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MicroReview

Recombination, repair and replication in the pathogenic *Neisseriae*: the 3 R's of molecular genetics of two human-specific bacterial pathogens

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Summary

Most of the detailed mechanisms that have been established for the molecular biological processes that mediate recombination, repair and replication of DNA have come from studies of the *Escherichia coli* paradigm. The human specific pathogens, *Neisseria gonorrhoeae* and *N. meningitidis,* are Gram-negative bacteria that have some molecular processes that are similar to *E. coli* and others that appear to be divergent. We propose that the pathogenic *Neisseriae* have evolved a specialized collection of molecular mechanisms to adapt to life limited to human hosts. In this MicroReview, we explore what is known about the basic processes of DNA repair, DNA recombination (genetic exchange and pilin variation) and DNA replication in these human specific pathogens.

Introduction

The genus *Neisseria* contains many species that are only found in humans including several commensal organisms that inhabit the nasopharynx. Two members of the *Neisseriae*, *N. gonorrhoeae* and *N. meningitidis*, have evolved into highly successful pathogens. *N. gonorrhoeae* (the gonococcus, Gc) colonizes the urogenital tract and is the sole aetiological agent of the sexually transmitted disease gonorrhea. Interpretation of Egyptian and Biblical writings suggests that gonorrhea has been found in humans for thousands of years (Wain, 1947; Morton, 1977). These observations coupled with the close association of the genus with humans suggest that most members of the *Neisseriae* have evolved within humans. *N. meningitidis* (the meningococcus, Mc) colonizes the nasopharynx, and while Mc also varies its surface molecules, it is less certain whether these changes also contribute to immune evasion or are variable solely for functional reasons. In the majority of people, Mc infections are asymptomatic and Mc behaves more like a commensal organism. However, Mc is a true pathogen as it is the most frequent cause of bacterial meningitis in young adults. If Mc exits the nasopharynx and travels through the blood stream, it can transit to the meninges to cause severe meningitis. Protection of the bacterium from complement and phagocytosis afforded by the polysaccharide capsule is one reason that this species is strongly associated with meningitis (McNeil *et al*., 1994; Vogel and Frosch, 1999). Moreover, systemic bacteremia can also result from Mc exiting the nasopharynx and can lead to septic shock without meningitis (Brandtzaeg and van Deuren, 2002). Factors believed to play a role in the transition from a carrier state to disease include the introduction of new antigenic types into individuals and unknown changes in the bacterium producing more virulent clones (Virji, 1996).

Like many bacterial pathogens, much research on Gc and Mc has focused on host–pathogen interactions, in hopes of identifying vaccine candidates and to better understand the mechanisms by which the bacteria interact with their host to elicit disease. In contrast, investigations into basic mechanisms of DNA replication, repair and recombination have been relatively sparse in the literature. One possible reason for the lack of research into these molecular processes is the assumption that all bacteria, and particularly all Gram-negative bacteria, are essentially the same as the *E. coli* paradigm. While it is true that many of the basic genetic mechanisms for replication, repair and recombination are conserved between bacterial species, there are many differences between species in the mechanistic properties, biological functions

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and molecular components involved in these processes. This review will outline the present status of research on basic replication, repair and recombination mechanisms in Gc and Mc.

Genetic exchange in the *Neisseriae*

As haploid organisms, all bacteria rely on horizontal transfer of chromosomal sequences to allow the mixing of different gene combinations and the acquisition of new genes. In fact, genetic exchange occurs so frequently in *Neisseria* that Gc are considered to be panmictic (Smith *et al*., 1993). DNA transformation is the only known means of genetic exchange of chromosomal markers by *Neisseria* (Norlander *et al*., 1979a; Spratt *et al*., 1989). While conjugal plasmids are found in the *Neisseriae* and contribute to antibiotic resistance (Eisenstein *et al*., 1977; Roberts and Falkow, 1977), they do not mobilize chromosome sequences (Norlander *et al*., 1979b; Steinberg and Goldberg, 1980). DNA transformation also contributes to the acquisition of chromosomally encoded antibiotic resistance (Spratt *et al*., 1989). Unlike other naturally competent organisms, such as *Haemophilus influenzae* and *Bacillus subtilis* that are competent only at certain stages of growth (Dubnau, 1999), both Gc and Mc are naturally competent and remain fully competent for DNA transformation throughout growth (Catlin and Cunningham, 1961; Sparling, 1966). For Gc, transformation frequencies are usually 10^{-3}–10^{-2} transformants per colony-forming unit under saturating conditions with whole chromosomal DNA (Sparling, 1966), but can reach frequencies as high as 50% when large amounts of cloned Gc DNA are provided (Stein, 1991; Boyle-Vavra and Seifert, 1996).

It has long been thought that the DNA available for genetic transformation is produced through cell lysis and death, which, in the *Neisseria* largely occurs during stationary phase. Recent work has provided an additional mechanism for transformation in Gc. A majority of Gc isolates carry a genetic island that encodes a type IV secretion system (Dillard and Seifert, 2001; Hamilton *et al*., 2001) that has been shown to provide transforming DNA into the medium without cell lysis (Dillard, 2001). The island-encoded type IV secretion system is most similar to the well-studied F-factor conjugation system (Frost *et al*., 1994). The genetic island carrying the type IV secretion system is found only in Gc and not in Mc or any commensal *Neisseriae* (Dillard, 2001), suggesting Gc acquired this genetic element after it diverged from the other *Neisseria*. Because the island is found in most isolates from disseminated gonococcal infections and certain forms of this island correlate with serum resistance (Dillard, 2001), it is likely that this genetic island directly influences pathogenicity. It is also possible that the type IV secretion system not only functions to mediate genetic transfer but may also transport other molecules that are important for Gc pathogenesis. The identification of this Gc type IV secretion system suggests that, in a majority of Gc strains, DNA released for transformation is not only obtained from lysing cells, but can also be secreted by growing bacteria that can continue on to cause disease and disseminate within and between hosts.

Once DNA is released by the type IV secretion system or by autolysis, the DNA is imported into the cytosol where it can recombine with resident homologous sequences. DNA uptake in the *Neisseriae* is directly correlated with piliation (Wolfgang *et al*., 1998). Recent work suggests that pilus expression exposes proteins on the bacterial cell surface that are required for transformation and allows them to interact with the DNA uptake sequence (Aas *et al*., 2002), a specific 10 base pair nucleotide sequence required for efficient transformation (Goodman and Scocca, 1988). There is no direct evidence that the pilus is required for transformation competence, but rather it appears that the pilus assembly apparatus is activated for DNA transport by the presence of the pilin subunit of the pilus (Aas *et al*., 2002) (Long *et al*., 2003). Once the DNA is internalized, it can be efficiently recombined with homologous sequences in the chromosome. Transformation absolutely depends on the RecA protein (Koomey and Falkow, 1987). In contrast, DNA transformation competence relies partially on the RecBCD enzyme (Mehr and Seifert, 1998) and RecN (Skaar *et al*., 2002), but is independent of the RecF-like pathway of recombination (Mehr and Seifert, 1998; Skaar *et al*., 2002). The biochemical function of RecN is unknown for any bacterium, but due to RecN similarity to SMC proteins has been hypothesized to play a role in positioning recombining segments of DNA to aid in recombination (Skaar *et al*., 2002). We have proposed that the partial dependence on the RecBCD enzyme for transformation may reflect two states of the transforming DNA, double stranded requiring RecBCD to unwind the DNA and single stranded not requiring RecBCD (Mehr and Seifert, 1998). Supporting this hypothesis are the observations that the type IV secretion system predominately secretes single-stranded DNA that efficiently transforms Gc cells (H. Hamilton, J. Skarie and J. Dillard, pers. comm.), that cloned single-stranded and double-stranded Gc DNA transform Gc with similar efficiencies (Stein, 1991) and that both single- and double-stranded DNA can be detected in the Gc periplasm after transformation with double-stranded DNA (Chaussee and Hill, 1998). However, others have reported that single-stranded DNA is about 100-fold less active for transformation of Gc (Biswas and Sparling, 1981) and could not be detected during transformation (Biswas and Sparling, 1981; Facius *et al*., 1996). More research is needed to understand how DNA is recognized, transported and

recombined during genetic transformation to determine the relative contributions of double- and single-stranded DNA molecules to transformation.

