

UV PROTECTION PRODUCED BY PLASMID pGW16 IN ESCHERICHIA COLI IS STRAIN DEPENDENT

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Plasmid pKM101 is included in some strains of Salmonella typhimurium used in the Ames mutagenicity test. pKM101 carries muc⁺ genes that are analogous to the chromosomal umu⁺ genes of Escherichia coli K12 (Walker, 1984). umu-deficient strains are immutable by DNA damaging agents such as ultraviolet irradiation (UV) and are more sensitive to UV than umu⁺-proficient strains. umu- or muc-gene products are therefore believed to be essential for error-prone DNA repair, but their mode of action is not fully understood. One way of investigating this is to study derivatives of pKM101 with altered mutator function. Plasmid pGW16 is a derivative of pKM101 selected for its increased spontaneous mutator effect. It increases post-UV mutagenesis and survival in S. typhimurium, but we now present evidence to show that although pGW16 increases mutagenesis in all strains tested, its UV-protecting effect in E. coli is dependent on the activity of chromosomal DNA-repair genes.

The strains of E. coli K12 used were AB1157 uvrB⁺ umu⁺, TK702 uvrB⁺ umu (deficient in error-prone DNA-repair), JC3890 uvrB umu⁺ (where the uvrB mutation abolishes error-free excision repair) and TK501 uvrB umu. Post-UV mutagenesis and survival were determined using methods described by Upton and Pinney (1983). All strains carry the his4 allele and mutagenesis was measured as the frequency of reversion to histidine independence in Vogel-Bonner medium. Survival was determined on nutrient agar. Both pKM101 and pGW16 increased post-UV mutagenesis in strains AB1157, TK702 and TK501 greater than ten-fold when compared with the plasmid-less (P⁻) strains and increased UV-induced mutation in JC3890 by three- and four-fold respectively.

Table 1 UV protection conferred by pKM101 and pGW16 in E. coli

<u>E. coli</u> strains	AB1157		TK702		JC3890		TK501	
UV dose (Jm ⁻²)	50	125	50	125	4	10	4	10
pKM101	2.3	25	18	567	4.4	27	34	739
pGW16	0.12	25	1.8	80	0.09	0.03	1.0	2.6

Figures are fold increase (or decrease) in survival produced by the plasmids in comparison to the P⁻ strains.

UV-dose survivor curves were produced for each strain and representative data are presented in Table 1. Plasmid pKM101 increased post-UV survival in all strains tested at both low and high UV doses. pGW16 also protected the umu-deficient E. coli strain TK702 at all UV doses, similar to the results of Podger and Hall (1984) with pGW16 in S. typhimurium, which is also deficient in umu functions (Walker, 1984). However pGW16 sensitised strain AB1157 umu⁺ to low UV doses but protected against higher doses. This difference in activities of pKM101 and pGW16 was even more apparent in JC3890 uvrB, which was sensitised by pGW16 to all UV doses. pGW16 had little effect on strain TK501, suggesting a balance in this uvrB umu double mutant between the protection of strain TK702 umu and the sensitisation of JC3890 uvrB.

All reported inactivations in the muc⁺ genes of pKM101 lead to simultaneous loss of UV protection and mutagenesis (Shanabruch and Walker, 1980). pGW16 still retains both activities but its strain-dependent UV protection suggests an alteration in either the function or regulation of its muc⁺ gene products.

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