# UV Stimulation of Chromosomal Marker Exchange in *Sulfolobus acidocaldarius*: Implications for DNA Repair, Conjugation and Homologous Recombination at Extremely High Temperatures

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ABSTRACT

The hyperthermophilic archaeon *Sulfolobus acidocaldarius* exchanges and recombines chromosomal markers by a conjugational mechanism, and the overall yield of recombinants is greatly increased by previous exposure to UV light. This stimulation was studied in an effort to clarify its mechanism and that of marker exchange itself. A variety of experiments failed to identify a significant effect of UV irradiation on the frequency of cell pairing, indicating that subsequent steps are primarily affected, *i.e.*, transfer of DNA between cells or homologous recombination. The UV-induced stimulation decayed rather quickly in parental cells during preincubation at 75°, and the rate of decay depended on the incubation temperature. Preincubation at 75° decreased the yield of recombinants neither from unirradiated parental cells nor from parental suspensions subsequently irradiated. We interpret these results as evidence that marker exchange is stimulated by recombinogenic DNA lesions formed as intermediates in the process of repairing UV photoproducts in the *S. acidocaldarius* chromosome.

PHYLOGENETIC analysis of small-subunit rRNA sequences has shown that a number of physiologically diverse prokaryotic lineages constitute a monophyletic, prokaryotic sister taxon to all eukaryotes; this taxon has been termed the domain Archaea (Woese *et al.* 1990). In support of this evolutionary relatedness to eukaryotes, archaea exhibit various molecular features previously considered uniquely eukaryotic, including incorporation of hypusine into a homologue of elongation factor 5A (Bartig *et al.* 1992), a PolII-type promoter structure (Reiter *et al.* 1990), complex RNA polymerase structure (Pühler *et al.* 1989), and functional TATA-binding protein and TFIIB transcription factors (Qureshi *et al.* 1997). Furthermore, key structural and functional aspects of several eukaryotic cell components [*e.g.*, the 26S proteasome, the TriC (TCP1) chaperonin, and the SPO11 meiotic nuclease] have been elucidated in recent years through analysis of the corresponding archaeal homologue (Sternlicht *et al.* 1993; Löwe *et al.* 1995; Bergerat *et al.* 1997).

In addition to their relevance for understanding the evolutionary origin and functional aspects of the eukaryotic nucleus and cytoplasm, many archaea provide striking examples of cellular function under environmental stress. For example, the most thermophilic organism known, *Pyrolobus fumarii*, is an anaerobic archaeon isolated from a deep-sea hydrothermal vent that grows optimally at 106° and can grow in temperatures as high as 113° (Blöchl *et al.* 1997). Indeed, nearly all "hyperthermophiles," *i.e.*, microorganisms that require temperatures at or >80° for optimal growth, are archaea isolated from geothermal habitats (Stetter 1996). The fact that high temperatures destabilize the primary structure of DNA focuses interest on the molecular mechanisms by which archaea from geothermal habitats minimize and repair DNA damage and on the efficacy of these mechanisms *in vivo* (Grogan 1998). Complete genomic sequences of hyperthermophilic archaea have revealed relatively few open reading frames that are similar to known DNA repair genes; furthermore, the few gene assignments that have been made on this basis generally have not been confirmed experimentally (Bult *et al.* 1996; Klenk *et al.* 1997). Indeed, provisional identification of the *Methanococcus jannaschii mutT* gene, based on sequence similarity to the 8-oxo-dGTP hydrolase gene of *Escherichia coli*, has been experimentally disproven (Sheikh *et al.* 1998). This latter result underscores the fact that functional relevance for DNA stability or repair must ultimately be demonstrated *in vivo*, which, in turn, implies a requirement for genetic assays in the organism itself.

*Sulfolobus acidocaldarius* is an aerobic archaeon that grows optimally at ~80° and pH 3 and has yielded a variety of analogue-resistant and auxotrophic mutants by classical isolation methods (Grogan 1991, 1995). In particular, 5-fluoro-orotic acid (FOA) efficiently selects pyrimidine auxotrophs of *S. acidocaldarius* (Grogan and Gunsalus 1993), and this selection has numerous applications for basic genetic analysis. It has been used, for

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example, to measure the rates of spontaneous, forward (*i.e.*, loss-of-function) mutation at two pyrimidine biosynthetic loci, *pyrE* and *pyrF* (Jacobs and Grogan 1997). The estimated rate of mutation per kilobase pair at these loci in *S. acidocaldarius* growing at temperatures up to 84° closely matches corresponding rates determined for *E. coli*. As a result, *S. acidocaldarius* extends the constant rate of mutation per microbial DNA genome, first noted by Drake, to the domain Archaea and to extremely high growth temperatures (Drake *et al.* 1998). The observed spontaneous mutation rates also imply effective mechanisms of DNA stabilization and repair in *S. acidocaldarius*, because spontaneous processes such as deamination and depurination should be accelerated ~1000-fold at the temperature and pH within a growing *S. acidocaldarius* cell compared to the temperature and pH within a growing *E. coli* cell. Additional (albeit indirect) evidence of efficient DNA stabilization and repair in *S. acidocaldarius* includes photoreactivation (Grogan 1997) and a significant level of resistance to UV irradiation in the absence of photoreactivation (Wood *et al.* 1997).

