Acta Biochimica Polonica

Vol. 45 No. 2/1998

523–533

QUARTERLY

Minireview

The role of mutation frequency decline and SOS repair systems in methyl methanesulfonate mutagenesis[★○]

Elżbieta Grzesiuk

Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland

Key words: methyl methanesulfonate, DNA alkylation, *Escherichia coli*, DNA repair, mutation frequency decline, SOS response, *umuDC*

Methyl methanesulfonate (MMS) is an S_N2 type alkylating agent which predominantly methylates nitrogen atoms in purines. Among the methylated bases 3meA and 3meG are highly mutagenic and toxic. The excision of these lesions leads to the formation of apurinic (AP) sites and subsequently to AT→TA or GC→TA transversions. The *in vivo* method based on phenotypic analysis of Arg^+ revertants of *Escherichia coli* K12 and sensitivity to T4 nonsense mutants has been used to estimate the specificity of MMS induced mutations. In the *E. coli* $arg^-his^-thr^-$ (AB1157) strain MMS induces *argE3*(oc) →Arg^+ revertants of which 70–80% arise by supL suppressor formation as a result of AT→TA transversions. The remaining 20–30% arise by supB and supE(oc) suppressor formation as a result of GC→AT transitions. The level of AT→TA transversions decreases during starvation. This is a consequence of action of the repair mechanism called mutation frequency decline. This system which is a transcription coupled variant of nucleotide excision repair was discovered in UV induced mutations. We describe the mutation frequency decline phenomenon for MMS mutagenesis. MMS is a very efficient inducer of the SOS response and a *umuDC* dependent mutagen. In MMS treated *E. coli* cells mutated in *umuDC* genes the class of AT→TA transversions dramatically diminishes. A plasmid bearing UmuD(D')C proteins can supplement chromosomal deletion of *umuDC* operon: a plasmid harbouring *umuD'C* is more efficient

---

[★]Paper presented at the Conference on "Mechanisms of DNA Repair and Mutagenesis" Commemorating the 100th Anniversary of the Discovery of Polonium and Radium, October, 1997, Warsaw, Poland.
[○]This work was supported in part by the State Committee for Scientific Research (KBN), grant No. 6 P04A 034 08.

[✉]Corresponding author: Dr. hab. Elżbieta Grzesiuk, Institute of Biochemistry and Biophysics PAS, A. Pawińskiego 5A, 02-106 Warszawa, tel: (48 22) 658 4499 ext. 3337; e-mail: elag@ibbrain.ibb.waw.pl

Abbreviations: AP, apurinic/apyrimidinic sites; BER, base excision repair; EMS, ethyl methanesulfonate; Fpg, formamidopyrimidine-DNA glycosylase; MFD, mutation frequency decline; MMS, methyl methanesulfonate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; MNU, *N*-methyl-*N*-nitrosourea; NER, nucleotide excision repair;.TRCF, transcription repair coupled factor.

in comparison to that harbouring *umuDC*. Moreover, plasmids isolated from MMS treated and transiently starved *E. coli* AB1157 cells harbouring *umuD(D')C* genes have shown the repair of AP sites by a system which involves the UmuD'C or at least UmuD' protein.

Procaryotic as well as eucaryotic cells are constantly exposed to endogenous and exogenous mutagens. In the last decade much attention was focused on DNA repair systems which enzymatically remove a modified base, sugar or phosphate from DNA. All these repair systems were first discovered and described in bacteria. The existence of a human DNA repair deficiency syndroms such as hereditary nonpolyposis colorectal cancer (HNPCC), xeroderma pigmentosum (XP) or Fanconi's anemia (FA) which lead to genetic instability and predisposition to cancer underscores the protective effect of DNA repair.

Alkylating agents are a group of mutagens and carcinogens that act through covalent modification of cellular DNA, producing at least 15 different lesions in DNA [1]. It is widely accepted that the mutagenicity of an alkylating agent is related to its ability to form O-alkylated *versus* N-alkylated adducts in DNA [1–3]. Alkylating agents can react with nucleophilic centers of organic macromolecules by either the S_N1 mechanism and be described as "high oxyphilic" agents or by the S_N2 mechanism, "low oxyphilic" agents [4, 5]. Chemicals like MNU or MNNG in the S_N1 reaction alkylate to a greater extent the oxygen atoms and are strongly mutagenic and carcinogenic, whereas MMS in the S_N2 reaction alkylates bases predominantly at the nitrogen atoms and is less mutagenic and carcinogenic [6].

There are several reasons for studying the biological activity of MMS. This methylating agent introduces into DNA a whole spectrum of lesions which can be repaired by different mechanisms allowing for mutation-avoidance and maintaining DNA stability. MMS is able to induce two DNA repair systems which are important for cell survival: the SOS system and adaptive response [7]. Both, very precisely described in bacteria, are also present in yeast and, highly probable, in human cells.

MUTAGENIC PROPERTIES OF MMS INDUCED BASE MODIFICATIONS

MMS is a monofunctional agent which reacts by the S_N2 mechanism. Relative proportions of methylated bases present in DNA after the reaction with MMS are as follows: 83% of N^7-methylguanine (7meG), 11% of N^3-methyladenine (3meA), 1.9% of N^7-methyladenine (7meA), 1.2% of N^1-methyladenine (1meA) and 0.3% of O^6-methylguanine (O^6meG) [8] (Fig. 1).

