Chapter 6
Roles of the BabA and the SabA Adhesins in Gastroduodenal Diseases

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Abstract Adhesion is an important prerequisite for colonization and it is the initial step in infections with pathogenic bacteria. Adherence to host epithelial surfaces is the result of bacterial surface proteins, called adhesins, and their specific interaction with cognate protein- or glycoconjugate receptors on the host cells. Often, the bacteria have a set of complementary adhesins that are specific for different host receptors. Alternative mechanism has been suggested to mediate *H. pylori* adhesion, and this chapter will focus on the two well-characterized adhesins BabA and SabA. In the healthy gastric mucosa, the Lewis b antigen (Leb) is present in the gastric epithelial lining of blood group O (H-antigen), B, and A individuals. *H. pylori* binding to ABO/Leb is mediated by the blood group antigen-binding BabA adhesin. As the inflammation develops, Leb is downregulated and the levels of sialylated antigens increase. Sialyl-Lewis x/a antigens (sLex/a) are specifically recognized by the *H. pylori* sialic acid-binding adhesin SabA. Even though bacterial adherence per se cannot cause disease, adherence is considered as a crucial step in pathogenesis since it is needed for bacterial delivery of effector molecules into the host cell. The presence of receptors and host-immune responses are two factors that differently affect adhesion. To achieve long-term colonization, *H. pylori* must regulate the expression of a cognate adhesin to fit the available receptors. Adhesion to the gastric epithelial cells promotes gain of nutrients, but too tight adhesion may be intimidating because of the risk of clearance by the bacteria for life-threatening immune responses. Thus, expression levels of the adhesins must be fine-tuned in accord to host receptor expression levels. This chapter will also discuss *H. pylori* adhesion in relation to severe gastric diseases.

Keywords *Helicobacter pylori* • Adhesion • Blood group antigen-binding adhesin BabA • Sialic acid binding adhesin SabA • ABO blood group antigen/Lewis b antigen (ABO/Leb) • Sialyl-Lewis x antigen (sLex) • Homologous recombination • Slipped-strand mispairing

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S. Backert, Y. Yamaoka (eds.), Helicobacter pylori *Research*,
DOI 10.1007/978-4-431-55936-8_6

6.1 Introduction

Bacterial colonization of the human host is the initial step to establish and maintain an infection. Bacterial adhesion to host cells is mediated via ligand-receptor interactions. Bacteria recognize and bind to certain receptors, including proteins, glycoproteins, or glycolipids on the host cell mucosal surface. Oligosaccharides present on the highly glycosylated secreted and membrane-bound mucins and to glycoproteins and glycosphingolipids on the host cell surfaces act as receptors for *H. pylori* attachment. Proteins on the bacterial cell surface that facilitated binding to the host receptor are called adhesins. A given bacterium often carries multiple adhesins. Adhesins interact with their cognate receptors with high specificity, which contributes to host and tissue tropism. Some adhesins are polymorphic and recognize several receptor moieties. The affinities of adhesin-receptor interactions are variable, from very high-affinity interactions to those of low affinity. Clustering of adhesins and/or receptors can cause multivalency effects and increase the binding. Expression of the host cell receptor repertoire exhibits a wide individual variation due to genetic predisposition, as well as variation in relation to healthy or inflamed mucosa.

In gastric biopsy material, the majority of *H. pylori* are found in the mucus layer but a subset is firmly attached to the epithelial cell surface (Hessey et al. 1990). *H. pylori* has also been identified intracellularly (Aspholm et al. 2006b; Semino-Mora et al. 2003). For *H. pylori*, it is a delicate balance to on the one hand stay close to the host cell to gain nutrients and on the other hand have the capacity to loosen the grip when the host cellular response becomes too vigorous or upon host cell shedding. Adhesion of *H. pylori* to the gastric mucosa also aids the bacteria to resist the shear force during the peristaltic movements. To persistently colonize the gastric mucosa for the lifetime is challenging, but obviously *H. pylori* have the ability to adapt to the continuous changes that occur in the stomach environment. The capacity to attach to gastric epithelial cells contributes to bacterial delivery of effector molecules to the host and subsequent host responses, events that sometimes lead to the development of disease. Therefore, adhesion is a significant step in the pathogenesis of *H. pylori*-related peptic ulcer disease and gastric cancer. This chapter discusses the two best-characterized *H. pylori* adhesins, the BabA adhesin that mediate binding to fucosylated blood group antigens in healthy gastric mucosa and the SabA adhesin that recognizes sialic acid sialyl-Lewis x antigen in inflamed mucosa, how their respective expression is regulated, and their role in gastric diseases.

