ULTRAVIOLET RADIATION-INDUCED MUTABILITY OF *uvrD*3 STRAINS OF *ESCHERICHIA COLI* B/r AND K-12: A PROBLEM IN ANALYZING MUTAGENESIS DATA

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Abstract—The involvement of the *uvrD* gene product in UV-induced mutagenesis in *Escherichia coli* was studied by comparing wild-type and *uvrA* or *uvrB* strains with their *uvrD* derivatives in B/r and K-12(W3110) backgrounds. Mutations per survivor (reversions to prototrophy) were compared as a function of surviving fraction and of UV fluence. While recognizing that both methods are not without problems, arguments are presented for favoring the former rather than the latter method of presenting the data when survival is less than 100%. When UV-induced mutation frequencies were plotted as a function of surviving fraction, the *uvrD* derivatives were less mutable than the corresponding parent strains. The B/r strains exhibited higher mutation frequencies than did the K-12(W3110) strains. A *uvrB* mutation increased the mutation frequency of its parental K-12 strain, but a *uvrA* mutation only increased the mutation frequency of its parental B/r strain at UV survivals greater than ~80%. Both the *uvrA* and *uvrB* mutations increased the mutation frequencies of the *uvrD* strains in the B/r and K-12 backgrounds, respectively. Rather different conclusions would be drawn if mutagenesis were considered as a function of UV fluence rather than of survival, a situation that calls for further work and discussion. Ideally mutation efficiencies should be compared as a function of the number of repair events per survivor, a number that is currently unobtainable.

INTRODUCTION

We wish to determine which of the bacterial repair pathways are error prone, and hence mutagenic. Of the two dark repair processes for UV-induced damage, postreplication repair appears to be the major mutagenic process. This is suggested by the fact that strains that are deficient in excision repair (i.e. Hcr⁻ strains), and therefore dependent on postreplication repair, appear to show an enhanced mutability over wild-type strains at equal UV fluences (Hill, 1965; Witkin, 1967). If postreplication repair is generally error prone, then strains deficient in this type of repair should show decreased mutability. In support of this, it has been reported that *recA* and *exrA(lexA)* strains are deficient in postreplication repair (Sedgwick 1975a; Youngs and Smith, 1976) and are refractory to the mutagenic effects of UV radiation (Witkin, 1967, 1969; Miura and Tomizawa, 1968).

The *uvrD* gene product has been shown, by DNA sedimentation analysis, to play a role in postreplication repair (Youngs and Smith, 1976). Actually, the *uvrD* gene appears to control two pathways of postreplication repair, one that is independent of the action of the *exrA* and *recB* genes, and one that is blocked by postirradiation treatment with chloramphenicol, and also requires the action of the *exrA* and *recB* genes (Youngs and Smith, 1976). Therefore, since chloramphenicol blocks one of the *uvrD* pathways of postreplication repair and also blocks the loss of photoreversibility of UV-induced mutations in a

uvrA strain (Sedgwick, 1975b), it suggests that the *uvrD* pathway of postreplication repair is mutagenic.

A *uvrD* strain is also deficient in the growth-medium dependent pathway of excision repair (E. Van der Schueren, D. A. Youngs and K. C. Smith, unpublished observations). This same pathway requires functional *recA* and *exrA* genes (Youngs et al., 1974), and appears to be mutagenic (Nishioka and Doudney, 1969, 1970).

Thus, the data on both postreplication repair and excision repair suggest that the *uvrD* gene product plays a role in UV-induced mutagenesis. However, Miura and Tomizawa (1968) reported that a *uvrD* mutation had no effect on the UV induction of clear plaque mutants of phage λ. Since their conclusion was only based upon data at a single UV fluence, we have undertaken a more extensive study on the mutability of *uvrD* strains. We have compared the relative UV-induced mutability (reversions to prototrophy) of *E. coli* wild-type, *uvrA* and *uvrB* strains with their corresponding *uvrD* derivatives in the B/r and K-12(W3110) backgrounds.

