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Review

Reversion of *argE3* to Arg$^+$ in *Escherichia coli* AB1157 — an informative bacterial system for mutation detection

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This review concerns reversion of the *argE3* (ochre) nonsense mutation to prototrophy in *E. coli* AB1157 strain as an informative system for mutation detection. Strain AB1157 bears the *argE3* (ochre), *hisG4* (ochre) and *thr-1* (amber) mutations, and the *supE44* amber suppressor on its chromosome. The Arg$^+$ phenotype can be restored by (i) any base substitution at the *argE3* site that changes the nonsense UAA codon to any sense nucleotide triplet or to UAG recognized by the *supE44* amber suppressor, or (ii) suppressor mutations enabling the reading of the UAA nonsense codon. The *argE3*→Arg$^+$ reversion-based system enables (i) determination of the spontaneous or induced mutation level; (ii) determination of base substitutions (suppressor analysis); (iii) examination of transcription-coupled repair (TCR) since targets for DNA damage are situated on the transcribed or coding strand of DNA; (iv) detection of mutations resulting from single stranded DNA damage. This review focuses on studies carried out since the early 1990s till now with the application of the AB1157-based mutation detection system. Recently, the system has been used to obtain new data on the processes of methyl methanesulfonate-induced mutagenesis and DNA repair in *E. coli alkB*$^-$ mutants.

Keywords: *Escherichia coli* K12, suppressor tRNA, prototrophy, Arg$^+$ revertants, T4 phages, mutations

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INTRODUCTION

Bacteria are simple and widely used models for examination of mutagenesis and DNA repair processes. Two representatives of *Enterobacteriaceae*, *Escherichia coli* and *Salmonella typhimurium*, are commonly used in studies on spontaneous and induced mutagenesis. The advantages of bacterial systems are their availability, ease of cultivation, short time of cell division, and haploidy. Many DNA-damaging agents and/or mutator genes cause mutations that are readily and clearly observable as changes of phenotype. Additional observations like (i) analysis of bacterial survival after treatment with mutagenic agents; (ii) microscopic examination of bacterial cells; (iii) induction of the SOS system measured by induction of β-galactosidase from *umuC*::*lacZ* fusion; (iv) examination of plasmid DNA isolated from MMS-treated cells for their sensitivity to the specific endonucleases Fpg and Nth that recognize abasic sites, all provide a simple and rapid yet highly informative characterization of the examined processes.

ESCHERICHIA COLI K12 AB1157 MODEL

Escherichia coli K12 was isolated from the stool of a convalescent diphtheria patient in the US (Palo Alto, California) in 1922 and deposited in the strain collection of the Department of Bacteriology of Stanford University. Many mutant derivatives of strain K12 have been obtained in numerous laboratories around the world. One of them is strain AB1157 with the relevant genotype: *thr-1 ara-14 leuB6* Δ*(gpt-proA)62 lacY1 tsx-33 supE44* amber, *galK2 hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1* and its derivatives (Bachman, 1987). The *argE3* (ochre), *hisG4* (ochre) and *thr-1* (amber) are nonsense point mutations in genes encoding enzymes involved in arginine, histidine and threonine biosynthesis, respectively. *supE44* encodes the *supE* amber suppressor reading UAG. However, it can only weakly suppress the *thr-1* mutation.

A suppressor mutation is a mutation that counteracts the effects of another mutation. One type of suppressor mutations are mutations that appear in the tRNA-encoding genes at the anticodon site. The changed tRNAs are able to recognize a nonsense codon that occurs elsewhere in protein-coding genes and incorporate the amino acids specific for them into the polypeptide chain during protein synthesis.

The bacterial test system of mutation detection described here is based on reversion of the auxotrophic *argE3* mutation to prototrophy. The marker is situated in the chromosome. The Arg$^+$ phenotype can be restored by (i) any point mutation at *argE3* that changes the nonsense UAA codon to any sense nucleotide triplet coding for any amino acid; (ii) an AT→GC transition at *argE3* that changes the UAA nonsense codon to the UAG nonsense codon recognized by the *supE44* amber suppressor; and (iii) suppressor mutations enabling reading of the UAA nonsense codon. The suppressors can be created *de novo* or as a result of a GC→AT transition at *supE44* (formation of *supE* ochre suppressor) (Sargentini & Smith, 1989; Śledziewska-Gójska *et al*., 1992).

