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# Archaeal RecA homologues: different response to DNA-damaging agents in mesophilic and thermophilic Archaea

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**Abstract** Two archaeal proteins, RadA and RadB, share similarity with the RecA/Rad51 family of recombinases, with RadA being the functional homologue. We have studied and compared the RadA and RadB proteins of mesophilic and thermophilic Archaea. In growing cells, RadA levels are similar in mesophilic *Methanococcus* species and the hyperthermophile *Methanococcus jannaschii*. Treatment of cells with mutagenic agents (methylmethane sulfonate or UV light) increased the expression of RadA (as evidenced by higher levels of both mRNA and protein) in all organisms tested, but the increase was greater in the mesophiles than in the thermophiles *M. jannaschii* and *Sulfolobus solfataricus*. Recombinantly expressed RadA proteins from the mesophile *M. voltae* and the thermophile *M. jannaschii* were similar in their ATPase- and DNA-binding activities. All the data are consistent with proposals that RadA plays the same role as eukaryotic Rad51. Surprisingly, the data also suggested that the thermophiles do not need more RadA protein or activity than the mesophiles. On the other hand, RadB is not coregulated with RadA, and its role remains unclear. Neither RadA nor RadB from a mesophile or from a thermophile rescued the UV-sensitive phenotype of an *Escherichia coli recA*⁻ host.

**Key words** DNA repair · RadA · RadB · Rad51 · Gene regulation

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## Introduction

Archaea are unique among organisms in that related species grow at remarkably different temperature optima. Hyperthermophiles such as *Methanococcus jannaschii* thrive at temperatures of 83°C, whereas mesophilic relatives such as *Methanococcus voltae* and *Methanococcus maripaludis* have optimal growth temperatures of 37°C. Although belonging to a phylogenetically coherent group, these organisms are more distantly related than the taxonomy implies (Boone et al. 1993), with an average of 70% sequence identity in pairs of aligned homologous proteins (Haney et al. 1999). Life at high temperatures poses severe challenges to the stability of DNA (Marguet and Forterre 1994; Grogan 1998, 2000). Spontaneous reactions such as deamination of cytosines and adenines, hydrolytic depurination, and strand breakage are all enhanced at high temperature, and organisms need to respond to these challenges by developing robust strategies to ensure the integrity of their chromosomes.

Powerful DNA repair systems are at work in thermophilic and hyperthermophilic Archaea. *Pyrococcus furiosus* cells are highly resistant to DNA break formation when exposed to temperatures in excess of 100°C (Peak et al. 1995) and are able to reassemble their genomes after extensive fragmentation by gamma irradiation (DiRuggiero et al. 1997). In *Sulfolobus acidocaldarius* growing at 75°C, the spontaneous rate of mutation is comparable to that in *Escherichia coli* growing at 37°C (Jacobs and Grogan 1997), and there is evidence for error-prone repair and genetic recombination after UV exposure (Wood et al. 1997).

Comparative analyses of whole genomes have revealed that, of the proteins involved in DNA repair, the only one that is universal is the Rec/Rad recombinase (Aravind et al. 1999; Eisen and Hanawalt 1999). These proteins, which include bacterial RecA, eukaryotic Rad51, and archaeal RadA, are crucial for double-strand DNA break repair and homologous

recombination (Roca and Cox 1997; Bianco et al. 1998; Baumann and West 1998). The proteins share a common origin (Leipe et al. 2000), and this history is underscored by functional similarities, including cooperative binding to DNA to form helical nucleoprotein filaments and DNA-stimulated ATPase activity (Roca and Cox 1990; Kowalczykowski 1991; West 1992). Mutants in the *recA* gene are deficient in homologous recombination and are sensitive to DNA-damaging agents; also, expression of the gene is induced by mutagenic treatment (Walker 1984). In yeast, *rad51* mutants are susceptible to ionizing radiation and chemical agents such as methylmethane sulfonate (MMS) that cause DNA breaks. As in the case of the bacterial gene, expression of *rad51* is increased by treatment with mutagens, such as alkylating agents (Basile et al. 1992; Ogawa et al. 1993).

In Archaea, there are two proteins, RadA and RadB, that share similarity with the Rec/Rad recombinases. RadA proteins contain about 330 residues and are more similar in sequence to eukaryotic Rad51 than to bacterial RecA (Sandler et al. 1996, 1999; Brendel et al. 1997). Archaeal RadB is a smaller protein of about 220 residues that retains only the core sequences common to all the Rec/Rad family (Rashid et al. 1996). Homologues of RadA and RadB have been found in all the archaeal genomes sequenced thus far (Aravind et al. 1999; Leipe et al. 2000). Although the Rec/Rad recombinases in Bacteria and Eucarya have been extensively studied, relatively little is known about the biochemical properties and in vivo functions of the archaeal proteins. The RadA proteins of *Sulfolobus solfataricus*, *Pyrococcus furiosus*, and *Pyrobaculum islandicum* have been shown to form nucleoprotein filaments on DNA and promote strand exchange (Seitz et al. 1998; Komori et al. 2000; Spies et al. 2000).

It has been reported that expression of this protein in *S. solfataricus* and *Pyrococcus furiosus* is not increased by DNA-damaging treatments (Sandler et al. 1996; Komori et al. 2000). In the halophile *Haloferax volcanii*, deletion of *radA* renders the cell recombination deficient and leads to increased sensitivity to UV light and ethylmethane sulfonate (Woods and Dyall-Smith 1997). In contrast, the smaller RadB protein of *Pyrococcus* sp. has been shown to have DNase activity and hydrolyze ATP to AMP and pyrophosphate instead of ADP and phosphate as do the RecA/Rad51 proteins (Rashid et al. 1997). Recently, the RadB protein from *P. furiosus* has been shown to interact with RadA and Hjc, a Holliday junction resolvase (Komori et al. 2000). Surprisingly, it has been reported that expression of RadB from *Pyrococcus* sp. KOD1 in an *E. coli recA1* strain can rescue the UV sensitivity of the host cell (Rashid et al. 1996).

The paucity of data about DNA repair systems in Archaea and the many unanswered questions arising from the problems posed by life at high temperatures prompted us to study the *radA* and *radB* genes and their proteins in mesophilic and thermophilic Archaea. In this article, we address the question of the effect of mutagenic agents in mesophilic and thermophilic Archaea on expression of the *radA* and *radB* genes, compare the meso- and thermophilic RadA proteins biochemically, and reinvestigate the ability of these proteins to complement the UV-sensitive phenotype of an *E. coli recA1* host expressing them.

## Materials and methods

### Bacterial strains and plasmids

*Escherichia coli* strain XL1-Blue MRF' (Stratagene, La Jolla, CA, USA) was used for all cloning experiments. Strain BL21(DE3) (Novagen, Madison, WI, USA) was used for expression of recombinant proteins. For in vivo complementation studies, host strain HMS174(DE3)pLysS (Novagen) was used. Plasmid pUC18 was used for library and partial library construction, as well as for subcloning gene fragments for sequence analysis. Plasmid pET-19b was used for subcloning the archaeal genes for expression of the recombinant proteins in *E. coli*. For in vivo complementation studies, relevant open reading frames (ORFs) were cloned into the plasmid pET-11b (Novagen).

