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Genetic Responses of the Thermophilic Archaeon *Sulfolobus acidocaldarius* to Short-Wavelength UV Light

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The archaea which populate geothermal environments are adapted to conditions that should greatly destabilize the primary structure of DNA, yet the basic biological aspects of DNA damage and repair remain unexplored for this group of prokaryotes. We used auxotrophic mutants of the extremely thermoacidophilic archaeon *Sulfolobus acidocaldarius* to assess genetic and physiological effects of a well-characterized DNA-damaging agent, short-wavelength UV light. Simple genetic assays enabled quantitative dose-response relationships to be determined and correlated for survival, phenotypic reversion, and the formation of genetic recombinants. Dose-response relationships were also determined for survival and phenotypic reversion of the corresponding *Escherichia coli* auxotrophs with the same equipment and procedures. The results showed *S. acidocaldarius* to be about twice as UV sensitive as *E. coli* and to be equally UV mutable on a surviving-cell basis. Furthermore, UV irradiation significantly increased the frequency of recombinants recovered from genetic-exchange assays of *S. acidocaldarius*. The observed UV effects were due to the short-wavelength (i.e., UV-C) portion of the spectrum and were effectively reversed by subsequent illumination of *S. acidocaldarius* cells with visible light (photoreactivation). Thus, the observed responses are probably initiated by the formation of pyrimidine dimers in the *S. acidocaldarius* chromosome. To our knowledge, these results provide the first evidence of error-prone DNA repair and genetic recombination induced by DNA damage in an archaeon from geothermal habitats.

Although prokaryotes represent the smallest and simplest cellular organisms, they typically have multiple and biochemically diverse mechanisms to repair damaged DNA. This can be rationalized in two ways. First of all, prokaryotes have simple haploid genomes with little or no functional redundancy. As a result, just one persistent (i.e., unrepaired) DNA lesion can be deleterious, if not lethal (13). Efficient detection and repair of DNA lesions should thus be critical for evolutionary fitness in prokaryotes; furthermore, multiple, independent repair mechanisms should be functionally superior to any single mode of repair. Second, DNA damage takes a variety of forms. Environmental agents such as UV radiation (3, 10), certain antibiotics produced by other microorganisms (24), desiccation (23), and heat (20–22) each produces its own characteristic type of damage in DNA. In addition, living cells, particularly those of respiring aerobes, produce endogenous activated species that cause yet other deleterious DNA modifications (20). Finally, DNA decomposes spontaneously at genetically significant rates, even under mild conditions (20, 21). Prokaryotic DNA is thus normally subjected to a variety of chemical alterations, and each of these alterations must be detected and repaired to ensure genetically faithful propagation of the species.

Prokaryotic mechanisms of DNA repair have been identified and studied in detail for a select few bacteria that are amenable to genetic analysis. In particular, several DNA repair systems of *Escherichia coli* have been studied intensively. Some of these repair only a particular form of DNA damage and do so by direct reversal. For example, photoreactivation repairs only cyclobutane pyrimidine dimers (3), whereas the adaptive response removes only alkyl groups from particular *O*-methyl or *O*-ethyl guanine or thymine adducts (34). In contrast, other systems appear to be general; both nucleotide excision repair and recombination (postreplication) repair, for example, can eliminate or circumvent the lesions described above as well as a variety of others, although prior processing by specialized enzymes (e.g., *N*-glycosylases) may be required for excision repair in some cases (11, 27).

As would be expected, study of bacteria only distantly related to *E. coli* has revealed a greater functional diversity of prokaryotic DNA repair. For example, *Deinococcus radiodurans* is a gram-positive, mesophilic bacterium related to the gram-negative extreme thermophiles of the genus *Thermus* and exhibits by far the highest resistance to UV and ionizing radiation known for any organism (23). Recent studies confirm that this bacterium has an extremely efficient mechanism to repair damaged DNA, due to the operation of some repair systems homologous to those of *E. coli* and others that appear fundamentally novel. *D. radiodurans* has at least two independent nucleotide excision pathways, only one of which appears homologous to the Uvr pathway of *E. coli. D. radiodurans* also has an extremely active general recombinase, which enables the repair of over 100 double-strand breaks per chromosomal equivalent; production of this RecA homolog is toxic to *E. coli* (23).