Considering all the theoretical possibilities of ochre suppressor formation in *E. coli* resulting from a single base substitution in tRNAs genes, it can be seen that such suppressors may arise from tRNA for tyrosine, lysine, glutamine, glutamate, leucine and serine. The fol-

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Abbreviations: BER, base excision repair; dsDNA, double-stranded DNA; EMS, ethyl methanesulfonate; MMS, methyl methanesulfonate; 1meA, 1-methyladenine; 3meA, 3-methyladenine; 7meA, 7-methyladenine; 3meC, 3-methylcytosine; 3meG, 3-methylguanine; 7meG, 7-methylguanine; O^{6}meG, *O*^{6}-methylguanine; MFD, mutation frequency decline; ssDNA, single-stranded DNA; TCR, transcription coupled repair

lowing tRNA species that may produce *de novo* an ochre suppressor by a single base substitution in the anticodon site are $tRNA^{Gln}_{UUG}$, $tRNA^{Lys}_{UUU}$, and $tRNA^{Tyr}_{GUA}$. The formed suppressors are, respectively, *supB*, *supL* (*supG*, *supN*) and *supC* (*supO*, *supM*), created as a result of GC→AT (*supB*), AT→TA (*supL*, *supG*, *supN*) or GC→TA (*supC*, *supO*, *supM*) base substitutions in *gln*-tRNA, *lys*-tRNA and *tyr*-tRNA genes, respectively (Table 1). The *supX* suppressor is also found in the Arg^+ revertants, but it has not yet been identified. This suppressor can be formed as a result of either GC→TA or AT→TA transversions (Sargentini & Smith, 1989; Śledziewska-Gójska *et al.*, 1992, and cited therein). Raftery and Yarus (1987) constructed the *gltT*(SuUUA/G) gene encoding $tRNA^{Glu}_{UUA}$ as a result of a GC→TA transversion in the *gltT* gene encoding $tRNA^{Glu}_{UUC}$. This construct was expected to explain the mystery of the *supX* suppressor. However, it failed to suppress the *argE3* ochre mutation in *E. coli* AB1157 strain (Plachta & Janion, 1992). Moreover, Prival (1996) identified three $tRNA^{Glu}_{UUA}$ suppressors: *supY*, *supW* and *supZ* that arose from the *gltW*, *gltU* and *gltT* genes, respectively. These suppressors were found in late-arising spontaneous Arg^+ revertants.

There are also theoretical possibilities of creating ochre suppressors from $tRNA^{Tyr}_{AUA}$, $tRNA^{Ser}_{UGA}$ and $tRNA^{Leu}_{UAA}$, but these suppressors have not been identified yet (Śledziewska-Gójska *et al.*, 1992). Figure 1 shows two schematic pictures of tRNA suppressors.

Arg^+ revertants can arise spontaneously or as a result of induced mutagenesis. The first step in the analysis of the Arg^+ revertants is the examination of their requirement for histidine and threonine for growth. Arg^+ revertants have been divided into four phenotypic classes (class I: Arg^+ His^- Thr^-, class II: Arg^+ His^+ Thr^-, class III: Arg^+ His^- Thr^+ and class IV: Arg^+ His^+ Thr^+). Because, as mentioned above, *supE44* suppresses to some extent the *thr-1* mutation, the Thr^+ phenotype may be wrongly read and thus the revertants of class I and class II may be incorrectly classified as class III and class IV, respectively. For this reason in practice only two groups of Arg^+ revertants have usually been considered: a sum of classes I and III, and a sum of classes II and IV (Todd *et al.*, 1979; Śledziewska-Gójska *et al.*, 1992).

The sensitivity of the Arg^+ revertants to tester T4 phages is the second step in mutational analysis. A set of five T4 phages carrying a defined nonsense mutation includes the following phage mutants: amber B17 and NG19, and ochre oc427, ps292 and ps205. Phage multiplication, observed as plaque formation on a lawn of tested bacteria, indicates that the host bacterium bears a specific suppressor mutation (Kato *et al.*, 1980; Shinoura *et al.*, 1983; Sargentini & Smith, 1989; Śledziewska-Gójska *et al.*, 1992).

Arg^+ revertants of class I are the result of back mutations at the *argE3* site, or *supB* or *supE* ochre suppressor formation. Arg^+ revertants of class II, III and IV occur as a result of *supL*, *supX* and *supC* suppressor formation, respectively. The details of the above analysis are contained in Table 1, in which species of tRNA producing the indicated suppressor by a single base substitution in the anticodon sites are also included. Sargentini and Smith (1989) constructed a set of AB1157 derivatives bearing all the mentioned suppressors.

