1

A Recombination Puzzle Solved: Role for New DNA Repair Systems in *Helicobacter pylori* Diversity/Persistence

Ge Wang and Robert J. Maier
Department of Microbiology, University of Georgia, Athens Georgia

1. Introduction

1.1 Helicobacter pylori pathogenesis

Helicobacter pylori is a gram-negative, slow-growing, microaerophilic, spiral bacterium. It is one of the most common human gastrointestinal pathogens, infecting almost 50% of the world's population [1]. Peptic ulcer disease is now approached as an infectious disease, and *H. pylori* is responsible for the majority of duodenal and gastric ulcers [2]. There is strong evidence that *H. pylori* infection increases the risk of gastric cancer [3], the second most frequent cause of cancer-related death. *H. pylori* infections are acquired by oral ingestion and is mainly transmitted within families in early childhood [2]. Once colonized, the host can be chronically infected for life, unless *H. pylori* is eradicated by treatment with antibiotics.

H. pylori is highly adapted to its ecologic niche, the human gastric mucosa. The pathogenesis of *H. pylori* relies on its persistence in surviving a harsh environment, including acidity, peristalsis, and attack by phagocyte cells and their released reactive oxygen species [4]. *H. pylori* has a unique array of features that permit entry into the mucus, attachment to epithelial cells, evasion of the immune response, and as a result, persistent colonization and transmission. Numerous virulence factors in *H. pylori* have been extensively studied, including urease, flagella, BabA adhesin, the vacuolating cytotoxin (VacA), and the cag pathogenicity island (cag-PAI) [5]. In addition to its clinical importance, *H. pylori* has become a model system for persistent host-associated microorganisms [6]. How *H. pylori* can adapt to, and persist in, the human stomach has become a problem of general interest in both microbial physiology and in pathogenesis areas.

1.2 Genetic diversity of *H. pylori*

H. pylori displays exceptional genetic variability and intra-species diversity [7]. Allelic diversity is obvious as almost every unrelated isolate of *H. pylori* has a unique sequence when a sequenced fragment of only several hundred base pairs is compared among strains for either housekeeping or virulence genes [8-10]. Approximately 5% nucleotide divergence is commonly observed at the majority of gene loci between pairs of unrelated *H. pylori* strains [11]. *H. pylori* strains also differ considerably in their gene contents, the genetic macro-diversity. The two sequenced strains 26695 and J99 share only 94% of their genes, whereas approximately 7% of the genes are unique for each strain [12, 13]. Supporting

studies using whole-genome microarray detected numerous genomic changes in the paired sequential isolates of *H. pylori* from the same patient [14, 15].

Mechanisms proposed to account for the observed genetic variability include mainly the high inherent mutation rate and high frequency of recombination [16]. The spontaneous mutation rate of the majority of *H. pylori* strains lies between 10^{-5} and 10^{-7} [17]. This is several orders of magnitude higher than the average mutation rate of *Escherichia coli*, and similar to that of *E. coli* strains defective in mismatch repair functions (mutator strains) [18]. While mutation is essential for introducing sequence diversity into the species, a key role in generating diversity is played by recombination.

H. pylori is naturally competent for DNA transformation, and has a highly efficient system for recombination of short-fragment involving multiple recombination events within a single locus [19, 20]. A special apparatus homologous to type IV secretion system (T4SS, encoded by *comB* locus) is dedicated to a DNA uptake role [21, 22] and a composite system involving proteins at the *comB* locus and ComEC mediates two-step DNA uptake in *H. pylori* [23]. T4SS systems are known to transport DNA and proteins in other bacteria, but *H. pylori* is the only species known to use a T4SS for natural competence [24]. Unlike several other bacterial species, *H. pylori* does not require specific DNA sequences for uptake of related DNA [25]. Instead, numerous and efficient restriction modification systems take over the function as a barrier to horizontal gene transfer from foreign sources [26, 27].

Population genetic analyses of unrelated isolates of *H. pylori* indicated that recombination was extremely frequent in *H. pylori* [9, 28]. There is evidence that humans are occasionally infected with multiple genetically distinct isolates and that recombination between *H. pylori* strains can occur in humans [29, 30]. Using mathematical modeling approaches on sequence data from 24 pairs of sequential *H. pylori* isolates, Falush et al. [31] estimated that the mean size of imported fragments was only 417 bp, much shorter than that observed for other bacteria. The recombination rate per nucleotide was estimated as 6.9×10^{-5}, indicating that every pair of strains differed on average by 114 recombination events. Compared to other bacteria studied in this way [32-34], the recombination frequency within *H. pylori* is extraordinarily high. The *H. pylori* genome also has extensive repetitive DNA sequences that are targets for intragenomic recombination [35].

2. Overview of DNA repair in *H. pylori*

Oxidative DNA damage represents a major form of DNA damage. Among the many oxidized bases in DNA, 8-oxo-guanine is a ubiquitous biomarker of DNA oxidation [36]. In addition, acid (low pH) conditions may result in DNA damage via depurination [37]. *H. pylori* survives on the surface of the stomach lining for the lifetime of its host and causes a chronic inflammatory response. Several lines of evidence suggest that *H. pylori* is exposed to oxidative damage soon after infection [38, 39]. Under physiological conditions, *H. pylori* is thought to frequently suffer oxidative and acid stress [40, 41]. In addition to diverse oxidant detoxification enzymes (e.g. superoxide dismutase, catalse, and peroxiredoxins) [42] and potent acid avoidance mechanisms (mainly urease) [43], efficient DNA repair systems are required for *H. pylori* to survive in the host.

2.1 DNA repair systems in *H. pylori*

The whole genome sequences of *H. pylori* revealed it contains several DNA repair pathways that are common to many bacterial species, while it lacks other repair pathways or contains

only portions of them. *H. pylori* encodes the homologues of all four members of the nucleotide excision repair (NER) pathway; these are UvrA, UvrB, UvrC, and UvrD, all of which are well conserved in bacteria. NER deals with DNA-distorting lesions, in which an excinuclease removes a 12- to 13- nucleotide segment from a single strand centered around the lesion; the resulting gap is then filled in by repair synthesis [44]. Loss of *uvrB* in *H. pylori* was shown to confer sensitivity to UV light, alkylating agents and low pH, suggesting that the *H. pylori* NER pathway is functional in repairing a diverse array of DNA lesions [45]. *H. pylori* UvrD was shown to play a role in repairing DNA damage and limiting DNA recombination, indicating it functions to ultimately maintain genome integrity [46].

The methyl-directed mismatch repair system (MMR), consisting of MutS1, MutH, and MutL, is conserved in many bacteria and eukaryotes, and it plays a major role in maintaining genetic stability. MMR can liberate up to 1000 nucleotides from one strand during its function to correct a single mismatch arising during DNA replication [47]. Notably, MMR does not exist in *H. pylori*, contributing to the high mutation rates observed in *H. pylori* [17]. *H. pylori* has a MutS homologue that belongs to the MutS2 family. *H. pylori* MutS2 was shown to bind to DNA structures mimicking recombination intermediates and to inhibit DNA strand exchange, thus it may play a role in maintaining genome integrity by suppressing homologous and homeologous DNA recombination [48]. In addition, *H. pylori* MutS2 appears to play a role in repairing oxidative DNA damage, specifically 8-oxo-guanine [49].

