Inducible Reactivation and Mutagenesis of UV-Irradiated Bacteriophage P22 in *Salmonella typhimurium* LT2 Containing the Plasmid pKM101

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The inducible (Weigle) reactivation of UV-irradiated bacteriophage P22 has been examined in strains of *Salmonella typhimurium* with and without the mutagenesis-enhancing plasmid pKM101. A large inducible reactivation was observed in the plasmid-containing strain, but only a small response was observed in the strain lacking the plasmid. An increased frequency of clear-plaque mutants was detected among the survivors. The efficiencies of the plasmid-mediated and cellular repair processes have been determined. The kinetics of induction of the phage reactivation have been investigated. The relationship of the observed results to the inducible reactivation of UV-irradiated λ in *Escherichia coli* and to error-prone repair is discussed.

A number of plasmids have been reported which confer upon their host both increased resistance to killing by UV irradiation (UV protection) and increased sensitivity to chemical and UV mutagensis. R46 (6) and its derivative pKM101 (22; K. E. Mortelmans and B. A. D. Stocker, Mol. Gen. Genet., in press), R-Utrecht (15), and ColI (7, 8) are representative examples in *Salmonella typhimurium* and *Escherichia coli*, and pMG2 is an example in *Pseudomonas aeruginosa* (10). The plasmid pKM101 has been incorporated into several of the Ames tester strains for the detection of carcinogens as mutagens and plays a major role in the success of the system (12–14). The observation of the UV-protective and mutagenesis-enhancing effects of R46 and pKM101 in mutants deficient in the various steps of excision repair (14, 16, 18, 21) and in certain branches of postreplicational repair (22) has suggested that these plasmids do not exert their effects by affecting the activity of these pathways. Instead an increasing amount of evidence has been presented suggesting that the plasmids act by increasing the capacity of the cell to carry out error-prone repair.

The mutagenesis- and repair-enhancing capacities of pKM101 appear to be coupled, as evidenced by the facts that (i) both capacities are lost concomitantly in pKM101 mutants (23) and (ii) both are dependent on the *recA*⁺ *lexA*⁺ genotype in *E. coli* K-12 (22) and on the *recA*⁺ genotype in *S. typhimurium* LT2 (14, 18). The dependence on the *recA*⁺ *lexA*⁺ genotype is the same as that observed for the hypothesized SOS repair system (19, 24). It does not appear that pKM101 or R46 simply causes the constitutive expression of the inducible cellular system, since (i) the inducible Weigle (W) reactivation (19) of UV-irradiated λ in unirradiated *E. coli* is still observed (22) and (ii) the presence of pKM101 in a *tif sfiA* mutant grown at 42°C causes a substantial increase in the amount of reversion occurring either spontaneously or upon methyl methane sulfonate or UV treatment (5, 17, 22). In addition, the presence of R46 or pKM101 does not cause the expression of protein X (11) or the induction of a λ lysogen (G. C. Walker, unpublished data). Nevertheless, a certain component of the pKM101-mediated repair and mutagenic effects appears to be constitutively expressed, since the plasmid enhances the spontaneous reversion rate (14, 18, 22) and also causes an increased survival of UV-irradiated phage even in unirradiated hosts (plasmid-mediated reactivation) (22, 23).

A limited amount of evidence suggests that there is also an inducible component to the pKM101-mediated enhancement of repair and mutagenesis. I have reported a pKM101-mediated inducible reactivation of UV-irradiated phage λ in *E. coli* (22), and Tweats et al. (21) have reported that postirradiation treatment of R46-containing *E. coli* with chloramphenicol abolishes the UV-protective effect of the plasmid. On the basis of physiological evidence, Tweats et al. (20) have postulated the existence of an inducible nuclease in strains containing R46. We have isolated and characterized a sin-

gle- and double-stranded endonuclease in pKM101-containing strains, but were not able to implicate it in the mechanism of plasmid-mediated repair and mutagenesis (9). Since pKM101 plays such a crucial role in the Ames strains, it was of particular interest to establish whether a plasmid-mediated inducible response could be detected in S. typhimurium LT2. In this paper I report the inducible reactivation and mutagenesis of UV-irradiated bacteriophage P22 in a strain of S. typhimurium containing pKM101.

MATERIALS AND METHODS

Media. Nutrient medium contained 0.8% nutrient broth with 0.5% NaCl. LB medium contained 1% tryptone, 0.5% yeast extract, and 0.5% NaCl. Nutrient and LB plates contained 1.5% agar (Difco). Top agar is 0.7% agar with 0.5% NaCl.