The domain *Archaea* (36) comprises prokaryotic lineages even more divergent from *E. coli* than *D. radiodurans* and includes microorganisms specifically adapted to extreme environments. In particular, the domain *Archaea* includes the great majority of prokaryotes isolated from geothermal habitats and the most-thermophilic organisms known (33). The extremely high temperatures required for optimal growth of archaea isolated from geothermal habitats (above 75°C in most cases and above 100°C in several [33]) greatly accelerate the spontaneous decomposition of DNA (20–22), which suggests a constitutive need for efficient DNA repair in these microorganisms. Gene

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TABLE 1. Strains used in the present study

Designation	Genotype	Source or derivation
S. acidocaldarius		
DG29	*pyrD1*	Replica-plating (9)
DG38	*cbp-2*	Replica-plating (9)
DG64	*pyrB4*	Replica-plating (9)
E. coli		
GI209	(HfrH?) *zjd*::Tn*10 pyrB289 relA1 spoT1* (*hsdR2?*)	This work[a]
KL185	F$^-$ *pyrD34 trp-45 his-68 thi-1 galK35 malA1 xyl-7 mtl-2 rpsL118*	K. B. Low via CGSC[b]
RC50	F$^-$ *carA50 tsx-273 rpsL135 malT1 xylA7 thi-1*	R. Lavalle via CGSC[b]

[a] P1(DF1062) (30) was used to transduce YA289 (1) to Tet^r and Pyr$^-$. The HfrH and *hsdR2* markers, which map in the region of *zjd*::Tn*10*, have not been scored in GI209.

[b] CGSC, Coli Genetic Stock Center.

sequences (4, 12, 18) and biochemical activities (12, 17) related to those of known bacterial DNA repair mechanisms have been reported for thermophilic archaea. However, the functional significance for DNA repair of novel nucleotide sequences or biochemical activities of thermophilic archaea promises to be difficult to directly demonstrate in the absence of biological assays. In particular, the development of quantitative assays for the genetic and physiological consequences of DNA damage in vivo is presently needed to provide the necessary foundation for functional studies of DNA repair at extremely high temperatures in a thermophilic archaeon.

To our knowledge, genetic manipulation of archaea from geothermal habitats has been reported only for the genus *Sulfolobus* (6, 7, 9, 28, 37), reflecting the relative responsiveness of *Sulfolobus* spp. to routine laboratory manipulation, compared to that of other thermophilic archaea. *Sulfolobus acidocaldarius* forms colonies from single cells on a variety of solid media (5) and has yielded several useful classes of mutants, including those which can be used for quantitative assays of forward and reverse mutation (6, 14) and of spontaneous genetic exchange between cells (7). To provide an initial characterization of basic, functional aspects of DNA damage and repair in a thermophilic archaeon, we took advantage of the available *S. acidocaldarius* mutants to develop assays of cell survival, mutation, and formation of genetic recombinants in suspensions of *S. acidocaldarius* cells irradiated with short-wavelength UV light. The quantitative relationships between each of these three responses and UV dose provide some of the first data relating basic cellular and genetic phenomena of an archaeon from geothermal environments to externally induced DNA damage.

MATERIALS AND METHODS

Strains and growth conditions. The *S. acidocaldarius* and *E. coli* strains used in the present study are all pyrimidine auxotrophs, as summarized in Table 1. Pyrimidine auxotrophs of *S. acidocaldarius* were chosen because their mutations have been characterized previously and provide selection for a prototrophic phenotype even on media heavily supplemented with amino acids, which facilitates selection. Corresponding pyrimidine auxotrophs of *E. coli* K-12 were those provided by the Coli Genetic Stock Center (Yale University, New Haven, Conn.) or strains derived from them (Table 1).

Each culture was grown from an isolated colony. *S. acidocaldarius* cultures were aerated at 80°C in mineral base (6) supplemented with 2 g of D-xylose, 1 g of NZAmine AS (Sigma), and 0.02 g of uracil, each per liter (XTura). *E. coli* cultures were aerated at 37°C in 5 g of yeast extract, 8 g of NZAmine AS, and 2.5 g of NaCl, each per liter of distilled water (YT medium). Growing cells of either species were harvested by centrifugation, resuspended in sterile, UV-transparent buffer, pelleted, and resuspended in fresh buffer to yield an optical density at 600 nm of approximately 0.2 (for *S. acidocaldarius*, this corresponds to about 1.5×10^8 cells/ml). Sdil buffer (6) was used for *S. acidocaldarius* cells, whereas SM buffer (26) was used for *E. coli*. UV spectra of both buffers were determined for a 1-cm path length; both spectra showed >90% transmission relative to distilled water at wavelengths of 250 nm and above.