An additional genetic property of *S. acidocaldarius* is exchange and recombination of chromosomal markers by a conjugational mechanism (Grogan 1996). This is one of only three examples of archaeal conjugation described so far; the others are a chromosomal marker exchange mechanism in *Haloferax volcanii* (Rosenshine *et al.* 1989) and transfer of plasmid pNOB8 among various Sulfolobus isolates (Schleper *et al.* 1995). In *S. acidocaldarius*, genetic exchange results in the formation of recombinant phenotypes at frequencies of $10^{-5}$ to $10^{-4}$ per cell (Ghané and Grogan 1998); a variety of biosynthetic loci can be exchanged by this mechanism (Grogan 1996), and no patterns have been observed which would imply surface exclusion or otherwise differentiate donor and recipient strains of *S. acidocaldarius* (Grogan 1996). This is the situation observed for *H. volcanii* as well (Rosenshine *et al.* 1989).

In *S. acidocaldarius*, the overall yield of recombinants per survivor increases dramatically as a function of UV dose if the two parental strains are irradiated before mating (Wood *et al.* 1997). This stimulation of marker exchange (hereafter termed SME) was photoreversed with an efficiency similar to that of photoreversal of UV-induced lethality and of UV-induced mutagenesis (Wood *et al.* 1997). Three properties argue that the SME phenomenon of *S. acidocaldarius* should be amenable to experimental manipulation and analysis: (i) rapid, accurate, and relatively nonperturbing introduction of the initiating damage via UV irradiation; (ii) rapid initiation of conjugation by simple mixing of parental suspensions; and (iii) arrest of metabolic processes in *S. acidocaldarius* during manipulation of cells at low (*i.e.*, room) temperature (Ghané and Grogan 1998). In the absence of mutants with specific genetic defects in DNA repair, conjugation, or homologous recombination, SME seems to provide a useful approach to investigating these processes and their interaction in a hyperthermophilic archaeon.

## MATERIALS AND METHODS

**Strains and growth conditions:** All strains used in this study are derivatives of *S. acidocaldarius* DG6 (ATCC 49426), a prototrophic strain routinely used in this laboratory as the wild type (Grogan 1991). The following pyrimidine auxotrophs and their corresponding alleles have been described (Grogan and Gunsalus 1993; Ghané and Grogan 1998): DG29 (*pyrD1*), DG64 (*pyrB4*), MR27 (*pyrF127*), MR39 (*pyrF139*), and MR41 (*pyrF141*). No other mutations are known in these strains, although it should be noted that strains MR27, MR39, and MR41 were derived from phenotypic revertants of amino acid auxotrophs.

All cultures to be mated were grown from a single colony in xylose-tryptone-uracil (XTura) medium (Jacobs and Grogan 1997) with continuous agitation at 80°, unless otherwise noted. Cultures were harvested at cell densities less than $\sim 4 \times 10^8$ cells/ml. Growth medium was removed by pelleting the cells, decanting the supernatant, washing cells once in Sdil buffer, and resuspending the cell pellet in Sdil buffer (Grogan 1996) to achieve the desired cell density ($2 \times 10^8$ cells/ml, unless otherwise noted). Aliquots (100 μl) of undiluted cell suspensions were plated on xylose-glutamine-casein hydrolysate medium (XGcaa) and incubated 1 wk at 75° to select $Pyr^+$ recombinants. The suspensions were also diluted in Sdil buffer and plated on solid XTura medium to enumerate total cfu.

**Assays of marker exchange:** Using a streamlined assay (Ghané and Grogan 1998), exchange and recombination of genetic markers was typically measured by mixing equal volumes of two cell suspensions and incubating 20–30 min at 22°. Suitable aliquots of the mixtures were then plated for enumeration of $Pyr^+$ and total cfu as described above. This procedure ensured that negligible metabolism occurred between the cellular treatments being evaluated and the initiation and assay of genetic exchange. Revertant frequencies were determined by plating pure suspensions on XGcaa medium; these frequencies were subtracted from the corresponding $Pyr^+$ titers of mated suspensions to yield the frequencies of recombinants. Typical revertant frequencies are less than $\sim 3 \times 10^{-7}$ revertants per cfu for strains DG29 and DG64, and $< 1 \times 10^{-7}$ for the *pyrF* strains used.

To test the effects of UV irradiation, suspensions of cells in 3–5 ml Sdil buffer were irradiated for 30 sec (unless otherwise noted) with intermittent agitation in a glass Petri dish (10 cm diameter), yielding an incident dose of ~105 J UV radiation/$m^2$ at short wavelengths (<300 nm, Wood *et al.* 1997). Irradiation of cells and all subsequent steps were performed under dim red lighting to prevent photoreactivation (Grogan 1997). Unless otherwise noted, uracil (20 μg/ml) was added to the cell suspensions after UV irradiation as a means of minimizing preferential survival or growth of $Pyr^+$ revertants or recombinants in the ensuing incubations. The effects of this supplementation were evaluated in several different experiments and appeared to be inconsequential for the analysis of SME (see results).

