umuC-Independent, *recA*-Dependent Mutagenesis

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Abstract

Although targeted UV-radiation mutagenesis appears to require both the *recA* and *umuC* genes in *Escherichia coli*, examples of *recA*-dependent but *umuC*-independent mutagenesis exist, e.g., gamma-radiation mutagenesis (Mutat. Res. 128, 1, 1984) and streptozotocin mutagenesis (Mutat. Res. 166, 229, 1986). Most of the information on *umuC*-independent mutagenesis comes from studies on ionizing radiation mutagenesis. These results will be reviewed here. Analyses of the various suppressor and back mutations that result in *argE3* and *hisG4* ochre reversion and an analysis of *trpE9777*(+1 frameshift) reversion were performed on *umuC* and wild-type cells gamma irradiated in the presence and absence of oxygen. In wild-type cells, the presence of oxygen enhances gamma-radiation mutagenesis. Although the *umuC* strain showed the gamma-radiation induction of base substitution and frameshifts when irradiated in the absence of oxygen, the *umuC* mutation blocked all oxygen-dependent base-substitution mutagenesis, but not all oxygen-dependent frameshift mutagenesis. For anoxically-irradiated cells, the yields of GC–>AT and AT–>GC transitions were largely *umuC* independent, while the yields of (AT or GC)–>TA transversions were heavily *umuC* dependent. Therefore, the data for anoxically-irradiated cells support the hypothesis that gamma irradiation produces two kinds of DNA lesions that require *recA*-dependent misrepair to induce mutations. For base-substitution mutagenesis, one kind of lesion requires the *umuC* gene and produces transversion mutations, while a second kind of lesion produces transition mutations and does not require the *umuC* gene. For cells irradiated in the presence of oxygen, there seems to be an additional kind of lesion whose mutagenic potential for base substitutions (but not frameshifts) is completely dependent on the *umuC* gene.

Introduction

Early studies on the effect of *recA* mutations on radiation and chemical mutagenesis suggested two mechanisms of mutagenesis in *Escherichia coli:* misreplication and misrepair (e.g., Kondo, 1968; Ishii and Kondo, 1975). Misreplication mutagenesis has been defined operationally as *recA*-independent mutagenesis, and is produced by agents (e.g., ethylmethane sulfonate) that alter the coding properties of the DNA template (e.g., Miller, 1983). Conversely, misrepair [or error-prone or SOS-repair (Witkin, 1976)] mutagenesis has been synonymous with *recA*-dependent mutagenesis, and is produced by agents (e.g., ionizing and UV radiations, and 4-nitroquinoline-1-oxide) that produce noncoding sites in the template.

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Therefore, it is not surprising that the concept has developed that the mechanism of mutagenesis (i.e., misreplication or misrepair) for any mutagen can be determined by testing whether its mutagenicity in *E. coli* depends upon the *recA* gene. However, *recA* mutations affect many phenomena in addition to mutagenesis (reviewed in Walker, 1984). With the independent discovery by Kato and Shinoura (1977) and Steinborn (1978) of the *umuC* and *umuD* mutations, which seem to only abolish misrepair, it has subsequently been considered preferable to test for *umuC* dependence rather than for *recA* dependence when one is trying to ascertain the basis of the mutagenicity of a new agent (e.g., Schendel and Defais, 1980; Shinoura et al., 1983).

However, this preference for a *umuC*-test over a *recA*-test may be leading to incorrect conclusions. Cases in point are the following: (i) The *recA* strain was not mutated by methyl methanesulfonate (Kondo et al., 1970; Walker, 1977), while the *umuC* strain showed 30% of the mutagenesis seen in the wild-type strain when assayed by reversion of the *argE3* mutation (Schendel and Defais, 1980). However, a *umuC* strain did not show the methyl methanesulfonate-induced reversion of the *hisG4* mutation (Walker and Dobson, 1979). (ii) The *recA* strain was not mutated (a rifampicin-resistance assay) by the alkylating agent streptozotocin (a monofunctional nitrosourea), while the *umuC* strain showed the wild-type level of mutagenesis (Fram et al., 1986). (iii) The *umuC* strain was mutated by gamma radiation when assayed for Arg reversion at the same radiation doses that showed no mutagenesis of the *recA* strain (Sargentini and Smith, 1989). Therefore, it is quite clear that the *umuC* gene does not control all types of *recA*-dependent mutagenesis. Most of the information on *recA*-dependent, *umuC*- independent mutagenesis has come from studies on ionizing radiation mutagenesis. A review of this information will constitute the major emphasis of this report.

