MISREPAIR MUTAGENESIS IN BACTERIOPHAGE T4

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ABSTRACT

The T4 mutations *px*, *y* and *1206* inactivate an error-prone recombination-like repair system, reducing or abolishing mutagenesis by UV irradiation, MMS, and white light irradiation in the presence of the photosensitizer 8MOP. Both *px* and *y* increase some spontaneous mutation rates and slightly enhance proflavin mutagenesis; neither mutation affects thymineless or 2AP mutagenesis appreciably, but both mildly enhance 5BU mutagenesis. The mutation *hm* promotes UV, MMS, photodynamic, thymineless, and base analog mutagenesis, in addition to spontaneous base pair substitution mutation. It does not, however, markedly affect proflavin mutagenesis. The *px* mutation maps in the vicinity of genes *41–56*, and the *hm* mutation maps in the vicinity of genes *rI–v*.

M ISREPAIR mutagenesis refers to a process of induced mutation in which a premutational lesion in DNA triggers the action of an error-prone repair process. The resulting mutations probably depend primarily upon the error tendencies inherent in the synthetic steps of the repair process, and not necessarily upon the chemical nature of the premutational lesion itself. Direct mispairing by an altered base, for instance, need not necessarily be involved, and the mutation may well arise at some distance from the premutational lesion.

Misrepair mutagenesis is inferred from the properties of certain repair-defective mutants. In the case of *Escherichia coli* these mutants harbor defects in the *recA* and *lex* (in K12 strains) or *exr* (in B strains) genes. Their effects on mutagenesis have been described by WITKIN (1969) and by KONDO *et al.* (1970). Strains defective in either gene show several simultaneous phenotypic modifications. They are defective in recombination, they usually exhibit a reduced growth rate and may generate numerous inviable cells, and they exhibit increased sensitivities to many different inactivating agents, including UV, ionizing and dye-sensitized white light irradiation, as well as alkylating agents, heat, and thymine deprivation (BRIDGES, ASHWOOD-SMITH and MUNSON 1968). The most important modification in the present context is a marked reduction—sometimes virtual abolition—of mutagenesis by these same inactivating agents. The involvement of repair in the mutagenic process is implied by the increased sensitivity to inactivation, and misrepair is impiled by the disappearance of mutagenesis in the repair-defective mutants. The noninvolvement of direct mispairing (as induced,

for example, by nitrous acid, hydroxylamine or EMS) is suggested by the loss of mutagenesis in the repairless mutants.

The recombination defects in these mutants suggest the operation of a recombination-like repair process, for which a specific model is available (RUPP et al. 1971). It is not yet clear, however, whether this model applies specifically to the misrepair process, nor, if so, whether the resulting mutations arise in donor or recipient strands. It does seem likely, however, that the error-prone repair system is inducible (Witkin and George 1973; Radman 1974).

We report here the properties of mutants of bacteriophage T4 which show similarities with recA and lex mutants of E. coli; some preliminary descriptions of these mutants have already appeared (DRAKE 1973, 1974; SMITH et al 1973). The y mutant, first described by BOYLE (1969) and BOYLE and SYMONDS (1969), carries an amber mutation which blocks DNA synthesis in an su⁻ host (S. MAYNARD SMITH and N. SYMONDS, personal communication) and which maps between genes 24 and 25 (MAYNARD SMITH and SYMONDS 1973), plus a suppressor mutation which allows it to grow on nonsuppressing hosts but does not abolish its repair-defective properties. The px mutant was isolated from T4Dx (HARM 1963, 1964), and has been separated from numerous other mutations in that strain, including the mutator mutation hm (DRAKE 1973). Both y and px have been backcrossed into T4B, since the rII mutations with which they are frequently coupled for reversion tests reside in a T4B background. A third mutant, 1206, also reduces recombination rates and increases UV sensitivity (VAN DEN ENDE and SYMONDS 1972); it may contain ts mutations in both gene 45 and gene 56 (N. SYMONDS, personal communication). The px and y and 1206 mutations are all epistatic and appear to affect the same repair system (SYMONDS, HEINDL and WHITE 1973). The hm mutant exhibits normal recombination rates, burst sizes and sensitivities to UV and MMS inactivation, but is likely to affect misrepair mutagenesis since it increases the rate of UV and of MMS mutagenesis (DRAKE 1973).