To test the effect of parental cell ratio on the yield of recombinants, series were set up in which each mating contained a different ratio (by volume) of two parental suspensions in a total volume of 160 μl. After being mixed and incubated 20 min at room temperature, aliquots of each mating were withdrawn and plated. Frequencies of $Pyr^+$ clones from the first and last suspension of each series (*i.e.*, pure parental suspensions) were used to correct for reversion, and the cfu

**TABLE 1**

**Temperature dependence of the rate of SME decay**

| Holding temperature | Strains mated | Trial 1 | | Trial 2 | |
|---|---|---|---|---|---|
| | | Rate constant ± SE | $r^2$ | Rate constant ± SE | $r^2$ |
| 55° | DG29 × DG64 | −0.085 ± 0.036 | 0.575 | −0.050 ± 0.010 | 0.870 |
| | MR27 × MR41 | −0.071 ± 0.020 | 0.705 | −0.009 ± 0.012 | 0.110 |
| | MR39 × MR41 | −0.138 ± 0.060 | 0.519 | −0.026 ± 0.017 | 0.314 |
| | Mean | −0.098 | | −0.011 | |
| 65° | DG29 × DG64 | −0.059 ± 0.028 | 0.523 | −0.092 ± 0.031 | 0.692 |
| | MR27 × MR41 | −0.120 ± 0.033 | 0.766 | −0.033 ± 0.027 | 0.222 |
| | MR39 × MR41 | −0.175 ± 0.035 | 0.830 | −0.024 ± 0.026 | 0.146 |
| | Mean | −0.118 | | −0.034 | |
| 75° | DG29 × DG64 | −0.274 ± 0.070 | 0.754 | −0.110 ± 0.037 | 0.690 |
| | MR27 × MR41 | −0.186 ± 0.022 | 0.931 | −0.074 ± 0.028 | 0.592 |
| | MR39 × MR41 | −0.184 ± 0.022 | 0.931 | −0.091 ± 0.047 | 0.488 |
| | Mean | −0.215 | | −0.092 | |
| 82° | DG29 × DG64 | −0.228 ± 0.034 | 0.901 | −0.160 ± 0.021 | 0.937 |
| | MR27 × MR41 | −0.179 ± 0.016 | 0.961 | −0.162 ± 0.051 | 0.670 |
| | MR39 × MR41 | −0.208 ± 0.026 | 0.927 | −0.170 ± 0.041 | 0.808 |
| | Mean | −0.205 | | −0.164 | |

Apparent first-order rate constants, standard errors, and linear correlation coefficients were calculated by linear regression as described in materials and methods.

of these suspensions (and, in some experiments, adjacent suspensions in the series) were used to calculate total cfu for each intermediate mixture in the series. Various series, which differed with respect to whether one or both of the strains had been previously irradiated with 70 J UV/m$^2$, were tested.

To test the effect of UV on surface-immobilized cell mixtures, cultures grown with aeration at 75° were harvested, washed, mated, diluted as necessary, and plated on XGcaa and XTura plates, as done for standard marker exchange assays, but without UV irradiation. Surfaces of the resulting dry plates were then irradiated with varying doses (9–70 J/m$^2$) of UV and incubated promptly. Selective plates spread with only one of the two parental strains measured the frequency of revertants, which was used to correct the frequency of recombinants. Controls in which dilutions of unirradiated cells were plated on nonselective (XTura) plates previously irradiated with 105 J/m$^2$ confirmed that possible photochemical toxicity or other indirect effects of UV radiation did not affect the efficiency of plating.

To measure time-dependent changes in marker exchange efficiency, UV-irradiated cell suspensions (typically 2–3 ml) were transferred to 8-mm-diameter screw-cap tubes or 10-mm-diameter screw-cap vials, sealed, and incubated at 75° (unless otherwise indicated). Aliquots were withdrawn at regular intervals and assayed for marker exchange as outlined above. In some experiments (*e.g.*, Figure 4), nonirradiated controls were incubated and analyzed in parallel with UV-irradiated suspensions. The effects of previous incubation and UV exposure on these kinetics (experiments in Figure 5) were as described above, except that no uracil was added to the suspensions. After several hours of incubation, the suspensions were transferred to Petri dishes, UV irradiated, and returned to clean vials for continued incubation (see text).

**Calculations:** Recombinant frequencies are reported as the number of Pyr$^+$ clones (less revertants) per cfu. Optimal parental ratios were estimated graphically by smooth curve fitting of recombinant frequency plotted as a function of log(cfu ratio), as shown in Figure 1. The data in Table 1 were calculated by linear regression of log(recombinant frequency) *vs.* hours of liquid holding time. Slopes (*i.e.*, rate constants), linear correlation coefficients, and standard errors of slopes were calculated by conventional spreadsheet functions.

## RESULTS

**Role of cell pairing in UV stimulation of marker exchange:** Although the known properties of chromosomal marker exchange in *S. acidocaldarius* imply a conjugational process (Grogan 1996; Ghané and Grogan 1998), the stage at which such a process is stimulated by UV has not been established (Wood *et al.* 1997). In *S. acidocaldarius*, conjugation initiates in dilute liquid suspension at room temperature, yet only ~7% of cells in a typical mixed suspension appear to be paired or otherwise aggregated, as judged by phase-contrast microscopy (Ghané and Grogan 1998). Furthermore, most of these are probably cells in the late stages of division (Poplawski and Bernander 1997). This low level of observed cell pairing is consistent with the fact that matings between unirradiated parent strains rarely yield recombinant frequencies much higher than $10^{-4}$ per cfu. Thus, even under favorable conditions, only a small minority of cells in a suspension appears to initiate conjugation. This raised the possibility that UV-induced SME could result from a corresponding increase in this otherwise small minority of cells, and, therefore, we sought evidence that UV significantly increases the proportion of viable *S. acidocaldarius* cells that conjugate.