Gamma-Radiation Mutagenesis is *recA*-Dependent, but can be Either *umuC*-Dependent or *umuC*-Independent.

Kato and Nakano (1981) reported that the *umuC* mutant was deficient in gamma-radiation mutability, when assaying for His^+, $ColE^R$ and Spc^R. In contrast, however, Steinborn (1978) showed that his *uvm* mutants, which were subsequently shown by Shinagawa et al. (1983) to be *umuC* and/or *umuD* mutants, were near normal in X-ray mutability when assaying for Arg^+.

In 1984, Sargentini and Smith attempted to resolve this apparent conflict in the literature concerning the role of the *umuC* gene in ionizing-radiation mutagenesis. Using the *umuC122*::Tn5 strain (Elledge and Walker, 1983), which is assumed to show a 'null' phenotype, and a number of different mutation assays [including some of those used by Kato and Nakano (1981) and Steinborn (1978)], they showed that the gamma-radiation mutability of this *umuC* strain varied from *no deficiency* to a 50-fold deficiency, depending upon the mutation assay and doses used (Sargentini and Smith, 1984). They concluded that both Kato and Nakano (1981) and Steinborn (1978) were correct for the mutation assays that they used, and that both *umuC*-independent and *umuC*-dependent

mechanisms exist for ionizing-radiation mutagenesis, while targeted UV-radiation mutagenesis seems to depend only on the *umuC*-dependent mechanism (Sargentini and Smith, 1984).

The *recA* strain was not mutated by gamma radiation when assayed either by *arg*(Am) reversion or by assays for the production of large deletions (Kondo, 1968; Ishii and Kondo, 1975), or when assayed by *arg*(Oc) reversion (Sargentini and Smith, 1989). However, the *umuC* strain showed mutagenesis after the same radiation doses and with the same *arg*(Oc) reversion assay that failed for the *recA* strain (Sargentini and Smith, 1989). These results reaffirm that gamma-radiation mutagenesis is totally dependent on misrepair, and they suggest that the *recA* gene controls gamma-radiation mutagenesis via both *umuC*-independent and *umuC*-dependent mechanisms.

How Can the Partial Requirement for the *umuC* Gene in Certain Mutation Assays be Explained?

One complexity with many mutation assays, and especially with nonsense-reversion assays (e.g., Kato et al., 1980), is that more than one base change can lead to the same phenotypic reversion. Therefore, the partial requirement for the *umuC* gene in gamma-radiation mutagenesis may be interpreted as either a partial dependence on *umuC* at each specific mutation site, or as the net effect of an 'all-or-none' dependence on *umuC* at the several mutation sites that are scored simultaneously in one mutation assay. Since no gamma-radiation mutagenesis was observed in the *umuC* strain with the assay for resistance to spectinomycin (Sargentini and Smith, 1984), the all-or-none hypothesis is favored.

This hypothesis was supported by analyzing mutagenesis at several base-pair sites, and showing that there is a large *umuC* dependence at some base-pair sites and little or no *umuC* dependence at other base-pair sites, when assaying for Arg^+ and His^+ ochre revertants. That is, for anoxically-irradiated cells, the requirement for the *umuC* gene in gamma-radiation mutagenesis was very small at the *supB*, *supE*(Oc), *argE3*, and *hisG4* sites (where transition mutations should be produced), while the requirement for the *umuC* gene was very large at the *supC* or *M*, *supL* or *N*, and *supX* sites (where transversion mutations should be produced). In addition, all of the mutant base pairs at the suppressor loci were AT, while the mutant base pairs at the back mutation sites were GC (Table 1).

The Effect of Oxygen and the *umuC* Gene on Gamma-Radiation Mutagenesis

The *umuC* strain showed less gamma-radiation mutagenesis than wild-type cells, whether irradiated oxically or anoxically (Fig. 1a-c). The *umuC* strain did not show the oxygen enhancement of gamma-radiation-induced base-substitution mutagenesis that was seen in the wild-type strain (Figs. 1a, b); however, both strains showed the oxygen enhancement of radiation-induced frameshift reversion (Fig. 1c) and cell killing (Fig. 1d).