MATERIALS AND METHODS

Strains, media and growth conditions are described in DRAKE (1966, 1973).

Scoring of genotypes: The px, y and 1206 mutations are readily scored in low-titer isolates in buffer by their increased UV sensitivities. The only known phenotype of the hm mutation, however, is its mutator activity. Isolates were therefore grown into stocks, diluted 100-fold in buffer, and UV-irradiated to a survival of about 0.1%. About 4000 plaques were then screened on BB cells: hm+ typically exhibited about 0.3% induced mutants, and hm about 0.9%.

Mutagenesis: Procedures for UV and MMS mutagenesis have been described previously (DRAKE 1966, 1973).

8MOP mutagenesis was performed by diluting T4 into M9 buffer containing 50 µg/ml 8MOP (Paul B. Elder Co.) and irradiating with white fluorescent light. Fresh 8MOP solutions must be used, but the irradiated samples can be stored in the refrigerator without modification of relevant properties.

Base analog mutagenesis was performed by infecting BB cells in M9CA medium with an average of about five T4 particles per cell at 37° on a rotary shaker at a cell density of 5×10^8/ml. After 5 minutes the samples were diluted fivefold into M9CA with or without 2AP or 5BU. After an additional 30 to 90 minutes lysis was completed with chloroform, and mutant frequences were assayed promptly.

Proflavin mutagenesis was performed by infecting BB cells in L broth, adjusted to pH 7.8, with an average of about five T4 particles per cell at 37° on a rotary shaker at a cell density of 5×10^8/ml. After five minutes the samples were diluted tenfold into broth with or without proflavin at 0.9–2.5 µg/ml, together with an additional five phage particles per cell to induce lysis inhibition. After an additional 25 or 30 minutes the complexes were diluted 50-fold into ordinary L broth. After an additional 15 to 25 minutes lysis was completed with chloroform, and mutant frequencies were assayed promptly.

Spontaneous reversion rates of *rII* mutants were measured by growing three or more stocks of the mutant in parallel in the various genetic backgrounds, using BB cells in L broth and an *rII* inoculum small enough to contain no pre-existing revertants.

RESULTS

UV and MMS mutagenesis: Forward mutation rates to the *r* phenotype are linear following UV or MMS treatments. The effects of *px*, γ and *hm* on these mutation rates have been described (DRAKE 1973, 1974; GREEN and DRAKE, manuscript in preparation), and are summarized in Table 1, together with new data on the effect of *1206*. Table 1 also lists the approximate specific UV and MMS sensitivities of these strains. The increases in sensitivity produced by *px*, γ and *1206* are less than the decreases in mutability, indicating that induced mutation rates are reduced regardless of whether the rates are expressed per lethal hit (as in the Table) or per unit of dose. Despite the very different types of lesions produced in DNA by these two agents, the *rII* mutations which they produce are very similar (DRAKE 1973), consisting mainly of G:C → A:T transitions, frameshift mutations, and a minority of other base pair substitutions. In an extended series of reversion tests with MMS, to be reported elsewhere, *rII* mutants carrying reverting A:T base pairs or frameshift mutations showed little or no response, whereas mutants carrying reverting G:C base pairs were reverted (typically about 20-fold at a dose producing a survival of about 20%). Furthermore, *px* and γ decreased the MMS-induced reversion rates of G:C mutants by variable but generally incomplete factors.

TABLE 1

Effects of T4 genotype on relative rates of inactivation and mutagenesis by UV and MMS

Genotype	UV		MMS	
	Inactivation	Mutagenesis	Inactivation	Mutagenesis
Wild type	1.0	1.0	1.0	1.0
px	1.9	0.15	2.6	0.25
γ	2.0	0.1	2.0	0.03
1206	1.0–1.2	0.04		
hm	1.0	3.2	1.0	1.7

Mutation rates to the *r* phenotype were determined by the extra-soft top agar method (DRAKE 1966). *1206* is partially *rII* in character (VAN DEN ENDE and SYMONDS 1972), and induced *r* mutants were scored on BB cells, which reveal only *rI* mutants. Induced *r* mutants for the other strains were scored on B cells, which reveal *rI*, *rII* and *rIII* mutants. Both UV and MMS mutation rates in the wild type are about 8×10^{-4} *r* per lethal hit. Relative mutation rates below about 0.2 are very approximate because of the small factors of increase over the spontaneous backgrounds. The UV sensitivity of *1206* is slightly host-dependent.