Bacterial strains and phage. The S. typhimurium LT2 strains GW45 (hisG46) and TA92 [GW45(pKM101)] were obtained from B. N. Ames and were stored as frozen permanents as previously described (22). The indicator strain GW19 (hisG46 recA ΔuvrB) was constructed by introducing a chl bio uvrB deletion into TS24 (hisG46 recA) using previously published procedures (1). The chlorate-resistant derivatives of TS24 were screened for UV sensitivity by streaking across a nutrient plate and irradiating half the plate with a dose of 2.5 J/m². Introduction of pKM101 into the indicator strain GW19 did not affect the UV sensitivity of this strain. Phage P22 int3 was obtained from D. Botstein.

W-reactivation of UV-irradiated P22. A recently prepared stock of P22 int3 was diluted to 10^7 plaque-forming units per ml in 10 mM MgSO₄. The phage (6 ml) were irradiated in glass petri plates (100 mm) under a 15 W G. E. germicidal lamp whose height from the sample could be varied. UV doses were measured by the use of a Latarjet UV meter. Cells were grown to 2×10^8/ml in nutrient or LB medium and then were suspended in 10 mM MgSO₄. After irradiation the bacteria were incubated for 10 min at 37°C without shaking. Then equal volumes of phage and bacteria were mixed (multiplicity of infection of 0.05) and were incubated for 20 min at 37°C without shaking. After the appropriate dilution in 10 mM MgSO₄, 0.1 ml of the phage-host mixture and 0.1 ml of a fresh stationary nutrient broth culture of the indicator strain GW19 were added to 2 ml of top agar, which was poured on nutrient or LB plates. Incubation was at 37°C overnight. A similar procedure was carried out using unirradiated phage. The UV survival of the bacteria was measured after the 10-min preincubation period by plating on nutrient plates. Reactivation factors could vary two- to threefold between experiments done on different days. Similar variability has been reported in E. coli (2). In kinetic experiments the length of the preincubation period was varied.

Clear-plaque mutation frequency. The same procedure was followed as in the W-reactivation experiment but with the following changes. The phage were irradiated at 10^8 plaque-forming units per ml.

The cells were grown to 2×10^8/ml and were irradiated as before, but after irradiation they were centrifuged and resuspended in 0.1 volume of 10 mM MgSO₄ prior to preincubation. The modification of the procedure was necessary to allow greater numbers of plaques to be screened. In general the reactivation factors obtained using the modified procedure were only 25 to 50% of those obtained using the above procedure.

RESULTS

I have examined the inducible W-reactivation of the lambdoid phage P22 int3 in the S. typhimurium LT2 strain GW45. Only a very slight W-reactivation of UV-irradiated P22 could be detected in this strain (Fig. 1). The reactivation factor ranged from two- to fourfold in several experiments. The maximum reactivation of the phage occurred at a UV dose causing about 40% survival of the bacteria. The survival of unirradiated P22 in the irradiated cells declined only slightly with the increasing dose of UV to the cells.

In contrast, a strong inducible reactivation of UV-irradiated P22 was observed when pKM101 was present in the cells (Fig. 1). The increased survival of the irradiated phage in the unirradiated cells is the plasmid-mediated reactivation (22) mentioned earlier. The maximum induced reactivation of the phage again occurred at a UV dose corresponding to about 40% survival of the cells. The higher dose necessary to cause an equivalent degree of killing in the plasmid-containing strain reflects the UV-protective effect of pKM101. Again the survival of unirradiated phage showed only a gradual decline with increasing UV dose to the bacteria, whereas the survival of irradiated P22 declined sharply at higher UV dose to the bacteria. In this respect the pKM101-mediated reactivation of P22 in S. typhimurium resembles that of the reactivation of λ in E. coli (3, 22).

