

## Short communication

# Induction of damage inducible (SOS) repair in *dam* mutants of *Escherichia coli* exposed to 2-aminopurine

Robert J. Craig\*, Judy A. Arraj, and M.G. Marinus

Department of Pharmacology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01605, USA

**Summary.** 2-Aminopurine induces damage inducible (SOS) repair in an *Escherichia coli dam-4* strain but not in a *dam-4 mutS456* derivative or in *dam*<sup>+</sup> bacteria.

The *dam* gene of *Escherichia coli* codes for a DNA adenine methylase which recognizes the sequence –GATC– in double stranded DNA (for review see Marinus 1984). One function of methylated –GATC– sequences is in mismatch repair of phage lambda heteroduplexes, where unmethylated strands are subject to repair whilst methylated chains are not (Pukkila et al. 1983). Such methylated/unmethylated regions may occur at the *E. coli* replication fork and thus allow correction of replication errors in the newly synthesized DNA strand (Wagner and Meselson 1976; Glickman and Radman 1980). Evidence for this model in *E. coli* consists of the phenotypic traits of *dam* mutants (Marinus 1984) and the hypermutability of bacteria containing high levels of *dam* methylase (Herman and Modrich 1981).

A phenotypic trait of *dam* mutants is that growth is inhibited in the presence of 2-aminopurine (2-AP; Glickman et al. 1978) and that second site mutations in *mutH*, *L* or *S* genes abolish sensitivity (Glickman and Radman 1980; McGraw and Marinus 1980). These *mut* genes are involved in mismatch repair (Rydberg 1978) suggesting that such repair is lethal in *dam* mutants which have incorporated 2-AP into DNA. Although the nature of the lesions induced by 2-AP in *dam* mutants is unclear (Marinus 1984) it might be expected that these would induce the *recA/lexA* damage inducible (SOS) repair system (Little and Mount 1982).

To test this possibility, we constructed *dam-4* and *dam-4 mutS456* derivatives of GW1040 which contains a *Mud* (Ap<sup>r</sup>, *lac*) operon fusion to the *dinD* promoter (Kenyon and Walker 1980). The *dam-4* mutation was introduced via conjugation between GM130 (Hfr, *dam-4*) with a Mal<sup>-</sup> derivative of GW1040 and selection for Mal<sup>+</sup>[Dam<sup>-</sup>] to yield GM1829. GM1874 (*mutS456*) was isolated as a spontaneous 2-aminopurine resistant derivative of GM1829 whose mutator phenotype was co-transducible with *cysC*. Induction of  $\beta$ -galactosidase in GW1040 is dependent on

the *recA* and *lexA* gene products and triggered by inducers (e.g., mitomycin C) of SOS functions. Addition of 2-aminopurine does not induce  $\beta$ -galactosidase in GW1040 or its *dam-4 mutS456* derivative (Table 1). In contrast, the *dam-4* derivative of GW1040 shows substantial induction of  $\beta$ -galactosidase after addition of 10–100  $\mu$ g 2-AP per ml and partial induction at 5  $\mu$ g 2-AP per ml (Table 1). In the absence of 2-AP a marginal increase in  $\beta$ -galactosidase activity in the *dam-4* derivative of GW1040 was noted (Table 1).

Further evidence for induction of the damage inducible (SOS) response in *dam*<sup>-</sup> mutants exposed to 2-AP was obtained by growing *dam*<sup>+</sup> (GM172) and *dam-3* (GM113) strains with <sup>35</sup>S-methionine in the presence or absence of 2-AP. Cell extracts were subjected to SDS-polyacrylamide gel electrophoresis and autoradiography. Figure 1 shows that *recA* protein is induced in the *dam-3* strain exposed to 2-AP for 2 h, but not in the *dam*<sup>+</sup> strain under similar conditions. There was no detectable *recA* induction in the *dam-3* strain in the absence of 2-AP. The *recA* protein was induced in both *dam*<sup>+</sup> and *dam*<sup>-</sup> strains after exposure to mitomycin C (data not shown).

Induction of the damage inducible response by 2-AP might be expected to increase the spontaneous mutation frequency in *dam*<sup>-</sup> cells. Although Glickman et al. (1978) reported that *dam* mutants are hypermutable by 2-AP, a subsequent genetic analysis of survivors after exposure to 2-AP did not support this hypothesis (Marinus 1984). In the absence of 2-AP, mutability in *dam*<sup>-</sup> strains or in cells overproducing *dam* protein is *recA* independent (McGraw and Marinus 1980; Herman and Modrich 1981), and *umuC* independent (data not shown).