6.2 The Blood Group Antigen-Binding Adhesin BabA

6.2.1 *Identification of the Blood Group Antigen-Binding Adhesin BabA*

Fucosylated H antigens are complex carbohydrates with a α1.2 fucose residue linked to a terminal Galβ (Fig. 6.1). The H-antigen forms the base for the ABO blood group antigens. Addition of a fucose residue to the H-antigen forms the difucosylated Lewis b antigen (Leb) (Fig. 6.1). In blood group A individuals, the H and Leb antigens are extended with terminal N-acetylgalactosamine (GalNAc), whereas in the blood group B individuals, H or Leb have instead been extended with galactose (Gal) (Fig. 6.1). Individuals with a so-called positive secretor status also expresses the secretor (fucosyl) transferase, in saliva, tears, milk and gastrointestinal mucus secretions, and epithelium, thus adding the α1.2 fucose residue to form the H-antigen that then can be extended into the ABO blood group antigens (reviewed by (Clausen and Hakomori 1989). Individuals of nonsecretor phenotype do not express the fucosyl transferase in gastric mucus and epithelium. The first functional host receptor for *H. pylori* to be identified was the fucosylated H and Lewis b antigens (Leb) (Borén et al. 1993). Since cell lines are transformed cells, the ABO/Lewis antigens are usually not expressed at all or only in limited amount. Therefore, the finding of Leb as a functional receptor for *H. pylori* was dependent on the usage of a newly developed in vitro adherence assay (Falk et al. 1993). Fluorescently labeled *H. pylori* were overlaid on to human gastric histo-tissue sections, which showed that *H. pylori* is bound to gastric surface mucous cells. Colostrum samples reflect the individual blood group phenotype. Later the same year, Borén and co-workers (1993) used the same in vitro adherence assay in combination with colostrum samples from individuals with different Lewis blood group antigens to show that the *H. pylori* receptor on human gastric surface mucous cells contains Leb (Borén et al. 1993). *H. pylori* is a human- and primate-specific pathogen and the Leb antigen is not expressed in mice. However, *H. pylori* mediates specific adhesion to the gastric and intestinal tract of transgenic mice that specifically expressed the human α-1,3/4 fucosyl transferase (Falk et al. 1995). When these transgenic mice were infected with *H. pylori,* it was demonstrated that Leb binding was associated with the development of chronic gastritis (Guruge et al. 1998).

Fig. 6.1 Composition of fucosylated H1 and Leb ABO blood group antigens. Addition of a fucose residue to the H1 antigen forms the Leb antigen. In blood group A and B individuals, the H1 or corresponding Leb antigens are either extended with an N-acetylgalactosamine (GalNAc) or a galactose (Gal)

The first study to analyze the prevalence of Leb-receptor binding activity in a collection of strains showed that 66 % of them exhibited Leb binding (Ilver et al. 1998). Receptor-binding activity was measured using ^{125}I-labeled Leb semi-synthetic HSA glycoconjugate (Aspholm et al. 2006a; Ilver et al. 1998). Affinity analysis using Scatchard assays, in which the binding affinity, the association constant *Ka*, is calculated, showed that Leb binding in strain CCUG17875 was of high affinity (the $K_a = 1 \times 10^{-10}$) (method is described in (Aspholm et al. 2006a). Based on using electron microscopy and Leb-receptor conjugate labeled with gold particles, the number of Leb-binding adhesins was estimated to be approximately 500 per bacterial cell surface. Far-western blot analysis, i.e., receptor overlay analysis with soluble Leb-receptor conjugate, was used to identify a Leb-binding protein. To isolate the Leb-binding protein, an affinity purification method called receptor activity-directed affinity tagging (retagging) was established (Ilver et al. 1998). An N-terminal amino acid sequence of the Leb-binding adhesin was determined and used to design degenerated PCR primers for PCR amplification of a DNA fragment, which then was used as a probe for the screening of a plasmid library. Initially, two sets of alleles were identified that encoded for proteins that displayed identical N- and C-terminal domains but contained different central domains. An additional, extended N-terminal sequence made it possible to distinguish the clones and determine which that encoded for the Leb-binding protein. The corresponding protein was named the blood group antigen-binding adhesin BabA, and the gene was consequently named *babA* (Ilver et al. 1998). The gene corresponding to the second set of clones was named *babB*. The function of the BabB protein is yet elusive.

At the time it was not known that the *H. pylori* strain that was used to identify the Leb receptor expressed a BabA adhesin with a specialist phenotype, meaning that its BabA adhesin specifically recognized H1 and Leb of blood group O individuals but not A-Leb or B-Leb. This strain, P466, originates from South America, where a majority of the population is of blood group O phenotype (Aspholm-Hurtig et al. 2004). Binding of strain CCUG17875 to gastric mucosa could be blocked with Leb, A-Leb, or B-Leb, while binding of strain P466 only was blocked with Leb. Close to 400 *H. pylori* strains from different geographic regions (Sweden, Germany, Spain, Japan, and Alaska) were analyzed for their receptor-binding phenotypes, and it then became evident that 95 % of them bound to A-Leb, B-Leb, and Leb. In contrast, strains from South America (Peru, Venezuela Amazons, and Colombian mestizo) displayed different binding patterns since 60 % of them only recognized Leb and not A-Leb or B-Leb (Aspholm-Hurtig et al. 2004). Strains that only bound Leb were named "specialists," and strains that exhibited the ABO/Leb phenotype were called "generalists." Receptor-overlay analysis (far-western probed with receptor glycoconjugate) and a DNA transformation approach to shuffle specialist and generalist *babA* alleles both showed that the BabA protein alone determines the "specialist"- or the "generalist"-binding modes. Moreover, phylogenetic analysis of *babA* sequences from specialist and generalist strains suggested that long-term adaptation of the *babA* alleles occurs in relation to

the types of receptors that are available in the local population (Aspholm-Hurtig et al. 2004).

Besides binding to the host epithelial cells, the BabA adhesin mediates binding to Lewis b antigen expressed on MUC5AC, MUC5B, and MUC1 mucins (Lindén et al. 2002, 2004). BabA also exhibits binding to MUC5B in saliva and the glycoprotein gp-340 (Walz et al. 2005, 2009). An update of BabA carbohydrate-binding specificities to further explore the structural requirements for carbohydrate recognitions was recently published (Benktander et al. 2012).

