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Recombination and DNA Repair in *Helicobacter pylori*

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Abstract

All organisms have pathways that repair the genome, ensuring their survival and that of their progeny. But these pathways also serve to diversify the genome, causing changes on the level of nucleotide, whole gene, and genome structure. Sequencing of bacteria has revealed wide allelic diversity and differences in gene content within the same species, highlighting the importance of understanding pathways of recombination and DNA repair. The human stomach pathogen *Helicobacter pylori* is an excellent model system for studying these pathways. *H. pylori* harbors major recombination and repair pathways and is naturally competent, facilitating its ability to diversify its genome. Elucidation of DNA recombination, repair, and diversification programs in this pathogen will reveal connections between these pathways and their importance to infection.

Keywords

Helicobacter pylori; DNA repair; Homologous Recombination; Natural competence; genetic diversity

INTRODUCTION

Helicobacter pylori is a ubiquitous bacterial pathogen. Infection rates have declined in economically developed countries throughout the 20th century, but even today, half the world's human population carries these bacteria in their stomach. *H. pylori* live in the viscous gastric mucous and in close apposition to the stomach epithelium. Infection is associated with development of life threatening chronic diseases of the stomach including peptic ulcers and gastric cancers. The pathophysiology of *H. pylori* associated diseases have been reviewed extensively (57, 102). Here, we focus on one aspect of the basic biology of this organism: the mechanisms by which it maintains and diversifies its genome.

Genetic diversity among *H. pylori* strains has been of considerable interest to those studying *H. pylori* pathogenesis because allelic variation in several genes, including the exotoxins CagA and VacA, correlate with disease risk and partially account for differing rates of *H. pylori* associated disease among human populations (25, 35, 102). Genetic diversity also creates an opportunity to study bacterial population structure. *H. pylori* strains show strong signatures of clonal descent that parallel the ancient migrations of its human host suggesting relative isolation of bacterial populations within closely related hosts (1, 30). But evidence for admixture among strains within geographic regions also exists suggesting that horizontal gene transfer and recombination between strains occur. Interestingly, the initial annotation of the genome suggested that *H. pylori* lacked many homologous recombination (HR) and mismatch repair genes required to maintain genome integrity in the face of polymerase errors as well as endogenous and exogenous DNA damaging agents (4, 81, 96). However recent work has revealed that many of these pathways do exist and were missed in part because *H. pylori*, an Epsilonproteobacteria, resides in an evolutionary group quite distinct from the model Gram (–) enteric pathogens, *Escherichia coli* and *Salmonella*. This review

focuses on describing pathways for DNA repair, recombination and horizontal gene transfer in *H. pylori*. We highlight overlap and interactions between these pathways as well as their role in promoting chronic colonization of *H. pylori* in its niche, the human gastric epithelium. In order to set the stage, we begin with an overview of the genetic diversity of this pathogen

GENETIC VARIATION: WHAT, WHEN, WHERE, HOW

A long association with humans allows accumulation of diversity

While the precise mode of *H. pylori* transmission remains unclear, no environmental or animal reservoirs have been identified. Acquisition of infection appears to occur in early childhood and requires close contact via an oral-oral (e.g. via vomit) or oral-fecal route. Colonization likely persists without intervention, previous infection does not protect against re-infection and super-infection with multiple strains occurs. Thus *H. pylori* transmission differs from that of many enteric pathogens that persist and travel within the environment (e.g. on contaminated food) allowing global outbreaks. Instead, *H. pylori* strains are transmitted within families or household groups (87). Consistent with a vertical mode of transmission, strains within families often show high genomic sequence similarity. In contrast, strains from unrelated individuals show extensive genetic diversity both in nucleotide sequence and gene content.

Comparison of strains from diverse geographic regions using multi-locus sequence typing (MLST) revealed distinct DNA sequence signatures associated with different ethno-geographic populations and decreasing genetic diversity among populations with increasing distance from Africa (30, 62). Indeed contemporary *H. pylori* strains fall into nine ethno-geographic groups with characteristic genetic signatures of five ancestral populations (ancestral Africa1, ancestral Europe1, ancestral Europe2, ancestral EastAsia, ancestral Africa2). These observations further support vertical transmission, suggest isolation of strains within human subpopulations and imply a long association of *H. pylori* with humans, at least since we migrated out of Africa 50,000–200,000 years ago. Recent analysis by whole genome sequencing of *Helicobacter acinonychis*, the closest known relative of *H. pylori* that colonizes large felines (cheetahs, lions, tigers), suggests the association of *H. pylori* with humans extends even further back in history (28). These authors posit *H. acinonychis* arose during a host jump from humans to a large cat that consumed humans. This idea is based on conservation of gene content and synteny between *H. pylori* and *H. acinonychis* and the high rate of gene inactivation by mutation. Synonymous differences in 612 genes that are conserved between an *H. pylori* strain of European origin (26695), an *H. pylori* strain of African origin (J99) and an *H. acinonychis* strain suggests that *H. pylori* diverged from *H. acinonychis* before humans left Africa (range: 100–400ky).

