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Complementation of sensitivity to alkylating agents in *Escherichia coli* and Chinese hamster ovary cells by expression of a cloned bacterial DNA repair gene

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Dual expression vectors derived from pSV2gpt and encoding all or part of the *Escherichia coli ada*$^+$ gene have been constructed. Following transformation into an *E. coli ada* strain or transfection and stable integration into the genome of Chinese hamster ovary (CHO) cells, plasmid vectors containing the whole *ada*$^+$ gene conferred resistance to both killing and mutagenesis by N-methyl-N′-nitro-N-nitrosoguanidine (MNNG). Thus, the bacterial DNA repair gene was functionally expressed in the mammalian cells. Plasmids containing an N-terminal fragment of the *ada*$^+$ gene which encoded only one of the two methyltransferase activities of the Ada protein did not significantly protect *E. coli* or CHO cells against MNNG. These results are consistent with the central role of the intact *ada*$^+$ gene in controlling the adaptive response to alkylating agents in *E. coli*. However, the data further suggest that some alkylation lesions in DNA, such as O^6-methylguanine, may exert partly different biological effects in *E. coli* and mammalian cells.

Key words: ada$^+$ gene/alkylating agents/complementation/Mex$^-$ phenotype/O^6-methylguanine/methylphosphotriesters

Introduction

The adaptive response to alkylating agents protects *E. coli* against killing and mutagenesis by agents such as N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) (reviewed by Walker, 1985). The response involves the coordinated induction of at least three genes. Central to the regulation of the response is the *ada*$^+$ gene. This gene encodes a protein of 39 kd which repairs O^6-methylguanine (O^{6}MeGua), O^4-methylthymine (O^{4}MeThy) and the Sp stereoisomers of methylphosphotriesters in DNA by two separate methyltransferase functions (McCarthy and Lindahl, 1985). Both methyltransfer reactions involve the suicidal transfer of a methyl group from DNA to a cysteine residue within the protein, although the acceptor cysteine residues for O^{6}MeGua (and O^{4}MeThy) repair and methylphosphotriester repair are situated at different loci in the protein; the methyl acceptor residue for O^{6}MeGua being Cys^{321} whereas the acceptor residue for methylphosphotriesters is probably Cys^{69} (Demple *et al.*, 1985). In addition to a direct role in reversing methylation damage in DNA, the *ada*$^+$ gene product acts as a positive effector both of its own synthesis and of the products of the proximal *alkB*$^+$ gene (Kataoka and Sekiguchi, 1985) and the distal *alkA*$^+$ gene. Genetic and biochemical evidence indicates that the induction of the AlkA protein (3-methyladenine-DNA glycosylase II) is responsible for protection against killing whereas methyltransferase mediated repair of O^{6}MeGua protects cells against mutagenesis by alkylating agents (Evensen and Seeberg, 1982; Karran *et al.*, 1982). The role played by DNA methylphosphotriesters in the biological effects of alkylating agents is uncertain although methyltransferase-mediated repair of these lesions has recently been implicated as an essential part of the regulatory mechanism of the adaptive response (Teo *et al.*, 1986).

Many mammalian (including human) cell lines contain a DNA methyltransferase which acts on O^{6}MeGua residues in DNA in a manner analogous to the bacterial enzyme. However, other cell lines lack this activity and exhibit a phenotype (Mex$^-$ or Mer$^-$) which includes the inability to remove O^{6}MeGua from DNA *in vivo* and hypersensitivity to killing by MNNG (Day *et al.*, 1980; Sklar and Strauss, 1981). Chinese hamster ovary (CHO) cells are similar to human Mex$^-$ cell lines in that they are sensitive to MNNG and do not express detectable methyltransferase activity against O^{6}MeGua in DNA. The biological role of DNA methylphosphotriesters in mammalian cells is not known although such lesions have been reported to persist for many generations (Shooter, 1978) and highly purified mammalian O^{6}MeGua-DNA methyltransferase is without effect on methylphosphotriesters in DNA (Myrnes *et al.*, 1986; J.Hall, unpublished).

We have used the known sequence and properties of the bacterial *ada*$^+$ gene (Demple *et al.*, 1985) to construct plasmids encoding either the entire Ada protein or a fragment of this protein which has retained the methyltransferase activity towards phosphotriesters but not that towards O^{6}MeGua. Expression of these methyltransferase activities in bacterial or mammalian cells has enabled us to identify the possible biological consequences of unrepaired O^{6}MeGua lesions in the DNA of both types of cell.

