

Neisseria gonorrhoeae DNA Recombination and Repair Enzymes Protect against Oxidative Damage Caused by Hydrogen Peroxide[∇]

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The strict human pathogen *Neisseria gonorrhoeae* is exposed to oxidative damage during infection. *N. gonorrhoeae* has many defenses that have been demonstrated to counteract oxidative damage. However, *recN* is the only DNA repair and recombination gene upregulated in response to hydrogen peroxide (H₂O₂) by microarray analysis and subsequently shown to be important for oxidative damage protection. We therefore tested the importance of RecA and DNA recombination and repair enzymes in conferring resistance to H₂O₂ damage. *recA* mutants, as well as RecBCD (*recB*, *recC*, and *recD*) and RecF-like pathway mutants (*recJ*, *recO*, and *recQ*), all showed decreased resistance to H₂O₂. Holliday junction processing mutants (*ruvA*, *ruvC*, and *recG*) showed decreased resistance to H₂O₂ resistance as well. Finally, we show that RecA protein levels did not increase as a result of H₂O₂ treatment. We propose that RecA, recombinational DNA repair, and branch migration are all important for H₂O₂ resistance in *N. gonorrhoeae* but that constitutive levels of these enzymes are sufficient for providing protection against oxidative damage by H₂O₂.

Neisseria gonorrhoeae is the sole causative agent of the sexually transmitted disease gonorrhea. The hallmark of symptomatic gonococcal infection is a massive influx of activated neutrophils into the urethra, resulting in a purulent discharge consisting of neutrophils and *N. gonorrhoeae* (42, 31). Neutrophils kill bacteria through the combined action of antimicrobial proteins and reactive oxygen species (ROS) (37), perpetrating a potent bactericidal oxidative burst that generates substantial amounts of superoxide anion, hydroxyl radical, and hydrogen peroxide (H₂O₂). These ROS can damage proteins, lipids, carbohydrates, and DNA (53). The ability of *N. gonorrhoeae* to survive oxidative damage is illustrated by its ability to survive among neutrophils (31, 42, 45). During infection, *N. gonorrhoeae* is also likely to encounter H₂O₂ produced by commensal lactobacilli, which inhibit the growth of *N. gonorrhoeae* in vitro (61, 49). Since *N. gonorrhoeae* is an obligate human pathogen, it is not exposed to typical environmental stresses such as UV light, ionizing radiation, or chemical mutagens. Therefore, the preponderant type of DNA damage *N. gonorrhoeae* is likely to encounter is oxidative damage from neutrophils and commensal lactobacilli, as well as oxidative damage caused by free radicals evolved during the normal process of oxidative phosphorylation (10).

The gonococcal genome contains many genes predicted to be involved in a number of DNA repair pathways, including base excision repair, nucleotide excision repair, mismatch repair, and recombinational repair (15). Recombinational DNA repair has been studied extensively in *N. gonorrhoeae* and requires the *recA* (19) and *recX* (52) genes, which act in concert with either the RecBCD pathway (*recB*, *recC*, and *recD* genes) (25) or the RecF-like pathway (*recO*, *recQ*, *recR*, and *recJ*

genes) (25, 46, 36). The Holliday junction processing enzymes encoded by *recG*, *ruvA*, *ruvB*, and *ruvC* also contribute to recombinational DNA repair in *N. gonorrhoeae* (36, 35). *N. gonorrhoeae* appears to use both DNA recombinational repair pathways simultaneously (25). This is in contrast to *Escherichia coli*, where mutants in the RecF pathway generally show phenotypes only in the context of *recBC sbcBC* mutations (21), leading to the conclusion that recombinational DNA repair is especially important for the repair of damaged DNA in *N. gonorrhoeae* (25).

In *E. coli*, *recA* (1) and many other DNA recombinational repair genes (12), have been shown to be important for the repair of oxidatively damaged DNA. *E. coli* RecA is important both directly for its functions in DNA repair and indirectly for its role in the induction of the SOS response of DNA repair (12, 18). However, since *N. gonorrhoeae* lacks a classical SOS response (2, 32), this indirect contribution of RecA to the repair of oxidatively damaged DNA is irrelevant in *N. gonorrhoeae*.

Microarray analysis has shown that only a single known DNA repair and recombination gene, *recN*, is upregulated after hydrogen peroxide treatment (51). The exact role of *recN* in DNA repair and recombination is unclear in any organism, but it appears to function in the repair of DNA double-strand breaks (29, 14, 33). Moreover, an *N. gonorrhoeae* *recN* mutant exhibits decreased survival to nalidixic acid (46) and hydrogen peroxide (51), both of which can result in DNA double-strand breaks.

Although several gonococcal genes have been identified that protect against oxidative damage, few of them are predicted to function in the repair of DNA. The *katA* gene product protects *N. gonorrhoeae* from H₂O₂ (48) by the reduction of H₂O₂ to H₂O and O₂. The *ccp* gene product (56) is also likely to act through the reduction of H₂O₂ (39), and the *sco* gene product may act as a thiol:disulfide oxidoreductase (38). Bacterioferitin (4) and azurin (60) appear to protect against oxidative stress by sequestering ions that exacerbate oxidative damage; a

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manganese uptake system (55) provides Mn ions that quench ROS; and the *msrAB* gene product repairs oxidatively damaged proteins (47). To date, only two genes that are involved in DNA repair and recombination have been found to protect against oxidative damage in *N. gonorrhoeae*. The previously described *N. gonorrhoeae recN* mutant (51) and a mutant inactivated in *priA*, which is involved in replication restart, both show decreased resistance to oxidative damaging agents (16). In contrast to *E. coli recA* (1), *N. gonorrhoeae recA* was reported to not protect against oxidative damage caused by H₂O₂ (9), suggesting that DNA repair and recombination enzymes may differ between *N. gonorrhoeae* and *E. coli* in their importance to the repair of oxidatively damaged DNA.

