TRANSITIONS AND TRANSVERSIONS INDUCED BY DEPURINATING AGENTS*

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Mutations which are caused by single base pair changes in DNA have been divided into two different classes:[1] (1) transitions, where a purine is replaced by the other purine or a pyrimidine by the other pyrimidine, and (2) transversions, where a purine is replaced by a pyrimidine or vice versa.

The existence of transitions has been proved.[2, 3] For a series of mutants it has even been suggested which base pair had been changed.[3–5] But it was still an open question whether transversions really exist or whether for those mutations which have been shown to be non-transitions, a different mechanism is responsible.

By using depurinating agents like ethyl ethane sulfonate[4] and treatment with low pH,[6, 7] it seemed possible to induce both transitions and transversions. These agents preferentially remove guanine from DNA. Across the resulting gap not only cytosine can be incorporated into a new DNA strand producing the original standard-type, or thymine, giving rise to a transition, but also one of the two purines should occasionally be incorporated into the new DNA strand and cause a transversion. Thus, transversions containing a G-C pair at their mutant site should be inducible by these agents to revert to the original genome.

Material and Methods.—T_4 rII mutants and the techniques of growing and selecting them have been described by Benzer,[8] Benzer and Freese,[9] Freese[1, 2] and Brenner *et al.*[10]

The method of measuring reversion induction from rII to standard type is given in detail in the paper of Freese and Freese[3] for reversion induction by nitrous acid. The procedure for induction with ethyl ethane sulfonate has been described by Bautz and Freese[4] and that for hydroxylamine by Freese *et al.*[5] For reversion induction with AP and BD, spot tests have been described by Freese.[1]

The only method used in this paper which has not been described so far was the treatment of phages with low pH. T_4 wild-type phages were suspended in 0.5 *M* sodium acetate buffer, pH 4.2, and incubated at 37°C. After various times (between time 0 and 10 hours treatment), aliquots transferred into Tris-buffer (0.5 *M*) of pH 7.8 at room temperature.

Results.—Abbreviations: Adenine = A, Guanine = G, Thymine = T, Cytosine or 5-hydroxymethylcytosine = C, Ethyl ethane sulfonate = EES, Hydroxylamine = HA, Nitrous acid = NA, Bromodeoxyuridine = BD, 2-aminopurine = AP; Bromouracil-induced mutants = N mutants,[10] 2-aminopurine-induced mutants = AP mutants,[3] Proflavine-induced mutants = P mutants,[10] and EES-induced mutants = EES mutants.

(1) *Phage T_4 rII mutants induced by ethyl ethane sulfonate and low pH:* Phage T_4 wild type was treated with EES (0.11 *M*) for 30 min followed by incubation at 37°C for 24 hours in the presence of 1 per cent sodium thiosulfate. Under these conditions, the survival was about 10^{-2}; the mutation frequencies were about 12 times above the spontaneous background. Forty-five rII mutants were isolated and tested for their ability to revert spontaneously and for reversion induction by the two base analogs BD and AP.

The same tests had been performed previously on mutants induced by low pH

(pH 5, 45°C).[7] Mutants were isolated at an inactivation of 3×10^{-5} and the mutation frequency was about seven times above the spontaneous level. All EES-induced mutants did revert spontaneously. Out of 115 low pH-induced mutants tested, six did not revert, while all the others did revert and were mapped as point mutations. Table 1 shows that EES and low pH produce both transitions and nontransitions, the ratio being about 3:1.

TABLE 1

INDUCTION OF REVERSE MUTATIONS OF EES AND LOW pH INDUCED MUTANTS

rII Mutants induced by	No. of mutants tested	Base Analog — Inducible (%)	Base Analog — Non-inducible (%)	Per cent spontaneous background of non-inducible mutants
Ethyl ethane sulfonate	47	70	30	10
pH 5, 45°C[8]	115	77	23	15

(2) *Reversion induction of transitions:* It is known from chemical analysis that EES preferentially removes guanine from DNA.[4] Low pH treatment is known to depurinate DNA. The data of Tamm, Hodes, and Chargaff[6] indicate that at pH 1.6 guanine was removed first but after some time adenine reacted too. From these data it could not be concluded whether pH 4.2, under the conditions used in our experiment, preferentially removes guanine or removes it at the same rate as adenine.