While currently there is a lack of general agreement as to the most appropriate way to analyze data on mutagenesis (see, e.g. Wohlrab and Tuveson, 1969; Clarke and Shankel, 1975), we prefer to compare mutation frequency as a function of survival, when survival is less than ~100%. By this type of analysis, our data suggest that the *uvrD* gene product plays a role in UV-induced mutagenesis, and are consistent, therefore, with the genetic and biochemical data cited

Table 1. Strains of *E. coli* used for mutation studies

Strain number	Genotype	Derivation or source
B/r		
WP2$_s$ *ilv*	*uvrA trp ilv malB*	E. M. Witkin (derived from WP2$_s$)
DY214	*uvrA uvrD3 trp malB*	{ P$_1$N14-4 × WP2$_s$ *ilv*
DY215	*uvrA trp malB*	{ (Select Ilv$^+$)
SR272	*trp*	{ P$_1$.K-12(Mal$^+$Uvr$^+$) × DY125
SR274	*uvrA trp*	{ (Select Mal$^+$)
SR275	*uvrD3 trp*	{ P$_1$.K-12(Mal$^+$Uvr$^+$) × DY214
SR276	*uvrA uvrD3 trp*	{ (Select Mal$^+$)
K-12 (W3110)		
N14-4	F$^-$ *uvrD3 trp gal str*	H. Ogawa
KH21	F$^-$ *leuB bio thyA thyR metE malB rha lacZ str*	R. B. Helling
DY174	F$^-$ *leuB bio thyA thyR malB rha lacZ str*	{ P$_1$.N14-4 × KH21
DY175	F$^-$ *uvrD3 leuB bio thyA thyR malB rha lacZ str*	{ (Select Met$^+$)
DY178	F$^-$ *uvrB5 leuB thyA thyR rha lacZ str*	Youngs and Smith, 1976
DY179	F$^-$ *uvrB5 uvrD3 leuB thyA thyR rha lacZ str*	Youngs and Smith, 1976

above, which indicate that the *uvrD* gene product functions in two pathways of repair that appear to be mutagenic.

MATERIALS AND METHODS

Bacterial strains. Table 1 lists the strains studied, their genotypes, and their sources. The transduction techniques have been described (Youngs and Smith, 1973). In each case the initial selection was for the nutritional requirement indicated. Presence or absence of the radiosensitizing mutation was determined by checking the UV sensitivity of the transductants.

Media. Cultures were grown in glucose minimal medium (Ganesan and Smith, 1968) supplemented with 0.5 μg thiamine hydrochloride/mℓ, and when required, with 2 μg thymine/mℓ, 1 μg biotin/mℓ and 10^{-3} *M* amino acids. Survival and reversion were assayed both on minimal plates lacking one required amino acid, and on such plates supplemented with 4% (by wt.) Difco nutrient broth (ΔNB plates).   *0.02%*

Procedure. An overnight culture was diluted 50-fold into fresh medium, and grown two to three generations to a density of ~2 × 10^8 cells/mℓ. The cells were centrifuged and resuspended in 0.067 *M* phosphate buffer at pH 7, and UV irradiated. Samples were concentrated 1-, 10-, 100-fold (depending on UV sensitivity and mutability of a particular
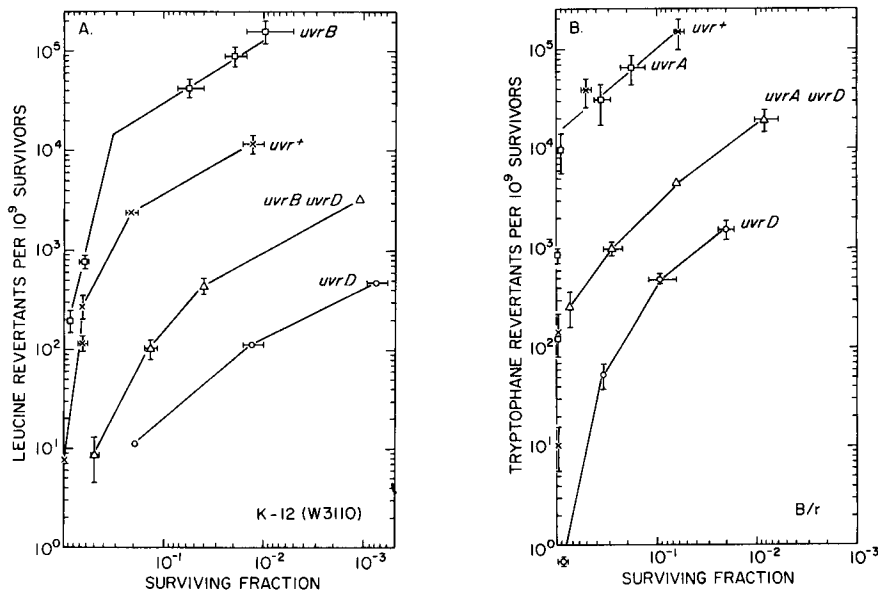