7meG does not appear to be significantly mutagenic, but it can generate secondary lesions by forming apurinic (AP) sites or by opening the imidazole ring, which yields 2,6-diamino-4-hydroxy-5-*N*-methylformamidopyrimidine (methyl-FapyG) [9]. Methyl-FapyG, 3meA and 3meG are highly toxic because each of them leads to formation of a replication block [10]. Enzymatic or spontaneous loss of 3meA, 3meG and 7meG leads to the formation of AP sites which may induce AT→TA or GC→TA transversions in a *umuDC*-dependent manner [11–13].

Of the methylated bases O^6meG has been the most extensively studied because it is a very effective premutagenic lesion which can mispair with thymine *in vitro* [1, 4]. Mutagenicity of O^6meG has been confirmed *in vivo* by site directed mutagenesis [14]. The results of these experiments have shown that O^6meG introduced into a precisely determined DNA sequence leads to GC→AT transition. This class is dominant among mutations induced by alkylating agents [15]. The efficiency of alkylation of O^6 position of guanine determines the mutagenicity of an alkylating agent: the

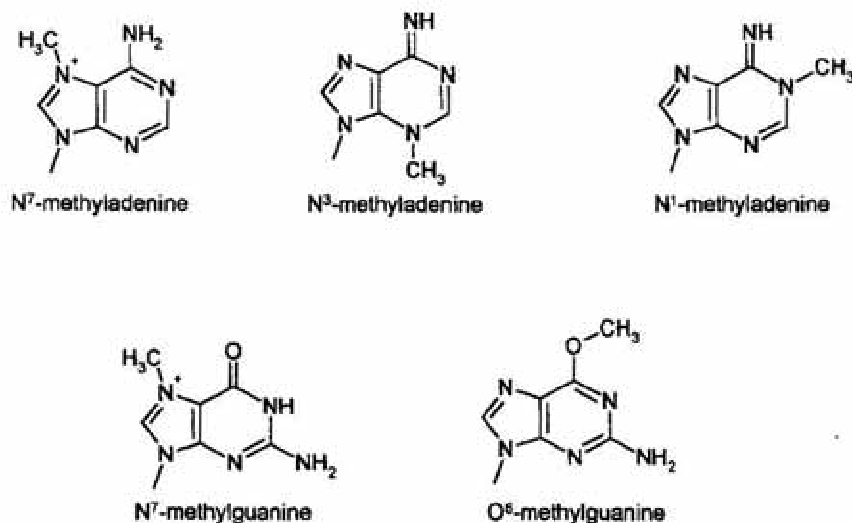

Figure 1. Principal DNA lesions formed by methyl methanesulfonate.

stronger mutagen, the higher is the proportion of O^6meG introduced into DNA [16].

IN VIVO METHOD FOR DETERMINING MUTAGENIC SPECIFICITY OF MMS

The mutagenic process taking place in *Escherichia coli* cells after MMS treatment can be characterised by analysis of tRNA suppressors [15]. One of the *in vivo* methods is based firstly upon phenothypic analysis of Arg^+ revertants of *E. coli* K12 bearing *argE3* (ochre), *hisG4* (ochre) and *thr-1* (amber) chromosomal mutations, and secondly on the sensitivity of these revertants to T4 nonsense mutants [17–21]. Introducing some modifications, we have used this method to estimate whether the revertants arose by suppressor formation or back mutations, and to identify the suppressor [15]. The pattern of suppression for tRNA ochre suppressors is shown in Table 1. The formation of supB or conversion of supE(am) to supE(oc) arises by GC→AT transitions. The remaining suppressors may be formed by GC(AT)→TA transversions.

Our studies show that, in *E. coli* AB1157 strain, MMS induces more than 70% of Arg^+ revertants which arise by supL suppressor formation as a result of AT→TA transversions. Approximately 30% of the mutants arise by formation of supB and supE(oc) suppressor formation as a result of GC→AT transitions [12, 13].

DECLINE IN MUTATION FREQUENCY – THE MFD PHENOMENON

The mutation frequency decline is defined as a rapid and irreversible decrease in the level of certain UV-induced tRNA suppressor mutations that occurs in *E. coli* when protein synthesis is transiently inhibited immediately after UV irradiation [22]. The mutations in the anticodon of a glutamine tRNA gene making it a suppressor are susceptible to MFD, but conversion type suppressor mutations (which converts a suppressor tRNA from recognizing an *amber* codon to recognizing an *ochre* codon) and true back revertants are not [23].

Analysis of the potential lesions in particular tRNA genes led to the conclusion that MFD is the process in which premutagenic lesions present in the transcribed strand of an active gene are repaired more rapidly compared to the nontranscribed strand or unexpressed DNA sequence [24]. The observation that UV induced pyrimidine dimers, as well as other lesions, effectively block transcription [25], has been of great importance for the current

model of preferential repair of the transcribed strand. The model provides for the existence of a factor able to interact with both, the stalled RNA polymerase-DNA complex and the excision nuclease. Such a protein called TRCF was purified from *E. coli*. It was a kind of surprise to realise that a defect in the transcription coupled repair observed in extracts from *E. coli mfd* strain could be complemented by the TRCF purified from wild type cells. A confirmation that the *mfd* gene encodes TRCF was obtained through cloning and functional analysis of the *mfd* gene and protein [26].