Impact of genetic diversity on gene function

H. pylori was among the first bacteria to be analyzed by whole genome sequencing (96) and in 1999 was the first bacterium for which sequences from two unrelated strains were completed (4). This landmark study provided evidence on a genome wide scale for the genetic diversity observed previously among unrelated strains but demonstrated that most nucleotide differences result in synonymous codons. Thus while only 8 genes share $\geq 98\%$ nucleotide identity, 310 proteins share $\geq 98\%$ amino acid identity. Similarly, while 7% of genes are specific to each strain, overall gene content and synteny are well conserved. Further sequence analysis suggest that slip strand mis-pairing at dinucleotide repeats and homopolymeric tracts affects protein expression in a large number of genes encoding surface structures (lipopolysaccharide biosynthesis, flagellar synthesis, outer membrane proteins, beta-lactamases), restriction modification genes, and hypothetical ORFs (85). As

the *H. pylori* genome encodes relatively few recognizable transcription factors, this mode of phase variation may allow *H. pylori* to adapt its surface during chronic colonization.

Gene content variation

Genome wide analysis of genetic diversity has been expanded to additional strains first using DNA microarrays and more recently using whole genome sequencing (32, 33, 49, 66, 69). Microarrays based on the 26695 (hpEurope) and J99 (hpAfrica1) strain sequences revealed that 22% of the genes from these two strains are variably present in 15 unrelated strains (83) leading to a concept of a core genome supplemented by strain specific genes. When a larger collection of geographically representative strains were analyzed, the core genome was further reduced to 1150 genes (34). Strain specific genes are enriched for restriction modification, transposon, outer membrane protein, and type IV secretion (T4S) annotations. Whole genome sequencing of mostly European strains, one African and one Amerindian strain (hpEastAsia), has expanded the strain specific gene pool to 847 and extrapolation predicts an open pan-genome (32). In these sequenced genomes, strain specific genes account for 4.9–12.4% of the genome and approximately a third are located in plasticity zones or the *cag* pathogenicity island (PAI), which encodes a T4S system that delivers the protein effector CagA from the bacterial cytoplasm, across the bacterial and host cell membranes and into the host cell cytoplasm. Outer membrane proteins and the *cag* T4S system mediate interactions with host cells while transposase and restriction modification genes may regulate genome diversification.

Gene content variation does not show the same strong phylogenetic signature observed with genomic sequence variation (34). Most strain specific genes show variable presence in all subpopulations suggesting their presence in a common ancestor and convergent evolution of gene loss. A notable exception is the *cag* PAI which was apparently acquired after separation of the dominant *H. pylori* from its nearest relative (Sheeba) and from HpAfrica2, suggesting horizontal gene transfer.

Like other strain variable genes, the rate of carriage of the *cag* PAI varies in different global populations (34), but 95% of hpAfrica1, hpEastAsia and HpAsia2 strains carry the island suggesting a selective advantage for persistence or transmission (77). On the other hand loss of the *cag* PAI has been observed during human infection through inter-strain homologous recombination (HR) between a *cag* PAI⁻ strain bearing an “empty site” allele and a *cag* PAI⁺ strain suggesting selection against carriage in some hosts (48). Phylogenetic trees based on the *cag* PAI genes are highly congruent with trees based on housekeeping genes further supporting a single acquisition event (77). Fixed polymorphisms and most rare polymorphisms are able to translocate CagA, indicating that they are functional. While most genes are under purifying selection, several known and putative surface exposed genes and the CagA effector showed signatures of diversifying selection consistent with adaptation to changes in hosts. Thus variable genes may be enriched for factors that interact with the host, as this interaction drives selection that can be both positive and negative.

Mutation rate

The original genome annotation suggested that *H. pylori* lacked mismatch repair (*mutHLSI*) and several HR genes involved in processing DNA damage intermediates (*recBCDFO*) suggesting a high mutation rate for this organism that may contribute to its genetic diversity. Analysis of the mutation rate among clinical isolates under laboratory growth conditions ranged from 10–100 higher than for *E. coli* (10^{-9} mutant/cell/generation) in some studies (16, 44) while being more similar to *E. coli* in other studies (15, 26). Mutation during human infection was first examined using sequential isolates obtained from patients that failed antibiotic eradication treatment after a mean interval of 1.8 years (29). MLST revealed many

changes, but most events appeared to have resulted from recombination, not de novo mutation. A more recent study re-examined these isolates as well as additional isolates obtained from families from both developed and developing countries where transmission of strains could be observed (72). The calculated short term mutation rate among patient serial isolates was 1.4×10^{-6} nucleotide⁻¹ year⁻¹ and 4.5×10^{-6} nucleotide⁻¹ year⁻¹ for isolates within families. Similar to the prior analysis, sites were three times more likely to be substituted by recombination than de novo mutation. One possible explanation for the three fold difference in rates is that an increased mutation rate can provide a selective advantage under conditions that require the bacterium to adapt (e.g. transmission). A more stable environment may select for a low mutation rate that will minimize adverse fitness effects. These data combined with the observed wide variation in mutation rates measured in vitro among *H. pylori* isolates suggest this organisms may actively regulate mutation rate.

Repetitive sequences as mediators of genome diversification

Recombination plays a major role in generating genetic variation among *H. pylori* strains and several classes of repetitive elements appear to drive intragenomic recombination events. Four related insertion sequence (IS) elements have now been described (IS605-IS609) and three show measurable transposition when expressed in *E. coli* (47, 50, 52, 53). These simple IS elements are unusual in having 2 annotated transposases, but their precise mode of actions remain to be elucidated. The distribution of these elements varies in different global populations but they often exist in multiple copies, have mediated rearrangements of the *cag* PAI, and serve as hot spots for integration of strain variable genes (17, 32, 49).