Damaged bases can be repaired by a variety of glycosylases that belong to the base excision repair (BER) pathway. All glycosylases can excise a damaged base resulting in an apurinic/apyrimidinic (AP) site, while some of them additionally nick the DNA deoxyribose-phosphate backbone (via an AP lyase activity). *H. pylori* harbors the glycosylase genes *ung, mutY*, *nth*, and *magIII*, whereas several other genes appear to be absent from the *H. pylori* genome, e.g. *tag*, *alkA*, and *mutM*. The *H. pylori* endonuclease III (*nth* gene product), which removes oxidized pyrimidine bases, was shown to be important in establishing long-term colonization in the host [50]. The *H. pylori* MutY glycosylase is functional in removing adenine from 8-oxoG:A mispair, and the loss of MutY leads to attenuation of the colonization ability [51-53].

To repair DNA double strand breaks and blocked replication forks, *H. pylori* is equipped with an efficient system of DNA recombinational repair, which is the main focus of this review (See section 4).

2.2 *H. pylori* response to DNA damage

Many bacteria encode a genetic program for a coordinated response to DNA damage called the SOS response. The best known *E. coli* SOS response is triggered when RecA binds ssDNA, activating its co-protease activity towards LexA, a transcriptional repressor [54]. Cleavage of LexA results in transcriptional induction of genes involved in DNA repair, low-fidelity polymerases, and cell cycle control. However, the *H. pylori* genome contains neither a gene for LexA homolog nor the genes for low-fidelity polymerases, and an SOS response pathway seems to be absent in *H. pylori* [12, 13].

To define pathways for an *H. pylori* DNA damage response, Dorer et al. [55] used cDNA based microarrays to measure transcriptional changes in cells undergoing DNA damage. In both ciprofloxacin treated cells and the Δ*addA* (a major DNA recombination gene, see section 4.4 below) mutant cells, the same set of genes were induced which include genes required for energy metabolism, membrane proteins, fatty acid biosynthesis, cell division, and some translation factors, although the contribution of these genes to survival in the face

of DNA damage is not understood. No DNA repair genes, a hallmark of the SOS response, were induced in either the antibiotic-treated cells or the recombination gene deleted strain. Surprisingly, several genes involved in natural competence for DNA transformation (*com* T4SS components *comB3*, *comB4* and *comB9*) were induced significantly. Indeed, natural transformation frequency was shown to be increased under DNA damage conditions. Another DNA damage-induced gene was a lysozyme-encoding gene. Experimental evidence was provided that a DNA damage-induced lysozyme may target susceptible cells in culture and provide a source of DNA for uptake [55]. Taken together, DNA damage (mainly DSBs in their experiments) induces the capacity for taking up DNA segments from the neighboring cells of the same strain (homologous) or co-colonizing strain (homeologous) that may be used for recombinational DNA repair.

3. Mechanisms of DNA recombinational repair known in model bacteria

Although the bulk of DNA damage affects one strand of a duplex DNA segment, occasionally both DNA strands opposite each other are damaged; the latter situation necessitates recombinational repair using an intact homologous DNA sequence [56, 57]. DNA double-strand breaks (DSB) occur as a result of a variety of physical or chemical insults that modify the DNA (e.g. DNA strands cross-links). In addition, if a replication fork meets damaged bases that cannot be replicated, the fork can collapse leading to a DSB. In *E. coli*, 20-50% of replication forks require recombinational repair to overcome damage [58].

Homologous recombinational repair requires a large number of proteins that act at various stages of the process [56]. The first stage, **pre-synapsis**, is the generation of 3' single-stranded (ss) DNA ends that can then be used for annealing with the homologous sequence on the sister chromosome. In *E. coli*, the two types of two-strand lesions (double strand end and daughter strand gap) are repaired by two separate pathways, RecBCD and RecFOR, respectively [57]. The second and most crucial step in DNA recombination is the introduction of the 3' DNA overhang into the homologous duplex of the sister chromosome, termed **synapsis**. This is performed by RecA in bacteria. RecA binds to ssDNA in an ATP-dependent manner, and RecA-bound ssDNA (in a right-handed helix structure) can invade homologous duplex DNA and mediate strand annealing, accompanied by extrusion of the other strand that can pair with the remaining 5' overhang of the DSB (called D-loop formation).

During DNA recombination, the single stranded DNA (ssDNA) is always coated (protected) by ssDNA-binding protein (SSB), which has a higher affinity to ssDNA than RecA. RecA needs to be loaded (during pre-synapsis stage), either by RecBCD or RecFOR, onto the generated ssDNA that is coated with SSB. During the third step in recombination, **post-synapsis**, RecA-promoted strand transfer produces a four-stranded exchange, or Holliday junctions (HJ) [59]. The RecG and RuvAB helicases are two pathways that process the branch migration of HJ. Finally, RuvC resolves HJ in an orientation determined by RuvB, and the remaining nicks are sealed by DNA ligase.

Several other genes (recJ, recQ, recN) are also required for recombination, although their functions are unclear [60, 61]. Single stranded exonuclease RecJ and RecQ helicase are sometimes needed to enlarge the gap for RecFOR to act [62]. RecN, RecO, and RecF were found to be localized to distinct foci on the DNA in *Bacillus subtilis* cells after induction of DSBs [63]. These proteins form active repair centers at DSBs and recruit RecA, initiating

homologous recombination. RecN was shown to play an important role in repairing DSBs, probably coordinating alignment of the broken segments with intact duplexes to facilitate recombination [64].

4. DNA recombinational repair factors in *H. pylori*

While some genes that are predicted to be involved in DNA recombinational repair, including *recA, recG, recJ, recR, recN, and ruvABC,* were annotated from the published *H. pylori* genome sequences, many genes coding for the components that are involved in the pre-synapsis stage, such as RecBCD, RecF, RecO, and RecQ, were missing. Considering that *H. pylori* is highly genetic diverse with a high recombination frequency, this has been a big puzzle over the past decade. Recent studies revealed the existence of both pathways, AddAB (RecBCD-like) and RecRO, for initiation of DNA recombinational repair in *H. pylori*. In the following sections we will summarize the current understanding of DNA recombinational repair in *H. pylori* by reviewing the literature accumulated in recent years.

4.1 The central recombination protein RecA

The RecA protein is a central component of the homologous recombination machinery and of the SOS system in most bacteria. The relatively small RecA protein contains many functional domains including different DNA-binding sites and an ATP-binding site. *E. coli* RecA has also coprotease activities for the LexA repressor and other factors involved in SOS response. However, *H. pylori* genome does not contain a LexA homolog and an SOS response pathway is likewise absent in *H. pylori*. Thus, a coprotease activity may be dispensable for the *H. pylori* RecA protein. Nevertheless, RecA is required for DNA damage response observed in *H. pylori*, although the underlying mechanism is unclear [55].

Before the genome era, the roles of *H. pylori* RecA in DNA recombination and repair have been studied genetically [65, 66]. *H. pylori* RecA (37.6 kDa protein) is highly similar to known bacterial RecA proteins. The *H. pylori* recA mutants were severely impaired in their ability to survive treatment with DNA damaging agents such as UV light, methyl methanesulfonate, ciprofloxacin, and metronidazole. *H. pylori* RecA also played a role in survival at low pH in a mechanism distinct from that mediated by urease [66]. Disruption of *recA* in *H. pylori* abolished general homologous recombination [65]. Interestingly, *H. pylori* RecA protein is subject to posttranslational modifications that result in a slight shift in its electrophoretic mobility [67]. One putative mechanism for RecA modification is protein glycosylation. *H. pylori* RecA protein was shown to be membrane associated, but this association is not dependent on the posttranslational modification. The RecA modification is required for full activity of DNA repair [67].