MFD is a special manifestation of nucleotide excision repair [27]. At the first step of the process TRCF recognises RNA polymerase stalled at a lesion and releases it together with the nascent RNA tail (see Fig. 2). Subsequently TRCF recruits the $UvrA_2UvrB$ complex to the damage site and facilitates the dissociation of UvrA from the $UvrA_2UvrB$–DNA complex. This step leads to the formation of the preincision UvrB-DNA complex and binding of UvrC protein. In the presence of UvrC, UvrB makes an incision at the fourth phosphodiester bond 3′, whereas UvrC by itself incises the seventh phosphodiester bond 5′ to the lesion. UvrC and the excised oligomer are released by UvrD protein (helicase II) and the UvrB is displaced from the repair gap by polymerase I, which synthesises a repair patch. The ligation of the patch done by DNA ligase completes the whole process [28].

For MMS induced mutations we have described a repair process very similar to that characteristic UV mutagenesis [13, 21]. Phenotypic analysis of Arg^+ revertants together with the results of T4 sensitivity test allows to conclude that the level of Arg^+ revertants decreases gradually in the course of starvation. Among the mutations only those which arose by AT→TA transversions are subject to MFD [13].

We have observed that the transformation of AB1157 strain with plasmids harbouring *umuD(D′)C* genes results in an increased level of the MMS induced mutations. The presence of the plasmid harbouring *umuD′C* genes leads to the most efficient MMS mutagenesis [13]. MMS treated AB1157 cells harbouring *umuD′C* plasmid are the source of Arg^+ revertants which arise mainly by AT→TA transversions. Under MFD conditions (transient amino-acids starvation) the level of these mutations drops but, unlike in the case of UV induced mutations, this MFD phenomenon is only slightly *uvrA* and *mfd* dependent.

We also described a simple method of direct observation of single stranded breaks in DNA [13, 21]. Plasmid DNAs have been isolated from MMS treated *E. coli* cells and digested with Fpg lyase. MMS action introduces AP sites into plasmid DNA predominantly after loss of 3meA. Fpg lyase forms single stranded breaks at AP sites and, as a consequence, the covalently closed circular (ccc) DNA undergoes transformation to the open circular (oc) form. The two forms can be easily distinguished on agarose gel electrophoresis. During starvation following MMS treatment the

Table 1. Suppression pattern for known tRNA suppressors of ochre mutation

Suppressor tRNA gene	Amino acid inserted	Base pair change	*E. coli* nonsense mutation		
			argE3 (oc)	*hisG4* (oc)	*thr1* (am)
supB	gln	GC→AT	+	–	–
supE$_{oc}$	gln	GC→AT	+	–	–
supC	tyr	GC→TA	+	+	+
supL	lys	AT→ TA	+	+	–

appearance of a growing amount of the Fpg resistant ccc form of plasmid DNA points to the repair of this DNA.

We have not observed the MFD phenomenon in the MMS treated *E. coli dnaQ49* strain at 37°C [29]. Interestingly, our recent results have shown that the level of MMS induced mutations drops in the course of starvation when *E. coli dnaQ49* is incubated at the permissive temperature of 30°C (E. Grzesiuk & A. Nowosielska, in preparation). This suggests that the proofreading ε subunit of DNA polymerase III encoded by *dnaQ* gene might be involved in MFD repair.

The association of mismatch repair with MFD is not clear yet. Some authors claim that there is no influence of the mismatch repair system on preferential transcription-coupled repair [30] but others have found that the defects in *mutS* or *mutL* genes which affect mismatch repair in *E. coli*, eliminate this rapid repair in the transcribed strand [31]. Recently, it has been shown that in UV irradiated *E. coli B mutS*$^-$ the kinetics of MFD is slower than in wild type strain [32]. Our studies show that in MMS treated *E. coli* K12 *mutS*$^-$ the level of GC→AT transitions increases and predominates over AT→TA transversions. Different specificity of the MMS induced mutations in *mutS*$^-$ and *mutS*$^+$ strains could explain the lack of MFD in the MMS treated *mutS*$^-$ cells [33]. It is also possible that the effect of mismatch repair deficiency on MFD is indirect, but the relationship between the two repair systems remains to be elucidated.

