SHORT COMMUNICATIONS

Spontaneous and Ultraviolet-induced Mutation in *Escherichia coli*: Interaction Between Plasmid and *tif-1* Mutator Effects

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INTRODUCTION

Certain plasmids increase ultraviolet (u.v.) resistance and spontaneous and u.v. mutagenesis in *Salmonella typhimurium* (Howarth, 1966; MacPhee, 1973; Mortelmans & Stocker, 1976). In *Escherichia coli*, thermally-induced expression of the *tif-1* mutation similarly causes enhancement of spontaneous and u.v.-induced mutagenesis (Witkin, 1974). The effects of both plasmid expression (MacPhee, 1973; Monti-Bragadin, Babudri & Samer, 1976) and *tif-1* expression (Castellazzi, George & Buttin, 1972) are *recA*⁺ dependent. In addition novel DNA synthesis characteristics are found in the presence of plasmids (MacPhee, 1974; Wilkins, 1975) and when *tif-1* is expressed (Radman *et al.*, 1976). These common features prompted us to examine the interaction between the plasmid and *tif-1* effects in an attempt to decide whether or not they act through the same mechanism.

METHODS

Plasmid pKM101 was transferred from *Salmonella typhimurium* TA100 (McCann *et al.*, 1975) into *Escherichia coli* WP2 *uvrA trp* (Hill, 1965) and its non-filamenting *tif-1 sfi* derivative WP44_{8NF} (Witkin, 1976) using ampicillin selection. Details of the mutation system of WP2 and its derivatives and general methods have been published elsewhere (Bridges *et al.*, 1973). Selective plates for Trp⁺ revertants contained Difco Casamino acids (0.4%, w/v), tryptophan (0.125 µg ml⁻¹) and adenine (75 µg ml⁻¹), which has been shown to potentiate *tif-1* mutability (Witkin 1974). Bacterial viability was determined by plating appropriate dilutions on the same medium.

Exponentially growing cultures were obtained by diluting an overnight broth culture 1:100 into fresh nutrient broth and incubating at 32 °C. The bacteria were harvested by filtration, resuspended in buffer and, where appropriate, u.v. irradiated (0.05 J m⁻²).

RESULTS AND DISCUSSION

Incubation of WP2 *uvrA* pKM101 (designated CM891) and WP44_{8NF} pKM101 (designated CM901) and their parental strains at 44 °C for several hours did not result in lethality. However, CM901, unlike the other strains, filamented at this temperature. This was attributed to the presence of the pKM101 plasmid, since acridine orange curing of CM901 resulted in reversion to the non-filamenting properties of WP44_{8NF}.

In Fig. 1, spontaneous mutation is plotted as a function of the period of incubation of plates at 44 °C. Strain WP2 *uvrA* was unaffected by this treatment (data not shown). Strains WP44_{8NF} and CM901 showed thermal enhancement of spontaneous mutability, although incubation of CM901 for longer than 2 h resulted in a reduction in the number of additional mutants. This loss was presumed to relate to the plasmid, since spontaneous mutability of CM891 was decreased by incubation at 44 °C (Fig. 1) and Stadler & Adelberg (1972) have reported that plasmid maintenance in *E. coli* is temperature sensitive. For u.v.-interaction

Fig. 1. Effect of incubation at 44 °C on the number of spontaneous mutants. Portions (0.1 ml) of exponentially growing cultures were resuspended in buffer (1×10^8 to 2×10^8 ml⁻¹) and spread on plates which had been pre-warmed to their subsequent incubation temperature. The plates were incubated at 44 °C for various periods before being transferred to 32 °C for subsequent incubation for 2 days. The numbers of pre-existing mutants per plate, as determined by plating 0.1 ml portions on to tryptophan-free plates followed by incubation for 2 days at 32 °C, were insignificant. The points are averages from several experiments. Because of differences in cell densities the inter-strain comparisons are not quantitative. ▲, WP44sNF; ■, CM89I; ●, CM90I.

Table 1. Effects of pKM101 and *tif-1* expression on the yields of spontaneous and u.v.-induced mutants

Values given are the average number of mutants per plate; normally four plates were scored for each variable. The values have been corrected for pre-existing mutants.

Variable*	Experiment					Average
	1	2	3	4	5	
Strain WP44sNF						
None	11	4	4	4	4	5.4
u.v.	17	12	15	10	5	11.8
<i>tif-I</i>	20	11	13	22	30	19.2
<i>tif-I</i> u.v.	32	20	32	36	102	44.4
Strain CM90I						
pKM	146	151	122	133	188	148.0
pKM u.v.	216	207	201	252	277	230.6
pKM <i>tif-I</i>	204	190	147	160	292	198.6
pKM u.v. <i>tif-I</i>	300	361	243	301	566	354.2
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Bacteria per ml plate d (CM90I)						
Bacteria per ml plated (WP44sNF)	1.10	1.16	0.91	0.76	0.73	

* u.v. indicates irradiation with 0.05 J m⁻²; pKM indicates the presence of plasmid pKM101; *tif-1* indicates that the bacteria were incubated at 44 °C for 1.5 h.

experiments a period of 1.5 h incubation at 44 °C was chosen as giving a full *tif-1* effect while minimizing the loss of the plasmid effect.

Experiments were performed to quantify the effects of pKM101 and *tif-1* expression on the yield of spontaneous and u.v.-induced mutants (Table 1).

If one can consider the events leading to a mutation in terms of conventional pathways, a combination of treatments which caused no increase in mutant yield above that expected

for one treatment would indicate that the treatments affected the same step in the process of mutation. An additive interaction might indicate that the treatments affected quite independent processes. For instance, one treatment might specifically enhance G:C to C:G transversions and another might enhance A:T to G:C transitions. A multiplicative interaction might indicate that the treatments affected separate components of the same process. For instance, one might affect fidelity of a DNA polymerase and another the number of DNA sites available to that polymerase. A departure from these specific models might reflect an interaction of separate steps in a common process.

Analyses of variance (Moroney, 1962) were therefore performed to test the nature of the interaction between the effects of pKM101, *tif-I* and u.v. To test for additive interaction, the original plate counts corrected for pre-existing mutants were used. For multiplicative interaction, these values were transformed to logarithms. Where necessary the variance relating to differences between experiments was pooled with residual variance and breakdown analyses were performed. The effects of *tif-I* × u.v., pKM101 × u.v., pKM101 × *tif-I*, and pKM101 × u.v. × *tif-I* were all significantly greater than additive. The effects of *tif-I* × u.v. and pKM101 × *tif-I* × u.v. were consistent with multiplicative interaction. The effects of pKM101 × u.v. and pKM101 × *tif-I* showed significantly less than multiplicative interaction. Apparent antagonism between the pKM101 and *tif-I* effects would have been predicted from the observations in Fig. 1.

Since the joint effects of pKM101 and *tif-I* are actually greater than additive, the presence of pKM101 is not equivalent to constitutive *tif-I* expression. Furthermore, the significant departure from additivity makes it unlikely that they affect separate pathways of mutagenesis. The most plausible interpretation is that they affect separate steps in the same pathway of mutagenesis. It is not certain whether the departure from multiplicative interaction with *tif-I* can be explained solely in terms of the effect of temperature on pKM101, or whether it indicates a degree of interaction between related pKM101 and *tif-I* steps in mutagenesis.

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