Figure 1. Auxotrophic reversion frequencies of strains of *E. coli* plotted as a function of surviving fraction. (A) Derivatives of *E. coli* K-12 (W3110) and (B) *E. coli* B/r. The points represent the average of at least three experiments. The associated standard deviations for the average mutation frequency and surviving fraction are indicated when greater than the breadth of the symbol. Although not immediately clear from this plot, the data in Fig. 1B demonstrate that, at survival levels *greater* then ~80%, the *uvrA* derivative of *E. coli* B/r exhibits a much higher mutation frequency than the parent strain. The data along the ordinate line are, from bottom to top, for 1.0 and 4.0 J m^{-2} for *uvr$^+$*, and 0.3, 0.5 and 1.0 J m^{-2} for *uvrA*. The mutation frequencies for these two strains are about the same, however, at survival levels *less* than ~80%.

Table 2. Comparison of fluence and mutation frequency at the 10% survival levels for strains of *E. coli*

B/r Derivatives	Wild-type	*uvrD*	*uvrA*	*uvrA uvrD*
UV fluence (J m^{-2})	32	6.4	3.3	0.8
Tryptophane revertants per 10^7 survivors	1000	4	1000	27
K-12 (W3110) Derivatives	Wild-type	*uvrD*	*uvrB*	*uvrB uvrD*
UV fluence (J m^{-2})	60	3.4	3.8	0.7
Leucine revertants per 10^7 survivors	38	0.2	300	1.6

strain), and were then plated either directly, to assay for revertants, or after dilution, to assay for viability. Plates were counted after incubating at 37°C for 2 days.

Irradiation. UV irradiation was performed with an 8-W General Electric germicidal lamp emitting primarily at 254 nm, exposing a 10-m𝓁 sample of ~2 × 10^8 cells/m𝓁 in an open 90 mm glass petri dish on a rotary shaker. The fluence rate was ~10 J m^{-2} s^{-1} and was checked before each experiment with a germicidal photometer (International Light, Inc., No. IL-254). All manipulations were carried out under General Electric 'gold' fluorescent lights to prevent photoreactivation.

Quantitation of mutation induction. The induced mutant frequency per survivor was calculated according to the formula of Sedgwick and Bridges (1972), which corrects for two types of spontaneous mutations, those preexisting in the population and those developing during growth on ΔNB plates.

RESULTS

The mutation frequencies of the *uvrD* derivatives and their parent strains were compared at equal survival values where the numbers of lethal mistakes in repair are probably the same (Fig. 1). The *E. coli* B/r strains were more mutable than the *E. coli* K-12 strains. The *uvrB* strains showed a greater mutation frequency relative to that of the wild-type strain of *E. coli* K-12 at all survival levels tested. In the B/r background, however, the *uvrA* derivative only showed a higher mutation frequency compared to its parent wild-type strain at survival levels greater than about 80%. This result could either be due to genetic differences between the B/r and K-12 strains, or to a difference in the effects of *uvrA* and *uvrB* mutations. What is more important in our present considerations, however, is that the introduction of *uvrD* into wild-type or excision deficient strains appears to substantially reduce the mutation frequencies.

Comparing the K-12 derivatives at the 10% survival level (Fig. 1A), the mutation frequency was reduced ~200-fold by introducing *uvrD* in both the *uvrB* and wild-type strains while the fluence reduction factor to yield equivalent survival was 5- and 18-fold, respectively. These data are summarized in Table 2. In the B/r background (Fig. 1B), again at the 10% survival level, the reduction in mutation frequency was 35- to 250-fold, respectively, while the reduction in fluence to yield equivalent survival was ~5-fold for both strains (Table 2). Thus, it seems unlikely that these large differences in mutation frequencies can be accounted for solely by differences in UV fluence, and initial amounts of damage.

In Fig. 2, auxotrophic reversion frequencies are plotted as a function of UV fluence for isogenic derivatives of *E. coli* K-12(W3110) (Fig. 2A) and *E. coli* B/r (Fig. 2B). Using this type of comparison, there appears to be at least a 100-fold increase in revertants in the *uvrA* and *uvrB* dervatives compared to their respective wild-type parent, as reported previously (see Witkin, 1967). On the other hand, the addition of *uvrD* to either an excision repair proficient or deficient strain appears to have no effect on revertant yield. This confirms the observation of Miura and Tomizawa (1968) for a *uvrD* strain, and adds data for the *uvrD uvrA* and *uvrD uvrB* strains.