Other short interspersed repetitive sequence elements have been identified using computational methods (10, 11). Short repeats in *cagY*, a component of the *cag* T4S apparatus and *amiA*, an amidase that cleave peptidoglycan, expand and contract among isolates. The mechanisms controlling these events are unclear, as engineered cassettes containing repeats of 5–100 bp undergo spontaneous deletion that does not depend appreciably on *recA* or the natural transformation machinery. Recently a new gene family of unknown function bearing variable regions encoding 7 and 11 amino acid repeats has been discovered at 2 genomic loci (FAR1 and FAR2) (88). Both the number of genes and repeats within these genes appear to vary by recombination events and slip strand mis-pairing.

Among the largest class of strain variable genes are outer membrane proteins (OMPs). *H. pylori* has more than 60 OMPs that have been grouped according to sequence similarity (3). The Hop class includes several adhesins and share similarity in their amino and carboxy termini while maintaining unique central regions. Sequence analysis of homologues and paralogues within and between strains demonstrates frequent recombination between *babA*, encoding an adhesin binding Lewis B blood group antigens, and a related gene, *babB* (80). Gene conversion events between *babA* and *babB* to occur both *in vitro* and during experimental infection (23, 90) and also included an additional locus (*babC*) (36). Thus intragenomic recombination likely affects both gene function and expression.

Horizontal gene transfer

In addition to recombination events within strains, the admixture observed in clinical isolates strongly suggests recombination among distinct strains occurs during co-colonization of hosts by multiple unique strains. Indeed several instances of mixed infection with accompanying recombination events have been documented (48, 84, 87). Horizontal transfer of DNA in bacteria can occur by natural transformation, conjugation and phage transduction. The vast majority of clinical isolates display measurable natural competence (105) which occurs via the com T4S system (37). Conjugation of plasmid DNA was

observed with an *oriT* containing RP4 plasmid that lacked *mob* genes and depended on a chromosomally encoded *traG* relaxase homologue (13). Whether this system can mobilize endogenous plasmids is not clear. Recently several T4S gene clusters have been observed in plasticity regions (PZ) of several strains (32, 51, 54). These clusters, dubbed TnPZs, contain direct repeats also present in one copy at unoccupied sites, a cluster of T4S genes, a tyrosine recombinase family gene (*xerT*) and a large ORF with helicase and methylase domains. Transfer of one TnPZ has been observed experimentally with concomitant *xerT* dependent detection of circular intermediates expected from TnPZ excision events. However, the experimental conditions used in the transfer experiment could not distinguish between conjugation and transformation (32). To date no convincing evidence for the presence of *H. pylori* phage has emerged. Thus *H. pylori* undergo horizontal gene transfer through highly efficient natural transformation and probably by conjugation of both plasmid and chromosomal elements.

MOLECULAR MACHINERY OF DNA REPAIR

All life must combat constant insults to DNA, and *H. pylori* is no exception. The starting point for identifying specific repair systems has been homology to proteins of known function in *E. coli*. Many of these proteins do not retain all the functions seen in their *E. coli* counterparts. Consistent with a reduced genome size, there is less redundancy in repair pathways and some homologs appear to be missing entirely due to sequence divergence. All of the genes referred to below are classified as core genes (conserved across all known strains) and are identified by the locus tags for strain 26695, the first sequenced strain.

Reversal of aberrant methylation

Chemical agents and wayward methyl transferases cause inadvertent methylation of DNA. Aberrantly methylated DNA can arrest polymerases performing replication and transcription, and thus must be repaired quickly. A methyl accepting peptide Ada (HP0676) has been identified and presumably functions like similar peptides in *E. coli* that accept methyl groups from DNA, eliminating the need to completely remove the altered base (82).

Base excision repair

Oxidative damage from oxygen radicals produced as byproducts of metabolism and by effectors of the host immune system can cause damage to the genome. Guanine residues are especially susceptible to reactive oxygen, with the predominant product being 8-oxoguanine. (58). Pyrimidines also undergo oxidation reactions to form products such as thymine glycol and 5,6-dihydrothymine (76). All of these products block progression of polymerases and must be excised before DNA replication or RNA transcription can proceed (22). Two endonuclease type III enzymes have been identified in *H. pylori* which can excise these mutated bases (75, 76). The endonuclease *nth* (HP0585), is responsible for repair of oxidized pyrimidines and has an AP lyase activity, shown in vitro to cleave the 3' end of the DNA backbone adjacent to the mutated base (76). Another type III endonuclease, AlkA (HP0602), is a 3-methyladenine DNA glycosylase, shown in *E. coli* to release 3-methyladenine from DNA, but not oxidized bases (75).

In *E. coli* the glycosylase MutY cleaves adenine mismatches (82). Most of these mismatches are derived from the oxidation of guanine to 8-oxo-guanine. Because 8-oxo-G can base pair with both cytosine and adenine, this reaction leads to mutations if it is not repaired before DNA replication. Inactivation of MutY in *E. coli* leads to a strongly increased rate of C to A transversion mutations. An *H. pylori* homolog of *mutY* (HP0142) has been recognized and plays a role in limiting transversion mutations (56). Inactivation of *mutY* results in a 3.7 fold

increase in the overall point mutation frequency, and a >300 fold increase at specific sites. The *mutY* mutant also displays a loss of fitness during stationary phase in vitro.