In recent years, the phenotypes of *H. pylori recA* mutants have been further characterized in comparison with other mutants. Among the mutants of DNA recombination and repair genes, *recA* mutants displayed the most severe phenotypes. For example, *recA* mutants were much more sensitive to UV or Gamma radiation than the *recB* or *recO* single mutants, and were similar to the *recBO* double mutant [68-70]. The *recA* mutants completely lost the ability to undergo natural transformation [68-70]. The intra-genomic recombination frequency of the *recA* mutant was also much lower than that of the *recR* or *recB* single mutants [68, 71]. Finally, the *recA* mutants completely lost the ability to colonize mouse stomachs [69]. In competition experiments (mixed infection with wild type and mutant

strains), *recA* mutant bacteria were never recovered, while some *addA* or *addB* mutant bacteria were recovered from mouse stomachs.

4.2 Post-synapsis proteins RuvABC and RecG

In addition to the synapsis protein RecA, the genes for post-synapsis proteins (RuvABC and RecG) are also well conserved among bacteria [72]. Genes for RuvABC proteins are present in *H. pylori*, thus *H. pylori* seems to be able to restore Holliday Junctions in a similar way to *E. coli*. RuvC is a Holliday junction endonuclease that resolves recombinant joints into nicked duplex products. A *ruvC* mutant of *H. pylori* was more sensitive (compared to the wild type) to oxidative stress and other DNA damaging agents including UV light, mitomycin C, levofloxacin and metronidazole [73]. As Macrophage cells are known to produce an oxidative burst to kill bacterial pathogens, the survival of *H. pylori ruvC* mutant within macrophages was shown to be 100-fold lower than that of the wild type strain [73]. Furthermore, mouse model experiments revealed that the 50% infective dose of the *ruvC* mutant was approximately 100-fold higher than that of the wild-type strain. Although the *ruvC* mutant was able to establish colonization at early time points, infection was spontaneously cleared from the murine gastric mucosa over long periods (36 to 67 days) [73]. This was the first experimental evidence that DNA recombination processes are important for establishing and maintaining long-term *H. pylori* infection. Further studies suggested that RuvC function and, by inference, recombination facilitate bacterial immune evasion by altering the adaptive immune response [74], although the underlying mechanisms remain obscure.

RuvAB proteins are involved in the branch migration of Holliday junctions. The annotated *H. pylori* RuvB (HP1059) showed extensive homology (52% sequence identity) to *E. coli* RuvB, particularly within the helicase domains. However, unlike in *E. coli, ruvA, ruvB*, and *ruvC* are located in separate regions of the *H. pylori* chromosome, which may predict possible functional differences. In contrast to *E. coli ruvB* mutants, which have moderate susceptibility to DNA damage, the *H. pylori ruvB* mutant has intense susceptibility to UV, similar to that of a *recA* mutant [75]. Similarly, the *H. pylori ruvB* mutant has a significantly diminished MIC (minimal inhibitory concentration) for ciprofloxacin, an agent that blocks DNA replication fork progression, to the same extent as the *recA* mutant. In agreement with these repair phenotypes, the *ruvB* mutant has almost completely lost the ability of natural transformation of exogenous DNA (frequency of $<10^{-8}$), similar to the *recA* mutant. In an assay measuring the intra-genomic recombination (deletion frequency between direct repeats), the *ruvB* mutants displayed significantly (four- to sevenfold) lower deletion frequencies than the background level. All four phenotypes of the *ruvB* mutant suggested that *H. pylori* RuvAB is the predominant pathway for branch migration in DNA recombinational repair [75].

In *E. coli*, an alternative pathway processing branch migration of Holliday junctions is the RecG helicase. In marked contrast to *E. coli, H. pylori recG* mutants do not have defective DNA repair, as measured by UV-light sensitivity and ciprofloxacin susceptibility [76]. Furthermore, *H. pylori recG* mutants have increased frequencies of intergenomic recombination and deletion, suggesting that branch migration and Holliday junction resolution are more efficient in the absence of RecG function [75, 76]. Thus, the effect of *H. pylori* RecG seems to be opposite to that of the RuvAB helicase. In the RuvABC pathway, the RuvC endonuclease nicks DNA, catalyzing Holliday junction resolution into double-stranded DNA. Although the resolvase in the RecG pathway has not been completely

elucidated, it has been hypothesized that RusA may serve this function in *E. coli* [77]. By introducing *E. coli rusA* into *H. pylori ruvB* mutants, the wild-type phenotypes for DNA repair and recombination were restored [75]. A hypothesis was proposed that RecG competes with RuvABC for DNA substrates but initiates an incomplete repair pathway (due to the absence of the RecG resolvase RusA) in *H. pylori*, interfering with the RuvABC repair pathway [75].

4.3 *H. pylori* RecN

Bacterial RecN is related to the SMC (structure maintenance of chromosome) family of proteins in eukaryotes, which are key players in a variety of chromosome dynamics, from chromosome condensation and cohesion to transcriptional repression and DNA repair [78]. SMC family proteins have a structural characteristic of an extensive coiled-coil domain located between globular domains at the N- and C-termini that bring together Walker A and B motifs associated with ATP-binding [79]. *E. coli* RecN is strongly induced during the SOS response and was shown to be involved in RecA-mediated recombinational repair of DSBs [64]. In *Bacillus subtilis*, RecN was shown to be recruited to DSBs at an early time point during repair [63, 80, 81]. In vitro, RecN was shown to bind and protect 3' ssDNA ends in the presence of ATP [82].

In the published *H. pylori* genome sequence [12], HP1393 was annotated as a *recN* gene homolog. The *H. pylori recN* mutant is much more sensitive to mitomycin C, an agent that predominantly causes DNA DSBs, indicating RecN plays an important role in DSB repair in *H. pylori* [83]. In normal laboratory growth conditions, an *H. pylori recN* mutant does not show a growth defect, but its survival is greatly reduced under oxidative stress which resembles the *in vivo* stress condition. While very little fragmented DNA was observed in either wild type or *recN* mutant strain when cells were cultured under normal microaerobic conditions; after oxidative stress treatment the *recN* mutant cells had a significantly higher proportion of the DNA as fragmented DNA than did the wild type [83]. Similar roles of RecN in protection against oxidative damage have been demonstrated in *Neisseria gonorrhoeae* [84, 85]. In addition, the *H. pylori recN* mutant is much more sensitive to low pH than the wild type strain, suggesting that RecN is also involved in repair of acid-induced DNA damage [83]. This could be relevant to its physiological condition, as *H. pylori* appears to colonize an acidic niche on the gastric surface [41].