DNA repair

The repair of damaged DNA is a critical process in maintaining genomic integrity. DNA repair mechanisms have been well-characterized in *E. coli*. Very little is known about DNA repair mechanisms in the *Neisseriae,* but with the completion of the genome sequences of one Gc and two Mc isolates, direct comparisons of potential DNA repair genes to the *E. coli* paradigm can be made. The pathogenic *Neisseriae* possess homologues of many genes known to be involved in DNA repair in *E. coli*, with several notable absences. Because the *Neisseriae* exist exclusively in the human body and are not believed to inhabit another environmental niche, they do not usually encounter UV irradiation, desiccation or other extreme environments. Therefore the repair capabilities of the *Neisseriae* are predicted to be specialized for damage that might occur within the human nasopharynx, blood stream or genital tract. One of the most important DNA repair systems in bacteria is the SOS response. In *E. coli*, the SOS system is induced in response to replication stalls and induces several factors that increase the probability that replication can continue (Goodman, 2002). There is no SOS system in the *Neisseriae*, as there are no LexA homologues or classic SOS boxes in the Neisserial genomes (Roe *et al.*, 1997; Black *et al.*, 1998). Moreover, in contrast to *E. coli*, treatment of Gc with the mutagens methyl methanesulfonate or UV light does not produce increased mutation frequency (Campbell and Yasbin, 1984a) or upregulation of *recA* (Black *et al.*, 1998). The possibility that *Neisseria* possess a damage inducible repair system different than the SOS system has not been ruled out.

Gc possesses an active excision repair system that can function on pyrimidine dimers (Campbell and Yasbin, 1984b). Excision repair systems are found in most organisms and can excise most types of severe damage except cross links. All of the genes encoding components of the nucleotide excision repair pathway (*uvrA*, *uvrB*, *uvrC*, *uvrD, mfd*) are present in the sequenced genomes of the *Neisseria* (Table 1). Inactivation of the gonococcal *uvrB* gene leads to increased sensitivity to UV light (Black *et al.*, 1995); however, because *Neisseria* are unlikely to encounter UV light within their human hosts, it is likely that the excision repair system repairs other types of damage.

Homologues of genes involved in base excision repair and very short repair can be identified in the *Neisseria* genomes including *nth, tag, mutMTY, xthA, ogt* and *fpg* (Table 1). The *Neisseriae* lack homologues of the DNA glycosylase *nei* and the endonuclease *nfo.* Molecules with redundant functions may compensate for the absence of these repair enzymes or the lesions recognized by these enzymes may be rare. *N. gonorrhoeae* has been reported to lack a photoreactivation system as survival rates of UV-irradiated gonococcal cells are similar whether incubated in the dark or exposed to photoreactivating light (Campbell and Yasbin, 1979). However, a potential homologue to the photolyase encoded by the *phr* gene is present in the sequenced genomes of both *N. gonorrhoea*e and *N. meningitidis*. The *phr* gene is in a potential operon with a LysR family regulator and a homologue of the repair associated helicase *dinG.* It is possible that expression of this putative operon is regulated by LysR and the lack of observable photoreactivation reflects non-expression-inducing growth conditions. Whether *phr* is a functional homologue for this repair system and is subject to regulation remains to be determined. Interestingly, both Gc and Mc lack homologues for any genes involved in the response to alkylative damage. The absence of this repair system suggests that these organisms never encounter alkylating agents in the human body.

The most studied of DNA repair systems in the *Neisseriae* is the recombinational repair system. Recombina-

Table 1. Classic repair pathways: homologues absent in the sequenced genomes of the pathogenic *Neisseria*.

Repair pathway	Genes comprising the *Escherichia coli* paradigmatic process	Gene homologues missing in the pathogenic *Neisseriae*
SOS repair	*recA*, *X, N*; *dinB*; *polB*; *lexA*; *umuC, D*	*polB*; *lexA*; *umuC, D*
Nucleotide excision repair	*uvrA*, *B*, *C*, *D*; *mfd*	none
Base excision repair	*nth; nei; tag; ung; mutM,T, Y; nfo; xthA; ogt*; *fpg*	*nei, nfo*
Photo reactivation	*phr*	none
Alkylation repair	*ada; alkA, B; aid B*	*ada; alkA, B; aid B*
Very short repair	*vsr; mutL, S*	none
Methyl-dependent mismatch repair	*mutH, L, S*	*mutH*
Recombinational repair	*recA*, *X*, *BCD*, *FOR*, *Q*, *J*, *N*, *G*, *E*, *T*; *sbcB*, *D*; *rusA*; *ruvABC*	*recF*, *E*, *T*, *sbcB*, *D*; *rusA*[a]
Replication restart	*priA*, *B*, *C*; *dnaA*, *B*, *C*, *G*, *T*; *rep*	*priC*, *dnaC*[a], *T*

a. The *rusA* and *dnaC* genes are present in the Gc FA1090 genome but missing in both Mc genomes.
Escherichia coli repair and recombination gene open reading frames (ORFs) were used to search for homologues in Gc strain FA1090 and Mc strains Z2491 and MC58 using the BLAST program. Genes with greater than 25% sequence identity or 40% sequence similarity over at least one-third of the gene were considered to be evidence for a homologue.

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tional repair in *E. coli* can be mediated by either the RecBCD or the RecF pathway of homologous recombination (Kowalczykowski *et al.*, 1994). Genes from both of these pathways have been found in Gc and have been shown to have a role in DNA repair. The *recA* (Koomey and Falkow, 1987) and *recX* (Stohl and Seifert, 2001) genes appear to act in both pathways and both are involved in DNA repair. The RecBCD pathway components *recB*, *recC* and *recD* have been shown to be involved in DNA repair in Gc (Mehr and Seifert, 1998). In addition, Mc possessing a naturally defective *recB* allele exhibit impaired repair of DNA lesions induced by UV treatment (Salvatore *et al.*, 2002). In contrast, a Gc *recD* mutant was reported to show no reduction in UV survival (Chaussee *et al.*, 1999). *Neisseriae* possess a *recN* homologue that is necessary for efficient DNA repair and potentially acts in the RecBCD pathway in Gc (Skaar *et al.*, 2002). RecN may be playing a similar role in DNA repair to that proposed for it in DNA transformation, that is, a structural role in positioning recombining DNA. Homologues of the RecF pathway components *recO*, *recR*, *recQ* and *recJ* are present in *Neisseriae*. However, the completely sequenced Neisserial genomes lack a RecF homologue, leading to the designation of a RecF-like pathway in these organisms (Mehr and Seifert, 1998). Finally, potential homologues of the recombinational repair genes *recG, recR, ruvA, ruvB and ruvC* can be identified in Neisserial genome sequences and we have found that *recG, recR* and *ruvA* all contribute to DNA repair (E. V. Sechman and H. S. Seifert, unpubl.) (Table 1). Interestingly, Mc differs from Gc in that Mc lacks a homologue to the Holliday junction resolvase encoded by *rusA* (Roe *et al.*, 1997; Parkhill *et al.*, 2000). Inactivation of individual Gc RecF-like pathway genes leads to severe reductions in DNA repair capability. This is in contrast to *E. coli*, where inactivation of individual RecF pathway genes leads to severe reductions in repair efficiency only in a *recBC sbcBC* mutant background (Kushner *et al.*, 1971; Lloyd and Buckman, 1985). Based on the lack of potential *sbcBC* homologues in Gc and the decreased repair phenotypes of RecF-like pathway mutants, it has been suggested that *N. gonorrhoeae* behaves similarly to an *E. coli sbcBC* mutant such that the RecF-like pathway is active in wild-type cells (Mehr and Seifert, 1998). This is probably due to the fact that the recombinational repair pathways are more important for the *Neisseriae* which lack an SOS response.