Identification of created suppressors allows deducing the specificity of mutation without DNA sequencing. However, such analysis does not say anything about the type of mutations in the *argE* gene creating a sense codon from the UAA stop codon. In this case DNA sequencing is required. The proportion of suppressor and

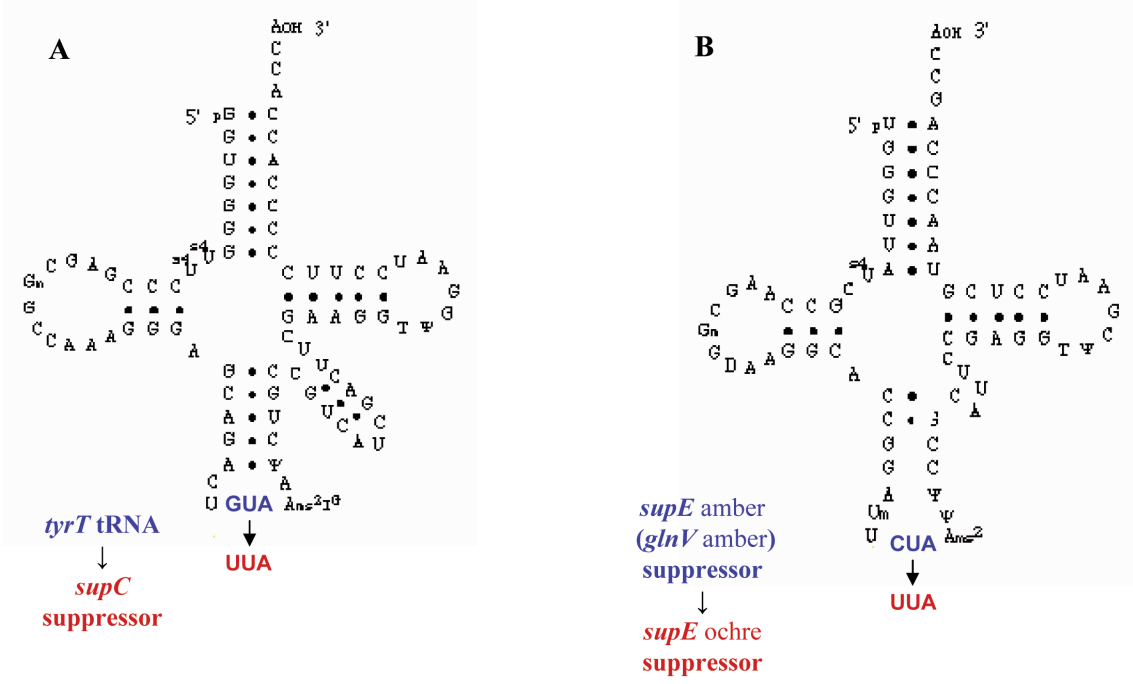

Figure 1. How mutations in the anticodon create suppressor tRNAs
(A) Tyrosine-specific tRNA recognizing the UAC codon in mRNA changes into *supC* suppressor reading UAA as a result of GC→TA transversion in the *tyrT* gene. Anticodon triplets are shown in blue and red for *tyrT* tRNA and *supC* suppressor, respectively. **(B)** Amber suppressor tRNA, *supE* amber (*glnV* amber), arising from the *glnV* gene (encoding tRNA for glutamine) recognizing the UAG codon in mRNA changes into *supE* ochre suppressor (reading UAA) as a result of GC→AT transition in the *supE* amber (*glnV* amber) gene. Anticodon triplets are shown in blue and red for *supE* amber (*glnV* amber) and *supE* ochre, respectively.

Table 1. tRNA suppressors counteracting effects of *ochre* and *amber* nonsense mutations in T4 phages and allowing formation of *E. coli* AB1157 Arg$^+$ revertants.

Suppressor	T4 phages					Arg$^+$ revertants			Recognized codon	tRNA charged with amino acid	Mutation leading to recognition of UAA nonsense codon
	amber		ochre			ochre		amber			
	B17	NG19	oc427	ps 292	ps205	argE3	hisG4	thr-1			
supB	+	–	+	+	+	+	–	–	CAA	Gln	GC→AT
supC (*supO*, *supM*)	+	+	+	+	–	+	+ t^S	+ t^S	UAC	Tyr	GC→TA
supL (*supG*, *supN*)	+	–	+	+	–	+	+	–	AAA	Lys	AT→TA
supX	+	–	+	-	–	+	–	+	?	XYZ	GC→TA or AT→TA
supE ochre	–	–	+	+	+	+	–	–	UAG	*supE* amber (gln)	GC→AT
AB1157 Arg$^-$ *supE* amber	+	–	–	–	–	–	–	–		–	

+ t^S, suppression is better at 30°C than at 37°C

back mutations in the *argE* gene depends on the type of mutagenic factor and bacterial background (examples are presented in Chapter: Studies with the use of the *argE3* →Arg$^+$ reversion-based system).

It is also possible to study the level of *hisG4*→His$^+$ revertants; however, only some suppressors may counteract the effect of the *hisG4* mutation so that many of the arising mutations are lost (Śledziewska-Gójska *et al.*, 1992).

