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Genetic battle between *Helicobacter pylori* and humans. The mechanism underlying homologous recombination in bacteria, which can infect human cells

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Abstract

Helicobacter pylori is a gram-negative pathogenic bacterium that colonises the human stomach. The chronic infection it causes results in peptic ulcers and gastric cancers. *H. pylori* can easily establish a chronic infection even if the immune system attacks this pathogen with oxidative stress agents and immunoglobulins. This is attributed to bacterial defence mechanisms against these stresses. As a defence mechanism against oxidative stresses, in bacterial genomes, homologous recombination can act as a repair pathway of DNA's double-strand breaks (DSBs). Moreover, homologous recombination is also involved in the antigenic variation in *H. pylori*. Gene conversion alters genomic structures of babA and babB (encoding outer membrane proteins), resulting in escape from immunoglobulin attacks. Thus, homologous recombination in bacteria plays an important role in the maintenance of a chronic infection. In addition, *H. pylori* infection causes DSBs in human cells. Homologous recombination is also involved in the repair of DSBs in human cells. In this review, we describe the roles of homologous recombination with an emphasis on the maintenance of a chronic infection.

Keywords: *Helicobacter pylori*; Reactive oxidative species; Double-strand breaks; Genetic recombination; Genome rearrangement; Antigenic variation

In higher eukaryotes, excluding plants, the immune system plays an important role in protection against pathogens. *Helicobacter pylori* is a pathogenic bacterium causing acute gastritis as a result of inflammatory responses against this pathogen, and chronic infection manifests itself as more serious clinical outcomes, such as atrophic gastritis, peptic ulcers, and gastric cancers [1–3]. The carcinogenic pathogenesis of *H. pylori* is quite unique because carcinogenic bacteria have not been fully identified yet. However, many carcinogenic viruses have been identified, namely, hepatitis B virus (HBV), hepatitis C virus (HCV), human T-lymphotropic viruses (HTLVs), human papillomavirus (HPV), and Epstein–Barr (EB) virus [4]. Clearly, the major interest in *H. pylori* is to understand its mechanism of gastric cancer induction. To date, the mechanism of how virulence factors of *H. pylori* stimulate inflammatory responses has been well characterised [1–3]. However, the one major question that remains how *H. pylori* succeeds in the long-term colonisation of the stomach even if the immune system of the host reacts against it. To eradicate infectious pathogens, the immune system attacks with two defencive agents: reactive oxidative species (ROS) and antibodies [5]. ROS are initially produced as nitric acid (NO), a free radical with an unpaired electron, from L-arginine by nitric oxide synthase (NOS) [5,6]. The biological role of NO is to act as a messenger in intra- and intercellular signal transductions, and NO signals contribute to cell communications, vasodilation, and immunological response. Since an unpaired electron is easily transferred to other molecules, NO can easily generate other oxidative species such as hydrogen radicals and superoxide, which are also ROS. Among various oxidative species, hydrogen radicals are

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particularly dangerous because they are highly reactive to carbohydrates, lipids, proteins, and nucleic acids, and they introduce cytotoxic damage. Therefore, the immune system utilises ROS to kill infectious bacteria [5]. Of all damages caused by hydrogen radicals, the most severe damage is DSBs, because even a single unrepaired DSB can cause cell death [7]. For microbes, a strong DSB repair pathway is necessary to successfully colonise a host. In bacteria, homologous recombination plays a central role in DSB repair [8,9]. Without homologous recombination, *H. pylori* cannot colonise its host; this was experimentally proven in mouse studies [10–13]. Other defencive factors are antibodies against *H. pylori*. Individuals infected with *H. pylori* immediately create anti-*H. pylori* immunoglobulins, such as IgM, IgG, and IgA. *H. pylori* colonises the mucus layer of the gastric epithelium and does not rely on tissue invasion for survival. This ability largely contributes to the evasion of the host's immune response. In this case, secreted IgAs are the only effective attack against *H. pylori*. In general, it is believed that the role of IgA is to inhibit the host–microbe interaction and bacterial colonisation by blocking the bacterial surface factors with IgAs. For chronic infection maintenance, microbes need to vary their bacterial surface factors, such as outer membrane proteins, flagella, and lipopolysaccharide (LPS) [14–16]. Some reports suggest that *H. pylori* are capable of varying the outer membrane protein BabA by homologous recombination [17]. Thus, homologous recombination plays important role in maintaining chronic infections by bacteria. In this review, we will describe the roles of homologous recombination, emphasising on the interaction between hosts and microbes.