Finally, a mismatch correction system has been identified in Mc, consisting of genes known to be involved in *E. coli* methyl-directed mismatch repair including *mutS, uvrD (mutU)*, *mutY* and *recJ* but lacking *mutH* (Richardson and Stojiljkovic, 2001) and *sbcB* (Exonuclease I) (Parkhill *et al.*, 2000) (Table 1). The same set of genes can also be found in the Gc FA1090 genome sequence (Roe *et al.*, 1997). Inactivation of *mutS* and *mutL*, and consequently the mismatch repair system of Mc, leads to an increase in overall mutability and elevated frequencies of phase variation (Richardson and Stojiljkovic, 2001). Because MutH is responsible for the initial nick of the newly synthesized, unmethylated strand in *E. coli*, the absence of a MutH endonuclease may explain why Mc *dam* mutants are not affected in mutability (Bucci *et al.*, 1999; Richardson and Stojiljkovic, 2001). Although lack of MutH homologues has been postulated to produce a mutator phenotype in *Neisseriae* (Radman *et al.*, 2000), it is probable that another mechanism provides strand discrimination during mismatch correction in these organism. The strand recognition system in the *Neisseriae* may be more similar to eukaryotic organisms and could be directly linked to the replication apparatus (Holmes *et al.*, 1990; Petranovic *et al.*, 2000).

Replication restart

DNA repair and recombination factors have a significant role in maintaining replication of the chromosome. During replication, unrepaired lesions in either template strand will hinder the progress of the replisome resulting in a stalling or complete dissociation of the replication machinery (McGlynn and Lloyd, 2002). In some cases, a replisome that encounters DNA damage can bypass it with the help of error-prone polymerases. Although bypass allows for cell survival, it can also result in mutation. Alternatively, recombination can be activated at stalled replication forks to allow for the repair of damaged DNA templates (Michel, 2000). Once the damage is repaired, the replication machinery must be reloaded onto the DNA template in order for replication to resume its progress.

Work in *E. coli* has led to the proposal that Holliday junctions form from stalled replication forks and it is through the processing of the junctions that restart of replication occurs (McGlynn and Lloyd, 2002). In *E. coli*, at least two recombination mechanisms can be employed to facilitate replication restart after repair of the DNA lesion. One requires RuvAB branch migration and RuvC cleavage of the Holliday junction to allow primosome assembly by PriA. A second pathway requires both the primosome assembly function and the helicase functions of PriA along with the helicase activity of RecG (Gregg *et al.*, 2002). One of several specialized molecular processes can then facilitate primosome loading and restart replication in an origin-independent manner in *E. coli*. Each of the replication restart mechanisms require reloading of the DnaB helicase by DnaC, which is targeted to branched structures at the stalled fork by different combinations of the primosome proteins PriA, PriB, PriC and/or DnaT (Sandler and Marians, 2000).

Little is known about replication restart in *Neisseriae.* In Gc, RuvA and RecG have been identified in a genetic screen for genes involved in other recombination-based processes (E. V. Sechman and H. S. Seifert, unpubl.). Additionally, an examination of the Gc and Mc genome sequences reveals homologues of PriA, PriB, DnaG (primase), DnaB and Rep (helicases), but no PriC or DnaT homologues (Table 1). It is also of note that Mc, but not Gc, lacks a DnaC homologue. Because DnaC is thought to be essential for the initiation of replication restart, it is likely that Mc have evolved a mechanism for DnaB loading independent of DnaC. Future investigations will reveal whether these Neisserial factors function similarly in replication restart to other bacterial species.

Polymorphic repeat changes

The pathogenic *Neisseriae* carry many genes which have been characterized as 'contingency loci', as changes in polymorphic repeats results in On/Off phase variation of these genes (Bayliss *et al*., 2001). The polymorphic sequences come in many different forms ranging from homopolymeric repeats to pentanucleotide repeats (Snyder *et al*., 2001). Phase variation through changes in a polymorphic repeat sequence was first described in the Gc Opa gene family as the molecular basis of phase and antigenic variation of these surface exposed adhesins (Stern *et al*., 1986; Murphy *et al*., 1989). Opa variation is mediated by translational control operating through changes in a pentameric repeat (CTCTT) located in the signal peptide coding sequence of each *opa* gene (Stern *et al*., 1986). Changes in the number of monomeric repeats in the coding sequence or in promoter sequences have been shown to alter the expression of LOS biosynthesis genes (Gotschlich, 1994; Jennings *et al*., 1995) and pilus-associated genes *pilC1* and *pilC2* (Jonsson *et al*., 1991) in both Gc and Mc, and capsule biosynthesis genes in Mc (Hammerschmidt *et al*., 1996). A number of other genes have also been demonstrated to undergo phase variation including *opc* (Sarkari *et al*., 1994), *porA* (van der Ende *et al*., 1995), *hmbR* and *hpuAB* (Lewis *et al*., 1999; Richardson and Stojiljkovic, 1999) and *fetA* (Carson *et al*., 2000). Whole genome analysis of Mc isolates MC58, Z2491 and Gc isolate FA1090 have identified numerous additional putative phase variable genes (Snyder *et al*., 2001). It is often assumed that all phase variable genes are directly involved in pathogenesis. Although extensive variation of surface molecules may prevent the host immune system from effectively eliminating and/or preventing subsequent infection, the role for phase variation of some of these factors in disease is not always obvious from their predicted functions.

The mechanisms used to alter the number of polymorphic repeats, and subsequent expression of the gene product in the *Neisseriae,* are presumed to be linked to DNA replication. It has been established that DNA polymerases can add or delete single repeat units during DNA replication (Levinson and Gutman, 1987a) and that the frequency of changes is influenced by the length of the repeat (Levinson and Gutman, 1987b). Analysis of the frequency of phase variation through polymorphic repeat changes supports this observation (Murphy *et al*., 1989) and demonstrates the predominance of single unit changes (Stern *et al*., 1986). Mismatch correction has been shown to negatively influence the frequency of formation of monomer and dimer repeat tracts in Mc (Richardson and Stojiljkovic, 2001), although the mechanisms by which mismatch correction prevents slippage is not well understood. There are substantial differences in the frequency of phase variation measured in different isolates of Mc, which has been correlated with virulence potential (Richardson *et al*., 2002). The possibility that the frequency of polymorphic repeat changes can be globally regulated by environmental conditions has not been reported.