### General DNA manipulations

Digestion of DNA with restriction enzymes (Gibco BRL; Invitrogen, Carlsbad, CA, USA) and agarose gel electrophoresis were performed under standard conditions (Sambrook et al. 1989). For Southern analyses and colony hybridizations, probes were labeled radioactively with [$\alpha$-$^{32}$P]dCTP (deoxycytidine triphosphate) using the Megaprime Labeling System (Amersham, Piscataway, NJ, USA). Small- and large-scale plasmid preparations used Qiagen (Valencia, CA, USA) systems and reagents. Recombinant plasmids were introduced into *E. coli* by electrotransformation using a Bio-Rad Gene Pulser (Bio-Rad, Hercules, CA, USA). Sequencing was carried out either with the Sequenase version 2.0 enzyme and system (USB; United States Biochemical, Cleveland, OH, USA) or the BigDye Terminator Cycle Sequencing kit (ABI Prism,; Applied Biosystems, Foster City, CA, USA). Oligonucleotide primers were synthesized by Operon (Alameda, CA, USA). All sequenced genes were cloned for expression by polymerase chain reaction (PCR) amplification of the relevant ORFs with oligonucleotide primers complementary to at least 17 positions at the 5′- and 3′-termini that incorporated appropriate restriction sites for in-frame cloning (*Nde*I and *Bam*HI).

### Growth and mutagenic treatment of archaeal cells

*Methanococcus jannaschii* strain JAL-1$^T$ (=DSM 2661) was grown in pressurized (27 psig) serum bottles at 83°C in an atmosphere of $H_2$:$CO_2$ (80:20) in mineral salts medium (Robb and Place 1995) supplemented with yeast extract (2 g/l) and tryptone (2 g/l), with periodic (every 2 h) flushing and replenishing of the headspace, until a density of ~2 × $10^8$ cells/ml was reached. For *M. voltae* strain PS$^T$ (=DSM 1537) and *M. maripaludis* strain JJ$^T$ (=DSM 2067), growth conditions were identical, except that incubations were done at 37°C. Cultures were transferred to an anaerobic chamber for the mutagenic treatments. For MMS treatment, 50-ml aliquots were transferred to 250-ml serum bottles, and

anaerobic MMS (or an equal volume of anaerobic water in control cultures) was added to 0.01%. For UV treatment, 50-ml aliquots of the culture were dispensed into sterile petri dishes (150 mm diameter) and irradiated for 5–25 s with a shortwave (254 nm) handheld UV lamp placed 20 cm above the cell suspension to deliver total doses from 10 to 50 J/m$^2$ (as measured with a Spectroline (Spectronics, Westbury, NY, USA) DM-254x ultraviolet meter) before transferring the cells to 250-ml serum bottles. Bottles were removed from the anaerobic chamber, crimped, repressurized, and incubated at their respective temperatures for 2 h before harvesting. *S. solfataricus* cells were grown aerobically in complex medium (Robb and Place 1995) at 65°C; mutagenic treatment was performed as described, except that an anaerobic chamber was not used. Cells were collected by centrifugation and frozen at –80°C.

## RNA isolation and analysis

Total cellular RNA was extracted from frozen cell pellets with the RNeasy Midi Kit (Qiagen), except initial resuspension of frozen cells was in TE buffer (10 mM Tris-HCl, pH 8.0/1 mM ethylenediaminetetraacetic acid [EDTA]), and cells were lysed by three cycles of freezing and thawing. Total RNA (8 µg) was separated by electrophoresis through 1.2% SeaKem agarose (FMC, Chicago, IL, USA) gels containing 10% formaldehyde at 3 V/cm (Sambrook et al. 1989). After transferring the RNA to Nytran Plus (Schleicher & Schuell, Keene, NH, USA), the damp membranes were UV cross linked (Stratalinker; Stratagene) and dried overnight at 65°C. RadA and RadB gene fragments were purified from a 1% SeaPlaque agarose (FMC) gel and labeled with [α-$^{32}$P]dCTP using the Megaprime DNA Labelling System (Amersham). The *S. solfataricus radA* transcript was probed with a 32-nucleotide oligomer complementary to positions 432–463 in the *S. solfataricus* coding sequence. Hybridizations were done overnight either in 6 × SSC at 65°C or in 6 × SSPE (150 mM NaCl, 10 mM $NaPO_4$, pH 7.4, 1 mM EDTA)/50% formamide at 45°C (Sambrook et al. 1989). Radioactivity was measured with a Molecular Dynamics SP phosphorimager, and pixel density was determined with ImageQuant 3.3 software (Molecular Dynamics, Sunnyvale, CA, USA). To correct for loading variation, data were normalized to 23S rRNA density, determined using NIH Image v1.61 and the photographic negative of the ethidium bromide-stained gel. Transcript mapping was performed as described by Ausubel et al. (1989).

## Purification of archaeal RadA proteins expressed in *E. coli*

*Escherichia coli* BL21(DE3), carrying pET-19b with an archaeal *radA* gene, was grown at 37°C to $OD_{600}$ = 0.7. Expression of the recombinant protein was induced by addition of isopropyl thiogalactoside (IPTG) to 1 mM and further incubation at 37°C for 5 h. Recombinant proteins from 150 ml of culture were purified by affinity to nickel-nitrilotriacetic acid (Ni-NTA) resin (Qiagen) as recommended by the manufacturer. Crude extracts with the thermophilic protein were heated to 65°C for 15 min and centrifuged at 12,000 *g* for 15 min before binding to the resin. Fractions containing the protein of interest were pooled and dialyzed overnight at 4°C against 1 l storage buffer (20 mM Tris-HCl, pH 8.0, 0.2 M KCl, 10% glycerol, 1 mM EDTA, 0.5 mM dithiothreitol [DTT]).

Protein concentrations were determined spectrophotometrically using a molar extinction coefficient of 17,669 $M^{-1}cm^{-1}$ for the *M. voltae* protein and 18,107 $M^{-1}cm^{-1}$ for the *M. jannaschii* protein. The proteins (>90% pure) were stored at –80°C at a concentration of 2 mg/ml. The *M. maripaludis* protein proved to be extremely unstable, losing activity hours after purification, with complete loss after 24 h. No significant loss of activity was detected on storage (for several months at –80°C) of the *M. jannaschii* and *M. voltae* proteins, and these were used for the comparison studies. Removal of the histidine tag from preparations of the recombinant proteins was performed with the Enterokinase Cleavage Capture kit (Novagen). Removal was monitored by Western analysis of the products with a penta-His antibody (Qiagen). As the His-tagged proteins were found to behave essentially identically to proteins without the tag in ATPase- and DNA-binding reactions, His-tagged versions were used routinely.

## Western blot analysis

Polyclonal antibodies were produced by the Immunological Resource Center at the University of Illinois Urbana/Champaign. Purified recombinant RadA protein from *M. voltae* was used to inoculate five mice intraperitoneally five times (50 µg per immunization) every third week. Four mice were tapped for ascites fluid; one mouse was given a final boost, then terminally bled and the immune serum collected. Archaeal cell pellets were resuspended in 10 mM Tris-HCl (pH 8.0), 150 mM NaCl, and lysed by addition of Triton X-100 to 1%. After removing cell debris by centrifugation, lysates (25 µg protein) were run on 12% discontinuous polyacrylamide gels and transferred to nitrocellulose membranes (Schleicher & Schuell) by electroblotting.

After washing and blocking the membrane (Ausubel et al. 1989), a 1:10,000 dilution of the polyclonal antibody preparation was added and incubated for 1 h at room temperature. After extensive washing, the antibody was detected with peroxidase-conjugated goat antimouse IgG (Jackson Immunoresearch, West Grove, PN, USA) and an enhanced chemiluminescence detection system (Pierce, Rockford, IL, USA). Pooled immune ascites fluid from injected mice, as well as immune serum, were reactive against the *M. voltae* protein as well as the *M. maripaludis* and *M. jannaschii* proteins, albeit with reduced affinity. We estimated the smallest amount of each protein that could be detected with the antibody as 0.25 ng, 1 ng, and 5 ng for the *M. voltae*, *M. maripaludis*, and *M. jannaschii* RadA proteins, respectively (data not shown). On Western blots of cell extracts, a single protein band was observed. The antibody preparation did not bind *E. coli* RecA protein.