6.2.2 *Location of the* babA *Gene*

The BabA adhesin was first described for strain CCUG17875 (Ilver et al. 1998). To identify its genomic location, screening of an ordered cosmid library constructed from the NCTC11638 strain (Bukanov and Berg 1994) identified two *babA* alleles and one *babB* gene (Ilver et al. 1998). DNA sequence analysis showed that the *babA1* allele in comparison with the *babA2* allele carried a 10 bp frameshifting deletion in its very 5′ end that causes a premature stop. Knockout deletion analysis confirmed that the *babA2* allele encoded for the BabA adhesin and that the *babA1* allele was silent. The results of DNA sequence analyses showed that the *babA* and the *babB* genes encode for proteins with a high level of amino acid identity in their N-terminal domains, display unique central domains, and share the ~300 most C-terminal amino acids. Both the BabA adhesin and the BabB protein belong to the Hop (*H. pylori* outer membrane porins) family that is characterized by a conserved N-terminal signal peptide, a variable middle domain, and a conserved C-terminal membrane-spanning β-sheet motif, of which the middle domain determines the specificity of the protein (Alm et al. 2000). Thus, the middle domain of BabA is likely to contain the Leb-binding domain.

Today, when the *babA* gene has been identified and sequenced in a large number of strains, it is evident that most strains carry only one *babA* allele. Comparison of the two first genome sequences of the 26695 and J99 strains showed that the *babA* and the *babB* alleles are located in reciprocal loci (Alm et al. 1999; Tomb et al. 1997). Then, the *babB* gene was found to be located in a third, alternative locus. The gene encoding for this third *bab* paralog has been named *babC* (Colbeck et al. 2006; Oh et al. 2006). The three loci where the *bab* genes are found are now called locus A, B, and C, respectively (Hennig et al. 2006). The *babA* gene is most often found in the A locus (Hennig et al. 2006; Oh et al. 2006). Some occasional strains have multiple copies of the *babA* allele, while some strains lack the gene. Noteworthy is that the majority of *H. pylori* strains seem to carry a *babB* gene.

6.2.3 Mechanisms That Switches BabA Expression On and Off

When the first genome sequences were published, high levels of homologous sequences in the 5′ and 3′ ends of the *hop* genes were found, indicating the possibility for homologous recombination events (Alm et al. 1999, 2000). In particular, Pride and Blaser reported about chimeric *babA*/*B* genes in 2 out of 42 strains (Pride and Blaser 2002). The same study suggested that *H. pylori* homologous recombination events were RecA dependent and DNase insensitive, which suggested that they probably were of intragenomic origin, i.e., gene conversion (Pride and Blaser 2002). Intragenomic changes of the *babA* gene were later found when rhesus macaques were experimentally infected with the BabA-expressing J166 *H. pylori* strain (Solnick et al. 2004). Output clones recovered 17 weeks postinfection had lost BabA expression, either by homologous recombination with *babB* creating a *babA*/*B* chimeric gene or by insertion or deletion of nucleotides in the dinucleotide CT repeat tract, 5′ of *babA*. One or two nucleotide changes in the CT repeat tract via slipped-strand mispairing cause frameshifts and thus a truncated protein. The opposite event, recombination of *babA* into the *babB* gene that results in a Lewis-binding *babB*/*A* chimera, was found in another study (Bäckström et al. 2004). Here, homologous recombination between the *bab* genes was demonstrated when Leb-binding clones were identified and isolated from an *H. pylori* strain carrying a silent *babA* allele. Clones of the Leb-binding phenotype were enriched by using biotinylated Leb conjugate and streptavidin-magnetic beads, and they were then identified using colony screening (Bäckström et al. 2004). The acquired Leb-binding clones were the result of homologous recombination of the silent *babA* allele into the *babB* gene (located in B locus). This chimeric *babB*/*A* gene carried a dinucleotide CT repeat tract in the 5′ end and was thus subjected to frequent reversible on/off phase shift variations. Phase shift variation via slipped-strand mispairing is a faster process than phase shift via homologous recombination. Homologous recombination of *babA* into the B locus in the 17875 strain was determined to occur with a frequency of 1×10^{-5} (Bäckström et al. 2004). The frequency for homologous recombination in the opposite direction, recombination of the *babB* gene into the A locus, occurs with similar frequency in strain J166, e.g., 3×10^{-5} or 3×10^{-6} per cell division (Amundsen et al. 2008). The same study showed that *bab* recombinations are promoted by RecA and the double break repair enzyme AddA. Phase shift via slipped strand occurs with a higher frequency. The *babA* gene situated in the B locus turns expression on and off with a frequency of 5×10^{-3}. Additional studies have also described homologous recombination event and slipped-strand mispairing between the *bab* genes (Colbeck et al. 2006; Hennig et al. 2006).

Besides gene conversion and slipped-strand mispairing, BabA expression can be switched off via mutations. Experimental infection of rhesus monkeys with strain J99 showed that BabA expression is turned off via single base-pair mutations (Styer et al. 2010). Another study where Mongolian gerbils were long-term infected with

H. pylori also showed that BabA expression was switched off (Ohno et al. 2011). Here output clones had switched off BabA expression due to either one base-pair insertions or deletions that introduced a premature stop codon. In addition, clones with larger deletions (31, 70, 84 bp, respectively) were also identified (Ohno et al. 2011).

Other recombination events can also switch off Leb binding. Output clones from Mongolian gerbils infected with strain 7.13 displayed BabA proteins with Leb-non-binding phenotype (Styer et al. 2010). Genetic analysis showed that the 7.13 strain carry two *babA* alleles, similar to strain CCUG17875, where a *babA2* allele encodes for a Leb-binding adhesin and the *babA1* allele is silent. In the Leb-non-binding 7.13 output clones, a DNA fragment encoding for six amino acids was replaced resulting in the expression of Leb-non-binding BabA proteins.