Pilus antigenic variation

Gc and Mc can alter the expression and antigenicity of several major surface molecules. As previously discussed, the LOS and Opa protein families can vary expression using changes in polymorphic repeat sequences. Pilin antigenic variation can affect pilus expression through a specialized recombination-mediated mechanism. Gc pili consist predominately of pilin monomers (encoded by *pilE*) and it is these pilin proteins that undergo high-frequency antigenic variation (Av), at rates between 10^{-4} and 10^{-3} events/cell/generation (Serkin and Seifert, 1998). Pilin Av occurs when sequence from a silent *pilS* copy recombines into the expressed *pilE* gene while the donor *pilS* sequence remains unchanged (Haas and Meyer, 1986; Segal *et al*., 1986), resulting in gene conversion. In addition to *pilE*, transcriptionally silent *pilS* loci are found at multiple sites throughout the chromosome and contain from 1 to 6 variable silent copies of pilin genetic information. Silent copies lack a promoter sequence, ribosomal-binding site and the 5′ portion of conserved pilin information and are therefore not expressed (Meyer *et al*., 1984). The 3′ two-thirds of pilin information found in all pilin copies contain regions of variable sequences flanked by conserved sequences. In some bacterial species such as *Mycoplasma synoviae*, *Anaplasma marginale* and *Borrelia hermsii* these silent storage copies of variant sequences have been called pseudogenes (Restrepo *et al*., 1994; Noormohammadi *et al*., 2000; Meeus *et al*., 2003). We argue that this is not the proper use of this term as these *pilS* storage copies of variant sequence have an evolved

role in antigenic variation and are therefore not evolving towards or away from complete genes.

Pilin Avenue results in a unidirectional transfer of pilin sequences, such that segments of *pilE* are replaced with *pilS* sequences of varying length and transfer occurs regardless of the extent of identity between the recombining silent and expressed copies. The newly recombined sequences are flanked by regions of exact identity as small as 5 base pairs that are shared between the silent copy and expressed gene (Howell-Adams and Seifert, 2000) (our unpubl. obs.). Numerous DNA sequences have been found to play an integral role in pilin antigenic variation. Several short sequence elements that are strictly conserved between silent and expressed pilin copies are known to be important for efficient antigenic variation (Hill *et al*., 1990; Howell-Adams *et al*., 1996; Howell-Adams and Seifert, 1999). In addition to sequences within the coding region of pilin copies, it has been reported that the conserved *Sma/Cla* repeat found at the 3′ end of all pilin loci (Meyer *et al*., 1984; Perry *et al*., 1987) is required for efficient antigenic variation (Wainwright *et al*., 1994; 1997). Recent work in our laboratory suggests that the requirement for the *Sma/Cla* repeat is not generalizable but may either be a strain-specific phenomenon or be constrained by the resident *pilE* sequence (E.V.S. and H.S.S., unpubl.). Taken together, these data suggest a role for conserved sequences in pilin Av, but further work is needed to determine whether these conserved sequence elements function to recruit specific enzymes or function during strand pairing reactions.

Pilin antigenic variation has been proposed to occur through two routes: DNA transformation (Norlander *et al*., 1979b; Seifert *et al*., 1988; Gibbs *et al*., 1989) or intracellular recombination (Hagblom *et al*., 1985; Haas and Meyer, 1986; Segal *et al*., 1986; Zhang *et al*., 1992). Regardless of the route, current models of pilin antigenic variation require at least two chromosomes to allow for gene conversion. For transformation-mediated variation, DNA could be delivered by autolysis or by the type IV secretion apparatus (Dillard and Seifert, 2001). For intracellular recombination, the process of pilin antigenic variation could be linked to replication or alternatively could depend on bacterial cells carrying more than one copy of the chromosome.

We have proposed a novel model that could explain how gene conversion is mediated in a bacterial chromosome (Fig. 1B) (Howell-Adams *et al*., 1996). In the first step of this model, recombination between the *pilE* locus and a *pilS* silent copy of the same chromosome results in the formation of a hybrid locus carrying *pilE* sequences fused to a *pilS* copy at a short region of shared sequence identity (Fig. 1BII). While we originally postulated that this intermediate would be in the form of a circular DNA molecule (Howell-Adams *et al*., 1996), extrachromosomal circles carrying genomic sequences, which have been found in *Neisseria* (Howell-Adams and Seifert, 2000; Barten and Meyer, 2001), do not form often enough to explain the high frequency of pilin variation (Howell-Adams and Seifert, 2000) and we have proposed that an undefined alternative structure is the true intermediate (Fig. 1BIII). The true test of the model will be the molecular or biochemical definition of this intermediate. In the second step of this model, extensive lengths of upstream or downstream homology between the *pil* loci would then target the donor hybrid for subsequent recombination with the recipient *pilE* of the second chromosome (Fig. 1BIV). This second step would require two recombination events: (i) homologous recombination between identical *pilE*-derived sequences of the hybrid and the recipient *pilE* and (ii) recombination at a short region of identity shared between the donor *pilS* hybrid sequences and the recipient *pilE* (Fig. 1BIV). This model allows for the incorporation of *pilS* sequences into *pilE* between short regions of identity shared between the donor *pilS* and the recipient *pilE* (Howell-Adams *et al*., 1996). We have shown that *pilE–pilS* hybrids are formed in Gc, can be isolated and recombine into a recipient *pilE* with a higher frequency upon transformation into Gc than insertion of other markers on the same molecule into *pilS* loci (Howell-Adams and Seifert, 2000). Therefore, these data strongly support the feasibility of the hybrid locus model to explain the apparent non-reciprocal nature of recombination during pilin variation. Hybrid pilin loci could also be formed after genome amplification via a recombination event between amplified pilin copies and/or resident pilin copies. Additionally, *pilE* gene conversion could result from more traditional models for gene conversion such as double-strand break repair leading to unequal crossing over (Fig. 1A) or through successive half crossing over (Fig. 1C) (Kobayashi, 1992). However, until a true molecular definition of the intermediate(s) of pilin variation is realized, all of these models remain unproven.

We have postulated that pilin antigenic variation should require both specific factors and generalized homologous recombination pathways. In support of generalized recombination factors participating in Av, it is known that the transfer of *pilS* sequences into *pilE* is absolutely dependent on the RecA protein (Koomey *et al*., 1987) and the RecA modulator, RecX, enhances the frequency of pilin Avenue (Stohl and Seifert, 2001). This is in contrast to *E. coli* where RecX inhibits RecA co-protease and recombinase activities during SOS (Stohl *et al*., 2003). The recombination events involved in antigenic variation at *pilE* requires the RecF-like pathway of homologous recombination genes, *recJ* (Skaar *et al*., 2002), *recO* and *recQ* (Mehr and Seifert, 1998). It is possible that the 3′ to 5′ DNA helicase function of RecQ, along with the 5′ to 3′ ssDNA exonuclease activity of RecJ, act to promote the formation of ssDNA intermediates for recombination

