Repair of UV damage in *Halobacterium salinarum*

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Abstract

*Halobacterium* is one of the few known Archaea that tolerates high levels of sunlight in its natural environment. Photoreactivation is probably its most important strategy for surviving UV irradiation and we have shown that both of the major UV photoproducts, cyclobutane pyrimidine dimers (CPDs) and (6–4) photoproducts, can be very efficiently repaired by photoreactivation in this organism. There are two putative photolyase gene homologues in the published genome sequence of *Halobacterium* sp. NRC-1. We have made a mutant deleted in one of these, *phr2*, and confirmed that this gene codes for a CPD photolyase. (6–4) photoproducts are still photoreactivated in the mutant so we are currently establishing whether the other homologue, *phr1*, codes for a (6–4) photolyase. We have also demonstrated an excision repair capacity that operates in the absence of visible light but the nature of this pathway is not yet known. There is probably a bacteria-type excision-repair mechanism, since homologues of *uvrA*, *uvrB*, *uvrC* and *uvrD* have been identified in the *Halobacterium* genome. However, there are also homologues of eukaryotic nucleotide-excision-repair genes (*Saccharomyces cerevisiae* RAD3, RAD25 and RAD2) so there may be multiple repair mechanisms for UV damage in *Halobacterium*.

Introduction

Organisms exposed to sunlight employ a range of strategies for surviving UV damage to their DNA. These include a variety of mechanisms for repairing DNA damage as well as tolerance mechanisms (including recombination and lesion bypass during DNA replication) that allow cells to survive in the presence of unrepaired lesions. Nucleotide excision repair (NER) is universal in the bacterial and eukaryotic domains and many organisms have additional forms of repair such as photoreactivation and alternative excision repair [1]. The Archaea have an intriguing combination of prokaryotic and eukaryotic repair gene homologues including homologues of both bacterial and eukaryotic NER genes [2–7]. There seem to be a number of different repair pathways represented but no obvious universal repair mechanism across the domain.

Excision repair

UV light induces two major photoproducts, cyclobutane pyrimidine dimers (CPDs) and (6–4) photoproducts [8], and the most widely occurring repair mechanism, NER, repairs both of these lesions. NER has been found in all bacteria and all eukaryotes [1]. However, although the basic process is common to prokaryotes and eukaryotes – involving dual incisions either side of the UV lesion, removal of the damaged DNA and resynthesis – the genes/proteins involved are different. In bacteria the key NER proteins are UvrA (for recognition), UvrB and UvrC (which act as an exonuclease to make incisions either side of the lesion) and UvrD (a DNA helicase II). In *Saccharomyces cerevisiae*, the model for eukaryotes, the incisions are made by the RAD1/RAD10 complex on the 5′ side and RAD2 (a member of the rad2/FEN1 family of flap endonucleases) on the 3′ side of the UV photoprodut. Other RAD proteins involved include RAD3 and RAD25, which are DNA helicases that work in opposite directions to open the repair bubble. These proteins are subunits of the RNA polymerase II transcription-initiation factor, TFIH. A large number of other proteins are also involved in NER in eukaryotes [9].

So far as we know, all bacteria and eukaryotes have NER capability. Some organisms also have additional repair mechanisms. These include UV excision repair [1,10], which is an alternative form of excision repair that depends on a UV endonuclease (‘uvde’) to perform an incision immediately 5′ to the UV photoprodut and a rad2 endonuclease that cuts off the single-stranded DNA ‘flap’ containing the lesion. This mechanism has been found in some fungi (*Neurospora crassa* [11] and fission yeast [10,12], but not budding yeast) and a few bacteria including *Bacillus subtilis* [10] and *Deinococcus radiodurans* [13]. In addition, *Micrococcus luteus* and bacteriophage T4 have UV endonucleases containing a CPD glycosylase and AP endonuclease activities that are used for repair of CPDs [14].

Excision repair in Archaea

Although NER is universal within the other two domains of life, as far as we can tell from examining sequence data, the Archaea do not seem to have a common excision-repair mechanism. A few Archaea, *Methanobacterium*, *Methanosarcina* and *Halobacterium*, have homologues of the bacterial *uvrA*, *uvrB*, *uvrC* and *uvrD* NER genes [4,15,16]. In *Methanobacterium thermoautotrophicum* and...
**Repair in *Halobacterium salinarum***

Early work suggested that *H. salinarum* was unable to repair UV-induced DNA damage in the dark [20,21]. However, it was shown that *Halobacterium* is extremely efficiently photoreactivated, and survival is restored to near 100% after relatively high UV doses [22–24]. So it was concluded that *Halobacterium* survives UV by photoreactivation alone. Later work, using a more sensitive assay for UV photo-products, showed that *Halobacterium* does have a dark repair capability and that both CPDs and (6–4) photoproducts are excised in the dark, at a rate roughly comparable with repair in yeast [25]. The nature of the excision-repair mechanism in *Halobacterium* has still to be established. The published genome sequence has now shown that *Halobacterium* has the bacterial NER genes *uvrA*, *uvrB*, *uvrC* and *uvrD*, so it is likely that it has a bacterial excision-repair mechanism. However it also has homologues of *RAD3*, *RAD25* and *RAD2* and it may, like fission yeast, *D. radiodurans* and *B. subtilis*, also have additional way(s) of excising UV photo-products [4].

Whatever excision repair mechanisms operate in *Halobacterium*, the fact remains that UV damage is very efficiently photoreactivated and, since cells are never exposed to UV light in the dark in the natural environment, it is likely that photoreactivation is biologically more important than excision repair for surviving UV light in sunlight. This has been shown to be the case in alfalfa seedlings: at low doses of UV (up to 30 photoproducts/Mb, which is in the range induced by natural sunlight), excision repair is insignificant and lesions are repaired by photoreactivation [26].

