DprA is required for natural transformation and affects pilin variation in *Neisseria gonorrhoeae*

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Natural transformation is the main means of horizontal genetic exchange in the obligate human pathogen *Neisseria gonorrhoeae* and drives the spread of antibiotic resistance and virulence determinants. Transformation can be divided into four steps: (1) DNA binding, (2) DNA uptake, (3) DNA processing and (4) DNA recombination into the chromosome. The DNA processing enzyme DprA has been shown to shuttle incoming ssDNA to the recombination enzyme RecA during transformation in *Bacillus subtilis* and *Streptococcus pneumoniae*. Here, we investigate the role of DprA during transformation in *N. gonorrhoeae*. Inactivation of dprA completely abrogated transformation of gyrB1-encoding DNA, which confers nalidixic acid resistance. The presence of the DNA uptake sequence enhances DNA uptake and transformation by binding to the minor pilus protein ComP. Loss of transformation in the *dprA* null mutants was independent of the DNA uptake sequence. DprA mutants exhibited increased RecA-dependent pilin variation suggesting that DprA affects pilin variation. Unlike the exquisite UV sensitivity of a *recA* mutant, inactivation of *dprA* did not affect survival following UV irradiation. These results demonstrate that DprA has a conserved function during transformation, and reveal additional effects of DprA in *N. gonorrhoeae* during pilin variation.

**INTRODUCTION**

Natural DNA transformation is a widespread mechanism of horizontal genetic exchange in numerous bacterial species [reviewed in Johnsborg et al. (2007)] and is a major driver of evolution of many bacteria. Transformation requires the ability to bind, take up, and recombine exogenous DNA from the environment and is defined as competence (Chen & Dubnau, 2004). With the exception of *Helicobacter pylori*, all currently identified DNA uptake systems use type IV pili (TFP), type II secretion systems, or machinery related to these systems (Chen & Dubnau, 2004). Transformation is the main means of genetic exchange in the obligate human pathogen *Neisseria gonorrhoeae*, which uses TFP for its uptake machinery [reviewed in Obergfell & Seifert (2015)]. TFP are long, thin (approximately 50–80 Å) appendages consisting of thousands of repeating subunits of pilin and function in bacterial adhesion, twitching motility, and transformation (Craig et al., 2004; Wolfgang et al., 1998). Twitching motility is mediated by the depolymerization of pilus oligomers to promote pilus retraction and DNA transport (Wolfgang et al., 1998). In *N. gonorrhoeae* and *Neisseria meningitidis*, TFP undergo pilus phase variation and pilin antigenic variation, which function in immune evasion [reviewed in Rotman & Seifert (2014)].

Pilus phase variation is mediated by two independent mechanisms: RecA-independent, slipped-stranded mispairing of the *pilC* gene and RecA-dependent, high-frequency gene conversion events at the pilE locus that create altered pilins failing to assemble into stable pili (Jonsson et al., 1991; Swanson et al., 1987). Pilin antigenic variation results from RecA-dependent, high-frequency gene conversion events at the pilE locus that create pilE variants forming stable pilin (Haas & Meyer, 1986).

Unlike most identified competent bacteria that regulate competence by quorum sensing, nutrient starvation and/or growth phase (Solomon & Grossman, 1996), *N. gonorrhoeae* is constitutively competent for DNA transformation during all phases of growth (Sparling, 1966). A lack of stable clonal lineages of *N. gonorrhoeae* and strong linkage equilibrium of genes indicate high levels of genome plasticity and that exchange of chromosomal DNA during mixed infections is common (Smith et al., 1993), similar to *Heli. pylori* (Levine et al., 2007). Furthermore, intergenic transfer of DNA has been identified between *Haemophilus* and *Neisseria* (Kroll et al., 1998) and surprisingly between *Neisseria* and humans (Anderson & Seifert, 2011a, b). Frequent DNA transformation is thought to aid in the spread of antibiotic resistance determinants in *N. gonorrhoeae*, which are increasingly refractory to antibiotic therapy. Currently, dual antibiotic therapy is indicated in the United States (Workowski et al., 2015).