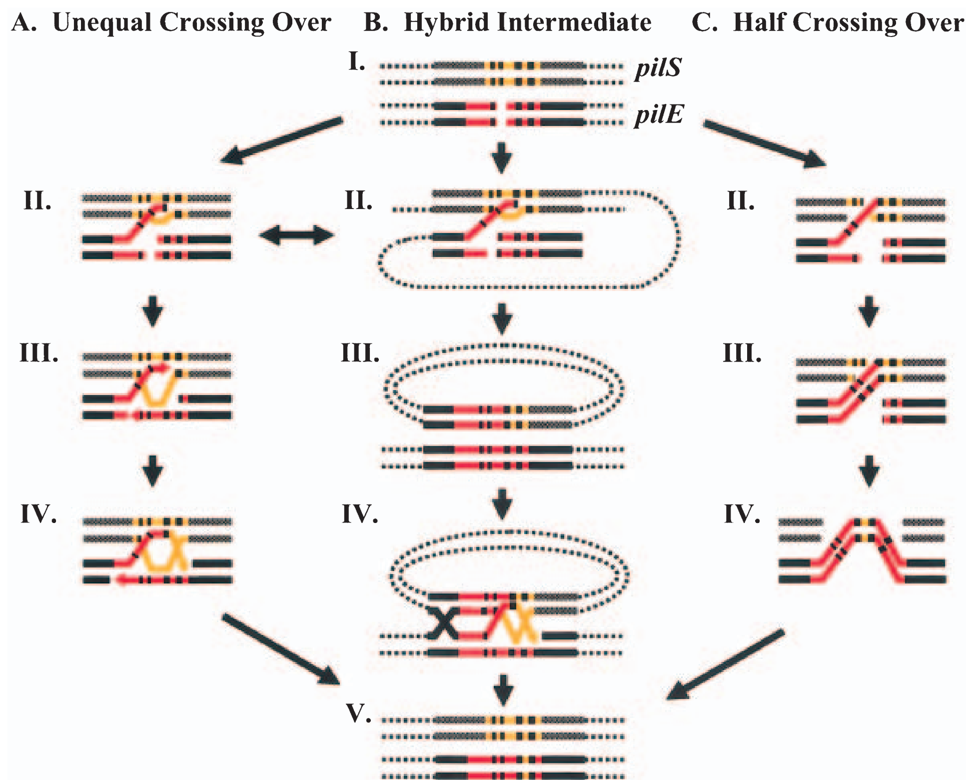

Fig. 1. Models for pilin antigenic variation in *Neisseria gonorrhoeae*. Models proposed to account for pilin antigenic variation include the previously proposed models to account for gene conversion (A and C) (Szostak *et al*., 1983; Rothstein, 1984; Kobayashi, 1992), as well as a more specialized model (B) (Howell-Adams *et al*., 1996). Each pair of lines represents a double-stranded DNA molecule. Yellow represents the donor *pilS* copy with *pilS* flanking sequence depicted as black and white hatched boxes. Red represents the recipient *pilE* copy with black boxes depicting *pilE* flanking sequence. Black boxes within *pilS* and *pilE* indicate areas of identical DNA sequence shared between the recombining pilin copies. Dotted lines signify double-stranded or partially single-stranded DNA. Two chromosomes are presumed for each model. Models A and B begin with a dsDNA break in the recipient (*pilE*) molecule followed by DNA degradation by an exonuclease such as RecJ to create a dsDNA gap (I). Unwinding by a helicase such as RecQ could also occur at this stage. Strand-pairing and exchange is likely mediated by the RecA, RecX and RecOR enzymes. Branch migration and resolvase activities in these models could be mediated by RecG/RuvAB, and RuvC, respectively.
A. In the unequal cross-over model, 3′ ssDNA invades and is paired with homologous donor (*pilS*) sequences (II). Displaced donor DNA becomes the template for repair of the damaged DNA (III). Branch migration may occur at this step. Holliday junction resolution (IV) results in the newly synthesized donor sequence in place of the degraded DNA in the recipient molecule (V).
B. The hybrid intermediate model consists of two steps. In the first step, invasion of the donor molecule by 3′ ssDNA and subsequent pairing occurs at short regions of identity between donor and recipient sequences (II). This recombination event creates a hybrid intermediate consisting of linked *pilE* and *pilS* sequences on a linear, closed double-stranded circular, or partially double-stranded circular molecule (III, IV). The second step of this model involves two more recombination events: one within the extended region of upstream homology between the hybrid intermediate and the final recipient *pilE* and a second at a short region of sequence identity between the hybrid and *pilE* (IV). The second recombination event of step (IV) could result via *pilS* (not depicted) or *pilE* (depicted) acting as the invading strand. Either would allow for the replacement of resident *pilE* sequences (V).
C. In the successive half-cross-over model, unwound 3′ ssDNA undergoes a RecF-like pathway-dependent recombination event with the donor DNA molecule, resulting in a direct link (a half cross-over) between the donor and recipient molecules (II, III), with little to no DNA synthesis occurring. The first half-crossing over results in one recombinant molecule and leaves free ends for a subsequent recombination event. In the second half cross-over, invasion by the remaining free end of the recipient molecule and linkage to the donor molecule a short distance from the first cross-over (IV) results in the replacement of donor sequence with recipient sequence (V).

(Mehr and Seifert, 1998; Skaar *et al*., 2002). The formation of single-stranded DNA is consistent with our current models describing antigenic variation, as these models propose the formation of partially single-stranded intermediates during pilin Av *in vivo* (Howell-Adams and Seifert, 2000). It is likely that RecO aids RecA in DNA strand exchange as it has been proposed that in *E. coli* the RecFOR or RecOR complexes assist RecA in synapsis (Umezu and Kolodner, 1994; Hegde *et al*., 1996; Madiraju *et al*., 1988; Morimatsu and Kowalczykowski, 2003). Additional recombination genes, such as *recR*, *recG* and *ruvA*, are also involved in pilin antigenic variation (E. V. Sechman and H. S. Seifert, unpubl.). While we did not find an effect of *recB*, *C* or *D* mutations on the frequency of pilin variation, two studies have reported an enhancement of variation frequencies when the Gc *recD* gene is inactivated (Chaussee *et al*., 1999) or when the Mc *recB* gene is mutated (Salvatore *et al*., 2002). These findings are

consistent with the RecBCD nuclease destroying recombination intermediates that are used during pilin Avenue. The results from two genetic screens for genes involved in pilin antigenic variation have not revealed any recombination factors that are specific for this specialized recombination system (Mehr and Seifert, 1997, 1998) (E. V. Sechman and H. S. Seifert, unpubl.). While these negative results do not rule out the existence of specific factors, there presently is no direct evidence for such factors and it is possible that general recombination factors mediate all the processes required for pilin variation.

Summary/future questions

The human-specific pathogens, *N. gonorrhoeae* and *N. meningitidis* are exquisitely adapted to certain environmental niches found within humans. While the high level of sequence similarity in the genomes of these two organisms suggests they could be considered as members of the same species, they contain a variety of genetic islands that differentiate them from one another (Perrin *et al*., 2002). This may help explain why these pathogens reside in different compartments within humans and cause distinct diseases. It is not clear whether the different anatomical sites that these two organisms localize to present sufficiently disparate environments to require diverse mechanisms for molecular processes. For example it is conceivable that different DNA repair mechanisms could provide advantages in distinct anatomical sites. The closely matched repertoire of recombination and repair genes suggest that there are not gross differences in the repair capabilities of these organisms (Table 1). However, gene expression data coupled with detailed biochemical analysis of the gene products will be required to determine whether the organism exhibit identical DNA repair capabilities.

Another environmental factor that might contribute to the contrasting molecular processes of these organisms is the bacteria with which they associate in nature. The meningococcus lives in close association with the commensal *Neisseriae*, while the gonococcus can associate with other STD pathogens (Joesoef *et al*., 1996; Rotchford *et al*., 2000; Cecil *et al*., 2001) and encounters a different set of commensal organisms when interacting with the vaginal flora (Saigh *et al*., 1978; Kraus, 1980; Bartlett and Polk, 1984). It is possible that unique DNA repair and/or transfer properties may be beneficial to organisms that coexist with other closely related species. All organisms utilize a subset of common replication, repair and genetic transfer mechanisms selected to promote life in different environments and for different selective pressures. During their co-evolution with humans, the pathogenic *Neisseriae* may have lost some genetic capabilities or diverged from other bacteria before these capabilities were acquired (e.g. SOS), but have enhanced other molecular capacities (e.g. transformation competence and surface antigen variability). Given the exclusivity of the pathogenic *Neisseriae* for humans, understanding the molecular processes that contribute to their virulence provides a unique perspective into how selective pressures of the human body can affect the evolution of these basic molecular and genetic processes in bacteria. It will be interesting in the future to compare these capabilities with the commensal *Neisseriae* that rarely elicit inflammation or disease.

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References

Aas, F.E., Wolfgang, M., Frye, S., Dunham, S., Lovold, C., and Koomey, M. (2002) Competence for natural transformation in *Neisseria gonorrhoeae*: components of DNA binding and uptake linked to type IV pilus expression. *Mol Microbiol* **46:** 749–760.

Barten, R., and Meyer, T.F. (2001) DNA circle formation in *Neisseria gonorrhoeae*: a possible intermediate in diverse genomic recombination processes. *Mol Gen Genet* **264:** 691–701.