SOS RESPONSE – UmuD(D′)C CONTRIBUTION TO MMS MUTAGENESIS

Methylation damages are repaired by various pathways among which error free repair systems are predominant. O^{6}meG is repaired by O^6-methylguanine–DNA methyltransferase [34]. Other methylated bases and AP sites are subject to base excision repair [34]. These lesions may be repaired also by the nucleotide excision repair system [35]. Besides, 3meA and/or AP sites, which arise as a result of MMS action, are very efficient inducers of the SOS system [36]. This system was called "SOS" because of its "response" to DNA damage [37]. There are more than 26 genes which are the members of SOS regulon. They are spread all over *E. coli* chromosome but they are under common control of Lex repressor which inhibits their transcription by binding to the operator sequences, called SOS boxes, located upstream of SOS genes [38]. Damage to DNA (for instance 3meA or AP site) leads to activation of RecA protein. The interaction between LexA and activated RecA (RecA*) results in the proteolytic cleavage of LexA repressor and derepression of the genes of SOS regulon [39]. The induction of these genes occurs with different kinetics. *uvrA*, *uvrB* and *uvrD* genes, being members of both: the SOS regulon and NER, are expressed first. *umuDC*, the only SOS operon that must be induced in *E. coli* to promote SOS mutagenesis [40], is fully derepressed as the last one [41] (see Fig. 2).

Error prone translesion DNA synthesis is a main issue of SOS mutagenesis. The current model suggests at least two steps of the mutagenic process [42]. In the first step DNA polymerase inserts any base opposite the miscoding lesion in the template strand. The second, "bypass", step requires the UmuDC proteins which together with RecA enable the DNA polymerase III holoenzyme to replicate through unrepaired DNA, but with a concomitant decrease in replication fidelity [43–45].

UmuD and UmuC proteins are encoded by *umuD* and *umuC* genes organized in an operon, with the upstream *umuD* gene overlapping the downstream *umuC* gene by one nucleotide [46]. Basal steady state level of UmuD is approximately 200 molecules per cell. The level of UmuC has been estimated to be as low as 16 molecules per cell [47]. Upon

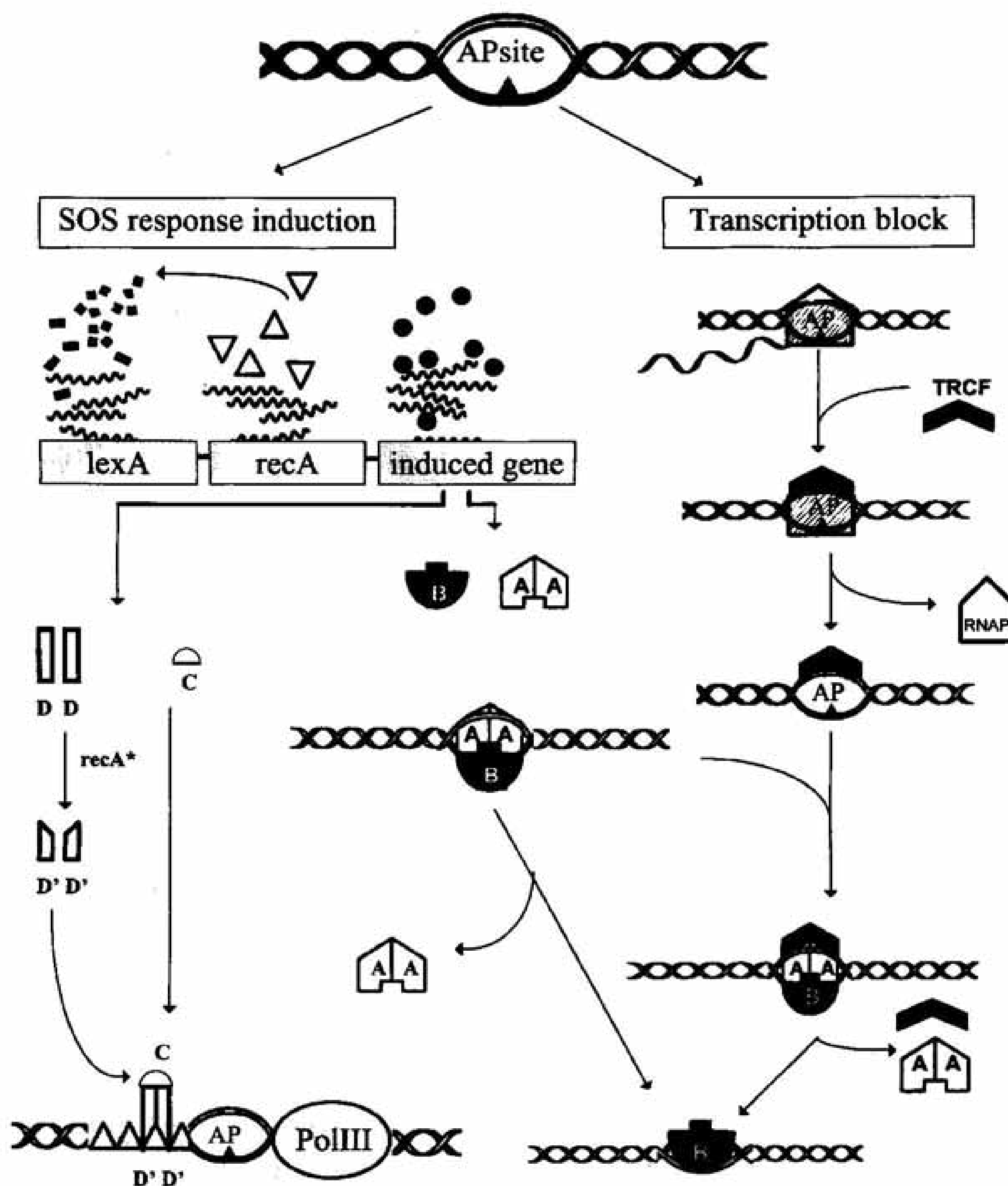

Figure 2. Mechanisms of apurinic/apyrimidinic (AP) sites repair.