STUDIES WITH THE USE OF THE *argE3*→Arg$^+$ REVERSION-BASED SYSTEM

In the era of intensive development of techniques of molecular biology and genetics studies based on reversion to prototrophy of the *argE3* mutation still provide new, interesting and valuable information: not only have they confirmed current knowledge but also supplied new data on the mutagenic specificity of different mutagens and mutator genes as well as on the mechanisms of mutagenesis and DNA repair. The applications of the described genetic system are presented below. This system is particularly useful for detection of GC→TA, GC→AT and AT→TA base substitutions and examination of transcription-coupled DNA repair.

Specificity of mutator genes

The system confirmed the mutagenic effects of mutator genes such as *mutT*, *mutY* and *fpg* (Wójcik *et al.*, 1996; Wójcik & Janion, unpublished; Nowosielska & Grzesiuk, 2000) or *dnaQ* (Nowosielska *et al.* 2004a; 2004b). MutT, MutY and Fpg (MutM), proteins belonging to the GO system, defend bacteria against the mutagenic action of 8-oxoG in DNA. MutT is a pyrophosphatase that hydrolyses 8-oxo-dGTP and prevents its incorporation into DNA. MutY is a DNA glycosylase excising from DNA adenine mispaired with A, 8-oxoG or G. Among others, Fpg excises from DNA 8-oxoG when it pairs with C (or T). The level of spontaneous transversions: AT→CG in *mutT*$^-$ and GC→TA in *mutY*$^-$ and *fpg*$^-$ mutants is, respectively, about 1000 to 10 000 and 10 to 100-fold higher than in the *w.t.* strain (Michaels & Miller, 1992). We have analyzed Arg$^+$ revertants arising spontaneously in *mutT*$^-$, *mutY*$^-$ and *fpg*$^-$ derivatives of *E. coli* AB1157 strain. In AB1157 *mutT*$^-$ strain a 1000-fold increase in the *argE3*→Arg$^+$ reversions was observed. All those reversions arose due to back mutations at the *argE3* site (probably as a result of AT→CG transversions). In *mutY*$^-$ and *fpg*$^-$ mutants all of the spontaneous *argE3*→Arg$^+$ reversions were due to GC→TA transversions by *supC* suppressor formation (Wójcik *et al.*, 1996; Wójcik & Janion, unpublished; Nowosielska & Grzesiuk, 2000).

DNA polymerase III, the main replicative polymerase in *E. coli*, comprises a *dnaQ*-encoded epsilon subunit responsible for proofreading activity. Mutants defective in this subunit chronically express the SOS response and exhibit a mutator phenotype (Echols *et al.,* 1983). Using the *argE3*→Arg$^+$ reversion, the effects of deletions in genes *polB* and *umuDC*, encoding, respectively, the SOS-induced DNA polymerases Pol II and Pol V, on the frequency and specificity of spontaneous mutations in the *dnaQ* background were studied. It was clearly shown that deletion of *umuDC* genes significantly decreased the level of spontaneous mutations in *dnaQ* strains (Nowosielska *et al.*, 2004a). The Arg$^+$ revertants in *mutD5* (allele of *dnaQ*) mutant occurred only as a result of tRNA suppressor formation, whereas those in *mutD5 polB* (Pol II deficient) strains arose at 81% by back mutation at the *argE3* ochre site (Nowosielska *et al.*, 2004b).

A mutator phenotype measured by an increased level of spontaneous *umuDC*-dependent *argE3*→Arg$^+$ reversions was one of the symptoms of the chronic induction of the SOS system in a *nth xth nfo* mutant deficient in base excision DNA repair system (BER). This mutant can not repair abasic sites. The chronic induction of the SOS system is due to accumulation of abasic sites left unrepaired in DNA (Janion *et al.*, 2003).

Specificity of mutagens

The mutagenic specificity of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), N^4-hydroxycytidine (oh^{4}Cyd), hydroxylamine (HA) (Śledziewska-Gójska *et al.*, 1992), ethylmethane sulfonate (EMS) (Grzesiuk & Janion, 1993), methylmethane sulfonate (MMS) (Śledziewska-Gójska & Janion, 1989; Grzesiuk & Janion, 1994) and UV light (Wójcik & Janion, 1997; 1999; Fabisiewicz &

Janion, 1998) has also been confirmed with the help of the *argE3*→Arg$^+$ reversion. Using this system it has been established that HA, a cytosine modifying agent, may also cause, apart from GC→AT transitions, a significant number of GC (or AT)→TA transversions (Śledziewska-Gójska *et al.*, 1992).