### ATPase activity

Reactions (20-µl volume) contained 12 µM (in nucleotides) single-stranded (ss) [+strand, positive strand] or double-stranded (ds) M13mp18 RF DNA (US Biochemical) and 4 µM recombinant RadA protein in buffer (50 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 20 mM KCl, 0.2 mM ATP, 1 mM DTT, 10 µg/ml bovine serum albumin [BSA]) supplemented with 0.2 µCi [α-$^{32}$P]ATP (3,000 Ci/mmol). At the indicated times, 1-µl aliquots were removed and spotted directly onto a cellulose PEI thin-layer chromatography plate (J.T. Baker; Mallinckrodt Baker, Phillipsburg, NJ, USA). After developing in 0.75 M $KH_2PO_4$, labeled ATP and released [$^{32}$P]$PO_4^{3-}$ were detected by autoradiography. For quantitative analysis, a Molecular Dynamics SP phosphorimager was used.

### DNA-binding assays

The DNA fragments used were a 251-bp fragment purified from a recombinant plasmid clone containing the tRNA$^{Val}$(GUY) gene from *Methanococcus vannielii* and the single-stranded noncoding strand derived from the same fragment and generated by asymmetrical PCR. Gel-purified DNA fragments, either ss or ds, were labeled with [γ-$^{32}$P]ATP and T4 polynucleotide kinase; unincorporated nucleotides were removed with the Nucleotide Removal Kit (Qiagen). Binding reactions (20 µl) were carried out in 50 mM Tris-HCl (pH 7.5), 15 mM $MgCl_2$, 40 mM KCl, 1 mM DTT, and 10 µg/ml BSA, either with or without 2 mM ATP. Typically, reactions contained 3.6 µM DNA (in nucleotides) and 0.4–3.6 µM RadA. After incubating the mixture for 20 min at the indicated temperatures, 1/5th volume 50% glycerol, 0.02 mg/ml bromophenol blue was added and the samples loaded immediately on 4% polyacrylamide gels in 1 × TBE (Tris-borate + EDTA) buffer containing 5% glycerol. Gels were run in 1 × TBE at 16.5 V/cm for 2 h at 4°C. Gels were transferred to 3MM paper and dried under vacuum for 1 h at 80°C. Labeled DNA was detected by autoradiography.

### Circular dichroism

The circular dichroism (CD) spectra of 5 µM and 10 µM RadA from *M. jannaschii* and *M. voltae*, respectively, were measured on a Jasco J-720W spectropolarimeter (Jasco, Easton, MD, USA), using a 0.4-mm cuvette in the far-UV region (200–250 nm) in 20 mM potassium phosphate buffer, pH 8.0. Spectra were collected in intervals of 5°C from 20° to 95°C and back to 20°C. Samples were allowed to equilibrate at the desired temperature before readings were taken.

### Complementation of an *E. coli recA* mutant

Recombinant pET-11b plasmids carrying copies of the *M. jannaschii radA* and *radB* genes, *M. maripaludis radA* and *radB* genes, and *E. coli recA* gene were transformed into *E. coli* strain HMS174(DE3)pLysS, which carries the *recA1* allele. Cells transformed with pET-11b vector plasmid (no insert) were used as the control. Cells were grown in Luria-Bertani (LB) broth to $OD_{600}$ = 0.35. Expression was induced by the addition of IPTG to a final concentration of 1 mM, and cells were grown for 2 h thereafter. Expression of the recombinant proteins was monitored by either Western blotting (for RadA) or by appearance of a protein band of the expected size in Coomassie-stained protein gels (for RadB and RecA). After serial dilutions of the cultures, 10-µl aliquots were spotted onto square-gridded LB plates in duplicate. The plates were irradiated with UV light (Stratalinker; Stratagene) at the indicated doses and incubated overnight at 37°C in the dark. Dose delivery was determined with a Spectroline DM-254x ultraviolet meter. Survival was calculated as the fraction of surviving colonies compared to the nonirradiated control.

## Results and discussion

### The *radA* genes of mesophilic and thermophilic Archaea

We cloned the *radA* gene cognates from the thermophile *M. jannaschii* and the mesophiles *M. voltae* and *M. maripaludis*. The thermophilic gene was cloned by PCR amplification based on the complete genome sequence of the organism (Bult et al. 1996). We had previously identified the *M. voltae* gene in a random genomic screen (GenBank accession number AF008421). The *M. maripaludis* gene was identified by heterologous hybridization to the *M. voltae* sequence in Southern blot analyses. The gene was sequenced on both strands (GenBank accession number AF322003).

Sequence comparisons of the mesophilic *M. voltae* and *M. maripaludis* RadA with the thermophilic *M. jannaschii* counterpart showed that the proteins follow the expected trends in amino acid abundance observed in large data sets comparing thermophilic and mesophilic proteins (Haney et al. 1999; Cambillau and Claverie 2000). There is an overall decrease in the content of uncharged polar residues and an increase in charged residues and hydrophobicity. Alignment of the protein sequences revealed that specific amino acid substitutions correlated with thermal adaptation are also observed, such as serine to lysine, serine to alanine, and lysine to arginine (Haney et al. 1999). A comparison between the *Pyrobaculum islandicum* RadA and the enzyme from the halophile *Haloferax volcanii* also revealed that helix-stabilizing substitutions occur (Spies et al. 2000), although this analysis is complicated by the dramatic difference in ionic strength milieu of the two organisms.

### Induction of radA expression in mesophilic and thermophilic Archaea by mutagenic agents

We investigated the response in evolutionarily related mesophilic and thermophilic methanococci to treatment

with MMS and UV light, two DNA-damaging agents. Cultures of *M. voltae* were exposed for different periods of time to increasing concentrations of MMS, an alkylating agent that causes double-strand breaks in DNA. RNA was prepared and levels of specific transcript were determined by Northern blot analyses. Treatment with 0.01% MMS increased mRNA levels more than 10-fold relative to the water-treated control (Fig. 1). Expression reached a maximum at 2 h and remained elevated for at least 8 h after treatment.

Lower doses of MMS resulted in lower levels of induction (data not shown); 10-fold-higher doses caused cell death. Cultures of the mesophile *M. maripaludis* subjected to a similar regimen showed similar behavior. Cells treated with 0.01% MMS showed 9.2-fold-increased levels of *radA*-specific transcript after a 2-h exposure (Fig. 1). In replicate experiments, expression of the *radA* gene in *M. maripaludis* was increased between 6- and 10-fold after exposure to 0.01% MMS for 2 h. Both *M. voltae* and *M. maripaludis* also showed increased levels of expression of the *radA* gene following exposure to UV light. *M. maripaludis* cells increased the level of *radA* mRNA by approximately 6-fold (Fig. 1); in *M. voltae*, the levels of induction were comparable (data not shown).

The sizes of the *radA*-specific transcripts were remarkably different in the two mesophiles. The *M. maripaludis* transcript is about 1.15 kb, whereas the *M. voltae* transcript was considerably larger, about 1.7 kb. To confirm this unexpected size difference, we mapped the 5′-end of the *M. voltae* transcript using reverse transcription from a $^{32}$P-labeled oligonucleotide complementary to positions 51–76 of the coding region of the *radA* gene. The size of the extension product (~670 nucleotides) confirmed the presence of an additional ~600 nt upstream of the *radA* coding sequence (Fig. 2). We sequenced 395 nt of this upstream region (GenBank accession number AF008421) without discovering any similarity to other DNA or protein sequences in the databases. The sequence, which contains multiple stop codons in all three frames, is unlikely to be translated. The significance of this long 5′-leader sequence in an organism with a compact genome is unknown.