The majority of antioxidants identified thus far in *N. gonorrhoeae* do not function in DNA repair and *N. gonorrhoeae recA* has been demonstrated to not protect from oxidative damage (9). Therefore, one hypothesis is that DNA repair is not important for protection from oxidative damage. Alternatively, since *recN* is the only DNA recombination and repair gene upregulated in response to H₂O₂ and both *recN* (51) and *priA* (16) protect against oxidative damage, it is possible that DNA repair is important for the repair of oxidative damage, but constitutive levels of DNA recombination and repair enzymes provide sufficient damage protection. To test the importance of gonococcal DNA recombination and repair genes in conferring resistance to oxidative damage, we measured the resistance of a *recA* mutant (40) and of several mutants with defects in recombinational DNA repair enzymes (25, 52, 46, 36, 35). We show that RecA, in addition to the RecBCD and RecF-like recombinational repair pathways and Holliday junction processing enzymes, contribute to the survival of *N. gonorrhoeae* to oxidative damage.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The gonococcal strains used in the present study were either strain FA1090 containing the *recA* gene under the control of its endogenous promoter or derivatives of this strain. FA1090*recA4* is a *recA* loss-of-function mutation that contains a tetracycline resistance [Tet^r] cassette inserted in the *recA* gene. FA1090*recA6* contains an IPTG (isopropyl-β-D-thiogalactopyranoside)-regulatable gonococcal *recA* allele under the dual control of *taclac* and *UV5* promoters and allows for the conditional control of *recA* expression and RecA-dependent processes (40). IPTG was used at a final concentration of 1 mM to provide induction of *recA* transcript (40). All strains contained the 1-81-S2 variant *pilE* sequence (41). Mutations in additional recombination genes were in the *recA6* background. All mutant strains were previously constructed, and the mutations were validated phenotypically by complementation for their effect on DNA repair of UV damage and on nalidixic acid- or gamma-ray-induced double-strand breaks (25, 52, 46, 36, 35). *N. gonorrhoeae* strains were grown at 37°C, unless otherwise indicated. Strains were propagated on *N. gonorrhoeae* (GC) medium base (GCB; Difco) plus Kellogg supplements I (22.2 mM glucose, 0.68 mM glutamine, 0.45 mM cocarboxylase) and II [1.23 mM Fe(NO₃)₃] (13) at 37°C in 5% CO₂ or in GC liquid (GCBL) medium (1.5% proteose peptone no. 3 [Difco], 0.4% K₂HPO₄, 0.1% KH₂PO₄, 0.1% NaCl) with Kellogg supplements I and II and 0.042% sodium bicarbonate. Unless explicitly stated, GCB and GCBL always contained Kellogg supplements I and II. All chemicals were obtained from Sigma unless otherwise indicated.

Hydrogen peroxide resistance assay. *N. gonorrhoeae* strains were grown from freezer stocks for approximately 20 h, and ~10 colonies were passaged onto GCB. After 12 h, colonies were collected with a Dacron swab (Puritan), resuspended in GCBL at an optical density at 550 nm (OD₅₅₀) of ≈0.1, grown 16 h at 30°C in 15-ml conical tubes (Sarstedt) in a drum rotator, diluted to an OD₅₅₀ of ≈0.3, grown 2.5 to 3 h at 37°C, and diluted to OD₅₅₀ of ≈0.06 in GCBL containing 1 mM IPTG to induce *recA* expression in the *recA6* background when appropriate. This culture was grown to mid-log phase (OD₆₀₀ of ≈0.5) and diluted 1:10 in GCBL containing IPTG where appropriate, and 1-ml aliquots

were placed into 15-ml conical tubes. H₂O₂ (Sigma-Aldrich) was added to the tubes at a final concentration of 0, 10, 20, or 50 mM, and the tubes were placed in a drum rotator at 37°C for 15 min. Cultures were immediately serially diluted into plain GCBL (no Kellogg supplements) containing 10 μg of catalase/ml and plated onto GCB agar containing IPTG where appropriate. Piliated colonies were counted after ~20× growth, and the survival of each strain at the 10, 20, and 50 mM doses of H₂O₂ was calculated relative to survival at the 0 mM dose.

Isolation of protein from *N. gonorrhoeae* and Western blot analysis. *N. gonorrhoeae* FA1090 and FA1090*recA6* were grown in liquid culture as described above or on plates as previously described (52). To monitor the effect of H₂O₂ on *N. gonorrhoeae* RecA protein expression, cultures were diluted 1:10 into GCBL containing 1 mM IPTG, where appropriate, split in half, and half received 5 mM hydrogen peroxide and half was mock treated with water. Samples (10 ml) were taken after 15 and 30 min, and catalase (10 μg/ml [final concentration]; Sigma) was added to the samples to degrade any remaining H₂O₂, followed by a 1-min incubation at room temperature. Cultures were quickly cooled on ice and pelleted by centrifugation (4,000 × g for 10 min), and the supernatant was removed. Pellets were resuspended in 500 μl of phosphate-buffered saline (PBS), and a 20-μl sample of the supernatant was used for determining the protein concentration using the BCA protein assay (Pierce Chemicals). A 5× sodium dodecyl sulfate protein sample buffer was added to the remainder of the sample, the genomic DNA was sheared by repeated passage through a small-bore needle, and the sample was stored at -20°C. Equal amounts of total protein were loaded onto each lane of a 12% protein gel, which was prepared and run by standard techniques (23). Prestained markers (Bio-Rad) were included on all gels. Gels were blotted using CAPS buffer [10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (pH 11.0); 10% methanol] onto 0.45-μm-pore-size polyvinylidene difluoride membrane (Millipore) using a Bio-Rad transfer cell at 75 V for 1.5 h at 4°C. Western blots were developed according to the chemiluminescent (ECL) Western blotting protocol (Amersham Life Sciences) with the following modifications. Blots were blocked overnight at 4°C in a 5% solution of Carnation powdered milk (Nestle Foods) dissolved in washing buffer (Tris-buffered saline [pH 7.6], 0.1% Tween 20). Primary polyclonal anti-RecA antibodies (a gift from Mike Cox, University of Wisconsin-Madison) and secondary goat anti-rabbit immunoglobulin G antibodies conjugated to horseradish peroxidase (Chemicon) were diluted 1:5,000 and 1:25,000, respectively, in blocking solution and then incubated with shaking for 1 h at room temperature.