Results

Construction of plasmids encoding whole or truncated Ada proteins

Figure 1 illustrates the construction of plasmids bearing all or part of the *E. coli ada*$^+$ gene. Plasmids pHJ2 and pHJ24 are dual expression molecules which can be read either from the endogenous bacterial promoter or from the SV40 early promoter on the pSV2gpt portion of the plasmid. Following transformation of each of these plasmids into *E. coli* BS24 (*ada5*) (Jeggo, 1979; Sedgwick, 1983), methyltransferase assays were carried out on crude cell extracts. Table I shows that pHJ2 transformants contained high levels of methyltransferase activity directed against both O^{6}MeGua and methylphosphotriesters in DNA, indicating that the endogenous *ada*$^+$ promoter was being effectively used in this new construct. Extracts of BS24(pHJ24) contained similar although somewhat lower levels of activity against methylphosphotriesters, but no measurable activity against O^{6}MeGua in DNA. These results confirmed the substrate specificities expected of the methyltransferase activities in the two cases.

Biological consequences of expression of the truncated Ada protein in E. coli

The resistance to cell killing and mutagenesis by MNNG was assessed for plasmid-carrying *E. coli* BS24 strains producing intact or truncated Ada proteins. Figure 2a shows that pHJ2 conferred MNNG resistance to the *ada5* strain BS24. In contrast,

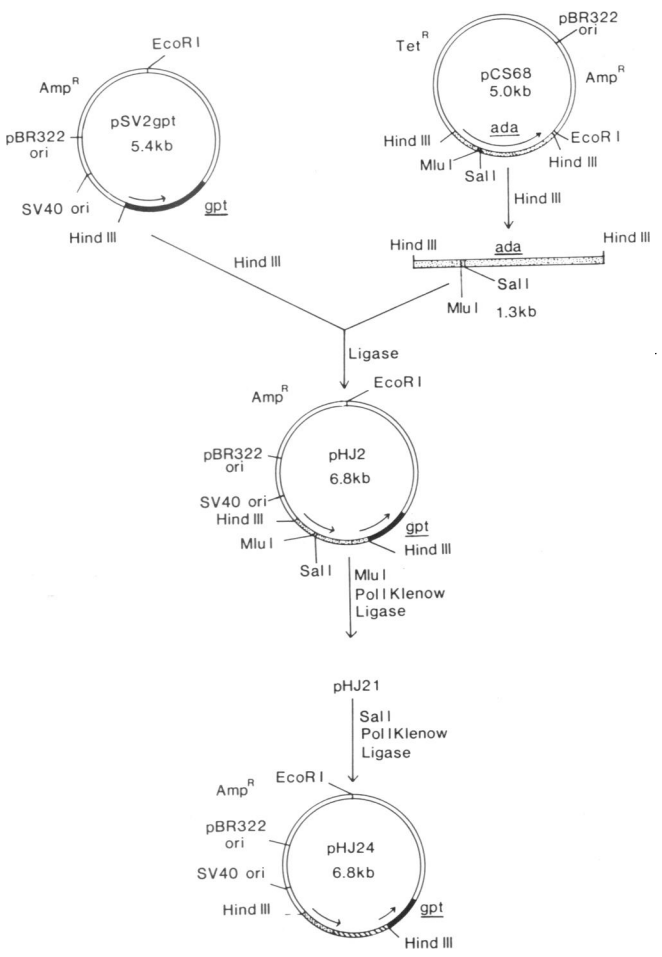

Fig. 1. Construction of the *ada*$^+$-containing expression vectors based on pSV2gpt. The solid regions indicate the *gpt*$^+$ coding sequence and the stippled regions the *ada*$^+$ coding sequence derived from the 1.3 kb *Hin*dIII fragment of pCS68. The sequence represented by the hatched region in pHJ24 is no longer in frame with the N-terminal sequences of the Ada protein due to the introduction of two frameshift mutations. The arrows indicate the direction of transcription.

Table I. Expression of methyltransferase activities in transformed *E. coli* BS24 (*ada5*)

Plasmid	Methyltransferase activity (units/mg protein)	
	O^{6}MeGua	Methylphospho-triester
None	0.6	0.3
pSV2gpt	0.7	0.5
pHJ2	40.0	40.0
pHJ24	0.7	15.5

1 unit of methyltransferase removes one pmol of methyl groups.