Bartlett, J.G., and Polk, B.F. (1984) Bacterial flora of the vagina: quantitative study. *Rev Infect Dis* **6 (**Suppl. 1): S67–S72.

Bayliss, C.D., Field, D., and Moxon, E.R. (2001) The simple sequence contingency loci of *Haemophilus influenzae* and *Neisseria meningitidis*. *J Clin Invest* **107:** 657–662.

Biswas, G.D., and Sparling, P.F. (1981) Entry of double-stranded deoxyribonucleic acid during transformation of *Neisseria gonorrhoeae*. *J Bacteriol* **145:** 638–640.

Black, C.G., Fyfe, J.A., and Davies, J.K. (1995) A promoter associated with the neisserial repeat can be used to transcribe the *uvrB* gene from *Neisseria gonorrhoeae*. *J Bacteriol* **177:** 1952–1958.

Black, C.G., Fyfe, J.A.M., and Davies, J.K. (1998) Absence of an SOS-like system in *Neisseria gonorrhoeae*. *Gene* **208:** 61–66.

Boyle-Vavra, S., and Seifert, H.S. (1996) Uptake-sequence-independent DNA transformation exists in *Neisseria gonorrhoeae*. *Microbiol* **142:** 2839–2845.

Brandtzaeg, P., and van Deuren, M. (2002) Current concepts in the role of the host response in *Neisseria meningitidis* septic shock. *Curr Opin Infect Dis* **15:** 247–252.

Bucci, C., Lavitola, A., Salvatore, P., Del Giudice, L., Massardo, D.R., Bruni, C.B., and Alifano, P. (1999) Hypermutation in pathogenic bacteria: frequent phase variation in meningococci is a phenotypic trait of a specialized mutator biotype. *Mol Cell* **3:** 435–445.

Campbell, L.A., and Yasbin, R.E. (1979) Deoxyribonucleic acid repair capacities of *Neisseria gonorrhoeae*: absence of photoreactivation. *J Bacteriol* **140:** 1109–1111.

Campbell, L.A., and Yasbin, R.E. (1984a) Mutagenesis of *Neisseria gonorrhoeae*: absence of error-prone repair. *J Bacteriol* **160:** 288–293.

Campbell, L.A., and Yasbin, R.E. (1984b) A DNA excision repair system for *Neisseria gonorrhoeae*. *Mol Gen Genet* **193:** 561–563.

Carson, S.D., Stone, B., Beucher, M., Fu, J., and Sparling, P.F. (2000) Phase variation of the gonococcal siderophore receptor FetA. *Mol Microbiol* **36:** 585–593.

Catlin, B.W., and Cunningham, L.S. (1961) Transforming activities and base contents of deoxyribonucleate preparations from various *Neisseriae*. *J Gen Microbiol* **26:** 303–312.

Cecil, J.A., Howell, M.R., Tawes, J.J., Gaydos, J.C., McKee, K.T., Jr, Quinn, T.C., and Gaydos, C.A. (2001) Features of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection in male Army recruits. *J Infect Dis* **184:** 1216–1219.

Chaussee, M.S., and Hill, S.A. (1998) Formation of single-stranded DNA during DNA transformation of *Neisseria gonorrhoeae*. *J Bacteriol* **180:** 5117–5122.

Chaussee, M.S., Wilson, J., and Hill, S.A. (1999) Characterization of the *recD* gene of *Neisseria gonorrhoeae* MS11 and the effect of *recD* inactivation on pilin variation and DNA transformation. *Microbiol* **145:** 389–400.

Dillard, J.P., and Seifert, H.S. (2001) A variable genetic island specific for *Neisseria gonorrhoeae* is involved in providing DNA for natural transformation and is found more often in disseminated infection isolates. *Mol Microbiol* **41:** 263–277.

Dubnau, D. (1999) DNA uptake in bacteria. *Annu Rev Microbiol* **53:** 217–244.

Eisenstein, B.I., Sox, T., Biswas, G., Blackman, E., and Sparling, P.F. (1977) Conjugal transfer of the gonococcal penicillinase plasmid. *Science* **195:** 998–1000.

van der Ende, A., Hopman, C.T., Zaat, S., Essink, B.B., Berkhout, B., and Dankert, J. (1995) Variable expression of class 1 outer membrane protein in *Neisseria meningitidis* is caused by variation in the spacing between the –10 and –35 regions of the promoter. *J Bacteriol* **177:** 2475–2480.

Facius, D., Fussenegger, M., and Meyer, T.F. (1996) Sequential action of factors involved in natural competence for transformation of *Neisseria gonorrhoeae*. *FEMS Microbiol Lett* **137:** 159–164.

Frost, L.S., Ippen-Ihler, K., and Skurray, R.A. (1994) Analysis of the sequence and gene products of the transfer region of the F sex factor. *Microbiol Rev* **58:** 162–210.

Gibbs, C.P., Reimann, B.Y., Schultz, E., Kaufmann, A., Haas, R., and Meyer, T.F. (1989) Reassortment of pilin genes in *Neisseria gonorrhoeae* occurs by two distinct mechanisms. *Nature* **338:** 651–652.

Goodman, M.F. (2002) Error-prone repair DNA polymerases in prokaryotes and eukaryotes. *Annu Rev Biochem* **71:** 17–50.

Goodman, S.D., and Scocca, J.J. (1988) Identification and arrangement of the DNA sequence recognized in specific transformation of *Neisseria gonorrhoeae*. *Proc Natl Acad Sci USA* **85:** 6982–6986.

Gotschlich, E.C. (1994) Genetic locus for the biosynthesis of the variable portion of *Neisseria gonorrhoeae* lipooligosaccharide. *J Exp Med* **180:** 2181–2190.

Gregg, A.V., McGlynn, P., Jaktaji, R.P., and Lloyd, R.G. (2002) Direct rescue of stalled DNA replication forks via the combined action of PriA and RecG helicase activities. *Mol Cell* **9:** 241–251.

Haas, R., and Meyer, T.F. (1986) The repertoire of silent pilus genes in *Neisseria gonorrhoeae*: evidence for gene conversion. *Cell* **44:** 107–115.

Hagblom, P., Segal, E., Billyard, E., and So, M. (1985) Intragenic recombination leads to pilus antigenic variation in *Neisseria gonorrhoeae*. *Nature* **315:** 156–158.

Hamilton, H.L., Schwartz, K.J., and Dillard, J.P. (2001) Insertion-duplication mutagenesis of neisseria: use in characterization of DNA transfer genes in the gonococcal genetic island. *J Bacteriol* **183:** 4718–4726.

Hammerschmidt, S., Muller, A., Sillmann, H., Muhlenhoff, M., Borrow, R., Fox, A., *et al.* (1996) Capsule phase variation in *Neisseria meningitidis* serogroup B by slipped-strand mispairing in the polysialyltransferase gene (*siaD*): correlation with bacterial invasion and the outbreak of meningococcal disease. *Mol Microbiol* **20:** 1211–1220.

Hegde, S.P., Qin, M.H., Li, X.H., Atkinson, M.A., Clark, A.J., Rajagopalan, M., and Madiraju, M.V. (1996) Interactions of RecF protein with RecO, RecR, and single-stranded DNA binding proteins reveal roles for the RecF-RecO-RecR complex in DNA repair and recombination. *Proc Natl Acad Sci USA* **93:** 14468–14473.

Hill, S.A., Morrison, S.G., and Swanson, J. (1990) The role of direct oligonucleotide repeats in gonococcal pilin gene variation. *Mol Microbiol* **4:** 1341–1352.