Photodynamic mutagenesis: White light irradiation in the presence of dyes such as psoralen or thiopyronin is mutagenic for bacteriophage T4 (DRAKE and McGUIRE 1967). The unavailability of psoralen prompted us to use the equally effective analogue 8MOP. Photodynamic mutagenesis with 8MOP is just as effective as with psoralen itself. It is reduced by *px*, *γ* and *1206* and enhanced by *hm* (Table 2).

Base analog and thymineless mutagenesis: Thymineless mutagenesis in T4 produces transitions and some transversions at A:T base pairs (SMITH *et al.* 1973). It is at most slightly reduced (average 14%) by *px* and *γ*, but is enhanced about 3.6-fold by *hm*.

The effects of *px*, *γ* and *hm* on base analog mutagenesis are shown in Table 3, where A:T mutants are reverted by 2AP and G:C mutants are reverted by 5BU. The rates of both 2AP and 5BU mutagenesis are more than doubled by *hm*. 2AP mutagenesis is not appreciably affected by *px* or *γ*, but the rate of 5BU mutagenesis is more than doubled by both mutations.

Proflavin mutagenesis: The effects of *px*, *γ* and *hm* on frameshift mutagenesis induced by proflavin are shown in Table 4. Both *px* and *γ* mildly enhance proflavin mutagenesis, but *hm* produces only a barely significant effect.

Spontaneous mutation: Data accumulated to date on the effects of *px*, *γ* and *hm* on spontaneous mutation rates are shown in Table 5. Only differences in excess of approximately two-fold are likely to be significant in measurements of this type. All three mutations exhibit a general pattern of weak to moderate mutator action on base pair substitution mutation rates, but show little or no significant effect upon frameshift mutation rates. The strongest mutator activity was usually seen with *hm*. The effects of *γ* were essentially identical when stocks were grown in BB (*su⁻*) or CR63 (*su⁺*) cells; thus suppression of the DNA synthesis defect in *γ* does not suppress the mutator phenotype, although it does suppress the repair defect.

Preliminary mapping experiments: The *γ* mutation has been mapped between genes *24* and *25* (MAYNARD SMITH and SYMONDS 1973).

Early crosses with *px* suggested the map order *px*–gene *42*–gene *43*, *px* produc-

TABLE 2

Effects of T4 genotype on relative rates of photodynamic inactivation and mutagenesis

Genotype	Inactivation	Mutagenesis
Wild type	1.0	1.0
px	1.6	0.22
γ	1.6	0.18
1206	1.1	0.01
hm	1.1	1.67

Mutation rates to the *r* phenotype were determined using the extra-soft top agar method (DRAKE 1966). The mutation rate in the wild type was 8×10^{-4} *r* per lethal hit, which is similar to the value (5×10^{-4}) obtained previously with psoralen (DRAKE and McGUIRE 1967). All inactivation rates were not obtained in parallel experiments, and relative inactivation rates are therefore only approximate at present.

TABLE 3

Effects of T4 genotype on base analog mutagenesis

Genotype		2AP	5BU	Net r^+	Factor of
rll	Other	μg/ml	μg/ml	$\times 10^6$	increase
rUV183	(wt)	400		22	
		500		101	
		800		25	
	px	500		113	1.1
	γ	500		111	1.1
	hm	400		47	2.1
		500		149	1.5
		800		90	3.6
rUV199	(wt)	500		69	
	px	500		90	1.3
	γ	500		46	0.7
	hm	500		152	2.2
rUV7	(wt)		200	361	
	px		200	961	2.7
	γ		200	1184	3.3
	hm		200	970	2.7
rUV13	(wt)		200	622	
	px		200	1133	1.8
	γ		200	1573	2.5
	hm		200	1962	3.2

rUV183 and *rUV199* are A:T mutants (reverted by 2AP but not by hydroxylamine). *rUV7* and *rUV13* are G:C mutants (reverted by hydroxylamine). Net revertant frequencies are corrected for spontaneous backgrounds. 2AP entries using 500 μg/ml and all 5BU entries are averages of four measurements; the remaining 2AP entries are single measurements.