It has been reported that treatment of thermophilic Archaea with DNA-damaging agents does not increase the levels of *radA* message significantly (Sandler et al. 1996; Komori et al. 2000). In view of our results showing *radA* induction in mesophilic Archaea, we reevaluated the response of thermophilic Archaea to mutagenic challenge. We measured levels of *radA*-specific transcript in *M. jannaschii* and *S. solfataricus* cells treated with MMS, exposed to UV light, or mock treated (Fig. 3). *M. jannaschii* cells treated with 0.01% MMS for 2 h showed a 2.6-fold increase of *radA* message compared to the control. In cells exposed to UV light (50 J/m$^2$), expression of the *radA* gene increased 2.5-fold. Although small, the differences between control and treated cells (by both MMS and UV light) were consistent and reproducible. When the experiments were performed with *S. solfataricus* cells, the results were essentially identical; both MMS and UV light treatments led to modest (~2-fold), but reproducible, increases in expression of the *radA* gene.

Our results are not necessarily at odds with those reported earlier. In the case of UV treatment of *S. solfataricus* cells (Sandler et al. 1996), the authors suggested the possibility of a modest increase in *radA* expression, but the assay used was not sensitive enough for confidence. *P. furiosus*, with an optimal growth temperature of 100°C, did not increase expression of the *radA* gene when subjected to UV light, gamma irradiation, or increased temperature (Komori et al. 2000). It is likely that this hyperthermophile, which constantly undergoes high rates of DNA damage, maintains high levels of repair enzymes and that exposure to mutagenic agents has little or no effect on their expression.

## Increases in *radA*-specific mRNA correlate with increases in RadA protein

To determine whether the increased levels of specific mRNA led to increased levels of the protein, we measured

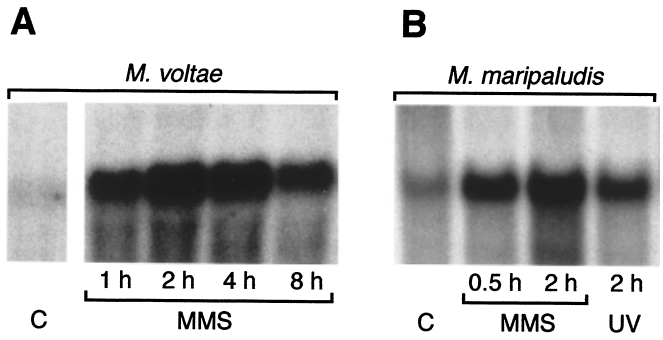


**Fig. 1.** Northern blot analysis of *radA* gene expression in mesophilic Archaea in response to treatment with DNA-damaging agents. Exponentially growing *Methanococcus voltae* (**A**) and *Methanococcus maripaludis* (**B**) cells were treated with either 0.01% methylmethane sulfonate (MMS) or UV light (50 J/m$^2$); at the indicated times after treatment, RNA was prepared, electrophoresed, and probed for specific *radA* transcript. Bound probe was quantitated and normalized for loading variation using 16S and 23S ribosomal RNA as standards. *C*, untreated cells

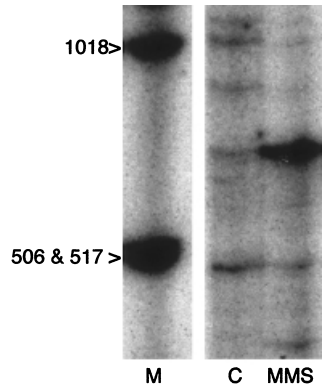


**Fig. 2.** Primer extension analysis of RNA isolated from *M. voltae* control cells and cells treated with 0.01% MMS for 2 h. A primer complementary to positions 51–76 of the *radA* gene coding region was annealed to 5 μg of total RNA and extended with reverse transcriptase. *M*, size markers (in base pairs, bp); *C*, untreated control cells

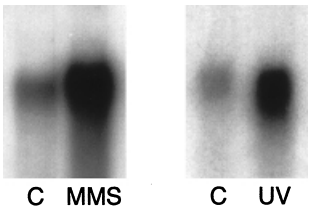


**Fig. 3.** Northern blot analysis of *radA* gene expression in the thermophile *Methanococcus jannaschii* in response to treatment with DNA-damaging agents. Exponentially growing cells were treated with 0.01% MMS or UV light (50 J/m$^2$). At 2 h after treatment, RNA was prepared, electrophoresed, and probed for *radA*-specific transcript. Bound probe was quantitated, and then normalized using 16S and 23S ribosomal RNA as standards. *C*, untreated control cells

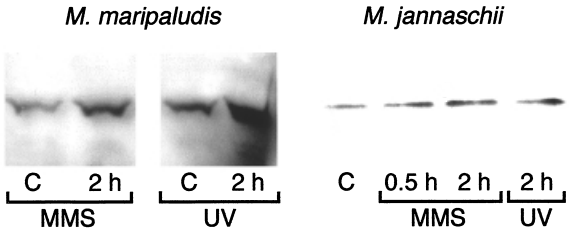


**Fig. 4.** Western blot analysis of RadA protein present in cell lysates. Exponentially growing cells of the mesophile *M. maripaludis* (*left*) and the thermophile *M. jannaschii* (*right*) were treated with 0.01% MMS or UV light (50 J/m$^2$). At the indicated times, cells were collected, lysed, and resolved by SDS-PAGE. Following transfer, RadA was detected with polyclonal antibodies. *C*, lysate from cells collected before treatment

RadA protein abundance immunologically in extracts of mesophilic and thermophilic Archaea before and after exposure to mutagenic agents. Treatment of *M. maripaludis* cultures with MMS or UV light led to increased amounts of RadA protein in extracts relative to the untreated control (Fig. 4, left side); the increases mirrored the increase of *radA*-specific transcripts. In a similar analysis of control and mutagen-treated *M. jannaschii* cells, the increase in RadA protein in cell extracts was very modest (Fig. 4, right side), mirroring the modest increase in the levels of *radA*-specific transcript observed in the thermophile.

Motivated by the possibility that the relatively modest increase in mRNA and protein in the thermophiles after mutagenic treatment might result from constitutive high levels of expression, we used Western blot analyses to determine the amount of RadA protein present in extracts of exponentially growing untreated cells. Cells were grown to a density of $2 \times 10^8$ cells/ml, lysates were prepared and resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and RadA was detected on Western blots. By comparison of band intensities to those of measured amounts of the corresponding recombinant proteins, we estimated that in *M. jannaschii*, *M. voltae*, and *M. maripaludis* RadA is approximately 0.1% of the total soluble protein. Therefore, and surprisingly, mesophilic and thermophilic methanococci have comparable amounts of RadA protein.

Recombinatorial repair is facilitated by maintaining multiple copies of the genome. The advantage of polyploidy has been dramatically demonstrated by the ability of heavily gamma-irradiated *Deinococcus radiodurans* and *Pyrococcus furiosus* to repair hundreds of double-strand breaks in their genomes (Minton 1996; DiRuggiero et al. 1997). *M. jannaschii* cells contain 1 to 5 genome copies in stationary phase and 3 to 15 copies during exponential growth (Malandrin et al. 1999); unfortunately, no data exist for mesophilic Archaea. The presence of multiple genome copies may make more efficient use of the available RadA, thereby decreasing their need for this protein. The lower genome content of stationary-phase cells might suggest the need for larger amounts of RadA. However, when we compared RadA content in cell extracts as a function of growth stage, we observed less RadA in stationary-phase cells than in exponentially growing cells (data not shown).