Holmes, J., Jr, Clark, S., and Modrich, P. (1990) Strand-specific mismatch correction in nuclear extracts of human and *Drosophila melanogaster* cell lines. *Proc Natl Acad Sci USA* **87:** 5837–5841.

Howell-Adams, B., and Seifert, H.S. (1999) Insertion mutations in *pilE* differentially alter gonococcal pilin antigenic variation. *J Bacteriol* **181:** 6133–6141.

Howell-Adams, B., and Seifert, H.S. (2000) Molecular models accounting for the gene conversion reactions mediating gonococcal pilin antigenic variation. *Mol Microbiol* **37:** 1146–1159.

Howell-Adams, B., Wainwright, L.A., and Seifert, H.S. (1996) The size and position of heterologous insertions in a silent locus differentially affect pilin recombination in *Neisseria gonorrhoeae*. *Mol Microbiol* **22:** 509–522.

Jennings, M.P., Hood, D.W., Peak, I.R., Virji, M., and Moxon, E.R. (1995) Molecular analysis of a locus for the biosynthesis and phase-variable expression of the lacto-N-neotetraose terminal lipopolysaccharide structure in *Neisseria meningitidis*. *Mol Microbiol* **18:** 729–740.

Joesoef, M.R., Wiknjosastro, G., Norojono, W., Sumampouw, H., Linnan, M., Hansell, M.J., *et al.* (1996) Coinfection with chlamydia and gonorrhoea among pregnant women and bacterial vaginosis. *Int J STD AIDS* **7:** 61–64.

Jonsson, A.B., Nyberg, G., and Normark, S. (1991) Phase variation of gonococcal pili by frameshift mutation in *pilC*, a novel gene for pilus assembly. *EMBO J* **10:** 477–488.

Kobayashi, I. (1992) Mechanisms for gene conversion and homologous recombination: the double-strand break repair model and the successive half crossing-over model. *Adv Biophys* **28:** 81–133.

Koomey, J.M., and Falkow, S. (1987) Cloning of the *recA* gene of *Neisseria gonorrhoeae* and construction of gonococcal recA mutants. *J Bacteriol* **169:** 790–795.

Koomey, M., Gotschlich, E.C., Robbins, K., Bergstrom, S., and Swanson, J. (1987) Effects of *recA* mutations on pilus antigenic variation and phase transitions in *Neisseria gonorrhoeae*. *Genetics* **117:** 391–398.

Kowalczykowski, S.C., Dixon, D.A., Eggleston, A.K., Lauder, S.D., and Rehrauer, W.M. (1994) Biochemistry of homologous recombination in *Escherichia coli*. *Microbiol Rev* **94:** 401–465.

Kraus, S.J. (1980) Resistance to gonorrhea possibly mediated by the genital microbial flora. *Arch Androl* **4:** 179–183.

Kushner, S.R., Nagaishi, H., Templin, A., and Clark, A.J. (1971) Genetic recombination in *Escherichia coli*: the role of exonuclease I. *Proc Natl Acad Sci USA* **68:** 824–827.

Levinson, G., and Gutman, G.A. (1987a) Slipped-strand mispairing: a major mechanism for DNA sequence evolution. *Mol Biol Evol* **4:** 203–221.

Levinson, G., and Gutman, G.A. (1987b) High frequencies of short frameshifts in poly-CA/TG tandem repeats borne by bacteriophage M13 in *Escherichia coli* K-12. *Nucleic Acids Res* **15:** 5323–5338.

Lewis, L.A., Gipson, M., Hartman, K., Ownbey, T., Vaughn, J., and Dyer, D.W. (1999) Phase variation of HpuAB and HmbR, two distinct haemoglobin receptors of *Neisseria meningitidis* DNM2. *Mol Microbiol* **32:** 977–989.

Lloyd, R.G., and Buckman, C. (1985) Identification and genetic analysis of *sbcC* mutations in commonly used *recBC sbcB* strains of *Escherichia coli* K-12. *J Bacteriol* **164:** 836–844.

Long, C.D., Tobiason, D.T., Lazio, M., Kline, K.A., and Seifert, H.S. (2003) Low level pilin expression allows for substantial DNA transformation competence in *Neisseria gonorrhoeae*. *Infect Immun* (in press).

Madiraju, M.V., Templin, A., and Clark, A.J. (1988) Properties of a mutant recA-encoded protein reveal a possible role for *Escherichia coli* recF-encoded protein in genetic recombination. *Proc Natl Acad Sci USA* **85:** 6592–6596.

McGlynn, P., and Lloyd, R.G. (2002) Recombinational repair and restart of damaged replication forks. *Nat Rev Mol Cell Biol* **3:** 859–870.

McNeil, G., Virji, M., and Moxon, E.R. (1994) Interactions of *Neisseria meningitidis* with human monocytes. *Microbial Pathogenesis* **16:** 153–163.

Meeus, P.F., Brayton, K.A., Palmer, G.H., and Barbet, A.F. (2003) Conservation of a gene conversion mechanism in two distantly related paralogues of *Anaplasma marginale*. *Mol Microbiol* **47:** 633–643.

Mehr, I.J., and Seifert, H.S. (1997) Random shuttle mutagenesis: gonococcal mutants deficient in pilin antigenic variation. *Mol Microbiol* **23:** 1121–1131.

Mehr, I.J., and Seifert, H.S. (1998) Differential roles of homologous recombination pathways in *Neisseria gonorrhoeae* pilin antigenic variation, DNA transformation, and DNA repair. *Mol Microbiol* **30:** 697–710.

Meyer, T.F., Billyard, E., Haas, R., Storzbach, S., and So, M. (1984) Pilus genes of *Neisseria gonorrheae*: chromosomal organization and DNA sequence. *Proc Natl Acad Sci USA* **81:** 6110–6114.

Michel, B. (2000) Replication fork arrest and DNA recombination. *Trends Biochem Sci* **25:** 173–178.

Morimatsu, K., and Kowalczykowski, S.C. (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.

Morton, R.S. (ed.) (1977) Gonorrhoea in Earlier Times. In *Gonorrhoea*. London: W.B. Saunders, pp. 1–24.

Murphy, G.L., Connell, T.D., Barritt, D.S., Koomey, M., and Cannon, J.G. (1989) Phase variation of gonococcal protein II: regulation of gene expression by slipped-strand mispairing of a repetitive DNA sequence. *Cell* **56:** 539–547.

Noormohammadi, A.H., Markham, P.F., Kanci, A., Whithear, K.G., and Browning, G.F. (2000) A novel mechanism for control of antigenic variation in the haemagglutinin gene family of *Mycoplasma synoviae*. *Mol Microbiol* **35:** 911–923.

Norlander, L., Davies, J., and Normark, S. (1979a) Genetic exchange mechanisms in *Neisseria gonorrhoeae*. *J Bacteriol* **138:** 756–761.

Norlander, L., Davies, J., Norqvist, A., and Normark, S. (1979b) Genetic basis for colonial variation in *Neisseria gonorrhoeae*. *J Bacteriol* **138:** 762–769.

Parkhill, J., Achtman, M., James, K.D., Bentley, S.D., Churcher, C., Klee, S.R., *et al.* (2000) Complete DNA sequence of a serogroup A strain of *Neisseria meningitidis* Z2491. *Nature* **404:** 502–506.

Perrin, A., Bonacorsi, S., Carbonnelle, E., Talibi, D., Dessen, P., Nassif, X., and Tinsley, C. (2002) Comparative genomics identifies the genetic islands that distinguish *Neisseria meningitidis*, the agent of cerebrospinal meningitis, from other Neisseria species. *Infect Immun* **70:** 7063–7072.

Perry, A.C., Nicolson, I.J., and Saunders, J.R. (1987) Structural analysis of the *pilE* region of *Neisseria gonorrhoeae* P9. *Gene* **60:** 85–92.