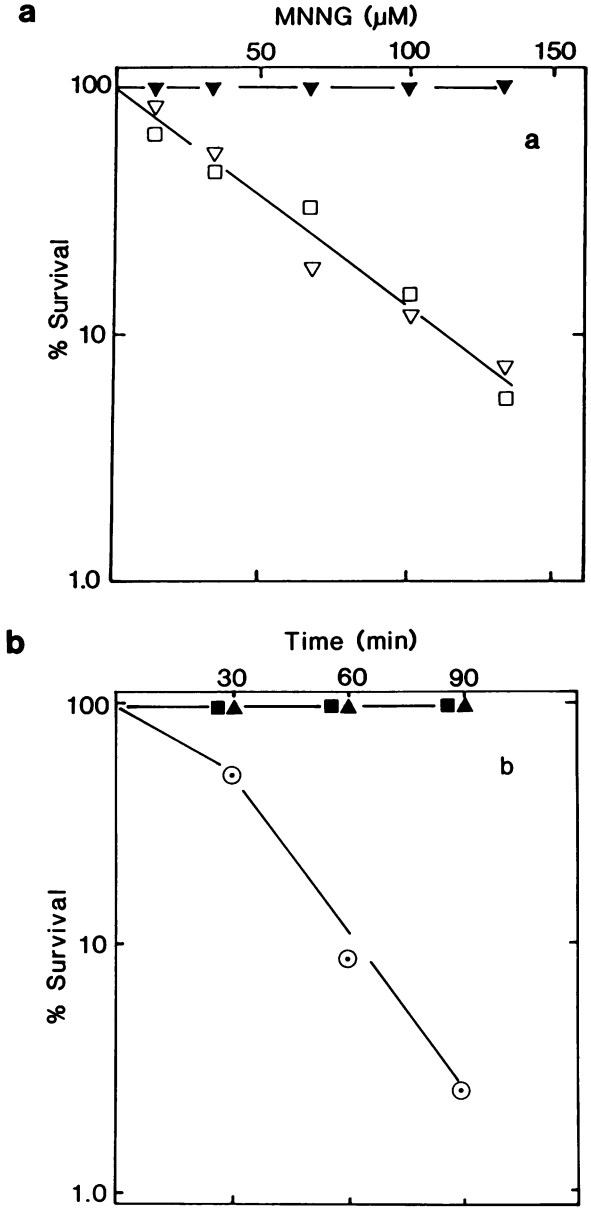

Fig. 2. (**a**) Survival of *ada*$^+$ plasmid-carrying BS24 after treatment with MNNG. 2 × 10^8 cells/ml in M9 salts solution were treated for 10 min with the MNNG concentrations shown. Appropriate dilutions were made into M9 salts and plated on nutrient agar plates containing ampicillin (100 μg/ml). Each point represents the mean of two experiments. [▼BS24 (pHJ2), □BS24 (pSV2gpt), ∇BS24 (pHJ24)]. (**b**) Survival of *ada*$^+$ plasmid-carrying AB1157 after treatment with MNNG. Cells were treated in M9 salts solution supplemented with appropriate amino acids and glucose (Sedgwick and Robins, 1980). MNNG was added to a final concentration of 35 μM and aliquots of cells removed for plating at the times shown. Each point represents the mean of two experiments [■AB1157 (pHJ2), ▲AB1157 (pSV2gpt), ⊙ AB1157 (pHJ24)].

pHJ24 did not alter the sensitivity of BS24 to killing and BS24(pHJ24) was as sensitive to MNNG as BS24(pSV2gpt) which contained only vector sequences. In order to investigate whether the absence of effect of pHJ24 could be due to the somewhat lower level of methylphosphotriester repair in BS24(pHJ24), we examined the sensitivity of AB1157(pHJ24) to killing by MNNG (Figure 2b). Expression of an increased amount of methylphosphotriester-DNA methyltransferase in this wild-type strain resulted in a decreased rather than an increased resistance to killing by MNNG. This result could be expected if methyl groups transferred from phosphotriesters to intact Ada protein molecules act as positive regulators of the adaptive response to alkylating agents and indicates that the truncated polypeptide is active *in vivo*. We assume that the removal of methylphosphotriesters from DNA by the truncated protein diminishes an important signal which results in an attenuated response and increased sensitivity to killing by MNNG. These data are in agreement with the role of methylphosphotriesters

Table II. Mutation induction by MNNG in BS24 (*ada5*) cells containing *ada*⁺ plasmids

MNNG (μM)	Arg⁺ revertants/10⁵ viable cells		
	pHJ2	pHJ24	pSV2gpt
0	0.007	0.005	0.014
33	0.015	67	72
66	0.102	45	234
133	0.180	1035	1189

Table III. Expression of methyltransferase in transfected CHO cell lines

Plasmid	Cell line	Methyltransferase activity (units/mg protein)		Methylpurine DNA glycosylase (units/mg protein)
		O⁶-MeGua	Methylphospho-triester	
pSV2gpt	CHOGPT	<0.01	<0.01	0.7
pHJ2	CHOCNU3	0.5	0.5	0.7
pHJ24	CHO623	<0.01	0.5	0.7

1 unit of methylpurine-DNA glycosylase removes one pmol methylpurines per hour.

in the induction of the adaptive response. Furthermore, our results indicate that methylphosphotriesters in DNA do not constitute an effective cytotoxic lesion in *E. coli*.

Table II shows the effect of the Ada proteins on mutation induction by MNNG. As expected, the *ada5* mutant BS24 was rendered resistant to mutation induction by the plasmid pHJ2 as measured by reversion of the *argE* mutation to Arg⁺. The mutability of BS24 was unchanged by the presence of pHJ24 indicating that the truncated protein encoded by this plasmid did not repair potentially mutagenic lesions.