Table 1. Effects of the *umuC* mutation and oxic irradiation conditions on gamma radiation mutagenesis of *E. coli*[*]

Comparison of mutant frequencies	Relevant genotype	Irradiation gassing condition	Mutants per 10^8 cells induced by 30 krads							
			supB (Arg^+)	*supE*(Oc) (Arg^+)	*argE3* (Arg^+)	*hisG4* (His^+)	*supC,M* (Arg^+)	*supL,N* (Arg^+)	*supL,N* (His^+)	*supX* (Arg^+)
	WT	Air	49	34	119	88	95	80	44	125
		N_2	35	10	70	35	36	18	5	66
	umuC	Air	31	8	62	12	3	2	2	21
		N_2	29	10	56	16	2	2	1	23
WT: Air/N_2			1	3	2	2	3	4	9	2
umuC: Air/N_2			1	1	1	1	1	1	2	1
Air: WT/*umuC*			2	4	2	7	32	40	22	6
N_2: WT/*umuC*			1	1	1	2	18	9	5	3
Putative base charges			GC->AT Transitions		AT->GC		(AT or GC) ->TA Transversions			

[*]From Sargentini and Smith (1989).

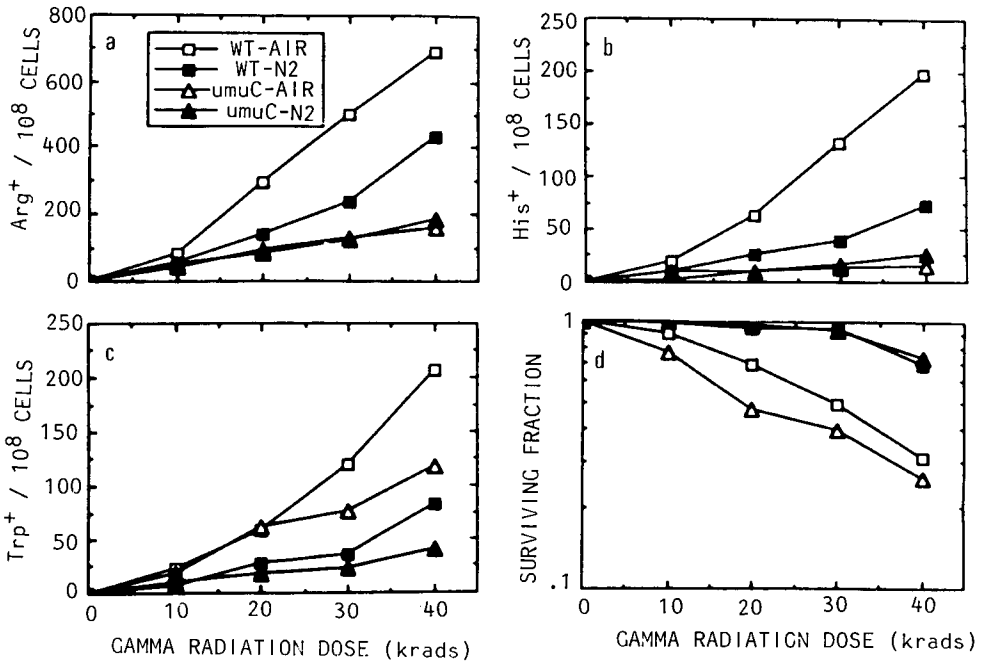

Figure 1. Gamma-radiation mutagenesis and survival for *E. coli umuC*$^+$ (squares) and *umuC122*::Tn*5* (triangles) cells, irradiated in the presence (open symbols) or absence (closed symbols) of O_2. Mutagenesis was assayed by reversion assays: *argE3*(Oc)->Arg$^+$, (a); *hisG4*(Oc)->His$^+$, (b); and *trpE9777*(+1 Fs)->Trp$^+$, (c). Survival (d) was determined by plating diluted cells on mutant-selection plates. Data are the means from three experiments per strain. (From Sargentini and Smith, 1989.)

The additional mutagenic lesions that are produced in the presence of oxygen all require the *umuC* gene for the production of base substitutions. The types of alterations produced in pyrimidines by gamma irradiation are affected markedly by the presence of oxygen (Teoule, 1987). For example, seven radiolytic products of thymine are produced in DNA only in the presence of oxygen: the hydroperoxides of thymine and their degradation products (e.g., urea), and 5-hydroxymethyl uracil (reviewed in Teoule, 1987). In fact, 5-hydroxymethyl-2'-deoxyuridine produces base substitutions at AT and GC sites when it is present in bacterial-culture media, and its mutagenicity depends on the presence of the *mucAB* genes, which are analogues of the *umuDC* genes (Shirname-More et al., 1987). Thus, 5-hydroxymethyl uracil seems to be one candidate for producing *umuC*-dependent, oxygen-dependent gamma radiation-induced base substitutions.