Both *N. gonorrhoeae* and the related pathogen *N. meningitidis* preferentially take up and transform their own DNA by virtue
of the DNA uptake sequence (DUS) (Ambur et al., 2007; Elkins et al., 1991; Goodman & Scocca, 1988). There are two forms of the DUS amongst Neisseria: DUS10 (5'-GCGCTGCTGAA-3') and DUS12 (5'-ATGCGCTGCTGAA-3') (additional two nucleotides are underlined), which function to bind the minor pilus protein ComP to induce efficient DNA uptake into the periplasm leading to efficient transformation (Berry et al., 2013; Cehovin et al., 2013). The DUS is abundant in Neisseria genomes (Ambur et al., 2007) and the magnitude of transformation enhancement by the DUS is dependent on the strain of Neisseria and the strand of the DUS when ssDNA is transformed (Duffin & Seifert, 2010, 2012).

Natural transformation can be divided into four basic steps: (1) DNA binding, (2) DNA uptake, (3) DNA processing, and (4) DNA recombination into the chromosome. Much work has identified the complex DNA binding, DNA uptake and DNA recombination machinery involved in these steps in Neisseria transformation, although less is understood regarding the processing of DNA in the periplasm and cytoplasm prior to recombination into the chromosome [reviewed in Obergfell & Seifert (2015)]. The factors that have been identified in DNA processing during transformation of Neisseria include the periplasmic proteins ComL and Tpc, which may aid DNA transport through peptidoglycan (Fussenegger et al., 1996a, b). Current models invoke DNA transport from the periplasm into the cytoplasm through the putative inner membrane protein ComA (Chaussee & Hill, 1998). Once delivered to the cytoplasm, RecA recombines sufficiently homogeneous DNA into the chromosome. A remaining question during Neisseria transformation is whether DNA is protected from cellular nucleases and whether the transforming DNA is delivered to RecA directly.

The DNA processing protein DprA has been shown to be required for transformation in several bacteria including Streptococcus pneumoniae, Helicobacter pylori, Bacillus subtilis and Campylobacter jejuni (Ando et al., 1999; Bergé et al., 2003; Dwivedi et al., 2013; Quevillon-Cheruel et al., 2012; Smeets et al., 2000; Takata et al., 2005; Yadav et al., 2013, 2014). Additionally, DprA was found to be required for transformation in N. meningitidis (Sun et al., 2005) which serves as a model for Neisseria transformation. The role of DprA in transformation is to bind to ssDNA (Mortier-Barrière et al., 2007). The binding of the ssDNA by DprA appears to protect the ssDNA from degradation by cellular nucleases and delivers the ssDNA to RecA to promote recombination into the chromosome (Bergé et al., 2003; Mortier-Barrière et al., 2007). The mechanism of DprA binding to ssDNA and dsDNA has been investigated in Helicobacter pylori (Dwivedi et al., 2015) and S. pneumoniae (Quevillon-Cheruel et al., 2012). Interestingly, DprA localizes to the DNA uptake machinery during transformation in B. subtilis (Tadesse & Graumann, 2007) presumably to receive incoming DNA to shuttle to RecA for recombination.

Based on the observation that DprA is required for transformation in N. meningitidis (Sun et al., 2005), here we investigate the role of DprA in transformation of two strains of N. gonorrhoeae. Inactivation of DprA by insertion mutagenesis completely abrogated transformation in strains FA1090 and MS11. Unexpectedly, the absence of DprA increased RecA-dependent pilin variation. Our data confirm the previous finding that DprA is required for transformation in Neisseria spp. (Sun et al., 2005) and additionally affects pilin variation. This work was presented by P. M. D. at the 19th International Pathogenic Neisseria Conference in 2014.

METHODS

Bacterial strains and growth conditions. Escherichia coli OneShot TOP10 competent cells (Invitrogen) were grown on Luria–Bertani (LB) agar or broth at 37°C for plasmid propagation. N. gonorrhoeae strains were grown on GC medium base (Difco) with Kellogg’s supplements I and II (GCB) (Kellogg et al., 1968) at 37°C in a 5% CO2 humidified atmosphere. The function of DprA was investigated in the laboratory N. gonorrhoeae strains FA1090 (Connell et al., 1998) and MS11 (Meyer et al., 1993) and in numerous mutants derived from these parent strains. The recA6 background contains an IPTG-regulated recA allele allowing for control of RecA expression by the addition of 1 mM final concentration of IPTG in the growth medium (Seifert, 1997). We utilized recA6 FA1090 and MS11 strains [a gift from H. S. Seifert (Northwestern University)]. The addition of 1 mM IPTG achieves RecA expression levels in the recA6 strains similar to the parental strains (Stohl & Seifert, 2006), and in the absence of IPTG, no pilin antigenic variation is observed. The concentration of antibiotics in GCB was as follows: nalidixic acid (Nal), 1 µg ml⁻¹ for FA1090 strains and 3 µg ml⁻¹ for MS11 strains; chloramphenicol (Cam), 0.75 µg ml⁻¹ for FA1090 strains and 10 µg ml⁻¹ for MS11 strains.