All four of the DNA bases are susceptible to spontaneous deamination reactions, with the most common being the hydrolysis of cytosine into uracil. Since uracil is not a standard base in DNA, it is recognized and removed via the base excision repair pathway (82). A uracil DNA N-glycosylase homologue (*ung*, HP1347) is present in the genome, but has not been characterized. The other deamination products are less common and no other specific glycosylases have been identified in *H. pylori*.

Nucleotide excision repair

The nucleotide excision repair pathway recognizes and repairs a wide variety of bulky lesions(82). UvrABC recognizes distortions in the DNA double helix rather than specific lesions, creating a versatile DNA repair system. Homologues of *uvrABCD* were annotated in the *H. pylori* genome (96), but unlike *E. coli*, these genes are scattered through out the genome, suggesting that there is either a mechanism for their coordinate regulation or they are constitutively expressed. In both *E. coli* and *H. pylori*, *uvrD* (HP1478) mutants are sensitive to UV radiation. (41) and the *E. coli* UV sensitivity can be partially restored by *H. pylori uvrD*. The Δ *uvrB* mutant(HP1114) is highly sensitive to UV, methylmethane sulfonate and acid induced DNA damage (95) suggesting that the nucleotide excision repair pathway is vital to maintaining DNA stability in the low pH environment of the human stomach.

During transcription, RNA polymerase occasionally encounters mutated bases which stall transcription. The transcription coupling factor Mfd interacts with stalled polymerase and actively recruits the UvrABC complex to engage in transcription-coupled repair (82). An *H. pylori* Mfd homologue (HP1541) was identified by homology and *mfd* mutants are more sensitive to a number of antibiotics including clarithromycin, amoxicillin and metronidazole, as well as the DNA damaging agent mitomycin C (MMC) indicating a possible additional role for Mfd in recombination (59).

Mismatch repair

Mismatch repair (MMR) identifies and corrects bases that are improperly inserted during DNA replication or recombination. MutHLS1 appears to be missing in the *H. pylori* genome. MutS2 (HP0621) has been identified, but its sequence diverges significantly from MutS1 (79). Multiple functions have been attributed to MutS2, but it does not alter mutation rate (our unpublished data), suggesting it is not important for MMR. MutS2 binds 8-oxo-G (97), indicating a potential role in base excision repair and other studies suggest it is a suppressor of recombination (79, 97). The potential lack of a MMR system may account for the high variability in some *H. pylori* strains. Alternatively *H. pylori* may retain a MMR system independent of MutL. Although specific evidence of the necessary endonuclease is absent, it has been suggested that MutS2 can recognize the errant nucleotide and provide the scaffolding for the enzyme which carries out the activity of MutH in *E. coli* (65). Given that some strains of *H. pylori* have low mutation rates (15, 26), additional screens to further elucidate an alternative MMR system are warranted.

Pre-synaptic pathways of HR

HR is an important DNA repair mechanism in *H. pylori*. HR is also responsible for enhancing genetic diversity via gene deletions, insertions, duplication. As described above, many outer membrane protein coding regions share conserved sequences on the 5' and 3' ends with a variable middle region allowing for gene conversion and locus switching. Such

gene conversion events occur between the adhesin genes *babA* and *babB*, depend on recombination proteins and are selected *in vivo* during chronic infection (6, 23).

The HR pathways found in *E. coli* are largely conserved in *H. pylori* with important mechanistic modifications (summarized in Figure 1). Distinct enzymes process DNA containing a double stranded break versus gapped single strand templates for loading of RecA. While in *E. coli* there seems to be some overlap in these presynaptic pathways, the available data suggest that *H. pylori* presynaptic pathways are independent (67, 98).

Stalled replication forks may cause double stranded DNA breaks during transcription and DNA synthesis (78). In *E. coli*, the RecBCD complex resects double strand breaks and releases a 3' ssDNA filament for RecA loading. In many Alpha- and Betaproteobacteria and all Epsilonproteobacteria including *H. pylori*, the AddAB complex carries out a similar activity (Figure 1A). Both subunits of the AddAB complex have a nuclease domain and AddA has an additional ATP dependant helicase activity (6). Nuclease activity in both subunits is both Mg²⁺ and ATP dependant and has been proposed to cut DNA and facilitate loading of RecA onto single stranded ends (7). Helicase activity is essential for exonuclease activity and the nuclease domains both contribute half of the exonuclease activity (7). RecBCD dependent recombination events in *E. coli* can be restored to near wild type levels by *H. pylori* AddAB, but only when RecA from *H. pylori* is also present (7). This provides compelling evidence that AddAB is essential for loading RecA onto the ssDNA ends to facilitate strand exchange and recombination. The *E. coli* RecBCD enzyme is regulated by *chi* sequences (8), but the *E. coli chi* sequence is not common in the *H. pylori* genome and the mechanism triggering AddAB to switch from nucleolytic activity to RecA loading activity remains unknown.