The effect of varying the UV dose to the P22 is shown in Fig. 2. Irradiated P22 were used to infect cells that were either unirradiated or had been irradiated with the dose giving maximal W-reactivation. In the case of the pKM101-containing strain, increasing doses of UV to the phage increased the relative degree of reactivation of the P22 (reactivation factor) (3). The efficiency (4) of this induced repair process was about 38% throughout most of the dose range tested. In contrast, in the same strain without the plasmid the efficiency of the induced process was only 11%. The efficiency of the pKM101-mediated repair was 28% in unirradiated cells. If the survival of UV-irradiated P22 in unirradiated GW45 was compared with the survival in irradiated GW45(pKM101), the efficiency of the overall pKM101-mediated repair (both constitutive and
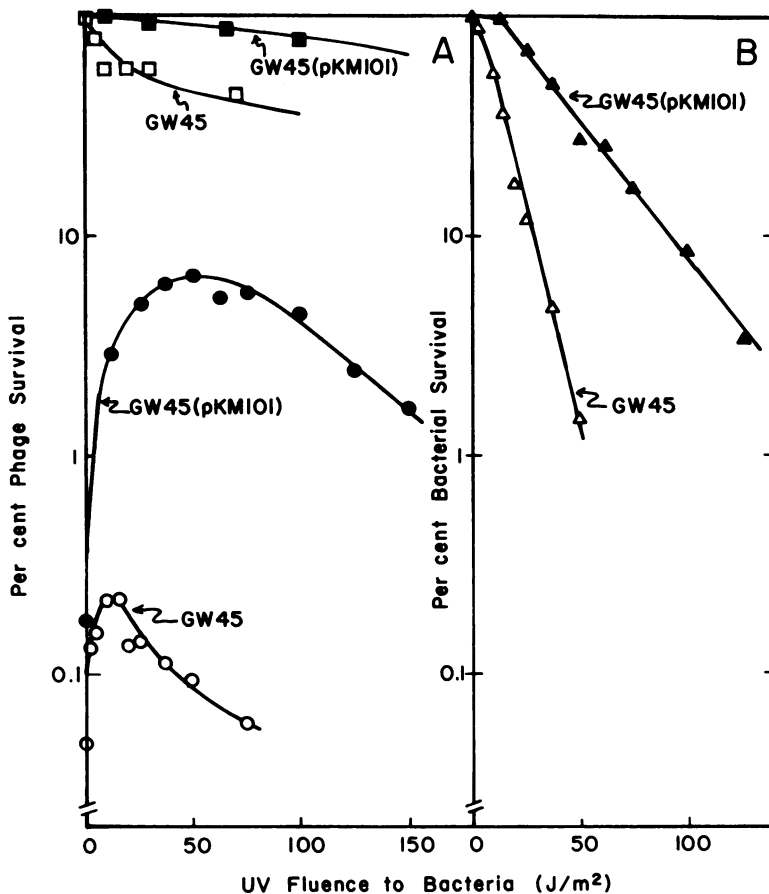
FIG. 1. *Effect of pKM101 on W-reactivation of phage P22. (A) Survival of UV-irradiated (300 J/m²) P22 on GW45 (O) and TA92 (●). Survival of unirradiated P22 on GW45 (□) and TA92 (■). (B) Survival of the bacterial strains GW45 (△) and TA92 (▲) after UV irradiation.*

inducible) was about 55%.

The kinetics of induction of the W-reactivation response have been examined under the conditions employed for these experiments. The strains GW45 and GW45(pKM101) were irradiated with the respective UV doses necessary to give maximal reactivation and then were incubated at 37°C in 10 mM MgSO₄ for varying lengths of time prior to the adsorption of the phage and plating for infective centers (Fig. 3). In the pKM101-containing strain, almost the maximal W-reactivation response was observed with no period of preincubation prior to the addition of the phage. The reactivation factor was approximately constant over the 2-h time course of the experiment. This could mean that a relatively short period is required for induction and that the system has been fully induced by the time the P22 adsorb to the cells, inject their DNA, and reach the stage of their life cycle where they are susceptible to the action of the

induced repair system. Once induced, the repair system would appear to be relatively stable under the experimental conditions employed. An alternative and perhaps less likely interpretation would be that no induction takes place in the 10 mM MgSO₄ and that the induction process is initiated by adsorption of the phage or the plating on rich medium. The constant W-reactivation factor over the 2 h of the experiment would then be viewed as reflecting the stability of the potential inducing signal. In the strain GW45 without pKM101, a small increase in plating efficiency was observed for irradiated and unirradiated cells up to 2 h while the W-reactivation factor slowly decreased.