Expression of the Ada proteins in CHO cells

Following transfection of pHJ2 into CHO cells, cloned cell lines were obtained which expressed the bacterial *gpt*⁺ gene (as determined by enzyme assay) and contained at least one copy of the intact *ada*⁺ gene, as determined by probing Southern blots of restriction enzyme digests of whole genomic DNA with radiolabelled intact *ada*⁺ DNA. However, less than one percent of such cell lines expressed the bacterial *ada*⁺ gene. Thus, direct assays of cell extracts for O⁶MeGua-DNA methyltransferase activity produced negative results as did probing such extracts on either dot blots or Western blots with polyclonal antisera raised against the authentic 39 kd bacterial Ada protein. A further stepwise selection with chloroethylnitrosourea (CNU) produced *gpt*⁺ CNU-resistant colonies at an approximate frequencey of 10^{-3}. Five such colonies were picked and recloned. All expressed methyltransferase activity at similar levels (~0.5 units per mg protein) and one of them (CHOCNU3) was chosen for further study. The level of methyltransferase activity expressed in CHOCNU3 cells is close to the mean level found in extracts of human Mex⁺ cells (Yarosh *et al.*, 1983).

The effect of the truncated form of the Ada protein representing the N-terminal sequences was also assessed in CHO cells. Following co-transfection of pHJ24 and pSV2gpt into CHO cells, we isolated by random screening a *gpt*⁺ clone which expressed methylphosphotriester-DNA methyltransferase activity. This clone (CHO623) is the only clone so far isolated which expresses this activity. Consequently, the frequency of expression of pHJ24 in CHO cells is not known.

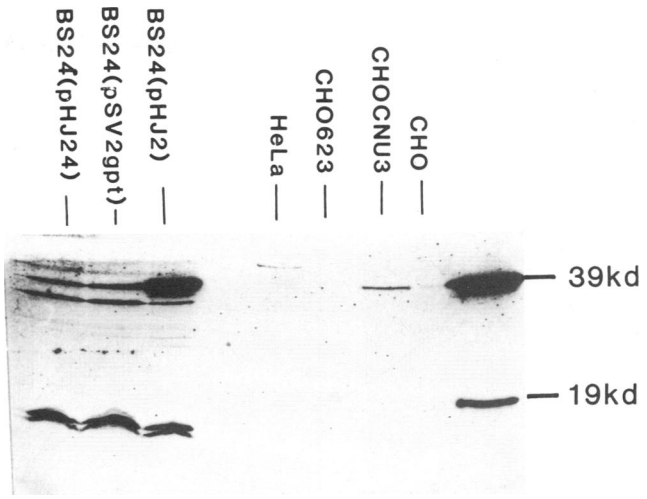

Fig. 3. Immunoblot of extracts (200 μg) of BS24-carrying *ada*⁺ plasmids and from the transfected CHO or control mammalian cell lines. Protein was electro-transferred from a 15% SDS–polyacrylamide gel to nitrocellulose which was subsequently incubated with rabbit antiserum raised against the purified 39 kd O⁶MeGua–DNA methyltransferase. Following incubation with peroxidase linked anti-rabbit IgG, bands were visualized by development with β-chloronaphthol and hydrogen peroxide. Homogeneous 19 kd (0.1 μg) and 39 kd (0.3 μg) Ada proteins were used as internal standard mol. wt markers.

The levels of methyltransferase activities in these transfected cell lines are shown in Table III. As expected, the specific methyltransferase activity acting on both O⁶MeGua and methylphosphotriesters in DNA was the same in extracts of the pHJ2 transfectant CHOCNU3 cell line. Moreover, the level of methylphosphotriester-DNA methyltransferase activity expressed from the N-terminal *ada*⁺ gene fragment in CHO623 was similar to that expressed from the whole *ada*⁺ gene, whereas no O⁶MeGua-DNA-methyltransferase was observed in this case.

The fact that the observed enzyme activities were due to the expression of the bacterial gene and not to the activation of a normally non-expressed endogenous CHO gene was confirmed by probing Western blots of extracts of the various cell lines with polyclonal antisera raised against the intact bacterial Ada protein. Figure 3 indicates that extracts of CHOCNU3 cells contain a protein of 39 kd which corresponds to the intact bacterial Ada protein produced in BS24(pHJ2). This material is absent from extracts of both the Mex⁻ parental CHO cell line and a Mex⁺ HeLa S3 cell line. (HeLa cell extracts contain an additional protein of ~44 kd which shows a weak cross-reaction with the antiserum against the Ada protein but is not associated with any methyltransferase activity.) No additional bands corresponding to the size of the truncated Ada protein (9.2 kd) were observed in Western blots of extracts of CHO623 cells. This absence apparently reflects the lack of antigenic determinants in this portion of the protein since no band of this size was obtained from extracts of BS24(pHJ24) which also expresses the truncated Ada protein (Figure 3).