Petranovic, M., Vlahovic, K., Zahradka, D., Dzidic, S., and Radman, M. (2000) Mismatch repair in xenopus egg extracts is not strand-directed by DNA methylation. *Neoplasma* **47:** 375–381.

Radman, M., Taddei, F., and Matic, I. (2000) Evolution-driving genes. *Res Microbiol* **151:** 91–95.

Restrepo, B.I., Carter, C.J., and Barbour, A.G. (1994) Activation of a *vmp* pseudogene in *Borrelia hermsii*: an alternate mechanism of antigenic variation during relapsing fever. *Mol Microbiol* **13:** 287–299.

Richardson, A.R., and Stojiljkovic, I. (1999) HmbR, a hemoglobin-binding outer membrane protein of *Neisseria meningitidis*, undergoes phase variation. *J Bacteriol* **181:** 2067–2074.

Richardson, A.R., and Stojiljkovic, I. (2001) Mismatch repair and the regulation of phase variation in *Neisseria meningitidis*. *Mol Microbiol* **40:** 645–655.

Richardson, A.R., Yu, Z., Popovic, T., and Stojiljkovic, I. (2002) Mutator clones of *Neisseria meningitidis* in epidemic serogroup A disease. *Proc Natl Acad Sci USA* **99:** 6103–6107.

Roberts, M., and Falkow, S. (1977) Conjugal transfer of R plasmids in *Neisseria gonorrhoeae*. *Nature* **266:** 630–631.

Roe, B.A., Clifton, S., and Dyer, D.W. (1997) *Gonococcal Genome Sequencing Project.*

Rotchford, K., Strum, A.W., and Wilkinson, D. (2000) Effect of coinfection with STDs and of STD treatment on HIV shedding in genital-tract secretions: systematic review and data synthesis. *Sex Transm Dis* **27:** 243–248.

Rothstein, R. (1984) Double-strand-break repair, gene conversion, and postdivision segregation. *Cold Spring Harb Symp Quant Biol* **49:** 629–637.

Saigh, J.H., Sanders, C.C., and Sanders, W.E., Jr (1978) Inhibition of *Neisseria gonorrhoeae* by aerobic and facultatively anaerobic components of the endocervical flora: evidence for a protective effect against infection. *Infect Immun* **19:** 704–710.

Salvatore, P., Bucci, C., Pagliarulo, C., Tredici, M., Colicchio, R., Cantalupo, G., *et al.* (2002) Phenotypes of a naturally defective *recB* allele in *Neisseria meningitidis* clinical isolates. *Infect Immun* **70:** 4185–4195.

Sandler, S.J., and Marians, K.J. (2000) Role of PriA in replication fork reactivation in *Escherichia coli*. *J Bacteriol* **182:** 9–13.

Sarkari, J., Pandit, N., Moxon, E.R., and Achtman, M. (1994) Variable expression of the Opc outer membrane protein in *Neisseria meningitidis* is caused by size variation of a promoter containing poly-cytidine. *Mol Microbiol* **13:** 207–217.

Segal, E., Hagblom, P., Seifert, H.S., and So, M. (1986) Antigenic variation of gonococcal pilus involves assembly of separated silent gene segments. *Proc Natl Acad Sci USA* **83:** 2177–2181.

Seifert, H.S., Ajioka, R.S., Marchal, C., Sparling, P.F., and So, M. (1988) DNA transformation leads to pilin antigenic variation in *Neisseria gonorrhoeae*. *Nature* **336:** 392–395.

Serkin, C.D., and Seifert, H.S. (1998) Frequency of pilin antigenic variation in *Neisseria gonorrhoeae*. *J Bacteriol* **180:** 1955–1958.

Skaar, E.P., Lazio, M.P., and Seifert, H.S. (2002) Roles of the *recJ* and *recN* genes in homologous recombination and DNA repair pathways of *Neisseria gonorrhoeae*. *J Bacteriol* **184:** 919–927.

Smith, J.M., Smith, N.H., O'Rourke, M., and Spratt, B.G. (1993) How clonal are bacteria? *Proc Natl Acad Sci USA* **90:** 4384–4388.

Snyder, L.A., Butcher, S.A., and Saunders, N.J. (2001) Comparative whole-genome analyses reveal over 100 putative phase-variable genes in the pathogenic Neisseria spp. *Microbiology* **147:** 2321–2332.

Sparling, P.F. (1966) Genetic transformation of *Neisseria gonorrhoeae* to streptomycin resistance. *J Bacteriol* **92:** 1364–1371.

Spratt, B.G., Zhang, Q.Y., Jones, D.M., Hutchison, A., Brannigan, J.A., and Dowson, C.G. (1989) Recruitment of a penicillin-binding protein gene from Neisseria flavescens during the emergence of penicillin resistance in *Neisseria meningitidis*. *Proc Natl Acad Sci USA* **86:** 8988–8992.

Stein, D.C. (1991) Transformation of *Neisseria gonorrhoeae*: physical requirements of the transforming DNA. *Can J Microbiol* **37:** 345–349.

Steinberg, V.I., and Goldberg, I.D. (1980) On the question of chromosomal gene transfer via conjugation in *Neisseria gonorrhoeae*. *J Bacteriol* **142:** 350–354.

Stern, A., Brown, M., Nickel, P., and Meyer, T.F. (1986) Opacity genes in *Neisseria gonorrhoeae*: control of phase and antigenic variation. *Cell* **47:** 61–71.

Stohl, E.A., and Seifert, H.S. (2001) The *recX* gene potentiates homologous recombination in *Neisseria gonorrhoeae*. *Mol Microbiol* **40:** 1301–1310.

Stohl, E.A., Brockman, J., Burkle, K.L., Morimatsu, K., Kowalczykowski, S.C., and Seifert, H.S. (2003) *Escherichia coli* RecX inhibits RecA recombinase and coprotease activities *in vitro* and *in vivo*. *J Biol Chem* **278:** 2278–2285.

Szostak, J.W., Orr-Weaver, T.L., Rothstein, R.J., and Stahl, F.W. (1983) The double-strand-break repair model for recombination. *Cell* **33:** 25–35.

Umezu, K., and Kolodner, R.D. (1994) Protein interactions in genetic recombination in *Escherichia coli*. Interactions involving RecO and RecR overcome the inhibition of RecA by single-stranded DNA-binding protein. *J Biol Chem* **269:** 30005–30013.

Virji, M. (1996) Meningococcal disease: epidemiology and pathogenesis. *Trends Microbiol* **4:** 466–469; discussion 469–470.

Vogel, U., and Frosch, M. (1999) Mechanisms of neisserial serum resistance. *Mol Microbiol* **32:** 1133–1139.

Wain, B.S. (1947) *The Unconquered Plague*. New York: International University Press.

Wainwright, L.A., Pritchard, K.H., and Seifert, H.S. (1994) A conserved DNA sequence is required for efficient gonococcal pilin antigenic variation. *Mol Microbiol* **13:** 75–87.

Wainwright, L.A., Frangipane, J.V., and Seifert, H.S. (1997) Analysis of protein binding to the Sma/Cla DNA repeat in pathogenic *Neisseriae*. *Nucleic Acids Res* **25:** 1362–1368.

Wolfgang, M., Lauer, P., Park, H.-S., Brossay, L., He'bert, J., and Koomey, M. (1998) PilT mutations lead to simultaneous defects in competence for natural transformation and twitching motility in piliated *Neisseria gonorrhoeae*. *Mol Microbiol* **29:** 312–330.

Zhang, Q.Y., DeRyckere, D., Lauer, P., and Koomey, M. (1992) Gene conversion in *Neisseria gonorrhoeae*: evidence for its role in pilus antigenic variation. *Proc Natl Acad Sci USA* **89:** 5366–5370.