As mentioned in the sections above, loss of *H. pylori* RecA, RuvB or RuvC functions results in a great decrease of DNA recombination frequency. Similarly, the *H. pylori recN* mutant has a significant decrease of DNA recombination frequency, suggesting that RecN is a critical factor in DNA recombinational repair [83]. In contrast, loss of UvrD or MutS2 in *H. pylori* resulted in an increase of DNA recombination frequency [46, 48]. Suppression of DNA recombination by UvrD or MutS2, and facilitation of DNA recombination by RecN, may play a role in coordinating DNA repair pathways. Recombinational repair could be mutagenic due to homeologous recombination or cause rearrangement due to recombination with direct repeat sequences. In addition, recombinational repair systems are much more complex and require more energy to operate, compared to nucleotide excision repair (NER) and base excision repair (BER) systems. Thus UvrD, as a component of NER, and MutS2 as a likely component of a BER (8-oxoG glycosylase) system [49], both suppress DNA recombination. Both NER and BER systems would be expected to continuously function in low stress conditions. Under a severe stress condition when large amounts of

DSBs are formed, RecN perhaps recognizes DSBs and recruits proteins required for initiation of DNA recombination.
The role of *H. pylori* RecN *in vivo* has been demonstrated, as the *recN*-disrupted *H. pylori* cells are less able to colonize hosts than wild type cells [83]. However, the mouse colonization phenotype of the *recN* strain seems to be less severe than those observed for the *recA* or *ruvC* mutants. In contrast to RecA or RuvC which are major components of DNA recombination machinery, RecN is a protein specific for repairing DSBs by linking DSB recognition and DNA recombination initiation. It was proposed that the attenuated ability to colonize mouse stomachs by *recN* cells was mainly due to the strain's failure to repair DSBs through a DNA recombinational repair pathway.

4.4 AddAB helicase-nuclease

DNA helicases play key roles in many cellular processes by promoting unwinding of the DNA double helix [86]. Bacterial genomes encode a set of helicases of the DExx family that fulfill several, sometimes overlapping functions. Based on the sequence homology, bacterial RecB, UvrD, Rep, and PcrA were classified as superfamily I (SF1) helicases [86-88]. In the well-studied *E. coli*, RecBCD form a multi-functional enzyme complex that processes DNA ends resulting from a double-strand break. RecBCD is a bipolar helicase that splits the duplex into its component strands and digests them until encountering a recombinational hotspot (Chi site). The nuclease activity is then attenuated and RecBCD loads RecA onto the 3' tail of the DNA [89]. Another bacterial enzyme complex AddAB, extensively studied in *Bacillus subtilis*, has both nuclease and helicase activities similar to those of RecBCD enzyme [90, 91].
The genes for RecBCD or AddAB were missing in the published *H. pylori* genome [12, 13]. However, HP1553 from strain 26695 was annotated as a gene encoding a putative helicase [12], and the corresponding gene from strain J99 was annotated as *pcrA* [13]. Amino acid sequence alignment of HP1553 to *E. coli* RecB (or to *B. subtilis* AddA) revealed 24% identity (to both heterologous systems) at the N-terminal half (helicase domain), and no significant homology at the C-terminal half (including nuclease domain). Thus, HP1553 could be a RecB (or AddA)-like helicase [69, 92]. Furthermore, by using the highly conserved AddB nuclease motif "GRIDRID" in BLAST search, HP1089 was identified as the putative AddB homolog [69]. Now it is accepted that HP1553 and HP1089 are termed *addA* and *addB* respectively in *H. pylori* with a reminder that previous *recB* [20, 68, 70, 92] was the equivalent of *addA* [69, 71, 93]. Both genes *addA* and *addB* are present in 56 *H. pylori* clinical isolates from around the world [94]; thus they are considered core genes that are not strain variable.
The biochemical activities of *H. pylori* AddAB helicase-nuclease have been demonstrated [69]. Cytosolic extracts from wild-type *H. pylori* showed detectable ATP-dependent nuclease activity with ds DNA substrate, while the *addA* and *addB* mutants lack this activity. Cloned *H. pylori addA* and *addB* genes express ATP-dependent exonuclease in *E. coli* cells. These genes also conferred ATP-dependent DNA unwinding (helicase) activity to an *E. coli recBCD* deletion mutant, indicating that they are the structural genes for this enzyme [69]. The roles of individual (helicase, exonuclease) activity of the AddA and AddB in DNA repair, recombination, and mouse infection have been further studied by site-directed mutagenesis approach [93].
H. pylori addA and *addB* mutant strains showed heightened sensitivity to mitomycin C and the DNA gyrase inhibitor ciprofloxacin, both of which lead to DNA ds breaks [69, 92]. The

level of sensitivity was similar to that seen for a *recA* mutant, but more severe than for the *recN* mutant. It is thus concluded that AddAB plays a major role in the repair of DNA ds breaks [69, 92]. On the other hand, the *addA* and *addB* mutants were markedly less sensitive to UV irradiation than a *recA* mutant, suggesting that AddAB does not play a major role in repair of UV damage in *H. pylori* [69]. AddA was shown to be important for *H. pylori* protection against oxidative stress-induced damage, as the *addA* mutant cells were significantly more sensitive to oxidative stress and contained a large amount of fragmented DNA [92]. Furthermore, loss of AddA resulted in reduced frequencies of apparent gene conversion between homologous genes encoding outer membrane proteins (*babA* to *babB*) [69]. Finally, it was shown that the *addA* and *addB* mutant strains display a significantly attenuated ability to colonize mouse stomachs, in both competition experiments and during single-strain infections [69, 92].

While *addA* and *addB* are adjacent in the chromosome in most bacteria, including other epsilon Proteobacteria, this is not the case in *H. pylori*. However, the phenotypes of *H. pylori addA* and *addB* mutants are indistinguishable. Thus, it was proposed [69] that the AddA and AddB act together in a complex, as do the RecBCD polypeptides and AddAB polypeptides of other bacteria. If so, the control of the unlinked *H. pylori addA* and *addB* genes to maintain the proper stoichiometry of the two polypeptides remains an interesting question.

Regarding the role of *H. pylori* AddA in DNA recombination during natural transformation, conflicting results were reported from different studies. The *addA* (note: it was named *recB* in certain references) mutant showed enhanced [68, 70], decreased [20, 71, 92], or no change [27, 69] in transformation frequency. Indeed, a high degree of variability (>100-fold) in transformation frequency in *H. pylori* was observed between different strains and different experiments. The use of different assay systems may partly explain the discrepancy in transformation results. For example, the total genomic DNA from antibiotic-resistant strain was used for the transformation assay in certain studies, while in others the defined linear DNA fragments of small size [92]. Use of the transformation frequency as an indicator of DNA recombination frequency is based on the assumption that the wild type *H. pylori* and its isogenic *rec* strains are equally competent for DNA uptake. However, it is now known that this assumption is not valid because DNA damage triggers genetic exchange in *H. pylori* [55]. *H. pylori addA* mutant cells suffered more DNA damage [92], and have an enhanced competence for DNA uptake [55]. Thus, the accumulation of unrepaired DNA damage and subsequent poor growth, as well as unknown strain differences, could be the main cause of the high degree of variability in *H. pylori* transformation frequency [27].

4.5 *H. pylori* RecRO pathway

RecFOR is a highly conserved DNA recombination pathway in bacteria, and is mainly used for ssDNA gap repair [72]. In the published *H. pylori* genome sequences, only the *recR* gene was annotated [12, 13]. Although RecF historically served as a reference for RecFOR pathway, it is absent from genomes of many bacteria including *H. pylori* [72]. By bioinformatics analysis, Marsin et al [68] identified HP0951 as a novel RecO orthologue, although its sequence identity with the *E. coli* protein is lower than 15%. Recent studies in *E. coli* indicated that RecOR in the absence of RecF can perform recombination by loading RecA [95, 96]. Whereas the RecO protein can displace ssDNA-binding protein (SSB) and

bind to ssDNA, RecR is the key component for loading RecA onto ssDNA [95, 97]. Likely, the RecRO pathway (with no RecF) is present in *H. pylori*.