Studies on *E. coli* AB1157 strain and its derivatives revealed that biological effects (survival, mutation induction and mutation specificity) of halogen light irradiation were very similar to those observed after UVC irradiation. The halogen light-induced mutations were GC→AT transitions (*supB* or *supE* ochre suppressor formation) and back mutations at *argE3* sites resulting from T-C 6–4 photoproducts or T<>T thymine dimers, respectively. The latter damage was observed only in *uvrA* mutants defective in nucleotide excision repair (NER), constituting less than 5% of the total number of Arg$^+$ revertants (Wójcik & Janion, 1997; 1999). Those results confirmed previous data showing that halogen light causes harmful effects such as DNA damage, mutations, genotoxicity and skin cancers in mice due to emission of a broad spectrum of UV light, particularly UVC (De Flora *et al.,* 1990; D'Agostini *et al.,* 1993; D'Agostini & De Flora, 1994).

Analysis of Arg$^+$ revertants supplied new data on the mechanisms of mutagenesis and processes of DNA repair. The mutagenic properties of DNA damaging agents and the spectra of the induced mutations depend on the bacterial background, i.e., the presence of mutations in genes encoding proteins involved in DNA repair systems.

It was known that EMS was an *umuDC*-independent mutagen and induced GC→AT transitions due to formation of O^6-ethylguanine in DNA. It has been shown that in the AB1157 strain, EMS-induced Arg$^+$ revertants arise by *supB* and *supE* ochre suppressor formation. However, in *mutS*$^-$, a mismatch repair-deficient strain, the specificity of the EMS-induced *argE3*→Arg$^+$ reversions was changed and formation of *supL* suppressor by AT→TA transversions was mainly observed. Moreover, these mutations were *umuDC*-dependent. It was suggested that the change in mutation specificity was due to 3meA lesions or creation of apurinic sites. These results also point to different processes of DNA repair in *mutS*$^+$ and *mutS*$^-$ strains (Grzesiuk & Janion, 1993).

MMS predominantly methylates nitrogen atoms in purines. This methylating agent creates the following adducts in double stranded DNA: 7-methylguanine (7meG), 3-methyladenine (3meA), 1-methyladenine (1meA), 7-methyladenine (7meA), 3-methylguanine (3meG), O^6-methylguanine (O^6meG), 3-methylcytosine (3meC), and methylphosphotriesters. In ssDNA, MMS induces the same lesions but in different proportions. In ssDNA, the participation of 1meA and 3meC increases significantly since the ring nitrogens at these positions are not protected by the complementary DNA strand (Wyatt & Pittman, 2006; Sedgwick *et al.,* 2007). Analysis of Arg$^+$ revertants in *E. coli* AB1157 strain without any additional mutations revealed that 70–80% of those revertants arose by AT→TA transversions in a *umuDC*-dependent process, whereas the rest occurred in a *umuDC*-independent manner either by GC→AT transitions (formation of *supB* or *supE* ochre suppressors) or by back mutations at *argE3* site. The latter ones were detected in less than 5% of the Arg$^+$ revertants. AT→TA transversions are thought to be the result of 3meA, abasic sites and 1meA, whereas GC→AT transitions come from O^6-meG and 3meC residues in DNA and from depurination of 7meG (Grzesiuk & Janion, 1994; Nieminuszczy *et al.*, 2006a; 2009; Wrzesinski *et al.*, 2010).

The spectrum of the MMS-induced *argE3*→Arg$^+$ reversions changes in various strains deficient in DNA repair systems. In the *mutS*$^-$ mutant Arg$^+$ revertants arose mainly by GC→AT transitions (*supB* and *supE* ochre suppressor formation) or back mutations at *argE3* site. The latter group constituted a few percent of the total number of the Arg$^+$ revertants (Grzesiuk & Janion, 1998). In the *dnaQ49* derivative of the AB1157 strain about half of the MMS-induced Arg$^+$ revertants occurred by AT→TA transversions (*supL* suppressor formation). In a double *dnaQ*$^-$ *umuDC*$^-$ mutant about 90% of the revertants possessed *supB* or *supE* ochre suppressors due to GC→AT transitions (Grzesiuk & Janion, 1996)