Bioinformatics and genome sequence databases. DNA sequences for strains FA1090 and MS11 were obtained from the websites STDOGEN (http://sttdgen.northwestern.edu/) and the Broad Institute Neisseria gonorrhoeae group database (http://www.broadinstitute.org/scientific-community/datacenter/page=1), respectively (accession nos NC_002946 for the FA1090 genome and NZ_AKCH10000007 for the MS11 genome). National Center for Biotechnology Information BLAST searches were conducted for annotation of sequences. Sequence comparisons were performed using the Vector NTI Express AlignX program (Invitrogen).

Construction of dprA mutants. PCR was used to amplify a 1324 bp fragment encompassing the FA1090 wild-type dprA allele. KOD Hot Start DNA polymerase (Novagen) was used with primers dprAupPac1 (5'-TTAATGAAGCATTGGAGAAATGCC-3') and dprAdownPme1 (5'-GTTTAAAAACAAATTTGCTAAGGTTAG-3') (PacI site in dprAupPac1 and Pme1 site in dprAdownPme1 are underlined) with an annealing temperature of 50°C according to the manufacturer’s instructions. The resultant PCR product was gel purified and ligated into pCR-Blunt (Invitrogen) and the ligation mixture was transformed into TOP10 E. coli (Invitrogen) according to the manufacturer’s instructions. Transformants were selected on LB agar containing 50 µg ml⁻¹ kanamycin. Positive clones harbouring pCR-Blunt containing dprA (pPMD1) were verified by DNA sequencing (Advanced Genetic Technologies Center, University of Kentucky).

The cat gene from pHSS6cat2-9 (a gift from H. S. Seifert) was inserted into the BglI site of dprA yielding the inactivated dprA::cat allele. Briefly, plasmid pHSS6cat2-9 was digested with NolI liberating a 1106 bp fragment containing the cat gene, which was gel purified and blunted-ended with Quick blunt (NEB). The pPMD1 plasmids were digested with BglI, blunted-ended with Quick blunt (NEB) and gel purified. The resultant linear DNA was treated with Antarctic Phosphatase (NEB) to remove the 5' overhangs prior to transformation in N. gonorrhoeae.
phosphates. The cat fragment was then ligated with the linear pPMD1 to yield plasmid pPMD2 (containing dprA::cat) and transformed into TOP10 E. coli. Transformants harbouring pPMD2 were selected on agar containing 20 µg ml⁻¹ Cam. Positive clones harbouring pPMD2 were verified via restriction digest analysis. N. gonorrhoeae strains FA1090 and MS11 were transformed with pPMD2 to create strains FA1090 dprA::cat and MS11 dprA::cat. Following passage on GCB Cam plates, we confirmed the presence of the dprA::cat allele by PCR. To construct strains containing dprA::cat and recA6 alleles, we isolated chromosomal DNA from dprA::cat strains and transformed it into strains harbouring recA6. The resulting dprA::cat recA6 FA1090 and MS11 strains were backcrossed and the dprA::cat allele was confirmed by PCR analysis.