RESULTS

***recA*, but not *recX*, mutants show decreased resistance to hydrogen peroxide.** *recA* has been demonstrated to confer protection against oxidative damage in *E. coli* (1), *Salmonella enterica* serovar Typhimurium (3), and *Lactococcus lactis* (7). Since *recA* is essential for recombinational DNA repair in *N. gonorrhoeae* (20, 40), it was puzzling that an *N. gonorrhoeae recA* mutant was previously reported to be more resistant to H₂O₂ than the parent strain FA1090 (9). The H₂O₂ sensitivity of two independent *recA* mutant strains was measured to determine whether recombinational DNA repair is important for the repair of damage caused by H₂O₂. The H₂O₂ sensitivity of the parental strain FA1090 and FA1090*recA4*, which is a *recA* loss-of-function mutant containing a Tet^r resistance cassette inserted in the *recA* gene (40), was assessed. FA1090*recA4* was up to fourfold less resistant to H₂O₂ than the parent strain FA1090 (Fig. 1A), as was an independent *recA* knockout construct containing an erythromycin resistance cassette, *recA9* (40; data not shown).

Since our current observation that a *recA* mutant showed decreased resistance to H₂O₂ was inconsistent with what has previously been reported (9), we wanted to verify our results by using a conditional *recA* mutant allele, FA1090*recA6*, where *recA* expression is controlled by IPTG and strains are phenotypically RecA⁻ in the absence of IPTG (40). FA1090*recA6* expressing *recA* (+IPTG) exhibited the same resistance to H₂O₂ as strain FA1090, which has *recA* under the control of its

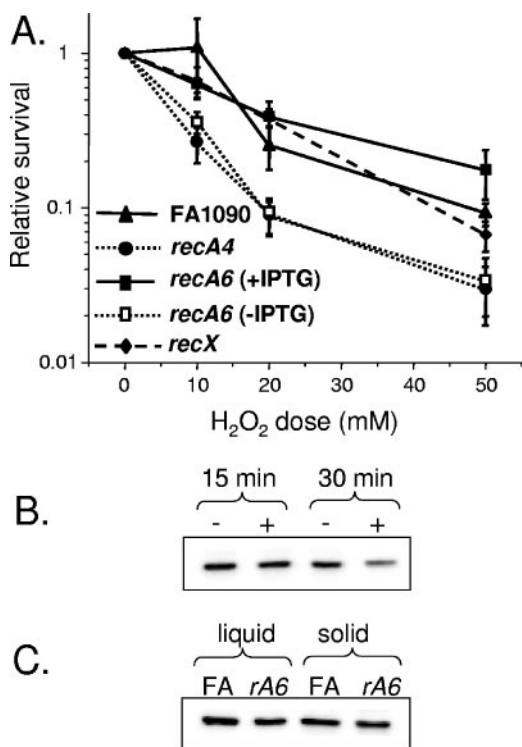


FIG. 1. (A) Effect of *recA* and *recX* mutation on H₂O₂ resistance. Error bars represent the standard error of the mean of three to six experiments. FA1090, FA1090*recA6* (+IPTG), and FA1090*recX* were statistically the same at all doses. FA1090*recA6* (+IPTG) was statistically distinct from FA1090*recA6* (-IPTG), at all doses ($P < 0.05$) as determined by the Student *t* test, and FA1090*recA4* was statistically the same as FA1090*recA6* (-IPTG), at all doses. (B and C) Western blot analyses of RecA levels. (B) RecA protein is not induced by H₂O₂. Strain FA1090 was grown as described and exposed to either no H₂O₂ (-) or 5 mM H₂O₂ (+) for 15 or 30 min. Then, 4 μ g of total protein was run per lane and transferred to a polyvinylidene difluoride membrane with subsequent Western blot analysis with anti-RecA antisera using the ECL kit. (C) RecA protein is expressed at equivalent levels in strain FA1090 (FA) and FA1090*recA6* (*rA6*) induced with IPTG when bacteria are grown in liquid or on solid media. Experiments for panels B and C were performed with three biological replicates on different days, and a representative blot is shown.

endogenous promoter. In contrast, FA1090*recA6* not expressing *recA* (-IPTG) showed the same resistance as the *recA4* loss-of-function mutant (Fig. 1A). Together, these data conclusively demonstrate that *recA* contributes to hydrogen peroxide resistance in *N. gonorrhoeae*.

The RecX protein inhibits RecA activity in vitro (57, 50, 6) and *recX* modulates a variety of RecA-dependent phenotypes in vivo (50, 44). An *E. coli* *recX* mutant shows decreased resistance to UV damage (50) and a *Herbaspirillum seropedicae* *recX* mutant shows decreased resistance to UV light and the mutagen methyl methanesulfonate (8). A *Deinococcus radiodurans* *recX* mutant shows an elevated frequency of recombination and decreased genetic stability (44) and appears to negatively regulate genes encoding the antioxidants catalase and superoxide dismutase through an unknown mechanism (43). Importantly, a *N. gonorrhoeae* *recX* mutant shows a small decrease in the ability to survive DNA damage caused by double-strand breaks (52), which is the major type of DNA

damage resulting from treatment with hydrogen peroxide. However, the contribution of *recX* to oxidative damage resistance has not been measured in any organism. In the present study, an *N. gonorrhoeae* *recX* loss-of-function mutant showed H₂O₂ resistance nearly identical to that of the *recA6* parent strain (Fig. 1A), suggesting that *recX* does not contribute to the repair of oxidative damage by H₂O₂ in *N. gonorrhoeae*.