6.2.4 Regulation of BabA Expression Levels

The first *H. pylori* genome analyses showed that there are a few RNA polymerase (RNAP) sigma (σ) factors and a few genes that encode for transcriptional regulatory proteins (Alm et al. 1999; Tomb et al. 1997). It was also shown that the *H. pylori* genomes contain many simple sequence repeats, which are typical hot spots for slipped-strand mispairing and thus to act as contingency loci that are known to contribute to generation of heterologous populations. BabA expression levels and Leb-binding activity vary between strains, but a few studies concerning regulatory mechanisms have been conducted. BabA expression in strain CCUG17875 was found to be higher when BabA was expressed from the A locus than when expressed from the B locus (Bäckström et al. 2004). The transcriptional start sites for the *babA* gene (located in the A locus) and the *babB* gene (located in the B locus) were determined with primer extension analysis. The −10 promoter region of *babA* (5′-**TATAAT**) had a perfect match to the −10 consensus sequence of *E. coli* σ^{70} housekeeping promoters (5′-**TATAAT**) compared to the −10 promoter region of the *babB* (5′-G**ATAA**G) (Bäckström et al. 2004). Even though a consensus sequence for *H. pylori* −35 promoter element has not been determined, the −35 region of the A locus (5′-A**TGACA**) in CCUG17875 have an almost perfect match to the *E. coli* −35 promoter region (5′-**TTGACA**). Besides the differences in nucleotide composition of the binding sequences for the RNAP σ-factor (i.e., the −35 and the −10 regions), the distance between these motifs are known to affect transcription initiation. The second *babA* allele of strain CCUG17875, *babA1*, has five extra As between the −10 and the −35 promoter regions. It seems possible that the length of this A tract affects promoter activity, but it has not been confirmed experimentally. Hennig and co-workers (2006) analyzed BabA expression levels relative the spacing between the ribosomal binding site and the *babA* ATG translational start codon in 35 strains. Although there was a variation, it did not correlate to the BabA expression levels (Hennig et al. 2006). They also did not find any sequence variations in the promoter regions that were associated with the BabA

expression levels. The recent mapping of the *H. pylori* transcriptome showed the presence of many small RNAs (sRNAs) and a massive antisense transcription, which suggested that *H. pylori* uses riboregulation to regulate gene expression (Sharma et al. 2010). However, no role of riboregulation on BabA expression has so far been demonstrated.

6.2.5 BabA Expression and Gastric Disease

There is a continuous interest in the BabA adhesin and its role in disease outcome. Over the years, a series of papers have reported about BabA and its association to severe mucosal inflammation and increased risk of peptic ulcer disease and gastric cancer (Aspholm-Hurtig et al. 2004; Colbeck et al. 2006; Fujimoto et al. 2007; Gerhard et al. 1999; Hennig et al. 2004; Ilver et al. 1998; Lehours et al. 2004; Odenbreit et al. 2009; Olfat et al. 2005; Oliveira et al. 2003; Sheu et al. 2006; Song et al. 2014; Yamaoka et al. 2002; Yu et al. 2002). Different approaches have been used to evaluate the association between BabA and disease. A series of studies applied PCR to detect the presence of the *babA* gene. Often, primers that amplify the *babA2* allele are used. Using such approach, *babA* located in other locus than the A locus will not be found unless additional primer pairs are used. Other studies have analyzed BabA expression by reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR), immunoblots, or assays that detect for BabA protein expression with Leb-binding activity such when RIA assay with ^{125}I-labeled Leb-receptor conjugate is used (Aspholm et al. 2006a; Ilver et al. 1998) or when enzyme-linked immunosorbent assay (ELISA) is used (Solnick et al. 2004). The collection of clinical isolates that has been assayed for BabA expression and binding to Leb has demonstrated differences in BabA expression levels as well as differences in binding affinity to the Leb receptor among strains. These differences probably mirror the ability of *H. pylori* to adapt local environmental conditions during the lifelong persistent infection (Aspholm-Hurtig et al. 2004; Fujimoto et al. 2007; Ilver et al. 1998; Yamaoka et al. 2002).

Already in 1999, Gerhard and co-workers reported that there was an epidemiologic association between *cagA*, *vacAs1*, and *babA2* genotypes and higher incidence of ulcer disease and gastric cancer (Gerhard et al. 1999). Later, BabA expression in combination with CagA- and VacAs1-expressing strains has been confirmed to be associated with severe gastric disease (Azevedo et al. 2008; Ishijima et al. 2011). Moreover, BabA-mediated binding to Leb plays an important role for the initiation of contact-dependent signaling mediated by the type IV secretion system (T4SS). When Leb-transfected cells were infected with a wild-type *H. pylori* strain and isogenic BabA and T4SS mutants, it was shown that BabA-Leb-binding induced T4SS-dependent host cell signaling, which increased mRNA levels of genes coding for pro-inflammatory cytokines as well as precancerous-related factors. These data were also supported by in vivo data from experimental *H. pylori* infection of Mongolian gerbils (Ishijima et al. 2011).

It was recently suggested that *H. pylori* has the capacity to jeopardize the host genome integrity via direct bacterial-host cell contact. *H. pylori* adhesion, via BabA-Leb binding, induced higher levels of double-strand breaks in the chromosomal DNA of infected host cells than a *babA* deletion mutant (Toller et al. 2011). Similarly, host cells, pre-incubated with soluble Leb prior to infection, exhibited reduced double-strand break induction. Other virulence-associated factors such as the VacA cytotoxin, the γ-glutamyl transpeptidase (GGT), and the *cag* pathogenicity island (*cag*PAI) were tested but did not promote double-strand breaks in host target cells (Toller et al. 2011). DNA damages that are not precisely repaired may increase carcinogenesis.