To establish whether the inducible processes described here are mutagenic, I examined the effect of pKM101 and the inducing treatments on the frequency of clear-plaque mutants in UV-irradiated P22. The conditions were modified slightly as described to allow for the plating of
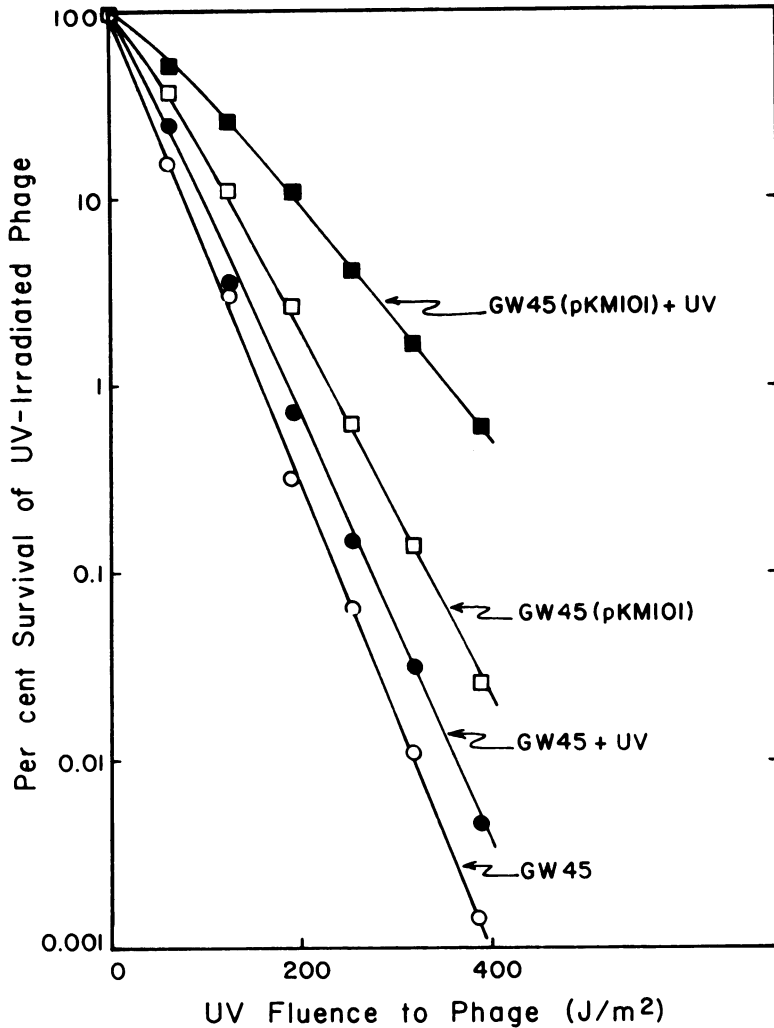
FIG. 2. *Survival of UV-irradiated P22 on unirradiated* (O) *and irradiated (7 J/m²;* ●) *GW45 and unirradiated* (□) *and irradiated (40 J/m²;* ■) *TA92.*

greater numbers of phage while still maintaining (i) the same multiplicity of infection and (ii) the same conditions for the irradiation of the bacteria. The phage were irradiated with a dose of 250 J/m², which gave about 0.08% survival on unirradiated GW45 and about 0.9% survival on unirradiated GW45(pKM101). Also, P22 irradiated with a dose of 400 J/m² were used to infect GW45(pKM101) to allow comparisons under conditions of approximately equal phage survival. The frequencies of clear plaques among unirradiated P22 adsorbed to the unirradiated strains GW45 and GW45(pKM101) and plated on the indicator strain GW19 (*recA ΔuvrB*) are shown in Table 1. The spontaneous mutation rate of unirradiated P22 was approximately the

same in both strains. With unirradiated strain GW45, irradiation of the phage to about 0.08% survival resulted in only a small increase in mutation frequency among the survivors. Irradiation of GW45 prior to infection resulted in an approximately 1.5-fold increase in both mutation frequency and phage reactivation.

If pKM101 was present in the cell, then UV light had a strongly mutagenic effect on the phage even when they were used to infect unirradiated cells. A UV dose of 250 J/m² to the phage increased the clear-plaque mutation frequency 27-fold if the phage were adsorbed to unirradiated GW45(pKM101), whereas the same UV dose caused only a 1.5-fold increase if the phage were adsorbed to unirradiated GW45.

Thus, phage P22 irradiated with this dose of UV showed a 10-fold higher survival rate and a 20-fold higher mutation frequency when adsorbed to unirradiated GW45(pKM101) rather than to unirradiated GW45.