In one approach, we measured the ratio of parental cells yielding the maximal number of recombinants. For two unirradiated suspensions, this optimum typically occurs at a ratio of ~1 (Ghané and Grogan 1998), as

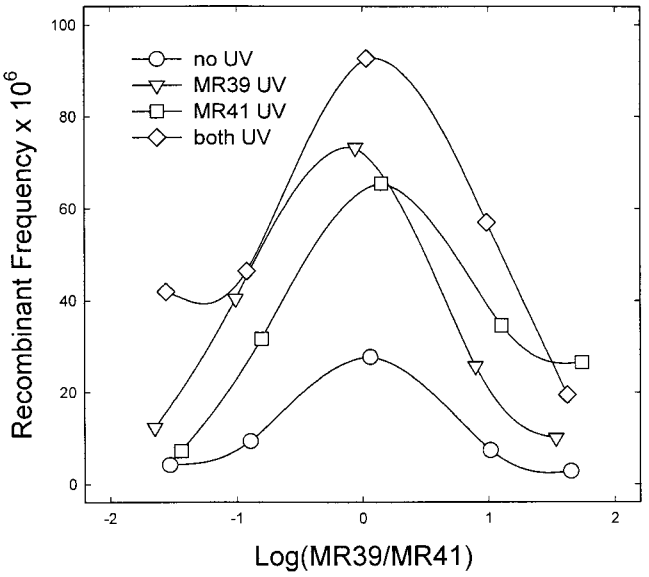


Figure 1.—Effect of UV on optimal parental ratios. Washed suspensions of strains MR39 (*pyrF139*) and MR41 (*pyrF141*) were mated at the indicated ratios with or without previous UV irradiation. $Pyr^+$ recombinants per total cfu in the mixture were determined as described in materials and methods.

predicted by a requirement for pairing between at least one cell of each parent (Maloy *et al.* 1994). We used this type of titration of one suspension against another in attempts to identify UV-induced changes in the relative number of conjugation-proficient cells. As shown in Figure 1, irradiation of one parent caused a significant increase in the maximal recombinant frequency (*e.g.*, 2- and 2.3-fold), but little shift in the optimal MR39: MR41 ratio, *i.e.*, 20% decrease (from 1.0 to 0.8) when strain MR39 was irradiated, *vs.* 40% increase (from 1.0 to 1.4) when MR41 was irradiated. Thus, the increased number of recombinants in response to UV could not be attributed to a corresponding increase in the number of conjugating cells. A separate, independent trial of this type (not shown) yielded greater stimulation with similar small shifts in the optimal parental ratio. In both cases, the modest shifts in optimal parental ratios could be explained by viability losses (~30%), assuming that a large proportion of UV-killed cells were nevertheless able to generate viable recombinants. The experiments supported two additional conclusions: (i) the stimulation achieved by irradiating both cultures (*vs.* one culture alone) was neither multiplicative nor fully additive under these conditions (Figure 1) and (ii) the yield of recombinants in the remaining experiments of our study (which used equal UV irradiation of both parents) should not have been sensitive to small differences in UV doses or survival of either parent.

Wood *et al.* (1997) observed SME in liquid suspensions incubated 16–20 hr at 75°. This prolonged incubation could have allowed long-term cellular changes in response to UV, as well as multiple rounds of cell pairing

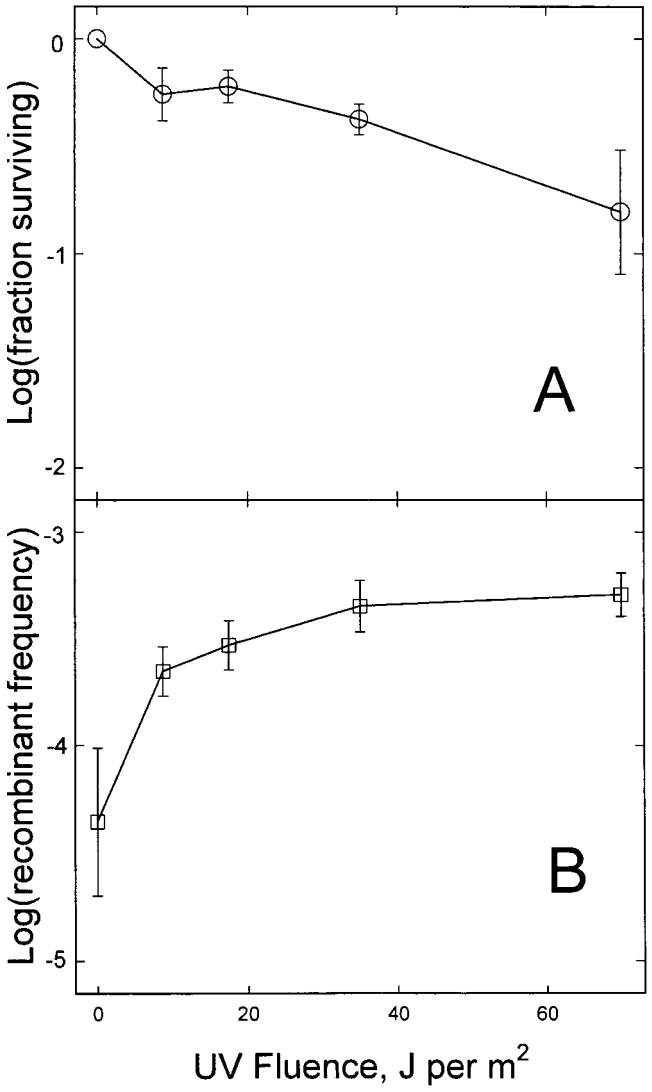


Figure 2.—Effects of UV on plated cells. Mixtures containing equal numbers of parental cells [DG29 (*pyrD1*) and DG64 (*pyrB4*)] were (A) diluted and plated on nonselective medium, or (B), plated on selective medium before irradiation with the indicated dose of UV and incubation, as described in materials and methods. The graph in A represents survival; B represents recombinant formation. Symbols represent mean values of four determinations; error bars are standard deviations.