UV irradiation. Cell suspensions (<5-ml volume) were transferred to 10-cm-diameter petri dishes, yielding an average depth of less than about 1 mm. The following operations were performed under dim red light.

Suspensions were gently agitated at a distance of 45 cm below a horizontal, 15-W germicidal lamp and reflector assembly. This device emits 70% of its light output at wavelengths shorter than about 300 nm, yielding a short-wavelength UV intensity of 3.5 W/m^2 at the surface of the cell suspension (8). Unless otherwise noted, all UV fluences (i.e., doses) refer to this short-wavelength (<300-nm) component of the lamp output.

Genetic assays. Samples of irradiated suspensions were withdrawn at appropriate intervals, transferred to 1.5-ml polypropylene centrifuge tubes, and stored and processed under dim red light. One aliquot of each sample was assayed for viability (see below), whereas two other aliquots (*S. acidocaldarius* suspensions only) were combined with similarly irradiated suspensions of the other two auxotrophs for assays of genetic exchange and recombination. The remaining cells were concentrated by centrifugation and plated to select phenotypic revertants. The viability of *S. acidocaldarius* was determined on solid XTura medium after 6 days of incubation at 75°C, whereas the viability of *E. coli* was determined after 1 day of incubation at 37°C. Pyr$^+$ recombinants or revertants of *S. acidocaldarius* auxotrophs were selected by spreading on solid minimal medium containing 2 g of D-xylose, 1 g of L-glutamine, and 1 g of acid-hydrolyzed casein, each per liter, whereas *E. coli* revertants were selected on M9 medium containing 4 g of D-glucose, 1 g of acid-hydrolyzed casein, and 0.05 g of any additional required amino acids, each per liter. Unless noted, all revertant and recombinant colony counts were normalized by the viable count from the same sample to yield the corresponding frequencies.

Conditions for detecting exchange and recombination of chromosomal markers were modified from those of a prior study (7). Aliquots of two cell suspensions were combined in a 13-mm-diameter glass culture tube containing 0.5 ml of solid, nonnutrient mating medium. The tubes containing these liquid overlays were then sealed inside a humid container and incubated without agitation for 20 h at 75°C. The cells were then resuspended and diluted by adding an additional 1.0 ml of Sdil buffer to each tube and agitating. Aliquots of the resulting dilute suspensions were plated on selective medium as described above. This modified procedure is more convenient and reproducible than that described previously (7). It also yielded higher frequencies of exchange and recombination; the basis of this latter observation remains to be established. Control experiments demonstrated that this mating assay itself does not select or enrich for Pyr$^+$ recombinants; mating tubes supplemented with 20 μg of uracil per ml yielded the same or higher ratios of Pyr$^+$ colonies to CFU plated as did unsupplemented tubes.

Tests for photoreversal of UV-induced responses. In some experiments, duplicate samples of UV-irradiated cells were withdrawn at the appropriate times; one sample was processed normally (i.e., under dim red light), whereas the duplicate (in a colorless polypropylene microcentrifuge tube) was illuminated for 2 h at about 13 W/m^2 in a foil-lined tray by a bank of fluorescent lamps. According to a previous study, this dose of visible light gives the maximal photoreactivation possible under these conditions, corresponding to a reversal of about 80% of the lethal lesions induced by UV (8).

RESULTS

UV sensitivity of *S. acidocaldarius*. Figure 1 shows the observed viability of *S. acidocaldarius* cells as a function of UV dose (i.e., fluence). Each of several independent trials evaluated three pyrimidine auxotrophs in parallel; each strain yielded a similar, triphasic survival curve. Low fluences caused relatively little inactivation, resulting in a short but distinct shoulder. At doses of 35 to 140 J/m^2, viability decreased exponentially and at a similar rate in all strains. At higher doses, the rate of killing slowed, particularly in strains DG29 and DG64. These features are commonly (though not universally) observed in survival curves of microorganisms (10). Under these same conditions, three corresponding *E. coli* auxotrophs gave more uniformly exponential kinetics and generally required higher UV doses to exhibit the same viability loss as *S. acidocaldarius*. These experiments covered a total viability loss of 5 to 6 orders of magnitude (Fig. 1).