Detection of mutations resulting from lesions in ssDNA

Examination of MMS-induced mutagenesis in AB1157*alkB*$^-$ derivatives indicates that the *argE3*→Arg$^+$ reversion system also enables detection of mutations arising from lesions in ssDNA (Nieminuszczy *et al.*, 2006a; 2009; Sikora *et al.*, 2010; Wrzesiński *et al.*, 2010). AlkB is an α-ketoglutarate-, O_2- and Fe(II)-dependent dioxygenase that oxidatively demethylates 1meA and 3meC in ds- and ssDNA and in RNA. However, ssDNA is repaired much more effectively than dsDNA (Trewick *et al.*, 2002; Falnes *et al.*, 2002). It has been shown that in *alkB*$^-$ mutants the level of MMS-induced mutagenesis depends on the test system used, and is several orders of magnitude higher when measured in the *argE3*→Arg$^+$ reversion test system in *E. coli* AB1157 in comparison to *lacZ*→Lac$^+$ reversion studied in CC101-CC106 strains (Nieminuszczy *et al.,* 2006a; 2006b; 2009; Kataoka *et al.*, 1983; Dinglay *et al.*, 2000). The CC101–CC106 tester strains are described in more detail in Chapter: Other enterobacterial systems for mutation detection. Briefly, the *lacZ*→Lac$^+$ reversion occurs only by a back mutation at one point in the structural gene encoding the β-galactosidase that if not expressed would be primarily in dsDNA form.

The *argE3*→Arg$^+$ reversion-based system showed that in AB1157 *alkB*$^-$ strain 95–98% of the induced mutations were *umuDC* (Pol V)-dependent AT→TA transversions (*supL* suppressor formation) and GC→AT transitions (*supB* or *supE* ochre suppressor formation). Back mutations in the *argE3* site constitute only about 2–5% of all types of Arg$^+$ revertants (Nieminuszczy *et al.*, 2006a). Genes encoding tRNA are heavily transcribed and exist mostly as ssDNA in cells. It facilitates methylation of A/C to 1meA/3meC. That is why we assume that in AB1157 *alkB*$^-$ strain the targets undergoing mutations leading do Arg$^+$ revertants are predominantly located in ssDNA. Reversion to Arg$^+$ occurs mostly by formation of a variety of *sup*tRNA ochre suppressors. The number of targets undergoing mutations and differences in the reactivity of MMS to form 1meA/3meC lesions in ssDNA *vs.* dsDNA are the main reasons of the great discrepancy in the frequencies of MMS-induced *argE3*→Arg$^+$ and *lacZ*→Lac$^+$ revertants observed (Nieminuszczy *et al.*, 2009).

In strains deficient in the BER system (abasic site repair), mutants in *nfo* (encoding endonuclease IV) and *xth* (encoding exonuclease III) genes with non-functional AlkB protein, an extremely high level of the MMS-induced *argE3*→Arg$^+$ reversions has been observed. This phenomenon can be explained by the involvement of abasic sites formed in DNA of the AB1157 *nfo*$^-$ *xth*$^-$ strain, causing a local relaxation of dsDNA structure. We

Table 2. Potential mutagenic targets for UV and MMS modification and mechanisms of mutation creation in *glnU, glnV* amber, *lys*-tRNA and *argE* genes, leading to Arg$^+$ phenotype in *E. coli* K-12 AB1157

DNA		→	tRNA	
glnU gene	5′----TT *TTG*AT----3′ 3′--- AAA*A*CTA----5′		5′--UUG--3′	gln-tRNA$_{CAA}$ — tRNA anticodon for glutamine reading 5′CAA3′ codon in mRNA
↓	↓	→	↓	↓
supB suppressor	5′----TT *TTA*AT----3′ 3′----AAA*ATT*A----5′		5′--UUA--3′	gln-tRNA$_{UAA}$ — tRNA anticodon reading nonsense *ochre* triplet 5′UAA3′ in mRNA
glnV$_{am}$ (*supE44*$_{am}$) suppressor	5′----T*C*TA----3′ 3′----A*G*AT----5′		5′--CUA--3′	gln-tRNA$_{UAG}$ — tRNA anticodon reading nonsense *amber* triplet 5′UAG3′ in mRNA
↓	↓	→	↓	↓
supE$_{OC}$ suppressor	5′----TTTA----3′ 3′----AAAT----5′		5′--UAA--3′	gln-tRNA$_{UAA}$ — tRNA anticodon reading nonsense *ochre* triplet 5′UAA3′ in mRNA
lys-tRNA genes	5′---*T TT*-----3′ 3′--*AA*A----5′		5′--UUU--3′	lys-tRNA$_{AAA}$ — tRNA anticodon for lysine reading 5′AAA3′ codon in mRNA
↓	↓	→	↓	↓
supL suppressor	5′---*TTA*----3′ 3′--- *AAT*----5′		5′--UAA--3′	lys-tRNA$_{UAA}$ — tRNA anticodon reading nonsense *ochre* triplet 5′UAA3′ in mRNA
argE3 mutation in *argE* gene	5′----TTTA*A*AT----3′ 3′---AA*A*TTTA---5′	→	No changes in tRNA encoding genes	
↓				
mutations leading to any sense nucleotide triplet or UAG nonsense codon recognized by *supE44* amber suppressor				