For experimental infection, the rhesus macaque is a highly relevant animal model since they are naturally infected by *H. pylori* and because it mimics the clinical outcome observed in humans. Several studies have focused on BabA expression and Leb binding (Mahdavi et al. 2002; Styer et al. 2010). Although not naturally infected by *H. pylori*, the Mongolian gerbil is an attractive alternative experimental model for studying *H. pylori* infection, and it has been used for studies of BabA (Ohno et al. 2011; Styer et al. 2010). Mongolian gerbils were infected with the BabA-expressing strain TN2GF4 for 18 months (Ohno et al. 2011). Besides BabA expression, the degree of inflammation was followed by histological examination and by scoring mononuclear cell (MNC) and polymorphonuclear cell (PMN) infiltration during the same time span. Cellular infiltration increased after 1 month and gradually increased to reach a peak after 6 months. Gastric ulcers developed to varying degree. Output clones were examined for BabA expression and Leb binding. Similar to what has been reported for rhesus macaques and gerbils previously, BabA expression was lost during the course of infection. After 1 month, 80 % of the output clones displayed BabA expression, but the levels declined to 33 % after 3 months. No output clones expressed BabA after 6 and 18 months postinfection. Among the 1-month output clones, BabA expression levels had increased significantly. The changes in expression levels could not be explained by any changes in DNA sequences in the promoter regions nor in the *babA* open reading frame (ORF). There was also a correlation between output clones isolated from gerbils with gastric ulcer, which displayed no BabA expression. In the same study, it was suggested that the BabA expression levels directly or indirectly contribute to cellular inflammation (Ohno et al. 2011). Gerbils were infected with isolated output clones with high or low expression levels. No differences in cellular infiltration occurred after 1 month, but after 3 months, the level of cellular infiltration of gerbils infected with output clones with low BabA expression levels was lower compared to those infected with output clones with high BabA expression levels (Ohno et al. 2011). Thus, the differences in BabA expression levels as well as in Leb-binding affinity among strains are likely to affect disease outcome.

6.3 The Sialic Acid-Binding Adhesin SabA

6.3.1 H. pylori *Binding to the Inflammation-Associated Sialyl-Lewis x/a Antigen Receptor*

A *babA* knockout deletion mutant was found to adhere to gastric histo-tissue sections from an *H. pylori*-infected patient, and the binding could not be fully blocked with soluble Leb-receptor conjugate (Mahdavi et al. 2002). In contrast, no binding to histo-tissue sections from healthy individuals was detected. Together these results suggested that *H. pylori* exhibit an additional receptor-adhesin interaction for binding to the gastric mucosa. Instead of the ABO/Leb as receptors, it was shown that *H. pylori* recognizes sialyl-Lewis x and a antigens (sLex/a) in inflamed gastric mucosa (Mahdavi et al. 2002). The sLex receptor was identified using thin-layer chromatography (TLC), mass spectrometry (MS), and ^{1}H nuclear magnetic resonance (^{1}H-NMR). First, glycosphingolipids were separated using TLC, which thereafter were overlaid with *H. pylori*. Binding to the sialyl-Lewis x (sLex) and sialyl-diLewis x (sdiLex) was confirmed with monoclonal antibodies. Stronger binding was obtained to sdiLex. The glycosphingolipid bound to *H. pylori* was confirmed to be sdiLex using MS and ^{1}H-NMR (Mahdavi et al. 2002). Only minute levels of sLex are found in healthy gastric mucosa, and there is a reciprocal regulation of the fucosylated ABO/Leb and sialylated sLex expression during *H. pylori* infection (Lindén et al. 2008; Mahdavi et al. 2002). An *H. pylori* infection alters expression of several genes involved in glycan biosynthesis and in particular upregulation of the gene encoding the *N*-Acetylglucosamine (GlcNAc) transferase β3GlcNacT5 (Marcos et al. 2008). Biosynthesis of Lewis antigens is dependent on β3GlcNacT5, and when β3GlcNacT5 was overexpressed, the expression levels of sLex increased. It has also been shown that *H. pylori* colonization density increases in patients with high expression levels of sLex (Sheu et al. 2006).

6.3.2 Identification of the Sialic Acid-Binding Adhesin SabA

The retagging technique was once again used for identification and purification of an *H. pylori* adhesin. The sLex-receptor conjugate was used in affinity purification of the sialic acid-binding adhesin, SabA (Mahdavi et al. 2002). Using mass spectrometry, four peptides were found to match one gene (JHP662 in strain J99), and two of these peptides also matched a related gene (JHP659 in strain J99). Deletion mutagenesis verified that JHP662 encoded the SabA adhesin since binding to sLex was abolished. The JHP659 gene was called *sabB*. Similar to the BabA and the BabB proteins, the SabA adhesin and the SabB protein share a high degree of homologies in their N- and C-terminal domains. The SabA and SabB proteins both belong to the Hop family of proteins. Mapping of the receptor epitope revealed that the NeuAcα2-3Gal-disaccharide is the minimal sialylated binding epitope that is

required for SabA-mediated binding (Aspholm et al. 2006b). Interestingly, when clinical SabA-expressing isolates were tested for binding to a series of sialylated glycans such as sialyl-di-Lex, sLea, and sialyl-lactosamine, it was found that the majority exhibited polymorphism in sialyl-binding properties and that it is an inherent property of the SabA adhesin itself (Aspholm et al. 2006b).

6.3.3 *The SabA Adhesin Is the* H. pylori *Hemagglutinin*

H. pylori was early described to hemagglutinate human erythrocytes (Emody et al. 1988). It was for many years a debate about the *H. pylori* hemagglutinin, i.e., the protein that is responsible for sialic acid-dependent hemagglutination of erythrocytes. Initially, the HpaA protein was described as the *H. pylori* hemagglutinin (Evans et al. 1993), but construction of a deletion mutant did not abolish the hemagglutination activity (O'Toole et al. 1995). However, when a *sabA* deletion mutant strain was tested for its function in sialic acid-dependent hemagglutination of erythrocytes, no agglutination was observed, which suggested that the SabA adhesin is the *H. pylori* hemagglutinin (Aspholm et al. 2006b). An additional role of SabA is its role in neutrophil activation via binding to sialic acid-containing receptors and thus to mediate nonopsonic activation of human neutrophils and oxidative burst (Unemo et al. 2005). Besides being a key player for oxidative metabolism, SabA has also been suggested to be important for the induction of phagocytosis (Petersson et al. 2006).

6.3.4 *Mechanisms for Regulation of SabA Expression*

Screening of single clones for sLex-binding activity showed that approximately 1 % switched off their binding (Mahdavi et al. 2002). The analysis of the *sabA* DNA sequence identified a simple dinucleotide CT sequence repeat, a typical hot spot for slipped-strand mispairing and thus phase variation. sLex-binding versus sLex-non-binding clones exhibited differences in the number of CTs which generated frameshifts that either put the *sabA* ORF in or out of frame (Mahdavi et al. 2002). Variation in the number of CT repeats and frameshifts were later confirmed with immunoblot analysis and α-SabA antibodies (Lehours et al. 2004; Yamaoka et al. 2006). Similar to the *babA* gene, *sabA* can also be located in alternative genomic loci without the CT repeats in the 5′ end of the *sabA* ORF (Sheu et al. 2006). Recently, the exact location of the *sabA* and the *sabB* genes was mapped in a thorough analysis of some fifty North American clinical strains (Talarico et al. 2012). Strain J99, in which the *sabA* gene corresponds to JHP0622 (*sabA* locus), the *sabB* gene corresponds to JHP0659 (*sabB* locus), and a third gene *omp27* (*hopQ*) corresponds to JHP1103, was used as reference. In this set of strains, 84 % of the *sabA* gene was located in the *sabA* locus and the

remaining was found in the *hopQ* locus. A significant number of strains lacked the *sabB* gene, while there seemed to be a selection to maintain the *sabA* gene. The same study showed that gene conversion events occur at the *sabA*, *sabB*, and *hopQ* loci. Similar as for *babA* and *babB* gene conversions, the *sabA* gene conversion events were affected by RecA, the nuclease-helicase AddA that is involved in double-strand break repair and RecG (Amundsen et al. 2008; Talarico et al. 2012). Thus, expression of the SabA adhesin can be switch on and off via phase variation, often by slipped-strand mispairing during replication of the CT nucleotide repeat tract but also via gene conversions.

There is a variation in SabA expression levels among strains (Aspholm et al. 2006b; Sheu et al. 2006; Yamaoka et al. 2006). Differences in SabA expression levels can often be explained by differences in the promoter strength (Åberg et al. 2014). In addition, a simple thymine (T) nucleotide repeat tract is located in close proximity to the −35 region of the *sabA* promoter and was suggested to affect promoter strength (Goodwin et al. 2008; Kao et al. 2012). There are wide differences in the length of the T-tract, from T_5 to T_{28} where T_{13} to T_{19} are to the most common length (Kao et al. 2012; Åberg et al. 2014). Variation of T-tract length changes along the course of infection. Output pools from mice as well as from human antrum, and corpus stomach regions displayed variation in sLex-binding phenotype as well as T-tract length (Åberg et al. 2014).

The actual effect of changes in the T-tract length was elucidated using site-directed in vitro mutagenesis to create a series of isogenic mutants with varying numbers of Ts (Åberg et al. 2014). The analysis of promoter strength, mRNA levels, SabA protein expression, as well as sLex-binding, both to receptor conjugate and human gastric histo-tissue sections, showed a multiphasic expression pattern. The maximum and minimum mRNA and protein levels were observed with a T-tract length interval of approximately ten base pairs, which corresponds to one turn of the DNA helix (Åberg et al. 2014). The effect of T-tract length variations and *sabA* mRNA levels has been confirmed by Harvey et al. (2014).

Simple sequence repeats between the −10 and −35 promoter elements can affect the docking of the RNAP σ-factor. The *sabA* T-tract has a promoter proximal location, and the effect of the T-tract length was likely to operate by an alternative mechanism. Exchange of specific lengths of the T-tract with adenine and guanine showed that the T-tract does not only work as a spacer to adjust the size and hence expression, but it probably affects DNA topology (Harvey et al. 2014; Åberg et al. 2014). In silico and functional biochemical analysis showed the T-tracts affect the local DNA structure and thus binding of the RNAP. A model where changes in the T-tract length affect the axial alignment between the core promoter and UP-like elements has been suggested (Åberg et al. 2014). Thus, without input from known trans-acting regulators, changes in the T-tract length act as a promoter rheostat to fine-tune SabA expression. Clones with optimal SabA expression levels, which best fit the host prerequisites, survive and continue colonization. In addition to *sabA*, other genes with simple sequence repeat motifs located at similar positions in other

genes were also shown to be affect promoter output in a similar way (Åberg et al. 2014).

6.3.5 SabA Expression and Regulation by Acidic Conditions

The majority of *H. pylori* reside in the mucus layer, and a minor population (about 20 %) is situated close to the gastric epithelium (Hessey et al. 1990). There is a pH gradient from very acidic conditions in the lumen through the mucus layer to be close to neutral at the epithelium. Thus, pH is an important environmental factor that *H. pylori* have to sense and respond to. Using DNA arrays and RT-qPCR, it was shown that *sabA* expression decreases as a response to acidic conditions and that the repression is regulated via the acid responsive regulon ArsRS (Bury-Moné et al. 2004; Merrell et al. 2003; Pflock et al. 2006; Yamaoka et al. 2006). The ArsR response regulatory protein probably acts to regulated SabA expression via its direct binding to the *sabA* promoter region located −20 to +38 relative the transcriptional start site (Harvey et al. 2014). Considering the neutral pH close to the epithelium where the bacteria make use of adhesion properties, it seems logical to downregulate adhesion properties in order to loosen the grip from the shedding host gastric epithelial cell when it reaches the lumen. Alternatively, when ArsRS senses acidic pH, downregulation of binding aids the bacteria to avoid attachment to receptor moieties present on mucins where the pH is acidic and instead promotes motility and thus the ability to swim toward the environment of neutral pH.

6.3.6 SabA and Gastroduodenal Diseases

The first study to assay for sLex binding among clinical *H. pylori* isolates showed that 37 % among 95 European isolates bound to sLex. sLex-positive strains often exhibited binding to Leb as well. Among the sLex-binding strains, almost half of them showed binding to sLea (Mahdavi et al. 2002). The analysis of SabA expression in a collection of 200 clinical isolates from the USA and Colombia diagnosed with either gastritis, duodenal ulcer, or gastric cancer showed that 66 % of the gastritis isolates, 88 % of the duodenal ulcer isolates, and 89 % of the strains isolated from patients with gastric cancer exhibited SabA expression (Yamaoka et al. 2006). A study based on 145 Taiwanese clinical isolates showed that all isolates expressed BabA and 31 % expressed SabA. Here, no differences in SabA expression and outcome of disease were found (Sheu et al. 2006).

Upon *H. pylori*-induced gastritis, the gastric mucosa is infiltrated with neutrophils. The SabA adhesin seems to have an essential function in adherence of *H. pylori* to sialylated neutrophils. SabA-mediated binding to neutrophils was a

prerequisite for nonopsonic activation of neutrophils and is thus likely to have a role in phagocytosis of *H. pylori* (Unemo et al. 2005).

6.4 BabA- and SabA-Mediated Binding to Mucins

The majority of individuals are of positive secretor phenotype, i.e., express ABO/Leb antigens in the gastric mucus. *H. pylori* that resides in the stomach mucus layer binds to secreted highly glycosylated mucins (Lindén et al. 2002). The secreted MUC5AC and the MUC6 mucins are the two major mucins expressed in the stomach mucus layer (Lindén et al. 2002; Van den Brink et al. 2000). The BabA adhesin mediates binding of *H. pylori* to MUC5AC mucin in individuals of positive secretor phenotype that expresses Leb glycans (Lindén et al. 2002). In addition, *H. pylori* mediate binding to mucins carrying sLex by the SabA adhesin (Lindén et al. 2008). Similar as in humans, the glycosylation and spatial distribution of mucins change in rhesus macaques as a consequence of the *H. pylori* infection (Cooke et al. 2009; Lindén et al. 2008). Experimental infection of rhesus monkeys showed that individuals that display a weak-secretor phenotype had more stable levels of fucosylation, lower degree of inflammation, and lower bacterial infection load, whereas individuals with a positive secretor phenotype displayed increased levels of inflammation-associated (sialylated) glycans and a transient decrease in fucosylated (Leb) glycans. This suggested that the secretor phenotype determines the dynamics of mucosal glycosylation in response to *H. pylori* infection and that *H. pylori* infection is associated with an increase in sialylated (sLex/a) mucosal antigens and a reciprocal decrease in fucosylated (Leb) mucosal antigens (Lindén et al. 2008). At acidic conditions, the BabA-mediated binding to Leb-containing glycans on the mucins is abolished (Lindén et al. 2004). Moreover, the bacterial-mucin interplay seems to co-regulate both *babA* and *sabA* expression as well as bacterial growth. The effects were host specific because mucins from different individuals with different disease state caused different responses (Skoog et al. 2012). The MUC1 mucin is present in the gastric glands. Adherence to the MUC1 mucin may limit the *H. pylori* infection and protect the gastric glands from *H. pylori* colonization (Lindén et al. 2009). Thus, MUC1 act as a decoy due to the release of the MUC1 extracellular domain upon *H. pylori* binding (Lindén et al. 2009). Mice that carried a deletion in MUC1 exhibited increased *H. pylori* colonization and increased inflammation relative wild-type mice (McGuckin et al. 2007).

6.5 BabA- and SabA-Mediated Adhesion of *H. pylori* Outer Membrane Vesicles to the Gastric Mucosa

Gram-negative bacteria shed outer membrane vesicles (OMVs) (Kulp and Kuehn 2010). The exact role of OMVs is yet elusive but it is clear that they function as vehicles to deliver bacterial components to host cells. Since the composition of the OMVs represents the outer membrane of the bacteria and also contain additional components from mainly the periplasmic space, they are carrier of host-effector molecules and thus disease-promoting factors (Kulp and Kuehn 2010). *H. pylori* OMVs carrying the VacA cytotoxin are present in human gastric biopsy specimens (Fiocca et al. 1999). Two-dimensional $^{31}P^{1}H$ NMR correlation spectra have been used to determine the phospholipid composition of *H. pylori* OMVs, and comprehensive mass spectrometry analyses have been used to identify their protein composition (Olofsson et al. 2010). Phosphatidylethanolamine and cardiolipin were the dominating phospholipids (Olofsson et al. 2010), and the majority of outer membrane proteins were found in the OMVs and among them the BabA and the SabA adhesins (Mullaney et al. 2009; Olofsson et al. 2010). Adhesion is a key step for the delivery of toxins and effector molecules to target tissues. Using electron microscopy in combination with soluble Leb- and sLex-receptor conjugates and gold particles confirmed that *H. pylori* OMVs carry the BabA and the SabA adhesins and that both bound their respective receptor. Receptor displacement assay showed that BabA on intact *H. pylori* bacterial cells and OMV-BabA are bound to the Leb receptor with the same affinity, which suggested that the BabA adhesins exhibit similar folding in the outer membrane as in the OMVs (Olofsson et al. 2010). Moreover, the same study showed that both the BabA and the SabA adhesins on OMVs mediate receptor-specific adhesion to human gastric mucosa.

6.6 Conclusion and Outlook

For *H. pylori* the life in the human stomach is similar to a roller coaster ride. Close attachment to the epithelium offers a nutrient-rich, replicative niche but with a high risk of eradication by host-immune responses or clearance by shear forces caused by the peristaltic movement. Life further out in the mucus layer offers a famine lifestyle and a high risk of acid exposure. The glycans on mucins and on the gastric epithelial cells that function as receptors for *H. pylori* vary from person to person. The glycans that are expressed depend on the individual expression of the transferase enzymes involved in glycan biosynthesis. In addition to this, the *H. pylori* infection per se alters regulation of the glycan transferases, which results in variable expression levels in different locations in the stomach. Therefore, it is absolutely essential for *H. pylori* to continuously adapt its adhesion properties to fit the local gastric environment in order to stay colonized (Fig. 6.2). Detailed knowledge about the molecular terms that operate to fine-tune the expression of the BabA and SabA

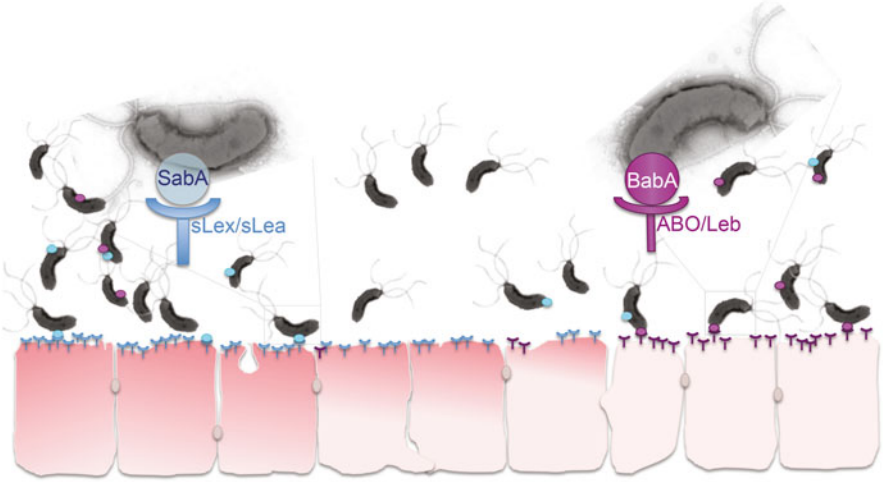

Fig. 6.2 Role of *H. pylori* adhesion in sickness and in health. The so far best-characterized *H. pylori* adhesins are the blood group antigen-binding BabA adhesin and the sialic acid-binding adhesin SabA. In the healthy gastric mucosa, the BabA adhesin binds to the fucosylated ABO/Leb antigens, while adhesion to inflamed gastric mucosa mainly is mediated by the binding of the SabA adhesin to the sLex and sLea antigens. The glycosylation of the gastric mucosa varies during the persistent *H. pylori* infection, and, therefore, the bacterium needs to adapt its adhesion properties accordingly. Expression of the BabA and the SabA adhesins can be switch on and off via homologous recombination or SSM. SSM also plays an important role in fine-tuning the expression levels of the SabA adhesin. A thymine (*T*) nucleotide repeat tract located close to the *sabA* −35 promoter element is a target for SSM, and changes in the T-tract length affect promoter strength and thus transcription initiation. Altogether, homologous recombination and SSM generate heterogeneous populations that include best-fit clones ready to adapt to any host changes such as changes in the glycosylation pattern and available receptor structures

adhesins in relation to continuously changes in the local environment during the persistent *H. pylori* infection remains to be solved.

Successful colonization is the first step for the development of chronic gastritis, peptic ulcer disease, and gastric cancer. Blocking attachment is an attractive target for future design of new drugs. Even though a large number of strains have been assayed for their Leb-binding activity and the nucleotide composition of the *babA* gene has been determined, no consensus for Leb binding in BabA has yet been determined (Aspholm-Hurtig et al. 2004). Further research to gain deep knowledge about the molecular details for ligand-receptor binding is crucial for design of drugs that can inhibit adhesion to the gastric mucosa. Therefore, structural determination will be essential to identify the Leb-receptor-binding domain. Recently, a crystal structure of a recombinantly expressed extracellular domain of the SabA protein (1–460 amino acids) was described to have a “club shape.” A conserved cavity in the SabA head domain that may function as binding site was identified. The authors made attempts to co-crystallize the protein together with sLex but did not succeed.

However, upon testing the expressed SabA protein in surface resonance experiments, they could detect binding to sLex but not to Leb, Lea, or Ley. Some binding of low affinity was also detected in Lex. This difference in binding specificity of the recombinantly expressed SabA and native SabA may be explained by differences in steric blocking, hydrophobicity, or charges in the local environment of the SabA protein (Pang et al. 2014). Based on the analysis of the SabA primary and tertiary structure of the suggested binding pocket, four highly conserved amino acid residues were mutated. A Q159A substitution reduced biding to sLex, while the Y148A and Q162A substitutions showed reduced binding to Lex, and the K152A substitution did not result affect binding to neither of sLex or Lex (Pang et al. 2014). The SabA carbohydrate-binding domain contains amino acid residues that are conserved both between SabA orthologs but also to the BabA protein (Pang et al. 2014). Further research is needed to fully determine the relation between structure and function.

Besides the BabA and the SabA adhesins, *H. pylori* have other adhesion properties that aid in attachments and probably pathogenesis, but their function is beyond the scope of this chapter. These additional attachment mechanisms have to be taken into account to fully describe the adhesion process as well as in design of future drugs.

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