## Comparison of the ATPase and DNA-binding activities of the mesophilic and thermophilic RadA proteins

The fact that mesophilic and thermophilic Archaea have comparable amounts of RadA protein raises the question of whether the protein is more active in the thermophile. To this end, we compared the meso- and thermophilic proteins biochemically. The purified enzymes were assayed for their ATPase activity, both intrinsic and in the presence of double-stranded (ds) and single-stranded (ss) DNA, after optimizing conditions. The enzymes have quite different ionic optima: 20 mM KCl for *M. voltae* and 140 mM KCl for *M. jannaschii*. The mesophilic protein has a temperature optimum for ATPase activity of 55°C, whereas the thermophilic protein shows increasing activity up to 83°C. Activity at higher temperatures was not assayed because the appearance of an unidentified protein-independent reaction product interfered with the measurements. Subsequently, activity of the mesophilic enzyme was assayed routinely at 37°C whereas the thermophilic enzyme was assayed at 83°C under mineral oil. The *M. jannaschii* protein gradually loses activity with incubation at high temperatures. After 1 h at 75°C, activity is reduced about threefold; no activity is detectable after 1 h at 90°C.

ATP hydrolysis as a function of time by the *M. voltae* enzyme in the absence or in the presence of ds- or ss DNA and by the *M. jannaschii* enzyme in the presence of ss DNA are shown in Fig. 5. At saturating (0.2 mM) ATP concentration, the mesophilic and thermophilic proteins do not differ significantly in their rate of ATP cleavage. The measured turnover rate in the presence of ss DNA (1.2 moles ATP hydrolyzed/mole RadA/min) is significantly lower than that of *E. coli* RecA (~30 mol/mol/min) (Kowalczykowski et al. 1994) but is comparable to that of eukaryotic Rad51 (Sung 1994). These turnover rates are about six times higher than that of the *S. solfataricus* enzyme (Seitz et al. 1998), but about three times lower than that of the *P. islandicum* enzyme (Spies et al. 2000). ATPase activity increased with increasing ss DNA concentration until a plateau was reached at a stoichiometry of three nucleotides per protein, as has been observed for RecA (Lauder and Kowalczykowski 1991), Rad51 (Sung 1994), and archaeal RadA (Seitz et al. 1998; Komori et al. 2000). Using [$\alpha$-$^{32}$P]- and [$\gamma$-$^{32}$P]-labeled ATP substrates, we

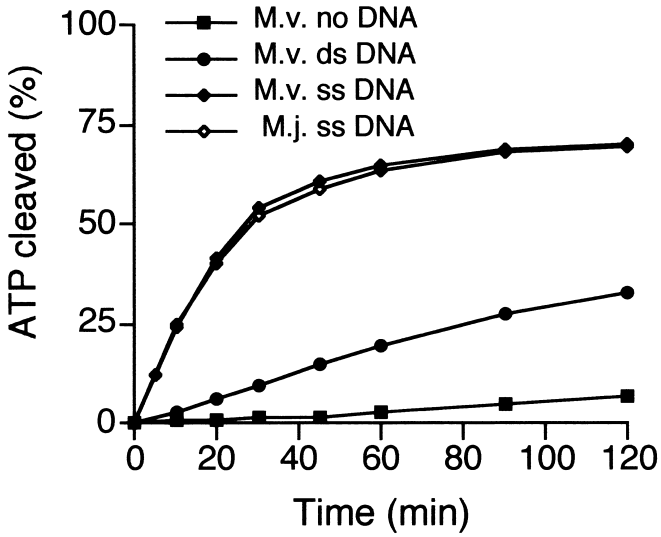


**Fig. 5.** ATPase activity of mesophilic and thermophilic RadA proteins. The proteins were assayed at the optimal growth temperatures (37°C for *M. voltae* and 83°C for *M. jannaschii*) and the respective salt optima of the organisms. The fractions of ATP cleaved as a function of time for the mesophilic protein in the absence or presence of double-stranded (*ds*) and single-stranded (*ss*) DNA and for the thermophilic protein in the presence of ss DNA are shown

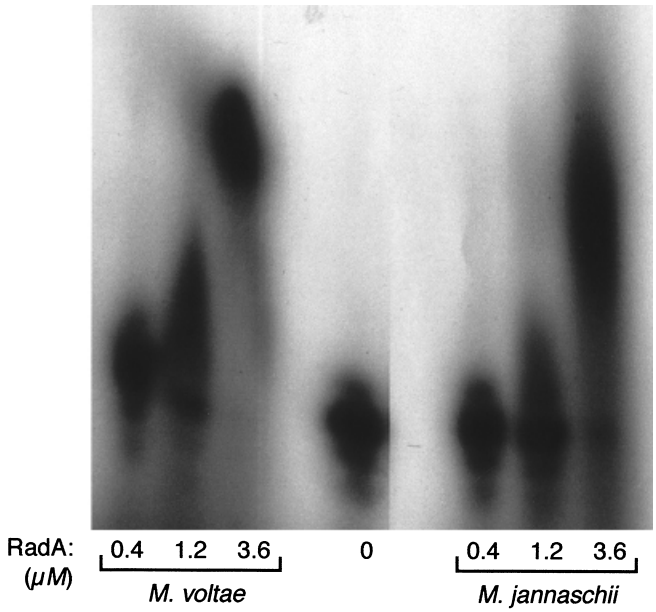


**Fig. 6.** ss DNA-binding activity of mesophilic and thermophilic RadA proteins. A radiolabeled 281-nucleotide ss DNA fragment (3.6 μM) was incubated with increasing amounts of either *M. voltae* or *M. jannaschii* RadA protein at 37°C and 75°C, respectively. Protein-bound DNA was separated from unbound DNA on a native polyacrylamide gel and the species was visualized by autoradiography. *0*, control with no RadA protein

confirmed that the hydrolytic products are ADP and phosphate (data not shown).

We analyzed the DNA-binding activity of the mesophilic and thermophilic proteins using electrophoresis gel mobility shift assays. Both proteins showed similar mono- and divalent cation optima for DNA binding, namely 40 mM KCl and 15 mM $MgCl_2$, respectively. The mesophilic and thermophilic proteins showed comparable ss DNA-binding activities (Fig. 6). Also, both archaeal RadA proteins bound ds- and ss DNA with similar efficiency, and this binding did not require ATP (data not shown). These features are similar to those of the eukaryotic proteins (Zaitseva et al. 1999 and references therein) and contrast with those of bacterial RecA (Pugh and Cox 1988). These observations are consistent with sequence comparisons indicating that the archaeal RadA proteins more closely resemble Rad51 than RecA (Eisen and Hanawalt 1999; Aravind et al. 1999). The ATP-independent binding of RadA to DNA is an important feature and suggests that DNA breaks in nongrowing cells may be bound by the protein and their repair deferred until growth resumes.