In *E. coli*, an alternate recombination initiation pathway exists to repair gaps in the DNA sequence and restart replication after a fork has stalled. The RecQ helicase and the RecJ nuclease create a ssDNA end similar to the end formed by RecBCD. RecJ and RecQ activity is sterically hindered by RecFOR, which then mediates RecA binding (20). Both the RecBCD and RecFOR pathways are at least partially redundant in *E. coli*, and the RecFOR pathway can repair double stranded breaks, albeit with lower efficiency than RecBCD, via HR when one or more components of the RecBCD pathway is absent (73).

H. pylori has *recO*, *recR* and *recJ*, but lacks *recF* and *recQ*. RecOR participates in gap repair and replication fork restart presumably in a similar manner to *E. coli* (68) (Figure 1B). Activity of RecOR is dependant on RecA, and presumably loads RecA onto ssDNA ends in a manner similar to the system described in *E. coli*. RecOR plays a significant role in resistance to ssDNA breaks (UV light) and intra-genomic recombination, with substrates including direct repeat sequences (67). Two groups have shown that *addA recO* double mutants have similar sensitivities to insults that lead to DSBs as *addA* single mutants, indicating the RecOR pathway does not participate in DSB repair, even in the absence of AddA (67, 98). This contrasts with the partial redundancy of the two pathways in *E. coli*.

Homologs of RecN and the RecJ the nuclease in *H. pylori*, but not RecQ. RecN is required for a high frequency of natural transformation and resistance to oxygen and acid stress, indicating it plays a role in DNA repair via recombination (99). Given that various double and triple mutant combinations of *recO*, *recR*, *addA* and *addB* show the same sensitivities to UV and ionizing radiation as the $\Delta recA$ mutant, it is unlikely that either *recJ* or *recN* participate in alternative presynaptic pathways (67). Further work will be needed to uncover how these proteins interface with the AddAB and RecOR pathways and the specific types of DNA damage to which they respond.

RecA, the master regulator of recombination

RecA is an essential protein for nearly all recombination events in *H. pylori*. It coats ssDNA and mediates synapsis, the pairing of homologous sequence. RecA is required for natural competence and resistance to many DNA damaging agents (6, 67, 86, 94). Early work showed that *H. pylori* *recA* could not complement an *E. coli* *recA* mutant and it was suggested that glycosylation may be important for RecA function (31, 86). However, recent work suggests that specificity between *H. pylori* RecA and AddAB is more likely the limiting factor in *E. coli* (7).

Post-synaptic pathways of HR

After synapsis, the highly conserved RuvABC complex uses an ATPase domain to drive migration of the Holliday junction (HJ), the intersection point between the four strands of DNA involved in recombination. The RuvC endodeoxyribonuclease activity cleaves DNA at the HJ, resolving the DNA into two separate dsDNA strands, allowing replication to restart. *H. pylori* has RuvABC homologues and the Δ *ruvC* mutant is sensitive to oxidative stress suggesting this pathway is preserved (64). In *E. coli*, either the RecG helicase in conjunction with the RusA nuclease or RuvABC resolve the HJ. Although *H. pylori* *recG* can complement UV induced lethality in the *E. coli* Δ *recG* mutant (43), the *H. pylori* Δ *recG* mutant is not sensitive to UV. Since *H. pylori* lacks a RusA homologue (42), it has been suggested that *H. pylori* RecG cannot resolve HJ, but instead stalls recombination when it binds, acting as a limiting regulator of recombination. The post-synaptic pathways exemplify the major theme of *H. pylori* repair pathways: the major functions are conserved, with differences from *E. coli*. As in the case of RecG, proteins may either have different functions from those elucidated in *E. coli* or most redundancy has simply been lost during genome reduction.

MOLECULAR MACHINERY OF COMPETENCE

Natural competence is the ability to take up DNA from the environment and recombine it into the genome, leading to genetic transformation. *H. pylori* uses novel pathways to bring DNA into the cell and to process this DNA for chromosomal integration, as outlined in Figure 2.

DNA transport across the cell envelope

H. pylori is unique among known competent organisms as it uses a T4S system (the Com apparatus) to transport DNA through the inner and outer membrane (37). T4S systems, including the *H. pylori* Cag apparatus, are better known as virulence factors in diverse bacterial species that inject both DNA and proteins into host cells (5). The Com apparatus is a simple T4S system – it requires only a single ATPase motor (45) in contrast with the three ATPases that drive transport in other systems (21). It also operates with opposite polarity from most systems; the Com T4S system brings DNA substrates into the cell instead of exporting macromolecule out of the cell.

H. pylori uses at least one conserved component for DNA transport. The ComEC protein is a cytoplasmic membrane channel that transports DNA in *Bacillus subtilis* (27, 91). An interesting question is how ComEC integrates with the T4S apparatus to achieve DNA uptake. One model suggests that the Com apparatus transports DNA through the outer membrane and then ComEC transports DNA through the inner membrane (91), but it is unclear whether the two systems are coupled. The preferred substrate for uptake is dsDNA (60) and DNA is thought to be unwound as it is transported. However, *H. pylori* lacks an obvious homolog of the ComFA helicase, required for DNA unwinding in *B. subtilis* (63).