The *recR* and *recO* mutants showed marked sensitivity to DNA damaging agents metronidazole and UV light, indicating roles of RecR and RecO in DNA repair. Unlike the *addA* (*recB*) mutant, the *recR* and *recO* mutants did not show significant sensitivity to ionizing radiation (IR) and to mitomycin C [68, 71], suggesting that RecRO pathway is not responsible for repairing DNA damage induced by these agents, most likely double strand breaks. This is in contrast to *E. coli* where the RecFOR pathway sometimes substitutes for the RecBCD pathway and in *Deinococcus radiodurance* where the RecFOR pathway plays a major role in double strand break repair [98, 99]. On the other hand, *H. pylori recR* and *recO* mutants were shown to be much more sensitive to oxidative stress and to acid stress than the wild type strain [71], indicating that *H. pylori* RecRO pathway is involved in repairing DNA damage induced by these stress conditions. The *addA recO* double mutant (deficient in both AddAB and RecRO pathways) was significantly more sensitive to atmospheric oxygen than the *recO* single mutant, indicating that both RecRO and AddAB pathways are important for survival of oxidative damage. Similar roles of the RecBCD and the RecFOR pathways for survival of oxidative damage were also observed in *E. coli* [57, 100] and in *Neisseria gonorrhoeae* [84]. In those bacteria, however, the RecBCD appeared to be the predominant (over the RecFOR) repair pathway for oxidative damage. Our results suggest that the two pathways in *H. pylori* play similarly important roles in repairing oxidative stress-derived DNA damage [71]. In accordance with the sensitivity to oxidative and acid stress in vitro, *H. pylori recR* and *recO* mutants were shown to be less able to colonize mouse stomachs [71]. Furthermore, the mouse colonization ability of the *addA recO* double mutant was significantly lower than that of the *addA* or *recO* single mutant. Therefore, both AddAB- and RecRO-mediated DNA recombinational repair in *H. pylori* play an important role in bacterial survival and persistent colonization in the host.

Although differing results regarding the effect of *addA* gene on transformation frequency were reported by different research groups, it was agreed that the RecRO-pathway is not involved in recombination of exogenous DNA into the *H. pylori* genome in the process of transformation [68, 71]. The RecRO pathway is known to have a major role in intragenomic recombination at repeat sequences [101]. Using an assay to assess the deletion frequency resulting from recombination on direct repeat sequences (358 bp long), Marsin et al [68] showed that the *recR* and *recO* mutants exhibited a statistically significantly lower deletion frequency than the wild type strain, suggesting a role of RecRO in intragenomic recombination. Recently we adopted a similar assay using DNA constructs (deletion cassettes) that contain identical repeat sequences of different length (IDS100 and IDS350) [71]. The results indicated that the intra-genomic recombination of 100 bp-long direct repeat sequences in *H. pylori* is partially dependent on RecR and RecA, yet a large portion of the recombination event is RecA-independent. This is basically in agreement (with small variance) with the results of Aras et al [35] who reported that the repeat sequences of 100 bp or shorter recombined through a RecA-independent pathway. For the deletion cassette containing repeat sequences of 350 bp in length, inactivation of *recR* or *recA* resulted in a significant 4-fold or 35-fold decrease respectively in deletion frequency, indicating that RecR plays a significant role in recombination of IDS350, while this recombination was highly dependent on RecA.

5. Concluding remarks and perspectives

Severe *Helicobacter pylori*-mediated gastric diseases are associated with the bacterium's persistence in the host and its adaptability to host differences, which in turn is associated with its remarkable genetic variability. DNA recombination is an extraordinarily frequent event in *H. pylori*, and this manifests itself into a bacterium with unusual flexibility in stress-combating enzymes, repair mechanisms, and other adaptability characteristics. Nearly every *H. pylori* recombination-related gene studied thus far by a gene directed mutant analysis approach has documented they are individually important in stomach colonization ability; this underscores the importance of these recombination repair processes in bacterial survival in the host. It is well recognized that homologous DNA recombination is a special system in bacteria for repairing stalled replication forks and double strand breaks, while generating genetic diversity as an advantageous byproduct [102]. *H. pylori* may be an especially fruitful organism in which to learn the ultimate boundaries in roles of recombination repair enzymes, as *H. pylori* is subject to intense and prolonged host mediated stress and it displays an enormous genetic diversity.

Substantial progress has been made recently in unraveling the complex systems of DNA recombinational repair in *H. pylori*. As expected, whole genome sequencing has been a powerful tool to aid in identifying recombination-related proteins in *H. pylori*. For example, *recA, recR, recN*, and *ruvABC* were identified and confirmed to play important roles in *H. pylori* as could be expected from results for other bacteria. Some recombination-related proteins (e.g. MutS2, RecG), however, play unique roles in *H. pylori*. Most of the genes for the major components of the two pre-synapsis pathways (RecBCD and RecFOR) were not annotated from *H. pylori* genome sequences, which drove researchers' interest to search for additional novel systems required for *H. pylori* DNA recombinational repair. Recent studies revealed the existence of both pathways, AddAB and RecRO, in *H. pylori*. Although they display a limited level of sequence homology to the known recombination enzymes, both AddAB and RecRO were shown to play important roles in *H. pylori* DNA recombinational repair, conferring resistance to oxidative and acid stress.

The major components of DNA recombinational repair machinery in *H. pylori* are listed in Table 1. *H. pylori* RecN protein may recognize DNA double strand breaks and recruits AddAB helicase-nuclease complex for further processing. While not being involved in repair of DNA double strand breaks, *H. pylori* RecRO proteins play a major role in intra-genomic recombination at repeat sequences. Both pre-synapsis pathways (AddAB and RecRO) require RecA for catalyzing DNA strand exchange (synapsis) and *H. pylori* RuvABC is the predominant pathway for DNA branch migration and Holliday Junction resolution (post-synapsis). Although the major functions of these components are similar to those observed in model bacteria, some novel attributes of these components have been discovered, which may be related to the highly-specific lifestyle of *H. pylori*. Additional new components that work synergistically with these pathways could be found in this unique bacterium via future biochemical and genetic approaches.

6. Acknowledgements

The work on *H. pylori* DNA repair in our laboratory was supported by NIH grant R21AI076569 and by the University of Georgia Foundation.

Gene	HP # (a)	Activity / function	Main phenotypes of mutant (b)	reference
recN	1393	Initiates DSB-induced recombination.	Sensitive to DSB damage; Sensitive to oxidative stress; Attenuated mouse colonization.	[83]
recJ	0348	5′-3′ ssDNA exonuclease.	Not studied experimently.	
addA	1553	AddAB Helicase-nuclease; Initiates DSB-induced recombination.	Sensitive to DSB damage; Sensitive to oxidative stress; Attenuated mouse colonization.	[69, 92]
addB	1089			
recR	0925	RecRO recombination pathway; Initiates ssDNA gap repair.	Not sensitive to DSB damage; Sensitive to oxidative stress; Attenuated mouse colonization.	[68, 71]
recO	0951			
recA	0153	DNA recombinase; Catalyzes DNA pairing and strand exchange.	Sensitive to DNA damaging agents; Decreased recombination frequency; Defective mouse colonization.	[65, 66, 69]
recG	1523	Holiday junction helicase.	Not sensitive to DNA damaging agents; Increased recombination frequency.	[76]
ruvA	0883	Holliday junction recognition.	Not studied experimently.	
ruvB	1059	Holiday junction helicase.	Sensitive to DNA damaging agents; Decreased recombination frequency.	[75]
ruvC	0877	Holliday junction resolvase.	Sensitive to DNA damaging agents; Decreased recombination frequency; Attenuated mouse colonization.	[73]

(a) HP# refers to the gene number in the genome sequence of strain 26695 [12].
(b) DSB (double strand breaks) damage refers to those damages caused e.g. by ionizing radiation, mitomycin C, or ciprofloxacin.