RecA protein levels are not upregulated in response to H₂O₂, and RecA expression is equivalent from its endogenous promoter and an IPTG-inducible promoter. In many bacteria, *recA* and other genes of the SOS regulon are upregulated after H₂O₂ exposure (62, 30, 28, 27), likely as part of the SOS response. *N. gonorrhoeae* lacks a classical SOS response, evidenced by its lack of LexA and UmuD homologues, LexA boxes, and the lack of induction *uvrA*, *uvrB*, and *recA* transcripts in response to the DNA-damaging agents methyl methanesulfonate and UV light (2). To determine whether RecA protein levels were affected by H₂O₂ treatment and to determine the relative levels of RecA protein expression when *recA* was expressed from its endogenous promoter (strain FA1090) or from an IPTG-inducible promoter (strain FA1090*recA6*), Western blot analyses were performed. Strains FA1090 and FA1090*recA6* (+IPTG) were grown in liquid culture, the culture was split, and half the culture was treated with 5 mM hydrogen peroxide, and the other half was left untreated. Aliquots were removed after 15 and 30 min, and the level of RecA protein expression was measured. Western blots containing equal amounts of total protein were probed with anti-RecA antiserum, and images were captured and analyzed by using a Gel-Imager (Alpha Innotech). RecA protein levels did not increase as a result of hydrogen peroxide treatment in either FA1090 (Fig. 1B) or FA1090*recA6* (data not shown). Comparison of the relative RecA protein levels in FA1090 and FA1090*recA6* (induced with 1 mM IPTG) showed the RecA levels to be equivalent in these two strains when cultures were grown either in liquid or on solid media (Fig. 1C). Together, these data show that RecA levels are comparable when *recA* is expressed from its endogenous promoter and from an IPTG-inducible *lac* promoter construct. They also demonstrate that RecA protein is not upregulated after hydrogen peroxide treatment. Since constitutive levels of RecA contributed to H₂O₂ resistance, we decided to expand our hypothesis by investigating the contribution of recombinational repair enzymes to H₂O₂ resistance.

The RecBCD pathway of DNA repair contributes to hydrogen peroxide resistance. In *E. coli*, recombinational DNA repair is mediated by RecA in conjunction with either the RecBCD or the RecF pathway (21, 24). Although both pathways are important for H₂O₂ resistance in *E. coli*, the RecBCD pathway appears to be the predominant oxidative damage repair pathway (11, 12), probably due to the fact that the RecBCD enzyme processes DNA double-strand breaks (22). In *N. gonorrhoeae*, the two pathways have been shown to both be important for the repair of UV-induced lesions and DNA double-strand breaks caused by gamma irradiation or nalidixic acid, with most mutants showing a level of resistance intermediate to the parental strain and the *recA* mutant (25, 46, 36), but the roles of the RecBCD and RecF-like pathways in the repair of oxidative damage have not been previously tested.

To determine the roles of members of the RecBCD pathway

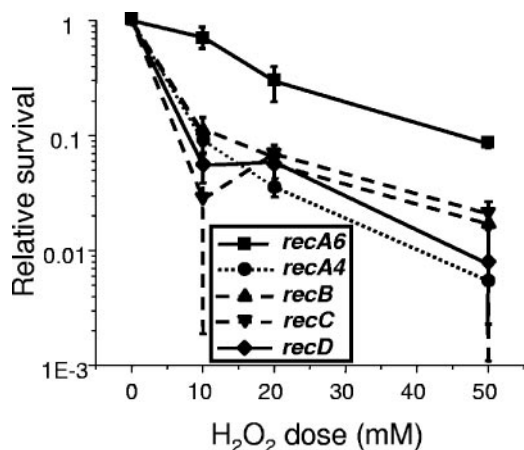


FIG. 2. Effect of RecBCD pathway mutants on H_2O_2 resistance. Strains were grown in the presence of 1 mM IPTG to induce RecA expression. Error bars represent the standard error of the mean of two to six experiments. Error bars for the *recC* strain are dashed to aid in distinguishing strains. All strains were statistically different from the parent *recA6* strain at the 50 mM dose ($P < 0.02$). FA1090*recA4* is included for comparison.

in H_2O_2 resistance, the H_2O_2 resistance of strains with individual mutations in *recB*, *recC*, and *recD* was measured. The individual *recBCD* mutants showed decreased resistance to H_2O_2 relative to the parent strain at all levels of H_2O_2 tested, particularly at the 10 mM dose. These results demonstrate that the *N. gonorrhoeae* RecBCD pathway is important for the repair of DNA damage caused by hydrogen peroxide (Fig. 2).

The RecF-like pathway of DNA repair contributes to hydrogen peroxide resistance. To determine the roles of members of the RecF-like pathway (designated "RecF-like" since *N. gonorrhoeae* lacks a *recF* homolog) in H_2O_2 resistance, strains with individual mutations in *recJ*, *recO*, and *recQ* (25, 46) (Fig. 3) were exposed to H_2O_2 , and the effect on survival was measured. All of the mutants showed the same pattern of sensitiv-

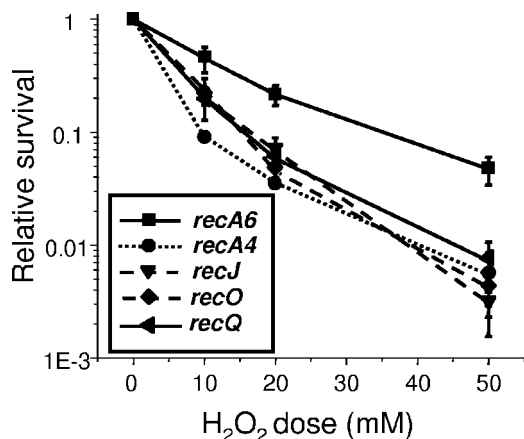


FIG. 3. Effect of RecF-like pathway mutants on H_2O_2 resistance. Strains were grown in the presence of 1 mM IPTG to induce RecA expression. Error bars represent the standard error of the mean of four to nine experiments. All strains were statistically different from the parent strain FA1090*recA6* at the 20 mM dose ($P < 0.04$) and 50 mM dose ($P \leq 0.002$). FA1090*recA4* is included for comparison.

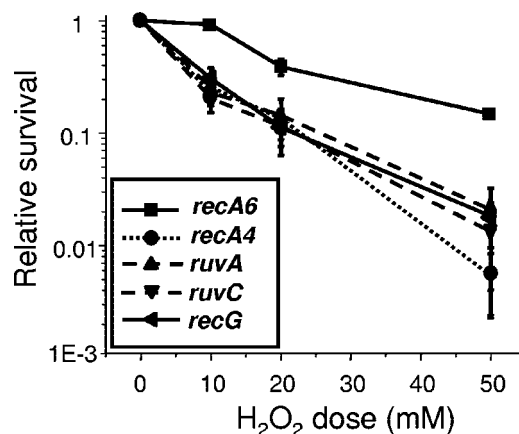


FIG. 4. Effect of Holliday junction processing mutants on H_2O_2 resistance. Strains were grown in the presence of 1 mM IPTG to induce RecA expression. Error bars represent the standard error of the mean of four experiments. All strains were statistically different from the parent strain *recA6* at the 50 mM dose ($P < 0.05$). FA1090*recA4* is included for comparison.

ity to H_2O_2 , exhibiting only small decreases in resistance (two-fold) relative to the parent strain FA1090*recA6* at low levels of H_2O_2 (5 mM) but showing greater decreases in resistance (~10-fold) relative to the parent strain FA1090*recA6* at the 50 mM dose of H_2O_2 . These results mirror what is seen in *E. coli*, with mutants in the RecF pathway being more sensitive to H_2O_2 damage but not as sensitive as mutants in the RecBCD pathway (12).

Branch migration machinery contributes to hydrogen peroxide resistance. The resolution of DNA intermediates formed during recombinational DNA repair by both the RecBCD and the RecF-like pathways requires the action of proteins that catalyze both the translocation (RuvAB and RecG) and the cleavage (RuvC) of Holliday junctions (59). Mutation of the *ruvA* gene in *E. coli* renders strains more sensitive to killing by H_2O_2 (18). Therefore, the H_2O_2 resistance of strains containing mutations in genes that are responsible for branch migration (*ruvA* and *recG*) (36) and cleavage of Holliday junctions (*ruvC*) (35) was measured. All three mutants showed decreased resistance to all tested doses of H_2O_2 , demonstrating that gene products involved in branch migration and Holliday junction resolution are also important for the repair of H_2O_2 -damaged DNA in *N. gonorrhoeae* (Fig. 4).

DISCUSSION

The data presented here represent the first comprehensive examination of the importance of DNA recombinational repair enzymes in resistance to oxidative damage by *N. gonorrhoeae*. We showed that *recA*, genes of the RecBCD and RecF-like recombination pathways, and genes whose products are involved in Holliday junction processing are all important for mediating repair of oxidative damage. Moreover, the data suggest that these genes are expressed at basal levels sufficient to mediate repair and do not need to be upregulated upon encountering DNA damage in order to function efficiently in *N. gonorrhoeae*.

In *E. coli*, RecA activity is central to the ability to repair damaged DNA. RecA participates directly in recombinational

DNA repair (24), and RecA regulates the induction of the SOS response of DNA repair and mutagenesis (58). It has been demonstrated that increased expression of both *recA* (12) and other SOS genes such as *ruvA* (18) contribute to H₂O₂ survival. Our data indicate that there is no increase in *N. gonorrhoeae* RecA protein levels after the exposure of cells to H₂O₂. The lack of RecA protein induction and the overall lack of induction of DNA recombination and repair genes by hydrogen peroxide (51) are consistent with the well-documented lack of an SOS response in *N. gonorrhoeae* (2).

An *N. gonorrhoeae* *recA* mutant was previously shown to be 10-fold more resistant to H₂O₂ than the isogenic parent strain (9), yet we found a *recA* mutant to be ~4-fold less resistant to oxidative challenge by H₂O₂. One reason for this difference could be that the *recA* mutants in the previous study (9) were selected through subculture in liquid, perhaps allowing the accumulation of compensatory mutations that could yield the *recA* mutant cells more resistant to H₂O₂. We used a conditional *recA* mutant in the present study, allowing us to modulate *recA* expression in a single strain, to measure the effect of *recA* on hydrogen peroxide resistance, so the chance of compensatory mutations affecting the results was virtually nonexistent. These results were further corroborated by using the *recA*-null *recA4* and *recA9* mutants, thereby demonstrating conclusively that *recA* is important for survival to oxidative damage in *N. gonorrhoeae*. We also showed that the levels of RecA protein expressed from the endogenous *recA* promoter and from an IPTG-inducible promoter (40) are equivalent.

recX mutation had been previously shown to influence all RecA-mediated processes in *N. gonorrhoeae*, but its effect on DNA repair was the most subtle phenotype (52). In *D. radiodurans*, a *recX* loss-of-function mutant showed both higher activities of the antioxidant enzymes catalase and superoxide dismutase and higher transcript levels of the genes encoding these activities (43). However, the effect of *recX* mutation on H₂O₂ resistance was not measured in *D. radiodurans*. In the present study, the effect of *recX* mutation on survival of *N. gonorrhoeae* to H₂O₂ damage was negligible, and an effect on antioxidant enzymes was not measured. We therefore conclude either that RecX has no role in H₂O₂ resistance or that the role of *N. gonorrhoeae* RecX in H₂O₂ resistance is small enough to not be manifest in our assays, which is consistent with the previously observed subtle phenotype of a *recX* mutant in DNA repair.

Both the *N. gonorrhoeae* RecBCD and the RecF-like pathways are important for survival of oxidative damage, although the increased sensitivity of the RecBCD pathway mutants relative to the RecF-like pathway mutants suggests that RecBCD is the predominant repair pathway for oxidative damage in *N. gonorrhoeae*. This is similar to what was previously observed in *E. coli* (12) and is likely attributable to the fact that the RecBCD pathway repairs DNA double-strand breaks (22), which are the lesions most often resulting from exposure to oxidative damage (5). In *E. coli*, a *recA* single mutant is more sensitive to oxidative damage than a RecBCD or RecF pathway single mutant, and a *recC recF* double mutant shows the same sensitivity as a *recA* mutant (12). In contrast, the RecBCD pathway mutants of *N. gonorrhoeae* were as sensitive as a *recA* mutant, and a *recB recO* double mutant was no more sensitive than the *recB* single mutant (data not shown). These data suggest that the two recombinational repair pathways of

N. gonorrhoeae may be intertwined during the repair of oxidative damage. The *E. coli* paradigm of recombinational repair is that the RecBCD pathway acts on double-strand breaks and the RecF pathway acts on single-strand DNA (ssDNA) gaps. Both double-strand breaks and ssDNA gaps result in stalled replication forks, which, if not repaired, will result in cell death. Our data suggest a model where the double-strand breaks caused by hydrogen peroxide are repaired by the RecBCD pathway and the ssDNA gaps which are repaired by the RecF-like pathway also feed into the RecBCD pathway through an unknown mechanism. It has been previously suggested that *N. gonorrhoeae* utilizes both DNA repair pathways simultaneously and that *N. gonorrhoeae* behaves like an *E. coli* *recBC sbcBC* mutant in its de facto use of the RecF-like pathway in wild-type cells (25). Interestingly, *E. coli* RecFOR proteins are needed for the repair of double-strand breaks in a *recBC sbcBC* mutant background (26), suggesting that there is cross talk between these pathways of DNA repair in *E. coli* as well.

Further steps common to both RecBCD- and RecF-catalyzed recombinational DNA repair are the migration and resolution of Holliday junctions, catalyzed by RuvABC and RecG, followed by replication restart. Replication restart in *E. coli* initiates through the action of DnaC, which reloads the DnaB helicase on stalled replication forks (34). Reloading of DnaB can occur either in a PriA-dependent manner, requiring PriB or PriC, as well as DnaT, or in a PriA-independent manner, requiring Rep, PriC, and possibly also DnaT. Studies on replication-restart in *N. gonorrhoeae* have demonstrated that *N. gonorrhoeae* lacks PriC and DnaT homologues (15), as well as a Rep-dependent replication restart pathway (17), suggesting that only the PriA-dependent pathway functions in replication restart in *N. gonorrhoeae* (16). The importance of replication restart in the repair of oxidative damage has not been investigated in *E. coli*. However, the sensitivity of an *N. gonorrhoeae* *priA* mutant to hydrogen peroxide (16) further demonstrates the importance of recombinational DNA repair in repairing oxidatively damaged DNA and predicts that additional genes involved in replication restart may be especially important for the protection of *N. gonorrhoeae* from oxidative damage.

Although we have demonstrated that RecA and DNA recombinational repair enzymes confer resistance to oxidative damage caused by H₂O₂, these mutants were not as sensitive as isogenic mutants inactivated in NGO1686 (51), which encodes an uncharacterized protein, or *kat* (catalase) (48) (E. Stohl, A. Criss, and H. Seifert, unpublished data), whose product directly degrades H₂O₂. Since oxidative damage is likely to be the main type of DNA damage *N. gonorrhoeae* is exposed to during infection of humans, possessing redundancy in genes conferring protection against H₂O₂ would be evolutionarily advantageous to this strict human pathogen. Moreover, the recent demonstration that *N. gonorrhoeae* is polyploid (54) suggests that, in the event of chromosomal damage, these additional copies of the chromosome could provide the genetic information present on the damaged copy, perhaps obviating the necessity of recombinational repair. Therefore, of the many mechanisms of resistance used by *N. gonorrhoeae* to combat oxidative insult, recombinational DNA repair appears to be one layer of resistance, and continued research will identify others.

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REFERENCES

- Ananthaswamy, H. N., and A. Eisenstark. 1977. Repair of hydrogen peroxide-induced single-strand breaks in *Escherichia coli* deoxyribonucleic acid. *J. Bacteriol.* **130**:187–191.
- Black, C. G., J. A. M. Fyfe, and J. K. Davies. 1998. Absence of an SOS-like system in *Neisseria gonorrhoeae*. *Gene* **208**:61–66.
- Buchmeier, N. A., S. J. Libby, Y. Xu, P. C. Loewen, J. Switala, D. G. Guiney, and F. C. Fang. 1995. DNA repair is more important than catalase for *Salmonella* virulence in mice. *J. Clin. Investig.* **95**:1047–1053.
- Chen, C. Y., and S. A. Morse. 1999. *Neisseria gonorrhoeae* bacterioferritin: structural heterogeneity, involvement in iron storage and protection against oxidative stress. *Microbiology* **145**:2967–2975.
- Demple, B., and L. Harrison. 1994. Repair of oxidative damage to DNA: enzymology and biology. *Annu. Rev. Biochem.* **63**:915–948.
- Drees, J. C., S. L. Lusetti, S. Chitteni-Pattu, R. B. Inman, and M. M. Cox. 2004. A RecA filament capping mechanism for RecX protein. *Mol. Cell* **15**:789–798.
- Duwat, P., S. D. Ehrlich, and A. Gruss. 1995. The *recA* gene of *Lactococcus lactis*: characterization and involvement in oxidative and thermal stress. *Mol. Microbiol.* **17**:1121–1131.
- Galvao, C. W., F. O. Pedrosa, E. M. Souza, M. G. Yates, L. S. Chubatsu, and M. B. Steffens. 2003. The *recX* gene product is involved in the SOS response in *Herbaspirillum seropedicae*. *Can. J. Microbiol.* **49**:145–150.
- Hassett, D. J., L. Charniga, and M. S. Cohen. 1990. *recA* and catalase in H₂O₂-mediated toxicity in *Neisseria gonorrhoeae*. *J. Bacteriol.* **172**:7293–7296.
- Imlay, J. A. 2003. Pathways of oxidative damage. *Annu. Rev. Microbiol.* **57**:395–418.
- Imlay, J. A., and S. Linn. 1986. Bimodal pattern of killing of DNA-repair-defective or anoxically grown *Escherichia coli* by hydrogen peroxide. *J. Bacteriol.* **166**:519–527.
- Imlay, J. A., and S. Linn. 1987. Mutagenesis and stress responses induced in *Escherichia coli* by hydrogen peroxide. *J. Bacteriol.* **169**:2967–2976.
- Kellogg, D. S., Jr., W. L. Peacock, W. E. Deacon, L. Brown, and C. I. Pirkle. 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to colonial variation. *J. Bacteriol.* **85**:1274–1279.
- Kidane, D., H. Sanchez, J. C. Alonso, and P. L. Graumann. 2004. Visualization of DNA double-strand break repair in live bacteria reveals dynamic recruitment of *Bacillus subtilis* RecF, RecO, and RecN proteins to distinct sites on the nucleoids. *Mol. Microbiol.* **52**:1627–1639.
- Kline, K. A., E. V. Sechman, E. P. Skaar, and H. S. Seifert. 2003. Recombination, repair and replication in the pathogenic neisseriae: the 3 R's of molecular genetics of two human-specific bacterial pathogens. *Mol. Microbiol.* **50**:3–13.
- Kline, K. A., and H. S. Seifert. 2005. Mutation of the *priA* gene of *Neisseria gonorrhoeae* affects DNA transformation and DNA repair. *J. Bacteriol.* **187**:5347–5355.
- Kline, K. A., and H. S. Seifert. 2005. Role of the Rep helicase gene in homologous recombination in *Neisseria gonorrhoeae*. *J. Bacteriol.* **187**:2903–2907.
- Konola, J. T., K. E. Sargent, and J. B. Gow. 2000. Efficient repair of hydrogen peroxide-induced DNA damage by *Escherichia coli* requires SOS induction of RecA and RuvA proteins. *Mutat. Res.* **459**:187–194.
- Koomey, J. M., and S. Falkow. 1987. Cloning of the *recA* gene of *Neisseria gonorrhoeae* and construction of gonococcal *recA* mutants. *J. Bacteriol.* **169**:790–795.
- Koomey, M., E. C. Gotschlich, K. Robbins, S. Bergstrom, and J. Swanson. 1987. Effects of *recA* mutations on pilus antigenic variation and phase transitions in *Neisseria gonorrhoeae*. *Genetics* **117**:391–398.
- Kowalczykowski, S. C., D. A. Dixon, A. K. Eggleston, S. D. Lauder, and W. M. Rehauer. 1994. Biochemistry of homologous recombination in *Escherichia coli*. *Microbiol. Rev.* **94**:401–465.
- Kuzminov, A. 1999. Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage lambda. *Microbiol. Mol. Biol. Rev.* **63**:751–813.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**:680–685.
- Lusetti, S. L., and M. M. Cox. 2002. The bacterial RecA protein and the recombinational DNA repair of stalled replication forks. *Annu. Rev. Biochem.* **71**:71–100.
- Mehr, I. J., and H. S. Seifert. 1998. Differential roles of homologous recombination pathways in *Neisseria gonorrhoeae* pilin antigenic variation, DNA transformation, and DNA repair. *Mol. Microbiol.* **30**:697–710.
- Morimatsu, K., and S. C. Kowalczykowski. 2003. RecFOR proteins load RecA protein onto gapped DNA to accelerate DNA strand exchange: a universal step of recombinational repair. *Mol. Cell* **11**:1337–1347.
- Mostertz, J., C. Scharf, M. Hecker, and G. Homuth. 2004. Transcriptome and proteome analysis of *Bacillus subtilis* gene expression in response to superoxide and peroxide stress. *Microbiology* **150**:497–512.
- Palma, M., D. DeLuca, S. Worgall, and L. E. Quadri. 2004. Transcriptome analysis of the response of *Pseudomonas aeruginosa* to hydrogen peroxide. *J. Bacteriol.* **186**:248–252.
- Picksley, S. M., P. V. Attfield, and R. G. Lloyd. 1984. Repair of DNA double-strand breaks in *Escherichia coli* K12 requires a functional *recN* product. *Mol. Gen. Genet.* **195**:267–274.
- Porwollik, S., J. Frye, L. D. Florea, F. Blackmer, and M. McClelland. 2003. A non-redundant microarray of genes for two related bacteria. *Nucleic Acids Res.* **31**:1869–1876.
- Rest, R. F., and W. M. Shafer. 1989. Interactions of *Neisseria gonorrhoeae* with human neutrophils. *Clin. Microbiol. Rev.* **2**(Suppl.):S83–S91.
- Roe, B. A., S. Clifton, and D. W. Dyer. 1997. Gonococcal Genome Sequencing Project. [Online.] <http://www.genome.ou.edu/gono.html>.
- Sanchez, H., D. Kidane, M. Castillo Cozar, P. L. Graumann, and J. C. Alonso. 2006. Recruitment of *Bacillus subtilis* RecN to DNA double-strand breaks in the absence of DNA end processing. *J. Bacteriol.* **188**:353–360.
- Sandler, S. J. 2000. Multiple genetic pathways for restarting DNA replication forks in *Escherichia coli* K-12. *Genetics* **155**:487–497.
- Sechman, E. V., K. A. Kline, and H. S. Seifert. 2006. Loss of both Holliday junction processing pathways is synthetically lethal in the presence of gonococcal pilin antigenic variation. *Mol. Microbiol.* **61**:185–193.
- Sechman, E. V., M. S. Rohrer, and H. S. Seifert. 2005. A genetic screen identifies genes and sites involved in pilin antigenic variation in *Neisseria gonorrhoeae*. *Mol. Microbiol.* **57**:468–483.
- Segal, A. W. 2005. How neutrophils kill microbes. *Annu. Rev. Immunol.* **23**:197–223.
- Seib, K. L., M. P. Jennings, and A. G. McEwan. 2003. A Sco homologue plays a role in defense against oxidative stress in pathogenic *Neisseria*. *FEBS Lett.* **546**:411–415.
- Seib, K. L., H. J. Tseng, A. G. McEwan, M. A. Apicella, and M. P. Jennings. 2004. Defenses against oxidative stress in *Neisseria gonorrhoeae* and *Neisseria meningitidis*: distinctive systems for different lifestyles. *J. Infect. Dis.* **190**:136–147.
- Seifert, H. S. 1997. Insertionally inactivated and inducible *recA* alleles for use in *Neisseria*. *Gene* **188**:215–220.
- Seifert, H. S., C. J. Wright, A. E. Jerse, M. S. Cohen, and J. G. Cannon. 1994. Multiple gonococcal pilin antigenic variants are produced during experimental human infections. *J. Clin. Investig.* **93**:2744–2749.
- Shafer, W. M., and R. F. Rest. 1989. Interactions of gonococci with phagocytic cells. *Annu. Rev. Microbiol.* **43**:121–145.
- Sheng, D., G. Gao, B. Tian, Z. Xu, Z. Zheng, and Y. Hua. 2005. RecX is involved in antioxidant mechanisms of the radioresistant bacterium *Deinococcus radiodurans*. *FEMS Microbiol. Lett.* **244**:251–257.
- Sheng, D., R. Liu, Z. Xu, P. Singh, B. Shen, and Y. Hua. 2005. Dual negative regulatory mechanisms of RecX on RecA functions in radiation resistance, DNA recombination, and consequent genome instability in *Deinococcus radiodurans*. *DNA Repair* **4**:671–678.
- Simons, M. P., W. M. Nauseef, and M. A. Apicella. 2005. Interactions of *Neisseria gonorrhoeae* with adherent polymorphonuclear leukocytes. *Infect. Immun.* **73**:1971–1977.
- Skaar, E. P., M. P. Lazio, and H. S. Seifert. 2002. Roles of the *recJ* and *recN* genes in homologous recombination and DNA repair pathways of *Neisseria gonorrhoeae*. *J. Bacteriol.* **184**:919–927.
- Skaar, E. P., D. M. Tobiason, J. Quick, R. C. Judd, H. Weissbach, F. Etienne, N. Brot, and H. S. Seifert. 2002. The outer membrane localization of the *Neisseria gonorrhoeae* MsrA/B is involved in survival against reactive oxygen species. *Proc. Natl. Acad. Sci. USA* **99**:10108–10113.
- Soler-Garcia, A. A., and A. E. Jerse. 2004. A *Neisseria gonorrhoeae* catalase mutant is more sensitive to hydrogen peroxide and paraquat, an inducer of toxic oxygen radicals. *Microb. Pathog.* **37**:55–63.
- St Amant, D. C., I. E. Valentin-Bon, and A. E. Jerse. 2002. Inhibition of *Neisseria gonorrhoeae* by *Lactobacillus* species that are commonly isolated from the female genital tract. *Infect. Immun.* **70**:7169–7171.
- Stohl, E. A., J. P. Brockman, K. L. Burkle, K. Morimatsu, S. C. Kowalczykowski, and H. S. Seifert. 2003. *Escherichia coli* RecX inhibits RecA recombinase and coprotease activities in vitro and in vivo. *J. Biol. Chem.* **278**:2278–2285.
- Stohl, E. A., A. K. Criss, and H. S. Seifert. 2005. The transcriptome response of *Neisseria gonorrhoeae* to hydrogen peroxide reveals genes with previously uncharacterized roles in oxidative damage protection. *Mol. Microbiol.* **58**:520–532.
- Stohl, E. A., and H. S. Seifert. 2001. The *recX* gene potentiates homologous recombination in *Neisseria gonorrhoeae*. *Mol. Microbiol.* **40**:1301–1310.
- Storz, G., and J. A. Imlay. 1999. Oxidative stress. *Curr. Opin. Microbiol.* **2**:188–194.
- Tobiason, D. M., and H. S. Seifert. 2006. The diplococcus, *Neisseria gonorrhoeae*, is polyploid. *PLoS Biol.* **4**:e185.