When the plasmid-containing strain GW-45(pKM101) was irradiated, increases in both the reactivation and the clear-plaque mutation frequency of the irradiated phage were observed. The reactivation factor was larger at greater UV doses to the phage, as has been discussed earlier. When the phage were irradiated with a dose of 400 J/m^2 and were plated on irradiated cells, a reactivation factor of about 10 was observed, but

the induced increase in mutation frequency was only about 2.5-fold. This larger induction of phage reactivation relative to the induction of the phage mutagensis in strain GW45(pKM101) differs from that which has been discussed above for strain GW45. It should be noted that in spite of the relatively small inducible mutagenic response in GW45(pKM101), quite high levels of phage mutagenesis (2.2% clear-plaque mutants) are obtained due to the additional plasmid-mediated mutagenic capacity seen even in unirradiated cells. Moreover, if comparisons are made under conditions of approximately equal phage survival in unirradiated cells with and without
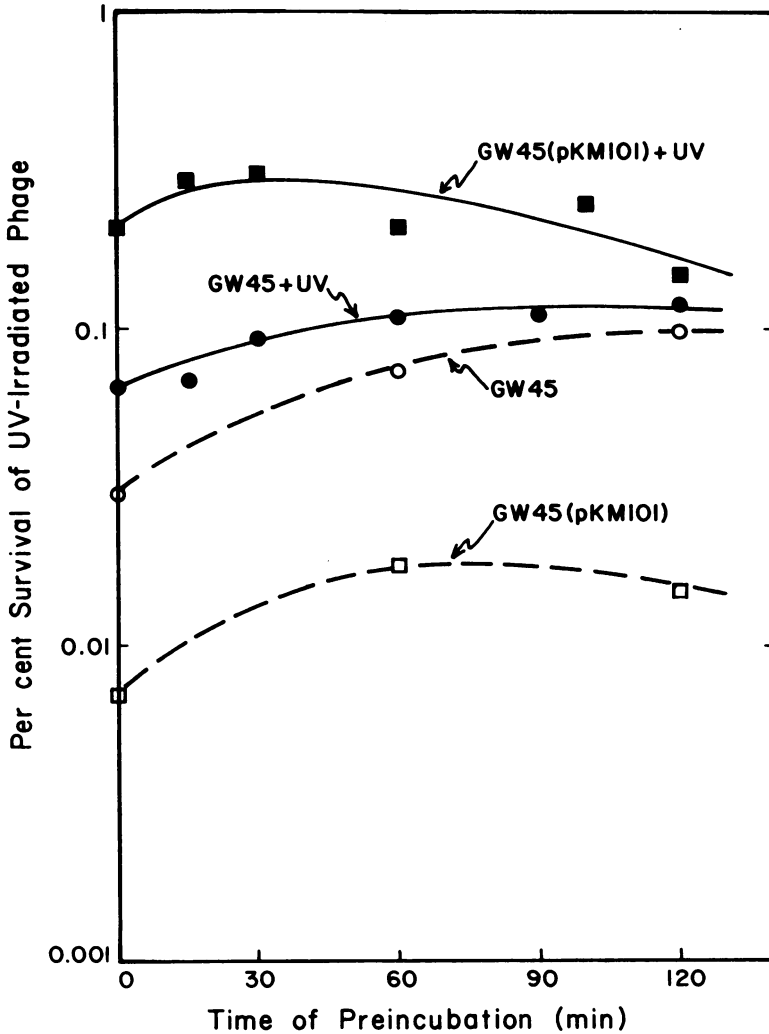

FIG. 3. *Effect of varying length of the preincubation period between irradiation of the bacteria and the adsorption of the phage. After the times shown, UV-irradiated (250 J/m^2) P22 were adsorbed to unirradiated (O- - -O) and irradiated (7 J/m^2; ●——●) GW45, and UV-irradiated (400 J/m^2) P22 were adsorbed to unirradiated (□- - -□) and irradiated (40 J/m^2; ■——■) TA92.*

TABLE 1. *Effect of pKM101 on frequency of clear-plaque mutants of P22*

Bacterial strain	UV to P22 (J/m²)	UV to bacteria (J/m²)	Percent P22 survival	Reactivation factor	Percent clear-plaque mutants among survivors[a]	Induced mutation factor
GW45	0	0	100		0.033	
GW45	250	0	0.085	1	0.049	1
GW45	250	2.5	0.13	1.5	0.080	1.6
GW45	250	5	0.13	1.6	0.075	1.5
GW45	250	13	0.10	1.2	0.064	1.3
GW45(pKM101)	0	0	100		0.036	
GW45(pKM101)	250	0	0.87	1	0.96	1
GW45(pKM101)	250	6	3.2	3.6	0.98	1.0
GW45(pKM101)	250	33	2.6	3.0	0.81	0.84
GW45(pKM101)	250	78	1.8	2.0	0.93	0.97
GW45(pKM101)	400	0	0.037	1	0.88	1
GW45(pKM101)	400	6	0.36	9.7	2.0	2.3
GW45(pKM101)	400	33	0.38	10	2.2	2.5
GW45(pKM101)	400	78	0.26	7.0	1.5	1.7