Table 1. *H. pylori* genes involved in DNA recombinational repair

7. References

[1] Dunn BE, Cohen H, Blaser MJ. Helicobacter pylori. Clin Microbiol Rev 1997;10(4):720-41.
[2] Suerbaum S, Michetti P. Helicobacter pylori infection. N Engl J Med 2002;347(15):1175-86.
[3] Uemura N, Okamoto S, Yamamoto S, *et al.* Helicobacter pylori infection and the development of gastric cancer. N Engl J Med 2001;345(11):784-9.
[4] McGee DJ, Mobley HL. Mechanisms of Helicobacter pylori infection: bacterial factors. Curr Top Microbiol Immunol 1999;241:155-80.
[5] Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. Clin Microbiol Rev 2006;19(3):449-90.
[6] Kang J, Blaser MJ. Bacterial populations as perfect gases: genomic integrity and diversification tensions in Helicobacter pylori. Nat Rev Microbiol 2006;4(11):826-36.
[7] Suerbaum S. Genetic variability within Helicobacter pylori. Int J Med Microbiol 2000;290(2):175-81.
[8] Falush D, Wirth T, Linz B, *et al.* Traces of human migrations in Helicobacter pylori populations. Science 2003;299(5612):1582-5.
[9] Suerbaum S, Smith JM, Bapumia K, Morelli G, Smith NH, Kunstmann E, Dyrek I, Achtman M. Free recombination within Helicobacter pylori. Proc Natl Acad Sci U S A 1998;95(21):12619-24.

[10] Kansau I, Raymond J, Bingen E, *et al.* Genotyping of Helicobacter pylori isolates by sequencing of PCR products and comparison with the RAPD technique. Res Microbiol 1996;147(8):661-9.
[11] Wang G, Humayun MZ, Taylor DE. Mutation as an origin of genetic variability in Helicobacter pylori. Trends Microbiol 1999;7(12):488-93.
[12] Tomb JF, White O, Kerlavage AR, *et al.* The complete genome sequence of the gastric pathogen Helicobacter pylori. Nature 1997;388(6642):539-47.
[13] Alm RA, Ling LS, Moir DT, *et al.* Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen Helicobacter pylori. Nature 1999;397(6715):176-80.
[14] Salama N, Guillemin K, McDaniel TK, Sherlock G, Tompkins L, Falkow S. A whole-genome microarray reveals genetic diversity among Helicobacter pylori strains. Proc Natl Acad Sci U S A 2000;97(26):14668-73.
[15] Israel DA, Salama N, Krishna U, Rieger UM, Atherton JC, Falkow S, Peek RM, Jr. Helicobacter pylori genetic diversity within the gastric niche of a single human host. Proc Natl Acad Sci U S A 2001;98(25):14625-30.
[16] Kraft C, Suerbaum S. Mutation and recombination in Helicobacter pylori: mechanisms and role in generating strain diversity. Int J Med Microbiol 2005;295(5):299-305.
[17] Bjorkholm B, Sjolund M, Falk PG, Berg OG, Engstrand L, Andersson DI. Mutation frequency and biological cost of antibiotic resistance in Helicobacter pylori. Proc Natl Acad Sci U S A 2001;98(25):14607-12.
[18] Horst JP, Wu TH, Marinus MG. Escherichia coli mutator genes. Trends Microbiol 1999;7(1):29-36.
[19] Lin EA, Zhang XS, Levine SM, Gill SR, Falush D, Blaser MJ. Natural transformation of helicobacter pylori involves the integration of short DNA fragments interrupted by gaps of variable size. PLoS Pathog 2009;5(3):e1000337.
[20] Kulick S, Moccia C, Didelot X, Falush D, Kraft C, Suerbaum S. Mosaic DNA imports with interspersions of recipient sequence after natural transformation of Helicobacter pylori. PLoS One 2008;3(11):e3797.
[21] Hofreuter D, Odenbreit S, Haas R. Natural transformation competence in Helicobacter pylori is mediated by the basic components of a type IV secretion system. Mol Microbiol 2001;41(2):379-91.
[22] Karnholz A, Hoefler C, Odenbreit S, Fischer W, Hofreuter D, Haas R. Functional and topological characterization of novel components of the comB DNA transformation competence system in Helicobacter pylori. J Bacteriol 2006;188(3):882-93.
[23] Stingl K, Muller S, Scheidgen-Kleyboldt G, Clausen M, Maier B. Composite system mediates two-step DNA uptake into Helicobacter pylori. Proc Natl Acad Sci U S A 2010;107(3):1184-9.
[24] Alvarez-Martinez CE, Christie PJ. Biological diversity of prokaryotic type IV secretion systems. Microbiol Mol Biol Rev 2009;73(4):775-808.
[25] Saunders NJ, Peden JF, Moxon ER. Absence in Helicobacter pylori of an uptake sequence for enhancing uptake of homospecific DNA during transformation. Microbiology 1999;145 (Pt 12):3523-8.
[26] Aras RA, Small AJ, Ando T, Blaser MJ. Helicobacter pylori interstrain restriction-modification diversity prevents genome subversion by chromosomal DNA from competing strains. Nucleic Acids Res 2002;30(24):5391-7.

[27] Humbert O, Dorer MS, Salama NR. Characterization of Helicobacter pylori factors that control transformation frequency and integration length during inter-strain DNA recombination. Mol Microbiol 2011;79(2):387-401.
[28] Go MF, Kapur V, Graham DY, Musser JM. Population genetic analysis of Helicobacter pylori by multilocus enzyme electrophoresis: extensive allelic diversity and recombinational population structure. J Bacteriol 1996;178(13):3934-8.
[29] Kersulyte D, Chalkauskas H, Berg DE. Emergence of recombinant strains of Helicobacter pylori during human infection. Mol Microbiol 1999;31(1):31-43.
[30] Salama NR, Gonzalez-Valencia G, Deatherage B, Aviles-Jimenez F, Atherton JC, Graham DY, Torres J. Genetic analysis of Helicobacter pylori strain populations colonizing the stomach at different times postinfection. J Bacteriol 2007;189(10):3834-45.
[31] Falush D, Kraft C, Taylor NS, Correa P, Fox JG, Achtman M, Suerbaum S. Recombination and mutation during long-term gastric colonization by Helicobacter pylori: estimates of clock rates, recombination size, and minimal age. Proc Natl Acad Sci U S A 2001;98(26):15056-61.
[32] Achtman M, Zurth K, Morelli G, Torrea G, Guiyoule A, Carniel E. Yersinia pestis, the cause of plague, is a recently emerged clone of Yersinia pseudotuberculosis. Proc Natl Acad Sci U S A 1999;96(24):14043-8.
[33] Guttman DS, Dykhuizen DE. Clonal divergence in Escherichia coli as a result of recombination, not mutation. Science 1994;266(5189):1380-3.
[34] Linz B, Schenker M, Zhu P, Achtman M. Frequent interspecific genetic exchange between commensal Neisseriae and Neisseria meningitidis. Mol Microbiol 2000;36(5):1049-58.
[35] Aras RA, Kang J, Tschumi AI, Harasaki Y, Blaser MJ. Extensive repetitive DNA facilitates prokaryotic genome plasticity. Proc Natl Acad Sci U S A 2003;100(23):13579-84.
[36] Martinez GR, Loureiro AP, Marques SA, *et al.* Oxidative and alkylating damage in DNA. Mutat Res 2003;544(2-3):115-27.
[37] Foster JW, Bearson B. Acid-sensitive mutants of Salmonella typhimurium identified through a dinitrophenol lethal screening strategy. J Bacteriol 1994;176(9):2596-602.
[38] Algood HM, Cover TL. Helicobacter pylori persistence: an overview of interactions between H. pylori and host immune defenses. Clin Microbiol Rev 2006;19(4):597-613.
[39] Chaturvedi R, Cheng Y, Asim M, *et al.* Induction of polyamine oxidase 1 by Helicobacter pylori causes macrophage apoptosis by hydrogen peroxide release and mitochondrial membrane depolarization. J Biol Chem 2004;279(38):40161-73.
[40] Ding SZ, Minohara Y, Fan XJ, *et al.* Helicobacter pylori infection induces oxidative stress and programmed cell death in human gastric epithelial cells. Infect Immun 2007;75(8):4030-9.
[41] Scott DR, Marcus EA, Wen Y, Oh J, Sachs G. Gene expression in vivo shows that Helicobacter pylori colonizes an acidic niche on the gastric surface. Proc Natl Acad Sci U S A 2007;104(17):7235-40.
[42] Wang G, Alamuri P, Maier RJ. The diverse antioxidant systems of Helicobacter pylori. Mol Microbiol 2006;61(4):847-60.