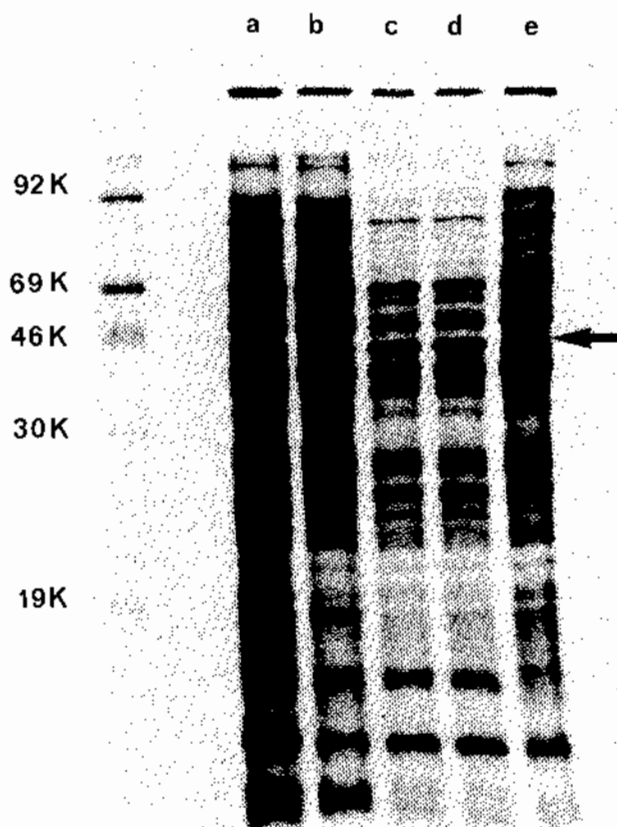
**Table 1.**  $\beta$ -Galactosidase levels in *dinD::lacZ* operon fusion strains

Strain	Genotype	2-AP ( $\mu$ g/ml)					MC ( $\mu$ g/ml)	
		0	1	5	10	100	0	1
GW1040	<i>dam</i> <sup>+</sup>	12	14	11	11	10	13	130
GM1829	<i>dam-4</i>	32	34	97	139	131	24	200
GM1874	<i>dam-4 mutS456</i>	12	8	14	14	16	23	175

Logarithmic phase cells were grown in the presence of 2-AP and mitomycin C (MC). Values are given as units of  $\beta$ -galactosidase (Miller 1972) after 5 h incubation during which time induction is linear

\* Present address: Biogen, 241 Binney St., Cambridge, MA 02142, USA

Offprint requests to: M.G. Marinus



**Fig. 1.** Induction of *recA* protein in *dam*<sup>+</sup> and *dam*-3 cells. GM113 (*dam*-3) and GM172 (*dam*<sup>+</sup>) were grown with <sup>35</sup>S-methionine for 5 min after growth in medium with or without 2-AP for 2 h. GM172 (*dam*<sup>+</sup>) grown without (lane a) or with (lane b) 2-AP. GM113 (*dam*<sup>+</sup>) grown in presence of 100 µg/ml (lane c), 50 µg/ml (lane d) or no 2-AP (lane e). The arrow indicates the position of *recA* protein. The migration of molecular weight standards is shown in the far left lane

The mechanism by which 2-AP inhibits growth of *dam*<sup>-</sup> cells is unclear (Marinus 1984) but as shown here involves production of lesions which induce the damage inducible (SOS) response. The induction is abolished in *dam*<sup>-</sup> *mut*<sup>-</sup> strains suggesting that absence of mismatch repair, or some other attribute of *mut*<sup>-</sup> strains, can prevent formation of potentially lethal lesions.

Strain GM1829 should also be useful to detect mutagens and carcinogens which, like 2-AP, would be expected to give a weak positive result in the Ames test or which like 5-bromouracil, require specialized strain constructions. Such agents should be rapidly and easily detected by their ability to induce  $\beta$ -galactosidase in strain GM1829.

**Acknowledgements.** This work was supported by grant GM30330 from the US Public Health Service. We thank Graham Walker for the gift of strain GW1040.

## References

- Glickman BW, Radman M (1980) *Escherichia coli* mutator mutants deficient in methylation-instructed DNA mismatch correction. *Proc Natl Acad Sci USA* 77:1063-1067
- Glickman BW, van den Elsen P, Radman M (1978) Induced mutagenesis in *dam*<sup>-</sup> mutants of *Escherichia coli*, a role of 6-methyladenine residues in mutation avoidance. *Mol Gen Genet* 163:307-312
- Herman GE, Modrich P (1981) *Escherichia coli* K-12 clones that overproduce *dam* methylase are hypermutable. *J Bacteriol* 145:644-646
- Kenyon CJ, Walker GC (1980) DNA-damaging agents stimulate gene expression at specific loci in *Escherichia coli*. *Proc Natl Acad Sci USA* 77:2819-2823
- Little JW, Mount DW (1982) The SOS regulatory system of *Escherichia coli*. *Cell* 29:11-22
- Marinus MG (1984) Methylation of prokaryotic DNA. In: Razin A, Cedar H, Riggs A (eds) DNA methylation. Springer Verlag, New York, in press
- McGraw BR, Marinus MG (1980) Isolation and characterization of *Dam*<sup>-</sup> revertants and suppressor mutations that modify secondary phenotypes of *dam*-3 strains of *Escherichia coli* K-12. *Mol Gen Genet* 178:309-315
- Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory, New York, p 466
- Pukkila P, Peterson J, Herman G, Modrich P, Meselson M (1983) Effects of high levels of DNA adenine methylation on methyl directed mismatch repair in *E. coli*. *Genetics* 104:571-582
- Rydberg B (1978) Bromouracil mutagenesis and mismatch repair in mutator strains of *Escherichia coli*. *Mutat Res* 52:11-24
- Wagner RW, Meselson M (1976) Repair tracts in mismatched DNA heteroduplexes. *Proc Natl Acad Sci USA* 73:4135-4139

Communicated by P.T. Emmerson

Received November 15, 1983