55. **Tseng, H. J., Y. Srikhanta, A. G. McEwan, and M. P. Jennings.** 2001. Accumulation of manganese in *Neisseria gonorrhoeae* correlates with resistance to oxidative killing by superoxide anion and is independent of superoxide dismutase activity. *Mol. Microbiol.* **40**:1175–1186.
56. **Turner, S., E. Reid, H. Smith, and J. Cole.** 2003. A novel cytochrome *c* peroxidase from *Neisseria gonorrhoeae*: a lipoprotein from a gram-negative bacterium. *Biochem. J.* **373**:865–873.
57. **Venkatesh, R., N. Ganesh, N. Guhan, M. S. Reddy, T. Chandrasekhar, and K. Muniyappa.** 2002. RecX protein abrogates ATP hydrolysis and strand exchange promoted by RecA: insights into negative regulation of homologous recombination. *Proc. Natl. Acad. Sci. USA* **99**:12091–12096.
58. **Walker, G. C.** 1996. The SOS response of *Escherichia coli*. ASM Press, Washington, D.C.
59. **West, S. C.** 1997. Processing of recombination intermediates by the RuvABC proteins. *Annu. Rev. Genet.* **31**:213–244.
60. **Wu, H. J., K. L. Seib, J. L. Edwards, M. A. Apicella, A. G. McEwan, and M. P. Jennings.** 2005. Azurin of pathogenic *Neisseria* spp. is involved in defense against hydrogen peroxide and survival within cervical epithelial cells. *Infect. Immun.* **73**:8444–8448.
61. **Zheng, H. Y., T. M. Alcorn, and M. S. Cohen.** 1994. Effects of H₂O₂-producing lactobacilli on *Neisseria gonorrhoeae* growth and catalase activity. *J. Infect. Dis.* **170**:1209–1215.
62. **Zheng, M., X. Wang, L. J. Templeton, D. R. Smulski, R. A. LaRossa, and G. Storz.** 2001. DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. *J. Bacteriol.* **183**:4562–4570.