Biological consequences of Ada protein expression in CHO cells

The transfectant which expressed both repair activities associated with the *ada*⁺ gene, CHOCNU3, was found to be considerably more resistant to MNNG cytotoxicity than the parental CHO cell line (Figure 4a). In order to investigate the possibility that, as in *E. coli*, the expressed *Ada*⁺ protein might be acting in CHO cells as an inducer of DNA glycosylase(s) specific for methylated bases, the levels of DNA glycosylase activity towards N-methyl-

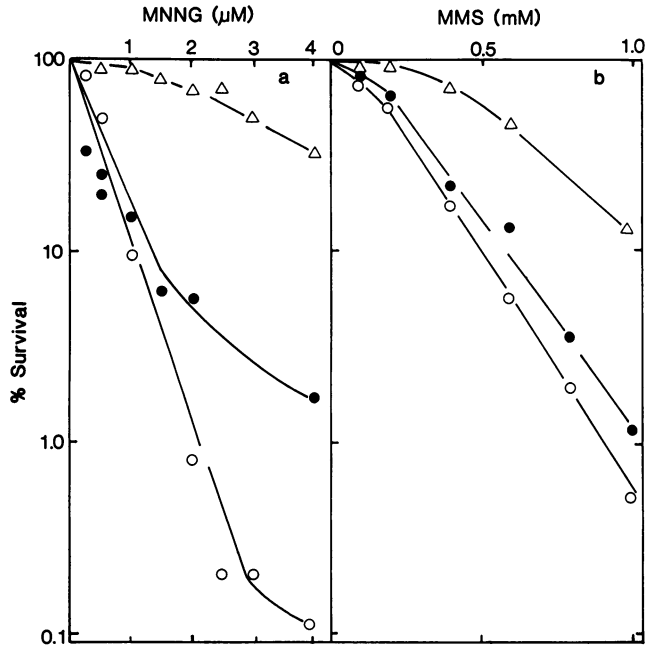

Fig. 4. Survival of CHO (○), CHO 623 (●) and CHOCNU3 (△) after treatment with MNNG (**a**) or MMS (**b**). Appropriate numbers of cells in 2 ml of medium were allowed to attach in 6 well plates for 2 h at 37°C and the medium was changed immediately before treatment with MNNG or MMS at the concentrations shown. MMS-containing medium was removed and replaced with fresh medium after 60 min incubation, whereas medium containing MNNG was not. Surviving colonies were stained and counted after 10 days. Each point represents the mean of three different experiments.

ated purines were determined. No differences in this activity were apparent between the transfected resistant cell line and CHO cells (Table III). These observations indicate that the protection against the cytotoxicity of MNNG was being conferred on CHOCNU3 by the ability to repair either O^{6}MeGua or methylphosphotriester (or both) lesions in DNA. The CHO623 cell line, in which expression of methylphosphotriester-DNA methyltransferase is at a level similar to that in CHOCNU3, was only slightly more resistant to MNNG cytotoxicity than control cells (Figure 4a). Consequently, these data indicate that the cytotoxic lesion which is being repaired more effectively in CHOCNU3 is probably O^{6}MeGua. CHOCNU3 cells were also resistant to killing by the unrelated monofunctional methylating agent methyl methanesulfonate (MMS) (Figure 4b). The magnitude of the resistance to MMS resulting from the expression of *ada*$^+$ gene functions was lower than that observed for MNNG, in agreement with the lower proportion of O^{6}MeGua produced by the former compound (Lawley, 1974).

The CHO cell line used here is hemizygous at the adenine phosphoribosyl transferase (APRT) locus (Bradley and Letovanec, 1982) and MNNG is a powerful inducer of APRT$^-$ mutants in these cells. Treatment of CHO cells with MNNG at 0.5 μM resulted in up to 20-fold increases in mutation frequency compared to untreated cells (from 4.1×10^{-6} to 52×10^{-6} in a typical experiment). In contrast, the same concentration of MNNG induced only a two-fold increase over the background mutation frequency in the CHOCNU3 cells (2.1×10^{-6} to 4.5×10^{6}). MNNG induced APRT$^-$ mutants in CHO623 at a frequency similar to that found with CHO cells (1.2×10^{-6} to 43×10^{6}). These data demonstrate the strong antimutagenic activity of the Ada protein when expresssed in CHO cells.

Discussion

The Ada protein of *E. coli* confers resistance to the biological effects of alkylating agents by virtue of its dual methyltransferase activities. We have introduced these activities either singly or together into *E. coli* and CHO cells in order to determine the biological roles of O^{6}MeGua and methylphosphotriesters in DNA. The abolition of MNNG-induced mutation in *E. coli* which expresses high levels of O^{6}MeGua-DNA- methyltransferase as part of the Ada protein is in agreement with the known mutagenicity of O^{6}MeGua in DNA and the protective effect afforded by the cloned *E. coli ada*$^+$ gene (Sedgwick, 1983). Efficient demethylation of this lesion prevents the induction of transition mutations due to miscoding of O^{6}MeGua during DNA replication (Loechler *et al.*, 1984; Hill-Perkins *et al.*, 1986).