Photoreactivation is a direct reversal of dimer formation and is accomplished by photolyases, enzymes that use blue light and cleave the covalent bonds between adjacent pyrimidines in dimers [27]. CPDs make up the majority of UV photoproducts (70–90%) and many prokaryotic and eukaryotic organisms have photolyases that repair CPDs [28,29]. A few organisms also have a (6–4) photoproduct photolyase, but these are much less common than CPD photolyases and no (6–4) photolyase has been demonstrated in a prokaryote. Organisms that have (6–4) photolyases include *Drosophila* [30] and *Arabidopsis* [31].

Since visible light restores viability of *Halobacterium* to virtually 100% even after relatively high UV doses (up to 40 J m⁻²; C. Vermont and S. McCready, unpublished work), it seems quite likely that it has a (6–4) photolyase.

In support of this, a sensitive immunoassay for UV photo-products has shown that both CPDs and (6–4) photoproducts are repaired more rapidly in the light than in the dark in *Halobacterium* [25]. However, this did not necessarily indicate the presence of a (6–4) photolyase. An equally likely possibility is that, in the light, as CPDs are photoreactivated, the excision repair proteins are free to repair (6–4) photoproducts more efficiently.

The *Halobacterium* genome contains two photolyase homologues, *phr1* and *phr2* [4]. Amongst the Archaea, photolyases are not common and only *Halobacterium* is known to have two photolyase gene homologues. These are very closely related, with 38% amino acid sequence identity, so presumably arose from a gene duplication. There is previous evidence that *phr2* encodes a CPD photolyase [32] but the function, if any, of *phr1* has not been investigated. It may code for a second CPD photolyase, or a (6–4) photoproduct photolyase or, since photolyases and cryptochromes are closely related proteins [28,29], an alternative possibility is that *phr1* encodes a cryptochrome (see Figure 3, below).

### Results

To find out whether (6–4) photoproducts are photoreactivated in *Halobacterium*, we have made a *phr2* deletion mutant and measured UV survival and repair of photoproducts. The *phr2* gene was amplified by PCR and just over a kb of the gene was replaced by the *Halobacterium* *ura3* gene. A linear DNA fragment containing the *ura3* gene flanked by the proximal 251 bases and 225 terminal bases, respectively, of the *phr2* gene, was used to transform a *ura3* deletion mutant of *Halobacterium* sp. NRC-1 [33,34]. Ura⁺ transformants were screened by PCR to identify mutants in which the resident *phr2* gene had been replaced by the deletion construct.

One of these deletion mutants was selected for further study and survival of UV irradiation measured with and without subsequent exposure to visible light. Survival curves are shown in Figure 1, which show clearly that photoreactivating light has little or no effect on UV survival in the mutant, in sharp contrast with the significant increase in survival of wild-type cells.

Figure 2 shows repair of CPDs and (6–4) photoproducts in wild-type and mutant cells with and without exposure to visible light. A high UV dose was chosen for this experiment to minimise excision repair. It is clear that CPDs are not
photoreactivated in the mutant but that (6–4) photoproducts are. Residual repair of CPDs is due to excision of lesions. Even after this high dose of UV, (6–4) photoproducts are repaired very rapidly indeed in the light. This strongly suggests that *Halobacterium* does possess a (6–4) photolyase. If this is so, it is the only archaeon, and possibly the only prokaryote, that has a (6–4) photolyase. Apart from *Halobacterium*, only *Sulfolobus, Methanopyrus, Methanobacterium* and *Methanosarcina* [5,15,35] have photolyase homologues and, since none of them has more than one, these are likely to be CPD photolyases rather than (6–4) photolyases. Alternatively, they may not be photolyases at all, but crytochromes (although the sequence of the *Sulfolobus* proteins at least, are more suggestive of a photolyase).

**Discussion**

These results strongly suggest that (6–4) photoproducts can be photoreactivated in *Halobacterium*. The most obvious inference from this would be that *phr1* probably codes for a (6–4) photolyase. We are therefore constructing a *phr1* deletion to find out whether this is the case. However, the sequence of the Phr1 protein does not predict that it would be a (6–4) photolyase. Photolyases are very closely related to cryptochromes, blue-light receptor proteins that are involved in circadian rhythms, and the (6–4) photolyases so far characterized are grouped, phylogenetically, with the animal cryptochromes [28,29] (Figure 3). Phr1, like Phr2, falls within the type I CPD-photolyase group. More significantly, the Phr1 protein does not have key histidine residues that have been specifically linked, by Hitomi et al. [36], to the activity of (6–4) photolyases. So the function of Phr1 is very much in question. If it is not a (6–4) photolyase, then it may be the first example of an archaeal cryptochrome. In this case, we would need to explain how (6–4) photoproducts are photoreactivated. It seems unlikely that the Phr1 protein has no function at all, since the *Halobacterium* genome, at half the size of the *Escherichia coli* genome, has little room for redundancy.
The survival curves in Figure 1 show that visible light exposure does not significantly increase survival in the *phr2* mutant, even though (6–4) photoproducts are photoreactivated. A similar result was observed when the introduction of a (6–4) photolyase into *E. coli* mutants lacking the CPD photolyase failed to increase survival under visible-light illumination. (S. Nakajima, personal communication). The explanation is almost certainly that, because CPDs are by far the majority UV lesion, in the absence of a functional CPD photolyase the lethal effect of unrepaired CPDs obscures any possible contribution to survival of repairing (6–4) photoproducts.

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References


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