UV mutability of *S. acidocaldarius*. The frequency of phenotypic reversion was determined concurrently with viability for both *S. acidocaldarius* and *E. coli* suspensions (Fig. 2). *S. acid-*

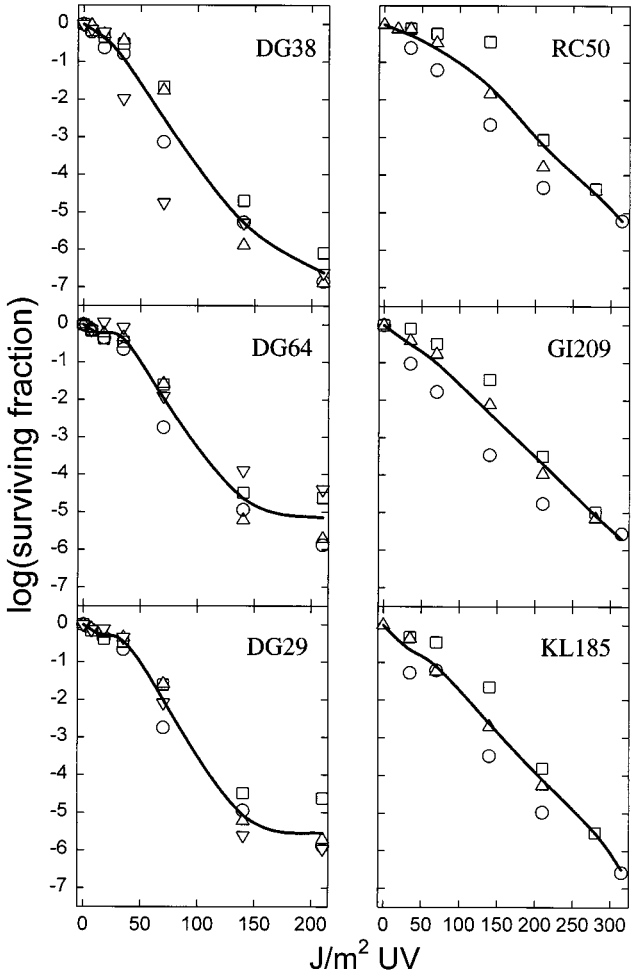

FIG. 1. Survival as a function of UV fluence. The viability of *S. acidocaldarius* (left panels) and *E. coli* (right panels) was determined by colony formation on nonselective media (see Materials and Methods). The results of independent determinations (different symbols) were averaged, and the mean values were fit by the solid lines shown. Note the different *x*-axis scales for *S. acidocaldarius* and *E. coli*. Upper two panels, *cbp* (=*car*) mutants; middle two panels, *pyrB* mutants; lower two panels, *pyrD* mutants (see Table 1 for summary of strains).

ocaldarius showed higher trial-to-trial variation in this assay, perhaps because of its greater sensitivity to the killing action of UV. On average, however, all six auxotrophic *S. acidocaldarius* and *E. coli* strains gave comparable increases in the proportion of phenotypic revertants among surviving cells as a function of UV fluence (Fig. 2).

Stimulation of genetic exchange and recombination. *S. acidocaldarius* cells appear intrinsically able to exchange and recombine regions of their chromosome, as indicated by the production of large numbers of prototrophs from mixtures of otherwise-stable auxotrophs (7). The process appears to be efficient even when the two strains are derived from a common parent (7); as a result, we were able to assay mating efficiencies among the same cell suspensions used for the viability and reversion assays described above. Figure 3 shows frequencies of Pyr^+ recombinants from three pairwise combinations of three pyrimidine auxotrophs as a function of UV fluence. Under the mating conditions employed (see Materials and Methods), unirradiated cell suspensions yielded at least 10^{-4} recombinants per CFU mated. Short-wavelength UV induced dramatic increases in recombinant frequency (Fig. 3). Under all conditions, recombinant frequencies were 2 to 4 orders of magnitude higher than the corresponding revertant frequencies (compare Fig. 2 and 3). As judged by the ratios of Pyr^+ to total CFU, a significant proportion (e.g., 10%) of survivors yielded genetic recombinants following high doses of UV (Fig. 3).