before recombinants were enumerated. Therefore, by irradiating cell mixtures on the dry surfaces of plates, we tested the ability of UV to stimulate marker exchange among cells unable to form new contacts. For this purpose, an undiluted aliquot of a cell mixture was plated on selective medium to enumerate recombinants, whereas a suitable dilution was plated on nonselective medium to enumerate survivors. The observed relationship between UV dose and frequency of recombinants among survivors was biphasic (Figure 2B). At relatively low doses, UV stimulated recombinant formation with an apparent exponential constant of $0.064 \pm 0.019$ $\log_{10}$ units/J/m$^2$ (mean $\pm$ standard deviation, $n = 4$). This

efficiency of stimulation is nearly twice that reported by Wood *et al.* (1997) (*i.e.*, 0.035 ± 0.010, $n = 4$) for liquid suspensions of the same two strains. This result thus argues that UV stimulated the formation of recombinants from cell pairs (or aggregates) that had already formed at the time the plate surface was irradiated. Furthermore, for plated cells, the increase in marker exchange efficiency stopped at UV doses >30 J/m$^2$ (Figure 2B). The plateau of Figure 2B could not be attributed to a general loss of UV effect at these doses, because survival declined more or less exponentially over the same range (Figure 2A) and declined even more steeply at doses >70 J/m$^2$ (data not shown). At high UV doses, therefore, plated cells gave different results than previously observed with liquid suspensions; in the latter case, recombinants per survivor continued to increase exponentially with doses of ≥70 J/m$^2$ (Wood *et al.* 1997).

**Kinetics of SME:** Studies from this laboratory have so far failed to identify a practical method of blocking or interrupting marker exchange once cell suspensions are mixed (Ghané and Grogan 1998), which currently limits the experimental strategies that can be applied to this phenomenon. However, the fact that conjugation initiates immediately upon mixing parental cells (Ghané and Grogan 1998), combined with the use of UV to introduce DNA damage and the use of low temperature (*e.g.*, 22°) to arrest metabolism, suggested a way to measure the kinetics and other physiological aspects of UV-induced SME. In a series of experiments, we UV irradiated two parental suspensions and separately subjected them to a common treatment (*e.g.*, incubation at 75°). We then combined the suspensions and enumerated recombinants; this genetic assay of the "fertility" of the cells allowed us to measure the effects of a variety of treatments on SME.

Figure 3 shows the effects of previous incubation in uracil-supplemented dilution buffer at 75°. The results showed that no preincubation at 75° was necessary to manifest SME. In fact, SME was highest when parental cells were not preincubated, and it decayed rather rapidly with increasing preincubation time. Similar kinetics of decay were obtained for both intergenic crosses (Figure 3A) and intragenic crosses (MR27 × MR41 and MR39 × MR41, Figure 3C) despite large differences in the absolute frequencies of recombinants for the two cases. The first few hours of decline could generally be approximated by exponential functions of time, as seen in semilog plots (closed symbols, Figure 3, B and D). We saw no evidence attributing this result to some severe physiological stress imposed by incubation, because viability either increased slightly or remained nearly constant (open symbols in Figure 3, C and D). Conversely, the decrease in recombinants per cfu could not be attributed solely to increased viability, as the exponential rates of decline were severalfold greater than those of viability increase (Figure 3, C and D).

In most cases, the yield of recombinants in UV-irradiated suspensions stabilized at a limiting value near that of unirradiated cell suspensions (open symbols in Figure

