Lack of Replication Disruption Following H2O2-induced damage in Escherichia coli.

Sierra S. Schmidt

Justin Courcelle

Charmain T. Courcelle

Abstract

Hydrogen peroxide (H_2O_2) toxicity has long been thought to be predominantly due to oxidative DNA damage that can disrupt DNA replication and result in lethality. Curiously and contrary to this view, it is also well established that the glycosylases responsible for repairing oxidized-base damage are as resistant as wild-type cells when treated with H_2O_2. The observation raises the possibility that H_2O_2-induced DNA damage does not disrupt or prevent replication. Thus, the sensitivity of recF mutants, known to be required to maintain and restore replication forks after disruption by DNA damage, to H_2O_2, was tested. Survival curves of mutants treated with either UV irradiation or H_2O_2 were generated and, ss expected, *recF* mutants were shown to die off quicker after UV exposure, relative to wild-type cells. However, *recF* mutants were not hypersensitive to H_2O_2. The results would be consistent with the idea that DNA damage induced by H_2O_2 does not disrupt DNA replication and may not factor significantly into its lethality.

Introduction

The most intrinsic element of much of cellular life, DNA, is under a seemingly constant barrage of damaging elements, particularly of interest are those agents which cause lesions in the DNA The two major forms of lesion-causing damage, UV irradiation and oxidative damage, are known to cause certain levels of genome instability, as a result of the lesions they leave. The sorts of lesions which these types of damage create are unique and distinct from each other, UV only creating two while oxidation has several different forms of damage. However, the major forms of oxidative damage are thymine glycols and 8-oxoguanine while the major forms of UV damage are 6,4 photoproducts and cyclobutene pyrimidine dimers (see Fig. 1).[1][10] Notably, oxidative damage, particularly through hydrogen peroxide (H_2O_2), is found to be lethal through DNA damage when the cell is metabolically active, suggesting that perhaps a byproduct of the breakdown of H_2O_2 is the reactive agent.[8]

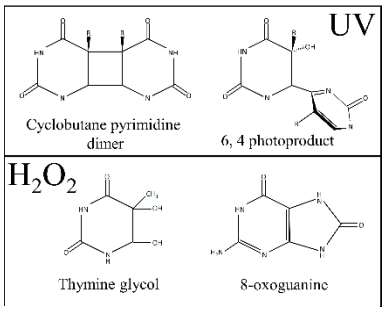

Fig 1. The major forms of both UV and oxidative DNA damage.

The difference immediately evident between these two types of damage is the size of these lesions, UV-lesions are characteristically bulky. This bulkiness can cause physical structural changes, 6,4 photoproducts are known for causing adjacent bases to move into a perpendicular position, contrary to normal parallel conformation.[2] While oxidative-lesions are less bulky, the differing structural can still cause certain interactions which may disrupt DNA structure.[7] Apart from the physical changes to the DNA structure, these lesions have the capacity to act as mutagenizing agents through mismatch, clearly as UV irradiation has been used for mutagenesis for some time. These taken together can explain some of the lethality of these agents to bacterial cells. Additionally, both types of lesions have been shown to block DNA polymerases in vitro within the *Escherichia coli* model[2][7] and are thought to disrupt replication in vivo as well (see Fig. 2). This stoppage of replication has been suggested to be followed by degradation of nascent DNA beginning at the replication fork, which could almost certainly prove fatal to the cell, and would most definitely prevent it from propagating.[3]