Table 2 provides quantitative summaries of the dose-response relationships depicted in Fig. 1 to 3. When expressed as first-order constants, all the biological responses measured in both *E. coli* and *S. acidocaldarius* were of comparable magnitudes. The largest consistent difference was observed with respect to killing: UV was twice as effective in killing *S. acidocaldarius* as it was in killing *E. coli* (Table 2). UV induced mutation (phenotypic reversion) with about equal efficiencies in *E. coli* and *S. acidocaldarius*, however (Table 2).

With respect to *S. acidocaldarius*, responses to UV light decreased in the order cell inactivation > recombination > reversion, but the observed differences were not large in view of the overall variation among individual measurements. When the lamp output was filtered through a 3-mm-thick glass plate, *S. acidocaldarius* exhibited none of the responses described

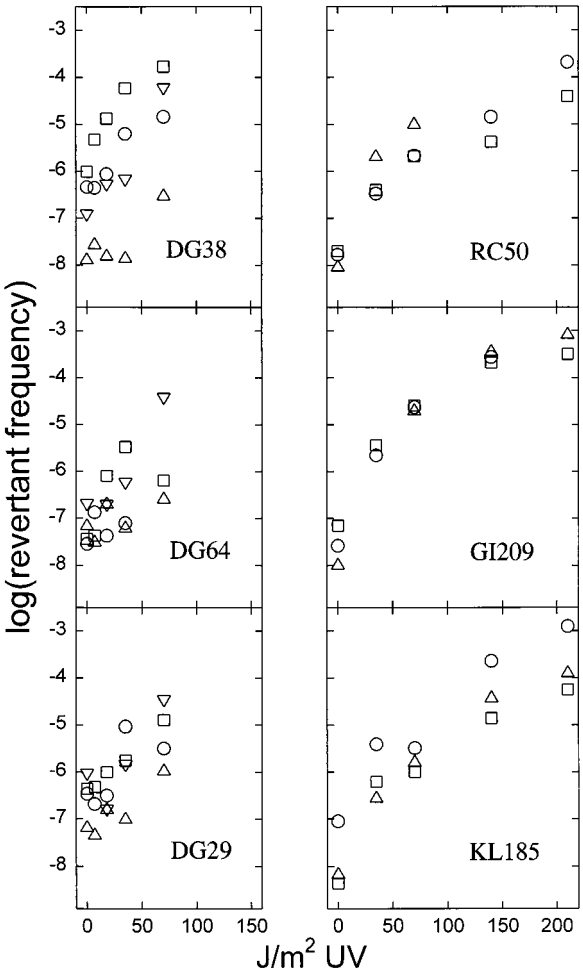

FIG. 2. Formation of phenotypic revertants. The number of Pyr^+ revertants of *S. acidocaldarius* (left panels) or *E. coli* (right panels) was determined by colony formation on selective media and normalized by the corresponding viable titer to yield the revertant frequencies shown. Each symbol shape represents an independent trial.