1. The role of bacterial homologous recombination

In bacteria, homologous recombination was originally discovered as an event of genome rearrangement during conjugation. In *Escherichia coli*, conjugation occurs between F+ and F− strains, and the F′-factor is transferred from the F+ strain to the F− strain. In addition, high frequency of recombination (Hfr) and certain plasmid vectors are transferred during conjugation. After conjugation, the transferred DNA fragment is inserted into the chromosome by homologous recombination. Some bacteria, including *Bacillus subtilis* and *H. pylori*, have the ability to incorporate environmental DNA, which is called natural transformation [18]. The DNA fragment obtained via natural transformation is usually integrated into the chromosome by homologous recombination. Homologous recombination is important because it increases genomic diversity, which in turn increases a chance of survival after various environmental changes, such as starvation, DNA damages, immunological attacks, or antibiotic stresses.

Homologous recombination plays an important role in DNA repair pathways, such as the repair of DSBs and stalled DNA replication forks. From genetic studies in *E. coli*, *B. subtilis*, yeast, knockout mice, and mammalian cells, many factors involved during homologous recombination were identified. About *H. pylori*, Dorer et al. summarised recent studies of homologous recombination in *H. pylori* [9]. Based on these knowledge, factors involved in homologous recombination was summarised in Table 1. Interestingly, *H. pylori* lacks several factors, such as RecQ, SbcCD, RecF, and RecG. Since both RecQ and SbcCD increase the accuracy of homologous recombination, chromosomes of *H. pylori* must possess higher potential of genomic diversity [19,20]. When a DSB occurs at a non-replication region, double-end breaks are formed. In this case, homologous recombination acts as a DSB repair pathway (Fig. 1A). On the other hand, if a DSB occurs at the DNA replication site, a single-end breakage could occur. In this case, homologous recombination encourages recombination-induced DNA replication to take place (Fig. 1B). Since the priA gene was discovered in *H. pylori* genome, *H. pylori* also possesses recombination-induced DNA replication.

For DSB repair, the first step of homologous recombination is to process the broken ends. At first, DNA ends are degraded by RecBCD complexes with their exo- and endonuclease activities [21,22]. When a RecBCD meets the χ-sequence, the processing mode of RecBCD switches to 5′ to 3′ exonuclease activity, resulting in the production of single-stranded DNA with a 3′-overhang [23]. In *H. pylori*, AddAB is a functional homologue of RecBCD [13]. As a RecBCD-independent pathway, the processing of DNA ends is occasionally carried out with a DNA helicase and a single-strand–specific exonuclease RecJ [24]. RecJ-dependent processing requires a DNA helicase, but the type of helicase utilised is species dependent [8,24]. In *E. coli*, RecQ is predominantly selected.

Table 1
Proteins involved in homologous recombination.

H. pylori	*E. coli*	*B. subtilis*	*H. sapiens*
End processing			
–	RecN	RecN	MRN-CtIP
AddAB	RecBCD	AddAB	
–	RecQJ	RecQJ/SJ	BLM? WRN?
UvrD	UvrD	PcrA/UvrD	
			MRN-CtIP
			EXO1
–	SbcCD	SbcCD	
Recombinase			
RecA	RecA	RecA	RAD51
			BRCA2
			RAD52
RecOR	RecOR	RecOR	
–	RecF	RecF	
			RAD54, RAD54B
			XRCC2-RAD51BD
			XRCC3-RAD51D
Holliday junction processing			
RuvAB	RuvAB	RuvAB	
RuvC	RuvC	RecU	GEN1
RecG	RecG	RecG	
?	RecQ-TopB	RecQ/S-TopB	BLM-TopoIII
			MUS81-EME1
			SLX1-SLX4
Replication restart			
PriA	PriA	PriA	
–	PriB		
–	DnaT	DnaDB	
Rep	Rep		