MMS-induced appearance of AP sites induces SOS response (left side of the model). As the first, *uvrA* and *uvrB* genes are induced; their products: UvrA (A) and UvrB (B) proteins are involved in nucleotide excision repair (NER) (middle). AP sites can also block transcription which leads to the induction of transcription coupled repair which is connected with NER (right). The transcription repair coupled factor (TRCF) interacts with RNA polymerase (RNAP) stalled at a lesion. RNAP dissociates leaving TRCF which recruits the $UvrA_2UvrB$ complex to the place of damage. Next steps after UvrB-damaged DNA complex formation are common for NER and transcription coupled repair (see text). Among genes of SOS regulon, *umuD* and *umuC* are induced as last one. For its function in SOS mutagenesis, UmuD protein (D) is posttranslationally modified in the presence of RecA* protein forming a shorter, active in mutagenesis, UmuD′ (D′) form of UmuD. $UmuD'_2UmuC$ (D′D′C) complex, RecA filament (Δ) and ε subunit of DNA polymerase III (pol III) form a mutasome which facilitates translesion synthesis made by DNA pol III (left).

SOS induction the level of UmuD and UmuC increases to approximately 2400 and 200 molecules per cell, respectively.

SOS mutagenesis also requires UmuD to be posttranslationally processed by a RecA* mediated proteolytic cleavage [48]. The cleavage occurs at the Cys-24-Gly-25 bond and as a result a shorter, active in SOS mutagenesis form of UmuD, UmuD′ arises. Under physiological conditions UmuD and UmuD′ proteins form homo- and heterodimers [49]. The heterodimer is formed preferentially but it is inactive in mutagenesis [50]. On the contrary, UmuD′ as a homodimer is relatively stable and active in SOS mutagenesis but it is formed only under conditions of UmuD′ excess. UmuD′ dimer preferentially binds UmuC protein generating the $UmuD'_2UmuC$ complex [51]. UmuC is very labile as a monomer, however it can be protected by Hsp60 and Hsp70 heat shock proteins while waiting for the appearance of a high level of UmuD′ [52].

UmuD′ homodimers also interact with RecA* nucleoprotein filament [53]. RecA protein, in addition to its action as a positive regulator of SOS response and the activator of UmuD protein, plays a direct role in the mutagenic process. There is evidence indicating that the third, direct role of RecA in SOS mutagenesis is to position the Umu proteins for a productive encounter with DNA polymerase III [54, 55].

DNA polymerase III, under normal conditions, is involved in translesion DNA synthesis [56]. It has been assumed that 3′→5′ proofreading activity of ε subunit of DNA polymerase III has to act less precisely in order to allow the enzyme to synthesize through a miscoding lesion. It is possible that Umu proteins are involved in the modulation of ε activity. In *E. coli mutD5* mutant, which shows reduced proofreading activity, the level of UV induced mutations is similar to that obtained for wild type control. This result suggests that during UV mutagenesis the ε subunit might be inhibited [57]. It is possible that the type of missincorporation and/or strand extension on damaged DNA template is very sensitive to the amount and the type of Umu proteins present [58].

MMS is a *umuDC*-dependent mutagen; the frequency of MMS induced mutations in *umuC*$^-$ strains is about 30% of that observed in *umuC*$^+$ [59]. Our results indicate that, in the *E. coli* AB1157 strain, 70–80% of the MMS induced Arg^+ revertants arise by supL suppressor formation as a result of AT→TA transversions [12, 13]. Obviously this class of mutations disappears in the MMS induced *E. coli* strain in which the *umuDC* operon is deleted but it can be restored by introducing a plasmid harbouring *umuDC* genes [13, 33]. The frequency of MMS induced AT→TA transversions is higher in *E. coli* harbouring *umuD′C* plasmid than in cells carrying plasmids with *umuDC, umuD* or *umuD′* genes. Electrophoretic analysis of plasmid DNAs isolated from MMS treated and temporarily starved *E. coli* cells shows large differences in the efficiency of the repair process. Plasmids harbouring umuD′C or umuD′ alone were repaired more efficiently in comparison to those bearing umuDC or umuD. The repair of pBR322 or pZ150 which contain none of the *umuD(D′)C* genes was very poor, if any [29]. This finding suggests that, in addition to taking a part in the SOS response, UmuD(D′)C proteins play a role in DNA repair. We propose that the MFD mechanism for MMS induced mutations differs from that described for UV mutagenesis. It is probable that UmuD(D′)C are invloved in this repair pathways and that, since the repair is delayed and copy number dependent ([29], and E. Grzesiuk, unpublished observations) this process takes place after processing of UmuD to UmuD′.