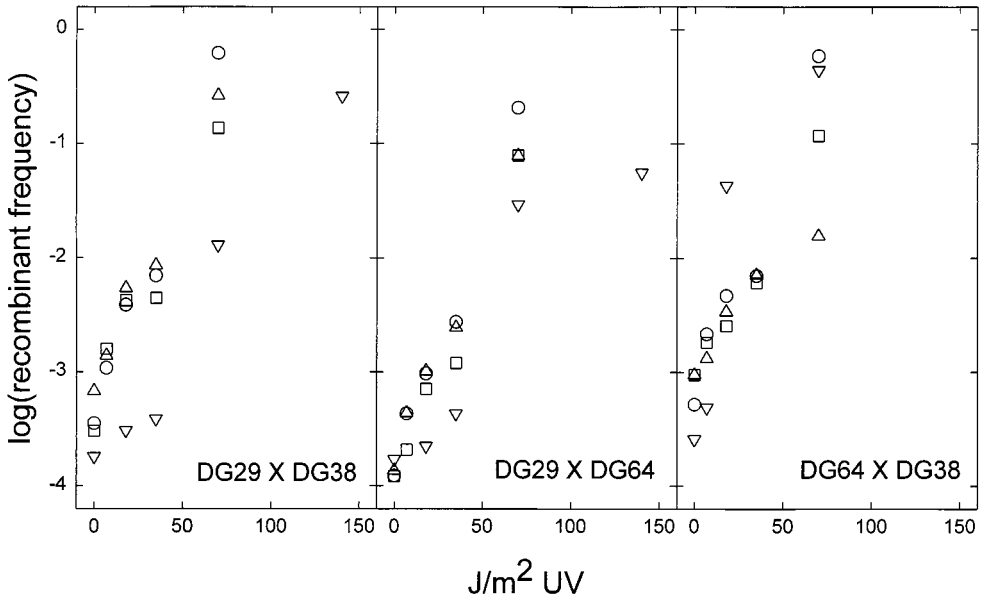

FIG. 3. Formation of genetic recombinants. All pairwise combinations of three *S. acidocaldarius* auxotrophs irradiated by a given dose of UV were mated, and the frequency of prototrophic recombinants per CFU mated was determined as described in Materials and Methods. Each symbol shape represents an independent trial.

above (Table 2). This confirms that the killing, mutagenic, and recombinogenic effects of UV observed in the present study for *S. acidocaldarius* are due to wavelengths shorter than about 300 nm; 97% of this short-wavelength portion of germicidal lamp emission normally occurs at 254 nm (10).

Reversibility of UV effects. *S. acidocaldarius* is efficiently photoreactivated at 24°C or higher temperatures following UV irradiation, which suggests specific photoenzymatic repair of pyrimidine dimers in the *S. acidocaldarius* chromosome (8). We therefore investigated the ability of subsequent illumination by visible light to reverse the genetic effects of UV in *S. acidocaldarius* cells. For this purpose, duplicate samples of UV-irradiated cells were withdrawn; one was stored in the dark, the other was illuminated for 2 h, and then both were processed normally (under dim red light) to assay viability, phenotypic reversion, and formation of recombinants. Table 3 shows the relative viability, revertant frequency, and recombinant frequency at several UV doses for control and photoreactivated suspensions. All three effects of UV were reversed by visible light. Although the magnitude of the effects, and thus their reversal, was modest at low fluences, at high fluence (where survival, reversion, and recombinogenic responses were all approximately exponential with dose), photoreactivation attenuated all three effects by similar factors (13×, 15×, and 15×, respectively) (Table 3). The inability of photoreactivation to completely reverse the effects of high fluence (e.g., 70 J/m^2) is consistent with prior indications that photoreactivation can repair only about 80% of the lethal UV damage to *S. acidocaldarius* cells under these conditions (8).

In other organisms, photoreactivation is only known to reverse those DNA lesions formed by UV light, and the available data regarding *S. acidocaldarius* are consistent with this generalization. In one set of experiments, for example, we treated strains DG29, DG38, and DG64 with 10 mM H_2O_2 for 25 min at 37°C, which yielded less than about 0.1% survival (comparable to the survival following 70 J/m^2 UV). Prompt removal of H_2O_2 followed by 2 h of illumination showed no recovery of viability in any of several trials. Thus, according to our data,

TABLE 2. Efficiencies of UV in eliciting responses

Strain or combination	Coefficient[a] for:		
	Mean viability ± SD (filtered[b])	Mean reversion ± SD (filtered[b])	Mean recombination ± SD (filtered[b])
DG38	−0.041[c] ± 0.010 (-1.5×10^{-4})	0.028 ± 0.009 (3.5×10^{-4})	
DG64	−0.040[c] ± 0.005 (-2×10^{-4})	0.017 ± 0.013 (1.9×10^{-3})	
DG29	−0.044[c] ± 0.006 (-5×10^{-5})	0.021 ± 0.004 (3×10^{-3})	
RC50	−0.021 ± 0.004 (ND)	0.025 ± 0.016 (ND)	
GI209	−0.020 ± 0.004 (ND)	0.021 ± 0.004 (ND)	
KL185	−0.023 ± 0.002 (ND)	0.018 ± 0.001 (ND)	
DG38 × DG64			0.033 ± 0.012 (2×10^{-4})
DG64 × DG29			0.035 ± 0.010 ($<1 \times 10^{-5}$)
DG29 × DG38			0.034 ± 0.008 (1×10^{-4})

[a] To facilitate comparison, each trial of Fig. 1 to 3 was fitted to an exponential function by least-squares approximation. The individual first-order rate constants for each trial were then averaged to give the means and standard deviations shown (values are $\log_{10}$ units per joule per square meter).
[b] Value when strain was irradiated for the corresponding times through a 3-mm-thick glass sheet. ND, not determined.
[c] Determined for the UV dose range of 35 to 140 J per m^2 (see Fig. 1).