In order to decide this question, a set of base analog induced mutants was tested for their reversion induction by low pH and the rate of induction was compared to the results obtained from experiments with EES.

As has been shown by Bautz and Freese,[4] EES induces the reversion of AP mutants. It does not induce or only very slightly induces the reversion of N mutants,[3] and some AP mutants. The same mutants were tested now for their reversion induction with pH 4.2. As can be seen from the data in Table 2, the same mutants that are highly inducible by EES to revert are also highly inducible by

TABLE 2

THE MUTAGENIC DIRECTION OF TRANSITIONS

Mutant	K_0/B_0 in 10^{-6}	α/β pH 4.2 in 10^{-6}	K_0/B_0 in 10^{-6}	α/β EES in 10^{-6}
AP 114	5.3	8.0	4.2	3.1
AP 275	1.7	7.6	1.07	1.5*
AP 72	1.29	5.2	0.76	1.0
AP 12	~0.5	~1.8	~0.85	~4.6*
AP 41	~0.3	~1.2	0.37	~2.3*
AP 156	0.007	0.01	0.04	0.08
AP 70	0.018	0.01	0.028	0.065*
N 29	0.013	0.01	0.04	0.03*
AP 83	0.011	$\lesssim$0.01	0.03	0.09
N 19	0.009	$\lesssim$0.01	0.07	0.016*
N 24	0.48	$\lesssim$0.08	0.32	$\lesssim$0.01*
AP 61	0.13	$\lesssim$0.03	0.18	$\lesssim$0.01*
N 31	0.028	$\lesssim$0.01	0.01	$\lesssim$0.01*
N 12	0.03	$\lesssim$0.01	0.03	$\lesssim$0.01*
N 7	0.016	$\lesssim$0.01	0.03	$\lesssim$0.01*
N 17	0.066	$\lesssim$0.01	0.02	$\lesssim$0.01*
r 320	0.013	$\lesssim$0.04	0.013	$\lesssim$0.02

The spontaneous reversion rates (K_0/B_0) and the absolute rates of reversion induction (α/β) per lethal hit are given for a number of transition mutants from reversion experiments with pH 4.2 and EES. $\alpha/\beta \lesssim$ means no detectable amount of induction.

* These data are from Bautz and Freese.[4]

treatment with pH 4.2. Actually, the separation of base analog induced mutants into the two possible classes (G-C → A-T and A-T → G-C) seems even clearer. The five AP mutants which can be highly induced to revert by both agents probably contain a G-C pair at their mutant site while the rest, N-mutants and also some AP mutants, are likely to have an A-T pair.

From these results, we conclude that under the conditions used, low pH preferentially removes guanine from DNA similar to EES. The data further suggest that the directional effect is even more pronounced.

(3) *Reversion induction of transversions:* Since EES and low pH remove guanine from DNA and thereby can induce transitions, it seems clear that they also should be able to induce transversions. Thus, EES and low pH can be used to prove the reversion induction of those transversion mutants which have a G-C pair at the mutant site. A mutant can be changed back to its original detectable standard type or into another rII mutant which cannot be recognized with our selective system.

Five EES mutants, 10 proflavine-induced mutants, and 15 spontaneous mutants were tested. By AP and BD spot tests and by reversion studies with nitrous acid, all these mutants were shown to be non-transitions. All of them did revert spontaneously, although with very different frequencies.