**Complementation.** Mutants harbouring the inactivated dprA::cat allele were complemented using the Neisseria insertional complementation system (NICS) (Mehr & Seifert, 1998). A functional copy of dprA with an IPTG-inducible lac promoter was inserted into the N. gonorrhoeae chromosome between the ietP and aspC genes by sub-cloning dprA into plasmid pGCC4 (https://www.addgene.org/37058/). Briefly wild-type dprA was liberated from pPMD1 by double digestion with PacI and Pmel and gel purified (Qiagen). Plasmid pGCC4 was linearized with Pacle and Pmel and gel purified (Qiagen). These fragments were ligated and transformed into E. coli TOP10 cells (Invitrogen) yielding plasmid pPMD3. E. coli transformants harbouring pPMD3 were selected on LB agar containing 100 µg ml⁻¹ kanamycin. Since the dprA::cat strains do not transform (Fig. 2), we could not introduce the complementing dprA locus into the dprA::cat strains. Therefore, to construct a complement containing the dprA::cat locus along with the wild-type dprA allele at the NICS, we first had to introduce the wild-type allele into the NICS and, subsequently, the dprA::cat allele at the dprA locus. FA1090 and MS11 were transformed with pPMD3, which introduced the IPTG-regulatable wild-type dprA allele into the NICS. To select for N. gonorrhoeae harbouring the wild-type dprA at the NICS locus, we plated 100 µl of the transformation mix on GCB containing 2 µg ml⁻¹ erythromycin for FA1090 and 10 µg ml⁻¹ erythromycin for MS11. Lastly, the dprA::cat allele was inserted into the native dprA locus by transforming the aforementioned FA1090 and MS11 cells with pPMD2 and plating as above; this yielded FA1090 and MS11 harbouring both the aforementioned FA1090 and MS11 cells with pPMD2 and plating as above; this yielded FA1090 and MS11 harbouring both the dprA::cat allele and the functional unlinked IPTG-inducible wild-type dprA allele referred to as FA1090 complement and MS11 complement, respectively. Both dprA loci were verified using PCR. FA1090 complement and MS11 complement were grown in the presence of 1 mM IPTG for all assays.

**Transformations and transformation assays.** Piliated N. gonorrhoeae were grown for 18 h on GCB plates and resuspended in liquid transformation medium [1.5% proteose peptone no. 3 (Difco), 0.1% NaCl, 0.4% K₂HPO₄, 0.1% KH₂PO₄, 5 mM MgSO₄ and Kellogg supplements I and II, pH 7.2] to an optical density at 600 nm of approximately 1.5. Thirty microlitres of the cell suspension was added to tubes containing 150 ng of transforming DNA and 200 µl transformation medium. DUS0, DUS10 and DUS12 containing plasmids of gyrB1, which confers resistance to Nal, were used as transformation DNA (Duffin & Seifert, 2010). Following incubation at 37°C for 20 min, we added transformation mixtures to pre-warmed 2 ml transformation medium and incubated at 37°C in the presence of 5% CO₂ for 4 h. For non-quantitative transformations, 100 µl of the mixture was plated on the appropriate selective medium and incubated overnight. For quantitative transformations, the mixtures were serially diluted 10-fold in transformation medium lacking MgSO₄ and Kellogg supplements and 20 µl serial 10-fold dilutions were spotted on GCB plates and GCB Nal plates and grown overnight. Transformation efficiencies are reported as antibiotic resistant c.f.u. divided by total c.f.u. and are the mean of at least three replicates. In cases where no resistant c.f.u. were observed, a value of 1 resistant c.f.u. was used to determine the limit of detection for the assay. Student’s t-test with Bonferroni correction was used for statistical analysis.

**UV sensitivity assays.** Approximately 10⁶ c.f.u. of N. gonorrhoeae strains were serially diluted onto GCB plates and exposed to 0–100 J m⁻² of UV irradiation using a Stratalinker 1800 (Stratagene). The c.f.u. were quantified after 24 h of growth. Survival was calculated relative to unirradiated cells of the same strain and is the mean of at least three replicates.

**Pilus-dependent colony morphology changes assay.** Pilin variation was measured using the previously described pilus-dependent colony morphology changes (PDMC) assay (Sechman et al., 2005), which monitors formation of faster-growing P⁺ colony outgrowths that emerge on the edge of P⁻ colonies over time with the following modifications. P⁺ and P⁻ colonies vary morphologically and are indicative of pilus expression. Colony variation of at least 15 P⁺ N. gonorrhoeae colonies grown on GCB was scored at the indicated times using a stereomicroscope. Colonies with zero outgrowths were given a score of 0, colonies with one outgrowth were given a score of 1 and so on. Colonies with four or more outgrowths were given a score of 4. Results are the means of at least three independent experiments per strain. Student’s t-test for matched time points was used for statistical analysis.