Thus, the same data plotted in two different ways lead to different conclusions concerning the role of the *uvrD* gene in mutagenesis. Arguments to resolve this dichotomy are presented in the Discussion.

DISCUSSION

A classic problem in photobiology is—what is the more appropriate basis for comparing the biochemical or physiological responses to radiation of two cell populations that show a marked difference in sensitivity to killing by radiation? Does one compare the two cell populations at equal fluence or at equal survival? This same problem exists when comparing mutation frequencies.

If the mutation frequencies for *uvrD* derivatives and their parent strains are compared as a function of UV fluence (Fig. 2) then one would conclude, as did Miura and Tomizawa (1968), that *uvrD* gene function is not required for mutation induction. However, if mutation frequencies are analyzed at equivalent survival levels (Fig. 1), one finds that the numbers of revertants are decreased substantially in the *uvrD* strains. Both forms of analysis are informative, yet, when they lead to conflicting conclusions, one must determine which form of comparison is more relevant.

In the absence of independent information suggesting which type of plot is most appropriate, one must analyze the meaning of the two types of plots. At the same fluence, the same amount and type of radiation-induced damage should be produced in the sensitive and resistant cell populations (Setlow *et al.*, 1963). However, at the same fluence these two cell populations will show markedly different survival levels, DNA synthesis kinetics and growth and division delays (Smith, 1969). Since the sensitive and re-
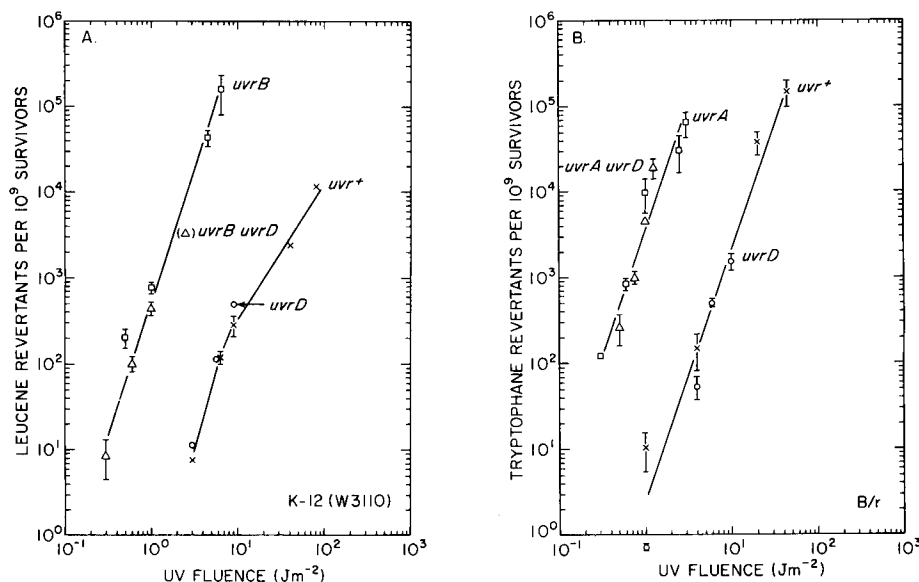
Figure 2. Auxotrophic reversion frequencies of strains of *E. coli* plotted as a function of UV fluence. (A) Derivatives of K-12 (W3110). (B) Derivatives of B/r. Except for (△) in part A which was calculated from only one experiment, the points represent the average of at least three experiments. The associated standard deviations from the average mutation frequency are indicated when greater than the breadth of the symbol. These data are taken from the experiments shown in Fig. 1.

sistant cell populations differ genetically in their ability to repair damaged DNA, *they will undergo different amounts and types of repair after exposure to the same UV fluence*. Thus, it seems inappropriate to compare, at equal fluences, the mutation frequencies of cell populations that have markedly different sensitivities to killing by radiation. This is an acceptable way of comparing mutation data, however, when there is no lethality from the radiation.