Nucleotide triplets corresponding to tRNA anticodon in *glnU*, *glnV* amber and *lys*-tRNA genes are in italics. Underlined sequences and grey letters show potential sites of photoproducts (6–4 photoproducts and thymine dimers) formation and targets for methylation, respectively.

assume that under these conditions more single-stranded DNA appears in the bacterial chromosome that facilitates methylation by MMS and results in "error catastrophy" in the triple AB1157 *nfo xth alkB* mutant (Sikora *et al.*, 2010). Analysis of MMS-induced Arg$^+$ revertants in *alkB*$^-$ and *alkB*$^-$BER$^-$ strains clearly points to a mutagenic activity of 1meA and 3meC (Nieminuszczy *et al.*, 2006a; Sikora *et al.*, 2010).

Determination of transcription-coupled DNA repair

The *argE3*→Arg$^+$ reversion system of mutation detection in *E. coli* AB1157 also enables studies on preferential removal of lesions from the transcribed strand of DNA. This type of DNA repair, called transcription-coupled DNA repair (TCR), requires Mfd protein that removes transcription elongation complexes stalled at non-coding lesions in DNA and recruits to these sites proteins involved in nucleotide excision repair (NER). TCR occurs under conditions of temporary inhibition of protein synthesis and results in a decrease in the frequency of induced mutations (Selby & Sancar, 1993; Savery, 2007). This phenomenon is called mutation frequency decline (MFD) and was discovered for UV-irradiated bacteria by Evelin Witkin (for review see Witkin, 1994). The MFD phenomenon has been studied by the Janion and Grzesiuk's group on UV (or halogen light)- and MMS-induced Arg$^+$ revertants in the AB1157 strain transiently incubated under non-growth conditions (amino acid starvation) after treatment with a mutagen (Grzesiuk & Janion, 1994; 1996; 1998; Wójcik & Janion, 1997; Fabisiewicz & Janion, 1998; Wrzesiński *et al.*, 2010). Table 2 shows all the mutagenic targets for UV- and MMS-induced DNA damage. Potential targets for UV-modifications (T-C and T-T sequences for creation of 6–4 photoproducts and pyrimidine dimers, respectively) are underlined. Potential targets (single bases) for MMS-modifications are shadowed. UV- or halogen light-induced Arg$^+$ revertants occur mainly as a result of a GC→AT transition forming the *supB* and *supE* ochre suppressors, respectively, at the transcribed DNA strand of the *glnU* and the coding DNA strand of the *glnV* amber (*supE44* amber) gene. In *mfd*$^-$ strains the formation of *supB* predominated over *supE* ochre suppressors and their number, in contrast to the *mfd*$^+$ strain, did not decrease during amino acid starvation. The MFD effect observed in *mfd*$^+$ strains is a reflection of repair of premutagenic lesions in the transcribed strand of the *glnU* gene leading to *supB* suppressor formation (Wójcik & Janion, 1997; Fabisiewicz & Janion, 1998).

Studies on the MFD effect in the AB1157 strain and its derivatives after MMS treatment involved both an analysis of Arg$^+$ revertants and examination of plasmid DNA isolated from cells treated with MMS for their sensitivity to the Fpg and Nth endonucleases. The decrease in the level of MMS-induced mutations during transient starvation was accompanied by repair of abasic sites in plasmid DNA. As it is shown in Table 2, potential targets for MMS damage are located on both the transcribed and coding DNA strands of *glnU*, *glnV* amber and *argE* genes and only on the transcribed strand of *lys*-tRNA genes. Lesions resulting from methylation of the transcribed DNA strand are subject to MFD repair. Previous studies on the MFD phenomenon after MMS treatment of the AB1157 strain and its derivatives focused on the preferential repair of transcribed-strand lesions of genes coding for *lys*-tRNA; this repair was manifested by a decrease in the number of *supL* suppressors (Grzesiuk & Janion, 1994; 1998). A recent study revealed a significantly slower and completely absent MFD effect in, respectively, AB1157*mfd* and double *alkB mfd* mutants. It was assumed that the former effect is the result of action of other DNA repair systems and the latter is a reflection of an accumulation of DNA damage and strong SOS induction. These results again have confirmed the strong mutagenic effects of 1meA/3meC lesions (Wrzesiński *et al.*, 2010).

Interestingly, in a *dnaQ* mutant no MFD repair was observed indicating that in this mutant the processes of DNA repair are different, probably due to chronic induction of SOS response and the presence of Pol V and Pol IV DNA repair polymerases induced within this response (Grzesiuk & Janion, 1996).