**RESULTS**

**Identification, mutagenesis and complementation of dprA in N. gonorrhoeae.**

One dprA homologue was identified in each of the annotated genomes of N. gonorrhoeae strains FA1090 and MS11. A BLAST search revealed no other copies of the gene in the genomes. The 1188 bp dprA gene (NGO1865 in FA1090 and NGFG_01781.2 in MS11) is predicted to encode a 395 amino acid protein and is the first gene in a putative operon with NGO1864 in FA1090 (Fig. 1). To inactivate dprA in N. gonorrhoeae strains FA1090 and MS11, we inserted a 1194 bp cat cassette into the BsgI site that cuts at dprA nucleotide 335 resulting in the knockout dprA::cat (Fig. 1). Given that dprA appeared to be in an operon, a wild-type dprA allele was inserted at an ectopic site in the chromosome unlinked to the original mutation under control of an IPTG-inducible promoter (Mehr & Seifert, 1998) to complement the dprA::cat allele. None of the mutants or complements exhibited any growth defects on solid medium (data not shown).

**DprA is absolutely required for transformation.**

The role of DprA in transformation [reviewed in Johnston et al. (2014) and Rotman & Seifert (2014)] has been studied in several species of naturally transforming bacteria including B. subtilis (Tadese & Graumann, 2007; Yadav et al., 2014), S. pneumoniae (Mirouze et al., 2013; Mortier-Barrière et al., 2007; Quevillon-Cheruel et al., 2012), Helicobacter pylori (Smeets et al., 2000; Zhang & Blaser, 2012) and C. jejuni (Takata et al., 2005). To test the role of DprA in transformation of N. gonorrhoeae, we performed quantitative transformation assays in parental (wild-type) and dprA strains of FA1090 and MS11. Transforming DNA contained...
the gyrB1 gene (confers resistance to nalidixic acid) with no DUS (DUS0) or containing the DUS10 or DUS12 sequence (Duffin & Seifert, 2010) (Fig. 2). DprA was absolutely required for transformation in both FA1090 and MS11 strains of *N. gonorrhoeae* as no transformation was detected in the *dprA* mutants regardless of the presence or absence of the DUS10 or DUS12 (Fig. 2). Complementation with a wild-type IPTG-inducible *dprA* allele at an ectopic locus restored transformation albeit to levels slightly lower than parental transformation efficiencies in both FA1090 and MS11 (Fig. 2, compare parental to complement *P* <0.016).

As previously reported (Duffin & Seifert, 2010), the DUS10 and DUS12 containing DNA transformed at higher efficiencies than DNA lacking the DUS (DUS0) in parental and complement FA1090 and MS11 (Fig. 2, *P* <0.05). Therefore, *dprA* is a functional gene in *N. gonorrhoeae* and is required for transformation.

**DprA affects pilin variation**

Pilin variation in *N. gonorrhoeae* has been extensively studied [reviewed in Cahoon & Seifert (2011)] and can occur through slipped-stranded mispairing of *pilC* (*PilC phase variation*) and the RecA-dependent antigenic variation of *pilE*. Given the well-studied interaction between DprA and RecA in non-*Neisseria* bacteria and the role of RecA in pilin variation, we hypothesized that DprA may play a role in pilin variation. To test this, we measured pilin variation of the *dprA* mutants using a colony morphology proxy for pilin variation. PDCMC, which are indicative of pilin variation (Sechman et al., 2005), were measured and quantified in our strains. Cells lacking functional DprA exhibited significantly higher pilin variation compared to parental cells in both FA1090 and MS11 (*P* <0.05, Fig. 3). Complementation of the mutants restored pilin variation to parental levels (Fig. 3). Thus, inactivation of *dprA* enhances pilin variation suggesting that DprA influences this process.

**DprA affects RecA-dependent pilin variation**

Two independent processes, RecA-independent slipped-stranded mispairing of *pilC* during DNA replication and RecA-dependent DNA recombination at the *pilE* locus, produce pilin variation. We wondered whether DprA affects both processes. Since PilC phase variation but not *pilE* variation occurs in the absence of RecA, we moved the *dprA*::*cat* allele into a *recA6* background. The *recA6* strains

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**Fig. 1.** *dprA* locus in *N. gonorrhoeae* strain FA1090. ORFs are indicated by block arrows and point in the direction of transcription. Names of ORFs are indicated below each arrow (1868 and 1864 encode hypothetical proteins). The triangle indicates the position of the *cat* cassette (black arrow) insertion in *dprA* yielding the loss-of-function allele *dprA*::*cat*. Drawing is to scale. Bar, 500 bp.