ing about 30% recombination with *amN55* in gene *42*. This order was confirmed by performing the cross *px-amE4322* (gene *43*) × *amN55* (gene *42*) under conditions of premature lysis (17 min at 37°, burst size about 5, 2.7% *am+* recombinants instead of the usual 8–10%) in order to increase linkage. Eighteen *am+* progeny were picked and scored for the *px* character: 17 were *px* and one was *px+*. The *px* mutation therefore resides in the approximate vicinity of genes *41* to *56*.

The three-factor cross *e-v* × *hm* $(- - + \times + + -)$ was performed with the following results from the analysis of 60 progeny: $- - + = 16, + + - = 15, + - + = 6, - + - = 0, - + + = 1, + - - = 1, - - - = 11, + + + = 10$. These results suggest the marker order *hm*–38%–*v*–13%–*e*, *hm* falling into the interval between *rI* and *v*.

DISCUSSION

The T4 mutations *px*, *γ* and *1206* abolish or sharply reduce mutagenesis induced by UV irradiation, MMS, and white light irradiation in the presence of 8MOP (photodynamic mutagenesis). While not markedly affecting mutagenesis induced by 2AP or by thymine deprivation, both *px* and *γ* do enhance 5BU muta-

TABLE 4

Effects of T4 genotype on proflavin mutagenesis

Reverting *rII* mutant	Mean relative induced r^+ revertants in indicated genetic backgrounds			
	Wild type	*px*	*γ*	*hm*
rUV58	1.0[10]	1.9[2]	2.0[6]	1.3[10]
rUV113	1.0[3]	1.7[3]	1.8[3]	1.3[3]

Induced revertant frequencies were obtained by subtracting spontaneous backgrounds. Relative induced rates are mean factors of increase compared to wild type, comparing only measurements performed in parallel. Superscripts indicate total numbers of measurements.

genesis. The antimutagenic effect of these mutations is quite similar to that of *recA* and *exr* or *lex* mutations of *E. coli*. Since all of these mutations also reduce recombination and increase sensitivities to inactivation by mutagenic agents, they are currently interpreted as inactivating error-prone repair systems which employ genes also involved in genetic recombination. The term "misrepair mutagenesis" therefore seems appropriate for this general process, in contrast to directly induced mispairing of bases induced, for instance, by base analogs, nitrous acid, hydroxylamine and EMS. The T4 mutants studied here produce less marked reductions in recombination rates than do the corresponding *E. coli* mutants, but highly recombination-defective mutations may be lethal in T4 because of a probable requirement for recombination in the T4 life cycle.

Misrepair mutagenesis occurs not only in *E. coli* and T4, but probably also in the virulent phage VIr of *Proteus mirabilis* (WITTE 1971), in the bacterium *Bacillus subtilis* (HILL, PRAKASH and STRAUSS 1972), in the yeasts *Schizosacch-*

TABLE 5

Effects of host genotype on spontaneous mutation rates

Reverting *rII* mutant	Probable reverting base pair	Revertants per 10^8 particles in indicated genetic background				
		wt	*px*	*γ*(BB)	*γ*(CR63)	*hm*
rUV7	G:C	42	97	76	73	360
rUV13	G:C	23[5]	40	170[5]	180	19
rUV48	G:C	30		95		
rSM94	G:C	14		77		
rUV183	A:T	67	690	480	590	2900
rUV188	A:T	28		49[2]		
rUV199	A:T	100[5]	170	36[6]	190	590
rUV27	(fs)	1200		800		
rUV58	(fs)	23[2]	53[2]	37[2]	12	39
rUV113	(fs)	51	76	69	31	98