TABLE 3. Reversal of UV effects by visible light[a]

UV dose (J/m^2)	Relative viability			Relative frequency of revertants			Relative frequency of recombinants		
	−PhR[b]	+PhR	+PhR/−PhR ratio	−PhR[b]	+PhR	+PhR/−PhR ratio	−PhR[b]	+PhR	+PhR/−PhR ratio
0	1	1	1	1	1	1	1	1	1
7	0.87	0.88	1.02	0.97	1.28	1.33	2.47	1.74	0.70
18	0.54	0.82	1.53	4.24	0.81	0.19	5.07	3.47	0.69
35	0.43	0.77	1.79	5.64	1.06	0.19	10.8	2.50	0.23
70	0.027	0.36	13.4	22.3	1.50	0.067	227	15.2	0.067

[a] Data are geometric means of the corresponding ratios from six independent assays (three pyrimidine auxotrophs, each assayed in two experiments on different days). −PhR, control suspension; +PhR, reactivated suspension.
[b] Includes data from trials summarized in Fig. 1 to 3.

visible light cannot be hypothesized to stimulate generalized DNA repair in *S. acidocaldarius.*

DISCUSSION

We have used stable auxotrophic mutants of *S. acidocaldarius* to quantify the genetic effects of a well-characterized DNA-damaging agent on an archaeon from geothermal environments. Short-wavelength UV is readily administered in precise doses, and its effects on DNA are well defined. It is also a biologically relevant DNA-damaging agent, as indicated by the widespread retention of UV-specific DNA repair mechanisms throughout evolution (10). Although several UV photoproducts of DNA have been identified, only two are known to accumulate in significant amounts in vegetative cells of prokaryotes, cyclobutane pyrimidine dimers and (6-4)pyrimidine-pyrimidone adducts (3, 10). Efficient photoreactivation of *S. acidocaldarius* at room temperature (8) suggests that one or both of these pyrimidine dimers mediates the observed lethal effects of UV, since they are the only UV photoproducts known to be repaired by DNA photolyases, including photolyases from archaea (3, 15, 27). To determine the genetic effects of UV photoproducts in the absence of this light-dependent repair, we used *S. acidocaldarius* strains that have been phenotypically, biochemically, and genetically characterized in previous studies (7, 9). Each strain has a single enzymatic defect in the de novo synthesis of UMP that has been identified and shown to cause the auxotrophic phenotype (9). Where feasible, we also compared the responses of the three *S. acidocaldarius* strains with those of corresponding *E. coli* mutants under these conditions.

To our knowledge, the present study provides the first quantitative assessment of genetic responses of an archaeon from geothermal habitats to externally induced DNA damage. The results reveal biological properties of *S. acidocaldarius* relevant for future studies of DNA damage and repair at extremely high temperature. The dose dependence of killing, measured over 5 orders of magnitude, suggests a significant capacity for dark repair of UV photoproducts in *S. acidocaldarius*. All strains exhibited a shoulder at low UV fluences and a modest rate of exponential decay (−0.042 log unit per J of UV/m^2) at higher fluences. For comparison, this rate was only twice that observed for the three dark-repair-proficient strains of *E. coli* included as controls in our study. This result contrasts with approximately 30-fold increases in killing rates caused by inactivating either of the two dark-repair systems of *E. coli*, the RecA pathway and the Uvr pathway, and the approximately 1,000-fold increase in *recA uvr* double mutants (13). The extreme UV sensitivity of the latter strains, which completely lack dark repair, demonstrate two important properties of short-wavelength UV relevant to all studies of its effect on prokaryotic cells: (i) one unrepaired UV photoproduct per chromosome can be lethal to a prokaryotic cell and (ii) the UV fluence required to form at least one photoproduct per prokaryotic chromosome is exceedingly small (13). In the absence of observed liquid holding recovery of *S. acidocaldarius* (8), the dose dependence of UV killing thus provides the first evidence in vivo that *S. acidocaldarius* has means other than photoreactivation for repairing (or perhaps preventing) UV-induced damage. The overall efficiency of repair appears to be about half that of the dark-repair systems of *E. coli.*