## Circular dichroism

Although biochemical measurements indicated that *M. jannaschii* RadA is active at higher temperatures than is *M. voltae* RadA, this method does not directly evaluate protein stability. Thus, we used circular dichroism (CD) to monitor the folding state of RadA as a function of temperature. CD spectra of purified recombinant *M. jannaschii* and *M. voltae* RadA proteins were collected at temperatures from 20° to 95°C (Fig. 7A,B). The downward peaks at 210 nm and 220 nm show that both proteins are largely alpha helical in structure, as is RecA (Wittung et al. 1995). On heating, the ellipticities become less negative (shift up), and the peaks flatten and shift. This change corresponds to a change in secondary structure and, ultimately, to the denatured state. When the ellipticities at 222 nm are plotted against temperature, the thermophilic protein shows a distinct transition with an inflection point at ~75°C (Fig. 7C). This shift may signal a change in conformation necessary for thermostability or function, as has been described by Spies et al. (2000). Whether this shift corresponds to different catalytic modes below and above a temperature threshold remains to be explored.

After holding the proteins at 95°C for 25 min, spectra were taken on cooling to 75°, 55°, and 20°C. Although the ellipticities became more negative, they never returned to their original magnitude or spectral shape, even after extended incubation (data not shown). This result suggests that the protein is unable to renature on cooling, consistent with the drop in ATPase activity observed after high-temperature incubations of *M. jannaschii* protein (see above).

In comparison to *M. jannaschii* RadA, the *M. voltae* protein showed a more gradual change in ellipticity with temperature (Fig. 7A,C). This result suggests a progressive unraveling of the protein structure with temperature, rather than cooperative unfolding. As with *M. jannaschii* RadA, once the shape of the CD spectrum shifts, it retains this shape on cooling.

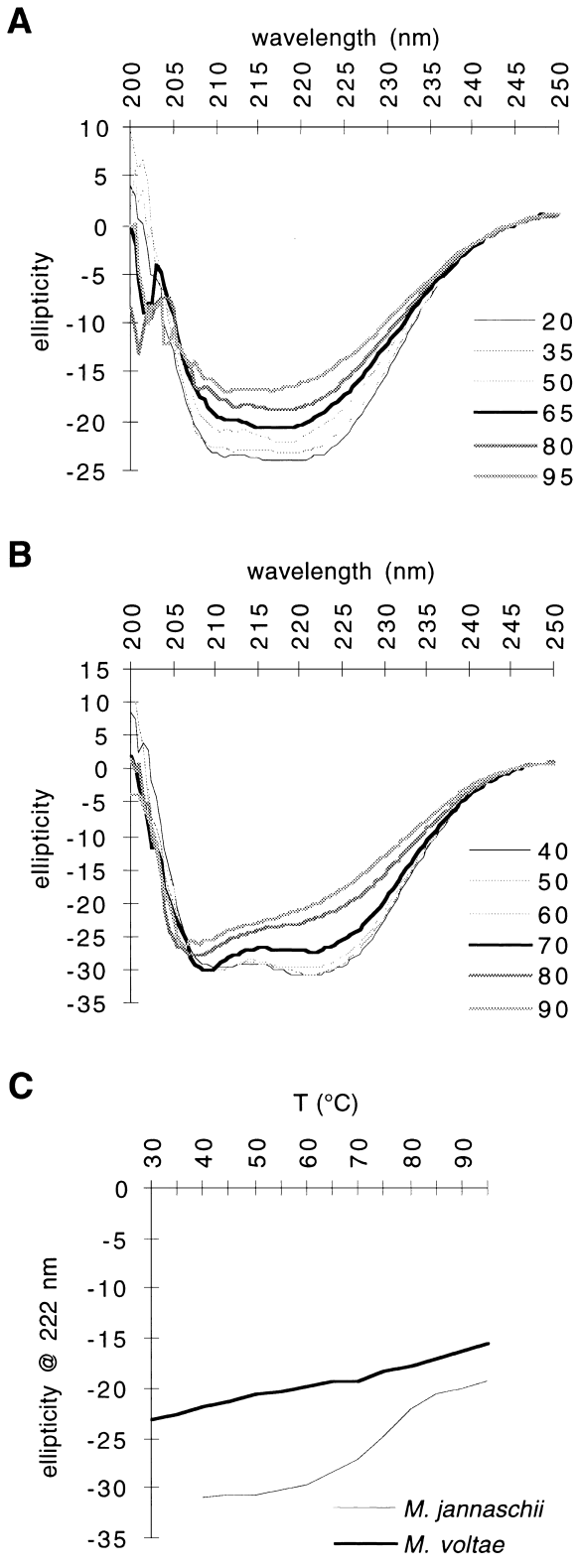


**Fig. 7A–C.** Circular dichroism (CD) analysis of mesophilic and thermophilic RadA proteins. **A** Far-UV CD spectra of 10 μM *M. voltae* RadA in phosphate buffer at increasing temperatures; measurements are shown at 15°C intervals between 20° and 95°C. **B** Far-UV CD spectra of 5 μM *M. jannaschii* RadA in phosphate buffer at increasing temperatures; measurements are shown at 10°C intervals between 40° and 90°C. **C** Temperature dependence of the residue molar ellipticity at 222 nm of *M. voltae* and *M. jannaschii* RadA proteins

## Expression of the *radB* gene is not induced by mutagenic treatment

In all Archaea sequenced to date, a second protein showing similarity to the RecA/Rad51 family has been identified. Named RadB, the function of this protein has not been elucidated. Phylogenetic analyses have related this protein to three groups of ATPases, all of which are composed of an RecA-like domain (showing similarity to archaeal RadB) fused to other functional protein domains (Aravind et al. 1999; Leipe et al. 2000). Although these analyses confirm the relatedness of the core ATPase sequences, they do not give clues as to the in vivo function of the RadB protein. To shed more light on its cellular roles, we have monitored expression of the *radB* gene in conditions where the *radA* gene is induced.

Northern blots of the same preparations of RNA used in the *radA* expression analyses (see earlier) were probed with $^{32}$P-labeled fragments of *radB* genes. We did not observe any significant increase in the level of *radB*-specific transcript in experiments in which the mesophilic *radA* gene was induced ninefold by MMS treatment and sixfold by UV-light treatment (Fig. 8). Similarly, the small but reproducible increases in *M. jannaschii radA* gene expression were not mirrored by concomitant increases in the level of *radB*-specific message (data not shown). Although some data suggest an interaction of RadB with RadA and an involvement in the resolution of the branched DNA structures that form during recombination (Komori et al. 2000), these proteins are neither present in similar stoichiometries (Komori et al. 2000) nor are they coregulated, as we demonstrated here. Although these latter observations do not vitiate the proposed interaction with RadA, they do nothing to support it.

Not only is there no evidence for induction, but in the case of MMS-treated cells (both meso- and thermophile), *radB*-specific RNA actually decreased while rRNA levels in the gel lanes were unchanged from those of the controls. Although we cannot readily explain this observation, we note that the MMS-treated cells showed a sharper decline in growth rate after treatment than the UV light-treated cells. In *Pyrococcus*, *radB* is in an operon with the replicative DNA polymerase, and it may play a role in DNA replication (Hayashi et al. 1999). This result would be consistent with the possible correlation between *radB* expression and growth rate observed after MMS treatment.

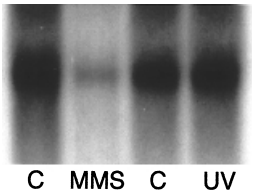


**Fig. 8.** Northern blot analysis of *radB* gene expression in the mesophile *M. maripaludis* in response to treatment with DNA-damaging agents. Exponentially growing cells were treated with 0.01% MMS or UV light (50 J/m$^2$). At 2 h after treatment, cells were collected and their RNA was prepared, electrophoresed, and probed for *radB*-specific transcript. *C*, untreated control cells

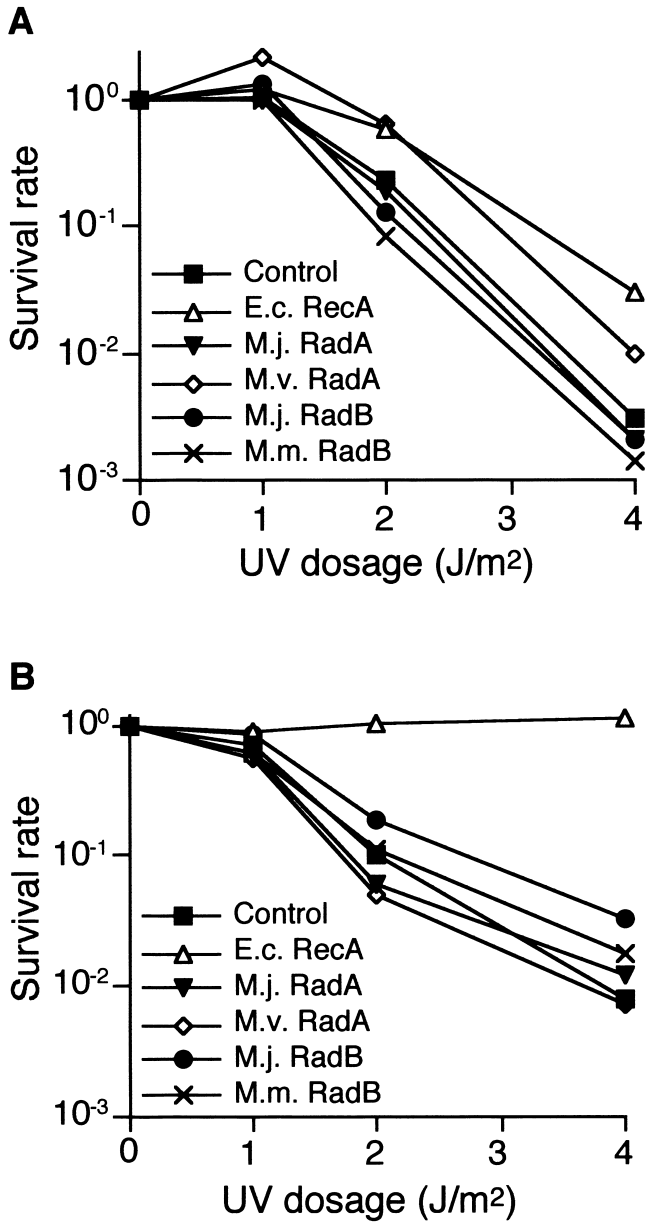


**Fig. 9A,B.** UV survival of *Escherichia coli recA1* strain HMS174(DE3)pLysS harboring plasmids expressing mesophilic and thermophilic *radA* and *radB* genes. Transformants were grown to $OD_{600} = 0.35$, and expression of the cloned genes was induced by addition of isopropyl thiogalactoside (IPTG). Aliquots were irradiated at the indicated doses, and fractional survival rate was determined by plating dilution series and counting colonies after overnight incubation at 37°C in the dark. **A** Survival of cells without addition of IPTG. **B** Survival of cells treated for 2 h with 1 mM IPTG

Neither RadA nor RadB complements the UV-sensitive phenotype of a *recA1 E. coli* host

It has been reported that expression of archaeal RadB protein in an *E. coli* host carrying the *recA1* allele restores tolerance to UV irradiation (Rashid et al. 1996). In view of the significant difference in biochemical properties between this protein and bacterial RecA and the failure of the *radB* gene to be induced by treatments that induce the *radA* gene, we reexamined the ability of the RadB proteins to complement the UV light sensitivity of an *E. coli recA1* strain. We also performed a similar analysis for the RadA proteins. As controls, untransformed *E. coli* cells and cells expressing the recombinant *E. coli recA* wild-type gene were used. Figure 9 shows the survival rates after UV exposure for the cell lines expressing the different proteins, both before and after induction. Expression of the wild-type *E. coli recA* gene in a *recA1* background rescued the mutant phenotype, but expression of the archaeal proteins had no significant effect on survival rates when compared with the untransformed *E. coli* control. A recent report examining the potential complementation of the *recA* phenotype by RadB from the hyperthermophile *Pyrococcus furiosus* yielded results similar to ours (Komori et al. 2000).

## Concluding remarks

Our results demonstrated that in the thermophiles *M. jannaschii* and *S. solfataricus radA*-specific mRNA and RadA protein increase modestly after treatment with UV light and MMS. The increases in mRNA and protein following treatment are much more pronounced in the mesophiles *M. voltae* and *M. maripaludis*. Surprisingly, the mesophiles and the thermophile *M. jannaschii* do not differ significantly in the amount of RadA protein present in untreated cells. Thus it appears that the need for very efficient DNA repair systems in the thermophiles is not explained by higher levels of RadA protein per cell, as compared to the mesophiles, nor is it explained by measurable differences in the activity of the proteins (see Results and discussion). This finding is even more enigmatic when one considers that after mutagenic treatment there will be more RadA in the mesophiles than in the thermophile. Conceivably, other factors or proteins could increase the activity of the RadA protein in vivo in the thermophile.

Organisms have evolved a multitude of mechanisms for coping with the harsh conditions imposed on DNA stability by life at high temperatures. Factors that stabilize duplex structure (such as high intramolecular salt concentrations, polyamines, and reverse gyrase) and biochemical strategies aimed at efficiently repairing damage to the primary structure of DNA contribute to the fitness of hyperthermophiles (Grogan 1998). RadA is just one of the players in efficient DNA maintenance and repair. A modest number of DNA repair proteins have been uncovered in the archaeal genomes sequenced to date (Aravind et al. 1999; Eisen and Hanawalt 1999; Grogan 2000). It is likely that other proteins involved in these processes are different enough from known repair proteins in both Bacteria and Eucarya to have eluded identification. Comparative analyses of complete archaeal genomes have revealed coding regions that are specific to Archaea and which are not represented in the other two domains of life (Makarova et al. 1999; Graham et al. 2000). It is likely that, among this pool of Archaea-specific genes, there are some directly involved in DNA repair and genome stability, especially in the thermophilic

lineages. Biochemical and genetic approaches are needed to reveal them.

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## References

Aravind L, Walker DR, Koonin EV (1999) Conserved domains in DNA repair proteins and evolution of repair systems. Nucleic Acids Res 27:1223–1242

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JD, Smith JA, Struhl K (1989) In: Current protocols in molecular biology. Wiley-Interscience, New York, pp 4.8.1–4.8.3

Basile G, Aker M, Mortimer RK (1992) Nucleotide sequence and transcriptional regulation of the yeast recombinatorial repair gene *RAD51*. Mol Cell Biol 12:3235–3246

Baumann P, West SC (1998) Role of the human RAD51 protein in homologous recombination and double-stranded-break repair. Trends Biochem Sci 23:247–251

Bianco PR, Tracy RB, Kowalczykowski SC (1998) DNA strand exchange proteins: a biochemical and physical comparison. Front Biosci 3:d570–d603

Boone DR, Whitman WB, Rouviere P (1993) Diversity and taxonomy of methanogens. In: Ferry JG (ed) Methanogenesis. Chapman & Hall, New York, pp 35–80

Brendel V, Brocchieri L, Sandler SJ, Clark AJ, Karlin S (1997) Evolutionary comparisons of RecA-like proteins across all major kingdoms of living organisms. J Mol Evol 44:528–541