Pre-synaptic events

While the precise mechanisms remain fuzzy, DNA import through the Com apparatus most likely yields single stranded cytosolic DNA fragments. These pieces of DNA have a very different structure from templates encountered during DNA repair of an intact chromosome and likely utilize a distinct presynaptic pathway for recombination. Consistent with this hypothesis, neither the RecOR or AddAB proteins significantly impact the frequency of transformation (38, 67)

DprA and RecA mediate presynaptic events of recombination leading to natural transformation (55). In *S. pneumoniae*, DprA and RecA cooperatively bind ssDNA and DprA lowers the threshold for RecA interaction with single stranded binding protein (SSB) coated DNA (74). Both DprA (89) and RecA (86, 94) are required for transformation of *H. pylori*, suggesting similar interactions. A putative resolvase homolog, DprB, is co-expressed with DprA (38). DprB is required for a high frequency of natural transformation and for efficient growth in broth culture, but is not required for DNA damage repair (38). Although the mechanism of DprB action is unclear, it is tempting to speculate that it might act in association with DprA to support formation or resolution of joint molecules during recombination of environmental DNA. Thus RecA, DprA and DprB likely comprise the presynaptic pathway for recombination substrates brought in through the Com system.

Post-synaptic events

The role of post-synaptic HR factors in controlling transformation frequency is controversial. RecG has been reported to decrease the transformation frequency (42, 43), but we have been unable to reproduce this observation in a different strain background (38). RuvC has also been reported to cause an increase in the transformation frequency (64), but we could not repeat this observation in the same strain background (38). Redundancy between these factors and DprB has not been investigated.

The length of DNA integrated into the genome is another measure of transformation efficiency. To measure the integration length, genomic DNA is mixed with recipient cells of a different strain and single nucleotide polymorphisms between the two strains are mapped by restriction digest and sequencing. Although mismatches decrease transformation frequency in other species, a 4% difference between strains has no effect on the transformation frequency of *H. pylori* (38). Longer integration lengths are measured for transfer of a whole gene (range 1022– 8397 bp) (38, 61) than for a point mutation (950– 1850 bp) (61). This difference is reflected in the transformation frequency of heterologous DNA. When strain Shi470 was mixed with heterologous DNA, transformation frequency for a point mutation was >1000-fold higher than that for an antibiotic resistance gene (49), supporting the idea that a shorter length of DNA is required for integration of a point mutation than a gene.

Post-synaptic HR factors control the length of DNA integrated into the genome (38). Consistent with its role in branch migration, RecG increases the length of DNA integrated into the genome. RuvC decreases the length of DNA integrated, suggesting that it is important for resolution of the HJ. Since RuvC has no adverse effect on transformation frequency in our system, it is possible that there are multiple routes to resolution of HJs formed during natural transformation. We propose HJs may be resolved in a RuvC-independent manner, as RecG may push the junctions to the end of the invading DNA. (Figure 2). Taken together, it appears there is a HR pathway leading to natural transformation that is distinct from DNA repair.

REGULATION

Both DNA repair and natural competence proteins are tightly regulated in many bacterial species. *H. pylori* is constitutively competent (14) and has few transcriptional regulators. However, recent work reveals regulation of competence activity and a regulatory circuit linking DNA damage and natural competence (26).

Transcriptional response to DNA damage

All organisms sense and repair DNA damage to prevent loss of genetic material. In many bacteria, the SOS response is the main transcriptional network that detects damage and induces or represses transcription of components required for repair. The SOS response is activated when RecA binds single stranded DNA, thus activating its co-protease activity towards the LexA repressor. The LexA protease cleaves itself and transcription of DNA repair proteins proceeds (71). The SOS response limits expression of repair proteins until they are necessary; however some species, including *H. pylori*, lack an obvious homolog of the LexA repressor, suggesting they do not regulate expression of DNA damage factors. One possibility is that *H. pylori* lost the SOS response as part of its genome reduction. In addition, DNA repair is required during growth in broth culture (26), so constitutive expression of DNA repair genes may be required for normal replication.

Transcriptional profiling of the *H. pylori* response to DNA damage revealed few changes in the expression of DNA repair proteins (26), supporting the idea that the SOS response is absent from this organism (4). Instead, DNA damage causes increased transcription of several classes of proteins including those required for natural competence (26).

The transcriptional circuit linking DNA damage and induced natural competence is not well understood. RecA is required for transcription of DNA damage-responsive genes, but since *H. pylori* lacks LexA, the connection between RecA and the transcriptional machinery is unclear (26). In an unusual twist, natural competence is required to stimulate the DNA damage regulon. As the DNA damage regulon induces transcription of natural competence, a positive feedback loop is created as natural competence stimulates itself. One possibility is that in *H. pylori*, RecA associates with the competence apparatus and this complex mediates transcriptional signaling. A second possibility is that DNA acts as a signal, since increased uptake of environmental DNA in the absence of DNA damage induces transcription of much of the DNA damage response (26). RecA binding to this incoming DNA may lead to transcriptional signaling.

A connection between natural competence and damage to the genome may be favored for two reasons. One possibility is that the organism acquires templates for repair through DNA uptake (101). This suggestion seems unlikely, since *H. pylori* cells lacking competence show wild-type resistance to DNA damaging antibiotics (26). An alternate possibility is that genetic exchange is favored under stressful conditions as it produces new variants with improved fitness. In support of this hypothesis, competent *H. pylori* increased their fitness faster during passage in the laboratory for 1000 generations (15).