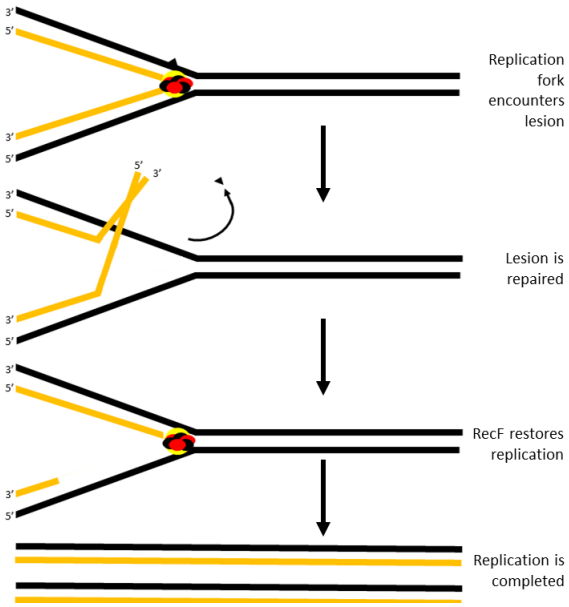

Fig 2. The propose operation of the replication fork upon encountering a UV lesion, followed byresolution of damage and restoration of replication. Adapted from Courcelle et al. 1999, *J Bacteriol,* 181:916.

Given the great threat to cells from these types of damage, it follows that cells have certain repair pathways which can be used to restore DNA to its proper state, and have, in fact, evolved many systems to deal with the different types of damage.[3] Two of these pathways, nucleotide and base excision repair, account for the repair of UV and oxidative-lesions, and act very differently on the enzymatic level. In the base excision repair pathway, the hydrogen bonds of a base are broken, and the incorrect base is subsequently rotated out from the DNA helix and excised, leaving the phosphate backbone is left intact. [9] Meanwhile, under the nucleotide excision repair pathway the nucleotide is fully excised out, and then the base resynthesized

and ligated [6]. A major nucleotide excision repair pathway which is integral to solving UV-lesions is the protein system made up of various *uvr* proteins.[8][11] Previously it has been shown that mutants in nucleotide base excision repair cannot remove DNA lesions after UV and are thusly hypersensitive, base excision repair mutants which cannot remove H_2O_2-induced lesions have comparable resistance to wild-type cells.

One enzyme which operates within the base excision repair pathway is *recF*, a part of the *recFOR* pathway. The pathway is triggered throughout the detection of a DNA lesion, at which point RecJ nuclease cuts out the base and proceeds upstream, continuing to cut out bases along the 5' strand. Single strand binding proteins (SSB) and RecQ helicase assist in allowing RecJ to cleave more of the 5' strand, following this the RecFOR complex loads RecA onto the ssDNA-dsDNA hybrid and recombinational repair is able to proceed. RecF is required to re-establish DNA replication following disruption. [4]

Understanding repair enzumes in a cellular system is vastly important to human health. Without any regards to the specific RecF pathway, repair mechanisms in general are important to understand. Mutations in these pathways can lead to serious health issues, including cancers. However, even beyond this simple general fact, the crystal structure of RecF has been identified as strikingly similar to a human protein, Rad50. The structure of RecF has been shown to have a strong similarity to the head domain of the Rad 50, despite the addition of a long coiled coil structure in Rad50. In spite of this difference, they have been shown to have similar subdomains in RecF's ATPase subdomain and the so-called Lobe II of Rad50. [10] Additionally, Rad50 has a very similar function as a part of a repair pathway.[5] Eukaryotic cells and *E.* coli are separated by vast evolutionary time, and yet this mechanism is seemingly conserved across domains, and so understanding the bacterial system can lead to greater understanding of the human system.[10] Furthermore, this eukaryotic protein Rad50 has been directly shown to be correlated to certain cancers when it is mutated. When it is not present in cells, DNA damage cannot be repaired and chromosomal instability follows. [5] Given the further understanding of this specific function in relation to human health could allow, at some point, the development of a more targeted therapeutic.

Given the inconsistencies between the literature and previous data, the *recF* mutants were exposed to UV- and H_2O_2-damage, as this is a known enzyme for DNA repair, with the expectation that recF would not be required for oxidative damage repair.

Methods

UV Survivals. In order to generate the survival curves of the various mutants and wild type cells; *E. coli* SR108 wild type, *xth*, *recF*, and *uvrA* mutants were struck out on LB plates enriched with thymine and left to incubate at 37°C overnight, and were then grown in 2 mL of DGC medium enriched with thymine overnight, also incubated at 37°C. These cultures were then diluted 1:100 in 5 mL of DGCthy, and were grown for 4-4.5 hours, at which point the cultures were serially diluted to 10^6 and spotted on LBthy plates in triplicate. The wild type, *xth*, and *recF* plates were then UV irradiated at 20, 40, 60, 80 and 100 J/m^2 on a rotating platform, keeping behind a control. *uvrA* was irradiated at 1, 2, 3, 4 and 5 J/m^2 due to the hypersensitive phenotype expected of this control, as with the other strains a control was set aside. The plates were then incubated at 37°C overnight. The colonies were counted, see Fig 3 for a representation of the plates.

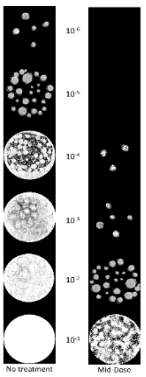

Fig 3: Depiction of a typical row of dilutions on a spot

H_2O_2 Survivals. *E. coli* SR108 wild type, *xth*, *recF*, and *uvrA* mutants were struck out on LBthy plates and left to incubate at 37°C overnight, and were then grown in 2 mL of DGCthy medium overnight, also incubated at 37°C. These cultures were diluted 1:100 in 5 mL of DGCthy, and were grown for 3.5-4 hours. Five sets of dilutions were set up for each strain kept on ice, one set for each was serially diluted with the subculture to act as the control. The subculture was then treated with 10 mM H_2O_2. 0.1-ml samples were taken from wild type, *recF*, and *uvrA* at 5, 10, 20 and 30 min post-H_2O_2 addition and serially diluted. The same procedure was completed for *xth*, except aliquots were taken at 2.5, 5, 7.5, and 10 minutes to account for the predicted hypersensitivity. The dilutions were then spotted in triplicate onto LBthy plates, incubated at 37°C overnight, and the colonies counted.

Results

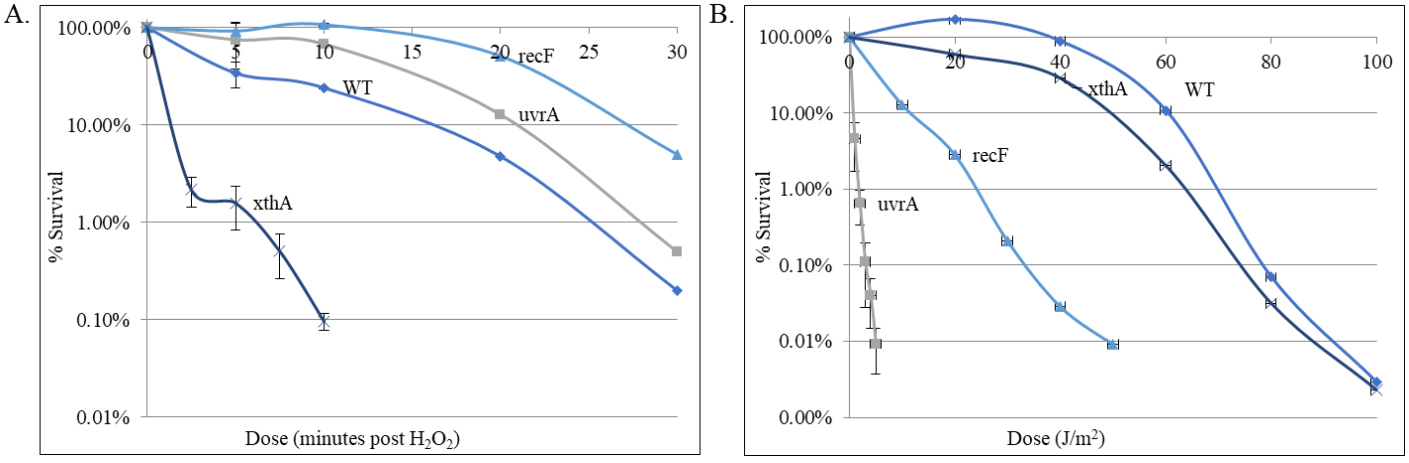

Fig 4. A. Logarithmically scaled percent survival of E. coli SR108 and mutant strains *recF*, *uvrA*, and *xthA* in increasing doses of hydrogen peroxide (minutes post H_2O_2). The hypersensitivity of *xthA* follows as expected, less than 0.1% survival at the highest dose showing hypersensitivity. *recF* and *uvrA* both show, interestingly, less sensitivity to the H_2O_2 than wild type cells. B. Logarithmically scaled percent survival of E. coli SR108 and mutant strains *recF*, *uvrA*, and *xthA* in increasing doses of UV irradiation (J/m^2). *uvrA* showed a high hypersensitivity to the UV irradiation, as expected. *recF*, while not hypersensitive, was obviously sensitive to the UV irradiation, particularly as compared to wild type.

Following the methodology listed above, the Colony Forming Units (CFU) of each treatment was calculated, and percent survival was calculated given the control plate taken as complete survival. These survival rates were then plotted against doses of the two DNA damaging units. *uvrA* showed no sensitivity, as compared to wild type, when exposed to increasing doses of hydrogen peroxide but showed a very drastic hypersensitivity to UV irradiation. This is expected due to the involvement of *uvrA* in UV lesion

nucleotide excision repair.[8][11] Opposed to this trend, *xthA* acted as expected; showing little sensitivity to UV while showing sensitivity to H_2O_2.[7]

As expected from previous literature, the *recF* mutant showed hypersensitivity, as compared to wild type *E. coli*, under increasing doses of UV irradiation (Fig. 4B). Specifically, the *recF* mutants are shown to die off quicker, the total average survival exponentially lower than wild type at the high end of the *recF* UV irradiation dose. Conversely, these *recF* mutants, when exposed to increasing doses of H_2O_2 survive markedly better than their wild type counterparts (Fig. 4A). The survival rate both decreases slower than wild type and ends with a higher average number of survived cells by the high end of the doage. Additionally, the *uvrA* and *xthA* mutants behaved as expected, with *uvrA* being hypersensitive to UV damage, even as drastically lower doses than the rest of the mutants, and *xthA* surviving well when compared to wild type. This converse is true, as expected, when exposed to increasing doses of hydrogen peroxide.

Discussion

The results listed above are consistent with the idea that, in contrast to UV-induced DNA damage, DNA damage induced by H_2O_2 may not disrupt DNA replication.[7] As discussed in the introduction, UV-induced DNA damage is repaired through the base excision repair pathway, which has previously been shown to be required to restore DNA replication.[3] More explicitly, it is expected that as DNA replication proceeds when UV-lesions are present, the replication fork is stopped at the lesion and the repair pathway proceeds before replication can resume, in particular recF was shown to be required for the resumption of replication.[3] Meanwhile, these base excision repair mutants are not shown to be hypersensitive to oxidative damage.[11] Since it appears that *recF* mutants have comparable survival to wild type cells when exposed to H_2O_2 damage, it would seem that this repair is not necessary for the replication to proceed. In fact, it is possible that replication fork simply goes past these oxidative-lesions, and replication can be completed without implementation of the *recFOR* pathway. This is quite peculiar, and adverse to decades of research.

The biochemistry and introduction of these lesions into DNA is dependent on the reactivity of oxygen, and byproducts of enzymatic processes which result in such harmful forms of oxygen such as free radicals or hydrogen peroxide. Oxygen is intrinsic to the life processes of cells and is also, mostly, readily available in the atmosphere.[1] UV-irradiation is also strongly relevant in the environment, most notably the sun. Given this information, it may, then, seem counterintuitive that the cell would have a system in place to stop at UV-lesions for repair, but not for oxidative-lesions, when both of these types of damage are readily taken on by the environment. Additionally, it may seem counter-intuitive based on how many types of oxidative-lesions exist. However, there are two highly speculative possibilities which could be interesting to explore in a future study. One of the major differences between oxidative- and UV-lesions is the size, UV-lesions tend to be quite bulky. In this sense, it is possible that the UV-lesions simply pose a greater threat to genomic stability and the ability for the DNA to function as intended. There may also potentially be some evolutionary explanation in that *E. coli* species are commonly found to be living within the intestinal tracts of mammals, wherein they would experience little UV irradiation, but would experience higher levels of oxidative stress. It could be some adaptation to somehow otherwise survive oxidative-lesions in order to expend less energy removing damage which constantly befalls the bacteria, particularly taken with the previous idea that oxidative-lesions are not nearly as bulky as UV-lesions and may not compromise the structure nor function of the DNA. Since this is highly speculative, it is very clear that

neither of these options may be true, and otherwise a novel mechanism exists which does not utilize RecF as do UV-lesions.

Regardless, this research stands as a starting off point for many other research questions in the future. There are many avenues which could be explored in regard to oxidative damage and its interaction within the cell. Of interest, many chemo therapeutics appear to cause oxidative damage, and approaching the interaction of these chemicals within the cell could prove to lead to interesting ends.

Acknowledgements

This research was funded by the NSF, grant number MCB1916625.

References

1. Cadet, J., & Davies, K. J. A. (2017). Oxidative DNA Damage & Repair: An Introduction. *Free Radical Biology & Medicine*, *107*, 2–12. https://doi.org/10.1016/j.freeradbiomed.2017.03.030

2. Chan, G. L., Doetsch, P. W., & Haseltine, W. A. (1985). Cyclobutane pyrimidine dimers and (6-4) photoproducts block polymerization by DNA polymerase I. *Biochemistry*, *24*(21), 5723–5728. https://doi.org/10.1021/bi00342a006

3. Courcelle, J., Crowley, D. J., & Hanawalt, P. C. (1999). Recovery of DNA Replication in UV-IrradiatedEscherichia coli Requires both Excision Repair and RecF Protein Function. *Journal of Bacteriology*, *181*(3), 916–922. https://doi.org/10.1128/JB.181.3.916-922.1999

4. Handa, N., Morimatsu, K., Lovett, S. T., & Kowalczykowski, S. C. (2009). Reconstitution of initial steps of dsDNA break repair by the RecF pathway of E. coli. *Genes & Development*, *23*(10), 1234–1245. https://doi.org/10.1101/gad.1780709

5. Heikkinen, K. (2003). Mutation screening of Mre11 complex genes: Indication of RAD50 involvement in breast and ovarian cancer susceptibility. *Journal of Medical Genetics*, *40*(12), 131e–1131. https://doi.org/10.1136/jmg.40.12.e131

6. Houten, B. V. (1990). Nucleotide Excision Repair in Escherichia coli. *MICROBIOL. REV.*, *54*, 34.

7. Ide, H., Kow, Y. W., & Wallace, S. S. (1985). Thymine glycols and urea residues in M13 DNA constitute replicative blocks in vitro. *Nucleic Acids Research*, *13*(22), 8035–8052.

8. Imlay, J. A., & Linn, S. (1987). Mutagenesis and stress responses induced in Escherichia coli by hydrogen peroxide. *Journal of Bacteriology*, *169*(7), 2967–2976. https://doi.org/10.1128/JB.169.7.2967-2976.1987

9. Koroleva, O., Makharashvili, N., Courcelle, C. T., Courcelle, J., & Korolev, S. (2007). Structural conservation of RecF and Rad50: Implications for DNA recognition and RecF function. *The EMBO Journal*, *26*(3), 867–877. https://doi.org/10.1038/sj.emboj.7601537

10. Krokan, H. E., & Bjørås, M. (2013). Base Excision Repair. *Cold Spring Harbor Perspectives in Biology*, *5*(4). https://doi.org/10.1101/cshperspect.a012583

11. Rastogi, R. P., Richa, Kumar, A., Tyagi, M. B., & Sinha, R. P. (2010). Molecular Mechanisms of Ultraviolet Radiation-Induced DNA Damage and Repair. *Journal of Nucleic Acids, 2010*. https://doi.org/10.4061/2010/592980

12. Schalow, B. J., Courcelle, C. T., & Courcelle, J. (2011). Escherichia Coli Fpg Glycosylase is Nonrendundant and Required for the Rapid Global Repair of Oxidized Purine and Pyrimidine Damage In Vivo. *Journal of Molecular Biology*, *410*(2), 183–193. https://doi.org/10.1016/j.jmb.2011.05.004