[43] Pflock M, Kennard S, Finsterer N, Beier D. Acid-responsive gene regulation in the human pathogen Helicobacter pylori. J Biotechnol 2006;126(1):52-60.
[44] Sancar A. Mechanisms of DNA excision repair. Science 1994;266(5193):1954-6.
[45] Thompson SA, Latch RL, Blaser JM. Molecular characterization of the Helicobacter pylori uvr B gene. Gene 1998;209(1-2):113-22.
[46] Kang J, Blaser MJ. UvrD helicase suppresses recombination and DNA damage-induced deletions. J Bacteriol 2006;188(15):5450-9.
[47] Modrich P. Mismatch repair, genetic stability, and cancer. Science 1994;266(5193):1959-60.
[48] Pinto AV, Mathieu A, Marsin S, Veaute X, Ielpi L, Labigne A, Radicella JP. Suppression of homologous and homeologous recombination by the bacterial MutS2 protein. Mol Cell 2005;17(1):113-20.
[49] Wang G, Alamuri P, Humayun MZ, Taylor DE, Maier RJ. The Helicobacter pylori MutS protein confers protection from oxidative DNA damage. Mol Microbiol 2005;58(1):166-76.
[50] O'Rourke EJ, Chevalier C, Pinto AV, Thiberge JM, Ielpi L, Labigne A, Radicella JP. Pathogen DNA as target for host-generated oxidative stress: role for repair of bacterial DNA damage in Helicobacter pylori colonization. Proc Natl Acad Sci U S A 2003;100(5):2789-94.
[51] Eutsey R, Wang G, Maier RJ. Role of a MutY DNA glycosylase in combating oxidative DNA damage in Helicobacter pylori. DNA Repair (Amst) 2007;6(1):19-26.
[52] Mathieu A, O'Rourke EJ, Radicella JP. Helicobacter pylori genes involved in avoidance of mutations induced by 8-oxoguanine. J Bacteriol 2006;188(21):7464-9.
[53] Huang S, Kang J, Blaser MJ. Antimutator role of the DNA glycosylase mutY gene in Helicobacter pylori. J Bacteriol 2006;188(17):6224-34.
[54] Butala M, Zgur-Bertok D, Busby SJ. The bacterial LexA transcriptional repressor. Cell Mol Life Sci 2009;66(1):82-93.
[55] Dorer MS, Fero J, Salama NR. DNA damage triggers genetic exchange in Helicobacter pylori. PLoS Pathog 2010;6(7):e1001026.
[56] Cromie GA, Connelly JC, Leach DR. Recombination at double-strand breaks and DNA ends: conserved mechanisms from phage to humans. Mol Cell 2001;8(6):1163-74.
[57] Kuzminov A. Recombinational repair of DNA damage in Escherichia coli and bacteriophage lambda. Microbiol Mol Biol Rev 1999;63(4):751-813, table of contents.
[58] Cox MM, Goodman MF, Kreuzer KN, Sherratt DJ, Sandler SJ, Marians KJ. The importance of repairing stalled replication forks. Nature 2000;404(6773):37-41.
[59] Liu Y, West SC. Happy Hollidays: 40th anniversary of the Holliday junction. Nat Rev Mol Cell Biol 2004;5(11):937-44.
[60] Fernandez S, Ayora S, Alonso JC. Bacillus subtilis homologous recombination: genes and products. Res Microbiol 2000;151(6):481-6.
[61] Skaar EP, Lazio MP, Seifert HS. Roles of the recJ and recN genes in homologous recombination and DNA repair pathways of Neisseria gonorrhoeae. J Bacteriol 2002;184(4):919-27.
[62] Courcelle J, Donaldson JR, Chow KH, Courcelle CT. DNA damage-induced replication fork regression and processing in Escherichia coli. Science 2003;299(5609):1064-7.
[63] Kidane D, Sanchez H, Alonso JC, Graumann PL. Visualization of DNA double-strand break repair in live bacteria reveals dynamic recruitment of Bacillus subtilis RecF,

RecO and RecN proteins to distinct sites on the nucleoids. Mol Microbiol 2004;52(6):1627-39.
[64] Meddows TR, Savory AP, Grove JI, Moore T, Lloyd RG. RecN protein and transcription factor DksA combine to promote faithful recombinational repair of DNA double-strand breaks. Mol Microbiol 2005;57(1):97-110.
[65] Schmitt W, Odenbreit S, Heuermann D, Haas R. Cloning of the Helicobacter pylori recA gene and functional characterization of its product. Mol Gen Genet 1995;248(5):563-72.
[66] Thompson SA, Blaser MJ. Isolation of the Helicobacter pylori recA gene and involvement of the recA region in resistance to low pH. Infect Immun 1995;63(6):2185-93.
[67] Fischer W, Haas R. The RecA protein of Helicobacter pylori requires a posttranslational modification for full activity. J Bacteriol 2004;186(3):777-84.
[68] Marsin S, Mathieu A, Kortulewski T, Guerois R, Radicella JP. Unveiling novel RecO distant orthologues involved in homologous recombination. PLoS Genet 2008;4(8):e1000146.
[69] Amundsen SK, Fero J, Hansen LM, Cromie GA, Solnick JV, Smith GR, Salama NR. Helicobacter pylori AddAB helicase-nuclease and RecA promote recombination-related DNA repair and survival during stomach colonization. Mol Microbiol 2008;69(4):994-1007.
[70] Marsin S, Lopes A, Mathieu A, Dizet E, Orillard E, Guerois R, Radicella JP. Genetic dissection of Helicobacter pylori AddAB role in homologous recombination. FEMS Microbiol Lett 2010;311(1):44-50.
[71] Wang G, Lo LF, Maier RJ. The RecRO pathway of DNA recombinational repair in Helicobacter pylori and its role in bacterial survival in the host. DNA Repair (Amst) 2011; 10: 373-379.
[72] Rocha EP, Cornet E, Michel B. Comparative and evolutionary analysis of the bacterial homologous recombination systems. PLoS Genet 2005;1(2):e15.
[73] Loughlin MF, Barnard FM, Jenkins D, Sharples GJ, Jenks PJ. Helicobacter pylori mutants defective in RuvC Holliday junction resolvase display reduced macrophage survival and spontaneous clearance from the murine gastric mucosa. Infect Immun 2003;71(4):2022-31.
[74] Robinson K, Loughlin MF, Potter R, Jenks PJ. Host adaptation and immune modulation are mediated by homologous recombination in Helicobacter pylori. J Infect Dis 2005;191(4):579-87.
[75] Kang J, Blaser MJ. Repair and antirepair DNA helicases in Helicobacter pylori. J Bacteriol 2008;190(12):4218-24.
[76] Kang J, Tavakoli D, Tschumi A, Aras RA, Blaser MJ. Effect of host species on recG phenotypes in Helicobacter pylori and Escherichia coli. J Bacteriol 2004;186(22):7704-13.
[77] Sharples GJ, Ingleston SM, Lloyd RG. Holliday junction processing in bacteria: insights from the evolutionary conservation of RuvABC, RecG, and RusA. J Bacteriol 1999;181(18):5543-50.
[78] Hirano T. At the heart of the chromosome: SMC proteins in action. Nat Rev Mol Cell Biol 2006;7(5):311-22.