**Fig. 2.** DprA is required for transformation in *N. gonorrhoeae* strains FA1090 (a) and MS11 (b). Parental (wild-type), *dprA* and complement *N. gonorrhoeae* were quantitatively transformed with *gyrB1* DUS0, DUS10 and DUS12 plasmid DNA (which confers nalidixic acid resistance). Transformation efficiencies are plotted as resistant c.f.u./total c.f.u. and are the mean of at least three replicates. White bars indicate no DNA added, mid-grey bars indicate DUS0 DNA, light grey bars indicate DUS10 DNA and dark grey bars indicate DUS12 DNA. In all cases where transformation was observed, transformation of the parent was significantly higher than the complement (*P* <0.016 by Student’s *t*-test, Bonferroni correction). Error bars are SEM. *, Below limit of detection (no transformation observed).
harbour IPTG-inducible recA alleles, which allow control of RecA expression (Seifert, 1997). In the absence of RecA expression, dprA mutants exhibited levels of pilin variation similar to the isogenic dprA wild-type recA6 strains, whereas the dprA mutants had increased levels of pilin variation in the presence of RecA expression ($P<0.05$, Fig. 4). These results indicate that DprA affects pilin variation resulting from pilE recombination but not PilC phase variation.

**DprA is dispensable for DNA repair following UV mutagenesis**

Given the aforementioned results suggesting that DprA functions alongside RecA in pilin variation and transformation, we wondered whether DprA affects DNA repair. Previous studies have shown that UV-induced DNA damage is repaired mostly by nucleotide excision repair in *N. meningitidis* (Davidsen et al., 2007). However, cells lacking RecA

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**Fig. 3.** Increased frequency of pilus-dependent colony variations in DprA-deficient FA1090 (a) and MS11 (b). Parental, isogenic dprA mutants and complemented strains were grown and colony variation was measured as an indicator of pilin variation. At the indicated times, colonies were scored as follows: 0, no blebs; 1, one bleb; and so on. Colonies with four or more blebs were given a score of 4. The variation scores for 100 colonies were averaged for each time point and graphed. *$P<0.05$ by Student’s $t$-test. Error bars are SEM.

**Fig. 4.** Increased frequency of RecA-dependent pilin variation in DprA-deficient *N. gonorrhoeae* strains FA1090 (a) and MS11 (b). Colony morphology changes were measured in FA1090 and MS11 recA6 parental strains and isogenic dprA mutants. The recA6 allele is IPTG regulatable, which allows for control of recA expression. IPTG was added to the medium to induce recA expression where indicated. Colony morphologies were scored as in Fig. 3. *$P<0.05$ by Student’s $t$-test. Error bars are SEM.
are exquisitely sensitive to UV, suggesting that RecA is involved in DNA repair following UV damage. To examine whether DprA functions in recombinational DNA repair, we measured the survival of dprA mutants following UV mutagenesis. Unlike the exquisite sensitivity of cells lacking recA (Criss et al., 2010; Kunkel & Erie, 2005), the dprA mutants were indistinguishable from the parental strain in both FA1090 and MS11 in survival following UV irradiation (Fig. 5). Therefore, DprA does not participate in survival against UV irradiation in N. gonorrhoeae.

**DISCUSSION**

The DNA processing protein DprA has been shown to be involved in transformation in many bacterial species. Here we show that DprA is absolutely required for transformation in two strains of *N. gonorrhoeae*. Given the interaction between DprA and RecA during transformation, we investigated a potential function of DprA in other processes involving RecA. Surprisingly, we uncovered that DprA affects RecA-dependent pilin variation in *N. gonorrhoeae*. Taken together, these data demonstrate the conserved function of DprA during transformation and reveal an additional effect of DprA in *N. gonorrhoeae*.

DprA has an established role during transformation of many bacteria species, as mutants deficient in DprA are completely or partially deficient in transformation (Bergé et al., 2003; Dwivedi et al., 2013; Karudapuram & Barcak, 1997; Mirouez et al., 2013; Mortier-Barrière et al., 2007; Quevillon-Cheruel et al., 2012; Yadav et al., 2013, 2014); collectively, these investigations demonstrate that DprA localizes to DNA uptake machinery and binds to incoming ssDNA, presumably protecting the DNA from degradation by cytoplasmic nucleases. The ssDNA–DprA complex has been shown to interact with RecA to form filaments, which promote recombination by RecA. Supporting previous models of DprA action, we observed no transformation in *N. gonorrhoeae* when dprA was inactivated (Fig. 2) indicating the conserved function of DprA in transformation.