Interesting results have been obtained for EMS mutagenesis [21, 60]. EMS is known as an alkylating agent acting independently of *umuDC* genes, however, under particular circumstances UmuDC proteins affect the specificity of EMS induced Arg^+ revertants. EMS alkylates O^6 of guanine forming highly muta-

genic O^6-ethylguanine which lead to GC→AT transitions arising by supB and supE(oc) suppressor formation [60]. Dramatic changes in specificity of Arg^+ revertants have been observed in *E. coli* strain with a chromosomal mutation in *mutS* gene. In this strain 60% of EMS induced mutants arose by supL suppressor formation as a result of AT→TA transversions. Moreover, an additional mutation in *umuD* or *umuC* led to the same mutation specificity as in the *E. coli* AB1157 $mutS^+umuDC^+$ strain [21, 60]. Unlike with MMS, in the case of EMS it is overproduction of UmuDC rather than UmuD′C that directs mutagenesis towards AT→TA transversions [60].

In the case of MMS, AT→TA transversions can be the main mutational event. Since these mutations are UmuDC dependent they most probably arise as a result of translesion DNA replication in a reaction involved DNA polymerase III, UmuD′, UmuC and RecA proteins. The MMS induced supL suppressor mutations monitored in Arg^+ revertants undergo the MFD effect due to the preferential repair of the transcribed strand. This suggestion has been supported by identification of AP sites and their subsequent repair under MFD conditions in the presence of UmuD′C proteins as described above.

REFERENCES

1. Singer, B. & Grunberger, D. (1983) *Molecular Biology of Mutagens and Carcinogens;* pp. 1-347, Plenum, New York.
2. Frei, J.V., Swenson, D.H., Warren, W. & Lawley, P.D. (1978) Alkylation of deoxyribonucleic acid *in vivo* in various organs of C57B1 mice by the carcinogens *N*-methyl-*N*-nitrosourea, *N*-ethyl-*N*-nitrosourea and ethyl methanesulfonate in relation to induction of thymic lymphoma. *Biochem. J.* **174**, 1031-1044.
3. Singer, B. (1976) All oxygens in nucleic acids react with carcinogenic ethylating agents. *Nature* **264**, 333-339.
4. Lawley, P.D. (1984) Carcinogenesis by alkylating agents; in *Chemical Carcinogenesis* (Searle, C.E., ed.) ACS Monograph 182, pp. 325-484, American Chemical Society, Washington.
5. Loechler, E.L. (1994) A violation of the Swain-Scott Principle, and S_N2 reaction mechanisms, explains why carcinogenic alkylating agents can form different proportions of adducts at oxygen versus nitrogen in DNA. *Chem. Res. Toxicol.* **7**, 277-280.
6. Lawley, P.D. & Tatcher, P.J. (1970) Methylation of DNA in cultured mammalian cells by MNNG: Influence of cellular thiol concentration on extent of methylation and O^6 of guanine as a site of methylation. *Biochem. J.* **116**, 693-707.
7. Janion, C. (1995) Mechanisms of action of methyl methanesulfonate on *Escherichia coli*: Mutagenesis, DNA damage and repair. *Adv. Biochem.* **41**, 308-313.
8. Pegg, A.E. (1984) Methylation of the O^6 position of guanine in DNA is the most likely initiating event in carcinogenesis by methylating agents. *Cancer Invest.* **2**, 223-231.
9. Tudek, B., Boiteux, S. & Laval, J. (1992) Biological properties of imidazole ring-open N7 methyl-guanine in M13mp18 phage DNA. *Nucleic Acids Res.* **20**, 3079-3084.
10. Boiteux, S. & Laval, J. (1983) Imidazole ring-opened guanine: An inhibitor of DNA synthesis. *Biochem. Biophys. Res. Commun.* **110**, 625-631.
11. Foster, P.L. & Eisenstadt, E. (1985) Induction of transversion mutations in *Escherichia coli* by *N*-methyl-*N*-nitrosoguanidine is SOS dependent. *J. Bacteriol.* **163**, 213-220.
12. Sledziewska-Gójska, E. & Janion, C. (1989) Alternative pathways of methyl methanesulfonate-induced mutagenesis in *Escherichia coli*. *Molec. Gen. Genet.* **216**, 126-131.
13. Grzesiuk, E. & Janion, C. (1994) The frequency of MMS-induced, *umuDC*-dependent,

mutation declines during starvation in *Escherichia coli*. *Molec. Gen. Genet.* **245**, 486–492.

14. Loechler, E.L., Green, C.L. & Essigmann, J.M. (1984) *In vivo* mutagenesis by O^6-methylguanine built into a unique site in a viral genome. *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6271–6275.

15. Śledziewska-Gójska, E., Grzesiuk, E., Płachta, A., Janion, C. (1992) Mutagenesis of *Escherichia coli*: A method of determining mutagenic specificity by analysis of tRNA supressors. *Mutagenesis* **7**, 41–46.

16. van Zeeland, A.A. (1988) Molecular dosimetry of alkylating agents: Quantitative comparison of genetic effects on the basis of DNA adduct formation. *Mutagenesis* **3**, 179–191.

17. Todd, P.A., Monti-Bragadin, C., Glickman, B.W. (1979) MMS mutagenesis in strains of *Escherichia coli* carrying the R46 mutagenic enhancing plasmid: Phenotypic analysis of Arg^+ revertants. *Mutation Res.* **62**, 227–237.

18. Kato, T., Shinoura, Y., Templin, A., Clark, A.J. (1980) Analysis of ultraviolet light-induced suppressor mutation in the strain of *Escherichia coli* K12 AB1157. An application for molecular mechanism of UV mutagenesis. *Molec. Gen. Genet.* **180**, 283–291.

19. Shinoura, Y., Kato, T., Glickman, B.W. (1983) A rapid and simple method for the determination of base substitution and frameshift specificity of mutagens. *Mutation Res.* **111**, 43–49.

20. Sargentini, N.J. & Smith, K.C. (1989) Mutational spectrum analysis of *umuC*-independent and *umuC*-dependent-radiation mutagenesis in *Escherichia coli*. *Mutation Res.* **211**, 193–203.

21. Janion, C. & Grzesiuk, E. (1990) Effect of *umuC* on EMS-induced mutagenesis in *Escherichia coli* deficient in mismatch repair; in *Mutation and Environment* (Mendelsohn, M.L. & Albertini, R.J., eds.) Part A, pp. 145–153, Wiley-Liss, New York.

22. Witkin, E.M. (1956) Time, temperature and protein synthesis: A study of ultraviolet-induced mutation in bacteria. *Cold Spring Harbor Symp. Quant. Biol.* **21**, 123–140.

23. Bockrath, R.C. & Palmer, J.E. (1977) Differential repair of premutational UV-lesion of tRNA genes in *E. coli*. *Molec. Gen. Genet.* **156**, 133–140.

24. Mellon, I., Spivak, G. & Hanawalt, P.C. (1987) Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. *Cell* **51**, 241–249.

25. Selby, C.P. & Sancar, A. (1990) Transcription preferentially inhibits nucleotide excision repair of the template DNA strand *in vitro*. *J. Biol. Chem.* **265**, 58–63.

26. Selby, C.P., Witkin, E.M. & Sancar, A. (1991) *Escherichia coli mfd* mutant deficient in "Mutation Frequency Decline" lacks strand-specific repair: *in vitro* complementation with purified coupling factor. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 11574–11578.

27. Witkin, E.M. (1969) Ultraviolet-induced mutation and DNA repair. *Annu. Rev. Microbiol.* **23**, 487–514.

28. Selby, C.P. & Sancar, A. (1994) Mechanisms of transcription-repair coupling and mutation frequency decline. *Microbiol. Rev.* **58**, 317–327.

29. Grzesiuk, E. & Janoin, C. (1996) MMS-induced mutagenesis and DNA repair in *Escherichia coli dnaQ49*: Contribution of UmuD′ to DNA repair. *Mutation Res.* **362**, 147–154.

30. Sweder, K.S., Verhage, R.A., Crowley, D.J., Crouse, G.F., Brouwer, J., Hanawalt, P.C. (1996) Mismatch repair mutants in yeast are not defective in transcription-coupled DNA repair of UV-induced DNA damage. *Genetics* **143**, 1127–1137.

31. Mellon, I. & Champe, G.N. (1996) Products of DNA mismatch repair genes *mutS* and *mutL* are required for transcription-coupled nucleo-

tide excision repair of the lactose operon in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 1292–1297.

32. Li, B.H. & Bockrath, R.C. (1995) Mutation frequency decline in *Escherichia coli*. I. Effects of defects in mismatch repair. *Molec. Gen. Genet.* **249**, 585–590.

33. Grzesiuk, E. & Janion, C. (1998) Mutation frequency decline in MMS-treated *E. coli* K12 *mutS* strains. *Mutagenesis* **13**, 127–132.

34. Lindahl, T., Sedgwick, B., Sekiguchi, M., Nakabeppu, Y. (1988) Regulation and expression of adaptive response to alkylating agents. *Annu. Rev. Biochem.* **57**, 133–157.

35. Friedberg, E.C., Walker, G., Siede, W. (1995) Nucleotide exision repair in procaryotes; in *DNA Repair and Mutagenesis* (Friedberg, E.C., Walker, G. & Siedge, W., eds.) pp. 191–225, ASM Press, Washington.

36. de Oliveira, R.C., Laval, J. & Boiteux, S. (1986) Induction of SOS and adaptive responses by alkylating agents in *Escherichia coli* mutants deficient in 3-methyladenine-DNA glycosylase activities. *Mutation Res.* **183**, 11–20.

37. Radman, M. (1974) Phenomenology of an inducible mutagenic pathway in *Escherichia coli*: SOS repair hypothesis; in *Molecular and Environmental Aspects of Mutagenesis* (Prakash, L., Scherman, F., Miller, M., Lawrence, C. & Tabor, H.W., eds.) vol. 3, pp. 128–142, Charles C. Thomas Publisher, Springfield.

38. Little, J.W. & Mount, D.W. (1982) The SOS regulatory system of *E. coli*. *Cell* **29**, 11–22.

39. Little, J.W. (1983) The SOS regulatory system: Control of its state by the level of RecA protease. *J. Mol. Biol.* **167**, 791–808.