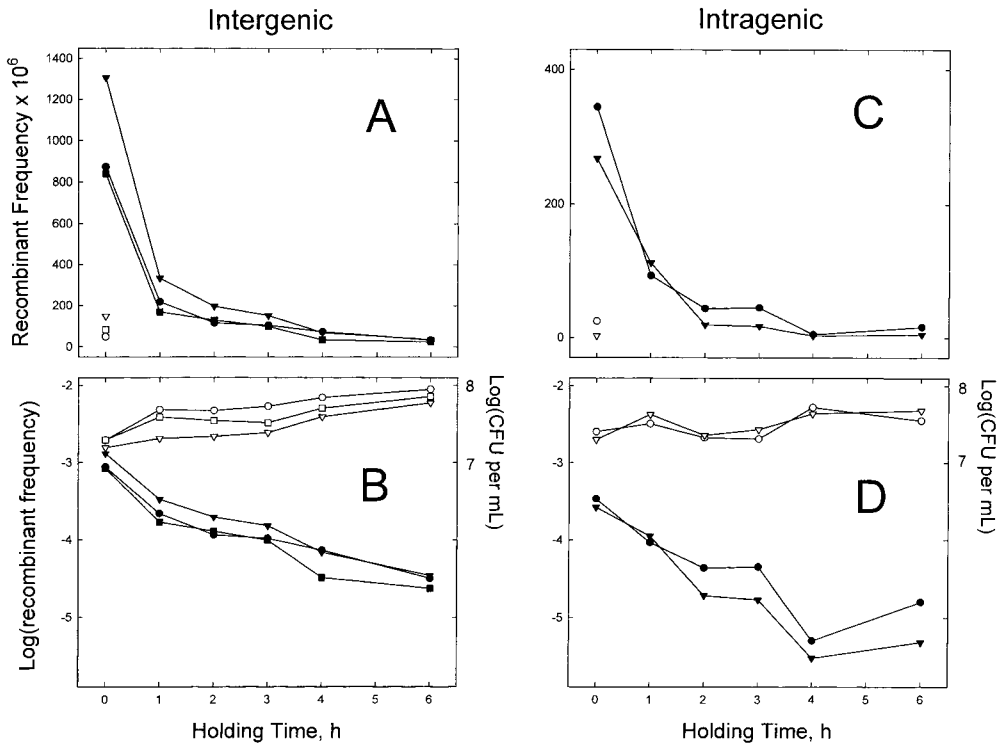


Figure 3.—Yield of recombinants as a function of liquid holding at 75°. Washed cell suspensions were irradiated with UV and were incubated separately at 75° for the indicated times. Aliquots of the suspensions were then mated, and recombinant frequencies were determined as described in materials and methods. Each symbol shape represents a different combination of strains. (A and B) Intergenic crosses: circles, DG29 × DG64 (*pyrD* × *pyrB*); triangles, DG29 × DG38 (*pyrD* × *cpb*); squares DG38 × DG64 (*cbp* × *pyrB*). (C and D) Intragenic crosses: circles, MR27 × MR41 (*pyrF127* × *pyrF141*); triangles, MR39 × MR41 (*pyrF139* × *pyrF141*). Solid symbols: recombination between UV-irradiated suspensions. Open symbols: (A and C) recombination between control suspensions; (B and D) viability of UV-irradiated cultures (scale on right).

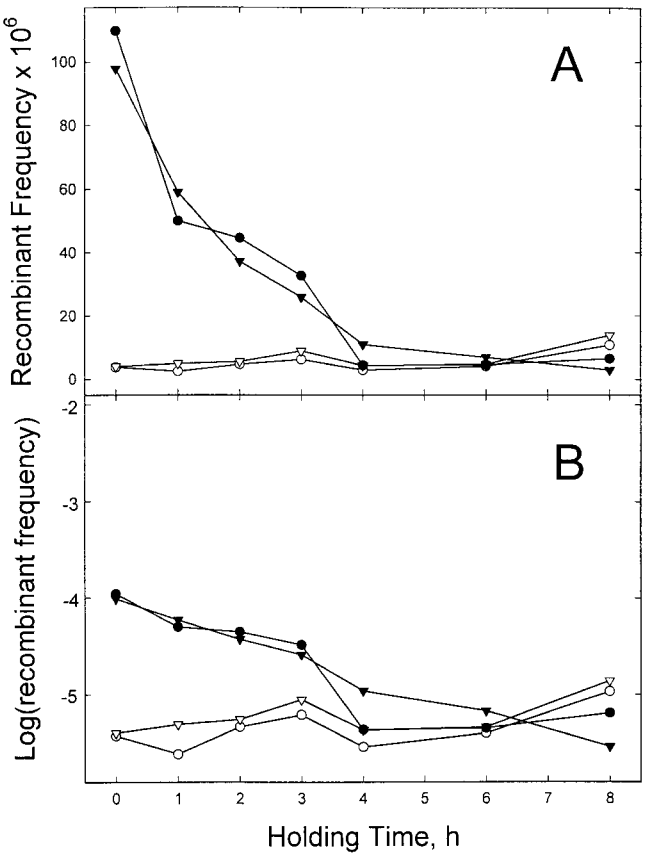


Figure 4.—Comparative kinetics for irradiated and nonirradiated suspensions. (A) Washed cell suspensions were split into two parts, one of which was UV irradiated. Both parts were then incubated and assayed for recombinant formation as described for Figure 3. Circles, MR27 × MR41(*pyrF127* × *pyrF141*); triangles, MR39 × MR41 (*pyrF139* × *pyrF141*); open symbols, unirradiated controls; closed symbols, UV-irradiated suspensions. (B) The graph is a semilogarithmic plot.

3A). In some experiments, however (*e.g.*, Figure 3C), we noted that the yield of recombinants actually declined below control levels. This raised the possibility that the observed decline could result from a general attenuation of fertility caused by incubation at 75°. We therefore repeated the intragenic crosses of Figure 3, assaying both irradiated and nonirradiated suspensions over an 8-hr period. As shown in Figure 4, the yield of recombinants from unirradiated suspensions remained reasonably constant, whereas that of irradiated suspensions started high and decreased rapidly to control levels. Similar results were obtained when uracil was omitted from the incubation medium, except that on average, the nonsupplemented control suspensions yielded twofold higher recombinant frequencies than uracil-supplemented controls (data not shown).