Although the recent elucidation of the mechanism of DUS enhancement of transformation via binding the minor pilus protein ComP (Cehovin et al., 2013) has clarified the complex transformation phenotypes reported when different DNA substrates are used, the possibility that the DUS plays a role during DNA processing remains possible (Duffin & Seifert, 2010, 2012). Here, no transformation was observed in dprA mutants regardless of the presence or absence of the DUS in the DNA substrate.

DprA has been identified in many bacterial species that are not known to undergo transformation leading to a hypothesis that DprA functions in other processes in these bacteria. To determine whether DprA from a non-transforming species is capable of functioning during transformation, Smeets et al. cloned dprA from *E. coli* into *Haemophilus influenzae* lacking the wild-type *Haem. influenzae dprA* (Smeets et al., 2006). *E. coli* DprA partially restored transformation in *Haem. influenzae*, demonstrating that the *E. coli* DprA retains the ability to do so. Interestingly, no phenotype was identified in the dprA mutant *E. coli*; thus, the function of DprA in non-transforming bacteria remains elusive.

**Fig. 5.** DprA is not involved in DNA repair mechanisms following UV irradiation in *N. gonorrhoeae* strains FA1090 (a) and MS11 (b). Parental and isogenic dprA mutant cells were exposed to the indicated dosages of UV and relative survival was measured. The mean relative survival from three replicates is graphed. No statistical differences were observed between the parent and dprA strains. Error bars are SEM.
Given the hypothesis that DprA may function beyond transformation, we investigated whether DprA was involved in pilin variation and DNA repair. Similar to investigations in S. pneumoniae (Bergé et al., 2003), we found that DprA was dispensable for DNA repair following UV mutagenesis (Fig. 5). However, we found that dprA mutants exhibited increased RecA-dependent pilin variation compared to isogenic parental strains (Figs 3 and 4) suggesting a possible role of DprA in modulating this process. RecA-dependent pilin variation at the pilE locus in Neisseria has been the focus of intense research for decades [reviewed in Cahoon & Seifert (2011) and Rotman & Seifert (2014)] and current models invoke numerous recombination proteins (RecA, RecX, RecO, RecR, RecJ, RecQ, RecG, RuvABC) and the formation of quadruplex DNA to initiate the non-reciprocal recombination process. Several components of the mismatch correction system have been shown to modulate pilin antigenic variation although the precise mechanism remains unknown (Criss et al., 2010). Similar to our work with DprA here, mismatch correction-deficient N. gonorrhoeae exhibited increased pilin variation and biased the type of changes occurring at the pilE locus (Criss et al., 2010). Both natural transformation and pilE variation require RecA-dependent strand invasion of ssDNA to form heteroduplex DNA leading to Holliday junctions that are processed by RuvABC. A key difference between transformation and pilin variation is that the recombination during pilin variation results from uni-directional gene conversion whereas transformation recombination utilizes bi-directional or reciprocal recombination.

Although the exact mechanism underlying our pilin variation data is not known, there are several possibilities. Given the observed binding of non-Neisseria DprA to single-stranded binding protein (SSB) coated ssDNA to recruit RecA for reciprocal recombination (Yadav et al., 2013), it is possible that, in the absence of DprA, recombination proteins assemble to bias strand invasion and Holliday junction intermediates that favour gene conversion, thus increasing the frequency of these events and pilin variation overall. Supporting this notion is the observation that purified N. gonorrhoeae RecA is more efficient at dislodging SSB from ssDNA to promote strand exchange than purified E. coli RecA (Stohl et al., 2011). Furthermore, differences in strand exchange were observed between E. coli RecA and N. gonorrhoeae RecA based on the DNA substrates. Thus, DprA loading of RecA onto ssDNA may bias RecA to favour reciprocal recombination that could explain the conserved function of DprA in transformation.

Another possible explanation of DprA modulating pilin variation relies on the observation that S. pneumoniae DprA binds ssDNA and interacts directly with RecA (Mortier-Barrière et al., 2007). Models of pilin variation invoke a double-strand break at the pilE locus resulting in a single strand invading the pilS locus (Rotman & Seifert, 2014). Therefore, it is possible that DprA binds this small single-strand fragment during strand invasion and inhibits the formation of heteroduplex thereby reducing gene conversion and pilin variation. Given the nature of interaction between DprA and RecA and the multifaceted role of RecA in many bacteria, it seems possible that DprA has additional interactions beyond its conserved role in transformation.

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DprA function in N. gonorrhoeae