In Figure 1, the linear increase of revertants per viable phage with the number of lethal hits by treatment with EES and pH 4.2 is shown for EES-induced mutant

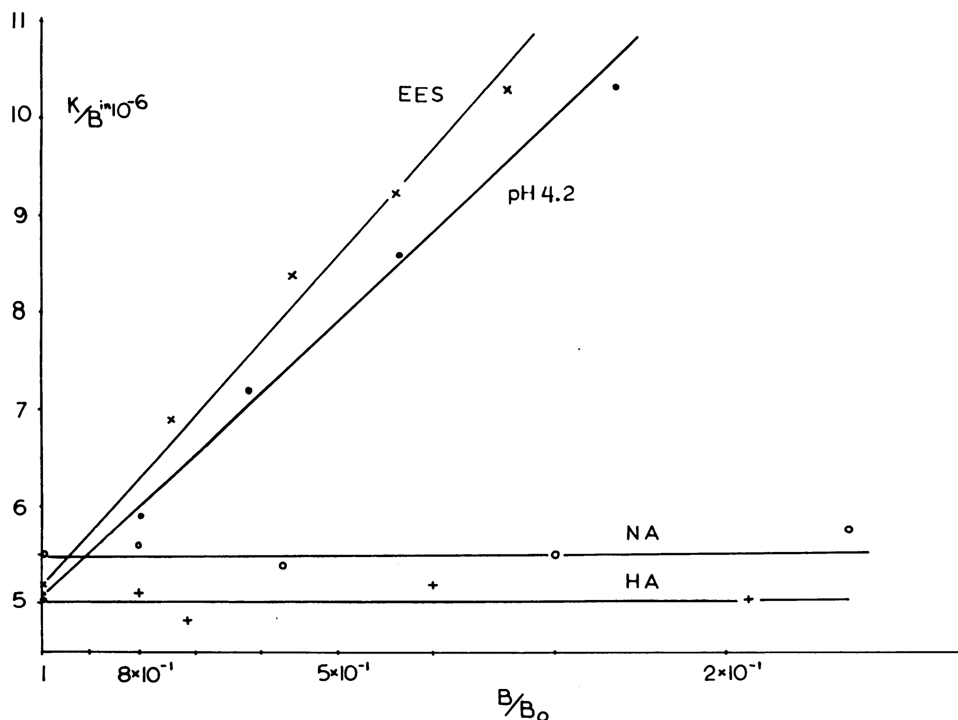

FIG. 1.—The values for the reversion induction of mutant EES 66 by EES and pH 4.2 show a linear increase over the spontaneous level with the number of lethal hits, i.e. (ln B/B_0) per viable phage. In contrast to this, there was no increase measurable after treatment with NA and HA.