In phenotypic reversion assays of six different auxotrophic mutants, distributed between the two species, *S. acidocaldarius* exhibited a UV mutability similar to that of *E. coli* on a surviving-cell basis. This result indicates the operation of an error-prone form of DNA repair, which is consistent with recent identification of a *umuC*-homologous DNA sequence in the related archaeon, *Sulfolobus solfataricus* (18, 31). Though common among microorganisms, error-prone repair is not universal and is not integral to the prokaryotic DNA repair mechanisms which it accompanies. *D. radiodurans*, for example, has very effective repair of UV lesions but lacks an error-prone form of repair and is not mutated by even lethal doses of UV (23). Indeed, even the phylogenetically close relatives within the enteric bacteria differ by factors of up to 200 in their ability to be mutated by UV (29). *E. coli*, used here as a control strain, is the most UV-mutable species of this group (29), yet its error-prone repair makes little contribution in wild-type cells to the overall survival of UV radiation (32, 34). As a practical matter, it should be noted that the overall yield of UV-induced revertants was significantly lower for *S. acidocaldarius* than for *E. coli*, despite a similar incremental effect on the frequency of revertants among survivors. This reflects the offsetting losses in viability, which are higher in *S. acidocaldarius* than in *E. coli* for a given UV dose.

Finally, the observation that UV radiation greatly stimulates formation of *S. acidocaldarius* recombinants in a simple genetic assay seems significant in several respects. UV irradiation has long been known to stimulate genetic recombination (2, 19, 35), but the mechanism does not involve pyrimidine dimers per se. Instead, it is attributed to strand scission and to daughter-strand gaps; the former occurs as part of excision repair, whereas the latter are formed opposite the UV photoproduct upon subsequent DNA replication (16, 19, 25). Our results seem consistent with either or both of these mechanisms in *S. acidocaldarius*. We note, for example, that the dose-response relationships of UV-stimulated genetic recombination which we observed resemble those of published studies on generalized transduction in bacteria (35) and on mitotic recombination in yeast (19). Alternatively, UV may trigger cellular responses that otherwise increase the efficiency of conjugation, DNA transfer, or homologous recombination in *S. acidocaldarius.*

Establishing the mechanism of the observed stimulation by UV will therefore require more precise characterization of the genetic exchange process itself. The available data nevertheless discredit one possible explanation of the apparent recombinogenesis by UV, namely, that it is solely an artifact of selection. This possibility arises from the ability of homologous recombination to construct one functional DNA molecule from two which have multiple, random lesions. As a result of this intrinsic property, pairs of genomes that have engaged in recombination after UV exposure can have a greatly enhanced probability of yielding a survivor compared to their nonrecombining peers in the same population (10). This bias is difficult to exclude in genetic assays of recombination such as ours, since the frequency of inviable genetic recombinants is not measured. However, we note that low doses of UV, which killed few *S. acidocaldarius* cells, increased not only the frequency of recombinants but also their absolute numbers by factors of about five (Table 3). A purely selective mechanism of enriching for recombinational events is thus not consistent with all our results.

At present, therefore, we conclude that formation of pyrimidine dimers in the *S. acidocaldarius* chromosome initiates various molecular processes leading to cell death, mutation, or recombination, based on (i) the requirement for short-wavelength UV and the similar dose dependencies of these three responses, (ii) the similar attenuation of these responses by subsequent treatment with visible light, and (iii) the fact that the only demonstrated mechanism of photoreactivation in vivo is resolution of pyrimidine dimers in DNA. On a practical level, the magnitude and reproducibility of UV stimulation of genetic recombination suggests its utility as an assay of UV-induced DNA damage in *S. acidocaldarius*, despite the fact that its mechanism has not been defined. In a more general sense, the observed relationships among this and other UV-induced responses of *S. acidocaldarius* provide a beginning point for experimental studies of the functional aspects of damage, repair, mutation, transfer, and recombination of DNA in archaeal cells at extremely high temperature.

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