Adaptive mutation

Reversion to prototrophy of the *argE3* mutation was also used as a method for determination of adaptive mutations. These mutations (also called "stationary phase" or "starvation associated") are a special kind of spontaneous mutations that occur in non-dividing cells and allow growth under selected conditions. Using the *argE3*→Arg$^+$ reversion system, AB1157 bacteria that mutated adaptively were selected on minimal plates devoid of arginine after 4 to 10 days of growth. Further phenotypic analysis and susceptibility to a set of amber and ochre T4 phages allowed the identification of stationary phase mutations in *mutY*$^-$ (Nowosielska & Grzesiuk, 2000) and *dnaQ*$^-$ (Nowosielska *et al*., 2004a; 2004b) strains. It has been shown that in the *dnaQ*$^-$ strain Pol IV and Pol V polymerases, in contrast to Pol II, influence the frequency and specificity of starvation-associated mutations.

OTHER ENTEROBACTERIAL SYSTEMS FOR MUTATION DETECTION

Reversions of *trpE65* to Trp$^+$ phenotype and *tyrA14* to Tyr$^+$ in *E. coli* B/r WP2 (Ohta *et al*., 2002) and WU3610 derivatives (Bockrath *et al*., 1987), respectively, are analogous mutation detection systems to that of *E. coli* K12 AB1157. Both *trpE65* and *tyrA14* are ochre mutations in genes coding for enzymes involved in tryptophane and tyrosine biosynthesis, respectively. The Trp$^+$ or Tyr$^+$ phenotype may be recovered by (i) any point mutation at *trpE65* or *tyrA14* leading to the formation of a sense nucleotide triplet, and (ii) ochre suppressor mutations. In the WP2 (*trpE65*) system the examined suppressors are *supB*, *supC*, *supG* and *supM* formed in the genes coding for tRNA: *glnU*, *tyrT, lysT* and *tyrU*, respectively (Ohta *et al*., 2002). In the WU3610 (*tyrA14*) strain the *de novo* ochre suppressor mutations in glutamine tRNA are studied. The WU3610-11 derivative bears an amber suppressor created from another glutamine tRNA gene that can be converted to an ochre suppressor (Bockrath & Palmer, 1977; Bockrath *et al*., 1987). Both systems have been used in MFD studies (Bridges *et al*., 1967; George & Witkin, 1974; Bockrath & Palmer, 1977; Bockrath *et al*., 1987).

A commonly used bacterial system allowing rapid detection of specificity of mutation is the *lacZ*→Lac$^+$ reversion in *E. coli* K12 CC101–111 strains. The *lacZ* gene encodes β-galactosidase and is part of the lactose operon. Mutants in *lacZ* gene are unable to grow on a medium containing lactose as the sole carbon source. A set of eleven mutants with a *lacZ* deletion in the chromosome and F' with cloned *lacZ* gene bearing defined mutations (six base substitutions and five frame shifts at the same coding position) have been constructed. Reversion to the Lac$^+$ phenotype is due to a specific mutation in the *lacZ* gene on F' (Coupples & Miller, 1989; Coupples *et al*., 1990). In this case the marker is episomal, in contrast to the AB1157 strain where the marker is situated on the chromosome.

E. coli K12 FC40 is another mutation detection system based on *lacZ*→Lac$^+$ reversion. This strain contains an F' episome carrying a *lacI-lacZ* fusion with a +1 base pair frameshift mutation, *lacI33,* in the *lacI* coding sequence. The Lac$^-$→Lac$^+$ reversion is due to a –1 frameshift mutation. FC40 was often used in studies on stationary-phase mutations (Foster, 1994).

The *Salmonella* mutagenicity assay was introduced by Bruce Ames and co-workers in the early 1970s and later modified (Ames *et al*., 1973). Briefly, the test involves reversion of a *his*$^-$ auxotrophic mutation to prototrophy by base substitutions in the *hisG46* allele or by frameshift in the *hisD3052* allele. In addition, the tester strains carry: (i) additional mutations, such as *rfa*, increasing the permeability of the bacterial cell wall and enabling better penetration of mutagenic agents to the cell, or *uvrB*, disturbing DNA repair; (ii) plasmid pKM101 — a mutagenesis-enhancing plasmid bearing *mucA* and *mucB* genes that code for proteins corresponding to *E.coli* UmuC and UmuD, responsible for translesion synthesis (TLS) (Mortelmans, 2006).

Antibiotic resistance to streptomycin, rifampicin or nalidixic acid are also often used markers for determination of the level of spontaneous and induced mutations in bacteria. Rifampicin-, streptomycin- and nalidixic acid-resistant mutants arise following mutations in the *rpoB* gene encoding β subunit of RNA polymerase, mutations leading to changes in the 30S subunit of the ribosome or mutations in genes encoding gyrase subunits, respectively.

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