Restriction-modification systems regulate interstrain recombination

Uptake of DNA from other strains or species allows diversification of the genome; however many competent organisms limit DNA uptake. *Haemophilus influenzae* and *Neisseria gonorrhoeae* encode short sequence tags in their genome that are recognized by the uptake machinery and most known competent species tightly regulate expression of competence (19). *H. pylori* shows no such specificity or regulation, taking up DNA throughout its lifecycle with no obvious sequence preference. Instead, cytosolic factors limit DNA uptake, including the post-synaptic recombination factors (discussed above) and restriction enzymes

(9, 12). *H. pylori* strains encode a varying group of 10–20 restriction enzymes, although many of these are inactivated (4, 38, 96). Expression of a restriction enzyme requires a cognate methyltransferase that modifies self-DNA at the enzyme target site, thus protecting the genome. Several groups have shown that restriction enzymes reduce the frequency of transformation with unmodified plasmid and genomic DNA, suggesting that they are an important block against foreign DNA (12, 39).

Restriction enzymes influence more than just natural transformation frequency. Unmodified DNA is not uniformly destroyed, but restriction enzymes reduce the length of DNA integrated into the genome, suggesting that they cut some, but not all cleavage sites. Restriction enzymes and strain specific genes combine to shape a variable *H. pylori* genome.

ROLES OF RECOMBINATION/REPAIR DURING INFECTION

DNA repair and recombination proteins play multiple roles during infection. *H. pylori* experiences DNA damage during host colonization that must be repaired. Additionally recombination may allow phenotypic diversification during infection to facilitate chronic infection and survive changes in the environment. Indeed changes in expression of OMPs through mutation and gene conversion have been observed in multiple infection models (90, 92). Many groups have tested the ability of various mutants to colonize a mouse model of infection to begin to explore the contribution of DNA repair and recombination to *H. pylori* infection.

DNA repair

In both human infection and the mouse model, *H. pylori* must escape the acidity of the lumen and penetrate the gastric mucus. In this extracellular niche, *H. pylori* faces oxidative damage from innate immune responses. *H. pylori* is a potent activator of the phagosome NADPH oxidase, leading to extracellular superoxide accumulation (2). *H. pylori* also induces both macrophages (18) and epithelial cells (103) to produce extracellular hydrogen peroxide by stimulating polyamine oxidase. Finally, isolated gastric pit cells show measurable constitutive extracellular superoxide production after exposure to *H. pylori*-derived lipopolysaccharide (93). These innate immune responses likely damage cells to the benefit of *H. pylori* through release of nutrients, but also necessitates bacterial mechanisms to combat oxidative damage to its DNA, proteins and lipids.

Loss of several *H. pylori* DNA repair proteins causes lower colonization load, usually tested after 1 week, or decreased persistence (beyond 1 month) in the mouse model. Proteins that recognize and repair DNA damage, including endonuclease III, which repairs oxidized pyrimidine residues (O'Rourke *et al.*, 2003), MutS2, which in *H. pylori* recognizes and binds 8-oxoguanine (Wang *et al.*, 2005), two DNA glycosylases (Baldwin *et al.*, 2007) are all required for high levels of colonization. Additionally, all the recombinational repair genes tested are required for high levels of stomach colonization including *addAB* (6, 100), *recN* (99), *recO* (98), *ruvC* (64) and *recA* (6). The $\Delta recA$ mutant displayed the most severe phenotype with essentially no detectable colonization, suggesting that RecA participates in multiple repair pathways.

In *H. pylori*, *in vitro* studies suggest little overlap between the major presynaptic recombinational repair pathways in contrast to *E. coli* (67) and AddAB and RecOR cannot substitute for each other (Figure 1). Since cells lacking *addAB* and *recOR* are impaired for colonization, the ability to process both strand breaks and gapped templates likely is required during infection. Interestingly, the $\Delta addA \Delta recO$ double mutant showed only slightly lower colonization than the single mutants and in contrast to the severe colonization defect of $\Delta recA$ mutants (6, 98). Point mutants in *addAB* that disrupt either nuclease or

helicase activity were also examined for their role in infection (7). All point mutants were defective relative to wild type for colonization. The two nuclease mutants colonized better than the null mutants, but the *addA* helicase mutant had a very severe defect compared to the $\Delta addA$ mutant. This result suggests that the AddA helicase mutant enzyme might act upon damaged DNA in such a way that an AddAB-independent repair pathway cannot be used for repair. This pathway would be functional in the *addA* null strains and contribute to colonization. The *B. subtilis* AddA helicase mutant enzyme binds tightly to dsDNA ends (104) and the same may be true for the *H. pylori* mutant enzyme. RecN may provide an alternative pathway for repair of double strand breaks through binding of DNA ends (70). Analysis of the $\Delta recN \Delta addA$ double mutant will reveal connections between these pathways for double strand break repair and infectivity. Additionally, an *H. pylori* *recJ* homologue has been annotated, but not characterized.

In summary, the available evidence suggests that *H. pylori* experiences multiple types of DNA damage during infection and requires multiple DNA repair pathways for optimal survival in the gastric niche. At this time, there is little evidence for redundancy between recombinational repair pathways, but further study is required to fully answer this question.