TABLE 3

RATES OF REVERSION INDUCTION FOR NON-TRANSITION MUTANTS

Mutant	K_0/B_0	α/β pH 4.2	α/β EES	α/β NA	m.o.p.	AP	BD
r 111	160.0	66.0	70.0	<3	0.002	—	—
r 207	69.0	57.0	55.0	<2.5	0.001	—	—
r 132	197.0	55.0	50.0	<2	0.0005	—	—
r 157	175.0	40.0	50.0	<5	0.001	—	—
r 315	5.0	5.0	4.5	$\lesssim 0.2$	0.05	—	—
r 101	14.6	2.2	3.8	$\lesssim 0.1$	0.02	—	—
r 101	4.0	....	3.9	....	....		
r 105	6.1	2.4	3.5	$\lesssim 0.1$	0.02	—	—
r 179	6.6	2.4	3.4	$\lesssim 0.05$	0.02	—	—
EES 66	5.3	4.0	4.5	$\lesssim 0.1$	0.02	—	—
EES 116	7.8	2.9	3.3	$\lesssim 0.1$	0.03	—	—
P 11	~ 4.0	~ 1.3	$\lesssim 0.8$	$\lesssim 0.2$	0.01	—	—
r 131	0.4	$\lesssim 0.1$	$\lesssim 0.1$	$\lesssim 0.1$	0.05	—	—
r 114	0.7	$\lesssim 0.2$	$\lesssim 0.1$	$\lesssim 0.1$	0.05	—	—
r 117	0.6	$\lesssim 0.1$	$\lesssim 0.1$	$\lesssim 0.01$	0.1	—	—
r 194	0.5	$\lesssim 0.1$	$\lesssim 0.1$	$\lesssim 0.01$	0.05	—	—
r 178	0.02	$\lesssim 0.01$	$\lesssim 0.01$	$\lesssim 0.01$	0.2	—	—
r 123	0.02	$\lesssim 0.01$	$\lesssim 0.01$	....	0.2	—	—
r 289	0.1	$\lesssim 0.01$	$\lesssim 0.01$	$\lesssim 0.01$	0.1	—	—
EES 64	1.3	$\lesssim 0.1$	$\lesssim 0.2$	$\lesssim 0.1$	0.05	—	—
EES 122	1.1	$\lesssim 0.3$	$\lesssim 0.2$	....	0.05	—	—
EES 32	0.3	$\lesssim 0.02$	$\lesssim 0.1$	....	0.05	—	—
P 5	0.9	$\lesssim 0.02$	$\lesssim 0.01$	$\lesssim 0.1$	0.02	—	—
P 12	0.5	$\lesssim 0.14$	....	....	0.05	—	—
P 13	0.3	$\lesssim 0.03$	....	....	0.1	—	—
P 33	0.2	$\lesssim 0.03$	....	....	0.1	—	—
P 4	0.1	$\lesssim 0.01$	$\lesssim 0.02$	....	0.1	—	—
P 36	0.2	$\lesssim 0.01$	....	....	0.1	—	—
P 14	0.1	$\lesssim 0.04$	....	....	0.1	—	—
P 3	0.02	$\lesssim 0.02$	$\lesssim 0.02$	....	0.1	—	—
P 8	0.02	$\lesssim 0.03$	$\lesssim 0.01$	....	0.2	—	—

The spontaneous reversion rates (K_0/B_0) and the absolute rates of reversion induction (α/β) per lethal hit are given for a number of nontransition mutants from reversion experiments with pH 4.2, EES, and NA. $\alpha/\beta \lesssim$ means no detectable amount of induction was measured. m.o.p. (multiplicity of plating) = number of phages per bacterium plated. A dash in the AP and BD columns means that no revertants could be detected by spot tests.

No. 66. In contrast to the induction with EES and low pH, there was no increase over the spontaneous background after treatment with nitrous acid and hydroxylamine, both of which induce mutagenic transitions exclusively.[4, 6] From these results, it is concluded that mutant EES 66 was caused by a mutagenic transversion.

Of five EES mutants tested, two were reversion-inducible by EES and pH 4.2 and three were not, while out of ten proflavine-induced mutants, only one showed any effect. Of fifteen spontaneous mutants, eight were reversion-inducible and seven were not. The data are given in Table 3. Among the eight spontaneous mutants which could be induced to revert, four showed very high induction values and had high spontaneous reversion rates (these high spontaneous rates rendered the tests with nitrous acid less sensitive). The other inducible transversion mutants had lower rates of reversion induction and also lower spontaneous backgrounds, but the rate of spontaneous reversions was still considerably higher than for the non-inducible mutants.

We can think of two interpretations for these findings: (1) The highly reversion-inducible mutants are caused by transversions having now a G-C pair at their mutant site, while the less inducible ones are caused by transversions having an A-T pair. The mutants that are not reversion-inducible at all (but revert spontaneously) would then have to be of a different type, e.g. deletions of a base pair.

(2) Both the highly and the less reversion-inducible mutants are transversions containing a G-C pair, and at least many of the non-inducible ones are transversions containing an A-T pair. In case (2), the difference in the rate of inducibility would depend on the genetic site of the G-C pair and would be caused by a "higher mutagenic specificity."

We have some experimental evidence for the second interpretation. The highly reversion-inducible transversion mutants also have high spontaneous reversion rates and belong to genetic sites at which also spontaneous forward mutations occur especially frequently. In contrast, the less reversion-inducible mutants recur very rarely at the same site.