**Temperature dependence:** The procedure of Figure 3 was repeated at several temperatures, utilizing both intergenic and intragenic crosses. To facilitate comparisons among the resulting decay kinetics, logarithms of recombinant frequencies as a function of holding time over the 0–6 hr interval were fitted into linear regressions. The results of two independent experiments are summarized in Table 1. Despite considerable variation at lower temperatures, both series show a trend in which the apparent first-order rate constant for SME decay increases with temperature. This provides evidence that the decay in SME may result from one or more enzymatic (*i.e.*, metabolic) processes.

**Effect of previous history:** The quantum efficiency and photoreversibility of UV-induced SME compared to those of other UV effects in *S. acidocaldarius* suggest that SME is initiated by the presence of pyrimidine dimers in the chromosome (Wood *et al.* 1997). We were therefore interested in further determining whether the decay of SME could be similarly attributed to the removal of DNA lesions *per se*, or whether it might instead reflect an indirect regulatory response, such as induction of a factor or cellular state that inhibits SME. We reasoned that a general regulatory change should not be reversed quickly, whereas removal of lesions from DNA could be reversed immediately and quantitatively by a second dose of UV. We therefore tested the ability of UV to induce SME after an initial UV exposure and several hours of preincubation at 75°. The results (Figure 5) indicate that neither previous UV and incubation nor prolonged incubation alone had a significant effect on (i) the lethality of the UV dose, (ii) the magnitude of SME, or (iii) the rate of SME decay. The results argue that the observed decay of SME does not result from a stable regulatory response of *S. acidocaldarius* cells induced by UV or incubation under the conditions we used.

## DISCUSSION

*S. acidocaldarius* is the only hyperthermophilic archaeon so far reported to exchange and recombine chromosomal genes. We note, however, that *S. acidocaldarius* is also the only hyperthermophilic archaeon for which various selectable markers allow chromosomal exchange and recombination to be detected genetically. The fact that other Sulfolobus species distantly related to *S. acidocaldarius* can transmit plasmid pNOB8 (Schleper *et al.* 1995) raises the possibility that conjugation may be a relatively common feature among the Sulfolobales. It may indeed be widely distributed among archaea, given that a similar type of marker exchange was first discovered in an extreme halophile (Rosenshine *et al.* 1989).

Although examples of conjugational genetic exchange among prokaryotes have proven to be mechanistically diverse (Wilkins 1995), the common features of the various systems argue that the *S. acidocaldarius* mechanism involves three distinct stages: (i) adherence of two or more parental cells to each other (cell pairing), (ii) cell fusion or other transfer of DNA between cells (zygote formation), and (iii) crossover events yielding

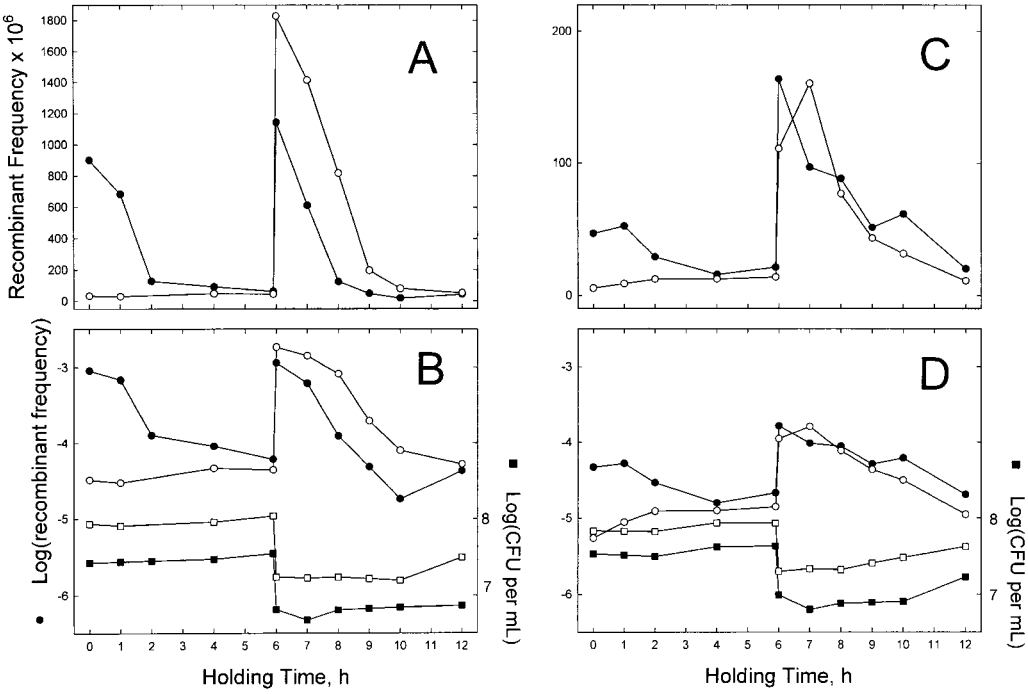


Figure 5.—Restimulation of marker exchange. Non-irradiated and irradiated suspensions were prepared and analyzed over a 6-hr period as described for Figure 4, except that uracil was omitted (see materials and methods). Both sets of suspensions were then UV irradiated, and the incubation and sampling procedure was continued for an additional 6 hr. (A and B) Intergenic cross (DG29 × DG64); (C and D) Intragenic cross (MR27 × MR41). Open symbols, suspensions first irradiated at 6 hr; closed symbols, suspensions irradiated both initially and at 6 hr.

a chromosome with a selectable genotype (homologous recombination). In principle, UV could increase the frequency of events (i)–(iii) or a combination of them. In the present study, however, we found no experimental evidence that UV substantially increases the probability that a cell will pair with another cell in liquid suspension, or that SME requires any cellular (*i.e.*, metabolic) responses to UV to occur before parental cells are mated. In contrast, we did find evidence that marker exchange between cells immobilized on the surface of a plate was stimulated as efficiently as between cells incubated overnight in liquid suspension (Wood *et al.* 1997) at relatively low UV doses. (The observed saturation of SME on plate surfaces at relatively high UV doses is consistent with a fixed number of cells on the plate surfaces able to transfer and recombine DNA.) We therefore interpret these results as evidence that UV does not stimulate marker exchange in *S. acidocaldarius* by increasing the proportion of cells that form cell pairs or aggregates.