[79] Haering CH, Lowe J, Hochwagen A, Nasmyth K. Molecular architecture of SMC proteins and the yeast cohesin complex. Mol Cell 2002;9(4):773-88.
[80] Kidane D, Graumann PL. Dynamic formation of RecA filaments at DNA double strand break repair centers in live cells. J Cell Biol 2005;170(3):357-66.
[81] Sanchez H, Kidane D, Castillo Cozar M, Graumann PL, Alonso JC. Recruitment of Bacillus subtilis RecN to DNA double-strand breaks in the absence of DNA end processing. J Bacteriol 2006;188(2):353-60.
[82] Sanchez H, Alonso JC. Bacillus subtilis RecN binds and protects 3'-single-stranded DNA extensions in the presence of ATP. Nucleic Acids Res 2005;33(7):2343-50.
[83] Wang G, Maier RJ. Critical role of RecN in recombinational DNA repair and survival of Helicobacter pylori. Infect Immun 2008;76(1):153-60.
[84] Stohl EA, Seifert HS. Neisseria gonorrhoeae DNA recombination and repair enzymes protect against oxidative damage caused by hydrogen peroxide. J Bacteriol 2006;188(21):7645-51.
[85] Stohl EA, Criss AK, Seifert HS. The transcriptome response of Neisseria gonorrhoeae to hydrogen peroxide reveals genes with previously uncharacterized roles in oxidative damage protection. Mol Microbiol 2005;58(2):520-32.
[86] Singleton MR, Wigley DB. Modularity and specialization in superfamily 1 and 2 helicases. J Bacteriol 2002;184(7):1819-26.
[87] Petit MA, Ehrlich D. Essential bacterial helicases that counteract the toxicity of recombination proteins. Embo J 2002;21(12):3137-47.
[88] Lestini R, Michel B. UvrD controls the access of recombination proteins to blocked replication forks. Embo J 2007;26(16):3804-14.
[89] Singleton MR, Dillingham MS, Gaudier M, Kowalczykowski SC, Wigley DB. Crystal structure of RecBCD enzyme reveals a machine for processing DNA breaks. Nature 2004;432(7014):187-93.
[90] Kooistra J, Haijema BJ, Hesseling-Meinders A, Venema G. A conserved helicase motif of the AddA subunit of the Bacillus subtilis ATP-dependent nuclease (AddAB) is essential for DNA repair and recombination. Mol Microbiol 1997;23(1):137-49.
[91] Yeeles JT, Dillingham MS. A dual-nuclease mechanism for DNA break processing by AddAB-type helicase-nucleases. J Mol Biol 2007;371(1):66-78.
[92] Wang G, Maier RJ. A RecB-like helicase in Helicobacter pylori is important for DNA repair and host colonization. Infect Immun 2009;77(1):286-91.
[93] Amundsen SK, Fero J, Salama NR, Smith GR. Dual nuclease and helicase activities of Helicobacter pylori AddAB are required for DNA repair, recombination, and mouse infectivity. J Biol Chem 2009;284(25):16759-66.
[94] Gressmann H, Linz B, Ghai R, *et al.* Gain and loss of multiple genes during the evolution of Helicobacter pylori. PLoS Genet 2005;1(4):e43.
[95] Sakai A, Cox MM. RecFOR and RecOR as distinct RecA loading pathways. J Biol Chem 2009;284(5):3264-72.
[96] Handa N, Morimatsu K, Lovett ST, Kowalczykowski SC. Reconstitution of initial steps of dsDNA break repair by the RecF pathway of E. coli. Genes Dev 2009;23(10):1234-45.
[97] Inoue J, Honda M, Ikawa S, Shibata T, Mikawa T. The process of displacing the single-stranded DNA-binding protein from single-stranded DNA by RecO and RecR proteins. Nucleic Acids Res 2008;36(1):94-109.

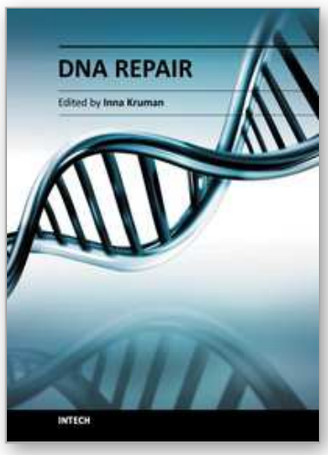
[98] Ivancic-Bace I, Peharec P, Moslavac S, Skrobot N, Salaj-Smic E, Brcic-Kostic K. RecFOR function is required for DNA repair and recombination in a RecA loading-deficient recB mutant of Escherichia coli. Genetics 2003;163(2):485-94.

[99] Bentchikou E, Servant P, Coste G, Sommer S. A major role of the RecFOR pathway in DNA double-strand-break repair through ESDSA in Deinococcus radiodurans. PLoS Genet;6(1):e1000774.

[100] Imlay JA, Linn S. Mutagenesis and stress responses induced in Escherichia coli by hydrogen peroxide. J Bacteriol 1987;169(7):2967-76.

[101] Galitski T, Roth JR. Pathways for homologous recombination between chromosomal direct repeats in Salmonella typhimurium. Genetics 1997;146(3):751-67.

[102] Cox MM. Historical overview: searching for replication help in all of the rec places. Proc Natl Acad Sci U S A 2001;98(15):8173-80.



DNA Repair

Edited by Dr. Inna Kruman

ISBN 978-953-307-697-3
Hard cover, 636 pages
Publisher InTech
Published online 07, November, 2011
Published in print edition November, 2011

The book consists of 31 chapters, divided into six parts. Each chapter is written by one or several experts in the corresponding area. The scope of the book varies from the DNA damage response and DNA repair mechanisms to evolutionary aspects of DNA repair, providing a snapshot of current understanding of the DNA repair processes. A collection of articles presented by active and laboratory-based investigators provides a clear understanding of the recent advances in the field of DNA repair.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Ge Wang and Robert J. Maier (2011). A Recombination Puzzle Solved: Role for New DNA Repair Systems in Helicobacter pylori Diversity/Persistence, DNA Repair, Dr. Inna Kruman (Ed.), ISBN: 978-953-307-697-3, InTech, Available from: http://www.intechopen.com/books/dna-repair/a-recombination-puzzle-solved-role-for-new-dna-repair-systems-in-helicobacter-pylori-diversity-persi

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen