Transcription-coupled repair of UV damage in the halophilic archaea

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Abstract

Transcription-coupled repair (TCR) is a subpathway of nucleotide excision repair (NER) in which excision repair proteins are targeted to RNA polymerase-arresting lesions located in the transcribed strand of active genes. TCR has been documented in a variety of bacterial and eukaryotic organisms but has yet to be observed in the Archaea. We used *Halobacterium sp. NRC-1* and *Haloferax volcanii* to determine if TCR occurs in the halophilic archaea. Following UV irradiation of exponentially growing cultures, we quantified the rate of repair of cyclobutane pyrimidine dimers in the two strands of the *rpoB2B1A1A2* and the *trpDFEG* operons of *Halobacterium sp. NRC-1* and the *pts* operon of *Haloferax volcanii* through the use of a Southern blot assay and strand-specific probes. TCR was observed in all three operons and was dependent on the NER gene *uvrA* in *Halobacterium sp. NRC-1*, but not in *Haloferax volcanii*. The halophilic archaea likely employ a novel mechanism for TCR in which an as yet unknown coupling factor recognizes the arrested archaeal RNA polymerase complex and recruits certain NER proteins to complete the process.

Introduction

The sun's energy is essential to life on Earth, however the genotoxic effect of solar ultraviolet (UV) radiation has been a strong selection pressure for species of organisms since the beginning of life. DNA repair mechanisms exist in all organisms to remove pre-mutagenic lesions, avoid mutations, and prevent many other deleterious consequences associated with exposure to UV light.

UV light induces a number of photoproducts in DNA, most notably the cyclobutane pyrimidine dimer (CPD). Among the first DNA repair mechanisms to be discovered was photoreactivation, a light-dependent repair mechanism catalyzed by photolyases that results in the direct reversal of CPD formation. Photoreactivation has been identified in species of archaea,

bacteria, fungi, and yeast, but does not occur in placental mammals (1). Nucleotide excision repair (NER) is a well-characterized, light-independent DNA repair mechanism. NER enzymes recognize helix-distorting lesions like the CPD, remove the nucleotides surrounding the damage, and DNA polymerases and ligases fill the gap with newly synthesized DNA (2). In bacteria, UvrABCD proteins coordinate NER, while a number of proteins from the XP complementation group manage eukaryotic NER (1).

Transcription-coupled repair (TCR) is a subpathway of NER that specifically targets DNA damage in the transcribed strands of active genes in *Escherichia coli*, yeast, and mammalian cells (3). RNA polymerase arrests at the site of a DNA lesion, like a CPD, on the transcribed strand and initiates TCR. The arrested RNA polymerase is displaced from the lesion and NER proteins are recruited by a Transcription-Repair Coupling Factor (TRCF). The canonical TRCF is Mfd in bacteria and CS-B/RAD26 in eukaryotes (3). The hallmark feature of TCR is the more efficient rate of repair of CPDs located in the transcribed strand (TS) compared to those in the nontranscribed strand (NTS).

In this study, we investigated TCR in halophilic archaea. The Archaea are evolutionarily distinct from the Bacteria and Eukarya yet share bacterial and eukaryotic features. For example, some core metabolic functions of Archaea closely resemble those of Bacteria, while information processing functions, including transcription, are homologous to those of Eukaryotes. The halophilic archaea thrive in high salt environments and are found in hypersaline ponds and salt lakes such as the Dead Sea. In these environments the halophilic archaea are exposed to large amounts of sunlight and, like bacteria and eukaryotes, these archaea have DNA repair mechanisms to combat the DNA damage caused by UV radiation. Photoreactivation is very active in these organisms (4, 5) yet, in *Halobacterium sp.* NRC-1 and *Haloferax volcanii*, sequence homologs of the bacterial UvrABC excision repair machinery are required to remove UV-induced DNA damage

in the absence of photoreactivation (4, 6). In addition to this bacterial-type NER machinery, the halophilic archaea also possess a subset of eukaryotic-like XP homologs, whose role in these organisms is unknown (7, 8). There are no obvious homologs to either bacterial or eukaryotic TRCFs in the halophilic archaea (7), and TCR has not yet been observed in any archaeal species (16,17).

In this study, *Halobacterium sp.* NRC-1 and *Haloferax volcanii* were used to determine if TCR occurs following UV damage in the halophilic archaea. We report that TCR does occur in both species but with different requirements for the NER protein UvrA.

Methods

Cultivation. *Halobacterium sp.* NRC-1 strains DJC501 (*ura3*) and DJC519 (*ura3 uvrA*) were grown with aeration at 42°C in CM+ complex medium as described (4) and were subcultured in CM salts + 0.5% casamino acids + 50 ug/ml uracil supplemented with 50 ug/ml tryptophan as needed for repair experiments. *H. volcanii* strains H26 (*pyrE2*) and H509 (*pyrE2, uvrA*; gift of T. Allers, (6)) were grown with aeration at 45°C in Hv-YPC complex medium and subcultured in either Hv-min (9) or fructose minimal media (Hv-Min with 20 mM fructose rather than the glycerol-based carbon source supplement) with 50 ug/ml uracil (10).

Repair Experiments. Log phase cells were harvested by centrifugation and resuspended in an equal volume of either CM+ salts (*Halobacterium sp.* NRC-1) or 18% sea water (*H. volcanii*) (9). To prevent photoreactivation, all subsequent manipulations were performed in yellow light. A shallow layer of cells was irradiated in a petri dish with 254 nm UV light (dose rate of 0.8 J/m²/sec; total dose = 65 J/m² for *H. volcanii*, 75 J/m² for *Halobacterium sp.* NRC-1) and gentle agitation. Zero time point samples were removed and the remainder of the cells were transferred to a flask containing pre-warmed, concentrated amounts of appropriate media supplements and then cultured at the appropriate temperature with aeration. At the indicated times, samples were

collected and genomic DNA prepared by immediate lysis in TE followed by phenol:chloroform extraction or DNeasy genomic preps (Qiagen).

Southern Hybridization. Genomic DNA samples were treated with RNaseA and restricted with appropriate enzymes (see Figures 1-3A) according to manufacturer's instructions (New England Biolabs). Samples were divided in two and mock treated (50% glycerol) or treated with T4 endonuclease V (TEV, Epicentre), an enzyme that specifically nicks DNA directly adjacent to CPDs, according to manufacturer's instructions. Samples were then loaded onto 1% alkaline agarose gels and electrophoresed at low voltage overnight. Gels were neutralized, visualized by ethidium bromide staining and transferred to Hybond N+ membranes (GE Healthcare). Blots were then hybridized using strand-specific RNA (*rpo* and *pts*) or DNA (*trpDFEG*) probes as described (11). RNA probes were synthesized by Riboprobe in vitro transcription (Promega) using plasmids engineered to carry 400-500 bp fragments of each targeted gene flanked by T7 and SP6 promoters. RNA probes were labeled with ^{32}P (*pts*) or chemifluorescent tags (Alkaline Phosphatase Direct Labeling; GE Healthcare). DNA probes were end-labeled with ^{32}P -CTP. Detection was performed using a Fuji Phosphorimager, Molecular Dynamics Typhoon or Molecular Dynamics Storm Imaging System and the associated software programs. The frequency of CPDs per restriction fragment was calculated from the percentage of fragments with no CPDs (zero class) using the Poisson expression ($-\ln$ of the zero class = average number of dimers per fragment).

RNA Isolation and Quantitative Reverse Transcriptase PCR (RT-qPCR). Total RNA was isolated from cells using RNeasy Plus Mini Kit (Qiagen) and treated with RNase-Free DNase (Qiagen). DNA-free total RNA was reverse transcribed into cDNA with iScript cDNA Synthesis Kit (Bio-Rad). The cDNA synthesis reaction protocol was 5 minutes at 25°C, 30 minutes at 42°C, and 5 minutes at 85°C. qRT-PCR was performed using iTaq Universal SYBR Green Supermix (Bio-Rad) and the CFX Connect Real-Time PCR Detection System (Bio-Rad). The qPCR protocol

was 10 seconds at 95°C, 30 seconds at 55°C, and 30 seconds at 72°C. This cycle was repeated 40 times. The ribosomal protein L10 gene (*rpl10*) was used as a reference gene for *Haloferax volcanii* (10, 12, 13) and the ferredoxin gene (*ferA2*) was used as a reference gene for *Halobacterium sp.* NRC-1 (14, 15). Primer sequences are listed in Table 1.

Table 1. Primers used for RT-qPCR

Gene	Primers for qRT-PCR	Sequence 5'-3'	PCR Product [bp]
<i>H. volcanii rpl10e</i>	rpl10-F-PCR	GATTACGAACCCGGCGTACA	132
	rpl10-R-PCR	GGCGAATCTGCACTTCCTCT	
<i>H. volcanii ptsI</i>	pts-F-PCR	CAAGGTCGCCTACGTGTTCT	138
	pts-R-PCR	TGGCGTTCTCGTACATCTCG	
<i>H. volcanii ptfC</i>	ptfC-F-PCR	TCGGCTTCTCGTTCATTACC	187
	ptfC-R-PCR	ATGAACATGAACGGCTGGTT	
<i>Halobacterium sp.</i> NRC-1 <i>ferA2</i>	ferA2-F-PCR	CTCAACTACGAAACGCTGGA	151
		AGAACGGCCAGTCGTAGC	
<i>Halobacterium sp.</i> NRC-1 <i>rpoB1</i>	rpo-F-PCR	GTTCCACCATCTCGGAGACCT	152
	rpo-R-PCR	ATCAGCATGGAAACGACATC	
<i>Halobacterium sp.</i> NRC-1 <i>trpD1</i>	trpD-F-PCR	ACGACGCTGTTCGAGGAC	159
	trpD-R-PCR	CGTTGATGGTGTCGTAGTCG	

Results

TCR of the *rpo* operon of *Halobacterium sp. NRC-1*.

TCR of UV-induced DNA damage is evidenced by a faster rate of CPD repair in the transcribed strand (TS) of an actively transcribed gene than in the nontranscribed strand (NTS). Repair is assayed by monitoring the rate at which full-length restriction fragments are restored in samples treated with TEV, an enzyme that nicks DNA at CPDs. Denaturing gel electrophoresis, Southern blotting and the use of strand-specific probes are used to determine these rates in the two strands. The rates of CPD repair in the TS and NTS of the *rpoB2B1A1A2* (*rpo*) operon *Halobacterium sp. NRC-1* were measured. This operon is expressed constitutively from a single

Figure 1. Transcription-coupled repair of CPDs in the *rpo* operon of *Halobacterium*. (A) The *rpo* operon. (B) Representative data from two independent experiments. Blots were hybridized, stripped, and reprobbed with chemifluorescent-labelled RNA probes for the transcribed (TS) and nontranscribed (NTS) strands. (C) Averages of at least three independent repair experiments. Error bars represent standard errors.

promoter (14-16), making it a good

candidate for assessing the effects of TCR.

Using RT-qPCR analysis, we found that the expression level of the *rpo* genes was consistent with expression of *ferA2*, a constitutively expressed gene used as a reference for qPCR analysis (15). Treating log phase cells with 75 J/m² of UV-C induced approximately one lesion per strand in the 3557 bp PvuII-EcoRI fragment of the *rpo* operon. This dose leads to less than one log of cell killing in this species (4). We

found that the TS of the *rpo* operon was repaired more rapidly and to a greater extent than the NTS (Figure 1) in the first 6 hours after UV exposure. Restoration of the full length 3557 bp band in the TEV-treated samples was evident over time in the TS, but not in the NTS (Figure 1B).

Quantitatively, a significant difference in repair kinetics was observed by 2 hours after UV exposure (or treatment), and by 4 hours, the TS was almost completely repaired. In contrast, the NTS did not exceed 50% repair even after 6 hours (Figure 1C).

TCR of the *trpDFEG* operon of *Halobacterium*

To confirm and extend our findings in the *rpo* operon of *Halobacterium* sp. NRC-1, CPD removal from the *trpDFEG* operon was measured. We predicted that cells cultured in the absence of tryptophan and actively expressing this biosynthetic operon (Table 2) would perform TCR. However, cells recovering from UV exposure in the presence of tryptophan maintain low levels of *trpDFEG* expression and would not perform TCR. Repair rates were measured in a 3574bp SmaI

Figure 2. Transcription-coupled repair of CPDs in the *trpDFEG* operon of *Halobacterium* cultures grown in the presence (+Trp) or absence (No Trp) of tryptophan. (A) The *trpDFEG* operon. (B) Data from two independent experiments using cells starved for tryptophan. Blots were hybridized, stripped, and reprobed with 32 P-labeled DNA oligonucleotide probes for the transcribed (TS) and nontranscribed (NTS) strands. (C) Averages of 2 independent repair experiments. Error bars represent standard errors. Error bars not shown for +Trp samples for clarity.

restriction fragment from the *trpDFEG* operon; the similarity in target sizes allowed us to keep the UV dosage consistent with that used to study the *rpo* operon (Figure 2A). CPDs were removed more rapidly from the TS of the *trpDFEG* fragment than the NTS, but only in cells starved for tryptophan after UV (Figure 2, B and C). A significant difference in repair of the two strands was observed after 2 hours and was maintained through 6 hours. The extent of repair of the TS was less in this operon than the *rpo* operon, reaching only 75% after 6 hours.

The NTS in the tryptophan-starved cells was repaired at the same rate as both strands of the operon in cells supplemented with tryptophan, with less than 50% of the CPDs removed after 6 hours.

Table 2. Transcription induction in the *trpDFEG* and the *pts* operons. Fold induction was calculated using $\Delta\Delta C_t$ methods and represent averages ($\pm$ standard errors) of repeated qPCR assays on RNA from two (*trpD*) or three independent experiments.

Gene	Operon	Growth condition	-fold induction ($\pm$ SE)
<i>trpD</i>	<i>trpDFEG</i>	tryptophan starvation	2.6 ($\pm$ 1.5)
<i>ptfC</i>	<i>pts</i>	fructose supplementation	8.7 ($\pm$ 3.8)
<i>ptsI</i>	<i>pts</i>	fructose supplementation	4.8 ($\pm$ 0.8)

TCR occurs in the fructose inducible *pts* operon of *Haloferax volcanii*

We also investigated TCR in the fructose inducible *pts* operon of *Haloferax volcanii*. The genes encoded in the *pts* operon are responsible for fructose transport and catabolism and are highly expressed when *Haloferax volcanii* is grown in the presence of fructose (Table 2, (10, 13)). The

EcoNI-HpaI restriction fragment in the *pts* operon is slightly larger than the *rpo* and *trpDFEG* fragments of *Halobacterium sp.* NRC-1. Therefore, cells were treated with a lower dose of 65 J/m² of UV (Figure 3A). Repair assays of the *pts* operon in *Haloferax volcanii* yielded results similar to those observed with *trpDFEG* in *Halobacterium sp.* NRC-1. For cells grown in the presence of fructose, recovery of the full-length *pts* fragment in the TEV-treated samples was evident sooner in the TS than in the NTS (Figure 3B). Quantitative analysis confirmed that the TS was repaired with more rapid kinetics than the NTS (Figure 3C). As soon as one hour after UV treatment there was a significant difference

between the two strands that was maintained at all time points. Repair of

the TS reached approximately 75% after 5 hours while the NTS remained around 50% repaired.

Cells grown without fructose repaired the TS and the NTS at similar rates for the 5 hours following UV treatment (Figure 3C).

TCR is absent in *Halobacterium* $\Delta uvrA$ but can still occur in *Haloferax volcanii* $\Delta uvrA$

To test whether NER is required for TCR in *Halobacterium sp.* NRC-1 and *Haloferax volcanii*, repair assays were performed using strains that carry deletions of the NER gene *uvrA*. This gene is required for all UV-induced lesion repair in the absence of photoreactivation in *Halobacterium sp.* NRC-1 (4). Likewise, in this study, repair was not detectable in the TS or the

Figure 3. Transcription-coupled repair of CPDs in the *pts* operon of *Haloferax volcanii* cultures grown in the presence or absence of fructose. (A) The *pts* operon. (B) Data from two independent experiments with fructose. Blots were hybridized, stripped, and reprobed with ³²P-labeled RNA probes for the transcribed (TS) and nontranscribed (NTS) strands. (C) Averages from two (no fructose) or four (+ fructose) independent repair experiments. Error bars represent standard errors and are not shown for No Fructose samples for clarity.

Figure 4. CPD removal from the *trpDFEG* operon of a *Halobacterium uvrA* mutant (left panels and graph) and from the *pts* operon of a *Haloferax volcanii uvrA* mutant (right panels and graph). Blots were hybridized, stripped, and reprobed with 32 P-labeled probes for the transcribed (TS) and nontranscribed (NTS) strands. Percent repair was calculated from averages of two (*Halobacterium sp. NRC-1*) or four (*Haloferax volcanii*) independent repair experiments. Error bars depict standard error.

NTS of the *trpDFEG* operon in

Halobacterium sp. NRC-1 $\Delta uvrA$

(Figure 4, left panels). However, in

Haloferax volcanii $\Delta uvrA$ cells grown

in the presence of fructose, the TS of

the *pts* operon was still repaired,

albeit at a rate slower than that

observed in wildtype cells (compare

Figures 3&4). The NTS, in contrast,

was not detectably repaired. This

indicates that *uvrA* is not required for TCR of CPDs in *Haloferax volcanii*.

Discussion

Our data demonstrates that the TS of an expressed gene is repaired at a faster rate than the NTS in both *Halobacterium sp. NRC-1* and *Haloferax volcanii*. This strand bias was observed in three different operons, the *rpoB2B1A1A2* and *trpDFEG* operons of *Halobacterium sp. NRC-1*, and the *pts* operon of *Haloferax volcanii*. When cells were grown in conditions that did not promote high levels of transcription of *trpDFEG* (*Halobacterium sp. NRC-1*) or the *pts* operon (*Haloferax volcanii*), the TS and the NTS were repaired at the same rate. These results indicate that both *Halobacterium sp. NRC-1* and *Haloferax volcanii* perform TCR to repair CPDs in active genes. This is the first time TCR has been documented in the Archaea.

Previous research into TCR in the thermophilic archaea *Sulfolobus solfataricus* found no evidence to suggest that CPDs in transcribed strands of actively expressed genes were repaired at a faster rate compared to nontranscribed strands or other areas of the genome (17, 18). However, the authors of these studies concluded that the very rapid rate of global genome repair of CPDs in *S.*

solfatarius might mask any detectable effects of TCR. In contrast, the relatively slow rate of global genome repair we have observed in this study facilitated the detection of TCR. Several factors may contribute to the relatively slow genomic repair of CPDs in the halophilic archaea. First, the halophilic archaea are polyploid with anywhere from 15-25 copies of the chromosome/cell in exponentially growing cultures like those used in this study (19). Therefore, in the first few hours post-UV exposure, DNA repair proteins have more DNA to scan and repair than do repair proteins in a monoploid species like *Sulfolobus solfataricus* (19), particularly in the absence of photoreactivation. Compounding this issue is the fact that the halophilic archaea do not appear to upregulate any genes thought to be involved in UV lesion removal following UV exposure, including the bacterial NER homologs, *uvrA*, *uvrB*, *uvrC* and *uvrD* (20-22). In contrast, *Sulfolobus solfataricus* performs rapid removal of UV damage from both strands of active genes, even in the absence of photoreactivation and after UV doses that induce CPDs to comparable levels as those measured in this study (17, 18). While it remains unclear which DNA repair systems are upregulated after UV and to what degree, *Sulfolobus solfataricus* has a more complex post-UV transcriptional response than what has been observed in the halophilic archaea (8, 20, 22, 23). Finally, *Sulfolobus solfataricus* does not possess *uvr* homologs, indicating that this species of archaea employs UV repair mechanisms that are distinct from the halophiles, perhaps involving homologs to the eukaryotic XP genes (8).

The mechanism of TCR in halophilic archaea remains to be elucidated. RNA polymerase plays an essential role in recognizing lesions in the transcribed strand of active genes because without induced levels of transcription both strands are repaired at the same rate (Figures 2&3). The particular fate of an archaeal RNA polymerase arrested by a DNA lesion remains an open and intriguing question. In both bacterial and eukaryotic cells, an RNA polymerase complex arrested by a CPD signals a TRCF, Mfd in bacteria or *CS-B/RAD26* in eukaryotes, to displace the arrested

RNA polymerase and recruit NER proteins to the lesion (24). The halophilic archaea do not possess Mfd or CS-B sequence homologs, and a putative TRCF has not been identified.

Recent investigations in *E. coli* reveal an Mfd-independent TCR pathway that involves the proteins UvrD and NusA (25, 26), both of which have sequence homologs in the halophilic archaea. In this alternative pathway of TCR, NusA and UvrD associate with *E. coli* RNAP during elongation and work collaboratively to push a stalled RNAP backwards and unveil the DNA lesion. DNA repair of the lesion is accelerated by clearing away the lesion-masking RNAP and by subsequent recruitment of UvrA and UvrB proteins to the DNA damage site (3, 27, 28). The *nusA* sequence homolog in both *Haloferax volcanii* and *Halobacterium sp.* NRC-1 genomes is located directly adjacent to the *rpoB2B1A1A2* operon, a location that implies it may be a functionally homologous transcription factor. It is important to note that bacterial UvrD and NusA facilitate TCR by associating with bacterial RNAP, a complex that is not particularly similar to the archaeal RNAP. In fact, archaeal RNAP shares significantly more sequence and structural homology with eukaryotic RNAPII than with bacterial RNAP. However, studies of transcriptional regulation in the archaea reveal more similarity to bacterial regulatory mechanisms than to those of eukaryotes (29). While it is tempting to speculate that the NusA and UvrD homologs are involved in TCR in the halophilic archaea, further research is required to test this hypothesis.

We investigated the role of the NER gene *uvrA* in TCR in halophilic archaea using *uvrA* deletion strains of *Haloferax volcanii* and *Halobacterium sp.* NRC-1. Previous studies have indicated that *Halobacterium sp.* NRC-1 *uvrA* is a functional homolog of *E. coli uvrA* and is required for removal of UV lesions in the dark in *Halobacterium* (4). Likewise, in *Haloferax*, cells deficient in *uvrA* are significantly UV sensitive, a result that supports a role for this gene in promoting repair of UV lesions (6). Presumably, the archaeal UvrA plays a role in the initial damage recognition step of both global NER and TCR, similar to its role in bacteria (3, 4).

Consistent with previous reports, *Halobacterium* sp. NRC-1 *uvrA* mutants did not efficiently repair UV-induced damage in either the TS or the NTS. However, the TS of the *pts* operon in *Haloferax volcanii* was repaired to greater extent than the NTS indicating that TCR can occur independent of UvrA. A similar phenotype has been observed in mammalian cells deficient in XPC, hHRAD23b, DDB1 and/or DDB2, all factors required for initial damage recognition in overall NER but dispensable for TCR (30). UvrA appears to be dedicated to recognizing lesions and initiating global NER in *Haloferax* but this protein is apparently not required for TCR, where RNA polymerase performs the initial lesion recognition. More investigations into TCR are needed to reveal the unique mechanism of this process in the Archaea.

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References

1. **Friedberg, EC.** 2006. DNA repair and mutagenesis, (ed.), ASM Press, Washington.
2. **Goosen, N, Moolenaar, GF.** 2008. Repair of UV damage in bacteria. *DNA Repair (Amst)* **7**:353–379.
3. **Spivak, G, Ganesan, AK.** 2014. The complex choreography of transcription-coupled repair. *DNA Repair (Amst)* **19**:64–70.
4. **Crowley, DJ, Boubriak, I, Berquist, BR, Clark, M, Richard, E, Sullivan, L, DasSarma, S, McCready, S.** 2006. The *uvrA*, *uvrB* and *uvrC* genes are required for repair of ultraviolet light induced DNA photoproducts in *Halobacterium sp.* NRC-1. *Saline Systems* **2**:11.
5. **McCready, S.** 1996. The repair of ultraviolet light-induced DNA damage in the halophilic archaeobacteria, *Halobacterium cutirubrum*, *Halobacterium halobium* and *Haloferax volcanii*. *Mutat Res* **364**:25–32.
6. **Lestini, R, Duan, Z, Allers, T.** 2010. The archaeal Xpf/Mus81/FANCM homolog Hef and the Holliday junction resolvase Hjc define alternative pathways that are essential for cell viability in *Haloferax volcanii*. *DNA Repair (Amst)* **9**:994–1002.
7. **Eisen, JA, Hanawalt, PC.** 1999. A phylogenomic study of DNA repair genes, proteins, and processes. *Mutat Res* **435**:171–213.
8. **Fröls, S, White, MF, Schleper, C.** 2009. Reactions to UV damage in the model archaeon *Sulfolobus solfataricus*. *Biochem Soc Trans* **37**:36–41.
9. **Dyall-Smith, ML.** 2009. The Halohandbook, (ed.), 7.2,
10. **Pickl, A, Johnsen, U, Schönheit, P.** 2012. Fructose degradation in the haloarchaeon *Haloferax volcanii* involves a bacterial type phosphoenolpyruvate-dependent phosphotransferase system, fructose-1-phosphate kinase, and class II fructose-1,6-bisphosphate aldolase. *J Bacteriol* **194**:3088–3097.

11. **Spivak, G, Pfeifer, GP, Hanawalt, P.** 2006. In vivo assays for transcription-coupled repair. *Methods Enzymol* **408**:223–246.
12. **Large, A, Stamme, C, Lange, C, Duan, Z, Allers, T, Soppa, J, Lund, PA.** 2007. Characterization of a tightly controlled promoter of the halophilic archaeon *Haloferax volcanii* and its use in the analysis of the essential *cct1* gene. *Mol Microbiol* **66**:1092–1106.
13. **Rawls, KS, Martin, JH, Maupin-Furlow, JA.** 2011. Activity and transcriptional regulation of bacterial protein-like glycerol-3-phosphate dehydrogenase of the haloarchaea in *Haloferax volcanii*. *J Bacteriol* **193**:4469–4476.
14. **Kixmüller, D, Strahl, H, Wende, A, Greie, JC.** 2011. Archaeal transcriptional regulation of the prokaryotic KdpFABC complex mediating K(+) uptake in *H. salinarum*. *Extremophiles* **15**:643–652.
15. **Losensky, G, Vidakovic, L, Klingl, A, Pfeifer, F, Fröls, S.** 2014. Novel pili-like surface structures of *Halobacterium salinarum* strain R1 are crucial for surface adhesion. *Front Microbiol* **5**:755.
16. **Leffers, H, Gropp, F, Lottspeich, F, Zillig, W, Garrett, RA.** 1989. Sequence, organization, transcription and evolution of RNA polymerase subunit genes from the archaeobacterial extreme halophiles *Halobacterium halobium* and *Halococcus morrhuae*. *J Mol Biol* **206**:1–17.
17. **Dorazi, R, Götz, D, Munro, S, Bernander, R, White, MF.** 2007. Equal rates of repair of DNA photoproducts in transcribed and non-transcribed strands in *Sulfolobus solfataricus*. *Mol Microbiol* **63**:521–529.
18. **Romano, V, Napoli, A, Salerno, V, Valenti, A, Rossi, M, Ciaramella, M.** 2007. Lack of strand-specific repair of UV-induced DNA lesions in three genes of the archaeon *Sulfolobus solfataricus*. *J Mol Biol* **365**:921–929.

19. **Soppa, J.** 2011. Ploidy and gene conversion in Archaea. *Biochem Soc Trans* **39**:150–154.
20. **Baliga, NS, Bjork, SJ, Bonneau, R, Pan, M, Iloanusi, C, Kottemann, MC, Hood, L, DiRuggiero, J.** 2004. Systems level insights into the stress response to UV radiation in the halophilic archaeon *Halobacterium NRC-1*. *Genome Res* **14**:1025–1035.
21. **Boubriak, I, Ng, WL, DasSarma, P, DasSarma, S, Crowley, DJ, McCready, SJ.** 2008. Transcriptional responses to biologically relevant doses of UV-B radiation in the model archaeon, *Halobacterium sp. NRC-1*. *Saline Systems* **4**:13.
22. **McCready, S, Müller, JA, Boubriak, I, Berquist, BR, Ng, WL, DasSarma, S.** 2005. UV irradiation induces homologous recombination genes in the model archaeon, *Halobacterium sp. NRC-1*. *Saline Systems* **1**:3.
23. **Salerno, V, Napoli, A, White, MF, Rossi, M, Ciaramella, M.** 2003. Transcriptional response to DNA damage in the archaeon *Sulfolobus solfataricus*. *Nucleic Acids Res* **31**:6127–6138.
24. **Ganesan, A, Spivak, G, Hanawalt, PC.** 2012. Transcription-coupled DNA repair in prokaryotes. *Prog Mol Biol Transl Sci* **110**:25–40.
25. **Cohen, SE, Lewis, CA, Mooney, RA, Kohanski, MA, Collins, JJ, Landick, R, Walker, GC.** 2010. Roles for the transcription elongation factor NusA in both DNA repair and damage tolerance pathways in *Escherichia coli*. *Proc Natl Acad Sci U S A* **107**:15517–15522.
26. **Epshtein, V, Kamarthapu, V, McGary, K, Svetlov, V, Ueberheide, B, Proshkin, S, Mironov, A, Nudler, E.** 2014. UvrD facilitates DNA repair by pulling RNA polymerase backwards. *Nature* **505**:372–377.
27. **Epshtein, V.** 2015. UvrD helicase: an old dog with a new trick: how one step backward leads to many steps forward. *Bioessays* **37**:12–19.
28. **Kamarthapu, V, Nudler, E.** 2015. Rethinking transcription coupled DNA repair. *Curr Opin*

Microbiol **24**:15–20.

29. **Jun, SH, Reichlen, MJ, Tajiri, M, Murakami, KS.** 2011. Archaeal RNA polymerase and transcription regulation. *Crit Rev Biochem Mol Biol* **46**:27–40.
30. **Adimoolam, S, Ford, JM.** 2003. p53 and regulation of DNA damage recognition during nucleotide excision repair. *DNA Repair (Amst)* **2**:947–954.