The inability of the plasmid encoding only the methylphosphotriester-DNA methyltransferase activity to confer resistance to MNNG-induced killing or mutation in *E. coli* indicates that the removal of methylphosphotriesters from DNA does not in itself affect mutation induction or cell killing but rather represents a specialized signal for induction of the adaptive response in *E. coli*. In agreement with this notion is the observation that uncoupled methylphosphotriester repair by the truncated Ada protein actually sensitizes wild-type *E. coli* AB1157 to MNNG. Presumably efficient induction of the adaptive response requires methylphosphotriester repair coupled to a conformational change in the intact Ada protein to activate it into a positive effector of transcription via its binding to regulatory DNA sequences (Teo *et al.*, 1986). The active repair of methylphosphotriesters by the truncated Ada protein, which lacks the structural information to undergo activation, either prevents repair by the endogenous intact protein or allows non-productive binding of the methylated fragment to the *ada*$^+$ and *alkA*$^+$ promoters and thus diminishes induction of the adaptive response.

The CHO cell line expressing both methyltransferase activities, CHOCNU3, was protected against mutation induction by MNNG. No protection was conferred on CHO623 cells by the ability to repair methylphosphotriesters alone. These data demonstrate a major role for O^{6}MeGua in mutation induction by MNNG in CHO cells and further indicate that methylphosphotriesters contribute little to MNNG-induced mutagenesis in these cells.

The expression of both methyltransferase activities in CHOCNU3 cells resulted in an enhanced resistance to MNNG-induced cell killing, indicating that either O^{6}MeGua or methylphosphotriesters (or both) in DNA are cytotoxic to mammalian cells. On the other hand, CHO623 cells, which express comparable levels of methylphosphotriester-DNA methyltransferase activity alone, are only slightly more resistant to the cytotoxic action of MNNG than control cells. Furthermore, the levels of a DNA glycosylase which acts on the known potentially cytotoxic lesions 3-methyladenine and 3-methylguanine are unchanged in the resistant cells. Thus, in contrast to *E. coli* in which the Ada protein regulates resistance to killing by alkylating agents via the induction of a second DNA repair protein (3-methyladenine-DNA glycosylase II), CHO cells apparently exhibit increased MNNG resistance simply as a result of the ability to repair O^{6}MeGua in DNA. As a direct test of this, we have constructed plasmids which bear a fragment of the *ada*$^+$ gene encoding the C-terminal portion of the protein under the control of either the *lac* promoter or the SV40 early promoter. In *E. coli*, high levels of O^{6}MeGua-DNA methyltransferase activity are produced following induction of the *lac* operon (data not shown). These high

levels have deleterious effects as cells cease growth immediately following induction. The reason for the apparent inability of cells to sustain growth in the presence of high levels of this C-terminal Ada protein fragment is currently being investigated.

Several other groups have recently reported increased resistance to alkylating agents following expression of the bacterial *ada*$^+$ gene in mammalian cells. Samson *et al.* (1986), observed increased resistance to 1,3 bis-chloroethylnitrosourea, and MNNG cytotoxicity and SCE induction following the expression of the *E. coli ada*$^+$ −*alkB*$^+$ operon in Mex$^-$ HeLa-S3 cells. The resistant cell line produced large amounts of a 39 kd methyltransferase. Although a contribution to protection by expression of the *AlkB*$^+$ gene cannot be ruled out in this particular case, Ishizaki *et al.* (1986) have shown that increased resistance to MNNG cytotoxicity and SCE induction is observed in a Mex$^-$ HeLa cell line expressing the Ada protein alone. Similarly, Brennand and Margison (1986) transfected Chinese hamster V79 cells with an expression vector incorporating the whole *E. coli ada*$^+$ gene. Cells which expressed high levels of O^{6}MeGua-DNA methyltransferase activity (and presumably methylphosphotriester activity) were resistant to the cross-linking nitrosourea CNU and MNNG, but not to unrelated cross-linking agents and MMS. A similar resistance to CNU has been observed following expression of the *ada*$^+$ gene in a CHO cell line (R.Day, personal communication). Again these data indicate that enhanced demethylation of O^{6}MeGua or methylphosphotriesters in DNA by the bacterial Ada protein, confers on mammalian cells a resistance to killing by MNNG. We can conclude from our data that unrepaired methylphosphotriesters in DNA do not represent a threat to mammalian cells whereas unrepaired O^{6}MeGua lesions may have either cytotoxic or mutagenic consequences.