DNA repair is a complex interaction of multiple pathways of excision repair (Youngs et al., 1974) and postreplication repair (Youngs and Smith, 1976), some of which appear to be error prone and some to be error free (see, e.g. Witkin, 1974). Therefore, if the mutation frequency of the wild-type strain is the result of the interaction of all of these pathways, it is difficult to understand how a strain deficient in a major pathway of repair [e.g. *uvrD* or *polA* (see, Clarke and Shankel, 1975)] could possibly have the same mutation frequency as the wild-type strain. A method of expressing data on mutation frequencies that generally yields this conclusion (i.e. at equal UV fluence) would, therefore, seem to be suspect.

At the same survival level, even though exposed to markedly different UV fluences, the DNA synthesis kinetics (Smith, 1969; Rude and Doudney, 1973) and growth and division delay (Smith, 1969; and unpublished observations) are the same for sensitive and resistant cell populations. Since the survival is the same, one may safely assume that the same number of *lethal mistakes* in repair have been made in the two cell populations. Unfortunately, this does not necessarily mean that the same total number of repair events has occured.

Because UV-induced mutagenesis does not appear to occur in the absence of DNA repair (see Introduction), how cells manipulate their radiation-induced damage seems relatively more important to mutagenesis than does the initial yield of damage. Since the survival of two strains is also primarily dependent upon their relative abilities to repair radiation-induced DNA damage, it seems more appropriate to plot mutation frequencies as a function of survival.

Although the arguments presented above favor the comparison of mutation frequencies at equal survival, there is independent information to substantiate this conclusion. On the basis of biochemical and genetic studies, it has been observed that the *uvrD* gene product participates in both excision and postreplication repair pathways that appear to be mutagenic (see Introduction). This being the case, then the direct measurement of mutation frequencies in *uvrD* strains should reflect the predictions of these independent data. Only when the mutation frequencies are compared at equal survival does this correlation exist.

When the present results on relative mutation efficients are analyzed at equal survival, they show (Fig. 1) that the wild-type *E. coli* B/r strain exhibits a much higher mutation frequency than the wild-type K-12 strain, as previously observed by Zampieri and Greenberg (1965) and by Sedgwick and Bridges (1972). The present results also indicate that a *uvrB* mutation in the K-12(W3110) background increased the mutation frequency markedly at all survival levels tested. The *uvrA* mutation in the B/r background, however, only increased the mutation frequency at fluences yielding less than about 10–20% killing (compare with Hill, 1965; Witkin, 1966). These data do

not permit us to say whether the differences in the results for the uvrA and uvrB mutations are characteristic for these two loci or are more a reflection of other genetic differences in the two strains (i.e. B/r vs K-12). It would be of interest to determine the molecular events (e.g. changes in the patterns of repair) that occur in E. coli B/r uvrA and/or E. coli B/r wild-type that produce this dramatic change in the relative mutation frequency as a function of survival. In the uvrD background, however, both the uvrA and the uvrB mutations increased the efficiency of mutagenesis to about the same extent whether in the K-12 or the B/r background.

The addition of a uvrD mutation to either a wild-type strain or to a uvrA or uvrB strain in the B/r or K-12 backgrounds, respectively, decreased the mutation frequencies of these strains (Fig. 1). Furthermore, these differences in mutation frequency are much greater than can be directly accounted for by the differences in the initial yield of radiation-induced damage (Table 2).

Three kinds of repair are relevant to survival and mutagenesis: (1) error free repair produces no lethality and no mutations; (2) error prone repair produces both lethal and non-lethal mutations; and (3) unsuccessful repair causes lethality and produces no mutations. Mutagenesis is due only to error prone repair, while survival depends upon the relative amounts of the three kinds of repair, which must certainly differ for each cell type. Thus, even the comparison of mutation frequencies at equal survival is not without problems, but it still appears superior to a comparison at equal fluences when survival is markedly different. What would be ideal, of course, is to be able to measure the total number of repair events performed by a surviving cell. Then, the true mutation efficiency would be the number of mutations produced per number of repair events. Unfortunately the latter number is presently unobtainable. The parameter of survival, however, at least encompasses repair efficiency, albeit to an undetermined degree that probably differs for each strain.

It is hoped that this paper may stimulate a renewed dialogue among workers in mutation research in order that a more general agreement can be reached concerning the most appropriate way to handle data on mutation frequencies. Such agreement is especially necessary when comparing several strains having markedly different survival characteristics in response to a given mutagen, and even for the same strain when comparing the efficiencies of several mutagens that have markedly different lethal effects on the given strain.

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