In addition, we found that UV-induced SME is reproducibly transient. Under typical experimental conditions (low-nutrient buffer supplemented with uracil at 75°), recombinant yields decayed to nearly basal (*i.e.*, unstimulated) levels within a few hours. This decay was found to be temperature dependent, and it apparently affected neither subsequent restimulation of marker exchange nor the decay of the restimulation. These results thus suggest that SME measures an internal "signal" or other condition that originates with UV photoproducts and that is continuously dissipated through metabolism. As a working hypothesis, we propose that this signal or condition consists of recombinogenic DNA lesions present in the *S. acidocaldarius* chromosome at the time that genetically marked parental cells are mixed at room temperature.

Short-wavelength UV (UV-C) irradiation is known to stimulate homologous recombination in other genetic systems, including bacteriophage, bacteria, and yeast (Wilkins and Howard-Flanders 1968; Wall and Harriman 1974; Ninkovic *et al.* 1994). This is attributed to secondary lesions (single-strand breaks, double-strand breaks, and daughter-strand gaps) formed by processes including excision repair and blocked DNA synthesis (Wilkins and Howard-Flanders 1968; Kowalczykowski *et al.* 1994; Ninkovic *et al.* 1994). In some systems, induction of recombinational proteins may also contribute to recombinogenesis (Simon and Moore 1988). However, synthesis of the DNA recombinase of *Sulfolobus solfataricus*, product of the *radA* gene, is reported to be unresponsive to UV radiation (Sandler *et al.* 1996), and our results suggest that induced changes in cellular protein levels do not play a major role in the kinetics of SME.

In conformity with the known properties of other systems, we presume that pyrimidine dimers formed in the *S. acidocaldarius* chromosome at room temperature must be metabolically processed to form the recombinogenic lesions detected by marker exchange. This processing may be mediated by dark-repair enzymes, whose existence can be inferred from the UV survival curves of *S. acidocaldarius* strains (Wood *et al.* 1997). Alternatively, formation of daughter-strand gaps, which presumes DNA synthesis and its blockage by unrepaired dimers, appears compatible with our observation that overall viability of UV-irradiated cells can increase during liquid

holding. With respect to photoproduct processing, however, one feature of SME seems to require explanation. In the kinetic experiments shown (*e.g.*, Figures 3 and 4), SME was always maximal in the absence of any preincubation of parental cells. Thus, no onset phase corresponding to the hypothesized conversion of nonrecombinogenic DNA photoproducts into recombinogenic DNA lesions was detected by the marker exchange assay. It seems plausible, however, that this reflects a situation in which the only metabolic conversion available for primary DNA photoproducts is via recombinogenic lesions. Thus, in our experiments (in which photoreactivation was excluded) every primary photoproduct may have been equivalent to a recombinogenic lesion because it would be eventually converted to one with high efficiency, either during premating incubation in liquid or during postmating incubation on plates. Thus, premating incubation could only decrease SME because it would allow proportionally more recombinogenic lesions to be completely repaired before homologous recombination could be initiated. We note that this makes the following prediction: if UV-irradiated parental cells are photoreactivated after being incubated various times at 75°, but before being mated, SME should be limited, but an onset (*i.e.*, ascending phase) should be apparent.

We also note that our working hypothesis (*i.e.*, that SME measures the abundance of recombinogenic DNA lesions and that decay of SME measures their repair) implies that DNA repair competes with the marker exchange mechanism of *S. acidocaldarius* for DNA lesions. The observed kinetics of SME decay indicate that the competing DNA repair must be fairly rapid at physiological temperatures. Therefore, either (i) the events preceding "capture" of recombinogenic lesions by the marker exchange process are comparably fast or (ii) a large fraction of the UV-induced damage is repaired before it can be detected by marker exchange. In either case, our ability to resolve the relatively rapid kinetics of SME decay by simply mixing and plating two *S. acidocaldarius* cell suspensions supports earlier evidence that *S. acidocaldarius* cells can rapidly initiate marker exchange in liquid medium (Ghané and Grogan 1998).

In this study, we also observed that irradiating cells immobilized on a plate limits the magnitude of SME obtained, whereas in a previous study, irradiating cells and incubating them in liquid suspensions did not show such a limit over the same dose range. This apparent discrepancy warrants investigation, because it could be explained mechanistically if genetic exchange between *S. acidocaldarius* cells initiated continuously in liquid suspension. Our results allow for the possibility that progressively heavier doses of UV should progressively prolong SME despite its ultimate decay. Therefore, if conjugational turnover in liquid suspension were sufficiently rapid, heavy UV doses would extend the "temporal window" of SME to include subsequent cohorts of newly established cell pairs and, thus, increase the overall yield of recombinants.

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