequency m (per viable phage) of phages producing mottled plaques would remain constant if no repair process would go on; with repair, it should change according to

$$\frac{dm}{d\mu} = -\rho\, a\, m \tag{1}$$

μ = number of inactivating DNA alterations; ρ = probability that such an alteration is repaired; a = probability that the repair region overlaps the site of the mutagenic DNA alteration. In half of the overlapping cases the excised DNA region contains the mutagenic alteration and the repair process produces standard type clones; but in the other half of the cases the complementary strand will be excised and the repair process produces pure r-mutant clones. Thus the frequency r of pure r-plaque producing phages will increase according to

$$\frac{dr}{d\mu} = +\tfrac{1}{2}\,\rho\, a\, m \tag{2}$$

Another simple equation can be obtained for the ratio $m/(2r+m)$, which has been used in earlier plots for this reason.

$$\frac{d}{d\mu}\, m/(2r+m) = -\rho\, a\, m/(2r+m) \tag{3}$$

One can express the number of inactivating DNA alterations by the known number of lethal hits $n = \ln P_0/P$

$$n = k\,\mu$$

where k depends on the repair efficiency of the phage. Replacing in equations (1), (2) and (3), μ by n/k, and solving the equations one obtains:

$$\ln \frac{m}{m_0} = -\frac{\rho\, a}{k}\, n \tag{4}$$

$$\frac{r}{r_0} = 1 + \tfrac{1}{2}\,\frac{m_0}{r_0}\,(1 - e^{-\rho\, a\, n/k}) \tag{5}$$

and

$$\ln \frac{m/(2r+m)}{m_0/(2r_0+m_0)} = -\frac{\rho\, a}{k}\, n \tag{6}$$

The experimental points scatter about curves which generally behave as expected from the equations (see Figures 4, 5, 6).

One can estimate, from the slope in Figure 6, that

$$\frac{\rho\, a}{k} = 0.35 \text{ for T4w} \tag{7}$$

whereas the corresponding value for v is much smaller. If one would use instead of Figure 6 only the initial slope of Figure 4a, one would obtain the lower value of

$$\frac{\rho\, a}{k} = 0.18 \text{ for T4w} \tag{8}$$

The value of k can be estimated from the inactivation rates of UV repairable and nonrepairable phage mutants. Instead of $T4v_1$ we use the results for the even more UV sensitive double mutant T4vx (HARM 1963a) whose inactivation rate is 2/0.45 that of T4v+. Hence $k \leq 0.225$.

The probability of repair is not known but it can be calculated from k if one assumes that the contribution of any especially vulnerable centers (which might have to be intact to allow repair enzymes being made) to the inactivation rate is small. One obtains then $k = 1 - \rho$, and therefore $\rho \geq 0.775$.

Using these values for ρ and k one obtains for the probability a, with which the repair region overlaps the mutagenic DNA alteration: $a \leq 0.10$ if one uses (7), and $a \leq 0.052$ if one uses (8). Considering the total amount of 2×10^5 nucleotide pairs in T4 DNA (HERSHEY and CHASE 1952), this repair region would correspond to $\leq 0.5\text{–}1 \times 10^4$ nucleotides which are excised from one DNA strand. This number is rather large compared to the estimates of 30 nucleotides excised per thymine dimer in bacterial DNA (SETLOW and CARRIER 1964). The actual size of the repair region may be smaller, if even T4vx phages should still benefit from a significant amount of repair. However, the repair region in phage T4 may actually be as large as estimated above, because even unirradiated phage DNA is known to be fragmented (into about 20 pieces) and dispersed onto the progeny (KOZINSKI 1961). Part of the repair process may therefore be identical to the normal mechanism of phage T4 DNA replication which involves recombination by breakage and repair (KOZINSKI and KOZINSKI 1964).

In contrast to the UV induced inactivating DNA alterations (thymine dimers) those induced by low concentrations of HA apparently can still be "repaired," even in $T4v_1$. For at 0.2 M HA, at which both mutagenic and inactivating DNA alterations are induced, a rapidly increasing fraction of induced mutants produced pure *r*-clones for both T4v+ and T4v (see Figure 2). The inactivating effect of HA has been shown to rupture and remove DNA bases (H. J. RHAESE, E. FREESE and M. MELZER, in preparation) and to break the sugar phosphate backbone (RHAESE and E. FREESE, unpublished results). Single DNA strands containing some of these alterations (in particular backbone breaks) may be eliminated (and repaired) even in phage T4v so that pure mutant clones can be produced from mutagenized DNA. The different response of T4v to inactivating alterations induced by UV and HA suggests that in T4v the first step of the repair process eliminating UV damage is blocked, i.e., the cutting of DNA near a thymine dimer.

We want to thank MRS. CYNTHIA MCALISTER for excellent technical assistance and DR. WALTER HARM for his UV sensitive mutant T4v_1.