40. Sommer, S., Knezevic, J., Bailone, A., Devoret, R. (1993) Induction of only one SOS operon, *umuDC*, is required for SOS mutagenesis in *Escherichia coli*. *Molec. Gen. Genet.* **239**, 137–144.

41. Levis, L.K., Harlow, G.R., Gregg-Jolly, L.A., Mount, D.W. (1994) Identification of high affinity binding sites for LexA which define new DNA damage-inducible genes in *Escherichia coli*. *J. Mol. Biol.* **241**, 507–523.

42. Bridges, B.A. & Woodgate, R. (1985) The two-step model of bacterial UV mutagenesis. *Mutation Res.* **150**, 133–139.

43. Bridges, B.A. & Woodgate, R. (1985) Mutagenic repair in *Escherichia coli*: Products of the *recA* gene and of the *umuD* and *umuC* genes act at different steps in UV-mutagenesis. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4193–4197.

44. Rajagopalan, M., Lu, C., Woodgate, R., O'Donnell, M., Goodman, M.F. & Echols, H. (1992) Activity of the purified mutagenesis proteins UmuC, UmuD', and RecA in replication bypass of an abasic DNA lesion by DNA polymerase III. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10777–10781.

45. Hagenesee, M.E., Timme, T., Bryan, S.K. & Moses, R.E. (1987) DNA polimerase III of *Escherichia coli* is required for UV and ethyl methanesulfonate mutagenesis. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4195–4199.

46. Kitagawa, Y., Akaboshi, E., Shinagawa, H., Horii, T., Ogawa, H. & Kato, T. (1985) Structural analysis of the *umu* operon required for inducible mutagenesis in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4336–4340.

47. Woodgate, R. & Levine, A.S. (1996) Damage Inducible Mutagenesis: Recent Insights into the activities of the Umu family of mutagenesis proteins; in *Cancer Surveys, Genetic Instability in Cancer*; vol. 28, pp. 117–136, Imp. Canc. Res. Fund.

48. Burckhardt, S.E., Woodgate, R., Scheuermann, R.H. & Echols, H. (1988) UmuD mutagenesis protein in *Escherichia coli*: Overproduction, purification, and cleavage by RecA. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1811–1815.

49. Woodgate, R., Rajagopalan, M., Lu, C. & Echols, H. (1989) UmuC mutagenesis protein

in *Escherichia coli*: Purification and interaction with UmuD and UmuD'. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7301–7305.

50. Battista, J.R., Ohta, T., Nohmi, T., Sun, W. & Walker, G.C. (1990) Dominant negative *umuD* mutations decreasing RecA-mediated cleavage suggest roles for intact UmuD in modulation of SOS mutagenesis. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7190–7194.

51. Bruck, I., Woodgate, R., McEntee, K. & Goodman, M.F. (1996) Purification of a soluble UmuD'C complex from *Escherichia coli*: Cooperative binding of UmuD'C to single-stranded DNA. *J. Biol. Chem.* **271**, 10767–10774.

52. Petit, M.A., Bedale, W., Osipiuk, J., Lu, C., Rajagopalan, M., McInerney, P., Goodman, M.F. & Echols, H. (1994) Sequential folding of UmuC by the Hsp70 and Hsp60 chaperone complexes of *Escherichia coli*. *J. Biol. Chem.* **269**, 23824–23849.

53. Peat, T.S., Frank, E.G., McDonald, J.P., Levine, A.S., Woodgate, R. & Hendrickson, W.A. (1996) Structure of the UmuD' protein and its regulation in the response to DNA damage. *Nature* **380**, 727–730.

54. Sweasy, J.B., Witkin, E.M., Sinha, N. & Roegner-Maniscalco, V. (1990) RecA protein of *Escherichia coli* has a third essential role in SOS mutator activity. *J. Bacteriol.* **172**, 3030–3036.

55. Bailone, A., Sommer, S., Knezevic, J., Dutreix, M. & Devoret, R. (1991) A RecA protein mutant deficient in its interaction with the UmuDC complex. *Biochimie* **73**, 471–478.

56. Bridges, B.A., Mottershead, R.P. & Sedgwick, S.G. (1976) Mutagenic repair in *Escherichia coli*, III. Requirement for a function of DNA polimerase III in ultraviolet light mutagenesis. *Molec. Gen. Genet.* **144**, 53–58.

57. Woodgate, R., Bridges, B.A., Herrera, G. & Blanco, M. (1987) Mutagenic repair in *Escherichia coli*. XIII, Proofreading exonuclease of DNA polymerase III holoenzyme is not operational during UV mutagenesis. *Mutation Res.* **183**, 31–37.

58. Woodgate, R., Singh, M., Kulaeva, O.I., Frank, E.G., Levine, A.S. & Koch, W.H. (1994) Isolation and characterization of novel plasmid-encoded *umuC* mutants. *J. Bacteriol.* **176**, 5011–5021.

59. Schendel, P.F. & Defais, M. (1980) The role of *umuC* gene product in mutagenesis by simple alkylating agents. *Molec. Gen. Genet.* **177**, 661–665.

60. Grzesiuk, E. & Janion, C. (1993) Some aspects of EMS-induced mutagenesis in *Escherichia coli*. *Mutation Res.* **297**, 313–321.