Revertant frequencies are means of three independent stocks (except when indicated by superscript) grown in parallel; *wt* = wild type and (fs) = frameshift. Stocks whose revertant frequencies were greater than 3.5 times the lowest value for a set of three stocks were excluded from the means except for *rUV199*-*γ*(BB), where the individual revertant frequencies of six stocks were 4, 11, 22, 58, 62 and 63. Mutants in *γ* backgrounds were sometimes grown both in BB (*su⁻*) and in CR63 (*su⁺*) cells.

aromyces pombe (NASIM 1968) and *Saccharomyces cerevisiae* (AVERBECK *et al.* 1970; LEMONTT 1971, 1972), and in the fungus *Neurospora crassa* (CHANG, LENNOX and TUVESON 1968; DE SERRES 1971). It does not, however, appear to occur in the UV- and/or MMS-immutable bacteria *Diplococcus pneumoniae* (J. G. TIRABY, personal communication), *Hemophilus influenzae* (R. F. KIMBALL, personal communication), or *P. mirabilis*, although the closely related *P. vulgaris* is UV-mutable (H. BÖHME, personal communication). Although widely observed, therefore, misrepair mutagenesis is probably not ubiquitous, and it is therefore important to determine whether it also occurs in higher eukaryotes.

The mutagens which trigger misrepair appear to be very diverse. UV and MMS mutagenesis apepar to depend entirely upon misrepair both in T4 and in *E. coli*, as do 4-nitro-quinoline-1-oxide, mitomycin C and X-rays in *E. coli* (BRIDGES, LAW and MUNSON 1968; KONDO *et al.* 1970). About 70% of N-methyl-N'-nitrosoguanidine and about 30% of EMS mutagenesis in *E. coli* also depend upon misrepair (KONDO *et al.* 1970), although we have thus far found no effects of *px* or γ upon EMS mutagenesis in T4 (unpublished results).

In contrast to mutagens which induce direct mispairing, the specificity of misrepair mutagenesis is rather broad. MMS, UV, X-rays and photodynamic irradiation produce similar (but not identical) mixtures of transitions, transversions, frameshift mutations and deletions in bacteriophage T4 (DRAKE 1966, 1973; DRAKE and McGUIRE 1967; and unpublished data from this laboratory), and a similarly wide spectrum is probably also produced in *S. pombe* (LOPRIENO 1966), *S. cerevisiae* (LEMONTT 1972) and *N. crassa* (KILBEY, DE SERRES and MALLING 1971). Since these diverse agents produce very different types of chemical lesions in DNA but tend to produce similar types of mutations, and since thymine dimers promote G:C → A:T transitions (MEISTRICH and DRAKE 1972), the specificity of misrepair mutagenesis appears to reside at least as much in the specific error tendencies of the repair system itself as in the nature of the premutational lesions.

Differences do, however, exist in the relative proportions of mutational types induced by different mutagens. UV irradiation, for instance, induces very few mutants reverting at G:C base pairs, whereas roughly equal numbers of mutants reverting at G:C and at A:T pairs are induced by EMS and by photodynamic irradiation; and about one fourth of the mutants induced by X-irradiation consist of deletions, in contrast to less than one tenth with the other mutagens. It is not clear at present whether these differences reflect a direct effect upon the misrepair process by the premutational lesion itself, or whether they represent differential action by two or more different misrepair systems, each with its own particular error tendencies.

While *px*, γ and *1206* reduce misrepair mutagenesis, *hm* promotes it. It also promotes base analog and thymineless mutagenesis, but not proflavin mutagenesis. It would be useful, in order to identify the specific error-prone step in the genetically complex process of recombination-like repair, to possess hypermutable variants, and *hm* may be such an example—as may also the *E. coli* mutations *mul* (WACKERNAGEL and WINKLER 1971), *uvr502* (SMIRNOV and SKAVRONSKAYA 1971), *mutH* (HILL 1972) and *mutU* (SIEGEL 1973). Since it

also promotes spontaneous, base analog and thymineless mutagenesis, however, *hm* may operate both during recombination-like repair and during normal DNA synthesis.

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