(4) *Difference in mutants which seem to belong to the same genetic site:* By locating the tested mutants on Benzer's genetic map of the rII region, the following was found: (1) Transitions and transversions can occur at the same genetic site as has been described earlier by Freese.[1] It has been shown for two transition mutants, by recombination tests, that each of them is located at the same genetic site as some transversion mutants. They were not reversion-inducible by EES and low pH and thus have an A-T pair at their mutant site. (2) Among the non-transitions of the same genetic site, we found mutants highly reversion-inducible by EES and pH 4.2 and non-inducible ones, corresponding to the high and low spontaneous reversion rates of these mutants (Table 4).[1, 9, 11] The highly inducible

TABLE 4

DIFFERENT MUTANTS AT THE "SAME GENETIC SITE"

Mutant	K_0/B_0	α/β pH 4.2	AP	BD
r 117	0.6	$\lesssim$0.05	—	—
r 132	197.0	55.0	—	—
N 17	0.07	$\lesssim$0.01	++	+
r 178	0.02	$\lesssim$0.01	—	—
r 207	69.0	57.0	—	—
r 320	0.01	$\lesssim$0.04	++	+
r 131	0.43	$\lesssim$0.06	—	—
r 157	175.0	40.0	—	—
?	....	....	..	..
r 114	2.7	$\lesssim$0.2	—	—
r 111	160.0	66.0	—	—
?	....	....	..	..

Two base analog non-inducible mutants available at each of the big spontaneous hot spots r 131, 114, and 117 showed very different spontaneous reversion rates (K_0/B_0) and also different α/β values, i.e. rates of reversion induction by pH 4.2. Mutant N 17, a base analog inducible mutant which had been located on the same genetic site as r 117 and r 132, was not inducible by pH 4.2 but showed strong reaction upon spot test with AP (++) and a weaker one with BD (+). r 178, r 207, and r 320 are three different mutants which seem to be at the same genetic site.

transversions are changes from a G-C pair back to the original C-G or T-A, whereas the non-inducible ones could be transversions with an A-T pair at the mutant site. Theoretically, these three kinds of mutants, the transition A-T, the transversion C-G, and a transversion T-A, could be derived from one base pair, G-C.

Summary.—(1) Low pH and EES induce either transitions or transversions.

(2) Both pH 4.2 and EES predominantly attack the G-C pairs; thus transitions mainly go from G-C to A-T and transversions from G-C to C-G or T-A.

(3) Transversions also exist among the spontaneous mutants.

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*FORMATION OF DUPLICATION-DEFICIENCY PRODUCTS BY ASYMMETRICAL EXCHANGE WITHIN A COMPLEX LOCUS OF DROSOPHILA MELANOGASTER**

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The possibility that pseudoalleles may represent closely linked genes having similar functions such that a position effect may exist between them, and the cytological evidence that such genes may occupy regions representing duplications, has led Lewis[1] to postulate that such cases may deal "with genes which were once (or are still) identical." The hypothesis states that before a gene with a new function can arise, there must first exist a pool of extra genes established through chromosomal duplication. One might expect to find pairing homologies within such a series of "old" and "new" genes in a manner comparable to such known duplications as Bar and Beadex-recessive, where unequal crossingover is a common feature. As yet, however, no clear case of other than strictly symmetrical pairing within complex loci has been demonstrated despite extensive studies of such loci in several organisms. Several examples of recovery of single products from a crossover event have been reported;[2-4] it is assumed in these cases that crossingover occurs in association with asymmetrical pairing and that the failure to recover one product is due either to its lethality or to a lack of distinctive phenotype necessary to distinguish it from one of the parental types.

Indication that asymmetrical pairing occurs within the white locus of *Drosophila melanogaster* first came from results obtained by MacKendrick,[5] who reported that w^{bl}/w^{aE} heterozygotes yielded both wild type and white recombinants, the latter through two different directions of crossingover. Judd[6,7] also reported unexpected white crossover products from several heterozygous combinations of white mutants. On the assumption that the reciprocal product was not being recognized the author attempted to use attached-X chromosomes to recover both