[a] For each entry for GW45, 45,000 to 80,000 plaques were screened for clear-plaque mutants. For each entry for GW45(pKM101), 25,000 to 75,000 plaques were screened.

pKM101, it is clear that the magnitude of the induced reactivation and mutagenesis is much greater in the pKM101-containing strain.

DISCUSSION

A UV-inducible reactivation of UV-irradiated P22 has been observed in a strain of *S. typhimurium* LT2 carrying the plasmid pKM101. Such an inducible response is seen only weakly in the same strain when it lacks the plasmid. The maximum response is induced in each case by a dose of UV giving about 40% survival of the bacteria. The plasmid could code for the inducible components itself or it could code for constitutively produced active components which somehow amplify the inducible cellular response. Experiments are currently in progress to determine whether protein synthesis is required for induction of plasmid-mediated W-reactivation.

The weak cell-coded response in *S. typhimurium* LT2 contrasts sharply with the strong cell-coded response observed in W-reactivation experiments in *E. coli* phage λ. Reactivation factors in excess of 100-fold can be achieved if the λ is sufficiently irradiated. The efficiency of the repair (4) is approximately constant (R. H. Rothman, L. J. Margossian, and A. J. Clark, submitted for publication; calculated from data in [3]) with increasing UV dose to the phage. In *E. coli* the presence of pKM101 also increases the extent of the inducible W-reactivation, but the differences between the strains with and without the plasmid are not as striking as in *S. typhimurium* LT2 (22).

It is clear from Fig. 1 and Table 1 that in addition to increasing the magnitude of the in-

ducible response the presence of pKM101 in cells also increases the reactivation of irradiated phage even in unirradiated cells. This plasmid-mediated reactivation has been described previously (18, 22). The survival of UV-irradiated P22 in unirradiated *S. typhimurium* LT2 compared to that in an irradiated *S. typhimurium* strain containing pKM101 can differ by a factor of several hundred fold.

I have previously reported the isolation and characterization of mutants of pKM101 deficient in their abilities to enhance mutagenesis and repair. Both the enhanced constitutive reactivation of UV-irradiated phage in unirradiated cells (plasmid-mediated reactivation) and the enhanced induced response observed in irradiated cells (W-reactivation) are absent in cells containing these mutant plasmids (23). This suggests that the constitutive and induced processes share at least one common step and may in fact represent the same process expressed at two different levels.

The kinetics of the induction of the W-reactivation response in *S. typhimurium* strains containing pKM101 described in this paper differ from those reported for *E. coli* strains lacking pKM101. Defais et al. (2) demonstrated a pronounced increase in the extent of W-reactivation of UV-irradiated λ upon a period of preincubation of the irradiated cells prior to infection with the phage. It should be noted that the procedure of Defais et al. (2) differed additionally from the experiments described in this paper in that the preincubation was carried out in rich medium rather than in the 10 mM MgSO₄ (3) employed here. We are currently investigating the influences of culture conditions on W-reactivation.

When the pKM101-containing strain was irradiated, the relative increases in phage reactivation and phage mutagenesis, while similar, were not completely equivalent. This is similar to the data of Defais et al. for the W-reactivation of UV-irradiated λ in *E. coli* (3). Also, the presence of pKM101 in unirradiated cells did not cause completely equivalent increases in reactivation and mutagenesis. At this stage I cannot eliminate the possibility that repair and mutagenesis are at least partially separable, even though they are evidently closely associated.

pKM101 apparently plays its critical role in the Ames tester strains by increasing the amount of error-prone repair activities available in the cells. Some compounds such as 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2) or aflatoxin B1 were either weakly detected or were not detected at all in the *S. typhimurium* strains lacking the plasmid, whereas they were potent mutagens in the pKM101-containing strains (14). *S. typhimurium* tester strains may benefit particularly from the introduction of pKM101 because of their relatively weak cellular inducible "error-prone repair" activity.

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