In apparent contradiction to the above conclusion, there are several lines of evidence to suggest that the lesions responsible for the mutagenic and cytotoxic effects of methylating agents in mammalian cells are different. Variant cell lines selected for resistance to the cytotoxic effects of alkylating agents do not exhibit any concomitant resistance to mutation induction (Baker *et al.*, 1979; Friedman and Huberman, 1980). This includes a number of human Mex$^-$ transfectant cell lines selected for resistance to MNNG of which only one (of 15) showed any increase in methyltransferase level (Yarosh *et al.*, 1984). Mutation induction in Chinese hamster V79 cells correlates well with the production of O-alkylated bases in DNA, whereas for cell killing, no such correlation is apparent (Newbold *et al.*, 1980). It has recently been shown that the O^{6}MeGua-DNA methyltransferase of human Mex$^+$ cells can be largely depleted by the inclusion of free O^{6}MeGua in the growth medium (Karran, 1985). Using this approach with a variety of cell lines, including a Burkitt's lymphoma line (Karran and Williams, 1985) and normal diploid human fibroblasts (Domoradzki *et al.*, 1985) depletion of O^{6}MeGua-DNA methyltransferase to 10−20% of normal levels was found to have no effect on the sensitivity to MNNG-induced killing. [This depletion is not a property peculiar to the mammalian methyltransferase, since a similar reduction in O^{6}MeGua-DNA methyltransferase activity may be brought about in the transfected CHOCNU3 cell line which expresses the *E. coli* protein, again without any sensitization to MNNG cytotoxicity (J.Hall, unpublished results).] However, the sensitivity to mutation induction can be increased by such transferase depletion (Domoradzki *et al.*, 1985) indicating that unrepaired O^{6}MeGua lesions in DNA exhibit their expected mutagenic effect. The most straightforward explanation of these data would be that O^{6}MeGua in DNA is a potentially mutagenic, but not cytotoxic, lesion in mammalian cells.

The apparent paradox presented by these two different approaches may conceivably be resolved along the following lines: Firstly, there will almost certainly be more than one type of potentially cytotoxic lesion introduced into the DNA of mammalian cells by methylating agents — the 3-methylpurines and O^2-methylpyrimidines would be likely candidates in this regard, particularly since the former lesions are known to be cytotoxic lesions in *E. coli* and the O^2-methylpyrimidines are efficiently excised from the DNA of *E. coli* by a DNA glycosylase which has no counterpart in mammalian cells (Hall and Karran, 1986). Selection of more resistant variant cell lines may not necessarily be linked to an increased level of any particular DNA repair enzyme. Secondly, a single DNA lesion may effect mutation induction and cell killing by unrelated mechanisms. In the particular case of O^{6}MeGua in template DNA there is now considerable evidence that while it will form base pairs and direct the incorporation of pyrimidines, the base pairs formed by the methylated purine are weak (Kuzmich *et al.*, 1983) and furthermore, it is an impediment to DNA synthesis (Snow *et al.*, 1984). Thus, on encountering O^{6}MeGua in DNA at a replication fork, the replication complex may either elongate past the methylated base — with a high probability of fixing a transition mutation — or pause. Such blocked replicons would eventually lead to cell death, although some time may elapse before the ultimate lethal event occurs. During this time, the methyltransferase may act to remove the blockage and DNA replication may resume. If this reversal occurs soon enough after the replication fork has encountered O^{6}MeGua in DNA, the cell will survive. Consequently, protection against cell killing, in contrast to mutation induction, may be achieved by depleted levels of O^{6}MeGua-DNA methyltransferase since a considerable time may elapse before the ultimate lethal event occurs whereas the mutagenic event is fixed immediately on replication.

E. coli has proved a useful model system for the study of the DNA repair reactions which protect cells against killing and mutation. The mechanisms by which a number of diverse types of DNA damage are repaired are closely similar in *E. coli* and mammalian cells. This implies that the biological consequences, that is, mutation or cell death, of a particular unrepaired lesion will be similar in both cell types. In the case of O^{6}MeGua in DNA, it appears that while it represents a potent pro-mutagenic lesion in both *E. coli* and mammalian cells, its effect on cell survival is much more profound in the latter. This probably reflects the greater complexity of DNA replication in mammalian cells and suggests that other types of DNA damage may have partially different biological effects in the two types of cell.

Materials and methods

Plasmid construction

All recombinant DNA techniques used were as described in Maniatis *et al.* (1982). The mammalian cell expression vector pSV2gpt (Mulligan and Berg, 1981) was obtained from Dr P.Berg and pCS68 (Teo *et al.*, 1984) containing the intact *ada*$^+$ structural gene and promoter sequences on a 1.3 kb *Hin*dIII fragment was obtained from Dr B.Sedgwick.

The construction of mammalian expression vectors containing the *ada*$^+$ gene fragments is outlined in Figure 1. pHJ2 was constructed by inserting the 1.3 kb *Hin*dIII fragment directly into the *Hin*dIII site of pSV2gpt bringing it under the control of the SV40 early promoter.

pHJ24 which encodes the methylphosphotriester-DNA methyltransferase portion of the *ada*$^+$ gene, was derived from pHJ2 in two steps. Firstly, a single base frameshift mutation was introduced by digestion of pHJ2 with *Mlu*I, filling in the overlapping ends with DNA polymerase I Klenow fragment and religa-

tion. The resulting plasmid, pHJ21, encoded the first 90 amino acids of the Ada protein along with 30 additional amino acids. The number of non-Ada protein amino acids at the C-terminus of the truncated Ada polypeptide was reduced by the creation of a second single base frameshift mutation. pHJ21 was digested with *SalI*, the end filled in with DNA polymerase I Klenow fragment and the resulting blunt-ended molecule religated to generate pHJ24. This plasmid has a termination codon 306 bp away from the start of the *ada*$^+$ structural gene and encodes the first 90 amino acids of the Ada protein plus an additional 12 amino acids.

Bacterial strains

BS24 is an *ada-5* derivative of AB1157 (Sedgwick, 1983). Measurements of cell survival and mutation after treatment with MNNG were performed essentially as outlined in Sedgwick and Robins (1980). MNNG (Sigma) was dissolved in 10 mM Na acetate pH 5.0 and stored in aliquots at −20°C. MMS (Aldrich) was diluted in sterile PBS before use.

Immunoblotting

The rabbit antisera against the purified *E. coli* methyltransferase was a kind gift from Dr T.McCarthy. SDS−PAGE was carried out as described by Laemmli (1970) using internal standard proteins of known mol. wts. Immunoblotting was performed as described by Teo *et al.* (1984). Southern blots were made essentially as described in Maniatis *et al.* (1982).

Enzyme assays

The substrate for the O^{6}MeGua-DNA methyltransferase reaction was *Micrococcus luteus* DNA treated with [^{3}H]MNU (28 Ci/mmol, Amersham International) and partially depurinated by heating at neutral pH (Karran *et al.*, 1979). Methylphosphotriester-DNA methyltransferase was measured using poly(dA-dT).poly(dA-dT) (Pharmacia) methylated at 80°C in 10 mM Na cacodylate pH 7.0/1 mM EDTA with [^{3}H]MNU (28 Ci/mmol). Alkylation at this high temperature produces a polymer containing [^{3}H]N^1-methyladenine and [^{3}H]methylphosphotriesters as its predominant radioactive constituents. N^1-methyladenine is stable in DNA in the presence of extracts of either bacteria or mammalian cells. Methylphosphotriester-DNA methyltransferase activity was assayed by the transfer of radioactivity from this methylated polynucleotide to a protease-sensitive form essentially as described in McCarthy *et al.* (1984). The DNA glycosylase acting on methylated purines was assayed as described in Harris *et al.* (1983). Xanthine phosphoribosyl transferase assays were carried out as described by Mulligan and Berg (1980). Protein concentrations were determined by the method of Bradford (1976).

CHO cells

The CHO cell line used was CHO.D422, kindly provided by Dr M.Meuth. Routine culture and cytotoxicity measurements were carried out according to Meuth (1981). The induction of mutation to aza-adenine resistance was determined as described by Nalbantoglu *et al.* (1983).

CHO cells were transfected by the calcium phosphate technique (Wigler *et al.*, 1979). 10 μg circular pHJ2 or pHJ24 plasmids were used with 2.5 μg pSV2gpt. In addition, pSVDHFR (kindly provided by Dr Y.Chernajovsky) which contains a mouse dihydrofolate reductase cDNA transcription unit (Chernajovsky *et al.*, 1984) and 5 μg sheared herring sperm DNA (Sigma) were used as carrier. Precipitated material was left in contact with exponentially growing CHO cells (2×10^5 per 10 cm tissue culture dish) for 4 h. Following removal of the precipitates and washing, cells were subjected to a DMSO shock (20% DMSO, 2 min) and subsequently maintained in MEM alpha medium containing 10% foetal bovine serum for 48 h before transfer to selective medium. *gpt*$^+$ cells were selected in MEM alpha medium (containing thymidine 0.02 mM, adenine 0.02 mM and xanthine 200 μg/ml) supplemented with aminopterin (0.02 μM), mycophenolic acid (Gibco) 5 μg/ml and 10% dialysed foetal bovine serum. Further selection for resistance to 1-(2-chloroethyl)-1-nitrosourea (CNU) was carried out by three treatments with 30 μM CNU at approximately two-weekly intervals followed by treatment of surviving cells with 60 μM CNU. CNU (a gift of Professor A.L.Harris) was dissolved in absolute ethanol immediately before use. Colonies of *gpt*$^+$ CNU-resistant cells were subcloned by transfer to fresh medium with a sterile pipette tip. Transfected cells were maintained in selective medium at all times.

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