Competence

As described above, competence can enhance the rate of selection during *in vitro* growth. Numerous studies have documented the generation of genetic variants during human infection (40, 48, 72, 84) that may promote adaptation during chronic colonization. The gastric niche undergoes marked changes during the long course of *H. pylori* infection (102). In addition to daily fluctuations due to epithelial turnover and pH changes during feeding, long term changes include innate and adaptive immune cell infiltration, hyperplasia of mucus producing cells and loss of parietal cells and zymogenic cells. This atrophy of the gastric glands leads to increased stomach pH that may allow competing bacteria to colonize the stomach. In addition to a role in adaptation to the changing environment, DNA taken up by the competence apparatus may serve as a template for DNA repair or as food.

Two groups have examined the role of competence during initial colonization using the Mongolian gerbil (46) and the mouse model (26) and found no significant effect of competence. Both studies used strains that had been pre-adapted to infect the rodent host and measured colonization at time points before significant changes in gastric gland architecture are observed. The latter study probed the contribution of competence to DNA repair by testing the $\Delta addA \Delta comB10$ double mutant in competition with each single mutant. Surprisingly, the double mutant colonized better than the $\Delta addA$ single mutant. While this result does not support a role for exogenous DNA in DNA repair, it suggests that competence is active during infection and creates a stress that AddA normally helps overcome. Though AddA is not required for natural transformation (38), double stranded DNA is the preferred substrate of the com apparatus and loss of AddAB may disrupt the balance of proteins that bind and process incoming DNA.

Further elucidation of the function(s) of competence during infection will require longer term or some experimentally induced selection (e.g. drug treatment). Further elucidation of the molecular connection between competence and recombinational repair will be necessary to understand the crosstalk between these pathways.

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List of important Abbreviations and Acronyms

PAI	Pathogenicity island
MLST	Multi-locus sequence typing
IS	Insertion sequence
OMP	Outer membrane protein
HR	Homologous recombination
dsDNA	Double stranded DNA
ssDNA	Single stranded DNA
HJ	Holliday junction
T4S	type IV secretion

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Summary points (up to 8)

1. *H. pylori* clinical isolates show high genetic diversity due to extensive intra and inter genomic recombination.
2. Most of the major DNA repair pathways have been identified in *H. pylori*, including those that repair ss- and dsDNA breaks, nucleotide excision repair, and base excision repair and there is little functional redundancy between these pathways
3. The mismatch repair pathway has not been identified in *H. pylori*, but as some strains have mutation rates as low as 10^{-9} mutation/cell/generation, some manner of mismatch repair may exist.
4. A poorly understood recombination pathway leads to natural transformation and is distinct from the pathway required for gapped DNA or double strand break repair.
5. All DNA repair pathways tested are required for infectivity.

Future Issues list

1. Elucidate the connection between DNA repair pathways and determine whether they are all acting during infection.
2. Identify mismatch repair pathways acting in *H. pylori*
3. Further characterize of the connection between competence and transcription, including identification of a transcriptional regulator of the DNA damage response.
4. Further define the path of incoming DNA during natural competence including the mechanism by which dsDNA is converted to ssDNA filament.
5. Determine the function(s) of natural competence during infection

Key Terms/definitions list (up to 10)

1. Natural competence: the ability to take up DNA from the environment and recombine it into the genome
2. Natural transformation: a genetic change conferred upon cells through natural competence
3. Homologous recombination: the exchange of highly similar DNA sequence through a cut and paste mechanism (recombination)
4. Mismatch repair: a system that identifies and correct bases are improperly inserted during DNA replication or recombination.
5. Holliday junction (HJ), the intersection point between the four strands of DNA involved in recombination
6. Synapsis: the pairing of homologous sequences
7. Strain specific genes: genes variably present among clinical isolates
8. Genetic diversity: the variable array of genes and alleles within the genome of a single species

Figure 1. (A) The double strand break repair pathway

The AddAB complex recognizes a double strand break or collapsed replication fork and uses its helicase and nuclease activity to expose a 3' tail. AddAB also loads RecA onto the 3' ssDNA end. RecA mediates synapsis, forming a Holliday junction (HJ). Replication fills gaps, RecG and RuvABC mediate branch migration and RuvC resolves the junctions. Resolution of junctions can lead to the formation of a replication fork and replication restart or invasion of a second 3' end (not shown) results in formation of double cross-over products. **(B) The gap repair pathway** (adapted from (24)). A lesion, such as those caused

by UV, blocks progression of the replication fork. UvrABC and UvrD remove damage. RecJ processes the fork and RecOR hinders its action, loading RecA. Replication resumes.

Figure 2. Natural competence is a linear process

Double stranded DNA is the preferred substrate for natural competence and is taken into the periplasm through the Com apparatus. Com apparatus (60) structure is based on that predicted for other systems (5). Transport into the cytosol likely occurs through the conserved ComEC channel (27,91). DNA may be unwound during this process as it is in *B. subtilis* (63), but how this occurs is unclear. If DNA is from an unrelated strain, REases cleave the DNA limiting the size of DNA available for import. Since REases prefer extracellular DNA the likely act prior to unwinding and perhaps extracellularly. In the cytosol, single-strand binding protein (SSB) protects DNA. RecA and DprA displace SSB and also protect DNA (74). RecA homology searching leads to the formation of a D-loop

structure that is modified and extended by replication and RecG-mediated branch migration. Resolution may occur in two different ways. RuvC may cleave the HJ. Alternately, we propose that RecG may push the HJ to the end of the invading piece of DNA, leading to RuvC-independent resolution of the junction.