Bult CJ, White O, Olsen GJ, Zhou L, Fleischmann RD, Sutton GG, Blake JA, FitzGerald LM, Clayton RA, Gocayne JD, Kerlavage AR, Dougherty BA, Tomb J-F, Adams MD, Reich CI, Overbeek R, Kirkness EF, Weinstock KG, Merrick JM, Glodek A, Scott JL, Geoghagen NSM, Wiedman JF, Fuhrmann JL, Nguyen D, Utterback TR, Kelley JM, Peterson JD, Sadow PW, Hanna MC, Cotton MD, Roberts KM, Hurst MA, Kaine BP, Borodovsky M, Klenk H-P, Fraser CM, Smith HO Woese CR, Venter JC (1996) Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. Science 273:1017–1140

Cambillau C, Claverie J-M (2000) Structural and genomic correlates of hyperthermostability. J Biol Chem 275:32383–32386

DiRuggiero J, Santangelo N, Nackerdien Z, Ravel J, Robb FT (1997) Repair of extensive ionizing-radiation DNA damage at 95°C in the hyperthermophilic archaeon *Pyrococcus furiosus*. J Bacteriol 179:4643–4645

Eisen JA, Hanawalt PC (1999) A phylogenomic study of DNA repair genes, proteins, and processes. Mutat Res 435:171–213

Graham DE, Overbeek R, Olsen GJ, Woese CR (2000) An archaeal genomic signature. Proc Natl Acad Sci USA 97:3304–3308

Grogan DW (1998) Hyperthermophiles and the problem of DNA instability. Mol Microbiol 28:1043–1049

Grogan DW (2000) The question of DNA repair in hyperthermophilic Archaea. Trends Microbiol 8:180–185

Haney PJ, Badger JH, Buldak GL, Reich CI, Woese CR, Olsen GJ (1999) Thermal adaptation analyzed by comparison of protein sequences from mesophilic and extremely thermophilic *Methanococcus* species. Proc Natl Acad Sci USA 96:3578–3583

Hayashi I, Morikawa K, Ishino Y (1999) Specific interaction between DNA polymerase II (PolD) and RadB, a Rad51/Dmc1 homolog, in *Pyrococcus furiosus*. Nucleic Acids Res 27:4695–4702

Jacobs KL, Grogan DW (1997) Rates of spontaneous mutation in an archaeon from geothermal environments. J Bacteriol 179:3298–3303

Komori K, Miyata T, DiRuggiero J, Holley-Shanks R, Hayashi I, Cann IKO, Mayanagi K, Shinagawa H, Ishino Y (2000) Both RadA and RadB are involved in homologous recombination in *Pyrococcus furiosus*. J Biol Chem 275:33782–33790

Kowalczykowski SC (1991) Biochemistry of genetic recombination: energetics and mechanism of DNA strand exchange. Annu Rev Biophys Biochem 20:539–575

Kowalczykowski SC, Dixon DA, Eggleston AK, Lauder SD, Rehrauer DM (1994) Biochemistry of homologous recombination in *Escherichia coli*. Microbiol Rev 58:401–465

Lauder SD, Kowalczykowski SC (1991) Asymmetry in the recA protein-DNA filament. J Biol Chem 266:5450–5458

Leipe DD, Aravind L, Grishin NV, Koonin EV (2000) The bacterial replicative helicase DnaB evolved from a RecA duplication. Genome Res 10:5–16

Makarova KS, Aravind L, Galperin MY, Grishin NV, Tatusov RL, Wolf YI, Koonin EV (1999) Comparative genomics of the Archaea (Euryarchaeota): evolution of conserved protein families, the stable core, and the variable shell. Genome Res 9:608–628

Malandrin L, Huber H, Bernander R (1999) Nucleoid structure and partition in *Methanococcus jannaschii*: an archaeon with multiple copies of the chromosome. Genetics 152:1315–1323

Marguet E, Forterre P (1994) DNA stability at temperatures typical for hyperthermophiles. Nucleic Acids Res 22:1681–1686

Minton KW (1996) Repair of ionizing-radiation damage in the radiation resistant bacterium *Deinococcus radiodurans*. Mutat Res 363:1–7

Ogawa T, Shinohara A, Nabetani A, Ikeya T, Yu X, Egelman EH, Ogawa H (1993) RecA-like recombination proteins in eukaryotes: functions and structures of *RAD51* genes. Cold Spring Harbor Symp Quant Biol 58:567–576

Peak MJ, Robb FT, Peak JG (1995) Extreme resistance to thermally induced DNA backbone breaks in the hyperthermophilic Archaeon *Pyrococcus furiosus*. J Bacteriol 177:6316–6318

Pugh BF, Cox MM (1988) General mechanism for RecA protein binding to duplex DNA. J Mol Biol 203:479–489

Rashid N, Morikawa M, Imanaka T (1996) A RecA/RAD51 homologue from a hyperthermophilic archaeon retains the major RecA domain only. Mol Gen Genet 253:397–400

Rashid N, Morikawa M, Nagahisa K, Kayana S, Imanaka T (1997) Characterization of a RecA/RAD51 homologue from the hyperthermophilic archaeon *Pyrococcus* sp. KOD1. Nucleic Acids Res 15:719–726

Robb FT, Place AR (1995) Media for thermophiles. In: Robb FT (ed) Archaea: a laboratory manual. Thermophiles. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp 167–188

Roca AI, Cox MM (1990) The RecA protein: structure and function. Crit Rev Biochem Mol Biol 25:415–456

Roca AI, Cox MM (1997) RecA protein: structure, function, and role in recombinational DNA repair. Prog Nucleic Acid Res Mol Biol 56:129–223

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Plainview, NY

Sandler SJ, Satin LH, Samra HS, Clark AJ (1996) *recA*-like genes from three archaean species with putative protein products similar to Rad51 and Dmc1 proteins of the yeast *Saccharomyces cerevisiae*. Nucleic Acids Res 24:2125–2132

Sandler SJ, Hugenholtz P, Schleper C, DeLong EF, Pace NR, Clark AJ (1999) Diversity of radA genes from cultured and uncultured Archaea: comparative analysis of putative RadA proteins and their use as a phylogenetic marker. J Bacteriol 181:907–915

Seitz EM, Brockman JP, Sandler SJ, Clark AJ, Kowalczykowski SC (1998) RadA protein is an archaeal RecA protein homolog that catalyzes DNA strand exchange. Genes Dev 12:1248–1253

Spies M, Kil Y, Maui R, Kato R, Kujo C, Ohshima T, Kuramitsu S, Lanzov V (2000) The RadA protein from a hyperthermophilic archaeon *Pyrobaculum islandicum* is a DNA-dependent ATPase that exhibits two disparate catalytic modes, with a transition temperature at 75°C. Eur J Biochem 267:1125–1137

Sung P (1994) Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. Science 265:1241–1243

Walker GC (1984) Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol Rev 48:60–93

West SC (1992) Enzymes and molecular mechanisms of homologous recombination. Annu Rev Biochem 61:603–640

Wittung P, Norden, Takahashi M (1995) Secondary structure of RecA in solution. The effects of cofactors, DNA and ionic conditions. Eur J Biochem 228:149–154

Wood ER, Ghane F, Grogan DW (1997) Genetic responses of the thermophilic archaeon *Sulfolobus acidocaldarius* to short-wavelength UV light. J Bacteriol 179:5693–5698

Woods WG, Dyall-Smith ML (1997) Construction and analysis of a recombination-deficient (*radA*) mutant of *Haloferax volcanii*. Mol Microbiol 23:791–797

Zaitseva EM, Zaitsev EN, Kowalczykowski SC (1999) The DNA binding properties of *Saccharomyces cerevisiae* Rad51 protein. J Biol Chem 274:2907–2915