SUMMARY

Inactivating DNA alterations convert mutagenized standard type phage T4 from mixed to pure mutant clone producers. For the ultraviolet-sensitive mutant T4v_1, this conversion is practically absent when UV is used but present when low concentrations of hydroxylamine are employed as the inactivating agent. The conversion has been explained by the repair of inactivating DNA alterations which involves copying of the mutated strand. The average size of the DNA strand eliminated per inactivating DNA alteration was estimated to be $\leq 1/20$ of the total phage genome, or $\leq 10^4$ nucleotides.

LITERATURE CITED

ADAMS, M. H., 1959 *Bacteriophages*. Interscience, New York, page 446.

BOYCE, R. P., and P. HOWARD-FLANDERS, 1964 Release of ultraviolet light-induced thymine dimers from DNA in *E. coli* K-12. Proc. Natl. Acad. Sci. U.S. **51**: 293–300.

FOLSOME, C. E., 1962 Specificity of induction of T4*rII* mutants by ultraviolet irradiation of extracellular phages. Genetics **47**: 611–622.

FREESE, E., 1966 Hereditary consequences of different DNA alterations. In *Mutation Process*, Vol. 2. *The Physiology of Gene and Mutation Expression*. Edited by M. KOHOUTOVÁ and J. HUBÁČEK. Academic Press, New York.

FREESE, E., and E. B. FREESE, 1965a The oxygen effect on deoxyribonucleic acid inactivation by hydroxylamines. Biochemistry **4**: 2419–2433. —— 1965b Mutagenic and inactivating DNA alterations. Radiation Res., Suppl. (In press.)

FREESE, E., E. B. FREESE, and E. BAUTZ, 1961 Hydroxylamine as a mutagenic and inactivating agent. J. Mol. Biol. **3**: 133–143.

FREESE, E., and H. B. STRACK, 1962 Induction of mutations in transforming DNA by hydroxylamine. Proc. Natl. Acad. Sci. U.S. **48**: 1796–1803.

FREESE, E. B., and E. FREESE, 1964 Two separable effects of hydroxylamine on transforming DNA. Proc. Natl. Acad. Sci. U.S. **52**: 1289–1297.

HARM, W., 1958 Zur Deutung der unterschiedlichen UV-Empfindlichkeit der Phagen T2 und T4. Naturwiss. **45**: 391–392. —— 1959 Untersuchungen zur Wirkungsweise eines die UV-Empfindlichkeit bestimmenden Gens der Bakteriophagen T2 und T4. Z. Vererb. **90**: 428–444. —— 1963a Mutants of phage T4 with increased sensitivity to ultraviolet. Virology **19**: 66–71. —— 1963b Repair of lethal ultraviolet damage in phage DNA. pp. 107–124. *Repair from Genetic Radiation*. Edited by F. H. SOBELS. Pergamon Press, Oxford.

HERSHEY, A. D., and M. CHASE, 1952 Independent functions of viral protein and nucleic acid in growth of bacteriophage. J. Gen. Physiol. **36**: 39–56.

KOZINSKI, A. W., 1961 Fragmentary transfer of P^{32}-labelled parental DNA to progeny phage. Virology **13**: 124–134.

KOZINSKI, A. W., and P. B. KOZINSKI, 1964 Replicative fragmentation in T4 bacteriophage DNA. II. Biparental molecular recombination. Proc. Natl. Acad. Sci. U.S. **52**: 211–218.

PRATT, D., and G. S. STENT, 1959 Mutational heterozygotes in bacteriophages. Proc. Natl. Acad. Sci. U.S. **45**: 1507–1515.

SCHUSTER, H., and VIELMETTER, 1961 Studies on the inactivating and mutagenic effect of nitrous acid and hydroxylamine on viruses. J. Chim. Phys. **58**: 1005–1010.

SETLOW, R. B., and W. L. CARRIER, 1964 The disappearance of thymine dimers from DNA: An error-correcting mechanism. Proc. Natl. Acad. Sci. U.S. **51**: 226–231. —— 1966 The *v*-gene controls dimer excision in T4 phage. Biophys. Soc. Abstr. **6**: 68.

STREISINGER, G., 1956 The genetic control of ultraviolet sensitivity levels in bacteriophages T2 and T4. Virology **2**: 1–12.

TESSMAN, I., 1959 Mutagenesis in phages ϕX174 and T4 and properties of the genetic material. Virology **9**: 375–385.

VIELMETTER, W., and C. M. WIEDER, 1959 Mutagene und inaktivierende W irkung salpetriger Säure auf freie Partikel des Phagen T2. Z. Naturforsch. **14b**: 312–317.

VISCONTI, N., and M. DELBRÜCK, 1953 The mechanism of genetic recombination in phage. Genetics **38**: 5–33.