When mutations were scored at different base-pair sites for the reversion of *argE3* and *hisG4* ochre mutations, the presence of oxygen during the gamma irradiation of wild-type cells either had no effect or it enhanced mutagenesis up to 9-fold, depending upon the specific base-pair site scored (Table 1). These results suggest that the nature of the base pair to be mutated and/or the neighboring bases have a profound effect on the role of

oxygen in mutagenesis. Neighboring bases have been shown to have a profound effect on chemical mutagenesis (Burns et al., 1987).

The Effect of the Absence of Oxygen on Gamma-Radiation Mutagenesis

When cells are gamma irradiated under anoxia, both *umuC*-independent and *umuC*-dependent mechanisms of mutagenesis exist. Furthermore, the yield of DNA lesions that cause *umuC*-independent mutagenesis is *not affected by oxygen* (Table 1). Among the thymine radiolysis products, only 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol) is produced both in the presence and in the absence of oxygen (Teoule, 1987). However, this type of damage does not seem to be mutagenic even though it does block the replication fork (e.g., Laspia and Wallace, 1988). Another possibility is *trans*-5,6-dihydroxy-5,6-dihydrouracil (uracil glycol). This cytosine-derived base damage is associated with the production of C–>T transitions (Ayaki et al., 1987), which were found to be *umuC*-independent in anoxically-irradiated cells (Table 1). Finally, the same kinds of purine radiolytic products are produced whether oxygen is present or not (R. Teoule, personal communication), which suggests that damaged purines must also be considered as a source of oxygen-independent, *umuC*-independent mutagenesis.

Regardless of which DNA lesions are responsible for *umuC*-independent mutagenesis (transitions) in anoxically-irradiated cells, another kind of lesion must be produced in anoxically-irradiated cells to explain the *umuC*-dependent transversions that are produced. Apurinic/apyrimidinic site mutagenesis is *umuC* dependent (Schaaper et al., 1982). These lesions are produced directly in DNA by gamma irradiation (Ullrich and Hagen, 1971), and they are also transiently present during the repair of gamma-radiation-induced base damage (e.g., Breimer and Lindahl, 1985). It would seem more than a coincidence that the spectral analysis shows that adenine is always part of the mutant base-pair for *umuC*-dependent anoxic gamma-radiation mutagenesis (Table 1).

It is known from studies on apurinic-site mutagenesis that the *umuC*-dependent mechanism shows a strong preference for inserting adenine when it encounters an apurinic/apyrimidinic site in the template strand (e.g., Kunkel, 1984). Furthermore, if the lesion relevant to *umuC*-dependent anoxic gamma-radiation mutagenesis is a damaged purine rather than an apurinic site, then the tendency for damaged purines to lead to transversions via SOS repair (Rabkin et al., 1983) provides an even better explanation for the data on *umuC*-dependent gamma-radiation mutagenesis in anoxically-irradiated cells.

Frameshift Mutagenesis

Even though base-substitution and frameshift mutagenesis are similar in being totally *umuC* dependent in UV-irradiated cells, and in being only partially *umuC* dependent in gamma-irradiated cells (Sargentini and Smith, 1984), the *umuC* gene seems to play a different role in base-substitution versus frameshift mutagenesis. The *umuC* gene is required for the oxygen effect on base substitutions but not for the oxygen effect on frameshifts (Fig. 1a-c). Also, the UV-radiation induction of base substitutions, but

not of frameshifts, is enhanced in *umuC* cells by a delayed photoreactivation procedure (Sargentini and Smith, 1987).

Conclusions

The data for anoxically-irradiated cells support the hypothesis that gamma radiation produces two kinds of DNA lesions that require *recA*-dependent misrepair to induce mutations. For base-substitution mutagenesis, one kind of lesion requires the *umuC* gene and produces transversion mutations, while a second kind of lesion produces transition mutations and does not require the *umuC* gene. For cells irradiated in the presence of oxygen, there seems to be additional kinds of lesions whose mutagenic potential for base substitutions (but not frameshifts) is completely dependent on the *umuC* gene.

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